KIDNEYWEEK 2013
Atlanta, GA | Nov 5 - 10

# JASN Abstract Supplement

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For example: Hsu CW, Mukherjee P, Kestenbaum BR, Himmelfarb J, Wurfel MM: The Association between Single Nucleotide Polymorphisms in the FAS Pathway with Acute Kidney Injury [Abstract]. J Am Soc Nephrol 24, 2013: 1A.

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Contact ASN
American Society of Nephrology
1510 H Street, NW, Suite 800
Washington, DC 20005
202-640-4660 phone, 202-637-9793 fax
email@asn-online.org, www.asn-online.org

**TH-OR001**

**The Association between Single Nucleotide Polymorphisms in the FAS Pathway with Acute Kidney Injury** Christine W. Hsu,[1] Paramita Mukherjee,[1] Bryan R. Kestenbaum,[1] Jonathan Himmelfarb,[1] Mark M. Wurfel.[2] *[1]Div of Nephrology, Univ of Washington, Seattle, WA; [2]ARDSNet Investigators, Div of Pulmonary & Critical Care Medicine, Univ of Washington, Seattle, WA.*

**Background:** Inflammation and apoptosis of renal tubular cells may be central to the pathophysiology of acute kidney injury (AKI). The Fas/Fas ligand pathway is a key initiator of both inflammation and apoptosis. Fas is a transmembrane protein in the tumor necrosis factor family that can induce inflammation and apoptosis upon binding Fas ligand.

**Methods:** We performed a retrospective cohort study of 401 patients who provided consent for genotyping while enrolled in the NHLBI ARDSNet Fluid and Catheter Trial (FACTT) from 2000-2005. We genotyped 376 single nucleotide polymorphisms (SNPs) on 45 genes of the Fas/Fas ligand pathway, and then used logistic regression, assuming an additive genetic model to identify associations between SNPs in Fas pathway genes and the development of AKI by day 2 after enrollment in FACTT. We classified levels of AKI using the Acute Kidney Injury Network (AKIN) criteria. In the primary analysis, we compared patients with AKI (AKIN stage 1 and above) to those without AKI (AKIN stage 0). In a sensitivity analysis, we compared patients with severe AKI (AKIN stage 2 and 3) to those without AKI (AKIN stage 0). Analyses were performed separately by race (Caucasian vs. African-American).

**Results:** In Caucasian patients, we identified associations between two SNPs and the incidence of AKI: rs1050851 and rs2233417. Both are found within the gene for nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (*NFKBIA*). For rs1050851 and rs2233417, the odds ratios (ORs) were 2.34 (95% confidence interval (CI)=1.58-3.46;p=$1.06x10^{-5}$) and 2.46 (CI=1.61-3.76;p=$1.81x10^{-5}$) for each minor allele, respectively. The associations were stronger still for AKIN stage 2-3 (severe AKI), with ORs 4.00 (CI=2.10-7.62;p=$1.05x10^{-5}$) and 4.03 (CI=2.09-7.77;p=$1.88x10^{-5}$) for each minor allele of rs1050851 and rs2233417, respectively.

**Conclusions:** In critically ill Caucasian patients with ALI, the presence of minor alleles in two SNPs in *NFKBIA* was strongly associated with the development of AKI.

*Funding:* NIDDK Support

**TH-OR002**

**Explaining the Temporal Trend in Dialysis-Requiring Acute Kidney Injury in the U.S.** Raymond K. Hsu, Charles E. McCulloch, Chi-Yuan Hsu. *Univ of California, San Francisco.*

**Background:** The U.S. population incidence of dialysis-requiring acute kidney injury (AKI-D) has increased rapidly, but reasons for this temporal trend are incompletely understood.

**Methods:** We analyzed data from the 2006-2010 Nationwide Inpatient Sample, a nationally representative sample of hospitalizations, and identified cases of AKI-D using validated ICD9 codes. To explore potential reasons for the temporal trend in risk of AKI-D among hospitalized patients, we identified the top 15 diagnoses and the top 15 procedures most strongly associated with AKI-D. We then used multivariate logistic regression to examine the degree to which accounting for the 15 diagnoses or for the 15 procedures attenuated observed temporal trends in AKI-D.

**Results:** From 2006-2010, the odds of developing AKI-D among hospitalized patients in the U.S. increased by 9% per year (unadjusted OR 1.09, 95% CI 1.07-1.11). After adding in sex, age, race, and the 15 top medical diagnoses as covariates, the temporal trend was completely accounted for (fully adjusted OR 0.97, 95% CI 0.95-0.98). Using backward selection, we determined that the increase in incidence of AKI-D appears to be driven by increases in both acute diagnoses (respiratory failure, septicemia, shock) and chronic diagnoses (chronic kidney disease, liver disease, hypertension) among hospitalized patients. In the second analysis, temporal trends in the top 15 procedures (such as cardiac and vascular surgeries) did not correlate well with changes in the risk of AKI-D.

**Conclusions:** Increasing rates of AKI-D observed in the U.S. appear to correlate with higher burden of acute and chronic conditions (such as infectious and cardiovascular disease) among hospitalized patients but not with increases in surgeries and procedures. This suggests that AKI prevention efforts should target these high-risk populations with multiple co-morbidities.

*Funding:* NIDDK Support

**TH-OR003**

**Pre-Operative Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blocker Use and Acute Kidney Injury in Patients Undergoing Cardiac Surgery** Steven G. Coca, Amit X. Garg, Madhav Swaminathan, Heather Thiessen Philbrook, Cary Steven Passik, Jay L. Koyner, Chirag R. Parikh. *TRIBE-AKI Consortium.*

**Background:** Using either an angiotensin converting enzyme inhibitor (ACEi) or an angiotensin receptor blocker (ARB) the morning of surgery may lead to 'functional' post-operative acute kidney injury (AKI), measured by an abrupt increase in serum creatinine. Whether the same is true for 'structural' AKI, measured with urinary biomarkers for tubular damage, is unknown.

**Methods:** The TRIBE-AKI study was a prospective cohort study of 1594 adults undergoing cardiac surgery at 6 hospitals between July 2007 and December 2010. We classified the degree of exposure to ACEi/ARB into three categories: "none" (no exposure prior to surgery), "held" (on chronic ACEi/ARB but held on the morning of surgery), or "continued" (on chronic ACEi/ARB and taken the morning of surgery). The co-primary outcomes were "functional" AKI based upon changes in pre to post-operative serum creatinine, and "structural AKI", based upon peak post-operative levels of 4 urinary biomarkers of tubular damage.

**Results:** Across the three levels (none, held and continued) of ACEi/ARB exposure, there was a graded-increase in functional AKI, as defined by a the incidence in a rise in creatinine of 50% or 0.3 mg/dL; (31%, 34%, 42%, p for trend 0.03) and by mean percentage changes in serum creatinine from pre to post-operative (25%, 26%, and 30%, p for trend 0.03). In contrast, there were no differences in structural AKI across the strata of ACEi/ARB exposure, as assessed by concentrations of four AKI biomarkers (NGAL, KIM-1, IL-18, or L-FABP; p for trend NS for all).

**Conclusions:** Pre-operative ACEi/ARB usage was associated with more functional but not more structural acute kidney injury. As AKI from ACEi/ARB in this setting is unclear, interventional studies testing different strategies of peri-operative ACEi/ARB use are warranted.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

**TH-OR004**

**Using e-Alerts for AKI – Recording Incidence, Measuring Outcomes and Guiding Education** Christopher J. Mulgrew, Allie Wilson, Barbara A. Flower. *Dept of Renal Medicine, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom.*

**Background:** AKI is common and complicates ~15% of hospital admissions. The UK NCEPOD report 'Adding Insult to Injury' evidenced suboptimal care given to some patients who died with AKI - 20% of AKI post-admission being predictable and avoidable. Accurate data on AKI in our hospital was unknown. Changes to antimicrobial prescribing resulting in a higher usage of gentamicin also led to reports of an increase in AKI. Two audits (one as part of a national audit) were then initiated.

**Methods:** Prospective data on all renal referrals over a six month period was collected, reviewing source of referral, AKI stage, if the AKI occurred pre- or post-admission and potential causes, length of stay and 90-day mortality. An e-alert was also added to our pathology system, generating AKI 'flags' for all cases of AKI, graded according to stage following the KDIGO AKI definition. Monthly reports were run and data analysed by AKI stage, location and clinical speciality.

**Results:** In the prospective audit, most referrals had AKI3 (76.7%), an average of 4 AKI risk factors and ~70% occurring pre-admission. Length of stay was significantly increased, from a hospital average of 5 days, to 14d for AKI3. AKI post-admission increased length of stay further to 22d (AKI3). Overall in-hospital mortality of those referred was around 15%. e-alerts from Aug 2012-Apr 2013 recorded 50-60 cases of AKI3 per month, 50% occurring pre-admission. Overall mortality was 32.3%, usually due to hypotension and/or sepsis. Analysis of AKI1-3 showed up to 500 cases/month, the majority being AKI1. 2/3 of the cases of AKI1 were post-admission, occurring where co-morbidities and sepsis were often seen.

**Conclusions:** AKI increases hospital length of stay and overall morbidity is high. The use of e-alerts increased awareness of the prevalence of AKI in our hospital. We can now target areas where AKI occurs most frequently, using local guidelines and our AKI outreach nurses to increase awareness of AKI amongst junior medical and nursing staff. The impact of these interventions will need to be re-audited but as reported elsewhere (e.g. Selby *et al* in Derby, UK), they can be effective at reducing mortality and cost.

*Funding:* Government Support - Non-U.S.

**TH-OR005**

**Acute Kidney Injury prior to End-Stage Renal Disease Predicts Poor Dialysis Outcomes** Timmy C. Lee,[1,2] Anthony Leonard,[1] Pratik Parikh,[3] Charuhas V. Thakar.[1] *[1]Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Medicine, Univ of Alabama at Birmingham, Birmingham, AL; [3]Biomedical, Industrial and Human Factors Engineering, Wright State Univ, Dayton, OH.*

**Background:** Previous studies have not evaluated the role of pre-end stage renal disease (ESRD) acute kidney injury (AKI) on major clinical outcomes at or after hemodialysis (HD) initiation.

**Methods:** We evaluated 47,327 adult incident hemodialysis patients (age ≥ 18) from the United States Renal Data System (USRDS) who initiated HD from January 1, 2008 to December 31, 2008 and had pre-ESRD Medicare claims during the two calendar years prior to incident HD. Using administrative records, pre-ESRD AKI was assessed as a risk factor for two separate outcomes: type of vascular access [catheter versus arteriovenous (AV) access] at HD initiation and one-year mortality after HD. Both unadjusted risks, and risks adjusted (by logistic regression) for demographic and comorbidity factors were calculated.

**Results:** Mean age at HD initiation was 72 years, 54% were male, 70% were white, and mean BMI was 29. 18% of patients initiated hemodialysis with an AV access (14% with AV fistula) and 82% with a catheter/others. 32% of patients died within 12 months of HD initiation. 53% of total patients had an AKI event, during inpatient stay in the pre-ESRD period. Patients with pre-ESRD AKI event were more likely to initiate HD with catheter compared to those without pre-ESRD AKI event (87% vs 74 %; p<0.0001) and less likely with an AVF (8% vs 20%; p<0.0001); the adjusted risk of starting HD with a catheter was higher in pre-ESRD AKI group [odds ratio (OR) = 2.2, 95% confidence interval (CI) = (2.1, 2.3)] After adjusting for access type and other covariates, pre-ESRD AKI was associated with higher risk of 1-year mortality in HD (OR = 1.6, 95% CI = (1.5, 1.6)).

**Conclusions:** AKI events prior to ESRD predict higher rates of catheter use in HD patients, and higher one year mortality when adjusted for access type. Preventing AKI and improving processes of care after AKI may improve dialysis outcomes. Biological and process-of-care relationships between these events need further analyses.

## TH-OR006

**De Novo Acute Kidney Injury and Subsequent Chronic Kidney Disease: Analysis of a National Sample of Veterans** Michael Heung,[1] Diane Steffick,[1] Tanushree Banerjee,[2] Neil R. Powe,[2] Meda E. Pavkov,[3] Desmond Williams,[3] Vahakn B. Shahinian.[1] *[1]Univ of Michigan, Ann Arbor; [2]Univ of California San Francisco; [3]Centers for Disease Control and Prevention, Atlanta, GA.*

**Background:** Acute kidney injury (AKI) is a risk factor for chronic kidney disease (CKD). We compared the odds of developing CKD within 1 year of discharge in patients with & without in-hospital AKI and also stratified by AKI type.

**Methods:** Patients with 1+ outpatient visit in 2005 and 1+ hospitalizations of >1 day with 2+ inpatient serum creatinine (SCr) tests in 2005-8 were selected from a 5% sample of national Veterans Affairs data. Baseline was the SCr closest to 1 week before admission but <1 year; if not available, then a SCr <7 days before admission or the first inpatient SCr was used. Patients with baseline MDRD-eGFR <60 ml/min/1.73m$^2$ and those who died within 1 year of discharge were excluded. CKD by 1 year was defined as: ICD9 codes, dialysis, transplant, or eGFR closest to 1 year <60. AKI was defined as a 0.3 mg/dL increase in SCr from baseline to peak. Subgroups of AKI were: pre-renal (SCr decrease within 2 days of peak), recovered AKI (last inpatient eGFR >60) or non-recovered AKI (eGFR <60). Logistic models estimated the association of AKI with CKD within 1 yr, after adjusting for age, race, sex, diabetes, and hypertension.

**Results:** Of the 13,563 patients in the sample, 1,937 (14.3%) had CKD by 1 year; 26.3% of those with AKI & 11.5% without AKI. Those with AKI had 2.5 times the odds of having CKD as those without. All AKI subgroups had elevated odds of CKD compared with those without.

Association of Acute Kidney Injury (AKI) with Development of Chronic Kidney Disease Within 1 Year, N=13,563

| | N | Adjusted OR | 95%CI | p-value |
|---|---|---|---|---|
| Model 1: | | | | |
| No acute kidney injury | 11,048 | 1.00 | ref | ref |
| AKI | 2,515 | 2.51 | 2.25-2.80 | <0.001 |
| Model 2: | | | | |
| No acute kidney injury | 11,048 | 1.00 | ref | ref |
| Pre-renal AKI | 1,425 | 2.14 | 1.87-2.46 | <0.001 |
| AKI with hospital recovery | 667 | 1.63 | 1.31-2.01 | <0.001 |
| AKI without hospital recovery | 423 | 6.32 | 5.15-7.76 | <0.001 |

Adjusted for age, race, sex, diabetes and hypertension.

*Chronic kidney disease within 1 year= serum creatinine (SCr) closest to 1 year post-discharge yielding MDRD eGFR<60, diagnosis of CKD by ICD-9-CM codes, maintenance dialysis, or transplant.*
*Acute kidney injury=rise in SCr > 0.3 mg/dL from baseline to peak inpatient level.*
*Pre-renal AKI=AKI with sCr decreasing in hospital to < baseline sCR+0.3 mg/dL within 2 days of peak inpatient level.*
*AKI with hospital recovery=AKI with last inpatient SCr yielding MDRD eGFR > 60 ml/min/1.73m$^2$*
*AKI without hospital recovery=AKI with last inpatient SCr yielding MDRD eGFR < 60 ml/min/1.73m$^2$*

**Conclusions:** De novo AKI is a strong risk factor for CKD. This relation persisted for AKI patients with rapid resolution of kidney function, suggestive of pre-renal AKI, often considered benign, and also for patients who appeared to recover from AKI during that hospitalization.

*Funding:* Other U.S. Government Support

## TH-OR007

**Community-Acquired Acute Kidney Injury: Hospital Admissions and Outcomes** Adil Hazara, Sunil Bhandari. *Hull and East Yorkshire Hospitals NHS Trust, United Kingdom.*

**Background:** Hospital-based emergency services are often the first point of contact for patients who develop Acute Kidney Injury (AKI) in the community. We have reviewed patterns of AKI presentation and their relationship with length of hospital stay, re-admissions and mortality.

**Methods:** Admissions with AKI were identified using a computerized algorithm developed in accordance with Acute Kidney Injury Network (AKIN) criteria. Baseline, admission, peak and final SCr values were reviewed retrospectively along with data relating to patient demographics, reasons for admission and outcomes.

**Results:** 172 patients were identified to have AKI in one month (1.8% of all hospital attendances). Mean age: 72 (range 19–100) years; Males: 76 (44%). Of these, 99 (58%), 50 (29%) and 23 (13%) patients had stage 1, 2 and 3 AKI respectively.

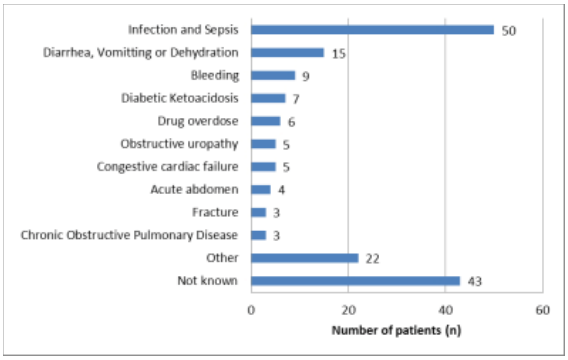



**Fig 1. Reasons for admission in patients with community-acquired AKI**

54 (31%) patients died within 30 days of admission. Mortality in AKI stage 1 (n=99), 2 (n=50) and 3 (n=23) groups was 23%, 42% and 43% respectively. Length of hospital stay also increased with AKI stage. After excluding deceased patients, mean length of stay for all (n=123), stage 1 (n=78), 2 (n=32) and 3 (n=13) patients were 10.2 +/-1.7, 9.1 +/-1.2, 10.7 +/-2.4 and 16.2 +/-5.3 (days +/-standard error) respectively.

AKI resolution (SCr return to 120% of baseline) was in-complete in 60 (49%) cases at the time of discharge. Of these, 21 (35%) were re-admitted or died within 30 days. The relative risk for '30-day re-admission or mortality' was 2.2 (95% confidence intervals 1.1 to 4.3, p=0.02) when compared to those whose AKI resolved.

**Conclusions:** Community-acquired AKI is associated with diverse range of medical presentations. Its severity can be a marker of increased length of hospital stay and mortality. Non-resolution of AKI at the time of discharge significantly increases risk of further re-hospitalization or death.

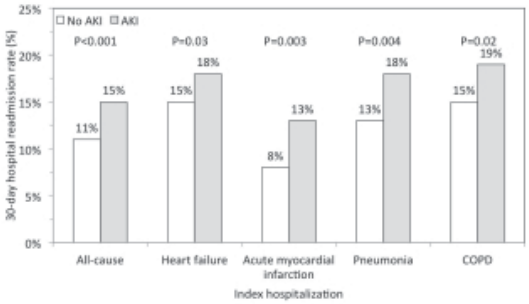
## TH-OR008

**Hospital-Acquired Acute Kidney Injury (AKI) and Early Readmission: A Cohort Study** Ioannis Koulouridis,[1,2] Lori Lyn Price,[3] Nicolaos E. Madias,[1,2] Bertrand L. Jaber.[1,2] *[1]Dept of Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Dept of Medicine, Tufts Univ School of Medicine, Boston, MA; [3]Clinical Research and Health Policy Studies, Tufts Medical Center & Tufts CTSI, Tufts Univ, Boston, MA.*

**Background:** AKI is associated with increased mortality and resource consumption. We examined whether survivors of hospital-acquired AKI are at an increased risk for early hospital readmission.

**Methods:** This was a single-center retrospective cohort study of 22,001 adults hospitalized for the first time (index) at an acute care facility and discharged alive. The nadir-to-peak serum creatinine difference during the index hospitalization was used to define AKI according to the AKI network criteria. Multivariable logistic regression analyses examined the association of AKI with 30-, 60-, and 90-day hospital readmission adjusting for age, sex, race, Charlson-Deyo comorbidity index, acute hospital-related factors, and baseline eGFR.

**Results:** The incidence of AKI was 15.2%. The 30-day hospital readmission rate was higher among patients with AKI for all-cause and condition-specific index hospitalizations (Figure).




Survivors of AKI episodes had 1.21 higher adjusted odds for 30-day hospital readmission (95% CI 1.08, 1.36), which persisted at 60 (OR 1.15; 95% CI 1.03, 1.27) and 90 (OR 1.13; 95% CI 1.02, 1.25) days. AKI was associated with higher odds for 30-day hospital readmission in patients originally hospitalized on medical services (OR 1.29; 95% CI 1.14, 1.46), younger patients (OR 1.54; 95% CI 1.26, 1.89), those with fewer comorbidities (OR 1.34; 95% CI 1.16, 1.56), and higher baseline eGFR (OR 1.20; 95% CI 1.04, 1.39).

**Conclusions:** Survivors of hospital-acquired AKI experience a higher risk of early hospital readmission. Transitions of care services may be warranted for such patients to prevent readmissions and reduce healthcare costs.

**TH-OR009**

**Acute Kidney Injury (AKI) Epidemiology in Very Low Birth Weight (VLBW) Infants** Neha Patil, Rajesh Koralkar, Susan Keeling, Namasivayam Ambalavanan, David J. Askenazi. *Pediatrics, Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** AKI is associated with mortality in neonatal and pediatric critically ill populations, but the true incidence, risk factors and outcomes in VLBW are yet fully known.

**Objective:** To determine incidence, risk factors and outcome of development of AKI in first two weeks of life in VLBW Infants.

**Methods:** Between Feb 2012 to Feb 2013, 91 VLBW Infants (birth weight <=1200 gm. or gestational age <31 weeks) were prospectively followed. Serum creatinine (SCr) values collected on days 1,2,3,4,and 14; and combined with clinically measured SCr were used to determine AKI status according to KDIGO. Stage 1 AKI was defined as SCr ≥ 0.3 mg/dl or ≥ 150-200% from lowest previous value. Stage 2: SCr > 200-300% from previous value and Stage 3 AKI: SCr > 2.5 mg/dl or SCr > 300% from previous value. Bronchopulmonary dysplasia (BPD) was defined if an infant was oxygen dependent at 28 days. Those who were alive at 36 weeks post-conception or discharged home were deemed survivors.

**Results:** Cumulative incidence of AKI over first two weeks of life was (27/87) 31%. Differences in infant and maternal demographics and co-morbidities and outcomes between AKI and No AKI showed that female, white race, lower gestational age, lower 5 minute Apgar and UAC were risk factors for AKI (p value <0.05), while pre-eclampsia was inversely associated with AKI (p value <0.001). AKI group had higher BPD, trend towards longer hospital stay, and lower survival.

| Cumulative Incidence of AKI by day of life | | | |
|---|---|---|---|
| **AKI Stage** | **Day 3** | **Day 7** | **Day 15** |
| **AKI 1** | 14 (16%) | 21 (24%) | 22 (25%) |
| **AKI 2** | 1 (1%) | 1 (1%) | 2 (2%) |
| **AKI 3** | 1 (1%) | 2 (2%) | 3 (4%) |
| **AKI Incidence** | 16 (18%) | 24 (27%) | 27 (31%) |
| **No AKI** | 75 (82%) | 65 (73%) | 60 (69%) |
| | | | |
| **Outcome for those with and without AKI at day 15** | | | |
| **Outcome** | **No AKI (N=60)** | **AKI (N=27)** | **P value** |
| **BPD** | 21 (37%) | 17 (71%) | 0.005 |
| **Survivor** | 58 (97%) | 22 (81%) | 0.01 |
| **Length of stay (days)** | 77 (15-232) | 92 (6-209) | 0.2 |

**Conclusions:** AKI is common in VLBW infants. Development of AKI in first two weeks of life can result in higher incidence of BPD and lower survival.

**TH-OR010**

**The Daily Burden of Acute Kidney Injury: A World Kidney Day Survey of U.S. Nephrologists** Jay L. Koyner,[1] Jorge Cerda,[1] Stuart Goldstein,[1] Bertrand L. Jaber,[1] Kathleen D. Liu,[1] Judy A. Shea,[2] Sarah Faubel.[1] *[1]AKI Advisory Group of the American Society of Nephrology; [2]General Internal Medicine, Univ of Pennsylvania.*

**Background:** This year, World Kidney Day (WKD, March 14, 2013) focused on raising awareness of the short- and long- term consequences of AKI.

**Methods:** To estimate the extent of in-hospital and outpatient nephrology effort devoted to the care of patients with AKI, we conducted an internet-based survey of all (n=4957) US-based ASN physician members, on WKD.

**Results:** We received survey responses from 598 nephrologists (12% response rate). 49% worked in a teaching hospital with a median of 398 beds. Nephrologists were asked about patients seen on WKD. Respondents saw a median (25th, 75th percentile) of 12 (5, 18) patients in the hospital compared to 8 (5, 12) patients seen in clinic. A median of 4 (1, 6) patients seen in the hospital had pre-existing ESRD while 5 (2, 8) patients had AKI (p=0.002). In total, most patients seen in the hospital had AKI, comprising 46% (n=1500) of the sample, followed by ESRD (38%; n=1233). Among patients seen in the hospital for AKI, a median of 2 (1, 3) were critically ill, and 1 (0, 2) required renal replacement therapy (RRT). The delivered dose of intermittent hemodialysis was measured in only 17%. Among patients seen in clinic, a median of 1 (0, 1) patient was seen for follow-up of in-hospital AKI; 55% reported that in the prior year, <10% of patients seen in clinic were for follow-up of in-hospital AKI.

**Conclusions:** AKI is the most common in-hospital diagnosis seen by US nephrologists. Patients with AKI are often critically ill and receive RRT. However, the delivered dose of intermittent hemodialysis is infrequently measured, and survivors of in-hospital AKI do not comprise a large part of outpatient clinic encounters, and might be lost to nephrology follow-up. These findings call for continued efforts to promote excellence in the delivery of RRT to patients with AKI, and reliable transition of care services following hospital discharge from an AKI episode.

*Funding:* Private Foundation Support

**TH-OR011**

**Id Proteins Regulate Capillary Repair and Perivascular Cell Proliferation following Ischemia-Reperfusion Injury** Matthew D. Plotkin, David H. Lee, Shantheri S. Shenoy, Yezina T. Nigatu. *Medicine, New York Medical College, Valhalla, NY.*

**Background:** Acute kidney injury (AKI) results in microvascular damage that may lead to fibrosis. The Id1 and 3 proteins promote angiogenesis during development and tumor growth by functioning as dominant negative regulators of bHLH transcription factors. The goal of this study was to determine if Id proteins regulate microvascular repair and if increased Id1 expression reduces capillary loss following AKI.

**Methods:** The effect of changes in Id expression *in vivo* on microvascular repair following ischemia-reperfusion injury (IRI) was examined using Id1 -/-, Id3RFP/+ (Id1/3 KO) and Tek (Tie2)-rtTA, TRE-lacz/TRE Id1 (TRE Id1) mice with doxycycline inducible endothelial Id1 and β-galactosidase expression.

**Results:** Id1 and 3 were co-localized in endothelial cells in adult kidneys and protein levels were increased at day 3 following IRI. In comparison with WT littermates, Id1/3 KO mice had decreased baseline capillary density and pericyte coverage and increased tubular damage following IRI but no fibrosis. TRE Id1 mice had no capillary rarefaction and dilated medullary peritubular capillaries. Following IRI, X-gal positive interstitial cells were located in areas of collagen deposition in doxycycline treated TRE Id1/ TRE-lacz mice while positive cells from doxycycline treated single transgenic TRE-lacz mice were confined to capillary lumens. TRE Id1 mice had increased proliferation of Id1/PDGFRβ positive interstitial cells and medullary collagen deposition. These differences were associated with increased Angiopoietin 1 (Ang1) and decreased Ang2 expression in TRE Id1 mice. Examination of gene expression in microvascular cells isolated from WT, Id1/3 KO and TRE Id1 mice showed increased Ang1 and αSMA in Id1 overexpressing cells and decreased endothelial and pericyte markers in cells from KO mice.

**Conclusions:** Transgenic endothelial Id1 expression resulted in reduced capillary rarefaction and increased perivascular fibroblast proliferation and matrix deposition following IRI. Id1 was expressed in perivascular cells in areas of fibrosis and was associated with increased Ang1 levels, suggesting a role in abnormal microvascular remodeling following injury.

**TH-OR012**

**Differentiated Kidney Epithelial Cells Repair Injured Proximal Tubule without Evidence for Intratubular Progenitors** Tetsuro Kusaba, Matthew A. Lalli, Akio Kobayashi, Benjamin D. Humphreys. *Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Whether kidney proximal tubule harbors a scattered progenitor population is a major unsolved question. Lineage-tracing studies, histologic characterization and ex vivo functional analysis results conflict.

**Methods:** To address this controversy, we analyzed the lineage and clonal behavior of terminally differentiated proximal tubule epithelial cells after injury. We knocked in a Cre-ERt2 cassette into the sodium-dependent inorganic phosphate transporter SLC34a1 locus, which is expressed only in fully differentiated proximal tubule.

**Results:** After crossing this new CreERt2 line to a reporter (R26Tomato), we validated that tamoxifen-dependent recombination was absolutely specific to proximal tubule. In vivo clonal analysis after injury and repair showed that the bulk of labeled cells proliferate after injury with increased clonal expansion after severe compared to moderate injury. For example, clone size-frequency analysis before and after injury revealed that the fraction of clones 5 cells or larger increased from 0 before injury to 24.1 +/- 8.5 % after injury,avg +/- SE, $p < 0.01$. Single labeled proximal tubule cells not only expressed injury (KIM-1) and proliferation (Ki67) markers but also transiently expressed vimentin and Pax-2, markers thought to identify intratubular epithelial progenitors in other species. This finding was recapitulated by ex vivo proximal tubule culture. After complete labeling of proximal tubule followed by injury and repair, there was no dilution of the fate marker despite substantial proliferation, strongly arguing against an undifferentiated intratubular progenitor. When single proximal tubular cells were labeled at e14.5, clone size expanded by P14, suggesting a similar mechanism of tubule elongation during growth as in adult repair.

**Conclusions:** These findings provide no evidence for a scattered intratubular progenitor population in mouse proximal tubule epithelia but indicate that fully differentiated epithelia re-express apparent progenitor markers during injury-induced dedifferentiation and repair.

**TH-OR013**

**Exploring the Origin and Limitation of Kidney Regeneration** Motoko Yanagita,[1] Tomomi Endo,[1] Tomohiko Okuda,[1] Jin Nakamura,[1] Misako Asada,[1] Masayuki Takase,[1] Ryo Yamada,[1] Sato Yuki,[1] Koji Takaori,[1] Akiko Oguchi,[1] Taku Iguchi,[1] Eri Muso.[2] *[1]Nephrology, Kyoto Univ, Japan; [2]Nephrology, Kitano Hospital, Osaka, Japan.*

**Background:** Recent epidemiological data indicates that acute kidney injury (AKI) increases the risk for chronic kidney disease (CKD), however the molecular mechanism remains unknown.

Genetic fate mapping experiments recently demonstrated that kidney tubular epithelial cells are repaired by surviving nephrons after AKI. However, the cell population in the nephron that repairs damaged kidneys has not been identified. It is also unknown whether there are repair capacity limitations in the kidney.

**Methods:** To answer these questions, we generated new transgenic mouse strain (*NDRG1*$^{CreERT2/+}$ mice) in which more than 90 % of mature proximal tubule, the segment mostly damaged during AKI, can be genetically labeled at desired time points by the administration of tamoxifen.

**Results:** Genetic fate mapping using this mouse strain showed the massive proliferation of mature proximal tubule cells during repair, with no dilution of genetic label being observed in the proximal tubules even after repeated repair processes. These results indicated that proximal tubules repair themselves by their own proliferation. We further demonstrated that repeated kidney injuries lead to significant shortening of the proximal tubules, renal atrophy and renal dysfunction, and clarified the limitations in the kidney's capacity to repair.

**Conclusions:** Our current findings demonstrate for the first time that repeated AKI leads to the shortening of proximal tubules, renal atrophy and renal dysfunction, and clarify the limited regenerative capacity of mature proximal tubule cells. These results indicate that

repaired kidney is, although similar, non-identical to healthy kidney, and these findings might explain, at least in part, why reversible episodes of AKI leads to the progression of CKD.

*Funding:* Government Support - Non-U.S.

**TH-OR014**

**KIM-1-Mediated Phagocytosis Induces Autophagy and MHC Antigen Presentation in Epithelial Cells** Craig R. Brooks,[1] Hui Chen,[2] Joel M. Henderson,[2] Takaharu Ichimura,[1] Joseph V. Bonventre.[1] *[1]Renal Div, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Dept of Pathology, Boston Univ, Boston, MA.*

**Background:** Phagocytosis of apoptotic cells (AC) by both professional and semi-professional phagocytes is required for resolution of organ damage and maintenance of immune tolerance. KIM-1 is the most up-regulated proximal tubule protein following kidney injury. KIM-1 is a phosphatidylserine receptor which transforms renal proximal tubule epithelial cells (PTC) into phagocytes. Here, we describe that KIM-1-mediated phagocytosis induces autophagy and antigen presentation on MHC in PTCs.

**Methods:** LLC-PK1 cells and primary PTCs expressing KIM-1 or phagocytosis-deficient KIM-1 mutants were exposed to AC. LC3 localization to the phagosome was measured by live cell imaging, phagosome isolation and western blotting. Activation of ULK1 and other autophagy proteins was also measured by immunoblots. MHC presentation in primary PTCs was measured by flow cytometry and activation of CD4+ and CD8+ T cells.

**Results:** Following uptake of AC, KIM-1 phagosomes localized to LC3-positive autophagosomes. The presence of KIM-1 phagosomes within double membrane bound autophagosomes was confirmed by electron microscopy. KIM-1-mediated phagocytosis induced activation of the essential autophagy protein ULK1. Autophagy induction was necessary for degradation of phagocytosed AC. Primary cultured PTCs expressing KIM-1 were found to have a high level of basal autophagy as measured by LC3 punctae formation and LC3 II formation. LC3 II formation was reduced in PTCs derived from mice expressing phagocytosis-deficient mutant KIM-1 and in primary PTCs from wild-type mice treated with shRNA to knockdown KIM-1 expresssion. Antigens phagocytosed by KIM-1 were processed and presented on MHC II and cross-presented on MHC I. Incubation of OVA liposomes with PTCs resulted in activation of OVA-reactive CD4+ and CD8+ T cells, which was inhibited by KIM-1 shRNA and/or autophagy inhibition.

**Conclusions:** These data reveal a novel mechanism linking a specific epithelial cell receptor (KIM-1) to phagocytosis, autophagy, antigen presentation and regulation of the inflammatory response.

*Funding:* NIDDK Support

**TH-OR015**

**KIM-1 Interacts with Gα12 and Suppresses Its Activity to Mediate Efferocytosis** Ola Ziyad Ismail, Xizhong Zhang, Lakshman Gunaratnam. *Western Univ, London, Canada.*

**Background:** The phagocytic clearance of apoptotic cells (efferocytosis) serves to attenuate inflammation and enable tissue repair following injury. Kidney injury molecule-1 (KIM-1) is a type I transmembrane protein that is upregulated on renal proximal tubular epithelial cells (PTECs) following acute kidney injury (AKI). It is a phagocytic receptor that specifically recognizes phosphatidylserine (PS), an "eat me" signal, displayed on the surface of apoptotic cells. The signalling mechanism by which KIM-1 converts PTECs into non-professional phagocytes is not known.

Previously we identified Gα12 as a novel KIM-1-interacting protein in PTECs using co-immunoprecipitation and mass spectrometry. Gα12 belongs to the G12 family of heterotrimeric G proteins that regulates multiple cellular responses, including proliferation, apoptosis, permeability and actin cytoskeleton reorganization. We hypothesized that KIM-1 interacts with Gα12 to mediate efferocytosis.

**Methods:** To test our hypothesis, we used porcine (LLC-PK1) PTECs overexpressing HA-tagged human KIM-1, and human embryonic kidney (HEK293) cells expressing high levels of endogenous Gα12 and overexpressing GFP-tagged human KIM-1.

**Results:** First, we established that Gα12 interacts with the cytosolic domain of KIM-1 via co-immunoprecipitation and GST-Gα12 pull-down assays. Using confocal microscopy, we observed the co-localization of KIM-1 with GFP-tagged Gα12 in PTECs. Next, using an active Gα12 (GST-TPR) pull-down assay, we determined that Gα12 activity (GTP-binding) is significantly reduced in PTECs upon exposure to apoptotic cells. To determine the impact of Gα12 activity on KIM-1-mediated efferocytosis, we overexpressed a constitutively active mutant of Gα12 in KIM-1-expressing PTECs and found a significant decrease in their phagocytic efficiency. Conversely, we found an increase in the uptake of apoptotic cells when Gα12 expression was silenced using siRNA in HEK293 cells stably expressing KIM-1. Finally, we observed that KIM-1 inhibited Gα12 activation in HEK293 cells loaded with non-hydrolyzable GTP in the absence of apoptotic cells.

**Conclusions:** Taken together, our experiments suggest that KIM-1 interacts with and inhibits Gα12 activation in PTECs to mediate efferocytosis.

*Funding:* Government Support - Non-U.S.

**TH-OR016**

**Elucidating the Mechanisms of Therapeutic Augmentation of Kidney Repair after Acute Kidney Injury** Lauren Brilli,[1] Takuto Chiba,[3] Nataliya Skrypnyk,[4] Lee McDermott,[2] Mark P. De Caestecker,[3,4] Neil A. Hukriede.[1] *[1]Developmental Biology, Univ of Pittsburgh, Pittsburgh, PA; [2]Pharmaceutical Sciences, Univ of Pittsburgh, Pittsburgh, PA; [3]Cell and Developmental Biology, Vanderbilt Univ Medical Center, Nashville, TN; [4]Medicine, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** Acute kidney injury (AKI) is a serious disorder for which there is no targeted clinical treatment. A promising approach to improve treatment options lies in developing novel post-AKI therapies that enhance innate renal regenerative processes. Our lab has used zebrafish to identify small molecules that enhance renal tubular epithelial cell (RTEC) regeneration after AKI. Using this approach we identified methyl-4-phenylthiobutanoate (m4PTB), an HDAC inhibitor that promotes renal progenitor expansion in zebrafish embryos and accelerates recovery after AKI in zebrafish and mice. The ability of m4PTB to expand the renal progenitor cell population in zebrafish embryos depends on intact retinoic acid (RA) signaling, but the mechanisms underlying how m4PTB accelerates recovery after AKI are unknown.

**Methods:** We utilize a nephrotoxic model of gentamicin-induced AKI in zebrafish larvae that demonstrates the same hallmarks of renal injury and regeneration post-AKI as the mammalian kidney. *Tg(12XRARE:GFP)* transgenic larvae are used to assess RA signaling, and heat shock is used to induce expression of a dominant negative RA receptor (RAR) in *Tg(hsp70:dnRARa)* larvae.

**Results:** Live imaging of *Tg(12XRARE:GFP)* larvae post-AKI shows that m4PTB stimulates RA signaling in the larval zebrafish kidney RTECs. m4PTB also increases expression of the RA pathway components *Aldh1a2* and *Ret* by qRT-PCR in a mouse model of ischemia reperfusion AKI. Finally, we show that reducing RA signaling by heat shock in *Tg(hsp70:dnRARa)* larvae abrogates m4PTB-stimulated RTEC proliferation and impairs larval survival post AKI.

**Conclusions:** These studies indicate that m4PTB activates RA signaling and that m4PTB-dependent renal regeneration requires intact RA signaling post-AKI. This work suggests that m4PTB action is mediated by activating RA-dependent regenerative responses after AKI. Funding support: R01 DK069403, RC4 DK090770.

*Funding:* NIDDK Support

**TH-OR017**

**Retinoic Acid Signaling Promotes Epithelial Cell Repair after Acute Kidney Injury** Takuto Chiba,[1] Lauren Brilli,[2] Nataliya Skrypnyk,[1] Neil A. Hukriede,[2] Mark P. De Caestecker.[1] *[1]Medicine, Vanderbilt Univ, Nashville, TN; [2]Developmental Biology, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** Restoration of tubular integrity is essential for functional recovery and reduction in post injury fibrosis after acute kidney injury (AKI). However the mechanisms promoting tubular epithelial cell (TEC) repair are poorly understood. We have used zebrafish and mouse model systems to explore the regulation and role of retinoic acid (RA) signaling in mediating regenerative TEC repair after AKI.

**Methods:** We used gentamicin-induced AKI in zebrafish larvae, and ischemia reperfusion AKI (IR-AKI) in mice; QRT-PCR to quantify RA target mRNAs in mouse kidneys, as well as mouse and zebrafish transgenic RA reporter lines to assess localization and kinetics of RA signaling post AKI. We also used heat shock to induce dominant negative RA receptor (DN RAR) expression in Tg(hsp70:dnRARa) larvae, and the RA antagonist BMS493 and agonist all-trans RA (ATRA) in mice.

**Results:** RA signaling is activated in de-differentiated, regenerating proximal TECs (PTEC) in the mouse and larval zebrafish kidneys post AKI. Inhibition of RA in zebrafish larvae and mice impairs functional recovery post AKI. In mice this is associated with a marked reduction in PTEC proliferation and increased post injury fibrosis. In addition, treatment with ATRA enhances recovery when administered 24 hours after injury in mouse IR-AKI, and enhances activation of RA reporters in zebrafish and mouse kidneys.

**Conclusions:** These studies show that RA signaling is activated in PTECs post AKI, and inhibition of RA impairs recovery and inhibits PTEC repair. Since high levels of RA signaling during kidney development are repressed postnatally, our findings suggest a novel molecular mechanism whereby reactivation of a conserved embryonic signaling pathway drives regenerative repair post AKI. Our data also indicate that RA agonists can be given post injury to enhance this intrinsic repair mechanism after AKI.

*Funding:* NIDDK Support

**TH-OR018**

**Extracellular Adenosine Controls the Kidney Microenvironment and Regulates Acute and Chronic Kidney Injury** Li Li,[1] Liping Huang,[1] Hong Ye,[1] Diane L. Rosin,[2] Mark D. Okusa.[1] *[1]Div of Nephrology, CIIR, Univ of Virginia, Charlottesville, VA; [2]Dept of Pharmacology, Univ of Virginia, Charlottesville, VA.*

**Background:** CD73, or ecto-5'-nucleotidase, is critical for generation of extracellular adenosine, which increases during organ ischemia/reperfusion injury (IRI).We hypothesized that regulation of the kidney adenosine microenvironment by CD73 could play a role in shaping both acute and chronic kidney injury.

**Methods:** To investigate kidney IRI inflammation, both (or one) kidney pedicles were clamped for 26 (or 28) min and then released for 24 hr.

**Results:** Acute kidney injury (AKI) after IR was the same in CD73$^{-/-}$ and WT (B6) mice (plasma creatinine, PCr; P>0.05). Because discrete tissue localization of CD73 may be important in regulating the adenosine microenvironment, we deleted CD73 on proximal tubule cells by generating PEPCK-CreCD73$^{fl/fl}$ mice and these mice had higher PCr than PEPCK-Cre controls (P<0.05) after IRI. Interestingly, conditional knockout of CD73 on CD11c+ dendritic cells (DCs) (CD11c-CreCD73$^{fl/fl}$) also resulted in more kidney inflammation compared with CD11c-Cre mice following IRI (P<0.05). These studies indicate that the expression of CD73 on hematopoietic and kidney epithelial cells is important for AKI.

Locally produced adenosine may activate its receptors. Our previous studies using CD11c-CreAdora2a$^{fl/fl}$ mice showed that activation of adenosine 2A receptors (A2aR) on DCs has a critical role in suppressing the immune response in kidney IRI . In this study to further investigate the downstream effects of CD73 in chronic kidney injury, we examined and demonstrated that CD11c-CreAdora2a$^{fl/fl}$ mice also have more kidney fibrosis with higher PCr (P<0.01; as revealed by contralateral nephrectomy on day 14) compared with CD11c-Cre mice 15 d after unilateral IRI.

**Conclusions:** Our studies reveal that hematopoietic and renal CD73 promote the production of adenosine that is important for AKI, and activation of A2aRs on DCs attenuate kidney IRI and fibrosis. Activation of CD73 or A2aR provides novel therapeutic targets for the disease.

*Funding:* NIDDK Support

## TH-OR019

### Sustained EGFR Activation Mediates Postischemic Acute Kidney Injury Progression to Chronic Kidney Disease

Shougang Zhuang. *Dept of Medicine, Rhode Island Hospital, Alpert Medical School of Brown Univ, Providence, RI.*

**Background:** Acute kidney injury (AKI) is frequently accompanied by maladaptive repair and renal fibrogenesis, however, the molecular mechanisms that mediate progression from acute kidney injury to chronic kidney disease remain poorly understood.

**Methods:** Here we examined the role of epidermal growth factor receptor (EGFR) in this process using waved-2 (Wa-2) mice, which have reduced EGFR activity, and their wild type (WT) littermates after renal ischemia.

**Results:** Renal EGFR phosphorylation was induced within 2 days after ischemia, increased over time, and remained elevated at 28 days in WT mice, but this was diminished in Wa-2 mice. At the early stage of post-ischemia (2 days), Wa-2 mice developed more severe acute renal tubular damage with less reparative responses as indicated by enhanced tubular cell apoptosis, and reduced dedifferentiation and proliferation as compared to WT animals. At the late stage of postischemia (28 days), Wa-2 mice exhibited less severe renal interstitial fibrosis as shown by reduced activation/proliferation of renal myofibroblasts and decreased deposition of extracellular matrix proteins. EGFR activation also contributed to cell cycle arrested at G2/M phase, a cellular event associated with production of profibrogenetic factors, in the injured kidney. Collectively, these results indicate that severe ischemic AKI results in sustained activation of EGFR, which is required for progression of AKI to CKD.

**Conclusions:** Thus, blockade of EGFR holds a novel therapeutic potential for attenuating or halting initiation/progression of CKD following ischemia/reperfusion injury.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## TH-OR020

### The Role of M2 Macrophage in the Progression of Chronic Kidney Disease following Acute Kidney Injury

Myung-Gyu Kim, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Dept of Internal Medicine, Korea Univ Anam Hospital, Seoul, Republic of Korea.*

**Background:** Acute kidney injury (AKI) is a major risk factor for progression of chronic kidney disease (CKD). However, the mechanism that link AKI to CKD remains unclear. Recently, macrophages were shown to shift their phenotype from M1 to M2 and these M2 macrophages facilitate recovery. In this study, we examined the alteration of macrophage phenotype during the extended period of recovery phase following ischemia/reperfusion injury (IRI) and tried to verify their roles in the development of renal fibrosis in a mouse model of I/R-CKD.

**Methods:** Renal pedicle was clamped unilaterally for 40 minutes and on day 7, 14 and 28 after IRI, phenotype of monocyte/macrophage, cytokine expression, renal fibrosis were assessed. To determine the role of monocyte/macrophage subset in renal fibrosis, liposome clodronate (LC) was injected or CD11c-DTR transgenic mice were used. Depletion was initiated on day 3 following IRI.

**Results:** Throughout the recovery phase of IRI, mRNA expression of M2 marker (Arginase-1) in kidney was markedly increased compared to M1 marker (iNOS). Flow cytometric analysis also revealed the predominant macrophage subset was F4/80$^{+}$CD206$^{+}$ M2-phenotype macrophages but not F4/80$^{+}$CD206$^{-}$ M1-phenotype macrophages during the recovery phase. On day 28 after IRI, renal fibrosis was clearly shown with increased type IV collagen and TGF-ß. Injection of LC resulted in preferential depletion of M2 macrophages and it was associated with significant reduction of kidney IL-6, type IV collagen, TGF-ß and ultimately improved renal fibrosis. Additionally, we found that TGF-ß were about 2.5 fold highly expressed in M2 macrophages than M1 macrophages, suggesting the important contribution of M2 macrophage in the development of renal fibrosis. On the other hand, injection of diphtheria toxin to CD11c-DTR transgenic mice resulted in depletion of CD11c-high cells with relatively preserving M2 macrophages and had minimal impact on renal fibrosis as well as tissue level of cytokines.

**Conclusions:** Although known to be important in facilitating recovery, M2 macrophages are thought to be main culprit in the development of renal fibrosis following IRI.

## TH-OR021

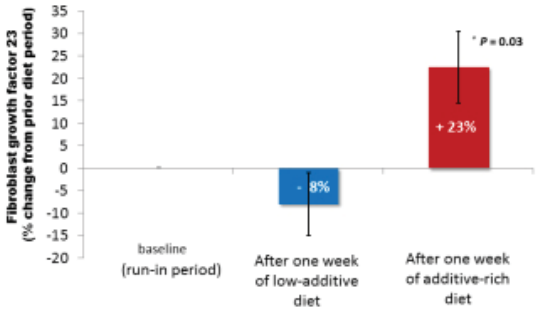
### Impact of Phosphorus-Based Food Additives on Bone and Mineral Metabolism in Healthy Volunteers

Orlando M. Gutierrez,[1] George R. Beck.[2] *[1]UAB; [2]Emory.*

**Background:** Phosphorus-based food additives substantially increase phosphorus intake in westernized diets. Few studies examined the impact of these additives on bone and mineral metabolism in humans.

**Methods:** 10 healthy volunteers participated in the study. Subjects needed to be 19-45 years old. Exclusion criteria included kidney disease, BMI ≥30 kg/m$^2$, or intake of medications known to affect phosphorus metabolism. After a two-week run-in period during which baseline biochemical data were obtained, subjects were fed standardized diets prepared by the metabolic kitchen over the following 2 weeks. During the 1st week, they were fed a diet designed to provide ~2,000 kcal and 900 mg of phosphorus (Pi) per day using foods known to be free of Pi additives. During the 2nd week, they consumed a diet containing the same food items as the low-additive diet only the foods contained Pi additives. Blood and urine specimens were collected at regular intervals throughout the intervention period.

**Results:** The mean age of subjects was 32±8 years old, 30% were male, and 70% black. The measured Pi content of the high-additive diet was ~617 mg higher than the low-additive diet, and the measured sodium content was ~1.3 grams higher; there were no other differences in the measured content of the two diets. There were no statistically significant changes in serum Pi, calcium, PTH, CTX, or P1NP during the dietary intervention period. FGF23 concentrations declined after one week of the low-additive diet, then significantly increased after 1 week of the high-additive diet (Figure).




Further, osteopontin levels significantly increased and sclerostin levels significantly decreased after one week of the high-additive diet.

**Conclusions:** One week of eating a diet rich in Pi additives significantly increased FGF23 and osteopontin concentrations, and significantly decreased sclerostin concentrations in healthy volunteers.

*Funding:* NIDDK Support

## TH-OR022

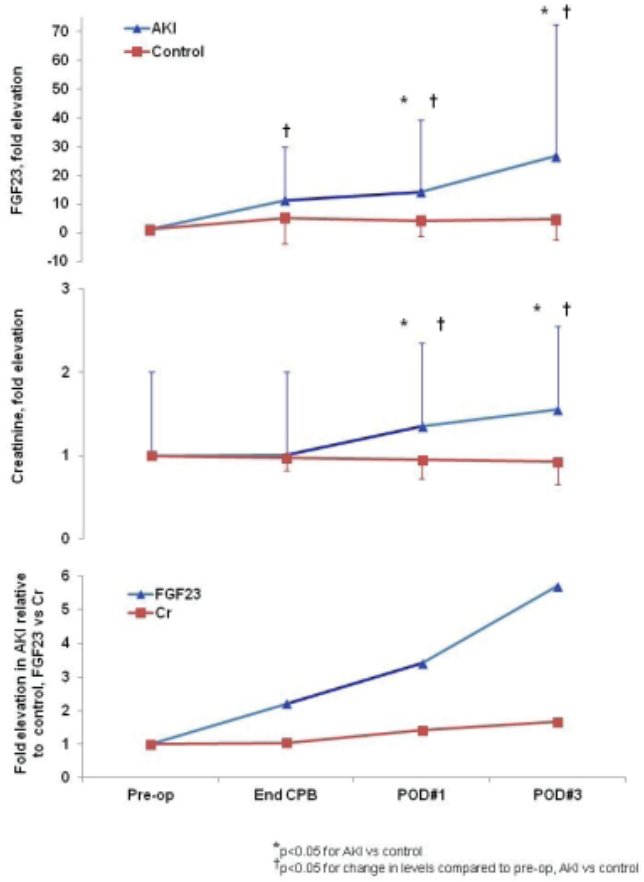
### Fibroblast Growth Factor 23 Rises Early in Acute Kidney Injury after Cardiac Surgery and Predicts Adverse Outcomes

David E. Leaf,[1] Marta Christov,[2] Harald Jüppner,[2] Myles S. Wolf,[3] Sushrut S. Waikar.[1] *[1]Brigham and Women's Hospital; [2]Massachusetts General Hospital; [3]Univ of Miami Miller School of Medicine.*

**Background:** FGF23 levels increase as CKD progresses and are strongly associated with increased mortality in both CKD and ESRD. The kinetics and prognostic value of FGF23 among patients with acute kidney injury (AKI), however, have not been well characterized. We hypothesized that FGF23 rises early in AKI and is associated with the composite outcome of hospital mortality or need for renal replacement therapy (death/RRT).

**Methods:** We performed a nested case-control study of 100 participants with and without AKI after cardiac surgery (N=50 in each group). Controls were matched based on age and eGFR. Plasma FGF23 and creatinine levels were measured at four timepoints: pre-op, end of cardiopulmonary bypass, and postoperative days 1 and 3. Logistic regression was used to assess the association between FGF23 area under the curve (AUC), calculated by the trapezoid method, and death/RRT among AKI cases. Secondary outcomes included post-op sepsis and post-op vasopressor requirement beyond 24 hours.

**Results:** FGF23 levels were significantly higher among cases than controls (figure1).




Among cases, FGF23 levels rose early in the course of AKI, becoming significantly elevated (11.3-fold) by the second timepoint (end of cardiopulmonary bypass). FGF23 AUC was significantly associated with death/RRT [OR 1.82, 95% CI 1.10-3.00, p=0.02] and remained significant after adjustment for baseline eGFR. FGF23 AUC was also significantly associated with post-op sepsis and post-op vasopressor requirement.

**Conclusions:** FGF23 levels are elevated in AKI after cardiac surgery, rise early in the course of AKI, and are associated with greater risk of death/RRT. Additional work is needed to determine potential mechanisms underlying these findings.

## TH-OR023

### Cinacalcet Decreases FGF23 Levels in Prevalent Dialysis Patients Compared to Placebo

Sharon M. Moe,[1] On Behalf of EVOLVE Investigators.
[1]*Indiana Univ, Indpls, IN.*

**Background:** Fibroblast Growth Factor 23 (FGF23) levels are associated with increased mortality in ESRD patients and FGF23 is a direct cardiac toxin in rodents. FGF23 is a phosphaturic hormone that is stimulated by 1,25(OH)2 vitamin D, parathyroid hormone (PTH), and elevated phosphorus (Pi), and inhibited by calcium (Ca). We hypothesized that treatment of hemodialysis patients with cinacalcet (which lowers PTH, calcium and phosphorus) would lower FGF23.

**Methods:** To test this hypothesis, we analyzed FGF23 in serum samples from 200 subjects in the EVOLVE trial (NEJM 2013). Subjects in the trial were randomized to treatment with cinacalcet or placebo, on a background of standard of care with calcitriol or its analogs and phosphate binders. In this study, 100 subjects in each arm were randomly selected from all subjects with baseline and week 20 blood samples and FGF23 was measured using a Millipore intact FGF23 assay.

**Results:** The demographics and baseline laboratory values in this cohort were reflective of the larger study: median age of 53 yrs; dialysis vintage median of 43.2 months (range 7.3 to 159); 37.5% female, 94% with cardiovascular disease; 61.5% receiving vitamin D sterols and 87% on phosphate binders. The baseline median PTH was 648 pg/ml (369-1602 p10, p90), Ca 9.8 mg/dl (9.1, 10.6), Pi 6.2 mg/dl (4.9, 8.2). The mean± SD baseline FGF23 levels were 8469 ± 9549 pg/ml in the cinacalcet patients and 8029 ± 8043 pg/ml in the placebo patients and was significantly greater in men and those patients with higher Pi or Ca (Odds ratio 4.55, 5.28, 7.28 respectively). After 20 weeks, FGF23 levels decreased by 25 ± 76% (mean ± SD) in the cinacalcet treated patients but increased by 31 ± 120% in the placebo treated patients ($p < 0.001$). These changes were similar in patients on or off vitamin D. The change in FGF23 was not related to PTH but correlated with change in Ca ($r = 0.34$) and Pi ($r = 0.33$, both $p < 0.001$).

**Conclusions:** In a secondary analysis of the EVOLVE study, patients treated with cinacalcet compared to placebo had significant lowering of FGF23. An analysis of whether the lowering of FGF23 leads to improved outcomes is ongoing.

*Funding:* Pharmaceutical Company Support - Amgen

## TH-OR024

### No Decrease in Albuminuria with Cholecalciferol in a Randomized Placebo Controlled Trial in Older Patients with Heart Failure

Amanda Gomes,[1] Brian Schmotzer,[3] Lavinia A. Negrea,[1] Rebecca S. Boxer.[2] [1]*Nephrology and Hypertension, Univ Hospitals Case Medical Center, Cleveland, OH;* [2]*Cardiovascular Medicine, Univ Hospitals Case Medical Center, Cleveland, OH;* [3]*Center for Clinical Investigaion, Case Western Reserve Univ, Cleveland, OH.*

**Background:** Proteinuria is an established risk factor for cardiovascular morbidity and mortality and adverse renal outcomes. Both microalbuminuria and macroalbuminuria are prevalent in heart failure. The mainstay for treatment of proteinuria is inhibition of the renin-angiotensin system. In vitro and animal studies have shown vitamin D to have a suppressive role on RAS. Activated vitamin D has been shown to reduce albuminuria in patients with type 2 diabetes and with chronic kidney disease. Oral cholecalciferol has also been shown to reduce albuminuria in type 2 diabetic nephropathy. The study objective is to determine the effect of oral cholecalciferol (D3) on urine albumin creatinine ratio (ACR) in an older adults with heart failure.

**Methods:** Single center, double blind, randomized placebo-controlled trial among 64 patients with heart failure greater than 50 years of age and low or low normal 25OH vitamin D were randomly assigned to receive 6 months of weekly treatment of 50,000 units of oral cholecalciferol (D3) (n=31) or placebo (n=33), both groups received calcium citrate 800mg daily. We measured change in ACR between 0 and 6 months.

**Results:** Treatment with 50,000 units of cholecalciferol resulted in an increase in serum 25OH vitamin D of 42 ± 16 ng/mL and was unchanged in the placebo group (0 ± 7 ng/mL) from baseline to 6 months. ACR levels were not associated with Vitamin D level at baseline ($r=0.08$, $p=0.56$). The treatment group did not demonstrate a reduction in urine ACR relative to the placebo group ($p=0.18$) from baseline to 6 months (treatment group change = 6 ± 52 mg/gm, placebo group change = -3 ± 32 mg/gm). There was also no difference in reduction in ACR between diabetics and non-diabetics ($p=0.54$).

**Conclusions:** 6 months of treatment with 50,000 units of weekly cholecalciferol did not result in reduction in albumin creatinine ratio in older patients with heart failure.

## TH-OR025

### Fasting Status, Serum Phosphorus, and Mortality: Results from NHANES III

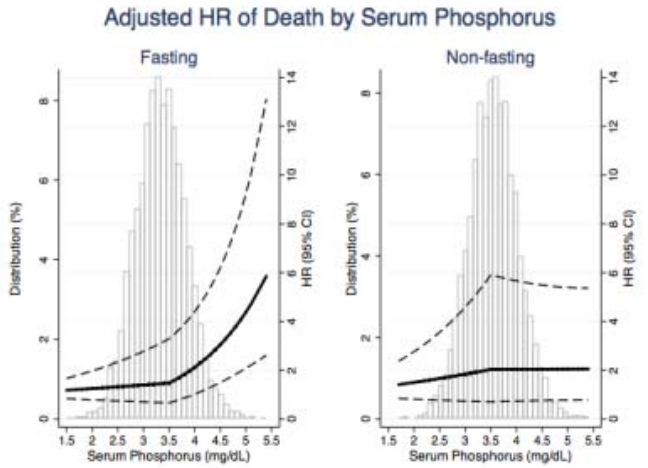
Alex Chang, M. Grams. *Nephrology, Johns Hopkins Univ.*

**Background:** Most but not all studies have found associations between elevated serum phosphorus levels and mortality; negative studies tend to use single random measurements. Since serum phosphorus levels may be affected by dietary phosphorus intake, we hypothesized that fasting status modifies the association between serum phosphorus and mortality.

**Methods:** The interaction between fasting status (≥12 hours without food or drink other than water), serum phosphorus, and all-cause and cardiovascular mortality was evaluated using participants from NHANES III (1988-1994). Cox proportional hazards models were adjusted for age, sex, race/ethnicity, time of examination, cardiovascular risk factors, eGFR, low vitamin D, and urine albumin/creatinine ratio.

**Results:** Fasting participants had lower serum phosphorus levels than non-fasting participants (3.3 vs. 3.6; $p<0.001$). Serum phosphorus was significantly associated with all-cause mortality among fasting participants (adjusted hazard ratio (aHR) per 1mg/dL:1.48; 95% CI 1.23-1.78) but not non-fasting participants (aHR 1.10; 95% CI 0.95-1.27; p for interaction=0.01). Similarly, serum phosphorus was significantly associated with cardiovascular mortality among fasting participants (aHR per 1mg/dL:1.67; 95% CI 1.28-2.17) but not non-fasting participants (aHR 1.25; 95% CI 0.98-1.61).

**Conclusions:** Fasting serum phosphorus levels have a stronger relationship with all-cause and cardiovascular mortality than non-fasting levels. This may explain the heterogeneity in previous studies regarding the association between serum phosphorus and mortality.




*Funding:* NIDDK Support

**TH-OR026**

**Cardiac FGF23 Expression Is Associated with Left Ventricular Hypertrophy in Pediatric Patients with Chronic Kidney Disease** Maren Leifheit-Nestler,[1] Kathrin Flasbart,[1] Robert Grosse Siemer,[1] Dagmar-Christiane Fischer,[2] Michael Klintschar,[3] Jan U. Becker,[4] Tomas Seeman,[5] Christoph Aufricht,[6] Dieter Haffner.[1] *[1]Dept of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Hannover, Germany; [2]Dept of Pediatrics, Univ Hospital Rostock, Rostock, Germany; [3]Institute for Forensic Medicine, Hannover Medical School, Hannover, Germany; [4]Institute of Pathology, Hannover Medical School, Hannover, Germany; [5]Div of Pediatric Nephrology, Univ Children´s Hospital Motol, Prague, Czech Republic; [6]Div of of Pediatric Nephrology, Univ Children´s Hospital Vienna, Vienna, Austria.*

**Background:** Left ventricular hypertrophy (LVH) is an important cause of cardiovascular disease (CVD) in patients with chronic kidney disease (CKD). Experimental and clinical studies demonstrated an association between elevated serum levels of fibroblast growth factor 23 (FGF23) and LVH in CKD.

**Methods:** We conducted a retrospective study in 19 deceased pediatric patients with end-stage renal disease (age 11±8y) and 23 age- and sex-matched healthy controls. Myocardial autopsy samples of the left ventricle (LV) were evaluated by immunohistochemistry and qPCR with respect to FGF23 expression, and signaling involved in calcineurin-NFAT associated pathway of hypertrophy. Expression of brain natriuretic peptide (BNP) served as a marker of LVH.

**Results:** FGFR1 was expressed in all samples to the same extent. Cardiac FGF23 protein expression tended to be enhanced in samples of CKD patients compared to controls. Cardiac FGF23 mRNA was 3-fold increased in LV of CKD patients versus controls ($p<0.05$), and associated with the duration of CKD ($r=0.636$, $p<0.05$). Cardiac BNP mRNA levels were enhanced in samples of CKD patients compared to controls, and associated with cardiac FGF23 gene expression ($r=0.982$, $p<0.05$). In line with a causative role of FGF23 in LVH, calcineurin mRNA expression was 19-fold higher in hypertrophic heart tissue compared to healthy tissue.

**Conclusions:** FGF23 expression as well as the FGF23-dependent calcineurin pathway is upregulated in heart tissue samples of pediatric CKD patients and significantly associated with duration of CKD and marker of LVH.

**TH-OR027**

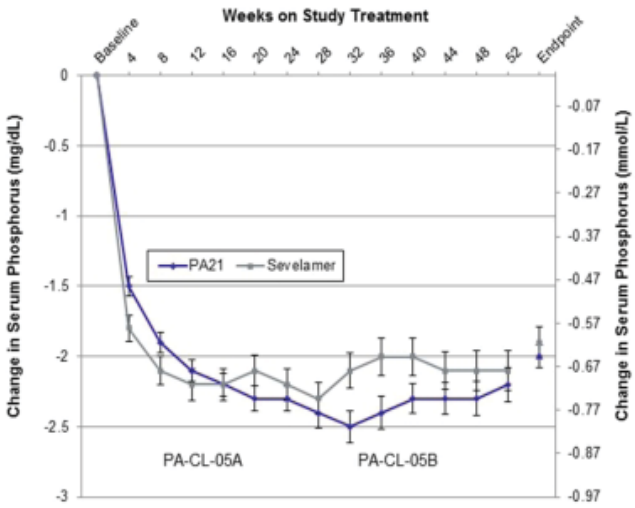
**Efficacy of PA21, a Novel Iron-Based Phosphate Binder, Maintained to 52 Weeks in Dialysis Patients with Hyperphosphatemia** Stuart M. Sprague,[1] Jürgen Floege,[2] Adrian Covic,[3] Markus Ketteler,[4] Anjay Rastogi,[5] Edward M.F. Chong,[6] Bruce S. Spinowitz.[7] *[1]NorthShore Univ Health System; [2]RWTH Univ Hosp Aachen; [3]Gr.T. Popa Univ of Med and Pharm; [4]Coburg Clinic and KfH-Dialysis Center; [5]Univ of California; [6]Vifor Pharma; [7]New York Hosp Queens.*

**Background:** A phase 3, open-label study investigated 1-year efficacy and safety of PA21, a novel polynuclear iron(III)-oxyhydroxide phosphate binder, vs sevelamer carbonate (SEV).

**Methods:** 1059 patients were randomized to PA21 (1.0–3.0 g/day; n=710) or SEV (2.4–14.4 g/day; n=349) for 12 weeks' dose titration then 12 weeks' maintenance; 99 hemodialysis patients receiving PA21 were re-randomized to evaluate PA21 maintenance-dose vs low-dose control (250 mg/day) from Weeks 24–27 (primary endpoint). Patients completing treatment, excluding those on low-dose PA21, were enrolled in a 28-week extension study. Secondary endpoints included change in serum phosphorus (sP; baseline–Week 12) to assess non-inferiority of PA21 vs SEV; long-term safety and efficacy of PA21 and SEV.

**Results:** Primary and Week 12 secondary efficacy endpoints were met (shown at ASN 2012). 658 patients were treated; 549 completed the extension. PA21 and SEV efficacy were maintained: mean [SD] change in sP concentration from baseline (7.6 [1.69] mg/dL and 7.4 [1.76] mg/dL) is shown (full analysis set, FAS; Figure). Mean daily tablet number over 52 weeks was lower for PA21 (3.3) vs SEV (8.7). Serum ferritin and transferrin saturation increased with PA21 over the first 24 weeks (mean change from baseline [SD]: 135.0 [340.33] ng/mL and 4.9 [16.12]%, respectively) then stabilized, with no evidence of iron accumulation with PA21 over 52 weeks. PA21 and SEV were well tolerated, with no notable difference in safety between groups.

**Figure:** Change (± standard deviation) in sP from baseline (FAS; N=1,041).




**Conclusions:** PA21 efficacy was maintained long-term, with a lower pill burden than SEV and similar safety profile.

*Funding:* Pharmaceutical Company Support - Vifor Pharma

**TH-OR028**

**Cinacalcet Induces Apoptosis of Parathyroid Cells through Increased Expression of FGF Receptor 1 in Hyperplastic Parathyroid Glands from Uremic Patients** Keiichi Sumida, Yoshifumi Ubara, Koki Mise, Masahiro Kawada, Tatsuya Suwabe, Junichi Hoshino, Kenmei Takaichi. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

**Background:** Decreased expression of both fibroblast growth factor receptor 1 (FGFR1) and its co-receptor Klotho was demonstrated in uremic parathyroid hyperplasia, particularly in nodular hyperplasia. However, the effect of cinacalcet hydrochloride (cinacalcet) on the expression of FGFR1 and Klotho in association with parathyroid cell proliferation and apoptosis remains unclear.

**Methods:** Four normal parathyroid glands and 71 hyperplastic parathyroid glands from 18 hemodialysis patients with refractory secondary hyperparathyroidism treated with ($n = 10$; cinacalcet group) or without ($n = 8$; conventional group) cinacalcet were examined immunohistochemically. The expressions of FGFR1 and Klotho, proliferative activity marker (Ki67), and apoptosis marker (M30 CytoDEATH) were analyzed semiquantitatively according to the areas of diffuse or nodular hyperplasia.

**Results:** Compared with normal glands, the immunohistochemical expressions of both FGFR1 and Klotho were decreased significantly in both the cinacalcet and conventional groups. In the cinacalcet group, the expression of FGFR1 was increased significantly only in areas of diffuse hyperplasia ($0.97 \pm 0.09$ vs. $0.25 \pm 0.11$, $P < .001$), but not in nodular areas, compared with the conventional group. The expression of Klotho was not different between the two groups or the two areas. In the cinacalcet group, Ki67 labeling indexes in both diffuse and nodular areas were significantly smaller than in the conventional group ($2.41 \pm 0.33$ vs. $3.94 \pm 0.54$ and $15.7 \pm 2.51$ vs. $23.4 \pm 2.64$, per 1000 cells, $P< .01$, respectively). The M30 labeling index was significantly higher in the cinacalcet group, particularly in areas of diffuse hyperplasia, than in the conventional group ($6.44 \pm 0.62$ vs. $3.65 \pm 0.57$, per 1000 cells, $P < .01$).

**Conclusions:** These results suggest that cinacalcet suppresses parathyroid cell proliferation and induces apoptosis in hyperplastic parathyroid cells through the increased expression of FGFR1, particularly in glands with diffuse hyperplasia.

*Funding:* Private Foundation Support

**TH-OR029**

**Temporal Trends in the Incidence, Treatment, and Outcomes of Hip Fracture after First Kidney Transplantation in the United States** Sumi Sukumaran Nair,[1] Colin R. Lenihan,[1] Maria E. Montez-Rath,[1] Glenn M. Chertow,[1,2] Wolfgang C. Winkelmayer.[1,2] *[1]Div of Nephrology, Dept of Medicine, Stanford Univ School of Medicine; [2]Dept for Health Research and Policy, Stanford Univ School of Medicine.*

**Background:** Kidney transplant recipients are at increased risk of hip fracture. Post-transplant bone disease is influenced by multiple pre- and post-transplant factors. The modern era has seen numerous changes in kidney transplant demographics, co-morbidities, wait-time and immunosuppression. It is currently unknown whether any secular trends exist in the hip fracture incidence, its treatment and case-fatality in kidney transplant recipients (KTR).

**Methods:** We identified first-time KTR (1997-2010) who had >1 year of Medicare coverage and no recorded history of hip fracture and followed them for 3 years. New hip fractures were identified from corresponding diagnosis and surgical procedure codes in

claims. Outcomes studied included time to hip fracture, type of surgery received (internal fixation; partial, or total hip replacement), and 30-day mortality. Cox and modified Poisson regressions were used to describe trends in study outcomes.

**Results:** Of 72,682 KTR transplanted in 1997-2010, 620 experienced a hip fracture event during 162,351 person-years of follow-up for an incidence rate of 3.81 per 1,000 person-years. While unadjusted hip fracture incidence did not change over 14 years, strong confounding by changes in case mix was present. Using year of transplantation as a continuous variable, the demographic-adjusted hazard ratio (HR) for hip fracture in 2010 compared with 1997 was 0.67 (95%CI: 0.50-0.89), further magnified with adjustment for dialysis and comorbid factors (HR=0.55; 95%CI: 0.41-0.74). No obvious trends in hip fracture treatment were noted. The 30-day mortality was lower than that of the general population at 2.09 per 100 events.

**Conclusions:** Adjusted post-transplant hip fracture rates declined substantially between 1997 and 2010 for reasons that further research will need to identify.

*Funding:* NIDDK Support, Private Foundation Support

## TH-OR030

**Reduced VEGFA Expression in Bone Marrow Cells Is Associated with Altered Bone Formation in CKD** Neal X. Chen,[1] Kalisha O'Neill,[1] Matthew R. Allen,[1] Christopher Newman,[1] Vincent H. Gattone,[1] Sharon M. Moe.[1,2] *[1]Indiana Univ School of Medicine, Indpls, IN; [2]VAMC.*

**Background:** Over suppression of osteoblastic bone formation is considered an adverse effect of aggressive management of elevated PTH in CKD-MBD. Osteoblasts, critical in bone formation, derive from bone marrow mesenchymal stem cells (MSC), a process in part regulated by vascular endothelial growth factor (VEGFA). Therefore, an alternative hypothesis is that CKD leads to impaired bone formation due to reduced VEGFA that is overcome in the setting of high PTH.

**Methods:** To test this hypothesis, we analyzed bone marrow cells (BMC) from CKD (Cy/+ rats) for expression of VEGFA and transcription factors of MSC differentiation pathways and determined the relationship to histomorphometry indices of bone turnover (BFR) and mineralization (MS/BS), and bone volume (%BV) by microCT. Normal (NL) rats were compared to CKD rats (CKD; high PTH and high BFR), CKD treated with calcium (CKD-Ca; low PTH and low BFR), and CKD treated with zoledronic acid, known to have anti-VEGFA activity (CKD-Zol; high PTH and low BFR).

**Results:** The expression of VEGFA was reduced in the BMC from CKD vs NL animals, and further reduced by both Ca and Zol ($p < 0.04$). In contrast, there was no difference in the VEGF-receptor expression between the groups. In CKD BMC VEGFA expression was correlated with BFR, MS/BS, and BV ($r = 0.80$, 0.65 and -0.85, respectively, all $p<0.01$). VEGFA is known to increase the differentiation towards osteoblast (RUNX2) rather than adipocyte lineage (PPARγ). The expression of these factors was strongly correlated with VEGFA expression, and the RUNX2 expression was equivalent in CKD animals compared to NL, but decreased in both CKD-Ca and CKD-Zol($p<0.001$). Similar results were obtained with PPARγ but CKD was slightly lower than NL.

**Conclusions:** We conclude that CKD is associated with decreased VEGFA expression in BMC. These results suggest that in untreated CKD animals, the elevated PTH could overcome the low VEGFA and increase RUNX2 and BFR. In contrast, the induction of low BFR and suppression of PTH with Ca is associated with reduced VEGFA and RUNX2 expression to levels similar to Zol treatment, a known VEGFA inhibitor.

*Funding:* Other NIH Support - NIAMS, Veterans Affairs Support

## TH-OR031

**LPA-LPA$_1$ Signaling Drives Renal Fibrosis by Inducing Fibroblast Accumulation through MRTF-SRF-Dependent Epithelial CTGF Expression** Norihiko Sakai,[1,3] Takashi Wada,[3] Andrew M. Tager.[2] *[1]Div of Rheumatology, Massachusetts General Hospital, Boston, MA; [2]Div of Pulmonary and Critical Care Unit, Massachusetts General Hospital, Boston, MA; [3]Div of Nephrology, Kanazawa Univ, Kanazawa, Ishikawa, Japan.*

**Background:** Renal fibrosis is a common pathway of progressive renal diseases and results in renal failure, but the mechanisms driving this pathway remain to be fully identified. We previously implicated the lipid mediator lysophosphatidic acid (LPA) and one of its receptors, LPA$_1$, in lung and skin fibrosis. Recently, we have demonstrated that LPA and LPA$_1$ drive peritoneal fibrosis by inducing connective tissue growth factor (CTGF)–dependent fibroblast accumulation. LPA signaling through LPA$_1$ on peritoneal mesothelial cells drives CTGF production in these cells by activating myocardin-related transcription factor (MRTF)-serum response factor (SRF)-dependant transcription. We therefore examined the role of this LPA-LPA$_1$-MRTF-SRF-CTGF pathway in renal fibrosis induced by unilateral ureteral obstruction (UUO).

**Methods:** Type I pro-collagen promoter-driven green fluorescent protein (GFP) mice were crossed with LPA$_1$-deficient mice (LPA$_1$ KO-GFP) or wild-type mice (WT-GFP).

**Results:** UUO-induced increases in renal hydroxyproline content were significantly attenuated in LPA$_1$KO-GFP compared with WT-GFP animals. Renal accumulation of a-smooth muscle actin$^+$ myofibroblasts and GFP$^+$ fibroblasts was also significantly reduced in LPA$_1$ KO-GFP mice. Renal CTGF expression was mainly detected in renal epithelial cells, and the levels of renal CTGF expression were suppressed in LPA$_1$ KO-GFP compared with those in WT-GFP mice. We also found that LPA directly induced CTGF expression dependent on an LPA$_1$-MRTF-SRF axis in mouse primary renal epithelial cells. Finally, supernatants of renal epithelial cells stimulated with LPA were found to contain CTGF protein, and to enhance fibroblast proliferation.

**Conclusions:** Our results suggest that LPA and LPA$_1$ drive renal fibrosis by inducing fibroblast accumulation through epithelial CTGFproduction, by activating MRTF-SRF-dependent transcription in epithelial cells.

## TH-OR032

**A Cationic-Independent Mannose 6-Phosphate Receptor Inhibitor (PXS64) Ameliorates Kidney Fibrosis Independent of Smad, AKT and ERK Signaling Pathways** Jie Zhang, May Yw Wong, Muh Geot Wong, Carol A. Pollock, Sonia Saad. *Kolling Institute of Medical Research, Univ of Sydney, St. Leonards, NSW, Australia.*

**Background:** Agents which completely inhibit transforming growth factor-β (TGFβ) have limited clinical use due to unwanted side effects. The cationic-independent mannose 6-phosphate receptor (CI-MPR) is a multifunctional protein which inhibits the activation of latent TGFβ1 without complete inhibition. We have previously shown that PXS25, has anti-fibrotic properties in human proximal tubular cells (HK-2) but is limited by its bioavailability. We aim to determine the role of PXS64, a pro-drug of PXS-25, in renal fibrosis and in HK-2 cells.

**Methods:** A 7 day unilateral ureteric obstruction (UUO) model was examined in 6-8 week old male C57BL/6 mice (20–25g, n=5) in the following groups: (i) Sham operated; (ii) UUO control; (iii) PSX64 (10mg/kg; by daily intraperitoneal injection) and (iv) Telmisartan (3mg/kg; in drinking water). Semiquantitative morphometric analyses of glomerulosclerosis and tubulointerstitial fibrosis were performed. Kidney tissue was analysed for fibrotic and inflammatory markers. HK-2 cells were exposed to TGFβ1 (1ng/ml) with or without PXS64 (10 μmol/L) for 24 hours. The mRNA expression of Collagen IV (ColIV) and Fibronectin (FN) was assessed by real time PCR. Phosphorylated and total protein expressions of signaling pathways downstream of TGFβ1 were assessed by western blot.

**Results:** The mRNA expression of ColIV and FN in kidney tissue was lower in animal treated with PXS64 as compared to the vehicle control (PBS) ($p<0.05$). The mRNA expression of TGFβ1 and MCP1 were suppressed by PSX64 and Telmisartan ($p<0.05$, both) compared to the vehicle. Mice treated with PXS64 had lower tubulointerstitial fibrosis score, FN and ColIV protein expression. In the *in vitro* study, PXS-64 inhibits TGFβ1-induced mRNA and protein expression of ColIV and FN compared to controls ($P < 0.05$) but did not inhibit TGFβ1-induced Smad2/3, AKT or ERK1/2.

**Conclusions:** PSX64 is as effective as Telmisartan as an antifibrotic agent in kidney fibrosis. PXS64 attenuates TGFβ1-induced matrix production independent of Smad2/3, AKT and ERK1/2 signaling pathways.

## TH-OR033

**MicroRNA Regulation of the Cell Cycle in Aristolochic Acid Nephropathy** Robert H. Jenkins,[1] Donald Fraser,[1] Luke C. Davies,[2] Philip R. Taylor,[2] Timothy Bowen.[1] *[1]Institute of Molecular and Experimental Medicine, Cardiff Univ, Cardiff, Wales, United Kingdom; [2]Institute of Infection, Immunity and Biochemistry, Cardiff Univ, Cardiff, Wales, United Kingdom.*

**Background:** Aristolochic acid nephropathy (AAN) is characterised by rapidly progressive tubulointerstitial nephritis culminating in end stage renal failure. A recent study causally links epithelial cell $G_2$M cell cycle arrest to fibrosis, following acute ischaemic, aristolochic acid (AA), and obstructive injuries (Yang L et al. Nat Med 2010; 16(5) 535). We have previously characterised microRNA expression in response to AA, including the induction of cell cycle associated microRNAs, miR-192, -194, -450a, and -542-3p. The purpose of this study was to investigate the mechanism of microRNA regulation of cell cycle arrest in AAN.

**Methods:** An in vitro study in proximal tubular epithelial cells in a model of AAN, using stable-expression cell lines, TaqMan RT-qPCR, Flow Cytometry, Western Blots, and Immunofluorescence.

**Results:** AA induced profound $G_2$/M arrest in proximal tubular cells via p53-mediated downstream inactivation of the maturation promoting complex CDK1/Cyclin-B1. This was associated with the formation of RNA stress granules, termed processing-bodies, indicative of translational repression. The enforced expression of miR-192 recapitulated the $G_2$/M arrest and translational repression. The mechanism of $G_2$/M arrest was due to miR-192 repression of the E3 ubiquitin ligase MDM2, a negative regulator of p53. The subsequent increase in p53 transcriptionally induced $p21^{cip1}$ and the growth arrest and DNA damage 45 (GADD45) proteins, which phosphorylated, inactivated and disassociated the maturation promoting complex CDK1/Cyclin-B1.

**Conclusions:** These data define a mechanism by which microRNAs control cell cycle arrest in epithelial cells in a model of AAN. This is of mechanistic importance in the recently described pro-fibrotic $G_2$/M arrest seen following a range of acute renal injuries.

## TH-OR034

**Nlrp3 Regulates Apoptosis in Renal Tubular Epithelial Cells** Akosua Vilaysane, Daniel A. Muruve. *Medicine, Univ of Calgary, Calgary, Canada.*

**Background:** Tubular cell apoptosis contributes to the pathogenesis of progressive chronic kidney disease (CKD). Caspases play an important role in this process that result in tubular atrophy, tubulointerstitial inflammation and fibrosis. Previous work from our lab showed reduced apoptosis in the renal epithelium of Nlrp3-/- mice undergoing unilateral ureteric obstruction when compared to wild-type controls. The mechanism behind this distinction in Nlrp3-/- renal cells is unknown but does not appear to be simply due to classical Nlrp3 inflammasome activation involving the adaptor protein ASC and caspase-1 that mediates the maturation of pro-inflammatory cytokines such as IL-1β.

**Methods:** Apoptotic responses were analyzed in primary mouse tubular epithelial cells from wild type and Nlrp3-/- mice.

**Results:** Mouse tubular epithelial cells (mTECs) were found to express Nlrp3 and ASC proteins, however active caspase-1 subunits and IL-1β were undetectable suggesting

inflammasome independent roles for Nlrp3 and ASC in these cells. Primary Nlrp3-/- mTECs displayed reduced caspase-8, -3 and -7 activation and reduced poly-ADP ribose polymerase (PARP) cleavage after incubation with extrinsic cell death ligands, TNFα and Fas ligand. The reduction in apoptosis in Nlrp3-/- cells occurred independent of ASC since apoptotic responses in these cells were comparable to wild type mTEC. Differences in cell death between Nlrp3 deficient and wild-type mTECs appear to be independent of death inducing signaling complex (DISC) mRNA and protein levels and NF-κB pro-survival signaling. Likewise, tumor necrosis factor receptor 1 (TNFR1) protein levels remained similar between both cell types. Consistent with these findings, overexpression of NLRP3 in HeLa cells increased levels of active caspase-8 over GFP controls. Initial experiments have localized NLRP3 to mitochondria in renal tubular epithelial cells suggesting that NLRP3 may modulate cell death by sensing or regulating mitochondrial function.

**Conclusions:** Taken together, these results support an inflammasome-independent role for Nlrp3 in renal cell death, which in addition to its roles in cytokine maturation and inflammation, identify this protein as a potential therapeutic target in progressive CKD.

*Funding:* Government Support - Non-U.S.

## TH-OR035

### Fibroblast αv Integrins Are Key Mediators of Renal Fibrosis – Potential Novel Targets for Anti-Fibrosis Therapy

Yongen Chang,[1] Dean Sheppard.[2] *[1]Medicine/Div of Nephrology, Univ of California San Francisco, San Francisco, CA; [2]Medicine/Div of Pulmonary and Critical Care, Univ of California San Francisco, San Francisco, CA.*

**Background:** Renal fibrosis is the common pathway of chronic kidney disease. So far, its molecular mechanism is not entirely clear. While epithelial αv integrins, i.e. αvβ6, were identified as key pro-fibrotic factors through activation of transforming growth factor β (TGFβ), the role of fibroblast αv integrins is not established.

**Methods:** Platelet-derived growth factor receptor β (PDGFRβ) is highly expressed in activated pericytes, a main source of fibroblasts. Using a PDGFRβ promoter driven Cre system, we deleted αv integrins specifically in activated pericytes in mice while turning on Tdtomato reporter fluorescence in these cells. We then performed unilateral ureteral obstruction (UUO) surgery to induce interstitial fibrosis in these mice. We isolated Tdtomato positive wild type or αv null renal fibroblasts using fluorescence-activated cell sorting. For TGFβ activation assays, isolated fibroblasts were co-cultured with mink lung epithelial cells expressing firefly luciferase downstream of a TGFβ sensitive portion of the plasminogen activator inhibitor 1 promoter. The assays were also performed in the presence of blocking antibodies or small molecule inhibitors specific for each αv integrin.

**Results:** We found that renal fibroblasts express several αv integrins including αvβ1, αvβ3 and αvβ5. Deletion of αv integrins in renal fibroblasts protected mice from interstitial fibrosis after UUO as evidenced by decreased collagen deposition in conditional αv null mice compared to wild type. Furthermore, isolated wild type renal fibroblasts are able to activate TGFβ in vitro. Specific small molecule inhibitors against αvβ1 inhibited TGF beta activation by renal fibroblasts. Similarly, function blocking antibodies against αvβ3, but not αvβ5, also impaired the ability of fibroblasts to activate TGFβ.

**Conclusions:** We found that specific fibroblast αv integrins, namely αvβ1 and αvβ3, mediate renal fibrosis through direct activation of TGFβ. Small molecule inhibitors or blocking antibodies against αv integrins may emerge as potential anti-fibrotic therapeutic agents.

*Funding:* Other NIH Support - T32 Training Grant from September 2012-September 2013, Private Foundation Support

## TH-OR036

### Impact of Connective Tissue Growth Factor (CCN 2) on Hypoxia Inducible Factor-1alpha (Hif-1α) Responsiveness

Leighton R. James,[1,3] Catherine Le.[2,3] *[1]Dept of Medicine, Nephrology Div, Univ of Florida, Jacksonville, FL; [2]Cell and Molecular Biology, Colorado State Univ, Fort Collins, CO; [3]Dept of Medicine, UT Southwestern Medical Center, Dallas, TX.*

**Background:** CTGF is implicated in the pathogenesis of diverse fibrotic conditions. Hif-1α mediates cellular adaptation to hypoxia, vasculogenesis, glucose disposal and intermediary metabolism. However, the interaction between CTGF and Hif-1α is contradictory. Hypoxia may induce CTGF via hif-1α in lungs, but inhibit CTGF expression in proximal tubular epithelial cells (protective effect of hypoxic preconditioning), while in some systems CTGF may regulate HiF1 stability. Recently, we have observed that *ctgf* expression may influence blood pressure in a mice model. The objective of the current study was to examine effect of *ctgf* gene dose on Hif-1α expression and downstream Hif-1α targets.

**Methods:** CTGF knockout and CTGF gene-duplicated mice, as well as, mouse embryonic fibroblasts (MEFs) derived from these lines, were studied. Expression analysis was also performed with Mouse Array Chip (Illumina). Expression of constructs containing Hif-1α responsive elements (HRE-Luc) and pro-apoptotic Nip3 promoter and Endothelin-1(Et-1) protein levels were assessed. Blood pressure was assessed using automated tail-cuff method.

**Results:** Disruption of *ctgf* gene enhances MEF growth. Expression array analysis revealed that relative to wildtype, there was increased expression of Hif-1α-responsive genes including stem cell growth factor, fifty percent reduction in Et-1 and, concurrently enhanced expression of Hif-1α antagonistic factors necdin and claudin 1. Expression of HRE-Luc and Nip3 were increased in ctgf-null MEF under normal oxygen conditions. Nip3 promoter mutation abrogated expression in wildtype MEF, but marginally influenced expression in ctgf-null MEFs. Et-1 protein was reduced in ctgf-null MEF and, in parallel with elevated blood pressure in mice, enhanced CTGF expression accentuated Et-1 levels.

**Conclusions:** Variation in *ctgf* expression impacts HiF-1α and potentially influences normal physiologic processes as well as cellular responses to perturbation and injury.

*Funding:* NIDDK Support, Private Foundation Support

## TH-OR037

### Systemic Deletion of CTGF Ameliorates Anti-Glomerular Basement Membrane Nephritis with Reduction of Macrophage Infiltration

Naohiro Toda,[1] Hideki Yokoi,[1] Masato Kasahara,[1] Kiyoshi Mori,[1] Takashige Kuwabara,[1] Hirotaka Imamaki,[1] Akira Ishii,[1] Kenichi Koga,[1] Keita P. Mori,[1] Yukiko Kato,[1] Shoko Ohno,[1] Akira Sugawara,[1] Taiji Matsusaka,[2] Kazuwa Nakao,[1] Masashi Mukoyama.[1] *[1]Dept of Medicine and Clinical Science, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [2]Dept of Internal Medicine, Tokai Univ School of Medicine, Kanagawa, Japan.*

**Background:** Connective tissue growth factor (CTGF/CCN2) regulates signaling of other growth factors and promotes fibrosis. Although we previously showed that drug-inducible systemic CTGF knockout mice attenuated anti-glomerular basement membrane (anti-GBM) nephritis, the molecular mechanism how systemic deletion of CTGF ameliorated nephritis was unclear. To address these questions, we generate podocyte-specific CTGF knockout (pod-CTGF cKO) mice and drug-inducible systemic CTGF knockout (Rosa-CTGF cKO) mice.

**Methods:** CTGF floxed mice were crossed with RosaCreER$^{T2}$ mice, which ubiquitously express a tamoxifen-inducible Cre recombinase, to generate Rosa-CTGF cKO mice. Nephrin-Cre mice were used to create pod-CTGF cKO mice. We evoked anti-GBM nephritis and investigated the progression of glomerular injury including macrophage infiltration for 28 days.

**Results:** Rosa-CTGF cKO mice and pod-CTGF cKO mice showed normal renal appearance and no proteinuria without nephritis. After induction of anti-GBM nephritis, severe proteinuria and glomerular injury were developed in control mice. Pod-CTGF cKO mice showed similar renal injury and proteinuria. In contrast, Rosa-CTGF cKO mice exhibited reduced proteinuria by half at day 7 with ameliorated histological changes. The number of MAC2-positive cells in glomeruli was reduced in Rosa-CTGF cKO mice but not in pod-CTGF cKO mice. Glomerular expressions of TGF-β1, fibronectin and F4/80 were upregulated in control mice, and these increments were significantly reduced in Rosa-CTGF cKO mice, but not in Pod-CTGF cKO mice.

**Conclusions:** These results indicate that systemic deletion of CTGF, but not podocyte-specific inhibition of CTGF, can reduce proteinuria and macrophage infiltration to ameliorate glomerular injury in anti-GBM nephritis.

## TH-OR038

### Molecular Mechanism for Asymmetric Dimethylarginine Dysregulation in Patients with Chronic Kidney Disease

Crystal A. Gadegbeku,[1,5] Subhasish Bose,[1] Wenjun Ju,[2] Louis G. D'alecy,[2] Kalyani Perumal,[3,5] Zeenat Yousuf Bhat,[4,5] Matthias Kretzler.[2,5] *[1]Temple Univ; [2]Univ of Michigan; [3]John H. Stroger Hospital; [4]Wayne State Univ; [5]Michigan O'Brien Renal Center (P30).*

**Background:** Endothelial dysfunction is implicated in accelerated atherosclerosis in patients with chronic kidney disease (CKD). Plasma asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase, is linked to cardiovascular morbidity and mortality and reaches high levels in CKD. ADMA is generated by protein methyltransferase 1 (PRMT1) and metabolized by dimethylarginine dimethylaminohydrolase (DDAH). This study was designed to characterize intra-renal molecular markers of ADMA metabolism in patients that have been implicated in experimental studies.

**Methods:** Renal biopsy tissue stored in the Michigan O'Brien Renal Center biobank from 29 consented individuals underwent gene expression analysis using Affymetrix 133 plus GeneChips®. Steady state gene expression levels of PRMT1, DDAH, and oxidative stress markers, cytochrome b-245 beta polypeptide (CYBB), neutrophil cytosolic factor 2 (NCF2), and superoxide dismutase 2 (SOD2), were measured from the micro-dissected tubulo-interstitial compartment of cortical samples. Estimated GFR (eGFR, CKD-EPI equation) at the time of biopsy was used for analysis. To explore relationships between molecular markers and eGFR, Pearson correlations were used with adjustment for multiple comparisons.

**Results:** Tubulo-interstitial gene expression of PRMT1 was inversely correlated with eGFR (-0.49, p=0.02) and DDAH1 (-0.65, p=0.0002) but not DDAH2. Also, positive correlations were observed with oxidative stress markers, CYBB and NCF2 (R=0.43; R=0.44, both p=0.04) and marginally, with SOD2 (R=0.41, p=0.06). Additionally, DDAH1 was inversely correlated with NCF2 (R=-0.59, p=0.001) and SOD2 (R=-0.44, p=0.04).

**Conclusions:** For the first time in humans, we demonstrate a potential mechanism for ADMA dysregulation that may contribute to CVD burden in CKD. These findings support animal model data demonstrating up-regulation of PRMT1 and down-regulation of DDAH1 associated with oxidative stress in CKD.

*Funding:* NIDDK Support

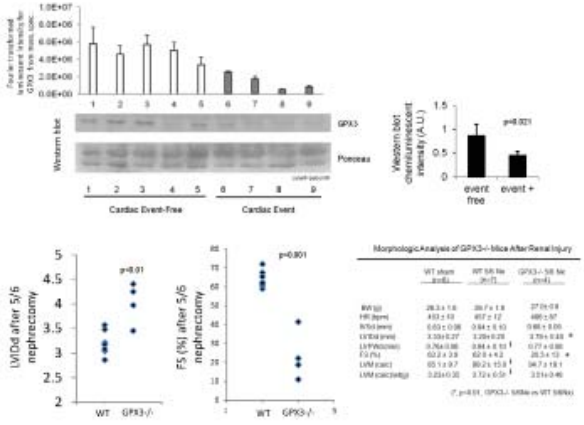
**TH-OR039**

**Uremic Cardiomyopathy Is Associated with Glutathione Peroxidase 3 Deficiency** Paul Pang, Rafael Kramann, Joseph Loscalzo, Joseph V. Bonventre, Andrew M. Siedlecki. *Medicine, Brigham and Women's Hospital, Boston, MA.*

**Background:** Glutathione peroxidase 3 (GPX3) is synthesized primarily in the kidney. Patients with chronic kidney disease (CKD) have deficient levels of circulating GPX3. This may lead to a form of cardiomyopathy due to the accumulation of reactive oxygen species that exacerbate inflammatory signaling in the myocardium.

**Methods:** 9 patients with CKD, had serum collected, were followed prospectively, and monitored for cardiac endpoints. Proteomic analysis was performed by LC-tandem mass spectrometry. GPX3-/- and wild type mice underwent 5/6 nephrectomy followed by echocardiography 4 weeks and 8 weeks after injury.

**Results:** We identified several circulating proteins of interest based on plasma proteomic analysis of patients with CKD and cardiac endpoints.




GPX3 showed the greatest absolute decrease in protein expression. Patients with an incident cardiac event on average had a 66.4% decrease in GPX3 plasma levels measured by mass spectrometry. This finding was validated by western blot analysis where GPX3 protein levels were decreased by 47.6±15.7% in CKD patients with a cardiac event (p=0.021) compared to CKD patients without a cardiac event. GPX3-/- mice underwent 5/6 nephrectomy compared to wild-types + 5/6nephrectomy. At 4 weeks after injury, LVIDd was increased, 4.02±0.42mm (n=4) vs 3.33±0.27mm (n=7) respectively (p<0.01). At the same time point fractional shortening was decreased to 23.5±11% in GPX3-/- compared to 62.0%±4 in injured wild-types (p<0.001).

**Conclusions:** For the first time we show that the mechanism of renal-induced cardiac disease is dependent on GPX3 deficiency. This was evident by the dramatic worsening of cardiac function after subtotal nephrectomy in GPX3-/- animals. GPX3 deficiency is also associated with increased frequency of cardiac events in patients with CKD and may offer a targeted therapy for renal-induced cardiomyopathy.

*Funding:* NIDDK Support

**TH-OR040**

**Activation of Aryl Hydrocarbon Receptor Mediates Suppression of Hypoxia Inducible Factor-Dependent Erythropoietin Expression by Indoxyl Sulfate** Hirobumi Asai, Junya Hirata, Sayaka Seki, Mie Watanabe-Akanuma. *Kureha Corporation, Tokyo, Japan.*

**Background:** Uremic toxins are considered to have a role in renal anemia which is caused by inadequate renal erythropoietin (EPO) production. Indoxyl sulfate (IS), a representative uremic toxin, has been reported to suppress the activation of hypoxia inducible factor (HIF) and subsequent EPO production. In this study, we investigated the involvement of aryl hydrocarbon receptor (AHR) on the suppressive effect of IS. Besides, effect of the other uremic toxins, p-cresyl sulfate (PCS) and phenyl sulfate (PhS), on EPO expression was also examined.

**Methods:** HepG2 cells were treated with cobalt chloride (50 μM) to induce hypoxic response in the presence or absence of IS, PCS, PhS or an AHR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). For the inactivation of AHR, cells were treated with an AHR antagonist CH-223191 or transfected with the specific siRNA prior to the IS treatment. EPO gene expression was examined by real time RT-PCR, and HIF transcriptional activity was assessed by hypoxia responsive element (HRE) luciferase reporter assay.

**Results:** IS significantly inhibited cobalt chloride-induced elevation of EPO expression at 100 μM or more. PhS slightly inhibited the EPO expression at relatively higher concentration (1.5 mM), whereas PCS (up to 1.5 mM) had no effect. IS also significantly inhibited the HIF activation at 20 μM or more, while PCS had no inhibitory effect. Inactivation of AHR by either CH-223191 or the specific siRNA completely abolished the inhibitory effect of IS. Similar to IS, TCDD also significantly inhibited the HIF activation.

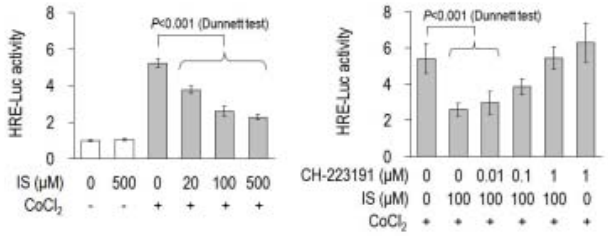



**Figure.** *Left*, effect of IS on cobalt chloride ($CoCl_2$)-induced HIF activation. *Right*, effect of CH-223191, an AHR antagonist, on the suppression of HIF activation by IS.

**Conclusions:** IS but not PCS and PhS suppresses HIF-dependent EPO expression at a concentration relevant to the serum levels in patients with chronic kidney disease, and AHR plays a crucial role in the suppressive effect of IS.

*Funding:* Pharmaceutical Company Support - Kureha Corporation

**TH-OR041**

**Impact of Genetic TNFR1 Modulation on Diabetic Nephropathy Course in a Murine Model of Diabetes** Roberto Bassi,[1] Andrea Vergani,[1] Masami Ikehata,[3] Jung Eun Lee,[2] Melissa Chin,[1] William Walker,[2] Moufida Ben Nasr,[1] Jackson Jeong,[2] Irene Garcia,[4] Maria Pia Rastaldi,[3] Paolo Fiorina,[1] Monika A. Niewczas.[2] *[1]Boston Children's Hospital, Boston; [2]Joslin Diabetes Center, Boston; [3]Ospedale Maggiore Policlinico, Milan, Italy; [4]Univ of Geneva, Switzerland.*

**Background:** Circulating TNFR1 was identified as a strong predictor of diabetic nephropathy (DN) progression in the Joslin Kidney Study. Aim of this work was to evaluate whether TNFR1 contributes to the injury of the diabetic kidney. We tested our hypothesis in a murine model of DN in which hyperglycemia was chemically induced with streptozotocin (STZ) and TNFR1 expression was genetically modulated.

**Methods:** Tnfrsf1atm1Imx knockout ($TNFR1^{-/-}$), TNFR1 transgenic (TNFR1tg) and control WT (WT) mice all in the C57BL/6 background (n=8 per group of 8 weeks of age) were used in our study. We confirmed their TNFR genotype with RT-PCR and their protein expression with ELISA. Repeated measurements of glycemia and albumin excretion rate (AER) were performed over a 12 week period following STZ injection.

**Results:** Serum TNFR1 levels were undetectable in $TNFR1^{-/-}$ mice, 1408±1634ng/ml in TNFR1tg mice and 0.89±0.16ng/ml in WT mice. Albuminuria course over time was significantly different among the 3 groups of mice, AER values were lower in $TNFR1^{-/-}$ and higher in TNFR1tg compared to the control group, respectively (p=0.007, significance obtained with mixed effect model). Survival of TNFR1tg mice was significantly reduced compared to the other groups (deaths: TNFR1tg: 5/8, vs. $TNFR1^{-/-}$: 2/8; WT: 2/8).

| **AER (mg/24hrs):** median (25th, 75th percentile) | | | |
|---|---|---|---|
| | **WT** | **$TNFR1^{-/-}$** | **TNFR1tg** |
| baseline | 7 (5-10) | 15 (8-18) | 7 (5-9) |
| 4 weeks | 17 (14-21) | 13 (11-13) | 13 (12-26) |
| 8 weeks | 17 (13-25) | 11(11-18) | 27 (20-32) |
| 12 weeks | 24 (19-44) | 13 (10-17) | 22 (18-32) |

**Conclusions:** We demonstrated that the modulation of TNFR1 expression impacts albuminuria course in an experimental model of murine DN. Our data suggest that TNFR1 may play a causative role in DN progression and warrants its consideration for therapeutic applications.

*Funding:* NIDDK Support

**TH-OR042**

**Tissue Transcriptome-Driven-Approach Facilitates Discovery of Non-Invasive Biomarkers for Chronic Kidney Disease** Wenjun Ju,[1] Viji Nair,[1] Shahaan Smith,[1] Jennifer Joyce Hawkins,[1] Ivan Formentini,[2] Maria Bobadilla,[2] Maria Chiara Magnone,[2] Crystal A. Gadegbeku,[3] Matthias Kretzler.[1] *[1]Univ of Michigan; [2]Hoffmann-La Roche; [3]Temple Univ, for the ERCB, C-PROBE and NEPTUNE Consortium.*

**Background:** Defining intra-renal molecular mechanism associated with CKD is critical for identification of therapeutic targets and molecular marker definition. We have employed a gene expression profiling study in renal biopsies of the European Renal cDNA Bank (ERCB) and identified a set of 68 intrarenal transcripts that was able to predict degree of CKD in ERCB training (n=164) and test cohorts (n=55), as well as in 42 patients of the Michigan O'Brien Renal Center C-PROBE cohort.

**Methods:** In this study we investigate if proteins encoded by the intrarenal CKD associated transcripts can be discovered in urine, if urinary proteins correlate with intrarenal transcript level and if they maintain their association with eGFR. Epidermal growth factor (EGF) was selected from the transcript panel as a proof of principle study.

**Results:** Intrarenal EGF mRNA correlates with eGFR in the ERCB test cohort (r=0.66) and C-PROBE cohort (r=0.42). In C-PROBE, matching urine samples at time of biopsy (n=34) were evaluated for EGF and demonstrated a significant correlation with intra-renal EGF mRNA (r=0.7, p<0.0001). Urinary EGF retained its correlation with eGFR in 93 C-PROBE patients irrespective of underlying disease type, stage, age, gender or race (r=0.82, p<0.0001). Robustness of the EGF-eGFR association in proteinuric patients was demonstrated in 213 patients from the Nephrotic Syndrome Study Network (NEPTUNE) (r=0.83, p<0.0001, urine protein level ranges from 0.01 to 66.8 g/g creatinine).

**Conclusions:** In conclusion, intrarenal transcripts with strong associations to eGFR can be used to define urinary protein markers for non-invasive stratification of CKD patients according to their intrarenal pathophysiology.

*Funding:* NIDDK Support, Pharmaceutical Company Support - F. Hoffmann-La Roche Ltd

### TH-OR043

**In Vitro Human Renal Primary Cells for Studies of Mineral Ion Homeostasis**

Rebecca M. Wadey,[1] Lydia E. Searchfield,[1] Sally A. Price,[2] Daniela Riccardi.[1]
*[1]Cardiff Univ, United Kingdom; [2]AstraZeneca, United Kingdom.*

**Background:** FGF23, PTH and Vitamin D play important roles in mineral ion homeostasis. The complex interactions between these hormones are difficult to investigate *in vivo*. We have characterised a human primary renal cell model in an attempt to specifically dissect out the role the kidney plays within the FGF23, PTH and Vitamin D axis.

**Methods:** Primary renal cells were isolated from ethically-consented kidney cortical tissue from patients undergoing nephrectomy. Cell monolayers were cultured on transwells without passaging and characterised in terms of viability (proliferation/apoptosis), morphology (electron microscopy) and expression of proximal and distal markers, N- and E-cadherin, respectively. Expression of NaPiIIa (type 2a $Na^+$-Pi cotransporter), NCX1 ($Na^+$-$Ca^{2+}$ exchanger), PMCA1 (plasma membrane $Ca^{2+}$ ATPase), FGFr1-4 and Klotho were determined by immunohistochemistry (on kidney sections) and western blotting (of kidney lysate) and then confirmed in isolated cells. Functional readouts included Pi-dependent NaPiIIa upregulation, ERK activation, 1-hydroxylase activity and Pi-induced mineralisation.

**Results:** Cell proliferation rates were high and apoptosis rates low throughout culture. Dual N- and E-cadherin immunostaining revealed that primary cultures consisted of 70% proximal and 30% distal cells after 5 days. When cultured on transwells, NaPi2a expression was present up to day 8 and NCX1, PMCA1, FGFr1-4 and Klotho proteins up to day 15. Proximal tubule brush border membranes were visible by electron microscopy at day 5. NaPiIIa expression was upregulated following 24h exposure to Pi-free medium. FGF23 treatment induced ERK activation, which could be blocked by inhibiting MEK. MEK inhibition also increased Pi-dependent mineralisation.

**Conclusions:** Primary human kidney cells retain the morphological and functional properties of kidney cells *in vivo* and express key proteins involved in mineral ion homeostasis. These observations suggest great potential for this preparation as a model for investigating the interplay between FGF23, PTH and Vitamin D in mineral ion homeostasis, and for understanding how mineral ion dysregulation occurs in chronic kidney disease.

### TH-OR044

**Urinary Proteomics: The Development of a Diagnostic Biomarker Panel for Pre-Eclampsia**

Kate Bramham,[1] Hiten Mistry,[1] Marta Hentschke,[1] Steven Lynham,[2] Malcolm Ward,[2] Lucilla Poston,[1] Lucy C. Chappell.[1] *[1]Div of Women's Health, King's College London, London, United Kingdom; [2]Centre of Excellence for Mass Spectrometry, King's College London, London, United Kingdom.*

**Background:** Early detection/diagnosis of pre-eclampsia allows appropriate monitoring and targeting of therapeutic strategies. Urinary proteomics is a rapidly developing field that allows detection and identification of individual proteins. Our hypothesis is that a distinctive pre-eclampsia urinary proteome profile can be identified.

**Methods:** A proteome profile for time-of-disease urine samples from twelve pre-eclamptic and twelve gestation-matched controls was established using a validated workflow, involving selective immunodepletion, 1D SDS-PAGE gel fractionation, in-gel digestion of gel sections, LC-MS/MS analysis, spectral analysis and selection of candidate proteins for Selective Reaction Monitoring (SRM) verification and quantification.

**Results:** 981 proteins were identified using minimal stringency in Scaffold, and 8 proteins were differentially expressed between cases and controls. Subsequent SRM area-under-curve peak analysis revealed 7 proteins which were present in significantly higher levels in pre-eclamptic urine samples than controls and 1 protein was significantly lower in pre-eclamptic samples. An example of spectral peaks are shown in Figure 1.

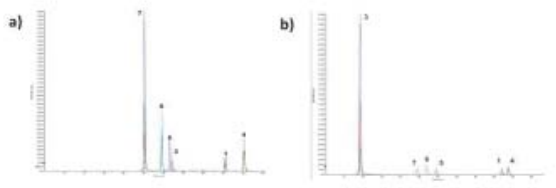



Figure 1: SRM peaks in a) one pre-eclampsia sample and b) one control sample for a single peptide from each of the seven proteins shown to be increased in 12 pre-eclampsia patients and one only present in the 12 controls

For protection of intellectual property protein identities are omitted.

**Conclusions:** A urinary proteomic signature can be identified in the urine of women with pre-eclampsia samples. Validation of this differential proteomic profile using a larger number of samples, including women with CKD and superimposed pre-eclampsia, chronic and gestational hypertension is currently underway. The use of a diagnostic urinary biomarker will be invaluable in low socioeconomic countries where facilities for blood taking and processing are limited.

### TH-OR045

**P-Cresol (PC) and P-Cresyl Sulfate (PCS) Stimulate Chemoattractant Protein-1 (MCP-1) Expression in Human Vascular Smooth Muscle Cells (VSMC)**

Rayana Ariane Pereira Maciel,[1] Lisienny Campoli Tono Rempel,[1] Alessandra Becker Finco,[1] Wesley M. Souza,[1] Roberto Pecoits-Filho,[2] Andréa Marques Stinghen,[1] Bruna Bosquetti.[1] *[1]Universidade Federal do Paraná, Curitiba, Brazil; [2]Pontifícia Universidade Católica do Paraná, Curitiba, Brazil.*

**Background:** Uremic toxins such as PC and its conjugate PCS are responsible for many of the uremia clinical consequences, such as atherosclerosis in CKD patients. In this study we investigate the *in vitro* role of PC and PCS in MCP-1 expression via transcription factor NF-κβ p65 in VSMC.

**Methods:** PCS was synthesized by PC sulfatation. VSMC were extracted by enzymatic digestion of umbilical cord vein and characterized by immunofluorescence against α-actin antibody. The cells were treated with PC and PCS in a kinetics of 0 and 3 h in their normal (PC1 0.60 and PCS1 2.87 mg/L), minimum (PC2 20.10 and PCS2 15.6 mg/L) and maximum uremic concentrations (PC3 40.7 and PCS3 47.20 mg/L). Cell viability was assessed by MTT. MCP-1 expression was investigated by ELISA in cells supernatant after toxins treatment in the presence or absence of NF-κβ p65 inhibitor.

**Results:** No significant difference was observed in cell viability after toxins treatment for all concentrations tested. After 3h there was a significant increase in MCP-1 expression in cells treated with PC2 (150±16 pg/mL, $P<0.001$), PC3 (144±28 pg/mL, $P<0.05$), PCS1 (145±24 pg/mL, $P<0.005$), PCS2 (150±13 pg/mL, $P<0.001$) and PCS3 (160±8 pg/mL, $P<0.001$), compared with control cells (37±3 pg/mL). When VSMC were treated with NF-κβ p65 inhibitor, we observed after 3h a pronounced decrease in MCP-1 levels, especially to PC2 (150±16 pg/mL *vs* 36±10 pg/mL, $P<0.001$) and PC3 (160±8 *vs* 50±18 pg/mL, $P<0.05$) treatment, but no difference was observed when the inhibitor was adding with PCS.

**Conclusions:** VSMC are involved in atherosclerosis lesion formation and cytokines production such as MCP-1, which contributes to the inflammatory response initiation and propagation to lipid. Our results suggest for the first time that PC mediates MCP-1 production in VSMC, probably through NF-κβ p65 pathway, although we hypothesize that PCS acts through a different subunit pathway since NF-κβ p65 inhibitor was not able to inhibit MCP-1 production.

*Funding:* Government Support - Non-U.S.

### TH-OR046

**Targeting the CRBN/DDB1/Cul4A E3 Ubiquitin Ligase Prevents Kidney Fibrosis**

Yuanyuan Shi,[1] Yanlin Wang,[2] William E. Mitch,[2] Zhaoyong Hu.[2] *[1]Nephrology Div, Second Hospital of Shanxi Medical Univ, Taiyuan, Shanxi, China; [2]Nephrology Div, Baylor College of Medicine, Houston, TX.*

**Background:** Cereblon (CRBN) interacts with damaged DNA binding protein 1 (DDB1) and Cullin 4A (Cul4A), forming an E3 ubiquitin ligase complex, the first step towards degradation by proteasomes (UPS). CRBN is the critical element that recognizes substrate proteins leading to their conjugation with ubiquitin and ultimately, UPS degradation. Notably, the CRBN/DDB1/Cul4A is activated by inflammation, in stress response and it can influence cell growth. Since CRBN is stimulated by inflammation, etc. we investigated whether blocking CRBN would ameliorate the progression of kidney fibrosis. The mechanisms of fibrosis are pertinent because the degree of fibrosis is closely associated with progression of chronic kidney disease (CKD). Unfortunately, there are few therapies that effectively block or suppress kidney fibrosis. We investigated whether blocking CRBN would ameliorate the progression of kidney fibrosis.

**Methods:** In mice with genomic or pharmacological inhibition of CRBN, we evaluated inflammatory mediators and the degree of kidney fibrosis following unilateral ureteral obstruction (UUO).

**Results:** At 7 days after UUO in wild type mice, there was increased expression of collagen, fibronectin and SMAa, as demonstrated by many investigators. Mice with CRBN knockout exhibited reduced expression of collagen I (60%), fibronectin (>80%) and SMAa (>50%). Importantly, there also was suppression of the tubulointerstitial fibrosis caused by UUO. Inhibition of CRBN suppressed TGFb1 mRNA expression as well as phosphorylation of Smad3. In cultured kidney fibroblasts and wild-type UUO mice, we found that thalidomide, a CRBN inhibitor, suppressed ubiquitin conjugation to SnoN, this results in an increase in SnoN with inhibition of TGFb1 expression. These responses reduced fibrosis in the kidney despite UUO.

**Conclusions:** The ubiquitin E3 ligase of the CRBN/DDB1/CUL4A E3 complex regulates the kidney expression of TGFb1 and ultimately, fibrosis. We have identified a molecular basis for an anti-fibrotic effect of blocking CRBN and this could become a treatment strategy for the fibrosis that damages the kidney in CKD.

*Funding:* NIDDK Support

### TH-OR047

**Resistance Exercise Prevents Muscle Wasting in Mice with Chronic Kidney Disease by Increasing microRNA-23**

Cong Zhang,[1] Li Hu,[1] Janet D. Klein,[1] Russ Price,[1,2] Xiaonan H. Wang.[1] *[1]Renal Medicine, Emory Univ, Atlanta, GA; [2]Renal Medicine, Atlanta VAMC, Decatur, GA.*

**Background:** There are 23 individual microRNAs that are significantly changed in muscle of chronic kidney disease (CKD) mice (Wang et al, JASN 2010). We previously showed that exercise improves muscle protein synthesis and prevents accelerated protein degradation in muscle of CKD mice (Wang at al, KI 2009). This study identifies In silico

analysis and other reports suggested that microRNA-23 (miR-23) targets several proteins associated with muscle atrophy. In this study, we evaluated whether exercise increases the level of miR-23 (miR-23) in CKD mice.

**Methods:** CKD was induced in 20-25g mice by 5/6th nephrectomy. Muscle overloading, produced by removing the gastrocnemius and soleus muscles from both hindlimbs of control and CKD mice, was used as an exercise model.

**Results:** Since atrogin-1, MuRF-1, YY1 and PTEN are putative targets of miR-23, and each of them can impact muscle wasting, we verified whether miR-23 changes the expression of each target using 3'-UTR-luciferase reporter assays. Overexpression of miR-23 in cultured muscle cells inhibits the reporter gene activities of atrogin-1 and MuRF1, suggesting that miR-23 attenuates the expression of its predicted targets. Exercise significantly increased the level of miR-23 in muscle of CKD mice vs. unexercised CKD mice. Consistent with the response, exercise decreased PTEN protein and increased Akt phosphorylation (2.3-fold, P<0.001) which would limit muscle atrophy. Exercise increased MyoD, myogenin and eMyHC, myogenic proteins that are linked to YY1 transcription factor in CKD mice. Finally, mRNAs for the muscle-specific E3 ubiquiin ligases, atrogin-1 and MuRF-1, were attenuated by exercise in CKD mice. Other muscle-specific miRs (i.e., miR-1, miR-133b and miR-206) were unchanged by exercise in muscle of CKD mice, indicating specificity of the miR-23 response.

**Conclusions:** Resistance exercise can prevent CKD-induced muscle loss by increasing miR-23. This leads to a suppression of multiple atrophy-related target proteins that may contribute to the muscle-sparing effects of exercise in CKD.

*Funding:* NIDDK Support, Other NIH Support - NIH AR060268 (XHW); DK095610 (SRP), Veterans Affairs Support

## TH-OR048

**GFR Decline as an Endpoint for Clinical Trials in CKD – A Meta-Analysis of Treatment Effects from Randomized Trials: Report of an NKF-FDA Workshop** Lesley Inker,[1] Hiddo Jan Lambers Heerspink,[4] Hasi Mondal,[1] Josef Coresh,[2] Tom Greene,[3] Andrew S. Levey.[1] *[1]Tufts Medical Center; [2]Johns Hopkins Univ; [3]Univ of Utah; [4]Univ Medical Center Groningen.*

**Background:** The FDA accepts doubling of serum creatinine (Scr) (halving of GFR), as a surrogate endpoint for kidney failure in trials of CKD progression. Substantial time is often required to reach this endpoint. Lesser declines in GFR could reduce the length of trials, increasing feasibility of RCTs. The NKF and FDA sponsored a workshop to examine data supporting alternative definitions of GFR decline as endpoints in RCT's.

**Methods:** Using a pooled dataset of 12821 patients from 43 CKD studies, we compared the treatment effect using established endpoints (EE) of dialysis, GFR<15 or 2XScr over the entire trial period to the alternative endpoints (AE) of a decline in GFR of 20, 30 or 40% over the entire period or 12, 18 and 24 months. Cox analyses were performed within study, followed by random effects analyses summarizing results across studies of same intervention.

**Results:** The table compares percent differences in hazard ratios for the treatment effect on AE's compared to the EE.

**Percent difference in HR of the AE relative to the HR of the EE**

| Intervention | All | | | 24 months | | | 18 months | | | 12 months | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 40% | 30% | 20% | 40% | 30% | 20% | 40% | 30% | 20% | 40% | 30% | 20% |
| RASB | 4 | 12 | 25 | -4 | 6 | 19 | -6 | 2 | 19 | -2 | 6 | 18 |
| RASB vs CCB | 15 | 22 | 43 | 9 | 24 | 39 | 4 | 34 | 49 | 10 | 39 | 65 |
| BP | -2 | 4 | 9 | -8 | 3 | 12 | -8 | -5 | -2 | 17 | 11 | 7 |
| LPD | -14 | -19 | -18 | -12 | -21 | -18 | -16 | -23 | -22 | -8 | -20 | -29 |
| IS | 24 | 32 | 47 | 46 | 52 | 45 | 83 | 98 | 68 | 94 | 86 | 70 |

Renin angiotension system blockade, RASB; Calcium channel blocker, CCB; intensive blood pressure, BP; low protein diet, LPD; immunosuppressive therapy, IS

Higher values indicate greater HR for the AE (less strong treatment effect) than HR of the EE. Pooled results may not apply to each studies. Solid shading: differences in HR of ≤± 10% (white), ±10-15% (gray), or >±15% (dark gray). Hatched shading: confounded results as intervention effected by nonGFR determinants of Scr.

**Conclusions:** For some interventions, 40 or 30% decline in GFR, appears to be a reasonable alternative endpoint for CKD trials, especially for shorter time periods. Studies in patients with high GFR, such as IS studies, may not show benefit due to the short duration. Scr based AE should not be used to evaluate interventions that effect nonGFR determinants of Scr.

*Funding:* Private Foundation Support

## TH-OR049

**GFR Decline as an Alternative Endpoint for Kidney Failure – Meta-Analysis of CKD Prognosis Consortium Cohorts: A Report from an NKF FDA Workshop** Josef Coresh, Kunihiro Matsushita, Yingying Sang, Shoshana Ballew, Lawrence J. Appel, Jamie Alton Green, Gunnar H. Heine, Lesley Inker, Areef Ishani, Angharad Marks, Tanvir Chowdhury Turin, Kunitoshi Iseki, Andrew S. Levey. *CKD Prognosis Consortium.*

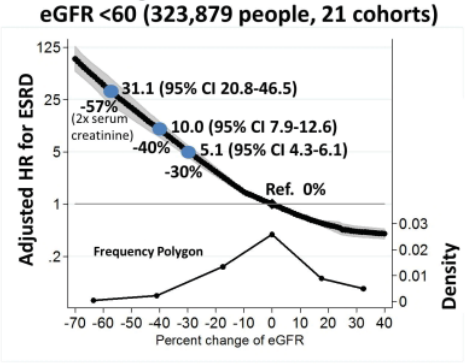
**Background:** The NKF and FDA convened a workshop to evaluate the evidence for using a change in GFR smaller than doubling (2x) of serum creatinine as an alternative endpoint to kidney failure in studies of CKD progression.

**Methods:** eGFR (CKD-EPI creatinine) % change from the first to last eGFR in a 2-year baseline period was related to subsequent ESRD in adjusted Cox Regression using splines. Random effects meta-analyses combined results across studies stratified by first eGFR (<60 & ≥60). Absolute ESRD risk is based on a pooled baseline risk.

**Results:** 21 cohorts with 722,221 participants reported 7,529 ESRD cases during 2.4 years average follow-up after 2-year baseline period. Log hazard ratio (HR) of ESRD increased linearly with % eGFR decline adjusted for baseline eGFR, age, sex, race, diabetes, history of CVD, cholesterol and blood pressure (Figure; eGFR<60 HR 5.1; eGFR 60+ HR 6.2 for -30% eGFR change). Association was strong and consistent even for modest, more common reductions in eGFR as well as length of baseline (1, 2 and 3 years), eGFR, age, diabetes, or ACR and slope of eGFR decline. Pooled absolute ESRD risk 10-years after baseline adjusted for covariates and competing mortality risk for eGFR changes of -57% (2x serum creatinine), -40%, -30% and 0% were 100%, 92%, 78%, vs. 28% respectively at baseline eGFR of 35.

**Conclusions:** Declines in eGFR, smaller than doubling of serum creatinine, are strongly and consistently associated with the risk of ESRD adjusted for the baseline eGFR and covariates supporting alternative endpoints for CKD progression.

**Figure**: Adjusted hazard ratio (HR) of ESRD vs. 2-year % change in eGFR: Baseline eGFR<60




*Funding:* Private Foundation Support

## TH-OR050

**Serum Level of Soluble (Pro)renin Receptor Is Modulated in Chronic Kidney Disease (CKD) and a Novel Valuable Biomarker for Progression of CKD: A Longitudinal Follow-Up Study** Kazu Hamada,[1] Yoshiko Shimamura,[1] Koji Ogata,[1] Kosuke Inoue,[1] Yoshinori Taniguchi,[1] Masayuki Ishihara,[1] Taro Horino,[1] Kenji Yuasa,[2] Yoshio Terada.[1] *[1]Dept of Endocrinology, Matabolism and Nephrology, Kochi Medical School; [2]Kochi-Takasu-Hospital.*

**Background:** Prorenin, the precursor of renin, binds to the (pro)renin receptor ([P]RR) and triggers intracellular signaling. The ligand binding sites of (P)RR are disconnected and are present in the soluble form of the receptor in serum. Given that the clinical significance of serum prorenin and soluble (P)RR in chronic kidney disease (CKD) is unclear, no longitudinal follow-up studies have been reported to evaluate soluble (P)RR as a biomarker for renal prognosis. We investigated the relation between serum prorenin, soluble (P)RR, and various clinical parameters in patients with CKD.

**Methods:** A total of 374 patients with CKD were enrolled. Serum creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA), hemoglobin (Hb), soluble secreted α-Klotho, and the urine protein/Cr ratio were also measured. Similarly, clinical parameters were also evaluated using serum and urine sample collected after 1 year (n = 204).

**Results:** Soluble (P)RR levels were positively associated with serum Cr, BUN, UA levels CKD stage and urine protein/Cr ratio. Soluble (P)RR levels were lower in patients treated with an angiotensin II receptor blocker (ARB) than in those without ARB therapy. Soluble (P)RR levels were slower in CKD patients with diabetes mellitus or primary hypertension than in those without these conditions. Soluble (P)RR levels did not correlate with prorenin levels.Finally, with respect to the relationship between basal soluble (P)RR levels and the progression rates of kidney function, soluble (P)RR levels were positively associated with ΔCr and inversely associated with ΔeGFR.

**Conclusions:** Serum levels of soluble (P)RR correlated with the stage of CKD, and had a positive correlation with the decline rate of eGFR. Our findings suggest that soluble (P)RR may be involved in renal injury and influence progression of CKD and become a novel valuable biomarker to predict the prognosis of kidney diseases.

## TH-OR051

**Elevated NT-proBNP and Kidney Function Decline** Meyeon Park,[1] Eric Vittinghoff,[1] Michael Shlipak,[1] Mary Whooley,[1] Nisha Bansal.[2] *[1]Univ of California, San Francisco; [2]Univ of Washington, Seattle.*

**Background:** Venous congestion may be an important contributor to kidney function decline even in patients without clinical heart failure (HF). N-terminal pro-brain natriuretic peptide (NT-proBNP) is released from cardiac myocytes in response to pressure or volume overload. We evaluated associations of NT-proBNP with longitudinal changes in kidney function among patients with stable coronary heart disease and without clinical HF.

**Methods:** Participants in the Heart and Soul Study had NT-proBNP measured from baseline serum samples. We excluded individuals with baseline HF and categorized NT-proBNP into quartiles, comparing the highest (>396 pg/mL) to the lowest (<72 pg/mL) quartile. We evaluated associations of NT-proBNP with longitudinal changes in estimated

glomerular filtration rate (eGFR), defined as a percent loss per year and as rapid eGFR loss (>3% eGFR loss per year). We adjusted for cardiovascular disease risk factors, echocardiography measures, and baseline kidney function.

**Results:** Among 810 participants without HF, mean age was 67 (SD 11); 82% were male; 60% were white. Median NT-proBNP was 150 pg/mL (IQR 67-377). Mean eGFR was 72 (SD 21) ml/min/1.73m$^2$. After multivariable adjustment, the highest quartile of NT-proBNP was associated with a 1.7% (0.5-3) faster loss in eGFR per year (p=0.007) compared with the lowest quartile. Participants in the highest quartile also had >5 times the odds of rapid eGFR loss compared with those in the lowest quartile (Table); findings were similar when restricted to participants with eGFR>60 ml/min/1.73m$^2$ at baseline (N=550).

| | Unadjusted OR (95% CI) | | Fully adjusted OR | | eGFR>60 only Fully adjusted OR | |
|---|---|---|---|---|---|---|
| Q1Odds of rapid eGFR loss by quartile of NT-proBNP | ref | p | ref | p | ref | p |
| Q2 | 3.0 (1.4-6.8) | 0.04 | 2.6 (1.1-6.3) | 0.04 | 2.8 (1.1-7.6) | 0.04 |
| Q3 | 3.7 (1.7-8.3) | 0.01 | 3.1 (1.2-7.7) | 0.02 | 2.6 (0.9-7.5) | 0.08 |
| Q4 | 7.8 (3.5-17.5) | <0.001 | 5.5 (2-15.1) | 0.001 | 7.4 (2.1-26.1) | 0.002 |

**Conclusions:** NT-proBNP is strongly associated with accelerated kidney function loss in individuals without HF, suggesting that venous congestion may be an important contributor to adverse kidney outcomes.

*Funding:* NIDDK Support

## TH-OR052

### Changes in Mortality Rates among Patients with End-Stage Renal Disease (ESRD) and Changes in Incident Rates of ESRD before and after the Great East Japan Earthquake and Tsunami 2011

Masaki Ohsawa. *Hygiene and Preventive Medicine, Iwate Medical Univ, Iwate Prefecture, Japan.*

**Background:** The Great East Japan Earthquake and Tsunami (March 11th 2011) caused significant damage to people in Iwate Prefecture (North-east area in Japan). Patients with end-stage renal disease (ESRD) are thought to be vulnerable for disasters and prolonged sedentary lifestyle during evacuation may contribute to worsening diabetic and hypertensive status of the refugees living in shelters. It is hypothesized that mortality rates in patients with ESRD increased and incident rates of ESRD increased in disaster area.

**Methods:** The Iwate ESRD registry program based on inventory survey was initiated in 2010 and this program has been continued to the present. We obtained data from the database of Iwate ESRD registry program from Iwate Medical Association. We counted total annual number of deaths in ESRD patients and total annual number of incident ESRD in Iwate Prefecture across the sea coast area (Tsunami disaster area) and inland region, respectively, in 2010 and 2011 (disaster year).

**Results:** The results are shown in the table. There were 4 ESRD patients who were missing and feared dead after Tsunami disaster in sea coast area.

| | 2009/12/31 | 2010/01/01-2010/12/31 | 2010/12/31 | 2011/01/01-2011/12/31 | 2011/12/31 |
|---|---|---|---|---|---|
| sea coast area total population | 128,635 | | 128,873 | | 124,982 |
| ESRD (prevalence /Million) | 318 (2472) | | 329 (2553) | | 360 (2880) |
| deaths | | 36 | | 27 | |
| incident of ESRD | | 47 | | 58 | |
| annual mortality (/1000 person-years (pys)) | | 113.2 | | 82.1 | |
| annual incidence (/1000 pys) | | 0.365 | | 0.450 | |
| inland region total population | 440,296 | | 439,762 | | 440,611 |
| ESRD (prevalence/Million) | 1,056 (2398) | | 1,074 (2442) | | 1,096 (2487) |
| deaths | | 121 | | 106 | |
| incident of ESRD | | 139 | | 128 | |
| annual mortality (/1000 pys) | | 114.6 | | 98.7 | |
| annual incidence (/1000 pys) | | 0.316 | | 0.291 | |

**Conclusions:** Mortality rates in patients with ESRD did not increase after The Great East Japan Earthquake and Tsunami. Incident rates of ESRD increased in Tsunami disaster area after the disaster.

*Funding:* Other U.S. Government Support

## TH-OR053

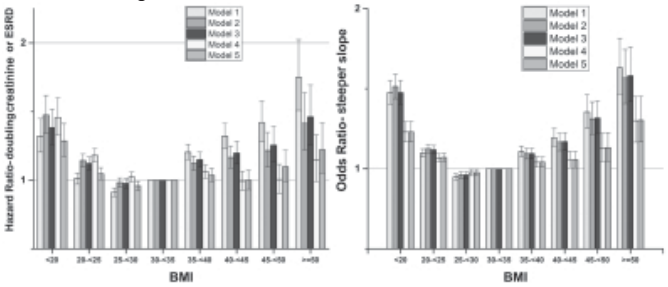
### Association of Body Mass Index with Progression of Kidney Disease in Patients with Non-Dialysis Dependent CKD

Jun Ling Lu,[1] Kamyar Kalantar-Zadeh,[2] Jennie Z. Ma,[3] Leigh Darryl Quarles,[1] Csaba P. Kovesdy.[1,4] *[1]Univ of Tennessee Health Science Center, Memphis, TN; [2]Harold Simmons Center for Chronic Disease Research and Epidemiology, Univ of California Irvine, Orange, CA; [3]Univ of Virginia, Charlottesville, VA; [4]Memphis VA Medical Center, Memphis, TN.*

**Background:** The association of obesity with progression of kidney disease in patients with non-dialysis dependent chronic kidney disease (NDD-CKD) is unclear.

**Methods:** We examined the association of BMI with CKD progression in a national cohort of 453,946 US veterans with estimated glomerular filtration rate (eGFR) <60ml/min/1.73m$^2$. CKD progression was defined as the incidence of the composite of doubling of serum creatinine or End Stage Renal Disease (ESRD), and as the risk of slopes of eGFR <-4 ml/min/1.73m$^2$/year. Associations were examined in crude (Model 1) and in multivariable adjusted Cox models and logistic regression models. Adjustments were made for age (Model 2), race (Model 3), comorbidities and medications (Model 4), and baseline estimated GFR (Model 5).

**Results:** The mean age (Standard Deviation [SD]) was 73.9(9.3) years, 9.1% were African American, and the mean estimated GFR (SD) was 47.8 (9.9) ml/min/1.73m$^2$. 148,276 patients died (mortality rate: 73.9/1000 Patient-Year (95% confidence interval [CI]:73.5-74.3)). BMI showed U-shaped association with both the incidence of the composite renal end point (Figure 1), and the risk of steeper slopes (Figure 2). Patients with BMI 25-30 kg/m$^2$ had the best outcomes.




**Conclusions:** The association of BMI with kidney disease is complex, with both relatively low and high BMI showing increased risk of progressive CKD. Overweight patients with CKD appear to have the best renal outcomes.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-OR054

### Percutaneous Coronary Intervention versus Optimal Medical Therapy for Stable Angina in Late-Stage Chronic Kidney Disease: A Decision Analysis

Ernest I. Mandel,[1] Matthew R. Reynolds,[2] David M. Charytan.[1] *[1]Renal Div, Dept of Medicine, Brigham and Women's Hospital, Boston, MA; [2]Dept of Cardiovascular Medicine, Lahey Clinic Medical Center, Burlington, MA.*

**Background:** Performing percutaneous coronary interventions (PCI) in the setting of stable symptomatic angina is often deferred in late-stage chronic kidney disease until necessitated by a more acute indication or until dialysis has been initiated. Whether immediate PCI or deferral of PCI in favor of optimal medical management (MM) improves quality of life or survival is uncertain.

**Methods:** Quality-adjusted life-years (QALYs) following PCI vs. MM in late-stage CKD and stable symptomatic angina were compared in a decision analysis. A Markov model was created to capture the possible disease states taking into account successful relief of symptomatic angina from the chosen intervention, dialysis-dependence as a result of CKD progression or contrast-induced nephropathy (CIN) from PCI, and procedural morbidity and mortality. Utilities and transition probabilities were derived from previous studies, literature review, and USRDS data. The Markov model was run in 3-month cycles for a total of three years of follow-up. Probabilistic sensitivity analyses on the likelihood of dialysis-dependent CIN and other transition probabilities were conducted using Monte Carlo simulation with 10,000 trials.

**Results:** The PCI strategy yielded a mean (SD) of 1.44 (0.04) QALYs over three years of follow-up while the MM strategy yielded a mean of 1.40 (0.04) QALYs (p<0.0001). The difference (95% CI) in QALYs between the two strategies was 0.038 (0.037 to 0.039). The probability of dialysis-requiring CIN at which the advanced CKD patient would be indifferent between MM and PCI, or over which would prefer MM, was 0.42.

**Conclusions:** A strategy of PCI for relief of stable symptomatic angina yielded higher expected QALYs than MM. The probability of CIN at which the decision would be indifferent or above which MM would be preferred is significantly higher than the observed probability of dialysis-requiring CIN in any previous studies. PCI should be considered despite concern over CIN in carefully selected late-stage CKD.

*Funding:* NIDDK Support, Private Foundation Support

## TH-OR055

### Monogenic Forms of Nephrotic Syndrome Occur in Diverse Genes across the Lifespan in North American Subjects

Matthew G. Sampson,[1,2,3] Edgar Otto,[1] J Troost,[1,2,3] Crystal A. Gadegbeku,[2,3,4] Matthias Kretzler.[1,2,3] *[1]Univ of Michigan, Ann Arbor, MI; [2]Nephrotic Syndrome Study Network (NEPTUNE); [3]The Michigan O'Brien Renal Center; [4]Temple Univ, Philadelphia, PA.*

**Background:** The frequency of causal mutations in children and adults with nephrotic syndrome (NS) in North American nephrology practices is unknown. This is in part because genetic and phenotypic heterogeneity of NS, and cost, precludes sequencing all known NS genes in all affected patients. To address this, we sequenced 21 monogenic NS genes in 103 unselected NS subjects from two prospective, multi-center glomerular disease cohorts.

**Methods:** NEPTUNE (n=67) enrolls at time of initial clinical biopsy and CPROBE (n=36) enrolls incident and prevalent patients. Recruitment is not based on age, suspicion of genetic NS, or steroid resistance. Subjects had either minimal change disease or FSGS on biopsy. All exons of 21 NS-associated genes were analyzed by microfluidic PCR paired with Next-Gen sequencing. After mapping and comparison to the Exome Variant Server, variants with minor allele frequency <1% (and *NPHS2* R229Q variant) were validated by Sanger sequencing. Variants deemed causal were based on previous reports of pathogenicity, <0.3% frequency or absence in control populations, and cross species conservation.

**Results:** 8/103 (8%) of NS subjects had a probable monogenic cause (5/8 > age 21; 0/8 with family history of NS). Variants were found in *NPHS2* (3), *ACTN4*, *CD2AP*, *LMX1B*, *MYO1E*, and *WT1*. These included a person in their 60's with compound heterozygosity of *MYO1E*, their 40s with an *LMX1B* variant, and a teen with a *CD2AP* variant. The five adult-onset subjects had variants in five different genes.

**Conclusions:** High-throughput sequencing reveals a diverse set of genetic forms of NS in 8% of this phenotypically varied cohort. This suggests that in sporadically affected subjects, targeting only subsets of genes based on age or other characteristics may result in missing genetic causes of NS. Discovery of additional monogenic NS genes will increase the prevalence of mongenic forms of NS. More frequent, or functional non-coding, NS-associated risk variants may explain a portion of the remaining heritability.

*Funding:* NIDDK Support

## TH-OR056

**Mutation-Dependent Recessive Inheritance in *NPHS2*-Associated Steroid-Resistant Nephrotic Syndrome** Kalman Tory,[1,2,3] Dora K. Menyhard,[4] Fabien Nevo,[1,3] Olivier Gribouval,[1,3] Andrea Kerti,[2] Pal Straner,[4] Christelle Arrondel,[1,3] Evelyne Huynh Cong,[1,3] Stephanie Woerner,[1,3] Tivadar Tulassay,[2,5] Geraldine Mollet,[1,3] Andras Perczel,[4] Corinne Antignac.[1,3] *[1]Inserm U983, Necker Hospital, Paris, France; [2]Ist Dept of Pediatrics, Semmelweis Univ, Budapest, Hungary; [3]Institut Imagine, Univ Paris Descartes, Paris, France; [4]MTA-ELTE Protein Modeling Research Group, Eötvös Loránd Univ, Budapest, Hungary.*

**Background:** *NPHS2*, encoding podocin, is the most frequently mutated gene in steroid-resistant nephrotic syndrome (SRNS). Patients who are compound heterozygous for an *NPHS2* mutation and the R229Q polymorphism ([R229Q];[mutation]) are expected to develop late-onset SRNS with a complete penetrance.

**Methods:** Based on epidemiological data, we hypothesized that the penetrance of late-onset SRNS in patients with [R229Q];[mutation] is incomplete. Therefore, we (1) screened 129 parents of affected children with *NPHS2* mutations for the R229Q variant, (2) compared the position of the *NPHS2* mutations between patients with two mutations and patients with [R229Q];[mutation], (3) coexpressed different R229Q-associated podocin mutants with podocin$^{R229Q}$ and podocin$^{wt}$ in human podocytes and (4) calculated the structure of different podocin dimers based on a homology model of P. horikoshii stomatin.

**Results:** Six of 129 unaffected parents carried [R229Q];[mutation], proving that the penetrance of late-onset SRNS is indeed incomplete. Though missense mutations of exons 7 and 8 are rare among *NPHS2* mutations (34/498, 6.8%), we found these mutations to be associated to R229Q in the vast majority of patients with late-onset SRNS (56/71, 78.9%, $p=1.4 \times 10^{-39}$). These podocin mutants retained podocin$^{R229Q}$ but not podocin$^{wt}$ in the cytoplasm and formed an abnormal heterodimer with podocin$^{R229Q}$. Podocin mutants that were non-pathogenic with R229Q did not change the subcellular localization of podocin$^{R229Q}$.

**Conclusions:** The R229Q variant is only pathogenic when associated to specific mutations. This is secondary to an altered dimerization and mislocalization of podocin dimers. The normal structure and localization of podocin$^{R229Q}$ homodimer explains the late-onset nature of SRNS in patients with [R229Q];[mutation].

## TH-OR057

**MiR-17-92 Is Required for Nephrogenesis and Renal Function** April Marrone,[1] Sheldon Bastacky,[3] Donna Beer Stolz,[2] Andrew J. Bodnar,[1] Jacqueline Ho.[1] *[1]Div of Nephrology, Dept of Pediatrics, School of Medicine, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Cell Biology, Univ of Pittsburgh, Pittsburgh, PA; [3]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** MicroRNAs (miRNAs) are small non-coding RNAs that act as novel regulators of gene expression through the repression of their target mRNAs. Although deletion of miRNAs in nephron progenitors demonstrates a critical role for miRNAs in progenitor survival, the specific miRNAs responsible have not been identified. Deletions in the *MIR17HG* cluster (*miR-17~92* in mice) represent the first miRNA mutations to be associated with a developmental defect in humans, leading to Feingold syndrome. While *MIR17HG* is expressed in the developing kidney, and a subset of patients with Feingold syndrome have renal anomalies, it remains unclear to what extent *MIR17HG* contributes to renal development and function.

**Methods:** To define the role of *miR-17~92*, we generated a conditional deletion of *miR-17~92* in nephron progenitors and their derivatives in mice, resulting in renal hypodysplasia.

**Results:** The nephron progenitor population is preserved; however, there are decreased developing nephrons. Cell division analyses throughout embryogenesis suggests that progenitors and their progeny divide less in the absence of *miR-17~92*, providing a possible mechanism causing low nephron endowment. Postnatally, the mice develop signs of renal disease by six weeks with albuminuria, and then focal podocyte foot process effacement and glomerulosclerosis at three months.

**Conclusions:** Taken together, these data support the first role for a single miRNA cluster in renal development, specifically in the regulation of nephron development, with subsequent consequences for renal function in adult mice.

*Funding:* NIDDK Support

## TH-OR058

**A Novel Variant of the Mitochondrial Heavy Strand Promoter Causes Renal Disease and Reduced Oxygen Consumption** Thomas Michael Connor,[1] Daniel P. Gale,[1] Phillippa J. Carling,[2] Gavin Hudson,[2] Patrick F. Chinnery,[2] Patrick H. Maxwell.[1] *[1]UCL Centre for Nephrology, Univ College London, London, United Kingdom; [2]Institute of Genetic Medicine, Univ of Newcastle, Newcastle, United Kingdom.*

**Background:** In the past few years, mitochondrial dysfunction has become a focus of research in human disease. Disorders of mitochondrial DNA (mtDNA) are an important cause of genetic disease and produce highly variable clinical features. There is a renal phenotype associated with a number of complex mitochondrial disorders, and evidence of an increased frequency of single nucleotide polymorphisms and deletions in end-stage renal disease. To date, the majority of pathogenic mutations have involved coding regions of the mitochondrial genome. We investigated a large Caucasian pedigree that included 17 individuals affected with adult-onset renal disease in the maternal line. There were no extra-renal signs in any of the affected individuals.

**Methods:** DNA was genotyped using the Illumina 300K chip. The mitochondrial genome was sequenced using Big Dye Terminator cycle sequencing and compared with the reference sequence. Primary dermal fibroblasts were cultured from four affected individuals and three healthy controls. Cybrids were created by fusion with 143B rho zero cells. Mitochondrial respiration was measured using the Seahorse XF bioanalyser.

**Results:** Linkage analysis demonstrated extra-chromosomal inheritance in this family. Mitochondrial sequencing showed the presence of a novel polymorphism located within the heavy strand promoter 1 (HSP1) and expressed at homoplastic levels. Primary fibroblasts showed a reduction in the ratio of transcripts from the HSP1 and a reduced rate of oxygen consumption. These findings were confirmed in mitochondrial cybrids, which showed an even greater reduction in HSP1 transcription and mitochondrial protein synthesis.

**Conclusions:** This is the first description of familial nephropathy due to a mutation in the mitochondrial heavy strand promoter. Reduced promoter function results in decreased transcription, and causes reduced oxygen consumption with a compensatory increase in glycolysis. These findings confirm the importance of mitochondrial function in renal disease.

*Funding:* Government Support - Non-U.S.

## TH-OR059

**Mutation Analysis in 30 Mouse Model Candidate Genes in 790 Humans with Isolated CAKUT** Stefan Kohl,[1] Gabriel C. Dworschak,[1,5] Alina Hilger,[2,5] Pawaree Saisawat,[2] Elijah O. Kehinde,[3] Radovan Bogdanovic,[4] Heiko M. Reutter,[5] Velibor Tasic,[6] Friedhelm Hildebrandt.[1,7] *[1]Dept of Nephrology, Boston Children's Hospital, Boston; [2]Dept of Pediatrics, Univ of Michigan, Ann Arbor; [3]Dept of Surgery, Kuwait Univ, Safat, Kuwait; [4]Medical Faculty, Univ of Belgrade, Belgrade, Serbia; [5]Institute of Human Genetics, Univ of Bonn, Bonn, Germany; [6]Dept of Pediatric Nephrology, Children's Hospital, Skopje, Macedonia, The Former Yugoslav Republic of; [7]Howard Hughes Medical Institute, Chevy Chase.*

**Background:** Congenital anomalies of the kidney and urinary tract (CAKUT) account for ~50% of the ESRD-cases in children. In order to identify single-gene causes of CAKUT, we performed exon resequencing in 790 children (690 families) with non-syndromic CAKUT examining 30 genes that (i) cause unilateral renal agenesis in mice and (ii) previously have not been implicated in pathogenesis of human CAKUT.

**Methods:** The coding sequences of 30 genes were re-sequenced using a high-throughput mutation analysis by array-based PCR (Fluidigm®) and next-generation sequencing (1). (1) Halbritter, J Med Genet, 49: 756, 2012.

**Results:** We detected homozygous or compound heterozygous variants in six genes. In the Fraser syndrome-causing genes, *FRAS1*, *FREM2*, and *GRIP1*, we detected recessive hypomorphic mutations in 17 unrelated individuals with isolated CAKUT. Furthermore, we identified a homozygous missense mutation in *GLI3* (c.316G>C, p.G106R) in an individual with left duplex ureter and UPJO. In *LHX1*, we found a homozygous missense mutation (c.159C>G, p.N53K) in an individual with left multicystic dysplastic kidney. A homozygous spice site mutation in *ITGA8* (c.2982+2T>C) was present in an individual with ureter duplex.

**Conclusions:** Our results indicate that in genes known to cause Fraser syndrome if truncating alleles are present, hypomorphic mutations cause isolated CAKUT. In 690 families with CAKUT we can molecularly "solve" ~2.5% with recessive hypomorphic mutations in *FRAS1*, *FREM2*, and *GRIP1*. Furthermore, we identified in 3 genes that cause CAKUT in mice mutations in 3 families, thereby making them candidates for mutation analysis in future studies.

## TH-OR060

**Copy-Number Variation in Children with a Solitary Functioning Kidney – The KIMONO-GENE Study** Rik Westland,[1,2] Brittany J. Perry,[1] Miguel VERBITSKY,[1] Michiel Schreuder,[3] Petra Jg Zwijnenburg,[4] Ali G. Gharavi,[1] Joanna Van Wijk,[2] Simone Sanna-Cherchi.[1] *[1]Div of Nephrology, Columbia Univ, New York City, NY; [2]Dept of Pediatric Nephrology, VU Univ Medical Center, Amsterdam, Netherlands; [3]Dept of Pediatric Nephrology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands; [4]Dept of Clinical Genetics, VU Univ Medical Center, Amsterdam, Netherlands.*

**Background:** Rare genic copy-number variations (CNVs) have been associated with congenital renal malformations. We investigated the role of rare CNVs in children with a solitary functioning kidney derived from the KIMONO study.

**Methods:** Genome-wide genotyping for CNV analysis was performed with the Illumina OmniExpress platform (730k markers). PennCNV was used to determine the CNV calls using generalized genotyping methods. To identify rare pathogenic CNVs, we compared data to 23,362 controls. Criteria for pathogenic CNVs were: 1) CNV-size ≥100kb, 2) confidence score ≥30 and 3) frequency of <1:5,000 in controls.

**Results:** 64 children with a solitary functioning kidney were included. Clinical phenotypes were renal hypodysplasia (48%), unilateral renal agenesis (30%), vesicoureteric reflux (14%) and obstructive nephropathy (8%). A total of 99 large, high-quality CNVs were identified (mean 1.55 per case), of which 58 (59%) were duplications and 41 (41%) were deletions. Median CNV-size was 173kb (IQR 133–250kb). CNVs overlapping significantly with known genomic disorders were identified in 6 (9%) patients (e.g. 3p29 microdeletion syndrome, 16p13.11 duplication and 16p12.1 distal deletion). Furthermore, 6 (9%) children were found to carry CNVs that were larger than 250kb, disrupting coding segments and absent or extremely rare in controls, representing potential novel genomic disorders. The identified genomic disorders occurred in all phenotypes.

**Conclusions:** The KIMONO-GENE study demonstrates that genomic disorders can be identified in almost one in five children with a solitary functioning kidney. As our findings underline the pivotal role of copy-number disorders in congenital kidney malformations, the identification of pathogenic CNVs should be considered in order to establish a molecular diagnosis in these patients.

## TH-OR061

**A Novel Mutation Specific Cause of Fanconi Syndrome and Nephrocalcinosis in *HNF4A*** Alexander J. Hamilton,[1,2] Coralie Bingham,[2] Sian Ellard,[1] Andrew Hattersley.[1] *[1]Univ of Exeter Medical School, Exeter, Devon, United Kingdom; [2]Renal Unit, Royal Devon and Exeter Hospital, Exeter, Devon, United Kingdom.*

**Background:** We have identified 5 individuals from 3 families with an *HNF4A* R76W mutation that co-segregates with Fanconi syndrome and nephrocalcinosis. *HNF4A* mutations cause maturity onset diabetes of the young (MODY), but are not among the 8 genes known to cause proximal tubulopathy. We aimed to describe this new phenotype and establish if this was a generalised or mutation specific effect by comparison with a cohort of patients with other HNF4A mutations.

**Methods:** We measured fasted urines in the R76W group and in 20 patients with other *HNF4A* mutations. Renal ultrasounds were performed in 15 patients. Results were compared using the independent samples median test or Z scores.

**Results:**

| | R76W (n=5) | Other *HNF4A* (n=20) | |
|---|---|---|---|
| Urinary α1-microglobulin mg/l, median | 264.0 | 6.0 | *p=0.005* |
| Urinary β2-microglobulin mg/l, median | 66.4 | 0.3 | *p<0.0001* |
| Urinary retinol binding protein mg/l, median | 128.0 | 2.0 | *p<0.0001* |
| Urinary glucose mmol/l, median | 62.3 | 0.3 | *p=0.005* |
| Serum urate umol/L, median | 102.0 | 268.0 | *p=0.002* |
| Serum magnesium mmol/l, median | 1.11 | 0.82 | *p=0.03* |
| Serum calcium mmol/l, median | 2.11 | 2.39 | *p<0.0001* |
| Urinary calcium:creatinine mmol(Ca)/m, median | 1.04 | 0.5 | *p=0.6* |
| Serum creatinine mg/dl, median | 1.33 | 0.95 | *p=0.004* |
| Nephrocalcinosis on ultrasound | 5/5 | 0/10 | |
| Amino acids, mean Z score | 24.3 | -0.16 | |

**Conclusions:** Patients with the *HNF4A* R76W mutation have a novel and specific phenotype of proximal tubulopathy and nephrocalcinosis characterised by aminoaciduria, massive low molecular weight proteinuria and glycosuria. These features were not seen in 20 patients with other *HNF4A* mutations. *In silico* modelling suggests that the R76 residue is involved in DNA binding to promoters and we hypothesize that the mutation may affect target specificity. This is the first description of a mutation specific effect in the MODY genes, hitherto not known to be related to renal tubular dysfunction or renal tract calcification.

*Funding:* Government Support - Non-U.S.

## TH-OR062

**Mutations in PAX2 Cause Adult-Onset Familial Focal and Segmental Glomerulosclerosis** Moumita Barua,[1] Emilia Stellacci,[2] Lorenzo Stella,[5] Astrid Weins,[3] Giulio Genovese,[4] Valentina Muto,[2] Hakan R. Toka,[1] Victoria Charoonratana,[1] Martin R. Pollak.[1] *[1]Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Dipartimento di Ematologia, Istituto Superiore di Sanità, Rome, Italy; [3]Pathology, Brigham and Women's Hospital, Boston, MA; [4]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; [5]Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, Rome, Italy.*

**Background:** FSGS is a histologically defined kidney injury characterized by sclerosis of some but not all glomeruli. Mutations in the transcription factor gene, *PAX2*, have been shown to cause congenital abnormalities of the kidney and urinary tract (CAKUT) as part of an autosomal dominant condition named papillorenal syndrome. We show that *PAX2* mutations leads to FSGS and demonstrate the mechanisms of disease.

**Methods:** Exome sequencing in a family with adult-onset FSGS revealed a suspicious *PAX2* variant. *PAX2* was sequenced in 175 FSGS families and 85 CAKUT individuals. *In silico* analysis and functional assays consisting of DNA binding and transactivation studies were performed. Immunohistochemistry to evaluate the impact of dysregulated PAX2 on expression of a target, WT1, was done.

**Results:** Six and seven heterozygous non-synonymous private variants were found in the familial FSGS and CAKUT cohort, respectively. There was an enrichment of rare variants in the patient groups compared to controls ($p<0.05$). In the CAKUT group, most variants localized within the transactivation domain while those in the FSGS group localized to outside of this region. As predicted, the PAX2 paired domain variant impaired DNA binding and transactivation activity while the PAX2 octapeptide variant resulted in enhanced repressor activity without affecting DNA binding. Immunohistochemical staining revealed reduced WT1 expression in both sclerosed and non-sclerosed glomeruli.

**Conclusions:** *PAX2* mutations leads to FSGS and CAKUT. Functional studies suggest that mutations lead to haploinsufficiency or dominant negative effects. Dysregulation of PAX2 targets expressed in the podocyte such as WT1 may lead to FSGS by disruption of this cell critical for filtration.

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## TH-OR063

**IQCJ Podocytopathy Causes Primary Renal Failure and De Novo Membranous Nephropathy Post Transplantation** Rachel Lennon,[1] Edward A. McKenzie,[1] Sarah B. Daly,[1] Helen Mary Stuart,[1] Jill Clayton-Smith,[1] Shelly Harris,[2] Lorna J. McWilliam,[2] Colin Short,[2] Laurence R. Solomon,[3] Ian Roberts,[4] Ron Korstanje,[5] Mario Schiffer,[6] Paul E. Brenchley.[1] *[1]Univ of Manchester, United Kingdom; [2]Central Manchester NHS Trust, United Kingdom; [3]Lancashire Teaching Hospitals, Preston, United Kingdom; [4]Univ of Oxford, Oxford, United Kingdom; [5]Jackson Labs, Bar Harbor; [6]Univ Medical School, Hannover, Germany.*

**Background:** We describe a novel podocytopathy causing primary renal failure in two siblings identified following "de novo" membranous nephropathy (MN) in their allografts.

**Methods:** A consanguineous family where parents and two children suffered CKD was investigated genetically following the post transplant development of de novo MN in both siblings resulting in allograft loss within two years. Autozygosity mapping identified a novel gene deletion of 129kb, near IQ Domain-Containing Protein J (IQCJ) on chr 3. Exome sequencing excluded known podocytopathies. Native kidney biopsy of an affected sibling showed a podocytopathy and excluded MN. In vitro and in vivo experimental systems were used to investigate the biological function of IQCJ.

**Results:** Parents were heterozygous and affected siblings homozygous for the gene deletion. Both affected cases became seropositive for anti-IQCJ antibodies detected by ELISA post transplantation. Immunostaining of normal kidney for IQCJ showed podocyte specific staining. In cultured normal podocytes, mRNA transcript for IQCJ was detected by PCR and immunostaining with anti-IQCJ located the antigen in transport vesicles with a distribution from perinuclear to cell membrane showing colocalisation with known transport proteins.Transient knock down of IQCJ mRNA in zebrafish caused edema consistent with a glomerular phenotype.

**Conclusions:** Alloimmunization post transplantation can reveal abnormal gene/protein expression causing the primary renal disease e.g. Alports Disease. Such a mechanism may explain two different rare phenotypes (in native and allograft kidney) occurring in this family and caused by a single podocyte gene, IQCJ. This gene may play a wider role in primary glomerulopathies and as a target for "de novo" MN post transplantation.

*Funding:* Private Foundation Support

## TH-OR064

**Mutations in *DGKE* Cause a Novel Form of Recessive Atypical Hemolytic-Uremic Syndrome without Complement Activation** Mathieu Lemaire,[1,2] Veronique Fremeaux-Bacchi,[3,4] Franz S. Schaefer,[5] Murim Choi,[1,2,6] Jack Tang,[1] Moglie Le Quintrec,[4] Fadi Fakhouri,[7] Sophie Taque,[8] Francois Nobili,[9] Weizhen Ji,[1,2] John Overton,[1,6] Shrikant M. Mane,[1,6] Gudrun Nürnberg,[10] Denis Morin,[11] Véronique Baudouin,[12] Brigitte Llanas,[13] Eva Simkova,[14] Peter Nuernberg,[10,15] Gilbert W. Moeckel,[1] John Hwa,[1] Chantal Loirat,[12] Richard P. Lifton.[1,2,6] *[1]Yale Univ; [2]Howard Hughes Medical Institute; [3]Hôpital Européen Georges-Pompidou; [4]Centre de Recherche des Cordeliers; [5]Heidelberg Univ; [6]Yale Center for Mendelian Genomics; [7]CHU Nantes; [8]CHU Rennes; [9]CHU Besançon; [10]Univ of Cologne; [11]CHU Montpellier; [12]Hôpital Universitaire Robert-Debré; [13]CHU Bordeaux; [14]Dubai Hospital; [15]ATLAS Biolabs GmbH.*

**Background:** Atypical Hemolytic-uremic syndrome (aHUS) is caused by pathological complement activation due to hereditary or acquired factors. Renal outcomes have improved since eculizumab (anti-C5) was introduced. Half of cases remain unexplained.

**Methods:** We performed exome sequencing on samples from two unrelated families each with 2 affected children. Bioinformatics analysis yielded a list of variants shared by each sibling set: gene(s) present in both lists were sequenced to find mutations in other aHUS patients. Expression of candidate genes in human and rodent tissues was tested with protein blotting and immunohistochemistry.

**Results:** We identified rare recessive damaging and missense mutations in *DGKE* (diacylglycerol kinase ε): mutations co-segregated with disease in 13 subjects from 9 unrelated kindreds. All patients presented before age 1, none had overt complement abnormalities, many developed chronic kidney disease with age, transplantation was uneventful in 3, and one had a HUS relapse while on eculizumab. DGKE protein is found in glomerular endothelium, podocytes, and platelets. DGKE terminates signaling by arachidonic acid-containing diacylglycerols, a potent activator of protein kinase C.

**Conclusions:** We infer that loss of *DGKE* function causes a novel form of renal thrombotic microangiopathy that does not require complement activation. The limited benefits of eculizumab for patients with *DGKE* nephropathy may lead to revision of aHUS treatment guidelines.

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## TH-OR065

**Differential Role of IL-6 Classical and Trans-Signaling in Crescentic Nephritis** Yoshikuni Nagayama,[1] Gerald S. Braun,[1] Claudia R.C. van Roeyen,[1] Luigi Villa,[1] Barbara Mara Klinkhammer,[1] Stefan Rose-John,[2] Tammo Ostendorf,[1] Jürgen Floege.[1] *[1]Div of Nephrology, RWTH Univ, Aachen, Germany; [2]Institute for Biochemistry, Christian-Albrechts-Univ, Kiel, Germany.*

**Background:** The role of IL-6 signaling in renal diseases remains controversial with data describing both anti- and pro-inflammatory effects. IL-6 can act via classical signaling engaging its two membrane receptors gp130 and IL-6R (anti-inflammatory potential). Alternatively, trans-signaling employs the soluble IL-6R (sIL-6R) to act on IL-6R negative cells (mostly pro-inflammatory). The aim of this study was to characterize the differential role of both pathways in crescentic nephritis (CN).

**Methods:** Human sera and urine samples from CN patients were analyzed. Nephrotoxic nephritis (NTN) was induced in Balb/c mice. Time-dependent IL-6 signaling was characterized. Specific blocking or activation of classical and trans-signaling was performed.

**Results:** Patients with CN had significantly elevated levels of serum IL-6 and of urinary IL-6, sIL-6R, and the endogenous trans-signaling inhibitor, sgp130. Consistent with this, NTN mice showed an early peak of both serum IL-6 and urinary IL-6, sIL-6R, and sgp130 levels. Serum sIL-6R and renal downstream signals (pSTAT-3, SOCS-3 mRNA) increased over time, suggesting increased trans-signaling. Simultaneous inhibition of both IL-6 signaling pathways using anti-IL-6 antibody did not impact on NTN severity when compared to treatment with isotype IgG. By contrast, specific inhibition of trans-signaling using recombinant sgp130fc (3 mg/kg) resulted in milder disease when compared to PBS-treated controls (crescents 4 ±6 vs. 10±7%, means±SD, $p<0.05$; necrosis score 0.5±0.6 vs. 1.2±0.8, $p<0.05$). Vice versa, specific activation of trans-signaling using a recombinant IL-6-sIL-6R fusion molecule ("hyper-IL-6") significantly aggravated NTN. This correlated with an increased synthesis of renal IL-17 mRNA.

**Conclusions:** Collectively our data suggest a central role for trans-signaling in crescentic nephritis. Global inhibition of IL-6 signaling was not beneficial during a model of NTN in Balb/c mice. On the other hand, specific antagonism of trans-signaling may represent a novel therapeutic approach.

*Funding:* Government Support - Non-U.S.

## TH-OR066

**Plasticity of Th17 Cells in Crescentic Glomerulonephritis** Christian Franz Krebs, Jan-Eric Turner, Hans-Joachim Paust, Sonja Kapffer, Sabrina Bianca Bennstein, Anett Peters, Rolf A. Stahl, Ulf Panzer. *UKE Hamburg, Nephrology, Hamburg, Germany.*

**Background:** $CD4^+$ T cells play a pivotal role in the pathogenesis of autoimmune disease, including human and experimental crescentic glomerulonephritis, by their ability to differentiate into pathogenic effector $T_H1$ and $T_H17$ cells or protective Treg cells. Classically, these $CD4^+$ T cell subsets have been viewed as terminally differentiated lineages with limited flexibility. The aim of the present study was to analyze the potential plasticity of $CD4^+$ $T_H17$ cells in experimental crescentic GN (nephrotoxic nephritis, NTN).

**Methods:** To study the fate of $CD4^+$ T cells during the course of NTN we performed three different approaches: (i) the adoptive transfer of in vitro polarized $T_H0$ or $T_H17$ cells in nephritic Rag-1-deficent mice; (ii) the use of a fluorescent acute activity reporter mice to directly detect, sort, and transfer IL-17A$^+$ ($T_H17$) $CD4^+$ T cells and (iii) the use of a IL-17A fate reporter mice to analyze renal 'ex-Th17' cells by flow cytometry.

**Results:** In vitro polarized 'bulk' $T_H17$ and $T_H0$ cells were transferred into Rag-1$^{-/-}$ mice, subsequently NTN was induced. As expected 10 days after nephritis induction renal tissue injury was aggravated in mice that received $T_H17$ cells. Interestingly, > 50 % of the transferred $T_H17$-polarized cells started to express IFN-gamma, a feature of $T_H1$ cells. To acquire a highly pure $T_H17$ population we FACS sorted IL-17A$^+$ cells from fluorescent acute reporter mice after $T_H17$ polarization in vitro (>95 % purity) and transferred them into nephritic Rag-1-deficient mice. The majority (>90%) of transferred $T_H17$ cells lost their IL-17A production and 25 % expressed IFN-γ. Furthermore, analysis of fate-mapped 'ex-$T_H17$' cells in IL-17A fate reporter mice (IL-17A Cre x Rosa26eYFP mice) with NTN showed that only a minor proportion of $T_H17$ cells (~15%) lost IL-17A production during the course of the nephritis, indicating that in vivo derived $T_H17$ cells are stable in NTN.

**Conclusions:** Here we show that in vitro polarized $T_H17$ cells have a high degree of plasticity during the course of the nephritis, whereas in vivo acquired $T_H17$ cells have a more stable phenotype in experimental crescentic glomerulonephritis.

*Funding:* Government Support - Non-U.S.

## TH-OR067

**Inhibition of IL-6 Receptor Attenuates Autoimmunity and Glomerulonephritis in Experimental ANCA Vasculitis** Sharon Lee Ford,[1] Michael Mülleneisen,[2] Stefan Rose-John,[3] Stephen R. Holdsworth,[1] Shaun A. Summers,[1] Oliver M. Steinmetz.[2] *[1]Centre for Inflammatory Diseases, Monash Medical Centre, Melbourne, Australia; [2]III. Med. Klinik, Univ of Hamburg, Germany; [3]Biochemistry, Univ of Kiel, Germany.*

**Background:** The pleiotropic cytokine IL-6, critically required for Th17 immunity, is elevated in patients with ANCA vasculitis. IL-6 signaling occurs via the classic pathway using membrane bound IL-6 receptor (mIL-6R) and the trans-pathway via soluble IL-6 receptor (sIL-6R). Anti-IL-6R therapy has recently been established as treatment for rheumatoid arthritis while the role in renal disease remains unclear.

**Methods:** We induced autoimmune anti-myeloperoxidase (MPO) glomerulonephritis (AIaMPOGN) by immunising C57BL/6 mice with murine MPO. Renal injury was triggered after 14 days by administration of a sub-nephritogenic dose of nephrotoxic serum. Effects of IL-6 inhibition administered during the induction phase (days -1 and 7) or during the effector phase (day 14) of injury were assessed using antibodies targeting IL-6, mIL-6R (classical and trans pathway), or sIL-6R (trans pathway only).

**Results:** mIL-6R blockade during the induction phase attenuated humoral and cellular autoimmunity. Anti-MPO IgG levels as well as spleen sizes and proliferative activity were significantly reduced. In line, MPO-stimulated splenocyte IL-17A and IFNγ production was impaired in anti mIL-6R treated mice. Importantly, renal injury was also attenuated in terms of histological damage and albuminuria. To clarify the role of IL-6 as effector cytokine, we also studied IL-6 pathway blockade in the effector phase of AIaMPOGN. However, neither systemic autoimmunity nor renal injury was altered by effector phase blockade of either IL-6, mIL-6R or sIL-6R.

**Conclusions:** IL-6 critically directs development of cellular and humoral autoimmunity in ANCA associated glomerulonephritis but does not seem to play a major role as effector cytokine. Early mIL-6R blockade significantly attenuated glomerulonephritis and therefore offers exciting therapeutic potential for ANCA vasculitis.

*Funding:* Government Support - Non-U.S.

## TH-OR068

**Functionally Active C3 and Other Complement Components Are Secreted by Human Podocytes** Anne Katrin Dettmar,[1] Isabell Kopka,[2] Christoph Licht,[3] Markus J. Kemper,[1] Peter F. Zipfel,[2] Jun Oh.[1] *[1]Univ Hamburg-Eppendorf, Hamburg, Germany; [2]Hans-Knöll-Institut, Jena, Germany; [3]The Hospital for Sick Children, Toronto, Canada.*

**Background:** The activation of the complement system plays an important role in various kidney diseases. The majority of the circulating complement components are produced in the liver. But in the last decade the local production of complement components by other cells is highly debated. The aim of our study was to proof the ability of human podocytes to produce and secrete complement components and the functionality of these produced components.

**Methods:** Immortalized human podocytes were analyzed by Western Blot (WB), immunofluorescence (IF) and PCR for their ability to produce components (C1/ C1q, C2, C3, C4, C5, C7, C9) inhibitors (factor H, CD46, CD55, CD59) and activators (factor B, properdin, factor D) of the complement system. Secretion of components was measured in the medium, hemolytic activity was tested in a hemolysis assay and functionality of C3 was tested in a specific C3-convertase assay. Stimulation of the cells was done with either interferon-γ or interleukin 6.

**Results:** PCR-studies revealed that human podocytes express on mRNA level the components C1, C2, C3, C4, C5, the inhibitors factor H, CD46, CD55 and CD59, and the activators properdin and factor B. Cells expressed CD55 and CD59 on protein level. In addition, we could show that podocytes secrete the factors C2, C3, C4, C5, C7 and factor H into the medium. As expected, because of the missing secretion of factor D and B, there was no functionally active complete complement cascade resulting in hemolysis of rabbit-erythrocytes, but the secreted C3 was clearly functionally active.

**Conclusions:** This is the first study showing the potential of human podocytes to secrete active complement components. These data suggest that podocytes might contribute to local complement activation and modify complement mediated renal diseases.

## TH-OR069

**Complement Pathway Inhibition by Antisense Oligonucleotide for Treatment of Renal Diseases** Tamar R. Grossman, Lisa A. Hettrick, Robert Benjamin Johnson, Brett P. Monia, Michael McCaleb. *Antisense Drug Discovery, Isis Pharmaceuticals, Carlsbad, CA.*

**Background:** Activation of the complement system plays a key role in normal inflammatory response to injury but may cause substantial injury when activated inappropriately. Complement contributes to injury in several forms of glomerulonephritis, chronic humoral rejection after renal transplantation and ischaemia/reperfusion injury. Genome-wide association studies have shown that polymorphisms at the complement factor H-related gene locus are associated with over activation of the alternative complement pathway and subsequent susceptibility to IgA nephropathy and systemic lupus erythematosus. Rare mutations in these genes and other complement genes are associated with familial forms of C3 glomerulopathy and hemolytic uremic syndrome.

**Methods:** Using second generation antisense technology, we identified and characterized Antisense oligonucleotides (ASO) to murine complement component 5 (C5) and complement factor B (CFB) and tested their efficacy in NZB/W and MRL/lpr mouse models for Lupus Nephritis as a representative of human renal disease.

**Results:** Systemic Treatment with CFB ASO (100 mpk/wk) resulted in 75-90% mRNA knockdown in the liver and 40-60% in the kidney, which led to improved survival, proteinuria, renal C3 accumulation and renal pathology in mouse models for lupus nephritis. In addition plasma C3 level, which is dramatically reduced by C3 turnover in the disease models, was corrected to normal level with treatment. Similarly, systemic treatment with C5 ASO (25 mpk/wk) resulted in 95% mRNA knockdown in the liver and 80% in the kidney and improved renal pathology and reduced renal C3 accumulation in MRL/lpr mice.

**Conclusions:** Our results suggest that the amplification of complement activation by the alternative pathway is necessary for glomerular C3 deposition and progression of proteinuria and that factor B and C5 play important roles for disease progression and pathology in lupus nephritis mouse models. Treatment with ASO to CFB or C5 may offer a novel treatment strategy to treat lupus nephritis and other complement related renal diseases, such as C3 glomerulopathy, hemolytic uremic syndrome and dense deposit disease.

*Funding:* Pharmaceutical Company Support - Isis Pharmaceuticals

## TH-OR070

**IgM Contributes to Glomerular Disease Progression in Complement Induced Glomerulopathy** Sarah E. Panzer,[1] Brandon Renner,[1] Danica Ljubanovic,[2] Dorin-Bogdan Borza,[3] Joshua M. Thurman.[1] *[1]Renal Div, Univ of Colorado Denver, Aurora, CO; [2]Dept of Pathology, Univ Hospital Dubrava, Zagreb, Croatia; [3]Renal Div, Vanderbilt Univ, Nashville, TN.*

**Background:** While glomerular IgM deposition is reported in a variety of glomerular diseases the mechanism of its deposition and the clinical significance of its presence remain controversial. Recent research demonstrates IgM binds damaged glomeruli. Clinical studies associate IgM deposition with more severe manifestations of nephrotic syndrome. We hypothesized natural IgM binds neo-epitopes exposed after insults to the glomerulus and exacerbates disease progression.

**Methods:** We used mice deficient in the complement regulatory protein factor H to model a non-sclerotic and non-immune-complex glomerular disease and assessed the contribution of glomerular IgM to disease expression. To test the functional importance of IgM in this model we crossed factor H deficient mice with mice that lack B cells and therefore do not produce IgM. We also injected B cell deficient mice with IgM from wild type mice to determine whether it has nephritogenic properties.

**Results:** We demonstrated glomerular IgM deposition occurred consistently in factor H deficient mice and progressed from a mesangial distribution into the basement membrane of capillary loops as mice aged. In contrast, glomerular IgG deposition was not observed. Factor H deficient mice lacking B cells were protected from renal damage, as evidenced by milder histologic lesions on electron microscopy and a trend towards reduced albuminuria. IgM from wild-type mice, but not IgG, bound to cultured murine mesangial cells. Injection of purified IgM into mice lacking B cells bound within the glomeruli and induced albuminuria ($P < 0.05$ by ANOVA, N=3).

**Conclusions:** These results suggest IgM plays an active role as a contributing factor to the development of glomerular damage in a non-sclerotic and non-immune complex model of glomerular disease. Natural IgM and complement may offer new therapeutic targets for slowing the progression of glomerular disease.

*Funding:* NIDDK Support

## TH-OR071

**Inflammasome in Renal Collecting Duct Cells Contributes to Inflammation and Fibrosis after Unilateral Ureteral Obstruction** Takanori Komada,[1,2] Shigeaki Muto,[1] Eiji Kusano,[1] Masafumi Takahashi.[2] *[1]Dept of Nephrology, Jichi Medical Univ, Tochigi, Japan; [2]Div of Inflammation Research, Jichi Medical Univ, Tochigi, Japan.*

**Background:** The inflammasome is a multiple protein complex containing Nod-like receptors (NLRs), ASC, and caspase-1. It regulates interleukin-1β (IL-1β) and contributes to the development of CKD. However, the role of each inflammasome component in the renal parenchymal cells remains unclear. We investigated the role of ASC using a murine unilateral ureteral obstruction (UUO) model and evaluated the localization of ASC.

**Methods:** C57BL/6J-background wild type (WT) and ASC-knockout (ASC-KO) mice underwent UUO. Primary mouse collecting duct (CD) cells were used in in vitro study.

**Results:** ASC-KO showed a significant reduction in tubulointerstitial injury and fibrosis histologically. On the 5 days after UUO, flow cytometric analysis showed that leukocyte infiltration was abolished in ASC-KO compared with WT, indicating that inflammatory response was remarkably suppressed in ASC-KO. The mRNA expressions of *IL1b*, *Ccl2*,*Col1a1*, *Col3a1*, and *Tgfb* showed less increase in ASC-KO. Immunohistochemistry showed that ASC expression was upregulated in the renal tubules after UUO. Double immunofluorescence analysis revealed that most ASC positive tubules were co-stained with AQP2, collecting duct marker. In *in vitro* study, we identified the presence of NLRP3 and ASC in primary mouse CD cells using western blotting. After 5mM ATP stimulation, IL-1β secretion and active caspase-1 were observed. This response in IL-1ß secretion was prevented significantly by $P2X_7$ inhibitor (A-438079), by blocking the $K^+$ efflux, by glibenclamide, or by antioxidant *N*-acetyl cysteine (NAC).

**Conclusions:** ASC was upregulated in CD cells after UUO and related to inflammation and fibrosis. Extracellular ATP stimulated inflammasome activation in CD cells, through $P2X_7$-potassium efflux and ROS-dependent pathways. These findings suggest that ASC in the CD cells contributes to kidney inflammation as a major component of the inflammasome.

## TH-OR072

**Macrophage $A_{2A}$ Adenosine Receptors Are Essential to Protect from Progressive Kidney Injury** Gabriela E. Garcia,[1] Luan D. Truong,[2] Jessica Helen Trostel,[1] Richard J. Johnson,[1] Lili Feng.[3] *[1]Medicine, Univ of Colorado Denver, Aurora, CO; [2]Pathology, The Methodist Hospital, Houston, TX; [3]Medicine, Baylor College of Medicine, Houston, TX.*

**Background:** $A_{2A}$ adenosine receptor ($A_{2A}R$) activation attenuates inflammation and induces tissue repair. Macrophages (Mφ) that are key effectors of kidney disease progression express $A_{2A}R$.

**Methods:** We investigated the role of Mφ $A_{2A}R$ in the progression of kidney injury in a model of severe, macrophage-mediated anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) using CD11b-DTR transgenic mice. In these mice, tissue Mφ can be selectively depleted by injection of diphtheria toxin (DT). GN was induced in CD11b-DTR mice and Mφ were selectively depleted in the established phase of the disease and reconstituted with Mφ from WT or $A_{2A}R$ deficient mice and then treated with an $A_{2A}R$ agonist and euthanized at day 8. Mice reconstituted with WT Mφ but not treated with $A_{2A}R$ agonist were used as a control.

**Results:** Mφ were tracked in vivo labeling them with PKH -26GL and found that Mφ were distributed in nephritic kidneys. In mice that received WT Mφ and were treated with $A_{2A}R$ agonist the glomerular cellularity, crescent formation, sclerotic glomeruli and tubulointerstitial (TIN) injury were significantly reduced compared with the control group. In contrast, mice reconstituted with $A_{2A}R$ deficient Mφ and treated with $A_{2A}R$ agonist, the kidney injury was worst with increased crescent formation, enhanced sclerotic glomeruli and higher TIN injury compared with the control group. In addition, collagen (Col)-I, Col-III and Col-IV deposition were increased in mice receiving Mφ deficient in $A_{2A}R$ compared with the control group. Importantly, the antigen-specific humoral immune response and the glomerular immunoglobulin G deposition were not affected by DT, Mφ depletion, or Mφ reconstitution. In vitro studies, higher levels of TGF-β2 and tissue inhibitor of matrix metalloproteinase-1 were induced in Mφ from $A_{2A}R$-deficiente mice compared with Mφ from WT mice.

**Conclusions:** These findings suggest that absence of Mφ $A_{2A}R$ increases Mφ pro-fibrotic activity and that endogenous Mφ $A_{2A}Rs$ are essential to protect from progressive kidney fibrosis.

*Funding:* NIDDK Support

## TH-OR073

**NLRP3 Is a Predictor of Renal Tubular Injury in Human IgA Nephropathy** Justin Chun, Xiangyu (Wendy) Wang, Hyunjae Chung, Matthew T. James, Kiril Trpkov, Brenda Hemmelgarn, Daniel A. Muruve. *Medicine, 1, Calgary, Canada.*

**Background:** Despite efforts to identify biomarkers to classify and prognosticate kidney disease, current markers are non-specific and unable to predict disease outcome. Our previous studies have demonstrated significant expression of a family of NOD-like receptors called NLRPs in various human kidney diseases. NLRP3 gene expression is increased in biopsies of patients with IgA nephropathy (IgAN), a slowly progressing form of CKD that is associated with tubulointerstitial fibrosis. Although NLRP3 mRNA expression has been linked with IgAN, little is known about the relationship between NLRP3 expression with IgAN progression.

**Methods:** Biopsies from a total of 67 patients with IgAN were selected for analysis of NLRP3 mRNA expression by quantitative real-time PCR. Patients with advanced renal failure (GFR≤15 ml/min) were excluded from the analysis to avoid patients with ESRD. Therefore, a total of 57 cases of IgAN were analyzed. NLRP3 gene expression was analyzed as a logarithmic variable due to its non-normal distribution, and displayed as tertiles in a survival curve. We used immunohistochemistry (IHC) and Western blot to study NLRP3 protein expression with correlation to a kidney injury molecule (KIM-1).

**Results:** Our results demonstrate that lower levels of NLRP3 gene expression is associated with a worse outcome in patients with IgAN. NLRP3 and KIM-1 had strong positive correlation both with high expression in IgAN tissue compared with normal tissue. With disease progression, there was increased tubular atrophy and fibrosis with a concomitant reduction of NLRP3 and KIM-1 in kidney tissue samples. Consistent with our IHC results, proximal tubular epithelial cells had an initial increase of NLRP3 and KIM-1 protein levels that diminished after prolonged treatment with TGFβ, a major profibrotic cytokine known to induce the expression of NLRP3.

**Conclusions:** NLRP3 is a promising marker of epithelial burden that can be used to predict IgAN progression. Higher levels of NLRP3 mRNA and protein expression are associated with earlier manifestation of IgAN. The presence of high levels of NLRP3 in IgAN may provide an opportunity to identify reversible or treatment responsive inflammation.

## TH-OR074

### Transcriptional Regulation of Endothelial Cell Proliferation Induced by Simultaneous Antibody Targeting of $AT_1$- and $ET_A$ Receptors

Aurélie Philippe, Rusan Catar, Philine Wagner, Duska Dragun. *Nephrology and Intensive Care Medicine, Univ Hospital Charité, Berlin, Germany.*

**Background:** Patients with systemic sclerosis (SSc) suffer from severe obliterative autoimmune vasculopathy affecting various vascular beds including kidney. Activating autoantibodies simultaneously targeting G-protein coupled receptors (GPCR) Angiotensin II type 1 receptor ($AT_1R$) and Endothelin-1 type A receptor ($ET_AR$) may predict velocity of vascular complications by mechanisms which remain to be clarified. We hypothesized that both activating antibodies may induce vascular dysfunction independent from receptor natural ligands, Angiotensin II and Endothelin-1.

**Methods:** Affinity purified IgG fraction derived from serum of SSc patients positive for $AT_1R$-/$ET_AR$-Abs (SSc P-IgG) served for functional, signaling and transcriptional regulation studies in human microvascular endothelial cells (HMEC-1). Mice were passively transferred with SSc P-IgG.

**Results:** Both autoantibodies induced ERK1/2 phosphorylation to a similar extent as natural ligands, indicative of their biologic activity, and induced further down-stream phosphorylation of the transcription factor Ets-1. SSc P-IgG transfer induced expression of Ets-1 in kidney arteries of mice. $AT_1$-/$ET_AR$ autoantibodies, but not natural ligands Ang II or ET-1 were able to induce endothelial cell proliferation. Respective receptor antagonists attenuated endothelial proliferation emphasizing receptor specific effect of both activating antibodies. These effects were attributed to GPCR since pharmacologic targeting of ERK1/2 achieved similar results. Same anti-proliferative effects could be achieved by siRNA mediated blockade of Ets-1. SSc P-IgG also induced increased DNA binding to Tissue factor (TF) promoter, which contains Ets-1 binding site, in EMSA and ChIP assays. Moreover, inhibition of TF obviated endothelial cell proliferation, illustrating that antibodies activating $AT_1R$ and $ET_AR$ contribute to pathogenesis of proliferative obliterative vascular lesion via enhanced Ets-1 mediated transcriptional regulation of TF.

**Conclusions:** Targeting pathways induced by $AT_1R$ and $ET_AR$ antibodies may offer new prospects in treating obliterative vascular lesions in autoimmune vasculopathies.

## TH-OR075

### Increased Hedgehog-GLI Signaling Causes Stromal Cell-Mediated Ureteropelvic Junction Obstruction

Norman D. Rosenblum,[1] Lijun Chi,[1] Brian Nieman,[3] Steven Potter.[2] *[1]Div Neph, Prog DSCM, Hosp Sick Child, U Toronto, Toronto, Canada; [2]Dev Biol, Child Hosp Med Centre, Cincinnati; [3]Toronto Centre Phenogenomics, Toronto, Canada.*

**Background:** Hedgehog (HH)-GLI signalling is required during kidney morphogenesis. While increased HH-GLI signalling causes cancer in nonrenal tissues, its pathogenic role in the embryonic kidney is undefined.

**Methods:** We generated and analyzed mice with a constitutive increase in HH-GLI signalling activity in the metanephric mesenchyme by genetically inactivating *Patched1* (*Ptch1*), that encodes a cell surface protein that constitutively inactivates HH signaling in the absence of ligand, using *Rarb2-Cre* and $Ptch1^{loxP}$ mouse strains.

**Results:** *Ptch1*-deficient (*Rarb2-Cre;*$Ptch1^{loxP/-}$) mice were characterized by hydronephrosis after E15.5. Dye injection into the renal pelvis revealed obstruction of dye passage beyond the ureteropelvic junction (UPJ). Serial 2 µm histologic sections revealed cells obstructing the UPJ. Analysis of *Ptch-lacZ* expression, a reporter of HH signaling activity, in *Ptch1*-deficient;*Ptch1-lacZ and Ptch1*-deficient;*Ptch2-lacZ* mice, revealed increased *Ptch1* and ectopic expression of *Ptch2* in the cells obstructing the UPJ. A 2-fold rescue of hydronephrosis, measured by MRI, was observed in *Ptch1*-deficient mice with constitutive expression of the $Gli3^{\Delta 699}$ allele that encodes GLI3 repressor, demonstrating that UPJO in *Ptch1*-deficient mice is GLI-dependent. *Ptch2*-positive cells (3 distinct batches of 2000 cells) were isolated distinct from *Ptch2*-negative cells in the region of the UPJ by tissue dissection and flow sorting and analyzed by RNAseq. In comparison to *Ptch2*-negative cells, *Ptch2*-positive cells exhibited a 2-9-fold increase in genes expressed in renal stromal cells or ureteric smooth muscle progenitors, or that promote smooth muscle development. Validation of these results using qPCR and double in situ hybridization with *Ptch2* mRNA established the stromal cell identify of obstructing *Ptch2*-positive cells.

**Conclusions:** Increased HH-GLI signaling in the metanephric mesenchyme causes UPJO due to ectopic stromal cells. Our results provide a basis to investigate human UPJO, presently understood only at the level of histopathology.

*Funding:* Government Support - Non-U.S.

## TH-OR076

### Wnt7b Signals to Pericytes/Perivascular Fibroblasts and Regulates Microvasculature Development in the Renal Medulla

LaToya Ann Roker, Jing Yu. *Cell Biology, Univ of Virginia, Charlottesville, VA.*

**Background:** Wnt7b is expressed in the ureteric trunk epithelium and essential for renal medulla formation. It elicits canonical Wnt signaling in interstitial cells of the prospective renal medulla, which is also vital for renal medulla formation. However, the identity of the Wnt7b target cells remains elusive. Determining the identity of these cells may help us to better understand *Wnt7b* functions in renal medulla development.

**Methods:** Therefore, we employed mouse genetics, Immunohistochemistry, and Transmission Electron Microscopy (TEM) to determine the identity of *Wnt7b* target interstitial cells and the effect of *Wnt7b* signaling on this cell population.

**Results:** We found that the in the nascent renal medulla, *Wnt7b*/canonical Wnt signaling target cells do not constitute the entire prospective medullary interstitium; rather, they are confined to 1-3 cell layers adjacent to the prospective medullary collecting ducts. Moreover, we found that they express pericyte markers and are intimately associated with endothelial cells of the microvasculature lining the prospective medullary collecting ducts, demonstrating that these cells are pericytes/perivascular fibroblasts. We further found that differentiation of this population of pericytes/perivascular fibroblasts did not depend on *Wnt7b* signaling from ureteric trunks; instead, ablation of *Wnt7b* signaling in the kidney led to their reduced *Pdgfrβ* expression and increased cell proliferation. Furthermore, *Wnt7b* mutants displayed an increase in the density of vascular coverage of prospective medullary collecting ducts and in endothelial cell proliferation. Further analysis identified a defect in endothelial cell flattening and lumen formation of this population of the microvasculature.

**Conclusions:** Taken together, our study revealed a novel function of *Wnt7b* in kidney development, that *Wnt7b* regulates peri-ureteric trunk microvasculature development in the developing renal medulla, most likely through its signaling to pericytes/perivascular fibroblasts lining the microvasculature.

*Funding:* NIDDK Support

## TH-OR077

### Wnt5a Is Crucial for Kidney Development

Liwei Huang, Soo Young Choi, Maria F. Chacon-Heszele, Weibin Zhou, Sarah McKenna, Xiaofeng Zuo, Yun Kyoung Ryu, Rejji Kuruvilla, Joshua H. Lipschutz. *Dept of Medicine, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** Wnt5a is a non-canonical secreted glycoprotein of the Wnt family that plays an important role in the development of various organs and postnatal cellular function. Little is known regarding the role of Wnt5a during kidney development.

**Methods:** The objective of the present study is to determine the role of Wnt5a during nephrogenesis using zebrafish and murine models.

**Results:** By Real-time RT-PCR and whole mount ISH (wmISH), wnt5a expression occurs in the zebrafish pronephros. Wnt5a knockdown by antisense wnt5a morpholinos results in abnormal glomerular structure and dilated renal tubules in zebrafish. In wt1-GFP fish, the pronephros can be visualized with fluorescence microscopy. Dilations are visualized at 48 hours post fertilization (hpf) in the glomerular and proximal tubular region of the wt1-GPF fish injected with wnt5a antisense morpholinos. Wnt5a knockdown resulted in decreased wt1 expression detected by wmISH and in the wt1-GFP fish detected by fluorescence microscope. Rescue with a mouse Wnt5a mRNA showed that this was not due to off-target effects. In the Wnt5a global knockout mouse, by wmISH, Wnt5a expression is localized to the entire intermediate mesoderm at embryonic day (E) 10.5, and its expression level is increased further at E11.5. At E13.5, Wnt5a is weakly expressed in the mesenchymal region of the metanephros. Wnt5a global knockout mice showed pleiotropic kidney phenotypes, including bilateral or unilateral kidney agenesis, hydronephrosis and fused kidneys. Further histologic examination, when kidney tissue was present, revealed reduced kidney tubules, glomeruli, and medullary zones in the Wnt5a -/- embryos.

**Conclusions:** A strength of this study is that we utilized two different animal models each of which has specific advantages that could be exploited to investigate the role of Wnt5a in nephrogenesis. In zebrafish and mice, Wnt5a was expressed very early in nephrogenesis. Disrupted Wnt5a resulted in abnormal glomerular and tubular growth and cyst formation. Our data indicate that Wnt5a is centrally involved in kidney development.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-OR078

### The WNT-Calcium Pathway Controls c-Ret Expression during Ureteric Bud Branching Morphogenesis

Diana Iglesias,[1] Jeremy A. Saban,[2] Rachel Corsini,[1] Paul R. Goodyer.[1,2] *[1]Pediatrics, Montreal Children's Hospital Research Institute, Montreal, Canada; [2]Human Genetics, Montreal Children's Hospital Research Institute, Montreal, Canada.*

**Background:** Normal nephrogenesis requires reciprocal signaling between the invading ureteric bud (UB) and the condensing metanephric mesenchyme (MM). Here, we examine the role of the non-canonical WNT/ $Ca^{2+}$/calcineurin (WNT-$Ca^{2+}$) signaling pathway in kidney development.

**Methods:** We used a transgenic mouse that carries a luciferase reporter downstream of an NFAT binding sensitive promoter.

**Results:** We found that WNT-$Ca^{2+}$ signaling peaks at embryonic days E13-E16 in wildtype embryonic mouse kidney and is downregulated perinatally as nephrogenesis comes to an end. The *Nfat*-luciferase reporter was responsive in vitro and ex vivo to non-canonical WNTs such as WNT5a and WNT11, but not to the canonical WNT3a. The effect of non-canonical WNTs is abrogated by cyclosporin-A (CsA) treatment in explants, which also causes reduced UB branching. *Nfat*-luciferase activity was significantly reduced in kidneys from E16 embryos when CsA was administered by gavage to pregnant mice between E13-E16. *In situ* hybridization for the luciferase transcript shows that WNT-$Ca^{2+}$ signaling activity is located in the outer nephrogenic zone. *Nfat*-luciferase activity was highest in *Hoxb7*/GFP-tagged UB cells isolated by FACS from E15 kidneys of mice bearing the reporter transgene. This unexpected finding suggests that WNT11 expressed in UB tips may play an autocrine role in branching morphogenesis. To ascertain whether the pathway regulates UB expression of RET (a receptor for MM-secreted GDNF) we treated E13 kidney explants with CsA vs control conditions for 48 hours and examined the levels of *Ret* and *Gdnf* by quantitative RT-PCR and IF microscopy. Inhibition of the WNT-$Ca^{2+}$ pathway by CsA caused striking reduction of explant *Ret* expression despite addition of excess soluble GDNF, which is proposed to activate *Ret* expression in response to paracrine WNT11 activity.

**Conclusions:** We propose a spatio-temporal regulatory role for the WNT-$Ca^{2+}$ pathway that ensures the expression of *Ret*, thereby mediating UB branching morphogenesis.

*Funding:* Government Support - Non-U.S.

## TH-OR079

### Gene-Environment Interactions in the Ureteric Bud Lineage Cause CAKUT

Lei Yan, Xiao Yao, Zubaida R. Saifudeen, Samir S. El-Dahr. *Pediatrics, Tulane Univ School of Medicine, New Orleans, LA.*

**Background:** Polymorphisms in the bradykinin B2 receptor (*BDKRB2*) are associated with susceptibility to human hypertension. In mice, targeted disruption of *Bdkrb2* predisposes to hypertension, senescence, and diabetic nephropathy. *Bdkrb2*$^{-/-}$ embryos are prone to congenital anomalies of the kidney and urinary tract (CAKUT) following gestational salt stress. We hypothesized that *Bdkrb2* inactivation in the ureteric bud (UB) lineage increases the susceptibility to CAKUT in mice.

**Methods:** 1. We generated mice harboring a conditional *Bdkrb2* allele; 2. To delete *Bdkrb2* in the UB lineage, Bdkrb2$^{f/f}$;R26R$^{tdTomato}$ were bred to *Hoxb7-Cre:GFP* mice, and females were placed on 5% NaCl diet during gestation; 3. GFP$^{+}$/tdTomato$^{+}$:Bdkrb2$^{UB-/-}$ and GFP$^{+}$:Bdkrb2$^{UB+/+}$ cells were isolated by FACS. RNA expression was analyzed by microarray hybridization, QPCR and ISH. Apoptosis was assessed by TUNEL and active caspase 3 IF. Double-stranded DNA breaks were assessed by γH2AX staining.

**Results:** 1. FACSorted *Bdkrb2*$^{UB-/-}$cells lack *Bdkrb2* DNA and mRNA.

2. Salt stressed *Bdkrb2*$^{UB-/-}$ kidneys exhibit hypoplastic papillae, dilated medullary collecting ducts, and occasional cortical cyst of UB origin.

3. The medullary collecting ducts of *Bdkrb2*$^{UB-/-}$ kidneys show a marked downregulation of Pax2, E-Cadherin, *Wnt7b, Wnt9b, and Wnt11.*

*4. Bdkrb2*$^{UB-/-}$ medulla exhibits extensive histone γH2AX phosphorylation, accumulation phosphorylated p53 (p-p53$^{Ser23}$), and upregulation of TUNEL/Caspase3-positive cells.

5. *Bdkrb2*$^{UB+/+}$ and *Bdkrb2*$^{UB-/-}$ cells exhibit d ifferential expression of 300 genes ($p<0.05$; >2-fold). The major categories of downregulated gene clusters are cell adhesion/ tight junction (claudin 4/7, E-cadherin, occludin), Wnt signaling (Wnt7b, Wnt10a, NFAT, cyclinD2), apoptosis (Caspase 4, Apaf1), transcriptional regulators of distal nephron fate (Irx2, Brn1), and collecting duct receptors/transporters/channels (ET1Rb, EP1, AVPR2, AQP3, AE1, UT-1).

**Conclusions:** 1. This study provides a proof-of-concept that gene-environment interactions act cell-autonomously to disrupt organogenesis.

2. *Bdkrb2* signaling opposes deleterious pathways leading to DNA damage, p53 activation, apoptosis, and impaired terminal epithelial differentiation.

*Funding:* NIDDK Support

## TH-OR080

### Heterochronic Transplantation of Mid- and Late-Gestation Metanephric Mesenchyme Revealed Age Related Differences

Shuang Chen, Raphael Kopan. *Developmental Biology, Washington Univ in St. Louis, Saint Louis, MO.*

**Background:** During development mammals build kidneys with a large surplus of nephrons from the cap mesenchyme (CM). Although stem-like in many respects, these embryonic progenitors differentiate en mass before or shortly after birth. It is unclear at present whether progenitor lifespan is regulated by progenitor-extrinsic signals (loss of niche cytokine, a powerful pulse of inductive signal) or intrinsic mechanisms (limited self-renewal capacity of progenitor cells).

**Methods:** To ascertain if there is an intrinsic difference in the self-renewal and differentiation capacity of young and old CM cells, we co-injected FACS sorted Six2+ cells from E12.5 (young) and P1 (old) kidneys at a 1:1 ratio into the CM of E12.5 recipient kidneys. To allow fate mapping of the injected progenitors, we collected young and old Six2+ cells from Six2$^{TGC+/tg}$; Rosa +/tdTom (red) and Six2$^{TGC+/tg}$; CAG-eCFP $^{+/tg}$ (blue) animals, respectively. Pax2Cre$^{+/tg}$, Rosa +/eYFP (green) kidneys were used as recipients to ensure positioning of injected cells into the CM. We then cultured the recipients for 4 days and compared the number of young and old cells within the CM and in epithelial compartment by immunostaining. In parallel, cells from the same sort were cultured in the presence of Fgf9 and BMP7 and the distribution of red and blue cells within cell aggregates were analyzed.

**Results:** Both young and old Six2+ cells readily integrate into the CM and/or the differentiating nephron epithelia when injected into the host kidneys. Interestingly, at the end of 4-day culture, most of the P1 progenitors have differentiated with few remaining in the CM. In contrast, 3-5 fold more E12.5 progenitor remained in the CM. When cultured in vitro, young and old progenitors sort away from each other, with the young cells in the periphery and old cells at the center. Switching the lineage label (red and blue) in young and old Six2+ cells does not alter these results.

**Conclusions:** These data suggest that young and old Six2+ CM cells are intrinsically different and the young niche did not "rejuvenate" older cells. This conclusion is further supported by their differential adhesion properties in vitro.

*Funding:* NIDDK Support

## TH-OR081

### Genetic Analysis of Nephron Segmentation Using the Zebrafish

Rebecca A. Wingert, Gary F. Gerlach. *Dept of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

**Background:** The zebrafish pronephros, or embryonic kidney, has become an increasingly popular model to study renal development and disease. Zebrafish form a pronephros comprised of two nephrons that consist of a series of proximal and distal functional segments, similar to mammals. How nephron segment cell types arise remains a major outstanding question in the field of nephrology.

**Methods:** We have performed a novel forward genetic screen to identify nephrogenesis genes using zebrafish. We screened approximately 700 genomes and observed 45 mutations that cause nephron cell type defects. To date, 15 recessive mutations have been recovered, and ongoing complementation suggests that these represent at least 12 different nephrogenesis genes.

**Results:** The kidney phenotypic classes include models of podocyte deficiency, as well as expansions or reductions in the domains of individual proximal and distal tubule segments. Thus far, we have characterized 5 mutant lines that evince podocyte formation defects. Mutants ND172 and ND233 have absent and reduced podocytes, respectively, and display normal development until 3-4 days, when they exhibit renal failure. These data suggest that ND172 and ND233 play specific roles in podocyte formation, while the other podocyte mutants have widespread defects suggesting a disruption in genes with pleiotropic functions. We have also recovered 5 mutant lines with tubule patterning defects that exhibit alterations in proximal or distal segment formation. For example, kidney mutant ND161 displays an abrogation of distal tubule formation, exhibiting a loss of romk gene expression. This finding suggests that ND161 may be a useful system relevant to the study of Barttner's syndrome. Ongoing efforts are aimed at further phenotypic characterization of ND161, and identification of the genetic lesion underlying this distal tubule mutant.

**Conclusions:** Taken together, our collection will provide a useful resource to delineate the genes that direct nephrogenesis pathways, and may provide new models to study human congenital kidney defects.

*Funding:* NIDDK Support

## TH-OR082

### Unraveling the Role of Podocyte Regeneration for Glomerular Homeostasis and Aging

Nicola Wanner,[1,2,3] Bjorn Hartleben,[1] Markus Gödel,[1] Natalie Stickel,[2,3,4] Gerd Walz,[1] Marcus J. Moeller,[5] Florian Grahammer,[1] Tobias B. Huber.[1,2,6] *[1]Renal Div, Univ Medical Center Freiburg; [2]Spemann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs Univ Freiburg; [3]Faculty of Biology, Albert Ludwigs Univ Freiburg; [4]Dept of Hematology and Oncology, Univ Medical Center Freiburg; [5]Div for Nephrology and Immunology RTWH Aachen; [6]BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-Univ, Freiburg, Germany.*

**Background:** Podocyte loss is a major determinant of progressive chronic kidney disease. Although recent studies have shown that a subset of parietal epithelial cells can serve as podocyte progenitors, the role of podocyte regeneration in aging and nephron loss remains unclear.

**Methods:** Here we combined genetic fate mapping with highly efficient podocyte isolation protocols to precisely quantify podocyte turnover.

**Results:** We demonstrate for the first time that parietal epithelial cells can give rise to fully differentiated visceral epithelial cells indistinguishable from resident podocytes, and found that limited podocyte renewal occurs during glomerular development as well as in a diphtheria toxin-model of acute podocyte ablation. In contrast, the compensatory programs in response to nephron loss mainly evoke podocyte hypertrophy, but not podocyte regeneration. In addition, under physiological conditions no turnover of podocytes could be detected in aging mice. In the absence of podocyte replacement, progressive accumulation of oxidized proteins, deposits of protein aggregates, loss of podocytes and glomerulosclerosis were characteristic features of aging mouse kidneys.

**Conclusions:** In summary, quantitative investigation of podocyte regeneration *in vivo* provides novel insights into the mechanism and capacity of podocyte regeneration in mice. Our data reveal that podocyte generation is mainly confined to glomerular development and occurs after acute glomerular injury, but fails to regenerate podocytes in aging kidneys or in response to nephron loss.

*Funding:* Government Support - Non-U.S.

**TH-OR083**

**A Transposon-Mediated System for the Generation of Nephron Progenitors from Adult Somatic Cells** Jessica May Vanslambrouck,[1] Lauren Elizabeth Woodard,[2] Norseha Suhaimi,[1] Matthew H. Wilson,[2] Melissa H. Little.[1] *[1]Institute for Molecular Bioscience, Univ of Queensland, Australia; [2]Dept of Medicine, Baylor College of Medicine, TX.*

**Background:** Novel treatments for chronic kidney disease are desperately needed and cellular reprogramming may represent one such option. As all nephrons in the human kidney form prior to birth, their regeneration may require the recreation of embryonic nephron progenitors (NPs). Using a lentivirus-mediated screen, we identified 6 transcription factors sufficient to re-impose a NP-like state when co-expressed in adult human proximal tubule cells (Hendry et al., JASN, 2013). Here we report the preliminary de-replication of this gene set and development of a multicistronic transposon construct to improve this reprogramming.

**Methods:** De-replication involved removal of individual lentiviral constructs from HK2 cell transductions. A transposon-mediated system was generated by engineering all 6 genes into a *piggyBac* construct with intervening 2A sequences, a tetracycline response element for doxycycline inducibility and a reporter (mCherry) for cell enrichment. FuGENE6 was used to co-transfect HK2 cells with this transposon, a tetracycline activator transposon and a *piggyBac* transposase construct. Gene expression was induced with 2μg/mL doxycycline. Reprogramming was assessed via morphological change (epithelial-to-mesenchymal transition) and expression of key NP genes.

**Results:** A NP-like state was achieved without *EYA1* or *HOXA11*. Transposon integration into HK2 cells was most efficient with the hyperactive *piggyBac* transposase (m7pB). Following integration, HK2 cells displayed tightly regulated doxycycline-inducible mCherry expression and NP-like characteristics, including increased expression of key NP markers. The expression of these genes was influenced by the length of doxycycline exposure.

**Conclusions:** These results further refine the genes required for NP reprogramming and demonstrate the feasibility of a transposon-based approach for inducible reprogramming to a NP. In the long term, this approach will facilitate patient-specific reprogramming to NP cells. Such cells may also prove invaluable for bioengineering and nephrotoxicity screening.

*Funding:* Government Support - Non-U.S.

**TH-OR084**

**Rapid and Efficient Differentiation of Human Pluripotent Stem Cells into Intermediate Mesoderm Cells That Form Tubules and Express Proximal Tubule Markers** Albert Q. Lam,[1,2] Benjamin S. Freedman,[1] Ryuji Morizane,[1] M. Todd Valerius,[1,2] Joseph V. Bonventre.[1,2] *[1]Renal Div, Brigham and Women's Hospital, Boston, MA; [2]Harvard Stem Cell Institute, Cambridge, MA.*

**Background:** Human pluripotent stem cells (hPSCs) have the capacity to generate the wide diversity of human cell types, making them ideal starting substrates for generating cells of the kidney lineage. Here we report a highly efficient system to differentiate human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) into cells of the intermediate mesoderm (IM), the embryonic tissue which gives rise to the adult kidneys.

**Methods:** hPSCs were cultured in mTeSR1 medium until they reached 50% confluence, then differentiated into mesendoderm using serum-free media supplemented with the GSK-3β inhibitor CHIR99021 (CHIR). To identify conditions to induce IM differentiation, we screened several growth factors at varying doses for the ability to induce expression of the IM markers PAX2 and LHX1.

**Results:** Treatment of hPSCs with CHIR induced BRACHYURY$^+$MIXL1$^+$ mesendoderm differentiation with nearly 100% efficiency. In the absence of additional exogenous factors, CHIR-induced mesendodermal cells preferentially differentiated into lateral plate mesoderm with minimal IM differentiation. Screening multiple growth factors for the ability to promote IM differentiation identified FGF2 and retinoic acid (RA) as potent inducers of the IM markers PAX2 and LHX1, respectively. The sequential treatment of hPSCs with CHIR followed by FGF2 and RA generated PAX2$^+$LHX1$^+$ cells after 3 days of differentiation with 70-80% efficiency as determined by immunocytochemistry and flow cytometry. These putative IM cells expressed other IM markers, including OSR1, PAX8, and WT1, without significant expression of lateral plate or paraxial mesoderm markers. After an additional 6 days of differentiation, these PAX2$^+$LHX1$^+$ cells gave rise to tubular structures co-expressing the proximal tubule markers LTL and Kidney-specific protein.

**Conclusions:** Our findings establish a system whereby hPSCs can be rapidly and robustly differentiated into PAX2$^+$LHX1$^+$ intermediate mesoderm cells which form tubules and express proximal tubule markers.

*Funding:* NIDDK Support, Private Foundation Support

**TH-OR085**

**Contrasting Roles for M1 and M2 Type Macrophages in Childhood IgA Nephropathy** Yohei Ikezumi,[1] Toshiaki Suzuki,[1] Takeshi Yamada,[1] Hiroya Hasegawa,[1] David J. Nikolic-Paterson,[2] Akihiko Saitoh.[1] *[1]Dept of Pediatrics, Niigata Univ Medical and Dental Hospital, Niigata, Japan; [2]Monash Univ Dept of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.*

**Background:** We have previously shown that M2-type macrophages (MQ) are dominant in chronic lesions, such as glomerular matrix expansion and interstitial fibrosis, in IgA nephropathy (IgAN). However, the role of M1-type activated MQ has not been elucidated. Therefore, we examined activated M1-type MQ in biopsies of childhood IgAN.

**Methods:** Biopsies taken from 81 cases of childhood IgAN (11±3 years) were examined for histologic changes and by immunofluorescence staining for activated M1-type MQ (CD86$^+$ cells) and M2-type MQ (CD163$^+$ cells). Nine biopsies from children with thin basement membrane disease (TBMD) were used as control.

**Results:** Histopathologic assessment of IgAN revealed significant accumulation of both M1 and M2 type MQ in glomeruli, while most interstitial MQ were CD163$^+$ (M2-type). CD86$^+$ M1-type MQ correlated with: endocapillary proliferation and cellular crescent formation (both $p<0.001$); the degree of hematuria ($p<0.001$) and proteinuria ($p=0.014$), but did not correlate with glomerular matrix expansion or interstitial fibrosis. In contrast, CD163$^+$ M2-type MQ correlated with glomerular matrix expansion ($p<0.01$) and interstitial fibrosis ($p<0.001$).

**Conclusions:** We have identified contrasting roles for M1 and M2 type MQ in childhood IgAN. Activated M1-type MQ appear to be involved in the development of acute lesions (endocapillary proliferation and cellular crescents), whereas M2-type MQ are involved in chronic lesions such as mesangial matrix expansion and interstitial fibrosis. The prognostic significance of these findings warrants further study.

*Funding:* Government Support - Non-U.S.

**TH-OR086**

**Mycofenolate Mofetil Therapy in IgA Nephropathy: Histological Changes after Treatment** Hannah Kate Sarah Beckwith, Nicholas R. Medjeral-Thomas, Jack W. Galliford, Megan Griffith, Jeremy B. Levy, Andrew B.D. Palmer, Candice A. Roufosse, H. Terence Cook, Tom Cairns. *Imperial College Kidney and Transplant Institute, London, United Kingdom.*

**Background:** Endocapillary hypercellularity independently predicts renal outcome in IgA Nephropathy (IgAN)[1]. Mycophenolate mofetil (MMF) treatment is offered to patients presenting to the Imperial College Kidney and Transplant Institute with IgAN and histological evidence of endocapillary inflammation. Clinical trials of MMF in IgAN have been inconclusive. Evidence of histological improvement following MMF treatment would support its therapeutic use. We therefore reviewed histological changes after MMF therapy in a cohort of IgAN patients.

**Methods:** Eleven IgAN patients with repeat native renal biopsies before and after MMF treatment were identified. Patients were excluded if they had received any other immunosuppression therapy, including corticosteroids. Based on the Oxford classification of IgAN[1], we reviewed histological changes after MMF treatment.

**Results:** Seven patients (60%) were male. At diagnostic renal biopsy, median age was 42 (range 19-67), serum creatinine was 127umol/L (56-233), and urine protein creatinine ratio (UPCR) was 157 mg/dl (67-224). The median time between biopsies was 29 months (14-41).

Following MMF treatment, repeat biopsy demonstrated statistically significant improvement in the mean percentage of glomeruli showing endocapillary hypercellularity and cellular/fibrocellular crescents. A significant reduction in IgA deposition ($p=0.04$) and a trend towards improved mesangial hypercellularity ($p=0.05$) was also demonstrated. There was no change in tubular atrophy. Median serum creatinine remained stable at 3 years follow-up at 110umol/L (59-421).

| | Biopsy 1 | Biopsy 2 | p-value |
|---|---|---|---|
| Glomeruli with endocapillary hypercellularity, mean % (SD) | 11.0 (14.3) | 1.60 (4.03) | <0.01 |
| Tubular atrophy, mean % (SD) | 21.8 (11.9) | 18.1 (16.4) | 0.23 |
| Glomeruli with crescents, mean % (SD) | 7.69 (8.77) | 1.60 (3.25) | <0.01 |

**Conclusions:** MMF treatment is associated with histopathological improvement in IgAN.

**References** 1.Cattran D, Coppo R, Cook T et al. The Oxford classification of IgA nephropathy: Rationale, clinicopathological correlations and classification. Kidney Int 2009;76:546-556.

**TH-OR087**

**Abnormal STAT3 Signaling Enhances Production of Autoantigen in an Autoimmune Disease, IgA Nephropathy** Colin Reily,[1] Koshi Yamada,[1,5] Zhi Qiang Huang,[1] Milan Raska,[1,4] Hitoshi Suzuki,[1,5] Bruce A. Julian,[1,2] Christopher D. Willey,[3] Jan Novak.[1] *[1]Microbiology, Univ of Alabama at Birmingham; [2]Medicine, Univ of Alabama at Birmingham; [3]Radiation Oncology, Univ of Alabama at Birmingham; [4]Palacky Univ in Olomouc; [5]Juntendo Univ Faculty of Medicine.*

**Background:** IgA Nephropathy (IgAN) is an autoimmune disease characterized by IgA1-containing mesangial immunodeposits. These deposits are likely derived from circulating immune complexes formed from IgA1 with galactose-deficient *O*-glycans (Gd-IgA1; autoantigen) and antiglycan autoantibodies. Disease activity (macroscopic hematuria) frequently manifests after an upper-respiratory-tract infection, suggesting that immune activation via cytokines may be involved in disease exacerbation. This concept is supported by the observation that serum IL-6 levels are elevated in some IgAN patients. Using immortalized IgA1-secreting cells derived from the circulation of IgAN patients and healthy controls (HC), we characterized signaling mechanisms involved in Gd-IgA1 production induced by IL-6.

**Methods:** IgA1-secreting cells were stimulated with IL-6, and IgA1 production and *O*-glycosylation were assessed. siRNA knock-down (k/d) and specific kinase inhibitors confirmed the role of IL-6-STAT3 pathway in Gd-IgA1 production. Association of a possible co-factor, HDAC6, with STAT3 was assessed by using immunoprecipitation and Western blot assay.

**Results:** IgA1-producing cells from IgAN patients but not HC stimulated with IL-6 secreted more Gd-IgA1 ($P<0.05$). IL-6 stimulation induced more robust and prolonged STAT3 phosphorylation in cells from IgAN patients than from HC. siRNA k/d and kinase

inhibitors confirmed the central role of STAT3 activation in the enhanced production of Gd-IgA1 in response to IL-6 (P<.05). HDAC6 showed no association with STAT3 regardless of IL-6 stimulation.

**Conclusions:** IL-6-mediated activation of STAT3 plays an important role in the enhanced production of Gd-IgA1 in IgAN. Further analysis is needed to determine a potential role of acetylation in STAT3 activation. STAT3 signaling is exacerbated in IgAN patients and may offer a future target for disease-specific therapy.

*Funding:* NIDDK Support, Other NIH Support - T32 training grant

## TH-OR088

**Tonsillar Cells in Patients with IgA Nephropathy Produce Aberrantly Glycosylated IgA1 and Anti-Glycan Antibodies** Hitoshi Suzuki,[1] Yusuke Suzuki,[1] Yuko Makita,[1] Bruce A. Julian,[2] Jan Novak,[2] Yasuhiko Tomino.[1] *[1]Nephrology, Juntendo Univ Faculty of Medicine, Tokyo, Japan; [2]Medicine and Microbiology, Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** IgA1 in circulating immune complexes and mesangial deposits of patients with IgA nephropathy (IgAN) is aberrantly glycosylated, galactose-deficient in *O*-glycans (Gd-IgA1), and is bound to anti-glycan IgG/IgA autoantibodies. However, the origin of cells producing Gd-IgA1 and the autoantibodies is not certain. Upper respiratory tract infections and tonsillitis are frequently associated with clinical flares of IgAN, suggesting a link with disease pathogenesis. Levels of gene transcription for specific glycosyltransferases are dysregulated in tonsillar B cells from patients with IgAN, resulting in production of Gd-IgA1. In some patients, tonsillectomy and glucocorticoids (TSP) may slow disease progression in early clinical stages. Therefore, we assessed whether tonsillar cells produce Gd-IgA1 or anti-glycan IgG.

**Methods:** Tonsillar cells obtained from 29 patients with IgAN were cultured 72 hours. Gd-IgA1 and anti-glycan IgG secreted by these cells were measured by ELISA. Proteinuria and hematuria, and serum levels of Gd-IgA1, Gd-IgA1-specific IgG and IgA, and IgG-IgA immune complexes (IC) were measured before and after TSP.

**Results:** Proteinuria and hematuria improved after TSP (P<0.05). Eighteen of 29 patients had proteinuria less 0.3 g/g and 5 red blood cells/HPF after TSP (Remission group). Eleven patients did not clinically improve (non-Remission group). Serum levels of Gd-IgA1, Gd-IgA1-specific autoantibodies, and IgG-IgA IC decreased during glucocorticoid therapy after tonsillectomy (P<0.01). The rates of decrease in the levels of Gd-IgA1, Gd-IgA1-specific antibodies and IgG-IgA IC were greater in the Remission group (P<0.01). Tonsillar cells from Remission group produced more Gd-IgA1 and anti-glycan IgG than those from non-Remission group (P<0.01).

**Conclusions:** Tonsillar cells may contribute to the circulating Gd-IgA1 and anti-glycan IgG in patients with IgAN. These biomarkers may be useful for guiding therapy of IgAN.

*Funding:* NIDDK Support

## TH-OR089

**Understanding the Structure of PLA2R: Insights into Formation of the Immunodominant Epitope Recognised by Autoantibodies in Patients with Idiopathic Membranous Nephropathy** Maryline Fresquet, Thomas A. Jowitt, Jennet O. Gummadova, Edward A. McKenzie, Rachel Lennon, Paul E. Brenchley. *Faculty of Human and Medical Sciences, Univ of Manchester, Manchester, United Kingdom.*

**Background:** 75% of patients with idiopathic membranous nephropathy (IMN) have autoantibodies against the phospholipase A2 receptor (PLA2R). The epitope(s) within PLA2R and its 3D structure are unknown. The objective is to understand how autoantibodies to PLA2R interact with the target receptor.

**Methods:** To identify the dominant epitope, PLA2R was trypsin digested, separated on unreduced gels and peptides reacting to patient sera were identified by western blotting. Bands of 16, 20 and 37kDa were excised, reduced and analysed by MS. The structure of both PLA2R and an immunoreactive PLA2R fragment, NCTLD3, were investigated using sedimentation, SAXS and 3D electron tomography. A SPR method was developed to assess binding kinetics of patient antibodies to PLA2R and NCTLD3.

**Results:** MS analyses suggest 3 peptides constitute part of the PLA2R epitope. The distribution of these peptides is discontinuous on the linear sequence but could be in proximity within the folded molecule.

Hydrodynamic experiments showed PLA2R exists in two distinct pH-dependent conformations similar to other mannose receptor family members. Low resolution *ab initio* structures from SAXS and EM have enabled us to construct a model of both full-length and NCTLD3 PLA2R.

SPR analysis of patient sera revealed equivalent autoantibody binding kinetics to both full-length PLA2R and NCTLD3. Antibody affinity was similar in all five patient sera tested.

**Conclusions:** We identified a specific region on PLA2R as the potential immunodominant epitope involved in the interaction with its autoantibody. This epitope appears to be solely within the N-terminal CTLD3 region as shown by SPR, and there appears to be no difference in affinity between patients. PLA2R fluctuates between an extended conformation at neutral pH and a bent conformation at lower pH possibly involved in the regulation of ligand recognition or receptor oligomerization. This has implications for formation of neo-epitopes that may become immunogenic and induce autoimmunity.

## TH-OR090

***APOL1* Risk Variants in PLA2R-Positive Membranous Glomerulopathy** Christopher Patrick Larsen, Patrick D. Walker, Josephine M. Ambruzs, Nidia Cordeiro Messias. *Nephropath, Little Rock, AR.*

**Background:** The APOL1 disease spectrum includes non-diabetic ESKD, FSGS and collapsing glomerulopathy (CG) associated with HIV and SLE. It is currently unknown if the presence of *APOL1* risk alleles modify the morphologic phenotype of other glomerulonephritides. We sought to determine if there were any morphologic alterations correlated with the presence of *APOL1* risk alleles in a homogeneous cohort of patients with PLA2R-positive membranous glomerulopathy (PLA2rMG).

**Methods:** 118 renal biopsies were identified in the case file of our institution from AA patients with PLA2rMG. A blinded morphologic evaluation was performed to evaluate for changes associated with the presence of 2 *APOL1* risk alleles. Features examined include global and segmental glomerulosclerosis, subtype of FSGS, degree of interstitial fibrosis and tubular atrophy, subtype of tubular atrophy, degree of interstitial inflammation, degree of arteriosclerosis, evidence of acute tubular injury, and the presence of microcystic tubular dilatation (MCTD). DNA was genotyped for *APOL1* risk alleles using TaqMan assays.

**Results:** Six (6) cases of CG were identified including 4/18 in the group with 2 risk alleles, 2/45 in the group with 1 risk allele, and 0/40 in the group with no risk alleles. *APOL1* associated with CG such that in a recessive model, *APOL1* risk alleles conferred 11.9 fold higher odds of developing CG ($P<0.01$, Odds ratio $CI_{95}=2.0\text{-}79.0$). *APOL1* risk alleles were also found to be associated with MCTD ($P<0.04$), interstitial inflammation ($P<0.03$), and segmental sclerosis ($P<0.01$). The cases with 2 risk alleles also showed a trend toward more interstitial fibrosis and tubular atrophy. Other morphologic features were not found to be associated with *APOL1*.

**Conclusions:** Collapsing glomerulopathy is an aggressive nephropathy with no currently available efficacious treatment. We have evaluated the changes associated with *APOL1* risk alleles in a cohort of patients with PLA2R-positive MG and found that there is significantly more CG with the presence of 2 *APOL1* risk alleles. These results raise the possibility that *APOL1* risk alleles modulate and potentially accelerate glomerular diseases though the development of collapsing lesions.

## TH-OR091

**The Prevalence of Circulating Monoclonal Protein in Proliferative Nephropathy with Monoclonal Deposits** Gauri Bhutani,[1] Fernando C. Fervenza,[1] Sanjeev Sethi,[2] Samih H. Nasr,[2] Nelson Leung.[1] *[1]Nephrology & Hypertension, Mayo Clinic; [2]Anatomic Pathology, Mayo Clinic, Rochester.*

**Background:** Monoclonal gammopathy of 'Renal' significance (MGRS) was recently described as a new classification of monoclonal gammopathy which results in renal disease but is not diagnostic of multiple myeloma. MGRS is defined as a kidney disease with monoclonal protein deposits or where the monoclonal protein acts as a C3 nephritic factor. This study was undertaken to evaluate the hematologic characteristics of these patients.

**Methods:** The pathology database at Mayo Clinic was reviewed for patients with MGRS associated proliferative kidney diseases from 2008 to 2012. Circulating monoclonal protein testing included serum protein electrophoresis with immunofixation (SPEP/IF) and serum free light chain assay (FLC). Methods to detect the pathological clone included bone marrow biopsy and PET scan. Fisher's exact test was used for statistical analysis.

**Results:** In a total 56 patients, biopsy displayed mesangioproliferative (8 = 14%), membranoproliferative (MPGN) (22 = 40%), diffuse proliferative (DPGN) (11 = 20%), a mixed MPGN- DPGN (10 = 18%) and proliferative GN with membranous features (5 = 9%). Monoclonal deposits were IgGK in 59%, IgGγ in 25% and IgAK / γ or IgMK / γ in the remaining 10%. IgG subtyping showed IgG3 in 14 (64%), IgG1 in 5 (22%) and IgG2 in 3 (14%) out of 22 biopsies. Table 1 notes pertinent diagnostic workup.

| All proliferative glomerulonephritides (56) | | | | | | |
|---|---|---|---|---|---|---|
| | Positive SPEP/IF (14 = 25%) | | Negative SPEP / IF (42 = 75%) | | | |
| FLC Ratio (0.37 – 3.1) | Clone detected (11 = 79%) | No clone detected (3 = 21%) | Clone detected (1 = 1.5%) | No clone detected (23 = 55%) | Unrelated clone (2 = 3.5%) | Clone not checked (16 = 38%) |
| Abnormal | 0 | 1 | 1 | 0 | 0 | 1 |
| Normal | 10 (91%) | 1 | 0 | 18 | 2 | 8 |
| Not known | 1 | 1 | 0 | 5 | 0 | 7 |

**Conclusions:** Majority of patients with a proliferative nephropathy and monoclonal deposits lack a detectable serum monoclonal gammopathy. Detection of a serum monoclonal protein is associated with significantly high detection rate of the pathologic clone ($p < 0.01$) and, hence, a more directed treatment. Serum free light chain ratio is normal in most patients and an abormal result does not appear to increase the likelihood of finding a pathologic clone.

## TH-OR092

**Toward a Working Definition of C3 Glomerulopathy by Immunofluorescence** Jean Hou, Glen S. Markowitz, Andrew S. Bomback, Gerald B. Appel, Leal C. Herlitz, Michael B. Stokes, Vivette D. D'Agati. *Columbia Univ Medical Center, New York, NY.*

**Background:** An impediment to the diagnosis of C3G is the lack of a working IF definition developed and validated in a disease cohort. While "C3 only" is applied as a theoretical construct, its practicality is untested. We aimed to test hierarchical IF criteria with varying stringency for C3G using dense deposit disease (DDD) as gold standard; determine if "C3 only" is a practicable criterion; apply these criteria to analyze the incidence of C3G in MPGN types 1 and 3; and explore clinical correlates.

**Methods:** A total of 807 biopsies (bx) diagnosed as MPGN from 1999-2012 were retrospectively reviewed. Excluding cases with known etiology (e.g. hepatitis C), we identified 319 cases of primary MPGN types 1-3. We analyzed IF reports and coded bx as glomerular deposits of: C3 only (Criterion 1); C3 dominant and ≤1+ IgM only (Criterion 2); C3 dominant and ≥2 orders of intensity stronger than any combination of IgG, IgM, IgA, C1q (Criterion 3); C3 dominant and >1+ IgG or IgM (Criterion 4).

**Results:** On analysis of initial bx in 319 patients (200 MPGN1, 42 DDD and 77 MPGN3), the most restrictive criterion of "C3 only" captured only 50% of DDD (compared to 8% MPGN1 and 10.4% MPGN3), whereas criterion 3 identified 88.1% of DDD (compared to 30.5% MPGN1 and 39% MPGN3). Broadening to criterion 4 added only 4.8% of DDD cases but added 26.5% MPGN1 and 13% MPGN3 cases, suggesting loss of specificity. Therefore criterion 3 was chosen as working definition of C3G. Among MPGN3, 90% of C3G cases were Strife and Anders variant. Repeat bx in C3G revealed a change in IF criterion class in 10 of 23 cases, indicating fluidity between subgroups. The prevalence of low serum C3 and/or C4 at bx did not significantly differ among the 3 proposed IF criteria. A nephritic presentation was more common in criterion 1 cases.

**Conclusions:** "C3 only" is an impractical definition of C3G. We propose a less restrictive definition of C3 dominant and at least 2 orders of magnitude more intense than any other immune reactant. These criteria provide a framework for identifying renal biopsies most likely to benefit from investigations into alternative pathway dysregulation.

## TH-OR093

**Biomarker Profiling of Dense Deposit Disease and C3 Glomerulonephritis** Yuzhou Zhang, Tara Maga, Bertha Martin, Nicole Meyer, Dingwu Shao, Xue Xiao, Carla M. Nester, Richard J. Smith. *Univ of Iowa, Iowa City, IA.*

**Background:** Dense Deposit Disease (DDD) and C3 Glomerulonephritis (C3GN) are prototypical C3 Glomerulopathies (C3G). On renal biopsy, both are C3-dominant by immunofluorescence while on electron microscopy DDD is defined by intramembranous glomerular basement membrane dense deposits while C3GN is associated with some combination of subepithelial, subendothelial and/or less dense, discontinuous intramembranous deposits. The pathophysiological basis of both diseases is dysregulation of the complement cascade at the level of the C3 and C5 convertases.

We sought to define the relative degrees of proximal and distal complement pathway dysregulation in a cohort of DDD and C3GN patients to test the hypothesis that these two diseases can be differentiated by biomarker profiling.

**Methods:** We examined a panel of biomarkers that are reflective of alternative and terminal complement pathway activity in 17 DDD patients and 17 C3GN patients.

**Results:** As compared to controls, in both DDD and C3GN patients, serum levels of C3, factor B, properdin and C5 are significantly reduced ($p<.005$) while levels of C3d are significantly elevated ($p<.005$). In C3GN patients but not in DDD patients, sMAC is elevated ($p<.005$) and C7 is reduced ($p<.01$) as compared to controls. Significant biomarkers differences between DDD and C3GN include a significant reduction in both properdin ($p<.005$) and C7 ($p<.01$) in C3GN and a significant elevation of sMAC ($p<.05$) in C3GN.

**Conclusions:** Biomarker profiling distinguishes DDD and C3GN and is a valuable metric for defining the degree of complement dysregulation that is present. These results have clinical implications and can be used to guide and follow response to different anti-complementherapies.

*Funding:* NIDDK Support

## TH-OR094

**Recurrent C3 Glomerulonephritis: Clinicopathological Findings and Outcomes** Ladan Zand,[1] Elizabeth C. Lorenz,[1] Fernando G. Cosio,[1] Fernando C. Fervenza,[1] Samih H. Nasr,[1] Richard J. Smith,[2] Sanjeev Sethi.[1] *[1]Mayo Clinic, Rochester, MN; [2]Carver College of Medicine, Iowa City, IA.*

**Background:** C3 glomerulonephritis (GN) is a proliferative glomerulonephritis characterized by bright C3 staining and the absence of significant Ig staining on IF microscopy. C3GN results from abnormalities in the alternative pathway of complement. In a significant number of C3GN patients, the disease progresses to end-stage renal disease (ESRD). The clinical, pathology findings and outcome of C3GN patients receiving a kidney transplant is largely unknown.

**Methods:** We identified 22 C3GN patients between 1996 and 2010 at our institution who received a kidney transplant.

**Results:** Fourteen of the 22 patients had recurrence of C3GN (63.6%). 6 were male and 8 were female patients. The median age at time of initial diagnosis of C3GN on native kidney biopsy was 23 years. Median time from diagnosis to ESRD was 82.6 months. Twelve patients received living donor transplants, 2 received deceased donors. Four transplants were pre-emptive. Median time to recurrence of disease was 9.2 months, with failure of 50% of the grafts (n=7), with a median of 16.6 months to graft failure. Majority of patients (n=11) had hematuria and/or proteinuria at time of recurrence; 3 patients had low C3 levels. Three patients (23%) of 13 tested were positive for a monoclonal gammopathy. All 3 patients had early recurrence of disease (within 3 months) with loss of graft in two patients. Kidney biopsy at time of diagnosis of recurrence showed mesangial proliferative GN in 8 patients and a membranoproliferative GN in 6 patients. All biopsies showed bright C3 staining (2-3+), 6 also showed trace-1+ staining for IgM and/or IgG. EM showed only mesangial deposits in 3 patients, mesangial and subendothelial deposits in 9 patients, 3 also showed subepithelial deposits. In 2 cases, EM studies were not done.

**Conclusions:** This is the first study of recurrent C3GN. Almost 2/3rds of C3GN recur. Despite standard therapy (combination of calcineurin inhibitors, mycophenolate mofetil and prednisone), the course is progressive and results in graft loss in 50% of patients. C3GN recurs early, particularly in patients with monoclonal gammopathy.

## TH-OR095

**Small Molecule Agonists of CD11b Reduce Leukocyte Activation and Recruitment to Promote Kidney Allograft Survival** Samia Khan,[1] Mohd Hafeez Faridi,[1] Anupam Agarwal,[2] James George,[2] Vineet Gupta.[1] *[1]Dept of Internal Medicine, Rush Univ Medical Center, Chicago, IL; [2]Biochemistry & Molecular Genetics, The Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** Graft loss remains a major obstacle in kidney transplantation and the severity of allograft rejection is often associated with inflammatory infiltrates of leukocytes including myeloid cells expressing β2 integrin CD11b/CD18. Recently, we reported that activation of CD11b/CD18 by a small molecule compound, leukadherin-1 (LA-1), increases leukocyte cell adhesion to the inflamed endothelium, prevents transmigration and inhibits leukocyte tissue recruitment, which resulted in improved kidney function in a murine model of experimental nephritis.

**Methods:** Here we tested whether targeting of CD11b/CD18 using LA-1 prolongs kidney allograft survival in a mouse model of fully major histocompatibility complex-mismatched orthotopic kidney transplant, where C57BL/6J (H-2b) recipients receive a kidney allograft from a Balb/c animal (H-2d) along with daily cyclosporine for 2 weeks to sustain the transplant (control group). In addition to standard cyclosporine therapy, the recipient C57BL/6 mice in the test group were administered LA-1 injections daily during week 1 and every other day from weeks 2-8 after receiving the transplant. Renal allograft rejection was considered at the time animals displayed signs of ill health and high serum creatinine levels.

**Results:** Combination therapy with LA-1 and cyclosporine resulted in a significant prolongation of median graft survival from 28 days (cyclosporine only) to 60 days (cyclosporine and LA-1). At week 5 after transplantation, cyclosporine treated controls had serum creatinine values near 0.6 mg/dl while LA-1 and cyclosporin treated mice had baseline values near 0.2 mg/dl. The mice in the combination therapy group showed significantly reduced leukocyte infiltration and neointimal hyperplasia.

**Conclusions:** These findings indicate a crucial role for CD11b/CD18 in the control of leukocyte migration to transplanted kidney and identify leukadherins as potential, novel therapeutics for use in renal allotransplantation.

*Funding:* NIDDK Support

## TH-OR096

**Myeloid Derived Suppressor Cells Induced by Apoptotic Donor Cells Suppress Transplant Rejection via Indolamine 2,3-Dioxygenase** Jane Bryant,[1] Nadine M. Lerret,[1] Jiaojing Wang,[2] Zheng Jenny Zhang,[2] Xun-Rong Luo.[1,2] *[1]Medicine, Northwestern Univ Feinberg School of Medicine; [2]Surgery, Northwestern Univ Feinberg School of Medicine.*

**Background:** We have previously shown that pre- and post-transplant infusions of apoptotic donor splenocytes induced by 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide treatment (ECDI-SPs) provide significant donor-specific protection to full MHC-mismatched donor cardiac allografts, and with a short course of rapamycin provides indefinite cardiac allograft survival in 100% of the recipients. The mechanisms of protection are not fully elucidated.

**Methods:** The current study focuses on the role of apoptotic donor ECDI-SPs in the induction of myeloid derived suppressor cells and their mechanism of suppression of subsequent transplant rejection in a murine cardiac transplant model. BALB/c cardiac grafts are transplanted to C57BL/6 recipients treated with infusions of donor (BALB/c) ECDI-SPs, and cells from the graft and the spleen are examined.

**Results:** We show that infusions of donor ECDI-SPs induce a significant increase of both the $CD11b^{+}Gr1^{hi}Ly6C^{int}$ and the $CD11b^{+}Gr1^{-}Ly6C^{hi}$ populations in the spleen in a PGE-2 dependent manner, and that depletion of these cell populations abolishes graft protection induced by donor ECDI-SPs infusions. Interestingly, both splenic $CD11b^{+}Gr1^{hi}Ly6C^{int}$ and the $CD11b^{+}Gr1^{-}Ly6C^{hi}$ populations suppress T cell proliferation *in vitro*, with more robust suppression towards $CD8^{+}$ T cells, whereas the same populations when trafficking to the cardiac allograft become less suppressive. Furthermore, the suppression by these myeloid-derived suppressor cells (MDSCs) is mediated through an interferon-γ dependent induction of the tryptophan metabolizing enzyme indolamine 2,3-dioxygenase (IDO). Consequently, treatment with the IDO inhibitor 1-MT abolishes T cell suppression *in vitro* as well as cardiac graft protection *in vivo*.

**Conclusions:** These findings reveal a novel link between apoptotic donor cell infusions and the induction of IDO-producing MDSCs as a mechanism for transplant graft protection, and provide several targets amenable to therapeutic manipulations for tolerance induction for cardiac transplantation.

*Funding:* NIDDK Support

## TH-OR097

**Protection from Apoptosis during Extreme Cold Ischemia and Reperfusion Is Mediated by XIAP and Akt** Swati Jain, Daniel Keys, Charles L. Edelstein, Alkesh Jani. *UC Denver.*

**Background:** Cold ischemia (CI) followed by warm reperfusion (WR) during hibernation is a natural model we have used to understand DGF. Hibernating ground squirrel (GS) kidneys survive CI for *several days* in torpor (LT) followed by WR in Arousal (IBA) without AKI or tubular cell (RTEC) apoptosis seen in mouse & human kidneys after CI/WR. The molecular mechanism of protection against apoptosis & AKI during hibernation has long been sought. We hypothesized that upregulation of anti-apoptotic proteins XIAP, pAkt & pBAD during hibernation prevents RTEC apoptosis.

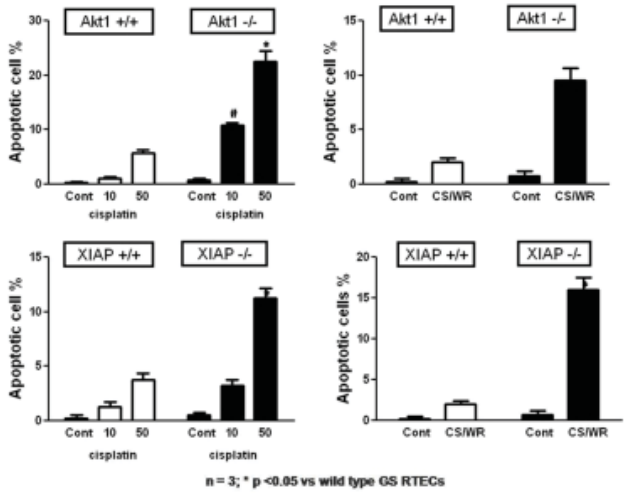
**Methods:** XIAP, pAkt & pBAD were examined: a)*in vivo* in LT & IBA GS kidneys (b)*in vitro* in GS & mouse RTEC under 2 apoptotic stimuli:1) cisplatin 2) cold storage followed by rewarming (CS/WR) to simulate IBA.

**Results:** XIAP, pAkt & pBAD increased after WR in IBA vs. LT kidneys, while cleaved caspase-3 (CC3) & apoptosis did not. Similarly, GS RTEC treated with cisplatin or CS/WR had less apoptosis, no CC3, & increased XIAP, pAkt & pBAD vs. mouse.

| | Mouse RTECs | | Squirrel RTECs | |
|---|---|---|---|---|
| | Cont | CS/WR | Cont | CS/WR |
| XIAP | +++ | + | +++ | +++ |
| pAkt | +++ | ++ | ++ | +++ |
| pBAD | + | - | ++ | ++++ |
| CC3 | + | ++++ | - | - |
| TUNEL + cells | 2.7±0.4 | 26±2.2* | 0.25±0.2 | 2.0±0.4 |

n = 3; * p <0.05 vs GS RTECs

To determine the mechanism of protection GS RTEC were treated with Akt1 shRNA & XIAP siRNA. GS RTEC deficient in Akt1 & XIAP had increased apoptosis & CC3.




**Conclusions:** We have shown for the first time a mechanism used by hibernators to survive prolonged CI/WR far in excess tolerable by human kidneys, using survival factors Akt1 & XIAP. Upregulation of anti-apoptotic factors may prevent apoptosis & AKI in donor kidneys subjected to prolonged CI/WR.

*Funding:* NIDDK Support

## TH-OR098

### Autoimmune Targeting of Protease Activated Receptor-1 (PAR-1) Deregulates VEGF and Disturbs Neoangiogenesis

Rusan Catar, Isa Annett Schramm, Michele Simon, Oskar Wischnewski, Aurélie Philippe, Angelika Kusch, Duska Dragun. *Univ Hospital Charite, Berlin, Germany.*

**Background:** Early loss of peritubular capillaries (PTC) with initiation of tubular atrophy and interstitial fibrosis is central to progressive nephron loss in native kidneys and transplants. VEGF is crucial for endothelial growth, differentiation and survival and mediates PTC homeostasis. Thrombin, a serin protease which elicits its cellular effects via the G-Protein Coupled Receptor (GPCR) PAR-1 is closely involved in VEGF regulation. Functional anti-GPCRs autoantibodies are able to induce endothelial dysfunction. We hypothesized that autoimmune GPCR targeting process may disturb VEGF induced angiogenesis and identified PAR-1 as a novel activating autoantibody target.

**Methods:** Human microvascular endothelial cells (HMEC-1) were stimulated with IgG isolated from patients' sera with transplant peritubular pathology (KTx-IgG). VEGF transcriptional regulation was studied by promoter deletion assay, transcription factor activation by qRT-PCR, western blot, EMSA and knockdown, VEGF secretion by ELISA. Tube formation on matrigel served to study endothelial neoangiogenic response.

**Results:** Treatment with KTx-IgG reduced ERK1/2 dependent VEGF secretion and tube formation. Upon treatment with several pharmacologic antagonists, VEGF secretion could be only normalized by pretreatment with specific PAR-1 inhibitor. Specificity was further confirmed by the use of PAR-1 2nd extracellular loop peptide which rescued endothelial tube formation. KTx-IgG increased cFos protein expression and its binding to VEGF-promoter contributing to deregulate neoangiogenesis by reducing VEGF-promoter activity. AP-1 inhibition or cFos siRNA-induced knockdown reconstituted VEGF levels and increased its promoter activity.

**Conclusions:** We identified the PAR-1 receptor as a new target for functional antibodies in the context of kidney transplantation with disturbed PTC homeostasis. We showed that KTx-IgG disturbs VEGF transcriptional regulation resulting in reduced VEGF secretion and inability of endothelial cells to form tubes. PAR-1 mediated VEGF regulation could offer new possibilities for treatment of kidney transplants to obviate loss of PTCs.

## TH-OR099

### Notch-2 Plays a Crucial Role in B Cell Development and Alloantibody Production

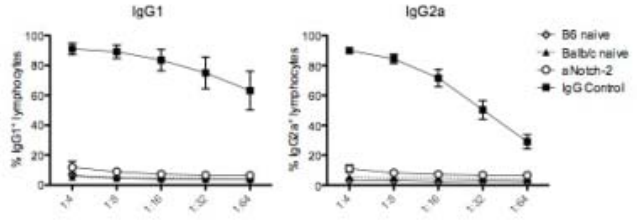
Ciara N. Magee, Tetsunosuke Shimizu, Kassem Safa, Nader Najafian, Leonardo V. Riella. *Transplantation Research Center, Brigham & Women's Hospital, Harvard Medical School.*

**Background:** The role of B cells in alloimmunity is increasingly recognized, while alloantibody production remains a major barrier to long-term graft survival. We have previously shown that Notch signaling critically affects T cell development; however, little is known about the influence of Notch in B cell differentiation and function in transplantation.

**Methods:** We examined the role of Notch-2 therein using a novel, selective antibody against the Notch-2 receptor (aNotch-2; Wu *et al.* Nature 2010) in a full MHC-mismatch cardiac transplant model (Balb/c->B6). B6 recipients treated with aNotch-2 for 6 days had significant, albeit modest, prolongation of graft survival compared to IgG-treated controls (MST 10 *vs* 7 days; $p$=0.0023).

**Results:** Treatment with aNotch-2 effected a 10-fold reduction in marginal zone B cells ($p$<0.0001), while follicular (FO) B cells were increased (71.9±1.45%, $n$=31.29±0.8 x$10^6$ *vs* 50.5±5%, $n$=11.01±0.2 x$10^6$ in controls; $p$=0.046 & $p$=0.06, respectively). Interestingly, despite this, there was significantly less proliferation of FO B cells in mice treated with aNotch-2. The plasma cell subset (B220$^-$CD138$^+$) was significantly reduced in both the spleen and bone marrow of aNotch2-treated mice (0.46±0.03 & 0.2±0.004 *vs* 1.7±0.3 & 0.3±0.01% in controls; $p$=0.047 & $p$=0.01, respectively); furthermore, a greater proportion of splenic plasma cells isolated from aNotch-2-treated mice were immature (CD138$^+$IgM$^+$). Most remarkable, however, was the profound inhibition of alloantibody production following aNotch-2 treatment (Figure; IgG1 & IgG2a).

**Conclusions:** These data reveal a crucial role for Notch-2 in B cell development and function in transplantation, particularly in alloantibody production. Selective Notch-2 blockade may be a useful therapeutic strategy to prevent the antibody-mediated injury so resistant to current therapies.




## TH-OR100

### Erythropoietin Inhibits Human Alloreactive T Cell Response

Joaquin Manrique,[1] Paolo Cravedi,[1] Anita Mehrotra,[1] Jessica A. Reid-Adam,[1] Katherine E. Hanlon,[2] Peter S. Heeger.[1] *[1]Renal Div, Dept of Medicine, Recanati Miller Transplant Institute and Immunology Institute, Mount Sinai School of Medicine, New York, NY; [2]Hematology and Medical Oncology, Mount Sinai School of Medicine, New York, NY.*

**Background:** EPO therapy has been associated with improved transplant outcomes but the mechanisms are unknown. Published data in murine systems indicate EPO has immunomodulatory properties prompting us to test the effects of EPO on in vitro human alloresponses.

**Methods:** Naïve and memory CD4$^+$ and CD8$^+$ T cells, and CD14+ monocytes were isolated from buffy coats or PBLs using magnetic beads. Monocytes were cultured for 5 days with IL-4 and GMCSF to induce DCs and then matured with LPS. Immune cell phenotyping, T cell proliferation in response to allogeneic DCs (CFSE dilution), and intracellular signaling (phosphoflow) were quantified using flow cytometry.

**Results:** CD4$^+$, CD8$^+$, and DCs expressed both the classic EPO receptor (EPOR) and the alternative receptor CD131. Addition of EPO caused a dose dependent decrease in proliferation of naïve and memory CD4$^+$ and CD8+ T cells responding to allogeneic DCs (-61.3±20.2% to 65.2±31.9%, for naïve CD4 cells, p<0.001 vs control). EPO also inhibited anti-CD3/CD28 stimulated T cell proliferation (1000-2000U/mL:-36.5±20.9% to -90±4.37%; p<0.001), the latter indicating a direct effect of EPO on the T cells. Addition of EPO blocked allo-induced IFNg production (-43.3±22.4% p<0.05 vs control) without altering IL-4, IL-17 or Treg induction under appropriate cytokine-polarizing conditions. While EPO lowered DC expression of CD40 (-32.2±11.8 vs. control, p<0.05), it had no effect on CD80/86 expression and EPO-treated DCs induced T cell proliferation to the same degree as controls. EPO inhibited T cell receptor (TCR) induced AKT (CD4$^+$ MFI= -47.1±2.6, p<0.05 and CD8$^+$ MFI= -50.8±6.2, p<0.05) and ERK (CD4$^+$ pERK1/2 MFI= -59.7±14.6, p<0.05; CD8$^+$ pERK1/2 MFI= -35.5±2.7, p<0.05) phosphorylation, linking the above functional effects to known biochemical pathways downstream of the TCR.

**Conclusions:** EPO directly suppresses human alloreactive T cells providing one potential mechanism underlying the observed improved outcomes of EPO treated kidney transplant recipients.

## TH-OR101

**CTLA4 Is Required for Rapamycin-Induced Augmentation of the CD8+ T Cell Response to a Latent Viral Infection** David F. Pinelli, Mandy L. Ford. *Emory Univ.*

**Background:** Latent viral infections are a major concern among immunosuppressed transplant patients. Treatment with belatacept, a CTLA4-Ig fusion protein, was shown to reduce toxicity in kidney transplant recipients compared to CNI therapy, but is contraindicated in EBV- patients, due to an increased risk of PTLD associated with primary EBV infection. Therefore, strategies to improve patients' protective immunity without increasing alloreactivity would be beneficial in this setting. Recent reports have shown that treatment with rapamycin (rapa) paradoxically enhances CD8 T cell responses to certain pathogen infections, raising the enticing possibility that rapa could be used to enhance responses to EBV in belatacept-treated patients.

**Methods:** Mice were infected with gammaherpesvirus 68 (gHV), the murine homolog of EBV, treated with rapa +/- CTLA-4 Ig, and MHC tetramers were used to track virus-specific CD8 T cells. We also performed ex vivo restimulation with gHV peptide to assess CD8 T cell IFN-γ production.

**Results:** Rapa treatment alone enhanced the T cell response to gHV, with a significant increase in the number of gHV tetramer positive CD8 T cells per $10^6$ PBMCs at Day 20 compared to mice that received no treatment (4.5E4±9E3 vs. 2.4E4±9E3) and also led to an increase in the percentage of IFN-γ producing CD8 T cells. In contrast, CTLA4-Ig treatment prevented rapa from significantly enhancing CD8 T cell responses (CTLA4-Ig 2.0E4±8E3 vs. +Rapa 2.6E4±6E3). Interestingly, when an anti-CTLA4 blocking antibody was used, which leaves CD28 signals intact, rapamycin was also unable to augment the response to gHV (Anti-CTLA4 4.2E4±5E3 vs. +Rapa 4.6E4±5E3).

**Conclusions:** Taken together, these results suggest that the ability of rapamycin to enhance virus-specific memory T cell formation requires signals through the CTLA-4 coinhibitor. Further work is necessary to dissect the interactions of these two signaling pathways, and thus to determine the mechanism by which CTLA4 blockade diminishes the ability of mTOR inhibition to promote the generation of CD8 T cell memory.

*Funding:* Pharmaceutical Company Support - Pfizer

## TH-OR102

**Gadolinium Accelerates Cardiac Allograft Rejection through Macrophage Activation and Blockade of Phagocytosis** Martina M. McGrath, Melissa Y. Yeung, Nader Najafian. *Renal Div, Brigham and Women's Hospital, Boston.*

**Background:** Phagocytosis is a crucial mechanism for the maintenance of self-tolerance. Defects in phagocytic mechanisms predispose to autoimmunity. Gadolinium has been used as a tool to impair macrophage phagocytosis in experimental models. In this study, we sought to examine the effect of blockade of macrophage phagocytosis by gadolinium, on allograft outcomes.

**Methods:** B6 recipients of fully mismatched heart transplants were treated with CTLA4-Ig 500ug on D0, ± 300ug gadolinium ip on D-2, D5 and D12 post transplant and assessed for survival and mechanistic studies.

**Results:** Treatment with gadolinium led to accelerated allograft rejection (MST 10d vs 30d, p<0.001). Mechanistic studies revealed increased AnnV+ apoptotic cells in spleens of gadolinium treated recipients (p<0.05). Furthermore, gadolinium treated recipients showed increased production of pro-inflammatory IFNg, IL4, IL6, IL17; and reduced IL10 levels in draining lymph nodes. Similarly, CD4+ and CD8+ T effector memory/T reg ratios were increased (p <0.05). [We next sought to more fully assess how gadolinium precipitated allograft rejection. In vitro and in vivo phagocytosis assays confirmed an incremental impairment in macrophage phagocytosis after treatment with gadolinium. DC phagocytosis was unaffected in vivo. Incubation with gadolinium increased macrophage expression of MHC Class II molecules and CD86. Macrophage production of pro-inflammatory TNFa, IL12 and IL17 also increased significantly in response to gadolinium treatment in vitro.

**Conclusions:** These data indicate that gadolinium promotes an activated macrophage phenotype with impaired phagocytosis, increased costimulatory molecule expression and proinflammatory cytokine production. In vivo treatment of recipients of fully mismatched cardiac allografts precipitates acute rejection, characterised by markedly increased pro-inflammatory T cell infiltrate in graft, spleen and draining lymph nodes and splenic accumulation of apoptotic cells. Further studies are required to fully elucidate the mechanisms of rejection induction and evaluate the translational significance of these findings in humans exposed to gadolinium.

*Funding:* Private Foundation Support

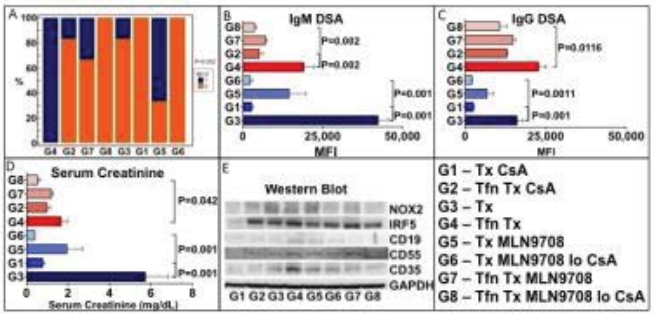
## TH-OR103

**Prevention of Acute Antibody Mediated Rejection by MLN9708, Alone and in Association with Low Dose Cyclosporine in Sensitized Rats** Shannon Reese,[1] Nancy A. Wilson Schlei,[1] Gengwen Huang,[1] Weixiong Zhong,[2] Arjang Djamali.[1] *[1]Medicine, Univ of Wisconsin; [2]Pathology, Univ of Wisconsin.*

**Background:** We designed a study to evaluate the activity of proteasome inhibitor Ixazomib Citrate (MLN9708) alone and in combination with low dose cyclosporine in the prevention of acute antibody mediated rejection (ABMR) in a rat kidney transplant model.

**Methods:** To develop a robust model of ABMR, full MHC mismatch (Brown Norway to Lewis) kidney transplants were performed in recipients that were sensitized to donors via blood transfusion 3 weeks prior to transplant (n=5-8 in all groups). Histopathological grading was performed in accordance with BANFF criteria using H&E, PAS and C4d staining. Flow-cytometry was used to evaluate the effect of treatment on circulating Donor Specific Antibody (DSA). The presence of graft infiltrating B cells, complement regulatory proteins (CD35 and CD55) and M1 macrophage transcription factor (IRF5) were assessed by western blot analysis. Serum creatinine was measured as an indicator of kidney function.

**Results:**




One week after transplant, MLN9708, alone or in combination with low dose CsA, was associated with a significant prevention of ABMR as evidenced by inhibition of microcirculation inflammation (MI score > 2), DSA (IgM and IgG) and improved kidney allograft function (Panels A,B,C & D). MLN9708 was also associated with reduced B-cell infiltration, lower levels of complement regulatory proteins, superoxide generating enzyme (Nox2) and IRF5 (Panel E).

**Conclusions:** Ixazomib citrate (MLN9708) + Low Dose CsA is effective in preventing acute ABMR in an experimental rat model. Further mechanistic studies are needed to understand the therapeutic synergy between proteasome inhibition and calcineurin inhibition in CNI minimization protocols.

*Funding:* Pharmaceutical Company Support - Takeda Millennium

## TH-OR104

**High Salt Intensifies Alloimmune Response** Kassem Safa, Tetsunosuke Shimizu, Ciara N. Magee, Anil K. Chandraker, Leonardo V. Riella. *Renal Div/ Transplantation Research Center, Brigham and Women's Hospital, Boston, MA.*

**Background:** Recent reports suggest that a high salt diet (HSD) exacerbates autoimmunity in mice (Nature, 2013). Whether HSD exerts a similarly deleterious effect in alloimmunity is unknown. Herein, we sought to examine the effect of NaCl on the alloimmune response both *in vitro* and *in vivo*, using mixed lymphocyte cultures (MLC) with allo-stimulation and a murine model of solid organ transplantation.

**Methods:** B6 mice were sensitized with fully MHC-mismatched BALB/c skin grafts; to perform MLCs, splenocytes were isolated from these mice and cultured with irradiated BALB/c splenocytes in media enriched with NaCl at gradual increments from 0 to 80 mM. To examine the effect of HSD *in vivo*, B6 mice were fed either HSD (3% Na chow, 0.9% saline) or normal salt diet (NSD; 0.3% Na chow, regular water) starting 3 days prior to receipt of a BALB/c cardiac graft; a single dose of CTLA-4-Ig was administered to control the initial alloimmune response.

**Results:** An increase in the *in vitro* [NaCl] from 5-10 mM effected a corresponding increase in IFNγ secretion, as measured by ELISPOT (311 vs 342 SFC; p=0.03); however, further increase in [NaCl] from 10-40 mM reduced IFNγ secretion (342 vs 275 SFC; p<0.001). Similarly, increased cellular proliferation, as measured by thymidine incorporation, was seen as [NaCl] increased from 0-20 mM (923 vs 1377 cpm; p=0.046), while further increase from 20-40 mM NaCl decreased proliferation (1377 vs 778 cpm; p<0.001).

*In vivo*, HSD-fed transplanted mice lost more weight than both NSD-fed transplanted mice and HSD-fed naïve controls (-7 vs +0.7 and -3.25g; p=0.002 and p=0.106, respectively). HSD precipitated earlier graft rejection compared to NSD (median survival 26 vs. 42 days; p=0.04). While the percentages of both CD4 and CD8 T cells increased in the HSD transplanted group (p<0.001), regulatory T cells (CD4+CD25+Foxp3+) were significantly reduced (5.9±0.4 vs 8.5±0.2% in NSD-fed recipients; p=0.006).

**Conclusions:** These results indicate that HSD may indeed influence the alloimmune response. Further mechanistic experiments will provide greater insight into this fascinating observation that has significant clinical implications in transplantation.

## TH-OR105

**Epigenetic Programing of Postnatal Renal Transcription Rates by Maternal Malnutrition** Oleg N. Denisenko,[1] Karol Bomsztyk,[1] Tom Patrick Fleming.[2] *[1]Medicine, Univ of Washington, Seattle, WA; [2]Univ of Southampton, Southampton, United Kingdom.*

**Background:** Adverse exposures during pregnancy result in intrauterine growth restriction, IUGR, and increased risk of chronic diseases later in life, a phenotype that can be transmitted through generations. A diversity of conditions that cause this phenomenon suggests that common mechanisms of programming may be operative. We recently reported that transcription rates were globally decreased in fetal kidneys in two animal models of IUGR, maternal protein restriction in pigs and placental insufficiency in sheep, together representing the two major causes of IUGR in humans. Here, we examined mechanisms of transcription changes in a mouse model of maternal malnutrition.

**Methods:** Pregnant animals were fed low protein (LPD, 9% protein) or normal protein (NPD, 18% protein) diets during the entire gestation period, after birth all offspring were fed normal diet. RNA and DNA purified from embryonic (17 dpc) and adult (6mo) kidneys were analyzed by RT qPCR and methylated DNA IP (MeDIP) respectively.

**Results:** We found that maternal LPD results in *increased* cellular RNA content in adult kidneys, in contrast to the *reduced* RNA levels in LPD fetal kidneys ($p<0.05$). Moreover, even brief exposure to LPD during the periconceptional period (0-3.5 dpc) was sufficient to consistently increase renal cellular RNA levels in kidneys after transition to normal diet. To explore epigenetic contribution, we examined levels of DNA methylation known to repress transcription. Ribosomal RNA transcribed from ribosomal DNA (rDNA) loci represents 80% of cellular RNA. Diet-induced changes in cellular RNA content inversely correlated with levels of rDNA methylation, and were matched by changes in expression levels of Rrn3, a key rDNA transcription factor.

**Conclusions:** These observations identify rDNA methylation and transcription factor Rrn3 as putative targets of dietary programming of rDNA transcription. Because ribosome production consumes up to 70% of cell resources, upregulation of rDNA transcription in LPD offspring may have adverse consequences for kidney function.

*Funding:* NIDDK Support, Private Foundation Support

## TH-OR106

**Effect of N-3 Fatty Acids on Kidney Function after Myocardial Infarction: The Alpha Omega Trial** Ellen K. Hoogeveen,[1] Johanna M. Geleijnse,[2] Daan Kromhout,[2] Theo Stijnen,[3] Eugenie Gemen,[1] Ron Kusters,[1] Erik Giltay.[3] *[1]Nephrology, Jeroen Bosch Hospital, Den Bosch, Netherlands; [2]Human Nutrition, Wageningen Univ, Wageningen, Netherlands; [3]Medical Statistics and Bioinformatics, Leiden Univ Medical Center, Leiden, Netherlands.*

**Background:** Kidney function gradually decreases with age, and a myocardial infarction accelerates this deterioration.

**Methods:** We examined in an ancillary study the effect of marine n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and plant-derived alpha-linolenic acid (ALA) on kidney function in the Alpha Omega Trial: a multicenter, double-blind, randomized, placebo-controlled trial with a 2x2 factorial design.

**Results:** We randomly assigned free-living Dutch patients with a history of myocardial infarction, aged 60-80 years (79% men), to margarines supplemented with targeted additional intake of 400mg/day EPA and DHA, 2g/day ALA, EPA-DHA plus ALA, or placebo for 40 months. In 2426 patients the treatment effect parameters cystatin C-, creatinine- and creatinine-cystatin C-based eGFR were assessed with CKD-EPI equations. Patients consumed 19.8g margarine/day, providing an additional amount of 238mg/day EPA with 158mg/day DHA, 1.98g/day ALA, or both, in active-treatment groups. In the placebo-group, cystatin C-based eGFR was 5.57 ml/min/1.73m$^2$ (SE=0.44) lower after 40 months. Compared to placebo the decline (95%-CI) in cystatin C-based eGFR was reduced by 1.50 (+0.26 to +2.74) for EPA-DHA, 1.03 (-0.21 to +2.28) for ALA, and 1.28 (+0.04 to +2.51) ml/min/1.73m$^2$ for EPA-DHA plus ALA. Creatinine- and creatinine-cystatin C-based eGFR in the placebo-group declined by 10.33 (SE=0.39) and 8.22 (SE=0.39) ml/min/1.73m$^2$, respectively. Decline of creatinine- and creatinine-cystatin C-based eGFR was not affected by EPA-DHA and/or ALA treatment.

**Conclusions:** Supplementation with modest amounts of EPA-DHA, with or without ALA, significantly attenuated the decline of cystatin C-based eGFR in post-myocardial infarction patients. However, a beneficial effect was not observed for creatinine-based and creatinine-cystatin C-based eGFR.

*Funding:* Other NIH Support - Dutch Kidney Foundation, Dutch Heart Foundation, US National Institutes of Health (NIH), Pharmaceutical Company Support - Unilever R&D

## TH-OR107

**Effects of a Low Protein Diet on Skeletal Muscle Protein Synthesis and Degradation in Patients with Chronic Kidney Disease** Giacomo Garibotto, Antonella Sofia, Valeria Cademartori, Emanuele L. Parodi, Daniela Verzola. *Div of Nephrology, Dyalisis and Transplantation, Genoa Univ and AOU San Martino-IST, Genoa, Italy.*

**Background:** Chronic kidney disease (CKD) is characterized by progressive loss of muscle mass, an effect which could be accelerated by a low protein diet (LPD). However, to what extent skeletal muscle protein metabolism adapts to a LPD in CKD patients is still unexplored.

**Methods:** To assess the effect of LPD on muscle protein metabolism in CKD patients, forearm [2H]phenylalanine kinetics and amino acid balance were evaluated in six CKD patients( 4M/2F, age 54±5 yrs, eGFR 18±2 ml/min) assigned to a usual-protein diet (1.2 g/kg/day, 32 kcal/Kg/day) (4 weeks), followed by a 4- week LPD (0.55 g/kg, 32 kcal/Kg/day) period. Studies were performed after an overnight fast (post-absorptive state) and results express basal rates of protein turnover.

**Results:** After LPD: (a) whole body protein turnover declined only slightly (from 0.65±2 to 0.57 ±2 μmol/min/kg, usual protein diet vs, LPD, $p<0.06$) ; (b) forearm protein net balance, i.e. the difference between protein synthesis and degradation, decreased markedly (-41%, from -21±2 to.-12 ±2 nmol/min.100 ml, $p<0.02$), (c) the efficiency by which amino acids are cycled back from protein degradation into protein synthesis increased by 30% ( $p<0.05$), d)muscle protein degradation underwent a marked decline (from 65±4 to 48 ±4 nmol/min.100 ml , $p<0.02$), c) Protein synthesis was unchanged (from 43±4 to 37±4 nmol/min.100 ml, p= NS).

**Conclusions:** Our data show that CKD patients achieve muscle protein metabolism adaptation to a LPD through a marked decrease in muscle protein degradation and enhanced recycling of amino acid derived from catabolism. Protein synthesis appears to be remarkably preserved, which suggests that a 0.55 g/kg LPD is nutritionally safe.

## TH-OR108

**Effect of Potassium Supplementation with and without Neutralization of Endogenous Acid Production on Markers of Insulin Secretion, Insulin Sensitivity and Blood Pressure in Human Subjects with Combined Glucose Intolerance ("Prediabetics")** Reto Krapf,[4] Katrin Conen,[1] Roberto Scanni,[2] Henry N. Hulter.[3] *[1]Medicine, Univ Hospital Basel, Basel, Switzerland; [2]Medicine, Univ of Basel, Bruderholz, Switzerland; [3]Medicine, Klinik St. Anna Hirslanden, Lucerne, Switzerland.*

**Background:** Potassium (K) depletion is known to inhibit insulin secretion, while metabolic acidosis is reported to decrease insulin sensitivity. Modern Western diets are characterized by comparatively low K and high acid loads. The effect of K supplements on beta cell function in the absence of overt K depletion and the effect of alkali on insulin metabolism without overt acidosis have not been reported.

**Methods:** Double-blind, randomized crossover design: 11 patients with CGI (fasting glucose > 5.7 to < 7.0 mmol/L and glucose levels >7.8 but < 11.1 mmol/L, 2h after 75 gr glucose) were supplemented with 90 meq K as KCl or Kcitrate or placebo daily for 14 days prior to measurements of insulin secretion (HOMA-Beta) and insulin sensitivity (HOMA-IR), oral glucose tolerance (oGTT) and 24 hour ambulatory blood pressure (ABPM) profiles. Washout periods of 2 weeks were interposed between the experimental periods.

**Results:** K supplementation significantly increased HOMA-Beta compared to placebo (from 74 + 7 % to 85 + 8% for KCl and to 84 + 7% for Kcitrate, p= 0.02 each). Only Kcitrate significantly decreased HOMA-IR from 2.76 + 0.23 to 2.41 + 0.19 (p = 0.03), while KCl induced a non-significant increase to 3.17 + 0.39. During Kcitrate, subjects significantly lost 1.5 kg + 0.4 kg (p = 0.18) bw (no change during KCl) and decreased their mean 24 hour BP by - 4.0 + 0.9 (syst) and – 2.7 + 0.4 (diast) mm Hg ($p < 0.02$ compared to KCl and placebo). Quantitative insulin sensitivity check index (Quicki) and measures of the insulin/glucose response after oGTTs confirmed the HOMA findings.

**Conclusions:** In prediabetic subjects, K supplementation improves beta-cell function even without overt K depletion. Only Kcitrate (alkali therapy) and not KCl improves insulin sensitivity and 24h ABPM. These novel results suggest for the first time, that K and/or alkali supplements may play a role in epidemic T2DM prevention or treatment.

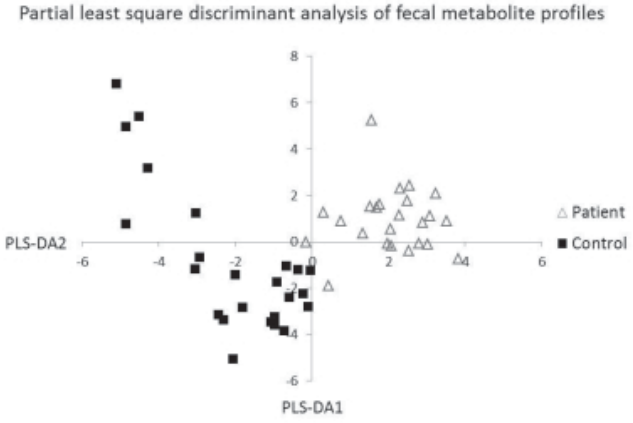
## TH-OR109

**Uremia Is Associated with a Distinct Gut Microbial Metabolism** Ruben Poesen,[1] Karen Windey,[2] Vicky De Preter,[2] Pieter Evenepoel,[1] Kristin Verbeke,[2] Bjorn Meijers.[1] *[1]Nephrology, Univ Hospitals Leuven, Belgium; [2]Translational Research in GastroIntestinal Disorders, Belgium.*

**Background:** The gut microbial metabolism contributes substantially to the human metabolome and is a well-known source of uremic retention solutes. Mounting evidence indicates that the gut microbial metabolism can be disease-specific. Whether uremia is associated with a different gut microbial metabolism has not been studied to date.

**Methods:** Fecal samples of patients on maintenance hemodialysis were collected (NCT01874210). A control group was composed of household contacts to minimize environmental differences. Subjects with gastro-intestinal disease were excluded. Untargeted fecal metabolic fingerprinting with characterization of individual volatile organic compounds was performed with a dedicated gas chromatography-mass spectrometry method. Differences in fecal metabolite profiles were examined with partial least square discriminant analysis.

**Results:** Fecal samples of 25 hemodialysis patients (median age 75y, IQR 64-81) and their household contacts (median age 70y, IQR 65-76) were included for analysis. There were no significant between-group differences in baseline demographics (age, gender, body mass index and presence of diabetes mellitus). A total of 228 different metabolites were identified. Partial least square discriminant analysis demonstrated a clear distinction between fecal metabolite profiles of hemodialysis patients and household contacts (see figure).




**Conclusions:** Uremia is associated with a distinct fecal metabolic fingerprint. Identification of discriminating metabolites is ongoing. Whether the different gut microbial metabolism is secondary to or also (partly) contributing to the syndrome of uremia needs further examination. The potential beneficial effect of therapeutics targeting the gut microbiota (e.g. prebiotics) in patients with renal disease has to be awaited.

*Funding:* Government Support - Non-U.S.

**TH-OR110**

**Effect of Restriction of Foods Containing Phosphorus Additives on the Phosphatemia of Patients with End-Stage Renal Disease**
Margareth Fornasari, Yvoty As Sens. *Santa Casa de São Paulo - School of Medical Sciences, Sao Paulo, SP, Brazil.*

**Background:** Hyperphosphatemia control in chronic renal disease involves restriction of foods which naturally contain phosphorus. However, this restriction must also be extended to processed foods with phosphorus additives, the percentage of which varies from country to country. The purpose of the study is to verify the effect of restricting foods containing phosphorus additives on the serum phosphorus in patients on hemodialysis.

**Methods:** This study was done on adults with end-stage renal disease on hemodialysis for ≥6 months at a single center in Sao Paulo, Brazil. A total of 248 patients with phosphorus > 5.5 mg/dL were evaluated, and 134 were selected according to the inclusion criteria and randomized into 2 groups: intervention (n=67) and control (n=67). Clinical-laboratorial, nutritional status, calories, protein, phosphorus intakes and nPNA evaluations were made at the beginning of the study and after 90 days. The intervention group received orientation to substitute processed foods with phosphorus additives for foods without these additives, but having similar nutritional value. The control group maintained the nutritional orientation given prior to the study. The prescription of phosphorus binders was not modified for either group.

**Results:** There was no initial difference between the groups for serum phosphorus, nutritional status, protein and calories intakes and nPNA. After 3 months, there was a decline in phosphorus levels in the intervention group (from 7.2±1.4 mg/dL to 5.0±1.3 mg/dL, $p<0.001$), while there was no significant difference in the control group (from 7.1±1.2 to 6.7 ±1.2 mg/dL, $p=0.650$). In the intervention group 65.7% of the patients reached the target of ≤5.5 mg/dL serum phosphorus versus 18.5% in the control group ($p<0.001$). There was no difference between groups for nutritional status, protein intake and nPNA.

**Conclusions:** The restriction of food with phosphorus additives reduced the serum phosphorus without interfering in the nutritional status of end-stage renal disease patients on hemodialysis.

**TH-OR111**

**Safety and Efficacy of Bariatric Surgery in Obese Patients with CKD: The London Renal Obesity Network (LonRON) Experience**
Helen L. MacLaughlin,[1] Iain C. Macdougall,[1] Ahmed R. Ahmed,[2] Ameet G. Patel,[1] Avril Chang,[1] Aine Burns,[3] Nick Finer,[4] Harvinder Chahal,[2] George Tharakan,[2] Andrea Pucci,[4] Frederick W.K. Tam,[2] Lina Johansson,[2] Jan Flint,[3] Andrew H. Frankel.[2] *[1]King's College Hospital, United Kingdom; [2]Imperial College Healthcare, United Kingdom; [3]Royal Free London, United Kingdom; [4]Univ College London Hospitals, United Kingdom.*

**Background:** Bariatric surgery is currently the most effective treatment for obesity. Recent evidence suggests the complication rate may be higher in those with CKD, than without.

**Methods:** We report a retrospective study of all obese patients with CKD (eGFR <60ml/min, or eGFR >60ml/min with evidence of kidney damage & under the care of a nephrologist) undergoing laparoscopic bariatric surgery in 3 major London teaching hospitals from 2007-2012. Patient demographics, surgery type, length of stay (LOS), weight loss, adverse events & mortality, were extracted from medical records during Oct 2012-Mar 2013.

**Results:** Of 74 patients (33M;41F), age (mean±SD) 52(±10)years, eGFR 48(±19)ml/min, pre-operative BMI 44.5(±5.7)kg/m$^2$, 38% underwent Roux-en-Y bypass (RYGB), 57% sleeve gastrectomy (SG), & 5% adjustable gastric banding (AGB). 11% were classified as CKD stages 1-2, 59% CKD stage 3, 12% CKD stage 4/stage 5 non-dialysis, & 18% were on haemodialysis at the time of surgery. Mean (95% CI) LOS for SG was 6 days (5, 7), 4 days (3, 5) for RYGB, & 3 days (0, 6) for AGB. Across all forms of surgery, excess BMI loss (>25kg/m$^2$) was 61% (55, 68), & BMI was 33.2 kg/m$^2$ (31.5, 34.8) 12 months post-surgery. There were 16 adverse events (16/74, 22%), including 2 deaths (3%) related to surgical complications. Acute kidney injury was most frequent (4%), followed by leak (3%), acidosis & hyperkalaemia (3%), post-operative chest infection (3%), B12/Fe deficiency (3%), fistula/graft failure (3%), & myocardial infarction (1%). A further 4 deaths occurred during the study period, including 2 related to cancer.

**Conclusions:** While bariatric surgery is effective for weight loss in obese patients with CKD, the adverse event and mortality rates are high. Identification of risk factors for adverse events & investigation of non-surgical alternatives remain priorities.

*Funding:* Government Support - Non-U.S.

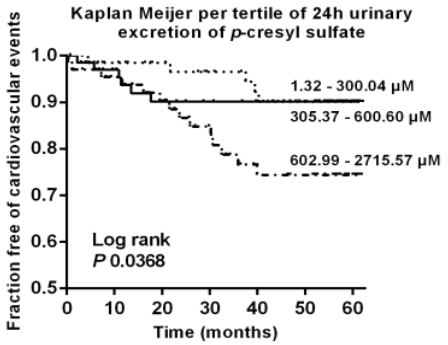
**TH-OR112**

**Cardiovascular Disease Relates to Intestinal Uptake of p-Cresol in Patients with Chronic Kidney Disease**
Ruben Poesen, Liesbeth Viaene, Bert Bammens, Kathleen Claes, Pieter Evenepoel, Bjorn Meijers. *Nephrology, Univ Hospitals Leuven, Leuven, Belgium.*

**Background:** Serum p-cresyl sulfate (PCS) associates with cardiovascular (CV) disease in patients at different stages of chronic kidney disease (CKD). PCS concentrations are determined by intestinal uptake of p-cresol (PC), human metabolism to PCS and renal clearance. Whether intestinal uptake itself is associated with CV disease in patients with CKD has not been studied to date.

**Methods:** We performed a prospective study in patients with CKD stage 1-5 (NCT00441623). Intestinal PC uptake, under steady state conditions, was estimated from 24h urinary excretion of PCS. Primary endpoint was time to first CV event, i.e. cardiac death, myocardial infarction/ischemia, ventricular arythmia, CV surgery, cerebrovascular accident or symptomatic peripheral arterial disease. Statistical analysis was done using Kaplan Meier estimates and Cox proportional hazard analyses.

**Results:** In a cohort of 200 patients, median 24h urinary excretion of PCS was 457.47 µM (IQR 252.68-697.17). After a median follow-up of 52 months, 25 patients reached the primary endpoint (tertile 1/2/3: 5/6/14 events, see figure). Higher urinary excretion of PCS was related with CV events (univariate HR per 100 µM increase: 1.112, P 0.0015). In multivariate analysis, urinary excretion of PCS remained a predictor of CV events, independent of markers of renal function (HR 1.120, P 0.0022) and in different models with other CV risk factors (Framingham, CV history, diabetes mellitus and biochemical parameters). The independent association between urinary excretion of PCS and outcome persisted after correction for serum PCS.




**Conclusions:** Intestinal uptake of PC associates with CV disease. Insights into mechanisms governing intestinal generation and absorption of PC may lead to identification of novel therapeutic targets to reduce CV disease risk in patients with CKD.

*Funding:* Government Support - Non-U.S.

**TH-OR113**

**Sagittal Abdominal Diameter Is an Independent Predictor of Mortality in Incident Peritoneal Dialysis Patients**
Mi Jung Lee,[1] Shin-Wook Kang,[1] Hyung Jung Oh,[1] Sung Jin Moon,[2] Tae Ik Chang,[3] Dae-Suk Han,[1] Tae-Hyun Yoo.[1] *[1]Brain Korea 21; Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea; [2]Internal Medicine, Kwandong Univ, Gyeonggi-do, Korea; [3]Internal Medicine, NHIC Ilsan Hospital, Gyeonggi-do, Korea.*

**Background:** Visceral fat plays a crucial role in the development and progression of cardiovascular (CV) disease. However, the impact of sagittal abdominal diameter (SAD), as an index of visceral fat, on clinical outcomes has never been explored in dialysis patients. In this prospective observational study, therefore, we elucidated the prognostic value of SAD in incident peritoneal dialysis (PD) patients.

**Methods:** SAD was determined by lateral abdominal X-ray at PD initiation in 418 incident PD patients. SAD was defined as the anterior-posterior distance, from skin to skin, at the L4-L5 intervertebral disc level. Cox proportional hazard analysis was performed to ascertain the independent prognostic values of SAD for all-cause and CV mortality.

**Results:** The mean SAD was 24.5±4.3 cm. During a mean follow-up duration of 39.4±21.3 months, 97 patients (23.2%) died. SAD was a significant independent predictor of all-cause [HR (hazard ratio) = 1.081, 95% CI (confidence interval) = 1.015-1.151, P=0.015] and CV mortality (HR = 1.119, 95% CI = 1.022-1.225, P=0.015). In addition, SAD provided significantly higher predictive values for all-cause (AUC: 0.691 vs. 0.547, P<0.001) and CV mortality (AUC: 0.644 vs. 0.483, P<0.001) than body mass index (BMI). Subgroup analysis revealed that higher SAD (≥24.2 cm) was significantly associated with all-cause mortality in men, women, younger patients (<65 years), and patients with lower BMI (<22.3 kg/m$^2$).

**Conclusions:** SAD on lateral abdominal X-ray at the time of PD start was a significant independent predictor of all-cause and CV mortality in incident PD patients, suggesting that estimating visceral fat by SAD could be useful to stratify mortality risk in these patients.

*Funding:* Government Support - Non-U.S.

**TH-OR114**

**Relationship between Body Composition Evaluated by Whole Body Bioimpedance and Survival in Hemodialysis Patients**
Daniele Marcelli,[1] Len A. Usvyat,[2,3] Cristina Marelli,[4] Michael Etter,[5] Jeroen Kooman,[6] Aileen Grassmann,[1] Laura Scatizzi,[1] Inga Bayh,[1] Peter Kotanko,[3] Bernard Canaud.[1] *[1]Fresenius Medical Care, Germany; [2]Fresenius Medical Care North America; [3]Renal Research Institute, N.Y.; [4]Fresenius Medical Care, Argentina; [5]Fresenius Medical Care Asia Pacific; [6]Maastricht Univ Medical Centre, Netherlands.*

**Background:** It is unknown which components of body composition contribute to the reported protective effect of high body mass index (BMI) in chronic HD patients. This international observational study explores the independent and joint associations of fat and lean tissue mass (FTM, LTM) with mortality.

**Methods:** The MONDO consortium comprises HD databases from 26 countries (Usvyat, Blood Purif 2013). Use of the Body Composition Monitor (BCM) (Fresenius Medical Care, Germany) in EMEA NephroCare clinics allowed analysis of data from 17 countries. Lean and Fat Tissue Index (LTI, FTI) were computed as the respective tissue masses normalized to height in $m^2$. The relationship between LTI, FTI and all-cause mortality was assessed by Cox regression adjusted for age, gender, and BMI.

**Results:** For the 10,331 HD patients (age 62.6±14.7; 57% male), baseline median (IQR) LTI and FTI were 12.3 (10.6-14.4) and 9.4 (6.8-12.2) $kg/m^2$, respectively. Analysed jointly, a 1 $kg/m^2$ increment of LTI or FTI was associated with a mortality reduction of 31% or 26%, respectively. Analysed separately, a 1 $kg/m^2$ increment of LTI and FTI was associated with a 13% decrease and a 9% increase in mortality, respectively. A 1-unit higher FTI/LTI ratio yielded a 70% increase in mortality (Table).

| | Hazard Ratio | 95% CI | | p-value |
|---|---|---|---|---|
| LTI independent of FTI (per 1 $kg/m^2$) | 0.87 | 0.84 | 0.90 | <0.001 |
| LTI jointly with FTI (per 1 $kg/m^2$) | 0.69 | 0.65 | 0.74 | <0.001 |
| FTI independent of LTI (per 1 $kg/m^2$) | 1.09 | 1.04 | 1.14 | <0.001 |
| FTI jointly with LTI (per 1 $kg/m^2$) | 0.74 | 0.68 | 0.80 | <0.001 |
| FTI / LTI ratio (per 1 unit) | 1.70 | 1.44 | 2.00 | <0.001 |

**Conclusions:** LTM, not FTM, seems to be the main determinant of the inverse relationship between BMI and mortality. FTM seems to be only protective if accompanied by an appropriately higher LTM. Patients with low LTM relative to FTM appear to be at highest risk.

## TH-OR115

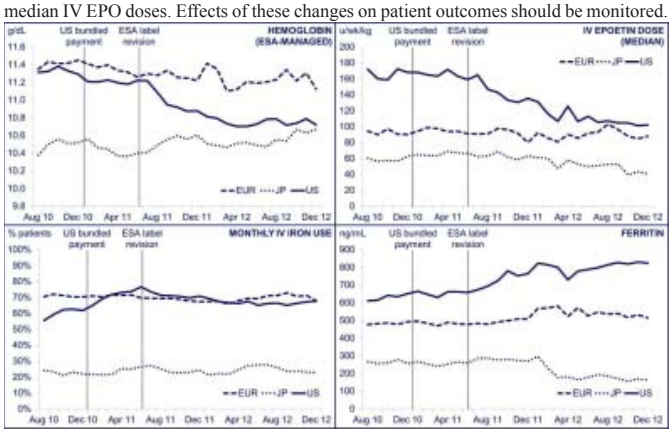
**International Comparisons Illustrate Effect of Payment and Regulatory Changes on Anemia Practice in U.S. Hemodialysis Patients: The Dialysis Outcomes and Practice Patterns Study** Douglas S. Fuller,[1] Bruce M. Robinson,[1] Brian Bieber,[1] Hal Morgenstern,[2] Tadao Akizawa,[3] Stefan H. Jacobson,[4] Francesco Locatelli,[5] Ronald L. Pisoni.[1] *[1]Arb Res Collab Hlth, Ann Arbor; [2]Univ of MI, Ann Arbor; [3]Showa Univ, Tokyo; [4]Danderyd Hosp, Stockholm; [5]Hosp A Manzoni, Lecco.*

**Background:** In the US in 2011, an erythropoiesis-stimulating agent (ESA) label change and Quality Incentive Program (QIP) update removed the 10 g/dL lower hemoglobin (Hb) target, and a new dialysis payment system began. To assess the impact, we compared recent anemia management practices in the US to Europe (EUR) and Japan (JP).

**Methods:** Linear spline regression adjusted for age, black race, sex, vintage, and catheter use was used to assess trends from Aug 2010 to Dec 2012 for ESA-managed Hb ($Hb^{ESA}$), serum ferritin, TSAT, and ESA and IV iron use in US, EUR, and JP.

**Results:** In Aug '10, mean $Hb^{ESA}$ in US (11.3 g/dL) was similar to that in EUR but then diverged after Jun '11 US ESA label change. By Dec '12, mean $Hb^{ESA}$ was 10.7, 10.7, and 11.1 g/dL in US, JP, and EUR. ESA use (any time over 3 months) changed from 95% to 89% in US, 91% to 89% in EUR, and 87% to 91% in JP. Median prescribed IV epoetin (EPO) dose fell 41% in US to 102 u/wk/kg (Dec '12) vs little change in EUR and JP (92 and 63 u/wk/kg, Dec '12). US monthly IV iron use over the study rose from 55 to 68% while stable in EUR and JP. US mean ferritin rose 35% to 825 ng/mL (Dec '12; median: 794), with an 8% rise in EUR to 514 ng/mL and no rise in JP. Higher ferritin in US may be due to higher mean IV iron doses used in US vs most other countries.

**Conclusions:** Recent US trends of lower ESA doses and Hb and higher ferritin levels have not been seen in EUR or JP, suggesting a large impact of payment and/or regulatory changes. In the US, mean Hb is lower than in EUR for first time in >10 yrs with similar median IV EPO doses. Effects of these changes on patient outcomes should be monitored.



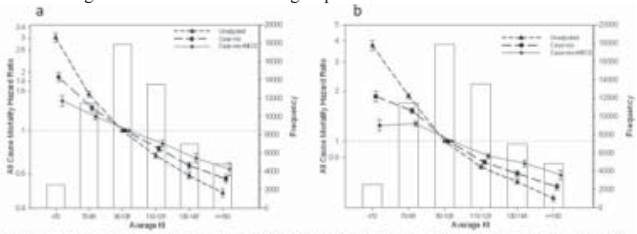

## TH-OR116

**Linear Relationships between Non-Volume-Scaled Dialysis Dose and Mortality Risk among Male and Female Hemodialysis Patients** Connie Rhee,[1] Vanessa A. Ravel,[1] Jongha Park,[4] Elani Streja,[1] Allen R. Nissenson,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Orange, CA; [2]DaVita Inc, El Segundo, CA; [3]Memphis VA Medical Center, Memphis, TN; [4]Univ of Ulsan College of Medicine.*

**Background:** Dialysis dose has typically been defined as the dialyzer clearance of urea multipled by dialysis duration (**Kt**) scaled to the volume of distribution of urea in the body (**V**). Using this classic metric (**Kt/V**), prior observational studies have shown that women receiving lower dialysis doses have higher mortality than women receiving higher doses, whereas men receiving higher doses have slightly increased mortality. However, **V** may have been a proxy for body size, which is associated with mortality independent of urea clearance. **KT** (dialysis dose non-scaled to **V**), may be a more optimal approach to examining the association between dialysis dose and hemodialysis (HD) patient mortality.

**Methods:** Using DaVita clinical data, we identified 104,219 maintenance HD patients who underwent treatment from 2001-2009. Associations between time-averaged **KT** and all-cause mortality were examined in the overall cohort and within subgroups of males (n= 57,156) and females (n=47,063) using Cox regression models.

**Results:** In the overall cohort, there was a graded inverse association between higher time-averaged **KT** levels and mortality risk in unadjusted, case-mix, and case-mix and malnutrition-inflammation-complex syndrome adjusted analyses (reference **KT**=90-109). A similar pattern of association between increased **KT** level and decreased mortality was observed among both male and female subgroups.




Death hazard ratios (and 95% CI) of 8-year average Kt levels for (a) 47,063 female and (b) 57,156 male HD patients (2001-2009)

**Conclusions:** Higher non-volume scaled dialysis dose defined by **KT** is associated with decreased mortality in HD patients, and contrary to findings in prospective randomized controlled trials, our results found no effect differences ascribable to gender.

*Funding:* NIDDK Support

## TH-OR117

**Effect of Medicare Part D Plan on Adoption on Renal Medications by Dialysis Patients on Renal Transplant Wait List** Marie D. Philipneri, Krista L. Lentine, Mark Schnitzler. *Saint Louis Univ, Saint Louis, MO.*

**Background:** Medicare Part D Prescription Drug Benefit Program came into effect on January 1, 2006. There is **l**imited data on the adoption of renal medications post Medicare Part D implementation among dialysis patients.

**Methods:** A novel database wherein OPTN identifiers for kidney transplant (KT) wait list patients were linked to pharmacy fill records from a large U.S. pharmaceutical claims clearinghouse (2005 to 2010) was evaluated. For this study, we selected dialysis patients with at least one Medicare pharmaceutical fill per quarter in 2006 and at least one pharmaceutical fill per quarter in 2005 (N=1,648).

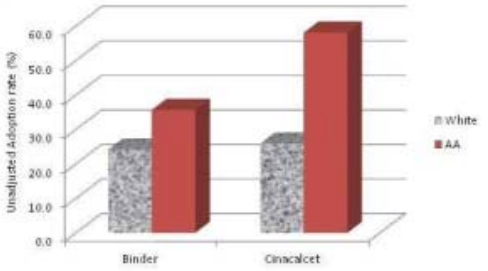
**Results:** 34.0% of the sample did not have pharmacy fills for any binder in 2005 and of these 27.8% filled at least one binder in 2006. African American patients were 1.65 times more likely to adopt binders after the start of Medicare Part D compared to Caucasians after adjusting for gender, education level, and employment status ($p<0.05$). 82.2% of the sample did not have pharmacy fills for cinacalcet in 2005 and of these 15.7% filled cinacalcet at least once in 2006. African American and middle-aged (31-65 year old) patients were more likely to adopt cinacalcet use compared to Caucasian and older (over 65 years) patients after adjusting for gender, level of education, and employment status. The patients who attained college education were 40% less likely to adopt use of cinacalcet compared to those who did not receive college education ($p< 0.05$).

**Association of Demographic Factors with Adoption of Phosphate Binders and Cinacalcet among Dialysis Patients after Medicare Part D Prescription Plan in 2006**

| Variable | Any Binder* [aOR (95% CI)] | Cinacalcet [aOR (95% CI)] |
|---|---|---|
| Age (years): >65 | Reference | Reference |
| 46-65 | 1.22 (0.67-2.23) | 2.10 (1.18-3.73)* |
| 31-45 | 1.36 (0.71-2.61) | 1.98 (1.09-3.63)* |
| 19-30 | 0.99 (0.43-2.28) | 1.01 (0.39-3.69) |
| 0-18 | 0.30 (0.04-2.53) | 0.45 (0.06-3.59) |
| Race: White | Reference | Reference |
| African Americans | 1.65 (1.06-2.58)* | 1.54 (1.09-2.16)* |
| Hispanics | 1.27 (0.65-2.49) | 1.11 (0.70-1.75) |
| Other | 1.46 (0.60-3.52) | 1.33 (0.72-2.43) |
| Female | 1.03 (0.70-1.51) | 1.30 (0.96-1.75) |
| Attained college education | 0.69 (0.46-1.03) | 0.60 (0.42-0.85)* |
| Employed | 0.82 (0.43-1.55) | 1.12 (0.69-1.84) |

*p<0.05

**Unadjusted Adoption Rates for Phosphate Binders and Cinacalcet by African Americans compared to Caucasians after Medicare Part D Prescription Plan in 2006**




**Conclusions:** The results of this study suggest that Medicare Part D Plan was particularly helpful for the African American, middle-aged, and those without college education among dialysis patients on renal transplant wait list. Future studies are needed to see if this trend is sustained and the findings could help policy makers focus their efforts on special populations in the implementation of new health care reforms.

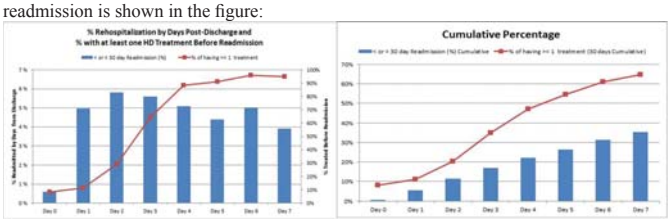
## TH-OR118

**Outpatient Dialysis Treatments in the Week Post-Discharge and Readmissions** Eduardo K. Lacson, Weiling Wang, Franklin W. Maddux. *Fresenius Medical Care, North America, Waltham, MA.*

**Background:** 30-day hospital readmissions in dialysis patients are thought to be influenced by outpatient dialysis facility care. Contact with facility staff to draw labs and implement interventions are necessary to influence outcome. Laboratory results are often received by the second (or third) treatment and could be acted upon. We evaluated the frequency of hemodialysis (HD) treatments within the 1st week post-discharge, before readmission events.

**Methods:** All adult in-center HD patients treated 3x/ week as of 1/1/2011 in Fresenius Medical Care, North America facilities with at least one hospital discharge in 2011 were included. Days to readmission and the number of HD treatments before readmission were recorded until 12/31/2011.

**Results:** There were 164,258 hospital events in 64,128 patients in 2011. 50,380 (30.7%) 30-day readmissions occurred in 23,575 patients. 17,797 (35.3%) out of 50,380 readmissions were within 7 days post-discharge, with only 5,810 (32.6%) of them having 1 treatment prior to readmission and 5,723 (32.2%) having at least 2 treatments. Within the first 3 days, only 34.8% (2975 of 8541 readmissions) received a treatment in the facility. The day-to-day (left) and cumulative (right) rate of treatments provided prior to readmission is shown in the figure:




**Conclusions:** The first week post-discharge from the hospital represents a critical actionable period for outpatient HD facilities to potentially impact 30-day readmission rates. However, over 35% of 30-day readmissions occur within the first week and may not be actionable, particularly since more than two-thirds of these readmissions did not have sufficient contact time to draw and result lab tests as well as respond with clinical intervention(s). Unlike hospitals, outpatient dialysis facilities should not be held accountable for hospital readmissions that occur in the first week post-discharge.

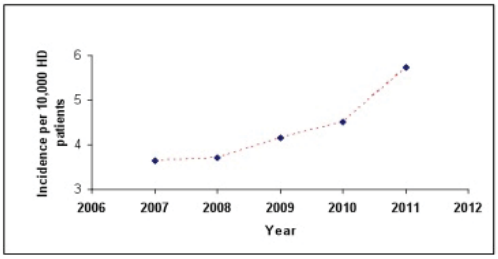
## TH-OR119

**Evaluation of Calciphylaxis Incidence in the United States Renal Data System** Sagar U. Nigwekar,[1] Craig Solid,[2] Elizabeth D. Ankers,[1] Ravi I. Thadhani,[1] Charles A. Herzog.[2] *[1]Massachusetts General Hospital; [2]CVSSC, US Renal Data System, Minneapolis, MN.*

**Background:** Exact incidence of calciphylaxis, a highly fatal condition seen in maintenance hemodialysis (MHD) patients, is unknown. This study is designed to develop a strategy to accurately identify calciphylaxis cases in the United States Renal Data System (USRDS) and to examine calciphylaxis incidence.

**Methods:** Using the USRDS database, we identified those receiving MHD during 2007-2011 (n=1,203,240). ICD-9 code 275.49 refers to *Other Disorders of Calcium Metabolism* with calciphylaxis included along with other diagnoses such as nephrocalcinosis, chondrocalcinosis, etc. Since accurate diagnosis of calciphylaxis requires a skin biopsy and other disorders covered under 275.49 do not require a skin biopsy for diagnosis, we theorized that applying ICD-9 code 275.49 plus skin biopsy procedure codes simultaneously to claims in the USRDS will accurately identify calciphylaxis cases. We planned a systematic validation of this strategy in Partners Healthcare System's Research Patient Data Registry (RPDR) that contains 11,451 MHD patients using natural language processing and by an independent query of pathology department records.

**Results:** Application of surrogate strategy to RPDR identified 69 calciphylaxis cases. Chart review confirmed 66 cases to be true positives. An independent query of pathology records identified 8 calciphylaxis cases missed by the surrogate strategy. This yielded sensitivity 87%, specificity 99%, and C statistic 0.96 for the surrogate strategy. Application of this validated strategy to the USRDS database identified 527 calciphylaxis cases. Annual incidence of calciphylaxis increased over the study period from 3.7 per 10,000 HD patients to 5.7 per 10,000 HD patients.




**Conclusions:** Calciphylaxis is rare but its incidence is rising. Further research is needed to investigate reasons for this rise.

*Funding:* Private Foundation Support

## TH-OR120

**Provider Characteristics and Hemodialysis Patient Outcomes** Yelena Slinin,[1] Haifeng Guo,[2] Suying Li,[2] Jiannong Liu,[2] Areef Ishani.[1,2] *[1]Veterans Administration Health Care System, Minneapolis, MN; [2]Chronic Disease Research Group, Minneapolis, MN.*

**Background:** Physician characteristics are associated with differential performance on quality measures and patient outcomes in several medical fields. We aimed to determine whether hemodialysis provider characteristics were associated with patient morbidity, mortality, and quality of care (defined by influenza vaccination and wait-listing for kidney transplant).

**Methods:** Using USRDS data for patients who initiated in-center hemodialysis 10/01/2003-9/30/2006 (n = 91,276), we defined patient characteristics and identified physicians from Part B Medicare claims for outpatient dialysis submitted during months 4-6 of hemodialysis. We obtained physician characteristics from the AMA Physician Master File. We determined associations of physician characteristics with 1-year patient mortality and first hospitalization using Cox proportional hazards analysis, and with vaccination and wait-listing using logistic regression.

**Results:** Physician characteristics were not associated with patient mortality. After adjustment for patient and other provider characteristics, longer practice duration (AHR 1.03(1.01-1.05) for 9-21 years in practice compared with 0-8), and administrative, research, or teaching practice (1.08(1.00-1.16) compared with office-based practice) were associated with greater risk of patient hospitalization. Practice in smaller metropolitan service areas (MSAs) was associated with lower risk of hospitalization (0.95(0.92-0.97)). Longer practice duration was associated with higher chance of wait-listing for kidney transplant (AOR(1.15(1.05-1.25) for ≥ 22 years in practice compared with 0-8), and practice in smaller MSAs was associated with lower chance (0.89(0.79-0.99) for population 100,000-249,999 and 0.84(0.74-0.95) for < 100,000, compared with ≥ 1,000,000). Graduation from a foreign medical school was associated with higher odds of influenza immunization (1.07(1.00-1.14)), as was practice in smaller MSAs (AOR 1.29(1.20-1.38) for population 100,000-249,999).

**Conclusions:** Several characteristics of physicians following patients for outpatient hemodialysis were associated with patient hospitalizations and quality of care.

*Funding:* NIDDK Support

## TH-OR121

**Comparison of Hospitalization between For-Profit and Nonprofit Dialysis Facilities** Lorien S. Dalrymple,[1] Kirsten L. Johansen,[2,3] Patrick S. Romano,[1] Glenn M. Chertow,[4] Yi Mu,[1] Julie H. Ishida,[3] Barbara A. Grimes,[3] George A. Kaysen,[1] Danh V. Nguyen.[5] *[1]UC Davis; [2]San Francisco VAMC; [3]UCSF; [4]Stanford; [5]UC Irvine.*

**Background:** The vast majority of US dialysis facilities are for-profit and profit status has been associated with processes of care and outcomes in patients on dialysis. Our study examined whether dialysis facility profit status was associated with the rate of hospitalization in patients starting dialysis.

**Methods:** We conducted a retrospective cohort study of Medicare beneficiaries starting dialysis between 2005 and 2008 using data from the United States Renal Data System. We examined and compared all-cause hospitalization between for-profit and nonprofit dialysis facilities through 2009 using Poisson regression. We conducted companion analyses of cause-specific hospitalization that are likely to be influenced by dialysis facility practices including hospitalizations for heart failure and volume overload, access complications, or hyperkalemia.

**Results:** The cohort included 150, 642 patients. Of these, 12,985 (9%) were receiving care in nonprofit dialysis facilities. In adjusted models, patients receiving hemodialysis in for-profit facilities had a 15% (95% CI 13 - 18%) higher relative rate of hospitalization compared to those in nonprofit facilities. Among patients receiving peritoneal dialysis, adjusted analyses showed a non-significant trend toward higher rate of hospitalization in for-profit facilities (RR 1.07, 95% CI 0.97 - 1.17). Patients on hemodialysis receiving care in for-profit dialysis facilities had 37% (95% CI 31 - 44%) higher rates of hospitalization for heart failure or volume overload and 15% (95% CI 11 - 20%) higher rates of hospitalization for vascular access complications.

**Conclusions:** Hospitalization rates were significantly higher for patients receiving hemodialysis in for-profit dialysis facilities.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Dialysis Clinic, Inc.

## TH-OR122

**Primary Care Nephrology versus Subspecialist Care for ESRD Patients: The Southern California Kaiser Permanente Experience** Dean A. Kujubu,[1] Miwa Takayanagi,[2] Jean Q. Wang,[2] Antoine C. Abcar,[1] Scott A. Rasgon.[1] *[1]Nephrology, Kaiser Permanente, Los Angeles, CA; [2]Research and Evaluation, Kaiser Permanente, Pasadena, CA.*

**Background:** Since most dialysis patients see their nephrologists more often than their generalists, many nephrologists serve as *de facto* primary care physicians. Alternatively, other nephrologists prefer to manage ESRD issues only and defer non-dialysis issues to the patients' primary care physicians. Which practice pattern results in better clinical outcomes, more efficient use of medical resources, or higher patient satisfaction is unclear.

**Methods:** We reviewed the number of emergency room (ER) visits, hospitalizations, hospital days, ambulatory visits, and mortality of ESRD patients in the Southern California Kaiser Permamente (SCKP) Health System from 1999-2009. In 2 of the 13 medical centers, nephrologists serve as primary care physicians (PC) for ESRD patients; in the other 11 medical centers, ESRD patients see nephrologists who do not provide primary care (NPC). Demographic data and the presence of co-morbidities (CAD, CHF, HTN, DM, cirrhosis) were collected.

**Results:** From 1999-2009, there were 1932 ESRD patients in the PC group and 9192 patients in the NPC group. The PC group had more PD patients (10.9% vs 9.1%), more men (60.2% vs 57.1%), fewer Blacks (14.5% vs 23.7%), more Asians (15% vs. 8.7%), and more cirrhotics (5.8% vs 3.7%) compared to NPC group. Age and the presence of other co-morbidities were not significantly different. Compared with NPC patients, PC patients had fewer ER visits/yr (4.0 v 4.7, p=.001), fewer hospitalizations (2.8 v. 3.1 p=.03), and fewer clinic visits (10.7 v 11.4, p<.0001). Hospitalization days, death rate, and time to death were not significantly different. Nephrologists who provided PC received higher patient satisfaction scores (9.55 v 9.46, p=.01).

**Conclusions:** Within SCKP, patients of PC nephrologists had significantly fewer ER and clinic visits, fewer hospitalizations, and better satisfaction with their physicians compared with those ESRD patients whose nephrologists who did not provide PC. An ESRD patient-centered medical home with nephrologists serving as primary care physicians may improve both patient care and patient satisfaction.

*Funding:* Clinical Revenue Support

## TH-OR123

**Cost Analysis of Provider-Patient Visit Frequency during In-Center Hemodialysis** Benjamin R. Morgan,[1] Suying Li,[3] Jiannong Liu,[3] Areef Ishani,[1,2,3] Yelena Slinin,[1,2] Haifeng Guo.[3] *[1]Univ of Minnesota, Minneapolis, MN; [2]Veterans Administration Health Care System, Minneapolis, MN; [3]Chronic Disease Research Group, Minneapolis, MN.*

**Background:** Beginning in 2004, CMS linked physician payment for HD services to the number of times/month providers see their patients. It is unknown whether increased HD provider visit frequency is associated with Medicare cost savings.

**Methods:** Using USRDS data, a retrospective cohort of 130,892 in-center HD patients was created, including patients starting HD between October 31st, 2003 & September 30th, 2006. During a 3 month run-in phase, provider visit frequency was defined as the average number of provider visits/month, and expressed as ≥4 or <4 provider HD visits/month. Total individual Medicare costs, inpatient Medicare costs, and hospital length of stay (LOS) were summed over the course of a 12 month observational phase. A multi-linear regression model was constructed to estimate the association of provider visit frequency on total Medicare monthly costs accounting for other patient demographics and comorbidities. For inpatient Medicare costs and LOS, a two step statistical model was generated to account for both the probability of hospitalization and the incurred inpatient costs and LOS.

**Results:** Using a multi-linear regression model, provider visit frequency corresponded to a non-significant 0.01% reduction in total Medicare monthly expenditures for patients with ≥4 provider visits a month. Logistical regression models showed a 4% reduction (C.I. 0.93-0.98) in the odds of incurring any inpatient costs or LOS among the ≥4 provider visits/month group as compared to <4 visits/month. On average, patients with <4 visits/month, incurred $232/month higher expenses and stayed in the hospital 0.21 days/month longer than patients with ≥4 provider visits/month.

**Conclusions:** A non-significant difference in total expenditures along with the observed reduction in hospitalization rates among the higher provider frequency group support the current CMS dialysis provider reimbursement policy strategy.

*Funding:* NIDDK Support

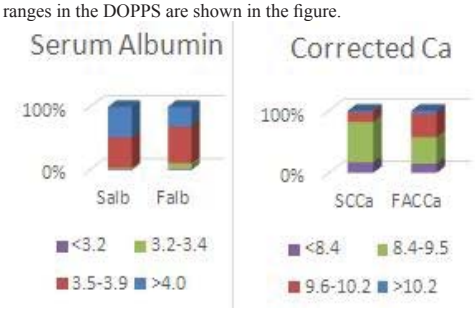
## TH-OR124

**Can We Compare Laboratory Results from Different Dialysis Units?** Panupong Lisawat,[1] Jeffrey M. Rimmer.[1] *[1]FAHC/UVM.*

**Background:** Calcium and albumin concentrations are important in management of hemodialysis patients and may be used as quality indicators. Difficulty achieving targets for serum calcium and albumin in our patients led to the hypothesis that differences in laboratory measurements influence assessment of population outcomes.

**Methods:** All patients at the our dialysis unit were offered inclusion in the study. Specimens were collected on the day of monthly testing in the usual manner. After routine testing was complete, the remaining sample in each specimen was divided equally into 2 plastic transport tubes. These samples were stored and then analyzed the same day at Fletcher Allen Laboratory (FAL) and Spectra Laboratories (SL). Both laboratories use similar indicator techniques for albumin and calcium but different analytic instruments and formulae for corrected calcium.

**Results:** Samples were obtained from 66 patients. Results for paired values of serum albumin, calcium and corrected calcium were significantly different between laboratories with p values of <0.001, 0.001 and <0.001. Bland-Altman analysis showed the systematic bias between laboratories for albumin, calcium and corrected calcium to be 0.08 g/dl, 0.24 mg/dl and -0.26 mg/dl. Albumin and corrected calcium values categorized according to the ranges in the DOPPS are shown in the figure.




Distributions of SL (Salb) and FAL (Faalb) albumin values are different (p=0.02) as are distributions of values for SL corrected calcium (SCCa) and FA corrected calcium (FACCa) (p=0.002).

**Conclusions:** Measurements of serum albumin, calcium and calculated calcium made on the same serum sample differ significantly between laboratories. A small systematic bias results in significant differences in categorized laboratory outcomes. Differences in quality of care may be mistakenly inferred from differences resulting from laboratory measurement.

*Funding:* Private Foundation Support

## TH-OR125

**In Vivo Modulation of Per1 Target Gene Expression in the Kidney by Pharmacological Blockade of Circadian Clock-Regulatory Kinases CK1δ/ε** Jacob Richards, Sean All, Kit-Yan Cheng, Michelle L. Gumz. *Medicine, Univ of Florida, Gainesville, FL.*

**Background:** Mounting evidence suggests that the circadian clock plays an important role in the regulation of renal function and blood pressure (BP). We previously showed that the circadian clock protein Per1 coordinately regulates expression of several genes encoding products that function in the regulation of sodium (Na) reabsorption in the kidney. Per1 positively regulates the alpha subunit of the renal epithelial Na channel (αENaC) and Fxyd5, a gamma-like subunit of the $Na^+ K^+$ ATPase. Per1 negatively regulates the lipid raft protein Caveolin-1 (Cav-1) and Endothelin-1 (ET-1), a potent inhibitor of ENaC channel activity. This coordinate regulation of Na transport genes predicts that knockdown of Per1 would result in renal Na wasting and subsequent decreased blood volume and BP. Indeed, we have shown that Per1 KO mice have significantly lower BP than control mice (Stow et al. *Hypertension* 2012). In order to enter the nucleus, Per1 must be phosphorylated by casein kinase 1 δ/ε (CK1δ/ε). Our previous *in vitro* data demonstrated that pharmacological inhibition of CK1δ/ε resulted in decreased αENaC expression and decreased ENaC Channel activity (Richards et al. *AJP Renal* 2012).

**Methods:** Wild type 129/sv mice were injected with vehicle or the CK1δ/ε inhibitor PF670462 every 12 hr for 2.5 days, a previously reported effective dose and time. Mice were euthanized at midnight and kidneys were harvested RNA was isolated, and real time PCR was used to assess changes in gene expression of αENaC, Fxyd5, Cav-1, and ET-1 in the renal inner medulla and cortex.

**Results:** Because the BP phenotype of Per1 KO mice suggests that Per1 is a viable target for the treatment of hypertension, we tested the *in vivo* effects of pharmacological inhibition of Per1 nuclear entry on the expression of Per1 target genes in the kidney. CK1δ/ε inhibition led to decreased αENaC and Fxyd5 and increased Cav-1 and ET-1 in the inner medulla and cortex, consistent with our previous *in vitro* data.

**Conclusions:** These results support the use of PF670462 for *in vivo* inhibition of Per1 activity and may have implications for the treatment of hypertension.

## TH-OR126

**A Novel Mouse Model Expressing Cre Recombinase along the Entire DCT** Juliette Hadchouel,[1,2] Christelle Soukaseum,[1,2] James A. McCormick,[3] Nicolas Picard,[1,2] David H. Ellison.[3] *[1]INSERM U970, Paris Cardiovascular Research Center, Paris, France; [2]Univ Paris-Descartes, Paris Sorbonne Cité, Paris, France; [3]Oregon Health and Science Univ, OR.*

**Background:** The distal nephron, composed of the cortical Thick Ascending Limb (cTAL), the Distal Convoluted Tubule (DCT), the Connecting Tubule (CNT) and the Collecting Duct (CD), is a key player in maintaining $Na^+$, $K^+$ and $Cl^-$ homeostasis. Since many important regulators of ion transport are expressed in several segments, the development of segment-specific models is critical. While transgenic mouse lines expressing Cre recombinase along the CNT and CD have been generated, only one transgenic line allowing targeting of the DCT is available, using the *parvalbumin* promoter. However, this model only targets the proximal part of the DCT. Our goal was to develop a transgenic line expressing Cre along the whole DCT, using the promoter of the Na-Cl cotransporter, NCC.

**Methods:** Our previous attempt with an NCC-based transgene was unsuccessful. We therefore chose to introduce the iCre cDNA into the endogenous *Slc12a3* locus, encoding NCC. In order to preserve NCC expression and activity, we used the viral 2A peptide, introduced between the iCre cDNA and the first coding amino acid of NCC. Using homologous recombination in ES cells, we have generated two independent mouse lines with the iCre-2A cassette targeted into the *Slc12a3* locus.

**Results:** Immufluorescence showed that iCre recombinase is expressed along the DCT in $NCC^{+/iCre}$ mice. We further demonstrated by RT-PCR that iCre is expressed only in the kidney. We are currently assessing whether the knock-in of iCre into the *Slc12a3* locus affects NCC activity by measuring NCC expression and phosphorylation, and by performing thiazide-response tests in $NCC^{iCre/iCre}$ mice.

**Conclusions:** Many studies have highlighted the crucial role of the DCT in the maintenance of normal sodium balance. However, characterization of the mechanisms regulating DCT function has been hampered by several issues, such as the lack of a good cellular model and the difficulties of microperfusing this segment *in vitro* in mice. The generation of NCC-iCre mice provides the first tool to specifically target the entire DCT.

*Funding:* Government Support - Non-U.S.

## TH-OR127

**A Western Diet Activates NCC to Promote Sodium Retention** Andrew Terker, Chao-Ling Yang, David H. Ellison. *Nephrology & Hypertension, Oregon Health & Science Univ, Portland, OR.*

**Background:** To maintain blood pressure homeostasis, the thiazide-sensitive Na-Cl cotransporter (NCC) is suppressed by diets that are high in salt, promoting natriuresis. NCC also plays a key role in potassium ($K^+$) homeostasis. Diets that are low in $K^+$ increase NCC abundance, reducing distal sodium delivery, and suppressing sodium-dependent potassium secretion along the distal nephron. A Western diet is typically high in salt and low in potassium. To determine the effects of a typical Western diet on NCC, we analyzed effects of opposing sodium and K+ signals.

**Methods:** Several models were used to generate opposing sodium/potassium signals: 1) Fludrocortisone (F) was administered via drinking water at a dose of 17 mg/L for one week; 2) Amiloride (A) was administered via drinking water at a dose of 50 mg/L for one week. 3) Indicated diets were administered for 7-10 days; high salt/normal K+ (HS/NK, 6% NaCl/0.8% K+); high salt/low $K^+$ (HS/LK, 6% NaCl/0% $K^+$).

**Results:** 1) We confirmed that F (to model primary aldosteronism/Liddle syndrome) increased total and phosphorylated NCC; when NCC activation could not occur (NCC knockout (KO)), F caused profound hypokalemia. 2) Surprisingly, A (to model psedohypoaldosteronism type I, PHA I), which caused hyperkalemia as expected, decreased total and phosphorylated NCC significantly. 3) HS/LK dramatically increased total and phosphorylated NCC, compared with HS/NK. Further, HS/LK increased WNK4 and SPAK, and redistributed WNK4 in DCT to cytoplasmic punctae. These effects were preserved in SPAK and angiotensin II receptor 1a (AT1a) KO mice. Lastly, a HS/LK diet decreased urinary sodium compared with a HS/NK diet in WT, but not in NCC KO mice.

**Conclusions:** Increased NCC caused by F attenuates $K^+$ wasting. Decreased NCC caused by A likely results from hyperkalemia; a similar effect may contribute to salt-wasting in PHA I. On a HS diet, LK greatly increases NCC, compared with NK, independent of SPAK or AT1aR. Changing from a HS/NK to a HS/LK diet promoted sodium retention, an effect that requires NCC. Lastly, HS/LK moves WNK4 into cytoplasmic punctae. The results suggest that a Western diet promotes hypertension in an NCC-dependent manner; this hypothesis should be tested in humans.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-OR128

**The Application of a Novel Phosphoprotein Technology to Study the WNK Kinase Signaling Network** Jesse Rinehart, Natasha L. Pirman. *Cellular and Molecular Physiology, Systems Biology Institute, Yale Univ, New Haven, CT.*

**Background:** Recent work has identified phosphorylation sites in the WNK kinases and has shown that mechanistic insight into WNK kinase phosphorylation will be a key to understanding their role in renal sodium transport and hypertension.

**Methods:** While many of the phosphorylation sites on mammalian proteins have been identified, and most others are within reach via phosphoproteomics; there remains a substantial knowledge gap in the connections between kinases and substrates. We have recently made substantial progress in transforming phosphoprotein research by introducing a new technology that enables site-specific incorporation of phosphoserine into proteins. Our technology adds phosphoserine to the genetic code of E. coli by using the rare TAG stop codon (Amber codon). Phosphoproteins can then be synthesized and studied without the corresponding upstream kinase.

**Results:** We have an new optimized version of our technology that enables robust production of stoichiometricly phosphorylated proteins at single or multiple sites. This new technology was used to produce and study WNK4 with its physiologically relevant phosphorylation sites and a combinatorial library of WNK1 variants with phosphoserine introduced at activation loop residues. Previous work showed that phospho-mimetic aspartate substitutions in these same positions produced an inactive WNK1. In contrast, our technology introduced native phosphoserines and produced fully activated WNK1. We used these activated WNK1 proteins to reconstitute the WNK-SPAK-NKCC sub-network which showed robust WNK1 dependent SPAK activation. Quantitative phosphoproteomic analysis of this network and WNK1 auto-phosphorylation have been investigated and suggest new functional roles for WNK phosphorylation.

**Conclusions:** Our technology provides unprecedented resolution into the role of protein phosphorylation in WNK kinase function, as well as recapitulates physiologically relevant signaling networks. Our study provides the basis for a general strategy to study the role of protein phosphorylation in kidney physiology and a novel platform to develop targeted therapies.

*Funding:* NIDDK Support

## TH-OR129

**WNK4 Modulates the WNK1/WNK3-Mediated Activation of the NaCl Cotransporter** Maria Chavez-Canales,[1] Christelle Soukaseum,[2] Chao-Ling Yang,[3] Erika Moreno,[1] Diana Pacheco-Alvarez,[4] Emmanuelle Vidal-Petiot,[2] Maria Castañeda-Bueno,[1] Norma Hilda Vázquez,[1] Lorena Leonor Rojas,[1] Xavier Jeunemaitre,[5] David H. Ellison,[3] Gerardo Gamba,[1] Juliette Hadchouel.[2] *[1]Molecular Physiology Unit, INNSZ-IIB, UNAM, Mexico City; [2]INSERM, Paris; [3]Oregon Health and Science Univ, Portland; [4]Universidad Panamericana, Mexico City.*

**Background:** Increased renal salt reabsorption is a key pathogenic factor in the development of Arterial Hypertension. Familial Hyperkalemic Hypertension (FHHt) is a monogenic form of salt-sensitive hypertension caused by mutations in genes encoding WNK1, WNK4, Cullin 3 or kelch-like 3 that belong to the same salt reabsorption regulatory cascade. However, the precise mechanisms by which WNK1 and WNK4 affect target transporters remain unclear. In the kidney, the most abundant WNK1 isoform lacks only exon 11, WNK1-Δ11.

**Methods:** We used *Xenopus* oocytes, HEK 293 cells and genetically altered mice.

**Results:** In oocytes and in HEK 293 cells transfected with NCC, WNK1-Δ11 increased NCC activity and phosphorylation, as previously shown for WNK3. In oocytes, the disruption of the WNK1-SPAK interaction, by removing a SPAK-binding motif in WNK1 (WNK1-Δ11-F316A), prevented WNK1-Δ11 stimulation of NCC and showed a dominant-negative effect on NCC activity, suggesting that activation of NCC occurs through a SPAK dependent pathway. We also demonstrated that WNK4 inhibited both the WNK1 and WNK3 activation of NCC and angiotensin II prevented this WNK4 inhibitory effect. These results suggest that WNK4 acts upstream of WNK1 and WNK3. To test whether WNK1 lies downstream from WNK4 in vivo, we crossed a $WNK1^{+/FHHt}$ mice, generated by deletion of the first intron of WNK1, with $WNK4^{-/-}$mice. $WNK1^{+/FHHt}$:$WNK4^{-/-}$ animals retained the full FHHt phenotype, caused by an increased in NCC expression and phosphorylation, as seen in $WNK1^{+/FHHt}$ mice.

**Conclusions:** Our results revealed that WNK kinases signalling cascade in the distal nephron is mastered by WNK4, which modulates the activity of WNK1 and WNK3 towards SPAK and NCC, an effect modulated by angiotensin II.This model of WNKs interaction provides a molecular understanding of blood pressure and distal salt reabsorption regulation.

*Funding:* Government Support - Non-U.S.

## TH-OR130

**WNK Regulates NKCC in the Fly Tubule through a SPAK Intermediate** Aylin R. Rodan, Chou-Long Huang. *Dept of Medicine, UT Southwestern, Dallas, TX.*

**Background:** The main segment of the *Drosophila melanogaster* renal tubule is $K^+$-secreting. We have previously shown that the fly NKCC, Ncc69, mediates approximately 1/3 of normal $K^+$ flux. In the mammalian nephron, the WNK/SPAK kinase cascade regulates the Ncc69-related transporters NCC and NKCC2. Here, we examined whether this pathway is conserved in the fly tubule.

**Methods:** We genetically manipulated the fly WNK and SPAK kinases in wild-type and *Ncc69* mutant flies and measured $K^+$ flux in isolated tubules. We measured *wnk* transcript levels using quantitative RT-PCR.

**Results:** Knockdown of *wnk* with RNAi in the cation-secreting cells of the fly renal tubule decreased $K^+$ flux (80±5 pmol/min/tubule in control vs 49±5 in *wnk* knockdown tubules, $p<0.0001$). Similarly, expression of $WNK^{D420A}$, a kinase-dead isoform with dominant-negative effects, also decreased $K^+$ flux (68±4 in control vs. 50±4 in kinase-dead WNK tubules, $p<0.05$). Fray is the fly SPAK homolog. Expression of constitutively active $Fray^{T206E}$ in *wnk* knockdown tubules restored $K^+$ flux to wild-type levels (control, 89±9; *wnk* knockdown 60±8; *wnk* knockdown with $fray^{T206E}$, 94±9, $p<0.05$ for *wnk* knockdown vs. control and $fray^{T206E}$ groups). Activated Fray expression on its own did not change $K^+$ flux (57±6 in control vs 68±8 in $fray^{T206E}$-expressing tubules; NS). *Wnk* knockdown in an *Ncc69* null mutant background did not result in decreased $K^+$ flux (65±5 in controls, 62±4 in *Ncc69* mutant tubules with *wnk* knockdown, NS). *Wnk* transcript levels were similarly decreased by *wnk* knockdown in both the wild-type and *Ncc69* mutant backgrounds, to 37±9% and 50±17% of control (NS).

**Conclusions:** Decreasing WNK activity in the fly renal tubule impairs $K^+$ flux. This phenotype is rescued by expression of constitutively active Fray (fly SPAK), suggesting that Fray acts downstream of WNK and that WNK activates Fray. Ncc69, the fly NKCC, appears to be the target of WNK/SPAK signaling, as the *wnk* knockdown phenotype is abolished in *Ncc69* mutant tubules. We have thus established that the WNK/SPAK/NKCC pathway is conserved in the fly renal tubule, laying the groundwork for further dissection of the mechanisms by which this occurs, as well as the discovery of new genes in this regulatory pathway.

*Funding:* NIDDK Support

## TH-OR131

### SPAK-Independent Activation of Na-(K)-Cl Cotransport by WNK4 Requires Cab39 as an Adaptor Protein

José Ponce-Coria,[1] Paul A. Welling,[2] Eric J. Delpire.[1] *[1]Anesthesiology, Vanderbilt Univ, Nashville, TN; [2]Physiology, Univ of Maryland, Nashville, TN.*

**Background:** SPAK (STE20/SPS1-related proline alanine rich kinase), OSR1 (oxidative stress kinase 1) and WNK (with no K-lysine) kinases modulate distal $Na^+$ transport by affecting the activities of the thick ascending limb of Henle Na-K-2Cl cotransporter (NKCC2) and of the distal convoluted tubule Na-Cl cotransporter (NCC). WNK4 is thought to affect the cotransporters through two different pathways: 1) by regulating their trafficking to the plasma membrane; and 2) by phosphorylating and activating SPAK which then phosphorylates and activates the cotransporters. Here, we demonstrate a third mode of action consisting of a direct stimulatory effect of WNK4 on the cotransporters.

**Methods:** We measured $K^+$ influx in *Xenopus* oocytes injected with cotransporter, wild-type and mutant kinase, and Cab39 cRNAs to assess the role of the kinases and adaptor protein in modulating cotransporter activity.

**Results:** When Cab39 or Cab39l cRNA was injected with WNK4 and NKCC1, we observed a significant increase in the level of $K^+$ influx. This increase was bumetanide-sensitive indicating that the flux was mediated by the cotransporter. We show that this stimulation requires the catalytic activity of WNK4 and is not affected by overexpressing dominant negative SPAK, suggesting that WNK4 is able to phosphorylate and activate the cotransporter in a SPAK-independent manner. When we mutated a phenylalanine residue in a WNK4 domain that resembles the SPAK/OSR1 binding domain, we prevented the Cab39/WNK4 activation of the cotransporter. When we mutated Cab39 residues known to promote interaction between Cab39 and Ste20 kinases, we observed WNK4 activation. Finally, we demonstrate that the WNK4 PHAII mutation, E599K, does not affect the ability of WNK4 to activate SPAK, whereas it completely abrogates the activation of NKCC1 by WNK4. These data were reproduced using a chimeric transporter consisting of the N-terminal regulatory tail of NCC fused to NKCC1, indicating that NCC is similarly activated by the Cab39/WNK4 interaction.

**Conclusions:** Our data demonstrate that WNK4 interact and activate the NaCl cotransporters in the absence of SPAK or OSR1.

*Funding:* NIDDK Support

## TH-OR132

### Af9 Targets Ring Finger Protein 2 (Rnf2) to the αENaC Promoter to Regulate Basal and Aldosterone-Stimulated αENaC Transcription

Zhiyuan Yu, Qun Kong, Bruce C. Kone. *The Univ of Texas Medical School at Houston, Houston, TX.*

**Background:** Af9 is an aldosterone-sensitive regulator of αENaC transcription required for enhanced renal tubular Na transport. Af9 binds to a cis-element in the αENaC promoter to recruit histone methyltransferase Dot1a to epigenetically repress basal αENaC transcription in collecting duct cells. Aldosterone downregulates this repressor complex to de-repress αENaC transcription. Rnf2, a central component of Polycomb repressive complex 1 involved in epigenetic silencing of target genes, lacks DNA-specific binding activity, so that other factors must mediate its site-specific chromatin recruitment. We tested the hypotheses that Af9 interacts with and recruits Rnf2 to the αENaC promoter and contributes to basal αENaC repression and aldosterone de-repression of αENaC transcription in mIMCD3 cells.

**Methods:** Immunofluorescence microscopy, co-immunoprecipitation, and GST pull-down assays were used to identify Af9-Rnf2 interactions in mIMCD3 cells. Chromatin immunoprecipitation (ChIP)/qPCR and re-CHIP assays, promoter-luciferase assays, overexpression and siRNA knockdown of Rnf2 were used to assay functionally relevant interactions of Rnf2 with the αENaC promoter in vehicle and aldosterone-treated mIMCD3 cells.

**Results:** Rnf2 and Af9 co-localized in the nucleus, and they interacted in co-immunoprecipitation and GST-pull-down assays. Rnf2 knockdown enhanced basal and aldosterone-stimulated αENaC mRNA levels and αENaC promoter activity. Conversely, Rnf2 overexpression inhibited aldosterone-stimulated αENaC mRNA expression and αENaC promoter activity. ChIP/re-ChIP assays showed that Af9 and Rnf2 co-occupy the R3 subregion (-57/+494) of the αENaC promoter. Aldosterone inhibited Rnf2 occupancy of the αENaC promoter and trimethylation of histone H3K27, a chromatin mark of repressed genes, coincident with induction of αENaC transcription.

**Conclusions:** We conclude that Af9 mediates site-selective physical and functional recruitment of Rnf2 to the αENaC promoter to constrain basal αENaC transcription. Aldosterone de-represses αENaC transcription by inhibiting Dot1a, Af9, and Rnf2 association and action at the αENaC promoter.

*Funding:* NIDDK Support

## TH-OR133

### Renal SGK1 Plays a Crucial Role in the Control of Potassium Homeostasis, by NEDD4-2 Dependent Regulation of ENaC and NCC

Lama Al-Qusairi,[1] Matteo Stifanelli,[1] Anne Debonneville,[1] Nourdine Faresse,[1] Johannes Loffing,[2] Olivier Staub.[1] *[1]Univ of Lausanne; [2]Univ of Zurich.*

**Background:** Dietary $K^+$ load results in increased kalemia, leading to aldosterone (aldo) release thereby stimulating $K^+$ secretion in the ASDN. However, the mechanisms of this regulation are unclear.

**Methods:** Here, we identified the specific role of the aldosterone inducible SGK1 kinase in the tight regulation of potassium secretion using an inducible nephron specific SGK1 KO ($SGK1^{Pax8/LC1}$) mouse model.

**Results:** We find that lack of SGK1 has no obvious effect on potassium homeostasis under normal diet. However, under high $K^+$ diet for 48 hours, mutant mice exhibit a 35% decrease in urinary $K^+$ excretion, accompanied with sever hyperkalemia and increased plasma aldo levels. At the molecular level, we observe a decrease in cleavage of alpha and gamma ENaC, indicating that ENaC activity is reduced. Moreover, phosphorylation of the Na-Cl-cotransporter NCC is strongly reduced under these conditions, indicating reduced NCC activity. The ubiquitin-protein ligase NEDD4-2, a well-known substrate of SGK1 kinase, and implicated in the positive regulation of both ENaC and NCC, is less phosphorylated on the SGK1-dependant phosphorylation sites S222 and S328, as evidenced by immunoblotting.

**Conclusions:** Our data indicate that renal SGK1 is a key regulator of potassium homeostasis in the kidney, which affects the activity of both NCC and of ENaC, likely via regulation of NEDD4-2.

## TH-OR134

### Calveolin-1 Is Required for Src-Family Protein Tyrosine Kinase (SFK)-Induced Regulation of the Basolateral K Channel in the Early Distal Convoluted Tubule (DCT1)

WenHui Wang. *Pharmacology, New York Medical College, Valhalla, NY.*

**Background:** The basolateral K channels in the DCT1 play an important role in determining the membrane potential which is essential for sustaining Cl exit across the basolateral membrane and Na-K-ATPase activity.

**Methods:** We performed the whole-cell recording to measure the $Ba^{2+}$-sensitive K currents in the DCT1 which represents the basolateral K channel activity since no apical K channel activity has been detected.

**Results:** The whole K currents in DCT1 are inwardly-rectifying and inhibited by blocking SFK with PP1. However, the effect of PP1 on K currents was absent in calveolin-1 knockout (KO) mice. Moreover, the K currents in KO mice were significantly lower than those in wt mice. This suggests that calveolin-1 is critically involved in modulating the effect of SFK on basolateral K channels in DCT1. This hypothesis was tested by investigating the effect of PP1 on K channel activity in HEK293T cells transfected with KCNJ10+KCNJ16 which are known to form heterotetramer K channel in the DCT1. We detected a 40 pS K channel in KCNJ10/16-transfected cells and a 20 pS K channel in the cells transfected with KCNJ10 alone. The whole cell recording demonstrated that K currents in KCNJ10/16-transfected cells were significantly larger than those in KCNJ10-transfected cells. However, inhibition of SFK had no effect on K currents in both KCNJ10/16- and KCNJ10-transfected cells. Co-transfection of calveolin-1 with KCNJ10/16 or KCNJ10 did not alter the K currents in comparison to those without calveolin-1 cotransfection. However, Inhibition of SFK significantly decreased the whole cell K currents in the cells transfected with KCNJ10/16+calveolin-1 or with calveolin-1+KCNJ10. Immunostaining showed that calveolin-1 and KCNJ10 are colocalized in the distal nephrons.

**Conclusions:** We conclude that SFK plays an important role in regulating the basolateral K channel activity and calveolin-1 is critically involved in modulating the effect of SFK on the basolateral K channels in DCT1.

*Funding:* NIDDK Support

## TH-OR135

**The Rapidly Growing Global Burden of End-Stage Renal Disease – An Analysis of the Change in Maintenance Dialysis Prevalence between 1990 and 2010** Bernadette A. Thomas,[1] Sarah Wulf,[2] Rajnish Mehrotra,[1] Jonathan Himmelfarb,[1] Mohsen Naghavi,[2] Christopher Jl Murray.[2] *[1]Kidney Research Institute, Univ of Washington, Seattle, WA; [2]Institute for Health Metrics and Evaluation, Univ of Washington, Seattle, WA.*

**Background:** Rapidly rising global rates of chronic diseases portend a consequent rise in end-stage renal disease (ESRD). However, the change in global burden of treated ESRD has never before been quantified. This study was undertaken to accurately report the trajectory of treated prevalent ESRD rates at the global and regional level between 1990 and 2010.

**Methods:** We extracted data from the Global Burden of Disease database, the largest existing database for global causes of morbidity and mortality. The prevalence estimates were calculated from data from national and regional ESRD registries and a structured literature review for years 1990 and 2010. Data from 26 countries that lack routine access to dialysis were excluded. Data from twenty-three countries providing 100% dialysis access and 138 countries providing partial dialysis access were included. A negative binomial Bayesian regression tool was used to estimate the prevalence of chronic dialysis in 161 countries stratified by year and sex.

**Results:** Within the past two decades, there has been a global increase in maintenance dialysis of 165%. The global prevalence of treatment with maintenance dialysis for countries with universal dialysis access increased by 134% after population growth and aging adjustment; 145% in women versus 123% in men. For countries whose populations lack universal dialysis access, adjusted prevalence increased by 102%; 116% for women versus 90% for men. The five world regions not experiencing substantial increase in maintenance dialysis prevalence include Oceania, South Asia, central sub-Saharan Africa, eastern Europe, and tropical Latin America.

**Conclusions:** The significant growth in maintenance dialysis therapy far out of proportion to population growth for a majority of regions in the world. This emphasizes the need for early chronic kidney disease detection and treatment targeting ESRD prevention, since continued rise in prevalence of maintenance dialysis may not be sustainable.

*Funding:* Private Foundation Support

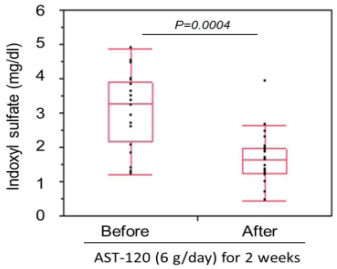
## TH-OR136

**Oral Activated Charcoal Adsorbent, AST-120, Induced Continuous Reduction of Protein-Bound Uremic Toxins in Maintenance Hemodialysis Patients: A Randomized Cross-Over Trial** Suguru Yamamoto,[1] Kentaro Omori,[2] Koji Matsuo,[1] Kazuko Kawamura,[1] Minako Wakasugi,[1] Hiroki Maruyama,[1] Junichiro J. Kazama,[1] Ichiei Narita.[1] *[1]Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Omori Clinic, Niigata, Japan.*

**Background:** An accumulation of protein-bound uremic toxins (PBUT) is one of the reasons why patients undergoing dialysis treatment have high risk of cardiovascular events. Oral activated charcoal adsorbent, AST-120, has been shown to decrease serum indoxyl sulfate in non-dialysis CKD patients. The aim of this study is to examine whether AST-120 decreases PBUT in maintenance hemodialysis patients.

**Methods:** Twenty maintenance hemodialysis patients were individually randomized in a crossover design between treatment with 6 g/day of AST-120 and non-treatment for 4-week periods. Ten participants followed the AST-120 treatment first for 2 weeks and then switched to non-treatment for another 2 weeks; the other 10 subjects followed the same treatment in reverse order. Serum level of indoxyl sulfate at pre/post dialysis session before and after the AST-120 treatment was measured by mass spectrometry. Data were presented as medians (interquartile range: IQR). Wilcoxon signed-rank test was used for the statistical analysis.

**Results:** At the baseline, serum levels of indoxyl sulfate were 3.27 (2.17 to 3.89) and 2.20 (1.38 to 2.89) mg/dl in pre- and post-dialysis sessions respectively while it returned to the previous level before the next dialysis sessions. However, AST-120 treatment for 2 weeks dramatically decreased serum indoxyl sulfate levels in both pre- [1.64 (1.25 to 1.98) mg/dl, P=0.0004 vs. baseline] and post- [1.24 (0.87 to 1.49) mg/dl, P= 0.002 vs. baseline] dialysis treatment.




**Conclusions:** Use of AST-120 showed a continuous and powerful effect to remove protein-bound uremic toxins in maintenance hemodialysis patients.

*Funding:* Government Support - Non-U.S.

## TH-OR137

**Prevalence and Predictors of Blood Pressure Variability in Hemodialysis Patients** Talal S. Alzahrani, Fangtang Yu, Samir S. Patel, Dominic S. Raj. *Dept of Medicine, Div of Renal Diseases & Hypertension, The George Washington Univ, Washington, DC.*

**Background:** Blood pressure variability (BPV) predicts cardiovascular mortality in general population and patients with kidney disease. We hypothesized that variation in pre-dialysis systolic blood pressure (Pre-SBP) and mean BP change are independent predictors of mortality in hemodialysis patients.

**Methods:** We used clinical and biochemical data derived from the DaVita database. The study population (N=420,255) included subjects undergoing hemodialysis in DaVita facilities across the US during the period of 2004 to 2011. Pre-SBP variability is described by the SD of SBP examined before every dialysis during a month; mean BP change during dialysis is described by the mean of the difference between post-dialysis mean BP and pre-dialysis mean BP over a month. Repeated measurement model were built to examine the factors that influence SBP variability. Cox proportional hazard model with time-dependent covariates were fitted to test if SBP variability is a significant predictor of time to death and time to hospitalization.

**Results:** The mean pre-dialysis systolic blood pressure was 148±53 mm of Hg. Low, medium and high BP variability was found in 35%, 34%, and 30% of the patients respectively. Patients' gender, race, BMI, weight gain during dialysis, lack of insurance, exposure to heart disease or Diabetes mellitus are significant predictors to both SBP variability and mean BP change during dialysis (p<0.001). Both Pre-SBP variability and mean BP change during dialysis were significant predictor of time to death (HR (pre-SBP)=1.009 95% CI (1.0081,1.0102) p<0.001, HR (BP change)=1.028, 95% CI (1.0273,1.0287), p<0.001) and time to hospitalization (HR (pre-SBP)=1.005 95% CI (1.0046,1.0058) p<0.001, HR (BP change)=1.010, 95% CI (1.0092,1.0100), p<0.001).

**Conclusions:** Pre-SBP variability and mean BP change are very common and they are associated with distinct clinical predictors. Both SBP variability and mean BP change during dialysis are significant predictors of time to death and time to hospitalization. Further studies are needed to confirm our results and to find ways to decrease BP variability in patient with ESRD.

## TH-OR138

**Greater Endotoxin Level of Dialysis Fluid Associated with Increased Mortality of Hemodialysis Patients: A Nationwide Cohort Study** Takeshi Hasegawa,[1,2,3] Ikuto Masakane,[3] Kunitoshi Iseki,[3] Yoshiharu Tsubakihara,[3] Tadao Akizawa.[4] *[1]Div of Nephrology, Showa Univ Fujigaoka Hospital, Yokohama, Japan; [2]Dept of Epidemiology and Healthcare Research, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [3]Committee of Renal Data Registry, The Japanese Society for Dialysis Therapy, Tokyo, Japan; [4]Div of Nephrology, Showa Univ School of Medicine, Tokyo, Japan.*

**Background:** There has been wide variation in targeted endotoxin level of dialysis fluid among countries, however, evidences for the effectiveness of dialysis fluid purity for mortality of patients on hemodialysis (HD) are lacking. The aim of this research is to evaluate the impact of water quality by dialysis fluid endotoxin level (dET) on mortality of HD patients.

**Methods:** The design of this investigation is a cohort study using a nationwide annual survey of the Japan Renal Data Registry. We analyzed 130,781 patients on HD (at the end of 2006: on HD > half a year, three times session per week, during 2007: no changing facilities and treatment modalities). The main outcome measure was all-cause death. Main exposure to be tested was dET reported by 2,746 facilities at the end of 2006 (categorized into 5 groups: <0.001, 0.001 to 0.01, 0.01 to 0.05, 0.05 to 0.1, and 0.1< EU/mL). Cox regression was employed to estimate the hazard of all cause death adjusted for potential confounders.

**Results:** During one year follow-up, 8,978 (6.9%) patients died of all-causes. The rate of all-cause mortality at one year was the highest of patients in the greatest dET (0.1EU/mL<) category (88.0 deaths/1,000 person-years). After multivariable adjustment, patients who were exposed to the greatest dET (0.1EU/mL<) displayed a 28% (95% CI, 15%-41%) increased risk of all-cause death compared to those who with the lowest dET (<0.001EU/mL).

**Conclusions:** These results suggested that greater dET was related to increased risk of all-cause death in HD patients. Correcting this modifiable water management practice may improve outcomes of HD patients.

## TH-OR139

**Convective Therapies *versus* Low-Flux Hemodialysis for Chronic Kidney Failure: A Meta-Analysis of Randomized Controlled Trials** Paweena Susantitaphong,[1,2] Bertrand L. Jaber.[1] *[1]Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Medicine, Chulalongkorn Univ, Bangkok, Thailand.*

**Background:** Convective therapies have gained popularity for the optimal removal of uremic solutes. However, their benefits and potential risks have not been fully elucidated. We conducted a meta-analysis of all randomized controlled trials (RCTs) comparing convective therapies vs. low-flux hemodialysis in patients with chronic kidney failure.

**Methods:** We searched MEDLINE (through December 2012), Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, scientific abstracts from meetings, and bibliographies of retrieved articles for randomized controlled trials comparing the effects of convective therapies (including high-flux hemodialysis, hemofiltration, and

hemodiafiltration) versus low-flux hemodialysis were included. Random-effects model meta-analyses were used to compute absolute net changes in mean values for continuous outcomes, and relative risks (RRs) for binary outcomes.

**Results:** Compared to low-flux hemodialysis, convective therapies resulted in a decrease in all-cause mortality (pooled RR 0.88; 95% CI 0.76, 1.02, P = 0.09; $I^2$ = 18%), cardiovascular mortality (pooled RR 0.84; 95% CI 0.71, 0.98, P = 0.03; $I^2$ = 0%), all-cause hospitalization (pooled RR 0.91; 95% CI 0.82, 1.01; P = 0.06; $I^2$ = 77%) and therapy-related hypotension (pooled RR 0.61, 95% CI 0.42, 0.89, P = 0.01; $I^2$ = 99%). Convective therapies also resulted in a signifcant improvement in the clearance, reduction ratio and/or pre-therapy serum levels of several low-molecular-weight solutes (urea, creatinine, and phosphate), middle-molecular weight solutes (beta-2 microglobulin and leptin), and protein-bound solutes (homocysteine, advanced glycation end-products, and pentosidine). There was no impact of convective therapies on cardiac structure and function, blood pressure parameters, and anemia parameters.

**Conclusions:** Although convective therapies are associated with decreased morbidity and mortality and improved clearance of uremic solutes, the potential long-term benefits of specific modalities require further study.

## TH-OR140

**Trends in Medicare Costs for Hemodialysis Patients, 2001-2011, and Estimated Change in Medicare Costs Associated with the New Medicare Bundle Payment Policy** Allan J. Collins,[1,2] Suying Li,[1] Jiannong Liu,[1] David T. Gilbertson,[1] Robert N. Foley,[1,2] James P. Ebben,[1] Craig Solid,[1] Shu-Cheng Chen.[1] *[1]USRDS Coordinating Center, MMRF, Mpls, MN; [2]Medicine, Univ of MN, Mpls, MN.*

**Background:** Starting in January 2011, several outpatient dialysis services, including laboratory services performed by nephrologists and injectable medications, were incorporated into the new Medicare prospective payment system (PPS). We aimed to examine trends in Medicare costs 2001-2011 and estimate change in Medicare costs associated with the new PPS in hemodialysis (HD) patients.

**Methods:** Yearly cohorts, 2001-2011, included point prevalent HD patients on January 1, with a 90-day rule applied and Medicare Parts A/B eligibility required. Patients were followed from January 1 to December 31 each year and censored at transplant, change in modality, death, or loss to follow-up. Medicare payment per person-year (PPPY) consisted of inpatient (IP), outpatient (OP)/physician services, and other costs. Dialysis facilities were characterized as 100% bundle or 25% blend based on their decisions regarding the new PPS in 2011. We estimated change in Medicare costs associated with the new PPS using the difference-in-difference approach.

**Results:** Total PPPY increased from $57008 in 2001 into $79758 in 2010 and decreased to $78864 in 2011. The cost changes (PPPY) from 2010 to 2011 were -$238 for IP, -$880 for OP/physician, and $224 for others. Among the change in OP/physician cost, the reduction in categories related to the new PPS (HD and routine lab services, injectable medications, and lab services performed by nephrologists) was $1,106 (PPPY). The estimated change (2011 vs. 2010) in Medicare cost (PPPY) associated with new PPS (100% bundle vs. 25% blend) was -$215 (95% CI, -$1617 to $1187).

**Conclusions:** The $894 (PPPY) saving in Medicare costs in 2011 implies an approximate saving of $220 million in total Medicare costs for HD patients included in this study. This reduction may contribute to a low rate of growth in per capita general Medicare spending in 2011. Although the new PPS bundle associates with stabilization of Medicare total costs in 2011, providers may reduce their expenses, generating margins previously unrealized.

*Funding:* NIDDK Support

## TH-OR141

**Factors Influencing Hospital Admissions and Deaths over the Two Day Gap in Three Times a Week Hemodialysis** James Fotheringham,[1] Damian G. Fogarty,[2] Michael J. Campbell,[1] Meguid El Nahas,[3] Ken Farrington.[2] *[1]School of Health and Related Research, Univ of Sheffield, Sheffield, South Yorkshire, United Kingdom; [2]The UK Renal Registry, Bristol, United Kingdom; [3]Global Kidney Academy, Sheffield, United Kingdom.*

**Background:** Excess mortality and hospitalization has been identified after the 2 day gap in 3 times a week hemodialysis (HD) patients compared to 1 day intervals. Thus far additional risk factors and potential interventions have not been formally explored in dialysis scheduling related events. Greater understanding of mortality and hospitalisation events is required.

**Methods:** Incident renal replacement therapy patients starting treatment between 2002 and 2006 in England with available data until 2009 from the UK Renal Registry were analysed. Hospitalisation data was used to identify HD attendance patterns, admissions and comorbidity. Laboratory and clinical measurements were obtained from the nearest recorded period in the 6 months before the week of analysis. Rates were compared with rate ratios of events after the 2 day gap compared to the rest of the week (RR).

**Results:** 9,925 patient years were identified in 5790 patients across 36 centres. Compared to the rest of the week, excess emergency admissions were seen after the 2 day gap (2.29 vs 1.36 per patient year, P<0.001). Admission RR remained constant for the first 2 years of dialysis, the 2 years approaching death and across centres. Greatest admission increases were seen in heart failure (RR 1.82 vs 1.64 without, P=0.012), chronic obstructive pulmonary disease (RR 1.97 vs 1.65 without, P=0.002) and Pre-HD systolic blood pressure >150mmHg (RR 1.79 vs 1.62 <150mmHg, P=0.038). Excess all-cause mortality after the 2 day gap (21.3 vs 15.7 deaths per 100 patient years, P<0.001) was primarily due to the 32% of deaths occurring at home (out of hospital mortality RR 1.59, in hospital RR 1.06, P=0.001).

**Conclusions:** In England, comorbidity and volume overload/hypertension were associated with excess 2 day gap admissions in 3 times a week HD. Excess mortality was driven by deaths out of hospital. Interventions which reduce the risk of volume overload and sudden death in susceptible patients merit exploration.

## TH-OR142

**Risk of Under-Dialysis: Use of Scaling Parameters Reflecting Metabolic Activity** Sivakumar Sridharan,[1] Jocelyn Berdeprado,[1] Neil Ashman,[2] Andrew Davenport,[3] Michael K. Almond,[4] Anindya Banerjee,[5] Enric Vilar,[1,6] Justin Roberts,[6] Ken Farrington.[1,6] *[1]Lister Hospital; [2]Royal London Hospital; [3]Royal Free Hospital; [4]Southend Hospital; [5]Arrowe Park Hospital; [6]Univ of Hertfordshire.*

**Background:** Patients with small body size have reduced survival on haemodialysis (HD). This may be due to total body water being used as the normalising factor for HD dose prescription.We explored the use of alternative scaling parameters reflecting metabolic activity.

**Methods:** We recruited adult in-centre HD patients.Anthropometric, demographic and HD adequacy details were obtained from direct measurements and medical records.Body surface area (BSA) was estimated using Haycock formula. Resting energy expenditure (REE) was estimated using a novel equation, derived and validated in HD patients.Total energy expenditure (TEE) was calculated from physical activity data obtained using Recent Physical Activity Questionnaire (RPAQ). Kt/BSA, Kt/REE and Kt/TEE were estimated for all subjects.

**Results:** 1500 HD patients (910 males) were recruited.Median BSA was 1.85 $m^2$, median REE 1530 kcal/day and median TEE 1781 kcal/day. For Kt/V of 1.2, there was a wide range of equivalent doses expressed as Kt/BSA, Kt/REE and Kt/TEE. The equivalent dose ranges of Kt/BSA for women ranged from 21.15 to 23.06 (median 21.80) and for men 22.04 to 29.72 (median 25.33). For Kt/REE these were 0.0240 to 0.0314 (median 0.0263) for women and 0.0265 to 0.0345 (median 0.0304) for men. For Kt/TEE they were 0.0118 to 0.0286 (median 0.0234) for women and 0.0116 to 0.0321 (median 0.0259) for men. The median equivalent dose was significantly lower in women with all 3 parameters (p<0.001). Kt/BSA was lower for women in each weight quartile compared to those in highest quartile (p<0.001).There was no difference in Kt/REE or Kt/TEE for women in lowest quartile compared to the highest quartile.In men, all 3 parameters were significantly lower in each weight quartile compared to the highest quartile (p<0.001).

**Conclusions:** Our data suggest that current dosing practices risk under-dialysis in women and men of lower body size.Using BSA, REE or TEE based dialysis prescription would result in higher dose delivery in these settings.

## TH-OR143

**Determinants of Potassium Kinetics during Hemodialysis** Baris U. Agar,[1] J. Ken Leypoldt,[1] Richard J. Fluck,[2] Bruce F. Culleton.[1] *[1]Medical Products, Baxter Healthcare Corportation, Deerfield, IL; [2]Dept of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom.*

**Background:** Both hyperkalemia and hypokalemia are associated with elevated mortality in hemodialysis (HD) patients, and low dialysate potassium (K) concentration (<2 mEq/L) during HD treatments are associated with a higher incidence of sudden cardiac arrest if predialysis serum K levels are ≤5 mEq/L. Improved control of serum K levels in HD patients, particularly those treated by frequent HD sessions, may benefit from improved understanding of K kinetics. We have recently shown (2013 ERA-EDTA Congress) that K kinetics during HD can be described using a pseudo one-compartment model and that K mobilization clearance ($K_M$) and predialysis K central distribution volume (V) are relatively independent of dialysate K concentration.

**Methods:** We determined $K_M$ and V from kinetic modeling sessions in 437 patients during the HEMO Study with a dialysate K concentration of approximately 2 (1.6-2.5) mEq/L by optimal fitting of pseudo one-compartment model predictions of serum K levels to those measured predialysis, after 1 hour of treament, postdialysis and 30 minutes after stopping the treatment. The determinants of these kinetic parameters were determined by univariate and multivariate linear regression.

**Results:** The patients were 59±14 (mean±standard deviation) years old with predialysis body weight (BW) of 72.6±15.1 kg; predialysis serum K concentration ($K_{pre}$) was 5.3±1.0 mEq/L and treatment time (T) was 206±29 minutes. Fifty-five percent of patients were female; 45% were diabetic and 34% were of black race. On univariate analyses, $K_M$ and V were associated (P<0.001) positively with BW and T but negatively with $K_{pre}$. $K_M$ was also lower for females than males (P=0.003). On multivariate analyses, $K_M$ and V were associated positively with BW and negatively with $K_{pre}$; only V was associated positively with T. In total, V was 27±19% of BW.

**Conclusions:** Faster K mobilization and a larger central distribution volume are expected for bigger patients, providing further support for the validity of a pseudo one-compartment model. The larger central distribution volume with longer treatment times may indicate more effective K removal.

*Funding:* Pharmaceutical Company Support - Baxter Healthcare Corporation

## TH-OR144

**Potential Application of Exhaled Breath Monitoring in Renal Replacement Therapy** Kelly M. Paschke,[1] Sevag Demirjian,[2] Jaime T. Newman,[1] Lauren L. Chen,[1] Raed A. Dweik,[1,3] Robert J. Heyka.[2] *[1]Pathobiology/Lerner Research Institute, Cleveland Clinic, Cleveland, OH; [2]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; [3]Pulmonary, Allergy, and Critical Care Medicine/Respiratory Institute, Cleveland Clinic, Cleveland, OH.*

**Background:** Uremic retention solutes are a systemic consequence of Chronic Kidney Disease (CKD) and contribute to disease progression. Urea removal is used for monitoring adequacy of solute removal in hemodialysis (HD). However, this does not always correlate with symptoms of uremia or removal of retained solutes. In end stage renal disease (ESRD), the unique breath profile composed of volatile organic compounds and inorganic amines represents accumulations of end products of metabolic reactions, gut bacteria, and exogenous volatiles. However, no standardized breath test exists for monitoring CKD or the efficacy of HD.

**Methods:** Selected ion flow tube mass spectrometry (SIFT-MS) was used to quantify the exhaled breath of 15 ESRD patients before and after HD. An additional 15 healthy subjects were recruited as a baseline control. Selected ion monitoring of 21 known breath volatiles and mass scanning for high intensity peaks of unknown volatiles was applied.

**Results:** ESRD patients pre HD had elevated breath ethanol, ammonia, trimethylamine, 2-propanol, and acetone compared to healthy controls ($p<0.05$) and 43 peaks in the mass spectra ($p<0.01$). HD reduced breath trimethylamine, 2-propanol, acetaldehyde, acetone, benzene, dimethyl sulfide, pentane, 1-nonene, 3-methylhexane ($p<0.01$), and 13 peaks in the mass spectra ($p<0.01$). Breath ammonia, ethanol ($p<0.05$), and 21 peaks in the mass spectra ($p<0.01$) remained elevated post HD compared to healthy controls. Post HD breath composition more closely resembled healthy controls.

**Conclusions:** Breath testing may serve as a novel modality for CKD monitoring, in particular for HD adequacy. A potential advantage of breath tests is the ability to measure multiple volatiles simultaneously for a disease state which may provide a more robust method for monitoring CKD and HD.

*Funding:* Pharmaceutical Company Support - American Society of Nephrology

## TH-OR145

**Gene-Targeted Deletion of the Angiotensin II Type 1 Receptor-Interacting and Functionally Modulating Protein Enhances Renal Sodium Reabsorption and Exacerbates Angiotensin II-Mediated Hypertension** Kouichi Tamura. *Dept of Medical Science and Cardiorenal Medicine, Yokohama City Univ Graduate School of Medicine, Yokohama, Japan.*

**Background:** The angiotensin II (Ang II) type 1 receptor (AT1R)-associated protein (ATRAP/Agtrap), a molecule which specifically interacts with the AT1R. We hypothesize that a down-regulation of ATRAP at local tissue sites that enhances the pathological activation of tissue AT1R signaling in response to certain stimuli without any evident effect on baseline physiological AT1R signaling. Functional significance of ATRAP in renal sodium handling and blood pressure (BP) regulation in response to pathological stimuli is not fully resolved.

**Methods:** We generated mice with a gene-specific disruption of ATRAP (ATRAP-KO mice). We examined the effects of chronic Ang II infusion on BP and renal sodium handling in ATRAP-KO mice and their littermate wild-type control mice (WT mice).

**Results:** Although ATRAP was abundantly distributed along the renal tubules in WT mice, there was no ATRAP expression in any of the nephron segments in ATRAP-KO mice. ATRAP-KO mice exhibited no apparent renal morphological alterations under baseline conditions and BP of ATRAP-KO mice was comparable to that of their littermate wild-type control mice (WT mice) at baseline. However, the Ang II-mediated BP elevation was significantly augmented in the ATRAP-KO mice. Consequently, the heart weight /body weight ratio and urinary albumin excretion in the Ang II-infused ATRAP-KO mice were significantly increased compared with those in Ang II-infused WT mice. Metabolic cage analysis disclosed the increased cumulative sodium balance during the Ang II infusion period in the ATRAP-KO mice, along with an increase in the expression of α-subunit of the epithelial sodium channel in the kidney of ATRAP-KO mice.

**Conclusions:** In summary, these results indicated that the gene-targeted deletion of ATRAP provoked the exacerbation of Ang II-mediated hypertension and its associated end-organ injury, along with an inhibition of natriuresis, without any evident influence on baseline physiological function. Therefore, ATRAP is a potential target of interest in the regulation of BP.

*Funding:* Government Support - Non-U.S.

## TH-OR146

**Somatic and Germline *CACNA1D* Calcium Channel Mutations in Aldosterone-Producing Adenomas and a Mendelian Syndrome Featuring Primary Aldosteronism** Ute I. Scholl,[1] Gerald S. Goh,[1] Gabriel Stölting,[2] Regina Campos de Oliveira,[3] Murim Choi,[1] Annabelle L. Fonseca,[4] Erum A. Hartung,[5] Matthew Benson,[6] Carol J. Nelson-Williams,[1] Steven Libutti,[7] Shrikant M. Mane,[1] Per Hellman,[8] Peyman Björklund,[8] Tobias Carling,[4] Christoph Fahlke,[2] Patricia Hidalgo,[2] Richard P. Lifton.[1] *[1]Genetics, HHMI, Yale School of Medicine, New Haven, CT; [2]Institute of Complex Systems, Forschungszentrum Jülich, Germany; [3]Neurophysiologie, Medizinische Hochschule Hannover, Germany; [4]Surgery, Yale School of Medicine, New Haven, CT; [5]Children's Hospital of Philadelphia, Philadelphia, PA; [6]Nemours Children's Clinic, Jacksonville, FL; [7]Surgery, Albert Einstein College of Medicine, Bronx, NY; [8]Surgical Sciences, Uppsala, Sweden.*

**Background:** Adrenal aldosterone-producing adenomas (APAs) are benign, hormone-producing tumors found in ~5% of patients referred to hypertension specialists. ~40% of these tumors are caused by recurrent mutations in the potassium channel *KCNJ5*, resulting in sodium permeability, depolarization and calcium influx.

**Methods:** We here use exome and targeted sequencing to identify additional recurrent mutations in APAs and evaluate their functional impact by using patch clamp electrophysiology.

**Results:** We describe seven novel somatic mutations in the voltage-gated calcium channel *CACNA1D* in 43 non-*KCNJ5*-mutant APAs (16.3%). Four alter G403, and one each alter I770, F767 and V1373. Six of seven mutations are located within the S6 domains of the channel and are shown by electrophysiology or inferred to be gain-of-function, resulting in activation at less depolarized potentials. G403 mutations also impair inactivation. Remarkably, we identify *de novo* mutations at the identical residues in two patients with a novel Mendelian syndrome featuring primary aldosteronism and neuromuscular abnormalities, including seizures. *CACNA1D* is expressed in human adrenal cortex. Excess calcium influx through mutant channels is expected to result in aldosterone production and proliferation by activation of the same pathways upregulated in *KCNJ5*-mutant tumors.

**Conclusions:** These findings explain a significant fraction of primary aldosteronism and have diagnostic and therapeutic implications.

*Funding:* Other NIH Support - Centers for Mendelian Genomics, Private Foundation Support, Government Support - Non-U.S.

## TH-OR147

**Mutation of Anti-Aging Gene Klotho Causes Hypertension via Upregulation of Adrenal CYP11B2 Expression and Plasma Aldosterone Levels** Zhongjie Sun. *Physiology, Univ of Oklahoma Health Sciences Center.*

**Background:** Klotho is a recently discovered anti-aging gene. The purpose of this experiment is to assess if klotho deficiency affects blood pressure.

**Methods:** One group of KL mutant heterozygeous (+/-) mice and one group of wild-type mice were used.

**Results:** Interestingly, klotho (+/-) mice demonstrated a significant and persistent increase in blood pressure starting form 3-4 months of age, indicating that klotho deficiency causes hypertension. Plasma level of aldosterone was elevated in KL(+/-) mice. Two groups of KL(+/-) mice and 2 groups of wild-type mice were used. When BP was elevated in KL(+/-) mice, 1 group of KL (+/-) mice and 1 group of WT mice were treated with aldosterone receptor blocker, eplerenone (6 mg/kg/day), while the remaining groups received DMSO and serve as controls. Eplerenone decreased hypertension to the control level. Eplerenone also abolished KL deficiency-induced kidney damage (glomerulus collapse, fibrosis). Klotho deficiency causes renal inflammation as evidenced by significant increases in inflammatory cytokines (IL-6, TNFα and MCP-1) and T cell and macrophage infiltration in kidneys of KL (+/-) mice. Eplerenone significantly attenuated KL deficiency-induced inflammation. Notably, NCC and Sgk1 levels were increased in kidneys in KL(+/-) mice, which could be abolished by eplerenone. Further analysis indicated that klotho co-localized with CYP11B2, a key enzyme for aldosterone synthesis, in the adrenal cortex. Klotho deficiency upregulated CYP11B2 protein expression.

**Conclusions:** KL deficiency caused hypertension via upregulating adrenal CYP11B2 protein expression and plasma aldosterone levels which led to inflammation and upregulation of NCC signaling in kidneys.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

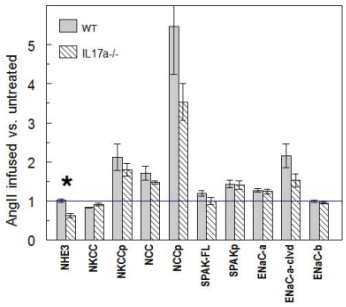
## TH-OR148

**Hypertensive Response to Angiotensin II (Ang II) Infusion Is Blunted in IL-17a$^{-/-}$ Mice Associated with Depressed Na/H Exchanger 3 (NHE3) Abundance** Nikhil Kamat,[1] David G. Harrison,[2] Alicia A. McDonough.[1] *[1]Cell and Neurobiology, Keck School of Medicine of USC, Los Angeles, CA; [2]Vanderbilt Vascular Biology Center, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** Previous studies (Harrison lab), establish that CD8 T cells are essential for the hypertensive response to AngII infusion and that CD8 cells accumulate in the kidney of hypertensive animals where they produce the cytokine IL-17a. AngII infusion hypertension is blunted in IL-17a $^{-/-}$ mice compared to wild type (WT) mice (18 vs. 38 mmHg rise). The aims of this study were to test the hypothesis that the blunted hypertensive response to AngII infusion in IL17a$^{-/-}$ mice is associated with blunted renal $Na^+$ transporter stimulation, and to define the transporter(s) involved.

**Methods:** WT and IL17a$^{-/-}$ mice were infused with AngII (490 ng/kg/min x 14 days), homogenates subjected to quantitative immunoblot analysis of $Na^+$ transporters' abundance, phosphorylation and processing. Density values were normalized to values in WT sham infused mice, defined as 1.00; * = p <0.05.

**Results:** In both WT and IL17a$^{-/-}$ mice, Ang II infusion increased thick ascending limb NKCCp, distal convoluted tubule NCCp, cortical SPAKp and collecting duct ENaCa cleavage, all evidence of transporter activation. However, in IL17a$^{-/-}$, PT NHE3 abundance fell to 62% of that in uninfused IL17a$^{-/-}$.




**Conclusions:** Since depressing NHE3 transport is key to pressure natriuresis, we postulate that AngII infusion stimulates IL17a production in infiltrating CD8 cells which blunts pressure natriuresis and ↑ blood pressure, and that in the IL17a$^{-/-}$ mice pressure natriuresis is enhanced leading to blunted hypertension.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

## TH-OR149

### Loss of Cilia Results in Increased Intra-Renal Angiotensinogen and Mislocalized Prorenin Receptor in the Collecting Duct of a Polycystic Kidney Disease Mouse Model

Takamitsu Saigusa,[1] Yujing Dang,[1] Marlene Amjad Bunni,[1] Stacy Steele,[1] P. Darwin Bell.[1,2] *[1]Nephrology/Medicine, Medical Univ of South Carolina, Charleston, SC; [2]Nephrology/Medicine, Ralph H. Johnson VA Medical Center, Charleston, SC.*

**Background:** The mechanism for early hypertension in polycystic kidney disease (PKD) has not been elucidated. Activation of the intra-renal renin-angiotensin-system (RAS) has been demonstrated from several human PKD studies. For example, angiotensinogen is primarily expressed in cysts of proximal tubule origin and may result in increased downstream intra-tubular Angiotensin II concentrations thus leading to increased distal tubular RAS activity, $Na^+$ reabsorption and elevated blood pressure. However, it is unknown whether primary cilium plays a role in intra-renal RAS in PKD. The purpose of this study was to determine whether loss of cilia results in increased intra-renal RAS in mouse model of PKD.

**Methods:** Adult *ift* 88 conditional floxed allele mice with or without cre were administered tamoxifen to induce cilia loss. Three weeks or 3 months after tamoxifen injection, kidney tissues were examined by histology, immunofluorescence, Western blot and mRNA to assess intra-renal RAS components. SV40 immortalized collecting duct cell lines from hypomorphic *ift*88 mouse were used to assess intra-renal RAS components in collecting duct cells.

**Results:** Loss of cilia significantly increased cyst formation, kidney angiotensinogen protein and mRNA levels but did not alter kidney renin, prorenin receptor, angiotensin-1 receptor expression. There was a trend in higher blood pressure in cilia (-) mice. Immunofluorescence revealed mislocalization of prorenin receptor to the basolateral membrane of principal cells in cilia (-) but not in cilia (+) kidneys. Prorenin was increased in cilia (-) collecting duct cell line and angiotensin II simulation resulted in increased levels of cAMP inhibited by losartan which may contribute to elevated prorenin levels in the collecting duct.

**Conclusions:** These data suggest that in the absence of cilia, there is an upregulation and mislocalization of intra-renal-RAS components, which may contribute to elevated blood pressure in PKD.

*Funding:* Veterans Affairs Support

## TH-OR150

### Renal ACE Causes Salt Sensitivity in Response to Renal Inflammation

Jorge F. Giani,[1] Tea Janjulia,[1] Jorge E. Toblli,[2] Romer Andres Gonzalez-Villalobos.[1] *[1]Depts of Biomedical Sciences and Pathology, Cedars-Sinai Medical Center, Los Angeles, CA; [2]Laboratory of Experimental Medicine, Hospital Alemán, Buenos Aires, Argentina.*

**Background:** Studies emphasize the importance of renal inflammation in causing defective sodium handling by the kidneys, the central feature of salt-sensitive (SS) hypertension. Yet, exactly how inflammation impairs renal sodium excretion is not fully understood. Our previous data show that local Ang II synthesis by renal ACE is a master switch for sodium transport along the nephron. Accordingly, we hypothesized that activation of the renal ACE/Ang II pathway by renal inflammation causes SS hypertension.

**Methods:** We studied the responses of mice lacking renal ACE but with normal circulating ACE (10/10 mice) to post L-NAME hypertension; a model in which the transient exposure to L-NAME triggers substantial renal inflammation and SS hypertension [L-NAME (4 weeks) → Washout (1 week) → high salt diet (HS 4%, 3 weeks)].

**Results:** Wild-type (WT) mice (n=12), previously salt resistant, became salt sensitive after L-NAME exposure. L-NAME increased WT systolic blood pressure (SBP) from 110±2 to 135±3 mmHg ($p<0.01$). SBP returned to baseline during the washout and increased again in response to HS diet (131±3 mmHg; $p<0.01$). Remarkably, ACE 10/10 mice did not develop post L-NAME salt sensitivity. This was despite having equivalent to WT levels of SBP (133±1 mmHg) and renal inflammation (macrophage infiltration, TNFα and IL-6, all by IHC) in response to L-NAME. At the end of HS phase, SBP of ACE 10/10 mice was 106±5 mmHg (NS). In addition, basal renal Ang II level (by IHC) was similar between WT and ACE 10/10. However, sodium load induced an accumulation of renal Ang II that was greater in WT compared to ACE 10/10 (26±2 vs. 13±2 %/area; $p<0.01$). Finally, sodium balance studies revealed an enhanced natriuretic response of ACE 10/10 vs. WT during the HS diet. For instance, sodium excretion after day 1 (before returning to balance) was 1076±67 μmol/day in WT vs. 1353±52 μmol/day in ACE 10/10 mice ($p<0.01$).

**Conclusions:** These results demonstrate that renal ACE is required to increase local Ang II, trigger anti-natriuresis and to induce SS hypertension in response to renal inflammation.

*Funding:* Other NIH Support - R00 DK083455

## TH-OR151

### Role of Collecting Duct Renin in Blood Pressure Regulation

Nirupama Ramkumar,[1] Deborah Stuart,[1] Sara Rees,[1] Curt D. Sigmund,[2] Donald E. Kohan.[1] *[1]Nephrology and Hypertension, Univ of Utah, Salt Lake City, UT; [2]Univ of Iowa, Iowa City, IA.*

**Background:** Recent studies propose that collecting duct (CD) renin is an important modulator of blood pressure regulation, especially in conditions such as angiotensin-II infused hypertension.

**Methods:** We used gene targeting to generate a CD-specific renin knockout (KO) to assess if CD derived renin can regulate BP. Utilizing the Cre lox P system, exon 1 of the renin gene was ablated specifically in the CD. BP was recorded via telemetry and plasma and urine were collected in metabolic cages on normal, high and low Na diets.

**Results:** DNA recombination showed kidney specific recombination in KO mice. Compared to floxed mice, CD renin KO mice had 70 % lower medullary renin mRNA levels and 90% lower renin mRNA in micro-dissected cortical and inner medullary CD tubules. Urinary renin levels were significantly lower in the KO mice on normal and low Na diets (45% of floxed levels) but not with high Na intake. Plasma renin concentration was significantly higher in the KO mice on all three diets. While BP was similar between the two groups on all three diets, infusion of Ang-II delayed the increase in BP in the CD renin KO group for at least 4 days post-infusion.

**Conclusions:** These findings suggest that CD renin likely plays a role in normal BP regulation (evidenced by an increase in PRC) and in response to AngII infusion.

*Funding:* Private Foundation Support

## TH-OR152

### Salt Overload Aggravates Hypertension and Promotes Severe Renal Injury in Rats Subjected to NF-κB Inhibition during Nephrogenesis

Victor F. Avila, Simone C.A. Arias, Orestes Foresto-Neto, Camilla Fanelli, Claudia R. Sena, Vivian L. Viana, Denise M.A.C. Malheiros, José E. Krieger, Roberto Zatz, Clarice K. Fujihara. *Univ of Sao Paulo, Brazil.*

**Background:** We showed recently that NF-κB inhibition by pyrrolidine dithiocarbamate (PDTC) during rat lactation leads to hypertension (HTN) in adult life, with no renal injury. Here we investigated whether challenging this new model with a high-salt diet (HS) would exacerbate HTN and promote renal damage.

**Methods:** Munich-Wistar dams were kept with their offspring (males only, n=49), receiving no treatment or PDTC, 280 mg/kg/day in drinking water during lactation only. The offspring underwent uninephrectomy (UNx) at 10 wks of age, and received normal salt diet (NS) until 12 wks of age, when they were divided into: **C+NS**, control (C); **C+HS**, C receiving 3.2% NaCl chow and 0.5% NaCl to drink; **PDTC$_{Lact}$+NS**, rats that had received neonatal PDTC; **PDTC$_{Lact}$+HS**, PDTC$_{Lact}$ on HS. Tail-cuff pressure (TCP, mmHg), glomerulosclerosis index (GSI), % cortical interstitium (% INT), % injured microvessels (%Vasc), INT macrophage infiltration (MΦ, cells/mm$^2$), plasma renin activity (PRA, ng/mL/h), and INT angiotensin II (AngII, cells/mm$^2$), were assessed 12 wks later.

**Results:**

| | TCP | GSI | %INT | %VASC | MΦ | PRA | AngII |
|---|---|---|---|---|---|---|---|
| C+NS | 144±2 | 3±1 | 1±1 | 0±0 | 33±3 | 2.7±0.6 | 2±1 |
| C+HS | 165±1$^b$ | 14±3 | 1±1 | 4±2 | 55±6 | 0.2±0.1$^b$ | 3±1 |
| PDTC$_{Lact}$+NS | 166±2$^a$ | 2±1 | 1±1 | 1±1 | 35±4 | 2.1±0.4 | 2±1 |
| PDTC$_{Lact}$+HS | 195±3$^{ab}$ | 55±21$^{ab}$ | 6±2$^{ab}$ | 16±6$^{ab}$ | 153±22$^{ab}$ | 0.2±0.1$^b$ | 5±1$^b$ |

Mean±SE, $^a p<0.05$ vs C; $^b p<0.05$ vs. NS)

Moderate HTN was seen in Groups C+HS and PDTC$_{Lact}$+NS, while Group PDTC$_{Lact}$+HS exhibited marked HTN, GS, glomerular thrombi and aneurysms, INT expansion, severe Vasc, intense MΦ infiltration, and INT AngII dissociated from PRA.

**Conclusions:** HS and UNx uncovered a renal dysfunction not apparent in PDTC$_{Lact}$ rats on NS, indicating that an intact NF-κB system is required to enable developing rat kidneys to avoid HTN and deal properly with salt excess in adult life.

FAPESP/CNPq.

*Funding:* Government Support - Non-U.S.

**TH-OR153**

**The Endoplasmic Reticulum Stress Inhibitor, 4-Phenylbutyrate, Reduces Hypertension in the Spontaneously Hypertensive Rat Model of Essential Hypertension** Kaitlyn Werner,[1,2] Victoria Yum,[1,2] Chao Lu,[1,2] Jeffrey G. Dickhout.[1,2] *[1]Medicine, Div of Nephrology, McMaster Univ, Hamilton, Canada; [2]Nephrology, St. Joseph's Healthcare Hamilton, Hamilton, Canada.*

**Background:** We have shown that endoplasmic reticulum (ER) stress is important in angiotensin II-mediated hypertension, and that the low molecular weight chemical chaperone, 4-phenylbutyrate (4-PBA), can inhibit endoplasmic reticulum stress in the kidney via oral dosing. Therefore, we tested the ability of 4-PBA to lower blood pressure in the most frequently used animal model of essential hypertension, the spontaneously hypertensive rat (SHR).

**Methods:** 12 week old male SHRs with established hypertension were used and treated with 4-PBA (1g/kg/day, orally). Blood pressure was measured 1 week before treatment using tail cuff plethysmography and was 181/118 initially. These animals were randomized into either 4-PBA or vehicle treatment groups. Treatment with 4-PBA proceeded for 5 weeks. Aortas and mesenteric resistant vessels were collected after 5 weeks for functional and structural analysis. Final blood pressures were measured directly through carotid artery cannulation.

**Results:** We found 4-PBA treatment significantly lowered blood pressure in the SHR (Vehicle 206.1 ±4.4 vs. 4-PBA 179.0 ±3.1, systolic; vehicle 143.6 ±4.4 vs. 4-PBA 121.2 ±4.3, diastolic) at 5 weeks. This was reflected in both tail cuff and direct blood pressure measurement. Examination of aortic and mesenteric resistant artery contractility found 4-PBA diminished mesenteric resistant artery contractility but had no effect on that of the aorta. 4-PBA treatment also augmented endothelial vasodilation in the resistant vessels, but not the aorta. 4-PBA treatment had no effect on the ability of nitric oxide to evoke vasorelaxation in either mesenteric vessels or the aorta, as shown by sodium nitroprusside treatment. 4-PBA treatment did not change the structure of mesenteric resistant vessels, as shown by three dimensional volume reconstructions.

**Conclusions:** The ER stress inhibitor, 4-PBA, reduces hypertension in the spontaneously hypertensive rat by improving resistant blood vessel endothelial dependent vasodilation without changing smooth muscle mass or vessel structure.

*Funding:* Government Support - Non-U.S.

**TH-OR154**

**Mesenchymal Stem Cells (MSC) Prevent the Progression of Renovascular Hypertension, Improved Renal Function and Architecture** Elizabeth B. Oliveira-Sales,[1] Edgar Maquigussa,[1] Patricia Semedo Kuriki,[1] Luciana Guilhermino Pereira,[1] Vanessa Meira Ferreira,[1] Niels O.S. Camara,[1] Cassia T. Bergamaschi,[2] Ruy Campos,[2] Mirian A. Boim.[1] *[1]Dept of Medicine, Federal Univ of Sao Paulo, Sao Paulo, Brazil; [2]Dept of Physiology, Federal Univ of Sao Paulo, Sao Paulo, Brazil.*

**Background:** Renovascular hypertension induced by 2 Kidney-1 Clip (2K-1C) is a renin-angiotensin-system (RAS)-dependent model, leading to renal vascular rarefaction and renal failure. RAS inhibitors are not able to reduce arterial pressure (AP) and/or preserve the renal function, and thus, alternative therapies are needed.

**Methods:** Three weeks after left renal artery occlusion, fluorescently tagged mesenchymal stem cells (MSC) ($2x10^5$ cells/animal) were injected weekly into the tail vein in 2K-1C hypertensive rats.

**Results:** Flow cytometry showed labeled MSC in the cortex and medulla of the clipped kidney. MSC prevented a further increase in the AP, significantly reduced proteinuria and decreased sympathetic hyperactivity in 2K-1C rats. Renal function parameters were unchanged, except for an increase in urinary volume observed in 2K-1C rats, which was not corrected by MSC. The treatment improved the morphology and decreased the fibrotic areas in the clipped kidney and also significantly reduced renal vascular rarefaction typical of 2K-1C model. Expression levels of IL-1β, TNF-α angiotensinogen, ACE, and Ang II receptor $AT_1$ were elevated, whereas $AT_2$ levels were decreased in the medulla of the clipped kidney. MSC normalized these expression levels.

**Conclusions:** In conclusion, MSC therapy in the 2K-1C model (i) prevented the progressive increase of AP, (ii) improved renal morphology and microvascular rarefaction, (iii) reduced fibrosis, proteinuria and inflammatory cytokines, (iv) suppressed the intrarenal RAS, iv) decreased sympathetic hyperactivity in anesthetized animals and v) MSC were detected at the CNS suggesting that the cells crossed the blood-brain barrier. This therapy may be a promising strategy to treat renovascular hypertension and its renal consequences in the near future.

*Funding:* Government Support - Non-U.S.

**FR-OR001**

**Loss of p47$^{phox}$ Ameliorates Kidney Fibrosis and Proteinuria** Xiwu Chen, Paisit Paueksakon, Ming-Zhi Zhang, Raymond C. Harris, Roy Zent, Ambra Pozzi. *Medicine, Vanderbilt Univ, Nashville, TN.*

**Background:** Reactive oxygen species (ROS) play an important pathogenic role in the development of various diseases, including kidney disease. The major generator of ROS in the glomerulus of the kidney is the NAPDH oxidase complex which consists of five different subunits. Among these subunits, the p47$^{phox}$ is a critical regulatory subunit of NADPH oxidase and its expression is upregulated in the course of renal injury. The contribution of p47$^{phox}$ has been widely investigated in diabetic nephropathy; however whether is also plays a role in non-diabetic-mediated glomerular injury is unclear.

**Methods:** To address this issue, we analyzed the contribution of the p47$^{phox}$ in glomerural disease since it is a critical regulatory subunit of NADPH oxidase. To this end, we crossed the p47$^{phox}$-null mice with integrin α1-null mice, a model of exacerbated NADPH-mediated ROS production and glomerulosclerosis after injury, to generate wild type, p47$^{phox}$-null, integrin α1-null, and p47$^{phox}$-null/integrin α1-null mice. These mice were then subjected to either ROS-dependent (adriamycin) or ROS-independent (partial renal ablation) renal injury and their proteinuria and kidney injury was analyzed over time.

**Results:** We provide evidence that in both models, deletion of p47$^{phox}$ significantly reduced proteinuria and kidney fibrosis, and these effects were more accentuated on the integrin α1-null background. This protection is accompanied by decreased production of free radicals, and consequent glomerular injury. Moreover, *in vitro* analysis of primary mesangial cells isolated from different types of mice revealed that loss of p47$^{phox}$ leads to reduced basal levels of superoxide and collagen production.

**Conclusions:** Our study suggests that the NADPH oxidase is major source of ROS in kidney glomeruli and contributes to kidney injury. Moreover, our study points to the p47$^{phox}$ a potential target for antioxidant therapy in fibrotic disease.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-OR002**

**miR-182 Inhibits FoxO3-Mediated Atrophic Signaling in Muscle, and Glucocorticoid Administration and Acute Diabetes Down-Regulate Muscle miR-182** Matthew B. Hudson,[1] Myra Woodworth-Hobbs,[1,2] Bin Zheng,[1] Jill Rahnert,[1] Harold A. Franch,[1] Russ Price.[1,3] *[1]Renal Div, Dept of Medicine, Emory Univ, Atlanta, GA; [2]Nutrition and Health Sciences Graduate Program, Emory Univ, Atlanta, GA; [3]Atlanta VA Medical Center, Decatur, GA.*

**Background:** Skeletal muscle atrophy occurs in response to a variety of conditions including chronic kidney disease, diabetes, metabolic acidosis, and elevated glucocorticoids. Previous studies demonstrate components of the Forkhead box O (FoxO) pathway are increased in multiple models of skeletal muscle wasting. FoxO transcription factors induce skeletal muscle atrophy by increasing the expression of components for the ubiquitin-proteasome and autophagy-lysosome systems.

**Methods:** To identify potential modulators of the atrophy process, an *in silico* target scan analysis of known microRNAs was performed. miR-182 was predicted to target the FoxO proteins. To test whether miR-182 regulates expression of the FoxOs, C2C12 myotubes were transfected with miR-182 and levels of FoxO1 and FoxO3 protein were evaluated.

**Results:** miR-182 reduced FoxO3 protein 64% ($P<0.05$ vs Con) whereas FoxO1 was unaffected. Treatment of C2C12 myotubes with dexamethasone (1 μM, 6 hr) to induce muscle atrophy decreased miR-182 expression by 46% ($P<0.05$ vs Con). Overexpression of miR-182 prevented the glucocorticoid-induced upregulation of FoxO3 gene targets including MAFbx/Atrogin-1, ATG12, Cathepsin L, and LC3. To determine if miR-182 is altered in an *in vivo* model of muscle atrophy, miR-182 was measured in the gastrocnemius muscle of rats with acute diabetes (3 d) induced by streptozotocin. miR-182 was suppressed by 43% in the gastrocnemius of diabetic mice ($P<0.05$ vs Con).

**Conclusions:** These data identify miR-182 is a new and important regulator of FoxO3-mediated signaling during muscle atrophy induced by catabolic disease states.

*Funding:* NIDDK Support, Other U.S. Government Support, Veterans Affairs Support

**FR-OR003**

**The Paraoxonase PON2 Modifies Lipid Peroxidation at the Slit Diaphragm** Henning Hagmann,[1] Dontscho Kerjaschki,[2] Stuart E. Dryer,[3] Bernhard Schermer,[1] Thomas Benzing,[1] Paul T. Brinkkoetter.[1] *[1]Dept of Nephrology, Univ of Cologne, Cologne, Germany; [2]Dept of Clinical Pathology, Univ of Vienna, Vienna, Austria; [3]Dept of Biochemistry, Univ of Houston, Houston.*

**Background:** The mammalian slit diaphragm is a site of highly active intercellular signalling between adjacent podocytes. Signalling pathways e.g. via TRPC6 depend on the distinct lipid composition of the plasma membrane. Interestingly the signalling-complex at the slit diaphragm shares close homology with the neuronal mechanosensory complex of *C. elegans.* The Podocin homologue MEC-2 orchestrates protein-lipid supercomplexes in the plasma membrane of *C.elegans* touch neurons to allow for proper signalling via the degenerin cation channel MEC-4/-10. Part of the mechanosensory complex is also the lipid modifying enzyme MEC-6, which interacts with MEC-4/-10 and enhances its channel activity.

**Results:** We identified the mammalian homologue of MEC-6, the paraoxonase family protein PON2, as a novel slit diaphragm protein. PON2 localizes to the slit diaphragm in immuno-fluorescence and immunogold-labeling studies. In addition it not only co-fractionates with Podocin in cholesterol-rich detergent resistant membrane domains but also interacts directly with slit diaphragm proteins like Nephrin, Podocin and TRPC6 in pull-down experiments. We were able to show that PON2 is an integral transmembrane protein that localizes to the plasma membrane with its enzymatically active domain facing extracellular. To address the functional role of PON2 in the plasma membrane we employed in vitro enzyme assays with recombinant protein and analysed the lipid profile of PON2-proficient and –deficient cells using mass spectrometry. We found that PON2 affects the content of glycolipids and the overall peroxidation state of membrane lipids.

**Conclusions:** In conclusion we show that PON2 is a novel slit diaphragm protein which modifies the lipid content and peroxidation state of the plasma membrane. It is therefore well conceivable that it is an important regulator of signal transduction e.g. via the cation channel TRPC6.

*Funding:* Private Foundation Support

**FR-OR004**

**Involvement of Nitric Oxide and Endothelin Systems in the Pathogenesis of Endothelial Dysfunction in Diabetic Nephropathy** Niroz Abu-Saleh,[1] Mogher Khamaisi,[2] Hoda Awad,[1] Suheir Assady,[3] Ravit Cohen,[1] Zaher Armaly,[4] Zaid Abassi.[1] *[1]Technion, Haifa, Israel; [2]Joslin Diabetes Center, Boston; [3]Rambam Medical Center, Haifa, Israel; [4]Nazareth Hospital-EMMS, Israel.*

**Background:** Endothelial dysfunction (ED) is a major cause of vascular complications characterizing diabetic nephropathy. Diabetic patients who also exhibit hyperlipidemia suffer from accelerated vascular complications. While the deleterious effects of hyperglycemia (HG) and hyperlipidemia alone on ED is well established, the effects of combined hyperlipidemia and HG has not been thoroughly studied.

**Methods:** We applied cultured human umbilical vein endothelial cell (HUVECs), and LDL receptor knockout mice ($LDLR^{-/-}$) to investigate the mechanisms underlying combined HG and hyperlipidemia-induced ED.Incremental doses of glucose in the presence or absence of oxidized LDL (oxLDL) were added to HUVECs. The status of nitric oxide (NO) and endothelin (ET)-1 systems as well as their signal transduction were assessed after 5 days of HG. The effects of chronic combination of HG and hyperlipidemia on endothelial integrity and function as well as alterations in NO and ET-1 systems in vascular, cardiac and renal tissues were examined in the $LDLR^{-/-}$ and wild type (BALB) mice.

**Results:** HUVEC exposed to HG and ox-LDL displayed enhanced ET-1 production, more than HG or Ox-LDL alone. Overproduction of ET-1 stems from upregulation of endothelin converting enzyme (ECE-1) immunoreactivity. Combination of HG and Ox-LDL dramatically decreased total and activated endothelial NO synthase (eNOS) and NRF2, a transcription factor for various antioxidant enzymes by ~50%. Likewise, $ET_B$ levels decreased by 64%. As compared with normoglycemic $LDLR^{-/-}$ and BALB mice, diabetic $LDLR^{-/-}$ mice displayed a higher blood pressure (135±2 vs. 113±4 and 97±4mmHg, respectively) plasma glucose (308±52.7 vs. 159.2±18.8 and 105±10.51mg%), ET-1 (14.9±0.9 vs. 12.31±2.097 and 9.57±0.497pg/ml) and NO2/NO3 levels (42.3±21.71 vs. 12.65±1.24 and 12.76±2.17μM).

**Conclusions:** Combined diabetes and hyperlipidemia activate the ET system and attenuate eNOS and Nrf2. These findings suggest that perturbations in these systems may contribute to diabetic nephropathy.

**FR-OR005**

**Human Kidney Proximal Tubular Epithelial Cells Are Not Protected against Oxidative Stress by PPARγ Agonists** David M. Small,[1] Christudas Morais,[1] David W. Johnson,[1,2] Glenda C. Gobe.[1] *[1]Centre Kidney Disease Research, Univ of Queensland; [2]Dept Nephrology, PA Hospital, Brisbane, Australia.*

**Background:** Peroxisome proliferator activated receptor-gamma (PPARγ) agonists are renoprotective in diabetic nephropathy but the mechanisms are not well-understood. Mitochondrial dysfunction and oxidative stress contribute to diabetic nephropathy. PPARγ upregulates proteins required for mitochondrial biogenesis. Our aim is to determine the role of PPARγ in protecting kidney proximal tubular epithelium (PTE) against mitochondrial destabilisation and oxidative stress.

**Methods:** HK-2 cells were treated with 0.2-1.0mM hydrogen peroxide ($H_2O_2$) for 2h and 18h. Treated and untreated cells were compared for: apoptosis, mitosis (morphology/biomarkers); cell viability (MTT); superoxide (dihydroethidium /DHE); mitochondrial function (MitoTracker Red; JC-1); ATP (luminescence); and mitochondrial ultrastructure (electron microscopy). Western immunoblotting was used to study PPARγ, phospho-PPARγ, PPARy-coactivator-1α (PGC-1α; mitochondrial biogenesis marker), and pak2, p62 and LC3-II (mitophagy markers). PPARγ agonists (rosiglitazone, pioglitazone, troglitazone) and the inhibitor (GW9662) were used to modulate PPARγ.

**Results:** At 2h and 18h, mitochondrial destabilisation increased with $H_2O_2$ concentration: MitoTracker Red and ATP decreased ($p<0.05$); JC-1 fluorescence altered red→green; and DHE increased (18h; $p<0.05$). Mitochondria were sparse and had disrupted cristae. Pak2 increased, p62 decreased (2h), and p62 and LC3-II increased (18h) (all $p<0.05$) indicating increased defective mitochondrial autophagy. PPARγ expression did not alter but phospho-PPARγ increased and PGC-1α decreased (2h) indicating aberrant PPARγ activation and reduced mitochondrial biogenesis. Cell viability decreased (18h; $p<0.05$), with further cell death when PPARγ agonists were used. PPARγ inhibition had negligible effect.

**Conclusions:** Oxidative stress promoted mitochondrial destabilisation in kidney PTE, in association with PPARγ activation. PPARγ agonists failed to protect the cells. Despite positive effects in other tissues, PPARγ activation appears to be detrimental to kidney PTE health when oxidative stress induces damage.

*Funding:* Government Support - Non-U.S.

**FR-OR006**

**Circulating Oxidized Albumin Is a Mediator of Endothelial Dysfunction in Uremia** Faiga Magzal,[2,3,4] Snait Tamir,[3,5] Andrea Szuchman-Sapir,[3,5] Shifra Sela,[2,4] Batya Kristal.[1,2] *[1]Nephrology and Hypertension Dept, Western Galilee Hospital, Israel; [2]Faculty of Medicine in the Galilee, Bar Ilan Univ, Safed, Israel; [3]Laboratory of Human Health and Nutrition Science, Migal, Israel; [4]Eliachar Research Laboratory, Western Galilee Hospital, Israel; [5]Nutrition Dept, Tel Hai College, Israel.*

**Background:** Oxidative stress and inflammation prevalent among hemodialysis patients underly atherosclerosis and cardiovascular (CV) morbidity and mortality. We suggest that modified human serum albumin (HSA) mediate endothelial dysfunction, a process that may initiate CVD. Our study aims to characterize the modifications of uremic HSA, reveal their effects on endothelial function and establish a "finger-print" of modified HSA as a potential novel tool to predict CVD.

**Methods:** Albumin enriched fraction from 5 healthy controls (HC) and 5 Hemodialysis (HD) patients were evaluated by electrospray ionization mass spectrometry (Q-TOF LC/MS). Human umbilical vein endothelial cells (HUVEC) were exposed to albumin separated from sera of HC or HD. Endothelial mRNA was extracted to determine expression of Interleukin 6 (IL-6), TNF-alpha and e-NOS.

**Results:** We revealed the existence of reversible and irreversible post-translational modifications, present in HC and HD patients. Each group is characterized by an unique profile with some of the post-translational modifications present only in HD patients. Unmodified Albumin showed a molecular mass of 66440.64 ± 0.14 Da. The known post-translational modifications observed were: Cysteinylation (66558.60 ± 0.74 Da), Nytrosylation (664676.80± 2.55 Da), Nitration (66483.57±3.31), Glycation (66601± 0.70 Da) and Sulphenic Acid formation (66655.49 ± 1.49 Da). Exposure of endothelial cells to HAS separated from HD patients resulted in an increase in the cytokine and e-NOS expression by 1.2, 1.5 and 2.4 fold, respectively, in comparison to HAS of HC.

**Conclusions:** We suggest that modified albumin plays a pivotal role in endothelial dysfunction underlying atherosclerosis. Further studies are needed to enable the use of these exclusive modifications as a "finger-print" for evaluation and follow-up of the atherosclerotic process.

*Funding:* Government Support - Non-U.S.

**FR-OR007**

**FGF23 Enhances Nitric Oxide Synthesis, and Reduces Production of Reactive Oxygen Species in Human Endothelial Cells *In Vitro*** Maren Leifheit-Nestler, Jacqueline Haller, Dieter Haffner. *Dept of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Hannover, Germany.*

**Background:** Oxidative stress and uremic toxins are well known risk factors for cardiovascular diseases (CVD), associated with comorbidity of patients with chronic kidney disease (CKD). Patients with CKD exhibit excessive serum concentration of phosphaturic hormone fibroblast growth factor 23 (FGF23) as a result of disordered calcium and phosphate homeostasis. FGF23 binds primarily to FGF-receptor (FGFR) 1 and its cofactor klotho. Klotho is a transmembrane protein and after cleavage the extracellular domain acts as a humoral factor (soluble a-klotho) as well. Klotho has been associated with aging and bone loss and klotho-deficient mice indicate accelerated arteriosclerosis suggesting an influence of klotho on the cardiovascular system.

**Methods:** To examine the impact of FGF23 on endothelial function, human coronary artery endothelial cells (HCAEC) were stimulated with FGF23 in a time- and concentration-dependent manner. Cells were evaluated for protein expression and activation of FGF23, FGFR1-4, klotho and endothelial nitric oxide synthase (eNOS). To analyze endothelial function, the formation of reactive oxygen species (ROS) was detected by fluorescence microscopy and FACS analysis and FGF23 dependent nitric oxide (NO) production was quantified using modified Griess assays.

**Results:** FGF23 receptors 1 and 2 as well as klotho were detected in HCAEC. Incubation of HCAEC with FGF23 (10-100ng/ml) resulted in (i) decreased formation of ROS, (ii) increased NO production, and (iii) enhanced eNOS activity, demonstrated by enhanced phosphorylation of serin1177, in a time-dependent fashion. Incubation of HCAEC with FGF23 for 60 minutes induced the release of soluble a-klotho (3.6-fold; $p<0.05$) in conditioned medium.

**Conclusions:** Vascular endothelial cells are a target of FGF23 as HCAEC express receptors required for FGF23 signaling. FGF23 reduces oxidative stress (i.e. ROS production) and increases eNOS activity, and NO production in cultured HCAEC. This might be at least partly mediated by increased secretion of soluble a-klotho. Thus, FGF23 has a positive impact on oxidative stress in HCAEC in vitro.

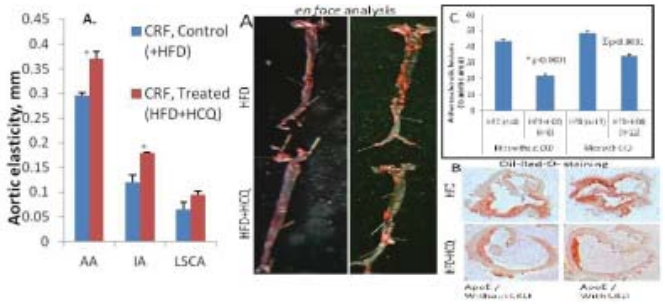
**FR-OR008**

**A Novel Approach for the Management of Cardiovascular Disease in CKD** Ashutosh M. Shukla,[1] Chhanda X. Bose,[2] Oleg K. Karaduta,[2] Sudhir V. Shah.[2] *[1]Div of Nephrology, Univ of Florida & NF/SG VA, Gainesville, FL; [2]Div of Nephrology, Univ of Arkansas for Med. Sci. & CAVHS, Little Rock, AR.*

**Background:** At the present time we lack an effective strategy to manage cardiovascular disease associated with chronic kidney disease(CKD). Atherosclerosis is an inflammatory process and hydroxychloroquine(HCQ) has multiple pleiotropic properties that are anti-inflammatory.

**Methods:** In the present study we examine the effects of HCQ in ApoE-/- mice with CKD. Males 6-8 weeks old ApoE-/- mice(C57BL/6 background) were grouped by 2 x 2 design into those with and without CKD, and those who received HCQ or placebo. CKD state was created by a two-step surgical technique. All mice remained on a high-fat diet for next 16 weeks and uremia was confirmed by serial lab values. Atherosclerosis was assessed through premortem(serial intravital ultrasound echography-IUE & cytokine studies), as well as postmortem studies(pathological-en face staining with Sudan IV of whole aorta and Oil Red O staining of aortic bulb; & immunohistochemical studies).

**Results:** In CKD model, HCQ reduced the progression of atherosclerosis and better preserved the vascular compliance for the aorta(0.37±0.01 Vs 0.29±0.01, $p<0.05$) and major vessels on serial IUE. Histopathological studies at the end of 16 weeks of therapy showed a significant reduction in the area of atherosclerosis(*en face* staining of whole aorta; treatment mice (34.1±3.1%), placebo (48.5±3.1%,$p<0.0001$) as well as the severity of atherosclerosis(Oil Red O examination).




Similar results were seen in non-CKD mice.

**Conclusions:** We conclude that HCQ reduces the extent & severity of atherosclerosis in CKD state & provide evidence for a novel approach toward the therapy of CKD-associated CVD. Additionally, detailed analysis of our data show the importance of nontraditional risk factors in the management of CVD, which may be important even in general population.

*Funding:* Other NIH Support - CTSA UAMS, Veterans Affairs Support

## FR-OR009

### Childhood Chronic Kidney Disease (CKD) Impairs Normal Vasoprotective Functions of High Density Lipoprotein (HDL)

Ryohei Kaseda, Kathy L. Jabs, Tracy E. Hunley, Deborah P. Jones, Valentina Kon. *Vanderbilt Univ.*

**Background:** HDL's beneficial effects encompass not only reverse cholesterol transport relevant to atherosclerosis but also modulation of inflammatory cells and protection of the endothelium relevant to vasculopathy. Since early intervention can impact CKD complications later in life, we evaluated if different degrees of CKD occurring in childhood disrupt these newly appreciated vasoprotective functions of HDL.

**Methods:** Inflammatory and chemotactic effects were assessed in THP-1 cells exposed to HDL isolated from children with end stage renal damage on peritoneal dialysis (ESRD-PD, n=14), CKD (stage III-IV, n=15) and normal kidney function (Control, n=7). Cytokine response of LPS-activated THP-1 cells ± HDL was assessed by mRNA expression of inflammatory markers. Chemotaxis of THP-1 cells to monocyte chemoattractant protein-1 (MCP-1) was assessed in a transwell chamber. THP-1 cells labeled calcein adhesion to HUVEC was assessed by fluorescence and microscopy.

**Results:** HDL of CKD and ESRD-PD heightened the inflammatory cytokine response: TNF-α: Control:0.47±0.04, CKD:1.03±0.08*, ESRD-PD:0.76±0.08*; MCP-1: Control:0.85±0.05, CKD:2.12±0.15*, ESRD-PD:1.70±0.19*; IL-1β: Control:1.41±0.20, CKD:2.60±0.24*, ESRD-PD:2.31±0.36#, (*P<0.01 and #P<0.05 vs Control). Control HDL reduced chemotaxis of THP-1 while HDL from CKD and ESRD-PD had no beneficial effect (ratio compared to medium: MCP-1:2.0±0.2, Control:0.9±0.2, CKD:1.9±0.2*, ESRD-PD:1.7±0.3#). HDL of CKD and ESRD-PD has reduced ability to protect HUVEC against macrophage adhesion (%decrease: Control:-7.9±2.3, CKD:-1.3±1.2*, ESRD-PD:-0.7±1.6*).

**Conclusions:** Even in the absence of long-standing concomitant diseases/risk factors prevalent in adults such as diabetes, obesity and smoking, children with CKD have HDL that dramatically increases the inflammatory and chemotactic response of macrophages while impairing its endothelial protective ability. The impairment is evident even pre-dialysis, in children with moderate CKD. The results indicate that CKD per se causes dysfunctional HDL. Dysfunctional HDL is thus an early therapeutic target that may forestall subsequent vascular complication of CKD.

*Funding:* NIDDK Support

## FR-OR010

### Podocyte B7-1 Inhibition as a Therapeutic Strategy for Diabetic Nephropathy

Roberto Bassi,[1] Andrea Vergani,[1] Monika A. Niewczas,[2] Marcus G. Pezzolesi,[2] Melissa Chin,[1] Maria Pia Rastaldi,[3] Jochen Reiser,[4] Andrzej S. Krolewski,[2] Peter H. Mundel,[5] Mohamed H. Sayegh,[1] Paolo Fiorina.[1] *[1]Boston Children's Hospital; [2]Joslin Diabetes Center, Boston; [3]Ospedale Maggiore Policlinico, Milan, Italy; [4]Rush Univ, Chicago; [5]Massachusetts General Hospital, Charlestown.*

**Background:** Glomerular podocytes, damaged during diabetic nephropathy (DN) progression, may express B7-1 under stress. We hypothesize that B7-1 upregulation leads to podocyte alteration and that B7.1-targeting (CTLA4-Ig) may protect podocytes from high glucose-mediated injuries.

**Methods:** Kidney biopsies were obtained from individuals with T2DN (n=30). 173 T2DN cases were screened for B7-1 single-nucleotide polymorphisms (SNPs). Epidemiologic studies were performed on the Joslin Clinic cohort of T2D and soluble (s) CD28 (B7-1 ligand) was measured by Platinum ELISA. Podocytes were cultured at 10mM (normal glucose) and 30 mM (high glucose) for 7, 14 days and treated with CTLA4-Ig at 100 μg/ml. Mice were treated with CTLA4-Ig using 500 μg at day 0 and 250 μg at day 2, 4, 6, 8, 10; 250 μg twice a week thereafter.

**Results:** Kidney biopsies from T2D patients showed glomerular B7-1 upregulation compared to controls. Two SNPs at the *B7-1* gene were associated with progression to end stage renal disease (ESRD; OR=1.56, p=0.008) and sCD28 serum baseline levels predicted ESRD progression in T2D patients. B7-1 was upregulated *in vitro* in murine podocytes when cultured in high-glucose and *in vivo* on glomerular podocytes of diabetic db/db and streptozotocined (STZ)-C57BL/6 mice. Pharmacological targeting of B7-1 with CTLA4-Ig, protected podocytes *in vitro* from high glucose-induced damage (deregulation of podocyte-specific cytoskeleton proteins) and CTLA4-Ig treatment of db/db and STZ-C57BL/6 mice was able to prevent urinary albumin excretion rise (UAE; db/db, STZ-C57Bl/6: untreated 7wks vs. 25wks; p<0.01, p<0.0001; CTLA4-Ig-treated 7wks vs. 25wks; p=ns) and glomerular alterations such as mesangial expansion and collagen I deposition.

**Conclusions:** Targeting B7-1 with CTLA4-Ig (clinically available as Abatacept) is a novel therapeutic strategy for diabetic nephropathy.

*Funding:* Private Foundation Support

## FR-OR011

### A Randomized, Double-Blind, Placebo Controlled Trial of FG-4592 for Correction of Anemia in Subjects with Chronic Kidney Disease in China

Jia Qi Qian,[1] Nan Chen,[2] Jianghua Chen,[3] Chuanming Hao,[4] Hong Li Lin,[5] Dalvin Ni,[6] Cameron S. Liu,[6] Thomas B. Neff,[6] Kin-Hung Peony Yu,[6] Frank H. Valone.[6] *[1]Renji Hosp., Shanghai; [2]Ruijin Hosp., Jiaotong Univ, Shanghai; [3]Zhejiang Univ, Hangzhou; [4]Huashan Hosp., Shanghai; [5]Dalian Med. Univ, Dalian; [6]FibroGen, Inc., San Francisco, CA.*

**Background:** FG-4592 is an oral inhibitor of hypoxia-inducible factor degradation being developed for treatment of anemia. This Phase 2b study tested the efficacy and safety of FG-4592 in anemic chronic kidney disease (CKD) subjects.

**Methods:** CKD subjects not on dialysis with hemoglobin (Hb) <10 g/dL were randomized 1:1:1 to FG-4592 low or high dose or placebo orally 3 times a week (wk) for 8 wks. The primary efficacy endpoint was maximum change of Hb ($\Delta Hb_{max}$) from baseline (BL) by Wk 9.

**Results:** 91 subjects enrolled, 90 completed 8 wks dosing; 88 were evaluable for efficacy. BL characteristics for FG-4592 and placebo groups are comparable. In FG-4592-treated subjects, Hb increased from BL in a dose-related fashion. The proportion of Hb responders (ΔHb >1 g/dL from BL) and subjects achieving Hb ≥11.0 g/dL was significantly higher in FG-4592 vs placebo subjects (Table). Significant decreases in total and low-density lipoprotein cholesterol (ΔTC & ΔLDL-C) occurred in FG-4592 subjects. The incidences of treatment emergent adverse events (AEs) and serious AEs (SAEs) were nearly identical for FG-4592-treated and placebo subjects. AEs were generally mild or moderate and typical for CKD patients.

| Cohort | 1 | 2 | FG-4592 | Placebo |
|---|---|---|---|---|
| N | 30 | 31 | 61 | 30 |
| Mean (SD) dose, mg/kg/Wk | 4.16 (0.52) | 5.03 (0.67) | 4.60 (0.74) | - |
| BL Hb, g/dL | 8.82 | 8.84 | 8.83 | 8.93 |
| Hb @9 wks, g/dL | 10.74# | 11.38# | 11.07# | 9.4 |
| $\Delta Hb_{max}$, g/dL | 1.75# | 2.69# | 2.22# | 0.57 |
| Hb responders (%) | 24 (80)# | 27 (93.1)# | 51 (86.4)# | 7 (24.2) |
| Subjects with Wk 9 Hb ≥11.0 g/dL (%) | 15 (50)^ | 22 (75.9)# | 37 (62.7)# | 2 (6.9) |
| ΔTC, mg/dL (%) | -32 [-19.7]# | -35 [-17.5]# | -33.9 [-18.5]# | +8 (+5.5) |
| ΔLDL-Cmg/dL, (%) | -23.7[-23.2]# | -32[-23.2]# | -27.9[ -23.2]# | +4 |
| Subjects w/ AEs (%) | 17 (56.7) | 19 (61.3) | 36 (59.0) | 19 (63.3) |
| Subjects w/ SAEs (%) | 4 (13.3) | 5 (16.1) | 9 (14.8) | 4 (13.3) |
| Subjects w/ cardiovascular SAE (%) | 0 | 0 | 0 | 1 (3.3) |

^p<0.01, #p < 0.0001

**Conclusions:** This study suggests that FG-4592 effectively corrects CKD anemia and is well-tolerated in CKD patients. FG-4592 may also lower cholesterol. P3 trials of FG-4592 in CKD patients are underway.

*Funding:* Pharmaceutical Company Support - FibroGen, Inc.

## FR-OR012

### Association between Sleep Quality and Cardiovascular Damage in Pre-Dialiysis Patients with Chronic Kidney Disease

Jun Zhang.[1] *[1]Div of Nephrology, Dept of Medicine, 3rd Hospital of Sun Yat-sen Univ, China.*

**Background:** Poor sleep quality, a novel risk factor of cardiovascular diseases (CVD), is highly prevalent in patients with chronic kidney disease (CKD). However, the association between poor sleep quality and cardiovascular damage is unclear. This study aimed to determine the relationship between sleep quality and cardiovascular damage and assess the prevalence and related risk factors of sleep disturbance among in Chinese patients with pre-dialysis CKD.

**Methods:** A total of 427 pre-dialysis CKD patients (mean age = 39±15 years, 260 male/167 female) were recruited in this study. Demographics and clinical correlates, including BMI and eGFR etc, were collected. The sleep quality was measured by Pittsburgh Sleep Quality Index (PSQI), while cardiovascular damage indicators (E/A ratio and left ventricular mass index (LVMI)) were determined by echocardiographic examination.

**Results:** 77.8% of the CKD patients were poor sleeper as defined by a PSQI score > 5. Logistic regression analysis revealed that left ventricular hypertrophy (LVH) were independently associated with PSQI score (OR = 1.092, 95% CI = 1.011-1.179, p=0.025), after adjustment for age, sex, and clinic systolic blood pressure, diastolic blood pressure, P, iPTH, HGB, eGFR. Linear regression analysis showed that E/A ratio were independently associated with PSQI score (β=-0.118, P=0.014) after adjusted for a series of potential confounding factors.

**Conclusions:** Poor sleep quality, which is commonly found in pre-dialysis CKD patients, is an independent correlated factor of cardiovascular damage in CKD patients. Our finding implies that the association between poor sleep and CVD might be mediated by cardiac remodeling and improvement of sleep quality might reverse this abnormality and subsequent CVD event.

**FR-OR013**

**A Systematic Exploration of Individual Haemodialysis Patient Prefrences for Treatment Outcome: Symptoms or Survival** Zoe C.L. Pittman,[1] Chris W. McIntyre.[1,2] *[1]Royal Derby Hospital, United Kingdom; [2]Univ of Nottingham, United Kingdom.*

**Background:** In many chronic diseases, patients treatment outcome preferences vary between symptom control and survival. This has not been formerly investigated in kidney disease. We therefore aimed to systematically explore haemodialysis (HD) patients' outcome preferences, utilising established methodology to compare and quantify the subconscious trade off in decision making.

**Methods:** Prevalent HD patients completed a full profile conjoint analysis outcome preference data set comprising 25 proposed treatment outcome profiles each scored for overall acceptability. All profiles contained the same 6 attributes (pain, tiredness, breathlessness, depression, survival and treatment burden (TB)), variable across 4 levels of severity. Standard conjoint analysis techniques were used to derive part worths (utilities) and importance weights for each attribute, allowing the relative contribution of each attribute to be determined per individual. Additional demographic data were collected.

**Results:** 22 patients were studied, mean age 63±15 years, 59% male, 52% diabetic. Overall importance weights (%) were higher for survival (20.6), pain (20.3) and breathlessness (18.2), than for tiredness (14.4), depression (13.7) or TB (12.8). Pain ranked 1st or 2nd in 15/22, survival 8, breathlessness 6, tiredness 5, depression 4, and TB 3. Reverse utility scores (indicating little or no influence over the decision), occurred for TB (9), depression (5), pain (3), survival (2) and breathlessness (2) but not for tiredness. There was clear separation in preference patterns between aversion to worsening symptoms with indifference to survival and/or indifference to increased TB compared to those patients that had a predominant preference for survival.

**Conclusions:** There are a variety of different outcome preferences in this cohort however the majority of patients showed a preference for symptom control, as opposed to survival, and were able to consider increased treatment burden in order to achieve this. Understanding of individual patient preferences for treatment outcome may allow a more informed discussion about changes to therapy, including potential for increased uptake of more intensive therapy.

**FR-OR014**

**Cross-Sectional Study of Hyponatremia Associated with Prescribed Medications in Chronic Kidney Disease** Diana Chiu, Darren Green, Smeeta Sinha, Philip A. Kalra. *Salford Vascular Research Group, Univ of Manchester, United Kingdom.*

**Background:** Medications prescribed to patients with Chronic Kidney Disease(CKD) may contribute to hyponatremia.We sought to explore which prescribed medication is most frequently associated with hyponatremia in this setting.

**Methods:** Cross-sectional baseline analysis of hyponatremia(<135mmol/l) and concomitant medication of patients enrolled in the Chronic Renal Insufficiency Standards Implementation Study(CRISIS),a prospective observational outcome study in all-cause CKD stages 3-5,managed in secondary care,was undertaken.We examined the association of drugs known to cause hyponatremia in a multivariate logistic regression(LR)adjusting for diabetes,eGFR,history of heart failure and liver disease.

**Results:** From 2093 patients,63% male,32% diabetic and mean eGFR was 33±16ml/min/1.72m$^2$.Mean sodium was 140±3mmol/l and 4.6% had hyponatremia(mean sodium was 132±2mmol/l,range 125-134mmol/l).Diuretics were associated with hyponatremia(OR 1.56,P=0.04),but when separated into loop(OR 1.20,P=0.40),thiazide(OR 1.31,P=0.36) and potassium sparing diuretics(spironolactone and amiloride),only the latter were statistically significant(OR 3.67,P<0.01).Proton pump inhibitors and Angiotensin receptor blockers were associated with hyponatremia whilst ACE inhibitors were not (OR 0.77, P=0.22). Significant predictors in the final LR model, are shown in the table.

| Medication | No. on medication | Odds Ratio | 95% CI | P value |
|---|---|---|---|---|
| Potassium sparing diuretic | 88 | 3.67 | 1.92-7.08 | <0.01 |
| Proton pump inhibitor | 538 | 1.67 | 1.08-2.56 | 0.02 |
| Angiotensin receptor blocker(ARB) | 550 | 1.72 | 1.11-2.65 | 0.01 |

Loop diuretics,thiazides,SSRIs,tricyclics,ACE inhibitors,sulphonylurea,sodium valproate and lamotrigine,benzodiazepines and thiazolidinediones were not statistically significant in the final model.

**Conclusions:** Potassium sparing diuretics,proton pump inhibitors and ARBs have important associations with hyponatremia in CKD.It is well known that spironolactone,amiloride and ARBs are associated with hyperkalemia,but their association with hyponatremia should not be ignored.The validity and nature of these relationships needs to be explored further.

**FR-OR015**

**Proteomic Analysis of the Plasma in Chronic Kidney Disease Related with Cardiovascular Disease** Maria Wanic-Kossowska,[3] Magdalena Luczak,[1] Dorota Formanowicz,[2] Elzbieta Pawliczak,[3] Lidia Koziol.[3] *[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland; [2]Dept of Clinical Biochemistry, Poznan Univ of Medical Sciences; [3]Dept of Nephrology, Transplantology and Internal Medicine, Poznan Univ of Medical Sciences.*

**Background:** Atherosclerosis is the most serious complications in chronic kidney disease (CKD) and the risk of cardiovascular events increase with degree of reduced renal function. However, molecular mechanisms that enhance the formation of plaque in CKD patients are still unclear. The major goal of this study was global analysis of plasma proteome to better understanding of molecular mechanisms of relation between cardiovascular disease (CVD) and chronic kidney disease (CKD).

**Methods:** We used 2DE and MS methods to study changes in the profiles of plasma protein accumulation during development of CKD. Study involved healthy volunteers and four group of patients: patients in stage 1-2CKD; 3-4CKD and 5CKD and patients with classical CVD with unstable coronary artery disease and after myocardial infarction, but without clinical signs of renal dysfunction. We analyzed three different plasma protein sets: high-abundant, low-abundant proteins and also low molecular proteins (?5kDa) to find differentially expressed proteins. The proteins were identified by MALDI/TOF-TOF mass spectrometry. Differentially expressed proteins were confirmed by selected reaction monitoring (SRM) analysis.

**Results:** Identified 13 proteins were used to analyze the functional interactions of all identified differential proteins. Created on this basis network revealed that at least three functional nodes are related with CKD and CVD: blood coagulation cascade, metabolism of lipoproteins and inflammatory processes.

**Conclusions:** Differential concentration of identified proteins in CKD and CVD indicate that different molecular mechanisms are involved in the development of CKD- and CVD-related atherosclerosis. These results suggest that CKDA is highly accelerated by the inflammatory processes compare to classical CVD which seems to be more affected by defects in cholesterol transport or metabolism.

**FR-OR016**

**Effects of Resistance Exercise on Akt Signalling and Protein Degradation in Chronic Kidney Disease** Emma Watson, Neil J. Greening, Nichakarn Ruttanaporn, Jonathan Barratt, Alice C. Smith. *Leicester Kidney Exercise Team, Univ of Leicester, United Kingdom.*

**Background:** Resistance exercise (RE) is anabolic in healthy individuals but its potential to overcome muscle wasting in Chronic Kidney Disease (CKD) has not been well studied. Akt is an important regulator of cellular survival via promotion of protein synthesis and inhibition of protein breakdown. In health, skeletal muscle Akt phosphorylation and protein synthesis are upregulated for 48 hours after RE. MAFbx and MuRF-1 are E3 ligases playing a key role in the regulation of muscle protein degradation. This study investigated molecular responses to RE in pre-dialysis CKD.

**Methods:** 18 patients CKD3b-4 (mean eGFR 23, range 16-36ml/min/1.73m$^2$; mean age 63, range 45-77years) were randomised to RE (3 sets 10-12 leg extensions at 70% maximum, 3/week for 8 weeks;n=11), or control (usual activity;n=7). Quadriceps cross-sectional area (CSA) was measured by ultrasonography at baseline (BL) and 8 weeks. Muscle biopsies were obtained at BL and 8 weeks in controls, and BL, 24h post first training session (PRE-RT) and 24h post final training session (POST-RT) in exercisers. Akt phosphorylation, MAFbx and MuRF-1 mRNA expression were analysed by Western blotting and RT-PCR.

**Results:** No changes were seen in the controls. In exercisers, 8 weeks RE significantly increased quadriceps CSA (P=0.001). After the first RE session, the normal Akt phosphorylation response failed to occur. In contrast, the expected increase was seen after the final RE session (BLvsPOST-RT P=0.04). Of the protein breakdown regulators, MuRF-1 initially increased in response to RT (BLvsPRE-RT P=0.02). However, after 8 weeks of RE, MAFbx fell below BL (BLvsPOST-RT P=0.004).

**Conclusions:** This study reveals a previously unknown failure of RE-stimulated Akt phosphorylation in CKD, which may be a novel contributor to muscle wasting. However, eight weeks RE restored Akt signalling, suppressed mRNA expression of E3 ligases MAFbx and MuRF-1, resulting in significant muscle hypertrophy. These results demonstrate that RE is effective in modifying molecular abnormalities in skeletal muscle in CKD and can overcome muscle wasting, which has important clinical implications in this population.

**FR-OR017**

**Increased Urine SEMAPHORIN 3A Is Associated with Renal Damage in Hypertensive and Diabetic Patients with Chronic Kidney Disease** Ganesan Ramesh,[2] Calpurnia Jayakumar,[2] Francesca Viazzi,[1] Giovanna Leoncini,[1] Debora Garneri,[1] Roberto Pontremoli.[1] *[1]Medicine, Univ of Genoa, Italy; [2]Vascular Biology Center, Georgia Regents Univ, Augusta, GA.*

**Background:** Semaphorins are guidance proteins that influence cellular morphology and function, and have been implicated in several pathophysiological processes such as angiogenesis, organogenesis, tumourogenesis, cell migration, cytokine release and immune modulation. Semaphorin3a (SEMA3A) administration in animals induces acute and transient massive proteinuria through distruption of podocyte foot process. However,its role in human disease has not been investigated so far.

**Methods:** To determine the role of sema3A in CKD, we performed a cross-sectional, nested, case-control study on a group of 151 age, gender, and body mass index (BMI) matched hypertensive patients with and without chronic kidney disease (CKD) or diabetes mellitus. SEMA3A was quantified in the urine by ELISA. CKD and its components, i.e., estimated glomerular filtration rate (eGFR) and albuminuria were measured by CKD EPI formula and albumin creatinine ratio (ACR), respectively. Data are expressed as mean ± SD. Statistical analysis was done using Statview for Windows.

**Results:** USEMA3A levels were positively correlated with urine albumin creatinine ratio (UACR) (P= 0.0038) and serum creatinine (P= 0.0013). USEMA 3A was significantly higher in patients with both components of renal damage as compared to those with only one and those with normal renal function (P<0.008 and <0.0001, respectively). In a logistic multiple regression analysis the presence of increased urine SEMA3A levels (i.e. top quartile) entailed a 14 fold (OR 95% CI 3.2-63, P <0.0005) higher risk of combined renal damage and at least a 5 fold higher risk of macroalbuminuria or of reduced eGFR even taking into consideration potential confounding factors such as serum uric acid (P<0.0079 and 0.0034, respectively).

**Conclusions:** This study demonstrates that USEMA 3A is significantly associated with renal damage both in diabetic and non diabetic hypertensive patients. USEMA 3A levels appear to be a marker of renal dysfunction, independent of eGFR, albuminuria and serum uric acid.

*Funding:* NIDDK Support

## FR-OR018

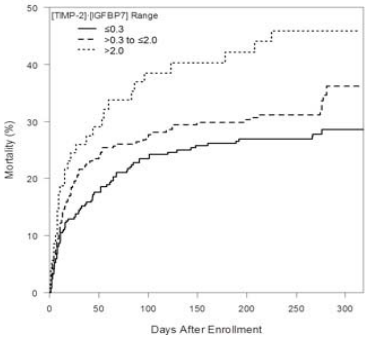
### Increased TIMP2•IGFBP7 Is Associated with Increased 9 Month Mortality in ICU Patients at Risk for AKI

Jay L. Koyner, Andrew Shaw, Lakhmir S. Chawla, Eric Hoste, Azra Bihorac, Kianoush Banaei-Kashani, Jing Shi, John A. Kellum. *On behalf of All Astute Medical Sapphire Investigators.*

**Background:** We recently reported a 728 patient multicenter study (Sapphire) where Insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2), were validated for risk stratification for moderate to severe Acute Kidney Injury (AKI, KDIGO stages 2 and 3).

**Methods:** We prospectively selected 2 clinical cutoffs for the TIMP2•IGFBP7 panel from the Sapphire dataset, one (0.3) with high sensitivity, 89% (specificity=50%) and one (2.0) with high specificity, 95%, (sensitivity 42%) for the development of KDIGO 2/3 within 12 hours of study enrollment. We then compared 9-month all cause mortality stratified by these cutoffs using a log rank test. A Cox proportional hazards model was used in which both TIMP2•IGFBP7 and serum creatinine were included as continuous variables.

**Results:** Baseline TIMP2•IGFBP7 results were available for 715 patients. KDIGO 2/3 occurred in 9 of 342 (2.6%) patients in the ≤ 0.3 cohort; 31 of 302 (10%) patients in the 0.3-2.0 cohort and 28 of 71 (39%) patients in the >2.0 cohort. Nine months following study enrollment 208 patients (29% of total) had died.




Nine-month mortality was significantly increased as TIMP2•IGFBP7 increased across the 3 strata (p=0.013). In the Cox model after adjusting for baseline creatinine, TIMP2•IGFBP7 remained significantly associated with 9 month mortality (p=0.018). Similarly, the >2.0 cohort had an increased risk of mortality compared to the ≤0.3 cohort, with a hazard ratio (95% CI) of 1.84(1.21-2.78), p=0.004. This risk was only slightly attenuated after adjusting for serum creatinine, HR=1.68(1.10-2.58), p=0.017.

**Conclusions:** We conclude that the TIMP2•IGFBP7 biomarker combination stratifies AKI severity and identifies patients with increased risk of mortality over the next 9 months.

*Funding:* Pharmaceutical Company Support - Astute Medical

## FR-OR019

### Renal Angina in the Pediatric Intensive Care Unit: The CHERUB Prospective Study

Shina Menon, Stuart Goldstein, Rajit K. Basu. *Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

**Background:** Delay in recognition of acute kidney injury(AKI) and inability to predict severe AKI delays institution of optimal therapy. We previously showed that fulfillment of renal angina(ANG) by renal angina index(RAI), a composite of AKI risk and early signs of injury, stratifies patients (pts) at risk for persistent AKI. In our multi-center retrospective study, RAI>8 (ANG+) in first 24 hours of pediatric intensive care unit(PICU) admission showed greater precision to identify pts with severe persistent AKI(doubling of serum creatinine) 3 days after assessment (Day3-AKI) than risk factors or signs of injury alone. We aimed to determine if fulfillment of ANG on PICU admission had greater predictive precision for Day 3–AKI than absence of ANG in this study of AKI in critically ill CHildren Evaluated by Renal angina and Urinary Biomarkers(CHERUB).

**Methods:** ANG was assessed in pts admitted to the PICU. 'Day0' was defined as the first 8 hours for admission after 12 pm or first 24 hours for admission prior to 12pm. RAI was calculated as previously described. Our primary outcome was Day 3–AKI.

**Results:** 94 pts(age 8.8+7 yrs) were enrolled from Sept 2012 to May 2013. 36 pts(38%) were ANG+ on Day 0.The incidence of Day 3-AKI was 30%. Day 3-AKI was higher for ANG+ pts and the area under the curve(AUC-ROC) for Day 3-AKI was 0.75(0.64-0.85). Negative predictive value for ANG+ was 80%(69-89). ANG+ pts had higher morbidity and mortality than ANG-.

| | ANG-(n=58) | ANG+(n=36) | p |
|---|---|---|---|
| Day 3-AKI | 12 (20%) | 16 (44%) | 0.01 |
| +LR for Day3-AKI | 0.51(0.29-0.87) | 1.89(1.16-3.07) | |
| -LR for no Day3-AKI | 2.21(1.38-3.54) | 0.61(0.39-0.97) | |
| ICU LOS | 8.2+6.6 | 10.2+9.8 | 0.07 |
| Organ Failure Days | 3.7+4.2 | 6.2+6.1 | 0.03 |
| RRT provision | 2(3%) | 6(17%) | 0.03 |
| ICU mortality | 1(2%) | 7(21%) | 0.001 |

**Conclusions:** In our initial analysis of the first prospective CHERUB study, ANG+ discrimination for subsequent severe AKI was superior to ANG-. Given a high negative predictive value of ANG for not progressing to severe persistent AKI, we suggest using ANG as a clinical adjunct to target biomarker testing. We plan to enroll more patients and incorporate biomarkers to validate our results and determine if biomarkers add to the prediction of AKI.

## FR-OR020

### Insulin-Like Growth Factor-Binding Protein 7, and Tissue Inhibitor of Metalloproteinases-2 Are Significantly Associated with 18 Month Decline in eGFR

Kianoush Banaei-Kashani,[1] John A. Kellum.[2] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Critical Care Medicine, Univ of Pittsburgh, School of Medicine, Pittsburgh, PA.*

**Background:** We recently reported Insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2) as validated markers for risk stratification for moderate to severe Acute Kidney Injury. We now report the 18-month follow up outcomes of the Mayo Clinic discovery cohort.

**Methods:** We enrolled 285 critically ill patients without known AKI and followed this cohort for 18 months for mortality and eGFR. Enrollment TIMP2•IGFBP7 was measured by immunoassay and was analyzed with respect to outcomes in a Cox proportional hazards model.

**Results:** In this cohort of 285 patients 162 (57%) were male, 267 (94%) were Caucasian and median age was 66 years (IQR 56-76). Incidence of moderate to severe AKI (RIFLE I-F) was 53 (19%) and 6 (2%), respectively. Eighty (28%) patients died within 18 months and 105 patients had at least one follow-up serum creatinine value measured between 3 and 18 months after enrollment. Of these patients with follow-up creatinine, 23 (22%) had at least a 25% drop in eGFR compared with baseline. Hazard ratio (HR) for incurring a 25% or greater decline in eGFR for the patients who had at least one serum creatinine measured more than three months after enrollment was significant with (HR, 2.52; 95% CI, 1.1-5.75, p 0.03) or without (HR, 2.49; 95% CI, 1.17-5.34, p=0.018) adjustment for enrollment sCr for each unit increment in the enrollment TIMP2•IGFBP7. HR for mortality was in the same direction but did not reach significance with (HR, 1.44; 95% CI 0.97-2.11, p 0.067) or without (HR, 1.42; 95% CI 0.97-2.08, p=0.074) adjustment for enrollment sCr.

**Conclusions:** TIMP2•IGFBP7 predicts worsening of renal function in 18 month follow up. These results show that TIMP2•IGFBP7 provides valuable information about kidney status in acutely ill hospitalized patients that is relevant to long-term health outcomes.

*Funding:* Pharmaceutical Company Support - Astute medical Inc.

## FR-OR021

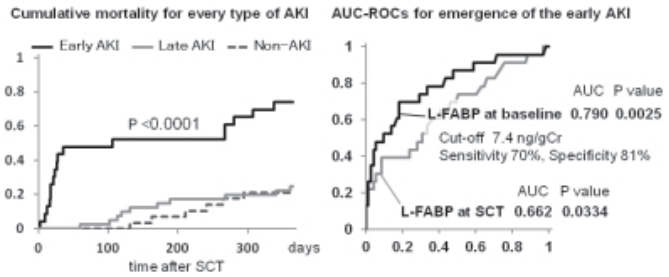
### Acute Kidney Injury prior to the Stem-Cell Engraftment Is a Harbinger of Short-Term Mortality in Hematopoietic Stem Cell Transplantation

Taku Morito,[1,2] Minoru Ando,[1,2] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Renal Div, Dept of Medicine, Tokyo Metropolitan Cancer Center, Komagome Hospital, Bunkyo-ku, Tokyo, Japan; [2]Dept IV of Internal Medicine, Tokyo Women's Medical Univ, Shinjuku-ku, Tokyo, Japan.*

**Background:** AKI that occurs before the stem-cell engraftment may be fatal in allogeneic hematopoietic stem cell transplantation (SCT). Prediction of such AKI may contribute to the improvement of prognosis in SCT recipients.

**Methods:** One-year prospective cohort study was conducted in 94 allogeneic SCT recipients, who had normal kidney function at baseline. Urinary Liver-type fatty acid binding protein (L-FABP) level was measured as a marker of tubular damage before conditioning therapy (baseline), and at days 0 (the morning of SCT). The "AKI prior to the stem-cell engraftment" was defined as the "early AKI" and the subsequently-occurred AKI was as the "late AKI". Cumulative mortality was analyzed by the Kaplan–Meier method. Discriminative ability of L-FABP for emergence of the early AKI was evaluated using AUC-ROC. Multivariate Cox hazards analysis was used to ascertain an association between the "early AKI" and the mortality.

**Results:** The early and late AKI developed in 23 patients (24.5%) and 41 patients (43.6%), respectively. The cumulative mortality of patients with the early AKI was the highest among the 3 groups: 73.9% in the early AKI; 24.7% in the late AKI; and 21.2% in the non-AKI. The emergence of the early AKI was the significant risk for the 1-year mortality (HR 4.02; 95% CI 1.50–12.0; P = 0.0051). The urinary L-FABP level at baseline had good discriminative ability for the early AKI.




**Conclusions:** The early AKI emerges in one-quarter of allogeneic SCT recipients, which has the significant impact on their short-term mortality. Potentially-existing tubular damage, which is identified by urinary L-FABP concentration, may be involved in the emergence of the early AKI.

## FR-OR022

**Development and Standardization of a Furosemide Stress Test to Predict the Severity of Acute Kidney Injury** Ermira Brasha-Mitchell,[1] Danielle Davison,[1] Jay L. Koyner,[2] Andrew Shaw,[3] John M. Arthur,[4] Divya M. Chalikonda,[1] Sharon A. Trevino,[2] Paul L. Kimmel,[5] James A. Tumlin,[6] Michael Seneff,[1] Lakhmir S. Chawla.[1] *[1]George Washington Univ; [2]Univ of Chicago; [3]Duke Univ; [4]Medical Univ of South Carolina; [5]NIH; [6]Univ of Chattanooga.*

**Background:** In the setting of early acute kidney injury (AKI), no test has been shown to definitively predict the progression to more severe stages.

**Methods:** We investigated the ability of a furosemide stress test (FST) (one-time dose of 1.0 or 1.5 mg/kg depending on prior furosemide-exposure) to predict the development of AKIN Stage-III in 2 cohorts of critically ill subjects with early AKI. Cohort 1 was a retrospective cohort who received a FST in the setting of AKI in critically ill subjecs as part of Southern AKI Network. Cohort 2 was a prospective multicenter group of critically ill patients who received the FST in the setting of early AKI.

**Results:** We studied 77 subjects; 23 from cohort 1 and 54 from cohort 2; 25 (32.4%) met the primary endpoint of progression to AKIN-III. Subjects with progressive AKI had significantly lower urine output following FST in each of the first 6 hours (p<0.001). The area under the receiver operator characteristic curves for the total urine output over the first 2 hours following FST to predict progression to AKIN-III was 0.87 (p=0.001). The ideal-cutoff for predicting AKI progression during the first 2 hours following FST was a urine volume of less than 200mls(100ml/hr) with a sensitivity of 87.1% and specificity 84.1%. Furosemide Stress Test Effect on Urine Flow:

| | Combined | Non-Progressors | Progressed to AKIN III | p |
|---|---|---|---|---|
| | *n=77* | *n=52* | *n=25* | |
| **Hour 1 (ml)** | 251(35.2) | 329(46.0) | 89(33.0) | .001 |
| **Hour 2 (ml)** | 296(35.8) | 392(42.2) | 96(46.6) | .001 |
| **Hour 3 (ml)** | 246(26.6) | 311(31.7) | 109(35.4) | .001 |
| **Hour 4 (ml)** | 207(24.1) | 265(31.1) | 88(23.4) | .001 |
| **Hour 5 (ml)** | 175(18.6) | 219(22.8) | 83(23.7) | .001 |
| **Hour 6 (ml)** | 155(17.4) | 194(22.3) | 75(17.4) | .001 |

**Conclusions:** The FST in subjects with early AKI serves as a novel assessment of tubular function with robust predictive capacity to identify patients with severe and progressive AKI. Future studies to validate these findings are warranted.

*Funding:* NIDDK Support

## FR-OR023

**Plasma Kidney Injury Molecule-1 Is a Sensitive and Specific Biomarker of Kidney Proximal Tubule Injury** Venkata Sabbisetti,[1] Sushrut S. Waikar,[1] Daniel J. Antoine,[2] Joseph V. Bonventre.[1] *[1]Dept of Medicine, Renal Div, Brigham and Women's Hospital, Harvard Medical School, Boston; [2]Dept of Molecular & Clinical Pharmacology, Univ of Liverpool, Liverpool, United Kingdom.*

**Background:** Kidney Injury Molecule-1 (KIM-1) is highly upregulated in dedifferentiated proximal tubular cells following kidney injury. The ectodomain of KIM-1 is shed and serves as a urinary biomarker of kidney injury. We report that shed KIM-1 is also leaked into the systemic circulation and serves as a sensitive and specific plasma biomarker of kidney proximal tubule injury.

**Methods:** Human and rodent plasma KIM-1 assays were developed. Mice were subjected to I/R injury for 10, 20, & 30 min and urine, plasma, and tissue specimens were collected for 14 days. Rats were given saline, 50, or 200 mg/kg of gentamicin i.p daily for 10 days. In humans, KIM-1 and creatinine were evaluated in spot urine and corresponding plasma samples from healthy control (HC), patients undergoing cardiac surgery with cardiopulmonary bypass (CPB), and patients admitted to the surgical ICU. To determine the time course of biomarker changes in prospective studies, plasma and urine samples were collected prior to surgery, at the end of CPB, then at 4h, 12h and then daily for 5 days after CPB. Urine and plasma samples were also collected from individuals with chronic kidney disease (CKD).

**Results:** In mice, plasma KIM-1 levels were elevated in a graded fashion with increasing periods of ischemia. In rats, plasma KIM-1 levels increased with gentamicin dose. In humans, plasma KIM-1 levels were higher in patients with acute kidney injury (AKI) than in HC or patients admitted to the ICU without AKI with AUC-ROC of 0.96. Plasma KIM-1 levels were elevated within 2 days (P = 0.008) after surgery in CPB in patients who developed AKI. Plasma KIM-1 levels were also elevated in patients with CKD and correlated positively with stage of CKD.

**Conclusions:** Plasma KIM-1 concentration is elevated in both acute and chronic kidney disease and serves as a novel plasma biomarker of kidney injury. To our knowledge, this is the first demonstration of a plasma biomarker with high specificity for kidney proximal tubule injury in rodents and humans.

*Funding:* Other NIH Support - DK03977, DK072381 , DK075941

## FR-OR024

**Effects of ABT-719 on Incidence of Acute Kidney Injury during Cardiac Surgery Procedures** Samina Khan,[1] Soren Nielsen,[2] Michael Beckert,[3] Mark T. Houser,[1] Deli Wang.[1] *[1]AbbVie; [2]Aarhus Univ, Denmark; [3]CaRACS, Germany.*

**Background:** Patients undergoing on-pump cardiac surgery are at increased risk for ischemia reperfusion (I/R)-induced acute kidney injury (AKI). ABT-719 is a novel melanocortin receptor agonist in development for prevention of I/R-induced AKI. We report here the proportion of patients developing postoperative AKI injury by surgical procedure.

**Methods:** In a double-blind study, patients undergoing on-pump cardiac surgery were randomized to placebo (PBO; n=26), ABT-719 given at either 600μg/kg (n=25), or 800μg/kg (n=26). Each dose was divided into 3 bolus infusions at fixed intervals. AKI was determined according to the AKIN and RIFLE scores.

**Results:** Baseline characteristics were: mean age, 71yrs; males, 64%; pre-existing kidney disease, 53%. Mean overall bypass duration was 2.3hrs and the majority of patients (53%) underwent combined CABG and valve surgery. ABT-719 (800μg/kg) reduced the incidence of AKI, compared with PBO, for the following surgery types: combined CABG and valve, multiple valve, and CABG with chronic kidney disease.

**Conclusions:** 800μg/kg dose of ABT-719 reduced the incidence of AKI in patients undergoing various cardiac surgeries compared to PBO. However, since the sample size of the study is limited, additional studies are in development to further evaluate ABT-719 in patients with a high risk of I/R-induced AKI.

**Table: % of patients with AKI based on AKIN and RIFLE scores with various cardiac surgery procedures**.

| Type of Surgery | PBO | PBO | ABT-719 (600 μg/kg) | ABT-719 (600 μg/kg) | ABT-719 (800 μg/kg) | ABT-719 (800 μg/kg) |
|---|---|---|---|---|---|---|
| | AKIN | RIFLE | AKIN | RIFLE | AKIN | RIFLE |
| Combined CABG and valve, n/N (%) | 11/15 (73) | 11/15 (73) | 14/18 (78) | 14/18 (78) | 2/8 (25) | 3/8 (38) |
| Multiple valve, n/N (%) | 2/4 (50) | 2/4 (50) | 1/1 (100) | 1/1 (100) | 2/5 (40) | 2/5 (40) |
| CKD and CABG or valve, n/N (%) | 4/6 (67) | 4/6 (67) | 2/2 (100) | 2/2 (100) | 3/7 (43) | 4/7 (57) |
| Others[a], n/N (%) | 0/1 | 0/1 | 3/4 (75) | 3/4 (75) | 2/6 (33) | 2/6 (33) |
| [a]Aortic root/aorta, and combined aortic root/aorta and CABG and/or valve cardiac surgeries | | | | | | |

*Funding:* Pharmaceutical Company Support - AbbVie

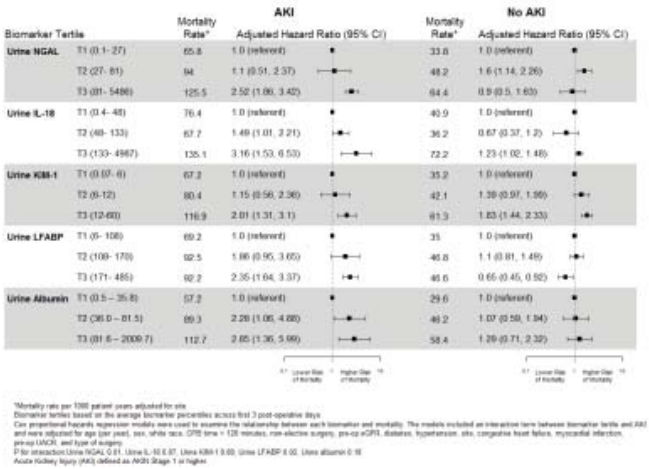
## FR-OR025

**Urinary Biomarkers of Acute Kidney Injury and Mortality 3-Years after Cardiac Surgery** Steven G. Coca, Amit X. Garg, Heather Thiessen Philbrook, Jay L. Koyner, Uptal D. Patel, Harlan M. Krumholz, Michael Shlipak, Chirag R. Parikh. *TRIBE-AKI Consortium.*

**Background:** The association between urinary biomarkers of acute kidney injury (AKI) and outcomes beyond hospital discharge is unknown.

**Methods:** 1199 patients that were discharged aliver after hospitalization for cardiac surgery in the TRIBE-AKI cohort study were followed prospectively for the occurence of death. The primary exposures were peak concentrations of five urinary biomarkers on post-operative days 1-3: neutrophil gelatinase-associated lipocalin (NGAL), interleukin(IL)-18, kidney injury molecule-1 (KIM-1), liver fatty acid binding protein (L-FABP), and albumin. Hazard ratios (HRs) of mortality according to tertiles of the five urinary biomarkers were adjusted for pre-operative clinical variables, cardiopulmonary bypass time, and change in serum creatinine from baseline.

**Results:** During a median follow-up of 3.0 years (IQR 2.2-3.6), 139 (11.6%) participants died (50 per 1000 person-years). Among patients with clinical AKI (serum creatinine change of 50% or 0.3 mg/dL), the upper tertiles of urinary NGAL, IL-18, KIM-1, L-FABP, and albumin were independently associated with a 2.0-3.1-fold higher risk for mortality compared with the lowest tertiles.




In patients without clinical AKI, the upper tertiles of IL-18 and KIM-1 were independently associated with long-term mortality and yielded a continuous net reclassification improvement of 26% and 37%, respectively, for the prediction of 3-year mortality.

**Conclusions:** Urinary biomarkers of kidney injury in the post-operative period provide additional prognostic information beyond that of changes in serum creatinine and clinical variables for risk of 3-year mortality.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

## FR-OR026

**The SAFE-T Approach to Collaborative Kidney Biomarker Qualification**

Patrick T. Murray,[3] Joe F. Keenan,[1] Frank Dieterle.[2] *[1]SAFE-T Consortium, EKF Diagnostics Ltd, Dublin; [2]SAFE-T Consortium, Novartis AG, Basel; [3]SAFE-T Consortium, UCD Mater Hospital, Dublin; [4]SAFE-T Consortium, Charite Hospital, Berlin, Germany; [5]SAFE-T Consortium, AstraZeneca, Wilmington, DE; [6]SAFE-T Consortium, Pfizer Inc, Cabmbridge, MA.*

**Background:** A European consortium (SAFE-T) was established in 2009 with the objective of providing a data submission package to the Health Authorities (FDA & EMA) to allow for the qualification of human kidney biomarkers to monitor drug induced kidney injury. The consortium has performed a first phase exploratory study of a larger list of candidate biomarkers through a number of healthy volunteer and drug induced kidney injury (DIKI) cohorts. Following statistical assessment of these exploratory studies SAFE-T will dismiss certain candidates that do not meet performance criteria and then perform a larger confirmatory DIKI study, the results of which are intended to serve as a convincing submission package for the qualification of a number of key kidney markers with the health authorities.

**Methods:** SAFE-T have established a candidate shortlist of 22 kidney biomarkers assembled from an extensive literature search of 50 biomarkers which were graded using a number of variables (including clinical publications, preclinical evidence, Marker stability, IP, etc.). Assays have been validated at 3 testing sites (the top 10 graded markers using dual technology i.e. both microtitre ELISA and Luminex). A healthy volunteer cohort of samples has already been assessed to establish marker variation and normative ranges. Clinical samples from three clinical studies have been collected and assessed: 1. Cisplatin Nephrotoxicity; 2. Acute Glomerulonephritis (GN); 3. Contrast Media.

**Results:** Performance of all 22 markers in an interim analysis of the key Cisplatin, acute GN and Contrast Media study cohorts as well as the healthy volunteer cohort will be assessed, presented and discussed. SAFE-T will also discuss critical aspects of this biomarker qualification including outlining the decision process for advancing successful biomarkers.

*Funding:* Government Support - Non-U.S.

## FR-OR027

**Does Choice of Renal Replacement Therapy Modality Impact Renal Recovery and Clinical Outcomes in Acute Kidney Injury in the Intensive Care Unit?**

Kelly V. Liang,[1] Raghavan Murugan,[2] Florentina E. Sileanu,[2] Paul M. Palevsky,[1,3] John A. Kellum.[2] *[1]Renal, Univ of Pittsburgh; [2]Critical Care, Univ of Pittsburgh; [3]Renal Section, VA Pittsburgh Healthcare System.*

**Background:** Observational evidence has suggested that renal replacement therapy (RRT) modality may affect renal recovery after acute kidney injury (AKI). However, the Kidney Disease Improving Global Outcomes (KDIGO) guideline recommends choosing RRT modality based on hemodynamics. It is unclear whether initial modality of RRT [intermittent (IRRT) or continuous (CRRT)] affects renal recovery, post-discharge survival, or development of end-stage renal disease (ESRD) in critically ill patients when modality choice is made primarily on hemodynamics.

**Methods:** We examined adults (≥18 years) admitted to University of Pittsburgh Medical Center intensive care units from 2000 to 2008 using the High Density Intensive Care (HiDenIC) electronic database. We defined AKI using the KDIGO definition. We analyzed patients who received RRT for AKI based on initial modality (IRRT vs. CRRT). We used propensity covariate adjustment with model selection and multivariable logistic regression. Renal recovery was being alive and free of RRT. Survival was analyzed from initiation of RRT. ESRD was determined by matching to United States Renal Data System.

**Results:** Out of 37,627 total patients, 22,953 (61.6%) had AKI of whom 5,282 (23.0%) were KDIGO Stage 3 and 1,397 (26.4% of stage 3 patients and 6.1% of all AKI) received RRT. 612 (43.8%) RRT patients survived to hospital discharge (293 CRRT and 319 IRRT). After adjusting for propensity score for CRRT and other covariates, there was no difference in recovery from AKI at 90 or 365 days between CRRT and IRRT (OR 0.88, 95% CI 0.56-1.39, p=0.58 at 90 days; OR 0.92, 95% CI 0.60-1.41, p=0.71 at 365 days). Among survivors, at one year, 31.1% vs. 32.0% (p=0.79) had died and 9.6% vs. 7.8% (p=0.43) were alive with ESRD.

**Conclusions:** There was no increased hazard for non-recovery, mortality or ESRD with IRRT vs. CRRT. These results suggest that when initial RRT modality is chosen based on hemodynamic stability per KDIGO guidelines, renal recovery and clinical outcomes are similar between IRRT and CRRT.

*Funding:* NIDDK Support, Other NIH Support - Gambro

## FR-OR028

**Measuring Glomerular Filtration Rate. A Systematic Review**

Inga Soveri,[1] Ulla B. Berg,[2] Jonas Björk,[6] Carl Gustaf Elinder,[7] Anders O. Grubb,[4] Ingegerd Anne Mejàre,[5] Gunnar Sterner,[3] Sten-Erik Bäck.[4] *[1]Dept of Medical Sciences, Uppsala Univ, Uppsala, Sweden; [2]Dept of Clinical Science, Intervention and Technology, Div of Pediatrics, Karolinska Institutet, Stockholm, Sweden; [3]Dept of Nephrology, Skåne Univ Hospital, Malmö, Sweden; [4]Dept of Clinical Chemistry, Lund Univ Hospital, Lund, Sweden; [5]Swedish Council on Health Technology Assessment, Stockholm, Sweden; [6]Dept of Occupational and Environmental Medicine, Lund Univ, Lund, Sweden; [7]Dept of Clinical Science, Intervention and Technology, Div of Renal Medicine, Karolinska Institutet, Stockholm, Sweden.*

**Background:** The accuracy of methods to measure GFR was assessed.

**Methods:** Original studies and systematic reviews comparing endogenous creatinine clearance, renal or plasma clearance of $^{51}$CrEDTA, DTPA, iohexol and iothalamate, and plasma clearance of inulin to renal inulin clearance measured under continuous inulin infusion and urine collection were included. Two reviewers assessed the quality of each study. When raw data was unavailable, individual clearance data were assessed from enlargements of index-reference method scatterplots. Bias, P30 and P10 were calculated. The quality of evidence across studies was rated (GRADE).

**Results:** Renal clearance of iothalamate measured GFR with sufficient accuracy (strong scientific evidence). Renal and plasma clearance of $^{51}$CrEDTA and plasma clearance of iohexol were sufficiently accurate to measure GFR (moderately strong scientific evidence). Renal clearance of DTPA, renal clearance of iohexol and plasma clearance of inulin had sufficient accuracy (limited scientific evidence). Endogenous creatinine clearance was an inaccurate method (strong scientific evidence) as was plasma clearance of DTPA (limited scientific evidence). There was insufficient scientific evidence to determine the accuracy of plasma iothalamate clearance.

**Conclusions:** Accurate methods to measure GFR are renal clearances of iothalamate and $^{51}$CrEDTA and plasma clearances of $^{51}$CrEDTA and iohexol. Endogenous creatinine clearance is an inaccurate method. The conclusions are supported by moderately strong to strong scientific evidence.

*Funding:* Government Support - Non-U.S.

## FR-OR029

**Relevance of Correction for Day-To-Day Variation in Cystatin C Measurement**

Priya Vart,[1] Stephan J.L. Bakker,[2] Hiddo Jan Lambers Heerspink,[3] Dick de Zeeuw,[3] Sijmen A. Reijneveld,[1] Ute Bültmann,[1] Ron T. Gansevoort.[2] *[1]Health Sciences; [2]Nephrology; [3]Clinical Pharmacology, UMC Groningen.*

**Background:** For epidemiological research it is important to obtain the most reliable measurement of filtration markers to estimate GFR. Despite standard laboratory quality control day-to-day variability in cystatin C (cysC) measurements can be observed. We investigated whether correction for day-to-day variation might help in obtaining more optimal cysC based GFR estimates.

**Methods:** Plasma samples of the PREVEND study (a general population-based observational cohort study, 8,592 subjects) were used to re-measure cysC (Gentian assay) over a period of 243 days (March 2010 to October 2012). Each measurement day two aliquots of a plasma pool were included ("controls"). First, we calibrated controls against the international reference standard for cysC. Second, using these control samples, we calculated for each measurement day a correction factor to adjust individual cysC values for day-to-day variation in cysC measurement. Estimated GFR with the cysC CKD-EPI equation.

**Results:** Coefficient of variation of cysC in control samples was 6.9%. Mean±SD eGFR using non-corrected cystatin C ($eGFR_{cysC}$) was 92.8±19.3 and using corrected cystatin C ($GFR_{cysC\ corr}$) 94.9±19.0 ml/min./1.73m$^2$. Subjects were categorized in 6 strata of $eGFR_{cysC}$ as reference:≥120, 90–119, 75–89, 60–74, 45–59 and <45 ml/min/1.73 m$^2$. Use of $GFR_{cysC\ corr}$ resulted in upward reclassification to a higher eGFR stratum in 0%, 4%, 36%, 30%, 31% and 25% of subjects and downward in 26%, 6%, 7%, 6%, 2% and 0%, respectively. Participants re-classified upward had significantly lower age, BMI, blood pressure, cholesterol, glucose and albuminuria, whereas opposite was true for participants reclassified downward. Renal risk factors explained more variance in $eGFR_{cysC\ corr}$ than in $eGFR_{cysC}$ ($p<0.001$). Importantly, $eGFR_{cysC\ corr}$ had a better association with incident cardiovascular events (n=789, follow-up 9.3±2.7 yrs.) than $eGFR_{cysC}$ (NRI 0.102, p=0.006).

**Conclusions:** In the absence of a gold standard measurement of GFR, these epidemiological data suggest that correction for day-to-day variation in cysC measurements improves GFR estimation using cysC.

**FR-OR030**

**Non-Glomerular Filtration Rate (GFR) Factors and Cystatin C**
Krishna Pothugunta,[1] Holly J. Kramer,[1] Lara Dugas,[1] Pascal Bovet,[2] Terrence Forrester,[3] Vicki Lambert,[4] Jacob Plange-Rhule,[5] Dale Schoeller,[6] Ramon Durazo,[1] Wolfgang Korte,[7] Amy Luke,[1] Franziska Lohrer.[7] *[1]Loyola Univ Chicago; [2]Lausanne Univ Hospital; [3]Univ of the West Indies; [4]Univ of Cape Town; [5]Kwame Nkrumah Univ of Science and Technology; [6]Univ of Wisconsin; [7]Centre of Laboratory Medicine St. Gallen.*

**Background:** Non-GFR factors may influence cystatin C variance but data on potential non-GFR factors among healthy adults remain limited.

**Methods:** The Modeling the Epidemiologic Transition Study is a cohort study of adults with African ancestry aged 25-44 years living in five different countries across the African Diaspora [Chicago (n=502), Jamaica (n=499), Ghana (n=500), South Africa (n=504) and Seychelles (n=500)]. Baseline exam was conducted during January 2010 –December 201; fasting blood samples were collected along with demographic and medical information using standardized surveys. Cystatin C was measured via nephelometry (Siemens). Characteristics were compared using ANOVA for continuous variables and Chi-square test for categorical variables by site. Spearman rank correlations coefficients were calculated to assess the correlation between c-reactive protein (CRP), thyroid stimulating hormone (TSH), waist circumference (WC) and Cystatin C by site. Linear regression was used to determine the amount of variance in cystatin C due to WC, CRP, age, gender and site.

**Results:** The mean age ranged from 33.4 years in South Africa to 35.3 years in Chicago. The mean BMI ranged from 24.1 kg/m$^2$ in Ghana to 31.9 kg/m$^2$ in Chicago. The mean CRP ranged from 0.47 mg/L in Ghana to 7.85 mg/L in South Africa and mean serum cystatin C level ranged from 0.57 mg/L in Seychelles to 0.70 mg/L in Ghana. In the pooled sample, age, gender, BMI, WC and CRP accounted for less than 3.1% of the total variance of cystatin C. Findings were similar when stratified by site. In the pooled sample, the Spearman correlation coefficient between cystatin C and CRP, TSH and WC was -0.17, 0.10 and 0.06, respectively. Results were similar across all sites.

**Conclusions:** Non-GFR factors account for only 3% or less of the total vairance of cystatin C in this young population with African ancestry regardless of site.

*Funding:* NIDDK Support

**FR-OR031**

**FAST Determination of Measured GFR (mGFR) in Normal Subjects: A Phase One Study**
Erinn Sheridan,[1] Daniel J. Meier,[1] James S. Strickland,[1] Ruben M. Sandoval,[2] Bruce A. Molitoris.[2,3] *[1]FAST Biomedical, Indianapolis, IN; [2]Medicine/Nephrology, IU School of Medicine, Indianapolis, IN; [3]Roudebush VAMC, Indianapolis, IN.*

**Background:** Development of a rapid technique to measure GFR (mGFR) has been a goal for many years as an aide in diagnosing AKI, determining severity of injury, drug dosing, CVVH initiation and discontinuation and determining risk for radiocontrast studies.

**Methods:** We have now conducted a prospective single blinded placebo controlled dose escalation (5 to 150mg, 0.3 to 3.0 ml injectate) study in 32 normal subjects. We used a patented technique employing large (150kDa-2-sulfohexamine rhodamine) and small (7kDa-fluorescein) fluorescent dextrans, optical quantification of plasma clearance, and two compartment modeling. Fluorescent dextrans were extremely water soluble and allowed for a high conjugation ratio with fluorescent molecules thus minimizing the milligram dose and administered volume when compared to other markers such as inulin.

**Results:** All doses were safe and well tolerated. Plasma volumes, determined by large dextran dilution 15 min post IV injection, were dose independent and consistent with standardized formula calculations. GFRs calculated by both continuous monitoring and four plasma sample optical determinations were agreeable and within the predicted range. The t1/2 for the 5kDa was as expected GFR dependent and for the 150kDa dextran marker 110 hours.

**Conclusions:** In summary, the technique provided a safe and rapid, less than 120 minute, determination of mGFR suitable for a wide variety of patients and purposes. This technique represents an important step in development of a reliable bedside mGFR technique for use in-hospital and in outpatient settings. Supported by a NIH SBIR phase II grant to FAST Biomedical and an NIH P-30 both to BAM.

*Funding:* Other NIH Support - SBIR phase and NIH P-30 both to Bruce A Molitoris

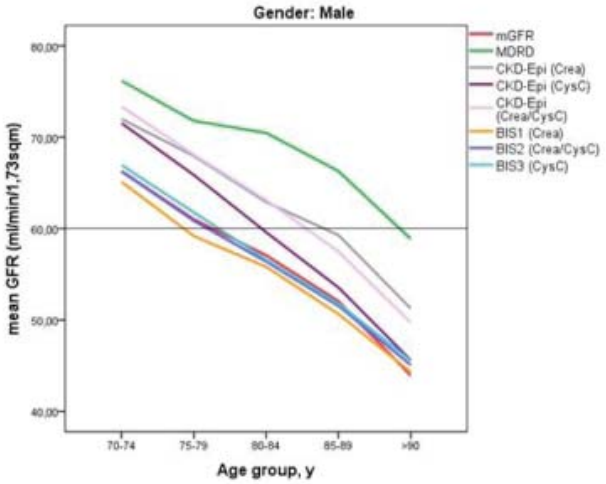
**FR-OR032**

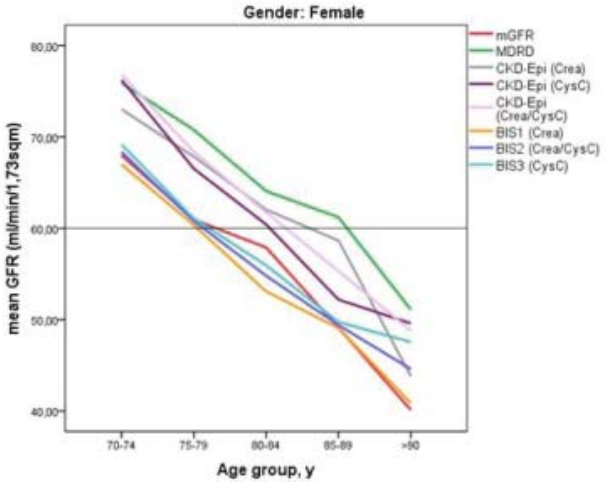
**Comparison of Current eGFR Equations with Measured GFR in an Elderly Population**
Natalie Ebert,[1] Olga Jakob,[2] Peter Martus,[3] Elke Schaeffner.[1] *[1]Nephrology, Charité, Berlin, Germany; [2]Biostatistics and Clin Epidemiology, Charité, Berlin, Germany; [3]Clin. Epidemiology and Medical Biostatistics, Eberhard Karls Univ, Tübingen, Germany.*

**Background:** Equations to estimate GFR have limitations, especially among older adults. The aim of this analysis was to compare current creatinine- and cystatin C-based equations with a gold standard in a subsample of the Berlin Initiative Study, a cohort of older adults.

**Methods:** We measured iohexol clearance in a non CKD-population of 570 participants of the Berlin Initiative Study (BIS) with a mean age of 78.5 yrs and compared the most recent 7 creatinine and/or cystatin C-based estimating equations to our measured GFR (mGFR). For iohexol clearance measurement blood samples were taken after 30, 60, 90, 120, 150, 180, 240, and 300 min (5h) after injection of 5 ml of iohexol. All comparisons were done separately for male and female patients and dependent on age.

**Results:** Compared to the mGFR (red line) the MDRD overestimates GFR by appr. 10-15 ml/min/1.73m$^2$ in all age groups, followed by the CKD-EPI equation and the new combined CKD-EPI$_{(Crea/CysC)}$ equation. The CKD-EPI$_{(CysC)}$ equation performs closer to the gold standard. The BIS-equations are the closest to the mGFR. The zig-zag-pattern of lines beyond the age of 85 in females might be due to the small numbers in that age group (n= 34). Overall the bias was largest for the MDRD equation (mean 11.2 ± 11.4 SD) followed by the CKD-EPI$_{(crea)}$ equation (mean 8.9 ± 10.1 SD) and CKD-EPI$_{(crea/cysC)}$ (mean 7.4 ± 9.2 SD). The BIS1$_{(crea)}$, BIS2$_{(crea/cysC)}$, and BIS3$_{(cysC)}$ and the CKD-EPI$_{(CysC)}$ showed lowest bias (mean -0.9, 0.1, 0.5, 4.8; SD ± 9.2, 8.1, 8.9, 10.7, respectively).




**Conclusions:** Clinicians and researchers should be aware of the fact that eGFR results differ considerably in older adults depending on the equation used. Current eGFR-equations mostly overestimate GFR in older adults.

*Funding:* Private Foundation Support

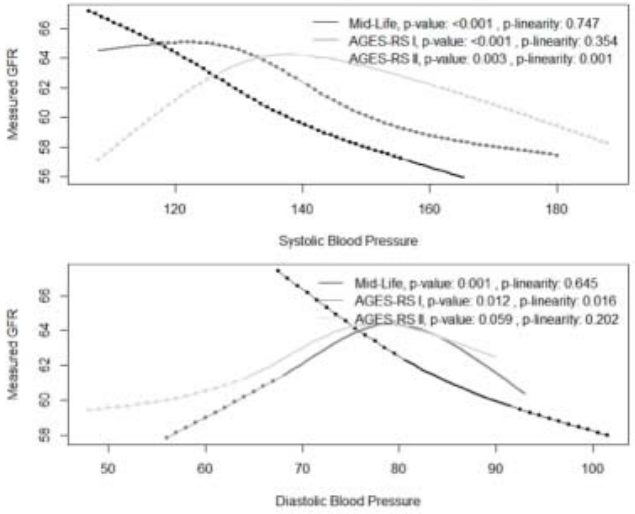
**FR-OR033**

**Kidney Function in the Elderly: A Population Based Study of Measured GFR**
Lesley Inker,[1] Aghogho A. Okparavero,[1] Hocine Tighiouart,[1] Margret B. Andresdottir,[2] Thor Aspelund,[3] Gudny Eiriksdottir,[3] Hrefna Gudmundsdottir,[2] Vilmundur Gudnason,[3,4] Tamara Harris,[4] Olafur S. Indridason,[2] Lenore J. Launer,[4] Gary F. Mitchell,[5] Hjalmfridur Lilja Nikulasdottir,[3] Runolfur Palsson,[2] Johanna Eyrun Sverrisdottir,[3] Andrew S. Levey.[1] *[1]Tufts Medical Center; [2]National Univ Hospital of Iceland; [3]Icelandic Heart Association; [4]National Institute of Aging; [5]Cardiovascular Engineering.*

**Background:** GFR declines with age, leading to a high prevalence of CKD in the elderly. Causes of this decline are not well understood due in part to absence of reliable information about GFR in a representative elderly population and its associations with midlife factors.

**Methods:** We measured GFR (mGFR) using plasma clearance of iohexol in 805 people participating in a community based prospective cohort study, Age, Gene/Environment Susceptibility Reykjavik Study (AGES-RS). We examined the associations of mGFR with age and systolic (SBP) and diastolic (DBP) blood pressure at 3 visits: midlife [mean(SD) age 51.0(5.8)], AGES-RS I [74.7(3.9)] and AGES-RS-II [79.4(3.8)].

**Results:** Mean mGFR was 62.3 (16.6, range 4-112) ml/min/1.73m$^2$. mGFR was 1.38 (1.09-1.66) ml/min /1.73m$^2$ lower per 1-y older age. The figure shows the relationships of mGFR at AGES-RS-II with SBP and DBP at the 3 visits. These relationships persisted after adjusting for age and creatinine at midlife, concurrent treatment, or BP at the other times.




Curves are cubic splines with 4 knots. Dots represent significant differences as compared to the reference point of SBP=140 and DBP=80. P-linearity <0.05 indicates nonlinearity of the curve. P-value < 0.05 indicates a significant association between BP and GFR.

**Conclusions:** Many elderly people have normal levels of GFR, suggesting decline in GFR may be secondary to pathological processes rather than aging. The strong associations of higher levels of SBP and DBP at midlife even less than 140/90 with lower mGFR in late life, suggest a possible mechanism for GFR related decline and may provide some directions for prevention of CKD in the elderly.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## FR-OR034

### Chronic Kidney Disease Diagnosis and Referral Patterns by International Classification of Diseases-9 Codes

Anju A. Oommen, Lynn E. Schlanger, James L. Bailey, Jeff M. Sands, Janice P. Lea. *Nephrology, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** There are an estimated 20 million persons with CKD in the U.S., which is associated with significant morbidity and mortality. Patients at high risk for CKD require screening including those with diabetes, hypertension, family history of CKD, and African Americans. The estimated glomerular filtration rate (eGFR) reporting has become standard practice in most labs to help identify persons with CKD stage 3-5. Despite having access to eGFR measurements, oftentimes ICD-9 CM codes may not reflect accurately the level of CKD.

**Methods:** We used cross-sectional analysis to evaluate the accuracy of renal related ICD-9 coding for CKD in our outpatient clinic populations across Emory Healthcare. Using the modified creatinine based eGFR, CKD was defined as having two or more eGFR values< 60ml/min/1.73m$^2$ at least 90 days apart. Exclusion criteria were ESRD patients, renal transplants, and presumed acute kidney injury.

**Results:** Of the 93,938 patients in our clinical data warehouse meeting criteria for CKD, 55% were males, 54% Caucasians and 33% African Americans. Of those 93,938 clinic patients, 17,584 (18.7%) had one or more specific renal diagnosis codes on record while 76,354 (81.3%) did not have any. Of those patients (17,584) with CKD by eGFR and ICD-9 coding reflecting a CKD diagnosis, 35.3% had been seen in nephrology clinic as compared to those patients (76,354) with CKD but no ICD-9 coding, only 0.8% was seen in nephrology clinic, p value< 0.0001. There were no differences observed according to race or sex in diagnosis or referral patterns.

**Conclusions:** In summary, ICD-9 codes used in clinic settings do not completely identify persons with all forms of CKD. Those patients with a CKD diagnosis reflected in ICD-9 coding are seen by nephrology more often than others, however, the majority of persons with CKD defined by eGFR are not identified by ICD-9 codes and are not under care of a nephrologist. More education is needed to emphasize importance of identifying all stages of CKD in order to provide proper risk stratification and interventions to improve outcomes.

## FR-OR035

### A Comparison of Slopes in Measured and Estimated GFRs: The CRIC Study

Dawei Xie, Wei Yang, Amanda Hyre Anderson, Harold I. Feldman. *Biostatistics and Epidemiology, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** This study compared the slopes in measured GFR (mGFR) and three estimated GFRs (eGFRs) from CRIC, MDRD and CKD-EPI equations.

**Methods:** Included were 1009 CRIC participants with >=2 mGFRs. Linear regression models estimated the slopes. Spearman correlations were calculated among the slopes. The slope difference of CRIC eGFR vs mGFR was regressed on baseline covariates.

**Results:** Mean length of follow up was 3.5 years (range=1.6-8.9). The Spearman correlations were >0.9 among the three eGFR slopes, and 0.59, 0.53, and 0.53 between mGFR and CRIC, MDRD, CKD-EPI eGFR slopes. Mean slope difference between CRIC eGFR and mGFR was 0.56 (SD=3.84, median=0.41, IQR: -1.33 to 2.37) ml/min/1.73m2/year. Model estimates which were the eGFR versus mGFR slope difference per unit change in the predictor were shown in Table.

| Baseline Predictors | Age, gender, race, center adjusted (95% CI) | p | Final multivariable model (95% CI) | p |
|---|---|---|---|---|
| CRIC eGFR-mGFR | -0.19 (-0.21, -0.17) | <.0001 | -0.19 (-0.21, -0.17) | <.0001 |
| mGFR | 0.04 (0.03, 0.06) | <.0001 | | |
| Log HOMA insulin sensitivity | -0.29 (-0.54, -0.04) | .03 | -0.26 (-0.47, -0.04) | .02 |
| Log TNF alpha | 0.31 (0.03, 0.60) | .03 | 0.27 (0.04, 0.51) | .02 |
| Log BNP | -0.29 (-0.57, -0.01) | .04 | | |
| Log urine urea nitrogen | 0.29 (0.02, 0.57) | .04 | | |
| Log Cystatin C | 0.26 (-0.01, 0.53) | .06 | | |
| Log IL-6 | 0.27 (-0.01, 0.55) | .06 | | |
| Log IL-1RA | -0.26 (-0.54, 0.02) | .07 | -0.38 (-0.60, -0.15) | .001 |
| Log fibrinogen | 0.20 (-0.06, 0.46) | .1 | | |
| Serum urea nitrogen | 0.20 (-0.06, 0.47) | .1 | | |
| CRIC eGFR | -0.01 (-0.03, 0.00) | .1 | | |
| Log hsCRP | 0.19 (-0.08, 0.46) | .2 | | |
| Log plasma homecysteine | 0.19 (-0.09, 0.47) | .2 | | |
| Wasit circumference | 0.18 (-0.09, 0.44) | .2 | | |
| Log Urine creatinine excretion | 0.19 (-0.10, 0.48) | .2 | -0.25 (-0.47, -0.04) | .02 |

**Conclusions:** The ability of eGFR slopes to approximate mGFR slope in the setting of CKD is impaired by inflammation, insulin sensitivity, creatinine generation, and poor agreement between baseline eGFR and mGFR.

*Funding:* NIDDK Support

## FR-OR036

### National Impact of Cystatin C on Safe and Appropriate Utilization of Metformin among Patients with Type 2 Diabetes

Delphine S. Tuot,[1] Michael Shlipak,[1] Jerry Yee,[2] Rajiv Saran,[3] Neil R. Powe.[1] *[1]Univ of California, San Francisco, San Francisco, CA; [2]Henry Ford Hospital, Detroit, MI; [3]Univ of Michigan.*

**Background:** Metformin may be underused among persons with diabetes (DM) and chronic kidney disease (CKD) because of concerns of side effects at low glomerular filtration rates (GFR). eGFRcr-cys correlates better with measured GFR than other measures and may be a more reliable measure on which to base medication dosing. We examined the potential impact of eGFRcr-cys on guiding safe and appropriate utilization of metformin among US adults with DM.

**Methods:** Self-reported metformin use was assessed among 397 diabetic adults using 1999-2002 National Health and Nutrition Examination Survey data. DM was defined by self-report or a glycosylated hemoglobin > 6.5mg/dL. eGFRcr was measured by the 4-variable MDRD equation; eGFRcr-cys by the CKD-EPI 2012 equation. We used proportions to reclassify: (1) the percentage of diabetics with eGFRcr >45ml/min not on metformin who could presumably safely be on metformin because a eGFRcr-cys >45ml/min and (2) the percentage of diabetics with eGFRcr < 45ml/min on metformin, who should presumably not be on metformin because of an eGFRcr-cys < 45ml/min. Results are weighted to the US population.

**Results:** Overall, 28% of patients with DM reported a metformin prescription, of whom 97% had an eGFRcr-cys <60ml/min. Nearly 16% of patients with DM and eGFRcr < 45ml/min not on metformin could safely be on metformin (Table 1) and 23% of persons with DM and eGFRcr >45ml/min on metformin should use the drug with caution based on eGFRcr-cys <45ml/min (Table 2).

Table 1: Percentage of patients with DM not on metformin who could safely use metformin by reclassification using eGFRcr-cys.

| | eGFRcr-cys ≥45 | eGFRcr-cys <45 |
|---|---|---|
| eGFRcr ≥45 | 94.1 | 6 |
| eGFRcr <45 | 15.7 | 84.3 |

Table 2: Percentage of patients with DM on metformin whose safety with metformin is concerning, by reclassification using eGFRcr-cys.

| | eGFRcr-cys ≥45 | eGFRcr-cys <45 |
|---|---|---|
| eGFRcr ≥45 | 77 | 23 |
| eGFRcr <45 | 14 | 86 |

**Conclusions:** eGFRcr-cys may help clinicians better identify individuals for whom metformin prescription is safe and appropriate. Prospective studies should further clarify the role of eGFRcr-cys for evaluation of safety and efficacy of medication dosing among CKD patients.

*Funding:* NIDDK Support, Other U.S. Government Support

**FR-OR037**

**GFR Decline as an Endpoint for Clinical Trials in CKD: Report of an NKF-FDA Workshop** Andrew S. Levey,[1] Josef Coresh,[2] Aliza M. Thompson,[3] Edmund J. Lewis,[4] Dick de Zeeuw,[5] Alfred K. Cheung,[6] Kerry Willis,[7] Norman Stockbridge.[3] *[1]Tufts Medical Center; [2]Johns Hopkins Univ; [3]Food and Drug Administration; [4]Rush Univ Medical Center; [5]Univ Medical Center Groningen; [6]Univ of Utah; [7]National Kidney Foundation.*

**Background:** The FDA accepts halving of GFR, assessed as doubling of serum creatinine (Scr) level, as a surrogate end point for the development of kidney failure in clinical trials of kidney disease progression. A Scr doubling is generally a late event in CKD and takes a long time to develop. There is great interest in considering alternative endpoints for clinical trials to shorten their duration and to extend their application to earlier stages of CKD. However, there is uncertainty about the associations of lesser declines in GFR with subsequent development of kidney failure. The NKF and FDA sponsored a scientific workshop to exam critically data that might new definitions of GFR decline as endpoints in clinical trials of CKD-related therapies. The anticipated outcome of the workshop was the identification of new definitions of GFR decline with sufficiently strong associations with important clinical outcomes of CKD so that they can be used as endpoints in CKD clinical trials.

**Results:**

Summary of performance of alternative time-to-event endpoints for 2-3 year trial*

| Acute effects** | Baseline GFR high Stage 2 | | Baseline GFR medium Stage 3 | | Baseline GFR low Stage 4 | |
|---|---|---|---|---|---|---|
| | 40% decline | 30% decline | 40% decline | 30% decline | 40% decline | 30% decline |
| Large negative | | | | | | |
| Moderate negative | | | | | | |
| None | | | | | | |
| Moderate positive | | | | | | |
| Large positive | | | | | | |
| Key** * | Type 1 error not acceptable (false positive for benefit) | Type 1 error not acceptable (false positive for harm) | Type 1 error acceptable but power not improved | Type 1 error acceptable and power improved | | |

*Acute effect refers to an initial GFR decline opposite in direction to the hypothesized long-term beneficial effect. **Assumptions: treatment effect is mixed proportional/uniform; acute effect attenuates to 0 at ESRD; trial duration 3 years for high GFR and 2 years for medium and low GFR. ***Key: acceptable type 1 error = 10% or less; improved power = smaller samples size for alternative vs. 57% decline (equivalent to a Scr doubling) in a trial of the same duration.

**Conclusions:** Based on a series of meta-analyses of 21 cohorts and 43 clinical trials and simulations of trial designs and analytic methods, the workshop concluded that a confirmed decline in eGFRcr of >30 or >40% may be an acceptable surrogate endpoint (acceptable type 1 error and power improved) in some circumstances, based on baseline GFR, magnitude of acute effects, duration of follow-up.

*Funding:* Private Foundation Support

**FR-OR038**

**Lower Creatinine Clearance Is Associated with Reduced Walking Speed and Muscle Atrophy among Older Adults** Baback Roshanravan,[1] Cassianne Robinson-Cohen,[1] Ian H. de Boer,[1] Ann M. O'Hare,[1] Jonathan Himmelfarb,[1] Kushang V. Patel,[2] Bryan R. Kestenbaum.[1] *[1]Nephrology, Univ of Washington, Seattle, WA; [2]Anesthesiology & Pain Medicine, Center for Pain Research on Impact, Measurement & Effectiveness, Seattle, WA.*

**Background:** CKD promotes malnutrition and inflammation that may lead to loss of skeletal muscle and reduced physical performance. Associations of kidney function with human skeletal muscle structure and longitudinal measures of physical performance are unknown.

**Methods:** We tested associations of 24hr creatinine clearance (CrCl) with physical function in the InCHIANTI study, a community-based cohort of adults ≥65yrs. We included 826 participants without stroke or disability who completed a 24hr urine collection. Linear regression was used to assess cross-sectional associations of CrCl with gait speed over 7 and 400 meters, and knee extension strength. We adjusted for age, sex, ht, wt, study site, smoking, education, diabetes, and CAD. We tested associations of CrCl with muscle and fat areas of the calf muscle by CT. Mixed effects models to estimate associations of baseline CrCl with longitudinal physical performance measures over 9yrs.

**Results:** Mean age was 74 ±7yrs. Mean CrCl was 79 ±25ml/min with 206 participants with CrCl<60ml/min. In cross-sectional analysis each 10ml/min lower CrCl was associated with an adjusted mean difference of -0.01m/s (95% CI: -0.014, -0.003; P=0.002) in usual gait speed and -0.009m/s (95% CI: -0.015, -0.004; P=0.004) in 400m gait speed. No difference was seen with knee strength at baseline (P=0.8). Each 10ml/min lower CrCl was associated with an adjusted mean difference of -30mm$^2$(95% CI: -58, -2; P=0.03) in calf muscle area and -0.16 mg/cm$^3$ (95% CI -0.27, -0.05; P=0.005) in muscle density. In longitudinal analysis each 10ml/min lower CrCl was associated with an adjusted difference of -0.025kg/year in knee strength (95% CI: -0.04, -0.01; P=0.001) but no difference in gait speed.

**Conclusions:** Lower CrCl is associated with slower 7m usual gait speed and 400m gait speed and diminished calf muscle area and muscle density. Knee extension strength declined more rapidly among those with lower CrCl.

*Funding:* NIDDK Support, Other NIH Support - National Institute on Aging support for InCHIANTI

**FR-OR039**

**Effects of Aging on Renal Arteriolar Hyaliosis in Autopsies: The Hisayama Study** Toshiharu Ninomiya,[1] Michiaki Kubo,[2] Masaharu Nagata,[1] Yoshinao Oda,[3] Takanari Kitazono,[1] Yutaka Kiyohara.[4] *[1]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [2]Laboratory for Genotyping Development, RIKEN Yokohama Institute, Yokohama, Japan; [3]Dept of Anatomic Pathology, Kyushu Univ, Fukuoka, Japan; [4]Dept of Environmental Medicine, Kyushu Univ, Fukuoka, Japan.*

**Background:** There were limited studies addressing the effect of aging on renal arteriolar hyalinosis by considering the status of other cardiovascular risk factors.

**Methods:** In 839 consecutive population-based autopsy samples, which were obtained between 1962 and 1994, the effect of aging on advanced renal arteriolar hyalinosis was examined, taking the status of other cardiovascular risk factors into account. The advanced renal arteriolar hyalinosis was determined based on the 95 percentile values of arteriolar hyalinosis index among subjects who had no clinical sign of kidney disease. The risk estimates were computed using a logistic regression analysis.

**Results:** Aging was significantly associated with the likelihood of advanced renal arteriolar hyalinosis after adjusting for other cardiovascular risk factors, being 1.00 (reference), 1.66 (95% confidence interval, 0.60-4.60), 2.03 (0.79-5.24), and 3.11 (1.21-7.98) for 40-59, 60-69, 70-79, and ≥80 years. In the analysis stratified by hypertensive status, however, the multivariate-adjusted odds ratios of the presence of advanced renal arteriolar hyalinosis increased significantly with aging in hypertensives, whereas there was no evidence of the association in normotensives: 1.00 (reference), 2.23 (0.64-7.72), and 1.97 (0.54-7.19) for 40-69, 70-79, and ≥80 years in normotensives: 3.37 (1.18-9.61), 4.35 (1.62-11.71), and 7.33 (2.75-19.49) in hypertensives. In the multivariate analysis, aging, hypertension, glucose intolerance, and proteinuria were significant risk factors for the presence of advanced renal arterial hyalinization, where hypertension had the greatest odds ratio.

**Conclusions:** Our findings suggest that aging is a significant risk factor for renal arterial hyalinization, but its magnitude of effect is less than that of hypertension.

**FR-OR040**

**Incidence of Dementia Based on Dialysis Modality** Dawn F. Wolfgram,[1] Qun Xiang,[2] Aniko Szabo.[2] *[1]Medicine, Medical College of Wisconsin and Zablocki VA Medical Center, Milwaukee, WI; [2]Biostatistics, Medical College of Wisconsin, Milwaukee, WI.*

**Background:** Dementia prevalence is increasing in our ESRD population. Although this may reflect their overall higher risk of vascular disease, dementia increases substantially after initiation of dialysis. This suggests that hemodialysis itself, with its rapid fluctuations in blood pressure and osmolality, contributes to initial cognitive changes. Currently, there is no data on the incidence of dementia in the peritoneal dialysis (PD) population. Since PD lacks the fluctuations seen in HD, we hypothesize that that persons on PD would be less likely to develop dementia than those on HD.

**Methods:** Using linked Medicare - United States Renal Data System data, we identified persons who initiated dialysis during 2006-2008 and did not have a dementia diagnosis code during the two years preceding dialysis initiation. We followed this cohort for up to 3 years to identify new dementia diagnoses. We estimated cumulative dementia incidence rates overall and for persons receiving HD vs PD using Nelson-Aalen estimators, censoring for mortality, and at December 31, 2008. We used Cox proportional hazards models to determine the independent effect of initial modality.

**Results:** We identified 144,244 eligible patients; PD was the initial modality in 6.2%. The overall mortality was 62.3% in 3 years. The cumulative incidence of dementia at 3 years was 6.1% overall; 6.8% and 3.5%, respectively, among those whose first modality was HD and PD. In bivariable analyses, African American race, older age, female sex, and hypertension or diabetes as primary ESRD cause were associated with increased risk for dementia. After adjusting for potential confounders, persons initiating dialysis with PD remained significantly less likely to develop dementia (HR 0.60, 95% CI 0.53-0.68).

**Conclusions:** Initial modality of PD is associated with lower risk of incident dementia than HD. Analysis taking into account further risk factor adjustment and time on dialysis modality is ongoing. These results suggest a need for more research regarding the potential risks of intradialytic events, including fluctuations in BP and osmolality.

**FR-OR041**

**Cognitive Impairment in Moderate Chronic Kidney Disease: The Brain IN Kidney Disease (BRINK) Study** Anne M. Murray,[1,2,3] Yelena Slinin,[2] Sarah L. Pederson,[3] Elizabeth Amiot,[3] David Tupper.[1,2] *[1]Hennepin County Medical Center, Minneapolis, MN; [2]Univ of MN; [3]MMRF.*

**Background:** In the longitudinal BRain IN Kidney disease (BRINK) study we are characterizing burden of cognitive impairment (CI) in 350 CKD and 100 non-CKD subjects. We describe the frequency of domain-specific CI in CKD and non-CKD cohorts after one year of recruitment.

**Methods:** Pts with CKD Stages 3b-5 (eGFR < 60 mL/min; dialysis pts exclude</a>d) and age, race, and education-matched controls (eGFR ≥ 60 mL/min) were recruited from three Minneapolis urban clinics. Domain- specific neuropsychological testing was performed at baseline using the following tests: 1) 3MS (Modified Mini-Mental State Exam) for global cognition, 2) Hopkins Verbal Learning Test-Revised (HVLT-R, Immediate and Delayed; verbal memory), 3) Controlled Oral Word Association Test (COWAT, semantic memory/language), 4) Color Trails 1 and 2 (executive function) (CTT2), 5) Symbol-Digit-

Modality Test (SDMT) (executive function), and 6) Brief Visuospatial Memory Test-Revised (BVMT-R, Immed and Delayed; visualspatial/memory) and the PHQ Depression Scale. We compared raw scores and T-scores using published norms.

**Results:** 201 participants (CKD N = 124, Control N = 77) were recruited. Mean ages of CKD and control groups were 67.5 ± 9.7 years and 65.1 ± 10.8 yrs, respectively (p=0.11). Baseline eGFR among CKD subjects was 29.8 ± 10.4 mL/min (Stage 4 CKD). CKD pts had a higher prevalence of HTN than controls (93.5% vs. 75.3%, p<0.01) but similar prevalence of prior stroke (15.3% vs. 13.0%, p=0.65) and diabetes (59.3 vs. 51.9%, p=0.30). CKD pts scored significantly worse on the 3MS (92.5 ± 6.5 vs. 94.4 ± 6.0, p = 0.04), HVLT-R delayed (6.9 ± 3.2 vs. 8.3± 2.7 words, p<.01), and SDMT (36.5 ± 12.2 vs. 42.2 ± 11.3, p<.01); associated T-scores were also significantly different. There were no significant differences on the CTT2, COWAT, Digit span, BVMT-R or PHQ9 </ a>depression scale between cohorts.

**Conclusions:** CKD pts had significantly lower levels of global cognitive function, verbal memory, and executive function/processing speed, consistent with possible mixed neurodegenerative and vascular cognitive impairment. Further analyses will measure factors associated with domain- specific CI.

*Funding:* Other NIH Support - NIA, Private Foundation Support

## FR-OR042

### Dizziness and Vestibular Disorders Are Associated with Falls in Chronic Kidney Disease (CKD)

Premila Bhat,[1,2] Ashok Valluri.[2] *[1]Atlantic Dialysis Management Services, LLC, Ridgewood, NY; [2]Wyckoff Heights Medical Center, Brooklyn, NY.*

**Background:** CKD is associated with increased risk of falls and fracture but the factors contributing to fall risk in CKD are not fully known. We hypothesize that vestibular disorders, including disorders of balance and dizziness, contribute to fall risk in CKD. To examine this association cross-sectional analysis of the continuous National Health and Nutrition Examination Survey (NHANES) was conducted.

**Methods:** Subjects ≥40 yrs of age and enrolled in NHANES 2001-2004 (n=21,161) were included. Screening for problems with dizziness, balance, or falls and results of a modified Romberg test were ascertained from the NHANES Balance Questionnaire and Exam. Uni- and multivariate logistic regression were used to compare the prevalence of balance associated symptoms or Romberg test failure across CKD categories. Continuous and categorical data are presented as means±SD and percents (%), respectively.

**Results:** Of the 4,878 subjects, 48.9% were female, 16.9% black, mean age was 60.7±13.4 yrs, mean BMI 28.2±5.3, 42.2% with HTN, 13.0% DM. Prevalence of CKD was: Stages 1-2 (eGFR≥60), 88.5%; 3A (45≤eGFR<60), 7.8%; 3B (30≤eGFR<45), 2.5%; and 4-5 (eGFR<30), 1.2%. Of 23.3% who reported dizziness, balance or falling problems, 24.2% reported falls. Compared to those with eGFR≥60, for CKD Stage 3A, 3B, and 4-5 unadjusted OR's for balance symptoms were 1.818 (95%CI: 1.452, 2.277), 2.475 (95%CI:1.709, 3.584), and 3.069 (95%CI: 1.829, 5.148), respectively. The relationship between advanced CKD (Stages 3B and 4-5) and balance symptoms was seen even after adjustment for age, gender, race, DM, HTN and BMI, with OR's 1.706 (95%CI: 1.151, 2.530) and 2.141 (95%CI:1.204, 3.808), respectively. Failure to pass level 2 or 3 Romberg exam remained predictive of falls after adjustment for CKD (level 2 OR=1.839, 95%CI: 1.224, 2.762; level 3 OR=2.575, 95%CI: 1.486, 4.463).

**Conclusions:** Problems with balance and dizziness are common in CKD. There is a graded relationship between CKD stage and balance symptoms. Among those with CKD, falls are associated with failure to pass sequential Romberg tests, suggesting that the Romberg test can identify individuals with CKD at high risk for falls.

*Funding:* Clinical Revenue Support

## FR-OR043

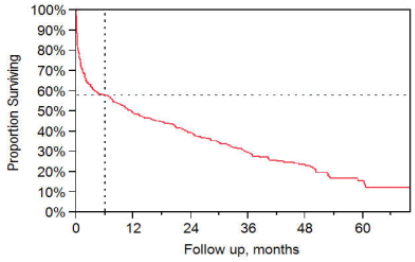
### Starting Dialysis at Age 75 Years or Older – Outcomes Data to Help in Shared Decision Making

Bjoerg Thorsteinsdottir,[1] Hanna L. W. Larson,[2] LaTonya J. Hickson,[1] Molly A. Feely,[1] Amy W. Williams.[1] *[1]Medicine, Mayo Clinic, Rochester, MN; [2]Univ of San Diego, San Diego, CA.*

**Background:** The intensity of medical care for the oldest old has escalated beyond population growth in the past decades driven by powerful moral and technological imperatives to treat. Many elderly patients and their families feel that they have no choice but to start renal replacement therapy (RRT) with several expressing regret of having initiated therapy. High HD withdrawal rates have been found in this group. Shared decision making regarding RRT in the elderly is hampered by the lack of evidence to guide practice in this age group.

**Methods:** Review of the medical record for all patients 75 years and older, initiating any form of RRT at Mayo Clinic Jan 1, 2007 - Dec 31, 2011.

**Results:** Of 390 patients, 66% were male, 94% were Caucasian, and mean age was 81.3 years (Interquartile range (IQR) 77.5-84.4), 40% had diabetes, and 27% had congestive heart failure. The mean Charlson comorbidity index was 7.9 (IQR 6-10). The majority, 290 (74%), started RRT in hospital. 147 (57%) began with continuous RRT in the ICU. Of 210 hospital-initiated patients admitted from independent living, only 70 (33%) were discharged home. Mean follow up was 17 months (range 0-74). Early mortality was high with 104 (27%) dying in less than 30 days and 140 (36%) dying in less than 90 days. The 6-month survival rate was 58% and 12 month survival 49% (Figure 1). Among hospital starters, 110 of 290 (38%) died during the index hospitalization.




**Conclusions:** This cohort study of elderly incident RRT patients suggests that most initiate RRT in the hospital following an illness or an event. Loss of independent living is frequently observed following hospitalization. Early and overall mortality is high. Patient awareness of these outcomes may allow for better informed discussions at time of RRT consideration.

## FR-OR044

### Determinants of Outcome of Conservatively Managed CKD Stage 5 Patients: Single Centre Review

Muhammad Nauman Hashmi, Mohammed Awais Hameed, Arvind Ponnusamy, Vijay Sundaram Thanaraj, Ajay Prabhakar Dhaygude. *Renal Medicine, Royal Preston Hospital, Preston, United Kingdom.*

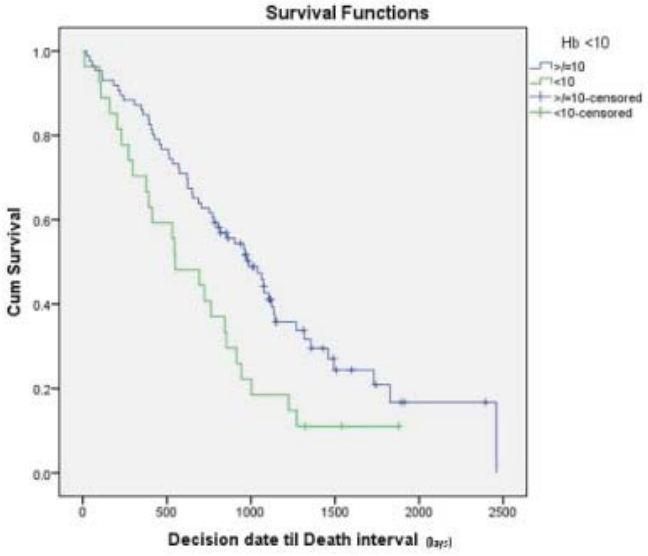
**Background:** Conservative treatment has become a recognized modality of treatment in end stage renal disease (ESRD) in selected group of chronic kidney disease (CKD) patients. These patients are managed in a multidisciplinary setting. However, there is lack of data regarding outcome of these patients.

**Methods:** This is a retrospective analysis of conservatively managed patients CKD stage 5 (Total number 119) patients followed up in a single centre from January 2005 till December 2010. Residual renal function (eGFR), laboratory parameters (calcium, phosphate, albumin & haemoglobin) were recorded from the time of decision to manage conservatively. These parameters were co-related.

**Results:** Median age of the study population was 85.5 years. The most common co-morbidity was hypertension. Mean haemoglobin at baseline was 11.3g/dl, calcium 2.30mmol/l, phosphate 1.31mmol/l and albumin 39 g/l. Median overall survival was 816 days.

| | Registration to death interval (days) | Decision date to death to interval (days) |
|---|---|---|
| Mean | 1660 | 825 |
| Median | 1531 | 816 |
| Mode | 1092 | 98 |

The number of patients died were 87. Haemoglobin >10 g/dl at presentation was associated with greater survival (p<0.001). Median survival of patients with haemoglobin >10 g/dl was 1116.5 days compared to 705.3 days for those with haemoglobin <10 g/dl.




Interestingly, other parameters did not predict outcome.

**Conclusions:** Conservative management in ESRD has become one of the modality in RRT. Increasing proportion of CKD patients, particularly the elderly, opt for conservative care. There should be a multidisciplinary approach to the management of such patients. Haemoglobin appears to be important predictor of survival in our cohort of patients.

## FR-OR045

**Non-Dialysis Management in the Elderly: Survival, Symptom Control and Quality of Life** Mark A. Brown, Gemma Collett, Elizabeth A. Josland, Frank Brennan. *Renal Medicine, St. George Hospital & Univ of NSW, Sydney, NSW, Australia.*

**Background:** Decisions concerning appropriateness of dialysis in the elderly require knowledge of expected survival and whether reasonable life can be maintained without dialysis. We compared prospectively symptom control, Quality of Life(QOL) and survival between ESKD patients managed without dialysis (NFD) and those planned for dialysis (Pre-Dx).

**Methods:** In a single centre *prospective observational study* over 4 years 290 Pre-Dx patients had usual nephrology care and 135 NFD patients also received Renal Supportive Care(RSC) adding the skills of a Palliative Medicine team. Patients were recommended a NFD pathway based upon co-morbidity, frailty, poor functional status or nutrition and their nephrologist's view that survival would be poor. Symptoms were measured using the validated MSAS survey and QOL using the SF-36 survey.

**Results:** NFD patients were older than the Pre-Dx group (median 83 vs. 69 yrs, $p<0.001$);median eGFR at 1st clinic visit was 16ml/min in both. 126(43%) of Pre-Dx patients commenced dialysis over the 4 years. Mean survival was greater in Pre-Dx (44 vs. 23 months, $p<0.001$). 71(53%) of NFD and 28(10%) of Pre-Dx patients died ($p<0.001$); death rates for patients > 75 yrs were 63/115(55%) and 15/99(15%) respectively ($p<0.001$). 48%(21/44) of NFD and 47%(46/97) of Pre-Dx patients had stable or improved symptoms over the 1st 6 months and 52%(12/23) vs.45%(23/52) at 12 months. Physical QOL was maintained or improved at 6 and 12 months in 38%(15/39) and 43%(9/21) of NFD and 51%(53/104) and 46%(25/54) of Pre-Dx patients.Mental QOL was maintained or improved at 6 and 12 months in 51%(20/39) and 52%(11/21) of NFD and 57% (59/104 and 31/54) of Pre-Dx patients.

**Conclusions:** As expected, elderly patients carefully selected for dialysis survive longer than those treated conservatively. However, a RSC service utilising the skills of palliative medicine ensures symptom control and QOL without dialysis to the level of those receiving usual nephrology care including dialysis. These data support a role for palliative (supportive) care and can assist nephrologists counselling elderly patients regarding appropriateness and likely outcomes if dialysis is not employed.

*Funding:* Pharmaceutical Company Support - Amgen Australia

## FR-OR046

**Preexisting Do Not Resuscitate Status Reduces Hospital-Centered Morbidity and Mortality at the End of Life in a University Hospital-Based Dialysis Population** Marc Richards,[1] Len A. Usvyat,[2] Philip J. Klemmer.[1] *[1]UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC; [2]Renal Research Institute, New York, NY.*

**Background:** Medical advances have extended life spans, but at the expense of more complex illnesses and comorbidities. This is particularly the case for end stage renal disease patients who have a 25% annual mortality, four times that of age-matched patients without kidney disease. Previous studies have suggested that end of life care in ESRD patients is often aggressive and expensive, yet frustratingly futile.

**Methods:** Records were obtained from the Renal Research Institute (RRI) detailing all patients who left one of three suburban or rural dialysis units affiliated with the UNC Kidney Center. 287 adult patients died or withdrew from dialysis. Data was compiled from an electronic medical record and the dialysis units to determine demographic data, comorbidities, aggressiveness of care at the end of life, advanced directives, and implementation of palliative measures.

**Results:** Please see table. Of note, black, ESRD onset age <70, and PD patients were more likely to receive aggressive end-of-life care (data not shown).

| N=287 | Hospitalization (last month of life) | ICU admission (last month of life) | CPR or CRRT or Intubation or Pressors (last month of life) | In-Hospital Death | Hospice Referral | With-drawal of dialysis as inpt. | With-drawal of dialysis as outpt. |
|---|---|---|---|---|---|---|---|
| Preexisting DNR/DNI prior to 1 month before death OR last admission (n=52, 22.1%) | 36 (69.2%) | 8 (15.4%) | 7 (13.5%) | 9 (17.3%) | 27 (51.9%) | 12 (23.1%) | 16 (30.8%) |
| No Preexisting DNR/DNI (n=235, 77.9%) | 196 (83.4%) | 106 (45.1%) | 115 (48.9%) | 135 (57.4%) | 37 (15.7%) | 24 (10.2%) | 10 (4.2%) |
| p-values -> | 0.009 | <0.001 | <0.001 | <0.001 | <0.001 | 0.006 | <0.001 |

**Conclusions:** Dialysis patients with established DNR/DNI orders were more likely to avoid aggressive intervention at the end of life, establish hospice care, and voluntarily withdraw from RRT. A prospective trial is warranted to see if earlier initiation of advanced directives in the ESRD population results in more compassionate, less costly care.

## FR-OR047

**Dignity and Quality of Life in End Stage Renal Disease Patients, Close to the End of Life** James M. Zacharias,[1] Sara N. Davison,[2] Harvey M. Chochinov.[3] *[1]Dept of Medicine, Section of Nephrology, Univ of Mantitoba, Winnipeg, Canada; [2]Dept of Medicine, Univ of Alberta, Edmonton, Canada; [3]Dept of Psychiatry, Univ of Manitoba, Winnipeg, Canada.*

**Background:** Maintaining dignity of patients in end-of-life (EOL) situations has been a foundation of palliative care. An empirical model of dignity (Chochinov et al, 2002), has been used to explore dignity in EOL cancer populations, but no work has yet been done to identify sources of dignity related distress in the End Stage Renal Disease (ESRD) population.

**Methods:** Entry criteria: ESRD patients on dialysis >3 months and between 65 and 80 years of age and their caregivers were consented. Demographics were collected and the disposition of the patient followed for up to two years. Various instruments measuring psycosocial and existential distress including the Patient Dignity Inventory (PDI), Structured Interview for Symptoms and Concerns. (SISC) and KDQOL, were administered at the onset of the study and 3 months later.

**Results:** 103 patients on dialysis a median of 1-3 years from two sites in Canada were enrolled. Sixty percent were male, 52% diabetic, mean age 72.5 years. Eighty-two percent had never had counseling or treatment for an emotional problem. SISC indicated 18.8% patients felt some loss of dignity; 51.6% some degree of suffering, 50.5% feelings of hopeless. 53.5% endorsed one of more items of the PDI. However more malignant expressions of distress such as; desire for death (9.9%) and thoughts of suicide (14.9%) were less frequent, comparable to terminal ill cancer populations.

**Conclusions:** Suffering and existential distress appears to be quite prominent in this patient population. However, more severe expressions of marked distress are less common. Further research must explore the full experiential landscape of distress, in order to determine appropriate therapeutic approaches.

*Funding:* Government Support - Non-U.S.

## FR-OR048

**Phospholipase $A_2$ Receptor Antibody Levels Predict Clinical Outcome and Therapeutic Response in Patients with Primary Membranous Nephropathy** Elion Hoxha, Ina Ellen Thiele, Gunther Zahner, Ulf Panzer, Sigrid Harendza, Rolf A. Stahl. *III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

**Background:** The prediction of clinical outcome and the decision for an immunosuppressive treatment in patients with Membranous Nephropathy (MN) currently relies on prolonged follow-up measurements of proteinuria. Yet, changes in proteinuria do not necessarily reflect the activity of immunologic disease. A marker which would reflect immunologic and clinical activity in real time and predict clinical outcome could substantially improve the care of patients with MN.Phospholipase $A_2$ Receptor antibody ($PLA_2R$-Ab) levels may serve as such a biomarker.

**Methods:** The correlation of $PLA_2R$-Ab levels with proteinuria and their predictive value for clinical response was analyzed in a prospective multicenter study including 133 patients with primary MN. The patients were followed for up to 24 months. $PLA_2R$-Ab levels were assessed at the time of inclusion in the study and at 3 months intervals.

**Results:** During the time of observation 101 patients were treated with immunosuppressants, 32 patients received supportive care only. Following immunosuppression $PLA_2R$-Ab level fell rapidly (81% in 3 months), while the decrease in proteinuria was protracted (39% after 3 months). Immunosuppressive regimens (calcineurin inhibitors, alkylating agents or rituximab) did not show differences in the efficacy to reduce $PLA_2R$-Ab levels. In patients with spontaneous remission of proteinuria, $PLA_2R$-Ab levels fell (from 197±279 U/ml at study start to 7±6 U/ml at 15 months) but remained high in patients who did not show a reduction in proteinuria (from 193±184 U/ml at study start to 98±88 U/ml at 15 months). At the time of inclusion in the study there was a significant difference in the $PLA_2R$-Ab levels between patients who experienced remission of proteinuria after 12 months and those with no remission of proteinuria (Remission: 179±207 U/ml; No remission: 311±297 U/ml).

**Conclusions:** $PLA_2R$-Ab levels reflect clinical disease activity and predict clinical response in patients with primary MN. $PLA_2R$-Ab are reliable real time markers of disease activity in patients with primary MN and may affect clinical outcome.

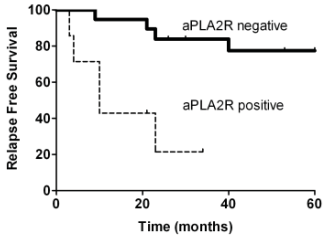
## FR-OR049

**Measurement of AntiPLA2R Antibodies Predicts Relapse Rate after Immunosuppressive Therapy in Patients with Idiopathic Membranous Nephropathy** Julia M. Hofstra,[1] Anneke Bech,[1] Paul E. Brenchley,[2] Jack F. Wetzels.[1] *[1]Nephrology, Radboud Univ Nijmegen Medical Center, Nijmegen, Netherlands; [2]Nephrology, Manchester Institute of Nephrology and Transplantation, Manchester, United Kingdom.*

**Background:** Antibodies against the phospholipase A2 receptor (PLA2R-ab) are present in 70% of patients with idiopathic membranous nephropathy (iMN). During immunosuppressive therapy a decrease of PLA2R-ab precedes remission of proteinuria. The clinical value of repeated PLA2R-ab measurements is unknown. Aim of this study is to evaluate if PLA2R-ab levels at the end of immunosuppressive treatment predict relapse rate in iMN.

**Methods:** We evaluated patients with iMN, and positive PLA2R-ab, who were treated with immunosuppression, and developed a remission. PLA2R-ab levels were measured with ELISA (Kanigicherla et al, 2013, KI) in serum samples collected at baseline and at the end of treatment.

**Results:** In total 26 patients were included (18 male). Treatment consisted of cyclophosphamide (n=11), MMF (n=8 ), or ACTH (n=7). At the start of treatment, the median eGFR was 59 ml/min/1.73m² (IQR 49-74), serum albumin 25 g/l (IQR 19-30) and PLA2R-ab 201 U/l (IQR 122-314). At the end of therapy, 27% of patients still were PLA2R-ab positive. During follow-up 35% of all patients developed a relapse of proteinuria (> 3.5 g/day with ≥ 50% increase from lowest level during remission) within 5 years after therapy. The risk of relapse in patients with PLA2R-ab positivity at end of treatment was significantly higher when compared to patients in whom PLA2R-ab had disappeared (relapse rate 21% versus 71%, p =<0.01).




**Conclusions:** PLA2R-ab remained elevated in a significant number of patients with iMN treated with immunosuppressive therapy. The persistence of antibodies was associated with a higher relapse rate. Our observations suggest that measurement of PLA2R-ab may guide duration of therapy in patients with iMN.

## FR-OR050

**Changes in Plasma Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) Levels Correlate with Naive T-Regulatory and Monocyte Subset Populations in Nephrotic Children with Focal Segmental Glomerulosclerosis (FSGS) following Rituximab Therapy** Wee Song Yeo,[1] Chang Yien Chan,[1] Changli Wei,[2] Jochen Reiser,[2] Subhra K. Biswas,[3] Hui Kim Yap.[1] *[1]Pediatrics, National Univ of Singapore, Singapore; [2]Internal Medicine, Rush Univ, Chicago, IL; [3]Singapore Immunology Network, A*STAR, Singapore.*

**Background:** Recent studies suggest suPAR as the plausible circulating factor in FSGS. In recent years, rituximab has been used in patients with nephrotic syndrome who have failed therapy with conventional immunosuppressants. This study aimed to examine the effect of rituximab in children with FSGS on plasma suPAR levels and identify the source of suPAR by correlating suPAR trends with various immunological subsets.

**Methods:** Eleven nephrotic children with FSGS were given 2-weekly doses of rituximab (375 mg/m²) for 1-4 doses. Urinary/blood biochemistry and immunological subsets (T-, B-, NK cells, and monocytes) with corresponding plasma suPAR levels were monitored throughout the study. Statistical analysis was done using Mann-Whitney U test and Spearman's correlation.

**Results:** Five (45%) patients achieved complete response defined as urine protein:creatinine ratio of <0.02 g/mmol and ability to wean off at least 2 immunosuppressants post-rituximab. Plasma suPAR levels did not differ significantly between complete responders (2622.02±298.10 pg/ml) and incomplete responders (3205.18±481.34 pg/ml) prior to initiation of rituximab therapy (p=0.47). Complete responders showed a negative mean-fold change in plasma suPAR levels (ΔsuPAR) (-0.033 ±0.077 pg/ml) compared to incomplete responders (0.436±0.173 pg/ml) (p=0.045). Significant correlation was shown between ΔsuPAR and mean change in naïve T-regulatory cells (CD25+CD45RA+CD4+) (ΔNTregs) (r=-0.85, p<0.001), as well as mean change in monocyte subsets (CD14dimCD16+ and CD14+CD16+)(Δmonocytes) (r=0.65, p=0.042). ΔNTregs also demonstrated a significant negative correlation with Δmonocytes (r=-0.75, p=0.013).

**Conclusions:** Our study suggested that the efficacy of action of rituximab in FSGS may be mediated via decrease in plasma suPAR levels, and this may be related to monocyte subset changes, regulated by naïve T-regulatory cells.

*Funding:* Government Support - Non-U.S.

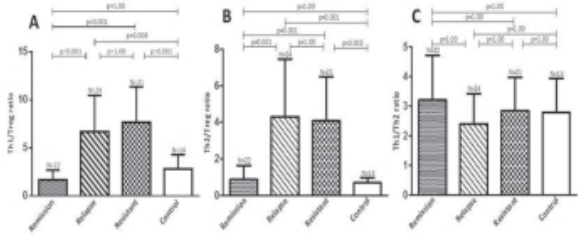
## FR-OR051

**Expression of Permeable Glycoprotein on Peripheral Blood T Lymphocytes with Regulatory and Effectors T Cells Imbalance Determines Steroid Response in Childhood Nephrotic Syndrome** Narayan Prasad,[1] Akhilesh Jaiswal,[1] Vikas Agarwal,[2] Brijesh Yadav,[1] Deepak Tripathi,[2] Amit Gupta,[1] Raj K. Sharma.[1] *[1]Nephrology, SGPGIMS, Lucknow, UP, India; [2]Immunology, SGPGIMS.*

**Background:** Permeable glycoprotein (Pgp) expression on lymphocytes may be related to cell mediated immune response and steroid response in Nephrotic syndrome (NS).We aimed to study the expression of Pgp on CD4+IFN-γ+ Th1, CD4+IL-4+ Th2 and CD4+CD25+FoxP3+ regulatory T lymphocyte (Treg) with their imbalance in steroid response in NS.

**Methods:** From patients of NS, 22 patients in sustained remission, 24 in relapse, and 21 steroid resistant patients and 14 healthy controls were included in the study .Circulating Treg, Th1 and Th2 lymphocytes and P-gp Expression on these T reg , Th1 and Th2 lymphocytes in patients in sustained remission, relapse,steroid resistant (SRNS) and healthy control were measured.

**Results:** The absolute expression of Pgp was greater in relapsed (83.51±37.22, P=0.001) and SRNS (101.72±44.91, P=0.001) compared to that of patients in remission (33.16±23.97) and controls (33.38±17.05). Th2 cells(%) in patients with remission (5.18±3.12) was significantly less than that of relapsed (9.89±5.23; P=0.006) or SRNS patients (10.74±5.91; P=0.001); and similar to that of control subjects (4.91±1.24) p=1.0. The % of Th1 cells was significantly lesser in patients with sustained remission (10.37±3.49) compared to that of patients during relapse (16.18±7.19; P=0.008); SRNS patients (20.24±7.01; P=0.001); and in controls (18.38±3.28; P=0.006). The Treg cells was significantly higher in controls and remission compared to that of SRNS and relapsed patients. The ratio of Th1/ Tregs, Th2/Tregs and Th1/ Th2 are shown in Figure 1 indicate that imbalance between Treg and Teff is responsible for remission and SRNS state.




**Conclusions:** The imbalance of Treg and Teff cells with expression of P-gp plays role in steroid response in NS.

*Funding:* Government Support - Non-U.S.

## FR-OR052

**Soluble Urokinase Receptor (suPAR) Is Not a Clinical Marker for Focal Segmental Glomerulosclerosis** Bjorn Meijers,[1] Ruben Poesen,[1] Markus Storr,[2] Kathleen Claes,[1] Dirk R. Kuypers,[1] Pieter Evenepoel.[1] *[1]Nephrology, Univ Hospitals Leuven, Belgium; [2]Gambro Dialysatoren GmbH, Hechingen, Germany.*

**Background:** Soluble urokinase receptor (suPAR) promotes proteinuria and induces glomerular focal segmental glomerulosclerosis (FSGS)-like lesions in mice. Furthermore, a serum suPAR concentration cut-off of 3000 pg/mL has been proposed as clinical biomarker for FSGS patients. Interestingly, several studies in patients with glomerulopathy demonstrated an inverse correlation between the eGFR and suPAR concentrations. As patients with FSGS present at different eGFR, we studied the relationship between eGFR and suPAR in a large cohort of non-FSGS patients.

**Methods:** We measured suPAR concentrations in patients with chronic kidney disease (CKD) from the Leuven mild-to-moderate CKD study (Clinical trials protocol NCT00441623) using the human uPAR enzyme-linked immune sorbent assay (ELISA, R&D systems). Variables determining suPAR concentrations were identified using multivariate regression analysis.

**Results:** We determined suPAR concentrations in 486 patients with known non-FSGS CKD (controls), and in 18 patients with biopsy-proven FSGS (cases: 6 active disease, 2 partial remission and 10 complete remission). In multivariate analyses, eGFR was the strongest determinant of suPAR concentrations (P<0.0001, $R^2$ 0.46). suPAR concentrations exceeded the proposed cut-off for FSGS of 3000 pg/mL in 17%, 39% and 88% in patients with eGFR of more than 60, 45-60 and 30-45 ml/min/1.73 m², respectively. In patients with eGFR of less than 30, suPAR concentrations exceeded the proposed cut-off in 95% of patients. suPAR levels of patients with biopsy-proven FSGS were interspersed with non-FSGS controls and for any given eGFR did not discriminate FSGS cases from non-FSGS controls.

**Conclusions:** This study does not support an absolute, i.e. eGFR independent, suPAR concentration cut-off as biomarker for FSGS. Our data moreover question the validity of relative, i.e. eGFR-dependent, suPAR cut-off values.

## FR-OR053

**Randomized Controlled Trial of Low-Dose Intravenous Cyclophosphamide versus Oral Mycophenolate Mofetil in Treatment of Lupus Nephritis** Ajay Goyal,[1] Manish Rathi,[1] Vivekanand Jha,[1] Aman Sharma,[2] Kusum Joshi,[3] Ritambhra Nada,[3] Vinay Sakhuja.[1] *[1]Nephrology, PGIMER, Chandigarh, India; [2]Internal Medicine, PGIMER, Chandigarh, India; [3]Pathology, PGIMER, Chandigarh, India.*

**Background:** Several recent controlled trials establish Mycophenolate Mofetil (MMF) as one of the first-choice drugs for inducing a remission in lupus nephritis. However, in most of these trials MMF was compared with either oral or high dose monthly pulses of intravenous cyclophosphamide (CYC). The current study was aimed at comparing the efficacy and safety of MMF with low-dose intravenous CYC (Euro-Lupus regimen) in these patients.

**Methods:** The study was an open label, prospective, randomized, two-arm study which included patients who had a kidney biopsy diagnosis of lupus nephritis (Class III/ IV/V). Out of 94 patients who were screened for inclusion, 53 patients were randomized to receive either low dose intravenous CYC (6 fortnightly pulses at a fixed dose of 500 mg) or MMF (target dosage 3g/day) as induction treatment for a period of 24 weeks in addition to steroids. The primary end point was a pre-specified decrease in proteinuria and

stabilization or improvement in serum creatinine. Secondary end points included complete renal remission, systemic disease activity (by Safety of Exogenous Estrogens in Lupus Erythematosus National Assessment/Systemic Lupus Erythematosus Disease Activity Index) and adverse events.

**Results:** At the end of 24 weeks, both groups had similar response rates. In the intention-to-treat analysis, 19 of the 26 patients (73.1%) who received CYC and 19 of the 27 patients receiving MMF (70.4%) showed a response (primary end point) (95% CI, 0.34 - 3.78%; *p*=0.83). Complete remission was achieved in 13 of the 26 patients (50.0%) and 13 of the 27 patients (48.1%), respectively (*p*=0.89). Gastro-intestinal symptoms and infections occurred in significantly lesser number of patients in CYC group as compared to MMF group. Four patients (1 in CYC group and 3 in MMF group) died during the study period.

**Conclusions:** MMF had similar efficacy in comparison to low dose intravenous CYC in terms of response and remission rates, however, it was associated with more adverse events.

## FR-OR054

### Long Term Follow Up of Patients Who Received Repeat Dose Rituximab as Maintenance Therapy for ANCA Associated Vasculitis (AAV)

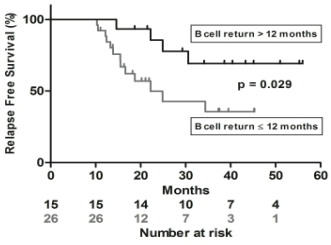
Federico Alberici, Rona M. Smith, Rachel B. Jones, David R.W. Jayne. *Vasculitis and Lupus Clinic, Addenbrooke's Hospital, Cambridge, United Kingdom.*

**Background:** Rituximab (RTX) is an established induction agent in AAV. We previously showed that repeat dose RTX for two years is a potential maintenance strategy. Relapse risk after repeat dose RTX discontinuation is not known.

**Methods:** We report long term follow up of patients who received a two year repeat dose RTX regimen for relapsing/refractory AAV (1g x 2, then 1g/6 months x 4).

**Results:** Sixty-nine patients completed the two year RTX course, during the treatment course 9 (13%) relapsed. Median post-treatment follow-up was 22.7 months (IQR12.5-38.6). 25 relapsed after a median of 30.6 months (95%CI 18.4-42.7). For relapse treatment, 20 received RTX and five other agents. By 6 months, 21/25 (88%) had regained remission.

54/69 were ANCA negative at the end of the RTX course. 12 (22%) became ANCA positive during follow-up, of which 9 (75%) relapsed a median of 1.6 months (0.5-4.6) after. 15 remained ANCA positive after the RTX course, of which 3 (20%) relapsed. Thus,12/25 (48%) had detectable ANCA at relapse. Switch of ANCA to positive conferred an 11.81 fold increased risk of relapse compared to those whom maintain their ANCA status (p=0.001). Of 56 patients (81%) with available B-cell counts, 42 (75%) had B-cell return a median of 11 months (IQR 9-13) after the RTX course. 17/25 (68%) had detectable B cells at relapse, and in 11/17 (65%) B cells had returned in the 6 months preceding relapse. The patients experiencing B-cell return within 12 months had a significant shorter relapse free survival compared to the ones whose B-cell returned after 12 months.




**Conclusions:** Following a two year RTX course, relapse risk was lower than that seen following a single RTX course for relapsing GPA. A switch from ANCA negativity to positivity and earlier B cell return were relapse predictors.

## FR-OR055

### Rituximab and Cyclophosphamide Decreases Dialysis and Death Rates in Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis

JulieAnne G. McGregor,[1] Susan L. Hogan,[1] Elizabeth Studstill,[1] Caroline J. Poulton,[1] Yichun Hu,[1] Roberto Negrete-Lopez,[2] Jason M. Kidd,[1] Suzanne L. Katsanos,[1] Donna O. Bunch,[1] Patrick H. Nachman,[1] Ronald J. Falk.[1] *[1]UNC Kidney Center, Chapel Hill, NC; [2]Hospital Universitario-UANL, Monterrey, Nuevo Leon, Mexico.*

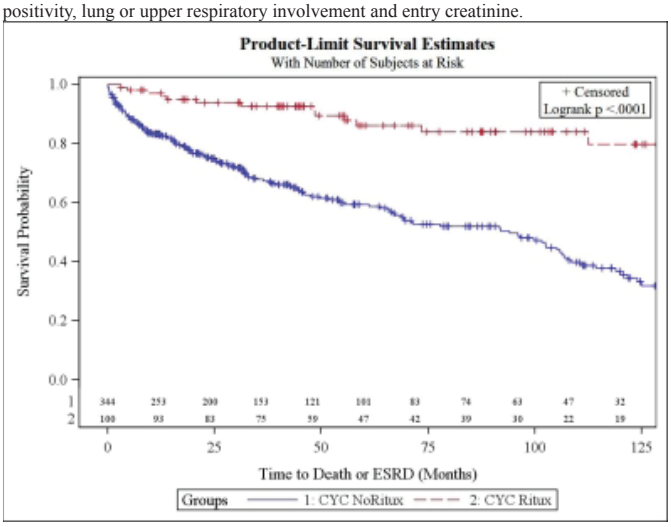
**Background:** Current evidence for use of rituximab (RTX) in Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis (AAV) has not shown improvement in hard outcomes including dialysis or death.

**Methods:** 100 patients with AAV (1991-2011) who received RTX, cyclophosphamide (CYC) and corticosteroids at any time were compared to 351 patients who received CYC and corticosteroids without RTX for treatment. End-stage renal disease (ESRD) at onset was excluded. Comparisons were done by Fisher's exact test for categorical and Kruskal-Wallis test for continuous variables. Multivariable Cox Regression was used. Incidence per person-year of follow-up was calculated.

**Results:** 166 RTX courses were given to 100 patients who had previously or concurrently received CYC. 4 patients had RTX at induction, 18 for resistance to CYC, 123 for relapse and 21 for remission maintenance.

Incidence of infection was higher in patients treated with CYC alone than patients also treated with RTX (0.20 95% CI [0.19, 0.21]; (0.13 95% CI [0.12, 0.15], respectively, p=<0.0001); likewise for severe infection (0.24 95% CI [0.21, 0.28], 0.17 95% CI [0.13, 0.22], respectively p=0.0150).

Those treated with RTX and CYC were less likely to reach death or dialysis than those treated with CYC alone which remained significant controlling for age, MPO/PR3 ANCA positivity, lung or upper respiratory involvement and entry creatinine.




HR for death or ESRD among patients who at some point received RTX was 0.27, 95% CI: 0.15-0.48, p=<0.0001.

**Conclusions:** Patients treated with RTX and CYC were 3.7 times less likely to reach ESRD and death, and had a lower incidence of infections and severe infections.

*Funding:* NIDDK Support

## FR-OR056

### Genetic Analysis of the Coagulation Pathway in Atypical Hemolytic Uremic Syndrome

Richard J. Smith, Fengxiao Bu, Nicole Meyer, Kai Wang, Carla M. Nester. *Univ of Iowa.*

**Background:** Atypical Hemolytic Uremic Syndrome (aHUS) is a complement-driven disease. Approximately 40% of patients carry single mutations in CFH, C3, CFI, CFB or CD46; multiple mutations are identified in 3% of patients. We hypothesized that additional genetic risk factors for aHUS could be identified by screening all genes in the coagulation pathway. We used targeted genomic enrichment and massively parallel sequencing (TGE+MPS) to screen all coding exons of the 85 genes that comprise the complement and coagulation pathways.

**Methods:** 47 patients with sporadic aHUS patients were included in this study. TGE was performed using an RNA-bait panel and MPS of captured libraries was completed on a HiSeq 2000 and a MiSeq. Data were analyzed using a local installation of the open-source Galaxy software running on a high-performance computing cluster at the University of Iowa.

**Results:** Mean total reads per sample approximated 9,000,000, with >97% mapping to the reference genome and >50% mapping to targeted regions. Average coverage-per-targeted-base was larger than 1600 reads; 99% of targeted regions had 30x coverage. Of the 9,966 variants identified, 9,424 (94.56%) passed quality control. After filtering by frequency and predicted functionality, 25 novel and 101 non-synonymous rare variants (nsRVs) remained. 55 of these variants were known or predicted to be deleterious and were carried by 32 patients. 27 of these 55 variants were found in previously reported aHUS genes, 18 were found in other complement-related genes, and 16 were found in coagulation pathway genes. The greatest number of deleterious variants was found in *CFH* and the second greatest number was found in *PLG*. Rare variants in *CFB* (rs143259927, Fisher Exact P = 0.001) and *PLAU* (rs55744193, Fisher Exact P = 0.01) were also significantly correlated with the disease.

**Conclusions:** As expected, most deleterious variants (39 of 55) were found in complement genes implicated in the pathogenesis of aHUS. However, variants in coagulation pathway genes, especially in antithrombotic genes *PLAU*, *PLG*, *PROC* and *ADAMTS13*, were also found in aHUS patients implicating this pathway in the pathogenesis of aHUS.

*Funding:* NIDDK Support

## FR-OR057

### Eculizumab (ECU) Inhibits Thrombotic Microangiopathy (TMA) and Improves Renal Function in Adult Atypical Hemolytic Uremic Syndrome (aHUS) Patients (Pts)

Fadi Fakhouri, Maryvonne Hourmant, Josep M. Campistol, Spero R. Cataland, Mario Espinosa, A. Osama Gaber, Jan Menne, Enrico E. Minetti, Francois Provot, Eric Rondeau, Piero Ruggenenti, Laurent E. Weekers, Masayo Ogawa, Camille L. Bedrosian, Christophe M. Legendre. *The C10-004 Study Group.*

**Background:** aHUS is a rare, severe, genetic, life-threatening disease of chronic complement-mediated TMA. ECU, a terminal complement inhibitor, is approved for the treatment of aHUS. Here, we report safety and efficacy results of ECU in adult pts from the largest prospective study performed in aHUS.

**Methods:** This was a single-arm, Phase 2 trial of ECU in adult pts (≥18 yrs) with aHUS and platelets <LLN at screening. Prior plasma exchange or infusion (PE/PI) was not required for inclusion. The primary endpoint was the proportion of pts with complete TMA response at 26 wks.

**Results:** 41 pts enrolled and 38 (93%) received 26 wks of treatment. 30 pts (73%) were newly diagnosed (median duration to treatment initiation of 2 weeks). Six pts had no PE/PI during the current clinical manifestation. At wk 26, 30 pts (73%) achieved the primary endpoint.

| Table: Wk 26 ITT Results (N=41) | |
|---|---|
| **Baseline Demographics and Disease Characteristics** | |
| Age (years) – mean (SD) | 40.3 (15.3) |
| Female sex – n (%) | 28 (68) |
| Identified complement regulatory protein mutation or auto-antibody – n (%) | 21 (51) |
| Time from aHUS diagnosis until screening (months) – median (range) | 0.8 (0–311) |
| Newly diagnosed pts – n (%) | 30 (73) |
| Duration of current clinical manifestation of aHUS (months) – median (range) | 0.5 (0.0–19.1) |
| PE/PI during current clinical manifestation of aHUS – n (%) | 35 (85) |
| Dialysis at baseline – n (%) | 24 (59) |
| Prior renal transplant – n (%) | 9 (22) |
| Platelet count <150x10⁹/L – n (%) | 27 (66) |
| LDH >ULN – n (%) | 32 (78) |
| eGFR ≤60 mL/min/1.73 m² – n (%) | 41 (100) |
| **Efficacy Outcomes** | |
| Complete TMA response – n (%) | 30 (73) |
| Hematologic normalization– n (%) | 36 (88) |
| Platelet count normalization – n (%) | 40 (98) |
| Platelet count increase (x10⁹/L) – mean (95% CI) | 119 (94; 145) *P*<0.0001 |
| eGFR increase from baseline ≥15 mL/min/1.73 m² – n (%) | 22 (54) |
| eGFR increase from baseline (mL/min/1.73 m²) – mean (95% CI) | 26.1 (19.8; 32.4) *P*<0.0001 |
| CKD improvement ≥1 stage from baseline – n (%) | 26 (63) |
| **Complete TMA response**: normalization of platelets and LDH, and <25% increase in serum Cr from baseline on 2 consecutive measurements ≥4 wks apart. **Hematologic normalization:** platelet and LDH normalized for ≥2 consecutive measurements ≥4 wks apart. **Platelet count normalization:** platelet count ≥150x10⁹/L ≥2 consecutive measurements ≥4 wks apart. | |

24/41 pts (59%) were on dialysis at BL, 20 of whom discontinued by wk 26. Mean (95% CI) eGFR increase from baseline was 26.1 mL/min/1.73 m² (19.8; 32.4: *P*<0.0001). Two pts who were not on dialysis at baseline initiated during the treatment period and remained on dialysis through 26 wks. QoL significantly improved. ECU was generally safe and well tolerated. Two pts had meningococcal infections; one pt continued ECU. No pts died.

**Conclusions:** ECU normalized hematologic parameters and significantly improved renal function and QoL. 83% of pts on dialysis at baseline were able to discontinue by wk 26. The results of this prospective study confirm that ECU inhibits complement-mediated TMA in adult aHUS pts. The study is ongoing.

*Funding:* Pharmaceutical Company Support - Alexion Pharmaceuticals

## FR-OR058

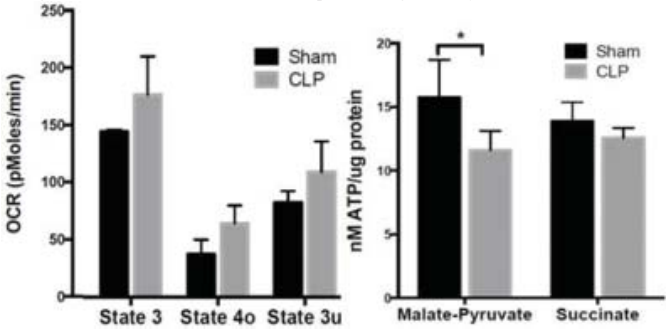
### Mitochondrial Dysfunction in Sepsis-Associated Acute Kidney Injury

Prabhleen Singh, Koji Aoyama, Joanna Thomas. *Medicine, UCSD & VASDHS, San Diego, CA.*

**Background:** Sepsis-associated AKI is frequently observed and has poor prognosis. The lack of complete understanding of its pathogenesis is a significant barrier to progress and fresh insights into its pathogenesis are critically needed.

**Methods:** We examined renal oxygenation (oxygen delivery and consumption) and mitochondrial function in the cecal ligation and puncture (CLP) model of sepsis in rats. RBF, GFR, and kidney oxygen consumption (QO2) were measured at 6 and 24 hours post CLP or sham surgery. Mitochondrial QO2, ATP generation, HIF1α expression and response to HIF-1α activation by DMOG was assessed. Data presented as mean±sem.

**Results:** At 6 and 24 hours, GFR was significantly lower in CLP (2.5±0.4 vs. 3.6±0.2 ml/min, p=0.03 at 6 hours and 1.2±0.3 vs. 3.3±0.2 ml/min, p<0.001 at 24 hours). While RBF and $O_2$ delivery was largely unchanged in CLP, renal $O_2$ extraction factored for GFR was higher compared to shams, (0.023±0.002 vs. 0.01±0.001 vol%ml/min, p=0.001) as was renal $QO_2$/GFR (0.11±0.01 vs. 0.05±0.01 ml/min, p=0.04). HIF-1a expression was seen in cortical and medullary tubules indicating hypoxia due to oxygen demand-supply mismatch. There was a trend for improvement in GFR at both 6 and 24 hours. In CLP mitochondria, we found elevated rates of both coupled and uncoupled respiration. However, ATP generation measured in the same samples was significantly lower.




**Conclusions:** We demonstrate increased renal oxygen utilization in the CLP rats sespite lower GFR and reabsorptive load leading to hypoxia. We also demonstrate dissociation between excess oxygen consumption and ATP generation in CLP mitochondria, which can explain the inefficiency in oxygen utilization. We are investigating alterations in mitochondrial dynamics which could explain mitochondrial dysfunction and tubular injury, and the role of HIF-1α pathway in ameliorating mitochondrial dysfunction and consequently global renal function.

*Funding:* NIDDK Support

## FR-OR059

### Even Delayed Therapy with the Mitochondria-Targeted Antioxidant Mito-TEMPO Ameliorates Sepsis-Induced Acute Kidney Injury

Naeem K. Patil, Lee Ann MacMillan-Crow, Philip R. Mayeux. *Pharmacology and Toxicology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

**Background:** Sepsis is characterized by severe systemic inflammatory response to a microbial infection. Acute kidney injury (AKI) is a frequently encountered complication of sepsis and leads to a high mortality rate ~70%. Human and animal studies suggest that mitochondrial dysfunction plays an important role in sepsis induced multi-organ failure, but the importance of mitochondrial dysfunction in renal injury during sepsis has not been well studied.

**Methods:** We used a clinically relevant cecal ligation and puncture (CLP) murine model of sepsis to assess renal mitochondrial function. High resolution respirometry was used to measure mitochondrial complex respiration. Intravital video microscopy was used to study oxidant generation and renal microcirculation.

**Results:** As early as 4h after CLP, the activity of manganese superoxide dismutase (MnSOD) was decreased by 50% and inhibition was sustained through 36h. This was associated with increased mitochondrial superoxide levels, implying compromised mitochondrial antioxidant defense. CLP also caused a time-dependent decrease in renal mitochondrial complex I and II/III respiration and a reduction in renal ATP levels. *Thus, we hypothesized that a mitochondrial specific antioxidant Mito-TEMPO (MT) could protect renal mitochondria and attenuate sepsis-induced AKI.* Dose-response studies with 3, 10 and 30mg/kg ip showed that 10mg/kg was the lowest most efficacious dose that decreased renal mitochondrial superoxide levels after CLP. Thus, this dose was used in a clinically relevant delayed dosing paradigm. MT given at 6h post CLP decreased mitochondrial superoxide levels, protected complex I and II/III respiration, restored MnSOD activity and improved renal microcirculation by 18h. Importantly, delayed therapy with MT significantly increased the 96h survival rate from 40% in untreated septic mice to ~83%.

**Conclusions:** Mitochondrial dysfunction is a critical event in sepsis induced AKI and even delayed therapy with mitochondria-targeted antioxidant MT represents a promising approach to treat septic AKI. Supported by NIH DK075991 (PRM) and AHA 12PRE12040174 (NKP).

*Funding:* NIDDK Support, Private Foundation Support

## FR-OR060

### Enhanced Glycolytic Activity and Mitochondrial Dysfunction in a Mouse Model of Sepsis-Induced Acute Kidney Injury

Joshua Andrew Smith, L. Jay Stallons, Rick G. Schnellmann. *Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC.*

**Background:** Sepsis is a common cause of acute kidney injury (AKI), accounting for approximately half of AKI cases in the ICU. Recent evidence indicates that septic AKI results in renal mitochondrial dysfunction. Because the mechanisms by which renal cortical ATP is maintained in the presence of mitochondrial dysfunction are unknown, we investigated glycolysis as a source of ATP production in a mouse model of sepsis-induced AKI.

**Methods:** Male C57BL/6 mice were treated with lipopolysaccharide (LPS, 10 mg/kg, i.p.) and kidneys harvested (3, 6, and 18 h post-LPS). Renal function was determined by measuring blood urea nitrogen (BUN) and urine output. Mitochondrial electron transport chain and glycolytic mRNAs and proteins in the renal cortex were assessed by qRT-PCR and immunoblot analysis. Standard biochemical assays were used to determine renal cortical hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) activities.

**Results:** LPS administration resulted in AKI with increased BUN and reduced urine output 18 h post-LPS. Transcript levels of mitochondrial markers (PGC-1α, ATPSb, NDUFS1, and COX1) were reduced >50% 18 h post-LPS treatment. Expression of HK2 mRNA increased (~2-fold) in these mice, while expression of other glycolysis-related mRNAs remained unchanged (glut1, pfkl, pkm, and ldha) or decreased (pfkm, pgk1, and pdk1). Immunoblot analysis revealed no changes in protein levels of glycolytic enzymes (HK1, HK2, PFKP, PKM1/2, and LDHA). However, a ~3-fold increase in HK activity was observed 3 h after LPS exposure and was maintained over 18 h.

**Conclusions:** Renal hexokinase activity rapidly increases in conjunction with the loss of mitochondrial function following LPS exposure. These findings suggest that glycolysis may be induced to maintain ATP levels and renal function in sepsis-induced AKI.

*Funding:* Other NIH Support - 5R01GM084147-04, Veterans Affairs Support

**FR-OR061**

**Muc1 Is Protective in a Mouse Model of Acute Kidney Injury** Rebecca P. Hughey,[1] Sheldon Bastacky,[3] Kenneth R. Hallows,[1] Nuria M. Pastor-Soler,[1] Timothy A. Sutton.[2] *[1]Dept of Medicine, Renal-Electrolyte Div, Univ of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Dept of Medicine, Div of Nephrology, Indiana Univ School of Medicine, Indianapolis, IN; [3]Div of Anatomic Pathology, Dept of Pathology, Univ of Pittsburgh School of Medicine, Pittsburgh, PA.*

**Background:** Ischemia-reperfusion injury (IRI) due to hypotension or sepsis is the most common cause of acute kidney injury (AKI). Human MUC1 is an apical transmembrane protein expressed in the distal tubules and collecting ducts of the adult kidney. MUC1 is induced by hypoxia and directly interacts with both HIF-1 and β-catenin in the nucleus where MUC1 transactivates these protective pathways and modulates metabolic adaptations and promotes cell survival.

**Methods:** To assess the function of Muc1 during AKI, we used a model of IRI in Muc1 knockout (KO) and congenic control C7BL/6 mice. Both renal pedicles of mice were clamped for 19 min prior to recovery periods of 0, 4, 24 and 72 h when blood and kidneys were recovered.

**Results:** Profiles of serum creatinine and BUN were significantly higher in the profile from Muc1 KO than control mice consistent with increased kidney damage and delayed recovery of kidney function in the absence of Muc1. This difference was readily apparent in the microscopic analysis of kidney morphology after H&E staining and scoring of tubule damage. Immunoblot analysis of kidney extracts revealed a significant increase in Muc1 levels consistent with its induction by hypoxia. We observed Muc1-dependent changes in glycolytic enzymes (e.g., LDHA) and kinases (e.g., PDK1 and PKM2) involved in the hypoxia-induced switch of glucose metabolism from oxidative to glycolytic, consistent with Muc1 transactivation of the HIF-1 pathway. This was supported by finding an accentuated and prolonged activation of AMPK in the kidneys of Muc1 KO mice after IRI when compared to controls, reflecting energy stress. As PKM2 also transactivates the β-catenin pathway we discovered significant Muc1-dependent increases in β-catenin and its downstream target consistent with a role for Muc1 in cell survival.

**Conclusions:** Altogether the data indicate that Muc1 is required for both efficient adaptation of glucose metabolism in response to IRI to avoid energy stress and for promoting tubule survival and recovery.

*Funding:* NIDDK Support, Other NIH Support - DCI, Inc.

**FR-OR062**

**Increasing cGMP-Dependent Protein Kinase I Activity Attenuates Cisplatin Induced Kidney Injury through Protection of Mitochondria Function** Shuxia Wang. *Nutritional Sciences, Univ of Kentucky, Lexington, KY.*

**Background:** Cisplatin is widely used to treat malignancies. However, its major limitation is the development of dose-dependent nephrotoxicity. The precise mechanisms of cisplatin-induced kidney damage remain unclear, and the renoprotective agents during cisplatin treatment are still lacking.

**Methods:** Both in vitro proximal tubular cell culture and in vivo ischemia-reperfusion induced acute kidney injury mouse model were utilized. In adidiiton, genetic as well as pharmacological approach was used to increase cGMP-dependent protien kinase activity in mice and the development of acute kidney injury in these mice was determined.

**Results:** We demonstrated that the expression and activity of cGMP-dependent protein kinase-I (PKG-I) was reduced in cisplatin treated renal tubular cells in vitro as well as in the kidney tissues from cisplatin treated mice in vivo. Increasing PKG activity by both pharmacological and genetic approaches attenuated cisplatin-induced kidney cell apoptosis in vitro. This was accompanied by decreased Bax/Bcl2 ratio, caspase 3 activity, and cytochrome c release. Cisplatin-induced mitochondria membrane potential loss in the tubular cells was also prevented by increased PKG activity. All of these data suggest a protective effect of PKG on mitochondria function in renal tubular cells. Importantly, increasing PKG activity pharmacologically or genetically diminished cisplatin-induced tubular damage and preserved renal function during cisplatin treatment in vivo. Mitochondria structural and functional damage in the kidney from cisplatin treated mice was inhibited by increased PKG activity. In addition, increasing PKG activity enhanced ciaplatin induced cell death in several cancer cell lines.

**Conclusions:** These results suggest that increasing PKG activity may be a novel option for renoprotection during cisplatin-based chemotherapy.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-OR063**

**Conditional Proximal Tubule Mitofusin 2 Knockout (cPT-MFN2 KO) Accelerates Renal Recovery and Improves Survival after Ischemia** Zhiyong Wang, Jonathan M. Gall, Ramon G. Bonegio, Andrea Havasi, John H. Schwartz, Steven C. Borkan. *Renal Section, Boston Univ Medical Center, Boston, MA.*

**Background:** Elimination of MFN 2, a major mitochondrial elongation protein, sensitizes proximal tubule (PT) cells to Bax-mediated death after *in vitro* stress. To assess its role in the intact kidney, the effect of cPT-MFN2 KO was evaluated after renal ischemia *in vivo.*

**Methods:** cPT-MFN2 KO mice were generated by crossing MFN2 floxed mice ($MFN2^{ff}$) with animals harboring a testosterone activated, PT-specific KAP2-Cre. These mice were supplemented with exogenous testosterone to enhance conditional PT MFN2 knockout prior to ischemia induced by bilateral renal pedicle occlusion. Testosterone treated parental line mice served as control. MFN2 KO in cultured cells was achieved by exposing primary PT cells harvested from $MFN2^{ff}$ mice to Cre expressing adenovirus.

**Results:** Compared to control, testosterone exposure in $MFN2^{ff}$–MFN2 KO mice selectively decreased PT MFN2 expression and resulted in short, punctate mitochondria in renal cortical PT cells. After transient renal ischemia induced AKI, only 28% of testosterone-treated control mice survived (n=18). In contrast, 86% of cPT-MFN2-KO animals survived (n=22; $P<0.001$). Enhanced survival in cPT-MFN2 KO mice was associated with a significantly lower peak BUN on days 1-2 post injury. Although histologic injury score did not differ between groups, cPT-MFN2-KO mice exhibited a four-fold increase in cell proliferation restricted to the renal cortex, the region targeted by the conditional MFN2 KO ($P<0.05$). Preliminary studies in primary PT cells suggest that MFN2 KO enhances proliferation by increasing RAS-dependent, ERK-1/2 activation. MFN2 binds p21 RAS, linking cell MFN2 to ERK-1/2 mediated PT cell proliferation.

**Conclusions:** These results support the hypothesis that PT MFN2 exerts independent, dual roles on: (1) mitochondrial morphology that regulates Bax-mediated apoptosis and; (2) ERK-1/2 dependent PT cell proliferation during renal recovery from ischemia. We speculate that MFN2 KO enhances survival by simultaneously increasing the removal of damaged PT cells by apoptosis and accelerating repair of injured tubular epithelium after ischemic AKI.

*Funding:* NIDDK Support

**FR-OR064**

**Activation of AMP-Activated Protein Kinase Contributes to Cisplatin-Induced Acute Kidney Injury** Yanlin Wang, Xiaogao Jin. *Deaprtment of Medicine, Div of Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** Cisplatin, a commonly used anti-cancer drug, has been shown to induce acute kidney injury, which limits its clinical use in cancer treatment. Emerging evidence has suggested that AMP-activated protein kinase (AMPK), which functions as a cellular energy sensor, is activated by various cellular stresses. However, the potential role of AMPK in cisplatin-induced acute kidney injury has not been studied.

**Methods:** We used mouse renal tubular epithelial cells to examine the role of AMPK signaling in cisplatin-induced apoptosis in vitro and we treated mice with Compound C, a selective AMPK inhibitor, to determine the role of AMPK in cisplatin-induced acute kidney injury in vivo.

**Results:** Our results demonstrated that cisplatin activated AMPK (Thr172 phosphorylation) in cultured renal tubular epithelial cell in a dose- and time-dependent manner, which led to p53 phosphorylation, Bax induction, caspase 3 activation, and apoptosis. Compound C, a selective AMPK inhibitor, abolished cisplatin-induced AMPK activation and p53 phosphorylation and apoptosis. Furthermore, silence of AMPK expression by siRNA attenuated cisplatin-induced apoptosis through inhibiting p53 phosphorylation, Bax induction, and Caspase 3 activation. Conversely, AICAR, a selective AMPK activator, dose-dependently induced p53 phosphorylation and subsequent Bax induction and apoptosis. AMPK is activated in the kidney in cisplatin treated mice. Cisplatin-induced renal dysfunction and tubular epithelial cell apoptosis in vivo were attenuated by treatment with Compound C.

**Conclusions:** Our results indicate that AMPK signaling plays a pivotal role in the development of cisplatin-induced renal injury through regulation of P53-Bax apoptosis pathway. These findings suggest that inhibition of AMPK signaling could be a novel target for the prevention and treatment of acute kidney injury.

*Funding:* NIDDK Support

**FR-OR065**

**Preconditioning Mouse Proximal Tubular Cells with Pharmacologic Activators of AMP-Activated Protein Kinase Ameliorates Apoptosis Induced by Subsequent Metabolic Stress** Wilfred Lieberthal,[1] Leiqing Zhang,[1] Jerrold S. Levine.[2] *[1]Medicine, Stony Brook Medical Center, Stony Brook, NY; [2]Medicine, Univ of Chicago at Illinois, Chicago, IL.*

**Background:** We have previously reported that AMP-activated protein kinase **(**AMPK) is activated when mouse proximal tubular (MPT) cells are subjected to metabolic stress, and plays a role in ameliorating stress-induced apoptosis of these cells (Lieberthal et. al.: AJP, 301:F1177, 2011). We now test the hypothesis that preconditioning MPT cells with pharmacologic activators of AMPK, can reduce apoptosis induced by subsequent metabolic stress. We also compare the efficacy of two novel AMPK activators (A768662 and RSVA314) with that of AICAR, an AMPK activator used for many years to elucidate the functions of AMPK.

**Methods:** MPT cells were preconditioned with AICAR, A-769662, or RSVA314. After preconditioning, the cells were subjected to metabolic stress by inhibiting mitochondrial function using antimycin A.

**Results:** Preconditioning MPT cells with AMPK activators was found to: 1) increase the expression of Glut1 and hexokinase II; 2) stimulate lactate production; 3) reduced the fall in cell ATP levels induced by metabolic stress and 4) ameliorate stress-induced apoptosis of MPT cells. All these effects of preconditioning were substantially greater with A769662 and RSVA314 than with AICAR.

**Conclusions:** Preconditioning MPT cells with AMPK activators substantially ameliorates apoptosis induced by subsequent metabolic stress. This beneficial effect of preconditioning appears due to the stimulation of glycolysis, and to a consequent conservation of cell energy stores during metabolic stress. Furthermore, A769662 and RSVA314 are more effective preconditioning agents than AICAR.

*Funding:* Veterans Affairs Support

**FR-OR066**

**Deleterious Role of the Polyol Pathway and Fructokinase Activation in the Proximal Tubule in Mice Undergoing Ischemic Acute Kidney Injury**
Miguel A. Lanaspa, Ana Andres-Hernando, Christina Cicerchi, Shinichiro Inaba, Sarah Faubel, Richard J. Johnson. *Medicine, Div of Renal Disease and Hypertension, Univ of Colorado Denver, Aurora, CO.*

**Background:** Despite important discoveries in the pathophysiology of acute kidney injury, no clinically applicable treatment to accelerate kidney recovery has emerged and new targets are needed. Our published data show that the metabolism of dietary fructose by fructokinase (KHK) results in ATP depletion and the generation of uric acid and oxidants causing acute tubulointerstitial injury. Here we show that besides dietary intake, fructose is endogenously produced from glucose by the polyol pathway in the renal cortex of mice undergoing ischemic acute kidney injury (iAKI). The aim of this study is to determine the specific role of this endogenous fructose and its metabolism by KHK in iAKI.

**Methods:** iAKI was induced by renal pedicle clamping in wild type (WT) or KHK knockout (KHK-KO) mice. Sham operation referred to renal pedicle exposure without clamping. iAKI was assessed by determination of parameters of renal function (serum creatinine and BUN), inflammation (serum and renal IL-6), and kidney tubular injury was assessed by NGAL urinary excretion and histology.

**Results:** Mice undergoing iAKI demonstrated a significant activation of the polyol pathway in the kidney cortex as noted by high levels of aldose reductase, sorbitol and fructose accumulation. Compared to sham, mice undergoing iAKI developed significantly higher serum creatinine and BUN as well as urinary NGAL. Also, iAKI induced elevated serum and renal levels of the pro-inflammatory cytokine IL6. Of interest, WT but not KHK-KO mice demonstrated renal ATP depletion, oxidative stress and uric acid generation. Furthermore, KHK-KO mice undergoing iAKI had improved renal function compared to WT mice with lower serum creatinine and BUN levels, reduced NGAL levels and improved proximal tubule histology.

**Conclusions:** Our data demonstrate that fructose generated and metabolized by KHK in proximal tubules during iAKI may have a deleterious role in the pathogenesis of this condition. Therefore, fructokinase (KHK) is a novel potential target for the prevention and treatment of iAKI.

*Funding:* NIDDK Support

**FR-OR067**

**Hypoxia-Inducible Factor-2 in Endothelial Cells Mediates Protection and Recovery from Ischemic Kidney Injury**
Pinelopi P. Kapitsinou,[1] Hideto Sano,[1] Timothy A. Sutton,[2] Volker H. Haase.[1] *[1]Nephrology, Vanderbilt Univ, Nashville, TN; [2]Nephrology, Indiana Univ, Indianapolis, IN.*

**Background:** Key mediators of cellular adaption to hypoxia are hypoxia-inducible factor (HIF)-1 and -2, which are regulated by the prolyl hydroxylases (PHDs). Although the PHD/HIF pathway has been shown to promote tolerance to acute ischemia, little is known about their cell type-specific functions in this context. Here, we used a genetic approach to specifically dissect the functions of endothelial HIF-1 and HIF-2 in renal ischemia-reperfusion injury (IRI).

**Methods:** To delete HIF-1 and -2 in endothelial cells (EC), Vecadherin (Cdh5)-Cre transgenic mice were crossed to mice carrying conditional HIF-1 and/or HIF-2 alleles. Systemic HIF activation was induced by a proly-hydroxylase-inhibitor (PHI), while EC specific HIF activation was achieved by crossing Cdh5-Cre transgenics to Phd2 floxed mice. IRI was induced by uni-/bilateral renal artery clamping.

**Results:** While at 2hrs and day1 post IRI, Hif1Hif2$^{-/-}$ showed similar injury to controls, at day3 they demonstrated impaired recovery as shown by higher morphologic injury score, an 1.6-fold increase in *Kim1* mRNA and a 47% increase in BUN levels compared to controls.Time course analysis of leucocyte infiltration by CD45 staining revealed an 8.5-fold increase in CD45$^{+}$area in Hif1Hif2$^{-/-}$ at day3 post IRI, which correlated with enhanced *VCAM1* expression. Renal IRI on single Hif1$^{-/-}$ or Hif2$^{-/-}$showed that Hif2$^{-/-}$ but not Hif1$^{-/-}$ mirror the changes observed in Hif1Hif2$^{-/-}$. To test whether EC-Hif-2 is required in PHI-mediated cytoprotection, wild type (WT) and Hif2$^{-/-}$mice were treated with PHI or vehicle, exposed to renal IRI and analyzed on day3 post IRI. In contrast to WT mice, PHI-induced cytoprotection was abolished in Hif2$^{-/-}$. Finally, EC-Phd2$^{-/-}$were protected from renal IRI as shown by a 2.2-fold reduction in *Kim1* mRNA compared to controls (n=3-4, P<0.01).

**Conclusions:** Our data establish a critical role for endothelial HIF-2 a) in the regulation of IRI-associated inflammation and recovery from kidney injury b) in the PHI-induced cytoprotection. Our findings identify the PHD2/HIF2 axis as a potential target for renoprotective therapies.

*Funding:* NIDDK Support

**FR-OR068**

**Tankyrase/β-Catenin Signaling: A Novel Regulatory Signaling Pathway for Vasopressin-Induced AQP2 Expression in Kidney Collecting Duct Cells**
Hyun Jun Jung, Eunjung Kim, Tae-Hwan Kwon. *Biochemistry and Cell Biology, School of Medicine, Kyungpook National Univ, Taegu, Korea.*

**Background:** Water channel aquaporin-2 (AQP2) mediates arginine vasopressin (AVP)-induced water reabsorption in the kidney collecting ducts. AVP increases the expression of AQP2 mainly via Gsα/cAMP/PKA signaling. Tankyrases, members of PARP family, are known to mediate Wnt/β-catenin signaling-induced transcription of Wnt-targeting genes. We aimed to examine as to whether tankyrase/β-catenin signaling play a role in AVP-induced AQP2 expression in kidney collecting ducts.

**Methods:** We performed 1) semiquantitative immunoblotting for AQP2 expression; 2) pull-down assay of ADP-ribosylated proteins using biotin-NAD$^{+}$; 3) live cell FRET imaging analysis for PKA activity; 4) siRNA-mediated β-catenin knockdown; and 5) laser scanning confocal microscopy for subcellular localization of phosphorylated β-catenin (S552).

**Results:** RT-PCR and immunoblotting showed expression of tankyrases in rat kidney and mpkCCDc14 cells. Tankyrase inhibitor XAV939 significantly decreased the AQP2 induction in response to dDAVP in mpkCCDc14 cells. ADP-ribosylation assay exhibited that ADP-ribosylated Gsα expression was increased by dDAVP treatment for 24 h or 48 h, whereas it was not observed in the presence of XAV939 co-treatment. FRET imaging analysis, however, revealed that XAV939 did not affect the dDAVP- or forskolin-activated PKA levels during 20 min-stimulation. Phosphorylation of β-catenin (S552) was significantly increased in response to dDAVP, but it was unchanged by XAV939 co-treatment. Importantly, siRNA-mediated knockdown of β-catenin was associated with attenuation of the dDAVP-induced AQP2 up-regulation.

**Conclusions:** We revealed that Gsα is a novel substrate protein of tankyrases in kidney collecting duct cells. Decrease of dDAVP-induced AQP2 up-regulation by tankyrases inhibition is not likely due to the decreased ADP-ribosylated Gsα expression, but possibly due to decreased β-catenin signaling to induce AQP2 transcription. Thus, tankyrase/β-catenin signaling could be a novel regulatory signaling for vasopressin-regulated AQP2 expression in kidney collecting duct cells.

*Funding:* Government Support - Non-U.S.

**FR-OR069**

**High Throughput Screening for Novel Drugs to Treat Nephrogenic Diabetes Insipidus by Stimulating Vasopressin-Independent Aquaporin-2 Membrane Trafficking**
Naohiro Nomura, Paula Nunes, Richard Bouley, Stanley Shaw, Hua Ann Jenny Lu, Dennis Brown. *Program in Membrane Biology, Massachusetts General Hospital, Boston, MA.*

**Background:** Nephrogenic diabetes insipidus (NDI) is characterized by loss of urine concentration with no response to vasopressin (VP). In most cases, NDI is caused by loss of plasma membrane accumulation of AQP2 in kidney principal cells due to defective VP signaling through the VP receptor (V2R). The amount of AQP2 on the plasma membrane is increased by VP, and is determined by a balance of exocytosis and endocytosis. To find novel therapeutic approaches for NDI treatment, we performed high throughput screening (HTS) for chemicals that stimulate AQP2 exocytosis independently of VP/V2R signaling.

**Methods:** HTS was performed with an exocytosis assay using LLCPK1 cells expressing soluble secreted YFP (ssYFP) and AQP2. We evaluated exocytosis of AQP2 by quantifying fluorescent ssYFP in the medium, and then tested whether identified compounds also induced AQP2 membrane accumulation by immunofluorescence in cell cultures, and rat kidney slices in vitro. Positive compounds were injected intraperitoneally into VP-deficient Brattleboro rats, and urine volume and osmolality were measured over 24 h.

**Results:** We screened 4000 compounds and identified 34 candidate exocytosis enhancers. Finally, 20 "novel" AQP2 enhancers were tested further. Three compounds increased AQP2 membrane accumulation in LLCPK1 cells and two were also effective in MDCK cells and rat kidney slices. Then we treated Brattleboro rats with each compound. One of them, AG490, a JAK2 kinase and EGFR inhibitor, decreased urine volume and increased urine osmolality significantly in the first few hours after a single injection.

**Conclusions:** By HTS screening and subsequent assays, we identified one compound that stimulates AQP2 exocytosis, induces AQP2 membrane accumulation, and stimulates urine concentration in a VP-independent manner. These data show that our chemical screening is effective in identifying novel and unsuspected strategies for the potential treatment of water balance disorders. Its mechanism of action is now under investigation.

*Funding:* NIDDK Support

**FR-OR070**

**Modulation of cAMP Signaling, AQP2 Phosphorylation and Osmotic Water Permeability in Response to DDAVP or FK under Tolvaptan Treatment in Renal Cells**
Grazia Tamma,[1] Annarita Di Mise,[1] Marianna Ranieri,[1] Peter M.T. Deen,[2] Maria Svelto,[1] Giovanna Valenti.[1] *[1]Dept of Biosciences, Biotechnologies and Biopharmaceutics, Univ of Bari, Bari, Italy; [2]Dept of Physiology, Univ, Nijmegen, Netherlands.*

**Background:** The vasopressin receptor antagonist tolvaptan has emerged as tool in the management of hyponatremia. However, no direct evidence that the aquaretic effect of tolvaptan is based on impairment of vasopressin stimulated AQP2 phosphorylation and targeting to the plasma membrane has been provided.

**Methods:** MDCK stably expressing hAQP2 or rat kidney slides were exposed to DDAVP or forskolin (FK) stimulation in the presence or in the absence of tolvaptan (10nM). The effect of these treatments on cAMP levels, AQP2 phosphorylation, intracellular calcium concentration and osmotic water permeability was analyzed.

**Results:** In MDCK cells, DDAVP treatment significantly increased cAMP levels paralleled by an increase in p256AQP2. Pretreatment with tolvaptan significantly reduced both effects. Surprisingly, tolvaptan pretreatment strongly reduced the increase in p256AQP2 elicited by FK, a direct activator of adenylyl cyclase. Similar results were obtained in rat kidney slides. In line, tolvaptan prevented the increase in the osmotic water permeability promoted by either DDAVP or FK in MDCK. We therefore analyzed whether tolvaptan had, *per se*, a cellular effect. Calibration of cellular calcium in MCDK cells revealed that tolvaptan caused a significant increase in intracellular calcium (tolvaptan 64.0±2 nM; ctr 32±1.7 nM). Since p256AQP2 can be de-phosphorylated by PP2A, a calcium depended serine/threonine phosphatase, rat kidney slides were pretreated with tolvaptan and exposed

to FK in the presence or absence of caliculyn (5pM) a specific inhibitor of PP2A. Under these conditions tolvaptan failed to prevent FK-induced increase in p256AQP2 suggesting that tolvaptan, activates PP2A.

**Conclusions:** Tolvaptan prevents vasopressin induced increase in p256AQP2, AQP2 trafficking and increase in osmotic water permeability. Moreover tolvaptan increases basal intracellular calcium, which might have relevant consequences in modulating p256AQP2 levels and therefore the clinical response to the drug.

*Funding:* Government Support - Non-U.S.

## FR-OR071

### Aquaporin2 (AQP2) Expression and Phosphorylation in Rats with Severe Salt Wasting and Volume Depletion

Kamyar A. Zahedi,[1] Sharon L. Barone,[1] Manoocher Soleimani.[1,2] *[1]Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Research Services, Veterans Administration, Cincinnati, OH.*

**Background:** Concomitant ablation of Na-Cl co-transporter (NCC) and pendrin leads to salt wasting, profuse diuresis and severe volume depletion. The carbonic anhydrase inhibitor acetazolamide (ACTZ) inhibits salt absorption in the proximal tubule and down regulates pendrin and is a mild diuretic. Hydrochlorothiazide (HCTZ), a specific inhibitor of NCC, is the most commonly used diuretic for control of mild hypertension. Our studies indicate that similar to the above mouse model, ACTZ pre-treated rats when given a combination of HCTZ and ACTZ develop severe salt wasting and diuresis. We propose that the increase in urine output in ACTZ/HCTZ treated animals that display severe salt wasting and volume depletion is due to AQP2 dysfunction.

**Results:** Compared to vehicle, ACTZ or HCTZ treated rats, ACTZ pre-treated rats given ACTZ and HCTZ excrete high amounts of salt and have significantly elevated urine output. The increased urine output in these rats was associated with decreased urine osmolality despite severe volume depletion. The latter alterations point to impaired water reabsorption. Therefore, we examined the expression of AQP2 in vehicle and ACTZ/HCTZ treated rats. Western blot analyses of kidney extracts from the vehicle and ACTZ/HCTZ treated animals revealed that the levels of total AQP2 and $^{Ser256}$p-AQP2 were significantly reduced while those of $^{Ser261}$p-AQP2 were only marginally affected in the ACTZ/HCTZ treated animals. These results were confirmed by immunofluorescent studies.

**Conclusions:** ACTZ/HCTZ treated animals have reduced levels of total and surface bound/cycling AQP2 ($^{Ser256}$p-AQP2, $^{Ser256-261}$p-AQP2) while their cytosolic AQP2 levels are significantly elevated. The inability to conserve water in ACTZ/HCTZ treated animals in the face of volume depletion mimics the situation in NCC/pendrin dKO mice and is due to reduced expression of AQP2, its diminished surface expression and impairment of water salvage mechanism. Despite being considered mild agents individually, we propose that the combination of ACTZ and HCTZ is a powerful diuretic regimen.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-OR072

### Abolished Renal Glucose Reabsorption in Euglycemic Mice Lacking SGLT1 and SGLT2

Volker Vallon,[1] Maria Gerasimova,[1] David R. Powell,[2] Hermann Koepsell,[3] Timo M. Rieg.[1] *[1]Div of Nephrology, UC San Diego & VA San Diego Healthcare System, La Jolla, CA; [2]Lexicon Pharmaceuticals, Inc., The Woodlands, TX; [3]Anatomy & Cell Biology, Univ of Wurzburg, Germany.*

**Background:** Gene knockdown of the sodium-glucose cotransporter (SGLT) 2 (Sglt2-/-) increases fractional renal glucose excretion (FGE) to ~55% in euglycemic mice (Vallon 2011), whereas deletion of SGLT1 (Sglt1-/-) increases FGE to ~3% (Gorboulev 2012). To further define the role of SGLT1 or of a possible non SGLT1/SGLT2 glucose transporter for renal glucose reabsorption, we generated and characterized mice lacking both SGLT2 and SGLT1 (Sglt1/2-/-).

**Methods:** All mice were fed a glucose-free diet to prevent glucose/galactose malabsorption and subsequent diarrhea due to complete absence of SGLT1. Food and fluid intake were determined in regular cages. Plasma glucose and urinary glucose to creatinine ratios (UGCR) were determined in non-fasted mice. Renal $^3$H-inulin clearance studies with precise determination of GFR/filtered and excreted glucose were performed under terminal anesthesia in Sglt1/2-/- to determine FGE. * P<0.05 vs WT.

**Results:** Absolute urinary glucose concentrations and UGCR were increased in Sglt1/2-/- compared with wild-type mice (WT)(402±34* vs 1.0±0.1mM; 4906±573* vs. 4±1µmol/mg; n=9/group; all male); previous studies reported UGCR in Sglt1-/- and Sglt2-/- of 87±5 and 1847 ± 119µmol/mg, respectively. Sglt1/2-/- had lower blood glucose levels vs WT (83±3* vs 115±5mg/dL), greater food and fluid intake (0.16±0.01* vs 0.10±0.01 g/[24h*g bw]; 0.57±0.02* vs 0.20±0.01 mL/[24h*g bw]), and higher values for hematocrit (49.8±0.3* vs 48.1±0.5%), plasma concentrations of sodium (158±2* vs 153±1mM) and aldosterone (1311±133* vs 276±42pg/mL), as well as urine vasopressin/creatinine ratios (192±34* vs 110±20nmol/mmol). Clearance studies revealed that fractional renal glucose reabsorption was not different from zero in euglycemic Sglt1/2-/-, which had a FGE of 110±7 % (n=4 female and 6 male mice).

**Conclusions:** The results indicate that in euglycemic mice 1) SGLT2 and SGLT1 account for renal glucose reabsorption, and 2) SGLT1 reabsorbs about half of the filtered glucose when SGLT2 is deleted.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-OR073

### S100A11, an EF Hand Calcium Binding Protein, Is a Novel NEDD4-Specific Substrate Protein in Kidney Inner Medulla

Eunjung Kim, Tae-Hwan Kwon. *Dept of Biochemistry and Cell Biology, Kyungpook National Univ, Taegu, Korea.*

**Background:** E3 ubiquitin-protein ligases confer the specificity to ubiquitylation by recognizing target substrates. Ubiquitylation by HECT E3 ligase, NEDD4, has gained interests as a central mechanism for understanding the regulation of the epithelial sodium channel (ENaC) in the kidney. Although NEDD4 is most abundantly expressed in the kidney inner medullary collecting ducts, most studies on NEDD4-mediated ubiquitylation to date are focused on NEDD4-specific substrate protein ENaC mainly expressed in the cortical collecting ducts. Herein, we aimed to identify novel substrate proteins of NEDD4 in rat kidney inner medulla.

**Methods:** All three WW domain-containing fragment protein of rat NEDD4 was made for substrate binding. Interactions between the WW domains and the rat kidney inner medullary homogenates were induced with post-chemical crosslinking (Sulfo-SMCC) and these were subjected to LC-MS/MS analysis. In vitro ubiquitylation assay of S100A11 was done.

**Results:** LC-MS/MS analysis identified nineteen candidate substrate proteins of NEDD4 in kidney inner medulla. Among them, S100A11, an EF hand calcium binding protein, was selected as a novel NEDD4 WW domain-binding protein. Immunohistochemistry revealed that S100A11 was abundantly localized at the inner medullary collecting duct cells. In vitro ubiquitylation assay showed that S100A11 was monoubiquitylated by NEDD4. When in vitro ubiquitylation reactions were carried out with increasing calcium concentrations, significant decreases in NEDD4-mediated monoubiquitylation of S100A11 as well as NEDD4 auto-ubiquitylation were detected.

**Conclusions:** We demonstrated that S100A11, an EF hand calcium binding protein, is a novel substrate protein for NEDD4-mediated ubiquitylation, which is affected by calcium levels. Further studies are needed to examine the role of monoubiquitylation of S100A11 by NEDD4 in translocation of S100A11 to the plasma membrane or to the nucleus for the regulation of water channel protein AQP2 and osmotic water permeability in the collecting ducts.

*Funding:* Government Support - Non-U.S.

## FR-OR074

### Protein Kinase C (PKC) Increases UT-A1 Urea Transporter Glycan Sialylation, Membrane Expression and Urea Transport Activity

Xuechen Li,[1] Janet D. Klein,[2] Jeff M. Sands,[1,2] Guangping Chen.[1] *[1]Physiology, Emory Univ, Atlanta, GA; [2]Renal Medicine, Emory Univ, Atlanta, GA.*

**Background:** UT-A1 urea transporter was initially identified as a vasopressin-regulated urea transporter and plays an important role in the urine concentration mechanism. However, accumulating evidence demonstrates that protein kinase C (PKC) also regulates urea permeability in the rat inner medullary collecting duct (IMCD). PKC particularly mediates hypertonicity-stimulated urea transport in kidney. In this study, we investigated the role of PKC alpha in UT-A1 protein membrane expression and glycosylation structure change.

**Methods:** UT-A1 MDCK cells, HEK293 cells and rat kidney IMCD suspension were used for PKC activator PDBu treatment or PKC alpha gene (pcDNA3-PKC alpha) transfection. Crude cell membrane from PKC alpha knockout mouse was isolated by sucrose gradient ultracentrifuge. Cell surface UT-A1 was measured by biotinylation and the glycan structure change of UT-A1 was examined using sugar-specific binding lectins.

**Results:** Treatment with 2 µM of the PKC activator PDBu significantly increases UT-A1 protein abundance in UT-A1 MDCK cells. Interestingly, activation of PKC by PDBu markedly increases UT-A1 glycan sialic acid. Consistently, activation of PKC increased UT-A1 sialylation from kidney inner medulla (IM). Functionally, increased UT-A1 sialylation has enhanced urea transport activity when the UT-A1 MDCK cells were incubated with 2 mM sialic acid for 24 h. To exclude the possible non-specific effect of the PKC activator, the PKC alpha gene was transfected into UT-A1 HEK293 cells. Cell surface biotinylation assay showed that PKC alpha directly increased UT-A1 membrane expression and the increased UT-A1 is highly sialylated as judged by SNA lectin pulldown assay. A PKC alpha knockout mouse has impaired urine concentrating ability. We examined UT-A1 glycosylation change and found that the UT-A1 sialylation is impaired from PKC alpha knockout mouse.

**Conclusions:** Our study revealed a novel, important mechanism of PKC-mediated UT-A1 membrane expression and bioactivity via enhancement of UT-A1 glycan sialylation.

*Funding:* NIDDK Support

## FR-OR075

### Transgenic Mice That Express UT-A1 but Lack UT-A3 Have Increased AQP3 and Normal Urine Concentrating Ability

Janet D. Klein,[1,2] Abinash C. Mistry,[1] Patrick A. Molina,[1] Priya Datta,[1] Richard T. Rogers,[1] Mitsi A. Blount,[1,2] Jeff M. Sands.[1,2] *[1]Renal Div, Dept of Medicine, Emory Univ; [2]Dept of Physiology, Emory Univ, Atlanta, GA.*

**Background:** Mice lacking the inner medullary collecting duct (IMCD) urea transporters, UT-A1 and UT-A3, are unable to concentrate their urine. To determine whether this defect results from the absence of UT-A1 (located intracellularly and at the apical membrane), UT-A3 (active at the basolateral membrane), or both, we made a mouse that expresses UT-A1 but not UT-A3 (UT-A1$^{+/+}$/A3$^{-/-}$). Since AQP3 has been reported to transport urea across the basolateral membrane, we assessed AQP3 levels and cellular location in the UT-A1$^{+/+}$/A3$^{-/-}$mice.

**Methods:** We generated a UT-A1 gene that could not be spliced to produce UT-A3, behind its original UT-A promoter. We introduced this transgene into UT-A1/A3 knock-out (KO) mice to yield a mouse expressing only UT-A1 (UT-A1$^{+/+}$/A3$^{-/-}$). Western blot and immunohistochemistry were used to assess transport and channel proteins in the UT-A1$^{+/+}$/A3$^{-/-}$ mice.

**Results:** Western blot demonstrated 1) UT-A1 protein in inner medulla (IM) of UT-A1$^{+/+}$/A3$^{-/-}$ and wild-type (WT) mice, but not in UT-A1/A3 KO mice and 2) UT-A3 absent from both mice. UT-A1 protein was not detected in extra-renal tissues from UT-A1$^{+/+}$/A3$^{-/-}$ mice. Immunohistochemisty showed negative UT-A3 staining and positive UT-A1 staining present only in the IM of the UT-A1$^{+/+}$/A3$^{-/-}$ mice. Basal urine osmolalities in WT (2844 mOsm) and UT-A1$^{+/+}$/A3$^{-/-}$ mice (2508 mOsm) increased upon overnight water restriction to 4469 mOsm in WT and 3656 mOsm in UT-A1$^{+/+}$/A3$^{-/-}$ mice. Both WT and UT-A1$^{+/+}$/A3$^{-/-}$ urine osmolalities were significantly higher than in UT-A1/A3 KO (basal: 920 mOsm; dehydrated: 1365 mOsm) mice. AQP3 was increased 61±13% (n=11, $p<0.05$), but its basolateral location was unchanged in the UT-A1$^{+/+}$/A3$^{-/-}$ mice compared with WT mice.

**Conclusions:** Mice expressing UT-A1, but not UT-A3, are able to concentrate their urine to similar levels as WT mice. This suggests that UT-A3 may not be essential for urine concentration. The increase in AQP3 suggests a potential role moving urea across the basolateral membrane to compensate for the lack of UT-A3.

*Funding:* NIDDK Support

## FR-OR076

### A Small Molecule Screen Identifies Selective Inhibitors of Urea Transporter UT-A1

Cristina Esteva-Font,[1] Puay Wah Phuan,[1] Marc O. Anderson,[2] Alan S. Verkman.[1] *[1]Depts of Medicine and Physiology, Univ of California San Francisco, San Francisco, CA; [2]Dept of Chemistry and Biochemistry, San Francisco State Univ, San Francisco, CA.*

**Background:** Urea transporter (UT) proteins, including UT-A in kidney tubule epithelia and UT-B in vasa recta microvessels, facilitate urinary concentrating function. UT-A1/A3 double knock-out mice have reduced urine-concentrating ability. We previously identified small-molecule UT-B inhibitors with low nanomolar potency using an erythrocyte lysis-based screen assay. UT-A is of greater importance in urinary concentration, with UT-A1 as the principal target. The goal of this study is to identify UT-A1 inhibitors for application as diuretics ('urearetics') with a novel mechanism of action.

**Methods:** A robust screen for UT-A1 inhibitors was developed in MDCK cells expressing UT-A1, aquaporin-1, and fluorescent chloride sensor YFP-H148Q/V163S. Changes in cell volume alter intracellular chloride concentration, producing a near-instantaneous change in YFP-H148Q/V163S fluorescence. Creation of an inwardly directed urea gradient produces rapid cell shrinking due to osmotic water efflux (decreased fluorescence), followed by UT-A1-dependent cell swelling due to water and urea entry (increased fluorescence).

**Results:** Screening of 100,000 synthetic small molecules yielded UT-A1 inhibitors of four chemical classes with low micromolar $IC_{50}$. The compounds fully and reversibly inhibited UT-A1-facilitated urea transport. Structure-activity analysis of analogs of the active compounds revealed compounds with high UT-A1 selectivity and compounds with comparable potency for UT-A1 and UT-B inhibition. Docking computations based on a homology model of UT-A1 suggested binding modes of the inhibitors into the hydrophobic channel region. Optimization of in vivo inhibitor pharmacology and testing in rodent models is in progress.

**Conclusions:** Small-molecule UT-A1 selective and non-selective inhibitors were identified by high-throughput screening. UT-A1 inhibitors may be useful as diuretics that may be effective in high-vasopressin, fluid-retaining conditions in which conventional salt transport-blocking diuretics have limited efficacy.

## FR-OR077

### Urea Transport in the Collecting Duct, Regulated by an Endothelin/Nitric Oxide Signaling Pathway, Is Required for Sodium Reabsorption

Richard T. Rogers, Sara K. Redd, Seongun M. Hong, Mitsi A. Blount. *Dept of Medicine - Renal Div, Emory Univ, Atlanta, GA.*

**Background:** Through several regulatory mechanisms, the kidney modifies blood pressure by controlling fluid homeostasis. Nitric oxide (NO) production in the collecting duct regulates sodium and water reabsorption to stabilize blood volume by regulating transporter function. Ablation of the collecting duct urea transporters, UT-A1 and UT-A3, induces polyuria in UT-A1/A3 null (KO) mice. The inability of these mice to reabsorb water leads us to speculate that NO production is altered.

**Methods:** We collected serum and 24-h urine samples then harvested renal medullary tissue from UT-A1/A3 KO mice and corresponding control littermates (WT). Samples were subjected to ELISA, qRT-PCR, Western, and electrolyte analysis accordingly. Blood pressure was measured in UT-A1/A3 KO and WT mice via tail cuff following 4 days of training.

**Results:** In corroboration with previous reports, urinary nitrate/nitrite levels were increased 3-fold in UT-A1/A3 KO mice. Investigation of nitric oxide synthase (NOS) mRNA expression levels revealed that NOS2 and NOS3 expression were unchanged in UT-A1/A3 KO medulla compared to WT; however, NOS1 expression was increased 40-fold in inner medulla of UT-A1/A3 KO mice. Because collecting duct endothelin (ET-1)/NO signaling pathway modulates pressure-natriuresis, we investigated this pathway in the inner medullary collecting ducts of UT-A1/A3 KO mice. We found that serum aldosterone levels and mineralocorticoid receptor protein expression, regulators of ET-1 concentration, were increased and endothelin type-B (ETB) receptor protein expression, the target of ET-1, was also increased in UT-A1/A3 KO mice compared to WT. Urinary sodium excretion in UT-A1/A3 KO was increased 57% and blood pressure was slightly lower in these mice compared to WT in accordance with the natriuretic and antihypertensive effects of ET-1 and ETB expression.

**Conclusions:** Renal handling of sodium and water has always been linked processes in regulation of blood volume. The present studies suggest that urea handling in the collecting duct, acting through endothelin-simulation of NOS1, also regulates sodium reabsorption.

*Funding:* NIDDK Support, Private Foundation Support

## FR-OR078

### Nephrology Curriculum Moving Forward in the Era of Team Based Learning

Rupal Mehta, James J. Paparello. *Nephrology, Northwestern Univ, Feinberg School of Medicine, Chicago, IL.*

**Background:** Team Based Learning (TBL) is an approach to medical education that focuses on active learning and application of knowledge in group collaboration. There are few studies to date that show correlations between how students perform on their TBL exercises to their own standardized final exam scores.

**Methods:** After being given preparation material, 162 first year medical students were given 10 acid base and 9 renal pathology questions as the individual readiness assurance test (IRAT). In groups of 10-12, students were then given the same questions as the group readiness assurance test (GRAT), followed by application to more complex clinical scenarios. The final exam given 2 weeks later consisted of 109 questions, 78 of which were related to the TBL material.


**Results:** The preliminary data suggests an improvement between IRAT and final examination scores with the average percentile on the IRAT score of 66% compared to 80.21% on the final exam. The average acid base physiology percentage was 64% vs 70% on the renal pathology component. This does not prove causation but shows that TBL can be a component of teaching nephrology.

**Conclusions:** TBL is an effective teaching tool in medical education. Ongoing steps include analyzing individual scores on the 78 questions testing TBL material and compare this to the 31 questions taught by other methods. This has the ability to show that TBL can be more successful than standard models. We are looking to identify subsets of students who benefited the most by TBL with qualifers such as baseline IRAT score percentile or gender. These groups can then be the focus of TBL exercises. Students identified the renal curriculum as ideal for the TBL model and this will broaden its use at Northwestern University. Team based learning is a rapidly growing educational strategy and the implementation will likely be seen throughout many levels of training.

## FR-OR079

### Palliative Care Training during Fellowship: National Survey of Second-Year U.S. Nephrology Fellows

Sara A. Combs,[1] Stacey Culp,[3] Jean L. Holley,[2,4] Alvin H. Moss.[3] *[1]Univ of Colorado, Aurora, CO; [2]Carle Physicians Group, Urbana, IL; [3]West Virginia Univ, Morgantown, WV; [4]Univ of Illinois, Urbana-Champaign, IL.*

**Background:** Dialysis patients have a high symptom burden and a limited life expectancy. Nephrologists are responsible for their care.

**Methods:** In 2013 we surveyed US second-year nephrology trainees to assess their attitudes toward and the quality of teaching in palliative care received during fellowship and their perceived preparedness to care for patients at end of life (EOL). 326 trainees were surveyed with a 64% response rate. These responses were compared to results from a similar fellows' survey in 2003.

**Results:** More fellows thought it was moderately to very important to learn to provide care to dying patients (95% in 2013, 54% in 2003, $p<0.001$). 99% of fellows in both surveys believed physicians have a responsibility to help patients at the EOL. On a 10-point scale in which 0 is "no teaching" and 10 is "a lot of teaching", fellows reported more teaching in managing a patient on dialysis (mean 9.0 ± 1.4) than a patient at the EOL (mean 4.5 ± 2.5) or with distal RTA (mean 6.5 ± 2.3), all $p<0.001$. On a similar 10-point scale for preparedness, fellows' self-assessments in managing a patient at the EOL (mean 6.1 ± 2.5) and with distal RTA (mean 7.3 ± 2.0) were also lower than managing a patient on hemodialysis (mean 8.9 ± 1.2), all $p<0.001$. Ranking of quality of teaching during fellowship in all areas (mean 4.1 ± 0.8 on a scale of 0-5 where 0 is poor and 5 is excellent) and specific to EOL care (mean 2.4 ± 1.1) did not change between 2003 and 2013, but knowledge of annual gross mortality rate for dialysis patients was worse in 2013, only 57.4% answered correctly ($p=0.048$). 86% reported that no palliative care rotation was offered during fellowship. To an open-ended question about what would most improve fellows' EOL care education, the most common response was a required palliative medicine rotation during fellowship.

**Conclusions:** The amount of teaching specific to palliative care has not improved over the last decade, but fellows increasingly believe that they should learn how to provide this care during fellowship.

**FR-OR080**

**Use of Simulated Patients for Teaching Core Communication Skills to Nephrology Trainees** Robert A. Cohen,[1] Jane O. Schell.[2] *[1]Dept of Medicine Nephrology Div, Beth Israel Deaconess Medical Center (BIDMC)/Harvard Medical School, Boston, MA; [2]Section of Palliative Care and Section Renal-Electorlyte, Univ of Pittsburgh School of Medicine/UPMC, Pittsburgh, PA.*

**Background:** Nephrologists face challenging conversations with patients about dialysis decision-making and end of life. Yet minimal training of nephrology fellows for how to engage in these discussions occurs. Other specialties have employed simulated patients to assist fellows in practicing communication skills for delivering bad news and discussing end of life transitions. We present a communication workshop for nephrology fellows using standardized patients to teach and practice key communication skills.

**Methods:** First-year nephrology fellows from the BIDMC, Brigham and Women's Hospital, and Massachusetts General Hospital and UPMC participated in a full-day interactive communication skills workshop for delivering serious news and promoting discussions about dialysis decision-making and end of life. This consisted of didactics followed by practice sessions. Simulated patients role-played cases giving each fellow an opportunity to practice newly learned skills. Faculty facilitated sessions, providing formative feedback. Surveys were completed prior to and at end of each workshop. Fellows' perceptions of preparedness for engaging in these discussions were rated on a Likert scale prior to and after the workshop.

**Results:** Nineteen fellows (9 females; 10 males) participated at two sites (13 in Boston and 6 in Pittsburgh). Each fellow had an opportunity to participate in facilitated role play with simulated patients at least once during the workshop. Perception of preparedness for engaging in these conversations increased for all fellows following the workshop.

**Conclusions:** Using nephrology specific cases, we report the use of simulated patients for teaching nephrology fellows communication skills for addressing dialysis decision-making and end of life issues. Simulated patients provide a novel approach to communication education in nephrology training. Future efforts include increasing the number and location of such workshops in an effort to train more nephrology fellows in core communication skills.

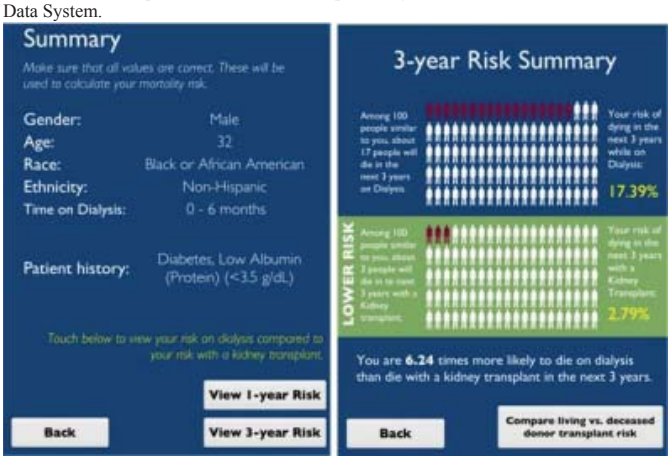
*Funding:* Clinical Revenue Support

**FR-OR081**

**Dialysis or Transplant: Use of a Shared Decision Making Tool for Treatment Options** Rachel E. Patzer,[1] Mohua Basu,[1] Janice P. Lea,[2] William M. McClellan,[2] David H. Howard,[3] Kimberly Arriola.[4] *[1]Emory Transplant Center, Atlanta, GA; [2]Dept of Medicine, Emory Univ, Atlanta, GA; [3]Dept of Health Policy and Management, Rollins School of Public Health, Atlanta, GA; [4]Dept of Behavioral Sciences and Health Education, Rollins School of Public Health, Atlanta, GA.*

**Background:** The decision to choose kidney transplantation (KTx) over dialysis is a life-altering decision that patients with end stage renal disease (ESRD) make with minimal and subjective data. Shared decision support tools may improve patients' decision-making.

**Methods:** In March 2013, we conducted a feasibility study of a decision support iPad Application tool (iChoose Kidney) that provided risks of death on dialysis compared to KTx based on risk prediction models developed using data from the United States Renal Data System.




The study was conducted at an urban dialysis center comprised primarily of African American (AA) patients, who historically have reduced KTx access.

**Results:** A total of 21 patients received standard education about treatment options plus iChoose Kidney (100% AA, mean age 54 yrs, 61.9% <high school education, 71.4% had income <$20,000, and all were within 1-year of ESRD). Patient knowledge and opinions were examined pre- and post-assessment. Nearly half (42.9%) of patients had never had a physician discussion about KTx. The majority (85.7%) agreed or strongly agreed the tool was useful in making treatment decisions, 90.5% said they learned something new, 71.4% said the tool encouraged them to speak with family about treatment, and 85.7% said they would recommend iChoose Kidney to other patients.

**Conclusions:** This study demonstrated the feasibility of iChoose Kidney to improve shared decision-making about treatment options for AA ESRD patients. A future randomized trial to assess clinical effectiveness is warranted.

*Funding:* Other NIH Support - National Center for Advancing Translational Sciences (NCATS)

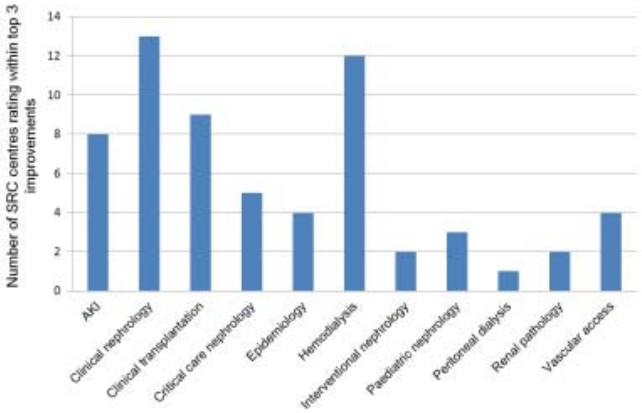
**FR-OR082**

**Successful Development of Worldwide Nephrology through the ISN Sister Renal Centre Programme** Matthew O. Brook,[2] Paul N. Harden.[1,2] *[1]International Society of Nephrology, Brussels, Belgium; [2]Oxford Kidney Unit, Oxford, United Kingdom.*

**Background:** The International Society of Nephrology (ISN) sister renal centre (SRC) programme seeks to advance nephrology by linking emerging centres (EC) in the developing world with supporting centres (SC) in the developed world.

**Methods:** Of 34 active links in 2012, 26 (76%) responded to a survey investigating each links focus for development and success.

**Results:** ECs were distributed throughout 4 continents and 19 countries whilst SCs originated from 11 countries. Developing general clinical nephrology (16/34 links), haemodialysis (12/34) and acute kidney injury (11/34) services were the three most prolific areas of activity although significant diversity was evident. The most improved aspects of nephrology were identified as general clinical nephrology (13/21 responding links), Haemodialysis (12/21) and transplantation services (9/21) (Figure).




SRC links had a positive impact on clinical training (mean impact rating 8.7/10), clinical services (8.1/10) and research (7.3/10) within the Ecs. SCs noted increased global health awareness and interest in global health issues (both 7.9/10) among staff.

Establishing new clinical services, education through training exchanges and direct interactions between SC and EC staff were identified as providing the greatest contribution to successful EC development.

The SRC link between Oxford, U.K. and Minsk, Belarus is a good example of sustained success. A new paediatric programme contributed to a >20 fold increase in transplantation in Belarus. The Minsk team have since used their experience to support the first ever paediatric transplants in Khazakstan and living donor transplants in Krygykstan.

**Conclusions:** The SRC programme provides a template that may be adopted by other specialities for substantial and sustained improvement in healthcare tailored to the demands and resources of the emerging unit.

**FR-OR083**

**NephMadness: Social Media Campaign to Promote Nephrology Education and Interest** Joel Topf,[1] Matthew A. Sparks,[3] Kenar D. Jhaveri.[2] *[1]Medicine, St. John Hospital and Medical Center, Detroit, MI; [2]Medicine, Hofstra North Shore LIJ School of Medicine, Great Neck, NY; [3]Medicine, Duke Univ Medical Center, Durham, NC.*

**Background:** In order to increase interest in nephrology we constructed an online social media campaign specifically designed to encourage an interactive educational experience of nephrology concepts called NephMadnes.

**Methods:** One of the cornerstones of social media is the rapid ability to share information and interact with colleagues. In March to April 2013 we created an online educational tournament coined NephMadness on the website http://www.ajkdblog.org" consisting of 64 nephrology achievements. The concept was to parody the NCAA men's Division 1 Basketball Tournament. We mirrored the traditions, language and structure of the basketball tournament to generate interest in our tournament of nephrology ideas.

**Results:** 64 nephrology concepts and achievements arranged in brackets were introduced on the initial day. Descriptions and educational material of all 64 achievements were divided across 8 blog posts and spanned 11,534 words. The achievements were divided into 8 groups: programs, drugs, research techniques, discoveries, learning tools, equations/lab values, diagnostics, and studies. Over the ensuing 4 weeks the field of 64 was narrowed until a winner was declared. While the first two rounds were scripted by the editors, subsequent narrowing was decided by the users through online voting. NephMadness generated 34 seperate blog posts. 40% (14/34) were posted from blogs other than eAJKD. Twitter was a primary source of interactivity in which 77 individuals tweeted about the project 473 times and generated 519,323 impressions using the hashtag #NephMadness.

**Conclusions:** To our knowledge this is the first educational social media campaign performed of this magnitude in medicine. The campaign was successful in drawing many people to interact and learn about nephrology. Traffic to the originating blog was the highest in it's history by a wide margin. Use of interactive campaigns are possible and deserve formal study. Such interactive games could spark interest in nephrology, at a time when interest in declining.

## FR-OR084

**Two Novel Educational Interventions to Navigate the Challenges of CKD Care** Stacey Jolly, Sankar D. Navaneethan, Jesse D. Schold, Susana Arrigain, Victoria Konig, Yvette K. Burrucker, Barbara H. Tucky, John W. Sharp, Joseph V. Nally. *Cleveland Clinic.*

**Background:** There is a scarcity of translational research in CKD that incorporates educational tools. We are 1 of 5 NIDDK R34 grants whose focus is improving outcomes for CKD patients through T2 research. We describe the development of two interventions, a CKD Patient Navigator program and a CKD specific enhanced personal health record (PHR).

**Methods:** To accomplish our specific aims, we assembled key members from our multidisciplinary CKD Team and enlisted new members from information technology and data management. Creation of the CKD Navigator Program encompassed three phases: hiring, training, and implementing. We hired a navigator who could provide "individual guidance, support, education, coordination of care, and other assistance to patients". For training, there were three key areas a) Harold Freeman Patient Navigator Institute b) CKD education and c) electronic health record (EHR) training. For implementation, we defined barriers of care, ensured randomization, and created EHR templates for which pertinent study data could be extracted. Enhanced PHR creation was a multi-step process. We selected educational materials specific to CKD Stage 3b/4 patients, developed a user guide for potential participants who may not be familiar with our PHR, and pilot tested including verifying our ability to collect PHR use data among participants.

**Results:** We created a CKD Patient Navigator program adapting the use of patient navigators successfully employed in other fields with the well-established chronic care model. We developed an EHR-based enhanced PHR that allows for CKD stage-specific education disseminated electronically and utilized many publicly available NKDEP and NKF education materials. We're performing an RCT to determine the effect of these interventions alone and in combination on CKD outcomes. Recruitment is underway and we have enrolled over half of our target population (n=208) and await future analyses.

**Conclusions:** CKD research that uses novel educational approaches is pressing and will require technology, stakeholders, and a multidisciplinary team to translate frameworks into adaptable interventions.

*Funding:* NIDDK Support, Other NIH Support - S.D.N. was supported by National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant RR024990

## FR-OR085

**Changes in Endothelial and Smooth Muscle Cells Morphology by Patient-Specific Disturbed Flow Patterns Derived from Autologous Arteriovenous Fistulae** Andrea Remuzzi,[1,2] Bogdan Ene-Iordache,[1] Marco Franzoni,[1] Irene Cattaneo.[1] *[1]IRCCS - Mario Negri Institute, Bergamo, Italy; [2]Univ of Bergamo, Dalmine, Italy.*

**Background:** Radial-cephalic arteriovenous fistula (AVF) is the first choice for haemodialysis ascular access. AVF surgery has significant early failure rates due to vessel stenosis in the draining vein. It has been proposed that oscillating wall shear stress (WSS) acting on endothelial cells (EC), is a trigger of intimal hyperplasia (IH), vessel stenosis and AVF failure. We investigated whether WSS acting on EC in regions of disturbed flow, that develop in AVF, may influence EC function.

**Methods:** We used magnetic resonance images obtained in two patients, 40 d post-surgery, to reconstruct 3D models of AVF and to estimate the blood flow field by computational fluid dynamics. We localized areas of disturbed flow using oscillatory shear index [He, 1996], and we derived time changes in WSS in these regions, and in regions exposed to pulsatile flow far from the anastomosis. We then exposed human umbilical EC (HUVEC) in vitro to oscillating and pulsatile WSS, using a cone-and-plate device, and exposed vascular smooth muscle cells (SMC) to culture media conditioned by HUVEC under different flow conditions.

**Results:** HUVEC exposed to pulsatile WSS (1.24 to 2.23 Pa, n=4) for 48 hrs elongated and aligned in the flow direction, while HUVEC exposed to oscillating WSS (1.28 to -1.62 Pa, n=4) did not align nor elongate. Morphology of SMC was importantly affected by medium pre-conditioned by oscillating WSS on HUVEC, while medium conditioned by HUVEC under pulsatile flow did not affect SMC phenotype.

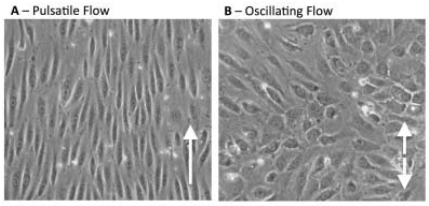


**Figure 1** – HUVEC after in vitro exposure to fluid flow for 48 hrs.

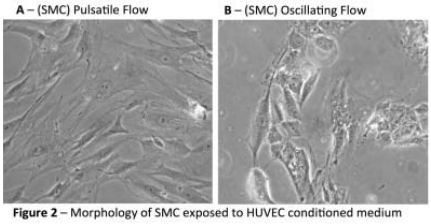


**Figure 2** – Morphology of SMC exposed to HUVEC conditioned medium

**Conclusions:** Our data demonstrate that exposure of EC to oscillating WSS that develop in the venous site in side-to-end AVF importantly affect EC and SMC function. These results confirm that disturbed flow conditions acting on EC may be responsible for IH and AVF stenosis.

## FR-OR086

**Pancreatic Elastase (PRT-201) Improves Radiocephalic Arteriovenous Fistula (AVF) Maturation and Patency** Bradley S. Dixon,[1] Robert J. Hye,[2] Michael R. Jaff,[4] Pamela Gustafson,[5] Francesca Lindow,[5] Marco D. Wong,[5] Laura M. Dember,[3] Steven K. Burke.[5] *[1]U Iowa; [2]Kaiser Permanente; [3]UPenn; [4]Mass General; [5]Proteon Therapeutics.*

**Background:** Over 50% of AVFs lose primary unassisted patency within one year of creation. A pilot study suggested PRT-201 is safe and may prolong AVF patency.

**Methods:** We conducted a randomized double-blind, placebo controlled trial of PRT-201 on primary unassisted patency (time from AVF creation to thrombosis or an intervention to maintain patency) of a new AVF. Secondary outcomes included secondary patency (time from AVF creation to fistula abandonment), and unassisted maturation assessed by duplex ultrasound (blood flow ≥ 500 mL/min and vein lumen diameter ≥ 4 mm).

**Results:** 169 patients were randomized and 151 patients treated with PRT-201 (10 µg, n=51 or 30 µg, n=49) or placebo (n=51) applied topically to the exposed artery and vein immediately following surgery. Primary unassisted patency at one year was 47% in placebo compared to 59% for PRT-201 (10 µg and 30 µg dose groups combined; p=0.11). In a Cox model adjusting for baseline covariates, the hazard ratio for time to primary unassisted patency loss was 0.76 (95% CI 0.43-1.35; p=0.35) for 10 µg and 0.59 (95% CI 0.32-1.10; p=0.10) for 30 µg. Unassisted maturation at 12 weeks was 67% for placebo compared to 87% (p=0.03) for 10 µg and 92% (p<0.01) for 30 µg. Stratifying by AVF location (Table 1), 30 µg PRT-201 significantly improved primary unassisted patency and unassisted maturation in radiocephalic but not brachiocephalic AVFs.

| Table 1 | Radiocephalic AVF (n=67) | | | Brachiocephalic AVF (n=84) | | |
|---|---|---|---|---|---|---|
| | Placebo | 10 µg | 30µg | Placebo | 10µg | 30µg |
| Primary Unassisted Patency (%) | 33 | 52 | 65* | 59 | 64 | 55 |
| Unassisted Maturation (%) | 47 | 74 | 93** | 80 | 83 | 77 |
| Secondary Patency (%) | 67 | 83 | 90† | 89 | 82 | 79 |
| *p=0.03; **p=0.009; †p=0.08 | | | | | | |

**Conclusions:** PRT-201 improved primary unassisted patency and unassisted maturation in radiocephalic AVFs.

*Funding:* Pharmaceutical Company Support - Proteon Therapeutics

## FR-OR087

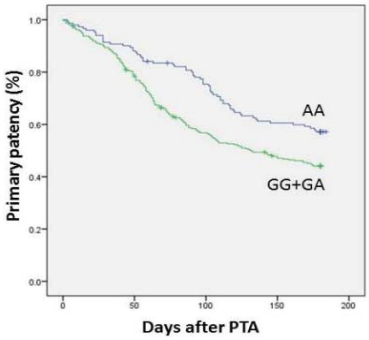
**Genotype Polymorphisms of Dimethylarginine Dmethyl Aminohydrolase 1 (DDAH1) Predict Restenosis of Vascular Access after Angioplasty in Hemodialysis Patients** Chih-Ching Lin. *Div of Nephrology, Dept of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.*

**Background:** Elevated plasma asymmetric dimethylarginine (ADMA) has been reported to be associated with restenosis after percutaneous transluminal angioplasty (PTA) of AVF in hemodialysis (HD) patients. Dimethylarginine dimethylaminohydrolase 1 (DDAH1) is the major enzyme eliminating ADMA, but the effect of genetic variations in DDAH1 on the outcome of vascular access after PTA in HD patients remained unknown.

**Methods:** We assessed the association between polymorphisms in DDAH1 and vascular access outcome in 473 maintenance HD patients, who were prospectively followed up for one year after PTA for vascular access dysfunction. Eleven single nucleotide polymorphisms (SNPs) in endothelial function related genes were analyzed and plasma ADMA levels were determined at baseline.

**Results:** After adjustment of demographic, access, and risk factors, individuals with high baseline plasma ADMA (>0.9µM) levels had higher rates of re-intervention at 3 and 6 months after PTA (three months, 56% vs. 36%, p=0.08; six months:74% vs. 53%, p=0.05). DDAH1 rs233112 was significantly associated with increased levels of plasma ADMA levels. Compared with individuals with rs233112 AA genotypes, individuals with rs233112 GA or GG genotypes had higher risks for re-intervention (58% vs. 45%, p=0.003) after

PTA at 6 months. In the same multivariate- adjusted model, the clinical factors predicting higher risk of re-intervention at 6 months include current smoker, graft access, and rs233112 GG+GA genotypes of DDAH1 gene (HR 2.302, 95% CI 1.557-3.407).




**Conclusions:** Our results provide the first evidence that rs233112 GG+GA genotypes of DDAH1 gene predict early and frequent restenosis of vascular accesses after PTA in HD patients.

*Funding:* Government Support - Non-U.S.

## FR-OR088

**The Impact of Arterial Micro-Calcification of Vascular Access on Early Access Failure in Hemodialysis Patients** Su Jin Choi,[1] Yu-Seon Yun,[2] Young Soo Kim,[1] Sunae Yoon,[1] Young Ok Kim.[1] *[1]Internal Medicine, College of Medicine, The Catholic Univ of Korea, Seoul, Korea; [2]Gyeonggi Province Geriatric Hospital of Dongducheon.*

**Background:** Vascular calcification is common in hemodialysis (HD) patients, and it is a significant predictor for cardiovascular mortality in HD patients. Also, vascular access calcification identified by plain radiography was reported as a risk factor for cardiovascular mortality in HD patients. But the relationship between arterial micro-calcification (AMiC) by pathologic study and patency of vascular access has been rarely reported. The aim of this study was to determine the impact of AMiC of vascular access on access patency in HD patients.

**Methods:** One-hundred six HD patients (Mean age; 59.6 ± 12.9 years, Male/Female; 66/40, Percent of diabetes mellitus; 73%) receiving vascular access operation were included in this study. During the operation, we obtained partial arterial specimen and performed pathologic examination by von Kossa stain to identify AMiC. We investigated early access failure (complete obstruction of blood flow or severe stenosis requiring radiologic intervention or surgical correction of AVF within 1 year after the operation) between the patients with AMiC and those without AMiC.

**Results:** Mean duration of follow-up was 42.5 ± 33.8 months and the incidence of AMiC was 38.7% (n=41). The form of vascular calcification is arterial medial calcification. Early access failure was occurred in 45 patients (42.5%), and mean time between the operation and access failure was 4.1 ± 3.1 months (range, 1 to 12 months). The access failure was associated with older age (62.7 ±11.8, 57.3 ± 13.2, p-value = 0.032) and low BMI (23.3±2.9, 24.5 ± 3.5, p-value = 0.043). The early AVF failure rate was higher in patients with AMiC than those without AMiC (56.1%, n=23/41, vs. 33.8%, n=22/65, p-value = 0.024). Diabetes, cardiovascular disease and cardiovascular death were not related to access patency. Kaplan-Meier analysis showed that the presence of AMiC significantly increased the risk for the access failure (Log rank = 4.98, p-value = 0.026).

**Conclusions:** This study demonstrates that AMiC of the vascular access is associated with early access failure in HD patients.

## FR-OR089

**Liposomal Delivery of Prednisolone Improves Outward Remodeling in Murine Arteriovenous Fistulas** ChunYu Wong,[1] Carolien Rothuizen,[1] Josbert Metselaar,[2] Floris Verbeek,[3] Erik Stroes,[4] Anton Jan Van Zonneveld,[1] Ton J. Rabelink,[1] Paul Quax,[3] Joris I. Rotmans.[1] *[1]Nephrology, Leiden Univ Medical Center, Leiden, Netherlands; [2]MIRA Institute, Univ of Twente, Enschede, Netherlands; [3]Surgery, Leiden Univ Medical Center, Leiden, Netherlands; [4]Vascular Medicine, Amsterdam Medical Center, Amsterdam, Netherlands.*

**Background:** Arteriovenous fistulas (AVFs) have a 1-year primary patency of only 60%, mainly as a result of maturation failure that is caused by insufficient outward remodeling (OR) and intimal hyperplasia (IH). The exact pathophysiology remains unknown, but the locally augmented inflammatory response is thought to play a role. Corticosteroids (CS) are powerful inhibitors of inflammation but result in adverse side effects when given systematically at a higher dose. In this study we evaluated the effect of CS combined with a targeted delivery method using liposomes in a murine AVF model.

**Methods:** AVFs between the jugular vein and common carotid artery were created in an end-to-side manner in C57bl6 mice. Subsequently, the animals were injected i.v. (dose 10 mg/kg) with either liposomal prednisolone phosphate (LPP), empty liposomal vehicle, prednisolone phosphate or PBS at days 0,2,5 and 10 after surgery. At 14 days, the labeled liposomes were visualized in mice *in vivo* by near infrared fluorescent imaging whereupon the animals were sacrificed for histomorphometric and immunohistochemical analysis.

**Results:** Liposomes accumulated in the anastomotic area of the AVF. Treatment with LPP resulted in a 27% increase in venous circumference and 47% increase in lumen ($p<0.01$;$p<0.03$) when compared to the PBS group. No significant difference in intimal hyperplasia was observed. Furthermore, we observed a 83% reduction in leukocyte infiltration in the anastomotic area in the LPP group ($p<0.01$).

**Conclusions:** Liposomal CS targeting proved to be an effective and selective method to ameliorate venous remodeling in AVFs by increasing the luminal area that was caused by augmented OR, rather than the reduction of IH formation. Treatment with liposomal prednisolone phosphate could be a valuable strategy to reduce the peri-anastomotic inflammatory response and improve maturation of AVFs.

*Funding:* Private Foundation Support

## FR-OR090

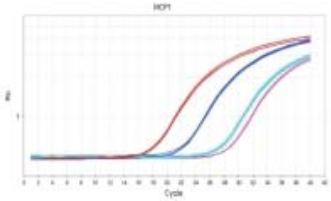
**Genomics of Arteriovenous Fistula Maturation** Timmy C. Lee,[1,2] Jing Chen,[1] Prabir Roy-Chaudhury,[1] Begoña Campos,[1] Mario Medvedovic.[1] *[1]Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Medicine, Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** Studies that evaluate the progressive effects of vascular injury and biological mechanisms leading to venous neointimal hyperplasia development and arteriovenous fistula (AVF) non-maturation have yet to be performed in vascular access. The objective of this study was to evaluate gene expression changes from vein specimens from subjects without chronic kidney disease, advanced chronic kidney disease (CKD) receiving new vascular access, advanced CKD subjects during second stage of basilic vein transposition, and stenotic early AVF failure.

**Methods:** Vein samples were isolated for RNA from non-CKD deceased donors (n=2), advanced CKD subjects receiving new vascular access (n=2), subjects at the time of second stage basilic vein transposition (n=2), and stenotic early AVF failure (n=2). We performed differential gene expression analysis using high throughput RNA-seq to compare gene expression at these various time points. We also performed qRT-PCR for selected genes of interests.

**Results:** We found significant and progressive increases in expression of genes regulating cellular proliferation, cell adhesion, chemokine and cytokine signaling, and extracellular matrix production with increased vascular injury to the vein. qRT-PCR for monocyte chemoattractant protein-1 (MCP-1), showed significantly progressive gene expression from non-CKD vein to stenotic AVF ($p<0.005$) (see figure).

**Conclusions:** Our results show significant and progressive increases in gene expression in pathways regulating inflammation, oxidative stress, and endothelial function from the non-CKD period to the time of venous stenosis. Future studies are needed to evaluate in greater detail the effect of different vascular injuries (uremia and hemodynamics) at different time points in the natural history of AVF maturation.




**qRT-PCR of MCP-1 from Vein Specimens.** MCP-1 expression curves of non-CKD veins (purple), CKD vein at time of AVF creation (aqua blue), vein from 2nd step of BVT (dark blue), and stenotic AVF vein (red). Note progressive increased expression of MCP-1 with vascular injury.

*Funding:* NIDDK Support

## FR-OR091

**Notch/FSP-1 Signaling Contributes to CKD-Induced Neointima Formation in AV Fistula** Jizhong Cheng,[1] William E. Mitch,[1] Yun Wang,[1] Anlin Liang.[1] *[1]Dept of Medicien/Nephrology, Baylor College of Medicine; [2]Dept of Medicien/Nephrology, Baylor College of Medicine; [3]Dept of Medicien/Nephrology, Baylor College of Medicine; [4]Dept of Medicien/Nephrology, Baylor College of Medicine.*

**Background:** Functional arteriovenous fistulas (AVF) are critical for hemodialysis patients but neointima formation causes 60% of AVF's to fail in 2 years. The migration and proliferation of smooth muscle cells (SMCs) are the major resources of neointima formation. Fibroblast specific protein 1 (FSP-1) regulate cell migration and could be associated with CKD-induced AVF failure.

**Methods:** AVFs were created in WT and FSP-1 KO mice with or without CKD, the neointima foramtion in AVFs was evaluated. The expression of FSP-1 and the underlying mechanism were investigated in cultured SMCs.

**Results:** FSP-1 expression was significantly increased in neointima area of AVFs from patient and CKD mice compared with that of WT mice. SMCs treated with FSP-1 showed enhanced potential of migration and proliferation. FSP-1 promoter contains RBP-Jk (a downstream transcription factor of Notch) consensus sites. Notch ligand, Jagged 1, increased FSP-1 expression and its promoter activity. Deletion of the RBP-Jkbinding sites inhibited Jagged 1-induced FSP-1 promoter luciferase activity. Moreover, CHIP and electrophoretic mobility shift assay (EMSA) analysis showed that Jagged 1 stimulated direct interactions of RBP-Jk with FSP-1 promoter sequences. Knockout of RBP-Jk in SMCs abolished these interactions. Similar responses were detected by using Notch inhibitor, DAPT. FSP-1-mediated migration and proliferation in SMCs were also suppressed by Notch inhibitor and by RBP-Jkknock out. Finally, AVFs created in mice with KO FSP-1 suppressed CKD-induced neointima formation. In these mice, neointima formation was ~ 40% below that in wild type, uremic mice.

**Conclusions:** Thus, uremia enhances FSP-1 expression through activation of Notch RBP-Jk signaling in SMCs and causes migration and proliferation of neighboring SMCs which accelerates neointima formation and AVF failure. FSP-1 could become a therapeutic target for suppressing neointima in AVFs.

*Funding:* NIDDK Support

## FR-OR092

**Preclinical Dataset Supports Initiation of Clinical Trials for Bioengineered Vascular Access Grafts** Shannon L.M. Dahl,[1] Jeffrey Lawson,[2] Heather L. Prichard,[1] Roberto J. Manson,[2] William Tente,[1] Alan P. Kypson,[3] Shawn Michael Gage,[2] Juliana Blum,[1] Laura E. Niklason.[4] *[1]Humacyte, Inc.; [2]Duke Univ; [3]East Carolina Univ; [4]Yale Univ.*

**Background:** End stage renal disease (ESRD) patients who require hemodialysis access would benefit from an alternative to synthetic grafts, which have high rates of thrombosis and venous intimal hyperplasia. Our bioengineered vascular graft is comprised of human extracellular matrix and is similar in strength to native human vein and artery. This acellular graft may be stored in a regular refrigerator, making it readily available for off-the-shelf surgical use. This graft is currently in clinical studies for evaluation of safety and efficacy for use as hemodialysis vascular access in patients with ESRD.

**Methods:** Banked smooth muscle cells were cultured in bioreactors in vitro to produce bioengineered tissue grafts, and a decellularization process was subsequently employed to remove cellular antigens and render grafts non-immunogenic. Canine-derived grafts were implanted as carotid artery bypass grafts in dogs, which provided directional guidance for evaluation of human grafts. Human-derived grafts were implanted as arteriovenous grafts in baboons. Evaluations included patency, durability, cannulation, immunogenicity, intimal hyperplasia, and remodeling.

**Results:** The mechanical integrity of these acellular grafts was retained throughout 1-year of refrigeration. Canine studies demonstrated long-term (1-year) safety and function of engineered tissues. Baboon studies showed that bioengineered grafts were safe, well tolerated, and functioned as intended. No significant intimal hyperplasia was observed in the graft or outflow vein in any recipient. Histologic analysis demonstrated evidence of host vascular cell migration into the graft.

**Conclusions:** These bioengineered grafts demonstrated high patency, could be cannulated repeatedly, and did not show significant intimal hyperplasia. These preclinical results support the evaluation of the grafts to provide dialysis access in patients with end stage renal disease.

## FR-OR093

**Taurolidine-Citrate-Hepatin Lock Solution Significantly Improves Inflammatory Profile in Hemodialysis Patients with Tunneled Catheters** Javier Donate,[1] Néstor Fontseré,[2] Celia Cardozo,[3] Mercedes Muros,[4] Alex Soriano,[3] Mercedes Pons,[5] Josep Mensa,[3] Josep M. Campistol,[1] Francisco Maduell,[1] Juan F. Navarro-Gonzalez.[1,6] *[1]Research Unit, HUNSC, S/C Tenerife; [2]Nephrology, Hosp. Clinic, Barcelona; [3]Infectious Diseases, Hosp. Clinic, Barcelona; [4]Clinical Analysis, HUNSC, S/C Tenerife; [5]CETIRSA, Barcelona; [6]Nephrology, HUNSC, S/C Tenerife.*

**Background:** The taurolidine-citrate-heparin lock solution (TCHLS) effectively eradicates pathogens from nontunneled and tunneled catheter biofilms and helped to maintain catheter lumen sterility. We aimed to evaluate the potential beneficial effect of this solution on the inflammatory profile of hemodialysis (HD) patients.

**Methods:** Thirty-one chronic patients, 18 males and 13 female, with a mean age of 72 years, and a mean time on HD of 82 months, were included in the study. All patients were on maintenance HD with tunneled catheters locked with a heparin filling were subsequently converted to a TCHLS. Serum concentration of inflammatory parameters [high-sensitive C-reactive protein (hsCRP), tumor necrosis factor-alpha (TNFa), interleukin (IL)-6 and IL-10], as well as mRNA expression levels of TNFa, IL6 and IL10 in peripheral blood mononuclear cells (PBMC) were analysed at baseline and after 6 months of TCHLS.

**Results:** Serum concentrations of hsCRP and IL-6 experienced a significant reduction (2.1±0.5 and 14.8±3.9 vs 1.5±0.4 and 10.6±2.7, respectively, p=0.01), whereas serum TNFa and IL10 did not change. Regarding inflammatory cytokine gene expression in PBMC, after 6 months of TCHLS, mRNA levels of IL-6 and TNFa significantly decreased by 20% and 19%, respectively ($p<0.05$), without changes in the expression levels of IL-10.

**Conclusions:** The use of TCHLS in maintenance HD patients with tunneled catheters resulted in an improvement of the inflammatory profile, with a significant decrease in the serum levels of hsCRP and IL-6, as well as a reduction in the mRNA gene expression levels of TNFa and IL-6 in PBMC.

## FR-OR094

**Safety of Small Bore Tunneled Central Venous Catheters in Chronic Kidney Disease Population** Gauri Bhutani,[1] Mireille El Ters,[1] Joe L. Klunder,[2] Sandra J. Taler,[1] Andrew Stockland,[2] Marie C. Hogan.[1] *[1]Nephrology & Hypertension, Mayo Clinic; [2]Interventional Radiology, Mayo Clinic, Rochester.*

**Background:** There is accumulating evidence that peripherally inserted central venous catheters (PICCs) are associated with high rates of venous thrombosis, remnant vascular injury and in the dialysis population, subsequent lack of functioning AV fistulas. Data from our center shows a PICC prevalance rate of 30% in the chronic hemodialysis population. A potential strategy to reduce vascular injury to arm veins may be to employ central neck veins for venous access.

**Methods:** Radiology database enquiry identified patients who received small bore tunneled central venous catheters (STCCs) at our center between January 1st 2010 and 31stMarch 2013. Demographics and line related characteristics from all available data are shown in table 1 below. Complication rate assessment is currently underway using chart audit.

**Results:** A total of 70 STCCs were placed in 62 adult patients under ultrasound guidance by interventional radiologists.

| | n = 62 patients ; 70 STCCs | Results |
|---|---|---|
| Age (years) | 62 patients | 21 to 84 (mean = 56.5) |
| Catheter diameter (Fr) | 48 lines (22 unknown) | 4 to 6 (4 Fr = 35.4% ; 5 Fr = 62.5% ; 6 Fr = 2.1%) |
| Number of lumens | 63 lines (7 unknown) | 1 to 3 (1 = 44.4% ; 2 = 52.4% ; 3 =3.2% ) |
| Site of placement | 67 lines (3 unknown) | Right Internal Jugular = 34 (50.7%)<br>Right External Jugular = 9 (13.4%)<br>Left Internal Jugular = 20 (29.8%)<br>Others = 4 (6 %)<br>(includes neck collaterals & subclavian veins) |

93 % lines were inpatient and for 72%, the indication was antibiotic administration (n=35). No placement complications were noted. Indwelling days ranged from 1 - 175 (mean 32). Follow up ranged from 4 - 1165 (mean 255) days. 69% of patients receiving these 35 STCCs had CKD, renal transplant or ESRD. There were no observed superficial or deep venous thrombosis sequelae.

**Conclusions:** We have observed a zero incidence of venous thrombosis over 918 indwelling days with STCCs.This is significantly lower than the 20 - 30% rate reported for PICCs in the current literature. STCCs may be a safer option in CKD patients in order to protect their arm veins for future dialysis access.

## FR-OR095

**Polycystic Kidney Disease without Apparent Family History (AFH)** Vinusha Kalatharan,[1] Alessia C. Borgo,[1] Young-Hwan Hwang,[1] Kairong Wang,[1] Jamie L. Sundsbak,[2] Christina M. Heyer,[2] Peter C. Harris,[2] York P. Pei.[1] *[1]Div of Nephrology, Univ Health Network, Toronto, Canada; [2]Dept of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** The absence of a positive family history in 15-20% of patients poses a diagnostic challenge for autosomal dominant polycystic kidney disease (ADPKD) and may be due to multiple causes.

**Methods:** In the Toronto Genetic Epidemiology Study of PKD (TGESP), 220 unrelated probands prospectively underwent renal function and imaging testing as well as a comprehensive *PKD1* and *PKD2* mutation screen. From this cohort, we identified all cases without AFH, examined their parental medical records, and performed renal imaging in any available parents of unknown disease status. From these results, we re-classified our cases into: (i) *de novo* disease (DND); (ii) positive family history (PFH) in retrospect; and (iii) indeterminate family history (IFH).

**Results:** We found that 53/220 (24%) of the TGESP probands did not have AFH. Among this patient subgroup, 26/53 (49%) had DND, 4/53 (8%) had a PFH in retrospect, and 23/53 (43%) had an IHF. Their re-classified family history and mutation types are shown below:

| Type of Mutation | *De Novo* Disease (n=26) | Positive Family History in Retrospect (n=4) | Indeterminate Family History (n=23) |
|---|---|---|---|
| Truncating PKD1 | 26.9% | 0% | 21.7% |
| Hypomorphic PKD1 | 26.9% | 75% | 30.4% |
| Truncating PKD2 | 3.9% | 0% | 8.7% |
| No mutation detected (NMD) | 42.3% | 25% | 39.1% |
| *% denotes column percent of n | | | |

Among the cases with *de novo* disease, we found one family (with two affected siblings and unaffected parents) suggestive of germline mosacism and 5 other with asymmetrical PKD suggestive of somatic mosacism. PKD1 and PKD2 mutation screen of the latter patients by Sanger sequencing was negative in all but one case (with a PKD1 frameshift mutation affecting the proband and her daughter).

**Conclusions:** PKD patients without AFH comprise a heterogeneous group resulting from *de novo* mutations, germline and/or somatic mosacism, mild disease from both hypomorphic *PKD1* and *PKD2* mutations, and unavailable parental medical records. Additionally, in ~1/3 of the cases, no pathogenic mutations could be found despite a comprehensive screen of both *PKD1* and *PKD2*.

## FR-OR096

**Identification of Mosaic Mutations in Mild, Mutation Negative ADPKD Patients Using Next-Generation Sequencing** Katharina Hopp,[1] Sandro Rossetti,[2] Maria V. Irazabal,[1] Binu M. Paul,[1] Jamie L. Sundsbak,[1] Christina M. Heyer,[1] Peter C. Harris.[1] *[1]Mayo Clinic; [2]Cardio-Renal Dept Otsuka Pharm.*

**Background:** In ~10% of ADPKD patients no *PKD1*/*PKD2* mutation is detected by conventional Sanger sequencing, and evidence for a third locus is not strong. The majority of these patients have mild disease and often a negative family history. An explanation consistent with these findings is mosaicism, which has rarely been described in ADPKD but might often be undetected since it is difficult to identify by Sanger sequencing.

**Methods:** We have combined imaging, family, and sequencing analysis to identify ~80 mild, mutation negative ADPKD patients with a negative/uncertain family history. Using next-generating sequencing we developed a protocol to deep sequence (>5000x) the *PKD1/PKD2* loci that reliably detects variants at the >3% level. This protocol takes into account equal read start site, additional allele call, and vicinity mismatch penalties, For/Rev read balance scores, and common false positive filters gathered from next-generation sequencing data of >200 ADPKD samples.

**Results:** In the first test run of 8 ADPKD probands we detected two promising variants, *PKD1* c.7006T>TG, p.Y2336D (6.6%, read depth 7911x) and *PKD1* c.12682C>CT, p.R4228X (7.4%, read depth 5601x). The first variant scored as highly likely pathogenic in the ADPKD Mutation Database and was found in a proband with only ~25 small cysts at 76 years of age and an unclear family history. The second variant was found in a patient with negative family history, who was diagnosed at 34 years with a few renal cysts and now at 73 years has a TKV of 1,417ml and serum creatinine of 1.5mg/dl. This variant was confirmed by allele specific PCR and Sanger sequencing, where the c.12682C>CT peak could be seen just above the background level.

**Conclusions:** We hypothesize that mosaicism explains a significant portion of ADPKD mutation negative patients with mild disease and the incidence is likely underestimated due to the lack of a protocol that allows reliable detection of these variants. Here, we have developed such a protocol using next-generation sequencing technologies and have shown its efficacy in an initial test run.

*Funding:* NIDDK Support, Private Foundation Support

## FR-OR097

**Prediction of GFR Endpoints in Early Autosomal Dominant Polycystic Kidney Disease** Michal Mrug,[1] Sylvie Mrug,[1] Doug Landsittel,[2] Vicente E. Torres,[3] Kyongtae Ty Bae,[2] Peter C. Harris,[3] Lisa M. Guay-Woodford,[4] Michael F. Flessner,[5] William M. Bennett,[6] Jared J. Grantham,[7] Arlene B. Chapman.[8] *[1]U AL Birmingham; [2]U Pittsburgh; [3]Mayo Clinic; [4]Children's Nat Med Ctr; [5]NIH; [6]Legacy Good Samaritan Hosp; [7]Kansas U; [8]Emory U.*

**Background:** There are no reliable formulas for predicting renal function over time in early autosomal dominant polycystic kidney disease (ADPKD).

**Methods:** We developed multivariable logistic models for reaching progression endpoints of CKD stage 3a and 3b and GFR decline by 30% and 50%. We developed the models and assessed their utility (with 10-fold cross-validation) using data collected by the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP), a prospective, observational, longitudinal, multicenter study of 241 ADPKD adults with preserved renal function. Demographic, physical, laboratory and imaging data were collected at baseline and during follow up visits.

**Results:** After a mean follow-up of 8.7 years, mean iothalamate clearance (GFR) decreased from 97.8 to 74.0 ml/min/1.73m$^2$. Baseline GFR alone was a poor predictor of GFR outcomes. However, prediction of the GFR endpoints has improved when ten baseline variables were used in multivariable models. GFR outcomes were significantly predicted by baseline age, BMI, hypertension, GFR, serum calcium and alkaline phosphatase, hemoglobin, white blood cell count, urinary MCP-1 and total kidney volume (TKV). Predicting the development of CKD stages 3a and 3b or GFR decline by 30% and 50% within 8.7 yrs using ROC analyses demonstrated an area under the curve (AUC) of 0.92, 0.93, 0.87 and 0.86, respectively for multivariable models. Similar AUCs were obtained using 10-fold cross-validation. The GFR-based modeling was repeated with MDRD-GFR, yielding similar outcomes.

**Conclusions:** Multivariable logistic models using routine clinical data predicted mild-moderate GFR decline endpoints in patients with early ADPKD. Such models may also facilitate the development of ADPKD therapies by informing selection of study participants with the highest risk for reaching FDA favored end-points.

*Funding:* NIDDK Support

## FR-OR098

**A New Algorithm to Predict Renal Outcome in Autosomal Dominant Polycystic Kidney Disease** Emilie Cornec-Le Gall,[1] Maryvonne Hourmant,[3] Marie-Pascale Morin,[4] Regine Perrichot,[6] Christophe Charasse,[7] Pascale Siohan,[5] Marie-Pierre Audrezet,[2] Claude Ferec,[2] Yannick Le Meur.[1] *[1]CHRU BREST, France; [2]INSERM 1078, CHRU Brest; [3]CHU Nantes; [4]CHU Rennes; [5]CH Quimper; [6]CH Vannes; [7]CH Saint-Brieuc.*

**Background:** As targeted therapies are under development in Autosomal Dominant Polycystic Kidney Disease (ADPKD), the selection of patients who should be treated is crucial. Herein, we present a new prognostic model to target patients at risk of poor renal outcome.

**Methods:** In a population of 1017 patients from 755 pedigrees, we studied the influence of 26 clinical, biological and genetic variables on renal outcome. We then built a model and tested it in a sub-population of 255 patients over 60 years or having reached ESRD before that age.

**Results:** The association of hypertension and at least one urologic complication (macroscopic hematuria, cysts infection or flank pain) before 35 yrs (group (g) 1) is associated with the poorest renal outcome (median age at ESRD=48 yrs, HR=3.8), the presence of either hypertension or urologic complication (g2) is of intermediate severity (54.4 yrs, HR=1.9), while patients without these characteristics (g3) have the best renal survival (67.9 yrs, $p<0.05$). Molecular data were available for 806 patients: truncating mutations (TM) of *PKD1* are associated with poorer renal outcome (53.9 yrs, HR=5.2) than non truncating (NTM) ones (62.6 yrs, HR=2.2) and *PKD2* patients have the the best renal survival (79.7 yrs, $p<0.05$). In a subgroup of patients over 60 yrs or having reached ESRD before that age, 96.3% of the patients belonging to g1 vs 79% of g2 and 44% of g3 reached ESRD before 60 yrs ($p<0.05$). Integrating molecular genetic and clinical data, we show that in g2 and g3, the proportion of patients reaching ESRD before 60 yrs is significantly higher in case of *PKD1* TM (g2:90.2%, g3:62%) than *PKD1* NTM (g2:62.9%, g3:37.1%) or *PKD2* mutations (g2:25%, g3:9.4%, $p<0.05$).

**Conclusions:** Based on the integration of simple clinical features with molecular genetic data, this algorithm might contribute to the development of a tailored approach of the therapeutic decision in ADPKD patients.

*Funding:* Government Support - Non-U.S.

## FR-OR099

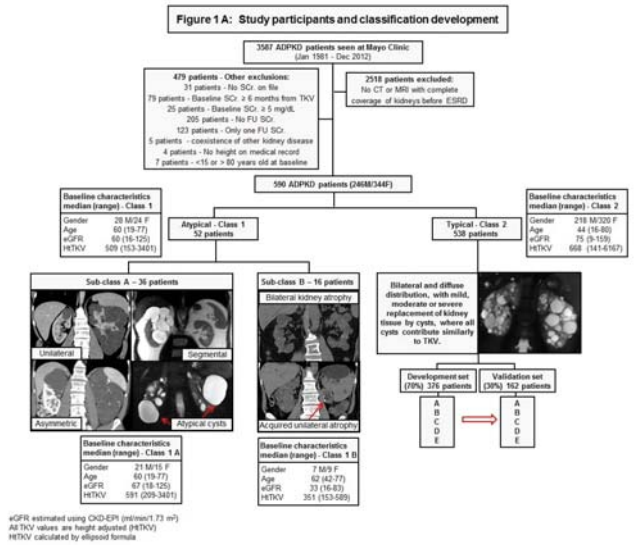
**Novel Imaging Classification to Predict Functional Disease Progression in Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Maria V. Irazabal, Laureano J. Rangel, Eric J. Bergstralh, Amber J. Harmon, Marie C. Hogan, Ziad El-Zoghby, Peter C. Harris, Bernard F. King, Vicente E. Torres. *Mayo Clinic, Rochester, MN.*

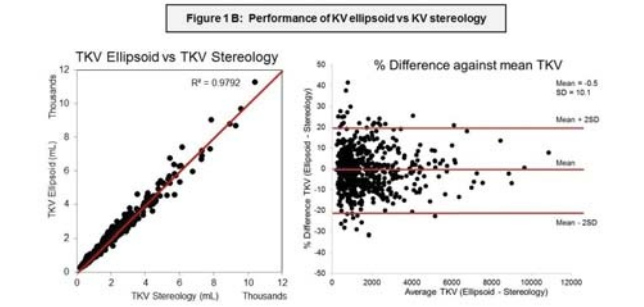
**Background:** CRISP showed that height adjusted total kidney volume (HtTKV) measured by stereology (TKVs) predicts eGFR decline in ADPKD. TKV can be used (1) as outcome measure in clinical trials and (2) for selection of patients more likely to exhibit treatment effect in a trial or benefit from an effective treatment. High precision but time consuming TKVs is essential for (1), but not for (2). Also, TKV and renal function correlate poorly in some patients. Our goal was to develop and validate a fast method to measure HtTKV that can be used for (2).

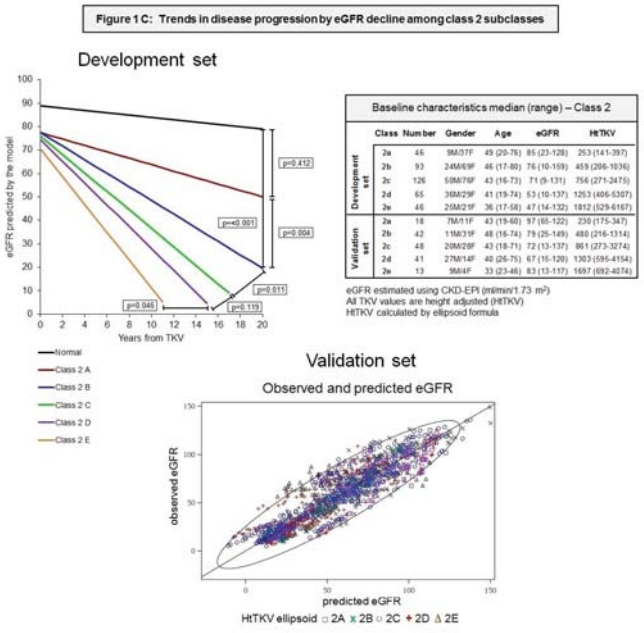
**Methods:** 590 ADPKD patients with CT/MR images and ≥3 eGFR measurements ≥6 months apart were classified as Atypical (class 1) or Typical (class 2) using pre-specified criteria (Figure 1 A). Class 2 patients were randomly partitioned into development and validation sets. TKV was calculated by stereology and ellipsoid formula (TKVe). Patients from the development set were subclassified (a-e) by age and HtTKV. A longitudinal mixed effects regression model was used to model eGFR decline.

**Results:** Figure 1 A shows baseline characteristics. TKVe correlated well with TKVs ($R^2$=0.98) without systematic bias (95% CI: -20.7%,19.6%) (Figure 1 B). HtTKV predicted eGFR decline in class 2 patients ($p<0.0001$), but not in class 1 ($p=0.07$). In the development set, patient subclasses showed significant differences in eGFR decline, with an average predicted error of=13.3 mL/min/1.73m$^2$ for the validation set (Figure 1C).

**Conclusions:** TKVe by CT/MR is an excellent predictor of TKVs and a fast way of estimating TKV for classifying class 2 ADPKD patients. The classification provides a practical tool for selecting the most appropriate patients for clinical trials and identifying patients with progressive disease likely to benefit from therapy.




*Funding:* NIDDK Support, Pharmaceutical Company Support - Otsuka Pharm., Private Foundation Support

## FR-OR100

**A Novel Agent That Inhibits Disease Progression in Models of Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Sorin V. Fedeles,[1] Bogdan I. Fedeles,[2] Seung H. Lee,[1] Stefan Somlo.[1] *[1]Internal Medicine, Yale School of Medicine, New Haven, CT; [2]Biological Engineering, MIT, Cambridge, MA.*

**Background:** ADPKD is a common monogenic cause of end-stage kidney disease for which no effective therapy exists. We examined the role of the novel antitumor agent 11**β** (Fedeles, et al., J Biol Chem, **286**:33910) in ADPKD. 11**β** has been shown to induce apoptosis in prostate cancer cells in vitro and to prevent growth of xenograft tumors in nude mice.

**Methods:** We used the orthologous Pkd1 model, Pkd1$^{fl/fl}$;Pkhd1-Cre. Mice were given daily intraperitoneal injections of 11**β** from P10 to P24. We determined kidney to body weight (KW/BW) ratio, cystic index, BUN, apoptosis, and proliferation at P24 in treated and control animals.

**Results:** 11**β** administration resulted in a decrease in KW/BW ratio as compared to vehicle injected controls (6.3+/-0.4 vs.15.1+/-2;***p,0.001); the body weight of treated animals was not statistically lower than controls. These changes were accompanied by a decrease in th cystic index (50%+/-1.1 vs. 70%+/-1.2;***p.0.001) and a decrease in BUN levels (41+/-1.4 vs. 76+/-1.5;***p<0.001). 11**β** specifically increased apoptosis as assessed by the cell-titer blue viability assay in cultured Pkd1 null cells compared to control (***p<0.001). The in vivo apoptotic index by TUNEL staining also showed significant decrease in treated mice (***p<0.001). Importantly, the effect of the drug was specific in vivo, as the vast majority of apoptotic cells stained positive were of collecting duct origin where the Cre is active; proximal tubule cells did not exhibit increased apoptosis. Interestingly, 11**β** did not have a statistically significant effect on the proliferation of Pkd1 null cells in vivo, as measured by Ki67 staining.

**Conclusions:** Our data show that 11**β** is a potent and specific inducer of apoptosis of Pkd1 null cells both in vitro and in vivo. In previous work 11**β** has been shown to have a pleiotropic effect on several cellular signaling pathways including mTOR, the unfolded protein response (UPR), and MAP kinase. Further studies are needed to elucidate the role of 11**β** in the apoptotic response seen on a Pkd1 knockout background.

*Funding:* NIDDK Support

## FR-OR101

**Inhibition of DNMT1 Delays Cyst Growth in Autosomal Dominant Polycystic Kidney Disease** Xia Zhou,[1,2] James P. Calvet,[2,3] Xiaogang Li.[1,2,3] *[1]Dept of Internal Medicine; [2]Kidney Institute; [3]Dept of Biochemistry and Molecular Biology, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Aberrant expression of DNA (cytosine-5)-methyltransferases (DNMTs) leads to promoter hypermethylation and the transcriptional silencing of a variety of tumor suppressor genes. Epigenetic alterations that result in dysregulated intracellular signaling pathways are proposed to promote cyst formation in ADPKD animal models. However, it is unknown whether DNMTs have a role in renal cyst formation.

**Methods:** To functionally test the role of DNMTs in renal cyst growth *in vivo*, *Pkd1* conditional knockout mice were treated with the DNMT inhibitor 5-azacytidine. To investigate how DNMTs might regulate cystic renal epithelial cell proliferation and apoptosis, cystic cells were treated with either DNMT1 siRNA or the inhibitor 5-azacytidine.

**Results:** We found that DNMT1 mRNA was upregulated in *Pkd1* mutant renal epithelial cells and tissues of *Pkd1*$^{flox/flox}$:Ksp-Cre mice vs *Pkd1* wild-type mice.Administration of 5-azacytidine to *Pkd1*$^{flox/flox}$:Pkhd1-Cre mice was found to significantly delay renal cyst growth *in vivo*. Knockdown of DNMT1 with siRNA or inhibition with 5-azacytidine in *Pkd1* mutant renal epithelial cells 1) decreased cystic epithelial cell proliferation as analyzed by the MTT assay, and induced G1 phase arrest as detected by FACS; and 2) decreased expression of Cyclin D1, phospho-Stat3, EGFR, and phospho-ERK, which were aberrantly upregulated and contribute to cystic epithelial cell proliferation in ADPKD. These results suggest that DNMT1 regulates cystic epithelial cell proliferation through these pathways. We also found that treatment with 5-azacytidine 1) induced cystic epithelial cell apoptosis as analyzed by Annexin V and PI double staining and TUNEL assay; and 2) increased the expression of p53, Bax and cleaved PARP, which suggested that the p53-Bax signaling pathway was involved in 5-azacytidine induced cystic renal epithelial cell death.

**Conclusions:** Inhibition of DNMT1 produces a potent anti-proliferative and pro-apoptotic effect in cystic renal epithelia and delays cyst formation in vivo, which suggests that targeting DNMT1 may function as a potential therapeutic strategy in ADPKD.

*Funding:* NIDDK Support

## FR-OR102

**Lanreotide Halts Polycystic Liver and Kidney Growth in Patients with Autosomal Dominant Polycystic Kidney Disease** Tom JG Gevers,[1] Jeroen C. Hol,[1] René Monshouwer,[3] Helena Dekker,[4] Jack F. Wetzels,[2] Joost P.H. Drenth.[1] *[1]Gastroenterology and Hepatology, Radboud Univ Nijmegen Medical Centre, Netherlands; [2]Nephrology, Radboud Univ Nijmegen Medical Centre, Netherlands; [3]Radiation Oncology, Radboud Univ Nijmegen Medical Centre, Netherlands; [4]Radiology, Radboud Univ Nijmegen Medical Centre, Netherlands.*

**Background:** We showed that the somatostatin analogue lanreotide reduced liver volume in patients with polycystic livers. However, this trial included patients with autosomal dominant polycystic kidney disease (ADPKD) and with isolated polycystic liver disease (PCLD). The aim of the present study was to assess the efficacy of lanreotide treatment in ADPKD patients with symptomatic polycystic liver disease.

**Methods:** This open-label clinical trial evaluated the effect of 6 months of lanreotide 120 mg subcutaneously every 4 weeks. Exclusion criteria were eGFR (MDRD) < 30 ml/min/1.73m2, use of oral contraceptives and PCLD. The primary outcome was change in liver volume determined by computerized tomography-volumetry. Secondary outcomes were changes in total kidney volume, eGFR and symptom relief. The severity of gastro-intestinal symptoms was measured with a 7-points scale questionnaire, and were dichotomized for absence or presence.

**Results:** We included 43 ADPKD patients with polycystic liver disease (84% female, mean age 51 years, eGFR 63 ml/min/1.73m2). Median liver volumes decreased from 4859 ml to 4595 ml (-3.2%, p<0.01). Lanreotide significantly relieved postprandial fullness (p<0.01), shortness of breath (p<0.012) and abdominal distension (p<0.01). Patients previously treated with somatostatin analogues had similar reductions in liver volumes as treatment-naïve patients. Although median kidney volumes decreased from 1023 ml to 1012 ml (-1.6%, p<0.01), this reduction did not improve eGFR.

**Conclusions:** Lanreotide reduces polycystic liver and kidney volumes and decreases symptoms in ADPKD patients with polycystic livers. Previous treatment with somatostatin analogues does not impair efficacy.

*Funding:* Pharmaceutical Company Support - We received an unrestricted grant from Ipsen Pharmaceuticals

## FR-OR103

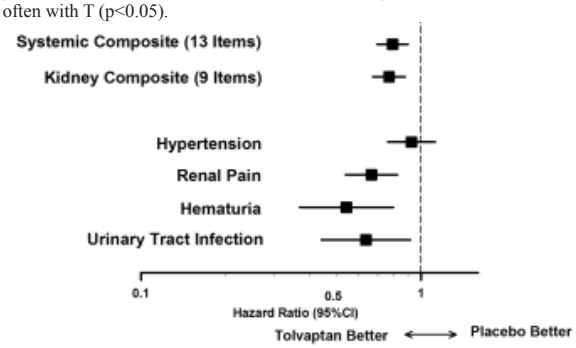
### Clinical Outcomes in ADPKD: Results from the TEMPO 3:4 Trial

Frank S. Czerwiec,[1] Arlene B. Chapman,[2] Olivier Devuyst,[3] Ron T. Gansevoort,[4] Eiji Higashihara,[5] Holly Krasa,[1] John Ouyang,[1] Ronald D. Perrone,[6] Vicente E. Torres.[7] *[1]Otsuka, USA; [2]Atlanta, USA; [3]Zurich, Switzerland; [4]Groningen, Netherlands; [5]Mitaka, Japan; [6]Boston, USA; [7]Rochester, USA.*

**Background:** ADPKD is a hereditary disease of progressive kidney destruction by cysts associated with systemic complications. The effect of tolvaptan (T) on systemic aspects of ADPKD was prospectively assessed in the TEMPO trial.

**Methods:** Clinical outcomes related to ADPKD were collected at each study visit and were analyzed by incidence and time to multiple events as a 13- (systemic) and 9-item (kidney) composite and the 4 most frequently observed events.

**Results:** Tolvaptan (T) subjects had less ADPKD-related outcomes in both disease composites (p<0.05). Of the most common events renal pain, hematuria, and UTI occurred less often with T (p<0.05).




For each of these events, T subjects reported less medical resource use (healthcare visits and/or medical procedures) and lost productivity due to the event. All 9 kidney-related events occurred less often with T.

| Event Type | Tolvaptan (N=961) % | Placebo (N=484) % |
|---|---|---|
| Hypertension* | 34.9 | 35.7 |
| Renal pain* | 26.2 | 38.0 |
| Urinary tract infection* | 9.8 | 14.6 |
| Hematuria* | 7.5 | 13.8 |
| Vascular/Cardiac Abnormalities | 4.6 | 4.7 |
| Anemia* | 2.2 | 4.5 |
| Abdominal/inguinal hernia* | 3.1 | 3.7 |
| Nephrolithiasis* | 1.7 | 3.5 |
| Other Cysts | 1.5 | 2.0 |
| Hepatic Cysts | 1.7 | 1.6 |
| Albuminuria* | 0.5 | 1.4 |
| Renal Function (eg, Dialysis)* | 0.9 | 1.0 |
| Colonic Diverticuli | 0.4 | 0 |
| * *Part of Kidney Composite* | | |

**Conclusions:** T treated subjects demonstrated fewer kidney related complications in an exploratory endpoint. These results support the impact of T beyond kidney cyst growth and functional decline.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceuticals

## FR-OR104

### Effect of Tolvaptan on Urine Osmolality versus Outcome in ADPKD: Results from the TEMPO 3:4 Trial

Olivier Devuyst,[1] Arlene B. Chapman,[2] Ron T. Gansevoort,[3] Jared J. Grantham,[4] Eiji Higashihara,[5] Ronald D. Perrone,[6] Vicente E. Torres,[7] Holly Krasa,[8] John Ouyang,[8] Susan E. Shoaf,[8] Xiaofeng Wang,[8] Frank S. Czerwiec.[8] *[1]Zurich, Switzerland; [2]Atlanta; [3]Groningen, Netherlands; [4]Kansas City; [5]Mitaka, Japan; [6]Boston; [7]Rochester; [8]Otsuka PDC.*

**Background:** The TEMPO 3:4 trial indicated that the vasopressin V2-receptor (V2R) antagonist tolvaptan (T) slowed the increase in total kidney volume (TKV) and the decline in kidney function (eGFR) over 3 years in ADPKD patients with preserved eGFR. We investigated whether this beneficial effect could be predicted by changes in urine osmolality (Uosm).

**Methods:** Post-hoc, exploratory analyses of a prospective, blinded RCT. Trough morning spot urine samples were collected at baseline (BL), and months (M) 12, 24 and 36. By protocol, water intake was encouraged and use of diuretics discouraged.

**Results:** Baseline Uosm was similar in placebo (N=333) and T (N=668) subjects (515 vs 497 mOsm/kg, respectively). It correlated with age, eGFR, and TKV. Placebo treated subjects had mean changes from BL Uosm ranging between -26 and -85 mOsm/kg over M36, reflecting increased water intake. A lower average Uosm during the trial was associated with a lesser increase in TKV. T treatment significantly reduced Uosm vs. placebo (by 249 mOsm/kg at week 3 and by 190 mOsm/kg at M36), with no loss of effect over time. T subjects with a greater suppression in Uosm had a 2-fold reduction in the occurrence of clinical progression events (severe renal pain and worsening kidney function) vs. those with less Uosm suppression (12.1% vs. 23.3%, highest vs. lowest quartile of Uosm change from BL, respectively), as confirmed by Kaplan-Meier analysis. T subjects with the greatest mean changes from BL Uosm were more likely to achieve no change in renal function.

**Conclusions:** These data in T treated patients (i) support the link between vasopressin V2R signalling and progression in ADPKD; (ii) suggest that monitoring Uosm could guide T titration and maintenance; and (iii) indicate that greater renoprotective effect may be achieved when Uosm is reduced by 300 mOsm/kg.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceuticals

## FR-OR105

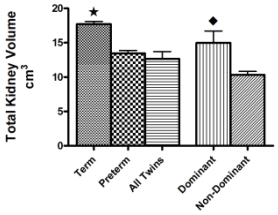
### Nephron Mass and the Intrauterine Environment: Twins from the Gerber Infant Kidney Study

Marissa J. Defreitas,[1] Teresa C. Cano,[1] Wacharee Seeherunvong,[1] Marta G. Galarza,[2] Chryso P. Katsoufis,[1] Vimal Master Sankar Raj,[1] Alcia D. Edwards- Richards,[1] Salih Y. Yasin,[3] Jayanthi Chandar,[1] Shahnaz Duara,[2] Gaston E. Zilleruelo,[1] Carolyn L. Abitbol.[1] *[1]Pediatric Nephrology, Univ of Miami/ Holtz Children's Hospital, Miami, FL; [2]Neonatology, Univ of Miami/ Holtz Children's Hospital, Miami, FL; [3]Obstetrics/Perinatology, Univ of Miami/ Holtz Children's Hospital, Miami, FL.*

**Background:** Nephrogenesis is active until 36 weeks' gestation. Preterm birth interrupts nephrogenesis and predisposes to a reduction in the total number of nephrons. Total Kidney Volume (TKV) is a surrogate for nephron mass. Multiple gestation imposes increased competition and provides a venue for studying a shared intrauterine environment.

**Methods:** Of 155 infants enrolled in the Gerber Infant Kidney Study, 53 were term infants (>37 weeks' gestational age) (GA), 86 were preterm (<37 weeks' GA) and 16 were preterm twins. Renal function was assessed by estimated glomerular filtration rate (eGFR) calculated from reference equations using serum creatinine and cystatin C. Renal length and volume were measured by ultrasound.

**Results:** eGFR was similar in twins and singletons of similar GA. Renal length and TKV were significantly less in preterm infants compared to term controls and correlated to GA (r=0.52; p<0.001). Among twin pairs, their was a significant discrepancy in TKV (15±5 versus 10±1 $cm^3$; p<0.01). One twin was dominant for kidney mass which was independent of gender or birth weight or length (p>0.5).




★ Significantly greater than others in same group; p<0.01

◆ Sigificantly greater than the non-dominant twin; p<0.05

**Conclusions:** Preterm birth imposes a restriction on nephron mass. Multiple gestation appears to impose an additional restriction in one of the twins that may predispose to adult cardiovascular and renal disease risks. Prospective longitudinal studies are needed to better understand the impact of twinning on nephron mass.

*Funding:* Private Foundation Support

## FR-OR106

**Mortality and Length of Stay Increase with Acute Kidney Injury (AKI) Severity Stage in Critically Ill Children** Scott M. Sutherland,[1] John James Byrnes,[2] Manish Kothari,[2] Pablo Garcia,[2] Stuart Goldstein.[3] *[1]Pediatrics, Stanford Univ, Stanford, CA; [2]SRI International, Menlo Park, CA; [3]Center for Acute Care Nephrology, Cincinnati Children's Hospital, Cincinnati, OH.*

**Background:** Recently, standardized AKI definitions have been developed (pRIFLE, AKIN, and KDIGO). These consensus definitions have not been correlated with outcomes in pediatric patients on a large scale.

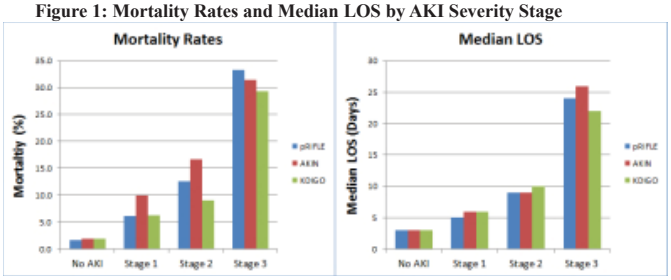
**Methods:** Patient data including age, height, and serial serum creatinine values were extracted anonymously from the electronic medical record. Mortality rates and length of stay (LOS) distributions were calculated for each stage of each definition. Likelihood ratios were calculated to assess the mortality risk associated with incremental AKI severity stages.

**Results:** 2365 intensive care unit (ICU) hospitalizations were analyzed. The median age was 6.0 (IQR 2.0-14.0). AKI rates varied between the pRIFLE (39%), AKIN (30%), and KDIGO (35%) definitions; across all three, AKI severity stage was associated with increased mortality rates and LOS (Figure 1). Compared to patients without AKI, patients with AKI experienced a greater likelihood of death; more severe AKI was associated with a greater mortality risk (Table 1, all p-values < 0.001).

**Table 1: Likelihood Ratio (LR) of Death by AKI Severity Stage**

| | Stage 1 vs. No AKI | Stage 2 vs. No AKI | Stage 3 vs. No AKI |
|---|---|---|---|
| **pRIFLE** | 3.8 | 7.8 | 20.5 |
| **AKIN** | 5.0 | 8.4 | 15.8 |
| **KDIGO** | 3.3 | 4.8 | 15.4 |

**Figure 1: Mortality Rates and Median LOS by AKI Severity Stage**




**Conclusions:** Within a large pediatric ICU cohort, AKI was significantly associated with poorer outcomes. Mortality rates and LOS increased incrementally as AKI severity increased. All three AKI definitions demonstrated strong outcome association. Further analysis is required to determine which definition is optimal.

## FR-OR107

**Microalbuminuria in Children with Chronic Kidney Disease** Dana F. Work,[1] Michael F. Schneider,[2] Susan L. Furth,[3] Jeffrey M. Saland,[4] Tom D. Blydt-Hansen,[5] Bradley A. Warady,[6] Marva M. Moxey-Mims,[7] George J. Schwartz.[1] *[1]Peds., Univ Rochester Med. Center, Rochester, NY; [2]Epi., J. Hopkins Bloom. Sch. Pub. Health, Baltimore, MD; [3]Peds., Child. Hosp. Phila., Phila., PA; [4]Peds., Mt. Sinai Sch. Med., New York, NY; [5]Peds., Univ Manitoba, Winnipeg, MB; [6]Peds., Child. Mercy Hospital, Kansas City, MO; [7]NIDDK, NIH, Bethesda, MD.*

**Background:** Children are generally screened for proteinuria by measurement of urine protein/creatinine ratio (Up/c). By this screening method those with microalbuminuria (urine albumin:creatinine between 30 and 300 mg/g) are classified as having clinically insignificant proteinuria. We sought to examine microalbuminuria, in the absence of elevated Up/c, as a potential marker for lower GFR in children with CKD.

**Methods:** We used data collected in the Chronic Kidney Disease in Children (CKiD) study. Linear regression was used to quantify the association of initial spot urine microalbuminuria, as well as Up/c, on GFR while controlling for age, diabetes, gender, hypertension, race and cause of CKD. GFR was measured by iohexol disappearance or estimated using CKiD-published formulas. Hypertension was defined as systolic or diastolic BP>95$^{th}$ percentile or on BP medication.

**Results:** In 682 subjects 1-21 years old, those with microalbuminuria did not have a significant difference in mean GFR (3.4±3.0 ml/min per 1.73m$^2$, p=0.64) when compared to subjects without microalbuminuria (64.9±23.7 vs. 69.5±24.5 ml/min per 1.73m$^2$). Individuals with elevated Up/c and > 300 mg albumin/g creatinine (albuminuria) had a mean GFR that was 19.0±2.2 ml/min per 1.73 m$^2$ less than those without proteinuria or microalbuminuria (p<0.001). Of 183 subjects who had Up/c < 0.2 and had >1 GFR value over a mean of 2 years of follow-up, 9 (5%) (7 without microalbuminuria) had a decline in GFR of at least 30%; this is compared to 307 subjects with Up/c>0.2 of which 74 (24%) had a decline in GFR of at least 30%.

**Conclusions:** While screening for microalbuminuria may be useful in some patient populations, our analysis suggests that when monitoring proteinuria in children with CKD, Up/c is sufficient. Urine albumin does not appear to provide additional information for the management of CKD in children.

*Funding:* NIDDK Support, Other NIH Support - T32 fellowship

## FR-OR108

**Does Nephrectomy of Failed Renal Transplant Affect Outcome in Pediatric Patients with a Second Transplant?** Marta Monteverde,[1] Alicia Beatriz Chaparro,[1] Liliana Margarita Briones,[1] Juan Carlos Ayus.[2] *[1]Hospital Garrahan, Bs As, Argentina; [2]Renal Consultants of Houston, Texas.*

**Background:** Patients with a failed renal allograft who return to dialysis have significant increase in morbidity and mortality, most likely related to chronic inflammation(Lopez JM;JASN 2004). Transplant nephrectomy in these patients is associated with decreased mortality (Ayus JC JASN 2010). However, a possible drawback of transplant nephrectomy could be an increase in transplant failure in retransplantation.**Purpose:** To evaluate the difference in outcome between pediatric patients who had a second transplant (STX) after nephrectomy of the failed allograft and patients with a first transplant (FTX), as no information regarding allograft nephrectomy and retransplantation exists in children.

**Methods:** We compared patient and graft survival and eGFR at last visit of follow-up between F TX and S TX children with allograft nephrectomy.

**Results:** Between December 1988 and March 2013, 696 TX were performed at a public tertiary pediatric referral center, 659 first renal TX **(FTX)** and 37 second TX **(STX)**. Thirty-one children from the STX group had their first failed graft removed. In 15, nephrectomy was early (<90 days post TX) and in 16 late. Median time of follow up after transplantation of patients in both FTX and STX groups was 5 years (IQR: 2-7.4). Except for panel reactive antibodies (PRA) levels pre-TX (<10% in 98% vs. 87%, 10 to 40% in 2.2% vs. 8.7%, and > 40% in 0% vs. 4% of FTX vs. ST X; p=0.004), and recipient age (FTX: 11.1 ±4.3 years, STX: 12.9±3 years; p=0.02), there were no other differences at baseline between both groups. Patient survival at 6 years was 95% for FTX and STX. Graft survival at 1 and 6 years was 93% vs. 90.3 % and 77% vs. 81% for FTX and STX patients with allograft nephrectomy (p= 0.82). Six-year graft survival was not different in those with late vs. early nephrectomy (78% vs. 86%; p=0.40). eGFR at last visit was 43±26 vs. 40±23 ml/min/1.73m2 (p=0.58).

**Conclusions:** Nephrectomy of the failed renal allograft did not affect either patient or graft survival and final eGFR in their second transplant.

## FR-OR109

**The Antimicrobial Activity of Ribonuclease 7 Is Blocked by an Endogenous Ribonuclease Inhibitor in the Human Urinary Tract** John David Spencer, Tad Eichler, David S. Hains. *Center for Clinical and Translational Research, The Research Institute at Nationwide Children's, Columbus, OH.*

**Background:** Although urine is considered sterile, little is known how the kidney defends against infection. We have previously demonstrated that the collecting duct produces antimicrobial peptides (AP). The collecting duct produce RNase7, one of the most potent human APs. To date, the regulation of RNase7's antimicrobial activity is not defined. Other research groups have demonstrated that an endogenous Ribonuclease Inhibitor (RI) binds to Ribonuclease A proteins and alters their function. The expression of RI in the urinary tract has not been previously evaluated and its on RNase7 in the urinary tract are unknown.

**Methods:** RNA from non-infected and pyelonephritic human kidney was used to quantify *RNASE7* and *RI* via real-time PCR. IF localized RNase7 and RI production. Western blot (WB) quantified RNase7/RI in kidney and urine samples. Native gel assays assessed RI-RNase7 binding. Bacterial binding and Live/Dead kill assays assessed the antimicrobial function of RNase7 on *E.coli* (UPEC) in the presence and absence of RI.

**Results:** With pyelonephritis, RNASE7 mRNA expression increased while RI mRNA expression decreased (p<0.05). IF localized RNase7 production to the apical surfaces of intercalated cells (ICs) in the renal collecting ducts and RI to the basolateral surfaces of ICs. WB demonstrated that kidney and urinary RNase7 peptide production increase with infection while RI peptide decreased with pyelonephritis. Recombinant RNase7 kills UPEC at micromolar concentrations. Protein binding assays demonstrate that RI tightly binds to RNase 7. In the presence of equal concentrations of RI, the antimicrobial effects of RNase7 significantly decrease against UPEC. RI blocks the binding of RNase 7 to LPS of Gram-negative uropathogens.

**Conclusions:** The renal epithelium is important in maintaining sterility of the urinary tract as it produces potent APs like RNase7. It also produces regulatory proteins like RI, which binds RNase7 and abrogates its antimicrobial effects. Further elucidation of the factors that regulate production/function of APs like RNase7 may lend insight into the UTI pathogenesis.

*Funding:* NIDDK Support

## FR-OR110

**Nephroprotective Efficacy of RAS Blockade in Mice Carrying R140Q Podocin Mutation** Tanja Wlodkowski,[1] Helga Denc,[1] Ivana Simic,[1] Mansoureh Tabatabaei Far,[1] Geraldine Mollet,[2] Corinne Antignac,[2] Franz S. Schaefer.[1] *[1]Pediatric Nephrology Div, Heidelberg Univ, Germany; [2]Inserm U983, Hopital Necker, France.*

**Background:** NPHS2 mutations cause hereditary nephrotic syndrome and progressive renal failure. We recently generated an inducible knock-in mouse model carrying R140Q, the analogue of the most common human mutation R138Q. These mice develop FSGS with nephrotic syndrome and progressive renal failure. Here we tested the efficacy of early and delayed RAS blockade in this model of hereditary podocyte disease.

**Methods:** In C57BL/6 mice with Nphs2$^{Flox/R140Q}$,Cre$^{+}$ genotype hemizygosity for mutant podocin was induced by tamoxifen injection. Animals received combined high-dose ACE inhibition and AT1 receptor blockade (ramipril+candesartan 10 mg/kg each,R+C) or

remained untreated. Treatment was started either prophylactically (P) at time of induction or with a two-week delay (D). Animals were sacrificed after 4 wks or observed open-end.

**Results:** RAS blockade reduced proteinuria to 6%(P,p<0.005) and 33%(D,p<0.05) of untreated animals 5 wks after induction. At this time point, the R+C animals retained normoalbuminemia(31 v.19 g/l), lower serum creatinine(0.14 v.0.16 mg/dl), a higher number of podocytes per glomerulus(73 v.39), and a lower glomerular sclerosis index(0.91 v.1.47). Podocin protein abundance was almost totally lost in both untreated and treated animals, despite preserved or even increased mRNA expression. At wk 9, proteinuria had gradually increased to 75% of controls in D(n.s.) but remained low in P animals(10% of controls,p<0.005). Three-month graft survival was 50.1% in the untreated animals, but 100% in all animals with RAS blockade(p<0.05).

**Conclusions:** In mice carrying the most common human podocin mutation, RAS blockade attenatues proteinuria, podocyte loss and glomerulosclerosis despite persistently increased degradation of mutant podocin protein. Renal failure is delayed and survival prolonged. Treatment is more effective when applied prophylactically than when started after 2 wks, the time when histopathological lesions first become detectable. Our findings indicate that early RAS blockade provides effective nephroprotection in this hereditary podocytopathy.

## FR-OR111

**Adeno-Associated Virus 9 Expression of GFP in Mice Using a Truncated Nephrin Promoter** Melissa A. Muff-Luett, Di Wu, Jason L. Picconi, Erik Bunchman, Patrick D. Brophy. *Div of Pediatric Nephrology, Univ of Iowa Children's Hospital, Iowa City, IA.*

**Background:** Structural renal diseases such as nephrotic syndrome have no specific curative therapy. Gene therapy offers the possibility of therapeutic intervention for diseases with single gene mutations. The kidneys especially glomeruli have proven difficult to target with previous experimental vectors. Recently the adeno-associated virus 9 (AAV2/9) has been shown to have broad tissue tropism in mice including the kidneys. A truncated 1.25kb promoter of NPHS1, the gene which encodes nephrin, has been shown to have renal specific expression. We developed a novel AAV2/9 with the truncated minimal nephrin promoter (MNP) to direct kidney specific gene expression and potential therapy. Our goal was to characterize GFP expression in mice after systemic tail vein injection with the AAV2/9-MNP-GFP vector.

**Methods:** We designed an AAV2/9-MNP-GFP vector with the 1.25kb nephrin promoter to drive GFP gene expression. A CMV promoter (AAV2/9-CMV-GFP) was used as the positive control vector and saline as a negative control. Eight-week old female C57 mice were injected with 3x10^11 viral particles of each vector. Tissue was harvested 8 weeks after injection. Kidney, brain, liver and heart were analyzed for the presence of viral DNA copies, GFP RNA expression and GFP protein expression.

**Results:** AAV2/9-MNP-GFP transfected kidneys showed 6 fold higher viral copy numbers than CMV controls with a similar trend in the other tissues. Renal GFP RNA expression was 5 fold higher in the AAV2/9-CMV-GFP transfected controls compared to the AAV2/9-MNP-GFP mice. AAV9-MNP-GFP livers showed GFP RNA expression but no protein expression. GFP RNA expression was absent in brain and heart of AAV9-MNP-GFP mice. AAV2/9-MNP-GFP transfected mice demonstrated sustained glomerular GFP protein expression within the kidneys by immunofluorescence with no protein expression in the liver, brain and heart.

**Conclusions:** The AAV2/9 vector utilizing the 1.25kb nephrin promoter provides a safe, viable tool for renal directed gene therapy in mice. These experiments provide a novel method for the investigation and potential therapy for congenital renal diseases in mice.

*Funding:* Private Foundation Support

## FR-OR112

**Chronic Epithelial Kidney Injury Molecule-1 Expression in Mice Causes Inflammation, Fibrosis and Kidney Failure** Benjamin D. Humphreys, Venkata Sabbisetti, Fengfeng Xu, Ivica Grgic, Said Movahedi Naini, Ningning Wang, Guochun Chen, Sheng Xiao, Dhruti D. Patel, Joel M. Henderson, Takaharu Ichimura, Shan Mou, Savuth Soeung, Andrew P. McMahon, Joseph V. Bonventre. *Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Acute kidney injury predisposes to future CKD and ESRD but the molecular details underlying this clinical association are unknown. We report that chronic expression of Kidney Injury Molecule-1 (KIM-1), an epithelial phosphatidylserine receptor expressed during acute injury and in fibrotic renal disease, promotes kidney fibrosis.

**Methods:** We generated a transgenic mouse line for conditional overexpression of KIM-1 in a cell specific manner. The KIM-1 conditional mouse line was crossed with a tubular Cre driver and we examined kidney phenotype over time. We also evaluated susceptibility to unilateral ureteral obstruction (UUO)-induced fibrosis in mice with a targeted deletion of the mucin domain of KIM-1, resulting in a mutant, KIM-1 polypeptide with markedly diminished phagocytic function.

**Results:** Mice with transgenic overexpression of KIM-1 in tubule epithelia, but no other cell type, developed spontaneous and progressive interstitial kidney inflammation with fibrosis leading to renal failure. Mice developed anemia, proteinuria, hyperphosphatemia, hypertension, cardiac hypertrophy and died of renal disease, analogous to progressive kidney disease in humans. KIM-1-transgenic kidneys had elevated expression of the pro-inflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) at early time-points. Heterologous expression of KIM-1 in an immortalized proximal tubule cell line triggered MCP-1 secretion and increased MCP-1-dependent macrophage chemotaxis. In KIM-1 mutant mice, experimental kidney fibrosis, secondary to UUO, was ameliorated and levels of MCP-1 were reduced, consistent with a pro-fibrotic role for native KIM-1.

**Conclusions:** Sustained KIM-1 expression, a characteristic of chronic kidney disease in humans, promotes kidney inflammation and fibrosis and provides a molecular link between acute injury and progressive chronic kidney disease.

*Funding:* NIDDK Support

## FR-OR113

**Myofibroblastic Transformation of Renal Erythropoietin-Producing Cells Governs Fibrosis but Is Reversible in Early Injury Stage** Tomokazu Souma,[1] Shun Yamazaki,[1] Takashi Moriguchi,[1] Norio Suzuki,[1] Sadayoshi Ito,[2] Masayuki Yamamoto.[1] *[1]Dept of Medical Biochemistry, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Dept of Medicine, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan.*

**Background:** Myofibroblasts are the culprit of renal fibrosis, and anemia develops along with scaring due to insufficient erythropoietin (Epo). We have recently characterized renal Epo-producing cells (REPs) as fibroblast-like cells with neural characters. They transform into myofibroblasts when injured, while exact contributions of REPs to the total myofibroblasts pool, mechanisms underlying this process and phenotypic reversibility are still unclear.

**Methods:** To analyze the myofibroblastic transformation process, we generated efficient monitoring and cell-fate tracing systems for REPs utilizing transgenic (Tg) complementary rescue method and Cre-loxP system. Genetically induced severe anemia in Tg-rescued mice enabled maximal recruitment and isolation of REPs. Transgenic Cre expressions, which are driven by full regulatory region of Epo, enabled tracking of cells with history of Epo expression.

**Results:** By adopting unilateral ureteral obstruction, we discovered that almost all myofibroblasts in cortex and outer medulla are derived from REPs. Transformed REPs were isolated by flow cytometry, and were found to lose their Epo-producing potential by 90%. On the contrary, they produced not only extracellular matrix, but also inflammatory mediators (such as Ccl2, Il6, and so on). By removing the obstruction in early injury stage, myofibroblasts reverted to physiological status, regaining normal Epo-producing ability, and stopped producing excess collagens. Microarray analysis showed inflammatory signaling cascades were the top canonical pathways induced in injured kidneys during the transformation process. Thereby, anti-inflammatory therapy accelerated the reversion of myofibroblasts to normal REPs.

**Conclusions:** Our findings demonstrate that REPs are the major source of myofibroblasts, and possess profound plasticity. Modulation of cellular status of REPs is an important strategy to control the balance between beneficial repair/regeneration and deleterious scaring of kidneys.

*Funding:* Government Support - Non-U.S.

## FR-OR114

**Multiple Genes of the Renin-Angiotensin System Are Novel Targets of Wnt/β-Catenin Signaling in Kidney Injury** Li Li Zhou,[1,2] Yingjian Li,[1] Dong Zhou,[1] Roderick J. Tan,[3] Jing Nie,[2] Fan Fan Hou,[2] Youhua Liu.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Div of Nephrology, Nanfang Hospital, Southern Medical Univ, Guangzhou, Guangdong, China; [3]Dept of Medicine, Univ Pittsburgh, Pittsburgh, PA.*

**Background:** Activation of the renin-angiotensin system (RAS) plays an essential role in the pathogenesis of chronic kidney and cardiovascular diseases. However, current anti-RAS therapy only displays limited efficacy, partly because of compensatory upregulation of renin expression. Therefore, developing a strategy to simultaneously target multiple RAS genes is necessary for the effective treatment of chronic kidney and cardiovascular diseases.

**Methods:** The promoter region of all RAS genes were analyzed by bioinformatics tool. The expression of RAS genes in cultured cells and kidney tissues were examined by qRT-PCR, Western blot, and immunostaining.

**Results:** By bioinformatics analyses, we uncovered that all RAS genes contained putative TCF/LEF-binding sites in their promoter regions, and β-catenin induced the binding of LEF-1 to these sites in kidney tubular cells. Over-expression of either β-catenin or different Wnt ligands induced the expression of all RAS genes. Conversely, a small molecule β-catenin inhibitor ICG-001 abolished RAS induction. In a mouse model of nephropathy induced by adriamycin, either transient therapy or late administration of ICG-001 was able to abolish an established proteinuria and kidney lesions. ICG-001 inhibited renal expression of multiple RAS genes in vivo, and abolished the expression of other Wnt/β-catenin target genes. As a result, ICG-001 therapy attenuated myofibroblast activation, repressed matrix expression, and inhibited renal inflammation and fibrosis.

**Conclusions:** Collectively, these studies identify all RAS genes as novel downstream targets of Wnt/β-catenin. Our results also indicate that blockade of Wnt/β-catenin signaling can simultaneously repress multiple RAS genes, thereby leading to the reversal of an established proteinuria and kidney injury.

*Funding:* NIDDK Support, Government Support - Non-U.S.

**FR-OR115**

**Blocking the TGF-Beta Type II Receptor in Interstitial Cells Does Not Ameliorate Renal Fibrosis** Leslie S. Gewin,[1,2] Roy Zent.[1,2,3,4] *[1]Dept of Research, Veterans Affairs Hospital, Nashville, TN; [2]Div of Nephrology, Dept of Medicine, Vanderbilt Univ, Nashville, TN.*

**Background:** TGF-beta exerts strong pro-fibrotic effects following injury, but the target cell responsible for mediating these effects is unclear. TGF-beta activates fibroblasts and induces their production of collagen I in vitro, but how TGF-beta signaling in fibroblasts modulates injury in vivo has not been examined.

**Methods:** To address this, we deleted the TGF-beta type II receptor in interstitial cells using two different tamoxifen-inducible Cre: COL1A2-Cre and TenascinC-Cre. The mT/mG reporter mouse localized Cre activity, and recombination after tamoxifen administration in vivo was confirmed using PCR. Characterization of these interstitial cells was performed by FACS analysis.

**Results:** Surprisingly, both $Tgfbr2^{floxed}$xCOL1A2-Cre and $Tgfbr2^{floxed}$xTenascin C-Cre mice had equivalent amounts of tubulointerstitial fibrosis compared to their $Tgfbr2^{floxed}$ littermate controls at 7 and 14 days after unilateral ureteral obstruction (UUO). Fibrosis was measured by picosirius red staining, immunohistochemistry for collagen I, IV, and fibronectin, and by immunoblotting renal tissue lysates for collagen I. Cells were isolated from both $Tgfbr2^{floxed}$xCOL1A2-Cre and $Tgfbr2^{wildtype}$xCOL1A2-Cre mice after UUO using FACS to sort out EGFP+ cells. Primary cells from both mice expressed markers typical for fibroblasts. Cells isolated from $Tgfbr2^{wildtype}$xCOL1A2-Cre mice had markedly increased collagen I expression after stimulation with TGF-b1 but those from $Tgfbr2^{floxed}$xCOL1A2-Cre mice had less collagen I at baseline and no significant response to TGF-b1.

**Conclusions:** These results suggest that interstitial cells with the type II receptor isolated from injured mice do upregulate collagen I expression in response to TGF-b1 in vitro, and this response is not observed in cells lacking the TGF-beta type II receptor. However, the absence of the TGF-beta type II receptor in interstitial cells does not protect against renal injury, implying that in vitro data may not reliably predict in vivo responses and that TGF-beta signaling in medullary interstitial cells may not be important in modulating UUO-induced tubulointerstitial fibrosis.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-OR116**

**Exploring the Function of Renal Fibroblasts of Extra-Renal Origin** Jin Nakamura, Akiko Oguchi, Motoko Yanagita. *Nephrology, Kyoto Univ, Graduate School of Medicine, Kyoto, Japan.*

**Background:** We previously reported that most fibroblasts in the renal cortex and outer medulla are myelin protein zero-Cre (*P0-Cre*) lineage-labeled cells (P0 cells)of extra-renal origin, and that some of them are erythropoietin (EPO) producing cells in the healthy kidney. In the diseased kidney, P0 cells transdifferentiate into myofibroblasts and predominantly contribute to fibrosis, with concomitant loss of EPO production. In this study, we further investigated the function of P0 cells and the crosstalk between the fibroblasts and tubular epithelial cells.

**Methods:** We utilized *P0-Cre* inducible simian diphtheria toxin receptor (DTR) transgenic mice (*P0-Cre:iDTR* mice). The binding of DT to DTR halts protein synthesis within P0 cells, inhibiting the crosstalk between fibroblasts and tubular epithelial cells.

**Results:** First we confirmed that renal fibroblasts were successfully labeled with DTR in *P0-Cre:iDTR* mice. DT administration ablated the expression of DTR and fibroblast markers, indicating the effective cessation of protein synthesis in P0 cells. Simultaneously, the expression of EPO was significantly reduced, and did not increase even after the induction of severe anemia. In addition, the expression of tubular injury markers, as well as the proliferation of proximal tubule cells was induced. The administration of DT to *P0-Cre:iDTR* mice with unilateral ureteral obstruction reduced the expression of fibrosis markers, and enhanced the expression of tubular injury markers in diseased kidney. Unlike the results of healthy kidney, tubular proliferation in diseased kidney was attenuated.

**Conclusions:** Cessation of protein synthesis in P0 cells reduced the expression of EPO in healthy kidney and the fibrosis markers in diseased kidney, supporting our previous findings. And this also induced the tubular injury and influenced the tubular proliferation, suggesting that fibroblasts inhibit tubular proliferation and injury in healthy kidney, while support the repair of injured tubule by promoting tubular proliferation in diseased kidney. These results indicate the possible interactions between the fibroblasts and tubular epithelial cells. We are currently searching for the molecules responsible for the interactions.

**FR-OR117**

**Tracing Tubular Cells' Fate in Folic Acid Induced Kidney Injury Model by Lineage Tagging** Jianling Tao,[1,2] Peng Guo,[3] Katalin Susztak.[1] *[1]Renal Electrolyte and Hypertension Div, Dept of Medicine, Perelman School of Medicine, Univ of Pennsylvania, Philadelphia, PA; [2]Div of Nephrology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing, China; [3]Analytical Imaging Facility, Albert Einstein College of Medicine, Yeshiva Univ, Bronx, NY.*

**Background:** Tubulointerstitial fibrosis (TIF) is the final common pathway leading to ESRD. Folic acid (FA) induced acute/chronic TIF model was used to follow the fate of different cell types in the kidney to address which tubule segment is injured and the origin of activated myofibroblast (epithelial, pericyte).

**Methods:** The tomato/GFP mouse model (mT/mG) and different cre recombinases were used. Tomato fluorescence turns to GFP (mG) after cre-mediated recombination. Tubule cells were traced by the Pax8rtTA/TREcre, proximal tubule epithelial cells by PEPCKCre, distal tubule epithelial cells by KSPCre and pericytes by Foxd1cre. GFP positive cells were followed by flourescence and FACS analysis. To detect activated myofibroblasts kidney was stained with alpha smooth muscle actin (aSMA).

**Results:** Pax8 positive cell number was decreased after FA injection. The proliferating cells re-expressed Pax8 and they were different from the original Pax8 expressing cells. KSP positive distal tubule cell number decreased on day 7(D7) and remained lower for 4 weeks, they proliferated and number increased at 12 weeks and returned to baseline by 20 weeks. PEPCK positive epithelial cell number did not significantly change on D7. Foxd1 positive pericyte number significantly increased on D7. aSMA, did not colocalize with epithelial lineage markers (PEPCK, KSP, PAX8). aSMA failed to fully colocalize with pericyte marker foxd1, indicating pericyte might not be the only origin of myofibroblast.

**Conclusions:** The collecting ducts and thick ascending limb of loop of Henle appear to be a key site in folic acid induced epithelial injury. Majority of activated myofibroblasts are not of epithelial origin and pericytes only partially contribute to the myofibroblast pool.

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**FR-OR118**

**Laminin 211-Mediated Focal Adhesion Kinase Activation Triggers Alport Glomerular Pathogenesis** Dominic E. Cosgrove, Brianna Johnson, Daniel T. Meehan, Duane C. Delimont, Marisa Zallocchi, Linda Cheung. *Genetics, Boys Town National Research Hospital, Omaha, NE.*

**Background:** It has been known for some time that laminins 211 and 111, normally restricted to the mesangial matrix, accumulate in the glomerular basement membranes (GBM) of Alport mice, dogs, and humans. Whether these abnormal laminins contribute to Alport glomerular pathology has remained unknown.

**Methods:** A double knockout mouse for laminin $\alpha$2 and COL4A3, and a podocyte-specific conditional FAK knockout Alport mouse were compared with wild type and Alport mice. SiRNA knocdown for FAK in glomerular pododytes was performed. The small molecule inhibitor for FAK, TAE226 was used in both podocyte cell cultures and *in vivo*. TAE226-treated Alport mice were assessed for glomeruloschlerosis, fibrosis, proteinuria and uremia. FAK activation for podocyte cultures on different laminin substrates was performed. Real time RT-PCR analysis for MMP-9, -10, and -12 as well as IL-6 and NFkappaBIA was assessed for RNA from both isolated glomeurli and cultured podocytes.

**Results:** Laminin 211, but not laminin 111, activates focal adhesion kinase (FAK) on glomerular podocytes *in vitro* and *in vivo*. Alport glomeruli showed significant induction of MMP-9, MMP-10, MMP-12 and IL-6. SiRNA knockdown of FAK in cultured podocytes significantly reduced expression of MMP-9, MMP-10 and IL-6, but not MMP-12. Glomerular mRNA analysis of podocyte-specific conditional FAK-null Alport mice also showed reduced expression of MMP-9, MMP-10, and IL-6 mRNA relative to age/strain matched Alport mice. MMP-12 expression was not affected. Treatment of Alport mice with TAE226, a small molecule inhibitor of FAK activation, ameliorated fibrosis and glomerulosclerosis, significantly reduced proteinuria and blood urea nitrogen levels, and partially restored GBM ultrastructure. Glomerular expression of MMP-9, MMP-10 and MMP-12 mRNAs was significantly reduced in TAE-226 treated animals.

**Conclusions:** This work identifies laminin 211-mediated FAK activation in podocytes as an important early event in Alport glomerular pathogenesis and suggests that FAK inhibitors might be employed as a novel therapeutic approach for treating Alport renal disease in its early stages.

*Funding:* NIDDK Support

**FR-OR119**

**Uromodulin Regulates Renal Calcium Channel TRPV5 via a Mechanism Synergistic with Secreted Klotho** Matthias Wolf,[1] Chou-Long Huang.[2] *[1]Pediatric Nephrology, Univ of Texas Southwestern Medical Center, Dallas, TX; [2]Internal Medicine, Univ of Texas Southwestern Medical Center, Dallas, TX.*

**Background:** Uromodulin (UMOD) is the most abundant urinary protein and protects against nephrolithiasis. We recently reported that UMOD acts extracellularly to upregulate TRPV5 by impairing its endocytosis. Secreted Klotho (sKL) cleaves the terminal sialic acid of TRPV5 N-glycan to allow the channel binding to galectin-1, which decreases endocytosis and increases surface abundance of the channel. We examined the mechanism of UMOD regulation of TRPV5, particularly the potential interaction with sKL.

**Methods:** TRPV5 surface abundance and activity were studied by biotinylation and whole-cell patch-clamp recording in HEK cells expressing TRPV5, UMOD and/or sKL.

**Results:** Coexpression of UMOD increased current density (pA/pF) of wild-type TRPV5 (446±37, -UMOD vs 802±45, +UMOD, $p<0.0001$), but not N-glycan deficient (N358Q) TRPV5 (453±45, -UMOD vs 422±43, +UMOD). Similarly, UMOD increased surface abundance of WT-TRPV5, but not N358Q mutant, supporting that UMOD upregulation of TRPV5 requires N-glycosylation. Upregulation of TRPV5 by UMOD was prevented by dominant-negative dynamin or by deletion of caveolin-1 in cells, as was the regulation by sKL. To examine the relationship between UMOD and sKL regulation of TRPV5, we showed that coexpression with sub-threshold dose of sKL or UMOD had no effect on TRPV5 whereas sub-threshold dose of sKL and UMOD in combination markedly increased its surface abundance and current density. The above finding was confirmed using application of purified UMOD and/or purified sKL to the culture medium, supporting the action of UMOD or sKL from the cell exterior. Similar to that for sKL, knockdown of galectin-1 by siRNA, but not control oligo, prevented UMOD regulation of TRPV5.

**Conclusions:** UMOD and sKL increase TRPV5 channel activity synergistically. UMOD forms polymers in urine. One potential mechanism for synergism between UMOD and sKL is stabilization and enhanced galectin-1 binding to TRPV5 on the apical membrane by UMOD, leading to decreased endocytosis of TRPV5. The effect of UMOD and sKL on TRPV5 may contribute to protection against nephrolithiasis.

*Funding:* NIDDK Support

## FR-OR120

**Mice with Kidney-Specific Deletion of the Sodium/Calcium Exchanger 1 Have Increased Intestinal Calcium Absorption** Olivier Bonny,[1,2] Candice Stoudmann,[1] Vlasta Zavadova,[1] Donald W. Hilgemann,[3] Orson W. Moe.[3] *[1]Dept of Pharmacology and Toxicology, Univ of Lausanne, Lausanne, Switzerland; [2]Service of Nephrology, Lausanne Univ Hospital (CHUV), Lausanne, Switzerland; [3]Depts of Internal Medicine and Physiology, Jane and Charles Pak Center of Mineral Metabolism, Univ of Texas Southwestern Medical Center, Dallas, TX.*

**Background:** Hypercalciuria constitutes a key risk factor in kidney stone formation and the role of the renal sodium/calcium exchanger 1 (NCX1/SLC8A1) in this process is not known. We established a mouse model in which we deleted NCX1 in the distal part of the nephron and showed that these mice are hypercalciuric, have higher urine volume and lower urine pH compared to their WT littermates. Their levels of $1,25(OH)_2$-vitamin D are elevated, but iPTH levels are normal. These mice displayed normal bone architecture at one year of age. We asked how these mice maintain calcium balance while being continuously hypercalciuric and we studied intestinal calcium absorption.

**Methods:** We used 8 wildtype (WT) littermates and 8 NCX1-kidney-specific KO (NCX1-KSKO) mice and performed $^{45}$Ca-absorption assay. The contribution of intestinal calcium load in hypercalciuria was studied by challenging the mice with a low calcium diet (0.12%) for one week and by measuring calcium balance in metabolic cages.

**Results:** We compared mRNA levels of the calcium transporters TRPV6, PMCA and NCX1 in the duodenum of NCX1-KSKO and WT mice and found increased TRPV6 expression in NCX1-KSKO mice. Then, we performed $^{45}$Ca absorption assay and found that NCX1-KSKO mice have significant increased intestinal calcium absorption (41% increase, 12 minutes after gavage). We finally exposed the mice to a low calcium diet and looked at complete calcium balance. Both WT and NCX1-KSKO mice stayed in balance even when challenged with low calcium diet.

**Conclusions:** In summary, we showed that NCX1-KSKO mice have increased intestinal calcium absorption due to higher 1,25(OH)2 vitamin D levels and are able to compensate for chronic hypercalciuria and maintain calcium balance.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## FR-OR121

**Expression of the Calcium-Sensing Receptor in Mouse, Rat and Human Kidney** Joao Z. Graca,[1,2] Martin Schepelmann,[2] Rebecca M. Wadey,[2] Sarah C. Brennan,[2] Simon Brocklehurst,[1] Daniela Riccardi,[2] Sally A. Price.[1] *[1]AstraZeneca, Macclesfield, United Kingdom; [2]Cardiff Univ, Cardiff, United Kingdom.*

**Background:** The calcium sensing receptor (CaSR), a key GPCR for calcium homeostasis, was cloned and characterized in kidney nearly two decades ago. Nevertheless, there is still no consensus in the literature regarding the renal expression pattern and discrepancies have been reported between species. This work aims to elucidate the localisation of the CaSR in mouse, rat and human kidney and thus allow a better understanding of its physiological role.

**Methods:** CaSR expression was analysed by *in situ* hybridization (ISH) using a branched-DNA methodology and by immunohistochemistry (IHC) using four antibodies raised against different CaSR epitopes [ADD (Thermo/Abcam); N-term (Anaspec); C-term (custom made, mouse/rat), whole protein (Novus, human)]. These antibodies were selected from a panel of seven antibodies by assessing CaSR immunofluorescence pattern in CaSR-HEK cells. The specificity of the selected antibodies was further confirmed by western blotting in mouse, rat and human kidney. The expression of CaSR along the nephron was assessed by dual labeling with specific markers for the descending thin limb (DTL, aquaporin 1), thick ascending limb (TAL, Tamm-Horsfall), distal tubule (DT, $Na^+$-$Cl^-$ cotransporter) and collecting duct (CD, aquaporin 2).

**Results:** By ISH, CaSR mRNA was detected in the TAL, and to a lesser extent in the DT and CD. By IHC, CaSR immunoreactivity was comparable in mouse, rat and human kidney with the four selected antibodies; generally CaSR immunoreactivity was stronger in the medulla/medullary rays and weaker in the cortex. Moreover, CaSR immunoreactivity was detected in proximal tubule (identified by the brush border), TAL, DT and CD, but not in the DTL.

**Conclusions:** Our data show that the CaSR is expressed along the nephron, including TAL, CD and DT, with the strongest expression being seen in the TAL. These findings clarify the renal CaSR expression pattern in mouse, rat and human, and therefore enable the determination of its full pathophysiological role.

*Funding:* Government Support - Non-U.S.

## FR-OR122

**Elucidation of the Distal Convoluted Tubule Transcriptome Identifies New Candidate Genes Involved in Renal Magnesium Handling** Jeroen H.F. De Baaij,[1] René J. Bindels,[1] Joost Hoenderop.[1] *[1]Dept of Physiology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Molecular Cancer Research, UMC Utrecht, Utrecht, Netherlands.*

**Background:** The kidney plays a key role in the maintenance of the magnesium ($Mg^{2+}$) homeostasis. Specifically, the distal convoluted tubule (DCT) is instrumental in fine-tuning of the renal $Mg^{2+}$ handling. In recent years hereditary $Mg^{2+}$ transport disorders have helped to identify important players in DCT $Mg^{2+}$ homeostasis. Nevertheless, several proteins involved in DCT-mediated $Mg^{2+}$ reabsorption remains to be discovered and a full expression profile of this complex nephron segment may facilitate the discovery of new $Mg^{2+}$-related genes.

**Methods:** Transgenic mice expressing eGFP under a DCT-specific parvalbumin promoter were subjected to $Mg^{2+}$-deficient or $Mg^{2+}$-enriched diets. Subsequently, the Complex Object Parametric Analyzer and Sorter (COPAS) allowed for the first time isolation of eGFP-positive DCT cells. RNA extracts thereof were analyzed by DNA microarrays comparing high vs. low $Mg^{2+}$ to identify $Mg^{2+}$ regulatory genes.

**Results:** Here, we report the $Mg^{2+}$-sensitive expression of the DCT transcriptome. Several known magnesiotropic genes, such as *Trpm6* and *Parvalbumin*, were upregulated under low dietary $Mg^{2+}$. First, examination of the $Mg^{2+}$ sensitive expression of all putative $Mg^{2+}$ transporters identified *Slc41a3* as potential $Mg^{2+}$ extrusion mechanism in the DCT. Additonally, based on statistical significance and a fold-change of at least two, 46 genes showed differential expression. Among these, *Pcbd1*, *Tbc1d4* and *Umod* were identified to be potentially involved in renal $Mg^{2+}$ handling. To confirm that the selected candidate genes were regulated by dietary $Mg^{2+}$ availability, the expression levels of *Slc41a3*, *Pcbd1*, *Tbc1d4* and *Umod* were determined by RT-PCR analysis. Indeed, all four genes show significant upregulation in the DCT of mice fed the $Mg^{2+}$-deficient diet. Furthermore, GO-term enrichment analysis identified 'EGF pathway' and 'Bone mineralization' among the enriched pathways in low $Mg^{2+}$-fed mice.

**Conclusions:** By elucidating the $Mg^{2+}$-sensitive DCT transcriptome new candidate genes in renal $Mg^{2+}$ handling have been identified.

*Funding:* Government Support - Non-U.S.

## FR-OR123

**New Missense Mutations Linked to Hypomagnesemia with Secondary Hypocalcemia** Joost Hoenderop,[1] René J. Bindels,[1] Sergio Lainez Vicente,[1] Karl P. Schlingmann,[2] Martin Konrad.[2] *[1]Physiology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands; [2]General Pediatrics, Univ Hospital Münster, Münster, Germany.*

**Background:** Despite recent progress in our understanding of renal magnesium ($Mg^{2+}$) handling, the molecular mechanisms accounting for transepithelial $Mg^{2+}$ transport are still poorly understood. Mutations in the *TRPM6* gene, encoding the epithelial $Mg^{2+}$ channel TRPM6 (transient receptor potential melastatin 6), have been proven to be the molecular cause of hypomagnesemia with secondary hypocalcemia (HSH). HSH manifests in the newborn period being characterized by very low serum $Mg^{2+}$ levels (<0.4 mmol/L) accompanied by low serum calcium ($Ca^{2+}$) concentrations. A proportion of previous described *TRPM6* mutations lead to a truncated TRPM6 protein resulting in a complete loss-of-function of the channel.

**Methods:** The study cohort comprises five families from different ethnic origin with seven individuals with HSH and at least one missense mutation leading to a non-conservative amino acid exchange in TRPM6. Human embryonic kidney (HEK) 293 overexpressing wild-type and mutant TRPM6 were analyzed by patch clamp analysis. Cell surface expression of TRPM6 was addressed by biotinylation assays.

**Results:** The aim of this study was to complement the current clinical picture by adding molecular data from six new missense mutations found in five patients with HSH. To this end, wild-type and mutated TRPM6 channels were expressed in HEK293 cells. Subsequently, patch-clamp analysis and cell surface measurements were performed to assess the effect of the various mutations on TRPM6 channel function. To determine the functional implications of the mutations on TRPM6 channel activity, HEK293 cells were transiently transfected with either mock, WT TRPM6 or the different TRPM6 mutants ($L^{708}P$, $E^{872}G$, $Y^{1053}C$, $L^{1143}P$, $Q^{1663}R$, $S^{1754}N$). All mutant channels, except $Q^{1663}R$, expressed in HEK293 cells, showed loss-of-function, while no significant trafficking impairment to the plasma membrane surface was observed.

**Conclusions:** We conclude that the new *TRPM6* missense mutations affect gating and permeation properties of the ion channel, leading to dysregulated intestinal/renal $Mg^{2+}$ (re)absorption.

*Funding:* Government Support - Non-U.S.

## FR-OR124

**Hepatectomy Hypophosphatemia: A Novel Phosphaturic Factor in the Liver-Kidney Axis** Kengo Nomura, Sawako Tatsumi, Atsumi Miyagawa, Yuji Shiozaki, Shohei Sasaki, Hiroko Segawa, Ken-Ichi Miyamoto. *Institution of Health Bioscience, The Univ of Tokushima, Japan.*

**Background:** Inorganic phosphate (Pi) absorption in the renal proximal tubules and small intestine is important for Pi homeostasis.

The incidence of liver transplantation has steadily increased, and, as a consequence, the incidence of partial hepatectomy (PH) has also increased. Marked hypophosphatemia is common after major hepatic resection, but the pathophysiologic mechanism remains unknown.

**Methods:** We used PH model rats to investigate the molecular basis of hypophosphatemia. PH rats exhibited hypophosphatemia and hyperphosphaturia.

**Results:** In renal and intestinal brush border membrane vesicles isolated from PH rats, The $Na^+$-dependent Pi (Na/Pi) uptake was decreased by 50% to 60%. PH rats also exhibited significantly decreased levels of renal and intestinal Na/Pi transporter proteins (NaPi-IIa, NaPi-IIb, and NaPi-IIc). Parathyroid hormone was elevated at 6 h after PH. Hyperphosphaturia persisted, however, even after thyroparathyroidectomy in PH rats. Moreover, DNA microarray data revealed elevated levels of nicotinamide phosphoribosyltransferase (Nampt) mRNA in the kidney after PH. Nampt catalyzes the first rate-limiting step in converting nicotinamide (NAM) to nicotinamide adenine dinucleotide (NAD). In addition, Nampt protein levels and the total NAD were significantly increased in the proximal tubules. PH rats also exhibited markedly increased levels of urinary NAM and NAM catabolites. In vitro analyses using opossum kidney cells revealed that NAM alone did not affect endogenous NaPi-IIa (NaPi-4) levels, but Nampt expression with the addition of its substrate (NAM) led to a marked decrease in the NaPi-4 protein levels. FK866, a specific Nampt inhibitor, completely blocked this reduction in Nampt-induced NaPi-4. Further, FK866-treated mice showed elevated renal Pi reabsorption and hypophosphaturia.

**Conclusions:** The findings of the present study indicate that hepatectomy-induced hypophosphatemia is due to abnormal NAM metabolism, including Nampt activation in the renal proximal tubular cells. The present study also suggests that NAM metabolism though the liver-kidney axis is important in Pi homeostasis.

*Funding:* Other U.S. Government Support

## FR-OR125

### Regulation of Renal Type IIa Sodium Phosphate Cotransporter (Npt2a) Trafficking by Na-H Exchanger Regulatory Factor 1 (NHERF1)

Eleanor D. Lederer,[1,2] Rebecca Murray,[2] Nina W. Lesousky,[2] Syed J. Khundmiri,[2] Edward J. Weinman.[3] *[1]Medical Services, Robley Rex VA Medical Center, Louisville, KY; [2]Medicine/Physiology, Univ of Louisville, Louisville, KY; [3]Medicine, Univ of Maryland, Baltimore, MD.*

**Background:** The PDZ domain protein NHERF1 plays a critical role as an anchor in the apical membrane (AM) for Npt2a, a proximal tubule transporter critical role for regulation of phosphate homeostasis. We and others have previously demonstrated that NHERF1 deficiency results in defective glycosylation and intracellular accumulationof Npt2a.

**Methods:** We hypothesized that NHERF1 associates with Npt2a prior to AM insertion and facilitates glycosylation. To test this hypothesis, we examined Npt2a-NHERF1 association in Golgi (G) by density gradient centrifugation of opossum kidney (OK) cells, a model of renal proximal tubule. Cells transfected with GFP-Npt2a wild type or C terminal –TRL deletion mutant constructs were treated with PTH to eliminate expression of surface Npt2a followed by exposure to low phosphate medium to stimulate Npt2a production for 8h. Cells were fractionated by sucrose gradient density centrifugation and the fractions representing G or plasma membrane identified by specific markers.

**Results:** Npt2a or GFP immunoprecipitation of G and PM fractions from cells expressing wild type GFP-Npt2a showed positive immunoblot for NHERF1. GFP-Npt2a lacking the terminal –TRL motif demonstrated failure to undergo complete glycosylation, associate with NHERF1 in the G, integrate into a membrane compartment, or traffic to the AM. Confocal imaging confirmed the diffuse intracellular distribution of the TRL deletion mutant.

**Conclusions:** We conclude that Npt2a associates with NHERF1 in the G through the C-terminal PDZ binding domain and that this association is essential for Npt2a glycosylation, membrane incorporation, and AM trafficking. These findings suggest a novel chaperone function for NHERF1 in Npt2a regulation.

*Funding:* Veterans Affairs Support

## FR-OR126

### Egr-1 Mediates FGF23-Induced Hypophosphatemia but Does Not Inhibit Renal 1,25 (OH)$_2$D Synthesis

Farzana Perwad, Martin Y.H. Zhang, Anthony A. Portale. *Pediatrics, Univ of California San Francisco, San Francisco, CA.*

**Background:** The transcription factor, early growth response 1 (egr-1), is a biomarker for activation of the MAP kinase/extracellular signal regulated kinase 1/2 (ERK1/2) signaling pathway by fibroblast growth factor 23 (FGF23). In *Hyp* mice, excess circulating FGF23 constitutively activates ERK1/2 signaling in the kidney, resulting in increased egr-1 expression, hypophosphatemia and 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) deficiency. These abnormalities are reversed by blockade of FGF23-dependent ERK1/2 signaling.

**Methods:** To test whether egr-1 itself mediates the renal actions of FGF23, we administered FGF23 to wild-type (WT) and egr-1 null *(egr-1$^{-/-}$)* mice. To explore the molecular mechanisms by which egr-1 mediates renal actions of FGF23, we performed chromatin-immunoprecipitation sequencing (ChIP-seq) with anti-egr-1 antibody.

**Results:** In WT mice, FGF23 induced hypophosphatemia and suppressed renal Npt2a and Npt2c mRNA expression. In FGF23-treated *egr-1$^{-/-}$* mice, hypophosphatemic effect was blocked and NPT2a/2c mRNA were not suppressed. In contrast, FGF23 induced equivalent suppression of renal Cyp27b1 mRNA and stimulation of Cyp24a1 mRNA expression in both WT and *egr-1$^{-/-}$* mice. Thus, downstream of FGF receptor binding and ERK1/2 signaling, we can distinguish the effector pathway that mediates inhibition of renal Pi reabsorption from the pathway that mediates inhibition of 1,25(OH)$_2$D synthesis. In kidneys from FGF23-treated WT mice, we demonstrate rapid binding of egr-1 to regulatory DNA elements in proximity to genes that encode key participants in renal Pi cotransport, specifically: renal Na/Pi cotransporters, Npt2a and Npt2c, scaffolding proteins (Nherf-1, Nherf-2, Ezrin, GABARAP and pdzk1IP), and vesicle trafficking proteins (Rab37 and sytl3). *In silico* gene network analyses identified the following significant functional pathways in the egr-1 ChIP-seq dataset; cytoskeleton remodeling, TGF/WNT signaling and FGFR signaling pathways.

**Conclusions:** These data suggest that the phosphaturic effect of FGF23 is mediated, atleast in part, by activation of the transcription factor, egr-1. We have thus identified the FGF23-dependent egr-1 cistrome in the kidney.

## FR-OR127

### PTH Increases FGF23 Transcription by Activating the Orphan Nuclear Receptor Nurr1 Both In Vitro and in Experimental CKD and This Is Prevented by the Calcimimetic R568

Tomer Meir,[1] Karina Durlacher,[1] Oded Volovelsky,[1] Gili Cohen,[1] William G. Richards,[2] Justin Silver,[1] Tally Naveh-Many.[1] *[1]Nephrology, Hadassah Hospital, Jerusalem, Israel; [2]Amgen, Thousand Oaks, CA.*

**Background:** PTH increases FGF23 mRNA and protein levels in vivo and in vitro. In experimental CKD parathyroidectomy both prevents and corrects the high levels of FGF23. We report that the effect of PTH to increase FGF23 is mediated by the orphan nuclear receptor Nurr1.

**Methods:** Adenine high Pi induced CKD rats were given the calcimimetic R568 to study calvaria FGF23 and Nurr1 mRNA and protein levels. UMR-106 rat osteoblast-like cells were incubated with PTH and transfected with Nurr1 expression plasmid to study the effect on endogenous FGF23 mRNA levels and on FGF23 promoter-luciferase expression. Chromatin immunoprecipitation (CHIP) using a Nurr1 antibody was performed on cell lysates.

**Results:** Nurr1 mRNA and protein levels were increased in calvaria from CKD rats together with the high PTH and FGF23 levels. Calcimimetics decrease PTH and FGF23 levels in CKD. Importantly, in CKD rats the calcimimetic R568 decreased PTH expression, calvaria Nurr1 mRNA and protein levels and FGF23 mRNA, suggesting that the decrease in Nurr1 by the calcimimetic mediates the decrease in FGF23. Nurr1 binds to cis-acting sequences in the promoter of its target genes and regulates their transcription. In UMR-106 cells PTH increased FGF23 transcription. Significantly, PTH increased Nurr1 mRNA levels at 30 min, before the increase in FGF23 mRNA levels. Nurr1 over-expression increased FGF23 mRNA levels also in the absence of PTH. Reporter gene experiments using different fragments of the FGF23 promoter showed that PTH and/or Nurr1 over-expression increased FGF23 promoter activity. Bioinformatic analysis identified several Nurr1 potential binding sites in the FGF23 promoter. The functionality of these response elements was demonstrated by FGF23 promoter-luciferase assays. CHIP analysis confirmed the binding of Nurr1 to the functional regions in the FGF23 promoter.

**Conclusions:** PTH increases Nurr1 to induce FGF23 transcription. In SHP the decrease in FGF23 by a calcimimetic is mediated by decreased PTH and Nurr1 levels.

*Funding:* Pharmaceutical Company Support - Amgen

## FR-OR128

### Phosphate Transport-Dependent and -Independent Functions of the Sodium Dependent Phosphate Transporter, PiT-1, in Vascular Smooth Muscle Cell Mineralization

Nicholas W. Chavkin, Matthew H. Crouthamel, Cecilia M. Giachelli. *Bioengineering, Univ of Washington, Seattle, WA.*

**Background:** Elevated serum phosphate is associated with increased vascular calcification in patients with chronic kidney disease. *In vitro*, elevated phosphate induces vascular smooth muscle cell (VSMC) mineralization, and this can be blocked by knocking down PiT-1. In this study, we aimed to determine the contribution of phosphate transport-dependent and-independent functions of PiT-1 on VSMC mineralization.

**Methods:** Wildtype (WT) and PiT-1 mutants containing three different point mutations of amino acids proposed to be required for phosphate uptake (E74K, S132A, and S623A) were generated and stably expressed in PiT-1 deficient mouse VSMCs derived from PiT-1 fl/fl SM22α Cre transgenic mice. Cell lines were characterized for gene and protein expression by Q-PCR and immunocytochemistry. Sodium-dependent phosphate uptake, ERK1/2 phosphorylation, and VSMC calcification were analyzed using published analytical methods.

**Results:** WT and mutant PiT-1 mRNAs were similarly expressed, and protein localized to cell membranes in transduced VSMCs. VSMCs containing the PiT-1 mutations had reduced sodium-dependent phosphate uptake compared with those containing WT PiT-1, with S132A and S623A having intermediate levels and E74K exhibiting the lowest levels. Phosphate-induced ERK1/2 phosphorylation was elevated to a similar extent in PiT-1 WT and E74K expressing VSMCs compared to vector control. SM22α mRNA levels were decreased to a similar extent in PiT-1 WT and E74K VSMCs compared to vector control. Phosphate-induced matrix mineralization was much higher in VSMC expressing WT PiT-1 compared to E74K, and extent of matrix mineralization correlated with transport uptake activity.

**Conclusions:** ERK1/2 phosphorylation and loss of SM22α expression are phosphate uptake-independent activities of PiT-1, whereas stimulation of VSMC matrix mineralization by PiT-1 is predominantly phosphate uptake-dependent. Together, the data suggest that both phosphate uptake-dependent and -independent functions of PiT-1 contribute to processes important for matrix mineralization.

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## FR-OR129

**Genetic Loss of Function Mutations in Zebrafish and Human CRB2, a Regulator of Epithelial Polarity, Are Associated with Podocyte Foot Process Effacement and Focal Segmental Glomerulosclerosis** Arindam Majumdar,[1] Shazia Ashraf,[2] Svjetlana Lovric,[2] Heon Yung Gee,[2] Friedhelm Hildebrandt.[2] *[1]Immunology, Genetics, and Pathology, Uppsala Univ, Uppsala, Sweden; [2]Div of Nephrology, Harvard Medical School, Boston Children's Hospital, Boston, MA.*

**Background:** Podocytes are polarized epithelial cells, a central component of the glomerular filtration barrier, and a major pathological target in kidney glomerular disease. In published work, we identified a requirement for zebrafish *crb2b*, a conserved regulator of epithelial polarity, in podocyte morphogenesis. The Crb proteins are EGF-like domain transmembrane proteins which lie at the intersection of epithelial polarity, cell signaling, and membrane biogenesis.

**Methods:** We study podocyte polarity in zebrafish *crb2b* mutants and we ask whether the CRB2 gene may be affected in human nephrotic syndromes.

**Results:** We present a null allele in zebrafish, *crb2b*$^{zm}$, which phenocopies our published morpholino results and independently confirms a role for *crb2b* in podocyte differentiation. In the absence of *crb2b*, Nephrin protein is mis-targeted to apical membranes. Biochemically, co-immunoprecipitation shows that Crb2b associates with Nephrin. Both Crb2b and Nephrin co-localize to vesicles in transient cell culture transfection assays.

We than extended our zebrafish Crb2 studies into nephrotic syndrome in humans. Podocyte Crb2 expression is conserved from zebrafish to humans. A combination of SNP homozygosity mapping and exome re-sequencing were used to screen for mutations in human CRB2 FSGS families. Analysis of the CRB2 sequence in 30 consanguinous sibling pairs identified a homozygous 1859 G > C missense mutation resulting in Cys 620 > Ser. The conserved Cys residue lies in an extracellular EGF like-repeat. The functional consequence of this mutation was tested in zebrafish *crb2b* mutants.

**Conclusions:** Our genetic analyses in both zebrafish and humans indicate a requirement for Crb2 function in podocyte cell structure and function. These results suggest that some nephrotic syndromes may be due to fundamental misregulation of podocyte apical basal polarity.

*Funding:* Government Support - Non-U.S.

## FR-OR130

**Gene-Gene Interactions in APOL1-Associated Nephropathy** Jasmin Divers, Nicholette D. Palmer, Lingyi Lu, Carl D. Langefeld, Michael V. Rocco, Pamela J. Hicks, Mariana Murea, Lijun Ma, Donald W. Bowden, Barry I. Freedman. *Wake Forest School of Medicine, Winston-Salem, NC.*

**Background:** Two APOL1 nephropathy variants confer risk for non-diabetic end-stage kidney disease (ESKD) in African Americans (AAs). Modifying factors likely contribute as not all genetically high-risk individuals develop ESKD.

**Methods:** We tested variants from a prior discovery GWAS aimed at detecting single nucleotide polymorphisms (SNPs) that potentially interact with *APOL1* to alter ESKD risk in 864 AA cases with non-diabetic ESKD and 612 non-nephropathy controls. The top 42 discovery interactive SNPs were re-examined in DNA from two new AA cohorts: 503 additional unrelated non-diabetic ESKD cases and 892 non-nephropathy controls (combined discovery + replication sample had 1,367 ESKD cases and 1,504 controls) and an independent family-based cohort including 608 1st degree relatives of non-diabetic ESKD probands. Logistic regression and mixed models were fitted to test for interaction effects with APOL1 on risk of ESKD and albuminuria/eGFR in families.

**Results:** Fourteen SNPs replicated *APOL1-interaction with ESKD in the same direction as the discovery GWAS analysis with interaction p-values <0.05 in combined replication + discovery ESKD samples. The strongest interactions were rs16854341 in podocin (NPHS2, odds ratio [OR] 0.5; p=5.0x10-4), rs2802723 in serologically defined colon cancer antigen 8 (SDCCAG8, OR 1.8; p=5.0x10-4), rs9533534 (ENOX, OR 0.7; p=8.0x10-4), and rs8014363 (near BMP4, OR 1.6; p=1.0x10-3).* Several SNPs demonstrated the same directions of *APOL1 interaction with nephropathy in family-based samples, including NPHS2 and SDCCAG8 for albuminuria (p=0.05 and 0.03, respectively).*

**Conclusions:** Variants in NPHS2 and SDCCAG8, and SNPs in other regions appear to interact with APOL1 to modulate risk for non-diabetic ESKD.

*Funding:* NIDDK Support

## FR-OR131

**Whole Exome Sequencing (WES) for Disease Gene Discovery in Familial IgA Nephropathy** Xuewen Song,[1] Nicole M. Roslin,[2] Bushra Joarder,[1] Meng Yi Xu,[1] Amirreza Haghighi,[1] Melody Ren,[1] Mitchell Li Cheong Man,[1] Andrew D. Paterson,[2] York P. Pei.[1] *[1]Div of Nephrology, Univ Health Network, Toronto, Canada; [2]Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Canada.*

**Background:** Multiple rare disease genes with high effect size have been implicated by genome-wide linkage analysis of familial IgAN (fIgAN) but none has been identified.

**Methods:** To identify disease genes for fIgAN, we performed WES using AB Solid 5500xL platform in 21 patients from 15 well-characterized families (each with at least one biopsy-proven case and one or more affected individuals with persistent hematuria/proteinuria). For 2 multiplex families each with 5 affected, we also performed genome-wide linkage scans and focused WES on regions of positive linkage signals. Standard algorithms for sequence alignment, base calling, and QC filtering were applied to identify rare putative deleterious variants of high and moderate impact as predicted by PolyPhen-2, Sift, and Provean.

**Results:** On average, our exome sequencing provided coverage for 99.8% of all the exons with a mean depth of 100X. We cataloged 19 regions with LOD >1 under a dominant model from our two moderately large families. From these studies, we identified a total of 24 rare deleterious variants from the following candidate disease genes: ITGA4, TNFRSF6B, CD81, FASLG, ITGB2, TNIP1, MSH6, MLH6, LIG4, LCP1, KIAA1704, TMEM52, TFRC, FCAR, DFFA4, OSM, MPDU1, CD68, CFH, and CFHR3. Five of these variants affected two or more independent families.

**Conclusions:** Our preliminary results are consistent with the notion of an underlying genetic heterogeneity in fIgAN with the possibility of multiple disease genes each only accounting for a small proportion of cases. To validate our findings, we are currently conducting exome sequencing in 12 more unrelated families. Future studies to identify deleterious mutations from the same candidate gene in multiple unrelated families as well as within-family segregation analysis will help to narrow a short list of most promising candidate disease genes for functional studies. Identification of disease genes for fIgAN has the potential to improve diagnosis and treatment.

## FR-OR132

**A CFHR2-CFHR5 Hybrid Protein Causes Complement Deregulation and Reveals a Novel Pathogenic Mechanism for Dense Deposit Disease** Christine Skerka,[1] Qian Chen,[1] Michael Sean Wiesener,[2] Hannes Uwe Eberhardt,[1] Kerstin U. Amann,[2] Maike Julia Buettner,[2] Tim Goodship,[3] Christian Hugo,[4] Peter F. Zipfel.[1] *[1]Leibniz Institute for Natural Product Research and Infection Biology; [2]Univ Erlangen-Nuremberg; [3]Univ Newcastle upon Thyne; [4]Univ Hospital Dresden.*

**Background:** Dense Deposit Disease (DDD) is a severe renal disorder that is caused by complement dysregulation. There is no effective treatment and patients often progress to end-stage renal failure. For a small fraction of DDD patient's genetic causes in form of *Factor H-* or *C3* gene mutations have been reported.

**Methods:** Gene copy number variations, genetic breakpoint amplification, gene sequencing. Complement activation assays, immune precipitation, recombinant expression.

**Results:** Genetic analysis of two DDD patients identified a chromosomal deletion in the *CFHR* gene cluster leading to the expression of a hybrid CFHR2-CFHR5 plasma protein, that has domains 1-2 (SCRs1-2) of CFHR2 fused to SCRs1-9 of CFHR5. The recombinant and the native hybrid protein stabilized the complement C3 convertase and also reduced Factor H mediated decay of the C3 convertase. Thus explaining why plasma addition to the patient *in vivo* enhanced complement activation and why the patient was refractory to plasma therapy. I*n vitro* assays performed with diverse complement inhibitors and C3 and Factor B supplemented serum of the patient revealed that the soluble complement inhibitor sCR1 was able to neutralize the C3 convertase stabilizing effect of the hybrid protein, but neither a tagged version of Compstatin nor the established C5 convertase inhibitor Eculizumab did restore the defective regulation of the C3 convertase.

**Conclusions:** Our findings provide first evidence that chromosomal rearrangements in the *CFHR* gene cluster leads to hybrid CFHR proteins that act as activators of the C3 convertase leading to DDD. Thus monitoring sequence variations in *CFHR2* and *CFHR5* genes is recommended for patients with DDD. Because of the permanently active C3 onvertase plasma infusion therapy is critical for these patients.

*Funding:* Government Support - Non-U.S.

## FR-OR133

**Genomic Disorders in the Chronic Kidney Disease in Children (CKiD) Cohort** Miguel Verbitsky,[1] Simone Sanna-Cherchi,[1] Krzysztof Kiryluk,[1] Brittany J. Perry,[1] Frederick J. Kaskel,[2] Susan L. Furth,[3] Bradley A. Warady,[4] Craig S. Wong,[5] Ali G. Gharavi.[1] *[1]Nephrology, Columbia Univ, New York, NY; [2]Pediatric Nephrology, Children's Hospital at Montefiore, Bronx, NY; [3]Pediatric Nephrology, Children's Hospital of Philadelphia, Philadelphia, PA; [4]Pediatric Nephrology, Children's Mercy Hospital, Kansas City, MO; [5]Pediatric Nephrology, Univ of New Mexico, Albuquerque, NM.*

**Background:** Previous studies reported a high prevalence of pathogenic copy number variants (CNVs) in children with Renal Hypodysplasia (RHD). We followed up these data in the Chronic Kidney Disease in Children (CKiD) cohort, an observational study of pediatric nephropathy.

**Methods:** PennCNV was used to determine CNV calls in 420 CKiD samples genotyped on the Illumina Omni2.5 beadarrays (N=420). To identify rare pathogenic CNVs, we compared data to 23,362 controls.

**Results:** Participants were phenotypically classified into Congenital Abnormalities of the Kidney and Urinary Tract (CAKUT; N=216), which includes Renal Hypodysplasia (RHD=69) and non-CAKUT (N=204, e.g. glomerular disorders). CNVs were diagnostic of 12 known syndromes in 15 individuals (3.6%): 10 (4.6%) in CAKUT and 5 (2.5%) in non-CAKUT participants, compared to 318 (1.4%) controls. Recurrent CNVs included 17q12 (*HNF1B*) and 16p11.2 deletions. Additionally, 20 large, likely pathogenic CNVs were identified in another 19 children (4.5% of cohort): 9 (4.2%) in CAKUT, and 10 in (4.9%) non-CAKUT. Genomic imbalances were most common in RHD patients (10.1% with known and 4.3% with likely pathogenic CNVs). In several cases, the microarray data indicated an alternative renal diagnosis.

**Conclusions:** As significant number of children with CKD have an unsuspected genomic imbalance, particularly those with RHD. Moreover, most of the identified genomic disorders have a known association with neurodevelopmental phenotypes. The diagnosis of CNV disorders has clinical implication for risk stratification, prediction of complications and personalized management of pediatric patients with CKD.

*Funding:* NIDDK Support

## FR-OR134

**Mutations in a Large VNTR of MUC1 Are Frequent in Autosomal Dominant Medullary Cystic Kidney Disease (MCKD)** Vincent Moriniere,[1] Simon Hollebecque,[1] Said Lebbah,[2] Andre Megarbane,[7] Andreas Gnirke,[3] Brendan Blumenstiel,[3] Anthony J. Bleyer,[5] Andrew Kirby,[6] Bertrand Knebelmann,[2] Corinne Antignac.[4] *[1]Genetic Dept, Necker Hospital, Paris, France; [2]Nephrology Dept, Necker Hospital, Paris, France; [3]Broad Institute, Cambridge, MA; [4]INSERM U983, Univ Paris Descartes, Paris, France; [5]Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; [6]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA; [7]Genetic Medical Unit, Saint Joseph Univ, Beirut, Lebanon.*

**Background:** MCKD is a rare autosomal dominant tubulo-interstitial kidney disease, sometimes associated with renal cysts and gout/hyperuricemia. UMOD mutations have been described in patients with hyperuricemia (MCKD2). Mutations in the MUC1 gene encoding mucin 1 were very recently identified in MCKD1 patients (Kirby et al, Nature Genet, 2013) using a sophisticated approach associating cloning of the genomic region, capillary sequencing and de novo assembly since next generation sequencing failed to identify them. All patients harbor a single cytosine insertion in one copy of the 60-base repeat unit of the long CG-rich coding VNTR of MUC1. The frameshift is predicted to result in a mutant protein which is expressed in the distal tubule and collecting duct.

**Methods:** Using an approach coupling PCR amplification of patient DNA cleaved by MwoI (that selectively cleaves the unmutated repeat sequence) and probe-extension assay (SNAPshot), we tested 26 new families.

**Results:** We detected the presence of the cytosine insertion in 14/26 families. In addition, we detected a new 5pb deletion, which is predicted to result in the same mutant protein as the cytosine deletion. Among 14/15 families with mutations, 101 individuals were affected, with detailed clinical information available for 75 patients. 58 reached ESRD at a mean age of 46 yrs (20-82 yrs) with high intrafamilial variability. Early gout occurred in only 1 case and hyperuricemia in 3 (2 from the same family). Renal cysts were present in 10/40 pts.

**Conclusions:** These results show that MUC1 mutations represent a frequent cause of MCKD.

## FR-OR135

**Functional Annotation of Single Nucleotide Polymorphisms Associated with Chronic Kidney Disease** Yi-An Ko, Katalin Susztak. *Renal Electrolyte and Hypertension Div, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** The eukaryotic transcription unit includes the promoter enhancer interaction, which is essential for transcription. Enhancers can be upstream or downstream of the transcription start site. What separate the transcript units are the insulators. These are the important regulatory elements in the genome. Each regulatory element is associated with certain specific histone tail modifications. Epigenetic modifications are characterized by their cell type specificity. We used different combination of histone modifications ChIP-seq data to annotate the single nucleotide polymorphisms (SNPs) that are associated with kidney function and chronic kidney disease (CKD).

**Methods:** The human kidney epithelial cell epigenome was generated from ChIP-seq using H3K4me1/2/3, H3K9me3, H3K27ac, H3K27me3, and H3K36me3 to targetthe human kidney epithelial cells (HKC8). The sequencing results were analyzed with the algorithm *ChromHMM*. The annotated human kidney epigenome map with annotated regulatory elements were used for mapping CKD associated SNPs (n=37) published in three different studies.

**Results:** Kidney specific epigenome was generated from HKC8 cell ChIP-seq. The outputs of the *ChromHMM* were states and patterns of regulatory elements, which are enhancers, promoters, transcribed regions, insulators, and repressed regions. Next, we compared the SNPs annotated with the kidney regulatory elements to nine cell types published in ENCODE project. The results revealed that CKD associated SNPs have strong cell type specificity enriched in human kidney enhancers. Furthermore, to exclude the possibility that all the SNPs are enriched in kidney specific enhancers, we used 36 control SNPs from unassociated studies. These SNPs did not show cell type specific gene regulatory region pattern.

**Conclusions:** CKD SNPs are enriched in the kidney specific gene regulatory regions, especially in the enhancers. This approach can potentially be used for prioritizing SNPs found in genome-wide association studies.

*Funding:* NIDDK Support

## FR-OR136

**The 4D Study: 11 Years of Follow-Up, Effects on Mortality and Morbidity of 4 Years Atorvastatin Treatment in 1255 Patients with Diabetes Mellitus on Hemodialysis, a Randomized, Controlled Trial** Vera Krane,[1] Kay-Renke Schmidt,[1] Lena Johanna Gutjahr-Lengsfeld,[1] Britta Stahl,[1] Monika Mehling,[1] Susanne Kempf,[1] Winfried März,[2] Christoph Wanner.[1] *[1]Dept of Medicine I, Div of Nephrology, Univ Hospital, Univ of Würzburg, Würzburg, Germany; [2]Synlab Academy, Synlab Services GmbH, Mannheim, Germany.*

**Background:** Findings of large randomized trials of lowering LDL-cholesterol in dialysis patients have shown controversial results, limited evidence exists about the long-term effects. The aim of this follow-up of the German Diabetes Dialysis Study (4D Study) is to assess long-term effects of atorvastatin treatment in hemodialysis (HD) patients.

**Methods:** 1255 patients with type 2 diabetes mellitus on HD were randomly allocated to either 20 mg atorvastatin daily or placebo, median follow-up was 4 years. Post trial statin treatment was at the discretion of the patients´ nephrologists. The primary outcome of the 4D study was a composite of death from cardiac causes, nonfatal myocardial infarction (MI) and stroke. Analysis was by intention to treat.

**Results:** In and post-trial follow-up yielded a mean duration of 11.5 years in both groups. Overall, 90% of patients died. Post-trial statin use (51% and 48%) and LDL levels were similar in both groups. Allocation to atorvastatin during the in-trial period yielded an average reduction in LDL cholesterol of 42%. Over the entire time period, a proportional non-significant 9% decrease in the combined endpoint of cardiac death, MI and stroke (95% CI, 0.78 to 1.07) was detected. However, the risk of all cardiovascular events combined was significanty reduced (RR 0.83, 95% CI 0.70-0.97) whereas the incidence of all cerebrovascular events combined did not differ between groups (RR 0.94, 95% CI 0.72-1.23). No significant effect of atorvastatin on fatal infection (RR 0.97, 95% CI, 0.76-1.23), or all-cause death (RR 0.93, 95% CI 0.82-1.04) was detected. No case of rhabdomyolysis was reported.

**Conclusions:** After 4 years of atorvastatin treatment, no significant effect on the 11 year endpoint of cardiac death, MI and stroke was detected. However, there was a significant 17% relative risk reduction in all cardiovascular events combined and no evidence of emerging hazards.

*Funding:* Government Support - Non-U.S.

## FR-OR137

**Unchanged Heart Function and Blood Pressure after Cholecalciferol Treatment–A Randomized Controlled Trial** Frank H. Mose,[1] Henrik Vase,[3] Thomas Larsen,[1] Anne Sophie Pinholt Kancir,[1] Renata Kosierkiewicz,[2] Bartlomiej B.J. Jonczy,[2] Anna Ewa Oczachowska-Kulik,[2] Ingrid Moeller Thomsen,[2] Jesper N. Bech,[1,2] Erling B. Pedersen.[1,2] *[1]Dept of Medical Research, Holstebro Hospital and Aarhus Univ, Denmark; [2]Dept of Medicine, Holstebro Hospital, Denmark; [3]Dept of Cardiology, Aarhus Univ Hospital, Denmark.*

**Background:** In dialysis patients vit-D3 improved biochemical endpoints, but the effect of vit-D3 supplementation on cardiovascular function and BP is unknown. The aim of this study was to test the hypothesis that vit-D3 supplementation improves cardiac function and reduces BP and pulse wave velocity (PWV) in patients on chronic dialysis.

**Methods:** In a randomized, placebo-controlled, double-blind study, we investigated the effect of 75 µg vit-D3 per day for 6 months, in patients on chronic dialysis. We performed two-dimensional echocardiography, with doppler and tissue-doppler imaging, 24-h ambulatory BP (24-h BP), PWV, augmentation index (AIx), central BP (cBP) and brain natriuretic peptide (BNP) at baseline and after 6 months.

**Results:** Sixty-four patients were allocated to the study, and 50 patients (42 on hemodialysis and 8 on peritoneal dialysis) completed the trial (25 in vit-D3 and 25 in placebo group). Mean age 68 years (range 46 to 88), 32 were men, BMI 23.9±4.4 kg/m$^2$ and baseline plasma 25-hydroxyvitamin D (p-25[OH]D) 28 (29;53) nmol/l. Age, sex, BMI and p-25(OH)D were not significantly different between groups at baseline. After 6 months, Vit-D3 had no impact on left atrial or ventricular structure. LV systolic and diastolic function was not changed by vit-D3. 24-hour BP decreased significantly in placebo group and in-significantly in vit-D3 group, but with no difference between treatments (p=0.314 for systolic BP and p=0.344 for diastolic BP). PWV, cBP, Aix and BNP were not changed in placebo or vit-D3 group at follow-up. P-25(OH)D increased significantly compared to placebo (88 (67;125) vs. 34 (21;56), p<0.001).

**Conclusions:** In conclusion, 6 months vit-D3 treatment in patient on chronic dialysis did not improve 24-h BP, arterial stiffness or cardiac function.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## FR-OR138

**Warfarin Initiation after Newly-Diagnosed Atrial Fibrillation Associates with Better Outcomes in U.S. Patients on Hemodialysis** Jenny I. Shen, Maria E. Montez-Rath, Tara I. Chang, Wolfgang C. Winkelmayer. *Div of Nephrology, Stanford School of Medicine, Palo Alto, CA.*

**Background:** Atrial fibrillation (AF) is increasingly common in patients on hemodialysis (HD). Although warfarin is indicated to prevent ischemic strokes in most patients with AF, evidence for its use in HD patients is limited and conflicting. In this observational cohort study, we examined the net safety of initiating warfarin for incident AF in a large, nationally-representative cohort of patients on chronic HD.

**Methods:** We identified from the USRDS all adult HD patients who were newly diagnosed with AF during a hospitalization from 2007-10, who participated in the low income subsidy program of Medicare Part D, and who had no history of warfarin use in ≥6 months prior to admission. Patients who filled a first prescription for warfarin within 30 days of discharge were considered users; all others were non-users. We excluded patients who died within 30 days of discharge. We applied Cox regression to a propensity-score matched cohort of warfarin users and non-users to estimate the hazard ratios (HR) for all-cause mortality and the combined outcome of gastrointestinal bleeding (GIB), any stroke, or death.

**Results:** Among 9316 patients, 1330 (14%) initiated warfarin. We matched 1329 warfarin users to 1329 non-users whose observed baseline characteristics were exquisitely balanced. Over 3163 person-years of follow-up, we observed 1083 deaths, 109 GIB, and 149 strokes. Warfarin initiation was associated with a decreased risk of both all-cause mortality [HR 0.85; 95% confidence interval (CI): 0.75-0.95] and the composite outcome (HR: 0.87, 95% CI: 0.78-0.98).

**Conclusions:** In HD patients with hospitalized incident AF, warfarin use was associated with a decreased risk of both all-cause mortality and a composite outcome of GIB, any stroke, and death, indicating net benefits of warfarin use in this indication. This is at odds with several previous studies that have suggested an increased risk of adverse outcomes in users. Given the uncertainty surrounding warfarin for AF in HD patients, further clinical trials are warranted.

*Funding:* NIDDK Support

## FR-OR139

### Irbesartan Shows No Beneficial Blood Pressure Independent Effects on Intermediate Cardiovascular Risk Markers in Hemodialysis Patients

Christian D. Peters,[1] Krista D. Kjaergaard,[1] J Dam Jensen,[1] Kent L. Christensen,[2] Charlotte Strandhave,[3] I. N. Tietze,[4] Marija K. Novosel,[5] Bo M. Bibby,[6] Erik Sloth,[7] Bente Jespersen.[1] *[1]Dept of Renal Medicine, Aarhus Univ Hospital (AUH), Denmark; [2]Dept of Cardiology, AUH, DK; [3]Dept of Nephrology, Aalborg Univ Hospital, DK; [4]Dept of Medicine, Region Hospital Viborg, DK; [5]Dept of Medicine, Fredericia Hospital, DK; [6]Dept of Biostatistics, Aarhus Univ, DK; [7]I-Research, AUH, DK.*

**Background:** Angiotensin II receptor blockers are frequently used in end-stage renal disease patients but it is unclear if they exert beneficial cardiovascular (CV) effects beyond blood pressure (BP) reduction. The aim of this study was to determine whether irbesartan improves well-established intermediate CV risk markers in hemodialysis (HD) patients.

**Methods:** A multicenter randomized placebo-controlled double-blinded trial initiated by the investigators with 1 year follow-up. A pre-defined systolic BP (SBP) target of 140 mmHg was used in all patients. ClinicalTrials ID: NCT00791830.

**Results:** Of the 82 patients randomized (placebo n=41/irbesartan n=41), 56 completed 1 year. Baseline mean±SD: Males 26/30, age 62±14/61±16 years, SBP 145±19/148±21 mmHg, HD vintage 168±95/171±93 days, HD time 10±2/11±3 hr/week, urine output 1.3±0.7/1.5±0.8 L/24 h. Comorbidity, antihypertensive drugs and HD parameters were comparable at baseline. Brachial SBP decreased significantly during the study but there was no significant difference between the groups (p=0.418). Mean decrease in SBP (baseline-1 year) was 8.2±4.1 mmHg, p=0.04 (placebo) and 10.0±4.3, p=0.02 (irbesartan). Use of additional antihypertensive drugs, ultrafiltration volume and HD dosage were similar during follow-up. Intermediate CV risk markers such as central aortic BP, carotid-femoral pulse wave velocity, left ventricular (LV) mass index, NT-proBNP, heart rate variability and plasma catecholamines were not significantly affected by irbesartan. Changes in SBP during the study period correlated significantly with changes in LV mass index (p=0.02) and arterial stiffness (p<0.001).

**Conclusions:** Effects of irbesartan on intermediate CV endpoints beyond BP reduction are absent in HD patients.

*Funding:* Pharmaceutical Company Support – Sanofi; NorDiaTech, Private Foundation Support, Government Support - Non-U.S.

## FR-OR140

### Association of Intradialytic Hypotension (IDH) with Interdialytic Weight Gain (IDWG) and Cardiovascular (CV) Disease

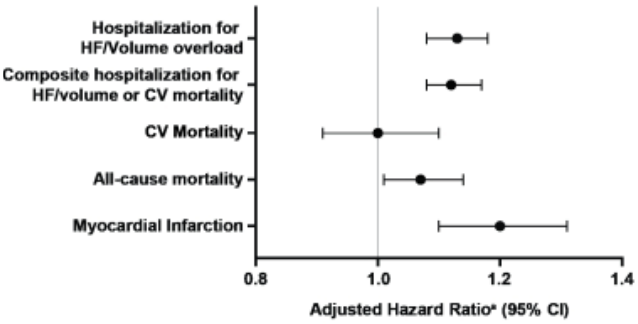
Claudia S. Cabrera,[1] Steven M. Brunelli,[2] David P. Rosenbaum,[3] Emmanuel A. Anum,[2] Karthik Ramakrishnan,[2] Donna E. Jensen,[2] Nils-Olov Stalhammar,[1] Bergur V. Stefansson.[1] *[1]AstraZeneca, Molndal, Sweden; [2]DaVita Clinical Research, Minneapolis, MN; [3]Ardelyx Inc, Fremont, CA.*

**Background:** Patients undergoing hemodialysis (HD) have elevated risk of CV disease-related morbidity and mortality compared to the general population. This burden may relate in part to episodic tissue hypoxia resulting from IDH. The aims of this study were 1) to estimate the associations of IDH with CV events/mortality and 2) to determine whether patients with greater IDWG (who require more ultrafiltration) are predisposed to IDH.

**Methods:** Patients incident to in-center HD (1 Jan 07–31 Dec 08) who remained on this modality for ≥181 days and had Medicare or Medicaid as primary insurer were assessed. IDWG and IDH were assessed over HD days 91-180. Outcomes were identified through US Renal Data Systems claims data, and were considered as those occurring on/after dialysis day 181 until death, care transfer, modality change or study end (31 Dec 09). IDH was defined as fall in systolic blood pressure of >20 mmHg from pre-dialysis to nadir intradialytic levels with at least 2 responsive measures (reduced ultrafiltration rate, reduced HD blood flow rate, saline administration, premature cessation of dialysis).

**Results:** The study included 39,497 patients; 35.7% of patients experienced IDH at least once during the baseline period. Cross-sectional analysis demonstrated a potent and statistically significant association between IDH and IDWG (p<0.001). IDH was independently associated with increased risk of heart failure (HF), composite HF/CV mortality, all-cause mortality and myocardial infarction.

**Adjusted Associations of Intradialytic Hypotension with Incident Clinical Outcomes**
(reference = IDH absent)




Abbreviations: CI, confidence intervals; CV, cardiovascular; HF, heart failure; IDH, intradialytic hypotension

*Hazard ratios were adjusted for age, race, sex, etiology of ESRD, prior renal transplant, access type, and baseline diabetes, uncontrolled hypertension, heart failure, myocardial infarction, atrial fibrillation, and ischemic/hemorrhagic stroke or transient ischemic attack.

**Conclusions:** These data show a significant association between IDWG and IDH; IDH was independently associated with CV events. Mitigating IDWG may reduce IDH and thereby improve CV outcomes in ESRD patients.

*Funding:* Pharmaceutical Company Support - Ardelyx, Inc

## FR-OR141

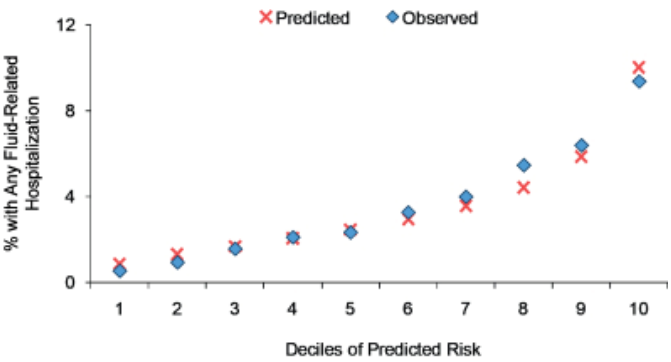
### Fluid Overload in Hemodialysis (HD) Patients: Development and Validation of a 'Big Data' Predictive Analytic

Steven M. Brunelli,[1] Karthik Ramakrishnan,[1] Scott Sibbel,[2] Irina Goykhman,[3] Chhaya B. Patel,[3] Robert Provenzano,[3] Stephen D. McMurray,[3] David B. Van Wyck,[3] Allen R. Nissenson,[3] Mahesh Krishnan.[1] *[1]DaVita Clinical Research, MN; [2]DaVita Labs, FL; [3]DaVita HealthCare Partners Inc, CO.*

**Background:** Fluid management is poor for many HD patients, with consequences that include increased morbidity, mortality, and utilization of healthcare resources. The ability to prospectively identify patients at high risk of imminent fluid-related hospitalization (FRH) would enable targeted implementation of avoidance strategies, and the more efficient allocation of resources. The aim of this study was to derive an empirical clinical risk algorithm using available clinical data to predict risk of FRH in the subsequent 3 months.

**Methods:** Demographic and clinical data were abstracted from the DaVita Clinical Data Warehouse. FRH events were identified from Centers for Medicare and Medicaid Services claims data. In the derivation set, predictor variables were considered as of Sept 2009 and used to predict risk of FRH in Q4 2009. In the validation set, predictors were considered as of June 2009; predicted and observed Q3 2009 FRH were compared.

**Results:** The derivation set contained 60,114 patients; the validation set contained 57,245 patients. Sixty-seven variables were identified and included in the model (C-statistic, 0.71). In the validation set, the model demonstrated excellent calibration over the 20-fold range of risk observed; patients in the highest decile of predicted risk demonstrated more than 3-fold greater risk of observed FRH (risk ratio, 3.2 [CI: 2.9, 3.7]; rate ratio, 3.4 [CI: 3.1, 3.8]).




**Conclusions:** The algorithm accurately and reproducibly predicts risk of FRH and can be tailored to accommodate more or less restrictive definitions of risk. Use of the algorithm will permit identification of high risk patients in whom to target interventions, and allow for the judicious allocation of resources.

*Funding:* Pharmaceutical Company Support - DaVita HealthCare Partners Inc

## FR-OR142

**Plasma Long-Chain Acylcarnitines Predict Cardiovascular Mortality in Incident Dialysis Patients** Sahir Kalim,[1] Clary B. Clish,[2] Julia Beth Wenger,[1] Sammy Elmariah,[3] Robert W. Yeh,[3] Joseph James Deferio,[1] Kerry A. Pierce,[2] Amy Deik,[2] Robert E. Gerszten,[2,3] Ravi I. Thadhani,[1] Eugene P. Rhee.[1,2] *[1]Nephrology Div, Massachusetts General Hospital, Boston, MA; [2]Broad Institute, Cambridge, MA; [3]Cardiology Div, Massachusetts General Hospital, Boston, MA.*

**Background:** The marked excess in cardiovascular mortality that results from uremia remains poorly understood.

**Methods:** In two independent nested case-control studies, we applied liquid chromatography-mass spectrometry based metabolite profiling to plasma obtained from participants of a large cohort of incident hemodialysis patients.

**Results:** First, 100 individuals who died of a cardiovascular cause within one year of initiating hemodialysis (cases) were randomly selected along with 100 individuals who survived for at least one year (controls), matched for age, sex, and race. Four long-chain acylcarnitines achieved the significance threshold adjusted for multiple testing ($P<0.0003$), and remained associated with 1-year cardiovascular death after multivariable adjustment: oleoylcarnitine (odds ratio (OR) per SD 2.3 [95% confidence interval, 1.4-3.8]; $P=0.001$), linoleylcarnitine (OR per SD 2.4 [1.4-4.1]; $P=0.002$), palmitoylcarnitine (OR per SD 1.8 [1.2-2.8]; $P=0.006$), and steaorylcarnitine (OR per SD 1.6 [1.1-2.5]; $P=0.025$). The association between oleoylcarnitine and 1-year cardiovascular death was then replicated in an independent sample (n= 300, OR per SD 1.4 [1.1-1.9]; $P=0.008$). Addition of oleoylcarnitine to clinical variables improved net reclassification (NRI, 0.38 [95% CI 0.20-0.56]; $P<0.0001$). In physiologic profiling studies, we demonstrate that the fold change in plasma acylcarnitine levels from the aorta to renal vein and from pre- to post hemodialysis samples exclude renal or dialytic clearance of long-chain acylcarnitines as confounders in our analysis.

**Conclusions:** Our data highlight clinically meaningful alterations in acylcarnitine homeostasis at the time of dialysis initiation which may represent an early marker, effector, or both of uremic cardiovascular risk.

*Funding:* NIDDK Support, Private Foundation Support

## FR-OR143

**Speckle Tracking Echocardiography Detects Uremic Cardiomyopathy Early and Predicts Cardiovascular Mortality in End-Stage Renal Disease** Rafael Kramann,[1] Vincent Brandenburg,[2] Thilo Krueger,[1] Jürgen Floege,[1] Georg Schlieper.[1] *[1]Nephrology, RWTH, Aachen, Germany; [2]Cardiology, RWTH, Aachen, Germany; [3]Clinical Epidemiology, Leiden Univ, Leiden, Netherlands.*

**Background:** Cardiovascular (CV) mortality in end stage renal disease is mainly driven by sudden cardiac death and recurrent heart failure due to uremic cardiomyopathy (UC). An early detection of UC might identify patients at risk and can have clinical impact in therapeutic decision-making. 2-dimensional strain echocardiography (**2DSE**) is a recently developed method to determine myocardial function in a multidimensional fashion.

**Methods:** In an animal study using two rat models of UC we tested whether 2DSE is superior to routine echocardiography to detect early changes in myocardial contractility. In a clinical study 2DSE parameters, ejection fraction (EF) and clinical characteristics were assessed in 171 ESRD patients, patients were followed up for 2.5 years. Primary endpoint was CV-mortality and secondary endpoint was all-cause mortality.

**Results: Animal study-**Using 2DSE in two rat models of UC early (4-6 weeks) after induction of kidney disease we observed that global radial and circumferential strain parameters were significantly decreased whereas fractional shortening (FS) and cardiac-output (CO) remained unchanged. Furthermore, compared to FS and CO, 2DSE parameters showed better correlations with histologic hallmarks of UC (grade of interstitial fibrosis and cardiomyocyte cross-sectional area). **Clinical study-** During the follow-up period, EF and various 2DSE parameters were significant risk factors for CV mortality in a multivariate cox model (EF hazard ratio-HR 0.97 95% confidence interval-CI 0.95-0.99, p=0.012, peak global longitudinal strain HR 1.17 95%-CI 1.07-1.28, p<0.001; peak systolic and late diastolic longitudinal strain rates HR 4.7, 95%-CI 1.23-17.64, p=0.023; HR 0.25, 95%-CI 0.08-0.79 p=0.019, respectively). Multivariate cox-regression analysis revealed circumferential early diastolic strain rate among others as an independent risk factor for all-cause mortality (HR 0.43; 95%-CI 0.25-0.74; p=0.002).

**Conclusions:** 2DSE can detect UC early in rats and predicts cardiovascular and all-cause mortality in ESRD patients.

*Funding:* Private Foundation Support

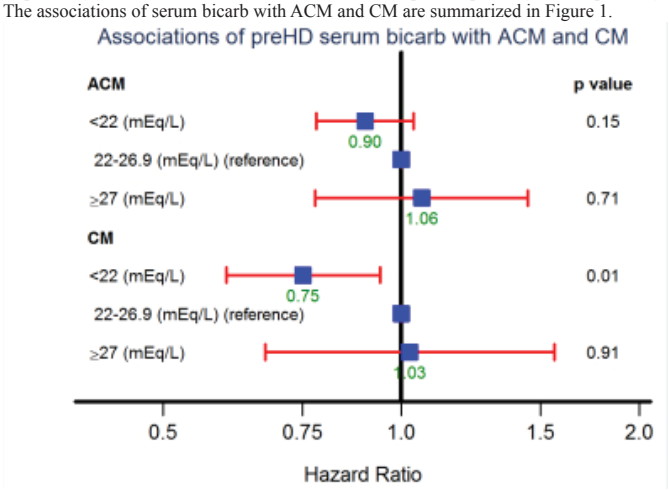
## FR-OR144

**Low Serum Bicarbonate Is Associated with Lower Cardiac Mortality (CM) in Hemodialyis (HD) Patients** Abinash C. Roy, G. Wei, R. Filipowicz, Tom Greene, Kalani L. Raphael, Alfred K. Cheung, Srinivasan Beddhu. *Dept of Medicine, Univ of Utah, Salt Lake City, UT.*

**Background:** Recent data suggest ↑ serum bicarbonate (bicarb) is associated with ↑ mortality. However, the associations of lower serum bicarb with mortality is controversial. Therefore, we examined the associations of lower than normal serum bicarb with mortality using the HEMO Study data.

**Methods:** The HEMO study was a 2X2 factorial design randomized controlled trial of dialysis dose and flux in 1846 prevalent HD pts. Patients were enrolled in the study from March 1995 until October 2000 and followed through December 2001. Causes of deaths were adjudicated by an outcomes committee. The associations of baseline pre-dialysis serum bicarb with all-cause mortality (ACM) and cardiac mortality (CM) were examined in Cox proportional hazard regression models adjusted for age, gender, race, diabetes, duration of dialysis, ICED score, Ktv group, flux group, enPCR, arrhythmia, pre HD dialysis serum albumin, K, Ca and P.

**Results:** Mean age was 58±14 yrs, 56% were female , 63 % were black and 69% had diabetes. Serum bicarb , Ca and K levels were 21.5±3.6, 9.29±.97 and 4.86±.77 mEq/L respectively. There were 16.6 ACM and 9.0 CM events per 100 patient years, respectively. The associations of serum bicarb with ACM and CM are summarized in Figure 1.




Interaction terms of serum bicarb with serum K and serum Ca were non-significant when added to the above models.

**Conclusions:** Elevated serum bicarb was not associated with ACM or CM in this study. Surprisingly, pre-HD serum bicarb below the normal range was associated with lower CM. Further studies are warranted to examine the mechanisms for the observed lower CM in those with lower serum bicarb.

*Funding:* NIDDK Support

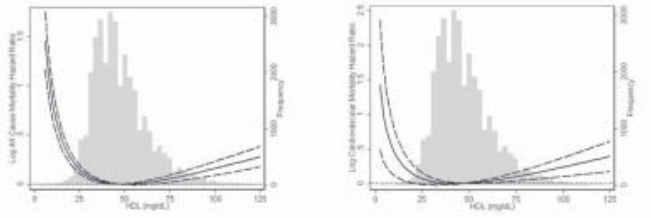
## FR-OR145

**Elevated Serum High Density Lipoprotein Cholesterol Level Is Paradoxically Associated with Increased Overall and Cardiovascular Mortality in Patients on Hemodialysis** Hamid Moradi,[1,2] Elani Streja,[1,2] Nosratola D. Vaziri,[2] Moti L. Kashyap,[2] Gregg C. Fonarow,[3] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Orange, CA; [2]Univ of California, Irvine, CA; [3]Univ of California, Los Angeles, CA.*

**Background:** High density lipoprotein (HDL) confers protection against atherosclerosis by several different mechanisms. Although in the general population, increasing levels of HDL are associated with reduced cardiovascular (CV) mortality, this association is not well known in patients with chronic disease states such as end stage renal disease. We hypothesize that the association of serum HDL concentration with all-cause and CV mortality in hemodialysis patients is different from the general population.

**Methods:** We examined the survival impact of serum HDL level in a 3-year (6/2004-6/2007) cohort of 33,109 maintenance hemodialysis patients being treated in clinics of a large dialysis organization using Cox models adjusted for demographics and case-mix variables and cubic splines were plotted.

**Results:** In this cohort the mean age (mean + SD) was 60+15 years old and included 45% women, 33% African-Americans, and 61% diabetics. In the fully adjusted models, higher HDL concentrations up to 50 mg/dL were associated with better overall survival, while HDL above 60 mg/dL was associated with a rise in all-cause and cardiovascular mortality. All-cause and CV mortality HR was 1.28 (1.20-1.38) and 1.08 (1.01-1.16) for HDL <30 mg/dL and 1.05 (1.00-1.10) and 1.08(1.00-1.16) for HDL≥60 mg/dL, respectively (reference: HDL 30-<60 mg/dL).



**Conclusions:** A U-shaped association between serum HDL cholesterol level and all-cause and CV mortality exists in HD patients with HDL around 50 mg/dL exhibiting the best survival. The underlying mechanisms responsible for these seemingly paradoxical associations await further investigation.

*Funding:* NIDDK Support

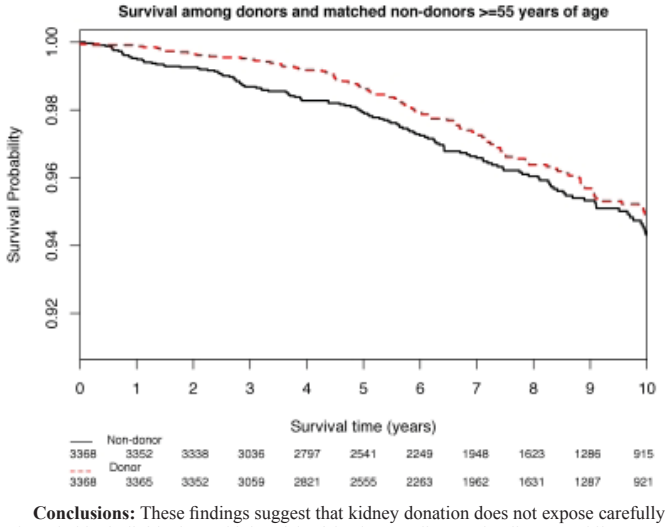
## SA-OR001

**Mortality, Cardiovascular and End-Stage Renal Disease Outcomes among Older Live Kidney Donors** Peter P. Reese,[1] Roy D. Bloom,[1] Harold I. Feldman,[1] Paul Rosenbaum,[1] Wei Wang,[2] Philip Saynisch,[2] Nicole Tarsi,[2] Nabanita Mukherjee,[2] Amit X. Garg,[3] Adam S. Mussell,[1] Orit Even-Shoshan,[2] Raymond R. Townsend,[1] Jeffrey H. Silber.[2] *[1]Univ of Pennsylvania; [2]Children's Hospital of Philadelphia; [3]Univ of Western Ontario.*

**Background:** Older individuals (≥55 y/o) have been accepted for live kidney donation more commonly over the past two decades. Given strong associations of both older age with worse renal function and cardiovascular (CV) disease, nephrectomy could make older donors vulnerable to death, CV events and end-stage renal disease (ESRD).

**Methods:** We performed a matched cohort study of older individuals who underwent nephrectomy between the years 1996–2006 and participants in the Health and Retirement Study (HRS). Each donor was matched to an HRS participant who was free of important comorbidities close to the date of the donor's nephrectomy. Among 5152 donors, 3368 (65%) were matched to healthy comparators using demographics, economic environment and body mass index. The primary outcome was death, ascertained from death registries. Secondary outcomes ascertained for pairs with Medicare claims were a) death/CV diagnoses, and b) ESRD.

**Results:** In each group, the mean age was 59 y/o and 6% were African-American. In median follow up of 7.8 years, mortality was not significantly different between donors and matched non-donors (p=0.21). The rate of death and CV events was not different for donors and matched non-donors (p=0.70). Among pairs in Medicare, the risk of ESRD was higher for donors (HR 7.4; 95% CI 2.91, 18.8; p<0.001), although the number of donors with ESRD was small (n=20).




**Conclusions:** These findings suggest that kidney donation does not expose carefully selected older individuals to a higher risk of death or cardiovascular disease. While ESRD was rare, older donors have an elevated risk of this outcome.

*Funding:* NIDDK Support, Private Foundation Support

## SA-OR002

**Donor Tubular Phosphate Handling Independently Predicts Recipient Outcomes after Living Kidney Donation** Marco van Londen, Jelmer Kor Humalda, Johannes S. Sanders, Stephan J.L. Bakker, Gerjan Navis, Martin H. De Borst. *Dept of Nephrology, Univ Medical Center Groningen, Netherlands.*

**Background:** Identification of pre-transplantation (pre-Tx) donor factors associated with recipient outcomes may help to improve prognosis after kidney transplantation. Tubular injury leads to reduced expression of NaPi cotransporters and reduced phosphate (Pi) transport. We investigated whether pre-Tx parameters of Pi metabolism in living donors are associated with recipient outcomes after transplantation (post-Tx).

**Methods:** In 130 donors, pre-Tx $^{125}$I-iothalamate GFR, serum and urinary Pi, plasma fibroblast growth factor 23 and parathyroid hormone, fractional Pi excretion and TmP-GFR (tubular maximum Pi reabsorption per GFR) were determined. In corresponding recipients GFR was measured 1 year post-Tx, and follow-up data documented. Associations between pre-Tx donor factors and recipient post-Tx GFR were tested by linear regression. Next, a multivariate Cox regression model for a composite endpoint of rejection, graft failure or recipient mortality was built using pre-Tx donor factors, recipient factors, and transplant characteristics.

**Results:** Pre-Tx GFR in donors (age 52±10 yrs, 48% men) was 118±24 ml/min. GFR one year post-Tx in recipients (age 48±19 yrs, 61% men) was 60±19 ml/min. In multivariate regression, pre-Tx donor TmP-GFR (standardized β 0.30, P=0.003) and donor age (st. β -0.28, P=0.01) were independent determinants of recipient 1-yr post-Tx GFR (final model $R^2$=0.20). During follow up of 2.4 [1.2-3.2] yrs, 29 recipients had rejection, 3 graft failure and 9 died. Donor pre-Tx TmP-GFR was independently associated with the composite endpoint (HR 0.25 [95% CI 0.08-0.72] per 1 mg/dL TmP-GFR, P=0.01), along with age, cold ischemia time and recipient gender. Other Pi-related factors were not associated with recipient outcome.

**Conclusions:** A lower donor pre-Tx TmP-GFR, indicating a reduced maximum tubular Pi reabsorption capacity, independently predicts a lower recipient GFR one year post-Tx and an increased risk of rejection, graft failure or recipient mortality. These data are the first to link donor tubular function to recipient outcomes, and warrant further dissection of underlying mechanisms.

*Funding:* Government Support - Non-U.S.

## SA-OR003

**New K/DIGO Guidelines and Kidney Transplantation: Is the Cystatin-C Based Recommendation Relevant?** Ingrid Masson,[1] Nicolas Maillard,[1] Pierre Delanaye.[2] *[1]Nephrology Dialysis Renal Transplantation, Univ Hospital, Saint-Etienne, France; [2]Dialysis, Univ Hospital, Liege, Belgium.*

**Background:** In 2013, K/DIGO guidelines published recommendations for evaluation and management of chronic kidney disease (CKD). These guidelines propose a new framework for the evaluation of CKD, especially in patients with altered glomerular filtration rate (GFR) .They suggest measuring cystatin C in adults with GFR estimated by the CKD-EPI creatinine-based equation (eGFRcreat) 45–59 ml/min/1.73 m² to confirm the diagnosis of CKD. Although derived from studies unrelated to kidney transplantation, the K/DIGO guidelines aim to target the transplant population as well. Herein, we sought to determine whether the new K/DIGO strategy to detect decreased GFR might be applicable in renal transplantation.

**Methods:** In 670 kidney transplant recipients (KTR), we analyzed the performance of the combined CKD-EPI equation (eGFRcreat-cys) to reclassify KTR in comparison with inulin clearances (mGFR) by using the analytical methodology developed in these guidelines. Serum creatinine was measured by an IDMS traceable enzymatic method; cystatin C by IFCC-traceable nephelometric method.

**Results:** In the whole cohort, 192 patients had eGFRcreat 45-59 mL/min/1.73m² but mGFR was above 60 mL/min/1.73m² in 39 of them (20%). When using eGFRcreat-cys in these 192 patients, only 181 (94%) had also eGFR below 60 mL/min/1.73 m². In the 39 patients with eGFRcreat <60 but mGFR >60 mL/min/1.73 m², only 8 were correctly reclassified by the eGFRcreat-cys equation. Estimating GFR by eGFRcreat led to 20% (39/192) of errors in CKD classification. Following the strategy suggested by the guidelines, errors in classification were actually marginally corrected (18%, 34/192).

**Conclusions:** The K/DIGO guidelines recommend the use of the eGFRcreat-cys to improve the detection of CKD in patients whom eGFRcreat 45-59mL/min/1.73m². In the present study, we show that this recommendation cannot be extended to transplant recipients.

## SA-OR004

**Differential Diagnosis of Acute Kidney Allograft Dysfunction Using Urinary Cell mRNA Profiles** Thangamani Muthukumar, Marie Matignon, Catherine Snopkowski, Carol Y. Li, John R. Lee, Darshana Dadhania, Manikkam Suthanthiran. *Cornell Univ.*

**Background:** A noninvasive diagnostic test that distinguishes acute rejection (AR) from acute tubular injury (ATN) is an unmet need in clinical renal transplantation.

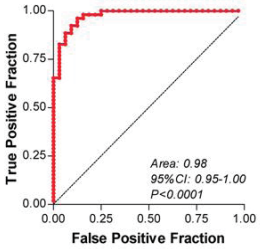
**Methods:** We tested the hypothesis that urinary cell mRNA profiles distinguish AR vs. ATN by profiling urine specimens collected at the time of allograft biopsy from 84 kidney transplant recipients with allograft dysfunction; 52 AR (ACR-26 and AMR-26) and 32 ATN. We measured absolute levels (copies/ug total RNA) of a mechanistically informative panel of 26-mRNAs using qPCR assays. We used discriminant analyses to develop a model to distinguish AR from ATN and validated it by a 10-fold cross-validation. We used decision curve analysis *(Vickers AJ. Med Decis Making 2006)* and determined whether the diagnostic test (urinary cell mRNA profile) is clinically useful.

**Results:** Neither s.creatinine nor tacrolimus levels differed between patients with AR or ATN. Urinary cell levels of 23 of the 26 mRNAs were statistically different (P<0.05) between AR and ATN. A urine composite score derived from a model of a combination of 6 of the 26 mRNAs assayed had a prediction accuracy of 94% (ROC-AUC: 0.98; 95%CI: 0.95-1.00).

| | | Predicted Category | |
|---|---|---|---|
| | | Acute Rejection | Acute Tubular Injury |
| Actual Category (N=84) | Acute Rejection (N=52) | 50 | 2 |
| | Acute Tubular Injury (N=32) | 3 | 29 |

Prediction Accuracy: 94.1%, Press's Q=65.2, P<0.0001
Acute rejection correctly classified: 96.2%
Acute tubular injury correctly classified: 90.6%

Hit ratio (percentage correctly classified) based on the discriminant function: 94.1%
Maximum chance criterion (MCC, predict everyone to be classified to the largest diagnostic category): 61.9%
Proportional chance criterion ($C_{pro}$, randomly classify proportionate to the two diagnostic categories): 52.8%




By 10-fold cross-validation, the prediction accuracy was 88.5% (ROC-AUC: 0.88, 95%CI: 0.78-0.97). The net benefit, over a wide range of physician's threshold probability for biopsy to diagnose AR, was higher when biopsy was done as informed by the urine composite score. By this approach, at 25% physician's threshold probability for biopsy, the number of biopsies avoided per 100 patients was 29.8, with resultant cost savings of about US$ 65,700.

**Conclusions:** In kidney transplant recipients with abnormal creatinine levels, urinary cell mRNA profile not only facilitates differential diagnosis of AR vs. ATN but also minimizes biopsy procedures and reduce health care costs.

*Funding:* Other NIH Support - NIAID

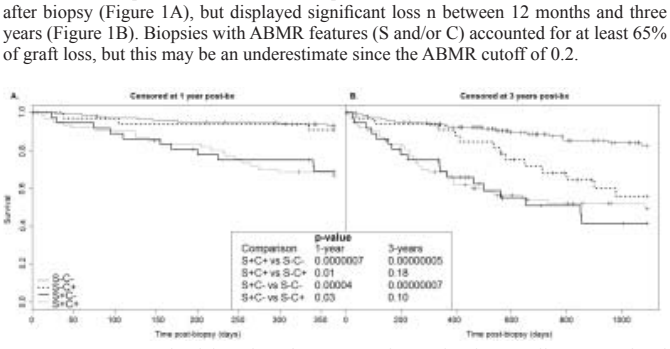
## SA-OR005

**Prediction of Failure in Antibody-Mediated Rejection Requires Molecular as Well as Conventional Assessment** Philip F. Halloran, Andre Barreto Pereira, Konrad S. Famulski. *Alberta Transplant Applied Genomics Centre, Edmonton, Canada.*

**Background:** Antibody-mediated rejection (ABMR) is the major determinant of transplant outcome but conventional assessment is problematic, particularly since the recognition of C4d negative ABMR.

**Methods:** We recently created and validated a microarray-based test for ABMR – the ABMR score - in 703 prospective indication biopsies in 579 patients. We now compared the ability of the conventional (C) tests – histology, C4d, HLA antibody - to the ABMR score (S>0.2) for predicting failure in the 342 patients undergoing late biopsies >1 year post transplant.

**Results:** Excluding glomerulonephritis and BK, there were four groups: 61 S+C+; 36 S+C-; 32 S-C+, and 144 S-C-. All 3 classes of classes of kidneys with ABMR features (S+C+, S+C-, S-C+) were at risk for failure, but only biopsies with positive scores (S+C- and S+C+) showed rapid deterioration. S-C+ biopsies showed no increased loss for 12 months after biopsy (Figure 1A), but displayed significant loss n between 12 months and three years (Figure 1B). Biopsies with ABMR features (S and/or C) accounted for at least 65% of graft loss, but this may be an underestimate since the ABMR cutoff of 0.2.




**Conclusions:** Optimal detection of ABMR requires molecular as well as conventional assessment. The molecular changes detect many missed cases and predict rapid deterioration after biopsy, and thus will be critical in developing new therapies and guiding their use.

*Funding:* Pharmaceutical Company Support - Roche; Novartis, Private Foundation Support, Government Support - Non-U.S.

## SA-OR006

**The Contribution of Post Transplant Disease Recurrence to Graft Failure in Glomerulonephritis** Maria Pippias,[1] Vianda S. Stel,[1] Fergus J. Caskey,[2] Kitty J. Jager.[1] *[1]On Behalf of the ERA-EDTA Registry Study Group, ERA-EDTA Registry, Amsterdam, Netherlands; [2]Richard Bright Renal Unit, Bristol, United Kingdom.*

**Background:** Glomerulonephritis (GN) continues to predominate as one of the leading causes of established renal failure and subsequent need for transplantation. Post-transplant disease recurrence (PTDR) is a significant cause of long term graft loss, however there is little consensus in the rate of graft loss due to PTDR. We studied the contribution of PTDR to graft failure in those with a primary renal diagnosis (PRD) of GN.

**Methods:** Data on all adult first kidney transplants (Tx) performed between 1991 and 2011 in 11 countries reporting to the ERA-EDTA Registry were used. In those with a PRD of GN [IgA, MPGN I, MPGN II, FSGS and membranous nephropathy (MN)] 5, 10 and 15 year death censored graft survival was studied using Kaplan Meier and Cox regression analysis whilst adjusting for gender, age at the start of RRT and age at Tx. Adult polycystic kidney disease (APKD) was the control group. Any additional risk of graft loss when compared to APKD was assumed to be due to PTDR.

**Results:** 12,646 individuals were included in the study, 6279 with GN and 6367 with APKD. Overall, 15 year adjusted graft survival was lowest in MN (56.3%) and highest in IgA (66.8%). No difference in 5, 10 or 15 year adjusted graft survival was found between APKD and IgA (15 year HR 1.04; 95% CI 0.92-1.16). Significantly worse graft survival was seen in all other GNs: MPGN II (HR 2.27; 95% CI 1.67-3.09), MPGN I (HR 1.88; 95% CI 1.58-2.23), MN (HR 1.4; 95% CI 1.18-1.67) and FSGS (HR 1.39; 95% CI 1.18-1.63).

**Conclusions:** This is the first study examining graft survival outcomes in a large European Tx population with various GNs over a 15 year period. We found no difference in graft survival in IgA compared to APKD but worse outcomes in other GNs. As short term graft function continues to improve further studies are required to understand the determinants of long term graft survival. Further analyses will focus on long term graft outcomes with different donor types as such information would aid the patient in Tx counselling particularly when considering donor type.

## SA-OR007

**Albuminuria and Cardiovascular Disease, Kidney Failure and Death in Stable Kidney Transplant Recipients** Alin A. John,[1] Andrew S. Levey,[1] Myra A. Carpenter,[2] Lawrence G. Hunsicker,[3] Roberto S. Kalil,[3] John W. Kusek,[4] Marc A. Pfeffer,[5] Scott D. Solomon,[5] Matthew R. Weir,[6] Daniel E. Weiner.[1] *[1]Tufts; [2]UNC; [3]Iowa; [4]NIDDK; [5]BWH; [6]Maryland.*

**Background:** Despite improvement in early graft loss, overall graft survival remains suboptimal. While albuminuria is a known risk factor for adverse outcomes in the general and CKD populations, the relationship between albuminuria and outcomes in kidney transplant recipients (KTRs) remains uncertain.

**Methods:** In a post-hoc analysis of the Folic Acid for Vascular Outcomes Reduction in Transplantation (FAVORIT) Trial, a large randomized trial of homocysteine lowering in stable KTRs, we evaluated the relationship between albuminuria and risk of cardiovascular disease, end-stage renal disease (ESRD) and all-cause death. Multivariable Cox proportional hazards models, adjusting for age, sex, race, treatment allocation, country, systolic and diastolic blood pressure, history of CVD, diabetes and smoking, cholesterol, BMI, eGFR, donor type, transplant vintage, and calcineurin inhibitor use, were used.

**Results:** In 3,460 participants with complete data, 1,912 (55%) had an albumin to creatinine ratio (ACR) <30 mg/g, 1,113 (32%) ACR of 30-299 mg/g, and 435 (12%) ACR of ≥300 mg/g at baseline. Median (IQR) ACR was 24 mcg/g (9-97) and mean eGFR 49 ± 18 ml/min/1.73m$^2$. Mean age was 52 ± 9 years, 17% were black, and 37% women; median graft vintage was 4.1 (1.7, 7.4) years and 57% received deceased donor kidneys. Variables associated with higher ACR included older graft vintage, deceased donor transplant, history of CVD and diabetes, higher blood pressure, and lower eGFR. Over mean follow-up of 4.0 ± 1.5 years, a total of 494 cardiac events, 281 kidney failure events and 395 deaths occurred. Following adjustment, each doubling of ACR was associated with significantly increased risk of cardiovascular outcomes [HR=1.05 (1.02, 1.09)], kidney failure [HR=1.43 (1.36-1.50)], and all-cause mortality [HR=1.14 (1.09-1.18)].

**Conclusions:** In stable KTRs, elevated baseline uACR was independently associated with risk of CVD, kidney failure and death. Future studies are needed to evaluate whether reducing albuminuria improves these clinical outcomes.

*Funding:* NIDDK Support

## SA-OR008

**5 Years Follow-Up on ZEUS: Improved Renal Function of an Everolimus/Enteric-Coated Mycophenolate Sodium Regimen after Calcineurin Inhibitor Withdrawal in De Novo Renal Transplant Patients** Wolfgang Arns,[1] Ute Eisenberger,[1] Oliver Witzke,[1] Claudia Sommerer,[1] Petra Reinke,[1] Martina Porstner,[2] Christoph May,[2] Eva-Maria Paulus,[2] F. Lehner,[1] Klemens Budde.[1] *[1]ZEUS Study Group, Germany; [2]Novartis Pharma, Germany.*

**Background:** Follow-up (FU) on renal function(RF), efficacy and safety after conversion to an Everolimus(EVR)/Enteric-Coated Mycophenolate Sodium(EC-MPS) regimen after cyclosporine A(CsA) withdrawal in *de novo* kidney allograft recipients at month (Mo)60 after transplantation (Tx).

**Methods:** In this prospective, open-label, controlled, multi-center study renal Tx patients (pts) were randomized (rdz) at Mo4.5 post Tx to an immunosuppressive (IS) regimen consisting of either EVR/EC-MPS or CsA/EC-MPS. After completion of 12Mo core-study pts were included in an observational FU.

**Results:** 300 pts were rdz to either EVR+EC-MPS(n=155) or CsA+EC-MPS(n=145). Mo60 visit was completed by 227(76%) pts. RF expressed as eGFR(Nankivell, ITT) was similar in both groups at baseline (4.5Mo post Tx) with an improvement by +3.6mL/min/1.73m2 (p=0.045) in favor of the EVE regimen at Mo60 (+9.8mL/min/1.73m2 at Mo12). All pts who remained on the assigned EVR regimen (on-therapy) had a higher improvement of RF, i.e.+7.3mL/min/1.73m2(p<0.001) at Mo60. An overall improved GFR was observed in 69% of EVR on-therapy pts at Mo60 vs 46% on CsA (p=0.007). 3deaths and 3graft losses were observed in CsA vs 4deaths and 4graft losses in EVR group (ITT). Number of pts with infections: 32(21%) in EVR, 21(15%) in CsA group; hospitalisation: 36(23%) in EVR, 21(15%) in CsA group between Mo48 and Mo60. No significant difference in BPAR was reported from rdz to Mo60 with 21(14%) in EVR vs. 11(8%) in CsA group (p=0.095).

| eGFR Nankivell (ml/min/1.73m²) | EVR | CsA | Difference EVR/CsA | P-Value |
|---|---|---|---|---|
| ITT population | n=99 | n=100 | Δ | |
| eGFR mean ±SD | 68.0±17.1 | 65.3±15.7 | +2.9 | 0.331[b] |
| eGFR change[a], mean ±SD | +5.1±13.7 | -0.4±12.9 | +5.5 | 0.003[b] |
| eGFR LS-mean[c] [95%CI] | 67.9 [63.7;72.1] | 64.2 [60.0;68.6] | +3.6 [0.1;7.2] | 0.045[c] |
| On-therapy population | n=71 | n=76 | Δ | |
| eGFR mean ±SD | 70.3±17.1 | 66.3±15.1 | +4.1 | 0.141[b] |
| eGFR change[a], mean ±SD | +8.3±13.1 | -0.9±11.8 | +9.2 | <0.001[b] |
| eGFR LS-mean[c] [95%CI] | 71.1 [66.4;75.9] | 63.8 [59.1;68.5] | +7.3 [3.4;11.3] | <0.001[c] |

[a]RandomizedTreatmentPeriod; [b]Wilcoxon Rank-Sum test; [c]ANCOVA

**Conclusions:** Early conversion to EVR in *de novo* KTx pts after CNI withdrawal maintains better RF over a period of 5years without compromising efficacy and safety.

*Funding:* Pharmaceutical Company Support - Novartis Pharma Germany

## SA-OR009

### Prevalence and Clinical Significance of C1q Binding Anti-HLA Antibodies in Patients with Functioning Allografts More Than 10 Years after Kidney Transplantation

Sumeyye Calp Inal, Adriana Colovai, Michal L. Melamed, Enver Akalin. *Renal Div, Albert Einstein College of Medicine, Bronx, NY.*

**Background:** We aimed to investigate the prevalence and clinical significance of C1q binding anti-HLA antibodies in patients with functioning grafts more than 10 years post-transplantation.

**Methods:** All recipients had negative complement-dependent-cytotoxicity cross-match at the time of transplantation. HLA antibody identification was performed using Luminex Single Antigen Bead assay. The capacity of serum HLA antibodies to fix complement was tested using the C1q assay.

**Results:** Of the 176 patients studied, %23 (n=41) had donor-specific anti-HLA antibodies (DSA), and %20 of the DSA+ patients (n=8) had C1q binding capacity. Mean fluoresence intensity values of DSAs were higher in DSA+/C1q+ group (7282 vs 3472). Comparing to DSA negative and DSA+/C1q- patients, DSA+/C1q+ patients had more history of previous kidney transplantation, higher class II panel reactive antibody levels, acute antibody-mediated rejection and transplant glomerulopathy. There was no difference in terms of allograft function between the groups.

**Conclusions:** Positive C1q assay was seen in 20% of the DSA+ patients 10 years after transplantation and it is significantly associated with AMR and transplant glomerulopathy.

| | DSA negative (n=135) | DSA+/C1q+ (n=8) | DSA+/C1q- (n=33) | p value |
|---|---|---|---|---|
| Mean age | 56(13) | 51(11) | 54(14) | 0.48 |
| Sex, % female | 38 | 75 | 39 | 0.11 |
| Race, % African-American | 21 | 38 | 15 | 0.36 |
| Transplant Type, % deceased-donor | 48 | 75 | 36 | 0.13 |
| Median follow-up, years | 13 (11-16) | 11 (10-18) | 12(11-16) | 0.96 |
| Previous transplant, % | 10 | 38 | 6 | 0.03 |
| Last mean creatinine | 1.8 ± 1.1 | 2.2±1.0 | 2.0±1.1 | 0.36 |
| Mean spot urine protein/creatinine, g/d | 0.9±2.0 | 1.1±1.4 | 0.45±0.69 | 0.37 |
| Acute cellular rejection , % | 8 | 13 | 15 | 0.45 |
| Acute antibody-mediated rejection, % | 3 | 50 | 3 | < 0.001 |
| Transplant glomerulopathy, % | 8 | 37 | 21 | 0.004 |
| Mean class I PRA, % | 11±22 | 9±12 | 34±34 | < 0.001 |
| Mean class II PRA | 13±25 | 60±41 | 44±26 | < 0.001 |

## SA-OR010

### Circulating MicroRNAs Correlate with Diabetic Nephropathy and Systemic Microvascular Damage and Normalize after Simultaneous Pancreas-Kidney Transplantation

Roel Bijkerk,[1,2] Meriem Khairoun,[1] Jacques Duijs,[1,2] Kristel C.J.H. ter Horst,[1] Aiko P.J. De Vries,[1] Eelco de Koning,[1] Johan W. De Fijter,[1] Ton J. Rabelink,[1,2] Anton Jan Van Zonneveld,[1,2] Marlies Reinders.[1,2] *[1]Nephrology, LUMC, Netherlands; [2]Einthoven Laboratory for Experimental Vascular Research, LUMC, Netherlands.*

**Background:** Simultaneous pancreas-kidney transplantation (SPK) is an advanced treatment option for type I diabetes (T1D) patients with extensive microvascular disease and nephropathy (DN). Circulating microRNAs (miRNAs) can be sensitive biomarkers and their functional properties could provide insight into disease state. By assessing miRNA profiles in healthy control subjects and T1D patients before and following SPK we aimed to identify differentially expressed subsets of miRNAs that associate with microvascular destabilization and disease state.

**Methods:** Circulating miRNA expression was determined using TaqMan® Human miRNA Array Card in plasma of DN (n=8) and SPK patients (n=8) and compared with healthy controls (n=3). In addition, the SPK patients were studied longitudinally before, 1, 6 and 12 months after SPK. Microvascular morphology and mean capillary density were visualized using sidestream darkfield imaging of the oral mucosa. Furthermore, circulating levels of angiogenic factors, including angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), VEGF and soluble thrombomodulin (sTM) were measured using ELISA.

**Results:** In our study we identified miR-25, 27a, 126, 130b, 132, 152, 181a, 320 and 660 to have elevated expression levels in plasma of DN patients as compared to healthy controls, whereas miR-223 and 574-3p expression was decreased. After SPK, expression levels of these miRNAs normalized and positively correlated with GFR and Hba1c levels. Interestingly, the expression of miR-126, 130b and 132, which are known to be pro-angiogenic, correlated with Ang2 levels. In addition, miR-130b showed a strong correlation with microvascular tortuosity and sTM levels.

**Conclusions:** Circulating miRNAs profiles correlate with DN and systemic microvascular destabilization. Following SPK these profiles normalized concomitant with microvascular stabilization supporting the potential use of miRNA profiles to assess disease progression.

## SA-OR011

### Impaired KLHL3-Mediated Ubiquitination of WNK4 Activates OSR1 and SPAK Kinases-NaCl Cotransporter (NCC) Signaling and Causes Hypertension

Mai Wakabayashi, Takayasu Mori, Kiyoshi Isobe, Eisei Sohara, Koichiro Susa, Yuya Araki, Motoko Chiga, Eriko Kikuchi, Naohiro Nomura, Yutaro Mori, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** Mutations in two WNK kinase genes, WNK1 and WNK4, cause pseudohypoaldosteonism type II (PHA II) characterized by hypertension, hyperkalemia and acidosis. Recently, mutations in Kelch-like 3 (KLHL3) and Cullin3 were also identified to cause PHAII. However, the pathogenic role of the mutations of KLHL3 and Cullin3 genes was not well understood. In this study, we investigated how these three genes interacted each other and how they were involved in the pathogenesis of PHAII.

**Results:** Methods and Results: KLHL3 was co-immunoprecipitated with WNK4, but not with OSR1, SPAK, or NCC. PHAII-causing mutations in either KLHL3 or WNK4 decreased the binding of two proteins. Overexpression of KLHL3 and Cullin3 with WNK4 dramatically decreased WNK4 protein expression, and this decrease was blunted by the PHAII mutations in WNK4, KLHL3, or Cullin3. KLHL3 coexpression with WNK4 significantly induced the ubiquitination of WNK4, which was also impaired by the PHAII mutations in WNK4 and KLHL3. In vitro ubiquitination assay confirmed that WNK4 is a direct substrate of KLHL3-Cullin3 E3 ubiquitin ligase. Transgenic mice overexpressing WNK4 showed PHAII phenotypes and the activation of OSR1/SPAK-NCC signaling in the kidneys, and WNK4 protein was indeed increased in *Wnk4*$^{D561A/+}$ PHAII model mice. WNK4 protein was also increased in KLHL3-mutated PHAII patients.

**Conclusions:** WNK4 is a target for KLHL3/Cullin3-mediated ubiquitination, and the impaired ubiquitination of WNK4 is a common mechanism of PHAII.

*Funding:* Government Support - Non-U.S.

## SA-OR012

### Analyses of KLHL3 Mutants That Cause Pseudohypoaldosteronism Type II

Yutaro Mori, Mai Wakabayashi, Takayasu Mori, Yuya Araki, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** Recently, we reported that WNK4 is a substrate of KLHL3-Cullin3 (CUL3) E3 ubiquitin ligase complexes, and that the impaired ubiquitination of WNK4 would be a common mechanism of pseudohypoaldosteronism type II (PHAII) caused by WNK4, KLHL3 and CUL3. Among various PHAII-causing KLHL3 mutants, we revealed that R528H mutant (a kelch domain mutant) showed less binding to WNK4, thereby causing less ubiquitination and increased abundance of WNK4 within cells. However, pathogenic mechanisms of other PHAII-causing KLHL3 mutants remained to be determined.

**Methods:** We transiently expressed four PHAII-causing KLHL3 mutants (E85A in BTB domain, C164F in BACK domain, S410L and R528H in kelch domains) in HEK293T cells. Stability of the KLHL3 mutants within cells was examined by cycloheximide chase assay. Immunoprecipitation and fluorescence correlation spectroscopy (FCS) were used to evaluate binding of the mutant KLHL3s with WNK4 or CUL3. In vitro and in vivo ubiquitination assays were performed to examine the ubiquitination of WNK4 by the KLHL3 mutants.

**Results:** The expression levels of the KLHL3 mutant proteins differed significantly. Especially, S410L expression was low even with increased amounts of the expression plasmid. Cycloheximide chase assay revealed that S410L was more rapidly degradated compared to wild-type KLHL3. E85A and C164F showed less binding to CUL3, and S410L as well as R528H showed less binding to WNK4. In both in vitro and in vivo ubiquitination assays, WNK4 was less ubiquitinated and increased within cells with all these mutant KLHL3s, compared with wild-type KLHL3.

**Conclusions:** PHAII-causing KLHL3 mutants examined in this study showed less ability to ubiquitinate WNK4 either by its own low stability and/or the decreased binding to CUL3 or WNK4.

*Funding:* Government Support - Non-U.S.

**SA-OR013**

**KLHL2 Interacts with and Ubiquitinates WNK Kinases** Daiei Takahashi, Takayasu Mori, Mai Wakabayashi, Yutaro Mori, Koichiro Susa, Moko Zeniya, Tatemitsu Rai, Eisei Sohara, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** Mutations in the WNK1 and WNK4 genes result in an inherited hypertensive disease, pseudohypoaldosteronism type II (PHAII). Recently, the KLHL3 and Cullin3 genes were also identified as responsible genes for PHAII. We reported that WNK4 is a substrate for the KLHL3-Cullin3 E3 ligase complex, and that the impaired ubiquitination and subsequent increase of WNK4 in the kidney would be a common mechanism of PHAII caused by mutations of WNK4, KLHL3 and Cullin3. Since WNK isoforms are widely expressed in the body, it is not clear whether all of the WNK isoforms are regulated only by KLHL3. To explore the interaction of WNKs and other kelch-like proteins, we focused on KLHL2 (Mayven), a human homolog of drosophila kelch that shares the highest similarity with KLHL3 (86% amino acid identity with KLHL3 in Kelch domain).

**Methods:** To determine the interaction between KLHL2 and WNK kinases, we performed immunoprecipitation and fluorescence correlation spectroscopy. The effect of KLHL2 coexpression on the abundance of WNK kinases was determined by immunoblotting. Ubiquitination of WNK isoforms by KLHL2 was studied in vivo (HEK293T cells) and in vitro.

**Results:** We found that KLHL2, as well as KLHL3, was co-immunoprecipitated with all four WNK isoforms. The direct interaction of KLHL2 with WNKs was confirmed on fluorescence correlation spectroscopy. Co-expression of KLHL2 and Cullin3 decreased the abundance of WNK1, WNK3 and WNK4 within HEK293T cells, and a significant increase of WNK4 ubiquitination by KLHL2 and Cullin3 was observed both in HEK293T cells and in an *in vitro* ubiquitination assay.

**Conclusions:** We have identified the function of KLHL2 as an E3 ubiquitin ligase for WNK kinases. Different combinations of KLHL2 and KLHL3 with WNKs could regulate WNK kinase signaling in different kinds of cells.

*Funding:* Government Support - Non-U.S.

**SA-OR014**

**WNK3 Regulates Blood Pressure through the Regulation of Vascular OSR1/SPAK-NKCC1 Phosphorylation Cascade** Moko Zeniya, Eisei Sohara, Katsuyuki Oi, Motoko Chiga, Koichiro Susa, Takayasu Mori, Daiei Takahashi, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Graduate School of Medical and Dental Sciences, Bunkyo-ku, Tokyo, Japan.*

**Background:** NKCC1 is involved in the regulation of vascular smooth muscle cell contraction. The WNK-SPAK-NKCC1 phosphorylation cascade in vascular smooth muscle cells was found to be important in the regulation of vascular tone. Moreover, we have recently reported that WNK3 knockout mice showed lower blood pressure only when mice were fed low-salt diet, through extra-renal mechanisms. In this study, we investigated whether the WNK-SPAK-NKCC1 cascade in mouse aortic tissue is regulated by dietary salt intake, and the mechanisms responsible, focusing on WNK3.

**Methods:** The phosphorylations of SPAK and NKCC1 were examined in the aorta of wild-type and WNK3 knockout mice fed high-salt, normal or low-salt diet. In addition, the effects of angiotensinII (AngII) on their phosphorylations and blood pressure were also examined.

**Results:** Phosphorylation of SPAK and NKCC1 was significantly reduced in the aorta in high-salt fed mice, and was increased in the aorta in low-salt fed mice, indicating that the WNK-SPAK-NKCC1 phosphorylation cascade in the aorta was indeed regulated by dietary salt intake. Acute and chronic AngII infusion increased phosphorylation of SPAK and NKCC1 in the mouse aorta. In addition, valsartan, an antagonist of angiotensin II type 1 receptor, inhibited low-salt diet-induced phosphorylation of SPAK and NKCC1, demonstrating that AngII activates the WNK-SPAK-NKCC1 phosphorylation cascade in aorta through the AT1 receptor. However, low-salt diet and AngII together did not increase phosphorylation of SPAK and NKCC1 in the aorta of WNK3 knockout mice, indicating that activation of the WNK-SPAK-NKCC1 phosphorylation cascade induced by low-salt diet and AngII is dependent on WNK3. Indeed, increases in blood pressure by AngII infusion were diminished in WNK3 knockout mouse.

**Conclusions:** We demonstrated that dietary salt intake regulates the WNK3-SPAK-NKCC1 phosphorylation cascade in mouse aortic tissue through AngII. We clarified for the first time the physiological role of WNK3 in vivo.

*Funding:* Government Support - Non-U.S.

**SA-OR015**

**mPGES-1 Deletion Increases Blood Lithium Concentration and Induces Colon Injury via Elevated Oxidative Stress** Zhanjun Jia,[1,2] Ying Sun,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, Univ of Utah, Salt Lake City; [2]VA Medical Center, Salt lake City, UT.*

**Background:** It is well established NSAIDs including specific COX-2 inhibitors elevated blood lithium levels in lithium treated patients. However, a specific prostanoid involved in this phenomenon is unknown.Present study attempted to study the role of microsomal prostaglandin E synthase-1 (mPGES-1) in lithium metabolism and toxicity.

**Methods:** Lithium at a low dose of 4 mmol/kg/day for 2 weeks or a high dose of 32 mmol/kg/day for 1 week was administered to mPGES-1 WT and KO mice.

**Results:** During 2-week low dose lithium treatment, KO mice showed a significant elevation of plasma lithium level compared with the WT (KO: 0.18±0.01 vs WT: 0.12±0.02 mmol/L, $p<0.05$), accompanied with reduced urine lithium excretion (KO: 30.0±2.9 vs WT:41.5±4.3 umol/24h, $p<0.05$). Meanwhile, the polyuria induced by lithium was blocked by 80% compared with WT mice. Interestingly, the high dose treatment in KO not only further elevated blood lithium level ((KO: 0.9±0.19 vs WT: 0.4±0.04 mmol/L, $p<0.05$), but also caused severe hematochezia with 50% lethal rate during 7-day lithium treatment. HE staining showed severe hemorrhage lesions without inflammatory cells infiltration. By qRT-PCR, none of inflammatory cytokines including IL-1β, TNF-α, MCP-1, were induced in colon irrespective of the genotype. Interestingly, the lithium overload induced remarkable elevations of SOD1 (3.5 folds) and SOD2 (4.8 folds) in colon of WT mice contrasting to the significant reductions of both SOD1 (-45%) and SOD2 (-44%) in KO. In contrast, colon expression of NADPH oxidase subunits, including $p47^{phox}$, $gp91^{phox}$, was similarly induced in two genotypes. Consistent with the change of SOD, administration of tempol (50 mg/kg/day), a SOD mimic, remarkably diminished the hematochezia and colon damage without affecting the blood lithium (0.86±0.11 mmol/L). Finally, the colon PGE2 content in WT exhibited a 50% increase ($p<0.05$) after lithium treatment contrasting to a marked decrease in KO mice (KOLi: 8.5±0.2 vs KO cont: 3.9±0.46 ng/mg protein, $p<0.01$).

**Conclusions:** mPGES-1 deletion enhanced blood lithium accumulation and resulted in lethal colon injury via oxidative stress.

*Funding:* NIDDK Support, Veterans Affairs Support

**SA-OR016**

**Acetazolamide: An Improved Treatment for Lithium-Induced Nephrogenic Diabetes Insipidus?** Theun de Groot,[1] Anne P. Sinke,[1] Marleen L.A. Kortenoeven,[1] Ruben Baumgarten,[2] Olivier Devuyst,[3] Johannes Loffing,[4] Jack F. Wetzels,[1] Peter M.T. Deen.[1] *[1]Radboud Univ Nijmegen Medical Centre, Netherlands; [2]St. Exp. Lab. Med, Netherlands; [3]Zurich Centre for Integr. Human Physiology, Switzerland; [4]Univ Zurich, Switzerland.*

**Background:** Lithium causes nephrogenic diabetes insipidus (Li-NDI) and hydrochlorothiazide (HCTZ) forms, together with amiloride, the mainstay treatment for Li-NDI patients. The antidiuretic action of HCTZ in Li-NDI is generally ascribed to increased proximal sodium and water uptake, compensating for sodium loss due to NaCl-co-transporter (NCC) inhibition. Earlier, we found that HCTZ also reduces Li-NDI in NCC knockout mice, coinciding with alkalinized urine, suggesting that inhibition of carbonic anhydrase by HCTZ plays a role. To test whether inhibition of only carbonic anhydrases could be a useful alternative therapy, the effect of acetazolamide (ACZ) in Li-NDI was tested.

**Methods:** Polarized mouse cortical collecting duct (mpkCCD) cells and mice were used to study the effect of ACZ on Li-NDI.

**Results:** Treatment of mpkCCD cells with ACZ attenuated the lithium-induced downregulation of endogenous AQP2. ACZ did not affect transcellular voltage, and, upon co-incubation with amiloride, resulted in significantly higher levels of AQP2, indicating that the action mechanism of ACZ differs from that of amiloride. Treatment of Li-NDI mice with ACZ revealed a significant antidiuresis and increased urine osmolality, which was indifferent from Li-NDI mice treated with HCTZ/amiloride. However, unlike HCTZ/amiloride, ACZ treatment did not result in hyponatremia, hyperkalemia, hypercalcaemia, metabolic acidosis and increased serum lithium concentrations. Moreover, ACZ rescued AQP2 expression over the entire length of the collecting duct, whereas HCTZ/amiloride only increased AQP2 levels in the renal papilla.

**Conclusions:** In conclusion, our data reveal that inhibition of carbonic anhydrases attenuates lithium-induced downregulation of AQP2 and NDI development. Moreover, as ACZ appeared as effective as the conventional treatment to rescue Li-NDI, but caused fewer side effects, ACZ may represent a better therapeutic agent than HCTZ/amiloride to treat Li-NDI.

*Funding:* Private Foundation Support

**SA-OR017**

**Mechanism of Acidosis-Induced Adaptive Proliferation of Collecting Duct Intercalated Cells** Lydie Cheval, Suresh K. Ramakrishnan, Christophe Klein, Alain Doucet. *Centre de Recherche des Cordeliers, Paris, France.*

**Background:** Kidneys adapt to acid load by increasing proton secretion by collecting duct a intercalated cells (AIC). This adaptation stems in part from increased number of AICs. We reported previously that acid loading induces proliferation of AICs in mouse outer medullary collecting duct (OMCD) and that this process depends on the production of growth and differentiation factor 15 (Gdf15), a member of TGFb super family (JASN, 19:1965-1974, 2008). Here we investigated the mechanisms of Gdf15 production and action in OMCDs.

**Methods:** Acidosis was induced by feeding mice a $NH_4Cl$-enriched diet (0.7M). OMCDs were microdissected from collagenase-treated kidneys. mRNAs were quantified by RT-quantitative PCR, number of AICs was counted after immunolabeling with an AE1 antibody. The angle of AICs doublets with the axis of the tubule was determined after confocal imaging of isolated OMCDs and 3D reconstruction.

**Results:** Within 2 days, acidosis induced a 3-fold over expression of p53. $NH_4Cl$-induced over expression of Gdf15 and cyclin D1 were abolished in $p53^{-/-}$ mice which, accordingly, developed stronger acidosis. Treatment with the pan-erbB tyrosine kinase inhibitor canertinib (60mg/Kg/day, IP) did not alter the over expression of Gdf15 but curtailed that of PCNA and cyclin D1, and accordingly mice developed stronger acidosis. In control mice, the angle of the doublets of AICs was evenly distributed from 0 to 90°. Conversely, in acidotic mice, the number of doublets increased and they were preferentially oriented in parallel with the tubule axis.

**Conclusions:** Gdf15 triggers axial proliferation of AICs through activation of ErbB receptor. This adaptive proliferation is dependent on p53 over expression.

*Funding:* Government Support - Non-U.S.

**SA-OR018**

**Multifunctional Role of the N-Terminal Variable (V1) Domain of the Sodium-Bicarbonate Cotransporter NBCe1-A: Patch Formation, Trafficking and Gating** Harry S. Gill,[1] Casey N. Watkins,[1] Anastas Popratiloff.[2] *[1]Dept of Medicine, The George Washington Univ & Div of Renal Diseases & Hypertension, Washington, DC; [2]Center for Microscopy & Image Analysis, The George Washington Univ, Washington, DC.*

**Background:** The electrogenic NBCe1-A is an integral membrane cotransporter that reabsorbs $Na^+$ and $HCO3^-$ across the basolateral membrane of the proximal tubule. Naturally occurring mutations (Q29X, R298S) in the cytoplasmic, N-terminal domain of NBCe1-A (Nt) lead to Type II acidosis. NBCe1 family members contain a large Nt with two variable regions, one at the extreme N terminus (V1).

**Methods:** The Nt structure was solved by X-ray crystallography. To investigate the role of the V1 domain, truncation mutants were rationally generated. Surface-plasmon-resonance (SPR) and light-scattering (MALS-SEC) techniques were used to evaluate pH-sensitive self-associations of the Nt. The mutants were also fused with the green fluorescent protein and expressed in cultured human proximal tubular (HK2) cells to evaluate self-associations at the plasma membrane by confocal microscopy.

**Results:** We present the Nt crystal structure at 2.4 Å resolution. The structure reveals that the Nt dimerizes by two-interlocking arms and that has two variable flexible regions, one at the extreme N terminus (V1) that gates substrate entry into the Nt and another large solvent accessible loop (V2) that extends ~20 A from the core Nt structure. The SPR and MALS-SEC experiments indicate that the V1 domain is responsible for Nt self-associations. In agreement, punctate clusters and large patches of full-length NBCe1-A are observed at the plasma membrane. Trafficking to the membrane is significantly affected in several Nt mutants.

**Conclusions:** We conclude that NBCe1-A molecules form patches in the plasma membrane of PT cells in the presence of intracellular $HCO3^-$. Secondly, we have discovered a novel role for V1 domain of NBCe1-A in cell membrane trafficking. These findings illuminate the role of the Nt of NBCe1-A in $HCO3^-$ sensing and transport and provide insight to the pathogenic processes observed in a subset of patients with truncating and point mutations in the gene encoding NBCe1-A.

*Funding:* NIDDK Support

**SA-OR019**

**Missense Mutation T485S Alters NBCe1-A Electrogenicity Causing Proximal Renal Tubular Acidosis** Quansheng Zhu,[1] Xuesi Max Shao,[1] Liyo Kao,[1] Rustam Azimov,[1] Alan Mark Weinstein,[2] Debra Newman,[1] Weixin Liu,[1] Ira Kurtz.[1] *[1]Dept of Medicine, Univ of California, Los Angeles, Los Angeles, CA; [2]Dept of Physiology and Biophysics, Weill Medical College of Cornell Univ, New York, NY.*

**Background:** The electrogenic sodium-base cotransporter, NBCe1-A plays a critical role in reabsorbing filtered bicarbonate in the proximal renal tubule. Eight missense mutations in NBCe1-A have been reported to cause proximal tubular acidosis (pRTA), of which the Thr485 to Ser mutation minimally perturbs the transporter structure and retains 50% of base transport. The disease causing mechanism of T485S is intriguing because Thr and Ser are structurally and chemically similar.

**Methods:** We expressed human NBCe1-A in HEK 293 cells and explored the molecular mechanism of the T485S pRTA mutation using a combined approach of intracellular pH assays, whole cell patch clamping, computational molecule simulation and small molecule probing.

**Results:** Wild-type NBCe1-A mediates electrogenic sodium-base cotransport with a 1:2 charge transport stoichiometry. Using nitrate as a surrogate for carbonate, our data suggests that wild-type NBCe1-A mediates electrogenic sodium carbonate cotransport. T485S causes the transporter to become electroneutral with a loss of sodium-coupled nitrate transport. The underlying mechanism appears to involve a change of carbonate for bicarbonate as the preferred substrate in the anion interaction site. The location of Thr485 in the anion interaction site was determined from [$^{14}$C]NEM labeling experiments, functional characterization with MTS reagents, and studies showing that the adjacent pRTA causing mutation, G486R, alters the position of Thr485 significantly impairing transporter function.

**Conclusions:** Our findings indicate that NBCe1-A cotransports sodium and carbonate (rather than bicarbonate), with 1:2 charge transport stoichiometry. In patients, electroneutral T485S is predicted to transport sodium-bicarbonate from blood into proximal tubule cells thereby impairing transepithelial bicarbonate absorption representing a potentially new pathogenic mechanism for generating human pRTA.

*Funding:* NIDDK Support, Private Foundation Support

**SA-OR020**

**Epithelial Sodium Proton Exchanger, NHE3, Activity Is Enhanced by a Physical and Functional Interaction with Carbonic Anhydrase II, CAII** Deivshree Krishnan, Emmanuelle Cordat, R. Todd Alexander. *Univ of Alberta.*

**Background:** Seventeen hundred mg of NaCl is filtered by the glomerulus daily, more than two thirds is reabsorbed from the proximal tubule. The molecular determinants of proximal tubular $Na^+$ reabsorption are coupled to bicarbonate reabsorption and the cytosolic C-terminus of NHE3 contains a putative CAII binding site. We therefore hypothesize that NHE3 and CAII; molecules involved in the combined flux of $Na^+$ and $HCO_3^-$, physically and functionally interact to increase $Na^+$ reabsorption from the proximal tubule.

**Methods:** Physical interaction studies included: immunostaining of mouse kidney, a proximity ligation assay and a microtitre plate assay with NHE3 GST-fusion constructs and recombinant CAII. NHE3 was stably over-expressed in opossum kidney cells (OK-$NHE3_{38HA3}$) and activity measured as the rate of intracellular pH recovery, induced by an acid load and detected with the fluorescent ratiometric probe BCECF-AM. Acidification was induced by switching cells from a bicarbonate free medium, to one containing bicarbonate and 5% $CO_2$.

**Results:** Murine renal sections immunostained for CAII and NHE3 demonstrate colocalization in the brush border. That the two proteins were closer than 40 nm apart was revealed by a proximity ligation assay. A direct physical interaction was confirmed with GST fusion constructs. OK-$NHE3_{38HA3}$ cells demonstrate significantly greater recovery of intracellular pH than controls that is both $Na^+$ dependent and inhibited by 100 uM EIPA. Inhibition of endogenous CAII activity with acetazolamide significantly decreased NHE3 activity, an effect that was not observed when acidification was induced in the absence of $CO_2$ and $HCO_3^-$. To ascertain whether CAII binding per se activates NHE3, we over-expressed $NHE3_{38HA3}$ with: CAII, a catalytically dead CAII mutant (CAII-V143Y) or a binding mutant (CAII-HEX). These studies revealed an increase of NHE3 activity induced by CAII coexpression that was absent in the presence of the V143Y or HEX mutant.

**Conclusions:** These studies confirm that CAII binds to and increases NHE3 activity likely augmenting $Na^+$ and $HCO_{3[sup]-[/sup]}$ reabsorption from the proximal tubule.

*Funding:* Government Support - Non-U.S.

**SA-OR021**

**Kruppel-Like Factor 6 Regulates Mitochondrial Function in Podocytopathy** Sandeep K. Mallipattu,[1] Ruijie Liu,[1] Vivette D. D'Agati,[3] Peter Y. Chuang,[1] Roel Sterken,[2] Natalia Papeta,[2] Ali G. Gharavi,[2] John C. He.[1] *[1]Nephrology, Mount Sinai Medical Center; [2]Nephrology, Columbia Univ; [3]Pathology, Columbia Univ.*

**Background:** Podocyte injury resulting from mitochondrial dysfunction has been implicated in glomerular disease. However, transcription factors mediating mitochondrial damage in podocyte injury have yet to be identified.

**Methods:** We performed quantitative trait loci (eQTL) analysis of recently identified HIVAN susceptibility loci. Kruppel-like Factor 6 (KLF6), a zinc-finger binding transcription factor, was highly predictive to be causal for glomerulosclerosis. KLF6 expression was compared in resistant and nonresistant murine models of HIVAN. Mice with podocyte specific loss of KLF6, $Cre^+$ podocin $KLF6^{flox/flox}$ (C57BL/6 background), were generated to assess the role of KLF6 in adriamycin (ADR)-induced nephropathy.

**Results:** First, we confirmed the eQTL findings by qPCR. KLF6 expression was reduced in HIVAN-sensitive Tg26 mice (FVB/n) as compared to HIVAN-resistant Tg26 mice (C57BL/6). $Cre^+$ podocin $KLF6^{flox/flox}$ mice exhibited a significant reduction in kidney weight with a 3-fold increase in proteinuria at 12 weeks of age. In addition, ADR-treated $Cre^+$ podocin $KLF6^{flox/flox}$ mice as compared to ADR-treated wild-typemice resulted in nephrotic range proteinuria with significant glomerulosclerosis, podocyte effacement, and structural mitochondrial defects in podocytes at 5 weeks post ADR treatment. Promoter analysis predicted multiple KLF6 promoter binding sites on mitochondrial genes. Genes involved in mitochondrial replication, transcription, and oxidative phosphorylation were reduced in isolated glomeruli from ADR-treated $Cre^+$ podocin $KLF6^{flox/flox}$ mice. These findings were confirmed in ADR-treated cultured murine podocytes with and without KLF6. Finally, KLF6 expression was reduced in human FSGS and HIVAN as compared to healthy donor biopsy specimens.

**Conclusions:** We showed a podocyte-specific loss of KLF6 resulted in podocyte injury and FSGS in a resistant mouse background of ADR-induced nephropathy. This was associated with mitochondrial injury specific to podocytes. Combined, this suggests a critical role of KLF6 in regulating mitochondrial function and protecting podocytes against injury.

**SA-OR022**

**The Regerative Potential of Parietal Epithelial Cells in Adult Mice** Marcus J. Moeller, Katja Berger, Kevin Schulte, Jürgen Floege, Bart Smeets. *Nephrology and Clinical Immunology, RWTH Univ of Aachen, Aachen, NRW, Germany.*

**Background:** Several years ago, we have shown in newborn PEC-rtTA transgenic mice that a small portion of podocytes is recruited from cells of Bowman's capsule. This finding has raised hope that parietal epithelial cells (PECs) might be a potential progenitor cell population for podocytes.

**Results:** To investigate whether PECs replenish podocytes, PECs were genetically labeled in an irreversible fashion in 5 weeks-old adult mice. No significant increase in labeled podocytes was observed even after more than one year.

In order to induce a relative podocytopenia, uninephrectomies or 5/6 nephrectomies were performed, which resulted in progressive glomerular hypertrophy. However, here also no significant recruitment of labeled PECs onto the glomerular tuft was observed.

Labeled PECs only invaded the glomerular tuft, when glomerulosclerosis was induced by 5/6 nephrectomy and DOCA/salt overload. Thus, in sclerotic lesions PEC invasion onto the glomerular tuft played a negative role, not a regenerative role.

To explain our findings of PEC recruitment in juvenile mice, these experiments were repeated. Again, we found significant recruitment of labeled cells from Bowman's capsule onto the glomerular tuft – confirming our initial findings. However, when we genetically labeled podocytes in juvenile newborn Pod-rtTA transgenic mice, cells on Bowman's capsule were also directly labeled. These labeled cells were later also recruited onto the glomerular tuft to become fully differentiated podoctes. This indicates that in newborn mice a fraction of the cells on Bowman's capsule is already committed to directly differentiate into podocytes. At this early stage, these cells are directly marked by the PEC-rtTA mouse as well as the Pod-rtTA mouse.

**Conclusions:** In summary, PECs do not differentiate into podocytes in models of glomerular hypertrophy or in aging mice. Instead, our findings further strengthen the notion that PECs play a major role in glomerular diseases, specifically in FSGS and crescentic glomerulonephritis.

*Funding:* Government Support - Non-U.S.

## SA-OR023

**Epigenetic Role of Wolf-Hirschhorn Syndrome Candidate 1-Like 1 in Glomerulogenesis** Yugo Ito,[1] Zentaro Kiuchi,[1] Yukino Nishibori,[1] Kunimasa Yan.[1] *[1]Pediatrics, Kyorin Univ School of Medicine, Tokyo, Japan; [2]Medical Biochemistry and Biophysics, Karolinska institute, Stockholm, Sweden.*

**Background:** We previously reported that Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) is a novel epigenetic molecule in the podocytes, which activates nephrin promoter through tri-methylating HistoneH3K4 (2012 ASN Kidney Week). The present study aimed to explore the functional role of WHSC1L1 in glomerulogenesis.

**Methods:** Zebrafish embryos depleted of WHSC1L1 by using the antisense morpholino oligonucleotides (MO) against the translation initiation site, the donor sites on exon1 and the acceptor sites on exon3 were established. The phenotypic changes of the WHSC1L1 morphants at 96 hpf were observed and compared with control zebrafish. PAS staining and electron microscopy were performed to verify the histological change of pronephros and morphology of the podocytes and their foot processes. RT-PCR was performed to compare the expression level of nephrin mRNA in WHSC1L1 morphants and control zebrafish. Dextran dye filtration assay with the WHSC1L1 morphants was used to evaluate the function of glomerular filtration barrier.

**Results:** Disruption of WHSC1L1 expression in MOs was confirmed by RT-PCR and Western blot study. Morphant embryos injected with WHSC1L1 MOs developed cardiac edema, dorsal body axis curvature and short stature in 89.9% of the translation initiatoion site MO, 59.2% of the exon1 MO and 65.5% of the exon3 MO compared with 5.3% of the control. Morphants lacking WHSC1L1 revealed collapsed glomeruli associated with dysgenesis of the podocytes and foot processes. Morphants lacking WHSC1L1 displayed the reduction of nepnrin mRNA. Finally, permeability studies of the glomerular filtration barrier demonstrated a disruption of the selective glomerular permeability filter in morphants.

**Conclusions:** The present study suggests that WHSC1L1 plays a pivotal role in glomerulogenesis.

## SA-OR024

**Regulation of Glomerular DAF by HO-1** Maria Detsika,[1] Pu Duann,[2] Elias A. Lianos.[2] *[1]Medicine, Univ of Athens, Greece; [2]Div of Nephrology, Univ of Medicine and Dentistry of New Jersey.*

**Background:** Rat glomeruli express Decay accelerating factor (DAF) exclusively in glomerular epithelial cells (GEC) attenuating complement (C) activation-mediated injury. C activation also induces Heme oxygenase (HO)-1. Although HO-1 induction affords cytoprotection via heme degradation products, an alternative mechanism may involve DAF regulation. This was explored in normal rat glomeruli.

**Methods:** hmox1$^{+/-}$ and hmox1$^{-/-}$ rats were obtained by Zinc Finger Nuclease (ZFN)-mediated HO-1 gene disruption. Rats with GEC targeted HO-1 overexpression (GEC$^{HO-1}$) were generated by Sleeping Beauty Transposon mediated transgenesis using a nephrin promoter. Glomeruli isolated from wild type (WT) or hmox1$^{+/-}$, hmox1$^{-/-}$ or GEC$^{HO-1}$ rats were treated for 18h with Metalloporphyrins (MPs): Heme or Cobalt Protoporphyrin (CoPP) (HO inducers), Zinc (ZnPP) or Tin (SnPP) (HO inhibitors). Co-incubations of Heme and cycloheximide (CHX, protein synthesis inhibitor) were also performed. HO-1 and DAF protein and mRNA levels were assessed by western blot analysis and Real-time PCR.

**Results:** A 60-70% reduction in constitutive HO-1 levels and complete HO-1 absence was observed in hmox1$^{+/-}$ and hmox1$^{-/-}$ glomeruli, respectively. Constitutive DAF levels decreased by 2-fold and 4-fold in hmox1$^{+/-}$ and hmox1$^{-/-}$ glomeruli, respectively. Heme and CoPP dose-dependently (50-800 μM) induced DAF in WT glomeruli. Heme-induced DAF in both WT and transgenic animals but DAF induction in hmox1$^{+/-}$ and hmox1$^{-/-}$ glomeruli was attenuated. In WT glomeruli co-incubated with Heme and CHX, heme-mediated DAF induction was also attenuated. In GEC$^{HO-1}$glomeruli, constitutive DAF expression was increased and heme-mediated DAF induction was augmented. ZnPP and SnPP, (5-100μM) increased DAF levels in WT glomeruli. In co-incubations with heme, ZnPP and SnPP had an additive effect on DAF induction.

**Conclusions:** Glomerular DAF expression is HO-1 dependent. As well as through HO-1, Heme induces DAF via a non-HO-1 mechanism. Non-heme HO inhibitory MPs upregulate DAF indicating a HO-activity independent mechanism. MP-mediated DAF induction may point towards novel therapeutic strategies in C-dependent glomerular forms of injury.

## SA-OR025

**TRPC5 Inhibition Protects the Kidney Filter by Preventing Podocyte Cytoskeletal Collapse** Thomas Schaldecker,[1] Sookyung Kim,[1] Constantine Tarabanis,[1] Samy Hakroush,[1] Philip M. Castonguay,[1] Lisa Maria Buvall,[1] Astrid Weins,[1,2] Anna Greka.[1] *[1]Dept of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA; [2]Dept of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

**Background:** Kidney filter damage results in urinary albumin loss, a hallmark of cardiovascular disease and kidney failure. However, the earliest molecular events leading to filter damage are not well understood. Our previous studies have implicated the ion channel TRPC5 in podocyte injury.

**Methods:** We utilized TRPC5 knockout mice and two models of acute podocyte injury to study the role of TRPC5 *in vivo*. Using a novel small molecule inhibitor of TRPC5, we also probed whether pharmacologic inhibition of the channel confers protection from albuminuria, filter barrier collapse, and podocyte injury. We developed a method for calcium imaging in isolated kidney glomeruli in addition to live imaging of podocyte actin dynamics to investigate the role of TRPC5 in the initiation of kidney filter damage.

**Results:** Loss of TRPC5 or its inhibition abrogates podocyte cytoskeletal collapse, by blocking Rac1 and stabilizing synaptopodin. Importantly, genetic deletion or pharmacologic inhibition of TRPC5 protects mice from filter damage.

**Conclusions:** This study reveals TRPC5 as the $Ca^{+2}$ permeable channel responsible for filter barrier collapse, and identifies TRPC5 inhibition as a therapeutic strategy for the prevention or treatment of albuminuric disease.

*Funding:* NIDDK Support, Private Foundation Support

## SA-OR026

**Rac1 Activation in Podocytes Induces Rapid Foot Process Effacement and Proteinuria** Haiyang Yu,[1] Hani Suleiman,[1] Alfred Hyoungju Kim,[1] Jeffrey H. Miner,[3] Shreeram Akilesh,[4] Andrey S. Shaw.[1,2] *[1]Dept of Pathology and Immunology, Washington Univ in St. Louis, Saint Louis, MO; [2]Howard Hughes Medical Institute, Washington Univ in St. Louis, Saint Louis, MO; [3]Dept of Medicine, Washington Univ in St. Louis, Saint Louis, MO; [4]Dept of Pathology, Univ of Washington, Seattle.*

**Background:** The exact nature of foot process effacement is not known, but recent studies proposed that it might reflect the Rac1-induced actin reorganization. Here we inducible expressed constitutively active Rac1 specifically in podocytes in a transgenic mouse model, and observed rapid onset of foot process effacement and proteinuria after doxycycline induction, which was validated as a direct effect of active Rac1 by a super resolution imaging method.

**Methods:** Dox-inducible knock-in mouse model stochastic reconstitution optical microscopy (STORM).

**Results:** To test the effects of Rac1 activation *in vivo*, we generated a transgenic mouse with inducible expression of active-Rac1. The transgene was knocked into the *Hprt1* locus in ES cells by homologous recombination. Transgenic mice were generated and bred to the *Podocin*-rtTA strain and a new strain we generated, *Nephrin*-rtTA. Proteinuria was detectable after 2 days of doxycycline induction, peaked at day 4, and gradually decreased. The transgene were patchy-expressed, but the magnitude of proteinuria correlated with efficiency of transgene expression with the *Nephrin*-rtTA more broadly expressed. We validated focal foot process effacement by TEM and showed that the amelioration of proteinuria correlated with the loss of transgene-positive podocytes. The patchy expression of transgene allowed us to test whether areas of focal foot process effacement correlated with transgene expression. Correlating STORM with freeze-etch electron microscopy, we showed that only transgene positive podocytes were effaced.

**Conclusions:** Here we show that activation of Rac acutely induces foot process effacement. Our data support the hypothesis that foot process effacement is the *in vivo* correlate of Rac activation and that Rac activation may lead to podocyte loss.

## SA-OR027

**Brain Derived Nuerotrophic Factor (BDNF) Repairs Podocyte Damage by Inducing Actin Polymeryzation through Brain-Specific microRNAs** Maria Pia Rastaldi,[1] Min Li,[1] Silvia Armelloni,[1] Cristina Zennaro,[2] Alessandro Corbelli,[1] Masami Ikehata,[1] Anna Mondini,[1] Deborah Mattinzoli,[1] Changli Wei,[3] Jochen Reiser,[3] Piergiorgio Messa.[1] *[1]Renal Research Laboratory, Fondazione IRCCS Ospedale Maggiore Policlinico, Milano, Italy; [2]Laboratory of Renal Pathophysiology, Univ of Trieste, Trieste, Italy; [3]Dept of Internal Medicine, Rush Univ Medical Center, Chicago, IL.*

**Background:** Podocyte damage is the hallmark of focal segmental glomerulosclerosis (FSGS), a devastating glomerulopathy that needs better treatment. Podocytes are ramified cells sharing numerous properties with neurons and BDNF improves neuronal cell morphology and synaptic function upon TrkB binding.

**Methods:** BDNF was added to control and damaged (protamine sulfate, PS. Puromycin aminonucleoside, PA) podocytes. 3D endothelial-podocyte co-cultures were used for permeability tests. In D. Rerio larvae and Balb/c mice BDNF was administered after pericardial oedema and nephrotic proteinuria were induced by adriamycin (Adr). TrkB was silenced in vitro and in vivo.

**Results:** Increased number and length of processes, via LIMK-1 overexpression and cofilin phosphorylation, were observed in control podocytes after BDNF incubation. LIMK-1 protein increase was not paralleled by mRNA changes, leading to investigate

miRNA involvement, which demonstrated BDNF opposite actions on brain-specific miRNAs; reduced miRNA134 and increased miRNA132 directly and indirectly (by reducing p250GAP) alleviated the blockade on LIMK-1 translation. Double transfection experiments confirmed opposite effects on process formation, actin polymerization and cofilin phosphorylation.

In vitro, BDNF repaired podocyte damage due to PS or PA and reduced albumin permeability in 3D co-cultures.

In vivo, BDNF reduced pericardial oedema, restored podocyte morphology and nephrin and TrkB levels in D. Rerio larvae exposed to adriamycin. In Balb/c mice, adriamycin-induced glomerular damage was repaired by BDNF and proteinuria diminished. All effects were mediated by BDNF-TrkB interaction, being abolished by TrkB silencing.

**Conclusions:** On the whole, our results support the possibility that BDNF could be a promising option for treating proteinuric diseases characterized by podocyte injury.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## SA-OR028

**Loss of Prohibitin-2 in Podocytes Leads to Proteinuria and Renal Failure in Mice** Christina Ising,[1] Bernhard Schermer,[1] Andreas Linkermann,[2] Sebastian Braehler,[1] Dontscho Kerjaschki,[3] Christine E. Kurschat,[1] Thomas Benzing,[1] Paul T. Brinkkoetter.[1] *[1]1Dept II of Internal Medicine and Center for Molecular Medicine Cologne, Univ of Cologne, Cologne, Germany; [2]Div of Nephrology and Hypertension, Christian-Albrechts Univ, Kiel, Germany; [3]Clinical Institute of Pathology, Medical Univ of Vienna, Vienna, Austria.*

**Background:** SPFH domain-containing proteins support the formation of functional lipid microdomains within cellular membranes. Prohibitin-2 (PHB2), a member of this family, has recently been reported to control mitochondrial cristae morphogenesis and biogenesis of oxidative phosphorylation system complexes. Genetic deletion of *Phb2* in mice results in embryonic lethality.

**Results:** Here, we studied the role of PHB2 at the kidney filtration barrier by conditional deletion of *Phb2* gene expression in podocytes and demonstrate development of progressive proteinuria, glomerulosclerosis and endstage renal failure. In contrast to previous observations in proliferating fibroblasts this phenotype was not associated with mitochondrial apoptosis because we did not detect increased caspase-3 cleavage in post-mitotic podocytes, suggesting an additional, previously undefined function for PHB2 outside the apoptotic pathway. Immunofluorescence stainings and immunogold labeling localized PHB2 not only in mitochondria but also at the slit diaphragm, a specialized cell contact of podocytes at the kidney filter. Here, PHB1 and PHB2 interacted with podocin, another SPFH domain-containing protein essential for the assembly of the slit diaphragm complex. Given the structural similarity of the slit diaphragm complex in mammals and the mechanosensory complex in *C. elegans*, we studied localization and function of the PHB2 ortholog (PHB-2) in touch neurons of the nematode. Immunofluorescence stainings revealed co-localization of PHB-2 and the podocin ortholog MEC-2. Moreover, knockdown of either *phb-1* or *phb-2* impaired the function of the mechanosensory complex.

**Conclusions:** These data indicated that besides its well established role at the mitochondrial inner membrane, PHB2 may have an additional function at mechanosensory protein complexes.

## SA-OR029

**Increased Podocyte $[Ca^{2+}]_i$ Correlates with Cell Motility in an In Vivo Imaging Model of Kidney Fibrosis** James L. Burford, Karie G. Villanueva, Anne Riquier-Brison, Janos Peti-Peterdi. *Physiology and Biophysics, Univ of Southern California, Los Angeles, CA.*

**Background:** Recent genetic and cellular studies highlighted the role of podocyte calcium ($[Ca^{2+}]_i$) signaling and cell motility in the development of glomerulosclerosis. However, our mechanistic understanding of podocyte $[Ca^{2+}]_i$ dynamics is limited to a few calcium channels and knowledge learned from *in vitro* approaches. Recently, we developed a serial multiphoton microscopy (MPM) approach to directly visualize the changes in podocyte $[Ca^{2+}]_i$ in vivo in the intact kidney of mice with podocyte-specific $[Ca^{2+}]_i$ indicator GCaMP3. We used MPM to study the role of podocyte $[Ca^{2+}]_i$ in a disease state using the unilateral ureteral obstruction (UUO) model of progressive renal fibrosis.

**Methods:** Podocin Cre GCaMP3 mice were subjected to UUO and serial MPM at days 7-14 after UUO. Vasculature were labeled red with the plasma marker Alexa594-albumin. Specific glomeruli were imaged repeatedly every 24 hours using MPM and full z-scans to visualize podocyte $[Ca^{2+}]_i$, morphology, dynamics and glomerular architecture during disease progression.

**Results:** Intravital MPM imaging of Podocin Cre GCaMP3 mice found low baseline $[Ca^{2+}]_i$before UUO but heterogeneous increases in podocyte $[Ca^{2+}]_i$ between and within glomeruli after UUO. Some podocytes in different regions within the same glomeruli showed low baseline $[Ca^{2+}]_i$(average fluorescent intensity (Fi) of GCaMP3 Fi= 5.16), while regions where visceral-to-parietal adhesions and parietal podocyte layers developed due to podocyte migration/cell dedifferentiation showed >3-fold increases in podocyte $[Ca^{2+}]_i$(Fi =18.93, P<0.01). Podocyte $[Ca^{2+}]_i$ was significantly reduced by the iv administration of the purinergic receptor inhibitor suramin.

**Conclusions:** This study is the first time visual demonstration of the *in vivo* importance of podocyte $[Ca^{2+}]_i$ in glomerular pathology and provides evidence for the link between high podocyte $[Ca^{2+}]_i$ and increased cell motility, and their causative role in progressive glomerulosclerosis. P2 receptor-mediated purinergic $[Ca^{2+}]_i$ signaling in podocytes maybe an important new mechanism in podocyte injury and a promising new therapeutic target in glomerular disease.

## SA-OR030

**Podocyte Detachment Induced by PAI-1 and uPAR-Mediated β1 Integrin Internalization** Namiko Kobayashi,[1] Toshiharu Ueno,[1] Kumi Ohashi,[1] Kazuo Sakamoto,[1] Satoshi Hara,[1] Yasutoshi Takashima,[1] Taiji Matsusaka,[2] Toshio Miyata,[3] Michio Nagata.[1] *[1]Renal Pathology, Univ of Tsukuba, Tsukuba, Japan; [2]Internal Medicine, Tokai Univ School of Medicine; [3]United Centers, Tohoku Univ.*

**Background:** We previously reported that transgenic mice with podocyte-specific injury (NEP mice) showed microangiopathy at the site of podocyte injury, and plasminogen activator inhibitor-1 (PAI-1) was increased prior to the reduction of podocytes. Both PAI-1 and uPA bind to uPAR, and make complex with integrin on cell membrane. Internalization of the complex induces the cell detachment as a result of reduction of cell-matrix adhesion molecules. The present study was aimed to show that PAI-1 was involved in podocyte detachment through the complex with uPAR-integrin by using NEP mice and podocyte cell line.

**Methods:** Two groups of NEP mice, with or without PAI-1 inhibitor (PI) were induced podocyte injury by LMB2 injection on day 0. PI was administered from day 0 to 12. Histological and clinical parameters were analyzed on day 12. Then, we treated cultured podocytes either with PAI-1/uPA complex (P/U), uPA (control), or antibody for blocking uPAR with P/U (B-P/U). After incubation, attached cells were counted, and localization of β1 integrin and uPAR was detected by immunofluorescence. Cytoplasmic β1 integrin was analyzed by Western blot.

**Results:** Proteinuria (P) and Thronbi score (T) in PI group were lower than the control (P; 64.29 ± 23.30 vs. 161.12 ± 34.0; p <0.05, T; 0.01 ± 0.01 vs. 0.23 ± 0.07, p <0.05), and podocyte numbers were preserved (9.41 ± 0.45 vs. 2.67 ± 0.41, p <0.0001). Glomerular morphology in PI group was preserved. In vitro, attached cells in P/U were reduced compared with the control and B-P/U (p <0.01). Confocal microscopy showed that β1 integrin and uPAR were colocalized (Pearson's coefficient (PC) = 0.50) and shifted to cytoplasm in P/U. In contrast, β1 integrin remained on the membrane and was not colocalized with uPAR in the control and B-P/U (PC = 0.12, 0.06, respectively). In Western blot, β1 integrin expression was increased in P/U.

**Conclusions:** PAI-1/uPA complex may act on the podocytes detachment via internalization of β1 integrin through the uPAR mechanism.

## SA-OR031

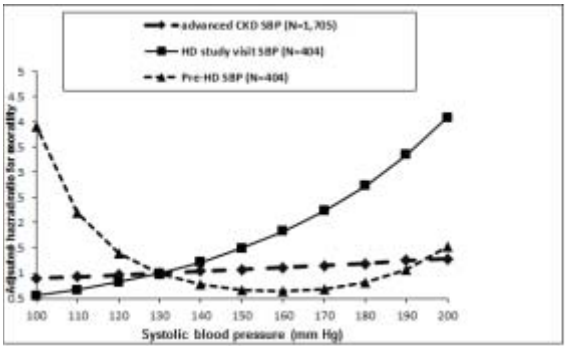
**Blood Pressure and Mortality at Advanced CKD and Incident ESRD: The CRIC Study** Nisha Bansal,[1] Raymond R. Townsend,[2] Mahboob Rahman,[3] Charles E. McCulloch,[1] Alan S. Go,[4] Arnold B. Alper,[5] Amanda Hyre Anderson,[2] John W. Kusek,[6] Claudia M. Lora,[7] Eva Lustigova,[5] Chi-Yuan Hsu.[1] *[1]UCSF; [2]UPenn; [3]Case Western; [4]KPNC; [5]Tulane; [6]NIDDK; [7]UIC.*

**Background:** In observational studies of hemodialysis (HD) patients, lower blood pressure (BP), even within the "normal" range, is associated with higher mortality. Whether this paradoxical association is present in advanced CKD is unknown.

**Methods:** Participants of the Chronic Renal Insufficiency Cohort had standardized BP measures at yearly study visits. We studied the association between mortality and systolic BP (SBP) measured at the: 1) first study visit after reaching eGFR of <30 ml/min/1.73m$^2$ ("advanced CKD SBP") (N=1,705); 2) first study visit after starting HD ("HD study visit SBP")(N=404); and 3) HD unit ("pre-HD SBP")(N=404). We performed a survival analysis, fitting a quadratic term for BP.

**Results:** Among 1,705 participants with advanced CKD, mean eGFR was 25±4 ml/min/1.73 m$^2$ and SBP was 131 (±24) mm Hg. There were 390 deaths over 4.7 (±2.3) years. Adjusting for demographics, tobacco use, BMI, diabetes, and cardiovascular disease, there was a linear association between advanced CKD SBP and mortality. Among the 404 participants who subsequently started HD, mean pre-HD SBP was 152 (±22) mm Hg and HD study visit SBP was 132 (±25) mm Hg. There were 98 deaths after the start of HD over 2.8(±1.7) years. A U-shaped association between pre-HD SBP and mortality was observed. There was a linear association between HD study visit SBP and mortality (**Figure**).

**Conclusions:** At advanced CKD, higher SBP was associated with higher mortality. Among those who started HD, a U-shaped association between pre-dialysis SBP and mortality was observed. In contrast, there was a linear association between mortality and SBP measured outside the HD unit, which may be a more appropriate target for therapy.




*Funding:* NIDDK Support

## SA-OR032

**Masked and Sustained Hypertension Are Associated with Increases in Left Ventricular Mass and Pulse Wave Velocity in CKD – The CRIC Study**

Paul E. Drawz,[1,2] Arnold B. Alper,[2] Amanda Hyre Anderson,[2] Denise C. Babineau,[2] Carolyn S. Brecklin,[2] Jeanne Charleston,[2] Jing Chen,[2] Yonghong Huan,[2] Susan P. Steigerwalt,[2] Jonathan J. Taliercio,[2] Raymond R. Townsend,[2] Matthew R. Weir,[2] Mahboob Rahman.[2] *[1]Univ of Minnesota; [2]CRIC Investigators.*

**Background:** The goal of this study was to evaluate whether white-coat (WCH), masked (MH), and sustained hypertension (SH) are associated with increases in left ventricular mass index (LVMI) and pulse wave velocity (PWV).

**Methods:** In the Chronic Renal Insufficiency Cohort study, 24 hour ambulatory blood pressure (ABP) was measured between 2008 and 2012. Clinic blood pressure (BP) was measured in triplicate. WCH was defined by a clinic BP ≥140/90mmHg and daytime ABP <135/85mmHg; MH by a clinic BP <140/90mmHg and daytime ABP ≥135/85mmHg; and SH by a clinic BP ≥140/90mmHg and daytime ABP ≥135/85mmHg. Echocardiograms were obtained and left ventricular mass was indexed to height$^{2.7}$. PWV was measured in the supine position using the right carotid and femoral arteries. Creatinine was measured annually. Urine protein was measured in a 24 hour sample at baseline.

**Results:** ABP was obtained in 1439 participants; echocardiography was obtained in 1230 and PWV in 1345 of these participants. In adjusted linear models, both MH and SH were associated with increased LVMI and PWV (table). In adjusted logistic regression models, MH was associated with increased odds for a PWV >10m/sec and SH was associated with increased odds for both left ventricular hypertrophy (LVH) and a PWV >10m/sec.

| | LVMI | LVH | PWV | PWV > 10m/sec |
|---|---|---|---|---|
| BP category | Beta (95% CI) | OR (95% CI) | Beta (95% CI) | OR (95% CI) |
| Normotensive | l | l | l | l |
| WCH | -1.04 (-3.9 to 1.9) | 0.84 (0.5 to 1.4) | 0.50 (-0.2 to 1.2) | 1.49 (0.8 to 2.8) |
| MH | **1.61 (0.1 to 3.1)** | 1.12 (0.9 to 1.5) | **0.82 (0.4 to 1.2)** | **2.48 (1.7 to 3.5)** |
| SH | **4.22 (2.5 to 6.0)** | **1.96 (1.4 to 2.7)** | **1.22 (0.8 to 1.7)** | **2.04 (1.4 to 3.0)** |

**Conclusions:** In a large cohort of participants with CKD, both MH and SH were associated with vascular injury and target organ damage. Ambulatory BP may better characterize the relationship between BP and adverse outcomes in patients with CKD.

*Funding:* NIDDK Support, Other NIH Support - Funding for the CRIC Study was obtained under a cooperative agreement from National Institute of Diabetes and Digestive and Kidney Diseases (U01DK060990, U01DK060984, U01DK061022, U01DK061021, U01DK061028, U01DK060980, U01DK060963, and U01DK060902). In addition, this work was supported in part by: the Perelman School of Medicine at the University of Pennsylvania Clinical and Translational Science Award NIH/NCATS UL1TR000003, Johns Hopkins University UL1 TR-000424, University of Maryland GCRC M01 RR-16500, Clinical and Translational Science Collaborative of Cleveland, UL1TR000439 from the National Center for Advancing Translational Sciences (NCATS) component of the National Institutes of Health and NIH roadmap for Medical Research, Michigan Institute for Clinical and Health Research (MICHR) UL1TR000433, University of Illinois at Chicago CTSA UL1RR029879, Tulane University Translational Research in Hypertension and Renal Biology P30GM103337, Kaiser Permanente NIH/NCRR UCSF-CTSI UL1 RR-024131, and a Career Development Award from NIDDK (PED-K23DK087919).

## SA-OR033

**The Clinical Impact of Renal Artery Denervation on Blood Pressure and Renal Function in Patients with Uncontrolled Hypertension: From the Global SYMPLICITY Registry**

Luis M. Ruilope,[1] Markus Schlaich,[2] Roland E. Schmieder,[3] Giuseppe Mancia,[4] Krzystof Narkiewicz,[5] Bryan Williams,[6] Felix Mahfoud,[7] Michael Böhm.[7] *[1]Universidad Autonoma, Madrid, Spain; [2]Baker IDI Heart and Diabetes Institute, Melbourne, Australia; [3]Univ of Erlangen-Nürnberg, Erlangen, Germany; [4]Univ of Milan-Bicocca, Milan, Italy; [5]Medical Univ of Gdansk, Gdansk, Poland; [6]Univ College London, United Kingdom; [7]Univ of Saarland, Homburg, Germany.*

**Background:** Renal artery denervation (RDN) has been demonstrated to safely lower blood pressure (BP) in controlled clinical trials. To further evaluate the safety and effectiveness of this minimally invasive treatment in "real world" patients with hypertension the Global SYMPLICITY Registry (GSR) was initiated.

**Methods:** The GSR is a prospective, multicenter (~200 sites), open-label study designed to evaluate the effect of RDN with the Symplicity™ renal denervation system in adults with uncontrolled hypertension. Data collected includes office BP measurements at each follow-up, renal function, procedural events and safety events possibly related to RDN.

**Results:** Baseline characteristics for 409 patients with baseline BP ≥160 mmHg (150 mmHg for diabetic patients) include mean age 60±12 yrs, 65% males, and 47% with diabetes. Mean baseline BP was 175.4± 17.6/ 94.3 ± 15.2 mmHg and baseline eGFR was 78±21 mL/min/1.73m$^2$; 97% of patients had an eGFR >45 mL/min/1.73m$^2$. BP dropped -16.5±21.5 /-7.1±13.3 mmHg (n=247) at 3 months and -17.1±19.5/-8.2±12.3 mmHg (n=134) at 6 months (p<0.001 for both). At 3 and 6 months post-RDN mean eGFR was 75±19 and 76±17 mL/min/1.73m$^2$. Patients with baseline eGFR 45-60 mL/min/1.73m$^2$ had similar significant drops in BP at 3 and 6 months (-15.6±20.5/-5.5±12.9 mmHg and -20.4±21.6/-9.3±13.2 mmHg) to those with baseline eGFR 60-90 (-13.8±21.5/-5.8±13.0 mmHg and -15.7±20.9/-7.7±12.0 mmHg). No serious procedural-related complications or adverse events related to RDN were reported.

**Conclusions:** RDN using the Symplicity catheter significantly and safely lowers BP in patients with uncontrolled hypertension. Renal function does not impact the drop in blood pressure.

*Funding:* Pharmaceutical Company Support - Medtronic, Inc

## SA-OR034

**Apolipoprotein L1 Gene Variants Associate with Increased Systolic and Diastolic Blood Pressure in Younger African Americans Independent of Renal Function**

Girish N. Nadkarni, Erwin P. Bottinger. *Dept of Nephrology, Mount Sinai Icahn School of Medicine.*

**Background:** Polymorphisms at the APOL1 locus confer a substantial increase in risk of non-diabetic renal diseases in African Americans, including renal disease associated with hypertension. Little evidence has emerged to date, to suggest that APOL1 variants can increase hypertension risk independent of kidney disease. We aimed to assess the association of these variants and blood pressure in African Americans.

**Methods:** We utilized PCR and ASPE APOL1 G1/G2 genotype testing. The clinical and biochemical parameters were abstracted using the electronic health record (EHR) from consented Mount Sinai Biobank participants. The primary outcome variables of interest were systolic and diastolic blood pressures (SBP and DBP) averaged over a nine-year period. We utilized multivariable logistic regression to assess this association.

**Results:** We had data on 4765 participants. Of these, 36% were male, 36% had a history of diabetes mellitus and 42% had a history of antihypertensive medication use. The mean age was 53 years. Homozygous APOL1 risk genotype was present in 700(14.7%). With participants less than 50 years of age (n=1478), the APOL1 risk genotype was present in 206(14%). Both the SBP and DBP were significantly higher with homozygous risk genotype (126 vs. 124 mmHg; p=0.01 and 76 vs. 74 mmHg; p=0.04 respectively). These participants were 2.5 times as likely to have a SBP>160 mm Hg (OR 2.54; 95% CI 1.25- 5.19; p=0.01) and 2 times as likely to have a DBP>90 mm Hg (OR 2.01; 95% CI 1.26- 3.21; p=0.01). This association remained significant after adjusting for potential confounders including the mean estimated glomerular filtration rate (eGFR).

**Conclusions:** We describe a significant association between homozygous APOL1 risk genotype status and higher SBP and DBP in African Americans < 50 years independent of kidney function. Whether this relationship is causative or mediated by a yet unknown action of APOL1 warrants further investigation.

## SA-OR035

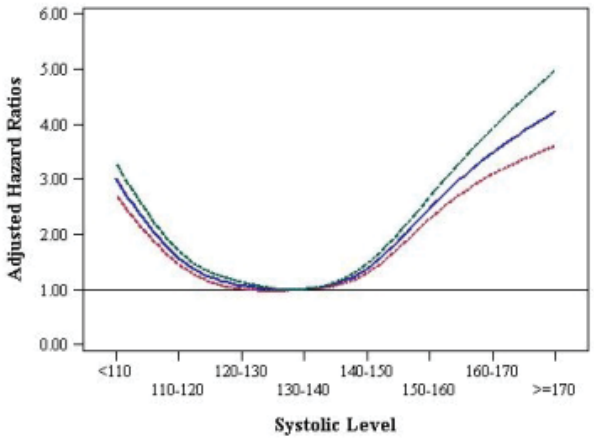
**Evaluation of Blood Pressure and Risk of ESRD/Mortality in Resistant Hypertension**

John J. Sim,[1] Jiaxiao Shi,[1] Csaba P. Kovesdy,[2] Kamyar Kalantar-Zadeh.[3] *[1]Nephrology & Hypertension, Kaiser Permanente Los Angeles Med Ctr, Los Angeles, CA; [2]Memphis VA Med Ctr; [3]UC Irvine Med Ctr.*

**Background:** Blood pressure (BP) goals in hypertension (HTN) have been challenged. Even less is known about resistant hypertension (RH) in terms of the comparative risk of BP on clinical outcomes. RH likely has chronic physiologic compensations including vascular remodeling and higher threshold perfusion BP. We hypothesize that a similar J shaped curve exists within RH and furthermore the nadir BP associated with best ESRD and mortality outcomes are higher than what is observed in the general HTN population.

**Methods:** Retrospective longitudinal study of Kaiser Permanente Southern California health system (1/1/2006-12/31/2009) performed. RH defined as HTN individuals with uncontrolled BP on 3 meds or requiring >/= 4 meds. Demographic, co morbidity, laboratory, medication, and outcomes data extracted from the electronic medical records. ESRD defined as eGFR<15, dialysis, or renal transplant. Serial BP during and up to 4 years follow up averaged and multivariable hazard ratios (HR) calculated for every 10 mm Hg increment systolic BP (SBP) using 130-139mm as reference.

**Results:** The RH cohort of 50,020 was comprised of 43% females, 46% whites, 19% blacks, 18% Hispanics, and 6% Asians. Mean age was 70 yrs. Adjusted HR (95% CI) for combined ESRD/mortality compared to SBP 130-139mm group were 3.0 (3.7-3.3), 1.6 (1.5-1.7), 1.1 (1.0-1.1), 1.4 (1.3-1.5), 2.5 (2.3-2.7), 3.2 (3.1-3.9), & 4.2 (3.6-5.0) for SBP <110, 110-119, 120-129, 140-149, 150-159, 160-169, & >169 respectively. Males, blacks, CKD, & DM also had increased HR for ESRD/mortality (not shown).




**Conclusions:** Among a large ethnically diverse RH cohort, we found a J curve in BP and risk for ESRD/Mortality with the nadir systolic BP at 130-139. This was not different to what was observed in our general HTN population (not shown).

*Funding:* Private Foundation Support

## SA-OR036

**Renal Artery Denervation Can Safely Lower Blood Pressure in Patients with Severe Treatment-Resistant Hypertension: Results from the Symplicity HTN Trials** Markus Schlaich,[1] Henry Krum,[2] Murray Esler,[1] Michael Böhm,[4] Roland E. Schmieder.[3] *[1]Baker IDI Heart and Diabetes Institute, Melbourne, Australia; [2]Monash Univ, Melbourne, Australia; [3]Univ Erlangen-Nürnberg, Erlangen, Germany; [4]Univ of Saarland, Homburg.*

**Background:** The sympathetic nervous system is an important contributor to the pathogenesis of hypertension. Renal artery denervation (RDN) via delivery of low power radiofrequency energy with the Symplicity™ RDN catheter reduces sympathetic nerve activity and blood pressure (BP). The Symplicity HTN-1 and Symplicity HTN-2 trials evaluated the safety and effectiveness of RDN in subjects with severe treatment-resistant hypertension (systolic BP >160 mmHg or >150 mmHg for subjects with diabetes while on ≥3 antihypertensive drugs, including a diuretic).

**Methods:** Inclusion criteria included an eGFR ≥45 mL/min/1.73 m$^2$. Renal imaging was performed pre-procedure and renal arteries <4mm in diameter or <20 mm in length or with significant abnormalities were excluded. Changes in office BP, serum chemistries, and all RDN-related safety events were followed every 6 months.

**Results:** A total of 239 subjects (mean age 57 yrs) were treated. Mean baseline BP was 178±18/98±15 mmHg and mean baseline eGFR was 82.7±19.9 mL/min/1.73 m$^2$. BP was significantly lower than baseline after RDN by -24±23/-10±13 mmHg at 6 months, -26±24/-12±13 mmHg at 1 yr and -30±24/-13±14 mmHg at 2 yrs (p<0.01 for all). Mean eGFR was 81.7±20.2 at 6 months and 80.8±18.6 mL/min/1.73 m$^2$ at 1 yr. Mean eGFR was 76.8 ± 22.8 mL/min/1.73 m$^2$ in 43 subjects followed through 2 yrs. Procedural complications included 2 renal artery dissections and 4 pseudoaneurysms all treated without further sequelae. Bradycardia required treatment during RDN in 10.7% of subjects. Transient acute renal failure deemed unrelated to RDN occurred in 3 patients. There was one progression of a pre-existing stenosis unrelated to RDN and one new moderate stenosis that did not require treatment.

**Conclusions:** RDN significantly lowers BP without adverse consequences on renal function. Further studies are warranted to assess a potential renoprotective role of RDN in patients with both normal and impaired renal function.

*Funding:* Pharmaceutical Company Support - Medtronic, Inc

## SA-OR037

**Preeclampsia and End-Stage Renal Disease: A United States Renal Data System Linkage Study** Andrea G. Kattah,[1] Sanket Agarwal,[1] Dawn C. Scantlebury,[4] Michelle M. Mielke,[2] Amy L. Weaver,[2] Wendy White,[3] Reem A. Asad,[1] Vesna D. Garovic.[1] *[1]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Div of Health Sciences Research, Mayo Clinic, Rochester, MN; [3]Dept of Obstetrics and Gynecology, Mayo Clinic, Rochester, MN; [4]Div of Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

**Background:** Preeclampsia has been implicated in the future development of hypertension (HTN) and renal disease. Recent data from registry-based studies suggest that preeclampsia is a risk factor for end-stage renal disease (ESRD). However, the magnitude of the association and the contributions of comorbidities remain unknown.

**Methods:** We performed a case-control study using a cohort of Olmsted County, MN residents who gave birth between 1976-1982 (n=8362). The ESRD cases were identified by linkage with the United States Renal Data System (USRDS); cases were matched to 2 controls based on maternal date of birth, age at first pregnancy, parity and length of follow-up. Medical records from all pregnancies were reviewed for evidence of preeclampsia, preeclampsia superimposed on HTN, or eclampsia and for comorbidities, including diabetes mellitus (DM) and HTN. Exclusion criteria were ESRD prior to pregnancy and refusal of research authorization.

**Results:** A total of 23 cases of ESRD were identified; 2 had ESRD prior to pregnancy and 1 denied authoriziation for medical record review, leaving 20 available ESRD cases for analyses. The mean (SD) age at diagnosis of ESRD was 52.6 (8.1) years. Per chart review, 8/20 (40%) cases vs. 5/40 controls (12.5%) had preeclampsia, preeclampsia superimposed on HTN or eclampsia (OR 4.5, 95% CI 1.1-21.4, p=0.04). DM and HTN were more common in cases than controls (50% vs. 15%, 80% vs. 45%, respectively). After adjusting for DM (OR 3.5, 95% CI 0.7-18.1, p=0.13) and HTN (OR 3.1, 95% CI 0.7-15.5, p=0.17), the OR for ESRD was attenuated.

**Conclusions:** Preeclampsia is associated with a higher odds of ESRD. However, after adjusting for DM and HTN, the association was attenuated and no longer significant. Larger population-based studies that rely on chart review or prospective studies are needed to confirm the association of preeclampsia and ESRD.

*Funding:* Other NIH Support - P50 AG 44170 from the National Institute on Aging, and the Society for Womens Health Research (SWHR) ISIS Network award

## SA-OR038

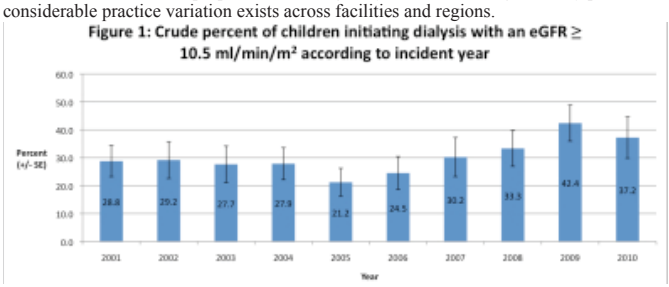
**Variability in Timing of Dialysis Initiation in Children** Allison Dart,[1,7] Susan M. Samuel,[2,7] Manish M. Sood,[1,7] R. Todd Alexander,[3,7] Steven Arora,[6,7] Robin L. Erickson,[4,7] Braden J. Manns,[2,7] Michael Zappitelli.[5,7] *[1]Univ of Manitoba; [2]Univ of Calgary; [3]Univ of Alberta; [4]Univ of Saskatchewan; [5]McGill Univ; [6]McMaster Univ; [7]Canadian Kidney Knowledge Translation and Generation Network (CANN-NET).*

**Background:** The benefits of initiating chronic dialysis in children must be balanced against increased complexity of care and potential complications. We assessed variability in eGFR at dialysis initiation and evaluated the association of patient, facility and regional factors with early vs. late dialysis initiation.

**Methods:** Incident Canadian dialysis patients <22 yo, between 2001-2010 from 9 Canadian provinces were identified from the Canadian Organ Replacement Registry. The outcome was early *vs.* late dialysis initiation (eGFR ≥ *vs.* < 10.5 ml/min/1.73m$^2$). We examined the associations of age at dialysis initiation, sex, ethnicity, ESRD etiology, distance from nearest pediatric facility, initial modality (peritoneal *vs.* hemodialysis), income quintile, era (2001-05 *vs.* 2006-10), pediatric *vs.* adult facility, and geographic region (West, Ontario, East), on the outcome using multiple variable logistic regression models.

**Results:** Thirty % of 540 children started dialysis with eGFR ≥10.5ml/min/1.73m$^2$ (21-31% across regions; 12-79% across facilities). This proportion increased from 29% in 2001 to 37% in 2010 (Figure 1). Mean eGFR at dialysis initiation by facility was also variable (7-15 ml/min/1.73m$^2$). Factors independently associated with early start were genetic cause of ESRD (OR=3.2; 95% CI 1.5-6.7) and later era (OR=1.5; 95% CI 1.0-2.3). Sensitivity analysis using eGFR threshold of 12 ml/min/1.73m$^2$ revealed similar results.

**Conclusions:** Approximately one third of children initiated dialysis with eGFR ≥ 10.5 ml/min/1.73m$^2$. The proportion initiating early increased during the study period and considerable practice variation exists across facilities and regions.




*Funding:* Private Foundation Support

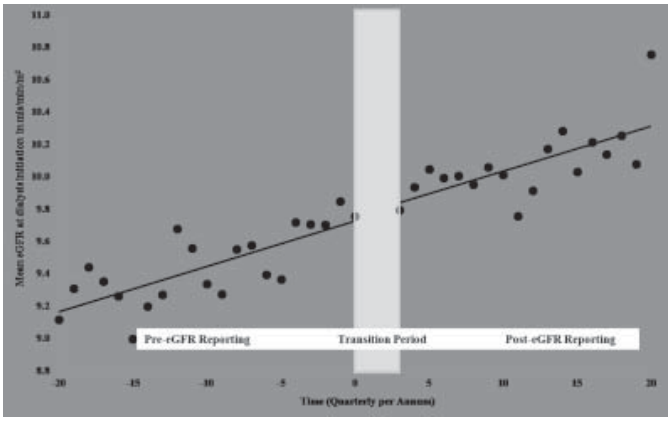
## SA-OR039

**The Impact of Estimated Glomerular Filtration Rate Reporting on the Timing of Dialysis Initiation** Manish M. Sood,[1] Paul Komenda,[2] Claudio Rigatto,[2] Brett M. Hiebert,[3] Navdeep Tangri.[2] *[1]Medicine, St. Boniface Hospital, Winnipeg, Canada; [2]Medicine, Seven Oaks Hospital, Winnipeg, Canada; [3]Cardiac Sciences, St. Boniface Hospital, Winnipeg, Canada.*

**Background:** Automatic reporting of the estimated glomerular filtration rate (eGFR) by laboratories has been widely implemented during the last decade. Concurrently, a steady increase in the eGFR at dialysis initiation has been reported. We hypothesize that automatic eGFR reporting may be leading to earlier dialysis initiation, based on level of eGFR, rather than clinical symptoms. The purpose of this study was to examine trends in the eGFR at dialysis initiation before and after eGFR reporting.

**Methods:** All incident dialysis patients from four Canadian provinces that implemented, province-wide, automatic laboratory reporting of eGFR were included in the study (N=27, 088). Data were obtained from the Canadian Organ Replacement Registry (CORR) from Jan 1, 2001 to Dec 31, 2010. The primary outcome was the mean eGFR level at dialysis initiation. An interrupted time series and adjusted multilevel models were used to determine the change in eGFR at dialysis initiation, pre- and post-reporting.

**Results:** We observed a linear increase in the mean eGFR at dialysis initiation from 9.1 to 10.8 mls/min during the study period. In an unadjusted interrupted time series analysis, there was no change in the trajectory of the eGFR at dialysis initiation, before or after eGFR reporting. In multi-level models, there was no change in the slope of the eGFR at dialysis initiation after adjustment for case-mix and facility characteristics (post-eGFR reporting X time interaction p=0.6).




**Conclusions:** Automatic laboratory-based eGFR reporting did not impact the eGFR at dialysis initiation in a large incident hemodialysis cohort. Concerns that widespread eGFR reporting leads to earlier dialysis initiation are not supported by this study.

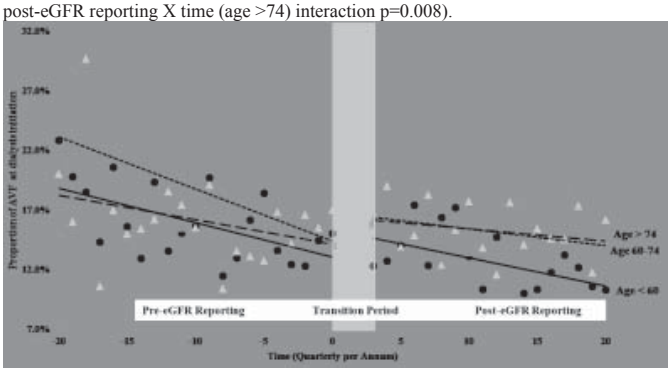
## SA-OR040

**The Impact of Estimated Glomerular Filtration Rate Reporting on the Initiation of Dialysis with an Arteriovenous Fistula** Manish M. Sood,[1] Paul Komenda,[2] Claudio Rigatto,[2] Brett M. Hiebert,[3] Navdeep Tangri.[2] *[1]Medicine, St. Boniface Hospital, Winnipeg, Canada; [2]Medicine, Seven Oaks Hospital, Winnipeg, Canada; [3]Cardiac Sciences, St. Boniface Hospital, Winnipeg, Canada.*

**Background:** The effect of automatic laboratory reporting of the estimated glomerular filtration rate (eGFR) on dialysis-related quality of care remains unknown. We set out to examine trends in the presence of an AVF at dialysis initiation before and after eGFR reporting.

**Methods:** All incident dialysis patients from four Canadian provinces that implemented province-wide, automatic laboratory reporting of eGFR with a known vascular access at dialysis initiation were included in the study (N=25,201). Data were obtained from the Canadian Organ Replacement Registry (CORR) from Jan 1, 2001 to Dec 31, 2010. The primary outcome was the proportion of AVF use at dialysis initiation. An interrupted time series and multilevel models adjusted for patient and facility characteristics were used to determine the change in AVF proportion pre- and post-reporting.

**Results:** The proportion of patients initiating hemodialysis with an AVF use decreased gradually over the study period from 19.0 to 14.6%. In an interrupted time series analysis, there was improvement in AVF usage at dialysis initiation after eGFR reporting, and this effect was predominantly seen in patients over 60 years. In multi-level models, there was improvement in the slope for AVF usage at dialysis initiation after adjustment for case-mix and facility characteristics, predominantly in patients over 60 (post-eGFR reporting X time (age < 60) interaction p=0.3, post-eGFR reporting X time (age 60-74) interaction p=0.01, post-eGFR reporting X time (age >74) interaction p=0.008).




**Conclusions:** Automatic laboratory-based eGFR reporting is associated with an increased likelihood of starting hemodialysis with an AVF in patients over 60.

## SA-OR041

**Early Nephrology Referral Reduces Economic Costs during the Period before and after Initiation of Renal Replacement Therapy: Comprehensive Prospective Study of the Clinical Research Center for End Stage Renal Disease in Korea** Jeonghwan Lee,[1] Jung Pyo Lee,[2] Hye Min Jang,[3] Ji-Young Choi,[4] Yong-Lim Kim,[4] Chul Woo Yang,[5] Shin-Wook Kang,[6] Yon Su Kim,[7] Chun Soo Lim.[2] *[1]Dept of Internal Medicine, Hallym Univ Hangang Sacred Heart Hospital, Seoul, Korea; [2]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea; [3]Dept of Statistics, Kyungpook National Univ, Daegu, Korea; [4]Dept of Internal Medicine, Kyungpook National Univ School of Medicine, Daegu, Korea; [5]Dept of Internal Medicine, The Catholic Univ of Korea College of Medicine, Seoul, Korea; [6]Dept of Internal Medicine, Yonsei Univ College of Medicine, Seoul, Korea; [7]Dept of Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea.*

**Background:** We evaluated the health care costs according to referral time of patients with CKD to a nephrology clinic.

**Methods:** A total of 941 patients who start dialysis from 2008 to 2011 were prospectively enrolled. Early referral was defined as patients who were referred to a nephrologist more than a year before dialysis and visited nephrology 2 or more times, and other patients whose referral time was less than a year were considered as late referral. Cost information was acquired from the claim data of Korea Health Insurance Review and Assessment Service.

**Results:** Total amount of medical costs during the first 12 months after initiation of dialysis was not different between the two groups. However, costs during the first 1 month of early referral patients were lower than those of late referral patients (3029 ± 2219 versus 3438 ± 2821 US dollars, P = 0.025). Total 12 months health care costs before initiation of dialysis were lower in the early referral group (6206 ± 5873 versus 8610 ± 7820 US dollars, P < 0.001). In multivariable analysis, early referral reduced significantly health care costs (2513 ± 443 US dollars, P < 0.001) during the 12 months before dialysis start and the first month (400 ± 182 US dollars, P = 0.028) after initiation of renal replacement therapy.

**Conclusions:** Early referral of CKD patients to a nephrologist is associated with decreased medical costs during the predialysis and early period of dialysis.

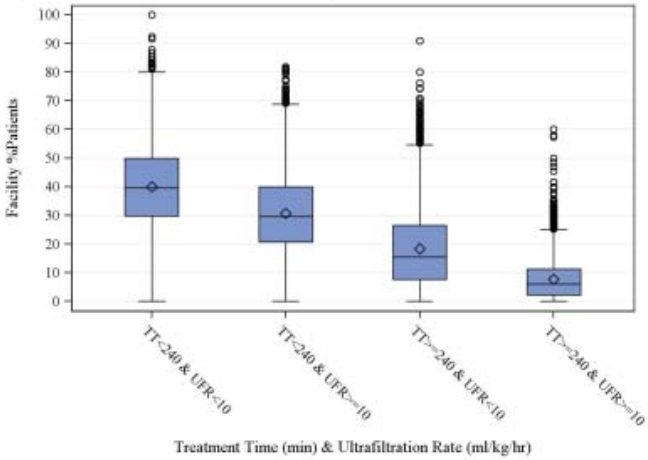
## SA-OR042

**Hemodialysis Facility and Patient Level Treatment Time and Ultrafiltration Rate Practices in the United States** Rajiv Saran,[1] Brett W. Plattner,[1] Chad M. Cogan,[2] Casey Parrotte,[1] Alissa Kapke,[2] Yi Li.[1] *[1]Univ of Michigan; [2]Arbor Research Collaborative for Health.*

**Background:** Longer dialysis treatment time(TT) and lower ultrafiltration rates(UFR) are associated with lower mortality in hemodialysis(HD) patients(pts). However, TT<240 min is still common in the US. We sought to examine US dialysis practice with respect to TT and UFR and characterizing pts receiving longer TT.

**Methods:** May 2012 CROWNWeb data on 235,629 pts from 4,313 dialysis facilities(≥10pts) were analyzed. 3x/week adult HD pts with residual urea clearance <2.0mL/min/1.73m² or not reported were included. TT was categorized: <180, 180-210, 210-240 and ≥240 min. UFR categories were <10, 10-13 and >13ml/kg/hr. A logistic regression model fitted via generalized estimating equations assessed the associations between pt characteristics and TT while adjusting for clustering in facilities.

**Results:** 66% of pts received TT <240 min. Based on the estimated regression coefficients, pt characteristics significantly associated with TT>240 min included younger age, men, African American race, shorter dialysis vintage, higher intradialytic weight loss, congestive heart failure, diabetes mellitus, higher BMI, and lower UFR.




The figure displays the variation in the distributions of clinically relevant TT/UFR combinations at the facility level. In a median US dialysis facility 15% of pts received TT>240 min and UFR<10. In contrast, 50% US dialysis facility had at least 40% pts receiving TT<240 min and UFR<10, and 30% pts receiving TT<240 min and UFR≥10.

**Conclusions:** In current US dialysis practice, the majority of pts on 3x/week dialysis have a TT<240 mins. Pt-level analysis suggests that longer TT in the US is utilized among pts with adverse prognostic factors. A proportion of facilities are able to provide TT>240 min and lower UFR to a sizable number of pts, demonstrating the potential feasibility of expanding this practice pattern.

*Funding:* Other U.S. Government Support

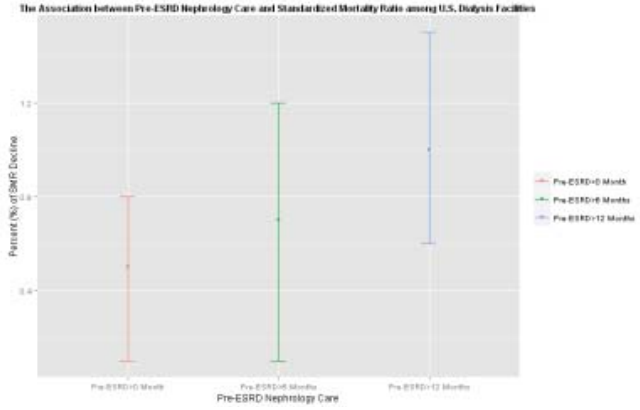
## SA-OR043

**Nephrology Care Influence Patient Mortality among United States Dialysis Facilities** Hua Hao,[1] Howard Chang,[2] Ritam Chowdhury,[1] Rachel E. Patzer.[3] *[1]Dept of Epidemiology, RSPH, Emory Univ; [2]Dept of Biostatistics and Bioinformatics, RSPH, Emory Univ; [3]Div of Transplantation, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** Pre-ESRD nephrology care is important for better clinical outcomes for patients with end stage renal disease (ESRD). The aim of this study was to determine the association between pre-ESRD nephrology care and the standardized mortality ratio (SMR) among dialysis facilities in the US.

**Methods:** A total of 5,387 dialysis facilities were identified from the Dialysis Facility Report data 2007-2010. A marginal generalized estimating equation model assuming exchangeable covariance structure was used to estimate the association between pre-ESRD nephrology care, timing of pre-ESRD nephrology care, and facility level SMR.

**Results:** The percentage of patients who received pre-ESRD nephrology care within a facility was significantly associated with facility-level SMR. A 10% increase in pre-ESRD nephrology care was associated with a 0.50% (95% CI, 0.10%-0.80%, *P*=0.0067) decline in facility SMR. After considering the timing of pre-ESRD nephrology care, for every 10% increase in the percentage of patients who received pre-ESRD nephrology care within a dialysis facility, there was a 0.70% (95% CI, 0.10%-1.20%, *P*<0.0001) and 1.00% (95% CI, 0.60%-1.50%, *P*<0.0001) decrease in facility SMR for patients who received pre-ESRD care >6 months and >12 months prior to ESRD, respectively.




**Conclusions:** As the percentage of patients who received pre-ESRD nephrology care within a facility increased, the facility-level SMR decreased. The timing also played a significant role in mortality reduction where earlier access to nephrology care resulted in a steeper decline in the mortality. This suggests that targeted interventions to improve access to pre-ESRD nephrology care could reduce mortality among ESRD patients in dialysis facilities.

## SA-OR044

**2012 National Survey of Hemodialysis Infection Prevention and Vascular Access Practices** Priti Patel, Katelyn Coutts, Ann Goding Sauer, Alicia Shugart, Anna M. Melville, Matthew J. Arduino, Nicola D. Thompson, Alexander Kallen. *Centers for Disease Control and Prevention (CDC), Atlanta, GA.*

**Background:** Infections cause many dialysis patient hospitalizations and deaths. To better understand practices related to infection risk, CDC administers an annual survey to dialysis centers via the National Healthcare Safety Network (NHSN).

**Methods:** The Outpatient Dialysis Center Practices Survey, required for NHSN participation, includes self-reported center characteristics, infection prevention strategies, and vascular access practices. Surveys completed in 2012 by U.S. hemodialysis centers were analyzed. Chi-square test was used for comparisons.

**Results:** Of 5666 centers, 589 (10.4%) were hospital-affiliated centers (HAC). Adherence to CDC recommendations for hepatitis C screening (16.2%), and catheter (CVC) exit site care using chlorhexidine (27.7%) or antimicrobial ointment (15.1%) was low. These practices were more common in HAC than freestanding centers (FSC).

| **Percent of Centers Reporting Select Practices** | | | |
|---|---|---|---|
| | All (5666) | HAC (589) | FSC (5077) |
| **CDC-recommended** | | | |
| Hepatitis C screening* | 16.2 | 33.5 | 14.1 |
| Chlorhexidine for CVC exit site care* | 27.7 | 73.3 | 22.4 |
| Antimicrobial ointment at CVC exit site* | 15.1 | 22.4 | 14.2 |
| | | | |
| **Other** | | | |
| Vascular access nurse* | 88.7 | 64.7 | 91.7 |
| Nurse does CVC care* | 67.2 | 82.7 | 65.4 |
| Antimicrobial-impregnated CVCs* | 2.4 | 5.3 | 2.0 |
| Chlorhexidine-impregnated CVC dressings* | 14.5 | 46.7 | 10.8 |
| CVC closed connector devices* | 46.3 | 39.4 | 47.1 |
| Buttonhole cannulation** | 61.2 | 59.1 | 61.5 |
| HAC vs. FSC: *p<0.001; **p=0.26 | | | |

Sodium hypochlorite was most often used for CVC exit site (47.4%) and hub (48.2%) care, despite no recommendation for its use. More HAC reported that nurses did most CVC care, but FSC more often had a dedicated vascular access nurse. Most centers performed buttonhole cannulation.

**Conclusions:** Infection prevention strategies vary and recommended practices are underused. HAC may be more aware of CDC recommendations than FSC. Understanding implementation barriers among FSC is important to reducing infections.

## SA-OR045

**Targeted Medication Therapy Management (MTM) Improves Outcomes for Dialysis Patients and the Healthcare System** Joshua K. Howland, May Hoang, Maricela Lara Nevarez, Kelly K. Chillingworth, Tracy Furgiuele. *DaVita Rx, Coppell, TX.*

**Background:** The Affordable Care Act expanded MTM services to become a core component of the Medicare Part D program. Identification of medication-related problems and partnership with interdisciplinary healthcare teams to achieve resolution can improve clinical outcomes, reduce hospitalizations, and reduce costs. Given the large number of medications prescribed for dialysis patients, a pharmacist medication review program targeted to patients in transitions of care should improve clinical and systems costs for these patients.

**Methods:** Data from 212 patients (new to dialysis [<90 days] or ≤14 days since hospital discharge) who received a medication review were assessed by review of patient records. Each participant received a comprehensive medication review conducted via telephone by a pharmacist. Interventions which resulted in an avoided hospitalization/ER visit were determined and independently verified by pharmacists based on the clinical impact of the medication-related problem. Systems costs avoided were calculated using data from the Agency for Healthcare Research and Quality (AHRQ) H-CUP database and the Kaiser Family Foundation database on Medicare enrollees based on probable ICD-9 codes.

**Results:** 279 unique interventions were identified that resulted in potential avoidance of hospitalization/ER visit. Modeled mean cost savings for the healthcare system were $642,436; median cost savings were $440,911.

| | | |
|---|---|---|
| Incidence of probable hospitalizations | 0.257 | |
| All-cause hospitalization % for ESRD patients | 0.36 | |
| Interventions avoiding ER/hospitalization | 279 | |
| Study period | 05-Mar-12 to 13-May-13 | |
| | | |
| | Mean | Median |
| Modeled cost of all discharge diagnoses | $2,496,673 | $1,713,494 |
| Total modeled costs avoided | $642,436 | $440,911 |

**Conclusions:** These data suggest that a pharmacist medication review program for vulnerable patient populations has the potential to improve patient outcomes and lower overall healthcare costs through avoided hospitalizations. Assessment of actual hospitalization rates for patients receiving program interventions will be needed to validate these findings.

*Funding:* Pharmaceutical Company Support - DaVita Rx

## SA-OR046

**Infrastructure for Patient Navigation and Timing of Referral and Evaluation for Kidney Transplant: A Survey of Nephrologists** Kevin C. Roe, Shahed Abbasi, Ahmad Aswad, Nasrollah Ghahramani. *Nephrology, Penn State College of Medicine, Hershey, PA.*

**Background:** Kidney transplant (KT) is the treatment of choice for majority of ESRD patients. Navigation of the evaluation process is a challenge, contributing to variations in timing for KT evaluation. We analyzed the association between the infrastructure for patient navigation and the timing of referral and evaluation for KT.

**Methods:** Invitations were sent to 3180 nephrologists in the eastern US. From 822 interested, 250 were randomly invited to complete a questionnaire about demographics, practice characteristics, and availability of infrastructure in their practice for patient navigation of the KT process. Excluding 9 with incomplete responses yielded 216 participants for a 2-step analysis: 1) association between practice characteristics and availability of infrastructure (formal protocol for KT referral, designated coordinator for KT workup, outreach activities by a transplant center (TC)); 2) association between infrastructure and timing of KT referral and evaluation. Chi-square and stepwise logistic regression were performed.

**Results:** A protocol (52%) was more likely among nephrologists in large groups (>10 members) (OR:3.08,p=0.003). Coordinator availability (46%) was more likely among academic nephrologists (OR:2.56,p=0.002) and those in large groups (OR:2.30,p=0.02). TC outreach was more likely for respondents (51%) attending transplant-related CME

(OR:2.37, p=0.004). Early referral (GFR:21-30) for KT evaluation (23%) was associated with coordinator availability (OR:2.09,p=0.04). A short (2 months or less) referral to evaluation time (52%) was more likely with TC outreach activities (OR:1.98,p=0.02). 44% indicated that the TC should only follow the KT patients 1 year; this was associated with coordinator availability (OR:1.87,p=0.04).

**Conclusions:** Availability of an infrastructure for patient navigation during the KT evaluation process is associated with referral at an earlier stage, a shorter referral to evaluation time and an earlier resumption of post-KT care by the primary nephrologist.

*Funding:* NIDDK Support

## SA-OR047

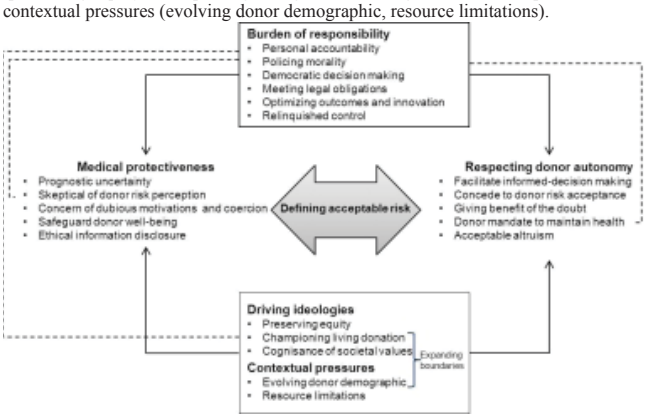
### Living Kidney Donor Assessment: Challenges, Uncertainties and Controversies among Transplant Nephrologists and Surgeons

Allison Tong,[1,2] Jeremy Chapman,[3] Germaine Wong,[1,2,3] Jonathan C. Craig.[1,2]
*[1]Sydney School of Public Health, The Univ of Sydney, NSW, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, NSW, Australia; [3]Centre for Transplant and Renal Research, Westmead Hospital, NSW, Australia.*

**Background:** The assessment of living kidney donors presents unique ethical challenges and complex psychosocial implications. We aimed to aimed ascertain the perspectives of transplant nephrologists and surgeons on living kidney donor assessment.

**Methods:** Semi-structured, face-to-face interviews were conducted with 110 transplant nephrologists and surgeons from 43 transplant units in 12 countries from Europe, Australasia, and North America.

**Results:** The challenge of defining acceptable risk to the donor was central to five themes identified: burden of responsibility (personal accountability, policing morality, democratic decision making, meeting legal obligations, optimizing outcomes and innovation, relinquished control); medical protectiveness (prognostic uncertainty, scepticism of donor risk perception, avoidance of undue coercion, concerns for dubious motivations and coercion, safeguard donor well-being, ethical information disclosure); respecting donor autonomy (facilitate informed-decision making, concede to donor risk acceptance, benefit of the doubt, donor mandate to maintain health, acceptable altruism); driving ideologies (preserving equity, championing living donation, cognisance of anti-paternalism); and contextual pressures (evolving donor demographic, resource limitations).




**Conclusions:** Living kidney donor assessment involves complex interactions between safeguarding the donors' welfare and respecting their autonomy. Authoritative and well-described transplant unit, hospital and public policy positions would make explicit the considerations which are often implicit, and reduce the uncertainty within which living donors are assessed today.

*Funding:* Government Support - Non-U.S.

## SA-OR048

### Impact of Hypertension Management in Patients with Chronic Kidney Disease on Outcomes after End-Stage Renal Disease

Elaine Ku,[1] David V. Glidden,[1] Barbara A. Grimes,[1] Kirsten L. Johansen,[1] Mark J. Sarnak,[2] Hocine Tighiouart,[2] Chi-Yuan Hsu.[1] *[1]UCSF; [2]Tufts Medical Center.*

**Background:** There is controversy regarding whether strict blood pressure (BP) control (BP < 140/90 mm Hg) is indicated in patients with chronic kidney disease (CKD) since primary results of randomized controlled trials showed this strategy does not reduce rates of kidney function loss. However, it is possible that strict BP control has other beneficial effects beyond reducing risk of end-stage renal disease (ESRD), such as lowering mortality rates after onset of ESRD (a possible mechanism for this would be reduced cardiovascular disease burden at the start of ESRD).

**Methods:** The Modification of Diet in Renal Disease (MDRD) study is a well-known CKD trial conducted from 1989-1993 that randomized 840 CKD patients to strict (mean arterial pressure <92 or <98 mmHg depending on age) vs. usual BP control (<107 mm Hg). We extended follow-up of MDRD study enrollees via cross-linkage with US Renal Data System (USRDS) and National Death Index to ascertain ESRD onset and death (prior to ESRD) through 12/31/2007. Deaths after onset of ESRD were ascertained via USRDS through 12/31/2009.

**Results:** Of the 840 MDRD enrollees, 616 developed ESRD by 12/31/2007. There were 306 cases of ESRD in the strict BP arm and 310 cases of ESRD in the usual BP arm (unadjusted Cox models hazard ratio [HR] 0.93; 95% CI 0.79-1.08; p = 0.34). Prior to ESRD, there were 66 deaths in the strict BP arm and 40 deaths in the usual BP arm (HR 1.48; 95% CI 1.00-2.19; p = 0.05). After onset of ESRD, there were 132 deaths in the strict BP arm and 179 deaths in the regular BP arm (HR 0.68; 95% CI 0.54- 0.85; p = 0.001) in a Cox model of time to event since dialysis initiation. When considering all deaths regardless of timing relative to ESRD, mortality risk was lower in the tight blood pressure control group (HR 0.81; 95% CI 0.67-0.99; p = 0.04).

**Conclusions:** Although strict BP control may not delay progression of CKD to ESRD, this strategy appears to be associated (even years after end of active intervention) with reduced risk of death after onset of ESRD. Post-ESRD outcomes should be considered when formulating BP targets for CKD patients.

*Funding:* NIDDK Support

## SA-OR049

### Physical Activity and Risk of Kidney Failure in the Singapore Chinese Health Study

Tazeen H. Jafar,[1] Jin Ai Zhen,[2] Woon-Puay Koh,[1] Khuan Yew Chow.[2] *[1]Duke-NUS Graduate Medical School, Singapore; [2]National Registry of Diseases Office, Health Promotion Board, Singapore; [3]Saw Swee Hock School of Public Health, National Univ of Singapore, Singapore.*

**Background:** The relationship between physical activity and risk of kidney failure is not clear. We analyzed data from the Singapore Chinese Health Study to investigate whether physical activity reduces the risk of kidney failure.

**Methods:** The Singapore Chinese Health Study is a population-based cohort of 63,257 Chinese adults enrolled between 1993 and 1998. Self-reported information on habitual physical activity at baseline. Incidence of kidney failure was identified via record linkage with the nationwide Singapore Renal Registry until 2008, defined by one of the following: 1) serum creatinine level of more than or equal to 500 µmol/l (5.7 mg/dl), 2) estimated GFR of less than 15 ml/min/1.73m$^2$, 3) undergoing hemodialysis or peritoneal dialysis, 4) kidney transplantation. Multivariable models were built and cox proportional hazard regression analysis was performed to assess the association between physical activity with kidney failure after adjusting for age, education, dialect, herbal medications, body mass index, sex, physician-diagnosed hypertension and diabetes.

**Results:** Mean age of subjects was 55.6 years; 44% were men. A total of 671 incident cases of kidney failure occurred during a median follow-up of 13.3 years. Individuals who were physically active had significantly reduced adjusted risk of kidney failure [hazard ratio (HR): 0.70; 95% CI: 0.58-0.83] compared to those who were inactive. The risk reduced with increase in intensity from moderate to vigorous/ strenuous (p for trend=<0.0001), and with increase in duration of moderate physical activity to 4-6 hours/week (adjusted p for trend =0.01). Vigorous activity or strenuous of 30 minutes to 2 hour/week independently reduced the risk of kidney failure (HR = 0.63, CI: 0.42-0.94).

**Conclusions:** Physical activity is associated with decreased risk of kidney failure. The risk reduction appears to be dose- dependent with increase in intensity and duration of moderate physical acitivity. Even a short duration of vigorous physical acitivity predicts additional risk reduction.

*Funding:* Other NIH Support - National Cancer Institute

## SA-OR050

### Race, Dietary Acid Load and Risk of ESRD among Low Income Americans with CKD

Deidra C. Crews,[1] Tanushree Banerjee,[2] Donald E. Wesson,[3] Hal Morgenstern,[4] Nilka Rios Burrows,[5] Rajiv Saran,[4] Desmond Williams,[5] Neil R. Powe.[2] *[1]Johns Hopkins Univ; [2]Univ of California, San Francisco; [3]Texas A&M College of Medicine; [4]Univ of Michigan; [5]Centers for Disease Control and Prevention.*

**Background:** Racial disparities in risk of ESRD are most profound among individuals of low socioeconomic status (SES). High dietary acid load (e.g., diet poor in base-producing fruits/vegetables) is positively associated with CKD progression to ESRD. Whether the effect of dietary acid load on risk of ESRD differs by race is unknown.

**Methods:** We examined data on 159 non-Hispanic black (NHB), and 760 non-Hispanic white (NHW) adults with CKD (eGFR=15-59 ml/min/1.73 m$^2$), aged >20 years, and with an annual household income <300% of the federal poverty guideline, from the 1999-2004 National Health and Nutrition Examination Survey. Dietary acid load was estimated using the net acid excretion (NAE) formula by Remer and Manz and 24h dietary recall data. ESRD events were ascertained via linkage with the Medicare ESRD registry through 2008. Cox regression was used to estimate the hazard ratio (HR) for ESRD, comparing NHBs with NHWs, adjusting for age, sex, diabetes, hypertension, baseline eGFR, urinary albumin:creatinine, daily caloric intake, and NAE. The modification of the NAE effect by race was examined by including an interaction term in the Cox model.

**Results:** Compared with NHWs at baseline, NHBs were younger, more likely to have diabetes and/or albuminuria and had greater NAE (49.1 vs. 45.3 mEq/d). Overall, 94 (12.4%) participants [36 (38.3%) NHWs and 58 (61.7%) NHBs] developed ESRD during an average of 6.4 years of follow up. NHBs had a higher risk of developing ESRD than NHWs with an unadjusted HR of 13.7 (95% CI 13.6-13.8) and adjusted HR of 3.04 (95% CI 3.02-3.07). On stratifying by race, each unit increase in NAE was more strongly associated with ESRD among NHBs [HR 1.035 (1.033-1.036)] than among NHWs [HR 1.018 (1.018-1.019)] in Cox models adjusted for age (P for interaction <0.001).

**Conclusions:** Among low SES adults with CKD, the detrimental effect of high dietary acid load on progression to ESRD appears to be greater for NHBs than for NHWs, and is worthy of further investigation in other populations.

*Funding:* Other U.S. Government Support, Private Foundation Support

**SA-OR051**

**Persistent Alkalosis Is Associated with High Risk of Heart Failure in Patients with CKD: A Report from the Chronic Renal Insufficiency Cohort (CRIC) Study** Mirela A. Dobre,[1] Wei Yang,[2] Lawrence J. Appel,[2] Keith A. Bellovich,[2] Harold I. Feldman,[2] Jing Chen,[2] Michael J. Fischer,[2] L. Lee Hamm,[2] Thomas H. Hostetter,[1] Bernard G. Jaar,[2] Radhakrishna Reddy Kallem,[2] Sylvia E. Rosas,[2] Julia J. Scialla,[2] Myles S. Wolf,[2] Mahboob Rahman.[1] *[1]Case Western Reserve Univ; [2]CRIC Study.*

**Background:** The aim of the study is to evaluate the effect of serum bicarbonate change over 1 year on clinical outcomes in a large cohort of participants with CKD.

**Methods:** Serum bicarbonate was measured at baseline and after 1 year in 3394 CRIC participants with CKD. Participants were divided in 4 groups of serum bicarbonate change: *Normal* [22-26mEq/L] at baseline and year 1, n=1288; *Improved* - abnormal at baseline who became normal at year 1, n=674; *Worsening/persistent acidosis* <22mEq/L at baseline and year 1, n=257 plus all who became <22mEq/L at year 1, n=349; *Worsening/persistent alkalosis* >26mEq/L at baseline and year 1, n=433 plus all who became >26mEq/L at year 1, n=393. Renal outcomes were defined as ESRD or 50% eGFR decline. Cardiovascular outcomes were defined as atherosclerotic (myocardial infarction, stroke, peripheral arterial disease) or CHF events. Multivariable Cox proportional hazards models were used to test the associations of interest.

**Results:** The risk of developing heart failure was 37% higher for participants in the alkalosis group (HR 1.37; 95%CI 1.02-1.84, p=0.04) compared to participants in the normal group, after adjusting for demographics, co-morbidities, medications including diuretics, eGFR and proteinuria.

| | Normal | Improved | | Worsening/persistent acidosis | | Worsening/persistent alkalosis | |
|---|---|---|---|---|---|---|---|
| | | HR(95%CI) | p | HR(95%CI) | p | HR(95%CI) | p |
| **CHF** | Ref | 1.12(0.81-1.53) | 0.50 | 0.93(0.68-1.29) | 0.70 | 1.37(1.02-1.84) | 0.04 |
| **Renal event** | Ref | 0.99(0.81-1.23) | 0.90 | 1.00(0.82 1.21) | 0.90 | 1.18(0.96-1.45) | 0.10 |
| **Death** | Ref | 0.87(0.64-1.18) | 0.40 | 1.01(0.75-1.34) | 0.90 | 1.00(0.75-1.32) | 0.90 |
| **Atherosclerotic event** | Ref | 1.17(0.84-1.63) | 0.30 | 0.88(0.62-1.25) | 0.50 | 1.02(0.73-1.42) | 0.90 |

**Conclusions:** In a cohort of participants with CKD, persistent alkalosis was an independent risk factor for heart failure events.

*Funding:* Other NIH Support - T32 Training Grant : 5T32DK007470 (PI: John Sedor)

**SA-OR052**

**The Impact of a Mediterranean Style Diet on Kidney Function** Minesh Khatri,[1] Yeseon Park Moon,[2] Nikolaos Scarmeas,[3] Yian Gu,[2] Consuelo Mora-McLaughlin,[2] Hannah Gardener,[4] Clinton Wright,[4] Ralph L. Sacco,[4] Thomas L. Nickolas,[1] Mitchell S.V. Elkind.[2] *[1]Nephrology, Columbia Univ, New York, NY; [2]Neurology, Columbia Univ, New York, NY; [3]Sergievsky Center, Columbia Univ, New York, NY; [4]Neurology, Univ of Miami, Miami, FL.*

**Background:** Adherence to a Mediterranean diet has been associated with improved cardiovascular risk through improved endothelial function and blood pressure, and decreased inflammation. Whether these benefits extend to kidney function is unclear.

**Methods:** The Northern Manhattan Study is a prospective, community-based, multi-ethnic cohort that was stroke-free at baseline. Serum creatinine was measured at baseline and at follow-up (mean 6.9 years). A dietary questionnaire was administered at baseline, from which elements of a Mediterranean diet were extrapolated into a previously used nine point scoring system (MeDi) that was associated with cerebrovascular disease outcomes in this cohort. The primary outcome was incident CKD, defined as a follow-up MDRD estimated glomerular filtration rate (eGFR) of <60 mL/min among subjects with eGFR>60 mL/min at baseline. A secondary outcome was defined as the upper quartile of annualized eGFR decline within this cohort (≥2.5 mL/min/year). Models were adjusted for demographics and baseline vascular risk factors.

**Results:** A total of 900 subjects had requisite data. At baseline, mean age was 64, 59% were women, 65% were Hispanic, 19% had diabetes and 69% had hypertension. Mean baseline eGFR was 83.1 mL/min, with a mean annualized decline of 1.1 mL/min; incident CKD developed in 14%. In multivariate analysis, every one-point increase in adherence to MeDi was associated with a 17% decreased odds (p=0.015) of incident CKD and a 14% decreased odds (p=0.009) of being in the upper quartile of eGFR decline.

**Conclusions:** This prospective analysis suggests that a Mediterranean style diet is associated with a reduced risk of CKD and slows the rate of eGFR decline. Randomized trials are needed to confirm these findings, and mechanisms by which a Mediterranean style diet may protect against kidney disease progression need to be determined.

*Funding:* Other NIH Support - NINDS R37 29993

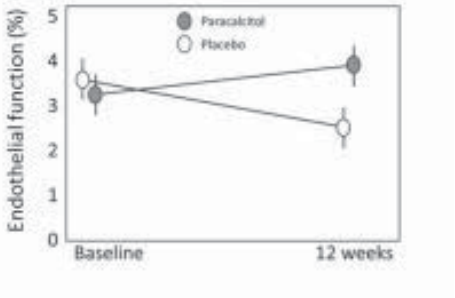
**SA-OR053**

**The Paricalcitol and ENdothelial FuNction in Chronic KidneY Disease (PENNY) Trial: Paracalcitol Improves Endothelium-Dependent Vasodilatation in Stage 3-4 CKD Patients** Carmine Zoccali,[1] Giuseppe Curatola,[1] Vincenzo Panuccio,[1] Rocco Tripepi,[1] Patrizia Pizzini,[1] Maria Carmela Versace,[1] Davide Bolignano,[1] Sebastiano Cutrupi,[1] Raffaele Politi,[1] Giovanni Tripepi,[1] Ravi I. Thadhani,[2] Francesca Mallamaci.[1] *[1]CNR-IBIM, Clin. Epid. and Physiopath. of Renal Dis. and Hypertens., CNR-IBIM, Reggio Calabria, Italy; [2]Massachusetts General Hospital, Harvard Medical Center, Boston.*

**Background:** An association between low vitamin D, endothelial dysfunction and atherosclerosis has been described in CKD pts but the effect of active forms of vitamin D on endothelial function has not been tested in a controlled trial in these pts.

**Methods:** PENNY is a double blinded RCT (Clinicaltrials.gov NCT01680198) testing the effect of paracalcitol (PCT, 2 µg/day x 12 weeks) on the forearm blood flow (FBF) response to ischemia (endothelium dependent vasodilatation) and to nitroglicerine (NTG, endothelium independent vasodilatation) by a standardized technique (JH 2012;30:1399) in 88 stage 3-4 CKD pts with PTH>65 pg/ml (PCT,n=44; Placebo,n=44).

**Results:** PCT reduced PTH from a median of 102 pg/ml (IQR:82-146 pg/ml) to 41 pg/ml (24-61 pg/ml). BP and HR remained unmodified in both study arms. PCT increased the FBF response to ischemia by 14% and the between groups difference in FBF changes across the trial (the primary end-point) was highly significant (P=0.01).




No effect of PCT on the response to NTG was registered indicating that PCT specifically affects endothelium dependent vasodilatation.

**Conclusions:** PCT improves endothelium dependent vasodilatation in stage 3-4 CKD pts. Such an effect indicates that endothelial dysfunction is a reversible phenomenon in this population. Since endothelial dysfunction is a strong predictor of death and CV events, findings in this study provide a rationale for designing randomized trials based on clinical end-points in CKD.

*Funding:* Pharmaceutical Company Support - AbbVie

**SA-OR054**

**The Effects of Allopurinol on Metabolic Acidosis and Endothelial Functions in the Hyperuricemic Patients with Stage 2-4 Chronic Kidney Disease** Mehmet T. Sezer,[1] Dilara Bayram,[2] Atila Altunta?,[1] Salih Inal,[1] Veysel Kidir.[1] *[1]Internal Medicine, Suleyman Demirel Univ School of Medicine, Isparta, Turkey; [2]Nephrology, Suleyman Demirel Univ School of Medicine, Isparta, Turkey.*

**Background:** An elevated serum uric acid level is reported to be associated with endothelial dysfunction which is common in chronic kidney disease (CKD). At the same time, endothelial dysfunction and metabolic acidosis have emerged as important risk factors for progression of kidney disease. In this study, we aimed to investigate the effects of allopurinol on metabolic acidosis and endothelial functions in hyperuricemic stage 2-4 CKD patients.

**Methods:** Thirty patients (14 male/16 female) with stage 2-4 CKD and serum uric acid levels over 5.5 mg/dL were included in the study group. They were prescribed 300 mg/day po allopurinol for 3 months. Age and gender matched CKD patients (n=30) with similar clinical characteristics (13 male/ 17 female) were taken as the control group and they were not given allopurinol. Endothelial functions were measured via flow mediated dilatation (FMD) over forearm. Additionally, pH and $HCO_3$ levels in venous blood, glomerular filtration rate (GFR) and proteinuria levels were calculated in all patients at the beginning and at the third month of follow up.

**Results:** Clinical and Laboratory findings were similar in both groups in the first visit. FMD was found to be significantly increased in allopurinol group (5,80 ± 2,56 vs 6,27 ± 2,70; p:0,006) whereas it decreased significantly in the control group (6,27 ± 1,62 vs 5,71 ± 1,90; p:0,005) between the two visits. When FMD variations within two groups were evaluated via the repeated ANOVA - general linear model, it was clearly significant. Additionally, uric acid levels were found to be inversely correlated with GFR and $HCO_3$ levels.

**Conclusions:** The primary finding in this study was that decreased uric acid levels with allopurinol in hyperuricemic CKD patients have beneficial effects over endothelial dysfunction and serum $HCO_3$ levels. We assume that decreasing uric acid levels seems to be helpful in order to restore endothelial functions, prevent metabolic acidosis and slow down the progression to end stage renal disease.

## SA-OR055

**Validation of the Kidney Failure Risk Equation in an International Consortium** Navdeep Tangri, Andrew S. Levey, M. Grams, Josef Coresh, Brad C. Astor, Allan J. Collins, Ognjenka Djurdjev, Carolyn Raina Elley, Stein I. Hallan, Lesley Inker, Csaba P. Kovesdy, Florian Kronenberg, Hiddo Jan Lambers Heerspink, Angharad Marks, Sankar D. Navaneethan, Robert G. Nelson, Mark J. Sarnak, Benedicte Stengel, Mark Woodward, Kunitoshi Iseki. *CKD Prognosis Consortium.*

**Background:** Predicting the progression of CKD can enable early and appropriate nephrology care. We previously developed and validated laboratory based kidney failure risk equations (KFREs) for the progression of CKD to kidney failure in patients referred for nephrology care in Canada. Evaluation in other countries and in non-referred CKD populations is needed.

**Methods:** We meta-analyzedindividual level data from 34,569 patients with CKD Stages 3-5 from 12 cohorts spanning 7 countries. We assessed models containing 3 (age, sex, estimated glomerular filtration rate [GFR]), 4 (+albuminuria) and 8 (+serum albumin, calcium, phosphorous, and bicarbonate) variables from the original publication. We compared hazard ratios for constituent predictors, discrimination, calibration, net reclassification index (NRI), and integrated discrimination index (IDI) for all models.

**Results:** The original 4- and 8-variable KFREs obtained similar hazard ratios for constituent predictors and achieved excellent discrimination (C statistic >0.84 – Table). Calibration of the original KFREs was adequate at 5 years, and improvements in NRI and IDI were observed in comparison with a 3-variable model (NRI 18%, IDI 5% for the 4-variable,and 25% and 6% for the 8-variable model). Performance in subgroups by age, race and diabetes status was similar.

**Conclusions:** The previously developed KRFEs accurately predict the progression of CKD Stages 3-5 to kidney failure across a wide range of studies. The abbreviated 4-variable equation is simple and highly accurate. Integration in routine clinical practice should be evaluated.

| Model | Difference of C Statistic | NRI with risk categories of 0-1%, 1-2%, 2%+ | IDI |
|---|---|---|---|
| | 1 year | | |
| 4-var vs. 3-var | 0.000 (-0.021, 0.022) | **0.183 (0.123, 0.243)** | **0.048 (0.021, 0.074)** |
| 8-var vs. 3-var | 0.001 (-0.020, 0.023) | **0.253 (0.198, 0.309)** | **0.060 (0.001, 0.118)** |
| 8-var vs. 4-var | 0.001 (-0.005, 0.007) | **0.093 (0.075, 0.112)** | 0.011 (-0.033, 0.055) |
| | 5 year | | |
| 4-var vs. 3-var | 0.008 (-0.025, 0.041) | **0.168 (0.130, 0.206)** | 0.037 (-0.007, 0.080) |
| 8-var vs. 3-var | 0.016 (-0.009, 0.041) | **0.194 (0.163, 0.225)** | 0.031 (-0.026, 0.088) |
| 8-var vs. 4-var | 0.008 (-0.005, 0.020) | **0.039 (0.017, 0.062)** | -0.004 (-0.024, 0.016) |

C-statistic for 3-variable model: 1y = 0.923 (0.889, 0.956); 5y = 0.843 (0.817, 0.868)
C-statistic for 4-variable model: 1y = 0.914 (0.865, 0.963); 5y = 0.851 (0.818, 0.883)
Bold indicates significance compared to 3- or 4-variable model

*Funding:* Private Foundation Support

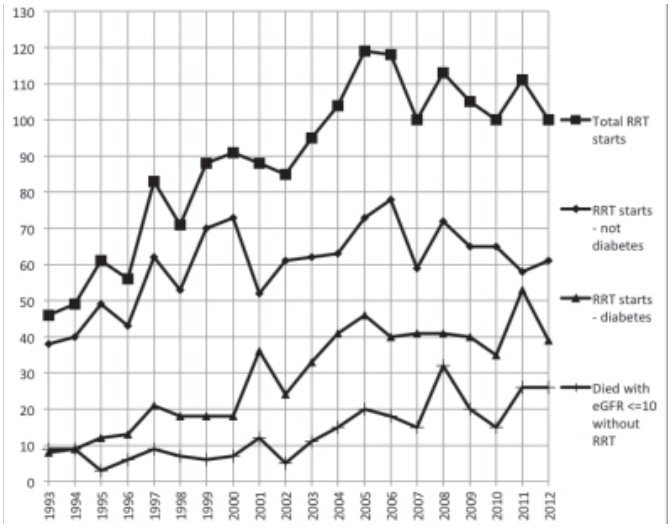
## SA-OR056

**CKD Management Program Improved Access to Nephrology Care and Stabilized Renal Replacement Therapy Incidence in Birmingham, UK** Hugh C. Rayner, Jyoti B. Baharani, Indranil Dasgupta, Vijayan Suresh, Robert Mark Temple, Mark E. Thomas, Stephen A. Smith. *Renal Medicine, Heart of England NHS Foundation Trust, Birmingham, West Midlands, United Kingdom.*

**Background:** Heart of England (HEFT) provides ESRD care for c.0.72m population with mixed ethnicity and large areas of deprivation. From 2001-11 the England unadjusted incident RRT rate increased 1.1% per year. In 2003/4, HEFT introduced a CKD management program with primary care education, multidisciplinary predialysis and conservative care (Nephron Clin Pract 2010;115:c283-8), routine eGFR reporting, and diabetes CKD surveillance (Rayner BMJ Qual Saf 2011). In 2006, national financial incentives for primary CKD care were introduced. We have studied access to nephrology care and trends in RRT and end of life care at HEFT.

**Methods:** Incident CKD5 patients >=75yrs (eGFR<15 x2 >3 mths apart Jan2010 - Jun2011) in HEFT pathology database linked with a nephrologist by UK Renal Registry. RRT = dialysis + pre-emptive transplant. Variation over time analyzed by statistical process control.

**Results:** In 2010/11 50/55 incident CKD5 patients >=75yrs were known to a nephrologist. From 1998, mean age at start of RRT (62 yrs) and eGFR at start of dialysis (7.8) were stable. From 2005 the unadjusted RRT incident rate stabilized (fig). RRT incidence at the neighboring centre was stable. Patients known to a nephrologist who died with eGFR <10 without dialysis increased (fig); mean age at death increased linearly from 70 in 1993 to 82yrs in 2012. From 2004, 105/189 patients (56%) who died with eGFR <10 without dialysis received multidisciplinary care; 67/189 (36%) received planned conservative care.




**Conclusions:** Following implementation of a CKD management program, >90% CKD5 patients aged >75 yrs were known to a nephrologist, the incident RRT rate stabilized and >50% patients dying with renal failure without dialysis received multidisciplinary care.

*Funding:* Government Support - Non-U.S.

## SA-OR057

**Change in GFR and Subsequent Mortality: Meta-Analysis of 32 Cohorts in the CKD Prognosis Consortium** Tanvir Chowdhury Turin, Kunihiro Matsushita, Josef Coresh, Hisatomi Arima, Steven J. Chadban, Massimo Cirillo, Ognjenka Djurdjev, Jamie Alton Green, Fujiko Irie, Joachim H. Ix, Csaba P. Kovesdy, Takayoshi Ohkubo, Chi Pang Wen, Paul E. de Jong, Kunitoshi Iseki, Benedicte Stengel, Ron T. Gansevoort. *CKD Prognosis Consortium.*

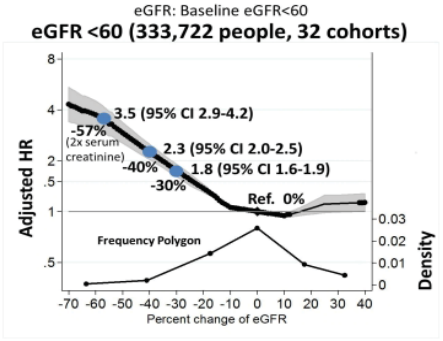
**Background:** Change in estimated GFR (eGFR) is frequently used to track CKD progression in clinical practice, trials and cohort studies but its association with mortality has not been studied extensively.

**Methods:** Change in eGFR was estimated as % change from the first to last eGFR (CKD-EPI creatinine) in a 2-year baseline period. We modeled the hazard ratios (HRs) of subsequent mortality as a spline function of %change in eGFR after adjusting for age, sex, race, first eGFR, and co-morbid conditions. We used random effects meta-analyses to combine results stratified by first baseline eGFR (<60 & ≥60) across studies.

**Results:** Mortality follow-up of 910,660 participants from 32 cohorts for a mean of 4.2 years after the 2-year baseline period showed 91,398 deaths for baseline eGFR <60 (n=333,722) and 45,063 deaths for baseline eGFR ≥60 (n=576,938). Change in eGFR had a non-linear association with mortality (Figure for eGFR<60). A decline in eGFR was consistently associated with higher subsequent mortality risk (adjusted HR for -30% vs. 0% change in eGFR were: 1.8 at eGFR <60; and 1.5 at eGFR ≥60; p<0.001). Similar results were obtained for a 1- or 3-year change in eGFR. Hazards ratios were largely similar for those with eGFR ≥60 or when stratified by ACR levels.

**Conclusions:** Declines in eGFR are strongly and consistently associated with subsequent risk of mortality adjusted for the first eGFR and covariates. These findings support using smaller changes than -57% (equivalent to doubling of serum creatinine) in clinical research.

**Figure.** Adjusted mortality hazard ratio vs. % 2-year Change in eGFR: Baseline eGFR<60




*Funding:* Private Foundation Support

**SA-OR058**

**EGFR Deletion in Podocytes Attenuates Diabetic Nephropathy**
Jianchun Chen,[1] Jian-Kang Chen,[2] Raymond C. Harris.[1] *[1]Medicine, Vanderbilt Univ, Nashville, TN; [2]Experimental Medicine, Georgia Regents Univ, Augusta, GA.*

**Background:** The generation of ROS, specifically superoxide ($\cdot O_2^-$), by damaged or dysfunctional mitochondria, has been postulated to be a primary initiating event in the development of diabetes complications. The glomerulus is a primary site of diabetic injury, and podocyte loss is one of the classic hallmarks of diabetic glomerular lesions. The current studies were designed to determine the potential role EGFR activation in podocytes in diabetic glomerular injury.

**Methods:** We generated podocyte-specific EGFR knockout mice ($EGFR^{podKO}$) by crossing $EGFR^{flox/flox}$ mice with podocin-Cre mice and induced diabetes in $EGFR^{podKO}$mice and their wild type littermates (WT) by streptozotocin injections.

**Results:** $EGFR^{podKO}$ and their WT control mice on a 129SVJ background developed similar levels of hyperglycemia and polyuria, but $EGFR^{podKO}$ developed significantly less albuminuria(urinary albumin/creatinine ratio at 32 weeks of hyperglycemia WT vs $EGFR^{podKO}$: 170.80 ± 12.93 vs. 80.8 ± 6.14 µg/mg, P<0.001, n =5-7). More podocytes were lost in WT diabetic mice compared with the $EGFR^{podKO}$ diabetic mice (podocyte number/glomerulus: WT vs $EGFR^{podKO}$: 9.71 ± 0.56 vs 12.71 ± 0.42 podocytes/glomerular section, P<0.01, n=4-6). Furthermore, increased fibronectin deposition in glomeruli was attenuated in $EGFR^{podKO}$ diabetic mice. Immunoblotting of isolated glomerular lysates revealed that up-regulation of cleaved caspase 3 and down-regulation of $EGFR^{podKO}$ mice. Our studies also revealed up-regulation of phospho-EGFR, phospho-smad2/3, and TGFβ1 expression were markedly inhibited in $EGFR^{podKO}$ diabetic mice. Furthermore, in WT diabetic mice, administration of either a mitochondria-targeted antioxidant, mito-TEMPO, or a cell-permeable NADPH-oxidase inhibitor, apocynin, also attenuated up-regulation of phospho-EGFR, phospho-smad2/3 and TGFβ1 expression, and blunted the altered expression of cleaved caspase 3 and Bcl-2 expression in glomeruli.

**Conclusions:** This study demonstrates that EGFR plays an important role in oxidative stress-induced podocyte loss and glomerular ECM expansion in the pathogenesis of diabetic nephropathy.

*Funding:* NIDDK Support, Veterans Affairs Support

**SA-OR059**

**Podocyte VEGF-A and eNOS Loss of Function Lead to Diffuse Glomerulosclerosis in Mice**
Alda Tufro,[1] Pardeep Kumar Aggarwal,[1] Gilbert W. Moeckel.[2] *[1]Pediatrics/ Nephrology, Yale Univ, New Haven, CT; [2]Pathology, Yale Univ, New Haven, CT.*

**Background:** Vascular endothelial growth factor-a (VEGF-A) and nitric oxide (NO) are essential for glomerular filtration barrier homeostasis, and are disregulated in diabetic nephropathy. While NO availability is consistently low, both high and low VEGF-A have been reported in patients with diabetic nephropathy.

**Methods:** Here we examined the effect of inducible podocyte VEGF-A knockdown (podocin-rtTA:tet-O-siVEGF = VEGF-$A^{KD}$) in diabetic mice and in endothelial nitric oxide synthase knockout mice (eNOS KO). Diabetes was induced with streptozotocin (STZ) using the ADMCC protocol. Proteinuria was measured by albumin ELISA, VEGF-A and total NO were measured in plasma and urine by ELISA and colorimetric methods, respectively.

**Results:** Podocyte VEGF-$A^{KD}$caused diffuse glomerulosclerosis associated with small glomeruli, foot process effacement and GBM thickening in mice with streptozocin-induced diabetes and in non-diabetic eNOS KO mice. In addition, VEGF-$A^{KD}$:eNOS KO mice developed microaneurisms, arteriolar hyalinosis, massive proteinuria (>30 fold above eNOS KO and >5 fold above VEGF-$A^{KD}$ diabetic mice) and renal failure. Conversely, VEGF-$A^{KD}$ diabetic mice had milder proteinuria and normal GFR, associated to extremely high NO urinary excretion (~10-fold above non-diabetic controls and eNOS KO mice).

**Conclusions:** Collectively, data indicate that glomerular VEGF-A and eNOS deficiency are necessary and sufficient to induce diffuse glomerulosclerosis in mice, and that compensatory NO generation prevents severe proteinuria and GFR loss in diabetic mice with intact eNOS, whereas in eNOS KO mice VEGF-$A^{KD}$ leads to severe diffuse glomerulosclerosis and renal failure mimicking the progression of diabetic nephropathy in humans.

*Funding:* NIDDK Support

**SA-OR060**

**Genetic Deletion and Pharmacological Inhibition of the NADPH Oxidase Nox4 Provides Renoprotection in Diabetes-Induced Nephropathy**
Jay C. Jha,[1,5] Stephen P. Gray,[1] Kirstin Wingler,[2] Cedric Szyndralewiez,[3] Freddy Heitz,[3] Rhian Touyz,[4] Mark E. Cooper,[1,5] Harald H. Schmidt,[2] Karin Jandeleit-Dahm.[1,5] *[1]Diabetic Complications Div, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; [2]Dept of Pharmacology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht Univ, Maastricht, Netherlands; [3]Genkyotex SA, Geneva, Switzerland; [4]Ottawa Hospital Research Institute, Ottawa, Canada; [5]Medicine, Monash Univ, Melbourne, Victoria, Australia.*

**Background:** Chronic kidney disease is a major complication of diabetes.Oxidative stress could contribute to the development of diabetic nephropathy(DN). NADPH oxidases (Nox) are a major source of reactive oxygen species(ROS) production in the kidney and contribute to renal damage in diabetes. However, the role of specific Nox isoforms in DN remains unresolved. Thus,we aimed to examine the role of Nox1 and Nox4 in DN using genetic deletion and pharmacological inhibition approaches in diabetic mice.

**Methods:** $Nox1^{-/y}ApoE^{-/-}$or $Nox4^{-/-}ApoE^{-/-}$and their respective wild type or $ApoE^{-/-}$ mice were rendered diabetic via streptozotocin injection. $ApoE^{-/-}$ mice were treated with the specific Nox1/4 inhibitor *GKT137831*. Animals were culled after 20 weeks and kidneys were obtained for assessment of renal structure and function as well as ROS production. In vitro, Nox4 was silenced in human podocytes and exposed to high glucose and TGFβ for gene expression and ROS measurement.

**Results:** Deletion of Nox4, but not of Nox1 resulted in renal protection from glomerular injury as evidenced by attenuated albuminuria, preserved renal structure, reduced glomerular accumulation of extracellular matrix proteins and attenuated glomerular macrophage infiltration. Administration of *GKT137831* to diabetic $ApoE^{-/-}$ mice conferred a similar degree of renoprotection as did deletion of Nox4. In human podocytes, silencing of Nox4 gene resulted in reduced ROS production and down-regulation of profibrotic markers that are implicated in DN.

**Conclusions:** Collectively, these results identify Nox4 is a key source of ROS responsible for kidney injury in diabetes and provide proof of principle for an innovative small molecule approach to treat and/or prevent DN .

*Funding:* Government Support - Non-U.S.

**SA-OR061**

**Altered Metabolic Substrate Utilization and Mitochondrial Dysfunction Characterizes Diabetic Nephropathy**
Pradeep Kayampilly, Kelli Sas, Jaeman Byun, Hongyu Zhang, Charles Burant, Frank C. Brosius, Subramaniam Pennathur. *Univ of Michigan, Ann Arbor, MI.*

**Background:** Diabetic nephropathy (DN) is the most common cause of end-stage renal disease in the United States. While it is well-recognized that diabetes leads to altered carbohydrate, amino acid and fatty acid metabolism, the utilization and flux of these substrates *in vivo* has not been systematically studied.

**Methods:** We developed a comprehensive targeted metabolomic platform for simultaneous quantitative analysis of glycolytic, tricarboxylic acid (TCA) and fatty acid oxidation intermediates by liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI/MS/MS) and gas chromatography-mass spectrometry (GC/MS). Animals which exhibit characteristic pathological features of DN (BKS db/db) were used as a model system to examine the dynamic changes in substrate utilization.

**Results:** Static metabolite levels of several metabolites including acylcarnitines, glycolytic, and TCA cycle were markedly and statistically significantly increased in diabetic renal cortex and mitochondria from renal cortex (1.5 to 6-fold). Diabetic urine, but not plasma, showed similar increases suggesting that urinary metabolites reflect renal cortical metabolism. Metabolic flux analysis was performed following incubation or intraperitoneal administration of isotopically labeled substrates ($U^{13}C$ glucose and 2, 3-$^{13}C$ pyruvate) for *ex-vivo* proximal tubule and *in vivo* whole-animal flux respectively. Proximal tubule and *in vivo* flux analyses showed a significant elevation of labeled glycolytic and TCA intermediates in diabetic renal cortex at 12 and 24 weeks compared to controls, suggesting increased flux through these pathways. Mitochondria from diabetic renal cortex exhibited reduced ATP production and increased proton leak indicating diminished function and compromised membrane integrity.

**Conclusions:** Taken together, these results show a metabolic reprogramming in the diabetic kidney leading to mitochondrial dysfunction and oxidative stress. The altered metabolic profile in mouse diabetic kidney is mirrored in urine samples of DN patients, suggesting that a subset of these metabolites may serve as potential biomarkers of DN.

*Funding:* NIDDK Support

**SA-OR062**

**Autophagy Is Beneficial for Podocytes during Diabetes-Induced Nephropathy**
Olivia Lenoir,[1,2] Magali Jasiek,[1,2] Pierre-Louis F. Tharaux.[1,2] *[1]Paris Cardiovascular Research Centre - PARCC, INSERM, Paris, France; [2]Univ Paris Descartes, Sorbonne Paris Cité, Paris, France.*

**Background:** Diabetic nephropathy (DN) is the major microvascular complication of diabetes and the leading cause of ESRD in industrialized countries. The development of CKD in patients with diabetes can take several decades and may manifest despite tight glycemic and blood pressure control. With the current standard therapies only partial renal protection is obtained, thus, it is of particular importance to understand more about the pathogenesis of DN and to identify novel therapeutic targets. Recently, autophagy pathway as emerged as a survival signal in several cell types during stress-induced cellular lesions.

Recently, Huber and coll. demonstrated the importance of autophagy in podocytes in the preservation of glomerular cells functions during aging. Moreover, we found that high glucose induces autophagy in podocytes. Thus, we hypothetized that autophagy could be important for podocyte cell survival during diabetes-induced nephropathy.

**Methods:** We evaluated the effects of high glucose on mouse primary podocytes cell function and survival and on the induction of autophagy. We also studied the development of diabetic nephropathy in streptozotocin-treated diabetic mice with a podocyte-specific disruption of the autophagy pathway.

**Results:** In freshly isolated podocytes, we demonstrated that high glucose induced podocyte autophagy. Furthermore, high glucose-induced autophagy is associated with a decrease in podocin and WT1 expression. Next, autophagy pathway was disrupted in mice using a genetic deletion of Atg5 specifically in podocyte using the Cre-lox system (NPHS2Cre). We found that genetic deletion of autophagy in podocytes dramatically sensitized mice towards the development of diabetic nephropathy in mice. Indeed,

NPHS2Cre-Atg5$^{lox/lox}$ mice present advanced diabetic nephropathy with mesangial thickening, glomerulosclerosis, podocyte loss, foot process effacement and GBM thickening, while control mice present only mild glomerular lesions.

**Conclusions:** These results point to a critical role of autophagy for the development of diabetes-induced glomerular disease representing a novel putative target to ameliorate diabetic nephropathy.

## SA-OR063

**miRNA Profiling in Human Diabetic Nephropathy** Francesca Conserva,[1] Paola Pontrelli,[1] Matteo Accetturo,[1] Anna Maria Di Palma,[1] Salvatore Di Paolo,[3] G. Grandaliano,[2] Loreto Gesualdo.[1] *[1]Dept of Emergency and Organ Transplants - Nephrology Unit, Univ of Bari, Italy; [2]Dept of Biomedical Sciences - Nephrology Unit, Univ of Foggia, Italy; [3]Nephrology and Dialysis Unit, Hospital Dimiccoli, Barletta, Italy.*

**Background:** Diabetic Nephropathy (DN) is a clinical complication of Diabetes. Up to date there is still no cure for DN and this condition has rapidly become the primary cause of End Stage Renal Disease (ESRD). As widely acknowledged, microRNA expression is tightly connected to protein synthesis as each microRNA can induce translational repression of hundreds of targets. Aim of our study was to identify a pattern of differentially expressed microRNAs in kidney biopsies of patients with DN and analyze the role of their putative targets in the pathogenesis and progression of the disease.

**Methods:** Using microarray technology we assayed microRNA expression in formalin fixed kidney tissues from 8 DN patients showing increased proteinuria and decreased glomerular filtration rate (GFR), and 4 control patients with no histological signs of nephropathy. Selected microRNAs were then validated using quantitative PCR.

**Results:** 76 miRNAs were differentially expressed in DN, of which 18 were further validated using quantitative PCR. The microRNA/mRNA joint target analysis revealed three proteins whose reduction could be crucial in the context of glomerular filtration: vascular endothelial growth factor (VEGF-A), essential for podocytes survival and preservation of the glomerular microvasculature; membrane-associated guanylate kinase inverted 2 (MAGI2), a newly discovered component of the slit diaphragm; and sulfatase 1 (SULF1), an enzyme that modulates the interaction of heparan sulfate glycosaminoglycans with growth factors, cytokines, chemokines and adhesion molecules.

**Conclusions:** To our knowledge there is no previous study reporting the microRNA profile of human DN kidney. The validation of these microRNAs in biofluids along with further functional studies in terms of mRNA target validation will soon allow the identification of novel disease biomarkers and untangle novel key players in DN progression.

## SA-OR064

**Systems Biology Approaches in Understanding Diabetic and Non-Diabetic Kidney Disease Risk Loci** Nora Ledo, Yi-An Ko, Ae Seo Deok Park, Katalin Susztak. *Perelman School of Medicine, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** Recent genome-wide association studies (GWAS) have identified multiple loci associated with the risk of chronic kidney disease (CKD) development. The functional role of these loci and the nearby genes is largely unknown. The aim of our study is to map the expression of these genes in normal and disease human kidney samples using systems biology approaches.

**Methods:** We interrogated the expression and regulation of 86 different transcripts that was associated with 211 single nucleotide polymorphisms. Baseline transcript expression was examined by whole human kidney RNA-sequencing. In addition, expression arrays of 92 microdissected (glomeruli and tubules) control, diabetic and hypertensive human kidney samples were analyzed.

**Results:** Among the 86 different transcripts, 57 genes were expressed in the kidney based on the RNA-sequencing data. Only 6 genes showed significantly higher expression in glomeruli compared to tubules ($p<0.05$, fold change>1.5), while 8 genes were specific for tubules in normal human kidney samples. In hypertensive CKD 18 genes were down-regulated, 2 were up-regulated ($p<0.01$). In diabetic kidney disease (DKD) 13 transcripts were down-regulated and 2 were up-regulated ($p<0.01$). Down-regulation of SORT1, DACH1 and SETDB1 and up-regulation of ALMS1 and TBX2 were observed exclusively in DKD, not in hypertensive CKD. Furthermore, of the 57 kidney expressed transcripts, 27 showed strong statistically significant linear correlation with eGFR levels; these included UMOD, NAT8, LASS2, SLC34A1, SLC6A13, ANXA9, SLC7A9 ($p<1E-04$). Network analysis of these eGFR-associated genes highlighted the enrichment for kidney development related pathways.

**Conclusions:** An important part of the post-GWAS functional characterization of the CKD risk loci is the analysis of the gene expression of nearby genes. We found that GWAS-associated genes are enriched for kidney developmental pathways. Our study did not find significant compartmental enrichment of these GWAS related genes. The expression of almost a third of these transcripts showed a strong association with renal function.

*Funding:* NIDDK Support

## SA-OR065

**Systems Genetics Identifies Drivers of Early Diabetic Kidney Disease** Claudiu V. Komorowsky,[1] Viji Nair,[1] E. Jennifer Weil,[2] Ann Randolph,[1] Berne Yee,[3] Kevin V. Lemley,[4] Robert G. Nelson,[2] Benjamin J. Keller,[1] Matthias Kretzler.[1] *[1]Nephrology, Univ of Michigan, Ann Arbor, MI; [2]Diabetes Epidemiology & Clinical Research Section, NIDDK, NIH, Phoenix, AZ; [3]Southwest Kidney Institute, Phoenix, AZ; [4]Nephrology, Children's Hospital LA, Univ of Southern California, Los Angeles, CA.*

**Background:** Genetic variation contributes to the risk of development and progression of diabetic kidney disease (DKD). We sought to elucidate the framework by which SNPs are linked via tissue-specific endophenotypic intermediaries to clinical outcome of human DKD.

**Methods:** 65 participants in the "Renoprotection in Early Diabetic Nephropathy in PIMA Indians Trial (NCT00340678)" underwent SNP genotyping. Matching protocol kidney biopsies were microdissected and profiled on an Affymetrix platform (HG-U133A/Plus2). Stepwise integration of expression quantitative trait locus analysis (eQTL), with weighted co-expression network analysis, quantitative morphometric analysis of renal biopsies (light- and electron microscopy) and deep clinical phenotypic data were leveraged for association analysis.

**Results:** Consistent with previous studies, the majority of eQTLs are located in non-coding regions of the genome. Joint eQTL and co-expression module analysis suggests that gene expression alterations in inflammatory processes are genetically determined and are major drivers of human DKD. Moreover, phenotypic data (morphometry, prospective clinical outcome measurements) was leveraged as functional and clinically relevant framework for variant and gene prioritization. Top eQTLs linked to clinical traits (SNP–Gene–Trait triads: 66 unique SNPs, 83 transcripts and 3 clinical traits) involve genes regulating cellular growth and metabolic adaptation (*e.g. SIRT3*), and inflammatory processes (*e.g. CD48, CSF1R*). Furthermore, we implicate genes not previously linked to diabetes or its complications (*e.g. ADAM12*).

**Conclusions:** Structured integration of information across the genome-phenome continuum offers unique insight into the genetic architecture of DKD. Moreover, it facilitates a systems-based prioritization of variants and linked transcriptional processes for further functional studies in humans and model organisms.

*Funding:* NIDDK Support, Other NIH Support - P30 DK081943

## SA-OR066

**Inhibition of EGFR Activity Protects against Diabetic Nephropathy by Decreasing ER Stress and Augmenting Autophagy** Ming-Zhi Zhang, Bing Yao, Raymond C. Harris. *Medicine, Vanderbilt Univ School of Medicine, Nashville, TN.*

**Background:** Previous studies have indicated activation of renal epidermal growth factor receptors (EGFR) in models of diabetic nephropathy (DN). In the present studies, we examined the effect of treatment with erlotinib, an inhibitor of EGFR tyrosine kinase activity, in the development of DN.

**Methods:** Male eNOS-/- mice on BKS background received daily injections of streptozotocin (STZ; 50 mg/kg i.p.) for 5 consecutive days. The mice were divided into 3 groups: non-diabetes, diabetes with vehicle (water), and diabetes with erlotinib (80 mg/kg/day, daily gastric gavage, 24 weeks).

**Results:** Inhibition of renal EGFR activation by erlotinib was confirmed by decreased phosphorylation of EGFR. Increased ACR in diabetic mice was markedly attenuated by erlotinib treatment (ACR: μg/mg: non-diabetes: 473 ± 96; diabetes: 1516 ± 234, $P < 0.01$ vs. non-diabetes; diabetes + erlotinib: 576 ± 112, $P < 0.01$ vs. diabetes. $N = 4$ in each group). Erlotinib protection against diabetic nephropathy was also indicated by less histological glomerular injury as well as decreases in renal expression of fibronectin and collagen 1. The mammalian target of rapamycin (mTOR) pathway, a key factor in the development of DN, has been reported to be inhibited by AMPK activation. Erlotinib treatment led to activation of AMPK and inhibition of mTOR pathway as evidenced by decreased phosphorylation of mTOR and its downstream target, S6K. Autophagy plays an important role in the pathophysiology of diabetes mellitus, and impaired autophagy may lead to increased endoplasmic reticulum (ER) stress and subsequent tissue injury. In diabetic mice, erlotinib treatment led to activation of renal autophagy as indicated by increased expression and activity of LC3A, a hallmark of autophagy, and was associated with decreased ER stress as indicated by decreased expression of CHOP, BIP and PERK.

**Conclusions:** These studies suggest that inhibition of EGFR with erlotinib attenuates the development of DN in type I diabetes, mediated in part by inhibition of mTOR pathway and subsequent activation of autophagy and inhibition of ER stress.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-OR067

**Role of Sirtuin-1 in Deacetylation of Transcription Factors during Diabetic Nephropathy** Yifei Zhong,[1] Ruijie Liu,[2] John C. He.[3] *[1]Nephropathy, Longhua Hospital, Shanghai Univ of Traditional Chinese Medicine, Shanghai, China; [2]Medicine, Mount Sinai School of Medicine, James J. Peters VAMC, New York City, NY; [3]Inst#2.*

**Background:** Acetylation and deacetylation of transcription factors such as NF-κB and STAT3 have been recognized as important events in pathogenesis of diabetic kidney disease (DKD). Sirtuin-1 (Sirt1) is an enzyme that mediates nicotinamide adenine dinucleotide-dependent deacetylation and can regulates several transcription factor's activity through deacetylation.

**Methods:** Diabetic db/db mice, podocyte-specific Sirt1 knockout diabetic db/db mice and human kidney biopsies were employed in the study. Kidney morphology and apoptosis were examined by PAS and TUNEL stainings respectively. Expression of acetyl-NF-kB and acetyl-STAT3 were examined by immnostaining.

**Results:** Acetylation of NF-κB and STAT3 were increased in the kidneys of both diabetic mice and patients.Moreover, when diabetic db/db mice were treated with pyridoxamine that restored Sirt1 expression, there were an increased level of Sirt1 and decreased acetylation of NF-kB and STAT3 in the kidney. In addition, pyridoxamine attenuated formation of proteinuria. It also improved kidney apoptosis. Furthermore, when Sirt1 was deleted in podocytes of db/db mice, there were aggravated proteinuria and kidney injury as compared to wild-type db/db mice. At the same time, acetylation of NF-kB and STAT3 was increased in podocte-specific Sirt1 knockout diabetic db/db mice.

**Conclusions:** Sirt1 could regulate acetylation of NF-kB and STAT3, which makes Sirt1 as a new therapeutic target for treatment of diabetic nephropathy.

*Funding:* Government Support - Non-U.S.

## SA-OR068

### Gene Expression Differences in Skin Fibroblasts (SF) of Type 1 Diabetic (T1D) Patients (pts) with and without Diabetic Nephropathy (DN)

Maria Luiza A. Caramori,[1] Youngki Kim,[1] Jason H. Moore,[2] Stephen Rich,[3] Andrzej S. Krolewski,[4] Allison B. Goldfine,[4] Helen D. Nickerson,[5] Michael Mauer.[1] *[1]U of MN; [2]Dartmouth College; [3]U of VA; [4]Joslin Diabetes Center; [5]JDRF.*

**Background:** The in vitro behaviors of SF from pts with and without DN have been associated with DN risk.

**Methods:** We performed research skin biopsies in 100 former Genetics of Kidneys in Diabetes (GoKinD) pts. SF were grown in high glucose (HG, 25 mmol/L) for 3-4 passages until 90% confluence and gene expression determined by RNAseq in 40 DN controls (long-standing T1D duration and normoalbuminuria; C) and 60 DN cases (35 proteinuric [P] and 25 ESRD). Directional Exploratory Visual Analysis determined whether differentially expressed genes were over represented in KEGG pathways.

**Results:** Cases were older (56±7 vs. 48±11 years; p<0.001) and had longer T1D duration (44±8 vs. 35±7 years; p<0.001) than C. Systolic blood pressure (BP) was higher in ESRD (137±20) than in P (127±17 mmHg; p<0.02) or C (121±14; p<0.001). There were no differences in sex distribution, $HbA_{1c}$ or diastolic BP among groups. Spliceosome ($p=8.4^{e-17}$), Cell cycle ($p=3.7^{e-15}$), DNA replication ($p=1.8^{e-14}$), Proteasome ($p=8.9^{e-10}$), Pyrimidine metabolism ($p=5.4^{e-7}$), Purine metabolism ($p=9.1^{e-7}$), Mismatch repair ($p=3.9^{e-6}$), RNA degradation ($p=2.1^{e-5}$), Base excision repair ($p=5.1^{e-5}$), Ribosome ($p=8.4^{e-5}$), Terpenoid backbone biosynthesis ($p=8.6^{e-5}$), Propanoate metabolism ($p=1.4^{e-3}$), Cysteine and methionine metabolism ($p=2.4^{e-3}$), Fatty-acid metabolism ($p=4.0^{e-3}$), Glycolysis-Gluconeogenesis ($p=4.9^{e-3}$), Nucleotide excision repair ($p=6.1^{e-3}$), Homologous recombination ($p=7.2^{e-4}$), Pyruvate metabolism ($p=7.2^{e-3}$), and TCA-citrate cycle ($p=1.7^{e-3}$) were up-regulated in C vs. DN cases. The results were similar when C were compared to P or ESRD pts only.

**Conclusions:** There are robust differences in gene expression in SF grown in HG between pts with and without DN. T1D pts without DN despite long T1D duration show increased expression of genes in pathways predominantly related to cell cycle, cell cycle regulation and cell repair mechanisms. These findings are consistent with previous observations indicating protective mechanisms in T1D pts without DN.

*Funding:* Private Foundation Support

## SA-OR069

### Renal Outcomes with Aliskiren in Patients with Type 2 Diabetes: A Pre-Specified Analysis from ALTITUDE

Hiddo Jan Lambers Heerspink,[1] Barry M. Brenner,[2] Frederik I. Persson,[3] Nish Chaturvedi,[4] Scott D. Solomon,[2] Marc A. Pfeffer,[2] Hans-Henrik Parving,[5] Dick de Zeeuw.[1] *[1]Clinical Pharmacology, Univ Medical Center Groningen, Netherlands; [2]Brigham Woman Hospital, Boston; [3]Steno Diabetes Center, Denmark; [4]Imperial College London, United Kingdom; [5]Aarhus Univ, Denmark.*

**Background:** The ALTITUDE (The Aliskiren Trial in Type 2 Diabetes Using Cardiorenal Endpoints) trial showed no benefit of aliskiren on renal outcomes (doubling of serum creatinine, ESRD or renal death) as an adjunct to angiotensin-converting-enzyme inhibitors or angiotensin receptor blockers. We performed a pre-specified analysis of the ALTITUDE trial analyzing the effects of aliskiren on intermediate renal outcomes.

**Methods:** In a double blind randomized controlled trial, 8561 patients were assigned to aliskiren 300 mg/day or placebo and followed for a median of 2.8 years. Intermediate renal endpoints were transitions in albuminuria-stages (i.e. normo-, micro, or macroalbuminuria) and rate of estimated glomerular filtration rate (eGFR) decline either calculated from baseline or from month 6, thus excluding the acute effect of aliskiren on eGFR.

**Results:** Aliskiren significantly decreased progression as well as increased regression of transitions in albuminuria classes by 14% (HR 0.86, 95%CI 0.77 – 0.95) and 27% (HR 1.27, 95%CI 1.17 – 1.37), respectively. Annual rate of eGFR decline was 3.1 ml/min/1.73m$^2$/year in the aliskiren and 3.0 ml/min/1.73m$^2$/year in the placebo group (p=0.52). However, subjects assigned to aliskiren had a significantly greater fall in eGFR during the first 6 months compared to placebo (2.5 vs. 1.4 ml/min/1.73m$^2$; p<0.001), but a slightly slower annual rate of eGFR decline thereafter (2.8 vs. 3.1 ml/min/1.73m$^2$/year; p=0.068).

**Conclusions:** Although the ALTITUDE showed no beneficial effect of aliskiren on hard renal outcomes, aliskiren did delay progression to micro- and macroalbuminuria, and improved regression to micro- and normoalbuminuria. In addition, aliskiren attenuated the (chronic) eGFR decline. The lack of renoprotection with aliskiren, as measured on hard clinical outcomes requires further examination.

## SA-OR070

### Protective Effect of Angiotensin Receptor Blockers on Stroke Attenuates with Lower Renal Function: A Post-Hoc Analysis of RENAAL, IDNT and LIFE

Jarno Hoekman,[1] Frank Holtkamp,[1] Hans Ibsen,[3] Hans-Henrik Parving,[2] Hiddo Jan Lambers Heerspink,[1] Dick de Zeeuw.[1] *[1]Dept Clinical Pharmacology, Univ Med Cent Groningen, Netherlands; [2]Dept Med Endocrinology, Rigshospitalet, Univ of Copenhagen, Denmark; [3]Div Cardiology, Holbæk Hospital, Holbæk, Denmark.*

**Background:** Recent clinical trials in type 2 diabetes and nephropathy assessing the effect of potential cardiorenal protective drugs, including dual Renin-Angiotensin-Aldosterone-System (RAAS) blockade, reported unexpected effects on stroke incidence. We performed a post-hoc analysis in three large trials to study whether specific baseline parameters modify the effect of RAAS-blockade on stroke.

**Methods:** Patients with type 2 diabetes were randomized to losartan or placebo (RENAAL), losartan or atenolol (LIFE), or irbesartan, placebo or amlodipine (IDNT). The total population comprised 4175 subjects. Median follow-up was 3.2 years. Stroke was part of a secondary endpoint in all trials. Homogeneity in treatment effect was tested by adding an interaction term (treatment*baseline parameter) to a multivariable Cox model that included age, sex, systolic blood pressure (BP), eGFR, and albuminuria. Treatment effects were compared in subgroups defined by thirds of the relevant baseline parameter.

**Results:** The effect of ARB therapy was modified by baseline eGFR (p for interaction = 0.03) and not by other baseline parameters. A total of 1215 had eGFR>60, 2267 had eGFR between 30 and 60, and 693 had eGFR <30 ml/min/1.73m$^2$. After 12 months, BP was reduced by 2.3 mmHg (95%CI -3.6 to -1.0) and albuminuria by 28% (35 to 20) in the overall population. Effects did not differ between baseline eGFR subgroups. The risk of stroke was reduced by 36% (-4 to 60) in patients with eGFR>60, reduced by 11% (-28 to 35) in those with eGFR 30 to 60 and increased by 38% (-31 to 174) in those with eGFR<30 ml/min/1.73m$^2$. The risk of other cardiovascular events did not differ between eGFR subgroups.

**Conclusions:** The protective effect of ARB therapy on stroke attenuates at lower eGFR in patients with type 2 diabetes despite similar reductions in BP and albuminuria. This suggests that other, yet unknown, mechanisms account for the lack of protection of ARB therapy in these patients.

*Funding:* Pharmaceutical Company Support - The RENAAL and LIFE trials were sponsored by Merck & Co. The IDNT trial was sponsored by Bristol-Meyers Squibb.

## SA-OR071

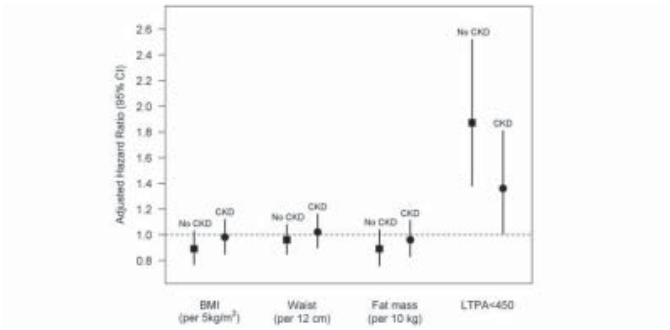
### Adiposity Measures, Physical Activity and Death in CKD: NHANES 1999-2004

Sankar D. Navaneethan,[1] John P. Kirwan,[2] Susana Arrigain,[3] Jesse D. Schold.[3] *[1]Nephrology; [2]Pathobiology; [3]Quantitative Health Sciences, Cleveland Clinic.*

**Background:** Few studies have examined the effects of various adipose tissue measures and leisure time physical activity (LTPA) on mortality.

**Methods:** Data from the National Health and Nutrition Examination Surveys (NHANES) 1999-2004 was used in this analysis. We studied the associations between adiposity measures (total fat mass measured by DEXA), waist circumference [WC], body mass index [BMI] and LTPA with all-cause mortality in those with and without CKD. LTPA was categorized into less than minimum goal (<450 MET/min/week) and at least meeting the minimum goal (>450 MET/min/week) as recommended by national guidelines. Multivariate models were adjusted for demographics, smoking, C- reactive protein, malignancy, eGFR and albuminuria.

**Results:** We included 2153 CKD participant and 9,433 non-CKD participants who had fat mass measured using DEXA, BMI, WC, LTPA and mortality data. After adjusting for covariates, there was no significant risk for death noted with higher WC, fat mass and BMI in those with and without CKD (Figure). When examining normal, overweight and obese groups based on BMI criteria, being overweight (BMI 25-29.9) was associated with lower risk of death in those without CKD (Hazard ratio 0.64, 95% CI 0.45, 0.92). There was a significantly higher risk for death among those who did not meet the minimum LTPA goals compared to those who met or exceeded the recommended activity levels in patients with and without CKD (CKD Hazard ratio: 1.36, 95% CI 1.01, 1.81; non-CKD HR 1.87, 95% CI 1.38, 2.52)(Figure).




**Conclusions:** Lower physical activity, but not adipose tissue measures is associated with higher mortality in those with and without CKD in the representative US population. Future studies should study the effects of improving physical activity levels particularly in the CKD population.

*Funding:* Other NIH Support - National Center for Research Resources: RR024990

## SA-OR072

**Versican and Mortality in Type 2 Diabetic Patients** Bernt Johan Illum von Scholten,[1] Maria Lajer,[1] Diana Julie Leeming,[2] Anne-Mari Gall,[1] Hans-Henrik Parving,[3] Lars M. Rasmussen,[4] Morten Asser Karsdal,[2] Peter Rossing.[1] *[1]Steno Diabetes Center, Denmark; [2]Nordic Bioscience, Denmark; [3]Rigshospitalet, Denmark; [4]Odense Univ Hospital, Denmark.*

**Background:** Extracellular matrix remodelling (ECMr) is a central part in many diseases, in which matrix metallo protease (MMP) driven proteolytic tissue destruction is central in the pathogenesis, such as kidney failure and cardiovascular related diseases. When tissue proteins are degraded by proteases, small fragments are released into the circulation where they may be used as quantitative biomarkers. Versican (VCAN) is an anti-adhesive proteoglycan involved in inflammation and associated with cardiovascular diseases. MMP-12 degraded VCAN circulates in plasma. We aimed to investigate the prognostic value of plasma MMP degraded VCAN in relation to all-cause and cardiovascular mortality in a cohort of type 2 diabetic patients.

**Methods:** In a prospective observational follow-up study, 165 type 2 diabetic patients (101 men; aged 53.9 ± 8.8 years) were followed for a median of 16.8 years (range 0.2-23.0). Baseline plasma VCAN concentrations were determined by ELISA assay. The assay recognises exclusively MMP degraded VCAN, and not intact version nor other closely related proteins.

**Results:** During follow-up, 71 (43%) patients died. Elevated levels of VCAN was borderline associated with all-cause mortality (covariate-adjusted for urinary albumin excretion rate [UAER], estimated glomerular filtration rate, p-NT-proBNP, and conventional risk factors); hazard ratio (HR) 2.64 [95% CI 0.93-7.52] p=0.069. Within groups divided according to level of UAER (normo-, micro- and macroalbuminuria), VCAN was predictive of all-cause mortality in microalbuminuria HR 14.96 (1.23 to 181) and macroalbuminuria HR 250 (3.2 to 19437) but not normoalbuminuria.

**Conclusions:** Elevated plasma VCAN is an independent predictor of all-cause mortality in type 2 diabetic patients with elevated UAER. The effect of VCAN on all-cause mortality was independent of conventional cardiovascular risk factors, UAER, and p-NT-proBNP levels.

## SA-OR073

**Identification of Urine Biomarkers to Predict Early Renal Function Decline in Patients with Type 2 Diabetes** Nishant M. Bhensdadia,[1] Kelly J. Hunt,[1] Joseph Alge,[1] Benjamin Neely,[1] Michael G. Janech,[1,2] John M. Arthur.[1,2] *[1]Nephrology, Medical Univ of South Carolina, Charleston, SC; [2]Nephrology, Ralph H Johnson VA Medical Center, Charleston, SC.*

**Background:** Diabetic nephropathy accounts for 44% of End Stage Renal Disease in United States. We have previously shown urine haptoglobin to urine creatinine ratio could predict patients at risk of renal functional decline. Here we have measured additional candidate markers in urine.

**Methods:** Urine samples of 88 randomly-selected type 2 diabetic patients with eGFR≥60 ml/min and ACR<300 mg/g were used to measure 9 candidate markers (Ceruloplasm, Non-secretory ribonuclease, VIP 36, CD 59 glycoprotein, Nidogen-1, Albumin, Alpha-2HS glycoprotein, Syndecan 4, Beta-hexosaminidase alpha subunit) using LC-MS/MS (Liquid Chromatography Mass Spectrometry) based PRM (Parallel Reaction Monitoring) method. Early Renal Functional Decline (ERFD) was defined as ≥3.3% eGFR decline per year.

**Results:** 16 out of 88 type 2 diabetic patients had ERFD. Creatinine normalized concentration of five candidate biomarkers (Ceruloplasm, Non-secretory ribonuclease, VIP 36, CD 59 glycoprotein, Nidogen-1) had AUC values to predict ERFD which exceeded 0.65 with p value <0.05. Ceruloplasm had the highest AUC value of 0.69 and a p value of 0.017. For comparison creatinine normalized albumin had an AUC value of 0.65 with p value of 0.055.

**Conclusions:** We have identified urine ceruloplasm to creatinine ratio as a biomarker along with 4 other urinary proteins (Non-secretory ribonuclease, VIP 36, CD 59 glycoprotein, Nidogen-1) which can predict ERFD with AUC values which are higher than the AUC for albumin to creatinine ratio in type 2 diabetic patients prior to the development of macroalbumiuria or reduced eGFR.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-OR074

**Urinary Monocyte Chemotactic Protein-1 and Early Diabetic Nephropathy (DN) Lesions** Michael Mauer,[1] Brad H. Rovin,[2] Jon B. Klein,[3] Vasan S. Ramachandran,[4] Harold I. Feldman,[5] Paul L. Kimmel,[6] John W. Kusek,[6] Robert G. Nelson.[7] *[1]Pediatrics, Univ of Minnesota, Minneapolis, MN; [2]Medicine, Ohio State Univ, Columbus, OH; [3]Medicine, Univ of Louisville, Louisville, KY; [4]Medicine, Boston Univ, Boston, MA; [5]Medicine, Univ of Pennsylvania, Philadelphia, PA; [6]NIDDK NIH, Bethesda, MD; [7]NIDDK NIH, Phoenix, AZ.*

**Background:** MCP-1 is a key chemokine regulating monocytes/macrophages migration and infiltration. We asked whether urinary MCP-1 levels are associated with DN lesion severity or the rate of change in DN lesions over 5 yrs in serial biopsy samples in normoalbuminuric normotensive, normal GFR patients (pts) with type 1 diabetes (T1D, duration 3-20 years) in the Renin Angiotensin System Study (RASS).

**Methods:** The progression of DN lesions was not slowed by RAS blockade in the trial. MCP-1 levels were measured by ELISA in baseline urines stored at -80°C and adjusted for urinary creatinine (MCP-1/UCr); 166 of 246 pts had measurable MCP-1/UCr levels.

**Results:** There was no statistically significant association between baseline MCP-1/UCr and the baseline biopsy severity of DN lesions. Higher MCP-1/UCr was associated with greater increases over 5 years in interstitial fractional volume [Vv(Int/cortex)] and glomerular basement membrane (GBM) width but not mesangial fractional volume [Vv(Mes/glom)] (Table 1). However, adjusted for T1D duration, HbA1c, age and sex, only change in GBM width remained statistically significant.

**Conclusions:** We have previously shown that GBM width is a strong and independent predictor of progression from NA to proteinuria and ESRD in normoalbuminuric T1D patients, (Caramori *et al*, JASN, in press). In summary, MCP-1/UCr may be an early biomarker of the progression of important DN lesions in normoalbuminuric, normotensive, normal GFR T1D patients consistent with the notion that inflammatory processes may be involved in the pathogenesis of early DN structural changes. FOR THE BIOMARKER CONSORTIUM.

| Dependent Variable: Delta 5 years | Predictor Variable: Urine MCP-1/Cr* |
|---|---|
| GBM Width | 0.0364 (0.02) |
| Vv(Mes/glom) | 0.0000 (0.37) |
| Vv(Int/cortex) | <0.0001 (0.01) |

*Beta coefficients and (p values)

*Funding:* NIDDK Support

## SA-OR075

**Clinical Significance of Linear Immunofluorescent IgG Staining in Patients with Diabetic Nephropathy** Koki Mise, Junichi Hoshino, Yoshifumi Ubara, Masahiro Kawada, Rikako Hiramatsu, Toshiharu Ueno, Keiichi Sumida, Tatsuya Suwabe, Shigeko Hara, Kenmei Takaichi. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

**Background:** The kidneys of diabetic patients usually exhibit a characteristic pattern of linear immunofluorescent staining for IgG along the glomerular and tubular basement membranes. However, the association between linear IgG staining and the clinical outcome remains unclear.

**Methods:** Among 233 patients with diabetes mellitus who underwent renal biopsy from 1985 to 2010 and were confirmed to have pure diabetic nephropathy according to the classification of Tervaert et al., 159 patients (glomerular class I to III) were enrolled in this study. Immunofluorescent staining was classified into three categories (0=none, 1=weakly positive, 2= positive) according to its intensity. Cox proportional hazards regression analysis was used to calculate the hazard ratio (HR) and 95% confidence interval (CI) for death-censored renal death, with each regression analysis examining two levels of multivariate adjustment. Renal death was defined as commencement of dialysis due to end-stage renal disease.

**Results:** After adjustment for age, gender, estimated glomerular filtration rate, type of diabetes, urinary protein excretion, systolic blood pressure, body mass index, HbA1c, diabetic retinopathy, and red blood cells in urinary sediment at the time of renal biopsy, the HRs for death-censored renal death of patients with IgG staining scores of 1 or 2 were 3.12 (95% CI: 1.08-9.14) and 4.50 (1.60-12.68), respectively, compared with patients whose staining score was 0.

**Conclusions:** More intense linear IgG staining is associated with higher HRs for renal death. This suggests that linear immunofluorescent staining for IgG may be a prognostic pathological finding in patients with diabetic nephropathy.

**SA-OR076**

**Serum and Urinary Levels of TNFRs and Decreasing Renal Function in Type 1 Diabetes (T1D)** Jung Eun Lee,[1,2,3] Tomohito Gohda,[1,2,4] Jan Skupien,[1,2,5] William Walker,[1] Kevin P. McDonnell,[1] Rita R. Holak,[1] Adam Smiles,[1] Andrzej S. Krolewski,[1,2] Monika A. Niewczas.[1,2] *[1]Research Div, Joslin Diabetes Center, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Dept of Medicine, Saumsung Medical Center, Sungkyunkwan Univ School of Medicine, Seoul, Korea; [4]Dept of Internal Medicine, Juntendo Univ School of Medicine, Tokyo, Japan; [5]Dept of Metabolic Disease, Jagiellonian Univ Medical College, Krakow, Poland.*

**Background:** Increased circulating levels of soluble TNFR1 and TNFR2 are strong predictors of renal decline and progression to ESRD, however, the mechanism underlying this increase is unknown. This study aimed to determine serum and urinary patterns of TNFRs in subjects with T1D and various stages of diabetic nephropathy.

**Methods:** Study groups comprised T1D subjects with normo- or microalbuminuria (n=667), with proteinuria (n=412) and 78 healthy subjects. We determined serum and urinary concentrations of TNFR1 and TNFR2 and their respective fractional excretion (FE) in the study group.

**Results:** Subjects with T1D and proteinuria had higher levels of serum and urinary TNFRs compared with subjects without proteinuria ($P < 0.001$). In proteinuria group serum and urinary TNFRs concentrations and FE increased progressively with advanced chronic kidney disease (CKD) stages ($P < 0.001$ for trend). Findings for both TNFRs were similar, so TNFR1 data are shown.

| | Healthy (n=78) | T1D (n=667) No proteinuria CKD 1-2 | T1D (n=261) Proteinuria CKD 1-2 | T1D (n=151) Proteinuria CKD 3-4 |
|---|---|---|---|---|
| Serum TNFR1 (ng/mL) | 1.2 (1.0-1.4) | 1.4 (1.1-1.7) | 2.1 (1.9-2.6) | 4.5 (3.6-5.7) |
| Urinary TNFR1 (ng/mg Cr) | 0.7 (0.5-1.1) | 1.3 (0.9-1.9) | 2.2 (1.4-3.9) | 6.4 (2.7-12.0) |
| TNFR1 FE (%) | 0.5 (0.3-0.7) | 0.8 (0.5-1.2) | 0.9 (0.6-1.5) | 2.7 (1.1-5.2) |

**Conclusions:** Pattern of fractional excretion of TNFRs in our study suggests that increased levels of these receptors in serum and urine in TID subjects at high risk of renal decline and rapid progression to ESRD result from their increased systemic production rather than being a simple reflection of impaired glomerular filtration.

*Funding:* NIDDK Support

**SA-OR077**

**The Impact of Vitamin D Nuclear Receptor on Inflammation of Type 2 Diabetic Nephropathy Patients** Bin Yi, Hao Zhang, Xiao-Fang Hu, Jing Huang, Wei Li. *Dept of Nephrology, The Third Xiangya Hospital of Central South Univ, Changsha, Hunan, China.*

**Background:** The development of chronic inflammation is closely related with the progress of diabetic nephropathy(DN). Studies in vitro have shown that nuclear vitamin D receptor(nVDR) has anti-inflammatory effect. In order to investigate the impact of VDR on the inflammatory state of DN patients, we will detect the nVDR and NF-κB expressions of peripheral blood mononuclear cells and urinary chemokines excretion levels of MCP-1 and RANTES.

**Methods:** 143 subjects were enrolled, including 113 diabetic patients and 30 healthy subjects (NC group). According to the uACR, diabetics were divided into normal albuminuria group(DN0 group), microalbuminuria group(DN1 group), and macroalbuminuria group(DN2 group).Urine MCP-1 and RANTES levels were measured by ELISA. VDR and NF-κB expressions were detected by realtime qPCR and western-blotting. One-way analysis of variance, Pearson correlation analysis and multiple stepwise regression analysis were used for statistical analysis.

**Results:** VDR mRNA and nVDR protein expressions in diabetic groups were gradually decreased with the uACR increase, and significantly lower than those in NC group($p<0.05$). However, NF-κB levels of diabetic patients were gradually increased with the uACR, and NF-κB expressions in DN2 group were significantly higher than those in DN0 andDN1 group($p<0.05$). Urinary MCP-1/Cr and RANTES/Cr levels were significantly different between thegroups, and those in DN2 group were higher than the other three groups. Multivariate linear regression analyses showed that uACR was closed related with SBP, eGFR, VDR and NF-κBp65 levles, andurinary MCP-1 and RANTES excretion rate; the urinary MCP-1/Cr and RANTES/Cr were independently associated with eGFR,SBP and VDR levels.

**Conclusions:** The expression of nVDR were decreased in type 2 DN patients, and negatively related with uACR and urinary MCP-1/RANTES excretion rate. The down-regulation of nVDR may induce inflammation by over-expressed NF-κB, which may play a role in DN pathogenesis.

*Funding:* Government Support - Non-U.S.

**SA-OR078**

**Klotho Genomic Variants Impact on Serum Klotho and Patients Survival in Hemodialysis: The Arnogene Project** Denis Fouque,[1,2] Delphine Maucort-Boulch,[3] Jocelyne Drai,[4] Leslie Genet,[1] Guillaume Jean,[5] Christophe Marçais.[4] *[1]Nephrology, Centre Hospitalier Lyon Sud, Pierre Benite, France; [2]Carmen Cens, Univ de Lyon, Lyon, France; [3]Biostatistics, Centre Hospitalier Lyon Sud, Pierre Benite, France; [4]Biochimie, Centre Hospitalier Lyon Sud, Pierre Benite, France; [5]Dialysis, Nephrocare, Ste Foy, France.*

**Background:** Klotho (KL) is a recently discovered FGF23 cofactor involved in phosphorus metabolism. KL overexpression is associated with extended longevity, whereas KL-KO animals die prematurely.

**Methods:** We prospectively followed 769 hemodialysis (HD) pts between 2009 and 2012 and studied 3 KL genomic polymorphisms, serum KL protein and survival. KL polymorphisms (KL-VS, rs577912 and rs526906) were genotyped through dHPLC analysis and DNA sequencing. Serum α-KL was measured by Elisa (IBL, Japan, normal values 239-1266 pg/mL).

**Results:** Patients were 69±14 yr old, male (58%), diabetic (35%), smokers (37%), dialysis vintage was 6.0 yr, BMI 25.2. Serum phosphorus was 4.7±1.6mg/dL, calcium 8.9±0.7mg/dL, 25OH vit D 56 nmol/L. Median serum a-KL was 127.4 pg/mL (IQ 117-272), significantly lower than in controls. Overall mortality was 13.3% per year. Cox analyses adjusted on dialysis vintage showed that KL-VS homozygotes had a 12% better survival compared to patients without the KL-VS allele (non significant trend). By contrast, homozygotes for rs577912 minor allele had a survival reduced by 20% ($p=0.05$) compared to homozygotes for rs577912 major allele. There was no trend of rs526906 polymorphism on survival. Accordingly, KL-VS homozygocity was associated with a higher serum a-KL concentration (245 vs 116pg/mL), whereas the detrimental rs577912 minor allele homozygocity was associated with a lower serum a-KL (116 vs 130pg/mL).

**Conclusions:** This is the first long term study showing that KL polymorphisms may impact on HD patients survival. Furthermore, KL expression differed depending on KL-VS and rs577912 genotypes, and genotypes with better survival had higher serum a-KL. Whether attempts to increase serum a-KL will improve survival should be tested in future trials.

*Funding:* Government Support - Non-U.S.

**SA-OR079**

**Oral Nutrition Supplements and Readmission Rate in Hypoalbuminemic Dialysis Patients** Eduardo K. Lacson, Weiling Wang, Franklin W. Maddux. *Fresenius Medical Care, North America, Waltham, MA.*

**Background:** Our group has shown that intradialytic oral nutrition supplements (ONS) associated with reduced hospitalization risk in hemodialysis (HD) patients. We evaluated whether early start of ONS within 30 days post-discharge will decrease readmission rates.

**Methods:** All adult HD patients, treated 3x/week on 1/1/2011 in Fresenius Medical Care, North America facilities with their 1st hospital discharge in 2011 were followed for up to 30 days. Patients with post-discharge albumin of ≤3.5 g/dL without ONS use during the 90 days prior to the index hospital admission were eligible. Patients prescribed ONS thereafter were in the ONS group, else were controls. Case-mix (age, gender, race, diabetes mellitus, vintage, access type, BMI, and hospitalization history during 10/1 -12/31/10 plus 3-month means for albumin, hemoglobin, phosphorus, and eKt/V) was recorded as of 1/1/11. Odds ratios (OR) for 30-day readmission were calculated by logistic regression in the overall cohort and in a 2:1 case-mix-based propensity score plus "time-to-albumin result" matched cohort.

**Results:** From 64,128 patients with ≥1 hospital discharge in 2011, 14,435 (22.5%) had no albumin result within 30 days post-discharge, 44,214 (68.9%) had albumin >3.5 g/dL and the remaining 5,479(8.5%) patients had at least one albumin ≤3.5 g/dl. Among the latter, 1,420 (25.9%) ONS patients and 4,059 (74.1%) controls were analyzed. Albumin was resulted within 7.3 ± 6.4 days (IQR: 2-11; median: 5) for ONS vs. 9.9 ± 8.6 days (IQR: 3-16; median: 7) for controls. The mean time from discharge to readmission was 20.3±6.5 days (IQR: 15-16; median: 21) for ONS vs. 15.8 ± 7.6 days (IQR: 9-22; median: 15) for controls. Among ONS patients, the mean time from albumin result to first ONS taken was 16.4±7.2 days (IQR: 10.5-22; median: 16). Readmission OR=2.64 (unadjusted) and 2.27 (adjusted) for controls vs. ONS, and remained at 1.69 for the matched comparison of 1,848 controls with 950 ONS patients (all $p<0.0001$).

**Conclusions:** ONS use was significantly associated with reduced 30-day readmission rate in chronic HD patients. There could be residual confounding from unmeasured factors. These results require confirmation in prospective clinical trials.

## SA-OR080

**Resistance Exercise Program Improves Protein-Energy Wasting and Inflammation in Hemodialysis Patients** Cristiane Moraes,[1] Sandra Mara Silva de Azevedo Marinho,[2] Milena Barcza Stockler-Pinto,[3] Wellington Seguins da Silva,[4] Maria Thereza Baptista Wady,[4] Antonio Nobrega,[1] Denise Mafra.[1] *[1]Graduate Program in Cardiovascular Sciences, Fluminense Federal Univ, Niterói, RJ, Brazil; [2]Graduate Program in Medical Sciences, Fluminense Federal Univ, Niterói, RJ, Brazil; [3]Carlos Chagas Filho Biophysic Institute, Federal Univ of Rio de Janeiro, Rio de Janeiro, RJ, Brazil; [4]Immunology Institute, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil.*

**Background:** Inflammation and protein-energy wasting (PEW) are highly prevalent in hemodialysis (HD) patients. We assessed the effects of reistance exercise (RE) on these conditions.

**Methods:** Patients (n=38,45.9±14.1yrs,61% men,BMI 23.8±4kg/m$^2$) underwent 6 months of RE (3x/week, intradialytic) with elastic bands and bilateral ankle loads. Adhesion molecules (ICAM-1, VCAM-1) were measured by EIA and, interleukin-6 (IL-6), C-reactive protein (CRP) and Tumor Necrosis Factor-Alpha (TNF-α) by ELISA. PEW was diagnosed if patient had simultaneously: BMI<23kg/m$^2$, albumin <4g/dL and,reduced mid-arm muscle circumference area.

**Results:** Adhesion molecules, CRP and, PEW reduced and, physical capacity improved significantly.

**Conclusions:** RE improved PEW and inflammation of HD patients.

Biochemistry and PEW of hemodialysis patients before and after resistance exercise program

| | Before | After |
|---|---|---|
| ***Biochemistry*** | | |
| **PCR (pg/mL)** | 2.3±0.9 | 1.6±0.6** |
| **ICAM (pg/mL)** | 2,204.9±1,460.5 | 1,653.0±1,193.6* |
| **VCAM (pg/mL)** | 5,500.8±1,553.8 | 3,367.3±1.998.6* |
| **IL-6 (pg/mL)** | 81.3±9.4 | 78.7±10.4 |
| **TNF-α (pg/mL)** | 21.0 (20.8 to 30.2) | 21.1 (20.8 to 25.4) |
| **Albumin (g/dL)** | 3.7±0.3 | 3.9±0.2* |
| ***Protein- Energy Wasting*** | 34% | 18.4%* |
| ***Physical Capacity*** | | |
| **STS-10, seconds** | 25.7±6.5 | 19.9±5.0* |
| **STS-60, repetitions** | 28.4±7.3 | 31.6±7.8* |

Median, interquartile range and PEW tested by Wilcoxon Signed Ranks Test; Mean, +-SD tested with paired sample T-test; Significances level, *p< 05; **p< 0001

*Funding:* Government Support - Non-U.S.

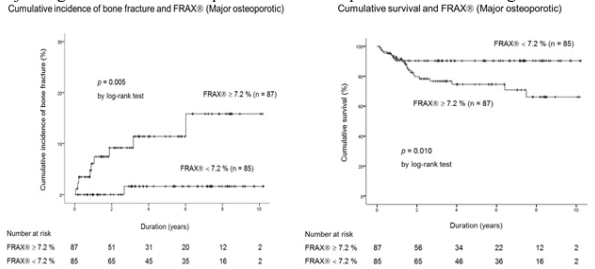
## SA-OR081

**Bone Fracture and Mortality Risk Prediction Using FRAX® in Male Japanese Hemodialysis Patients** Toshihide Hayashi, Nobuhiko Joki, Yuri Tanaka, Hiroki Hase. *Div of Nephrology, Toho Univ Ohashi Medical Center, Tokyo, Japan.*

**Background:** Individuals with bone fracture are at substantially increased risk of mortality. Recently, The World Health Organization Fracture Risk Assessment Tool (FRAX®) has been developed to estimate a 10-year absolute risk of major osteoporotic fracture among general population; however the evidence in CKD patients has been lacking. We, therefore, conducted this hospital-based prospective cohort study to evaluate the predictive ability of FRAX®, not only for fracture but also for mortality, in hemodialysis patients.

**Methods:** We studied a total 172 Japanese men (mean ± SD age; 67 ± 14 years), who had been instituted maintenance hemodialysis from March 2003 to November 2012. Patients were divided into two groups according to the median value of FRAX® for major osteoporotic fracture. The endpoint was defined as all-cause bone fracture and death. The Cox proportional hazard model was used to calculate the hazard ratio (HR) and 95% CI.

**Results:** The baseline mean (± SD) FRAX® for major osteoporotic fracture was 8.5 ± 5.4 %. Total 10 fractures during a mean (± SD) follow-up period of 4.1 ± 3.0 years, and 29 deaths during which of 4.3 ± 3.0 years were observed. Cumulative incidence of fracture in patients with FRAX® below and above the median was 1.2 and 10.3 % ($p$ = 0.005, log-rank test), and cumulative survival was 90.6 and 75.9 % ($p$ = 0.010, log-rank test), respectively. In multivariate Cox regression analysis, FRAX® was significantly associated with both fracture (HR 1.14, 95% CI 1.05 to 1.23) and mortality (HR 1.11, 95% CI 1.04 to 1.18), adjusting for covariates except for clinical component of FRAX® algorithm.




**Conclusions:** These results indicate that FRAX® predict bone fracture risk as well as general population, and mortality risk in hemodialysis patients.

## SA-OR082

**Soluble Ferric Pyrophosphate (SFP) Administered via Dialysate Reduces ESA Requirements in CKD-HD Patients with ESA Hypo-Response** Vivian H. Lin, Tasha M. Farmer, Carrie D. Guss, Raymond D. Pratt, Ajay Gupta. *Rockwell Medical Inc.*

**Background:** SFP added to bicarbonate concentrate crosses the dialyzer membrane and binds to apotransferrin during hemodialysis.

**Methods:** In this double-blind RCT, 103 iron replete, CKD-HD patients were randomized to either SFP dialysate (2 μM or 110 μg iron/L) or placebo, (premixed liquid bicarbonate). Two strata were prospectively defined (Table). ESA was managed by a centralized Anemia Management Center to facilitate consistent adherence to the hemoglobin target range of 95 to 115 g/L. IV iron administration was protocol defined.

**Results:** The primary end-point was change in prescribed ESA dose from baseline to end of treatment (EoT). The SFP group required 35% less prescribed ESA compared to placebo (p=0.045). The subgroup analysis examined the effect of SFP in patients with relative ESA resistance (baseline ESA doses ≥ 13,000 U/week)compared to those who were normo-responsive to ESA.

| | Stratum 1 (ESA< 13,000 U/wk) N=80 | | Stratum 2 (≥13,000 U/wk) N=23 | |
|---|---|---|---|---|
| | SFP N=40 | Placebo N=40 | SFP N=12 | Placebo N=11 |
| Hgb (g/L) BL | 109.2 | 111.3 | 110.7 | 110.2 |
| Hgb (g/L) EoT | 103.7 | 103.9 | 108.5 | 104.5 |
| ESA (U/wk) BL | 7083.2 | 7300.0 | 17,483 | 16,134 |
| ESA (U/wk)) EoT | 7913.6 | 9251.8 | 16,386 | 24,909 |
| LS Mean % change Mean ± SE | 7.6 ± 12.08 | 34.0 ± 12.08 | -8.5 ± 33.4 | 65.9 ± 34.83 |
| LS Mean Difference Mean ± SE (95% CI) | -26.4 ± 17.18 (-60.6, 7.8) | | -74.4 ± 48.23 (-175, 26.2) | |

SFP reduced ESA utilization in both subgroups, compared to placebo. Subgroup size was not suffiicient for statistical significance, however the results were similar in each group; a reduction in prescribed ESA favoring SFP. The effect was numerically larger in the hypo-responsive group. Reticulocyte Hgb was better maintained in the SFP group. The adverse events in the SFP group were similar in frequency and severity to placebo.

**Conclusions:** SFP, delivered via dialysate, reduces ESA dose approximately 75% in hypo-responders compared to placebo while maintaining Hgb and CHr. SFP is well-tolerated with a safety profile similar to that of placebo patients.

*Funding:* Pharmaceutical Company Support - Rockwell Medical Inc.

## SA-OR083

**Ferric Citrate, a Phosphate Binder, Increases Iron Stores but Is Not Associated with Evidence of the Malnutrition-Inflammation-Cachexia Syndrome** John Paul Middleton,[1] Balaji Padmanaban Athreya,[2] Olayiwola B. Ayodeji,[3] Rafael Burgos-Calderon,[4] Ingrid J. Chang,[5] A. Kaldun Kaldun Nossuli,[6] Juan Jose Olivero,[7] Mohammed Sika,[8] Kotagal Shashi Kant,[9] The Collaborative Study Group.[10] *[1]Duke Univ; [2]Pioneer Valley Neph; [3]Peninsula Kidney Assoc; [4]Univ Puerto Rico; [5]Western Neph; [6]Washington Neph Assoc; [7]Med Cent Dialysis; [8]Vanderbilt Univ; [9]Univ Cincinnati; [10]CMM.*

**Background:** A cornerstone of care in hemodialysis (HD) patients is controlling serum phosphorus (P) by use of oral binders. Oral iron in the form of ferric citrate (FC) can reduce serum P concentrations. However, elevated iron stores may create adverse clinical effects by favoring growth of invasive pathogens or by modulating immune pathways. The purpose of this study was to determine whether FC was associated with evidence for the malnutrition-inflammation-cachexia syndrome (MICS) in HD patients.

**Methods:** We conducted a multicenter, randomized, clinical trial of oral FC in 441 dialysis subjects. We did a 2-week washout period and a 52-week Safety Assessment Period (SAP). Subjects were randomized 2:1 to FC or active control (AC; sevelamer, calcium acetate, or both). Intravenous iron usage was at the discretion of the site, but IV iron was not administered if the serum ferritin was >1000 ng/mL or transferrin saturation (TSAT) was > 30%.

**Results:** 292 subjects received FC and 149 subjects received AC. During the SAP, serum P was similar between the FC and AC groups (mean difference at SAP end 0.036mg/dL; p=0.82). We examined laboratory markers associated with iron stores and MICS.

| Variable | | Ferric Citrate | Active Control | Rx Effect P Value |
|---|---|---|---|---|
| Serum Albumin (mg/dL) | Baseline | 4.02 ± 0.33 | 4.00 ± 0.33 | 0.7 |
| | Week 52 | 3.98 ± 0.41 | 3.98 ± 0.35 | |
| Serum Ferritin (ng/mL) | Baseline | 593 ± 293 | 609 ± 308 | <0.001 |
| | Week 52 | 896 ± 489 | 624 ± 361 | |
| TIBC (mcg/dL) | Baseline | 233 ± 40 | 227 ± 39 | <0.001 |
| | Week 52 | 225 ± 43 | 235 ± 39 | |
| Mean Periph WBCs (x10$^{-3}$) | Baseline | 6.7 ± 2.0 | 6.5 ± 2.2 | 0.9 |
| | Week 52 | 6.6 ± 2.0 | 6.5 ± 2.0 | |
| Lymphocytes (%) | Baseline | 22.4 ± 7.7 | 22.3 ± 8.6 | 0.5 |
| | Week 52 | 22.1 ± 7.9 | 21.7 ± 8.2 | |

**Conclusions:** Thus oral FC controls serum P concentrations and is associated with evidence of increased iron stores. However, FC is not associated with the appearance of markers of inflammation or malnutrition. These data suggest that FC will not predispose to MICS in HD patients.

*Funding:* Pharmaceutical Company Support - Keryx

## SA-OR084

**Intravenous Iron for Functional Iron Deficiency Anemia in Hemodialysis Patients: A Meta-Analysis**

Paweena Susantitaphong,[1,2] Fahad S. Alqahtani,[1] Bertrand L. Jaber.[1] *[1]Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Medicine, Chulalongkorn Univ, Bangkok, Thailand.*

**Background:** Studies on benefits of intravenous iron therapy among hemodialysis patients with functional iron deficiency anemia have shown conflicting results. We conducted a meta-analysis to assess the efficacy and safety of intravenous iron in hemodialysis patients with functional iron deficiency anemia.

**Methods:** We searched MEDLINE (through December 2012), Cochrane Central Register of Controlled Trials, and ClinicalTrials.gov for single-arm studies and randomized controlled trials (RCTs) that examined the effect of intravenous iron for functional iron deficiency anemia in hemodialysis patients on anemia parameters and markers of oxidative stress and inflammation. Studies of absolute iron deficiency were excluded. Random-effect model meta-analyses were used to compute changes in outcomes of interest.

**Results:** We identified 41 studies (3,202 patients), representing 27 single-arm studies, and, 2 crossover and 12 parallel-arm RCTs. The results of the single arm studies are shown in the Table.

| Outcome variable | No. study arms | No. patients | Mean change (95%CI) | P-value |
|---|---|---|---|---|
| Hemoglobin, gm/dL | 32 | 3242 | 0.5 (0.3, 0.7) | <0.001 |
| Hematocrit, % | 16 | 1524 | 1.3 (0.4, 2.2) | 0.006 |
| Ferritin, ng/mL | 51 | 4798 | 138 (89, 186) | <0.001 |
| TSAT, % | 60 | 4880 | 15 (11, 18) | <0.001 |
| Erythropoietin dose, units/week | 29 | 2404 | -1506 (-2360, -653) | 0.001 |
| C-reactive protein, mg/dL | 10 | 652 | -0.01 (-0.02, 0.001) | 0.06 |
| Oxidized LDL, U/L | 5 | 152 | 9.1 (-11.4, 29.7) | 0.38 |
| Total anti-oxidant capacity, mmol/L | 4 | 156 | -0.4 (-0.6, -0.1) | 0.002 |
| MDA, µmol/L | 2 | 52 | 0.12 (0.01, 0.23) | 0.04 |
| Neutrophil respiratory burst, RLU | 2 | 28 | -453 (-736, -170) | 0.02 |

Similar trends were observed in RCTS.

**Conclusions:** Intravenous iron administered to hemodialysis patients with functional iron deficiency anemia results in an increase in hemoglobin and a decrease in erythropoietin dose. The observed effects on markers of oxidative stress are of unclear clinical significance. Studies with long-term clinical outcomes are needed.

*Funding:* Clinical Revenue Support

## SA-OR085

**Hepatitis C Serostatus Modifies Anemia Control in Chronic Hemodialysis Patients: Results from an International Study**

Lim Paik Seong,[1] Len A. Usvyat,[2] Michael Etter,[4] Peter Kotanko.[4] *[1]Nephrocare and Tungs Taichung Metroharbour Hospital, Taiwan; [2]Fresenius Medical Care North America, Waltham, MA; [3]Fresenius Medical Care Asia Pacific, Hong Kong, Hong Kong; [4]Renal Research Institute, New York, NY.*

**Background:** Recent basic research indicates that hepatitis C core+1/ARFP, a novel protein produced by a second functional open reading frame within the core gene, decreases hepcidin expression in hepatoma cells (Kotta-Loizou, *J Gen Virol* 2013). Since hepcidin is a key regulator of iron availability, we hypothesized that hepatitis C positive chronic hemodialysis (HD) patients experience better anemia control and less resistance to erythropoietin (EPO) compared to their hepatitis C negative fellow patients.

**Methods:** We tested our hypothesis in two diverse large HD populations from December 2012, namely in patients treated in Nephrocare facilities in Taiwan, and in patients dialyzed in US Renal Research Institute (RRI) clinics. We extracted hepatitis C serology, indicators of anemia control, and iron status (RRI only). EPO resistance was calculated as EPO dose divided by hemoglobin level.

**Results:** We analyzed data from 14,589 Taiwanese and 6,238 US patients. In the cohort from Taiwan 16.8% of patients were hepatitis C positive, in the US cohort 9.3%. Hepatitis C positive patients in both populations showed higher hemoglobin levels (Taiwan: 10.5 vs. 10.4 g/dL; US: 10.9 vs. 10.8 g/dL) and lower EPO doses (Taiwan: 3637 vs. 4352 U/HD; US: 4978 vs. 5199 U/HD). Compared to hepatitis C positive patients, EPO resistance was increased in hepatitis C negative patients by 5% in the US and by 20% in Taiwan. In the US cohort the average iron dose per HD was lower in hepatitis C positive patients (73.9 vs. 77.0 mg/HD).

**Conclusions:** In two large diverse dialysis populations a positive hepatitis C serostatus is associated with improved anemia control and EPO responsiveness. This observation is possibly related to the recently described interaction between hepatitis C virus and hepcidin expression.

## SA-OR086

**Uremic Toxicity Induces Eryptosis and Atypical Monocyte Transformation: Erytrophagocytosis as a Novel Pathway to Renal Anemia**

Natalia Borges Bonan,[1] Thiago Maass Steiner,[1] Peter Kotanko,[2] Viktoriya Kuntsevich,[2] Roberto Pecoits-Filho,[1] Andrea Novais Moreno-Amaral.[1] *[1]PUCPR, Curitiba, Brazil; [2]RRI, New York.*

**Background:** Anemia is common in chronic kidney disease (CKD) and hemodialysis (HD). Uremia alters red blood cell (RBC) membrane resulting in increased phosphatidylserine (PS) exposure and increased RBC clearance (eryptosis). CD14+/CD16+ monocytes represent an atypical inflammatory phenotype that mimics a macrophage, potentially capable of erythrophagocytosis. Here we investigate the effects of uremic serum and p-Cresol (pC) on eryptosis, monocytes transformation and erythrophagocytosis.

**Methods:** Cells from CKD and HD patients and healthy controls (HC) (5 subjects each) were collected. HC cells were treated with RPMI, pC (concentrations of 10 and 50µg/ml) or uremic serum. Cells were stained with annexin V to detect PS expression, and anti-CD14-CD16 to detect monocyte transformation. Erythrophagocytosis was evaluated using control monocytes and RBC stained with anti-CD14 and antiGlycophirin-A, previously incubated with pC or uremic serum and after co-incubated with control cells for 2 hours: control monocytes against pretreated RBC or control RBC against pretreated monocytes.

**Results:** The fraction of annexinV+ RBC was increased in CKD (6%) and HD (6%) when compared to HC (1.9%). Incubation of HC RBC with uremic serum or pC 50µg/ml, resulted in 5.9% and 8.5% annexin V+ cells. The fraction of CD14+/CD16+ monocytes was greatly increased in CKD (40%) and HD (19%), when compared to controls (3.4%). We observed an increase in CD14+/CD16+ cells following addition of pC at concentrations of 10µg/ml (8%) and 50µg/ml (18%). Erytrophagocytosis was increased when RBC were pretreated with pC 50µg/ml (74%) and uremic serum (62%), and co-incubated with monocytes. HC monocytes pretreated with pC 50µg/ml or uremic serum, and co-incubated with RBC showed increased erythrophagocytosis ( 73% and 35%, respectively).

**Conclusions:** This is the first description that that uremic serum and pC can (a) increase RBC PS expression (and thus eryptosis); (b) transform monocyte phenotype; and (c) increase erythrophagocytosis. Taken together, our observations indicate a novel pathway in the pathogenesis of CKD anemia.

## SA-OR087

**Hypoxia Inducing Factor Prolyl Hydroxylase Inhibitor FG-4592 Corrects Anemia in Peritoneal Dialysis**

Anatole Besarab,[1] Daniel Tak Mao Chan,[3] Sohan L. Dua, Marietta Franco,[2] Eugenia Henry,[2] Robert Leong,[2] Lona Poole,[2] Ming Zhong,[2] Lynda Szczech,[2] Kin-Hung Peony Yu,[2] Thomas B. Neff.[2] *[1]Henry Ford Hosp; [2]FibroGen; [3]Univ of Hong Kong; [4]Valley Renal Medical Group.*

**Background:** FG-4592 inhibits prolyl hydroxylase and transiently stabilizes hypoxia-inducible factor being developed for CKD anemia treatment. Endogenous erythropoietin levels are transiently normalized to physiologic levels. The safety and efficacy of FG-4592 using various iron regimens was tested in a phase 2 study FGCL4592053 in hemo (HDP) and peritoneal (PDP) dialysis patients and reported. In that ~10% of patients with end stage renal disease (ESRD) globally receive PD, it is important to characterize the efficacy of FG-4592 in this group.

**Methods:** 48 ESA-naïve HDP were randomized to FG-4592 and no, oral or IV iron. All PDP (12) received FG-4592 with oral iron. The cohorts of PDP and HDP receiving oral iron were compared (n=24). Hb target was 11-13 g/dL.

**Results:** There were no clinically or statistically significant differences in demographics, medical history or baseline labs btw PDP and HDP. Among PDP, median age was 51.0 y (range 30-68). At baseline, Hb was 8.8 g/dL (0.7), ferritin level was 144.3 ng/mL (79.2) and TSAT was 19.4% (5.8) in the PD cohort. All PDP had hypertension while none had DM. PDP had a median baseline:peak change in Hb of 3.1 g/dL (range 2.4-4.7). All PDP and 11/12 HDP on oral iron responded (a change in Hb>1 g/dL from wks 3-13). Comparing PDP to HDP controlling for baseline Hb and iron stores, no difference in Hb change from baseline or in time to Hb response was detected btw groups (P=0.90 and 0.42, respectively). Median time to response was 4 wks (range 3-8). Adverse Event rates were similar between HDP and PDP and were generally mild-moderate and typical for ESRD patients.

**Conclusions:** While the power afforded by the number of patients receiving PD is limited, these data do not suggest a difference in efficacy or safety for FG-4592 among patients with ESRD based on modality. Given these data and safety signals demonstrated in trials of ESAs, FG-4592 could represent a safer and more convenient option for anemia treatment in ESRD.

*Funding:* Pharmaceutical Company Support - FibroGen, Inc.

## SA-OR088

**Validation of Rare Genetic Variants Associated with Focal Segmental Glomerulosclerosis by Using an ES Cell-Based RNAi Mouse Model**

Haiyang Yu,[1] Andrey S. Shaw.[1,2] *[1]Dept of Pathology and Immunology, Washington Univ in St. Louis, Saint Louis, MO; [2]Howard Hughes Medical Institute, Washington Univ in St. Louis, Saint Louis, MO.*

**Background:** We sequenced 2,500 podocyte genes in over 200 FSGS patients of European ancestry. Based on the distribution of variants, we identified candidate genes that have a variant pattern that is distinct from an ethnically matched control group. To validate potential FSGS disease genes in an animal model, we developed a mouse ES cell model, which will allow us to efficiently and physiologically test candidate genes.

**Methods:** A new ES cell line that is sensitized to FSGS, and express podocyte specific rtTA. Laser assisted micro-injection that facilitates generation of 100% chimeric mice. Inducible RNAi in podocytes to knock down candidate genes in podocyte *in vivo*.

**Results:** We developed a FSGS disease candidate gene list based on the statistical analysis of the sequences of 2500 podocyte genes that we sequenced in over 200 FSGS patients of European ethnicity. To efficiently validate these genes, we developed a mouse ES cell strategy to test individual genes. We first established a mouse ES cell line that is sensitized to the development of FSGS based on our previous finding that haploinsufficiency in both *Cd2ap* and *Synpo*, leads to FSGS over 6-12 months. We designed a Mir30 based shRNA expression cassette that allows tetracycline inducible expression of the shRNA and facilitates efficient homologous recombination into the *Hprt1* locus. The recombinant ES cells were then injected using a method that generates mice with near 100% chimerism. Chimeric mice were directly used for screening by doxycycline-induced shRNA expression. The method was validated by showing that a CD2AP shRNA can induce nephrotic syndrome. Using this method, we will test each of our candidate genes.

**Conclusions:** We established a mouse model to induce RNAi in podocyte in vivo, and validated that this method is applicable for screening gene candidates that were identified by the large-scale genomic analysis. Now we are screening for the genes that induces proteinuria when they are knocked down in the chimeric mice.

## SA-OR089

### Lack of Murine Double Minute (MDM)-2 in Podocytes Causes Dysregulation of Autophagy and Focal Segmental Glomerulosclerosis

Dana Thomasova,[1] Hauke A. Bruns,[1] Helen Liapis,[2] Hans J. Anders.[1] *[1]Renal Div, Medizinische Klinik und Poliklinik IV, Univ of Munich, Munich, Germany; [2]Dept of Pathology & Immunology, Washington Univ School of Medicine, St. Louis, MO.*

**Background:** Podocytes are terminal differentiated epithelial cells of the glomerular filtration barrier that can hardly be replaced upon loss. Like neurons, they seem to survive many decades and manage to stand the hemodynamic, toxic, and immunologic insults that occur during lifetime. The E3-ubiquitin ligase murine double minute (MDM)-2 is a non-redundant element of NF-kB signaling and the master negative regulator of tumor suppressor gene p53-mediated cell cycle arrest. MDM2 blockade can prevent adriamycin-induced podocyte loss by restoring p53 and preventing podocyte mitotic catastrophe, because podocyte mitosis leads to podocyte death. The role of the intense MDM2 expression in non-stressed podocytes, however, is unknown today. We hypothesized, that MDM2 would be required to prevent p53 overactivation, a state that may cause premature podocyte loss.

**Methods:** We generated and characterized podocyte-specific MDM2-knockout mice (Podocin-Cre/MDM2flox).

**Results:** Comparison of podo-MDM2 -/- mice with their heterozygous +/- littermates revealed lack of MDM2 in podocytes. Nephrin/WT-1+ podocyte counts were identical in 3 weeks old mice of both groups. The podocyte/glomerulus ratio increased with aging in the control mice while it declined in podo-MDM2 KO mice by week 14 of age. Relative podocytopenia in podo-MDM2 KO mice resulted in proteinuria and progressive FSGS. Electron microscopy of podo-MDM2-/- renal tissue displayed the typical abnormalities of glomerulosclerosis but also specific ultrastructural changes in the podocytes, such as massive vacuolization and ER abnormalities suggesting involvement of dysregulated autophagy. Upregulation of p53 expression concurrently with no increase of TUNEL staining in the podo-MDM2 KO podocytes indicates involvement of p53-dependent alternative, nonapoptotic programmed cell death mechanism in podocyte loss.

**Conclusions:** In contrast to the pathogenic role of MDM2 in podocyte injury, podocytes need MDM2 during homeostasis to avoid dysregulated autophagy and podocyte loss.

## SA-OR090

### Direction of Transdifferentiation between Podocytes and Parietal Epithelial Cells, and Its Role for Focal Segmental Glomerulosclerosis

Kazuo Sakamoto,[1,2] Toshiharu Ueno,[1] Namiko Kobayashi,[1] Satoshi Hara,[1] Taiji Matsusaka,[3] Michio Nagata.[1] *[1]Pathology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Nephrology, Iizuka Hospital, Iizuka, Hukuoka, Japan; [3]Medicine, Tokai Univ, Isehara, Kanagawa, Japan.*

**Background:** Although transdifferentiation (TD) between podocytes and parietal epithelial cells (PECs) has been suggested in focal segmental glomerulosclerosis (FSGS), its role for FSGS is still unknown. Using genetic tagging system combined with cell-marker staining, we are able to determine the direction of TD between two cells and its frequency/localization.

**Methods:** Mouse model of FSGS (LMB2-treated NEP25 mouse) carrying genetic tagging on podocyte (FSGS mice; n = 9) and NEP25 without LMB-2 (controls; n = 7) were analyzed. TD from podocyte to PEC was determined when cell expressed LacZ, a podocyte lineage-marker, and Pax-8, PEC marker, by double staining. TD from PEC to podocyte was estimated by comparison of frequency between Nestin, a podocyte marker, and Pax8-double positive (DP) cells, and that of LacZ-Pax8-DP cells. When frequency of each-DP cells are not different, majority of TD is podocyte to PEC. When Nestin/Pax-8 DP cells are more, some fraction of cells is supposed to be PEC to podocyte. We counted the number of DP cells per 100 glomeruli to examine the frequency. From localization of DP cells, we speculated role of epithelial TD in FSGS. Statistical analysis was done by Student's T test.

**Results:** Totally 795 glomeruli in FSGS and 546 glomeruli in control were analyzed. 1. The frequency of DP cells for Nestin/Pax8 was significantly increased in FSGS mice (1.6 ± 0.4% vs. 5.8 ± 0.5%, control vs. FSGS, P < 0.01) and those of LacZ/Pax8 were also significantly higher in FSGS mice (1.5 ± 0.6% vs. 4.3 ± 0.6%, control vs. FSGS, P < 0.01). 2. Incidence of Nestin/Pax8 and LacZ/Pax8-DP cells showed no difference. Suggesting majority was podocyte to PEC. 3. Most of DP cells were in glomerular tufts without sclerotic lesion of FSGS, and few (less than 1 %) of them were detected in extra-capillary hyperplasic lesions in FSGS mice.

**Conclusions:** Epithelial transdifferentiation in FSGS was much more likely from podocyte to PEC than PEC to podocyte in FSGS and its role is mimal in FSGS lesion.

## SA-OR091

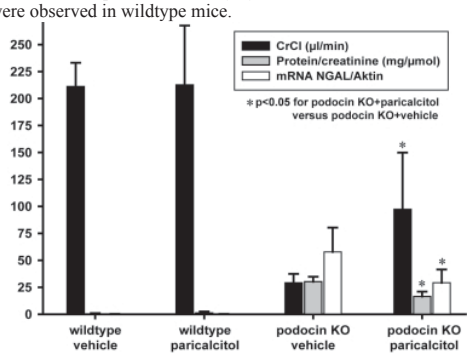
### Paricalcitol Reduces Proteinuria and Improves Renal Function in Podocin Deficiency and Nephrotic Syndrome

Henrik Birn,[1] Corinne Antignac,[2] Rikke Nielsen,[1] Erik I. Christensen,[1] Kathrin Weyer.[1] *[1]Dept of Biomedicine, Aarhus Univ, Aarhus, Denmark; [2]Inserm U983, Hôpital Necker Enfants Malades, Paris, France.*

**Background:** Mutations in the podocin gene, *NPHS2*, have been shown in familial and sporadic forms of steroid-resistant nephrotic syndrome with focal segmental glomerulosclerosis (FSGS). Studies indicate that the vitamin D analog paricalcitol ameliorates proteinuria and prevents kidney injury in acquired podocyte injury. So far the renoprotective effect of vitamin D in genetic nephrotic syndromes has not been addressed. We examined the effect of paricalcitol in a genetic FSGS model caused by inactivation of podocin in the mouse kidney using the Cre-loxP technology.

**Methods:** Mature podocin knockout mice ($Nphs2^{lox/lox}$; $Cre^{+}$) and littermate controls ($Nphs2^{lox/lox}$; $Cre^{-}$) were induced by injection with tamoxifen. Subsequently, the knockout and control mice were receiving either paricalcitol (0.15 µg/kg BW) or vehicle using osmotic minipumps for 5 weeks. Plasma samples, 24-hour urine samples, and kidney tissue were collected.

**Results:** Paricalcitol attenuated weight-loss of the podocin knockout mice and reduced the urinary protein/creatinine ratio by 43 +/- 3% when compared to vehicle treated knockout mice. At 5 weeks creatinine clearance in vehicle treated podocin knockout mice was reduced to 29 +/- 8 µl/min or 13% of wildtype (211 +/- 22 µl/min, p<0.0001). Paricalcitol treated knockouts sustained a creatinine clearance of 97 +/- 53 µl/min (p=0.02 compared to vehicle treated knockout mice), and had a reduced renal expression of the injury marker NGAL and the pro-inflammatory cytokine IL-6. No renal effects of paricalcitol treatment were observed in wildtype mice.




**Conclusions:** Paricalcitol protects renal function and reduces proteinuria in a mouse model of genetic nephrotic syndromes involving mutations of the podocin gene. This may be be further explored in human disease.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## SA-OR092

### Induction of CCAAT/Enhancer-Binding Protein Delta (CEBPD) Links Inflammation and Hypoxia to Injury by Regulating Hypoxia-Inducible Factor 1 (HIF-1)

Junna Yamaguchi, Tetsuhiro Tanaka, Nobuaki Eto, Masaomi Nangaku. *Div of Nephrology and Endocrinology, The Univ of Tokyo, Tokyo, Japan.*

**Background:** Tubulointerstitial hypoxia is a critical determinant in the pathogenesis of ischemic kidney diseases. HIF-1 is a master regulator of defense against hypoxia, and we designed this study to identify novel HIF-1 regulators in kidney.

**Methods:** An shRNA library was created based on our microarray analysis of chronic kidney hypoxia model. The impact of candidate genes on HIF-1 was evaluated by immunoblotting and hypoxia-inducible reporter assays in vitro, leading to identification of a novel upregulator of HIF-1. Its canonical regulation of HIF-1 and the underlying mechanisms were investigated by real-time PCR, immunoblotting, HIF-1α promoter assay and chromatin immunoprecipitation (ChIP) in human proximal tubular cells (HK-2).

**Results:** An shRNA library experiment consisting of 150 genes identified CEBPD, a transcription factor, as a novel HIF-1 regulator. CEBPD was induced in kidneys subjected to systemic hypoxia, as well as in models of various acute and chronic ischemic kidney injuries (ischemia reperfusion injury, cisplatin nephrotoxicity, renal artery stenosis and 5/6 nephrectomy), with predominant expression in the nuclei of tubular cells. In vitro, CEBPD siRNA knockdown and overexpression mediated down- and upregulation of HIF-1α as well as its target genes, respectively. Mechanistically, CEBPD directly promoted the transcription of HIF-1α by binding to its promoter. Furthermore, we characterized the inflammatory nature of CEBPD and linked inflammation and the HIF response. CEBPD was rapidly inducible by inflammatory cytokines, such as interleukin-1β (IL-1β), in an NF-κB-dependent manner, and was indispensable for the non-hypoxic induction of HIF-1α.

**Conclusions:** These results demonstrate CEBPD as a novel HIF-1 regulator in kidney. CEBPD was up-regulated in tubular epithelial cells via NF-κB-dependent pathway and regulated HIF-1 expression and its transcriptional activity. Given the overall protective roles of HIF-1, modulation of CEBPD activity may offer a novel therapeutic strategy in kidney diseases characterized by co-existent hypoxia and inflammation.

*Funding:* Government Support - Non-U.S.

## SA-OR093

**mTORC1 and -2 Induced Signalling Pathways Regulate Renal Proximal Tubular Endocytosis** Franziska Theilig,[1] Patricia Matthey,[1] Brigitte Scolari,[1] Florian Grahammer,[2] Tobias B. Huber.[2] *[1]Medicine, Anatomy, Fribourg, Switzerland; [2]Medicine, Nephrology, Freiburg, Germany.*

**Background:** Receptor-mediated endocytosis is a pivotal function of the renal proximal tubule (PT) to reabsorb proteins from the ultra filtrate. PT dysfunction occurring e.g. in nephrotic syndrome results in an impairment of this process. The regulation of endocytosis and signaling pathways involved remain unknown. The aim of the study was to identify possible kinase-induced signaling pathways involved in the regulation of endocytosis.

**Methods:** Therefore OK cells were incubated with various kinase inhibitors and endocytosis assays using rhodamine-coupled albumin were performed. Transgenic mutants of mTORC1 and -2 were used to elucidate their role in endocytosis.

**Results:** Inhibitors for mTORC1, mTORC2, PI3-kinase and PKC significantly inhibited receptor-mediated endocytosis, whereas Inhibitors for MAPK, JNK and ERK did not alter albumin uptake. To further elucidate the role of mTORC1and -2; Raptor $^{fl/fl}$ and Rictor $^{fl/fl}$ Pax8rtTa*TetOCre as well as double mutants were analyzed. Low molecular weight proteinuria was only observed in the double knockout. Injections of horse reddhish peroxidase demonstrated a significantly reduced uptake in PT of transgenic mice compared to the respective controls. Electron microscopic analysis revealed a diminished content of endocytic vesicles which was mostly pronounced in the Raptor$^{fl/fl}$/Rictor$^{fl/fl}$;Pax8$^{Cre}$. Silencing of S6-kinase 1 and rictor by adenoviral transduction strongly reduced early endosome formation, the actin cytoskeleton remained unaltered. Western blot analysis demonstrated a knockout degree of approx. 65 ± 11 % and reduced clathrin, EEA1 and Rab11 expression for clathrin vesicles, early endosomes and recycling endosomes, respectively. However, PI3-kinase and PKC were unchanged between groups and may not be involved as a downstream target of mTORC1/-and 2 to affect endocytosis.

**Conclusions:** In conclusion, mTORC1 and -2 are important regulators to alter PT endocytic functions.

*Funding:* Other NIH Support - SNF

## SA-OR094

**Anti-miR21 Protects Collagen 4A3 Deficient Mice from Progression of Alport Disease by Decreasing Oxidative Stress** Ivan G. Gomez,[2] Deidre Mackenna,[1] Allie M. Roach,[2] Bryce Gordon Johnson,[2] Tai-He Xia,[4] Vivek Kaimal,[1] Dorin-Bogdan Borza,[3] Jie Zhang,[4] Shiguang Liu,[4] B. Nelson Chau,[1] Jeremy Stuart Duffield.[2] *[1]Regulus Therapeutics; [2]Medicine, Univ of Washington, Seattle, WA; [3]Vanderbilt Univ; [4]Sanofi-Genzyme R&D Center.*

**Background:** Alport syndrome in humans is an inherited form of kidney disease caused by a mutation in the gene coding the capillary basement membrane collagen IV. The disorder is characterized by progressive glomerulonephritis, leading to glomerulosclerosis, tubulo-interstitial disease and organ failure. Col4a3$^{-/-}$ mice also spontaneously develop severe kidney disease highly similar to human disease. Recent studies have shown that miR-21 promotes interstitial kidney disease with fibrosis by silencing metabolic pathways, particularly fatty acid metabolism, and by promoting ROS formation. We hypothesized that treating Col4a3$^{-/-}$ mice with silencing anti-miR21 oligonucleotides would prevent progression of disease.

**Methods:** Col4a3$^{-/-}$ mice were given anti-miR21 (25mg/kg q4d) or control from wk3 to wk9. Urine, plasma and kidneys were harvested at the end of wk9. All samples were analyzed to determine changes in kidney function and fibrosis.

**Results:** Anti-miR21 treatment attenuated the loss of kidney function and development of albuminuria. Glomerulosclerosis and interstitial fibrosis were markedly attenuated, proximal tubules were preserved, and crescent formation reduced. Anti-miR21 reduced infiltrating macrophages, myofibroblast appearance, and tissue and urinary ROS production. Global transcriptional analysis confirms our previous observations implicating a role for PAPRa as a mediator of anti-miR-21 in models of chronic kidney disease; additional analyses are ongoing and will be discussed.

**Conclusions:** Anti-miR21 prevents progressive loss of kidney function in the Col4a3$^{-/-}$ mouse, attenuates both glomerular and tubulo-interstitial disease by reducing oxidative stress as one of the potential mechanisms. Anti-miR21 is a potential new therapy for Alport Syndrome.

## SA-OR095

**APOL1 Is an Innate Immunity Effector That Induces Stress Autophagy by Interacting with the SNARE Protein, VAMP8** John F. O'Toole,[1] Sethu M. Madhavan,[1] David B. Thomas,[2] L. Barisoni,[2] Leslie A. Bruggeman,[1] John R. Sedor.[1] *[1]Case Western Reserve Univ; [2]Univ of Miami.*

**Background:** Apolipoprotein L1 (APOL1) prevents African Sleeping Sickness, a trypanosomal disease. However, some species express a serum resistance associated protein (SRA) that binds and blocks APOL1 function. Genetic variants in APOL1 (variant APOL1s) that occur in the SRA-binding domain of APOL1 circumvent parasitic resistance, but associate with non-diabetic kidney diseases in African Americans. The mechanisms by which variant APOL1s promote nephropathy are unknown. We hypothesized that the SRA-binding domain of APOL1 interacts with a human protein(s) orthologous to SRA and that identification of APOL1's binding partner(s) would reveal its function(s).

**Methods:** APOL1 and VAMP8 localization and expression were examined in cultured cells and normal human kidney sections. Transfection, immunoblotting, immunoprecipitation (IP) and pull-downs were performed with standard methods.

**Results:** APOL1 localized in podocytes of normal human kidney. We used a bioinformatic structural homology search to identify human orthologs of the trypanosomal SRA protein and elucidate the function of the SRA-binding domain of APOL1. VAMP8, the cognate receptor for an autophagosomal SNARE protein, was the top hit; its interaction with wild type APOL1 was confirmed by pull-down and IP. APOL1 risk variants attenuated this interaction and deletion of the SRA-binding domain abolished it. APOL1 and VAMP8 colocalized in podocytes of normal kidney. Expression of wild-type APOL1 in cell lines stimulated autophagy, which was less when variant APOL1s were expressed. Expression of wild-type or variant APOL1s in VAMP8 knockdown cells also increased the appearance of LC3II on immunoblotting, but the small change with the addition of bafilomycin A with variant APOL1s suggests a block in autophagy maturation.

**Conclusions:** Wild-type APOL1 stimulates autophagy and interacts with VAMP8, a structural ortholog of the trypanosomal SRA protein. Variant APOL1s attenuate the APOL1-stimulated autophagy, as well as, the APOL1 interaction with VAMP8. The interaction of VAMP8 with the SRA-domain of APOL1 may promote autophagic maturation as a defense against environmental stress.

*Funding:* NIDDK Support, Clinical Revenue Support

## SA-OR096

**Variants in Genes Encoding Complement Regulation Proteins CFH, CFHR3 and CFHR1 Affect the Complement Activation and Susceptibility of IgA Nephropathy** Li Zhu,[1,2,3] Yaling Zhai,[1,2,3] Fengmei Wang,[1,2,3] Jicheng Lv,[1,2,3] Damin Xu,[1,2,3] Sufang Shi,[1,2,3] Lijun Liu,[1,2,3] Feng Yu,[1,2,3] Minghui Zhao,[1,2,3] Hong Zhang.[1,2,3] *[1]Renal Div, Dept of Medicine, Peking Univ First Hospital; [2]Peking Univ Institute of Nephrology; [3]Key Laboratory of Renal Disease, Ministry of Health of China.*

**Background:** Complement activation was common in patients with IgAN and further associated with disease severity. Our recent GWAS study identified 1q23 as a IgAN associated locus, containing complement regulatory protein genes, CFH and the related CFHR3, CFHR1, CFHR4, CHFR2, CFHR5, with rs6677604 in CFH as the top signal SNP. But the underlying genetic mechanism was still unknown. In the present study, we explore the genetic pathogenesis of CFH, CFHR3 and CFHR1 in IgAN susceptibility.

**Methods:** In total, 428 IgAN patients and 902 healthy controls were enrolled. Genotyping of rs6677604 were detected by TaqMan SNP Genotyping Assay. The copy number variation of the CFH-CFHRs region were detected by multiplex ligation-dependent probe amplification (MLPA). Additionally, plasma levels of factor H, complement activation split products C3a and C5a, and sC5b-9 were detected by ELISA in IgAN patients with different copy number of CFHR3-1.

**Results:** In our present population, we confirmed the association of rs6677604 with IgAN susceptibility. Moreover, the linkage disequilibrium of A allele in rs6677604 with CFHR3-1Δ deletion were also confirmed. Furthermore, we found that IgAN patients with CFHR3-1Δ deletion presented with higher plasma factor H and lower complement activation split products C3a and C5a, compared to those without CFHR3-1Δ deletion (CFH: 425.94±137.12ug/ml Vs 337.49±101.87 ug/ml, p=0.002; C3a: 468.89 (276.41-687.59) ng/ml Vs 2469.53 1051.97-3556.53) ng/ml, p<0.001; C5a: 8.34 (6.24-10.42) ng/ml Vs 11.69 (6.56-20.18) ng/ml, p=0.037). However, Plasma sC5b-9 levels showed no difference.

**Conclusions:** Our findings implied a possible genetic mechanism of 1q23 in IgA nephropathy: genetic CFHR3-1Δ deletion, which tagged by A allele of rs6677604, was associated with up-regulation of factor H, and through its inhibition effect on complement activation, at last resulted in the protection to IgAN susceptibility.

*Funding:* Government Support - Non-U.S.

## SA-OR097

**Mesangial Cell Hypersensitivity to Galactose Deficient IgA, a Key to the Development of IgA Nephropathy** Kerstin Ebefors,[1] Peidi Liu,[1] Johannes Elvin,[2] Borje Haraldsson,[2] Jenny C. Nystrom.[1] *[1]Physiology, Neuroscience and Physiology, Gothenburg, Sweden; [2]Molecular and Clinical Medicine, Medicine, Gothenburg, Sweden.*

**Background:** The key feature of IgA nephropathy (IgAN) is deposition of galactose deficient IgA (gd-IgA) in the mesangial matrix. These depositions can also be found in asymptomatic individuals. There are also individuals who have gd-IgA in their circulation without developing the disease. We therefore hypothesize that patients developing IgAN have mesangial cells that are hypersensitive to gd-IgA. Since IgAN is a disease that can reoccur in the transplant (around 40% of cases), we also hypothesize that around half of the population have mesangial hypersensitivity. However, since most of us do not have gd-IgA in our circulation, we have no risk of developing the disease.

**Methods:** We developed a unique method for culturing mesangial cells from patients with IgAN and from controls. IgA1 was purified from serum from patients with IgAN and healthy controls using the method of jacalin purification. Mesangial cells were stimulated with purified IgA1 and the expression of matrix genes and release of cytokines were investigated.

**Results:** First, we investigated the gene expression of selected matrix genes in cells from patients and controls, and found that untreated cells from patients had a different expression profile compared to cells from controls. Stimulation of cells with purified IgA1 did not affect the expression of selected matrix genes further. Secondly, we investigated the release of cytokines after stimulation with IgA1 from patients and controls. We found that the cells responded with release of several cytokines and cells from patients with IgAN released more PDGF-bb than most controls. Stimulation with gd-IgA gave a significantly stronger response than control IgA. In line with our hypothesis we also found control cells (cells from 3 out of 8 control subjects) responding the same way as cells from patients with IgAN.

**Conclusions:** Mesangial cells from patients with IgAN are hypersensitive to gd-IgA, likely depending on their matrix composition (as shown previously by our group). We believe that this hypersensitivity is necessary for the IgA depositions to be pathogenic.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## SA-OR098

**Toll Like Receptor 9 on Regulatory T Cells Regulates Acute Kidney Injury** Shaun A. Summers, Joanna R. Ghali, A. Richard Kitching, Stephen R. Holdsworth. *Medicine and Nephrology, Monash Univ and Monash Health, Melbourne, Victoria, Australia.*

**Background:** Acute kidney injury (AKI) is a significant cause of renal disease. Cisplatin nephrotoxicity is mediated by leukocytes and cytokines. Toll like receptors (TLRs) are innnate immune cells which actively control inflammation.

**Methods:** We treated C57BL/6 wild type (WT) and TLR9-/- mice with cisplatin and assessed renal inflammation and injury. CD25 negative (-), effectors, and CD4+CD25+ splenocytes were isolated by magnetic bead separation. For reconstitution experiments effector cells and whole splenocytes, from WT or TLR9-/- mice were administered to recombinant activation gene deficient mice (RAG1-/-) which lack adaptive immune cells. Administration of diphtheria toxin to Depletion of Regulatory T cell (DEREG) mice successfully depleted endogenous regulatory T cells (Tregs), which were then reconstituted with CD4+CD25+ cells from WT or TLR9-/- mice.

**Results:** Renal injury and inflammation was enhanced in the absence of TLR9. Blood urea nitrogen (BUN) was increased (WT 43.6±11.1 vs. TLR9-/- 98.9±15.0 mmol/L, $P<0.01$), as was histological injury, interstitial neutrophil recruitment and kidney CXCL1 and CXCL2 expression. Kidney mRNA expression of FoxP3 was decreased in TLR9-/- mice. Renal injury was similar in RAG1-/- mice reconstituted with WT and TLR9-/- effectors and treated with cisplatin. However after reconstitution with whole splenocytes functional (BUN; WT recon 32.3±8.1 vs. TLR9-/- recon 63.6±11.6 mmol/L, $P<0.05$) and histological injury (Injury Score [0-4], WT recon 2.3±0.3 vs. TLR9-/- recon 3.0±0.2, $P<0.05$) was increased in RAG1-/- mice reconstituted with TLR9-/- splenocytes. Finally, we depleted endogenous Tregs in DEREG mice and reconstituted them with CD4+CD25+ cells from WT or TLR9-/- mice, followed by cisplatin. Compared to DEREG mice reconstituted with WT CD4+CD25+ cells functional renal injury was enhanced after reconstitution with TLR9-/-CD4+CD25+ splenocytes (BUN; WT recon 50.3±13.7 vs.TLR9-/- recon 93.8±11.3 mmol/L, $P<0.05$) as was histological injury (Injury Score [0-4] WT recon 2.6±0.3 vs. TLR9-/- recon 3.7±0.1, $P<0.01$).

**Conclusions:** Endogenous TLR9 regulates Treg mediated suppression of cisplatin induced acute kidney injury.

*Funding:* Government Support - Non-U.S.

## SA-OR099

**Blockade of Death Ligand TRAIL Inhibits Renal Ischemia Reperfusion Injury** Takaomi Adachi,[1,3] Noriyuki Sugiyama,[2] Takahiko Yokoyama.[3] *[1]Dept of Nephrology, Kyoto Prefectural Univ of Medicine, Kyoto, Japan; [2]Dept of Anatomy and Cell Biology, Osaka Medical College, Takatsuki, Osaka, Japan; [3]Anatomy and Developmental Biology, Kyoto Prefectural Univ of Medicine, Kyoto, Japan.*

**Background:** Acute kidney injury (AKI) remains a significant clinical problem because of high mortality and morbidity rates. However, the mechanisms of AKI has not been fully elucidated. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor superfamily, and induces apoptosis and inflammation. In the field of nephrology, recent studies suggest that TRAIL plays a role in the pathogenesis of diabetic nephropathy. However, the role of TRAIL in renal ischemia reperfusion injury (IRI) is unclear.

**Methods:** To induce AKI, the left renal pedicle was occluded by clamping the left renal artery for 60 min, followed by reperfusion. Kidneys were collected and weighed at the indicated times after reperfusion and examined. Neutralizing anti-TRAIL monoclonal antibodies were given 24 hours before ischemia, and a half-dose booster injection was administered into the peritoneal cavity immediately after reperfusion.

**Results:** We observed that the expression of TRAIL and its receptors were highly upregulated in renal tubular cells in renal IRI. To clarify the function of TRAIL in renal IRI, we treated the IRI kidney with anti-TRAIL antibodies. We found that TRAIL blockade reduced tubular injury and inhibited tubular apoptosis. Moreover, TRAIL blockade reduced the accumulation of neutrophils and macrophages. Furthermore, TRAIL blockade abrogated renal fibrosis and atrophy after IRI.

**Conclusions:** We suggest that TRAIL plays a critical role in renal IRI, and that TRAIL blockade ameliorates renal IRI. Our findings may have clinical relevance and provide new insights into the pathogenesis of renal IRI. This study also suggests that targeting TRAIL may be a novel significant therapeutic approach to overcome renal IRI.

*Funding:* Government Support - Non-U.S.

## SA-OR100

**Mesenchymal Stem Cells Ameliorates Rhabdomyolysis-Induced Acute Kidney Injury through Activation of M2 Macrophage** Xiang-Mei Chen, Li Zhang, Yanqiu Geng. *Dept of Nephrology, State Key Laboratory of Kidney Disease, State Key Laboratory of Kidney Disease, Beijing, China.*

**Background:** Acute kidney injury (AKI) associated with myoglobinuria is the most serious complication of rhabdomyolysis. The mortality of critical AKI is still high since there is no effective therapy. Several studies have shown that administration of bone marrow-derived mesenchymal stem cells (MSCs) leads to the amelioration of AKI, but the mechanisms are still unknown. It was showed that MSCs could induce macrophage both in vivo and in vitro, which mediates MSCs protection in other experimental inflammation related organ injury. However, whether macrophage is also involved in the protective effect of MSCs in rhabdomyolysis-induced AKI needs to be clarified. In this study, we investigated the effects of MSCs on macrophage activation in rhabdomyolysis-induced AKI.

**Methods:** Rhabdomyolysis-induced AKI was induced in male C57BL/6 mice by intramuscular injection with 50% glycerol solution following deprivation of water for 24 hours. Then, we administered MSCs ($10^6$ per mouse) via tail vein.

**Results:** We found that intravenously delived MSCs to Rhabdomyolysis- induced AKI may significantly decrease serum creatinekinase, creatinine and blood urea nitrogen levels, improve tubular injury and lowered acute tubular necrosis score. Serum proinflammatory cytokines TNF-$\alpha$ and IL-6 decreased and anti-inflammatory cytokines IL-10 increased. Immunofluorescence revealed that renal macrophages accumulation with an increasing percentage of "M2" ($CD206^+$) phenotype. Western Blot demonstrated that MSCs caused an advanced expression of CD206. We double labeled $CD206^+$ macrophages with the proliferation marker Ki67. The increases in M2 macrophages resulted largely from in situ proliferation in the kidney. Depletion of M2 macrophages with clodronate delayed the recovery of AKI.

**Conclusions:** This study demonstrates that MSCs-mediated expansion and activation of M2 macrophages is a novel and important mechanism in the protection of rhabdomyolysis-induced AKI.

*Funding:* Government Support - Non-U.S.

## SA-OR101

**Myeloid Cells Are Essential Mediators of Renal Endotoxin Preconditioning** Takashi Hato, Rakesh Kumar, Pierre C. Dagher. *Medicine, Indiana Univ, Indianapolis, IN.*

**Background:** We have previously demonstrated that systemically administered endotoxin directly interacts with renal proximal tubules and results in severe tubular oxidative stress. Here we examined whether it is possible to precondition the kidney in vivo and curtail endotoxin-induced tubular injury.

**Methods:** We compared preconditioned (0.25 mg/kg followed by 5 mg/kg lipopolysaccharide ip) and non-preconditioned animals (single-dose 5 mkg/kg ip). A combination of tools was used including mutant and bone marrow chimeric mice, qPCR, flow cytometry, and intravital 2-photon microscopy.

**Results:** We found that preconditioning completely abolished tubular oxidative stress upon subsequent high-dose endotoxin challenges. Renal KIM-1 and NGAL mRNA expression levels were also markedly decreased in the preconditioned kidneys. While preconditioning did not affect the total number of infiltrating renal macrophages, it favored an M2 phenotypic switch. In addition, preconditioning upregulated cytoprotective heme oxygenase-1 and sirtuin-1 in infiltrating macrophages. In chimeric mice lacking hematopoietic TLR4, preconditioning failed to prevent tubular oxidative stress indicating that $TLR4^+$ hematopoietic cells are indispensable for successful preconditioning. Along with TLR4, CD14 is a co-receptor for endotoxin that is primarily expressed on myeloid cells and is essential for TRIF pathway signaling. Chimeric mice lacking hematopoietic CD14 also showed complete failure to exhibit tolerance after preconditioning suggesting that TRIF pathway is involved in endotoxin tolerance. Indeed, the administration of poly (I:C), which stimulates exclusively the TRIF pathway, significantly restored endotoxin tolerance in CD14KO/WT chimeras.

**Conclusions:** These data support a model in which myeloid cells are essential mediators of the protective effects of endotoxin preconditioning on the kidney. Understanding the mechanisms underlying endotoxin preconditioning can have important clinical applications in the prevention of sepsis-induced acute kidney injury.

*Funding:* NIDDK Support, Private Foundation Support

## SA-OR102

**Forskolin, a Potent Cyclic-AMP Pathway Activator, Protect Mice from Acute Pyelonephritis Induced by Uropathogenic *Escherichia Coli*** Ke Li,[1] Yang Wei,[1] Wuding Zhou.[1,2] *[1]Core Research Laboratory, The Second Affiliated Hospital, Xi'an Jiaotong Univ, Xi'an, China; [2]MRC Center for Transplantation, King's College London, London, United Kingdom.*

**Background:** Pyelonephritis caused by uropathogenic Escherichia coli (UPEC) are very common infections that can cause severe kidney damage. Uncontrolled or dysregulated innate immune responses are well recognized as a contributor to the pathogensis. In this study, we investigated the effect of forskolin (a potent activator of cyclic-AMP pathway) on susceptibility/severity of acute pyelonephritis and innate immune responses to pathogen using an established experimental model of ascending UTI and primary cell cultures (i.e. renal tubular epithelial cells(RTEC), mono/macrophages).

**Methods:** Forkolin (10mg/kg) was given before the induction of infection by i.p. injection. Kidney infection was assessed in forskolin or control reagent treated mice at 6,

24, 48h after bladder inoculation of UPEC (J96). Bacteria load in kidneys was analyzed by the agar plate assay. Tissue damage was assessed by histopathology. Leukocytes infiltration was analysed by immunochemical staining, tissue MPO activity assay and flow cytometry.

**Results:** Administration of forskolin significantly reduced bacterial load in kidneys and protected tissue damage at different time points, this was associated with reduced intrarenal production of pro-inflammatory cytokines/chemokines (e.g. TNF-α, KC, MCP-1) and attenuated intrarenal infiltration and accumulation of leukocytes (i.e. CD45+. Gr-1+, F4/80+) as well as intrarenal MPO activity. In vitro, forskolin inhibited LPS or UPEC mediated pro-inflammatory cytokine/chemokine production by primary RTECs and monocytes/macrophages. Furthermore, forskolin reduced bacterial uptake by RTECs (a potential reservoir for bacteria), but had no apparent effect on phagocytosis by professional phagocytes.

**Conclusions:** These findings demonstrate that administration of forskolin is beneficial for controlling the development of UPEC mediated acute pyelonephritis in mice. The protective effect of forskolin (via cAMP activation) can be explained at least in part by limiting excessive inflammatory responses through acting on both renal parenchymal and inflammatory cells.

*Funding:* Government Support - Non-U.S.

## SA-OR103

### DNA Methylation in Acute Kidney Injury and Kidney Repair

Zheng Dong.[1,2] *[1]GRU; [2]VA.*

**Background:** To evaluate the changes of DNA methylation in acute kidney injury (AKI) and kidney repair by a global methylation analysis.

**Methods:** To examine DNA methylation during AKI, B6 mice were treated with cisplatin ( i.p.) for 3 days or subjected to 30 minutes of bilateral renal ischemia followed by 48 hours of reperfusion. To study DNA methylation during kidney repair, B6 mice were subjected to 25 minutes of bilateral renal ischemia followed by 1 week or 1 month of reperfusion. Genomic DNA was purified from freshly frozen cortex tissue for Reduced representation bisulfite sequencing (RRBS) to evaluate DNA methylation changes during and after AKI.

**Results:** Totally 1.4-1.9 million of CpG sites in the sham and injured kidney genome were analyzed. Comparing the methylation profiles of sham control and AKI kidneys, there were 296-359 differentially methylated regions (DMRs). The numbers of hypermethylated DMRs and hypomethylated DMRs were similar between sham and cisplatin 3days, sham and 30 minutes ischemia /48 hours reperfusion, sham and 25 minutes ischemia/1week reperfusion groups., suggesting that the overall DNA methylation does not change under these conditions. In contrast, the 25 minutes ischemia/1month reperfusion group showed more hypermethylated DMRs than hypomethylated DMRs, indicating an increase of DNA methylation. The distribution of DMRs was very similar in the 4 AKI groups comparing with sham; relatively few (6-9%) DMRs were located in the promoter or 5'-UTR regions of genes, while most DMRs were intergenic. 381-491 genes showed significant DNA methylation changes from DMRs. These genes were enriched in several functional pathways including Wnt, Jak-stat and TGF-beta pathways. Several genes showing significant DNA methylation changes following AKI were chosen to for pyrosequencing to confirm the global methylation sequencing results for further functional study.

**Conclusions:** Collectively, the results demonstrate DNA methylation in specific genes during and after AKI.

## SA-OR104

### Telomerase Deficiency Delays Renal Recovery after Ischemia Reperfusion Injury (IR)

Huifang Cheng,[1] Xiaofeng Fan,[1] William E. Lawson,[1] Paisit Paueksakon,[2] Raymond C. Harris.[1] *[1]Medicine, Vanderbilt Univ Medical Center, Nashville, TN; [2]Pathology, Microbiology and Immunology, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** Increasing morbidity and higher mortality of acute kidney injury (AKI) are reported in the aged populations. Aging may be directly linked to telomere shortening; both (TerT) and telomerase RNA (TerC) are essential to maintain telomere length.

**Methods:** To investigate molecular and genetic mechanisms underlying susceptibility of AKI in seniors, we induced renal IR in mice from three genotypes: wild type (Wt); and fourth generation (G4) mice with TerC or TerT deletion (TerC KO and TerT KO respectively) by clamping both renal pedicles for 25 mins., using sham operated mice with each genotype as control.

**Results:** Our results suggested that IR induced similar renal injury from day 1 in each genotype, indicated by plasma BUN (Wt: 112.9±2.6; TerC KO: 119.6±3.0; TerT KO: 122.6±6.0 mg/dl), albuminuria and acute tubular injury score (ATI) according to the percentage of damaged tubules (eg., loss of brush border, tubular dilation, "blebbing", cast formation and epithelial cell lysis), injury predominantly in proximal tubules. Increased apoptotic tubular epithelial cells detected by TUNEL Assay and renal Kim-1 were found in all mice by day 1 after IR. However, compared to Wt, both TerC KO and TerT KO mice had significantly delayed recovery, based on evaluation of BUN, albuminuria, ATI score and calculation of tubular epithelial cell apoptosis / proliferation (measured by Ki 67). Electron microscopy demonstrated that IR stimulated autophagosome formation in renal tubular epithelial cells from each group, but the peak in Wt was at day 3 post IR, while it was markedly delayed in TerC / TerT KO mice (peak at day 14). Immunoblotting and immunohistochemistry also demonstrated that the ratio of LC III-2/1 significantly increased at day 3 in Wt, but only at day 14 in TerC / TerT KO. Similar patterns were noted with up-regulation of P62 and ubiquitin.

**Conclusions:** Our study suggested that deletion of either TerC or TerT in mice led to delayed kidney recovery from IR, possibly via impairment of autophagy.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-OR105

### Proximal Tubule Specific Deletion of p53 Reduces Ischemic Renal Injury in Mice

Yuan Ying, Babu J. Padanilam. *Cellular & Integrative Physiology, Univ of Nebraska Medical Center, Omaha, NE.*

**Background:** The key features of Ischemic renal injury (IRI) are vascular damage, inflammation and proximal tubular cell apoptosis and necrosis, particularly in the outer medulla. The transcription factor p53 plays an important role in conducting cell cycle arrest, apoptosis, and even necrosis after various types of cell stress. Although a key role for p53 is implicated in IRI, there are conflicting reports on its benevolent and malevolent effects. We hypothesize that specific deletion of p53 in the proximal tubule protects kidneys from ischemic injury.

**Methods:** Mice with the p53 gene specifically deleted in the proximal tubule underwent either sham surgery or clamping of the renal pedicle for 30 min followed by reperfusion for different time periods. Renal function was assessed by creatinine and blood urea nitrogen (BUN) assays, histological damage by Periodic acid-Schiff staining, inflammation by neutrophil and macrophage (F4/80) staining, and fibrosis by Sirius red staining. Apoptosis was measured by TUNEL staining and Western blot analysis for apoptotic proteins.

**Results:** Plasma levels of creatinine and BUN were significantly decreased 24 h after reperfusion in p53 knockout mice compared with wild type. Knockout mice had less renal histological damage, infiltration of leukocytes and apoptosis. Deletion of p53 attenuated the expression of its target gene, the cyclin-dependent kinase inhibitor, p21 after injury. Consistently, renal fibrosis was significantly reduced in p53 knockout mice 16 d after reperfusion.

**Conclusions:** Specific knockout of p53 in the proximal tubule protects kidneys from functional and histological deterioration after IRI by decreasing apoptosis, inflammation, and fibrosis.

*Funding:* NIDDK Support

## SA-OR106

### Primary Cilia-Dependent NOS1 in the Macula Densa (MD) Protects against Ischemia-Reperfusion-Induced Acute Kidney Injury (I/R-AKI)

Yan Lu, Luis A. Juncos, Ying Ge, Yiling Fu, Jin Wei, Richard J. Roman, Ruisheng Liu. *Univ of Mississippi Medical Center, MS.*

**Background:** The macula densa has prominent primary cilia that may be important in regulating NOS1 activity in the MD, and consequently renal function.

**Methods:** Animal: Tissue specific knockout mice: 1). NKCC2-cilia KO 2). NKCC2-NOS1 KO mice. Surgery:IRI was induced by bilateral renal pedicel clamping for 18 min at 37oC. NO measurement: fluorescent TGF was measured with micropuncture and microperfusion AKI marker: Serum Creatinine; Urine KIM-1; Histology (fluorescent H&E staining).

**Results:** Increasing flow across a MD cell-line increased NO (from 175 ± 22 to 276 ± 24) in the presence of intact primary cilia, but had no effect when the cilia were removed by siRNA (48.6 ± 17.5 vs. 62.7 ± 15.2). Similarly, increasing tubular flow in isolated JGA obtained from control mice, increased NO in the MD from 120±22 to 164±14. Subjecting C57BL/6 mice (WT) to 18 minutes of bilateral I/R-AKI decreased the number and length of primary cilia and prevented flow-induced increases in NO in isolated JGA (72±16 vs. 86±15). We then tested whether specifically inhibiting primary cilia-dependent NO production in the MD would exacerbate I/R-AKI. For this we developed 2 strains of NKCC2-tissue-specific KO mice (thus targeting the MD and thick ascending limb). In the first, the primary cilia was deleted in the MD and thick ascending limb, while the second resulted in MD specific deletion of all the splice variants of NOS1. Twenty-four hours post I/R-AKI, the plasma creatinine (mg/dl) was 0.075±0.03 in sham, 0.56±0.02 in WT, 1.25±0.05 in Cilia KO and 0.89±0.03 in NOS1 KO. Urine KIM-1(pg/24h) was 10.7±2.9 in sham, 31.2±5.5 in WT, 274.7±9.3 in Cilia KO and 1340.3±362.2 in NOS1 KO. Morphologically, we observed mild injury in WT and more severe cortical injury in both the Cilia KO and NOS1 KO while no injury in sham. Tubuloglomerular feedback (TGF) was enhanced (4.5±0.3 vs 8.4±0.7 mmHg) and NO in the MD was absent in the NOS1 KO compared to WT mice.

**Conclusions:** We conclude renal IRI damages cilia and reduces NO, and that reduction of primary cilia-dependent NO in the MD exacerbates I/R-AKI, perhaps via enhanced TGF.

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## SA-OR107

### Polyamine Catabolism in Renal Ischemia/Reperfusion (I/R) Injury

Kamyar A. Zahedi,[1] Sharon L. Barone,[1] Tracy Murray-Stewart,[2] Yang Wang,[1] Prabir Roy-Chaudhury,[1] Robert A. Casero,[2] Manoocher Soleimani.[1,3] *[1]Univ of Cincinnati, Cincinnati, OH; [2]Johns Hopkins Univ, Baltimre, MD; [3]Veterans Administration, Cincinnati, OH.*

**Background:** The activity of Spermidine/Spermine-N[1]-Acetyltransferase (SSAT) increases in kidneys subjected to I/R injury. We propose that increased SSAT expression in proximal tubule epithelial cells (PTEC) contributes to I/R induced tubular injury.

**Methods:** The severity of renal I/R injury was compared in wild type (wt) and proximal tubule specific SSAT knockout (PT-SSAT-Cko) mice.

**Results:** The severity of renal I/R injury, as determined by renal histopathology and serum creatinine levels, is reduced in PT-SSAT-Cko compared to wt mice. The ablation of SSAT also leads to significant decreases in the expression of spermine oxidase (SMO), tumor necrosis factor-a, and monocyte chemoattractant protein 1 in the injured kidneys. Animals treated with MDL72527, an inhibitor of polyamine oxidases, had less severe renal injuries than those treated with vehicle only, suggesting that polyamine oxidation plays an

important role in the mediation of renal I/R injury. In order to assess the basis of SSAT-mediated cell injury, we examined the effect of its expression on HEK cells. Our results indicate that SSAT expression leads to the loss of mitochondrial integrity, activation of caspase3, cleavage of poly (ADP-ribose) polymerase 1 and apoptosis. In addition, SSAT expression leads to increased expression of high mobility group B 1 (HMGB 1) protein. Based on our in vivo and in vitro results we examined the effect of SSAT ablation on the onset of innate immune response and apoptosis. Our results indicate that, compared to wt mice, PT-SSAT-Cko animals had: 1) lower HMGB 1, toll-like receptor-2 and -4 levels; 2) increased serine 637 phosphorylated dynamin-related protein 1 levels, indicative of reduced mitochondrial fission; and 3) reduced cleaved caspase 3 levels.

**Conclusions:** 1) Enhanced polyamine catabolism by PTEC plays a maladaptive role in kidney I/R injury; 2) reduced tissue damage in PT-SSAT-Cko mice is in part due to the modulation of innate immune and apoptotic responses; 3) Enzymes involved in polyamine catabolism can be targets for the treatment of renal I/R injury.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-OR108

**The Cystic Kidney Disease Protein ANKS6 Is a Novel Binding Partner and Substrate of the NEK8 Kinase**

Peter G. Czarnecki,[1,2,5] Danielle K. Manning,[3] Mikhail Sergeev,[2,5] Maija Garnaas,[3] Wolfram Goessling,[3] David Beier,[3,4] Jagesh V. Shah.[2,5] *[1]Div of Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Renal Div, Brigham and Women's Hospital, Boston, MA; [3]Genetics Div, Brigham and Women's Hospital, Boston, MA; [4]Center for Developmental Biology and Regenerative Medicine, Children's Research Institute, Seattle, WA; [5]Dept of Systems Biology, Harvard Medical School, Boston, MA.*

**Background:** Cystic kidney disease is a consequence of mutations and knockouts of many ciliary genes. How these gene products exert their effect on kidney epithelium to promote cystogenesis remains unknown. Here we investigated the cystic kidney disease gene NEK8, which is mutated in the juvenile cystic kidney (*jck*) mouse and in a subset of patients with nephronophthisis (NPHP9).

**Results:** Analysis of NEK8 co-immunoprecipitates by mass spectrometry revealed ANKS6, the protein mutated in the cy/+ rat model of cystic kidney disease, as a novel binding partner of NEK8. The ANKS6-NEK8 interaction is mediated by the binding of the ankyrin domain of ANKS6 to the kinase domain of NEK8. The ankyrin domain of ANKS6 is also required for its localization to the Inversin compartment in ciliated cells. Morpholino mediated disruption of ANKS6 in zebrafish results in the formation of pronephric cysts and pericardial edema, a phenotype that is characteristic of many ciliopathy genes. However, unlike loss of function mutations in NEK8, INVS and NPHP3, three other core constituents of the Inversin compartment, defects in L/R asymmetry were not observed. We found ANKS6 to be readily phosphorylated by NEK8 and identified a number of phosphorylation sites in the C-terminal region including the SAM-domain. Deletion of the SAM domain results in enhanced oligomerization of ANKS6 and NEK8, and hyperaccumulation of these proteins in the Inversin compartment.

**Conclusions:** Our work reveals a novel interaction network between cystic kidney disease proteins that may represent the basis of a new cystogenic pathway.

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## SA-OR109

**Defects in the IFT-B Component IFT172 Cause Nephronophthisis with Skeletal Abnormalities**

Jan Halbritter,[1,10] Jonathan Porath,[1,10] Albane A. Bizet,[2,10] Miriam Schmidts,[3,10] Daniela A. Braun,[1] Peter G. Czarnecki,[4] Emilie Filhol,[2] Heon Yung Gee,[1] Pauline Krug,[2] Rannar Airik,[1] Patrick Nitschke,[2] Christine Bole-Feysot,[2] Anna M. Lehman,[5] Edgar Otto,[6] Bertrand Knebelmann,[2] Stephane Burtey,[2] Attila J. Szabo,[7] Kalman Tory,[7] Markus Schueler,[1] Hulya Kayserili,[8] Dan Doherty,[9] Peter Scambler,[3] Philip L. Beales,[3] Jagesh V. Shah,[4] Hannah Mitchison,[3] Sophie Saunier,[2] Friedhelm Hildebrandt.[1] *[1]Med, BCH, Boston; [2]INSERM U983, NH Paris, France; [3]MMU, UCL, United Kingdom; [4]Med, BWH, Boston; [5]Med Gen, U BC, Vancouver, Canada; [6]Ped, U MI, Ann Arbor; [7]Ped, SW U, Budapest, Hungary; [8]Med Gen, Istanbul U, Turkey; [9]Ped, U WA, Seattle; [10]Equally Contributed.*

**Background:** Intraflagellar transport (IFT) is an evolutionarily conserved mechanism, essential for cilia assembly and maintenance. All components of IFT subcomplex A, have been implicated in human skeletal ciliopathies including Sensenbrenner, and Mainzer-Saldino syndrome (MSS). Additionally, the IFT B-component IFT80 and the IFT-A motor DYNC2H1 have been shown to cause Jeune syndrome (JATD) when mutated. The role of other IFT-B proteins in human disease remains obscure.

**Methods:** To identify additional IFT-B complex products as defective in ciliopathies, we performed array based multiplex PCR of 13 IFT-B components in 1,056 patients with nephronophthisis-related ciliopathies (NPHP-RC) and whole exome resequencing in 56 individuals with JATD.

**Results:** This approach led to the detection of 8 families with recessive mutations in the gene *IFT172*. All affected individuals showed NPHP and skeletal abnormalities of thorax and long bones, combined with hepatic or retinal involvement, consistent with the diagnosis of JATD or MSS. Injection of *ift172* or *ift80* antisense-morpholino in zebrafish resulted in formation of glomerular cysts. A similar phenotype resulted from injection of subphenotypic doses of *ift172* and *ift80* in combination, indicating genetic interaction.

**Conclusions:** We show that mutation of an additional IFT-B component, *IFT172*, is a novel cause of NPHP-RC with skeletal involvement, and we demonstrate genetic epistasis between *ift80* and *ift172*.

## SA-OR110

**Cdc42 Knockdown/Knockout Results in Ciliary Abnormalities and Cystic Kidneys**

Soo Young Choi,[1] Maria F. Chacon-Heszele,[1] Liwei Huang,[1] Sarah McKenna,[1] Francis P. Wilson,[1] Xiaofeng Zuo,[1] Joshua H. Lipschutz.[1,2] *[1]Dept of Medicine, Univ of Pennsylvania; [2]Philadelphia VAMC, Philadelphia, PA.*

**Background:** Primary cilia are found on renal tubule cells, and cilia dysfunction is linked to polycystic kidney disease (PKD). We previously demonstrated that the exocyst, a conserved eight-protein trafficking complex, is required for ciliogenesis and cystogenesis, most likely due to its role in trafficking ciliary proteins. We also showed, in culture, that Cdc42, a small GTPase, co-localizes with the exocyst at primary cilia, and biochemically interacts with Sec10, a crucial exocyst component. Knockdown of Cdc42 and Tuba, a Cdc42 GEF, inhibited ciliogenesis and resulted in MAPK pathway activation, something observed in cells with dysfunctional cilia.

**Methods:** Given the importance of Cdc42 for cell function, to study how *cdc42* affects ciliogenesis and cystogenesis *in vivo*, we used two different animal models and techniques: antisense morpholinos (MOs) to knockdown *cdc42* in zebrafish, and the Cre-Lox system to knockout Cdc42 in a kidney-specific manner in mice. Zebrafish embryos were injected with *cdc42* start-site MOs. Cdc42 renal tubule specific knockout mice were generated by breeding Cdc42 floxed and Ksp-cadherin Cre mice.

**Results:** We show cdc42 knockdown in zebrafish phenocopies many aspects of sec10 knockdown—including tail curvature, glomerular expansion, and MAPK activation. Other ciliary phenotypes include hydrocephalus and loss of photoreceptor cilia. We also observe a synergistic genetic interaction between zebrafish *cdc42* and *sec10*, suggesting that *cdc42* and *sec10* function in the same pathway. Cdc42 kidney-specific knockout mice died within weeks of birth of renal failure. Histology revealed cystogenesis in distal tubules and collecting ducts, decreased ciliogenesis in cyst cells, increased tubule cell proliferation, increased apoptosis, increased fibrosis, and MAPK activation, all features of PKD, especially nephronophthisis.

**Conclusions:** These data support a model in which Cdc42 localizes the exocyst to primary cilia, whereupon the exocyst targets and docks vesicles carrying ciliary proteins, and, if this does not occur, the result is abnormal ciliogenesis and PKD.

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## SA-OR111

**Deletion of S6 Phosphorylation Exacerbates Renal Cystogenesis in Conditional Tsc1 Knockout Mice**

Huijuan Wu,[1] Jianchun Chen,[2] Jian-Kang Chen.[1] *[1]Experimental Medicine, Georgia Regents Univ, Augusta, GA; [2]Medicine, Vanderbilt Univ, Nashville, TN.*

**Background:** Tuberous sclerosis (TSC) is caused by *Tsc1* or *Tsc2* gene mutations and characterized by proliferative lesions in many organs, with kidney involvement being the leading cause of death in adult TSC patients. Phosphorylation of the40S ribosomal protein S6 is implicated in regulation of cell growth, but its role in the renal lesions of TSC has not yet been explored.

**Methods:** We generated renal proximal tubule-specific *Tsc1* knockout mice ($Tsc1^{ptKO}$) by crossing *Tsc1*-floxed mice with γGT-Cre mice. We then bred $Tsc1^{ptKO}$ with S6 knock-in mice carrying unphosphorylatable S6 ($S6^{P-/-}$) to produce $Tsc1^{ptKO}$ mice lacking S6 phosphorylation ($Tsc1^{ptKO}$; $S6^{P-/-}$).

**Results:** By 1 week of age, $Tsc1^{ptKO}$ mice developed enlarged kidneys, which were blunted in $Tsc1^{ptKO}$; $S6^{P-/-}$mice (kidney-to-body weight ratios (K/Bwt): 1.14±0.2 vs. 0.71±0.02%; p<0.01, n=5), with indistinguishable body weight. Surprisingly, by 2 weeks of age, $Tsc1^{ptKO}$; $S6^{P-/-}$ kidneys outgrew $Tsc1^{ptKO}$ kidneys (K/Bwt: 3.0±1.49 vs. 1.4±0.16%; p<0.05, n=5). By 4 weeks of age, $Tsc1^{ptKO}$;$S6^{P-/-}$ mice developed severe polycystic renal lesions, with increased BUN (74.29±12.36 vs. 25±3.38 mg/dl in $Tsc1^{ptKO}$; p<0.01, n=7). 67% of $Tsc1^{ptKO}$;$S6^{P-/-}$ mice died by 9 weeks of age when $Tsc1^{ptKO}$ mice were all alive, with far fewer renal cysts. Triple immunofluorescence staining confirmed that all cysts originated from proximal tubules. $Tsc1^{ptKO}$ mice showed increased S6 phosphorylation in involved renal tubules compared with WT littermates, but $Tsc1^{ptKO}$;$S6^{P-/-}$ mice and $S6^{P-/-}$mice showed no S6 phosphorylation. Compared with $Tsc1^{ptKO}$ mice, Ki67-positive renal cells in $Tsc1^{ptKO}$;$S6^{P-/-}$ mice were significantly fewer at 1 week of age but more by 2 weeks of age. Immunoblotting revealed aberrantly heightened phosphorylation of EGFR and STAT3 in $Tsc1^{ptKO}$;$S6^{P-/-}$ mice by 2 weeks of age.

**Conclusions:** This study, for the first time, reveals that blocking S6 phosphorylation transiently inhibits Tsc1 deletion-induced renal growth and then unexpectedly exacerbates cystogenesis and renal function. This may explain why recent clinical trials for treatment of polycystic kidney disease with mTOR inhibitors, which block S6 phosphorylation, failed.

*Funding:* NIDDK Support

## SA-OR112

**Loss of Cilia Suppresses Cyst Growth in Genetic Models of Autosomal Dominant Polycystic Kidney Disease** Ming Ma,[1] Xin Tian,[1] Mingfeng Li,[5] Christopher Y. Chen,[1] Yiqiang Cai,[1] Peter Igarashi,[2] Gregory J. Pazour,[3] Stefan Somlo.[1,4] *[1]Dept of Internal Medicine, Yale Medical School, New Haven, CT; [2]Dept of Medicine, Univ of Texas Southwestern Medical School, Dallas, TX; [3]Program in Molecular Medicine, UMass Medical School, Worcester, MA; [4]Dept of Genetics, Yale Medical School, New Haven, CT; [5]Dept of Neurobiology, Yale Medical School, New Haven, CT.*

**Background:** Inactivation of a single cilia membrane component protein, polycystin, in ADPKD or complete removal of cilia by inactivation of intraflagellar transport-related proteins in ciliopathies both give rise to kidney cysts, yet the interrelationship of these two mechanisms of cyst formation is not understood.

**Methods:** We combined inactivation of *Pkd1* and *Pkd2* with loss of *Kif3a* and *Ift20* in kidney and liver to investigate the role of cilia in ADPKD. We examined epithelial cell proliferation and activation of several known ADPKD associated pathways in polycystin-only and cilia/polycystin double mutants. We also defined genotype dependent changes in the transcriptome at the pre-cystic stage of inducible adult onset ADPKD models using RNASeq with loss of polycystin alone or of cilia/polycystin together.

**Results:** We found that loss of intact cilia suppresses cyst growth following inactivation of polycystins. The severity of cystic disease and the associated epithelial proliferation was directly related to the length of time between the initial loss of polycystins and the subsequent involution of cilia. This effect was not explained by activation of the MAPK/ERK, mTOR or cAMP pathways. RNASeq analysis of wild type and pre-cystic polycystin-1 (PC1) single and PC1/Kif3a double mutant kidneys 6 weeks after adult gene inactivation showed 407 transcripts whose expression was altered in the PC1-only mutants when compared to both wild type and PC1/Kif3a double mutants.

**Conclusions:** The data establish the existence of a novel signaling pathway showing polycystin-dependent inhibition and cilia-dependent activation that is central to the pathogenesis of ADPKD. Alterations in the transcriptome of polycystin-only mutants compared to cilia/polycystin double mutants should identify transcriptional targets which will help in defining these pathways and in identifying therapeutic targets.

*Funding:* NIDDK Support

## SA-OR113

**$Pkd1_{extra}$ Transgenic Mice Implicate Pc2 Gene Dosage as a Pathogenetic Mechanism** Marie Trudel, Almira Kurbegovic. *Molecular Genetics and Development, Institut de Recherches Cliniques de Montréal, Montreal, Canada.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is characterized by slow progression of multiple cysts in both kidneys that lead to renal insufficiency in mid-life or later. ADPKD is associated with mutations mainly in the PKD1 gene (encoding polycystin-1 or PC1) and less frequently in the PKD2 gene (encoding polycystin-2 or PC2).

**Methods:** To mimic naturally occurring human PKD1 mutations and gain insight into the PC1 extracellular domain function, four transgenic mouse lines were established with exclusively the extracellular domain of the Pkd1 gene ($Pkd1_{extra}$) under endogenous transcriptional regulation.

**Results:** Expression of the $Pkd1_{extra}$ transgene was 2- to 80-fold above endogenous levels. Strikingly, the $Pc1_{extra}$ protein was more abundant than expected based on proportion to the Pc1 endogenous levels. All four transgenic mouse lines consistently displayed progressive renal cystic phenotype. Consequently, these transgenic mice reproducibly developed altered renal function similar to human ADPKD with proteinuria, renal insufficiency, anemia and died of renal failure late in life. Substantial levels of $Pc1_{extra}$ protein were detected in exosomes from $Pkd1_{extra}$ MEFs and from urine of $Pkd1_{extra}$ mice. In precystic kidneys, the $Pkd1_{extra}$ transgene modulated Pc2 expression. Furthermore, we identified increased interaction of $Pc1_{extra}$/Pc1 with Pc2 in $Pkd1_{extra}$ kidneys relative to controls. This study uncovered a potential Pc1-mutant/Pc2 pathogenic crosstalk mechanism. Moreover, the pathophysiologic mechanism also implicates c-myc, a major modulator of cystogenesis.

**Conclusions:** Altogether, the novel $Pkd1_{extra}$ mouse model is the first Pc1 extracellular mutant that reproduces human ADPKD clinical progression and physiopathology.

*Funding:* Government Support - Non-U.S.

## SA-OR114

**Scattered Single-Cell Deletion of Pkd1 in Mouse Kidneys Recapitulates Human Polycystic Kidney Disease and Refines the Model of Cystogenesis** Wouter N. Leonhard,[1] Malu Zandbergen,[2] Kimberley Veraar,[2] Emile De Heer,[2] Martijn H. Breuning,[1] Dorien J.M. Peters.[1] *[1]Dept of Human Genetics; [2]Pathology, Leiden Univ Medical Center, Netherlands.*

**Background:** ADPKD patients carry a germline mutation in *PKD1* or *PKD2* which leads to the formation of thousands of kidney cysts. The relatively rapid progression of the disease is preceded by a lag-phase of several decades. Although the cause of this rapid progression is poorly understood, extensive research led to the presumption that somatic inactivation of the remaining allele initiates the formation of cysts, and the progression is further accelerated by renal injury. This hypothesis is based primarily on animal studies in which the gene is inactivated in large percentages of kidney cells. In this study we lowered this percentage to mimic human ADPKD more precisely.

**Methods:** To inactivate *Pkd1* in fewer renal cells in adult tamoxifen-inducible kidney-specific *Pkd1*-deletion mice, we lowered the tamoxifen dose. Quantification and visualization of $Pkd1^{del,del}$ cells was done by eMLPA and by crossbreeding to LacZ reporter mice. Renal injury was applied by nephrotoxicant (DCVC) injection, unilateral nephrectomy, or combinations. Western blot and IHC analysis was used to assess the expression of several PKD-related proteins.

**Results:** Interestingly, no pathological changes occurred for six months after scattered single-cell *Pkd1* deletion in adult mice and additional renal injury did not trigger rapid PKD. However, in the next few months (i.e. 6-10 months following *Pkd1*-disruption) the mice developed rapidly progressing PKD that was remarkably similar to human ADPKD. This shift was not caused by general aging but was preceded by increased pSTAT3, Ki-67 and LCN2 expression in the vicinity of the initial cysts.

**Conclusions:** Our data argue against the presumption that acute renal injury plays a major role in triggering cyst formation. We show that the initial cysts themselves, by imposing persistent stress on surrounding tissue, are the principal trigger for a 'snow-ball' effect driving the formation of new cysts. In addition, our approach is a suitable model for mimicking human ADPKD and will provide more accurate predictions of clinical outcome.

## SA-OR115

**A Human Pathogenic Single Amino Acid Deletion Affecting Polycystin-1 (PC1) G-Protein Signaling Prevents Activation of the Polycystin-2 (PC2) Channel** Stephen C. Parnell,[1] Tengis S. Pavlov,[2] Brenda S. Magenheimer,[1] Robin L. Maser,[1] Alexander Staruschenko,[2] James P. Calvet.[1] *[1]Kidney Inst, Univ of Kansas Med Ctr, Kansas City, KS; [2]Dept Physiol, Med Coll Wisc, Milwaukee, WI.*

**Background:** We previously reported that a single amino acid deletion in mouse polycystin-1 (PC1) disrupts the ability of PC1 to signal via heterotrimeric G-proteins while preserving the ability of PC1 to interact with PC2. Introduction of this mutation, which corresponds to human ADPKD-associated L4132Δ (ΔL, Afzal et al Hum Genet 1999 105:648), causes a severe PKD phenotype in Pkd1cond/ΔL knock-in mice. To determine if the ΔL mutation affects the ability of PC1 to regulate PC1/PC2 channel activity, we co-expressed PC1/PC2 and measured whole-cell macroscopic currents under voltage-clamp conditions.

**Methods:** HA-tagged human full-length wild-type, L4132Δ, or C-terminally truncated S4213X PC1 were co-transfected with human Myc-tagged PC2 and eGFP in CHO cells. S4213X lacks the PC2-interacting coiled-coil region. Whole-cell current recordings were made with eGFP-positive cells. Currents were elicited by test pulses of 20 mV steps from 80 to -80 mV from a holding potential of 0 mV to assess current–voltage (I-V) relationships and to measure current at -80 mV. After initial recordings cells were superfused with bath solution containing 0.5 mM $LaCl_3$ to block PC1/PC2 in order to determine $La^{3+}$-sensitive cation current density.

**Results:** As shown by co-immunoprecipitation, transfected wild-type PC1 and L4132Δ interacted equally well with PC2. However, compared to wild-type PC1, both L4132Δ and S4213X failed to elicit PC2-dependent channel activity in CHO cells (pA/pF: 30.9±8.6 vs 10.2±1.9 and 6.5±1.6; $P<0.05$).

**Conclusions:** Previous work showed that PC1/PC2 channel activity is stimulated by Gβγ subunits in a PC1-dependent manner (Kwon et al PNAS 2012 109:21462); and we showed that the PC1 L4132Δ mutation disrupts G-protein signaling and causes a severe PKD phenotype in mice. Here we show that this mutation inhibits PC1/PC2 channel activity, without affecting PC1/PC2 interaction, suggesting that PC1-dependent heterotrimeric G-protein signaling is critical for normal PC1/PC2 channel complex function.

*Funding:* NIDDK Support

## SA-OR116

**Polycystin Activity Is Regulated by Cellular Oxygen Sensing Pathways and Influences Mitochondrial Energy Production** Valeria Padovano, Ivana Y. Kuo, Benjamin J. Flaherty, Hans R. Aerni, Ming Ma, Stefan Somlo, Barbara E. Ehrlich, Jesse Rinehart, Michael J. Caplan. *School of Medicine, Yale Univ, New Haven, CT.*

**Background:** ADPKD is caused by mutation in the genes encoding polycystin-1 (PC1) and polycystin-2 (PC2). Loss of polycystin expression leads to perturbations in energy production, although the mechanisms responsible for this effect are unclear. We find that the activity and subcellular localization of the PC1-PC2 complex is sensitive to $O_2$ levels and that this $O_2$ sensitivity plays a role in regulating mitochondrial energy production.

**Methods:** Mitochondrial function was measured using Seahorse analysis. Mass spectrometry was used to identify proline hydroxylation. PC2 channel activity was studied by single channel recording in bilayer membranes.

**Results:** Seahorse analyses showed that $Pkd1^{-/-}$ cells have decreased $O_2$ consumption and oxidative phosphorylation (OXPHOS) rates. Moreover, pyruvate dehydrogenase (PDH), a key enzyme in OXPHOS regulation, is less active (higher pPDH/PDH ratio) in $Pkd1^{-/-}$ cells and in pre-cystic $Pkd1^{-/-}$ mouse kidneys, suggesting that OXPHOS reduction precedes cyst formation. $Ca^{2+}$ release from the ER positively regulates PDH and OXPHOS activity. Interestingly, we found that a cellular $O_2$ sensor, prolyl hydroxylase domain protein 3 (PHD3 or EGLN3), interacts with PC1 and that PHD3 knockdown led to increased PC1 localization at the cell surface; the same effect was also induced by hypoxia and the hypoxia-mimicking compound $CoCl_2$. Mass spectrometry revealed the presence of hydroxylated prolines in PC1, the extent of which was reduced by treatment with DMOG, a PHD inhibitor. Furthermore, DMOG induced a reduction in the activity and the $Ca^{2+}$ sensitivity of the PC2 channel. Finally, inhibition of PDH phosphorylation by dichloroacetate reduced the formation of cysts in 3D cultures of $Pkd1^{-/-}$ cells.

**Conclusions:** Our data suggest the intriguing possibility that changes in $O_2$ concentration alter the channel properties of the PC1-PC2 complex, leading to changes in ER calcium release that in turn affect mitochondrial function. This study also suggests that PDH may be a possible novel target for PKD pharmacotherapy.

*Funding:* NIDDK Support, Other U.S. Government Support

## SA-OR117

### Transcriptional Targets of STAT6 in Polycystic Kidney Disease

Erin E. Olsan, Nicholas Doerr, Thomas Weimbs. *Molecular, Cellular and Developmental Biology, Univ of California, Santa Barbara, CA.*

**Background:** The majority of ADPKD patients have mutations in the PKD1 gene, encoding the large, multi-pass transmembrane protein polycystin-1 (PC-1). Previously, we showed that a soluble fragment of PC-1, consisting of the c-terminal cytoplasmic tail, interacts with STAT6 and co-activates STAT6-dependent reporter transcription. Additionally, we reported increased phosphorylated STAT6 in several PKD mouse models, and showed that genetic removal or pharmacological inhibition of STAT6 decreases disease severity. To understand how STAT6 activation may contribute to renal cyst growth we identified several potential transcriptional targets of STAT6 signaling in PKD including IL24, periostin and galectin-3. IL24 is an IL10 family cytokine that functions in wound healing and inflammation in the skin and has been shown to be transcriptionally regulated by STAT6 in immune cells. Periostin, an extracellular matrix protein, and galectin-3, a β-galactoside binding lectin, both have previously been implicated in PKD.

**Methods:** Kidney epithelial cells treated with IL4 or IL13 or transfected with the PC-1 cytoplasmic tail were analyzed by RT-PCR. Microarray analysis was performed on cells overexpressing the PC-1 cytoplasmic tail. Total RNA or protein extracts from normal or cystic mouse kidneys were analyzed using RT-PCR or Western blot.

**Results:** We found that treatment of human and mouse kidney epithelial cells with IL4 or IL13, cytokines that signal through STAT6, increases IL24 gene expression. Overexpression of the PC-1 cytoplasmic tail also increases IL24 transcript levels. Importantly, we show that IL24 is upregulated in kidneys from several cystic mouse models. As predicted, we found increased levels of periostin and galectin-3 in cystic mouse kidneys compared to normal, and their expression levels are diminished in STAT6 KO cystic kidneys, suggesting that gene regulation is dependent on STAT6. Overexpression of the PC-1 cytoplasmic tail increased transcript levels of a related protein, galectin-8.

**Conclusions:** STAT6 has been shown to be an important signaling molecule in PKD. Here we identify several genes involved in the pathogenesis of PKD that can be transcriptionally regulated by STAT6.

## SA-OR118

### Evidence for Abnormal Glucose Homeostasis in Idiopathic Calcium Stone Formers

Kristin J. Bergsland, Elaine M. Worcester, Fredric L. Coe. *Dept of Medicine, Nephrology Section, Univ of Chicago, Chicago, IL.*

**Background:** Calcium (Ca) stone formers with idiopathic hypercalciuria (IHSF) have an exaggerated calciuric response to glucose administration. We and others have found that the calciuric effect of insulin does not differ between IHSF and normal people, so what mediates this response is unclear.

**Methods:** In the General Clinical Research Center, we studied renal mineral handling and glucose homeostasis in 21 normal controls (C), 12 female, and 31 IHSF (20 calcium oxalate [ICSF], 7 female; 11 with stones >50% calcium phosphate [IPSF], 5 female). We collected 15 urines and 20 blood samples over a 15 hour day, both fasting and with 3 meals of known composition. Subjects were selected for hypercalciuria and were not diabetic.

**Results:** By ANOVA adjusted for age, sex and creatinine excretion (UCreat/hr), all IHSF were hypercalciuric, fasting and fed (Table). Adjusted for age, sex and BMI, all IHSF had elevated serum glucose vs C, fasting and fed. In the fed state, ICSF had increased serum insulin vs. IPSF. BMI did not differ by subject type (26.7±1.1, 25.9±1.1, and 24.4±1.4 kg/m$^2$ for C, ICSF and IPSF, respectively, all p, NS). Gender differences were not detected in any of the analyses.

| | Glucose (mg/dL) | | Insulin (pM) | | UCa (mg/hr) | |
|---|---|---|---|---|---|---|
| | Fast | Fed | Fast | Fed | Fast | Fed |
| C | 91.5 ± 1.3 | 103.7 ± 1.3 | 39 ± 3 | 245 ± 15 | 5.7 ± 0.4 | 11.0 ± 0.4 |
| ICSF | 96.5 ± 1.3* | 110.6 ± 1.3* | 48 ± 3 | 274 ± 14† | 9.0 ± 0.4* | 20.0 ± 0.5* |
| IPSF | 97.5 ± 1.8* | 111.5 ± 2.0* | 39 ± 5 | 194 ± 22 | 10.4 ± 0.6* | 19.4 ± 0.6* |

Mean ± SEM adjusted for age, sex and BMI (for glucose, insulin) or UCreat/hr (for UCa).*, differs from C;†, differs from IPSF; all p<0.05

**Conclusions:** Glucose homeostasis in both male and female IHSF is markedly different than in C. Higher glucose in all IHSF, and higher insulin in ICSF after eating, cannot be explained by differences in diet or body size, since subjects were non-diabetic, ate the same meals, and analyses were corrected for BMI. Alterations in insulin sensitivity or pancreatic function in IHSF may contribute to Ca stone disease, and possibly to differences in stone type, and will be the subject of future research.

*Funding:* NIDDK Support

## SA-OR119

### Distinguishing Characteristics of Idiopathic Calcium Oxalate Kidney Stone Formers with Low Amounts of Randall's Plaque

Xiangling Wang,[1] Amy E. Krambeck,[1] James Williams,[2] Andrew D. Rule,[1] Eric J. Bergstralh,[1] Loren Paola Herrera Hernandez,[1] John C. Lieske.[1] *[1]Mayo Clinic, Rochester, MN; [2]Indiana Univ School of Medicine, Indianapolis, IN.*

**Background:** Overgrowth of calcium oxalate on Randall's plaque, composed of apatite that extends from the medullary interstitium to the papillary surface, has been hypothesized to be the main mechanism of stone formation among idiopathic CaOx stone formers (ICSF). However, it is less clear how stones form in ICSF that have little or no plaque.

**Methods:** In the current study a cohort of ICSF who underwent percutaneous nephroscopic papillary mapping and papillary tip biopsy during a stone removal procedure were divided into those with high (>5%; mean 10.5%) versus low (<5%; mean 1.5%) amounts of Randall's plaque coverage per papillum. Demographic and laboratory features were compared between these two groups.

**Results:** Low plaque ICSF were more likely to be female and obese and had a significantly lower 24-hr urine calcium excretion (Table). Their stones were more likely to have a positive bacterial culture and to have an atypical morphology by micro CT (lacked a CaP core consistent with origin upon plaque). Papillary biopsies of low plaque patients had less severe interstitial and basement membrane punctate crystallization (P<0.05).

| | High Plaque ICSF (n=10) | Low Plaque ICSF (n=31) | P value |
|---|---|---|---|
| Age (yr) | 61±7 | 62±10 | 0.76 |
| Female | 4 (40%) | 25 (74%) | 0.05 |
| Body Mass Index>30 | 4 (10%) | 15 (48%) | 0.03 |
| History of prior stone event | 9 (82%) | 15 (43%) | 0.04 |
| 24-hr Urine Volume (L) | 2.5±0.06 | 1.9±0.8 | 0.03 |
| 24-hr Urine Calcium (mg) | 291±99 | 196±87 | 0.01 |
| Positive Stone Culture | 0 (0%) | 6 (19%) | 0.06 |
| Atypical Stone Morphology | 0 (0%) | 9 (29%) | 0.04 |

**Conclusions:** These findings suggest that the pathogenesis of CaOx stone formation differs in patients with low amounts of Randall's plaque. Those ICSF with low plaque lack hypercalciuria, more commonly have stones with atypical morphology, and appear to have distinct clincial features (more likley to be female, obese, and have infected stones). Thus, there may be differnt pathways to stone formation amongst ICSF with and without abundant plaque.

*Funding:* NIDDK Support

## SA-OR120

### Modulators of Urinary Stone Formation: Transcriptional Study in an Animal Model of Hyperoxaluria

Saeed R. Khan, Sunil Joshi, Wei Wang, Ammon B. Peck. *Pathology, Univ of Florida, Gainesville, FL.*

**Background:** Role of macromolecular modulators (MMs) in stone formation has been investigated in animal models, one MM at a time. We used an unbiased approach of gene expression profiling to determine differential gene expression of all MMs shown to be involved in stone formation, in hyperoxaluric rats, with and without treatment with NADPH oxidase inhibitor apocynin.

**Methods:** Male Sprague-Dawley rats were fed rat chow or chow supplemented with 5% w/w hydroxy-L-proline (HLP) with or without apocynin-supplemented water. After 28 days, rats were euthanized, kidneys explanted and dissected to separate cortex and medulla. Total RNA was isolated and microarray analysis was conducted on the renal cortical and medullary tissues using the Illumina bead array reader™ . Gene ontology (GO) analysis and the pathway analysis of the genes was done using DAVID (Database for Annotation, Visualization of Integrated Discovery) enrichment analysis tool. Immunohistochemistry was used to confirm expressions of selected gene products.

**Results:** Administration of HLP led to crystal deposition in the kidneys. Deposits ranged from few scattered crystals to heavy deposit in the cortical tubules. Apocynin treatment resulted in near complete absence of crystals, despite showing similar levels of hyperoxaluria. Genes encoding for alpha-1and alpha-2-microglobulin, fibronectin, CD 44, osteonectin, fetuin, osteopontin, and matrix-gla protein, were up-regulated in both cortex and medulla. On the other hand, genes encoding for inter-alpha-inhibitor 1, 3 and 4, calgranulin B, prothrombin, and Tamm-Horsfall protein were down-regulated in both cortex and medulla. HLP-fed rats receiving apocynin had a significant reversal in gene expression profiles, those that were up-regulated came down while those that were down-regulated stepped up.

**Conclusions:** Our study provides a unique introspection into oxalate induced metabolic pathways in the cortex and medulla of hyperoxaluric kidneys. It appears that NADPH oxidase is involved in regulation of MM gene expression. Clearly, there are two distinct types of MMs, one is down-regulated and the other up-regulated during hyperoxaluria and crystal deposition in the kidneys.

*Funding:* NIDDK Support

## SA-OR121

### High Prevalence of CKD among Genetic Stone Formers

Lada Beara Lasic,[1] Vidar O. Edvardsson,[2] Runolfur Palsson,[2] David S. Goldfarb,[1] Eric J. Bergstralh,[3] Ramila A. Mehta,[3] John C. Lieske,[3] Dawn S. Milliner.[3] *[1]New York Harbor VAMC, New York, NY; [2]Landspital Univ Hospital, Reykjavik, Iceland; [3]Mayo Clinic, Rochester, MN.*

**Background:** Kidney stone disease is a multisystem disease associated with reduced kidney function. Patients with primary hyperoxaluria (PH), cystinuria (Cys), APRT deficiency (APRTd), and Dent disease (DD) have lifelong recurrences of stones. Due to their rarity, understanding of the impact of these disorders on kidney function is limited. We performed a cross-sectional study of estimated glomerular filtration rate (eGFR) in patients with each of these disorders.

**Methods:** A total of 380 patients with PH, 223 with Cys, 46 with APRTd, and 108 with DD were enrolled in the observational registries of the Rare Kidney Stone Consortium. Serum creatinine at first presentation was used for calculation of first eGFR by the MDRD equation in adults and modified Schwartz equation in children. The prevalence of CKD at presentation and end stage renal disease (ESRD) ever was compared between diseases.

**Results:** Stage 3 CKD was common at presentation, in 21-35% of patients. Stage 4 CKD was also common at presentation in PH, APRTd and DD (14-19%), but less so in Cys (3%). ESRD was documented in 0% (Cys) to 42% (PH) of the patients.

**Conclusions:** Stage 3 and 4 CKD is common at presentation of patients with PH, Cys, APRTd and DD. Development of ESRD at any time during registry follow up is common in all but Cys. Whether kidney damage is related to crystal nephropathy, stone burden or to other factors remains to be determined.

| | APRT deficiency (n=46) | Dent disease (n=108) | Cystinuria (n=223) | PH (n=380) |
|---|---|---|---|---|
| Age at first eGFR (yrs) | 37.2 ± 18.9 | 12.2 ± 10 | 37.4 ± 18.1 | 15.7 ± 17.1 |
| Age at last follow up (yrs) | 42.5 ± 19.4 | 18.5 ± 13.9 | 41.8 ± 18.7 | 26.1 ± 19.9 |
| Stones at diagnosis | 0.6 ± 0.8 | 3.0 ± 3.6 | 1.7 ± 1.5 | 3.9 ± 8.3 |
| N (First eGFR available) | 42 | 80 | 58 | 220 |
| First eGFR ml/min/1.73 $m^2$ | 73.4 | 74.4 | 78.8 | 71.9 |
| eGFR < 60 ml/min/1.73 $m^2$ | 14 (33%) | 27 (34%) | 12 (21%) | 78 (35%) |
| eGFR < 30 ml/min/1.73 $m^2$ | 8 (19%) | 12 (15%) | 2 (3%) | 30 (14%) |
| Patients with ESRD | 9 (20%) | 11 (10%) | 0 | 159 (42%) |
| Age at ESRD | 38.0 ± 18.8 | 34.3 ± 11.4 | - | 29.8 ± 18.5 |

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## SA-OR122

### Proximal Tubule Is a Site of Injury in Oxalate Nephropathy

Dawn S. Milliner, Eric J. Bergstralh, Ramila A. Mehta, John C. Lieske. *Mayo Clinic, Rochester, MN.*

**Background:** Loss of kidney function leading to kidney failure occurs in most patients with primary hyperoxaluria (PH). Oxalate (ox)-induced parenchymal kidney injury is felt responsible. Plasma ox (Pox) is freely filtered, concentrated in tubular fluid, and may also be secreted by the proximal tubule (PT), leading to high levels in the S3 segment where nephrocalcinosis could occur. Patients with higher Pox will have higher PT ox concentrations, favoring injury by ox ion or Caox crystals. We thus examined the relationship between Pox, PT ox, and markers of PT injury (urinary retinol binding protein (RBP) and alpha 1 microglobulin (A1M)). Urine supersaturation (SS) was used as a marker for Caox crystal formation in the collecting duct.

**Methods:** 110/276 patients enrolled in the PH Registry of the Rare Kidney Stone Consortium had Pox prior to ESKD and were included for study. eGFR was estimated by modified Schwartz equation in children and MDRD in adults. RBP and A1M were measured in 24 hr urine collections. Urine SS was by EQUIL2. Maximal PT ox was estimated assuming an average 75% PT fluid reabsorption. Associations with Pox, PT ox, and eGFR were assessed by Spearman's rank correlation.

**Results:** Age at PH diagnosis was 12.8 (3.3,16.7) yrs, mean (25th, 75th %ile), eGFR 88 (66,105) ml/min/BSA, Pox 6.7 (2.1, 8.2) umol/L, PT ox 47 (29,72) umol/L, and Uox 1.56 (0.94, 2.06) mmol/24 hrs/BSA.

| Spearman rank correlations: * .01<p< 05, **p<= 01 | Pox, umol/L (n=110) | PT ox, umol/L (n=88) | eGFR ml/min/BSA (n=98) |
|---|---|---|---|
| Uoxalate, mmol/24 hrs/BSA (n=89) | 0.51** | 0.81** | -0.13 |
| UCaox SS (n=58) | -0.03 | 0.10 | 0.24 |
| Pox, umol/L (n=110) | n/a | 0.67** | -0.47** |
| RBP, ug/gm creat (n=21) | 0.76** | 0.65** | -0.38 |
| A1M, mg/gm creat (n=21) | 0.55** | 0.32 | -0.12 |
| PT ox,umol/L (n=88) | 0.67** | n/a | -0.54** |

**Conclusions:** Uox and Caox SS, reflecting distal tubule events, did not correlate with eGFR. However, the correlation of Pox with A1M (p=0.0093), and both Pox and PT ox with RBP (p<0.001) and eGFR (p<0.001) suggest that ongoing PT injury could mediate loss of kidney function in PH.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## SA-OR123

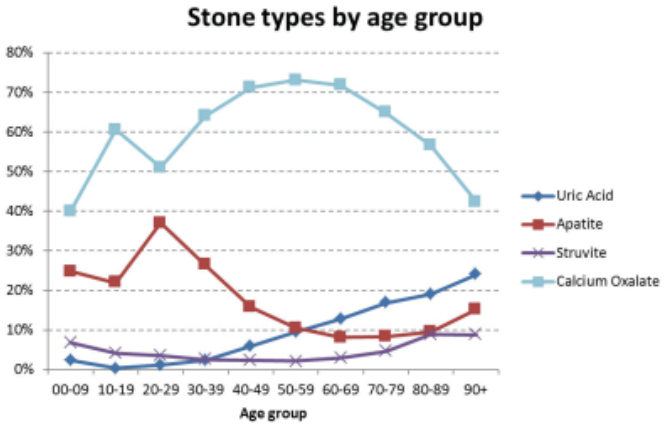
### Characteristics Associated with Stone Composition in a Large Referral Laboratory

John C. Lieske, Eric J. Bergstralh, Xujian Li, Thomas P. Moyer, Andrew D. Rule. *Mayo Clinic, Rochester, MN.*

**Background:** The prevalence of stone disease appears to be rising in the United States. We examined the case mix of stone composition in a large referral laboratory in order to discern characteristics that may explain this trend.

**Methods:** There were 48,446 stones submitted for analysis in the Mayo Clinic metals laboratory for the year 2010, but analysis was restricted to the first stone submitted per person (n=43,545). Each stone former was classified into mutually exclusive groups using the following order: any struvite (ST), any cystine (Cy), any uric acid (UA), any brushite (BR), majority (≥50%) Calcium oxalate (CaOx), or majority (≥50%) hydroxyapatite (HA).

**Results:** CaOx (67%) were most common, followed by HA (16%), UA (8%), Struvite (3%), BR (0.9%) and cystine (0.35%). Women were more likely to have a HA (65%) or ST (65%) stone, while men were more likely to have a CaOx (64%) or UA (72%) stone (P< 0.001). There were striking age trends in stone type, with HA stones being more common among those 20-29 yrs old, CaOx among those 40-70 yrs old, and UA among those 70+ yrs old (P<0.001, Figure). More CaOx and UA stones occurred in July and August (P<0.001), while calendar month did not influence other stone types.




**Conclusions:** Overall, CaOx stones are the most common, as is generally accepted. However, age and gender have marked influence on the type of stone formed. The change in composition amongst the elderly favoring UA has not previously been appreciated. These data also suggest a summer effect of increased stone risk, although this is restricted to CaOx and UA stones.

*Funding:* NIDDK Support

## SA-OR124

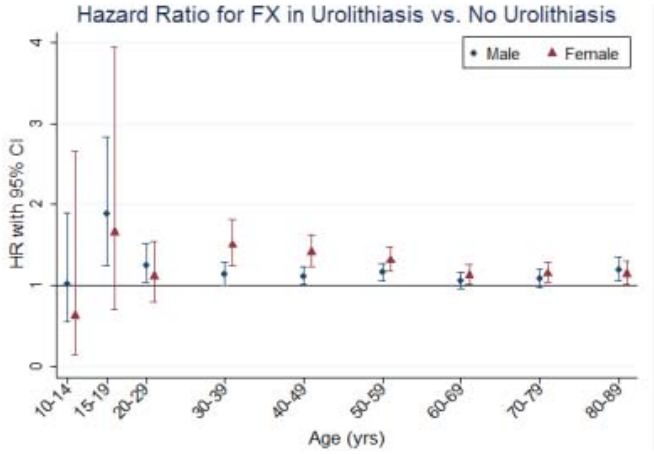
### Urolithiasis and Fracture Risk over the Lifespan: A Population-Based Study Using the Health Improvement Network (THIN)

Michelle Denburg,[1,2] Lawrence A. Copelovitch,[1,2] Kevin Haynes,[1] Shamir Tuchman,[3] Gregory Edward Tasian,[2] Mary B. Leonard.[1,2] *[1]Perelman School of Medicine at the Univ of Pennsylvania; [2]Children's Hospital of Philadelphia; [3]Children's National Medical Center.*

**Background:** Studies have shown decreased bone mineral density in patients with urolithiasis, but the fracture (FX) burden is unclear. The objectives of this study were to determine if urolithiasis is associated with increased FX risk and to explore age, sex and site-related differences.

**Methods:** In a retrospective cohort study using THIN, a primary care electronic medical records database, we identified 60,629 subjects with urolithiaisis and randomly selected 605,264 age, sex, and practice-matched subjects (10:1). Cox regression analysis was used to estimate the hazard ratio (HR) for first FX.

**Results:** Median age was 51 yrs, and 68% were male confirming their 2-fold greater urolithiasis burden. Over a median follow-up of 5 yrs (IQR 2-10), 4321 first FX events occured in those with urolithiasis (113/10,000 person-yrs) vs. 36,367 FX events (98/10,000 person-yrs) in those without urolithiasis. Distribution of FX site was comparable between groups with forearm/wrist, hand, and lower leg/ankle being the most common sites in both. Median time from initial urolithiasis diagnosis to FX was 10 yrs (IQR 4-19). The effect of urolithiasis on FX risk differed by sex and age (p for interactions ≤0.003). Urolithiasis was associated with significantly increased FX risk in women after age 30 and in young, middle-aged and elderly men, with the greatest risk in the 3rd decade for females and late adolescence for males.




**Conclusions:** Urolithiasis was associated with increased FX risk, and the distribution of FX sites did not differ from that of the general population. While the HR was greatest in young adults, the significantly increased risk in the elderly has important public health implications.

*Funding:* NIDDK Support, Other NIH Support - This work was supported by the National Institutes of Health Clinical Translational Service Award: UL1-RR024134, Private Foundation Support

## SA-OR125

**Metabolic Acidosis as a Risk Factor for Mortality in Peritoneal Dialysis Patients** Tae Ik Chang,[1] Seung Hyeok Han,[2] Hyung Jung Oh,[2] Tae-Hyun Yoo,[2] Dae-Suk Han,[2] Shin-Wook Kang.[2] *[1]Dept of Internal Medicine, NHIC Ilsan Hospital, Gyeonggi-do; [2]Dept of Internal Medicine, Yonsei Univ College of Medicine, Seoul, Korea.*

**Background:** Metabolic acidosis is common in patients with end-stage renal disease (ESRD) and is associated with increased mortality in hemodialysis (HD) patients. Given the continuous provision of dialysis treatment with PD, PD is more effective in correcting metabolic acidosis than HD, and thus the impact of metabolic acidosis on clinical outcomes may differ between the two dialysis modalities. However, there have been few studies on the relationship between serum bicarbonate levels and the risk of death in PD patients.

**Methods:** This prospective observational study included a total of 441 incident ESRD patients who started PD from Jan 2000 to Dec 2005. Using time-averaged serum bicarbonate levels (TA-Bic), we elucidated whether metabolic acidosis could predict all-cause mortality in these patients.

**Results:** Multivariable linear regression analysis revealed that serum bicarbonate concentrations were positively associated with hemoglobin levels ($\beta = 0.187$; $P < 0.001$) and residual renal function (RRF) ($\beta = 2.038$; $P = 0.042$), while they were negatively associated with serum albumin ($\beta = -0.235$; $P < 0.001$) and C-reactive protein (CRP) levels ($\beta = -2.804$; $P = 0.005$). During a median follow-up duration of 34.8 months, 149 deaths were recorded. After adjustment for age, diabetes, coronary artery disease, serum albumin, ferritin, CRP, RRF, normalized protein catabolic rate, and percentage of lean body mass, TA-Bic levels were associated with a significantly lower risk for mortality (HR per 1 mEq/L increase, 0.83; 95% CI, 0.76-0.90; $P < 0.001$). In addition, compared to patients with TA-Bic concentrations of 24-26 mEq/L, TA-Bic levels < 22 and 22-24 mEq/L groups conferred 15.9- and 2.1-folds increased risk of death, respectively. Similar findings were observed for cardiovascular mortality.

**Conclusions:** Metabolic acidosis was an independent risk factor for mortality in incident PD patients. This relationship between low bicarbonate levels and adverse outcome might be partly related to enhanced inflammation associated with metabolic acidosis.

## SA-OR126

**The PD-Biosensor: A New, Quick and Easy Method to Assess the Functions of the Peritoneal Membrane** Giuseppe Pontoriero, Vincenzo La Milia, Francesco Locatelli. *Nephrology and Dialysis, A. Manzoni Hospital, Lecco, Italy.*

**Background:** During peritoneal dialysis (PD) glucose (G) is absorbed from dialysate (D) to blood (B) and the others solutes as urea, creatinine (Cr), etc, diffuse from B to D. During a dwell with a high osmolarity PD solution (S) a marked reduction in the D sodium concentration ($\Delta$Na) may occur. To evaluate the functions of peritoneal membrane (PM) we use the peritoneal equilibration test (PET). The PET is complicated and requires a considerable expenditure of time and resources.

**Methods:** The PD-Biosensor (PD-B) is based on the ionic conductivity (Cd). Since the Cd of a S is a function of electrolytes concentration and of viscosity, due the G concentration, it is possible to evaluate changes in the Cd of the PD S during a dwell with a 3.86% G concentration, during which there is a dilution of the electrolytes and G absorption present in PD S. This difference in Cd are measured by the PD-B and can be used to evaluate the functions of PM. We evaluate the functions of PM during a 3.86%-modified-PET by the PD-B and the classical PET parameters in 69 PD pts (38 M, 31 F).

**Results:** The values of Cd, in the D at 240' of dwell, was well correlated with transport of G ($D/D_0$) ($r^2$=-0.62), small solutes transport (D/PCr) ($r^2$=0.72) and $\Delta$Na ($r^2$=-0.63). Twelve pts (17%) had ultrafiltration failure (UFF). All the pts with UFF had a Cd, in the D at 240' of the dwell, more then 12.5 mS/cm. The ROC curve for UFF of Cd measured by PD-B at 240' had an area under curve (AUC) of 0.907 (chiSquare=10.20, p<0.002), the AUC of D/PCr was 0.879 (chiSquare=10.87, p=0.001) and the AUC of $D/D_0$ was 0.810 (chiSquare=8.50, p<0.01). The changes of Cd, measured by PD-B, during a 3.86%-modified-PET, are expression of changes in the peritoneal transport of G, small solutes (Cr) and Na: all these parameters can be measured together and with a single numeric parameter. The changes of Cd, measured by PD-B, are predictive of UFF.

**Conclusions:** The PD-B is a new, inexpensive and easy method to evaluate the functions of PM in real time without laboratory assay, calculation and interpretation of data. The PD-B can be used to evaluate the functions of PM more frequently, also at home, and may open new perspectives in PD.

## SA-OR127

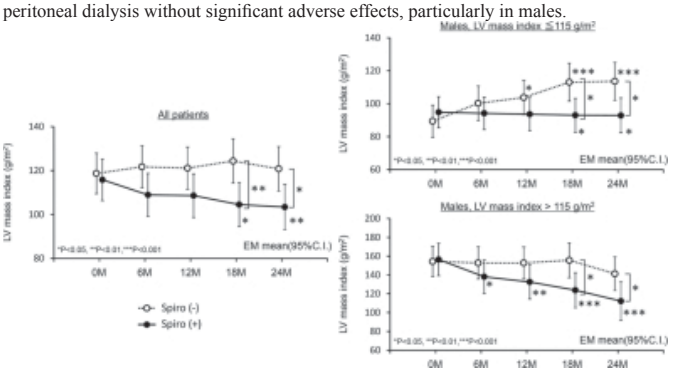
**Spironolactone Prevents Cardiac Hypertrophy in Peritoneal Dialysis Patients without Significant Adverse Effects** Yasuhiko Ito,[1] Masashi Mizuno,[1] Yasuhiro Suzuki,[1] Hirotake Kasuga,[2] Shoichi Maruyama,[1] Yukio Yuzawa,[2] Seiichi Matsuo.[1] *[1]Nephrology, Nagoya Univ, Nagoya, Japan; [2]Nagoya Spiro Study Group.*

**Background:** End-stage renal diseases treated by dialysis are associated with a high prevalence of increased left ventricular hypertrophy, which is associated with high mortality. Mineralocorticoid receptor antagonists are reported to improve the survival of patients with chronic heart failure (NEJM 1999, 2003, 2011); however, these effects remain uncertain in patients on dialysis.

**Methods:** We conducted a multicenter, open-label, prospective randomized trial in 158 patients under treatment with either angiotensin converting enzyme inhibitor or angiotensin type 1 receptor antagonist and on peritoneal dialysis with and without spironolactone (control group) for 2 years.

**Results:** Left ventricular mass index (LVMI) was significantly suppressed in patients taking spironolactone after 18 months. As normal values of LVMI differ between males and females, we separately analyzed the effects. LVMI for males with a normal LVMI of ≤115 g/m$^2$ at baseline did not change in the group given spironolactone, but significantly increased in the control group. The LVMI for males with a higher LVMI at baseline did not change in the control group but was significantly reduced in the spironolactone group. These effects were unclear in females. The effects of spironolactone were obvious in patients with reduced residual renal function. Effects on ejection fraction were also confirmed in patients with reduced residual renal function. Renal Kt/V and dialysate/plasma creatinine ratio did not significantly differ between the groups during the observation period. Serious adverse effects such as hyperkalemia were not observed.

**Conclusions:** Spironolactone may help to prevent cardiac hypertrophy in patients on peritoneal dialysis without significant adverse effects, particularly in males.




## SA-OR128

**Urgent-Start Peritoneal Dialysis versus Other Modalities of Dialysis: A Prospective Cohort Study** Arshia Ghaffari, Gbemisola Adeseun, Ushir V. Patel. *Div of Nephrology, Univ of Southern California, Los Angeles, CA.*

**Background:** Several reports have described the feasibility of urgent initiation of peritoneal dialysis (PD) in unplanned end-stage renal disease (ESRD) patients using low-volume recumbent PD soon after PD catheter placement. While short-term clinical outcomes are similar to other dialysis modalities, there is a lack of data about longer-term outcomes.

**Methods:** This is a single-center prospective cohort study comparing hospitalizations, central venous catheter (CVC) exposure and infectious complications amongst 111 incident ESRD patients (17 planned HD, 13 planned PD, 53 urgent HD, 28 urgent-start PD) at our dialysis center between Jan 1, 2010 and December 31, 2011 with a median followup of 365 days (range 90-365 days).

**Results:** The differences in hospitalization rates (planned PD 0.92 ± 1.16; urgent-start PD 0.89 ± 1.17; planned HD 1.2 ± 2.1; urgent HD 1.6 ± 1.8) and length of stay (planned PD 5.7 ± 8.17 days; urgent-start PD 4.39 ± 6.06; planned HD 9.4 ± 17.6; urgent HD 9.6 ± 11.5) were not significantly different amongst different dialysis modalities (p>0.05). As compared to the urgent HD group, the average duration of CVC use was significantly lower in all other groups (planned HD 33.4 ± 64.1 days; planned PD 31.9 ± 85.3; urgent-start PD 19.1 ± 44.13; urgent HD 248.3 ± 99.4, p<0.001). The bacteremia rate were significantly

higher in the urgent HD group as compared to all other groups (planned HD 0, planned PD 0, urgent HD 0.39 ±0.77, urgent PD 0.07± 0.26, (p=0.01). Peritonitis rates were comparable between the urgent-start PD and planned PD groups at 1 to 100.9 patient-months versus 1 to 25.4 patient-months respectively. There were 2 deaths in the urgent HD group while no deaths occurred in the other groups.

**Conclusions:** Our study demonstrates that patients urgently started on PD have significantly lower CVC exposure and bacteremia rates than patients urgently started on HD, while having comparable rates to planned PD and HD. Our study suggests that urgent-start PD is feasible in late presenting patients with outcomes that warrant more widespread adoption of this modality in clinical practice.

## SA-OR129

**Peritoneal Dialysis Start and Mortality** Arsh Jain,[1] Jessica M. Sontrop,[1] Jeffrey Perl,[2] Peter G. Blake,[1] William F. Clark,[1] Louise M. Moist.[1] *[1]Nephrology, London Health Sciences Centre, London, Canada; [2]Nephrology, St. Michael's Hospital, Toronto, Canada.*

**Background:** Several observational studies of hemodialysis patients show an association between early dialysis initiation and increased mortality. Few studies have examined this association among peritoneal dialysis patients.

**Methods:** We analyzed a cohort of 8,047 peritoneal dialysis patients and evaluated the association between timing of dialysis initiation and mortality (Canada, 2001-2009). We calculated estimated glomerular filtration rate (eGFR) at dialysis initiation and defined early, mid, and late starts as eGFR >10.5, 7.5–10.5, and <7.5 mL/min per 1.73 $m^2$, respectively. We used multivariable piece-wise Cox regression to evaluate mortality (overall and annually).

**Results:** Between 2001 and 2009, the proportion of patients starting peritoneal dialysis as early starts increased from 29% (95% CI: 26% to 32%) to 44% (95% CI: 41% to 47%). Compared with late starts, the overall mortality rate was not higher among early starts (adjusted HR 1.08; 95% CI: 0.96, 1.23) nor mid starts (adjusted HR 0.96; 95% CI: 0.86, 1.09). However, when examined annually, early starts were significantly more likely to die within the first year of starting dialysis compared with late starts (adjusted HR 1.38; 95% CI: 1.10, 1.73), but not in subsequent years.

**Conclusions:** In Canada, patients are initiating peritoneal dialysis at increasingly higher levels of eGFR. Contrary to most observational studies assessing hemodialysis, the early initiation of peritoneal dialysis, at an eGFR above 10.5 mL/min per 1.73 $m^2$, is not associated with increased mortality.

## SA-OR130

**Chondroitin Sulfate Attenuates Peritoneal Fibrosis Induced by Chlorhexidine Gluconate in Mice** Shinichi Abe,[1] Tomoya Nishino,[1] Kumiko Io,[1] Yoko Obata,[1] Takehiko Koji,[2] Shigeru Kohno.[1] *[1]Second Dept of Internal Medicine, Nagasaki Univ School of Medicine, Nagasaki City, Nagasaki Prefecture, Japan; [2]Dept of Histology and Cell Biology, Nagasaki Univ School of Medicine, Nagasaki City, Nagasaki Prefecture, Japan.*

**Background:** Long-term peritoneal dialysis causes peritoneal fibrosis in submesothelial areas. Previousreports suggest that the angiogenesis and inflammation play crucial roles in peritoneal fibrosis. Chondroitin sulfate (CS) is a typical glycosaminoglycan distributed in a wide variety of tissues, and recent studies suggest the effect to various inflammatory diseases by suppressing NF-kB activation. Therefore, we examined the effect of chondroitin sulfate on the progression of peritoneal fibrosis in mice.

**Methods:** 10 week-old male ICR mice were divided into three groups, chlorhexidine gluconate (CG), CG + CS, and control group. Peritoneal fibrosis was induced by the injection of CG into peritoneal cavity in mice every other day for 3 weeks. CS or vehicle was administered every day orally from the start of CG injection for 3 weeks. The mice were sacrificed 3 weeks after the first CG injection and peritoneal tissues were dissected out. Morphologic peritoneal changes were assessed by Masson's Trichrome staining. Inflammation and fibrosis associated factors were assessed by immunohistochemically.

**Results:** In CG-injected mice, the marked thickening of the submesothelial compact zone was shown. In contrast, the administration of CS suppressed the progression of submesothelial thickening and type III collagen accumulation in CG-injected mice. The numbers of a smooth muscle actin-positive cells and phosphorylated-Smad 2/3-positive cells were decreased to a significantly greater extent in CG + CS group than CG group. Also, the numbers of F4/80-positive cells and MCP-1-positive cells in CG + CS group were significantly fewer than that in CG group.

**Conclusions:** Our results indicate that CS can suppress peritoneal thickening and fibrosis in mice. These results suggest that CS may have therapeutic potential for peritoneal fibrosis.

## SA-OR131

**The Impact of Interaction of Advanced Glycation End-Products and Its Receptor for the Development of Peritoneal Fibrosis in Peritoneal Dialysis with Renal Failure** Akira Onishi,[1] Yoshiyuki Morishita,[1] Hiromichi Yoshizawa,[1] Ichiro Hirahara,[1] Shigeaki Muto,[1] Eiji Kusano.[2] *[1]Div of Nephrology, Dept of Medicine, Jichi Medical Univ, Shimotsuke, Tochigi, Japan; [2]Utsunomiya Social Insurance Hospital, Utsunomiya, Tochigi, Japan.*

**Background:** The interaction of advanced glycation end-products (AGEs) and its receptor; RAGE was suggested to contribute to the development of peritoneal fibrosis in peritoneal dialysis (PD); however, the impact of this interaction in renal failure (RF) that is the actual clinical condition of PD has not been fully elucidated. In this study, we investigated the impact of AGEs-RAGE interaction for the development of peritoneal fibrosis in PD with RF.

**Methods:** RF was induced by feeding 0.75% adenine-containing food for 2 weeks in Sprague-Dawley rats. Subsequently, 100 ml/kg of PD fluid containing 5 mM of methylglyoxal (MGO) was intraperitoneally injected every day for 3 weeks to induce peritoneal fibrosis. In some RF rats, PD fluid containing AGE inhibitors; 50 mg/kg of aminoguanidine (AG) or 1 mg/kg of pyridoxamine (PX) with MGO was injected. The rats with normal renal function (non-RF) that were injected with MGO-containing PD fluid served as control. Finally, the peritoneal tissue were obtained to investigate morphological changes by light microscopy, the expression of an epithelial marker; cytokeratin and a mesenchymal marker; α-smooth muscle actin (α-SMA), AGEs, and RAGE, and the signal transduction by immunofluorescence and/or qRT-PCR.

**Results:** Peritoneal fibrous thickening with decreased expression of cytokeratin and increased expression of α-SMA were more strongly observed in RF rats than those in non-RF rats. The increased expression of AGEs and RAGE was also more strongly detected in RF rats compared with those in non-RF rats. The increased expression of TGF-β1 and Snail was highly detected in RF rats compared with those in non-RF rats. These changes in RF rats were significantly inhibited by AG and PX.

**Conclusions:** The results of present study suggested that AGEs-RAGE interaction was enhanced and contribute to peritoneal fibrosis in PD with RF. The AGE inhibitors may be a potentially good therapeutic option for peritoneal fibrosis in PD with RF.

## SA-OR132

**Cell-Free Plasma DNA as Index of Apoptosis and Tissue Damage in Peritonitis** Grazia Maria Virzì, Sabrina Milan Manani, Alessandra Brocca, Sonya Day, Ilaria Tantillo, Carlo Crepaldi, Claudio Ronco. *Nephrology Dept-IRRIV, Vicenza, Italy.*

**Background:** Cell-free plasma DNA (cfDNA) is a circulating acellular DNA fragments and originates from necrotic and apoptotic cells reflecting inflammation. High levels of cfDNA have been reported in many clinical conditions as Chronic Kidney Disease. Its application has increased in diagnostic field. However, there is no data on cfDNA in peritoneal dialysis (PD) patients with peritonitis. The objective of this study was to evaluate the variation of cfDNA subsequent to peritonitis as index of apoptosis and tissue damage.

**Methods:** cfDNA was extracted from plasma in 54 PD patients: 33 without peritonitis, 29 with peritonitis recovered for more than 3 months (mo), and 8 with peritonitis during the last 3mo. cfDNA was quantified by Real-Time PCR for the β-globin gene, in triplicate. We compared patients with peritonitis in the last 3mo and without by Mann-Whitney test. We applied a linear transformation of cfDNA. Furthermore, we performed a univariate linear regression of log(cfDNA) on days from the start of last peritonitis. A p<.01 was considered statistically significant.

**Results:**

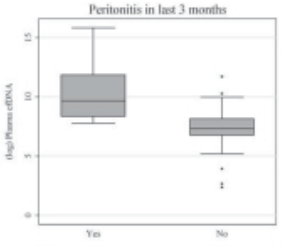



Figure1a:The median concentrations of plasma cfDNA of patients with and without peritonitis in the last 3 months

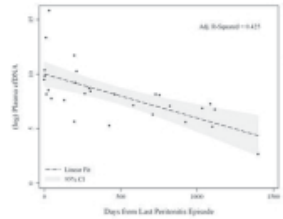


Figure1b: A significant negative correlation was observed between plasma cfDNA concentration and days from the start of last peritonitis (p<.01).

The median concentrations of patients cfDNA with and without peritonitis in the last 3mo were 16025 vs 1518.37 pg/µl, respectively (p<.01). A significant negative correlation was observed between cfDNA concentration and days from the start of last peritonitis (p<.01). Each one-unit increase in days from the start of peritonitis was associated with .402% decrease in cfDNA level.

**Conclusions:** Our data have demonstrated that cfDNA subsequent to peritonitis is increased in PD patients as index of tissue damage. Decreasing levels of cfDNA were observed in patients with a longer peritonitis-free period. cfDNA could be a marker of peritoneal membrane repair. Our results indicate the potential future role of cfDNA in the prognosis of tissue recovery from peritonitis.

**SA-OR133**

**The Development of *In Vivo* Small Interfering RNA Delivery System with Nanoparticles to Peritoneum for the Treatment of Peritoneal Fibrosis**
Hiromichi Yoshizawa,[1] Yoshiyuki Morishita,[1] Akira Onishi,[1] Shigeaki Muto,[1] Eiji Kusano.[2] *[1]Div of Nephrology, Dept of Medicine, Jichi Medical Univ, Shimotsuke, Tochigi, Japan; [2]Utsunomiya Social Insurance Hospital, Utsunomiya, Tochigi, Japan.*

**Background:** Peritoneal fibrosis is an intractable complication without any established therapies in peritoneal dialysis (PD). Gene therapies would be a potentially good strategy for treatment of peritoneal fibrosis; however, the delivery methods of treatment genes to peritoneum remain to be established. We attempted to develop in vivo small interfering RNA (siRNA) delivery system with non-viral nanoparticles (NPs) to peritoneum for treatment of peritoneal fibrosis.

**Methods:** Peritoneal fibrosis mice were produced by intraperitoneally injection of PD fluid containing 40mM of methylglyoxal to C57BL/6 mice every day for 2 weeks. For delivery of anti-fibrotic (TGFβ1) siRNA to peritoneum, 500nmol of lyophilized NPs composed with phospholipids were rehydrated by 100 μl of water containing TGF β1-siRNA (5nmol). Then, TGF β1-siRNA encapsulated with NPs (TGF β1-siRNA-NPs) were dissolved in 1000 μl of PD fluid and injected intraperitoneally to peritoneal fibrosis mice 3 times a week for 2 weeks. Peritoneal fibrosis mice those were injected with TGF β1-siRNA alone or non-targeted-siRNA encapsulated with NPs served as controls. Finally,the peritoneal tissues were obtained after peritoneal equilibrium test. to investigate the expression of TGF β1, epithelial cell markers (E-cadherin and occludin) and mesenchymal cell markers (α-SMA and vimentin) by qRT-PCR and/or immunohistocmestory, and morphological changes by light microscopy.

**Results:** TGFβ1-siRNA-NPs significantly knock downed TGF β1 expression in peritoneum compared with those of other groups. TGFβ1-siRNA-NPs also inhibited peritoneal fibrous thickening with decreases expression of epithelial cell markers and increases expression of mesenchymal cell markers in peritoneum compared with those of other groups. Furthermore, TGFβ1-siRNA-NPs maintained better peritoneal functions than those of other groups.

**Conclusions:** We developed in vivo anti-fibrotic siRNA delivery system with NPs to peritoneum in the therapeutic setting for peritoneal fibrosis in PD.

**SA-OR134**

**Mediators of Inflammation and Fibrosis in Peritoneal Dialysis**
Michael E. Ullian, Thomas Morinelli, Linda Walker, Megan Hicks. *Medical Univ of South Carolina.*

**Background:** Fibrosis of the peritoneal membrane limits the efficacy of peritoneal dialysis (PD), and angiotensin II (AngII) and glucose, the osmotic agent in PD fluid, appear to cause cycles of inflammation and fibrosis. We examined AngII-glucose interactions on the intracellular signals that mediate inflammation [cyclooxygenase 2 (Cox2)] and fibrosis [fibronectin (FN)] in cultured peritoneal mesothelial cells (PMC) and experimental PD.

**Methods:** Cells harvested from rat peritoneum were studied in culture passages 0 and 1, when characteristic cobblestone appearance was maintained. Cox2 and FN protein content (b-actin content for normalization) were quantitated by immunoblotting. Experimental PD was performed in 100 gm rats by intraperitoneal injection twice daily of 5 ml of saline or 4.25% glucose-containing PD fluid for 5 weeks, followed by parietal mesothelium fixation and Masson's Trichrome staining.

**Results:** In PMC, 10 nM AngII increased Cox2 expression 9.8-fold at 6 hr and 2.1-fold at 24 hr (n = 4); in contrast, FN expression occurred more slowly: 0.6-fold increase at 6 hr and 1.3-fold increase at 24 hr (n = 2). Glucose (2.5%) increased Cox2 expression 9.4-fold at 6 hr and 24.1-fold at 24 hr, and the addition of 10 nM AngII to the glucose increased Cox2 expression to 33.9-fold at 6 hrs but did not increase it at 24 hr. Exposure of PMC to maximal stimulation of glucose (4.25%, 24 hr), the osmotic control mannitol (4.25%, 24 hr), or AngII (100 nM, 6 hr) resulted in 17- to 26-fold increases in Cox2 expression that were 90% prevented by inclusion of 5 μM Ro-1069920, an inhibitor of NF-kB, which is a well-described mediator of inflammation (n = 2-4). Experimental PD in rats resulted in thickening of the mesothelium: [13 μm (saline) vs 24 μm (4.25% glucose-containing PD fluid), n = 8], which was completely prevented by inclusion of 10 μM losartan (AngII type 1 receptor antagonist), $p < 0.05$.

**Conclusions:** Glucose in PD fluid appears to cause cycles of inflammation and fibrosis of the peritoneal membrane, via mechanisms that include AngII and osmotic stress. The AngII type 1 receptor and NF-kB may be loci whose inhibitors could limit inflammation/fibrosis of the peritoneum and prolong PD as a renal replacement modality.

*Funding:* Pharmaceutical Company Support - Dialysis Clinic Incorporated

## TH-PO001

**RIFLE Classification in Geriatric Patients with Acute Kidney Injury in the Intensive Care Unit** Eun Young Seong,[1] Min Ji Shin,[1] Harin Rhee,[1] Il Young Kim,[2] Dong Won Lee,[2] Soo Bong Lee,[2] Byeong Yun Yang,[1] Ihm Soo Kwak.[1] *[1]Dept of Internal Medicine, Pusan National Univ School of Medicine; [2]Dept of Internal Medicine, Pusan National Univ Yangsan Hospital.*

**Background:** The RIFLE (risk, injury, failure, loss, and end-stage) classification is widely used to gauge the severity of acute kidney injury(AKI), but its efficacy has not been formally tested in geriatric patients. We studied 256 elderly patients who developed AKI in intensive care unit(ICU) in accordance with the RIFLE creatinine criteria.

**Methods:** Patients were eligible if they were ≥65 years and developed AKI in ICU between between January 2009 and April 2013. Exclusion criteria included patients on maintenance dialysis and stayed in ICU for <48 hours. We used the sRIFLE classification system in which only creatinine levels are used to classify patients.

**Results:** The mean age was 74.4±6.3 years. The cause of AKI was dehydration(11.7%), infection(47.7%), bleeding(4.3%), contrast(3.1%), drugs(5.1%), obstruction(2.3%), cardiogenic shock(17.2%), renal infarction(0.8%), rhabdomyolysis(3.9%). They were categorized into R (n=53,20.7%), I (n=102,39.8%), and F (n=101,39.5%) groups. Amon 256 patients, 97(37.9%) died during index hospitalization (19 days, interquartile range 10-34). Age was not significant factor discriminating between survivors and non-survivors. The in-hospital mortality rates for R, I, and F groups were 26.4%, 42.2%, and 39.6%,respectively. A logistic regression analysis was performed to identify the independent predictors for in-hospital mortality. There was no significant associations between RIFLE groups (for I vs. R: odds ratio (OR), 2.03; 95% confidence interval (CI), 0.98-4.19; p=0.56; for F vs. R: OR, 1.82; 95% CI, 0.88-3.78; p=0.11). After adjustment, hemoglobin and number of failing organs were significantly associated with higher in-hospital mortality rates, however, R,I, or F classifications were not significantly associated with increased hospital mortality.

**Conclusions:** RIFLE classification might not be associated with mortality rate in geriatric patients in ICU. In geriatric patients with acute kidney injury in ICU, various factors should be considered other than serum creatinine.

## TH-PO002

**The Effect of Minor Episodes of Acute Kidney Injury on Progressive Renal Dysfunction in Early Stage of Chronic Kidney Disease Patients** Eunjung Cho,[1] Myung-Gyu Kim,[1] So-Young Lee,[2] Won-Yong Cho,[1] Sang-Kyung Jo.[1] *[1]Korea Univ Anam Hospital, Republic of Korea; [2]Eulji Univ Hospital, Republic of Korea.*

**Background:** Several recent studies performed in in-hospital or ICU settings revealed that dialysis requiring severe acute kidney injury (AKI) episode is a well-known risk factor for progression of chronic kidney disease (CKD). However, the impact of minor episodes of AKI in ambulatory, outpatient setting in patients with early stages of CKD has not been well studied.

**Methods:** We retrospectively assembled a cohort of stable, 391 ambulatory patients with stage 2 or 3 CKD who had more than three values of serum creatinine and visited outpatient clinic longer than one year. Our primary outcome was a 15 ml/min/1.73m$^2$ decline of eGFR and we analyzed the risk of CKD progression as a function of AKI episodes. In addition, we also assessed the effect of eGFR variability defined as degree of deviation from the patient specific slope of eGFR on CKD progression.

**Results:** Over a mean follow-up of 905 days, 100 (25.6%) patients had AKI episodes. Only one patient needed renal replacement therapy and most patients (74%) showed less than two fold increase of eGFR. Patients with AKI episodes were older and more likely to have diabetes or hypertension and lower baseline GFR. More patients were taking diuretics or beta blocker than patients without AKI episodes. Mean follow up period was similar in both groups. We observed no mortality in our cohort during the follow-up period. Significantly more patients with AKI episodes reached primary outcome than patients without AKI episodes [19 (19.0%) vs. 18 (6.2%), p=0.001]. In multivariate Cox proportional hazard model, AKI episode and baseline eGFR were independent predictors of CKD progression (hazard ratio 5.515 for AKI vs. 1.039 for baseline GFR). However, eGFR variability was not found to be associated with faster progression of renal dysfunction.

**Conclusions:** Even minor AKI episodes in stage 2 or 3 CKD patients are thought to be still a powerful risk factor for progression of CKD and a multi-sided approach to develop tools for prevention, risk stratification or early detection, should be continued to improve ultimate prognosis.

## TH-PO003

**Effect of Acute Kidney Injury on Chronic Kidney Disease Progression and Proteinuria: Initial Results from a Pilot Study** Robert A.D. Scott,[1] Nitin V. Kolhe,[1] Chris W. McIntyre,[1,2] Richard J. Fluck,[1] Nicholas M. Selby.[1,2] *[1]Dept of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]Div of Medical Sciences and Graduate Entry Medicine, Univ of Nottingham, United Kingdom.*

**Background:** The long term sequelae of AKI on renal function and mortality are well reported, but there remains the need for prospective studies to reinforce current evidence, allow determination of risk factors for poor outcome and to ensure that generalisable populations including all severities of AKI are studied.

**Methods:** We report the baseline results from a pilot study to test proposed methodology for a long-term, observational case-control study. Cases (AKI) and controls were identified from a hospital-wide electronic reporting system for AKI. Controls were matched to AKI patients on a 1:1 basis for CKD stage and age ± 5yrs. Renal function and proteinuria were measured at 3 months and will also be measured at 1 and 3 years. Survival data will be tracked to five years.

**Results:** 298 cases and controls were matched successfully with no differences in age (71 ± 11yrs versus 72 ± 12yrs, p=0.48) or baseline CKD stage. Baseline eGFR and serum creatinine were also similar between groups (p=0.22). At hospital discharge and at three months, serum creatinine was significantly higher in the AKI group. At three months, the mean delta eGFR was -4.5 ± 14ml/min in AKI group as compared with +3.4 ± 16ml/min in controls (p<0.001). A significantly greater proportion of patients in the AKI group demonstrated an increase of at least one CKD stage (29.7% versus 6.3%, p<0.001). Proteinuria was more common in the AKI group as compared to controls; in a significant proportion of cases this appeared non-glomerular in origin (as evidenced by a disproportionately low ACR:PCR ratio).

**Conclusions:** AKI is associated with a decline in renal function that persists to at least three months. Proteinuria in AKI patients may reflect renal parenchymal damage at the time of AKI or reflect pre-existing proteinuria that predisposed to AKI. These findings, along with longer term outcomes, will be further examined in a larger study following the successful pilot of this recruitment, consent and matching methodology.

## TH-PO004

**Community Acquired Acute Kidney Injury: Incidence and Outcomes** Robin G. Parry,[1] Aileen Coupe,[1] Anna L. Barton,[2] Angela Susan Mallard,[2] Paul Johnston.[1] *[1]Renal Unit, RCHT, Truro, Cornwall, United Kingdom; [2]Clinical Chemistry, RCHT, Truro, Cornwall, United Kingdom.*

**Background:** Acute Kidney Injury (AKI) is an important health issue. It is described in up to 20% of hospital admissions; however there are is a paucity of data concerning patients who develop AKI within the community (cAKI). We report an 8 month observational study of cAKI with respect to incidence and outcomes.

**Methods:** All General Practice (GP) serum creatinine results that exhibited a change of +/- 26 umol/L, or at least 50% change from previous results, were flagged by the pathology IT system for review to determine if AKI was present.

**Results:** A total of 606 samples were identified as having AKI between April-Nov 2012. 72 were removed as they were either repeat flags during the same AKI episode, did not meet the criteria or were not from GP locations. This left a total of 534 separate AKI episodes, identified on 528 patients. For the same period there were 159,280 samples received on 103,362 patients giving an incidence of 0.3% of samples in 0.5% of patients. AKI stage, age/sex split and follow up data are shown below.

| | AKI Total | Stage 1 | Stage 2 | Stage 3 |
|---|---|---|---|---|
| No of Patients (% of Total) | 534 | 313 (58.6%) | 165 (30.9%) | 56 (10.5%) |
| Female(%) | 51.7 | 52.7 | 49.1 | 53.6 |
| Median Age (years) (range) | 79 (19-102) | 79 (19-99) | 79 (47-102) | 80 (38-94) |
| Repeat creat within 14 days | 59.6% | 50.5% | 70.3% | 78.7% |
| Repeat creat within 28 days | 72.3% | 67.1% | 77.6% | 85.7% |

Of the repeat samples, 64.2% were taken in the community and 16.7% in acute hospital units. Worsening AKI was identified in 9.5% of patients. 28% did not have a repeat sample within 28 days (including 20% of patients who died).

**Conclusions:** The incidence of cAKI was low; however, these patients had a high mortality and high rate of hospital admission.The absence of repeat blood tests within 4 weeks in 28% of patients raises the possibility of under-recognition of the importance of AKI and a need for ongoing education.

## TH-PO005

**Effects of Renin-Angiotensin System Blockers on the Outcomes of AKI** Mark Dominic Uniacke,[1,2] Robert Lewis,[1] Scott Harris,[2] Paul J. Roderick.[2] *[1]Wessex Renal and Transplantation Service, Portsmouth, United Kingdom; [2]Univ of Southampton, United Kingdom.*

**Background:** We studied the effect of Renin-Angiotensin System Blockers (RASB) usage on the clinical features of AKI in hospitalized patients and the effect on outcomes 6 months later in those with and without pre-existing CKD.

**Methods:** A single-center prospective observational study of unselected hospital admissions between Nov. 2009 and Apr. 2011. Patients with AKI were recruited into two groups – Group 1: without pre-existing CKD and Group 2: with pre-existing CKD. Historic records of eGFR were used to identify baseline renal function. AKIN criteria were used to identify cases of AKI. 6 months later, mortality and residual functional loss (eGFR >25% fall from baseline) was assessed. Logistic Regression was used to assess the impact of RASB use on outcomes.

**Results:** 375 patients were recruited: 190 in Group 1 and 185 in Group 2. RASB use at baseline was 52%(n=99) in Group 1 and 74%(n=137) in Group 2. RASB was stopped in 90% and 92% of Groups 1 and 2 respectively at onset of AKI. There was no difference in AKIN staging between those taking and not taking RASB in either group.

In Group 1 those taking RASB at baseline were older, more likely male, and had more comorbidities. RASB had no influence on length of hospital stay (LOS), hospital or 6 month mortality, and no independent effect on recovery of function after adjustment for comorbidity.

In Group 2 there was no age, sex or comorbidity differences between those taking RASB at baseline compared to those who were not. LOS was longer in those not on RASB but there were no differences in hospital or 6 month mortality or recovery of function after adjustment for comorbidity.

After AKI, RASB were restarted in only 77% of Group 1 and 66% of Group 2. A clear clinical indication for continued used of RASB was identified in 33% and 65% of those who did not have them restarted.

**Conclusions:** Taking a RAS blocker prior to AKI does not appear to impact on the severity of AKI or its outcomes. The known clinical benefits of RASB, particularly in people with CKD, are denied to many patients following AKI.

*Funding:* Private Foundation Support

**TH-PO006**

**Community and Hospital Acquired Acute Kidney Injury in the General Hospital Population** Mark Dominic Uniacke,[1,2] Robert Lewis,[1] Scott Harris,[2] Paul J. Roderick.[2] *[1]Wessex Renal and Transplantation Service, Portsmouth, United Kingdom; [2]Univ of Southampton, United Kingdom.*

**Background:** Little is known about the natural history of AKI initiated in the community compared to AKI acquired in hospital. This is a study of hospitalized patients comparing the characteristics, natural history and outcomes of hospital and community acquired AKI.

**Methods:** A single-center prospective observational study of unselected hospital admissions between Nov. 2009 and Apr. 2011. The cohort consisted of two groups who sustained an AKI: Group 1 without pre-existing CKD and Group 2 with pre-existing CKD. Each group was further subdivided into those who had community acquired AKI evident on hospital admission and those who acquired an AKI in hospital. Historic records of eGFR were used to identify baseline renal function. AKIN criteria were used to identify cases of AKI. 6 months later, mortality and residual functional loss (eGFR >25% fall from baseline) was ascertained.

**Results:** 375 patients with AKI were recruited (Group 1 n =190, Group 2 n=185). AKI was community acquired in 62% of cases (n=118) in Group 1 and 39% of cases (n=72) in Group 2. In Group 1 community cases had a higher Charlson Comorbidity Score, were more likely to be medical admissions, to have underlying sepsis and to be of greater severity by AKIN staging. Hospital and 6 month mortality was higher in community cases while hospital acquired cases had a longer length of hospital stay. The odds of death within six months was increased in community AKI (OR 3.5, 95% C.I. 1.135 – 10.6, p=.03) but this was attenuated with adjustment for AKIN stage. No influence was found on recovery of renal function. In Group 2, community acquired AKI had higher AKIN stage and shorter hospital stay than hospital acquired AKI. There were no differences in AKI cause, mortality or in recovery of function.

**Conclusions:** Peak severity by AKIN staging was higher in community acquired AKI. In community cases without pre-existing CKD, sepsis was the most frequent cause of AKI and follow up mortality was higher. Sepsis in the community should be a target for preventive strategies.

*Funding:* Private Foundation Support

**TH-PO007**

**Angiotensin Converting Enzyme Inhibitors/Angiotensin Receptor Blockers and Risk of Contrast Induced Acute Kidney Injury: Systematic Review and Meta-Analysis** Jeffrey Ma,[1] Jesse Heyland,[1] Brenda Hemmelgarn,[1] Pietro Ravani,[1] Neesh I. Pannu,[2] Merril Knudtson,[3] Matthew T. James.[1] *[1]Div of Nephrology, Univ of Calgary, Calgary, Canada; [2]Div of Nephrology, Univ of Alberta, Edmonton, Canada; [3]Libin Cardiovascular Institute, Univ of Calgary, Calgary, Canada.*

**Background:** Contrast induced acute kidney injury (CI-AKI) is associated with adverse short and long-term outcomes. The effect of concurrent use of angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARB) on the risk of CI-AKI is uncertain. The purpose of this study is to determine the risk of CI-AKI associated with ACE-I/ARB use at the time of coronary angiography.

**Methods:** Electronic databases (MEDLINE and Embase from inception to September 2011) were searched for eligible studies. Two reviewers independently identified eligible articles and extracted data from randomized controlled trials (RCTs) and cohort studies. The random effects model of DerSimonian and Laird was used to obtain pooled estimates.

**Results:** A total of eight studies (5 RCTs and 3 cohort studies) including 1699 patients undergoing coronary angiography were identified. The mean age of participants ranged from 53.6 to 71.8 years, and mean baseline serum creatinine ranged from 80.0-141.6 umol/L. Among 5 RCTs identified, allocation concealment and blinding were reported in only one trial, and none reported an intention-to-treat analysis. All 3 cohort studies reported results following multivariable adjustment. Significant differences in the risk of CI-AKI associated with ACE-I/ARB use were identified based on study design (meta-regression p=0.04). Based on pooled results from cohort studies, ACE-I/ARB use was associated with an increased risk of CI-AKI (Relative Risk [RR] 2.84, 95% CI 1.71 – 4.69, $I^2$ 0%). Results from RCTs were heterogeneous, with no significant effect of ACE-I/ARB use on CI-AKI (Pooled RR 0.82, 95% CI 0.33 – 2.02, $I^2$ 48.9%).

**Conclusions:** Estimates of the risk of CI-AKI associated with concurrent use of ACE-I/ARB use differ significantly by study design, suggesting confounding effects among observational studies. The effects of ACE-I/ARB use on the risk of CI-AKI remain uncertain.

**TH-PO008**

**Albumin for Fluid Resuscitation Is Independently Associated with Acute Kidney Injury in Patients Undergoing Cardiac Surgery** Josee Bouchard,[1] Anne Julie Frenette,[2] Pascaline Bernier,[2] Annie Charbonneau,[2] Jean-Philippe Rioux,[1] David R. Williamson,[2] Stephan Troyanov.[1] *[1]Medicine, Hôpital du Sacré-Coeur de Montréal, Canada; [2]Pharmacy, Hôpital du Sacré-Coeur de Montréal, Canada.*

**Background:** The risk of acute kidney injury (AKI) with the use of albumin-containing fluids in the intensive care setting is not well characterized. We evaluated the risk of AKI associated with albumin (5 and/or 25%) following cardiac surgery.

**Methods:** We performed a retrospective cohort study including patients undergoing cardiac surgery in a tertiary care center from 2008 to 2010. AKI was defined by the RIFLE creatinine criterion. We performed a multivariate logistic regression (LR) to predict AKI and a propensity score predicting the likelihood to receive albumin. Our LR model included reduced left ventricular ejection fraction (LVEF), duration of extracorporeal circulation (ECC), hemodynamic instability, and use of pentastarch (HES 10%), red blood cells (RBC) or albumin.

**Results:** Our cohort included 984 patients, with a majority of Caucasian men, and a baseline eGFR of 72±19 mL/min. Twenty-three percent had a reduced LVEF and 23% underwent valvular surgery. Sixteen percent of patients received albumin, with a median dose of 1.4 g/kg (IQR 1.1-2.2). The incidence of AKI was 5.3% and was independently associated with a reduced LVEF, duration of ECC, hemodynamic instability, use of albumin and HES 10%, and transfusion of RBCs. In our model, albumin administration was associated with the highest odds ratio (OR), 2.43 (95% CI 1.28-4.61). We derived a propensity score, matching 141 cases to 141 controls. In this analysis, albumin was still associated with an increased AKI risk (OR 1.94 95% CI 1.21-3.11). The risk for AKI was higher as the albumin dose increased.

**Conclusions:** In our study, albumin administration was an independent risk factor of AKI after multivariable adjustment and remained significant in a propensity score model. We also identified a dose-relationship between albumin administration and the risk for AKI. Future studies should address the safety of albumin-containing fluids on kidney function in patients undergoing cardiac surgery.

**TH-PO009**

**Assessment of Nephrotoxic Medications in ICU Patients with Acute Kidney Injury** Kyle Gillis, Linda Awdishu, Ravindra L. Mehta. *Medicine, UCSD Medical Center, San Diego, CA.*

**Background:** AKI complicates up to 25% of ICU admissions and is associated with a high hospital and long-term mortality and adverse outcomes. Critically ill patients are exposed to multiple medications that can contribute to drug induced kidney injury. Improperly dosed medications may contribute to nephrotoxicity and further worsen underlying kidney function. We evaluated the prevalence of nephrotoxic agent exposure to test the hypothesis that nephrotoxic agent exposure would be present in greater than 25% of ICU patients who develop AKI.

**Methods:** We reviewed medical records of 94 patients enrolled in a prospective study of patients who developed AKI within the first 7 days of ICU admission. We examined exposure of nephrotoxic agents classified as antibiotics, NSAIDS, chemotherapy, ACE/ARB, immunosuppressive medications and contrast agents. Outcomes included AKI severity stage (AKIN criteria), duration, renal functional recovery and mortality.

**Results:** Nephrotoxic agent exposure was seen in > 65% of patients with over 25.27% of patients receiving multiple agents. Nephrotoxic antibiotics (58%), immunosuppressive agents (19%) and ACE/ARB agents (18%) were the commonest contributors. Patients with nephrotoxic exposure had a greater dialysis requirement, higher hospital mortality and were more likely to be dialysis dependent at hospital discharge. Patients with underlying CKD had similar hospital outcomes as those with de novo AKI. A multivariate regression model showed renal recovery was predicted by AKI severity stage and nephrotoxic exposure.

**Conclusions:** Nephrotoxic agents are important contributors to the development and resolution of AKI in critically ill patients. Additional studies are required to determine the susceptibility for drug induced kidney injury and establish the best techniques for medication dose adjustment.

*Funding:* NIDDK Support

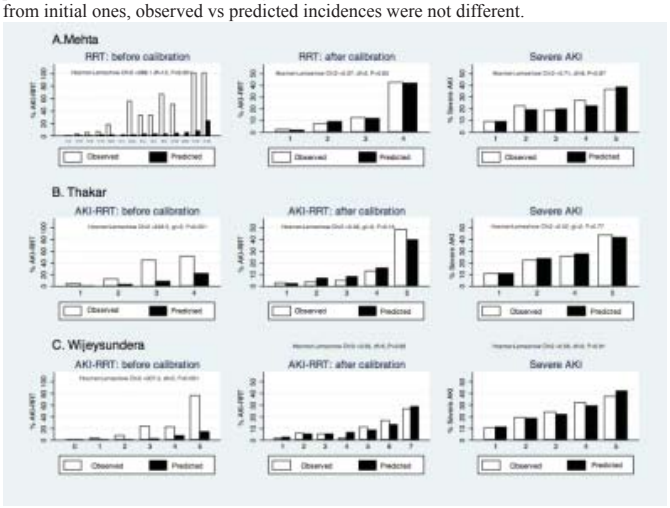
**TH-PO010**

**Validation of Three Clinical Scores to Predict Renal Replacement Therapy and Severe Acute Kidney after Cardiac Surgery** Cesar Flores-Gama, Maribel Merino, Armando Vazquez-Rangel. *Nephrology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico.*

**Background:** Different scores to predict acute kidney injury requiring renal replacement therapy (AKI-RRT) after cardiac surgery (CS) have been developed, different criteria for starting RRT suggest that predicting severe AKI could be more useful. The aim of this study was to validate three clinical scores (Mehta, Thakar and Wijeysundera) to predict AKI-RRT and severe AKI.

**Methods:** Prospectively all patients over 17 years old undergoing CS with cardiopulmonary bypass at the Instituto Nacional de Cardiología Ignacio Chávez in Mexico City from March 2010 to June 2012 were included. RRT onset was not standardized. Severe AKI was defined as KDIGO stage ≥2 using urinary output and SCr criteria. Discrimination analysis was described using area under curve (AUC), and Hosmer-Lemeshow statistic. For calibration, logistic regression models were developed.

**Results:** A total of 752 patients (55 [42-65] years,eGFR 87±22 ml/min/1.73 m$^2$) were analyzed for Thakar and Wijeysundera scores, only 499 patients with valvular and/or coronary arterial bypass surgeries for Metha score. Forty five percent patients developed AKI, 16% severe AKI and 6.9% requiring RRT. AUCs for RRT for Mehta, Thakar and Wijeysundera scores were 0.85(0.78-0.92), 0.75(0.66-0.83) and 0.77(0.70-0.83) respectively. For severe AKI AUCs were 0.68, 0.64 and 0.66 respectively. Observed vs predicted incidences differ from original articles. After calibration, AUCs were not different from initial ones, observed vs predicted incidences were not different.




**Conclusions:** Clinical scores evaluated tend to underestimate the risk of RRT and severe AKI in our cohort. Result can be explained by a higher prevalence of RRT and severe AKI in our cohort, due to differences between cohorts. It is advisable to evaluate and calibrate scores in any population where they are meant to be used.

## TH-PO011

### Risk Factors for Acute Kidney Injury in Mechanically Ventilated Patients

Raul Lombardi,[1] Nicolas Nin,[2] Alejandro Ferreiro.[3] *[1]Dept of Critical Care Medicine, SMI, Montevideo, Uruguay; [2]CIBERES, Hospital Universitario de Torrejon, Madrid, Spain; [3]Dept of Nephrology, CASMU, Montevideo, Uruguay.*

**Background:** Few clinical studies have explored risk factors (RF) for AKI in patients under mechanical ventilation (MV). Therefore we carried out a study with the hypothesis that respiratory and ventilators variables, among others, are implicated in AKI in this setting.

**Methods:** The database of the 3th International Study of MV was used. Inclusion criteria: MV >24 hrs; two or more serum creatinine (SCr); a SCr ≤1.2 mg/dl at start of MV. AKI was diagnosed according to AKIN criteria. Variables known to be associated to AKI were included for the analysis. The study was divided in two periods: EARLY: first 48 hrs of MV; and LATE: day 5 to day 7. Accordingly, early AKI ($AKI_E$) and late AKI ($AKI_L$) were investigated. Demographic, reason for MV, physiological variables and ventilator parameters were recorded. Continuous variables are presented as mean (SD) or median IQR. Categorical variables as proportions. Comparison between groups was made by *U* Mann-Whitney or *t* test, or Fisher Exact test where appropriate. RF were assessed by a conditional logistic regression model. Variables with p<0.10 were retained in the model.

**Results:** 3520 pts were included. Mean age 58.4 (17.7). Male 58.5%. SAPS II 40 (30,52). GCS 10 (5,15). Main reasons for MV: coma and postoperative (PO). ICU mortality 21.8%. Total cases of AKI 699 (19.8%), $AKI_E$ 575 and $AKI_L$ 124. $AKI_E$ pts were older: 61.5 (17.2) vs. 58.7 (19.1); with more frequent PO reason for MV and less frequent pneumonia and coma; also higher proportion of cardiovascular (CV) SOFA ≥3; greater fluid balance; lower pH at admission. $AKI_E$ was independently associated to age, SAPS II, COPD, PO and cardiac arrest as reason for MV, CV SOFA, $Pa/FiO_2$, peak pressure, VT/kg, pH, SCr, platelet count at admission and 24 hrs fluid balance. $AKI_L$ to pneumonia pre-ICU, and CV SOFA, pH, Pa/FiO2, PEEP, plateau and platelet count all of them 24 hrs preAKI.

**Conclusions:** AKI is frequent in MV and the RF pattern is different according to the onset of AKI. $AKI_E$ was related to age, severity of illness, MODS at admission and injurious MV, while $AKI_L$ to MODS and injurious MV in ICU but not at admission.

## TH-PO012

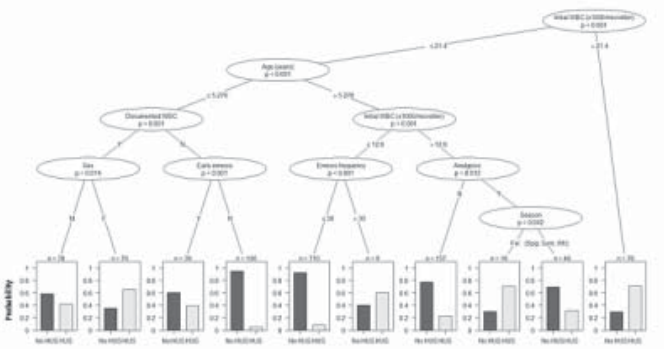
### Early Predictors of Hemolytic Uremic Syndrome (HUS) in Patients with Shiga Toxin Producing *Escherichia coli* (STEC) O157 Infection

Von Nguyen, Magdalena Kendall, Rajal K. Mody. *Centers for Disease Control and Prevention, Atlanta, GA.*

**Background:** Most studies examining risk factors for STEC-related HUS focus on children; information applicable to patients of all ages is limited.

**Methods:** We assessed 46 candidate predictors of HUS among a cohort of patients of all age groups with STEC O157 infection from 10 states during 2006–10. Confirmed HUS was defined as anemia, thrombocytopenia, serum creatinine ≥1.0 mg/dL if <13 years old ( ≥1.5 mg/dL if ≥13), and schistocytes. Probable HUS met the first 3 criteria. We built a classification tree for HUS using recursive partitioning; splitting criterion was the level of Bonferroni-adjusted significance of a permutation-based test.

**Results:** Among 1315 patients, 137 (10%) developed HUS (102 confirmed). The tree separated patients into 10 groups. Predictors identified were, in order, initial white blood cell count (WBC) (>21,400 cells/μL regardless of age and >12,800 cells/μL in patients >5.2 years old) and age ≤5.2 years. Among patients ≤5.2 years old, other predictors were any documented WBC, female sex, and emesis during the first 3 days of illness. Among patients >5.2 years old, other predictors were >30 emesis episodes in 24h, use of non-prescription analgesics, and illness during September–November. The probability of HUS varied from 6–71% across groups. The tree's sensitivity was 54%; specificity was 91%.



**Conclusions:** Distinct clinical profiles which varied by age, were associated with risk of HUS in STEC O157 infection. Profiles may help guide early clinical management of STEC O157 infection.

*Funding:* Other U.S. Government Support

## TH-PO013

### The Incidence and Risk Factors of Kidney Injury after Hepatobiliary Surgery

Won-Yong Cho,[1] So-Young Lee,[2] Eunjung Cho,[1] Myung-Gyu Kim,[1] Sang-Kyung Jo,[1] Hyoung-Kyu Kim.[1] *[1]Korea Univ Anam Hospital, Republic of Korea; [2]Eulji Univ Hospital, Republic of Korea.*

**Background:** Although intraperitoneal procedure is known to be one of major operations associated with postoperative acute kidney injury (AKI), the incidence, risk factors or its long-term renal outcome have not been well known. This study aimed to determine the risk factors and six month renal outcome after hepatobiliary surgery along with evaluating the role of urine neutrophil gelatinase-associated lipocalin (NGAL) in early detection of AKI.

**Methods:** Data were collected from 135 consecutive liver surgeries. Urine samples for NGAL determination were collected before, 6, 12, and 24 hr after surgery. AKI was defined according to the AKIN criteria. Outcome was defined as the decrease of eGFR at 6 month after surgery.

**Results:** AKI was developed in 9% after hepatobiliary surgery. Renal replacement therapy was required in only one patient. Urine NGAL at 12 hr post surgery was most predictive for the development of AKI (AUROC 0.769, p=0.003). Subclinical AKI (urine NGAL+, AKIN-) was diagnosed by the highest urine NGAL level (cutoff value, 92.85 ng/mL) during the first 24 hr after surgery, and additional 42 patients were included in AKI group. AKI group showed higher level of urine NGAL at all time points, SOFA score, prothrombin time (INR), lower albumin, and had longer operation time than those of non-AKI group. Older age and higher preoperative SOFA score were risk factors for the development of postoperative AKI. AKI group had longer hospital stay after surgery, but there was no difference in in-hospital mortality. AKI group showed significantly decreased eGFR at 6 month after surgery. Liver transplantation, baseline eGFR, maximum value of urine NGAL, and intraoperative fluid balance were associated with the decrease of 6 month eGFR.

**Conclusions:** In hepatobiliary surgery, urine NGAL was useful to diagnose AKI early and to predict 6 month renal outcome. To assess the impact of postop AKI or subclinical AKI on progressive decline of renal function, longer term follow up is needed.

## TH-PO014

### Acute Kidney Injury – What Happens after Discharge?

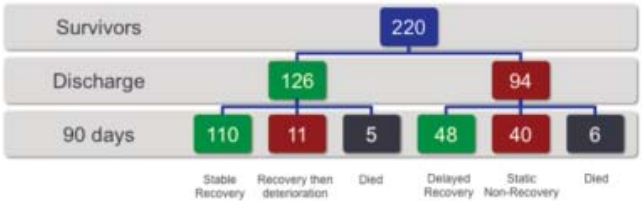
Simon Sawhney, Nick Fluck. *Renal Unit, Aberdeen Royal Infirmary, United Kingdom.*

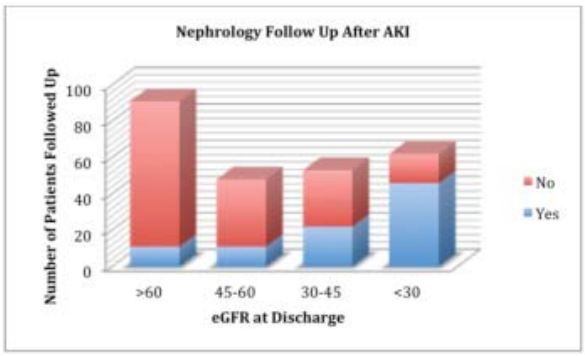
**Background:** Acute Kidney Injury (AKI) affects 1 in 5 hospital admissions. Mortality is high, but most survive to discharge although some recover incompletely. Severe AKI is linked with future CKD and mortality. KDIGO guidelines recommend outpatient surveillance. In practice, there is little data, but one study suggested as few as 10% receive outpatient renal review.

**Methods:** We audited outpatient referral in Northeast Scotland (population 500,000) over 6 months (July 2011-January 2012). Data collected included demographics, clinical context, renal function, dialysis, renal recovery (UK Renal Association criteria), mortality and follow-up.

**Results:** 333 referred patients had severe AKI with 26% hospital mortality. Of survivors, 58.1% recovered to within 20% baseline at discharge. Half of those with incomplete recovery improved over 90 days. Of hospital survivors, 36.8% received outpatient renal review. This was greater in young patients, those with incomplete recovery or renal impairment at discharge(all p 0.000). Follow up was not related to AKI severity, acute dialysis or ITU stay.

**Conclusions:** Only a minority of AKI is followed up. Nephrologists seem to prioritize by renal function but not AKI severity. Future studies should assess whether practice is consistent and supported by prognostic models.




| Follow Up | Yes | No | P-value |
|---|---|---|---|
| Baseline eGFR | 56.2 | 67.9 | 0.005$ |
| Discharge eGFR | 35.1 | 65.3 | 0.000$ |
| 3 Month eGFR | 47.4 | 71.0 | 0.000$ |
| | | | |
| AKIN1 | 35(51%) | 24(49%) | |
| AKIN2 | 11(25.5%) | 32(74.5%) | |
| AKIN3 | 52(35.3%) | 95(64.7%) | 0.13* |
| RRT | 11(37.9%) | 18(62.1%) | 0.895* |
| ITU | 3(33.3%) | 6(66.7%) | 0.818* |
| | | | |
| Recovery at Discharge | 33(25%) | 99(75%) | |
| Not Recovered | 49(53.3%) | 43(46.7%) | 0.000* |
| | | | |
| Recovery at 3 Months | 47(30.5%) | 107(69.5%) | |
| Not Recovered | 31(62%) | 19(38%) | 0.000* |
| *$ANOVA *Chi-Square* | | | |

## TH-PO015

**Hantavirus Infection – Acute Course of the Disease and Long-Term Outcome in 456 Patients from Southern Germany** Joerg Latus, Mark Dominik Alscher, Martin Kimmel, Niko Braun. *Nephrology, Robert- Bosch Hospital, Stuttgart, Germany.*

**Background:** Puumala virus (PUUV) is the most common Hantavirus species in Europe. Nephropathia Epidemica (NE), caused by PUUV, is characterized by acute renal failure (AKI) with thrombocytopenia. Data to predict a severe course of the disease, including acute course and and long-term outcome, are missing.

**Methods:** In cooperation with the local health authorities of the region, all patients with serologically confirmed NE between 2001 and 2012 were contacted (n = 1570). Between September 2012 and April 2013, altogether 456 patients were included in the study. Clinical and laboratory data were collected retrospectively from medical reports, a biobank, including blood samples, tissue and urine, was installed. During follow-up visit at our outpatient clinic, a careful PE, blood pressure measurement and lab investigations were done. During acute course, severe AKI was defined as RIFLE I and F.Long-term outcome focused on proteinuria and hypertension.

**Results:** Regarding renal dysfunction, serum levels of creatinine were elevated in 72%. 86% of the patients had AKI based on RIFLE classification. Abdominal pain, hematuria and proteinuria showed statistically significant ($p<0.01$) odds ratios (OR) (standardized OR=2.3 (95% CI, 1.4-3.7), OR=2.7 (95% CI,1.5-4.9) and OR=2.8 (95% CI,1.5-5.2) concerning severe AKI. Remarkably, neither platelets, hemorrhage, gender and age nor splenomegaly, hepatomegaly or pathological findings using x-ray were predictors for severity of AKI. None of the clinical symptoms or laboratory findings during acute course of the disease were predictors for hypertension at time of follow-up (median follow-up 17 (7-35 months). Hematuria and C-reactive protein levels during acute course were strong predictors for proteinuria within the long-term follow-up ($p<0.001$, OR=6.4 (95% CI, 3.5-12) and OR=1.3 (95% CI, 1.2-1.5).

**Conclusions:** Risk factors for a severe course of the disease were abdominal pain, proteinuria and hematuria. Regarding long-term outcome, development of hypertension seems to be independent of the severity of the acute disease whereas hematuria and the inflammatory response were strong predictors of proteinuria.

## TH-PO016

**Incidence, Risk Factors, and Outcomes of Pregnancy-Related Acute Kidney Injury Treated with Dialysis** Ainslie M. Hildebrand,[1] Kuan Liu,[2] Salimah Z. Shariff,[2] Joel G. Ray,[3] Michelle A. Hladunewich,[4] William F. Clark,[1] Amit X. Garg.[1,2] *[1]Div of Nephrology, Western Univ, London, Canada; [2]Institute for Clinical Evaluative Sciences at Western, London, Canada; [3]Dept of Medicine, Univ of Toronto, Toronto, Canada; [4]Div of Nephrology, Univ of Toronto, Toronto, Canada.*

**Background:** Acute kidney injury is a rare complication of pregnancy, but may be associated with significant morbidity and mortality in young and often otherwise healthy women. As renal dysfunction in pregnancy may be missed or misclassified using serum creatinine based definitions of acute kidney injury, we report the incidence of acute kidney injury treated with dialysis and describe the associated risk factors and maternal and fetal outcomes.

**Methods:** We used data from universal health care databases to study all consecutive pregnancies in Ontario over a 15-year period. Maternal outcomes included acute dialysis during the peripartum period, 90-day mortality, and dialysis dependence among women who survived beyond 90 days. Fetal outcomes included low birthweight, small for gestational age or intrauterine growth restriction, premature birth, stillbirth, neonatal death, and perinatal mortality.

**Results:** There were a total of 1,918,789 pregnancies in Ontario between 1997 and 2011. The cumulative incidence of acute dialysis during pregnancy was 1:10,000 over the study period. Among women who received acute dialysis, 4.3% died within the 90-day postpartum period and 3.9% of survivors were dialysis dependent. Adverse fetal outcomes associated with acute kidney injury treated with dialysis during pregnancy included low birthweight (unadjusted relative risk [RR] 4.7, 95% confidence interval [CI] 3.6 – 6.0), small for gestational age (RR 3.2, 95% CI 1.9 – 5.3), and premature birth (RR 2.5, 95% CI 2.0 – 3.1).

**Conclusions:** The incidence of acute kidney injury treated with dialysis is low, but adverse maternal and fetal outcomes exist. These results provide important prognostic information for patients and reinforce the role of the nephrologist in pre-conception counseling, risk factor reduction, and monitoring during pregnancy for women at risk.

## TH-PO017

**Iloprost: As an AKI Triggering Agent in Severe Atherosclerotic Patients** Mehtap Erkmen Uyar,[1] Piril Yucel,[2] Zeynep Bal,[1] Saliha Yildirim,[2] Emre Tutal,[1] Tankut Akay,[3] Siren Sezer.[1] *[1]Dept of Nephrology, Baskent Univ Medical School, Ankara, Turkey; [2]Dept of Internal Medicine, Baskent Univ Medical School, Ankara, Turkey; [3]Dept of Cardiovascular Surgery, Baskent Univ Medical School, Ankara, Turkey.*

**Background:** Iloprost, a stable prostacyclin analog, is used as a rescue therapy for severe peripheral arterial disease (PAD). Prostacyclin has important effects on microvascular blood flow, inhibition of platelet aggregation, leucocyte-vessel interaction and it is used frequently in the treatment of obstructive PAD. It has systemic vasodilatation and antiaggregant influence while severe vasodilatation might cause organ ischemia when severe atherosclerosis is the underlying cause. In this study we retrospectively analysed the renal outcome after iloprost infusion therapy in 87 patients.

**Methods:** 87 patients with PAD who received iloprost infusion with 1 ng/kg/min dosage for the last 6 months were retrospectively analyzed. All patients' clinical and biochemical parameters before (baseline, Cr1), during (third day, Cr2) and after (2 weeks after infusion, Cr3) the iloprost treatment were recorded. Acute kidney injury (AKI) was defined as ≥0.3 mg/dL increase in creatinine levels from baseline within 48 hours according to KDIGO guidelines.

**Results:** C2 (1.46±0.1 mg/dL) and C3 (1.53±0.12 mg/dL) levels were significantly higher from baseline Cr (1.15±0.6 mg/dL) values. AKI was observed in 36 patients (41.37%) on the third day of iloprost infusion. Binary logistic regression analysis revealed that smoking and no ASA use were the primary predictors (p: 0.02 and p:0.008 respectively) of acute kidney injury during iloprost treatment. In the third day of the infusion urinary output of patients was significantly increased from the initiation of therapy (1813.30±1123.46 cc vs. 1545.17±873.00 cc). 74.14±9.42 mm Hg vs. 70.07±15.50 mm Hg.

**Conclusions:** Even though the iloprost treatment is effective in peripheral arterial disease patients who are not suitable for surgery, severe systemic vasodilatation might cause renal ischemia ending up with non-oliguric acute kidney injury. Smoking, no ASA use and lower diastolic BP are the clinical risk factors for AKI during iloprost treatment.

## TH-PO018

**Low 25-Hydroxyvitamin D Level at Continuous Renal Replacement Therapy Initiation Predicts In-Hospital Mortality** Kyung Don Yoo, Hajeong Lee, Seung Seok Han, Jung Nam An, Yon Su Kim, Dong Ki Kim. *Internal Medicine, Seoul National Univ Hospital, Seoul, Jongno-gu, Korea.*

**Background:** The low 25-hydroxyvitamin D (25(OH)D) level has been known to be associated with the development and prognosis of acute kidney injury (AKI) in critically ill patients. However, the association between serum 25(OH)D level and outcome of AKI remains to be clarified in patients initiating continuous renal replacement therapy (CRRT).

**Methods:** This prospective observational cohort study included patients with severe AKI requiring CRRT, from Nov. 2011 to Nov. 2012. Patients with end-stage renal disease were excluded. Biochemical data including 25(OH)D were measured at the time of CRRT initiation. Patients with 25(OH)D level below the median were included in the Low group

and those with 25(OH)D level above the median were included in the High group. The primary outcome was in-hospital mortality, and the secondary outcome was duration of ventilator support and RRT.

**Results:** A total of 131 patients were analyzed. The mean age was 66.3 years and male patients were 73 (55.7 %). The mean APACHE II score was 29.18 ± 8.07. The median of serum 25(OH)D level was 6.90 ng/ml , and the mean serum 25(OH)D level were 3.97 ± 1.56 ng/mL and 12.47 ± 5.48 ng/mL in the Low group and the High group, respectively. There was no significant difference in baseline clinical characteristics including causes of AKI, Charlson comorbidity index between the groups. There was no significant difference in ventilator free days and RRT free days between two groups. However, the mortality rate was significantly higher in the Low group than in the High group (86.6 vs. 71.9 %, p=0.038). In survival analysis, low 25(OH)D level was proved to be an independent risk factor for in-hospital mortality after adjustment for by age, sex, mean arterial pressure, APACHE II ,SOFA score, c-reactive protein, hemoglobin, BUN and serum albumin (Hazard Ratio 1.63, 95% confidence interval 1.08–2.45;P=0.018).

**Conclusions:** In this study, we demonstrated that critically ill patients with AKI requiring CRRT showed severe 25(OH)D deficiency. Moreover, low 25(OH)D levelat the time of CRRT initiation might be associated with higher in-hospital mortality.

## TH-PO019

**Mean Platelet Volume Is a Prognostic Factor in Patients with Acute Kidney Injury Requiring Continuous Renal Replacement Therapy** Ji Suk Han,[1] Ji Sun Paeng,[2] Hye-Young Kang,[2] Sung Jin Moon,[3] Tae-Hyun Yoo,[1,2] Shin-Wook Kang,[1,2] Dae-Suk Han,[1] Hyung Jung Oh.[1] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul; [3]College of Medicine, Kwandong Univ, Gyeonggi-do, Korea.*

**Background:** Increased platelet size has been demonstrated to reflect platelet activity and to predict clinical outcome in patients with cardiovascular disease. However, the prognostic value of platelet size for mortality has never been explored in patients with acute kidney injury (AKI).

**Methods:** This study investigated the relationship between platelet size and mortality in 361 AKI patients who received continuous renal replacement therapy (CRRT) in an intensive care unit between August 2009 and October 2011. We retrospectively collected the data for mean platelet volume (MPV) representing platelet size, platelet count, and other biochemical parameters at the time of CRRT initiation. Kaplan-Meier (K-M) plots were constructed to determine and to compare 28-day mortality rates and multivariate Cox analyses were conducted to elucidate the independent prognostic value of MPV for mortality.

**Results:** The mean age was 61.1 years and 224 patients (62.0%) were male. The median MPV was 0.12 fL. At the start of CRRT, MPV was negatively correlated with platelet count ($r = -0.315$, $P < 0.001$), whereas it was positively associated with APACHE II score ($r = 0.135$, $P = 0.010$). During the study period, 239 deaths (66.2%) occurred. K-M curve revealed that all-cause mortality was significantly higher in patients with MPV ≥ 0.12 fL compared to those with MPV < 0.12 fL ($P < 0.001$). In addition, Cox regression analysis found that MPV was an independent predictor for mortality after adjustment of age, gender, platelet count, serum albumin, APACHE II score, and the cause of AKI (HR, 1.093; 95% CI, 1.023-1.167; $P = 0.008$).

**Conclusions:** MPV at the time of CRRT initiation might be a simple and useful predictor for mortality in AKI patients requiring CRRT.

## TH-PO020

**Minimal Changes in Postoperative Creatinine Values for Prediction of Mortality and Cardiovascular Events after CABG** Daniel Olsson, Marcus Liotta, Ulrik Sartipy, Martin Holzmann. *Karolinska Institutet, Stockholm, Sweden.*

**Background:** Acute kidney injury (AKI) is related to an increased risk of long-term mortality, end-stage renal disease and heart failure in patients with coronary heart disease. Coronary artery bypass grafting (CABG) is one of the most common surgeries and performed as often as 1.000.000 times annually world-wide. CABG is associated with a 5-30% risk of developing AKI The aim was to investigate the prognostic value of small, subclinical increases in postoperative serum creatinine (SCr), in patients undergoing CABG.

**Methods:** From the SWEDEHEART registry all 25.686 patients who underwent primary, isolated, non-emergent CABG in Sweden between 2000 and 2008 were included. Postoperative absolute SCr changes were categorized into 3 stages: stage 1, 0-0.3 mg/dL; stage 2, 0.3-0.5 mg/dL and stage 3, > 0.5 mg/dL. No AKI (reference group) was defined as no increase or decrease in postoperative SCr. Hazard ratios were calculated using logistic and Cox proportional hazard regression for 30-day mortality, long-term mortality and a composite of death, myocardial infarction, stroke and heart failure by AKI stage.

**Results:** During a mean follow-up of 4.5 years there were 4.350 (17%) deaths and 7.095 (28%) hospitalizations for MI, stroke, heart failure or death combined. The adjusted odds ratios with 95% confidence intervals (CI) in AKI stages 1, 2 and 3 for 30-day mortality were 1.37 (0.84-2.21), 3.64 (2.07-6.38) and 15.4 (9.98-23.9), respectively. The adjusted hazard ratios for long-term mortality in AKI stages 1, 2 and 3 were 1.07 (95% CI 1.00 to 1.15), 1.33 (95% CI 1.19 to 1.48) and 2.11 (95% CI 1.92 to 2.32), respectively. The corresponding results for the composite end-point were 1.09 (95% CI 1.03-1.15), 1.39 (95% CI 1.29-1.52) and 1.99 (95% CI 1.84-2.16), respectively.

**Conclusions:** Already subclinical increases in postoperative creatinine values are related to long-term mortality and cardiovascular events in patients undergoing CABG.

## TH-PO021

**Impact of Acute Kidney Injury Requiring Renal Replacement Therapy on Mortality and Renal Survival** Silvia Gonzalez S, Pedro J. Labrador, Jesús P. Marin, Clarencio Javier Cebrian Andrada, Maria C. Jimenez, Ines Castellano, Sandra Gallego, Vanesa Garcia-Bernalt, Juan R. Gómez-Martino. *Nephrology, San Pedro de Alcantara Hospital, Caceres, Spain.*

**Background:** The aim was to assess characteristics, renal survival and mortality of patients who developed acute kidney injury (AKI) stage 3, according to KDIGO guidelines, and needed renal replacement therapy (RRT).

**Methods:** All patients who required RRT due to AKI stage 3 along one year were included, excluding patients in intensive care unit. Demographic and personal history data, previous renal function, cause of AKI, renal function, renal survival, and mortality at one, three and six months after AKI were recorded.

**Results:** A total of 60 patients were enrolled (incidence 150 patients/$10^6$ population/year). Mean age 73.6±13.6 (range 25-91), 53.3% men. Patient's characteristics: 80% hypertensive, 32% diabetics, 42% dyslipemics, 43% obese, 23% smokers, and 58% with chronic renal failure (28.1% stage 3, 21.1% stage 4, and 8.8% stage 5). Cause of AKI: renal disease 60%, prerenal 32%, obstructive causes 8%.

Renal function (RF): Serum creatinine before AKI 1.78±1.2mg/dL; maximum serum creatinine during AKI hospitalization 7.48±4.04mg/dL; at discharge, 2.88±1.63mg/dL; one month later, 2.25±1.49mg/dL; three months later, 2.62±1.73mg/dL; and six months later, 2.53±2.09mg/dL.

During hospitalization, 20% died, 15% kept on RRT at discharge, and 65% recovered partial or completely RF. One month after AKI, 28.3% had die, 11.7% kept on RRT, and 60% preserved RF. Three months later, 41.7% died, 13.3% kept on RRT, and 45% preserved RF, 6.7% was missing. Six months later, 48.3% had died, 10% kept on RRT, 33.3% preserved RF, 8.3% were missing.

Previous RF according to KDOQI classification of patients who survived six months after AKI, was: stage 1 4.8%, 2 19%, 3 38.1%, 4 9.5% and 5 28.6%; at discharge, RF was: stage 1 2.5%, 2 7.5%, 3 17.5%, 4 45% and 5 27.5%.

**Conclusions:** In our health area AKI stage 3 requiring RRT has an incidence similar to other studies. Mortality in AKI patients increase in time, near to 50% six months after AKI episode, while renal survival decreases in this period. So nephrology follow-up must be established in these patients.

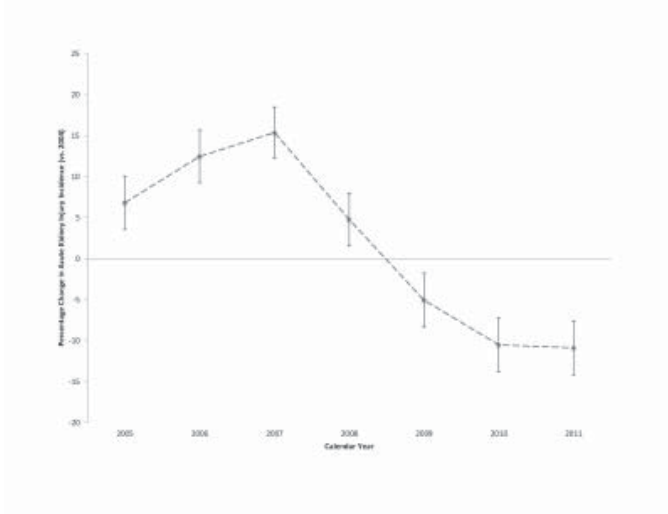
## TH-PO022

**Community-Based Trends in Hospitalized Acute Kidney Injury between 2004-2011: The Kaiser Permanente Experience** Alan S. Go,[1] Sijie Zheng,[2] Chi-Yuan Hsu,[3] Jingrong Yang,[1] Thida Tan,[1] Kathleen D. Liu,[3] Juan Daniel Ordonez.[2] *[1]Div of Research, Kaiser Permanente Northern California, Oakland, CA; [2]Div of Nephrology, Kaiser Oakland Medical Center, Oakland, CA; [3]Div of Nephrology, Univ of California, San Francisco, San Francisco, CA.*

**Background:** Administrative claims data suggest that the incidence of acute kidney injury (AKI) has increased over time, but few data exist about community-based temporal trends in AKI using changes in serum creatinine (SCr).

**Methods:** In Kaiser Permanente Northern California, a large integrated health care delivery system, we identified all hospitalized cases of AKI between 2004-2011 based on AKIN criteria (change in SCr of >=0.3 mg/dL or >50% relative change within 48 hours). We calculated the age-sex-race-adjusted incidence of AKI per year and examined the association of calendar year with AKI incidence using Poisson regression adjusting for age, sex, race, and other risk factors (diabetes, hypertension, GFR, and proteinuria) in the source population.

**Results:** Among 3,910,697 adults, mean age=48 yr; 52% were women, and 40% minorities. Adjusted incidence (per 100,000 person-years, p-y) of AKI increased from 352 in 2004 to 408 in 2007 but then decreased steadily to 322 in 2011, with similar trends in each AKI stage except for dialysis-treated AKI which increased over time (24 to 30 per 100,000 p-y). After adjustment for age, gender, race and other risk factors, compared with 2004, there was a 15% relative increase in 2007 and 11% relative decrease in 2011.



**Conclusions:** In a large, diverse community-based population, incidence of hospitalized AKI increased between 2004 and 2007 but decreased through 2011, which was not explained by population demographic and clinical factors. Studies are needed to evaluate how changes in targeted management practices may explain recent favorable trends in AKI.

*Funding:* NIDDK Support

## TH-PO023

**Urine Output Criteria for Defining Acute Kidney Injury: Association with Length of Stay and 5-Year Mortality in Critically Ill Children**

Rami Ali,[1,2] Jacques R. Lacroix,[2] Veronique Phan,[1] Philippe Jouvet,[1] Jean-Philippe Lafrance,[1] Ronald Gottesman,[2] Shari Ann Segal,[2] Ana Palijan,[2] Michael Pizzi,[2] Mariana Dumitrascu,[1] Nikki J. Rink,[2] Susan M. Samuel,[3] Michael Zappitelli.[2] *[1]U. Montreal, Montreal, Canada; [2]McGill U., Montreal, Canada; [3]U. Calgary, Calgary, Canada.*

**Background:** The relevance of acute kidney injury(AKI) urine output(UO) criteria in children is unclear. We determined impact of UO criteria on AKI incidence and outcome associations.

**Methods:** Retrospective cohort study (chart, administrative data) of children in to 2 intensive cares units(PICU) (excluded:no health number, serum creatinine(SCr), outcome data; dialysis, renal transplant). We compared AKI by Kidney Disease Improving Global Outcomes (KDIGO) SCr(AKI-SCr), UO(AKI-UO) and both(AKIfull) criteria for associations with outcomes: hospital[Hosp-LOS]; PICU length of stay[PICU-LOS] (multiple linear regression); 5-yr mortality(multiple logistic regression). We evaluated cardiac surgery(CS) subgroups (a priori).

**Results:** 850 children were included: (median[interquartile range,IQR]) 4.5[11.3] yrs, 54% male, 16% CS, 8% death, 31% AKI-SCr, 8% AKI-UO, 36% AKIfull. AKI-SCr and AKIfull were associated with Hosp-LOS(AKI-SCr: 14±22 *vs.*Non-AKI: 8 ±9d, p<0.0001) and PICU-LOS(AKI-SCr: 5±7 *vs.*Non-AKI: 2±2d,p<0.0001); in adjusted(adj) analyses, both with p<0.001. AKI-UO was associated with PICU-LOS(4±6d *vs.*3±4d, p<0.001), but less strongly with Hosp-LOS(11±19 *vs.* 9±12d, p<0.05); these remained significant after adjustment. The LOS associations were only significant in non-CS(not shown). All AKI methods predicted 5-yr mortality; this was strongest using AKI-UO in non-CS(Table).

| AKI Definition | All Patients 5-yr Mortality | | Non-CS 5-yr Mortality |
|---|---|---|---|
| | Unadjusted OR (95% CI) | Adjusted OR (95% CI) | Adjusted OR (95% CI) |
| AKI-SCr | 2.7 (1.6-4.4) | 1.7 (1.0-3.1) | 1.8 (1.0-3.3) |
| AKI-UO | 2.5 (1.2-4.9) | 2.5 (1.2-5.3) | 3.0 (1.3-6.7) |
| AKI-full | 2.8 (1.7-4.6) | 1.9 (1.1-3.3) | 2.0 (1.1-3.6) |

Adjustment for age, gender, PRISM score, trauma, neurologic disease, infection and vasopressors.

**Conclusions:** AKI-UO was more strongly associated with 5-yr mortality, but more weakly associated with LOS than AKI-SCr was. Studies should elucidate how this may impact on AKI definition in future research.

## TH-PO024

**Defining Renal Angina for the Prediction of Acute Kidney Injury in Patients with Septic Shock**

David D. Leedahl,[1] Erin N. Frazee,[1] Garrett E. Schramm,[1] Lakhmir S. Chawla,[2] Kianoush Banaei-Kashani.[3] *[1]Pharmacy Services, Mayo Clinic, Rochester, MN; [2]Renal Diseases and Hypertension, George Washington Univ Medical Center, Washington, DC; [3]Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** The concept of renal angina (RA) has been recently proposed to enhance early identification of critically ill patients at high risk for acute kidney injury (AKI). The primary objective of this study was to define RA in a cohort of patients with septic shock.

**Methods:** This was a retrospective analysis of 390 adult patients admitted to the medical intensive care unit (ICU) of a tertiary, academic medical center with septic shock. We collected hourly urine output from septic shock recognition (hour 0) to the earlier of hour 96, urine catheter removal, or ICU discharge. Serum creatinine (SCr) measurements were collected until hour 96. The definition of RA was determined during the first 12 hours of resuscitation by evaluating the initial episode of oliguria, increase from baseline to peak SCr level, and Acute Physiology and Chronic Heath Evaluation (APACHE) III score in a multivariable receiver operator characteristic (ROC) analysis. The primary outcome was incidence of moderate to severe AKI according to Acute Kidney Injury Network (AKIN) criteria. Secondary outcomes included the need for renal replacement therapy and 28-day mortality.

**Results:** Ninety eight (25%) patients developed moderate to severe AKI after septic shock recognition. APACHE III score and SCr level increase in the first 12 hours were not statistically associated with moderate to severe AKI in multivariable ROC analysis. Consecutive oliguria for 3 hours had fair predictive ability for moderate to severe AKI (area under ROC curve = 0.73), and five hours of consecutive oliguria demonstrated optimal accuracy (82%, 95% CI 79%-86%).

**Conclusions:** The novel concept of RA, defined as 3 to 5 hours of consecutive oliguria in septic shock patients, may provide a valuable measure of AKI risk. Further validation to support this definition is needed.

*Funding:* Private Foundation Support

## TH-PO025

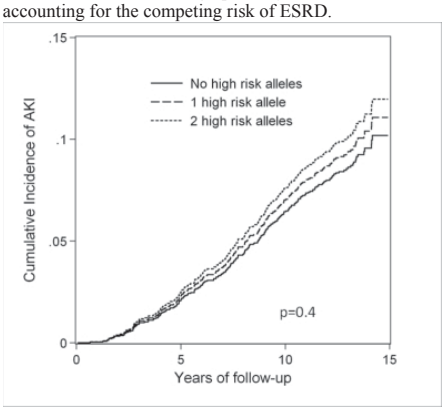
**Racial Disparity in Acute Kidney Injury: The Atherosclerosis Risk in Community Study**

M. Grams, Kunihiro Matsushita, Yingying Sang, Michelle M. Estrella, Adrienne Tin, Meredith C. Foster, Wen Hong Linda Kao, Josef Coresh. *Johns Hopkins Univ.*

**Background:** African-Americans face higher risk of acute kidney injury than Caucasians. The extent to which this increased risk is due to differences in clinical, socioeconomic, or genetic risk factors is not known.

**Methods:** We evaluated 10,589 African-American and Caucasian participants in the Atherosclerosis Risk in Community (ARIC) study, a community-based prospective cohort of middle-aged individuals. Participants were followed from baseline study visit (1996-1999) until first hospitalized AKI (defined by billing code), end-stage renal disease (ESRD), death, or December 31, 2010, whichever came first.

**Results:** African-American participants were slightly younger (61.8 years vs. 63.1 years, p<0.001), more often female (63.9% vs. 53.5%, p<0.001), and had higher baseline eGFR and prevalence of microalbuminuria. Annual family income, presence of health insurance, and education level were lower among African-Americans compared with Caucasians. The unadjusted incidence of hospitalized AKI was 8.1 cases per 1000 person-years among African-Americans and 6.2 cases per 1000 person-years among Caucasians. The elevated risk of AKI persisted after adjustment for demographics, cardiovascular risk factors, kidney markers, and time-varying number of hospitalizations (adjusted hazard ratio (HR) for African-American race: 1.27, 95% CI:1.07-1.50, p=0.007); however, after accounting for differences in income and/or insurance by race, the association was no longer significant. High risk variants of APOL1 were not significantly associated with AKI among African-Americans (demographic-adjusted HR 1.21, 95% CI: 0.80-1.82, p=0.4) even after accounting for the competing risk of ESRD.




**Conclusions:** African-Americans face higher risk of AKI than Caucasians, a difference which may be related to disparities in socioeconomic status.

*Funding:* NIDDK Support

## TH-PO026

**Renal Function Testing and Nephrology Follow-Up after Acute Kidney Injury: Update from a National Sample of Veterans**

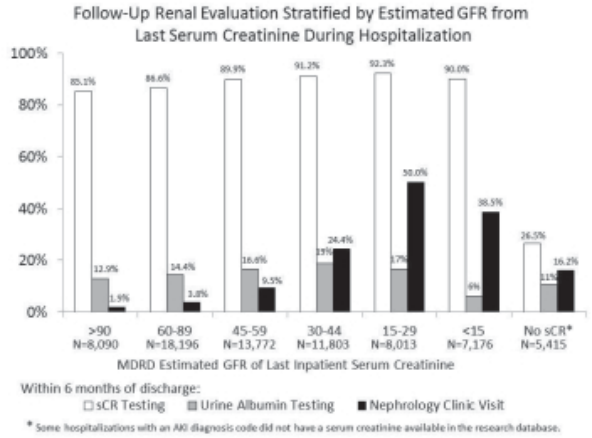
Diane Steffick,[1] Michael Heung,[1] Neil R. Powe,[2] Chi-Yuan Hsu,[2] Meda E. Pavkov,[3] Nilka Rios Burrows,[3] Rajiv Saran.[1] *[1]Univ of Michigan, Ann Arbor; [2]Univ of California San Francisco; [3]Centers for Disease Control and Prevention, Atlanta, GA.*

**Background:** Improving rates of follow-up renal evaluation after acute kidney injury (AKI) has been identified as an objective by the US Healthy People (HP) 2020 initiative. However, renal evaluation is not defined and optimal targets remain unclear. Using 3 different metrics, we look at renal evaluation from a health system perspective.

**Methods:** We examined all hospitalizations in the Veterans Affairs data in 2010 with AKI – either an ICD-9 code or a 0.3mg/dL increase in serum creatinine (sCr) from pre-admission baseline to peak. Hospitalizations where the patient died within 6 months of

discharge were excluded. Post-hospitalization renal evaluation was defined 3 ways: (1) sCr testing, (2) urine albumin testing, and (3) nephrology clinic visit. Post-AKI evaluation was assessed at 6 months and stratified by eGFR from the last inpatient sCr.

**Results:** The study found 72,465 hospitalizations with AKI where the patient was alive at 6 months after discharge. 84% of these hospitalizations had sCr, 14.5% had urine albumin and 17.5% had nephrology visits within 6 months of discharge. 15% of hospitalizations had no patient sCr or urine albumin testing and 82.5% had no nephrology follow-up. Follow-up evaluation increased with decreasing eGFR estimated by the last inpatient sCr (see Figure).




**Conclusions:** The vast majority of US VA patients underwent sCr testing during the 6 months after an AKI hospitalization. The proportion with nephrology follow-up visits was higher among those with poorer kidney function at discharge. Additional studies may determine the impact of renal follow-up on outcomes and cost-effectiveness.

*Funding:* Other U.S. Government Support

## TH-PO027

**Evaluation of a Clinical Risk Assessment Scoring System for Acute Kidney Injury in Critically Ill Patients** Jane S. Kim, Ravindra L. Mehta. *School of Medicine, Univ of California, San Diego, San Diego, CA.*

**Background:** Acute kidney injury (AKI) is a major complication of hospitalization and is an independent risk factor for in-hospital mortality. Because clinical manifestations can vary widely, AKI is under-recognized. Several studies have evaluated risk scores for AKI in specific settings (e.g. cardiac surgery, contrast exposure). However, few studies identified the clinical risk factors for developing AKI in the intensive care unit (ICU). We evaluated the performance of a clinical risk score based on clinical and demographic factors to predict AKI in an ICU population.

**Methods:** Medical records of 327 patients who were admitted to the ICU at UCSD Hillcrest Medical Center and were screened for enrollment in a prospective registry for AKI were reviewed. Patient information within the first 48 hours of ICU admission was recorded, and AKI risk scores were calculated based on the presence of 5 co-morbidities (CKD, liver disease, CHF, hypertension, atherosclerotic CVD) and 5 acute clinical events (arterial pH, nephrotoxin exposure, sepsis, mechanical ventilation, and anemia). Point scores were applied to each variable, and univariate and multivariate analyses were performed to evaluate how well the risk factors in the model could predict AKI. Area under the curve (AUC) and Hosmer-Lemeshow were used to determine the model's discrimination and calibration.

**Results:** AKI developed in 153 (46.8%) of patients, and overall dialysis requirement was 7.3% with an in-hospital mortality of 9.8%. Development of AKI in the ICU was associated with higher scores for the variables included in the AKI risk score. Length of stay, dialysis requirements, and in-hospital mortality were also greater among patients with AKI. Higher scores predicted the development of AKI with an ROC curve with AUC of 0.778 ($p < 0.001$) and with Hosmer-Lemeshow test of $p = 0.334$.

**Conclusions:** A clinical risk score combining chronic co-morbidities and acute events within the first 48 hrs after ICU admission has a good performance in predicting development of AKi in critically ill patients. This score can allow physicians to identify patients at high risk for AKi at ICU admission and implement preventive strategies to improve outcomes.

*Funding:* NIDDK Support

## TH-PO028

**Association between Perioperative Blood Transfusions and Acute Kidney Injury in Patients Undergoing Joint Replacement Surgeries: A Retrospective Cohort Study** Ghanshyam Palamaner Subash Shantha, Ravi Gurusamy, Robert M. Corey, Himanshu Sharma, Jayakrishna Chintanaboina, John E. Prior. *Internal Medicine, The Wright Center For Graduate Medical Education, Scranton, PA.*

**Background:** Blood transfusions (BT) increase risk for perioperative acute kidney injury (AKI) in cardiac surgeries. This association has not been well studied in patients undergoing surgeries that involve minimal hemodynamic changes like joint replacement surgeries. In this retrospective cohort study, we assessed the association between BT and AKI among patients undergoing joint replacement surgeries.

**Methods:** Case records of 425 consecutive adult patients who underwent joint replacement surgeries, in 2 community hospitals in Scranton, Pennsylvania, from January - December 2011 were reviewed. Peri-operative (pre, during and post operative) red cell transfusions (BT) was considered exposure. Acute kidney injury (AKI) was defined by Acute Kidney Injury Network Criteria (AKIN). Cox proportional hazards regression was performed to assess the association between BT (coded as categorical variable) and AKI, after adjusting for age, gender, pre-operative hemoglobin (PH), eGFR, diabetes diagnosis, hypertension diagnosis, NSAIDS use, type of anesthesia, average intraoperative mean arterial pressure (MAP) and the type of joint replaced.

**Results:** The mean age was 68.7 years. The population was 58% male and 8% black. Baseline creatinine was $0.9 \pm 0.3$ mg/dl. Mean PH was $12.1 \pm 1.2$ g/dl. Three hundred and twelve (73%), 97 (23%) and 16 (4%) had knee, hip and shoulder replacements respectively. MAP was $93 \pm 6$ mmhg. Forty seven (11%) developed AKI. AKI developed $24 \pm 6$ hours post surgery. Of these, 39 (83%), 6 (13%) and 2 (4%) had stages 1, 2 and 3 AKI respectively. None of these AKI's were dialysis requiring. Sixty three (15%) received BT. BT was independently associated with AKI (Hazards ratio: 1.39, 95% C.I: 1.07 – 1.62, $P = 0.032$).

**Conclusions:** BT is associated with increased risk of AKI in patients undergoing joint replacement surgeries, independent of MAP and PH. AKI was predominantly stage I and non-dialysis requiring. Randomized trials are required to confirm our observational data.

## TH-PO029

**Association of Vitamin D and Acute Kidney Injury in Patients with Hematological Malignancies: A Retrospective Cohort Study** Ghanshyam Palamaner Subash Shantha,[1] Qasim Malik,[1] Manoj Das,[1] Pavan Kumar Irukulla,[1] Lawrence J. Cheskin.[2] *[1]Hematology/Oncology, The Wright Center For Graduate Medical Education, Scranton, PA; [2]Health Behavior and Society, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

**Background:** Association of vitamin D and acute kidney injury (AKI) in patients with hematological malignancies (HM) has not been well studied. In addition to inflammation, vitamin D deficiency has been associated with increased cell survival and poor outcomes in HM. In this retrospective cohort study, we assessed the association between vitamin D and AKI in HM.

**Methods:** Case records of 346 consecutive adult patients with HM from hematology/oncology divisions of 5 community hospitals in Scranton, Pennsylvania, from 2007 - 2012 were reviewed. Vitamin D levels (25-hydroxy cholecalciferol) at cancer diagnosis were considered exposure. AKI was defined by Acute Kidney Injury Network Criteria (AKIN). Cox proportional hazards regression was performed to assess the association between vitamin D levels and AKI, after adjusting for age, gender, eGFR, diabetes diagnosis, NSAIDS use, HsCRP, cancer stage, cancer type and type of chemotherapy.

**Results:** Mean age was 67.5 years. The population was 64% male and 27% black. Baseline creatinine was $1.0 \pm 0.2$ mg/dl. One hundred seven (31%), 57 (16%), 55 (16%), 43 (12%), 39 (11%), 38 (11%) and 7 (3%) had a diagnosis of chronic myeloid leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, B cell lymphoma, T cell lymphoma, multiple myeloma and miscellaneous HM respectively. Mean follow-up period was $8 \pm 4$ months. Seventy six (22%) developed AKI [stage 1: 34 (45%), stage 2: 20 (26%), stage 3: 22 (29%)] within a mean of $4 \pm 2$ months. Six (8%) required dialysis. The unadjusted cumulative AKI incidence across quartiles 1-4 ($\leq 10$, $11 - 20$, $21 - 30$, $> 30$ mg/dl) of vitamin D concentration were 31, 30.5, 25.5, and 13% respectively. After adjustment, 1st vitamin D quartile had a 1.53 times the hazard of the fourth quartile (95% CI: 1.10, 1.72; P-trend = 0.022) for AKI.

**Conclusions:** Low vitamin D at cancer diagnosis is associated with incident AKI in patients with HM. Further studies are needed to elucidate the underlying mechanism.

## TH-PO030

**Severe AKI Increases Platelet Adhesion and Aggregation in Cancer Patients** James Hung,[1] Tania Rubia Flores Rocha,[2] Elbio Antonio D'amico,[2] Luis Yu.[1] *[1]Instituto do Cancer do Estado de Sao Paulo, Universidade de Sao Paulo, Brazil; [2]Hospital das Clinicas, Universidade de Sao Paulo.*

**Background:** The association between renal dysfunction (uremia) and bleeding due to impaired platelet function has been previously described in AKI patients. However, patients with cancer usually present hypercoagulable state. Thus, we sought to evaluate the effect of uremia on platelet function in cancer patients.

**Methods:** We prospectively analysed patients admitted to the Intensive Care Unit of a Cancer University Hospital with sepsis. Renal dysfunction was classified according to the AKIN criteria. DiaMed Impact R was used to evaluate platelet function under near physiological flow conditions using cone and plate technology. In this test, an image analyser quantifies the adhered platelets and results are expressed as percentage of well surface covered by aggregates (SC %) as an index of adhesion and average size of the aggregates (AS microm$^2$) as an index of aggregation.

**Results:** From August 2012 to February 2013, 103 patients were included: mean age 61±15 y, 57% male, 85% solid tumors, 15% hematological tumors, 67% on vasopressors, 68% developed AKI (AKIN 1 - 19 patients, AKIN 2 - 20 patients, AKIN 3 - 31 patients).

| | Without AKI[#] | AKIN 1 | AKIN 2 | AKIN 3 | p |
|---|---|---|---|---|---|
| Creatinine (mg/dl) | 0.80 (0.54-0.97) | 1.29 (0.96-1.84) | 1.40 (0.94-2.18) | 2.59 (1.98-3.78) | <0.05, # vs AKIN 1, # vs AKIN 2, # vs AKIN 3 |
| Urea (mg/dl) | 44 (33-60) | 66 (43-118) | 87 (65-140) | 142 (108-193) | <0.05, # vs AKIN 2, # vs AKIN 3 |
| Calcium (mg/dl) | 4.6 (4.0-4.8) | 4.7 (4.6-4.8) | 4.5 (4.4-4.8) | 4.6 (4.4-4.8) | 0.72 |
| Hemoglobin (g/dl) | 8.7 (8.2-11) | 9.8 (8.5-10.5) | 8.5 (7.8-9.2) | 9.7 (8.8-11.7) | <0.05, AKIN 2 vs AKIN 3 |
| Platelets ($x10^3$/mm3) | 184 (66-274) | 214 (118-296) | 236 (122-285) | 160 (68-284) | 0.56 |
| SC | 10.3±4.6 | 13.5±3.8 | 13.9±5.1 | 14.1±5.5 | <0.05, # vs AKIN 2, # vs AKIN 3 |
| AS | 44.9±13.9 | 51.3±13.5 | 52.6±13.7 | 54.9±14.2 | <0.05, # vs AKIN 3 |

**Conclusions:** Severe AKI (AKIN 3) increases platelet adhesion and aggregation in cancer patients.

*Funding:* Government Support - Non-U.S.

## TH-PO031

**Association between Pre-Operative Statin Use and Acute Kidney Injury Biomarkers in Cardiac Surgery** Amber O. Molnar,[1] Chirag R. Parikh,[2] Steven G. Coca,[2] Heather Thiessen Philbrook,[6] Jay L. Koyner,[3] Michael Shlipak,[4] Mary Lee Myers,[5] Amit X. Garg.[6] *[1]Div of Nephrology, Dept of Medicine, Univ of Ottawa, Ottawa, Canada; [2]Section of Nephrology, Yale Univ School of Medicine, VA CT Healthcare System, and the Program of Applied Translational Research, New Haven, CT; [3]Section of Nephrology, Dept of Medicine, Univ of Chicago, Pritzker School of Medicine, Chicago, IL; [4]Div of General Internal Medicine, San Francisco VA Medical Center, Univ of California, San Francisco, CA; [5]Div of Cardiac Surgery, Western Univ, London, Canada; [6]Div of Nephrology, Dept of Medicine, Western Univ, London, Canada.*

**Background:** Acute kidney injury (AKI) is a serious complication of cardiac surgery for which there remains no specific therapy. Animal data and several observational studies suggest that statins prevent AKI, but the results are not conclusive.

**Methods:** We conducted a multi-centre prospective cohort study of 625 adult patients undergoing elective cardiac surgery. All patients were on statins and were grouped on whether statins were continued or held in the 24 hours prior to surgery. The primary outcome was AKI defined by a doubling of serum creatinine or dialysis. The secondary outcome was the peak level of several kidney injury biomarkers. Results were adjusted for demographic and clinical factors.

**Results:** Continuing (vs. holding) a statin prior to surgery was not associated with a lower risk of AKI defined by a doubling of serum creatinine or dialysis, [adjusted relative risk (RR) 1.09 (95% confidence interval (CI) 0.44, 2.70)]. However, continuing a statin was associated with a lower risk of elevation of the following AKI biomarkers: urine interleukin-18, urine neutrophil gelatinase-associated lipocalin (NGAL), urine kidney injury molecule-1 (KIM-1), and plasma NGAL [adjusted RR 0.34 (95% CI 0.18, 0.62), adjusted RR 0.41 (95% CI 0.22, 0.76), adjusted RR 0.37 (95% CI 0.20, 0.76), adjusted RR 0.62 (95% CI 0.39, 0.98), respectively].

**Conclusions:** Statins may prevent kidney injury after cardiac surgery as evidenced by lower levels of kidney injury biomarkers.

*Funding:* Other NIH Support - The study was supported by the NIH grant RO1HL085757 (CRP) to fund the TRIBE-AKI Consortium to study novel biomarkers of acute kidney injury in cardiac surgery. SGC is supported by National Institutes of Health Grants K23DK080132, R01DK096549, and R01HL085757. CRP is also supported by NIH grant K24DK090203. SGC, AXG, and CRP are also members of the NIH-sponsored ASsess, Serial Evaluation, and Subsequent Sequelae in Acute Kidney Injury (ASSESS-AKI) Consortium (U01DK082185)., Pharmaceutical Company Support - Abbott Diagnostics and Sekisui Diagnostics Inc

## TH-PO032

**Risk Factors for Non-Steroidal Anti-Inflammatory Drugs Induced Acute Kidney Injury in Patients with Chronic Kidney Disease** Teruhiko Yoshida, Keita Hirano, Masahiko Nagahama, Yasuhiro Komatsu. *Div of Nephrology, Dept of Internal Medicine, St. Luke's International Hospital, Chuo-ku, Tokyo, Japan.*

**Background:** Acute Kidney Injury (AKI) is one of the major adverse effects of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), which restricts the prescription of NSAIDs for Chronic Kidney Disease (CKD) patients; however, clinical studies on the risk factors of NSAIDs-induced AKI are scarce. The aim of the present study is to clarify risk factors for NSAIDs-induced AKI to propose appropriate NSAIDs use in CKD patients.

**Methods:** In a retrospective cohort study, we identified 770 patients with CKD (eGFR (estimated glomerular filtration rate) <60ml/min/1.73m$^2$) who received Flurbiprofen axetil (Ropion®) between December 2006 and December 2011. We used the AKI criteria from KDIGO (Kidney Disease: Improving Global Outcomes) for defining AKI patients. Risk factors for AKI was evaluated by multivariate analysis.

**Results:** Mean (Standard Deviation) of age was 69.3(±13.1) years old, creatinine (Cr) and eGFR were 1.07(±0.59) mg/dl and 47.7(±11.7) ml/min/1.73m$^2$. Among all patients in the analysis (N= 325), 81 patients (25.0%) developed AKI. Diuretic use (Odds ratio(OR) 1.91, P=0.03), diabetes mellitus (OR 1.83, P=0.05), low eGFR (OR 1 (30<eGFR<60), OR 2.83, P=0.02 (eGFR<30)) were risk factors for AKI. We also found that AKI itself was a risk factor for CKD worsening (more than 50% of serum Cr increase in later than 3 months after NSAIDs prescription) (OR 3.02, P=0.04).

**Conclusions:** Our study suggests that NSAIDs should be used with caution in CKD patients with diuretic use, diabetes mellitus and low eGFR. NSAIDs-induced AKI might be irreversible disease resulting in CKD worsening.

## TH-PO033

**Risk Factors and Predictive Score of Acute Kidney Injury in Patients with Acute Myocardial Infarction** Tong Yue, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital, Capital Medical Univ, Beijing, China.*

**Background:** Although an acute kidney injury (AKI) commonly occurs among patients hospitalized for acute myocardial infarction (AMI), its risk factors is unknown. The article aims to develop a clinical predictive score in order to predict the AKI after AMI. The early identification of patients with risks of developing AKI can reduce its incidence.

**Methods:** We analyzed data on consecutive patients with AMI from January, 2010 to July, 2011 at Beijing Anzhen Hospital. All the patients were divided into two groups, AKI group and non-AKI group, with the definition of AKI by Acute Kidney Injury Network. The univariable analysis and logistic regression model were used to establish the predictive score.

**Results:** The total cohort consisted of 1429 patients. The derivation cohort consisted of 1033 patients and the validation cohort consisted of 396 patients.In the derivation cohort, the rate of AKI was 14.3%. Mortality was significantly higher in the AKI group (10.1% vs 0.6% in those without AKI, P=0.000). In the validation cohort, the rate of AKI was 15.7 %.A lot of in-patients occur AKI within a week, patients with AKI stay longer than without AKI.Univariate analysis disclosed age,hypertension, diabetes mellitus, chronic kidney disease(CKD), heart rate, lower estimated glomerular filtration rate (eGFR), anemia, severe Killip class, extensive anterior myocardial infarction, troponin I(TNI) ≥50ng/ml ,left ventricular ejection fraction (LVEF)<50%,shock, Ventricular Fibrillation,β-blocker non-use and longer time before admission to hospital as risk factors to develop AKI. After adjusting for other factors associated with AKI, reduced GFR at presentation, severe Killip class, extensive anterior myocardial infarction,hypertension, β-blocker non-use and longer time before admission to hospital were independently associated with AKI.

**Conclusions:** we developed a clinical predictive score for AKI after AMI. This predictive score presented good discrimination and calibration. It would help the clinicians to make decision for preventive intervention. Further studies on interventions to minimize AKI or to more aggressively treat patients developing AKI should be tested.

*Funding:* Government Support - Non-U.S.

## TH-PO034

**Cardiorenal Syndrome in Hospitalized Patients with Mild versus Moderate-to-Severe Concomitant Hyponatremia on Admission: A Retrospective Study** Rainer U. Pliquett, Katrin Schlump, Matthias Girndt. *Clinic of Internal Medicine, Nephrology, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany.*

**Background:** Hyponatremia on admission has been related to a worse outcome among different co-morbidities including chronic heart failure. Regarding cardiorenal syndrome (CRS), a detrimental role for hyponatremia has been extrapolated from chronic heart failure studies (CJASN 2009;4:2013). Here, the degree of (acute) renal failure, (acute) cardiac failure on admission, duration of hospital stay, and 1-year mortality were determined for hospitalized CRS patients with mild or moderate-to-severe hyponatremia on admission.

**Methods:** Retrospective study of consecutive patients hospitalized for CRS in "University Hospital Halle" of Martin-Luther University Halle-Wittenberg from 01/01/2007 to 12/31/2011. Diagnosis was derived from diagnosis information using hospital documentation software. Among all CRS patients, patients with mild hyponatremia (<135 mmol/l; >=130 mmol/l) and moderate-severe hyponatremia patients (<130 mmol/l) were analyzed and entered this study. Information on plasma laboratory values including sodium, creatinine, brain-natriuretic peptide (BNP) on admission, duration of hospitalization and 1-year mortality (city-council inquiry) was gathered. Results were given as median with 95% confidence interval (CI). Significance was assumed for p<0.05 (one-tailed Mann-Whitney u test).

**Results:** Over a 5-year period, 386 CRS patients were admitted. In 262 patients, CRS diagnosis was confirmed. Hyponatremia occurred in 90 or 34.4% of CRS patients. Thereof, mild hyponatremia occurred in 59 (22.5%) patients, moderate-severe hyponatremia in 31 (11.8%). BNP was significantly higher in CRS patients with mild hyponatremia (754.5 pg/ml, CI: 799.5 - 2106) versus moderate-severe hyponatremia (491.5 pg/ml, CI: 300.2 - 1934; p<0.0001). Creatinine on admission was not different between groups (moderate-severe hyponatremia: Crea=226 µmol/l; mild hyponatremia: Crea=256 µmol/l; p ns). Likewise, duration of hospital stay (14.8 vs. 14.9 days) and 1-year mortality (25 of 56 patients or 44.6 % vs. 14 of 30 patients or 46.7 % in mild vs. moderate-severe hyponatremia) were not different. In a subgroup, 9 CRS patients with severe hyponatremia (Na ≤ 125 mmol/l) had a 1-year mortality of 66.7 %.

**Conclusions:** CRS patients with hyponatremia on admission had a considerably poor outcome (1-year mortality). Interestingly, irrespective of volume state, mild hyponatremia associates with worse cardiac function than moderate-severe hyponatremia on admission in CRS patients. The role of arginine vasopressin peptide (AVP) release as a compensatory mechanism and the role of renal failure for AVP action need to be further investigated.

*Funding:* Clinical Revenue Support

## TH-PO035

### Incidence of Acute Kidney Injury following Left Ventricular Assist Device Placement: A Meta-Analysis of Prospective Cohort Studies

Reejis Stephen,[1] Chandrashekar Kashyap,[2] Sevag Demirjian,[2] Sankar D. Navaneethan.[2] *[1]Medicine, Bridgeport Hospital, Bridgeport, CT; [2]Nephrology, Cleveland Clinic, Cleveland, OH.*

**Background:** Left Ventricular Assist Device (LVAD) placement is an increasingly common therapy for bridge-to-transplant or destination therapy for patients with heart failure. Although some reports noted improvement in renal function following LVAD, it is associated with a high risk of acute kidney injury (AKI). We conducted a meta-analysis to determine the incidence of AKI with LVAD and whether this risk differs based on the type of the device.

**Methods:** We searched MEDLINE and Scopus (April 2013) for relevant prospective cohort studies and trials of LVAD therapy. Incidence rates for AKI reported in individual studies were pooled using an inverse of variance method. AKI was defined as rise in serum creatinine or requirement of dialysis as defined by individual studies. A random effects model was used, and subgroup analyses were performed to explore the influence of type of LVAD device (continuous vs. pulsatile) on the incidence of AKI.

**Results:** 15 prospective studies including 2586 patients were included in this meta-analysis. Mean follow-up of these studies varied from 28 days to 657 days. The incidence of AKI after LVAD was 21% (15 studies, 2586 patients, 95% CI 15%-30% p<0.001) with significant heterogeneity between the studies ($I^2$=92%) and the device type accounted for 40% of this heterogeneity. The incidence of AKI was higher in patients receiving pulsatile LVAD device placement at 25% (7 studies, 1217 patients, 95% CI 15%-38%, p<0.001) compared to 15.6% when continuous-flow devices were used (7 studies, 983 patients, 95% CI 10%-23%, p<0.001).

**Conclusions:** The incidence of AKI has varied widely in patients receiving LVAD therapy. Newer continuous-flow devices have reduced this risk which still remains substantial. Studies focused on renal function and potential risk factors are needed to better understand this phenomenon.

## TH-PO036

### Acute Kidney Injury and Mortality following Ventricular Assist Device Implantation

Jay L. Koyner, Abhijit S. Naik. *Nephrology, Univ of Chicago.*

**Background:** Ventricular assist devices (VADs) are increasingly common and their surgical implantation predispose patients to acute kidney injury (AKI). We sought to evaluate the incidence, risk factors and short and long term mortality of patients with AKI following VAD implantation.

**Methods:** We identified all patients who underwent VAD implantation at the University of Chicago from Jan. 2008 to Jan. 2012. Using demographic and lab data we evaluated the prevalence of AKI, defined as a ≥50% increase in serum creatinine over the first 7 post-op days (RIFLE Risk). Using a cox proportional hazards model (model: age, gender, race, preoperative eGFR, AKI, Diabetes Mellitus (DM), Body Mass Index (BMI), cardiopulmonary bypass time and intra-operative blood transfusion) we examined the factors that were associated with 30 and 365 day mortality.

**Results:** A total of 157 eligible patients had VADs, with 44 (28%) developing post-implantation AKI. There was no difference between baseline serum creatinine or eGFR for those with and without AKI (p= 0.57 and 0.87 respectively). In a multivariate analysis only DM (OR= 2.55(1.12-5.78), P=0.03) was identified as a significant predictor of postoperative AKI. Using a multivariate model (censoring for heart transplantation), DM [HR=2.98(1.02-8.68), P= 0.05], and AKI [HR= 3.02(1.13-8.03),P=0.03] were both independently associated with 30-day mortality. At 365 days, 21(48%) of those with AKI had died compared to 34(30%) without AKI (p= 0.037).

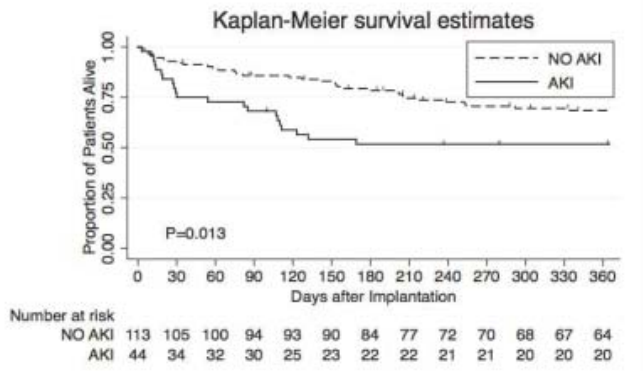



Figure 1: Patient Survival Following VAD Implantation

Pre-operative BMI (HR=0.93 (0.89-0.98)p=0.01), DM (HR= 2.01(1.13-3.60), P=0.02) and AKI (HR=1.85(1.03-3.30);P=0.04) were independently associated 365-day mortality. In survivors, there was no difference in serum creatinine or eGFR values at 365-days when comparing those with and without AKI(p=0.56 and 0.83).

**Conclusions:** AKI is common following VAD implantation. AKI and DM are independent predictors of 30 and 365 day mortality following VAD implantation.

*Funding:* NIDDK Support

## TH-PO037

### Independent Risk Factors in Common for Elderly Patients of Cardiorenal Sydrome Type I by Different Criteria

Lu Cai, Xinling Liang, Zhilian Li. *Div of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

**Background:** Cardiorenal sydrome type I reflects an abrupt worsening of cardiac function (eg. Acute heart failure) leading to acute kidney injury and is associated with substantial morbidity and mortality, especially in elderly patients. But there are no consensus diagnostic criteria for it. Thus, the objective of present study was to explore independent risk factors in common for elderly patients of cardiorenal sydrome type I by RIFLE, AKIN and KDIGO criteria of acute kidney injury.

**Methods:** Data was retrospectively collected from elderly patients (age ≥60 years old) with acute heart failure in Guangdong General Hospital between July 2002 and July 2012. The primary outcome was regarded as AKI defined as RIFLE, AKIN and KDIGO criteria, and baseline serum creatinine was defined as the lowest serum creatinine in the three months before admission.

**Results:** Among 808 elderly participants, more patients were diagnosed as AKI by KDIGO (46.2%) than RIFLE (37.9%) and AKIN (48.6%) . Logistic regression indicates that albumin<30g/L (OR 1.746), uric acid>436umol/L(OR 1.621), use of low-dose dopamine (2-3ug/kg/min) (OR 2.283) within 48 hours after admission were independent factors for cardiorenal sydrome type I by RIFLE and so were albumin<30g/L (AKIN:OR 1.798; KDIGO:OR 1.745), uric acid>436umol/L (AKIN:OR 2.658; KDIGO:OR 2.464), use of diuretics, angiotensin-converting-enzyme inhibitor/Angiotensin receptorinhibitor(ACEI/ARB) and low-dose dopamine(AKIN:OR 2.010; KDIGO: OR 2.173) within 48 hours after admission by AKIN and KDIGO.

**Conclusions:** Albumin<30g/L, uric acid>436umol/L and use of low-dose dopamine within 48 hours after admission were independent risk factors in common for elderly patient of cardiorenal sydrome type I by RIFLE, AKIN and KDIGO criteria.

*Funding:* Government Support - Non-U.S.

## TH-PO038

### Relationship of the Time Interval between Coronary Angiography and Elective Off-Pump Coronary Artery Bypass Surgery with Postoperative Acute Kidney Injury

Yan Zhang, Nan Ye, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital, Capital Medical Univ, Beijing, China.*

**Background:** To investigate whether there are effects of time intervals between coronary angiography (CAG) and elective off-pump coronary artery bypass graft (OPCABG) on postoperative acute kidney injury (AKI).

**Methods:** Clinical data of patients undergoing CAG and OPCABG from June, 2010 to December, 2011 in Beijing Anzhen Hospital were retrospectively analyzed. All the patients were divided into AKI group and non-AKI group Univariate analysis was performed to find possible factors associated with AKI. Multivariate logistic regression analysis was used to identify whether the short time interval was one of the independent risk factors of AKI after adjusting for potential confounding variables.

**Results:** Of the 1513 patients, 436 patients (28.8%) developed AKI. The mortality rate in AKI group (5.7%) was 6 times higher then that in non-AKI group (0.9%). The incidence of AKI was highest (48.8%) in patients in whom OPCABG was performed ≤24h after CAG. Multivariate logistic regression analysis showed that the time interval less that 24h between CAG and OPCABG did increase the risk of AKI (OR=2.44, 95%CI为1.19, 4.99) after adjusting for the following confounding variables: male, advanced age, hypertension,

diabetes, NYHA heart function class III and IV, lower estimated glomerular filtration rate, numbers of coronary artery bypass grafts>3, intraoperative or postoperative intra aortic balloon pump, postoperative hypotension time ≥30min, dosage of furosemide ≥60mg/d.

**Conclusions:** It was one of the independent risk factors of postoperative AKI that the OPCABG was performed ≤24h after CAG.

*Funding:* Government Support - Non-U.S.

### TH-PO039

**Risk Factors and Clinical Predictive Score for Acute Kidney Injury after Off-Pump Coronary Artery Bypass Surguey** Yan Zhang, Nan Ye, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital, Capital Medical Univ, Beijing, China.*

**Background:** To investigate the risk factors of acute kidney injury (AKI) after off-pump coronary artery bypass graft (OPCAB), develop a clinical predictive score and validate its discrimination and calibration.

**Methods:** Clinical data 0f 1513 patients undergoing coronary angiography(CAG) and OPCAB from June, 2010 to December, 2011 at Beijing Anzhen Hospital were retrospectively analyzed. All the patients were divided into AKI group and non-AKI group. Univariable analysis and multivariate logistic regression analysis were used to establish the predictive score.

**Results:** 436 patients (28.8%) developed AKI. In a multivariable model about AKI included: increased age, male, hypertension, diabetes, New York Heart Association class III or IV, lower estimated glomerular filtration rate (eGFR), shorter time interval between CAG and elective OPCAB, much numbers of grafts, intraoperative or postoperative intra aortic balloon pump, longer postoperative hypotension time, larger dosage of loop diuretics. The predictive model was discriminated well (ROC=0.71) and had well calibrated according to the Hosmer-Lemeshow test (P=0.30).

**Conclusions:** We developed a clinical predictive score for AKI after CAG and OPCAB. This predictive score presented good discrimination and calibration.

*Funding:* Government Support - Non-U.S.

### TH-PO040

**Serum Hepcidin – A Protective and Inverse Biomarker of CI-AKI in Patients Undergoing Percutaneous Coronary Interventions-PCI** Jolanta Malyszko,[1] Jacek S. Malyszko,[1] Hanna Gajewska,[2] Ewa Koc-Zorawska,[1] Slawomir Dobrzycki.[2] *[1]Nephrol. Dept, Med Univ, Poland; [2]Invasive Cardiol., Med Univ, Bialystok, Poland.*

**Background:** Contrast-induced acute kidney injury (CI-AKI) is a common and potentially serious complication of percutaneous coronary interventions-PCI. Hepcidin, a peptide hormone that regulates iron homeostasis. Urinary hepcidin-25 has been shown to be elevated in patients who do not develop AKI.

In this study we tested the hypothesis whether serum hepcidin could represent an early protective biomarker of CI-AKI in 26 patients with normal serum creatinine undergoing PCI. In addition, we assessed serum and urinary NGAL, cystatin C, eGFR serum and urinary creatinine in these patients.

**Methods:** Hepcidin, serum and urinary NGAL, cystatin C, urinary L-FABP, KIM-1, and IL-18 were evaluated before, and after 2, 4, 8, 24 and 48 hours after PCI using commercially available kits. Serum creatinine was assessed before, 24 and 48 hours after PCI. ANOVA or Kruskall-Wallis ANOVA for repeated measurements were used in statistical analysis.

**Results:** We found a significant rise in serum hepcidin as early as after 4 hours when compared to the baseline values. It was also significantly higher 8 and 24 hours after PCI. Serum NGAL increased after 2, 4 and 8 hours, and in urinary NGAL after 4,8 and 24 hours after PCI. We found a significant rise in serum NGAL after 2, 4 and 8 hours, and in urinary NGAL and IL-18 after 4, 8&24 hours after PCI. Serum cystatin C increased significantly 8 hours, reaching peak 24 hours after PCI and then decreased after 48 hours. L-FABP and KIM-1 increase significantly after 24 and 48 hours after PCI. When contrast nephropathy was defined as an increase in serum creatinine by >25% of the baseline level 48 hours after PCI, the prevalence of CI-AKI was 4/26. Hepcidin were significantly lower 8 and 24 hours after PCI in patients with CI-AKI, while serum and urine NGALwere significantly higher in patients with CI-AKI . Hepcidin correlated negatively with NGAL ($r=-0.42$, $p<0.05$).

**Conclusions:** Our findings suggest that serum hepcidin might be an early predictive biomarker of ruling out CI-AKI after PCI, thereby contributing to early patient risk stratification.

*Funding:* Government Support - Non-U.S.

### TH-PO041

**Risk Factors of Non-Early Recovery after an Episode of Acute Kidney Injury** Carlos Enrique Arias, Eva Rodriguez, Sheila Bermejo, Adriana Sierra, Julio Pascual. *Nephrology, Parc de Salut Mar-IMIN, Barcelona, Spain.*

**Background:** Acute kidney injury (AKI) might be a risk factor for the future development or accelerated progression of chronic kidney disease (CKD).

The aim is to determine risk factors are related to non-recovery after an AKI episode.

**Methods:** Retrospective observational cohort study during a follow-up period of 9 years. Potential subjects were identified and enrolled by systematic chart review using the hospital coding system (ICD-9). Patients were included after exclusion of pre-renal and obstructive etiologies or known CKD. We used the ADQI definition and classification.

**Results:** We included 228 patients, 64% men, age 64±19 years. Background revealed hypertension (53%), diabetes (28%), ischemic heart disease (17%) and peripheral vascular disease (23%). The most common etiology was ischemic AKI (42.5%), followed by septic (22.8%).Distribution of cases was: Risk 22.3%, Injury 25.9%, Failure 51.8%. Median duration of AKI episode was 32±44 days, and 30 (13.2%) patients needed renal replacement therapy during hospitalization. In our study, 185 patients (81.1%) recovered renal function during the first 4 weeks after diagnosis, compared with 43 (18.9%) patients who did not. In univariate analysis, the predictors of non-recovery of renal function after an episode of AKI were: age>64 years (OR 2.5, 95%CI 1.17-5.40), hypertension (OR 2.09, 1.04-4.22), AKI secondary to nephrotoxicity (OR 5.18, 1.68-16.0), and serum creatinine at admission >4mg/dl (OR 2.70, 1.37-5.3). In multivariate analysis, independent risk factors for lack of recovery of renal function in four weeks were: age>64 years (OR 2.54, 1.01-6.37), AKI secondary to nephrotoxicity (OR 3.58, 1.07-11.93) and serum creatinine during hospitalization >4mg/dl (OR 3.58, 1.07-11.93).

**Conclusions:** Age older than 64 years, serum creatinine >4mg/dl and that the AKI was related to nephrotoxicity were the independent risk factors for lack of recovery of renal function during the first 4 weeks after an episode of AKI.

### TH-PO042

**Retrospective Analysis of 30 Years' Data Base, over 68000 Patients Using IMIS (Integrated Medical Information System in Kochi University) to Search Risk Factor of Acute Kidney Injury** Taro Horino,[1] Kazunori Otomo,[2] Kazu Hamada,[1] Yoshiko Shimamura,[1] Koji Ogata,[1] Kosuke Inoue,[1] Yoshinori Taniguchi,[1] Yoshio Terada,[1] Yoshiyasu Okuhara.[2] *[1]Dept of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi Univ, Nankoku, Kochi, Japan; [2]Center of Medical information Science, Kochi Medical School, Kochi Univ, Nankoku, Kochi, Japan.*

**Background:** In Japan, the etiology of acute kidney injury (AKI) remains unclear. Therefore, we estimated risk factors of acute kidney injury by using large medical data base in Kochi University, or Integrated Medical Information System (IMIS) data base for 30 years.

**Methods:** 68504 patients who had hospitalized and were more 18 years old in Kochi Medical School from 1981 to 2010 were enrolled. AKI was diagnosed according to AKIN criteria. We investigated odds ratio for AKI of underlying diseases which developed within 30 days from AKI onset and continued during AKI course. All data were estimated by analysis software, Retrieval sYstem for Open Medical Analysis (RYOMA2).

**Results:** AKI patients were 8979 (13.1%); male 5541 (61.7%). In elderly patients especially over 70years, the risk of AKI up to 15.5%. The odds ratios of classical risk factors; septic shock, cardiogenic shock, liver failure, acute respiratory distress syndrome, pulmonary edema and acute lung injury were 16.45 (95%CI, 12.21-22.17), 9.96 (7.31-13.58), 9.36 (7.70-11.39), 9.12 (6.86-12.13), 7.97 (6.21-10.23), and 7.93 (5.94-10.58), respectively. Noteworthy, we detected also novel risk factors; the odds ratios of hemorrhagic gastric ulcer and hyperuricemia were 2.50 (2.15-2.90) and 1.76 (1.60-1.95), respectively. Furthermore, the incidence of AKI was elevated in patients, especially in female, with hyperuricemia (more 7.0 mg/dl in male and more 5.5 mg/dl in female) at dose-dependent manner. Interestingly, the risk of AKI is also increased hypouricemia (less than 3.0 mg/dl), so the curve of odds ratio for AKI risk shows "J-shaped" curve.

**Conclusions:** This is the first data investigated about the risk factors for AKI with using large scale medical data base in Japan. Our data revealed that hyperuricemia might be an important risk factor for AKI in addition to classical risk factors.

### TH-PO043

**Association between Mortality Risk and Severity of Acute Kidney Injury among Critical-Care Patients from Remote Islands: A Single Center Prospective Cohort Study** Junichi Ishigami,[1,2] Masato Tajima,[1] Ayako Motomura,[1] Tatemitsu Rai,[2] Shinichi Uchida,[2] Sei Sasaki.[2] *[1]Dept of Nephrology, Tokyo Metropolitan Hiroo Hospital, Tokyo, Japan; [2]Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** The prevalence of acute kidney injury (AKI) is high among critical-care patients in emergency department and their outcome remains poor. Tokyo Metropolitan Hiroo Hospital plays a major role to provide emergency medical care for people in the remote islands and patients are transferred to the hospital if immediate intensive care is required. We conducted a prospective observational cohort study to assess the prevalence of AKI and the outcome of the patients transferred from the islands.

**Methods:** 476 patients who were transferred from 10 remote islands to the hospital between December 1st 2010 and November 31st 2012 were included to the study. Patients were stratified according to the severity of AKI using AKIN (Acute Kidney Injury Network) definitions: Stage1, 2 and 3. Kaplan-Meire survival curve was used to assess the longitudinal risk of mortality. Logistic regression analysis was used to assess the multiple factors affecting the outcome.

**Results:** Among 476 patients, 54(11.3%), 17(3.6%) and 32(6.7%) developed AKIN 1, 2 and 3 respectively. 34 patients (7.1%) died within 30 days from ICU admission. Patients who developed AKI had longer length of ICU stay compared to patients who did not (6.4 versus 3.5 days; $p<0.001$). Patients developed either AKIN 2 or 3 had higher mortality risk compared to patients who did not develop AKI (HR 5.66, 95%CI 1.79 to 17.93; $p<0.0001$). In logistic regression model, development of AKI was not associated with age, sex, use of radiocontrast agents, history of diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, ischemic heart disease and congestive heart disease.

**Conclusions:** The patients developed AKI had longer length of ICU stay compared to those without AKI. Since AKIN 2 and 3 patients had highest mortality risk among the cohort, these patients should be transferred preferentially to a hospital with adequate emergency medical service.

**TH-PO044**

**Should We Consider Initial Insertion of Tunneled Hemodialysis Catheter in the Patients Who Need Renal Replacement Therapy in Intensive Care Unit?** Hoon Suk Park, Kyungyoon Chang, Hyung Wook Kim, Bum-Soon Choi, Cheol Whee Park, Chul Woo Yang, Dong Chan Jin. *Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** Non-tunneled hemodialysis (HD) catheter was known to be used safely without infectious complication up to 1 month, but recent trend is to recommend using tunneled catheter in the patients requiring renal replacement therapy (RRT) for more than one week. Nevertheless, there exist difficulties following such recommendation in ICU, because the patients in ICU have poor survival so that it is difficult to predict RRT duration, so we investigated the propriety of current recommendation for catheter choice in ICU.

**Methods:** Forty-five ICU patients receiving tunneled HD catheter insertion and 105 ESRD patients were respectively assigned to ICU and ordinary ESRD groups. Patient survival and tunneled catheter survival, in addition to duration of catheter use and immediate and long term complication rates were compared.

**Results:** Immediate complication rate including exit site bleeding, bruise and hematoma was rather higher in ordinary ESRD group (32.9 % vs. 8.1 %, p = 0.003). Long term complication rates including catheter dysfunction and catheter related infection were comparable (5.4 % in ICU group vs. 12.7% in ordinary ESRD group, p = 0.33). Duration of catheter use was significantly shorter in ICU group (39 ± 53 days vs. 73 ± 50 days, p < 0.001), although catheter survivals were comparable between 2 groups (p = 0.8). Patient survival in ICU group was significantly poor, compared with ordinary ESRD group (p = 0.002).

**Conclusions:** ICU patients receiving tunneled catheter insertion for RRT showed poor life survival rate with short span in catheter use, compared with ordinary ESRD group, although catheter survival was not inferior and its insertion was safely performed in ICU setting. Therefore, considering patient survival and span of catheter use besides infection risk, separate guideline for catheter choice in ICU setting is required.

**TH-PO045**

**Better Prediction Using Ratio of Contrast Volume to eGFR without BSA Normalization for Contrast Nephropathy** Hoon Suk Park, Kyungyoon Chang, Hyung Wook Kim, Bum-Soon Choi, Cheol Whee Park, Chul Woo Yang, Dong Chan Jin. *Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** Considering contrast is excreted through kidneys, use of estimated glomerular filtration rate (eGFR) without body surface area (BSA) normalization is expected to be appropriate for evaluating the risk of contrast-induced acute kidney injury (CI-AKI). No studies have thus far suggested use of eGFR without BSA normalization for predicting CI-AKI. We aimed to demonstrate eGFR without BSA normalization is more appropriate than eGFR with BSA normalization for predicting CI-AKI.

**Methods:** This study included 433 myocardial infarction patients treated with primary percutaneous coronary intervention. Demographic data and clinical findings were compared between groups with and without CI-AKI. Logistic regression analysis was performed to identify independent risk factors. We used receiver-operating characteristic curves to compare the prediction of CI-AKI between eGFRs with and without BSA normalization.

**Results:** The ratio of contrast volume to raw MDRD eGFR, which is the value of MDRD eGFR without BSA normalization (odds ratio [OR], 1.352; 95% confidence interval [CI], 1.140–1.604; p = 0.001) was an independent risk factor for CI-AKI in multivariate logistic regression analysis besides cardiogenic shock (OR, 4.509; 95% CI, 1.055–19.267; p = 0.042). Ratio of contrast volume to MDRD eGFR without BSA normalization exhibited 94.7% sensitivity and 64.1% specificity whereas ratio of contrast volume to MDRD eGFR with BSA normalization exhibited 88.9% sensitivity and 51.5% specificity. Difference between 2 area under curves was significant (0.0276; p = 0.004).

**Conclusions:** Ratio of contrast volume to eGFR without BSA normalization better predicts CI-AKI than does ratio of contrast volume to eGFR with BSA normalization.

**TH-PO046**

**Acute Kidney Injury in Emergency Hospital Admissions: Are We Missing the Middle?** Dhaneesha Senaratne,[1] Yvonne Chan,[1] Sanjay Kartikay Ojha,[2] Amir Bhanji.[2] *[1]School of Clinical Medicine, Univ of Cambridge, Cambridge, United Kingdom; [2]Dept of Renal Medicine, Addenbrooke's Hospital, Cambridge, United Kingdom.*

**Background:** Acute kidney injury (AKI) in acute admissions is common and associated with poor outcomes, independent of co-morbidities. The 2009 UK NCEPOD Report: Adding Insult to Injury showed that good assessment and management can improve outcomes but is only provided in half of patients. We aimed to assess the detection and management of AKI in our centre and whether this is influenced by AKI severity.

**Methods:** Acute admissions between 28/08/12 and 12/10/12 were retrospectively analysed. Patients with creatinine ≥150µmol/L and who fulfilled criteria for Stage 2 (2-3x baseline) or Stage 3 (>3x baseline) AKI were selected. These admissions were judged against standards established from The Renal Association guidance.

**Results: Patient characteristics**: 72 patients fulfilled inclusion criteria; 26 (36.1%) were Stage 2 and 46 (63.9%) were Stage 3. Stages were similar for age (p=0.397) and number of co-morbidities (p=0.442). **Assessment**: 2 patients (2.8%) fulfilled 6/6 assessment criteria; 1 (3.8%) was Stage 2 and 1 was (2.2%) Stage 3. **Management**: 43 patients (59.7%) fulfilled 3/3 management criteria; 14 (53.8%) were Stage 2 and 29 (63.0%) were Stage 3. 18 (25.0%) patients received renal team input during the admission; 2 (7.7%) were Stage 2 and 16 (34.8%) were Stage 3. **Outcome**: 16 patients (22.2%) died during the acute admission; 8 (30.8%) were Stage 2 and 8 (17.4%) were Stage 3 (p=0.190).


**Conclusions:** As both patient groups were balanced for age and number of co-morbidities one would expect better outcomes with lower severity of AKI. Although outcomes were not statistically significant, the trend suggests poorer assessment & management of less severe AKI at our centre. This has led to the local introduction of an AKI risk assessment tool and AKI care pathway.

**TH-PO047**

**Are Bicarbonate-Based Solutions Better in the Treatment of Patientes with Established Acute Kidney Injury?** Rolando Claure-Del Granado, Vania Prudencio. *School of Medicine, IIBISMED, Universidad Mayor de San Simon, Cochabamba, Bolivia.*

**Background:** Fluid administration constitutes an important part of the treatment of establish acute kidney injury (AKI). The optimal hydration strategy for AKI treatment remains unknown. AKI is often associated with metabolic acidosis, and acidosis has been linked to several adverse effects that are deleterious to kidney function. We hypothesized that the use of bicarbonate-based solutions will facilitate AKI recovery.

**Methods:** We analyzed data from 59 hospitalized non-ICU patients; who developed AKI. Patients with CKD K-DIGO 4 and 5 were excluded from the analysis. Twenty-nine patients received bicarbonate-based solutions for the management of AKI and 30 patients received different types of bicarbonate-free solutions. We assessed the effect of bicarbonate-based solutions on Δ serum creatinine (sCr), urine output (UO), days to achieve basal sCr, and the amount of fluids been administered on each group.

**Results:** Of the 59 patients included in the study, 29 patients received bicarbonate-based solutions and 30 patients received other types of IV fluids. Median baseline sCr was higher in the patients who received bicarbonate-based solutions (1.12 IQR[0.9 – 1.3] vs. 1.08 IQR[0.9 – 1.23] mg/dL; p<0.001). sCr reduction rate was higher in the group treated with bicarbonate-based solutions (mean Δ sCr -0.29±0.47 vs. -0.07±0.42 mg/dL; p=0.007). Patients treated with bicarbonate-based solutions; returned to their baseline sCr in fewer days (5.6±2.1 vs. 7.6±2.8 days; p<0.001). In patients where diuretics were not employed, no difference was found in 24 hour UO between patients who received bicarbonate-based solutions and those who received other types of solutions (1,592 IQR[1,409 – 1,905] vs. 1,647 IQR[1,296 – 2,192] ml; p=0.294). No difference also was found in the amount of fluids that each group received (bicarbonate-based 1,000 IQR[500 – 2,000] vs. bicarbonate-free 1,000 IQR[1,000 – 2,000] ml; p=0.903).

**Conclusions:** Bicarbonate-based solutions for the treatment of established AKI could improve renal function, accelerating renal recovery. An adequately powered randomized controlled trial is warranted to support the use of bicarbonate-based solution in patients with AKI.

**TH-PO048**

**Management of Acute Kidney Injury in Hospitalised Patients Remains a Challenge** Jennifer R. Joslin, Daniel Zubli, Hannah R. Wilson, Nathan Gauge, Mark Andrew Yates, Katherine E.S. Warren, Joanna Sutherby, Sabrina Rossi, Charlotte Masterton-Smith, Cornelia Junghans Minton, Tracy Ellimah, Anna Buckingham, Taryn Pile, Maria Ostermann. *Guy's & St. Thomas' NHS Hospital Trust, London, United Kingdom.*

**Background:** When managing patients with acute kidney injury (AKI), a multidisciplinary approach with good communication between medical teams and nursing staff is essential, especially when patients are transferred between wards and medical teams, and health care staff work shifts. In 2012, an AKI 'care bundle' was developed, in conjunction with the London AKI network. The aim of this audit was to evaluate whether there were differences in appreciation and management of AKI on the 1st day of AKI compared to the following days.

**Methods:** During a 7 day period in April 2013, electronic patient records of all level 1 medical and surgical patients in a London teaching hospital were screened. Medical notes of patients with AKI as defined by the KDIGO criteria were reviewed by doctors independent from the treating team to assess compliance with recommended management. Patients receiving palliative care were excluded.

**Results:** 64 patients presented with or developed AKI within the data collection. Data were available for the 1st day of AKI for all 64 patients and for 180 inpatient hospital days beyond the 1st day.

| | Management on 1st day of AKI | Management after 1st day of AKI | p-value |
|---|---|---|---|
| AKI recognised by managing team | 70% | 75% | NS |
| Documented AKI management plan | 87% | 83% | NS |
| Clinical assessment of fluid status | 63% | 67% | NS |
| Complete fluid balance chart | 33% | 43% | NS |
| IV fluids given if vulnerable to hypovolaemia | 74% | 76% | NS |
| Timely antibiotics given if septic | 71% | 68% | NS |
| Nephrotoxic drugs discontinued, if appropriate | 72% | 77% | NS |

**Conclusions:** Patients with AKI did not receive optimal care on a daily basis. However, care did not significantly deteriorate on subsequent days when compared to Day 1 of AKI. More work and education of all members of staff is necessary to achieve full compliance with the AKI bundle on a daily basis.

## TH-PO049

**Exertional Acute Kidney Injury in U.S. Military Personnel Is Not Always due to Rhabdomyolysis** John S. Hammes, Matthew J. Wauson, Joseph D. Hebreo, Daniel J. Dean. *Medicine, Naval Medical Center San Diego, San Diego, CA.*

**Background:** Exertion leading to acute kidney injury (AKI) is well described. It may be due to rhabdomyolysis (AKI-R) or patchy ischemia (AKI-ALPE) or exertional heat illness (AKI-EHI). We sought to determine why AKI-ALPE is appearing in U.S. military personnel (ADSM). The U.S. Marine Corps instituted a Combat Fitness Test (CFT) which involves intense bursts of exertion in 2008.

**Methods:** We reviewed inpatient and outpatient electronic health records of all ADSM's hospitalized with discharge diagnosis of AKI from 2008-2012. We evaluated demographic, clinical and laboratory parameters of patients (pts) for cases of AKI-R, AKI-ALPE, and AKI-EHI. AKI-ALPE was defined as AKI occurring after exertion, accompanied by loin or abdominal pain, and with a creatine kinase (CK) value less than 3000 IU/L which did not resolve with 24 hours of volume repletion.

**Results:** There were 15 cases of AKI-ALPE, 9 cases of AKI-R, and 11 cases of AKI-EHI. AKI-ALPE cases had similar peak SCr (3.7 vs 4.2 p=.7) to AKI-R. There was no difference in the need for dialysis between groups (7% vs 33%, p=.139). The mean number of days til recovery of kidney function (10.4 vs 13.4 p=.61) did not differ between AKI-ALPE and AKI-R. Of 15 AKI-ALPE cases, 9 had recently completed a Combat Fitness Test (CFT) compared with 0 of 20 non AKI-ALPE cases. Pts with AKI-EHI were quickly identified, cooled, and had lower severity of illness (mean peak SCr 1.6, 0 pts dialyzed). No patients died.

**Conclusions:** AKI-ALPE is an important cause of AKI in ADSM who have completed CFT. It may be severe enough to warrant dialysis and has a similar morbidity burden to that of AKI-R. Clinicians caring for AKI pts after exertion should consider ALPE when typical features of rhabdomyolysis are absent. Rapid recognition and cooling of patients with exertional heat illness has mitigated the morbidity of AKI-EHI in this population.

*Funding:* Other U.S. Government Support

## TH-PO050

**Acute Kidney Injury due to Vitamin D Intoxication in Kashmiri Population** Muzafar Maqsood Wani, Imtiyaz Ahmad Wani. *Nephrology, SKIMS, Soura, Srinagar, J&K, India.*

**Background:** Vitamin D deficiency is highly prevalent in Kashmir. Most of the elderly get Vit D, given mostly as injections; at times dose prescribed is far above the permissible limit, resulting in many cases of Vit D toxicity with hypercalcemia and reversible acute kidney injury (AKI). We report 50 patients with malpractice-related Vit D intoxication with hypercalcemia and AKI.

**Methods:** 58 cases of Vit D toxicity were seen over 24 months (2011-2012). Detailed investigations and follow up was available in 50 cases. The diagnosis of Vit D intoxication was made on basis of history of multiple vitamin D injection intake (600,000 IU/injection), toxic blood levels of 25 OH Vit D and after ruling out common causes of hypercalcemia (malignancy and hyperparathyroidism). Their presentation was either de novo AKI (Group 1) or acute on top of chronic kidney disease (Group 2).

**Results:** Demographic and lab data is shown in table 1.

| Parameter | Group 1 - 39 (27;12) | Group 2- 11 ( 5;6) |
|---|---|---|
| Age (years) | 60.33±14.48 | 62.11±13.01 |
| Admission days | 7.05± 3.03 | 7.77± 3.86 |
| No of injections (million units) | 2-28 (1.2-16.8) | 3-24 (1.8-14.4) |
| Creat at presentation (mg/dl) | 3.25±.96 | 4.53±1.17 |
| Creat at follow up(mg/dl) | 1.41 ± .27 | 3.32 ± 1.06 |
| Calcium(mg/dl) | 13.76 ±1.47 | 13.68 ±2.02 |
| Calcium on follow up (mg/dl) | 10.76 ±1.23 | 11.11 ±1.08 |
| Vit D level (nmol/L) | 313.33 ± 54.84 | 303.73 ± 48.41 |
| PTH-(pg/ml) | 18.13 ± 9.62 | 42.31 ± 12.69 |

The average follow up was of 7.2± .6 months. The clinical presentation was weakness in 100%, constipation in 80%, abdominal pain in 60%, nausea/vomiting in 60%, anorexia in 50%, oliguria in 20% and altered sensorium in 20%. The treatment received was IV fluids in all, normal saline and steroids (short course) in 38 and bisphosphonates in 5.

**Conclusions:** This case series elucidates the alarming trend of AKI secondary to Vit D toxicity in Kashmiri population. It is a cause of reversible AKI which responds to conservative measures in most cases.

## TH-PO051

**Pre-Operative Fibroblast Growth Factor 23 (FGF23) Levels Predict Post-Operative Acute Kidney Injury in Pediatric Cardiac Surgery Patients: A Prospective Study** Mark Hanudel, Barbara Gales, Isidro B. Salusky, Katherine Wesseling-Perry. *Dept of Pediatrics, UCLA, Los Angeles, CA.*

**Background:** In CKD patients, FGF23 levels increase early in the CKD course, predict renal disease progression, and are associated with excess morbidity and mortality. Less is known about FGF23 in AKI; small, retrospective studies have shown higher FGF23 levels in AKI patients.

**Methods:** To prospectively assess the role of FGF23 in pediatric AKI, plasma FGF23 levels (2nd generation C-terminal, Immunotopics®) were measured pre-operatively and at 2, 6, 12, 24, 48, and 96 hours post-reperfusion in 20 patients without underlying CKD undergoing cardiopulmonary bypass (CPB). Serum creatinine was obtained at baseline and daily post-reperfusion. AKI was defined by the AKIN criteria; eGFR was calculated using the Schwartz formula; and cardiac surgery complexity was classified via RACHS-1 score.

**Results:** Of the 20 enrolled patients, 13 developed at least Stage 1 AKI post-operatively. The AKI group was younger than the non-AKI group; other baseline characteristics were similar between the two groups. Pre-operative FGF23 levels were inversely related to age ($r = -0.67$, $p=0.001$). Pre-operative FGF23 levels were higher in patients who developed AKI than in those who did not; this finding remained significant upon correcting for age. Post-operatively, FGF23 levels increased in all patients.

**Conclusions:** Pre-operative FGF23 levels predict the development of post-operative CPB-associated AKI. FGF23 levels increase in pediatric non-CKD patients undergoing CPB, even in patients without significant post-operative changes in serum creatinine, suggesting that FGF23 may be a more sensitive marker of AKI than serum creatinine.

| | AKI Patients | Non-AKI Patients | p-value |
|---|---|---|---|
| Number | 13 | 7 | |
| Gender (% male) | 77 | 43 | NS |
| Age (months) | 30.5 +/- 52.0 | 100.4 +/- 69.9 | <0.05 |
| Weight SDS | -1.14 +/- 1.80 | -1.78 +/- 2.68 | NS |
| Height SDS | -1.51 +/- 1.71 | -1.28 +/- 1.89 | NS |
| RACHS-1 score | 2 (2, 3) | 2 (2, 2.5) | NS |
| CPB time (min) | 46 (36, 68) | 59 (33, 76) | NS |
| Pre-op eGFR (ml/min/1.73m²) | 129 +/- 42 | 127 +/- 33 | NS |
| Pre-op Creatinine (mg/dl) | 0.3 +/- 0.1 | 0.5 +/- 0.2 | <0.01 |
| Peak Fold Creatinine Increase | 1.86 +/- 0.52 | 1.16 +/- 0.11 | <0.01 |
| Pre-op Calcium (mg/dl) | 10.3 +/- 0.4 | 9.7 +/- 0.7 | NS |
| Pre-op Phosphorous (mg/dl) | 5.3 +/- 1.0 | 4.1 +/- 0.7 | <0.01 |
| Pre-op Phosphorous SDS | -1.55 +/- 2.05 | -1.95 +/- 1.60 | NS |
| Pre-op FGF23 (RU/ml) | 437 (356, 551) | 102 (72, 136) | <0.05 |

Data presented as mean +/- SD or median (IQR). SDS denotes standard deviation score.

## TH-PO052

**Small Molecule-Mediated Enhancement of Post Injury Repair Mechanisms after Acute Kidney Injury** Nataliya Skrypnyk,[1] Tatiana Novitskaya,[1] Takuto Chiba,[1] Lauren Brilli,[2] Lee McDermott,[2] Subramaniam Sanker,[2] Neil A. Hukriede,[2] Mark P. De Caestecker.[1] *[1]Div of Nephrology, Vanderbilt Univ, Nashville, TN; [2]Dept of Developmental Biology, Univ of Pittsburgh, PA.*

**Background:** Methyl-4-phenylthiobutanoate (m4PTB) is an HDAC inhibitor (HDACi) that promotes renal progenitor expansion in zebrafish embryos and accelerates recovery after ischemia reperfusion acute kidney injury (IR-AKI) in mice. In these studies we generated series of m4PTB analogues and compare efficacy with that of the HDACi SAHA in accelerating recovery and reducing post injury fibrosis after AKI.

**Methods:** m4PTB analogues substitute the zinc chelating carboxylic acid in m4PTB, with other HDACi warheads: 1) Carboxylic acid UPHD22 and 25 (m4PTB); 2) Hydroxamic acid (from TSA) UPHD29; and 3) Benzamide (Class 1 HDACi) UPHD186 and 36. We used zebrafish reporters to evaluate activity; mass spectrometry and quantitative analysis of renal histone H4 acetylation to assess pharmacokinetics (PK); IR-AKI and unilateral ureteral obstruction (UUO) mouse models. Compounds administered IP at 50mg/kg/day starting 1-day post injury for 7 days. Renal function assessed by serum creatinine, and post injury fibrosis with Sirius red and QRT-PCR for fibrotic markers.

**Results:** All 5 analogues show similar activity in zebrafish assays (ED50 600-1000nM). PK studies identified two patterns: long acting (SAHA, UPHD22 and 36) and short acting (UPHD25, 186 and 29). UPHD22, 25, 29 and SAHA reduce creatinine vs. vehicle in IR-AKI. UPHD186 further decreases creatinine vs. other analogues. UPHD25 and 186 reduce post injury fibrosis after IR-AKI. Treatment with UPHD186 3 days after injury

also reduces serum creatinine and post injury fibrosis after IR-AKI. In UUO, UPHD186, 22, 29 and 36 reduce fibrosis and expression of fibrosis markers. SAHA and UPHD25 are ineffective in this model.

**Conclusions:** The short acting PTBA analogue, UPHD186 with a Class 1 HDAC selective warhead, most effectively ameliorates AKI and reduces post injury fibrosis in IR-AKI and UUO models, and UPHD186 is still effective when administered 3 days after IR-AKI. UPHD186 has a broad range of applications that could be useful for delayed treatment of patients with AKI.

*Funding:* NIDDK Support

## TH-PO053

**Class I Histone Deacetylase Activity Is Required for Proliferation of Renal Epithelial Cells** Shougang Zhuang. *Dept of Medicine, Rhode Island Hospital, Alpert Medical School of Brown Univ, Providence, RI.*

**Background:** The process of renal regeneration after acute kidney injury is thought to recapitulate renal development, and proliferation of renal proximal tubular cells (RPTC) is a critical step in the regenerative response. Recent studies indicate that class I histone deacetylases (HDACs) are required for embryonic kidney gene expression, growth and differentiation. The role and underlying mechanisms of class I HDACs activation in RPTC proliferation, however, remains unclear.

**Methods:** In this study, we used cultured RPTC to examine this issue since four class I HDAC isoforms (1, 2, 3, and 8) are abundantly expressed in this cell type.

**Results:** Blocking class I HDAC activity with a highly selective inhibitor, MS-275, induced global histone H3 hyperacetylation, reduced RPTC proliferation, and diminished expression of cyclin D1 and proliferating cell nuclear antigen (PCNA). Silencing HDAC1, 3 or 8 with siRNA resulted in similar biological effects. Activation of epidermal growth factor receptor (EGFR) and signal transducers and activators of transcription 3 (STAT3) was required for RPTC proliferation, and STAT3 functioned downstream of EGFR. Treatment with MS-275 or knockdown of HDAC1, 3 or 8 suppressed EGFR expressionand phosphorylation, and silencing HDAC1 and 3 also reduced STAT3 phosphorylation. However, HDAC2 down-regulation did not affect RPTC proliferation, or phosphorylation of EGFR and STAT3.

**Conclusions:** Collectively, these data reveal a critical role of class I HDACs in mediating proliferation of renal epithelial cells through activation of the EGFR/STAT3 signaling pathway.

*Funding:* NIDDK Support

## TH-PO054

**Contribution of Thio Group in PTBA Analogues for Potency and Specificity of HDAC Activity Inhibition** Subramaniam Sanker,[1] Lee McDermott,[1] Mark P. De Caestecker,[2] Neil A. Hukriede.[1] *[1]Dept of Developmental Biology and Pharmaceutical Sciences, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Cell and Developmental Biology, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** Acute Kidney injury (AKI) can result in irreversible kidney damage and fibrosis. Currently there are no established post AKI therapies to treat the resultant damage. Based on a zebrafish larval screen we identified that, histone deacetylase (HDAC) inhibitor phenylthiobutanoic acid (PTBA) and analogues initiate embryonic proliferation of renal progenitor cells (RPC) and expand larval kidney field. The methylated PTBA analogue UPHD-25 exhibits an EC-50 value of 800nM. UPHD-25 also activates proliferation of RPCs and enhances renal function following tubule injury in our zebrafish AKI model by inducing de-differentiation and proliferation of proximal tubule epithelial cells. In our mouse AKI models UPHD-25 reduces post-injury fibrosis and increases the regenerative capacity of renal tubular cells.

**Methods:** The Kidney field expansion is assayed by in situ hybridization using lhx1a antisense probes and RPC proliferation by quantification of GFP expression in Tg(lhx1a:eGFP)pt303 transgenic embryos.

**Results:** PTBA analogues target the zinc-dependent catalytic domains of class 1 and 2 HDACs. Bi-dentate $Zn^{++}$ chelators, N-(2-aminophenyl)-4(phenylsulfanyl)butanamide or N-(2 hydroxy phenyl)-4(phenylsulfanyl)butanamide do not significantly enhance the EC-50 values (EC-50 = 600nM). Class I HDACs have a similar binding pocket with small changes in the outer rim where the "thio" group possibly interacts. To understand the contribution of the "thio" group to the potency and specificity of inhibition, we synthesized and tested analogues where the "thio" moiety was shifted over the fatty acid chain in UPHD-188, UPHD-190 and UPHD-193. This "thio" moiety shift results in about 100-fold change in the EC-50 to low nM values.

**Conclusions:** Using this strategy we describe identification of the next generation of PTBA class HDAC inhibitors that enhance RPC proliferation and kidney field expansion at low nM values. This work was supported by NIDDK grants DK069403, DK090770, and DK079307.

*Funding:* NIDDK Support

## TH-PO055

**Lysophosphatidic Acid (LPA) Transactivates Epidermal Growth Factor Receptors (EGFR) via LPAR1/Gαi/o Signaling to Potentiate LPAR2/Gαq/αvβ6 Integrin Dependent TGFβ Signaling and Increase the Production of PDGF-B and CTGF by Proximal Tubule (PT) Cells** Hui Geng,[1] Rongpei Lan,[1] Prajjal Kanti Singha,[1] Pothana Saikumar,[1] Joel M. Weinberg,[2] Manjeri A. Venkatachalam.[1] *[1]U. Texas; [2]U. Michigan.*

**Background:** In cultured PT cells, LPA stimulates the production and secretion of fibrogenic peptides PDGF-B and CTGF via LPAR2-Gαq-αvβ6 integrin mediated TGFβ transactivation; *in vivo*, during fibrosis after ischemia-reperfusion injury (IRI), TGFβ dependent PDGF-B and CTGF production by PT is associated with LPAR2 and αvβ6 integrin overexpression (Am J Pathol. 181:1236, 2012).

**Methods:** We studied LPAR1 mediated PDGF-B and CTGF production by cultured PT cells and investigated the localization of LPA signaling molecules in kidneys after IRI.

**Results:** *In cultured PT cells*, LPA transactivated EGFR via LPAR1-Gαi/o signaling. This was inhibited by LPAR1 shRNA, LPAR1/3 inhibitor Ki16425 or Gαi/o inhibitor pertussis toxin (PTX). After LPAR1 mediated activation, EGFR and downstream MEK-ERK synergized with LPAR2 dependent TGFβ signaling to increase PDGF-B/CTGF production. Conversely, PTX, EGFR inhibitor AG1478 or Erk inhibitor U0126 synergized with TGFβ inhibitor SB431542 to optimally suppress net TGFβ signaling output and PDGF-B/CTGF production, and to promote PT differentiation. *In vivo after IRI*, PT expression of LPAR1, phospho-EGFR and phospho-ERK increased progressively and persisted in "atrophic" tubules associated with fibrosis. Expression of lysophospholipase D (autotaxin), the enzyme producing LPA from precursor lysophospholipids, also increased progressively in a similar time course, localizing in "atrophic" tubules associated with fibrosis.

**Conclusions:** LPAR2 mediated TGFβ transactivation and potentiation of TGFβ signaling through LPAR1 dependent EGFR activation may be a powerful mechanism to stimulate PDGF-B and CTGF fibrogenic peptide production by PT cells. Together with prior work (Am J Pathol.174:1291,2009 & 181:1236, 2012), the findings after IRI suggest that autotaxin mediated LPA production and signaling via LPAR2 and LPAR1 receptors is a candidate autocrine mechanism for sustained TGFβ signaling and PDGF-B and CTGF peptide production by PT in a context of IRI that causes fibrosis.

## TH-PO056

**HMGB1 Is a Novel Target of Sirt1 Deacetylation: Implications for Stress-Induced HMGB1 Translocation** May M. Rabadi, Sandhya Xavier, Michael S. Goligorsky, Brian B. Ratliff. *New York Medical College, Valhalla, NY.*

**Background:** During AKI, alarmins are released by stressed organs as part of the innate immune response to unleash a signaling cascade responsible for systemic inflammation causing potential multi-organ failure. During cellular stress, the alarmin high mobility group box 1 (HMGB1) undergoes nuclear-to-cytoplasmic translocation and release and this requires its acetylation. HMGB1 deacetylation is unclear, although, partially under the control of HDAC. We hypothesized that deacetylase activity of SIRT1 could participate in regulating nuclear retention of HMGB1, modulating the degree of damage signaling initiated by HMGB1 secretion during stress.

**Methods:** Acetylated HMGB1 was immunoprecipitated and incubated it with SIRT1. Proteomic analysis was used to show that SIRT1 deacetylates HMGB1, which in turn may affect its nuclear retention.

**Results:** We immunoprecipitated acetylated HMGB1 and incubated it with SIRT1, revealing that HMGB1 acetylation was decreased by 49% within 60 minutes. Proteomic analysis showed that SIRT1 deacetylates HMGB1 at four lysine residues positioned within the pro-inflammatory and NLS domain of HMGB1 (lysines 55, 88, 90 & 177). I*n vivo*, resveratrol in AKI led to decreased HMGB1 acetylation, nuclear retention, decreased systemic release and improved renal function. Conversely, genetic ablation and/or pharmacological inhibition of SIRT1 in endothelial cells stressed with LPS increased HMGB1 translocation, an effect significantly enhanced by inhibition of SIRT1 activity, as demonstrated using immunocytochemistry and real-time intravital imaging of HMGB1-GFP transfected cells. In contrast, deletion of SIRT1 or its inhibition both led to enhanced translocation and release of HMGB1. Results indicated SIRT1 regulates the acetylation of HMGB1 and its release during basal and pathophysiologic conditions.

**Conclusions:** In summary, our findings 1) establish HMGB1 as a target for SIRT1 deacetylation; 2) increased cytoplasmic translocation and circulating levels of HMGB1 after inhibition of SIRT1 activity; and 3) attenuation of cytoplasmic translocation and renal injury by pretreatment with an activator of SIRT1.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO057

**a(E)-catenin Expression Regulates N-Cadherin and N-CAM Expression and Migration in NRK-52E Cells** Alan R. Parrish, LaNita A. Nichols, Elizabeth A. Borgmann, Xinhui WANG. *Medical Pharmacology and Physiology, Univ of Missouri School of Medicine, Columbia, MO.*

**Background:** The aging kidney is associated with a decreased ability to repair following acute kidney injury. We have shown a loss in expression of a-catenin and N-cadherin in the aging rat kidney and hypothesize that loss of a-catenin expression in tubular epithelial cells may elicit changes that result in the decreased repair capacity. In these studies, we demonstrate that a-catenin and N-cadherin are decreased in the aging kidney, with decreased protein expression detectable as early as 18 months in male Fischer 344 rats. Loss of the proteins is also observed in aged non-human primate kidneys, suggesting that this is not a species-specific response.

**Methods:** In an effort to elucidate alterations due to the loss of a-catenin, we generated NRK-52E cell lines with stable knockdown of a(E)-catenin (C2 cells).

**Results:** These cells exhibited decreased cell-cell adhesion, and increased monolayer permeability. Interestingly, C2 cells had decreased expression of N-cadherin. Increased proliferation in serum, and serum-free, was seen in C2 cells. Using a wound repair model, C2 cells had a deficits in wound repair, due to alterations in cell migration. Analysis of gene expression in the migrating control cells indicated that expression of N-cadherin and N-CAM, was increased during repair. Similar to N-cadherin, N-CAM expression was reduced in C2 cells, and the aging kidney.

**Conclusions:** Taken together, these data suggest that the loss of a-catenin, and subsequent down-regulation of N-cadherin and N-CAM expression, is a mechanism underlying the decreased migration of tubular epithelial cells that contributes to the inability of the aging kidney to repair following injury.

*Funding:* Other NIH Support - NIA

## TH-PO058

**Renal Denervation Prevents Epithelial Cell Cycle Arrest and Interstitial Fibrogenesis after Ischemia Reperfusion Injury**

Jinu Kim,[1] Babu J. Padanilam.[1,2] *[1]Cellular and Integrative Physiology, Univ of Nebraska Medical Center, Omaha, NE; [2]Medicine, Div of Nephrology, Univ of Nebraska Medical Center, Omaha, NE.*

**Background:** Cell cycle arrest and inflammation after renal ischemia reperfusion injury (IRI) contributes to development of tubulointerstitial fibrosis. We previously reported that renal nerves drive tubulointerstitial fibrogenesis in obstructive nephropathy. Here, we hypothesized that renal nerve-derived neuropeptide/neurotransmitter may promote tubular cell cycle arrest and tubulointerstitial fibrosis after IRI in mouse kidneys.

**Methods:** Unilateral ischemia was performed for 30 minutes followed by reperfusion for up to 14 days.

**Results:** IRI induced tubulointerstitial fibrosis demonstrated by collagen deposition and profibrotic protein expression from 4 days after the injury. Kidney inflammation demonstrated by leukocyte influx and proinflammatory protein expression was enhanced after IRI. Kidney denervation at the time of injury or up to 1 d post-injury, however, decreased the proinflammatory and profibrotic response. Furthermore, administration of norepinephrine and calcitonine gene-related peptide (CGRP) in denervated IRI kidneys restored inflammation and fibrosis. Norepinephrine or CGRP-administration increased the number of tubular cells at G2/M phase after IRI. Consistent with the in vivo study, treatment with norepinephrine or CGRP induced G2/M arrest in HK-2 human kidney proximal tubule cells, whereas antagonists against their respective receptors inhibited the induction of cell cycle arrest.

**Conclusions:** These data demonstrate that kidney denervation prevents tubular cell cycle arrest and inflammation to attenuate interstitial fibrogenesis after IRI.

## TH-PO059

**Cell-Specific Role of ERK in Kidney Repair following Kidney Ischemia/Reperfusion Injury**

Hee-Seong Jang,[1] Jee In Kim,[1] Joshua H. Lipschutz,[2] Kwon Moo Park.[1] *[1]Anatomy and Cardiovascular Research Institute, Kyungpook National Univ School of Medicine, Daegu, Korea; [2]Medicine, Univ of Pennsylvania and Philadelphia Veterans Affairs Medical Center, Philadelphia, PA.*

**Background:** Extracellular signal-regulated kinase (ERK) signals have shown to involve in kidney pathogenesis. However, the role of ERK in the repair process after kidney injury remains to be defined. Here, we investigated the role of ERK in proliferation and differentiation of tubular epithelial cells, and proliferation of interstitial cells following ischemia/reperfusion (I/R) injury in mouse kidney.

**Methods:** Mice were subjected to 30 minutes of renal ischemia. Some mice were administered with U0126, a specific inhibitor of ERK, daily during the recovery phase, beginning at 2 days after I/R until sacrifice.

**Results:** I/R caused severe tubular cell damage and functional loss in the kidney. Nine days after ischemia, the kidney was restored functionally even though the fibrotic lesion expanded. ERK was activated by I/R immediately and the activated ERK was sustained for 9 days. U0126 treatment inhibited the proliferation of tubular epithelial cells, basolateral relocalization of Na,K-ATPase and lengthening of primary cilia in tubular epithelial cells, whereas it enhanced the proliferation of interstitial cells, the accumulation of extracellular matrix, and the expansion of interstitial area. Furthermore, U0126 treatment elevated the expression of cell cycle arrest markers. U0126 treatment mitigated the post-I/R increase of Sec10, which is a crucial component of exocyst complex and an important factor in ciliogenesis and tubulogenesis. U0126 treatment also enhanced expression of fibrosis markers, TGF-β1 and phosphorylated NF-κB after ischemia.

**Conclusions:** The results demonstrate that activation of ERK is required for both the restoration of damaged tubular epithelial cells, and the inhibition of fibrosis progression following I/R injury.

*Funding:* Government Support - Non-U.S.

## TH-PO060

**Late Inhibition of Inhibitor of IkB Kinase Attenuates Acute Kidney Injury**

Nimesh Patel,[1] Florence Lilian Johnson,[1] Massimo Collino,[2] Mara Rogazzo,[2] Magdi Yaqoob,[1] Christoph Thiemermann.[1] *[1]The William Harvey Research Institute, Queen Mary Univ of London, London, United Kingdom; [2]Dept of Drug Science & Technology, Univ of Turin, Turin, Italy.*

**Background:** Acute kidney injury (AKI) caused by ischemia-reperfusion injury (IRI) is being increasingly regarded as a risk factor for chronic kidney disease (CKD). Activation of nuclear factor-κB (NF-κB) is known to play a key role in the production of various cytokines and chemokines, and seen to be a significant contributor to injury following IRI. NF-κB is a diverse family of transcription factors that can be activated by IκB kinase (IKK). We hypothesised that the specific inhibition of IKK with IKK16 will aid in the attenuation of renal, glomerular and tubular dysfunction.

**Methods:** Forty-three male Wistar rats underwent a right nephrectomy and unilateral renal ischemia by clamping the left renal artery with non-traumatic vascular clips for 30min. The rats were randomised into 5 groups; sham, control, 0.1mg/kg IKK16, 0.3mg/kg IKK16, 1mg/kg IKK16. IKK16 was administered i.v. 24h after the onset of reperfusion. Twenty-four hours prior to termination of the experiment, rats were placed into metabolic cages for the collection of urine at 48h. The experiment was terminated 48h after the commencement of reperfusion for the collection of serum and urine.

**Results:** When compared to rats subjected to sham-operation, rats subjected to unilateral renal IRI (control) demonstrated a significant increase in serum creatinine, creatinine clearance and fractional excretion of sodium indicating the development of renal, glomerular and tubular dysfunction, respectively. The administration of IKK16 demonstrated a dose dependant attenuation of dysfunction, which was seen to be significant in all 3 parameters when administered at a dose of 1mg/kg 24h into reperfusion.

**Conclusions:** We have shown here, for the first time that the late administration of an IKK inhibitor accelerates the rate of recovery of renal, glomerular and tubular dysfunction. The late inhibition of IKK may, therefore, have therapeutic potential in the recovery of AKI and the prevention of CKD. Further investigations are required to determine the exact role of IKK in the development of CKD.

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## TH-PO061

**The Antidepressant Fluvoxamine Protects against Renal Ischemia/Reperfusion Injury**

Judit Hodrea,[1] Adam Hosszu,[1] Sandor Koszegi,[1] Zsuzsanna Antal,[3] Nora Fanni Banki,[3] Adam Vannay,[2] Laszlo J. Wagner,[4] Attila J. Szabo,[3] Tivadar Tulassay,[2,3] Andrea Fekete.[1,3] *[1]"Momentum" Diabetes Research Group, HAS-Semmelweis Univ, Budapest, Hungary; [2]Research Laboratory for Pediatrics and Nephrology, HAS-Semmelweis Univ, Budapest, Hungary; [3]1st Dept of Pediatrics, Semmelweis Univ, Budapest, Hungary; [4]Dept of Transplant Surgery, Semmelweis Univ, Budapest, Hungary.*

**Background:** The Sigma-1 receptor (S1R) agonist Fluvoxamine (FLU) diminishes heart ischemia/reperfusion (IR) injury through S1R – nitric-oxide synthase (NOS) system. Here we tested the effect of FLU pretreatment on IR survival, renal damage and expression of Sigma-Akt-NOS axis.

**Methods:** Male Wistar rats pretreated with FLU (20mg/bwkg); FLU+S1R antagonist NE-100 (1mg/bwkg; FN); FLU+ non-selective NOS blocker L-NAME (10mg/bwkg); FLU+eNOS blocker L-NIO (20mg/bwkg) or FLU+ nNOS blocker 7-NI (25mg/bwkg) had left renal pedicle clamped for 50 min. followed by 24h of reperfusion (T24) and were compared to vehicle-treated (VEH) rats and shams (n=8/group). We measured renal function, histology, and protein level of S1R, pAkt, peNOS and nNOS. Intrarenal capillary hemodynamics was determined by multiphoton microscopy. Additional groups were followed for 7 day survival.

**Results:** FLU pretreatment improved survival and resulted in less functional and histological kidney damage. IR induced renal vasoconstriction at T24 was ameliorated by FLU vs. VEH and FN respectively. This FLU effect was neutralized both by the S1R inhibitor and all NOS blockers. Protein levels of S1R, pAkt, peNOS and nNOS at T24 were more elevated in FLU vs. VEH and FN.

Moreover at 30 min after pretreatment, FLU produced intrarenal vasodilation in shams without IR. Parallel to this, protein levels of S1R, pAkt and peNOS were increased, while nNOS remained unchanged.

**Conclusions:** The antidepressant FLU improves survival and is protective against postischemic renal damage. This better outcome may be attributed to the S1R-NOS mediated intrarenal vasodilator effect of FLU in a time and NOS isoform specific manner. Fundings: LP2011-008; OTKAPD83431-NK84087/2010; TÁMOP4.2.2.B10/1.

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## TH-PO062

**Nephroprotective Effects of TVP1022 in Experimental Model of Diabetic Renal Ischemic Injury**

Niroz Abu-Saleh,[1] Hoda Awad,[1] Mogher Khamaisi,[2] Ravit Cohen,[1] Samuel N. Heyman,[3] Zaher Armaly,[4] Zaid Abassi.[1] *[1]Technion, Haifa, Israel; [2]Joslin Diabetes Center, Boston; [3]Hadassah Hebrew Univ Hospital, Jerusalem, Israel; [4]Nazareth Hospital, Israel.*

**Background:** Ischemic acute kidney injury (iAKI) in diabetes mellitus is associated with a rapid kidney dysfunction. Both diabetes and iAKI are characterized by increased oxidative stress. TVP1022, non–MAO inhibitor S-isomer of rasagiline, has been shown

to possess anti-oxidative and anti-apoptotic proprieties in experimental models of cardiac and neuronal injuries. The current study examines the effects of TVP1022 and Tempol, on iAKI in diabetic rats.

**Methods:** Control and streptozotocin-injected rats were studied, with unilateral iAKI performed by clamping of the left renal artery for 30 min. Animals were also subjected to Tempol and TVP1022 (10 and 7.5 mg/kg/day, P.O. for 7 days, respectively), or to their vehicles. Urinary flow (V), sodium excretion (UNaV) and glomerular filtration rate (GFR) were determined 48h following iAKI. Renal morphology was assessed in H&E stained slides, and 4HNE immunofluorescent and nitrotyrosine mmunohistochemistry that were carried out in the various experimental groups.

**Results:** As compared with normoglycemic rats, kidney dysfunction was most prominent in the Lt. iAKI kidneys of diabetic vs. Control animals: V reductions (from 19.6±1.6 to 1.5± 0.4 vs. 7.2±1.2 to 5.5±1.5 µl/min, respectively) and GFR (from 1.17±0.056 to 0.026±0.01 vs. 1.16±0.04 to 0.43±0.1ml/min). iAKI induced outer and inner medullary damage (necrosis, congestion and cast formation), which was most prominent in diabetic animals, and provoked renal immunoreactive 4HNE and nitrotyrosin staining in both groups. Tempol and TVP1022 treatment increased GFR by two (0.044±0.003) and five (0.139±0.06) fold in diabetic ischemic kidneys, respectively. Tempol and TVP1022 treatment also decreased nitrotyrosin and 4HNE staining, whereas TVP1022 but not Tempol decreased necrosis and casts in the renal medulla.

**Conclusions:** TVP1022 treatment ameliorates renal dysfunction and histological changes in the iAKI diabetic model, suggesting a role for TVP1022 therapy in diabetic kidney protection under warm ischemic settings.

## TH-PO063

**Formoterol-Induced Mitochondrial Biogenesis Restores Renal Function Post Ischemic-AKI** Sean Robert Jesinkey,[1] Jason A. Funk,[1] Lauren P. Wills,[1] L. Jay Stallons,[1] Judit Megyesi,[2] Craig Cano Beeson,[1] Rick G. Schnellmann.[1,3] *[1]Center for Cell Death, Injury, and Regeneration, Dept of Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC; [2]Medicine, Univ of Arkansas for Medical Sciences, Little Rock, AR; [3]Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC.*

**Background:** At this time there are no drug therapies for acute kidney injury, resulting in high mortality. We hypothesized that stimulation of mitochondrial biogenesis 24 hours (h) after ischemia/reperfusion (I/R)-induced acute kidney injury (AKI) in mice would accelerate recovery of mitochondrial and renal function.

**Methods:** C57BL/6 mice were subjected to either sham or I/R-induced AKI followed by once daily injection (ip) with either vehicle or formoterol, a stimulator of mitochondrial biogenesis (Wills et al., 2012), 24 h after injury for 5 days. Renal function was measured by serum creatinine and renal injury was determined histologically and by measuring renal KIM-1. Mitochondrial injury and biogenesis was determined by measuring the abundance of nuclear- and mitochondrial-encoded mitochondrial proteins and by evaluating state 2 and 3 respiration in isolated mitochondria.

**Results:** On day 6, I/R alone resulted in increased serum creatinine and KIM-1 protein expression, and renal necrosis. Mitochondrial proteins NDUFB8 (nuclear-encoded) and COX1 (mitochondrial-encoded), and state 3 respiration were decreased. Formoterol treatment after I/R mice restored serum creatinine to control levels, and decreased renal necrosis and KIM-1 expression. Mitochondrial protein levels and state 3 respiration returned to control levels.

**Conclusions:** These results represent the first proof-of-principle study of a novel drug therapy to treat AKI by restoring mitochondrial function and accelerating the recovery of renal function after AKI has occurred.

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## TH-PO064

**Disruption of Mitochondrial Homeostasis and Suppression of Mitochondrial Biogenesis in Folic Acid-Induced AKI** Ryan Whitaker, L. Jay Stallons, Rick G. Schnellmann. *Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC.*

**Background:** Recent studies demonstrate that mitochondrial dysfunction is a mediator of AKI development and progression. We demonstrated persistent disruption of mitochondrial homeostasis and inhibition of mitochondrial biogenesis (MB) following I/R-induced AKI. These studies examine the mitochondrial dysfunction associated with folic acid (FA)-induced AKI.

**Methods:** Male CD1 mice were injected with a single IP dose of 250 mg/kg FA dissolved in 300 mM $NaHCO_3$. Sera and urine samples were collected and mice were euthanized at 0, 1, 2, 6, and 14 d. Serum and urine creatinine, and blood urea nitrogen (BUN) were measured using commercially available kits. Kidneys were removed and preserved for histology, qPCR and biochemical analyses.

**Results:** FA caused a rapid decrease in renal function in mice as serum creatinine and BUN levels increased 3-fold and 8-fold over controls at 2 d, respectively. Renal cortical NGAL expression was increased at 1-2 d after FA. All of these markers recovered to near control levels 6 d after FA. In contrast, renal morphology as assessed by PAS staining revealed continued injury through 14 d after FA. Mitochondrial electron transport chain expression and biogenesis remained disrupted 14 d after FA with decreased mRNA expression of PGC-1α, Tfam, NDUFβ8, COX1 and ATPSβ. Protein expression of components of the mitochondrial electron transport chain and transcriptional mediators of MB were decreased out to 6 d, but recovered to control levels by 14 d after FA. Mitophagy and mitochondrial dynamics were also disrupted with changes in PINK1 and mitochondrial fission/fusion proteins.

**Conclusions:** We conclude that mitochondrial dysfunction and disruption of mitochondrial homeostasis are key components of FA-induced AKI. Similar disturbances have been observed in other models of AKI and may represent a conserved mechanism of persistent renal dysfunction. The incomplete recovery of renal morphology after AKI may be linked to a persistent suppression of mitochondrial number and function and restoration of mitochondrial function following AKI may be key to the recovery of kidney function.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO065

**Modulation of the Early Innate Immune Response by Heme Oxygenase-1 in Acute Kidney Injury** Ahmed I. Kamal,[1] Subhashini Bolisetty,[1] Ravindra Boddu,[1] James George,[2] Anupam Agarwal.[1] *[1]Medicine; [2]Cardiothoracic Surgery, Birmingham, AL.*

**Background:** Ischemia reperfusion injury (IRI) is a major cause of acute kidney injury. Heme oxygenase-1 (HO-1) is cytoprotective and anti-inflammatory by regulation of the innate immune response. We hypothesized that HO-1 regulates renal macrophage recruitment, infiltration and phenotype in renal IRI. We utilized HO-1 knockout (KO) mice and wild-type (WT) littermates to avoid nonspecific effects of pharmacologic modulators of HO activity.

**Methods:** Age and sex-matched HO-1 KO and WT mice were subjected to renal IRI by bilateral renal pedicle clamping for 10 minutes. This time was chosen due to increased sensitivity and mortality in HO-1 KO mice subjected to renal IRI at ischemic times of 15 or more minutes. Both kidneys were harvested at 1 or 7 days post IRI. Renal infiltrating cells were analyzed by flow cytometry and immunofluorescence staining.

**Results:** Even with 10 min IRI, 50% mortality was observed in HO-1 KO mice by 7 days. Renal function measured by serum creatinine at 24 hrs showed significantly increased levels in the HO-1 KO IRI group compared to all other groups (1.0±0.3 vs. 0.12±0.01,0.16±0.05 and 0.17±0.02 mg/dL for HO-1 KO IRI,WT sham,WT IRI and KO sham,respectively),(n=4-8,P= 0.025 Kruskal-Wallis test). Renal resident monocytes ($CD45^+CD11b^+MHCII^+Ly6C^-CD62L^-$) were significantly decreased in the HO-1 KO IRI kidneys compared to HO-1 WT IRI and sham groups (7.4±1.05 vs. 29±4.3,34.6±2.9 and 31.1±4.8 % of $CD45^+$ ,P<0.01). There was a significant increase in CDD11b cells (P<0.01), nonresident ($CD45^+CD11b^+MHCII^+Ly6C^+CD62L^+$) macrophages (P<0.01) and neutrophilic infiltration in the HO-1 KO IRI group (P<0.05). At 7 days, the HO-1 KO IRI mice exhibited increased serum creatinine (n=3,P=0.6).

**Conclusions:** Lack of HO-1 resulted in increased sensitivity to IRI and intra-renal infiltration by macrophages and neutrophils suggesting that the exaggerated inflammatory responses are a consequence of HO-1 deficiency. These results underscore an important role for HO-1 in modulating inflammatory responses in acute kidney injury and may represent a key target for therapeutic intervention.

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## TH-PO066

**Interleukin-10 Deficiency Modifies Repair after Ischemic Acute Kidney Injury** Yu Chen,[1,3] Sanjeev Noel,[1] Samatha Bandapalle,[1] Maria Noel Martina Lingua,[2] Abdel Hamad,[2] Lorraine C. Racusen,[2] Hamid Rabb.[1] *[1]Dept of Medicine, Johns Hopkins Univ, Baltimore, MD; [2]Dept of Pathology, Johns Hopkins Univ, Baltimore, MD; [3]Dept of Medicine, Nanjing Medical Univ, Nanjing, Jiangsu, China.*

**Background:** The role of inflammation in repair process following AKI is largely unknown. Previous studies in rats and cultured mouse cortical tubule cells have shown that interleukin-10 (IL-10) treatment protects from ischemic AKI (Deng, Star et al, *Kidney Int*, 2001). In this current study we hypothesized that IL-10 plays an important role in the repair following ischemic AKI.

**Methods:** C57BL/6 wild type (WT) and IL-10 deficient ($IL\text{-}10^{-/-}$) mice underwent unilateral renal pedicle ischemia for 45 minutes followed by reperfusion. Kidneys were harvested at 3 days or 10 days to examine histological, proliferative and inflammatory changes.

**Results:** Ischemic kidneys from $IL\text{-}10^{-/-}$ mice had significantly (p<0.05) less necrotic tubules than WT in the cortex at day 3 (31±4.7 vs 66.8±13.1) and day 10 (1±0 vs 7.3±3.8) as compared to WT mice. Outer medulla showed significantly (p<0.05) less necrosis in $IL\text{-}10^{-/-}$ mice at day 10 as compared to WT mice (12.5±5.9 vs 46.7±18.0). The epithelial cell proliferative marker, KI-67 staining, revealed significantly (p<0.05) less proliferating epithelial cells in $IL\text{-}10^{-/-}$ mice as compared to WT mice at day 3 (314.3±, 25.4 vs 667.1±32.5) and day 10 (84.5±6.5 vs 128.3±8.0). Higher number of kidney mononuclear cells (KMNCs) was found in $IL\text{-}10^{-/-}$ mice as compared to WT mice (15.8±2.0 vs 6.86±1.19), 10 days after ischemic injury. Percent of $CD25^+FoxP3^+$ (Treg) cells in $CD4^+TCR\beta^+$ cells was significantly (p<0.05) lower in $IL\text{-}10^{-/-}$ mice as compared to WT mice (14.3±1.6 vs 25±6.3) at day 10. There were no significant differences in CD4+, CD8+, NK1.1 cells between $IL\text{-}10^{-/-}$ and WT mice either at day 3 or 10.

**Conclusions:** These data demonstrates that IL-10 deficiency leads to abnormalities in kidney structure, epithelial cell proliferation, and leukocyte trafficking in the repair phase of ischemic AKI. While IL-10 has been previously shown to be protective in the initiation phase of AKI, it might have negative consequences during repair.

*Funding:* NIDDK Support

**TH-PO067**

**Hydrogel Delivery of Co-Embedded EPC-MSC for Treatment of AKI and Modulation of Macrophage Proinflammatory Cyto-/Chemokine Release**
Ellen Nadel, Joseph Alexander Zullo, Matthew J. Baskind, Rishi Rajdev, May M. Rabadi, Cameron M. Demaree, Radovan Vasko, Michael S. Goligorsky, Brian B. Ratliff. *New York Medical College, Valhalla, NY.*

**Background:** We have shown that endothelial progenitor cells (EPC) embedded in hydrogels confers improvement in renal function during AKI and promotes angiogenesis. We attempted to improve EPC delivery by co-embedding with renal mesenchymal stem cells (MSC), while examining their paracrine mechanisms that affect proinflammatory macrophages.

**Methods:** A live/dead cell assay determined if EPC-MSC co-embedding improved viability during LPS treatment. EPC-MSC co-embedded in hydrogels were delivered to LPS-induced AKI mice and animals were assessed for effects on short-/long-term blood pressure (BP), renal blood flow (RBF), serum creatinine, proteinuria and angiogenesis (femoral ligation). The cyto-/chemokine release from embedded stem cells was examined including effects on modulating the release of proinflammatory molecules from RAW 264.7 macrophages.

**Results:** Hydrogel co-embedding improved stem cell viability during LPS exposure, an effect augmented by MSC hypoxic preconditioning. Delivery of co-embedded EPC-MSC to AKI mice demonstrated additive improvement (as compared to EPC delivery alone) only in short-term proteinuria, with no statistical differences observed for long-term proteinuria and short-/long-term serum creatinine, BP, RBF or angiogenesis. However, hypoxic preconditioning modestly enhanced the therapeutic efficacy of MSC, as compared to untreated MSC. Incubation of co-embedded EPC-MSC with macrophages significantly blunted the release of proinflammatory cyto-/chemokines (INFγ, TNFα, IL-1α, IL-1β, IL-6) from macrophages treated with 100 uM $H_2O_2$.

**Conclusions:** Hydrogel EPC-MSC co-embedding increased their viability during LPS exposure and improved proteinuria during AKI. However, co-embedding did not provide additive improvement in serum creatinine, BP, RBF and angiogenesis, presumably because EPC delivery alone normalized these parameters during AKI or femoral ligation. Paracrine molecules released from co-embedded EPC-MSC dramatically attenuated the release of proinflammatory cyto-/chemokines from macrophages during stress.

*Funding:* NIDDK Support, Private Foundation Support

**TH-PO068**

**Directional Migration of CXCR4 Gene-Modified Bone Marrow-Derived Mesenchymal Stem Cells to the Kidney Area after Acute Kidney Injury**
Huiling Wang. *Div of Nephrology, Jiming Hospital, Shanghai, China.*

**Background:** Bone marrow-derived mesenchymal stem cells (BMSCs) can migrate to the injured kidney after acute kidney injury (AKI) but with limited efficiency. Here we investigated the effect of CXCR4 overexpression on BMSC migration to the AKI kidney and discussed the possible mechanisms.

**Methods:** CXCR4 gene-modified BMSCs (CXCR4-BMSCs, CXCR4-BMSCs/eGFP) and null-BMSCs (BMSCs/eGFP) were prepared by infecting BMSCs with lenti-CXCR4-eGFP or lenti-eGFP, mice AKI model were divided into 5 groups and sacrificed at 1-14 d after transplantation. Blood indicators,histology, expression of stromal cell-derived factor 1 (SDF-1) and BMSC migration were investigated. Hypoxia/re-oxygenation pretreated renal tubular epithelial cells (HR-RTECs) were prepared to generate AKI in vitro (to culture RTECs first in hypoxia condition for 12 hrs and then in re-oxygenation condition for 12hrs). SDF-1 levels in the HR-RTECs and in the supernatant of HR-RTECs were detected. The chemotaxis experiment was performed using the transwell chamber.he phosphorylation of AKT and MAPK in the BMSCs was also investigated.

**Results:** CXCR4-BMSC transplantation sharply increased the accumulation of BMSCs in the renal tissue, especially at day 3 and day 7. The AKI mice in the CXCR4-BMSCs group showed a greater improvement of renal function and the ATN scoring. HR pretreatment significantly enhanced the SDF-1 protein level in the RTECs and in the culture supernatant. The transwell chamber-based migration assay showed that migration of BMSCs to the HR-RTEC culturing chamber was CXCR4-dependent, and the number of migrating CXCR4-BMSCs was the highest. In addition, the migration could be fully inhibited by AMD3100, a CXCR4-specific antagonist. The migration could also be partly blocked by LY294002 (PI3K inhibitor) and PD98059 (MAPK inhibitor). Phosphorylated Akt and MAPK were increased in the BMSCs co-cultured with HR-RTECs and their expression was the highest in the CXCR4-BMSCs.

**Conclusions:** CXCR4 gene overexpression could enhance BMSC migration to the kidney area after AKI. The SDF-1/CXCR4 axis and the activation of PI3K/AKT and MAPK in BMSCs are the possible mechanisms underlying this function.

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**TH-PO069**

**Deleterious Effects of Fibrate-Treated eEOCs in Acute Ischemic Kidney Injury**
Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology & Rheumatology, Univ Hospital of Göttingen, Göttingen, Niedersachsen, Germany.*

**Background:** Early Endothelial Outgrowth Cells (eEOCs), a major subpopulation of EPCs (Endothelial Progenitor Cells) have reliably been shown to protect mice from acute ischemic renal failure. In addition, different exogenous strategies have been established for improving renoprotective competence of eEOCs in AKI. PPAR-alpha can promote EPC differentiation. Since fibrates act as PPAR-alpha agonists we aimed to analyze consequences of fibrate treatment of eEOCs in murine AKI.

**Methods:** Male, 8-12 weeks old C57/Bl6N mice were subjected to bilateral renal ischemia followed by systemic injection with either untreated or clo-/fenofibrate (CF/FF) pretreated syngeneic murine eEOCs. Cellular consequences of fibrate treatment were evaluated by different in vitro assays (TGF-beta induced eEOC apoptosis/necrosis, eEOC migration, secretion of pro-/antiangiogenic mediators).

**Results:** Administration of CF treated eEOCs dramatically aggravated postischemic kidney dysfunction while FF treatment of the cells neither improved nor aggravated renal function after ischemia. Both substances significantly reduced cellular secretion of proangiogenic VEGF, such effect was more pronounced in the presence of CF. Migration analysis showed a complete CF induced disruption of cultured eEOCs. TGF-beta mediated eEOC apoptosis/necrosis was intesified by FF but not by CF.

**Conclusions:** Fibrates do not act as eEOC agonists in murine AKI. Contrasting to the literature on other cell types, fibrate induced PPAR-alpha activation decreases the functional competence of cultured eEOCs in vitro.

**TH-PO070**

**VEGF Transduced Amniotic Fluid-Derived Stem Cells in the Ischemic Acute Renal Injury in Rats**
Marina Gabriela M. Carvalho Mori Cunha,[1,3] Silvia Zia,[1] Fanny Oliveira Arcolino,[1] Marianne Carlon,[1] Diego Vilibaldo Beckmann,[2] Ney Luis Pippi,[2] Dominguita Luhers Graca,[2] Elena N. Levtchenko,[1] Jan A. Deprest,[1] Jaan Toelen.[1] *[1]KU Leuven, Leuven, Belgium; [2]Universidade Federal de Santa Maria, Brazil; [3]Universidade Estadual do Ceará, Fortaleza, Brazil.*

**Background:** We have shown that hAFSC (human amniotic fluid derived stem cell) has a nephroprotective effect when delivered in rats with renal ischemia and reperfusion (IR) injury, preventing extensive fibrosis as a late complication. The aim of the present study was to evaluate whether the overexpression of VEGF in the hAFSCs improves their nephroprotective effect in the IR models.

**Methods:** hAFSCs were isolated and characterized for immunophenotypic and differentiation properties. A cell line expressing CD24, c-kit and the renal progenitor markers Six2 and Lim1 was transduced with VEGF. IR injury was induced in 30 male 8w-old Wistar rats and treated 6h later via arterial injection. The control group received only vehicle, the naïve group received $1x10^6$ hAFSCs, VEGF+naïve group received $0,5x10^6$ VEGF and $0,5x10^6$ hAFSCs and VEGF group received $1x10^6$ VEGF cells. Serum creatinine and proteinuria levels were measured at 24h, 48h and 2 months after IR injury. Histological analysis was performed to quantify tubular necrosis and hyaline cast formation at 48h and interstitial fibrosis at 2 months. Additionally, the expression of Ki-67, ED-1 and α-SMA was assessed using immunohistochemistry at 48h.

**Results:** We observed improvement of nephroprotective effect only in the VEGF+naïve group. At 48h, we observed significantly less tubulointerstitial injury in the naïve and VEGF+naïve groups, which was characterized by a higher tubular proliferation, lower inflammatory response and inhibition of myofibroblast expression. The VEGF group aggravated the tubulointerstitial injury, renal function and the inflammatory response. The VEGF+naïve group showed less fibrosis at 2 months than the naïve group and the VEGF group showed more fibrosis than control.

**Conclusions:** VEGF-hAFSCs are more nephroprotective than naïve hAFSC when delivered in rats with renal ischemia and reperfusion injury, however it can have a dose dependent toxic effect and aggravate this injury.

*Funding:* Government Support - Non-U.S.

**TH-PO071**

**Human Cord Blood-Derived Endothelial Colony Forming Cells Are Protective in Ischemic Acute Kidney Injury in Mice**
Dylan Burger, Shareef Akbari, David Allan, Rhian Touyz, Kevin D. Burns. *Div of Nephrology, Dept of Medicine, Kidney Research Centre- Ottawa Hospital Research Institute, Univ of Ottawa, Ottawa, Canada.*

**Background:** Endothelial colony forming cells (ECFCs) diminish injury in ischemia/reperfusion (I/R) models, through unknown mechanisms. The purpose of this study was to determine the direct effect of human ECFCs in acute kidney injury (AKI) in mice.

**Methods:** ECFCs were isolated from human umbilical cord blood and expanded in culture. Non-obese diabetic severe combined immunodeficient (NOD-SCID) mice underwent bilateral renal I/R and ECFCs ($10^6$/mouse) were injected via the jugular vein at reperfusion.

**Results:** Renal I/R caused significant increases in plasma creatinine, associated with tubular necrosis and apoptosis, interstitial macrophage infiltration, loss of proximal tubule brush border and renal production of reactive oxygen species (ROS) ($P<0.05$ for all vs sham, n=6-10). Administration of ECFCs decreased plasma creatinine and all indices of tubular injury ($P<0.01$ vs I/R alone). In ischemic kidneys, ECFC treatment increased expression of endothelial nitric oxide synthase (eNOS), suggesting endothelial protection (134% of I/R, $P<0.001$). ECFCs also significantly decreased renal macrophage infiltration (~5-fold vs I/R, $P<0.01$) and ROS production (~3-fold vs I/R, $P<0.05$). In ischemic kidneys after 72 hrs, endothelial cell proliferation was markedly increased, as determined by proliferating cell nuclear antigen (PCNA) staining. By contrast, ECFC treatment completely prevented the increase in endothelial cell proliferation ($P<0.001$). ECFCs were detectable in the blood, kidneys, and other organs 1 hr after infusion, but were absent after 24 hrs, suggesting that they act primarily through released factors.

**Conclusions:** These data indicate that human cord-blood derived ECFCs attenuate endothelial injury in a mouse model of I/R, associated with protection of renal function. The results suggest that human ECFCs may represent a therapeutic strategy for the treatment of ischemic AKI.

## TH-PO072

**Human Antigen-Expressing Rat and Human Marrow Stromal Cells or Human Adipose-Derived Stem Cells Induce an Antibody Response That Abolishes Their Robust Renoprotective Activity in Rats with Acute Kidney Injury** Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Florian Toegel,[3] Christof Westenfelder.[1,2] *[1]Medicine, U of Utah and VA Medical Centers, Salt Lake City, UT; [2]Physiology, U of Utah, Salt Lake City, UT; [3]Brigham and Women's Hospital, Boston, MA.*

**Background:** Our pre-clinical (AJP Renal 2005) and Phase I Clinical studies (Nat Rev Neph 2010) showed that allogeneic rat and human Marrow Stromal Cells (MSCs) effectively protect renal function post IRI Acute Kidney Injury (AKI) in rats and in on-pump cardiac surgery patients. Allogeneic cells do not elicit an antibody response, confirming MSCs' immune privileged properties. The present study tested whether the expression of human antigens such as Alkaline Phosphatase by Fischer344 rat (hPAP F344) rMSCs, by human MSCs (hMSC) or by human Adipose-derived Stem Cells (hASC) elicits an antibody response, and whether the presence of such antibodies affects the kidney-protective efficacy of cells in rats with AKI.

**Methods:** Bilateral IRI AKI was induced in F344s (n=6 /group). Post reflow, rats were infused with hPAP-ratMSC, hASCs, hMSC, or vehicle. At endpoint, renal ourcomes were examined, and sera were FACS analyzed for anti-hPAP and anti-human MSC-or ASC-specific IgG antibodies. Two groups of F344s (n=6 each) received hASC i.p., inducing an antibody response by d 14, at which time IRI AKI was induced, followed by a single infusion of hASCs or vehicle.

**Results:** Vs. controls, all 3 cell types significantly protected renal function and hastened recovery from AKI. Although robust renoprotective actions were observed by d 1-3, within 14 days, each treatment elicited a significant IgG antibody response (57-99%) directed at the infused cell type. Rats inoculated with hASCs and confirmed to have antibodies to hASCs were no longer renoprotected by hASCs when treated with them for AKI.

**Conclusions:** Xenogeneic hMSCs and hASCs are renoprotective in rats. However, the induction or presence of antibodies to xenogeneic antigens (e.g. Fetal Bovine Serum or bovine antigens) could render them unsafe and ineffective clinically, mandating the use of non-animal based culture media in clinical applications.

*Funding:* Veterans Affairs Support

## TH-PO073

**Novel Prostacyclin Analog ONO-1301 (SR-ONO) Ameliorates AKI in a Rat Experimental Contrast-Induced Nephropathy Model** Haruyo Ujike,[1] Yohei Maeshima,[1] Norikazu Hinamoto,[1] Hiroyuki Watatani,[1] Kana Masuda,[1] Katsuyuki Tanabe,[1] Hitoshi Sugiyama,[1] Sakai Yoshiki,[2] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Okayama, Japan; [2]ONO Pharmaceutical Co., LTD., Osaka, Japan.*

**Background:** Contrast-induced nephropathy (CIN) is the third most common cause of acute kidney injury. Multiple factors including renal vasoconstriction, direct renal tubular toxicity, and oxidative stress contribute to tubular damage in CIN. ONO-1301(SR-ONO) is a novel sustained-release prostacyclin analog, possessing thromboxane $A_2$ ($TXA_2$), synthase inhibitory activity.We previously observed the inhibitory effects of SR-ONO on diabetic nephropathy as well as tubulointerstitial injuries in the unilateral ureteral obstruction (UUO) model. In the present study, we studied the therapeutic efficacies of intermittent administration of SR-ONO on renal alterations in the rat experimental CIN model, and examined therapeutic mechanisms.

**Methods:** Male SD rats were divided into four groups (n=10-14/group): sham, control CIN, and SR-ONO-treated CIN groups. CIN was induced by intravenous injection of indomethacin, N ω-nitro-L-arginine methyl ester after uninephrectomy. SR-ONO-treated CIN groups received a single subcutaneous injection of SR-ONO (1 or 3 mg/kg) following the administration of Oypalomin, and animals were sacrificed on Day 1.

**Results:** A single injection of SR-ONO was sufficient to maintain serum concentrations of ONO within the therapeutic range (Day 1). Treatment with SR-ONO resulted in significant improvement of CIN-induced renal dysfunction (BUN and Cr), albuminuria and elevation of urinary NAG. Urinary levels of prostaglandin $E_2$ ($PGE_2$), $PGI_2$ metabolite (2,3-dinor-6-OXO-$PGF_{1\alpha}$) and $TXA_2$ metabolite (11-dehydro-$TXB_2$) were not affected by SR-ONO treatment. SR-ONO treatment significantly suppressed CIN-induced tubulointerstitial injuries (histological score), interstitial ED-1+ monocyte/macrophage infiltration, the elevation of renal mRNA for TNF-a and CCL-2 (real-time PCR) and apoptosis (TUNNEL).

**Conclusions:** These results implicate the potential therapeutic efficacies of SR-ONO in ameliorating tubulointerstitial injuries in CIN.

## TH-PO074

**Protective Effects of Relaxin against Cisplatin-Induced Acute Nephrotoxicity and Subsequent Fibrosis in Rats** Takuya Yoshida,[1] Naoki Ikegaya,[2] Hiromichi Kumagai.[1] *[1]Dept of Clinical Nutrition, Univ of Shizuoka, Shizuoka, Japan; [2]Dept of Medicine, Yaizu Municipal Hospital, Yaizu, Japan.*

**Background:** Cisplatin (CDDP) induced acute kidney injury (AKI) involves pro-inflammatory responses, apoptosis of renal tubular epithelial cells and vascular damage. Furthermore, AKI can increase the risk of developing incident chronic kidney disease (CKD). Relaxin (RLX), a pregnancy hormone, has anti-apoptotic and anti-fibrosis properties. The aim of this study was to investigate the effects of RLX on nephrotoxicity by CDDP.

**Methods:** We investigated the mitigating effects of RLX on AKI induced by CDDP, and then also examined anti-fibrotic effect of RLX on renal fibrosisafter AKI. In the short-term experiments, rats were divided into three groups: 1) control group, 2) CDDP group, 3) CDDP+RLX group. In the third group, RLX was infused at 500ng/ hr via subcutaneous osmotic minipump for 5 or 14 days. CDDP wasadministered intraperitoneally (6mg/kg) after RLX or saline infusion. At 5, and 14 days after CDDP administration, renal function was assessed and kidneys were removed for analysis. In addition, the effect of RLX on renal fibrosis after AKI was evaluated at 6 weeks after CDDP administration as a long-term experiment.

**Results:** In the short-term experiments, CDDP transiently increased plasma levels of creatinine and urea nitrogen with the peak at the fifth day, and RLX provided protection against the increases of creatinine (-32%) and urea nitrogen (-28%). Semi-quantitative assessment of the histological lesions showed marked structural damage and apoptotic cells in CDDP group, those lesions being lessened by RLX treatment (TUNEL:-49%). Overexpression of interleukine-6 observed in the kidneys of CDDP group was reduced in the CDDP+RLX group. In the long-term experiment, the same treatment with RLX significantly reduced renal interstitial fibrosis when compared to CDDP group (-50%).

**Conclusions:** These results suggested that RLX provided protection against CDDP-induced acute kidney injury and the subsequent fibrosis by reducing apoptosis and inflammation.

## TH-PO075

**Activation of G-Protein-Coupled Receptor 40 Attenuates Cisplatin-Induced Apoptosis** Seong Kwon Ma,[1] Soo Yeon Joo,[1] Sang Heon Suh,[1] Chang Seong Kim,[1] Joon Seok Choi,[1] Eun Hui Bae,[1] Jongun Lee,[2] Soo Wan Kim.[1] *[1]Internal Medicine, Chonnam National Univ Medical School, Gwangju, Republic of Korea; [2]Physiology, Chonnam National Univ Medical School, Gwangju, Republic of Korea.*

**Background:** G-protein-coupled receptor 40 (GPR40) plays diverse physiological functions including cellular proliferation and inflammation. We investigated the role of GPR40 in the cisplatin-induced kidney injury.

**Methods:** We determined the protein expression of GPR40 and apoptotic markers in rats with cisplatin-induced kidney injury and cisplatin-treated human renal proximal tubule (HK-2) cells. HK-2 cells were cultured with cisplatin in the absence or presence of GW9508, a selective GPR40 agonist.

**Results:** After cisplatin treatment, the protein expression of GPR40 in the kidney was decreased in association with the increase of serum creatinine levels and the ratio of Bax/Bcl-2 expression. In HK-2 cells, cisplatin treatment increased the number of cells with condensed nuclei, which was ameliorated by GW9508 pretreatment. TUNEL stain showed that the pretreatment of GW9508 ameliorated cisplatin-induced apoptosis. Cisplatin treatment increased the ratio of Bax/Bcl-2 and cleaved caspase-3 expression, which were ameliorated by GW9508 pretreatment. Cisplatin-induced activation of reactive oxygen species/Src/caveolin/epidermal growth factor receptor was counteracted by the pretreatment of GW9508. The pretreatment of GW9508 also counteracted the cisplatin-induced phosphorylation of mitogen-activated protein kinases (MAPKs) and activation of nuclear NF-kB.

**Conclusions:** Activation of GPR40 attenuates cisplatin-induced apoptosis by the inhibition of pro-apoptotic factors, MAPKs and nuclear NF-kB.

## TH-PO076

**5-Aminolevulinic Acid (ALA) Protects against Cisplatin-Induced Acute Kidney Injury via Protection of Mitochondrial Viability and Prevention of Tubular Apoptosis without Compromising Its Anticancer Efficiency In Vitro and In Vivo** Yoshio Terada,[1] Masayuki Ishihara,[1] Kazu Hamada,[1] Tatsuki Matsumoto,[1] Yoshiko Shimamura,[1] Kosuke Inoue,[1] Yoshinori Taniguchi,[1] Taro Horino,[1] Keiji Inoue,[2] Taro Shuin,[2] Koji Ogata.[1] *[1]Dept of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Nankoku, Kochi, Japan; [2]Dept of Urology, Kochi Medical School, Nankoku, Kochi, Japan.*

**Background:** Nephrotoxicity is major limitation in cisplatin(CDDP)-based chemotherapy. 5-Aminolevulinic acid (ALA) is widely distributed, and it is a precursor of tetrapyrole compounds such as heme that is important in energy metabolism. The aim of this study is to evaluate protective role of ALA in CDDP-induced acute kidney injury (AKI). We also evaluated the effects of ALA on anticancer efficiency of CDDP in rats.

**Methods:** We used CDDP-induced AKI rats and cultured renal tubular cells (NRK-52E). We divided four groups of rats control, CDDP only, CDDP+ALA(post);(ALA 10mg/kg+Fe) after CDDP, CDDP+ALA(pre&post);ALA is started 5 days before CDDP.

**Results:** ALA treatments protect CDDP-induced AKI. CDDP increased Cr up to 6.5mg/dl, BUN up to 230mg/dl, ALA significantly reduced these changes (Cr2.1 and 1.8, BUN 55 and 50 in ALA pre and pre&post, respectively). ALA ameliorates CDDP-induced morphological renal damages, and reduced tubular apoptosis evaluated by TUNEL staining and cleaved caspase 3. Protein and mRNA levels of ATP5a, COX-IV, UCP2, PGC-1α in renal tissue were significantly decreased by CDDP, and ALA ameliorates these reduction. Heme Oxigenase (HO)-1 level is induced by CDDP, ALA treatment further up-regulates HO-1 both in vivo and in vitro. The CDDP-induced reduction of mitochondrial enzymes was significantly recovered by ALA+Fe than ALA only in vitro. We evaluated the size of transplantated renal carcinoma to the rat skin, ALA did not change the anti cancer effects of CDDP.

**Conclusions:** These data suggested that the protective mechanisms of ALA in CDDP-induced AKI are via protection of mitochondria and prevention of tubular apoptosis. Also there are no significant effects of ALA on anticancer efficiency of CDDP. Thus, ALA has the potential to prevent CDDP nephrotoxicity without compromising its efficacy.

*Funding:* Government Support - Non-U.S.

### TH-PO077

**Inhibition of Poly (ADP-RIBOSE) Polymerase-1 (PERP-1) Activity Ameliorates Renal Proximal Tubular Epithelial Cells (PTEC) Injury Induced by Cisplatin** Hideaki Yamabe, Michiko Shimada, Reiichi Murakami, Takeshi Fujita, Ikuyo Narita, Yoshiko Shutto, Norio Nakamura. *Dept of Nephrology, Hirosaki Univ School of Medicine, Hirosaki, Aomori, Japan.*

**Background:** Cisplatin is a chemotherapy drug which is widely used to treat cancers. It is platinum-based and inhibits DNA production of cancer cells. However, it is well known cisplatin sometimes injures PTEC and induces acute kidney injury (AKI). PARP-1, which is nuclear protein, plays an important role in not only DNA repair but apoptosis or cell necrosis. This study investigated the effect of PARP-1 inhibitor on PTEC injury induced by cisplatin in vitro.

**Methods:** Human cultured PTEC of 4th passage were used. Cultured PTEC were incubated with cisplatin or cisplatin plus olaparib, which is PARP-1 inhibitor for 24 hours. The cytotoxicity by cisplatin or olaparib was evaluated by LDH release from PTEC. The amount of LDH in the cell supernatants and PTEC lysed by mellitin after removing the cell supernatants was measured. The cytotoxicity was expressed as ratio of the LDH amount in the cell supernatants to total LDH. PARP-1 expression was evaluated by immnofluorescent study using anti-PARP-1 antibody.

**Results:** Cisplatin (5.0-100 μM) injured PTEC for 24 hours incubation. Olaparib (0.1-10.0 nM) ameliorated cell injury induced by cisplatin (control: 37.6±3.04%, 5.0 μM cisplatin: 52.2 ±8.0%, $p<0.001$ vs control, 5.0 μM cisplatin plus 5.0 nM olaparib: 32.0±2.1%, $p<0.001$ vs 5.0 μM cisplatin, 5.0 nM olaparib: 29.1±0.8%). PARP-1 expression was enhanced by 5.0 μM cisplatin and the enhanced PARP-1 expression was inhibited by 5.0 nM olaparib.

**Conclusions:** We demonstrated that PARP-1 inhibitor ameliorated PTEC injury induced by cisplatin and enhanced PARP-1 expression by cisplatin was blocked by PARP-1 inhibitor. These data suggested that PARP-1 inhibitor might be useful for the prevention of AKI induced by cisplatin.

*Funding:* Private Foundation Support

### TH-PO078

**Magnesium Deficiency Worsens and Magnesium Treatment Attenuates Cisplatin-Mediated Renal Cell Apoptosis and Acute Kidney Injury** Malvika H. Solanki, Prodyot K. Chatterjee, Madhu Gupta, Xiangying Xue, Andrei Plagov, Pravin C. Singhal, Christine N. Metz. *The Feinstein Institute for Medical Research, Manhasset, NY.*

**Background:** Cisplatin is an effective chemotherapeutic agent for treating ovarian, bladder and breast tumors. However, >25% of patients experience acute kidney injury (AKI), particularly the elderly with females>males. Magnesium (Mg) deficiency is common among the elderly and because renal Mg handling is impaired by cisplatin, we examined the effects of Mg deficiency and supplementation on cisplatin-AKI.

**Methods:** In vitro: The effect of Mg status on cisplatin-cell death was examined using LLC-$PK_1$ renal epithelial cells. In vivo: Older female C57BL/6 mice (10/group, 10mo old) were fed either normal (100%Mg) or 10%Mg-deficient diets for 2wks and then injected with saline or cisplatin (12mg/kg, i.p.). In addition, one group of Mg-deficient mice was supplemented with $MgCl_2$ in water and $MgSO_4$ s.c. prior to cisplatin. Kidney injury was assessed 48hrs post-cisplatin by blood urea nitrogen (BUN) levels, renal tubular damage (H&E staining), oxidative stress, apoptosis (TUNEL assay) and renal p53 expression (Western Blotting).

**Results:** Cisplatin-induced LLC-$PK_1$ cell death was significantly enhanced by Mg deficiency ($P<0.001$) and decreased by Mg replacement ($P<0.001$). In mice, Mg deficiency elevated BUN levels ($P< 0.05$), renal *Ncf1* and *Bak* mRNA expression ($P<0.05$) and renal cell apoptosis following cisplatin compared to 100%Mg controls ($P<0.05$). Mg deficient mice following cisplatin showed increased oxidative stress, renal damage ($P<0.01$) and enhanced renal p53 expression/phosphorylation compared to controls ($P<0.01$). Mg replacement significantly reversed the damaging effects of cisplatin ($P<0.01$).

**Conclusions:** Mg deficiency significantly exacerbates cisplatin-AKI and promotes renal oxidative stress and apoptosis in older female mice; Mg replacement reverses this effect. Mechanistic studies support the renoprotective role of Mg in regulating p53 expression and activation, a critical pathway involved in cisplatin-induced apoptosis. These findings warrant future studies examining Mg status and aggressive Mg replacement therapy in cisplatin-treated cancer patients.

### TH-PO079

**Remote Ischemic Precondition Provide Renal Protection against AKI** Pu Duann,[1] Ling-Mei Chiang.[2] *[1]DHLRI, Ohio State Univ Medical Center, Columbus, OH; [2]Paediatrics, Chang-Gung Memorial Medical Center, Keelung, Taiwan.*

**Background:** TRIM72 (tripartite motif 72), primarily expressed in muscles, was newly identified in nephron. TRIM72 is capable to form a membrane patch in maintaining the the integrity of plasma lemma. Primary culture of proximal tubule epithelium cells transfected with GFP-TRIM72 showed fluorescence storage in microvessicle and tether to cytoplasma inner plasma membrane. It implied TRIM72 excute its cyto-regenerating role and physiological function in a form of microvesicles traffickling manner. We therefore explore the cell biology of TRIM72 in form of microvessicle and exosome.

**Methods:** Ischemia-reperfusion induced AKI was conducted on Sprague Dawley rats using non-occlusive clamping on renal arteries for 35 minutes followed with reperfusion. Microvessicles (100-650 nm) or exosomes (30-70 nm) were isolated from plasma/urine of rats by differential centrifugation. Remote ischemia preconditioning (RIP) was conducted via alternate femoral arterial non-occlusive clamping. TRIM72-containg microvessicles / exosomes were isolated from kidney or muscle by sucrose gradient fractionation.

**Results:** Western blot analysis revealed both TRIM72-containg microvessicles and exosomes increased after RIP in plasma. The TRIM72 containing microvessicles and exosomes prior to acute kidney injury were recruited to injured nephron and reduced proteinuria. AKI induced prior RIP failed to obtain any beneficial effects. The TRIM72(+)-microvessicles and exosomes released from RIP induced after AKI were not reabsorbed by the injured kidney thereby excreted to urine. Renal brush border isolated by ultracentrifuge-based fractionation showed an abundance of TRIM72 and was further upregulated after AKI. TRIM72(+)-microvessicles isolated from a multitude of RIP-pretreated donor rats were isolated for adoptive transfer. These microvessicles were shown to successfully protect recipients from AKI as seen by reduced proteinuria. Microvessicles from sham-treated donors failed to show any salutary effects.

**Conclusions:** RIP release circulating TRIM(+)-microvesicles and exosomes. It protect host from Ischemia reperfusion mediated AKI. The injury nephron recruited RIP-released TRIM(+)-microvesicles.

### TH-PO080

**Meclizine Preconditioning Protects against Ischemic Kidney Injury** Seiji Kishi,[1] Gabriela Campanholle,[2] Mohit Jain,[3] Craig R. Brooks,[1] Vishal M. Gohil,[4] Takaharu Ichimura,[1] Joseph V. Bonventre.[1] *[1]Renal Div, Brigham and Women's Hospital, Boston, MA; [2]Dept of Nephrology, Univ of Washington, Seattle, WA; [3]Dept of System Biology, Harvard Medical School, Boston, MA; [4]Biochemistry & Biophysics, Texas A&M Univ, TX.*

**Background:** Meclizine, a commonly used anti-histamine, has been identified in a nutrient-sensitized screen for drugs that shift energy metabolism from mitochondrial respiration to glycolysis. Meclizine has cardioprotective and neuroprotective effects in cellular and animal models.

**Methods:** C57BL/6 mice were pretreated with meclizine or vehicle, and subjected to bilateral renal ischemia (30 min) followed by reperfusion (I/R injury). Serum, urine and kidney tissue samples were collected at 24 hours after injury. Experiments in vitro involved cell viability and intracellular metabolite analyses on meclizine-treated kidneys and renal tubular epithelial cells (RPTEC) subjected to chemical hypoxia.

**Results:** Meclizine pretreatment 17hr, but not 3 or 8hr, prior to ischemia protected mice from I/R injury in a dose dependent manner as reflected by reduced BUN, serum creatainine, tubular necrosis, neutrophil and macrophage infiltration. Inflammatory cytokines, IL-1b, IL-6, TNFa, MCP-1, were also decreased. Oxygen consumption of isolated kidney mitochondria pre-treated with meclizine was decreased without increasing energy metabolism pathways and glycolytic enzymes. Meclizine preconditioning of RPTEC decreased LDH release after chemical hypoxia in a dose-dependent manner. NaCN-induced cytochrome C release from mitochondria was inhibited by meclizine pretreatment. Furthremore, meclizine administration increased intracellular phosphoethanolamine. Ethanolamine pretreatment protected against chemical hypoxia-induced cell death.

**Conclusions:** Meclizine pretreatment protects against mouse I/R injury and chemical hypoxia in vitro. Meclizine induces intracellular phosphoethanolamine accumulation which inhibits mitochondrial respiration, downregulates cytochrome c release and promotes cell protection. Meclizine, a drug which has been used extensively clinically without major side effects, may be an important candidate as a pharmacological renal preconditioning agent.

*Funding:* NIDDK Support

### TH-PO081

**Remote Ischemic Pre-Conditioning Mobilizes Mesenchymal Stem Cells and Endothelial Progenitor Cells and Has Therapeutic Potential for Acute Kidney Injury** Jon D. Ahlstrom,[1] Zhuma Hu,[1] Ping Zhang,[1] Christof Westenfelder.[1,2] *[1]Depts of Medicine, Univ of Utah and VA Medical Centers, Salt Lake City, UT; [2]Depts of Physiology, Univ of Utah and VA Medical Centers, Salt Lake City, UT.*

**Background:** Acute kidney injury (AKI) is a common and serious syndrome for which there is currently no effective and approved treatment. One novel therapy for AKI that has shown potential both in animal models and in clinical trials is the use of Remote Ischemic Pre-Conditioning (RIPC). RIPC therapy consists of several brief episodes of ischemia/reperfusion at a remote location such as a limb, where short periods of ischemia are generated with a simple non-invasive procedure. Our laboratory has previously shown in animal models and in a Phase I Clinical Trial that Mesenchymal Stem Cells (MSCs) are a safe and effective therapy for AKI. This study examined the hypothesis that RIPC mobilizes therapeutically beneficial progenitor cells into the circulation.

**Methods:** To evaluate whether or not RIPC increases the numbers of MSCs mobilized into the circulation, male Sprague Dawley rats weighing 200-300 g were randomized into either RIPC or sham procedure groups (n=6 per group). RIPC was performed on both hind limbs with 3 cycles of 4 min ischemia/4 min reperfusion. One day following RIPC,

whole blood was sampled and evaluated by flow cytometry for the relative abundance of MSCs, endothelial progenitor cells (EPCs), and hematopoietic stem cells (HSCs). MSCs were defined as CD45-/CD271+, EPCs as CD45-/CD34+ and HSCs as CD45+/CD34+.

**Results:** At 24 hours following the RIPC procedure, the numbers of circulating MSCs were increased 3.7 fold (from 195±57 to 732±112 MSCs per mL of whole blood, p<0.001), and the numbers of EPCs in circulation were increased 3.8 fold (from 29.5±1.1 to 114.6±14 EPCs per mL of whole blood, p<0.001). RIPC did not significantly change the numbers of circulating HSCs.

**Conclusions:** RIPC significantly increases the numbers of circulating MSCs and EPCs. Since both are known to afford renoprotection post AKI, our data identify an important mediator mechanism that explains, at least in part, whereby RIPC promotes renal repair following AKI.

*Funding:* Veterans Affairs Support, Private Foundation Support

## TH-PO082

**The Water Soluble Triptolide Derivative PG490-88 Protects against Cisplatin-Induced Acute Kidney Injury** Hyun-Jung Kim,[1] Kameswaran Ravichandran,[1] Zhibin He,[1] Danica Ljubanovic,[2] Charles L. Edelstein.[1] *[1]UC Denver; [2]Univ Hospital Croatia.*

**Background:** Triptolide, a traditional Chinese medicine, has anti-inflammatory, anti-proliferative and pro-apoptotic activities in pre clinical studies. As interstitial inflammation and tubular apoptosis are features of cisplatin-induced AKI and Triptolide has anti-inflammatory and pro-apoptotic actions, we determined the effect of the water soluble Triptolide derivative PG490-88 (PG) in a mouse model of cisplatin-induced AKI.

**Methods:** Mice injected with Cisplatin (C), (20mg/kg) develop apoptosis on day 1, ATN on day 2 and renal failure on day 3. Mice were injected with PG (0.5 mg/kg) prior to C.

**Results:** PG protected against renal failure and ATN despite having no effect on tubular apoptosis. SCr (mg/dL) was 0.25 in vehicle (V), 0.23in PG, 1.53 in C (P<0.001 vs. V), 0.77in PG+C (P<0.01 vs. C). BUN (mg/dL) was 30 in V, 26 in PG, 206 in C (P<0.001 vs. V), 136 in PG+C (P<0.05 vs. C). ATN score was 0 in V and PG, 4.3 in C (P<0.001 vs. V), 2.4in PG+C (P<0.01 vs. C). Apoptotic tubular cells (/10 HPF) was 0 in V and PG, 10.3 in C (P<0.001 vs. V), 15.3 in PG+C (P<0.01 vs. V, NS vs. C). Triptolide is known to inhibit pro-inflammatory cytokines and chemokines. IL-1α, IL-1β, IL-6, IL-8, IL-33 and TNFα were significantly increased in C, but unaffected by PG. MAPKpathways are activated in cisplatin-induced AKI and known to be inhibited by PG. On immunoblot and densitometry analysis p-p38was not increased by C norinhibited by PG (P=NS vs. V), p-JNK was 1.6 fold increased by C (P=NS vs. V) and 1.2 fold inhibited by PG (P=NS vs. V) and p-ERK was 3.6 fold increased by C (P<0.0001 vs. V) and 2.0 fold inhibited by PG (P<0.05 vs. V). On densitometry analysis, the p-NFκB/t-NFκB ratio was 10.5 fold increased in the nuclear extracts in AKI (P<0.001 vs. V) and the increase in nuclear extracts was completely inhibited by PG.

**Conclusions:** The protection of PG against cisplatin-induced AKI was associated with a decrease in ERK and NFkB pathways. Inhibition of NFkB in cisplatin induced AKI merits further study.

*Funding:* Other NIH Support - 5R01DK074835-04

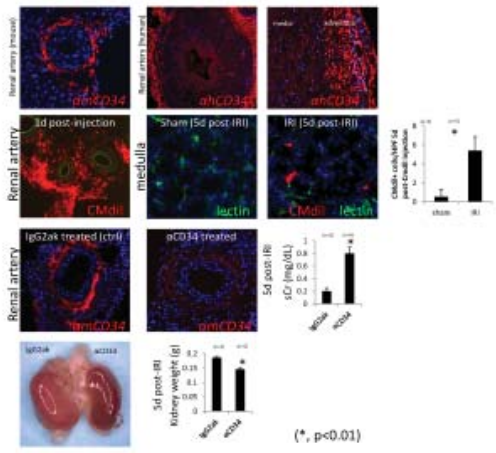
## TH-PO083

**Endothelial Progenitor Cells Exist in the Adventitia of the Renal Artery and Migrate to Repair Kidney Microvasculature** Paul Pang, Padsawan Khamlue, Vanesa Bijol, Andrew M. Siedlecki. *Medicine, Brigham and Women's Hospital, Boston, MA.*

**Background:** Endothelial progenitor cells resident in arterial vessels are defined by the expression of CD34 in the adventitial layer; distinct from mature endothelial cells present in the intima. Presence and function of endothelial progenitor cells in adventitial layer of renal arteries has not been described.

**Methods:** Renal artery tissue was digested and sorted for CD34+ cells. Animals underwent ischemia/reperfusion injury followed by isolation of CD34+ cells from renal arteries 5 days after injury.

**Results:** The adventitia of renal arteries from adult mice and humans contained CD34+ cells.




Mouse renal arteries were digested and cells were sorted (CD34+, CD105-, CD45.2-). Isolated cells formed tube-like structures, and expressed CD34, CD133 but not CD144 or CD31. Cmdil cytotracker was locally injected to the adventitia of the renal artery with care to avoid intra-arterial injection . 5d post-IRI, Cmdil+ cells were present in peritubular capillaries in the outer medulla. Cmdil+ cells were not identified in tubular epithelial cells and were not present in the parenchyma 5 days after sham injury. αCD34 antibody was injected to the same region as above. Compared to IgG2ak control injection, kidneys with αCD34 were decreased in size 5 days after IRI. Serum creatinine was increased on day 5, 0.8mg/dL±0.05 vs 0.2±0.03 ( p<0.01), compared to IgG2ak control injection. By microarray 5d post-IRI, CD34+, CD45.2-, CD105- cells upregulated mir-125, mir-214, and mir-145, mir-532 >2.5-fold (p<0.01) compared to shams and downregulated mir-152 3.9-fold(p<0.01); each of which are associated with tissue repair or cell differentiation.

**Conclusions:** We show that EPCs are present in the adventitia of the renal artery and migrate to the kidney after injury. Further, their deletion delays recovery from IRI.

*Funding:* NIDDK Support

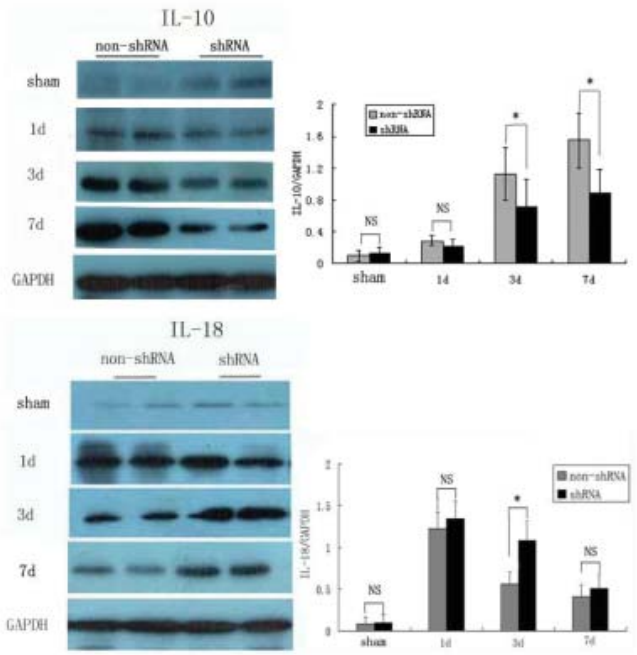
## TH-PO084

**IKKα-Dependent NF-kB p52/RelB Alternative Pathway Enhances Inflammation Resolution in Renal Ischemia-Reperfusion Injury** Qian Zhang, Changchun Cao, Xin Du, Binbin Pan, Qian Zhang. *Nanjing Hospital Affiliated to Nanjing Medical Univ.*

**Background:** Renal ischemia-reperfusion(IR) injury is a major cause of acute kidney injury(AKI). Recent evidence suggests inflammation play an important role in pathophysiology of AKI, which is thought to be regulated by nuclear factor-κB (NF-κB). Activation of NF-κB is dependent on the activation the inhibitor of NF-κB kinase(IKK) β and IKKα . Our previous study indicated targeted silencing of IKKβ prevents renal ischemia injury and inflammation induced by ischemia-reperfusion(IR). While the effect of IKKα and its potential targets are not well understood.

**Methods:** We have examined the role of IKKα in IR injury by shRNA. Mice were divided into four groups: sham-operated**,** IR injury , shRNA-treated and shRNA-treated scrambled. All were subjected to IR injury by bilateral pedicle clamping for 25 min after pentobarbital anesthesia. Application of shRNA or saline started 2 weeks before IR. All mice were sacrificed 24h, 72h and 7d. Serum and kidney tissue samples were collected for further evaluation.

**Results:** Higher blood urea nitrogen, serum creatinine levels and severe renal function were noted in IR injury mice as compared with sham-operated mice. After induction of IR injury, IKKα was increased from 24h to day 7, with a peak on day 3 compared with sham-operated mice. IKKα activation was paralleled by an increase in p52, RelB and IL-10,IL-18. shRNA-tre.ated mice significantly decreased IKKα activity, inhibited p52, RelB, IL-10 and promoted IL-18 at all time points (Figure 1).




**Conclusions:** IKKα-Dependence NF-κB p52/RelB alternative pathway could be activated after IR injury, low-expression of IKKα may block inflammation resolution via down-regulated NFκB alternative pathway family members of both p52 and RelB.

## TH-PO085

**A Mouse Model of Nephrectomy-Induced Renal Repair: An Elegant Tool to Study Renal Regeneration** Benjamin Arthur Vervaet, Nathalie Le Clef, Patrick C. D'Haese. *Biomedicine, Univ Antwerp, Belgium.*

**Background:** Enhanced renal repair is defined as the remarkable repair of an acutely injured kidney upon removal of the healthy contralateral kidney. If the latter kidney is left in place, repair is only marginal and the injured kidney turns fibrotic. The molecular mechanism of this phenomenon is unknown. As most original observations were done in species less accessible to genetic manipulation for mechanistic studies, i.e. dogs and rats, we aimed at optimizing an "enhanced renal repair" model in C57BL/6J mice.

**Methods:** Acute kidney injury was induced by left ischemia/reperfusion (I/R) after which either right nephrectomy (Nx) or mock-Nx was performed 3 days later. Two critical ischemic parameters (i.e. duration of and body temperature during ischemia) were varied. Wild type mice (25g, n=4-6 per group) underwent either 30 min of ischemia at 35, 36 or 37°C or, alternatively, 21, 23, 25 or 30 min of ischemia at 36°C. Control mice underwent mock-I/R and mock-Nx surgery. Mice were euthanized 6 weeks after I/R. Kidneys were weighed and qPCR analysis of the profibrotic genes Col1, Col4, TGFbeta and CCN2 was performed.

**Results:** In the groups undergoing I/R in combination with mock-Nx, no mortality was noted. Of the groups undergoing I/R in combination with Nx, only the mice undergoing 21 min. of ischemia at 36°C survived. Others perished within 48 hours after Nx. In the I/R (21min) without Nx group the median left kidney-to-body weight ratio (mg/g) was 2.8 (range 2.1-3.1), whereas that of its right healthy kidney was 6.7 (range 6.4-7.0). In the Nx group, left kidney-to-body ratio was 6.9 (range 6.0-7.3) and that of its right kidney at the time of Nx 6.5 (range 5.9-7.5). When no Nx was performed, Col1, Col4, TGFbeta and CCN2 were upregulated 18-, 5-, 7- and 3-fold compared to controls, respectively. In case of Nx, this decreased to 5-, 2-, 2-, and 0-fold upregulation, respectively.

**Conclusions:** We clearly demonstrated in C57BL/6J mice that Nx performed 3 days after I/R (21 min, 36°C) is able to strongly attenuate development of renal atrophy and fibrosis. This is indicative of enhanced renal repair and provides a useful tool to study the cell biological mechanisms driving renal regeneration.

*Funding:* Government Support - Non-U.S.

## TH-PO086

**Delayed Administration of a Single Dose of Lithium Promotes Recovery from Cisplatin-Induced Acute Kidney Injury** Hui Bao,[1] Yan Ge,[1] Lance D. Dworkin,[1] Ai Peng,[2] Rujun Gong.[1] *[1]Nephrology, Brown Univ, Providence, RI; [2]Nephrology, Shanghai Tenth People's Hospital, Shanghai, China.*

**Background:** Evidence suggests that glycogen synthase kinase (GSK)3β is a key regulator of tissue injury, repair and regeneration. Lithium, a selective inhibitor of GSK3β and FDA approved first-line mood stabilizer, has been well known for its pro-reparative activities in multiple organ systems. However, its effect on renal repair following acute kidney injury (AKI) remains uncertain.

**Methods:** The role of GSK3β in post-AKI kidney repair was examined in a murine model of cisplatin-induced nephrotoxicity and in cultured renal proximal tubular cells *in vitro*.

**Results:** In a murine model of cisplatin-induced AKI, delayed treatment with a single low dose of lithium accelerated recovery of kidney function, promoted repopulation of renal tubular epithelia and improved kidney repair. This was associated with reduced GSK3β activities and elevated expressions in renal tubular epithelia of pro-proliferative molecules, including Cyclin D1, c-Myc and HIF-1α. In cultured renal tubular cells, GSK3β activity was transiently repressed by cisplatin followed by a prolonged upregulation. Rescue treatment with lithium reduced GSK3β activity, enhanced nuclear expression of Cyclin D1, c-Myc and HIF-1α, and boosted cellular proliferation. Moreover, ectopic expression of a kinase dead mutant of GSK3β enhanced the expression of Cyclin D1, c-Myc and HIF-1α and amplified cellular proliferation following cisplatin injury, reminiscent of the effect of lithium; whereas forced expression of a constitutively active mutant of GSK3β abrogated the lithium's effect, suggesting that GSK3β inhibition is sufficient and indispensable for lithium's pro-reparative effect. Mechanistically, GSK3β colocalized and physically interacted with Cyclin D1, c-Myc and HIF-1α in renal tubular cells. *In silico* analysis revealed that Cyclin D1, c-Myc and HIF-1α all harbor putative GSK3β consensus phosphorylation motifs, inferring a GSK3β directed phosphorylation and subsequent degradation of these molecules.

**Conclusions:** Pharmacological targeting of GSK3β by lithium might represent a novel therapeutic strategy to improve renal salvage after AKI.

*Funding:* NIDDK Support

## TH-PO087

**Resolution of Neutrophilic Inflammation during the Recovery Phase in Ischemia/Reperfusion Injury** Sang-Kyung Jo, Won-Yong Cho, Myung-Gyu Kim, Hyoung-Kyu Kim. *Internal Medicine, Korea Univ Medical Center, Seoul, Republic of Korea.*

**Background:** Although effective clearance of leukocytes seems to be prerequisite for restoration of normal function and structure, mechanisms underlying resolution in IRI are not known. To examine the possible mechanisms of resolution, we hypothesized that infiltrated neutrophils might be removed by processes of 1) apoptosis, 2) reverse transendothelial migration (rTEM) and 3)urinary expulsion via leukocytes-epithelial interaction.

**Methods:** The role of neutrophil apoptosis in resolution and recovery was determined in Bax KO bone marrow chimera in which only bone marrow derived cells are resistant to apoptosis. To examine the role of rTEM, number of circulating ICAM-$1^+$ reverse transmigrated neutrophils, as well as the effect of in vivo blockade of junctional adhesion molecule-C (JAM-C), a negative regulator of rTEM on functional, histologic kidney recovery were determined. Urinary expulsion of neutrophils was examined by flow cytometric detection of Gr-$1^+$ neutrophils in urine and also the effect of MMP-9 inhibitor, that suppress leukocytes-epithelial interaction was tested.

**Results: apoptosis** While initial Scr and neutrophil influx were not different, clearance of neutrophils was significantly delayed in Bax $KO^{BM}$ chimera and it was also associated with delayed functional, histologic recovery, higher tissue cytokines, impaired regeneration and accelerated fibrosis. **rTEM** Number of ICAM-$1^+$ reverse migrated neutrophils increased on day 2 and in vivo blockade of JAM-C resulted in not only increase in circulating ICAM-$1^+$ neutrophils, but also faster resolution of neutrophilic inflammation and functional, histologic recovery. Presence of Tomm-Horsfall protein in circulating neutrophils confirmed that these neutrophils are derived from injured kidneys. **Urinary expulsion** Gr-$1^+$ neutrophils were identified in urines and treatment with minocycline or MMP-9 inhibitor was associated with decrease in urinary MPO, as well as delayed resolution of inflammation, impaired recovery.

**Conclusions:** We suggest that apoptosis, rTEM and urinary expulsion are critically involved in natural resolution mechanisms of neutrophilic inflammation and also in recovery following IRI.

*Funding:* Government Support - Non-U.S.

## TH-PO088

**Activation of the miR-17/106 Family and miR-21 during Murine Kidney Ischemia-Reperfusion Injury** Peter Hamar, Mária Godó Godó, Csaba Revesz, Tamás Kaucsár. *Institute of Pathophysiology, Semmelweis Univ, Budapest, Hungary.*

**Background:** Ischemia induced acute tubular necrosis (ATN) is a main cause of acute kidney injury. MicroRNAs (miRNAs) are posttranscriptional regulators of gene-expression. Our aim was to investigate the miRNA profile and time course of selected miRNAs to characterize the miRNA network activated during recovery from AKI.

**Methods:** At 1, 3, 6, 15, 24 and 48 hours after sublethal (20 min) or lethal (30 min) renal ischemia (I/R) of C57BL/6 mice, renal function (urea, NGAL) and morphology (PAS stain) were assessed. MiRNA profile was evaluated at 24 hours post-ischemia (Luminex multiplex assay) and was further analyzed during reperfusion (real-time PCR). Renal cell proliferation was evaluated by CyclinD1 immunohisto on tissue microarray.

**Results:** Three miRNAs (miR-21, miR-17-5p, miR-106a) were significantly elevated 24 h after I/R (3.0, 1.5 and 1.4 fold, respectively, $p<0.05$). Real-time PCR demonstrated, that these miRNAs started to elevate after 24 hours of reperfusion further increasing at 48 hours (miR-21: 2.3 fold, $p<0.01$; miR-17: 2.2 fold, $p<0.01$; miR-106a, 1.9 fold, $p<0.01$). After sublethal I/R, renal miR-17-5p increased until the 3. day of reperfusion, while the miR-21 increase started later and lasted longer. Interestingly miR-17-5p and miR-21 correlated with each other over the course of recovery from I/R. This correlation was dependent on the severity of ischemia. Histology was pathologic already at 1, blood urea started to elevate at 3, renal NGAL mRNA at 6 and plasma NGAL at 15 hours. CyclinD1 nuclear staining decreased significantly after 1 hour (0.51 fold, $p<0.01$), but returned to control at 48 hours.

**Conclusions:** Our results demonstrated that besides miR-21, the miR-17/106 family is additionally activated during the maintenance and recovery phases of renal I/R. A correlation between these miRNAs warrant further investigations. Cyclin-D1 staining revealed recovery of renal tubular epitehlium. Thus, our results suggest that these miRNAs may be involved in the regeneration processes and could represent possible therapeutic tools in the treatment of I/R. Support: OTKA:K81972.

## TH-PO089

**Activation of Tim-3/Gal-9 Pathway Promotes the Proliferation of Foxp3$^+$ Treg in Mice with Renal Ischemia Reperfusion Injury** Yamei Wang, Yuhong Tao. *Dept of Pediatrics, West China Second Univ Hospital, Sichuan Univ, Chengdu, Sichuan Province, China.*

**Background:** Foxp3$^+$regulatory T cells (Treg) participate in the repair of renal ischemia reperfusion injury(IRI). Other studies have shown that galectin-9 (Gal-9), a beta galactoside binding mammalian lectin, binds to the regulatory molecule T cell immunoglobulin mucin-3 (Tim-3) on T cells and regulates immune responses by increasing Foxp3$^+$ Treg . This study is to investigate whether Tim-3/Gal-9 pathway promotes the proliferation of Foxp3$^+$Treg in mice with renal IRI for the first time.

**Methods:** The left renal pedicle was clamped in C57BL6 male mice for 45 min, followed by reperfusion. Expression of Gal-9 was detected by qRT-PCR, immunohistochemistry staining and Western blot. Tim-3 in kidney mononuclear cells (KMNCs) was determined using qRT-PCR and flow cytometry. The percentage of Foxp3$^+$Treg in CD4$^+$T cells and expression of Foxp3 mRNA were measured with flow cytometry and qRT-PCR, respectively. To explore the influence of Tim-3/Gal-9 pathway on the proliferation of Foxp3$^+$Treg and the protection from renal IRI, recombinant adeno-associated virus(RAAV) carrying Gal-9 was injected into mice two days before kidney IRI surgery in order to overexpress Gal-9 and activate Tim-3/Gal-9 pathway. Then, the percentage of Foxp3$^+$ Tregs, the expression of Foxp3 mRNA and cytokines were evaluated at day 3,10 and 21.

**Results:** The expression of Gal-9 and Tim-3 in the injured kidney at day3,10 and 21 increased significantly compared with that in injured kidney at day 1 or baseline. The percentage of Foxp3$^+$Treg in CD4$^+$T cells and Foxp3 mRNA was upregulated with time. Mice treated with RAAV carrying Gal-9 were significantly protected from renal IRI. Overexpression of Gal-9 decreased the levels of inflammatory cytokines (TNF-alpha and IFN-gamma) and increased the levels of cytokines (IL-10 and TGF-beta) in injured kidney . Furthermore, the proportion of Foxp3$^+$Treg cells and the level of Foxp3 mRNA in injured kidney were significantly higher than those in uninjured kidney or adenovirus group.

**Conclusions:** Tim-3/Gal-9 pathway involves the proliferation of Foxp3$^+$Treg in mice with renal IRI. Gal-9 becomes a potential novel immunotherapeutic target in renal IRI.

*Funding:* Government Support - Non-U.S.

## TH-PO090

**Thrombospondin-1 Mediates ECFC Recruitment after Renal Ischemia-Reperfusion Injury In Vivo** Simon Paul Parmentier,[1] Jan Sradnick,[1] Vladimir T. Todorov,[1] Bernd Hohenstein,[1] Christian Hugo.[1] *[1]Dept of Internal Medicine III, Div of Nephrology, Dresden, Germany; [2]Dept of Internal Medicine III, Div of Nephrology, Dresden, Germany; [3]Dept of Internal Medicine III, Div of Nephrology, Dresden, Germany; [4]Dept of Internal Medicine III, Div of Nephrology, Dresden, Germany; [5]Dept of Internal Medicine III, Div of Nephrology, Dresden, Germany.*

**Background:** Thrombospondin-1 (TSP-1) is an important mediator of renal I/R injury. Induced early on TSP-1 as an anti-angiogenic molecule might not only relate to tubular injury, but could also mediate indirect -progenitor cell driven- effects. Various studies suggest a role of mainly circulating EPC following I/R injury. The present survey investigated the role of TSP-1 in the recruitment of EPCs after I/R in the mouse kidney.

**Methods:** Unilateral I/R (25min) was induced in left kidneys (after right kidney nephrektomy) of 25 C57/Bl6 and 12 TSP-1 deficient mice. Five mice served as healthy controls. Tissues were harvested 4, 24 and 72 hours after I/R and processed for histology. CD34+/Flk-1+/CD133-/CD45-ECFC and CFU were measured using FACS analysis.

**Results:** I/R injury led to profound changes in renal morphology after 72h in TSP-1 deficient and Wt mice. PAS staining showed less tubular damage in TSP-1 deficient mice (by scoring, $p<0.01$). Compared to WT kidneys, ECFCs were significantly decreased in TSP-1 deficient mice after 24 and 72h (24h=$p<0.01$;72h=$p<0.01$), matching histological differences. Surprisingly, differences in renal ECFC numbers could be detected after 4 hours of I/R, which is before the occurrence of major histological changes (TSP-1 -/- vs wt $p<0.05$).

**Conclusions:** TSP-1 deficiency protects from I/R injury. Less This ECFC were recruited, either as consequence or cause of histological differences in TSP-1 -/- vs. WT mice. The early (4h) difference supports a direct link between TSP-1 and progenitor cell activation. Further studies should reveal the concise biochemical interactions.

*Funding:* Private Foundation Support

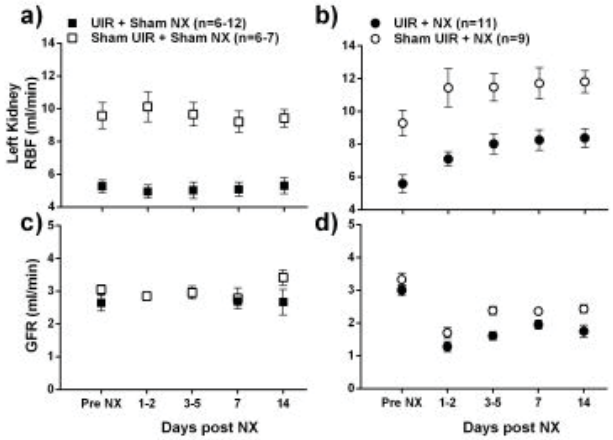
## TH-PO091

**The Hemodynamic Correlates of "Renal Counterbalance" Phenomenon in Conscious Rats** Aaron J. Polichnowski,[1] Hector Licea-Vargas,[2] Rongpei Lan,[3] Maria M. Picken,[2] Jianrui Long,[4] Geoffrey A. Williamson,[4] Karen A. Griffin,[1] Manjeri A. Venkatachalam,[3] Anil K. Bidani.[1] *[1]Medicine, Edward Hines Jr. VA Hospital and Loyola Univ Chicago, Hines, IL; [2]Medicine/Pathology, Loyola Univ Chicago, Maywood, IL; [3]Pathology, Univ of Texas Health Scie Ctr, San Antonio, TX; [4]Electrical and Computer Engineering, Illinois Institute of Technology, Chicago, IL.*

**Background:** In contrast to bilateral ischemia reperfusion (IR) injury, unilateral IR (UIR) results in progressive kidney fibrosis and atrophy. However, contralateral nephrectomy (NX) even 2 wks after UIR results in striking structural and functional recovery (*Circ Res*, 1980, 46:440) through poorly understood mechanisms.

**Methods:** Chronically instrumented male Sprague-Dawley rats (BP radiotransmitters, Transonic renal flow probes) underwent left RBF and 24 hr GFR measurements (FITC inulin, osmotic minipumps) at 2 weeks after 40 min of left UIR or Sham UIR. Both UIR and control rats underwent NX or sham NX at 15 days after UIR. After multiple RBF and GFR measurements, rats were sacrificed at 4 wks after UIR (2 wks after NX).

**Results:** Mean±SE. UIR was associated with a persistent reduction ($P<0.05$) in RBF (Fig 1a). NX increased RBF within 1-2 days in a pattern qualitatively similar to that in sham IR rats (Fig 1b). Although differences in total GFR were not seen in the absence of NX (Fig 1c), the pattern of recovery after NX was similar to RBF (Fig 1d).




Striking beneficial effects ($P<0.05$) of NX were also seen on left kidney weights after UIR (10.4±0.9 vs 4.7±0.3 g/kg without NX). Compensatory hypertrophy was also seen in sham UIR rats (5.7±0.2 vs 4.2±0.2 g/kg), but of lesser magnitude.

**Conclusions:** These data indicate that NX associated hemodynamic improvements occur early and likely play a role in subsequent structural recovery after UIR (*Am J Phys Renal*, 2012, 302:1210).

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support

## TH-PO092

**Tissue Engineering of Microvessels to Perfuse Isolated Glomeruli In Vivo** William Gee Chang,[1] Alessia Fornoni,[2] Jordan S. Pober.[3] *[1]Medicine, Yale Univ, New Haven, CT; [2]Medicine, Univ of Miami, Miami, FL; [3]Immunobiology, Yale Univ, New Haven, CT.*

**Background:** More than 400,000 patients receive treatment for end stage renal disease in the United States. Kidney transplantation is the most effective therapy, but there are not enough donor organs to meet the rising demand. Tissue engineering of a kidney is a potential solution to this organ shortage. However, kidneys are more anatomically complicated than other tissues that have been produced by bioengineering because they contain structures such as glomeruli and tubules that are composed of distinct cell types with distinctive relationships within the vascular system.

**Methods:** Our laboratory has previously reported that human umbilical vein endothelial cells transduced with the anti-apoptotic protein Bcl-2 (designated Bcl-2-EC) can spontaneously organize into perfused microvessels within type I collagen/fibronectin gels when implanted in immunodeficient mice. As a first step in tissue engineering of renal microvasculature, we combined Bcl-2-ECs with microdissected intact whole rat glomeruli in type I collagen gels and implanted them within immunodeficient mice.

**Results:** We observed that rat glomeruli remained viable for up to two weeks, and maintained podocyte specific nephrin staining. Using GFP-expressing rat glomeruli and intravital rhodamine dextran injection, we saw that ~30% of glomeruli were perfused by microvessels derived from Bcl-2-ECs. However, in the absence of Bowman's capsule and tubular outflow, several glomeruli lost their glomerular capillary tuft morphology and became perfused capillary plexi. Transmission electron microscopy revealed endothelial swelling, loss of endothelial fenestrae, and podocyte foot process effacement after two weeks *in vivo*.

**Conclusions:** Anastomosis of capillaries derived from Bcl-2-ECs with isolated glomeruli provides proof of concept that self-assembled microvessels can perfuse specialized organ structures. We hypothesize that addition of tubular outflow would

ameliorate some of the morphological changes observed, and that the tissue engineered constructs described here could be further modified to engineer functional kidney tissue in the future.

*Funding:* Other NIH Support - Clinical and Translational Science Award KL2-RR024138 from the National Center for Advancing Translational Science; National Institutes of Health (NIH) Grants R01-HL085416

## TH-PO093

**Development of the Kidney Peritubular Microvascular Niche *Ex Vivo***

Giovanni Ligresti,[1] Takahide Aburatani,[1] Sijie Sun,[2] Kimberly A. Muczynski,[1] Susan K. Anderson,[1] Jonathan Himmelfarb,[1] Jeremy Stuart Duffield,[1] Ying Zheng.[1] *[1]Dept of Medicine, Univ of Washington, Seattle; [2]Dept of Bioengineering, Univ of Washington, Seattle.*

**Background:** Dysfunction or loss of peritubular capillaries (PTCs) features chronic kidney disease and fibrosis where pericytes (PCs) dissociate from the endothelium and become scar-forming myofibroblasts, which promote the formation of scar tissue. Much remains unknown about the interactions between endothelial cells (ECs) and pericytes in normal and injured kidney, partially because kidney vasculature has complex architecture, flow dynamics and pleiotropic functional properties. Studies are also limited due to the lack of ex vivo models that recapitulate such a biological complexity, particularly in human kidney. In this study we overcome these challenges by engineering a microphysiological system (MPS) for the kidney peritubular microvascular niche ex vivo to study the interactions between Ecs and PCs under varying flow conditions.

**Methods:** We isolated kidney microvascular endothelial cells (KMECs) and pericytes from both mouse and human kidneys by flow cytometry, and seeded them in the engineered MPS.

**Results:** KMECs self-organize into a vascular network resembling kidney blood microvessels. Confocal microscopy shows a continuous endothelial monolayer with CD31 and VE-cadherin staining at cell contacts. Scanning EM reveals that KMECs have a flat cell shape with perforating fenestrations suggesting they preserve their normal function ex vivo. When kidney pericytes are added into the system they migrate to the microvasculature and participate in basement membrane matrix deposition on the abluminal side of the endothelium, whereas KMECs seeded alone into the MPS are unable to secrete basement membrane components. We also showed that the engineered kidney vascular bed is very sensitive to shear with highly aligned ECs and pericytes.

**Conclusions:** We have developed a microphysiological system that recapitulates the kidney perivascular niche ex vivo and allows for the study pericyte interaction wit kidney endothelium. The system should allow identification of key molecular mechanisms that can be exploited for therapeutic purposes in kidney fibrosis.

## TH-PO094

**A Bioinformatics Approach Identifies an Intricate Transcriptional Network Regulating the Uromodulin Gene**

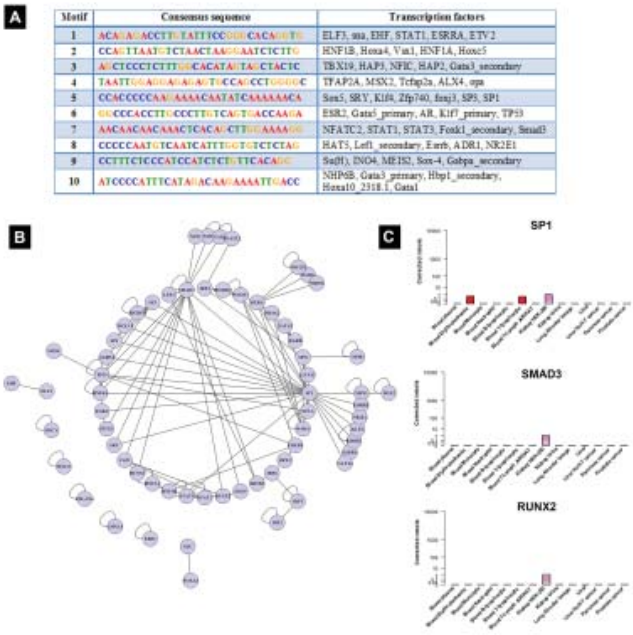
Rajneesh Srivastava,[1] Radmila Micanovic,[2] Sarath Chandra Janga,[1] Tarek M. El-Achkar.[2] *[1]School of Informatics, IUPUI, Indianapolis; [2]School of Medicine, Indiana Univ and Indianapolis VA, Indianapolis.*

**Background:** Uromodulin is a glycoprotein uniquely produced by the kidney. Although its expression is thought to modulate renal homeostasis, the set of sequence specific transcription factors (TFs) which regulate the Uromodulin gene (UMOD) and their upstream binding locations are not well characterized. Our study aims to build a high resolution map of the transcriptional regulation of UMOD.

**Methods:** Phylogenetic foot-printing on the upstream regulatory regions of a diverse set of human UMOD orthologs was performed using MEME-SUITE. This allowed the identification of 10 high confidence conserved binding motifs (BMo) and the corresponding position specific weight matrices. We further analyzed the predicted BMo by TOMTOM, which identified TFs likely to bind these discovered motifs. Predicted TFs were then integrated with existing protein-protein interaction databases like Biogrid and tissue-specific protein expression resources to delineate the important regulators controlling the expression of UMOD.

**Results:** Based on the reliable set of BMo in the upstream regulatory regions of UMOD (Fig. 1a) , we build a high confidence list of transcription factors that could potentially bind to these discovered motifs such as GATA3, HNF 1, SP1, SMAD3, RUNX2 and KLF4. Construction of a manually curated protein interaction network between the predicted TFs controlling UMOD regulatory region in human revealed several highly connected TFs such as SP1 and SMAD3 and the likely protein complexes formed between them (Fig. 1b). The significant expression of these in Kidney cells compared to other tissues suggests a central role in controlling UMOD expression (Fig. 1c).

**Conclusions:** Our findings will form a roadmap for understanding the regulation of Uromodulin expression in health and disease.




*Funding:* Veterans Affairs Support

## TH-PO095

**The Shape of the Curve of Protein Concentration as a Function of Distance across the Glomerular Basement Membrane: Analysis and Implications for Fundamental Mechanisms**

Douglas L. Somers.[1,2] *[1]Univ of Iowa, Iowa City, IA; [2]Iowa City VA, Iowa City, IA.*

**Background:** Fujigake et al (KI 1993) measured protein distribution across the Glomerular Basement Membrane (GBM) using immuno-gold labeling of in-situ fixed glomeruli. Transferrin concentration declines steeply from endothelial to epithelial interfaces, whereas albumin concentration increases to a peak (albumin peak anomaly) partway through the GBM, then declines steeply to low concentrations at the epithelial interface. These results also demonstrate the podocyte paradox -podocytes are important to prevent proteinuria, but proteins never get to the podocyte. No model of GBM protein transport has yet explained the variation between different proteins, the steepness of protein concentration decline, the podocyte paradox, nor the albumin peak anomaly.

**Methods:** Protein concentrations are calculated from probability distribution functions. Assumptions include: i. Endothelial glycocalyx (EG) is the initial barrier to protein translocation, providing an energy trap for some proteins (i.e. albumin), but not others (i.e. transferrin), ii. Proteins escape from EG to GBM with a thermal energy distribution, but those proteins with binding to EG are emitted with less energy, and iii, proteins emitted from the EG travel a certain distance (depending on emission energy) into GBM under constant epithelial-towards-endothelial acceleration, so turning around and returning to the EG.

**Results:** This model reproduces the variation between different proteins, the albumin peak anomaly, and the sharp decline in concentration toward the epithelial interface. Protein deceleration in the GBM represents a decrease in protein momentum. There are few realistic physical mechanisms available to change protein momentum; momentum transfer from a different molecular species emitted from the podocyte provides a force and resolves the podocyte paradox.

**Conclusions:** The results of Fujigake et al suggest: i. Endothelial glycocalyx is the initial protein barrier in the glomerular capillary wall for some, though not all, proteins, ii. The glomerulus appears to be a diffusion pump.

## TH-PO096

**Clearance and Hemocompatibility Assessment of a Parallel Plate Mini-Scale Dialyzer Based on Silicon Nanopore Membranes**

Steven Kim,[1,2] Zohora Iqbal,[2] James A. Heller,[2] Eun Jung Kim,[2] Rishi Kant,[2] William Fissell,[3] Shuvo Roy.[2] *[1]Nephrology, UCSF; [2]Bioengineering, UCSF; [3]Nephrology, Vanderbilt Univ.*

**Background:** Silicon nanopore membranes (SNM) have previously shown a remarkable capacity for hemofiltration. Their compact geometry and uniform pore size distribution offer potential advantages over polymer hollow fiber membranes. To assess these advantages for hemodialysis, we designed a prototype parallel plate mini-scale dialyzer and evaluated its clearance and hemocompatibility.

**Methods:** Computational fluid dynamics was used to refine a parallel-plate design (4.2*w* x 7.6*l* x 1*h cm*) for enhanced blood flow and minimal pressure drop, and subsequently machined from titanium. Polyethylene glycol (PEG)-coated SNM with 4.4 nm pores and effective surface area of 3.46cm$^2$ were assembled in a parallel-plate arrangement. Artificial serum (PBS with Cr: 9mg/dL, BUN: 88mg/dL, alb: 3g/dL) or heparinzed porcine blood was

recirculated (40ml), while dialysate (140mEq NaCl) was recirculated in a counter-current fashion (40ml). Dialysis was performed with zero transmembrane pressure at $Q_d \sim Q_b \sim 30$ml/min. Solute clearance (K) was calculated by fitting concentrations measured hourly for 4hr (n=3) to an exponential decay function: $C(t)=C_i e^{-Kt/V}$. C(t): concentration at time $t$ ,$C_i$: initial concentration, t: time, V: volume. Platelet adhesion and activation on SNM were evaluated by immunohistochemistry (IHC) after flowing heparinized porcine blood (<1hr post-collection) for 4 hours at 5ml/min.

**Results:** The mini-dialyzer was tested without complications for a total of ~24 hours and exhibited ~4 mmHg pressure drop. Creatinine and urea clearances were calculated to be 49.4±1.9, 73.0±2.2 ml/min/m$^2$ (artificial serum) and 38.6±0.7, 67.2±1.5 ml/min/m$^2$ (blood), respectively. There was no significant change in albumin. Diffusion of solutes into dialysate was confirmed. Single-pass changes were below the level of detection. IHC showed a qualitative reduction in platelet activation on PEG-coated SNM and no thrombi were visualized after 4 hours.

**Conclusions:** These preliminary results confirm that SNM exhibit clearance consistent with mathematical models, while PEG coatings offer a hemocompatible interface for blood flow.

*Funding:* NIDDK Support, Other NIH Support - NIH T32 Training Grant; NIH Quantum Grant

## TH-PO097

**Multi-Detector Computed Tomography to Identify Device Defects and Failure Modes in an Implantable Silicon Hemofilter** Steven Kim,[1,2] Olufoladare G. Olorunsola,[3] Charvi Shetty,[3] James A. Heller,[2] Zohora Iqbal,[2] Rishi Kant,[2] Steven Hetts,[3] Maythem Saeed,[3] William Fissell,[4] Youngho Seo,[3] Mark Wilson,[3] Shuvo Roy.[2] *[1]Nephrology, UCSF; [2]Bioengineering, UCSF; [3]Radiology, UCSF; [4]Nephrology, Vanderbilt.*

**Background:** New innovations in membrane technology using microelectromechanical systems techniques have led to the development of a silicon nanopore membrane (SNM) based hemofilter for use in an implantable bioartificial kidney. Such a device will require non-invasive imaging methods to monitor device functionality, identify potential problems, and detect failure modes. This study aims to address these challenges by developing imaging techniques to non-invasively visualize a hemofilter prototype.

**Methods:** The hemofilter was constructed with a parallel plate design (3.8*w* x 1.8*h* x 8*l* cm). A titanium plate measuring 3.8*w* x 0.3*h* x 6.5*l* with a blood path height of 2.4mm was mounted with SNM. A bottom plate without SNM served as an internal control. The non-blood-contacting plates were constructed from aluminum. A peristaltic pump infused iodinated contrast through the blood paths (1-20ml/min) and deionized water through the filtrate path (h=0.2mm). Serial imaging was performed using multi-detector computed tomography (MDCT) before and during contrast infusion. Images (140kVP & 250mA) were acquired in a plane parallel to the direction of flow and reformatted in orthogonal planes.

**Results:** Several potential device-related malfunctions were correctly identified using MDCT. Qualitative assessment of contrast in the blood path showed areas of inhomogeneous flow. Trapped loculated gas was easily visible and allowed for localization and tracking without disassembly. Microscopic leaks were detectable, manifesting as active extravasation of contrast from the device. Finally, membrane fracture leading to device failure was also confirmed using MDCT.

**Conclusions:** Despite the challenges of imaging a predominately metallic object, we were able to non-invasively image flow and identify device defects within a hemofilter prototype. The imaging method allows for internal evaluation of the device without requiring disassembly, which will be invaluable in future studies for the development of the bioartificial kidney.

*Funding:* Other NIH Support - T32 Training Grant; NIH RO1 Biocompatibility of Implantable Renal Replacement Devices

## TH-PO098

**Therapeutic Plasma Exchange Using High Cut-Off Membrane Plasma Separator** Atsushi Ohkubo,[1] Naoki Kurashima,[1] Ayako Nakamura,[1] Satoko Miyamoto,[1] Hiroshi Seshima,[1] Soichiro Iimori,[2] Naofumi Yui,[2] Tatemitsu Rai.[2] *[1]Dept of Clinical Engineering, Tokyo Medical and Dental Univ, Japan; [2]Dept of Nephrology, Tokyo Medical and Dental Univ, Japan.*

**Background:** Therapeutic plasma exchange (PE) using albumin solution as replacement fluid is a treatment of choice for acute exacerbation of inflammatory demyelinating disorders of the central nervous system (CNS-IDDs). Though it is important to remove IgG for treatment, coagulation factors are also potentially removed by this modality. Recently, a newly designed high cut-off membrane plasma separator with smaller pore size have become available. The purpose of this study is to evaluate the efficacy of high cut-off membrane plasma separator by investigating the removal rate of IgG and coagulation factors during treatment by PE with albumin solution replacement.

**Methods:** A retrospective analysis of patients treated with PE with albumin solution replacement between May 2011 and April 2013 in our hospital was performed. They were either treated with the high cut-off membrane plasma separator (high cut-off group) or the conventional type of membrane separator (control group). Seven and nineteen patients were assigned to the high cut-off and control groups, respectively. The removal rates of IgG and coagulation factors were compared during the PE procedures. Data were expressed as group means ± standard deviations and a *p* value under 0.05 was considered statistically significant.

**Results:** The amount of plasma replaced was significantly larger in the high cut-off group compared with that in the control group (1.21 ± 0.01 vs 0.87 ± 0.09 plasma volume, respectively). The removal rates of IgG showed no significant differences between the two groups (high cut-off group 55.9 ± 5.6 % vs control group 65.0 ± 4.7 %). The removal rate of Fib was significantly lower in the high cut-off group (high cut-off group 19.2 ± 8.0 % vs control group 62.8 ± 10.9 %).

**Conclusions:** High cut-off membrane plasma separator with smaller pore size is equally effective in removal of IgG while retaining coagulation factors compared to the conventional type of membrane separator in the treatment of CNS-IDDs by PE with albumin solution replacement.

*Funding:* Government Support - Non-U.S.

## TH-PO099

**Protein Bound Toxin Removal by Increased Ionic Strength Hemodiafiltration** Eric Devine,[1] Horst-Dieter Lemke,[1] Joachim Jankowski,[2] Christoph Wanner,[3] Detlef H. Krieter.[3] *[1]eXcorLab GmbH, Obernburg; [2]Dep. of Medicine IV (CBF), Charité-Universitätsmedizin, Berlin; [3]Nephrology, Univ Hospital, Würzburg, Germany.*

**Background:** Protein bound uremic toxins (PBT) are poorly removed by dialysis. Aim was to develop a strategy based on ionic strength modification in plasma to enhance PBT removal.

**Methods:** In *ex vivo* pre-dilution hemodiafiltration (pre-HDF) using freshly donated human blood and substitution fluid differing in NaCl content, the hemocompatibility of 0.15, 0.36, and 0.50 M NaCl concentration in blood ($[NaCl]_{blood}$) was assessed during 120 min. Two different setups of two serial dialyzers (PUREMA®) in pre-HDF also were tested: i) standard infusion and increased [NaCl] in dialysate of the 1st filter (pre-SDial); ii) transmembrane infusion through the 1st dialyzer of substitution fluid increased in [NaCl] (TM-SDial). The removal rate (*RR*) of indoxyl sulfate (IS) was assessed with the same setups over 30 min in conditioned (100 µM IS) human plasma (n=3) at 0.15 and 0.50 M [NaCl]. Animal hemocompatibility experiments were performed in 4 sheep applying pre-HDF (0.36 and 0.45 M $[NaCl]_{blood}$) and TM-Sdial (0.36 and 0.60 M $[NaCl]_{blood}$) randomized and crossover during 90 min after a 30 min baseline period at physiologic $[NaCl]_{blood}$.

**Results:** Not till $[NaCl]_{blood}$ was 0.50 M, significant hemolysis (free Hb 17.6±7.2 and 2.1±1.6 %; P<0.05) and a decrease of the white blood cell count (WBC; 57.7±20.4 and 52.3±12.8 %; P<0.05) were noted at 120 min with both pre-HDF and pre-SDial, resp. Less cell damage was seen with TM-SDial. Red blood cell (RBC) and platelet (PLT) counts, complement C5a and thrombin-antithrombin III concentrations were always unchanged. Independently of the setup, the *RR* of IS was significantly higher at 0.50 M vs. 0.15 M [NaCl] (18±1 vs. 13±1 %/run; P<0.001). In sheep, free Hb and LDH concentrations at 120 min were increased only with pre-HDF at 0.36 M and TM-SDial at 0.60 M $[NaCl]_{blood}$ compared to baseline (range 0.12-0.14 vs. 0.18-0.22 % and 449-462 vs. 490-506 U/L, resp.; P<0.05). RBC, WBC, PLT and blood pressure were constant. No adverse events were observed.

**Conclusions:** Ionic strength HDF enhances the removal of PBT and can be safely applied at the conditions studied.

*Funding:* Government Support - Non-U.S.

## TH-PO100

**Toxicity of Bisphenol A in Hemodialysis: *In Vitro* Study** Mauro Neri, Jeong Chul Kim, Grazia Maria Virzì, Alessandra Brocca, Francesco Garzotto, Claudio Ronco. *Nephrology Dep-IRRIV, Vicenza, Italy.*

**Background:** Bisphenol A (BPA) is an environmental hormones or endocrine disrupting molecular compound. It is used in the production of polycarbonate (PC) housing (hous.) and polysulfone (PS) membranes of many hollow-fiber hemodialyzers.

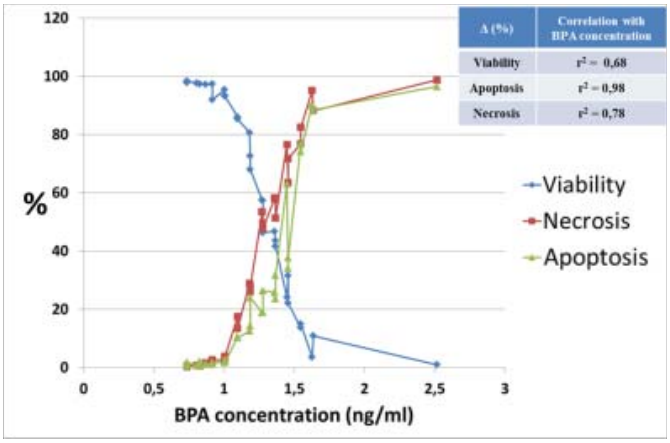
In our *in vitro* study we compared the BPA elution from 3 hemodialyzers and the BPA cytotoxic effect on a monocyte cell line.

**Methods:** We considered these dialyzers: Nipro Elisio 17H (Polypropylene hous., Polynephron membranes), B-Braun Diacap (PC hous., PS membranes), Nipro Elisio 170H (PC hous., Polynephron membranes). We circulated 600ml of cell culture media for 4 hours through dialysis circuit. For each dialyzer, we measured the eluted BPA mass through ELISA kit. By cytometer we evaluated the effect of BPA between viability, necrosis and apoptosis of monocytes incubated for 24 hours in samples taken before and after the treatment. We measured the correlation between BPA concentration and its toxicity on cells.

**Results:** The results are summarized as follow (Median (IQR))

| Dialyzer | BPA eluted (ng/treatment) | Δ% Viability | Δ% Necrosis | Δ% Apoptosis |
|---|---|---|---|---|
| Elisio 17H | 83.2 (42.9,123.4) | -0.93 (-0.95,-0.90) | 0.72 (0.33,1.11) | 0.61 (0.53,0.69) |
| Diacap | 255.4 (255.3,255.5) | -12.83 (-13.30,-12.36) | 2.52 (2.49,2.56) | 2.34 (2.27,2.41) |
| Elisio 170H | 230.1 (228.3,232.0) | -12.37 (-13.2,-11.56) | 2.33 (2.26,2.40) | 2.45 (2.43,2.48) |

Elisio 17H released less BPA than the other dialyzers: the viability of the monocytes is higher, necrosis and apoptosis are lower. The BPA citotoxicity is evident in the correlation curve with viability, necrosis and apoptosis:




**Conclusions:** Viability, necrosis and apoptosis of monocytes are influenced by BPA concentration. Use of alternative polymers for dialyzer's components may reduce BPA elution during dialysis. However, more experiments are needed to confirm these results.

*Funding:* Pharmaceutical Company Support - Nipro

## TH-PO101

### Monitoring Intravascular (Vb), Interstitial (Vi), and Intracellular (Vc) Volume Changes during Hemodialysis (HD) in Patients (pts) with End-Stage Renal Disease (ESRD) in Chronic Out-Patient (CU) and Acute In-Patient (AU) Unit Settings

Susie Q. Lew,[1] Manuel T. Velasquez,[1] Julian M. Stewart,[2] Leslie Montgomery,[3] Wayne A. Gerth,[3] Richard W. Montgomery.[3] *[1]Medicine, George Washington Univ, Washington, DC; [2]Medicine, New York Medical College, Valhalla, NY; [3]LDM Associates, San Jose, CA.*

**Background:** A multi-frequency electrical impedance spectroscopic (EIS) technique was developed that enables non-invasive measurement of compartmental volume (CV) changes. The aims: 1) to evaluate the feasibility & precision of EIS to quantify acute changes in Vb, Vi, & Vc during HD, & 2) to assess differences in CV changes in relation to the total amount of fluid removed (Vt) in CU & AU.

**Methods:** Using EIS, we evaluated changes in Vb, Vi, & Vc in ESRD pts in CU & AU. Each HD was completed according to the nephrologist's prescription. Volume changes were obtained by analysis of electrical impedance spectra taken on the left calf of each pt at approximate 1-min intervals throughout each HD by a quadrapolar EIS device as previously described. (Montgomery, et al:, *Med. Biol. Eng. Comput* 3 April 2013).

**Results:** Table 1 shows the mean volumes of fluid removed during HD from Vb, Vi, Vc, & Vt in CU & AU. Table 2 shows the mean pre & post HD CVs & the associated % distributions in CU & AU. A negative value indicates a gain in volume.

| Table 1 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Vb (%) | Vc (%) | Vi (%) | Vt (%) | Vb (mL) | Vc (mL) | Vi (mL) | Vt (mL) |
| CU (n=53) | | | | | | | |
| 12 +/-8 | -11 +/-12 | 30 +/-13 | 9 +/-5 | 96 +/-65 | -44 +/-52 | 81 +/-44 | 133 +/-70 |
| AU (n=22) | | | | | | | |
| -21 +/-73 | -6 +/-13 | 15 +/-20 | 1 +/-4 | -4 +/-58 | -51 +/-81 | 72 +/-81 | 16 +/-46 |
| Table 2 | CU | | | AU | | | |
| | Vb | Vc | Vi | Vb | Vc | Vi | |
| mL pre | 717 | 407 | 270 | 379 | 654 | 425 | |
| | 51% | 29% | 19% | 26% | 45% | 29% | |
| mL post | 621 | 451 | 189 | 383 | 705 | 353 | |
| | 49% | 36% | 15% | 27% | 49% | 25% | |

**Conclusions:** Summary: 1-The patterns of CV changes differed between CU & AU. 2-AU pts retained a larger % of total fluid in the Vi & Vc compartments. 3-Vc increased during HD in pts at both centers. The EIS technique was able to track acute fluid volume changes in the 3 principal fluid compartments of ESRD pts' calves during HD. Results indicate that Vc in the monitored calves tended to increase during HD at both centers as overall calf volumes were being reduced.

*Funding:* Other NIH Support - SBIR

## TH-PO102

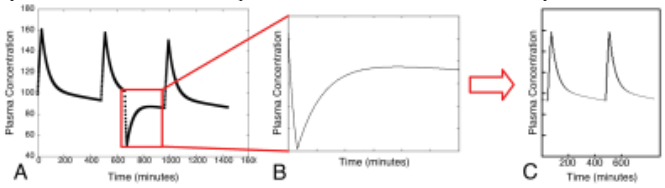
### Use of the Hemodialysis Procedure to Measure Individual Pharmacokinetics

Rueben Banalagay,[1] Don Mitchell Wilkes,[1] William Fissell.[2] *[1]Electrical Engineering, Vanderbilt Univ, Nashville, TN; [2]Nephrology and Hypertension, Vanderbilt Univ, Nashville, TN.*

**Background:** Hemodialysis is an underappreciated opportunity to assess individual pharmacokinetics. As dialysis is started and stopped, drug clearance suddenly changes, disturbing drug equilibration between pharmacokinetic compartments. Individual response to this perturbation reveals individual pharmacokinetics. Here, we utilize linear systems theory to create individualized pharmacokinetic models from intradialytic data.

**Methods:** A simple two compartment pharmacokinetic model was simulated, incorporating a patient receiving regular drug doses and one dialysis session. Equally timed samples of the plasma concentration and dialysate during the transient response of the dialysis perturbation were extracted from the dataset. The samples were used to estimate a linear systems model of the patient. The model was then excited with a signal representing the original dosing schedule of the patient. The resulting output signal was compared with the original dataset to assess prediction accuracy. In addition, the effect of decreasing the sample size and adding varying levels of white Gaussian noise were studied to evaluate the robustness of our approach.

**Results:** In ideal cases of large sample sizes with little noise, the produced linear systems model was shown to reproduce the simulated data almost exactly.




In simulations of more real-world scenarios, robust estimates in the presence of noise were still achievable with as few as 10 samples.

**Conclusions:** Our simulations suggest that the dialysis procedure can be used to create individualized pharmacokinetic models of the patient. By reformulating the effect of dialysis in the language of linear systems, one could easily characterize a particular patient's pharmacokinetics and make reasonable predictions of the patient's drug levels over time.

## TH-PO103

### Ion Exchange Resin and Conductivity Measure Approach for Sodium Concentration Measurement during Dialysis (HD): In Vitro Experiments

Emanuele Mambelli,[1] Andrea Tura,[2] Stefano Sbrignadello,[2] Giovanni Pacini,[2] Antonio Santoro.[1] *[1]Nephrology Dialysis and Hypertension Unit, Policlinico S. Orsola-Malpighi, Italy; [2]Institute of Biomedical Engineering, National Research Council, Padova, Italy.*

**Background:** The measurement of sodium concentration is important to preserve the HD patient from possible events of hypo- or hyper-natremia during the dialysis session. Usually, the sodium measurement is performed by direct or indirect potentiometry, or by flame photometry, through laboratory equipment which is typically expensive, and also requires intervention of the clinical personnel to perform the measurement. Thus, continuous and automatic sodium measurement during dialysis is not possible with that approach. We propose a new method, where the conductivity measurement occurs after treatment of the ultrafiltrate through a ion-exchange resin.

**Methods:** We performed some in vitro experiments. We prepared 40 ml sodium chloride (NaCl) samples at 280, 140, 70, 35, 17.5 mEq/l, and some "mixed samples", i.e., with added potassium chloride (KCl) at different concentrations (4.375-17.5 mEq/l), to simulate the confounding factors in a conductivity-based sodium measurement. We measured the conductivity of all samples. Afterwards, each sample was treated for 1 min with 1g of DOWEX G-26 cation exchange resin, and conductivity was measured again (figure1).

| NaCl → KCl ↓ | 280 | 140 | 70 | 35 | 17.5 |
|---|---|---|---|---|---|
| | | | Pre-treatment | | |
| 17.5 | $\varepsilon_{pre}$ = 17.28 | - | - | - | - |
| 8.75 | $\varepsilon_{pre}$ = 15.27 | $\varepsilon_{pre}$ = 19.93 | $\varepsilon_{pre}$ = 31.53 | - | - |
| 4.375 | $\varepsilon_{pre}$ = 12.78 | $\varepsilon_{pre}$ = 13.09 | $\varepsilon_{pre}$ = 20.54 | $\varepsilon_{pre}$ = 24.09 | $\varepsilon_{pre}$ = 33.13 |
| | | | Post-treatment | | |
| 17.5 | $\varepsilon_{pre-post}$ = 6.35 | - | - | - | - |
| 8.75 | $\varepsilon_{pre-post}$ = 7.45 | $\varepsilon_{pre-post}$ = 7.07 | $\varepsilon_{pre-post}$ = 12.83 | - | - |
| 4.375 | $\varepsilon_{pre-post}$ = 2.94 | $\varepsilon_{pre-post}$ = 0.41 | $\varepsilon_{pre-post}$ = 17.13 | $\varepsilon_{pre-post}$ = 23.07 | $\varepsilon_{pre-post}$ = 11.71 |

**Table 1**. Concentration (mEq/l) of mixed samples (NaCl+KCl), and corresponding difference ε in the conductivity (%) compared to the samples with NaCl alone at the same concentration, without resin treatment, and with resin treatment (pre-post resin conductivity difference).

**Results:** On average, the difference in the conductivity between mixed samples, and corresponding pure NaCl samples (at the same NaCl concentration) was 20.9%. After treatment with the exchange resin, when considering the pre-post resin variation in conductivity, the difference between mixed samples, and corresponding pure NaCl samples was 9.9% only.

**Conclusions:** Ion-exchange resin treatment coupled with conductivity measures can be a possible simple approach for continuous and automatic measures of sodium during dialysis session.

## TH-PO104

### A Machine-Based Method to Anticipate Intradialytic Morbid Events

Camille Linick Stewart,[1,3] Jane Mulligan,[2] Greg Grudic,[2] Michael Bronsert,[1] Mark Talley,[1] Steven L. Moulton.[1,2,3] *[1]Surgery, Univ of Colorado School of Medicine, Aurora, CO; [2]Flashback Technologies, Boulder, CO; [3]Surgery, Children's Hospital Colorado, Aurora, CO.*

**Background:** Intradialytic morbid events (IME) due to ultrafiltration-induced hypovolemia are commonplace during hemodialysis (HD). The compensatory reserve index (CRI) is a novel mathematical algorithm originally developed for noninvasive monitoring of blood loss on the battlefield. CRI continuously monitors pulse oximetry waveform features and in real-time interprets how these features change with central volume loss,

from normovolemia (CRI=1) to decompensation (CRI=0). We hypothesized that changes in CRI would anticipate future IME in chronic end-stage renal disease (ESRD) patients undergoing HD.

**Methods:** Demographic, clinical information and continuous measurements of CRI, blood pressure (BP) and heart rate (HR) were collected from chronic ESRD patients during HD. IME were defined as a decrease in systolic BP or symptom (e.g. cramps, dizziness) triggering intervention. Univariate and multivariate logistic regression generalized estimating equation models were designed to evaluate correlation with future IME.

**Results:** 85 ESRD patients had CRI and HR recorded during HD; 56 also had continuous BP recorded. There were 44 IME among 33/85 (38%) patients. There were no differences in age, gender, race, weight gain, duration of treatment, volume of ultrafiltrate removed, heart disease or diabetes status among patients who sustained an IME versus those who were event-free ($p>0.05$). In multivariate analysis, a 0.15 decrease in CRI over a 15-minute interval was independently associated with an IME in the upcoming 15 minutes (4.076, 95% CI 1.748-9.508, $p=0.003$), whereas 10-point changes in HR and BP were not ($p>0.05$). Patients with this type of change in CRI were 4 times more likely to experience an IME.

**Conclusions:** Decreasing CRI is associated with future IME. Demographic, clinical and traditional vital sign parameters did not correlate with IME. CRI is a novel, noninvasive parameter that is able to anticipate and may help to prevent intradialytic morbid events in chronic ESRD patients who undergo HD.

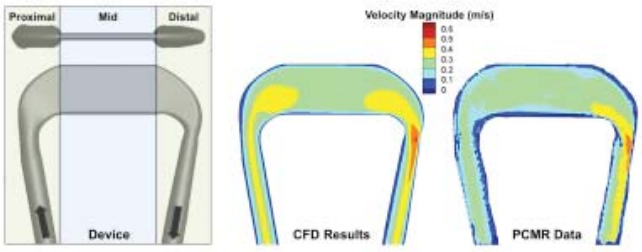
## TH-PO105

**Simulation and Validation of Flow in a Hemofilter Device** Amanda Buck,[1] Daniel Colvin,[1] Mark S. Goodin,[2] Joseph J. Groszek,[1] Shuvo Roy,[3] William Fissell.[1] *[1]Vanderbilt Univ; [2]SimuTech Group; [3]Univ of California, San Francisco.*

**Background:** When designing biomedical devices for use in vasculature, it is vital that flow through the device not initiate or exacerbate blood clotting, which may lead to device failure. Computational fluid dynamics (CFD) simulations can predict pathophysiologically relevant variables; however, they require assumptions related to flow conditions. *In vitro* studies require fewer assumptions and can be used to validate CFD results. The purpose of this study was to compare MR-measured and CFD-simulated flow fields in a hemofilter device.

**Methods:** Steady flow in the device was measured using phase contrast MR (PCMR). The PCMR data were acquired using a 9.4 T Varian scanner. Citrated bovine blood was circulated through the device at 100 ml/min using a peristaltic pump (Cole Parmer) with offset pump heads to mitigate pulsatility. CFD simulations were conducted using CFX (Ansys, Inc.) at conditions matching the *in vitro* flow experiment.

**Results:** Velocity maps demonstrate excellent agreement between measured and simulated flow fields in the mid and distal portions of the device.




The CFD- (0.51 m/s) and PCMR-determined (0.56 m/s) peak velocities in the distal constriction differed by 9%; however, calculated velocities were higher in the proximal region. At the inlet, the CFD results show fully developed flow while PCMR data show a flatter velocity profile. This may reflect slight variations in flow due to the pulsatile nature of the pump. The similarity of the distal flow fields suggests the channel region in the middle of the device may mitigate the effect of inlet flow profile on the distal portion of the hemofilter.

**Conclusions:** These results demonstrate good agreement between measured and simulated velocity fields and suggest that these complementary approaches can yield information valuable for device design.

*Funding:* Other NIH Support - NIH 1R01EB014315-01A1 Biocompatibility of Implantable Renal Replacement Devices

## TH-PO106

**A Longitudinal Study on the Hemodynamic and Lumen Changes in Human Arteriovenous Fistulas** Christi M. Terry,[1] Michelle Fitts,[1] Daniel B. Pike,[1] Yan-Ting E. Shiu,[1] Yong He,[2] Scott A. Berceli,[2] Alfred K. Cheung.[3,1] *[1]UofU, SLC, UT; [2]U of FL, Gainesville, FL; [3]VASLHCS, SLC, UT.*

**Background:** Arteriovenous fistulas (AVF) often fail to mature, likely partly due to aberrant wall shear stress (WSS). We have developed and begun to use an MRI-to-computational fluid dynamics (CFD) pipeline for in-depth serial assessment of AVF hemodynamics and lumen geometry in an on-going NIDDK-sponsored multicenter study.

**Methods:** At 1-3 days, 6 wks, and 6 mos post-AVF creation, non-contrast black-blood and cine phase-contrast MR images were acquired and used for reconstructing AVF lumen geometry and measuring artery inlet and vein outlet blood flow, in 3 patients. Using these data, WSS throughout a long segment of the AVF was simulated by CFD in 1 patient. Early WSS was co-registered with later vein lumen cross-sectional areas at 1-mm intervals using the anastomosis as an anatomical landmark.

**Results:** Table 1 shows the longitudinal changes in lumen area and flow for the inflow artery and outflow vein are shown. Venous blood flow rapidly increased after AVF creation, but decreased by 6 mos (§$p<0.05$), whereas arterial inflow rapidly increased and remained increased (*$p<0.05$). Inflow artery lumen areas decreased at 6 wks and 6 mos compared to 1-3 d (†$p<0.05$), whereas outflow vein lumen areas increased over time (‡$p<0.05$). WSS was assessed in 1 patient: Vein lumen area at 6 wks correlated with 1-3 d vein WSS ($r=-0.49$, $p<0.05$), with higher WSS (>30dyn/cm$^2$) correlating with decreased lumen areas.

| Table 1. | Flow (ml/min) | | | | Avg total vessel area (mm$^2$) | | |
|---|---|---|---|---|---|---|---|
| | baseline | 1-3 d | 6 wks | 6 mos | 1-3 d | 6 wks | 6 mos |
| inflow artery | ~75 | 724 ± 385.7 | 933.0 ± 274.9* | 1001.8 ± 194.3* | 25.1 ± 5.4 | 23.6 ± 8.0† | 19.5 ± 5.0† |
| outflow vein | ~5 | 425.5 ± 67.0 | 392.4 ± 201.0 | 341.1 ± 122.6§ | 22.7 ± 13.0 | 44.0 ± 17.1 | 59.2 ± 40.7‡ |

**Conclusions:** The pipeline is a robust tool and provides a means to evaluate the impact of WSS on AVF maturation. Correlational analyses of WSS and other hemodynamic profiles with lumen area changes, in a large number of patients from our multi-center study, should provide novel insight into factors that contribute to AVF failure and success.

*Funding:* Other NIH Support - NIDDK

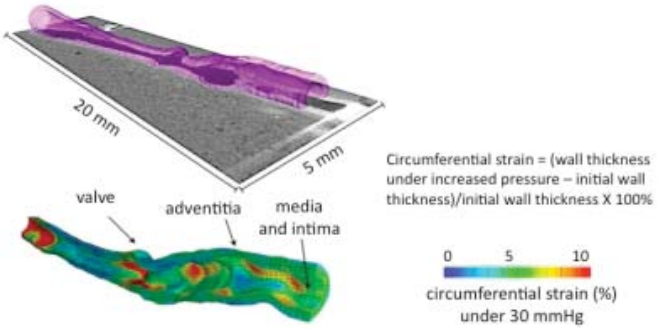
## TH-PO107

**A Novel Method for High-Resolution Characterization of Vein Deformation under Arterial Blood Pressure** Yan-Ting E. Shiu,[1] Arnold David Gomez,[1] Huan Li,[1] Ilya S. Zhuplatov,[1] Alfred K. Cheung,[1,2] Edward Hsu.[1] *[1]U of Utah, SLC, UT; [2]VASLCHCS, SLC, UT.*

**Background:** Hemodialysis arteriovenous grafts often fail due to neointimal hyperplasia (NH) at the venous anastomosis (VA). Analogous to the known association between abnormal wall deformation and NH in artery, aberrant venous wall deformation at the VA induced by increased hydrostatic blood pressure may play a role in the focal nature of NH formation, but a clear correlation has not yet been demonstrated. We have developed a new method to characterize venous wall deformation, laying the foundation to investigate this correlation.

**Methods:** Techniques for characterizing arterial wall deformation are seldom useful to measure changes in the thickness of thin venous wall, which are often ≤ 100 μm - below the resolution of conventional MRI or ultrasound. Our method uses micro-computed tomography (μCT) and deformable image registration to measure strain. A μCT-compatible device was built to image a freshly explanted porcine vein under hydrostatic pressure (0-160 mmHg). An iodine-based staining protocol was optimized to provide soft-tissue contrast for visualization while preserving the tissue's morphological structure. μCT images (17 μm resolution) were segmented to reconstruct the vein (**Fig. 1**, top), which was used to calculate the strain.

**Results:** Best contrast and negligible tissue shrinkage of the vein was obtained using 10% Lugol solution and a 3-hour soaking. The strain map (**Fig. 1**, bottom) shows highly heterogeneous strain values in the vein, in addition to complex anatomical features such as non-uniform wall thickness and a valve.




**Conclusions:** We have developed a robust method to assess the strain of thin vein under hydrostatic pressure. Serial analyses using this method should illuminate the relationship between early wall deformation and subsequent focal NH formation and have implications in the design of anti-NH strategies for vascular accesses.

*Funding:* NIDDK Support

## TH-PO108

**Correlation between Wall Shear Stress and Subsequent Lumen Area Change at Venous Anastomosis Is Different from That at Arterial Anastomosis** Yan-Ting E. Shiu,[1] Daniel B. Pike,[1] Christi M. Terry,[1] Yong He,[2] Huan Li,[1] Ilya S. Zhuplatov,[1] Alfred K. Cheung.[3,1] *[1]UofU, SLC, UT; [2]U of FL, Gainesville, FL; [3]VASLHCS, SLC, UT.*

**Background:** Neointimal hyperplasia (NH) occurs much more commonly at the venous anastomosis (VA) than at the arterial anastomosis (AA) of arteriovenous grafts (AVG). Aberrant wall shear stress (WSS) may play a role in increased susceptibility of the vein to NH formation. We examined this hypothesis by comparing the WSS and subsequent lumen area between the venous and arterial anastomoses in a swine model of AVG stenosis.

**Methods:** AVG were implanted between the common carotid artery and external jugular vein in swine. At 1, 2, 3, or 6 wk (n=5 at each wk) after graft implantation, contrast-free black-blood and cine phase-contrast magnetic resonance images were acquired and used

to assess the lumen geometry and blood inflow and outflow rates. These parameters were then used for computational fluid dynamics simulations. WSS magnitudes and lumen area changes were calculated for 4 cm lengths of arterial and venous segments, upstream and downstream from their respective anastomoses.

**Results:** Although WSS at wk 3 was higher in the artery than in the vein (116 ± 57 vs. 36 ± 21 dyne/cm$^2$; p<0.05), subsequent lumen area changes from wk 3 to wk 6 were larger in the vein than in the artery (44 ± 35 vs. 12 ± 4 mm$^2$; p<0.05). No linear correlation was found in the artery between WSS at wk 3 and lumen area changes from wk 3 to wk 6 (r=0.12; p=0.50). In contrast, a biphasic correlation was found in the vein: when WSS was low (<20 dyne/cm$^2$) at wk 3, lumen area increased from wk 3 to wk 6 (r=0.98; p<0.05); when WSS was high (>40 dyne/cm$^2$) at wk 3, lumen area decreased from wk 3 to wk 6 (r=-0.93; p<0.05); no correlation was found where WSS was in the 20-40 dyne/cm$^2$ range (r=0.47; p=0.69).

**Conclusions:** The observation of differential WSS-lumen area correlations between the vein and the artery is novel. The finding of a biphasic relationship between early WSS and later lumen area change in AVGs is also novel. These differential hemodynamics relationships enhance our understanding of the pathogenesis of NH in AVG and have implications in the design of anti-NH strategies.

*Funding:* Other NIH Support - NIDDK

## TH-PO109

**Antimicrobial Peptide Coating Prevents Bacteria Survival and Protein Fouling on Catheter Materials** Jennifer A. Neff, Danir F. Bayramov, Fatima Alim, Burke Chan, Esha A. Patel. *Allvivo Vascular, Inc., Lake Forest, CA.*

**Background:** Catheter related bacteremia associated with dialysis access is a major cause of morbidity and mortality. Technologies to combat infection that will not select for resistant bacteria are needed. A catheter coating containing a cationic antimicrobial peptide (CAP) was tested for its effect on bacteria attachment and survival, fibrinogen (Fb) deposition, and CAP release profile.

**Methods:** Polyurethane (PU) discs coated with a CAP/polymer formulation were tested for bacteria attachment and survival. Samples were challenged with 5x10$^7$ cfu/mL *Staphylococcus Epidermidis* following incubation in heat inactivated 50% serum (HIS) for 96 hrs with HIS being replaced daily. Discs were sampled every 24 hr for live, attached bacteria by plating after washing and sonicating in a CAP neutralizing agent. Resistance to Fb adsorption was evaluated by incubating samples in PBS containing 2 mg/mL of Fb, followed by ELISA. Peptide content and release were quantified by RP-HPLC.

**Results:** Within 2 hrs, the CAP/polymer coating resulted in a 3 log reduction in bacteria attachment relative to uncoated PU followed by nearly complete sterilization of samples after 96 hrs in HIS (Figure 1). Placebo coated (polymer only) samples showed nearly 2 log reduction in bacteria attachment at early incubation times in HIS (Figure 1), and a 70% reduction in Fb adsorption by 1 week. Sustained release of CAP at therapeutically effective levels was confirmed by RP-HPLC over a period exceeding 1 week.

**Conclusions:** The formulated polymer coating displayed improved non-fouling properties and reduced Fb deposition. Incorporating CAP and tailoring its release profile further led to a significant reduction in bacteria attachment and survival. Antimicrobial coatings often facilitate protein deposition, making it difficult to achieve a nonfouling surface. This CAP/polymer formulation provides a promising approach to reduce both infection and occlusion of catheters without contributing to development of drug resistant bacteria.

*Funding:* NIDDK Support

## TH-PO110

**Proteomic Analysis of Nephron Segments of Formalin-Fixed Paraffin-Embedded Human Kidney Tissues** Tadashi Yamamoto,[1] Shigeru Miyazaki,[2] Hidehiko Fujinaka.[1] *[1]Dept of Structural Pathology, Institute of Nephrology, Niigata Univ, Niigata, Japan; [2]Shinrakuen Hospital, Japan.*

**Background:** Nephrons are the functional units of the kidneys and consist of several parts (glomerulus, proximal tubule, descending and ascending loop of Henle and distal tubule) connected to collecting ducts. Knowing of proteome profile of each nephron segment will contribute to complete understanding of kidney functions.

**Methods:** We separated nephron segments (glomeruli, proximal tubules, descending and ascending loops of Henle and distal tubules) and collecting ducts by laser microdissection of FFPE-human kidney sections after immune-labeling of each part with antibodies against unique proteins in each part. Tissue sections of total area of ~1 mm$^2$ each were digested with trypsin after autoclave treatment (On-site direct digestion, OSDD method). Peptides were collected from the sections and purified by C-18 Stage-Tip and analyzed by LC-MS/MS (Thermo Orbitrap LTQ) and proteins were identified by Mascot with FDR less than 5%.

**Results:** More than 1000 proteins were identified by a single MS analysis of each sample and proteins uniquely expressed in each nephron segment were selected: glomerulus; 153, proximal tubule; 306, distal tubule; 58, and collecting duct; 40. Gene Ontology annotation analysis showed that the most enriched cellular components were cytoskeleton proteins in the glomerulus, cytoplasm proteins in the proximal tubule, mitochondrion proteins in the distal tubule, and cytoplasmic part proteins in the collecting duct. The protein quantity was compared by label-free normalized spectral index. Among the uniquely expressed proteins the highest were proteins translated from S100A6 gene in the glomerulus, MPDZ in the proximal tubule, HIST1H2B in the distal tubule, and MAST4 in the collecting duct.

**Conclusions:** The proteome analysis of nephron segments demonstrated their significant features and provided useful knowledge of their functions.

*Funding:* Government Support - Non-U.S.

## TH-PO111

**MRI Characterization of UUO Kidney Injury Using CEST and MTR Techniques** Feng Wang,[1,2] Keiko Takahashi,[2] Zhongliang Zu,[1] Raymond C. Harris,[2] Christopher Chad Quarles,[1,2] Takamune Takahashi.[2] *[1]Vanderbilt Univ Institute of Imaging Science; [2]Vanderbilt O'Brien Kidney Disease Center, Nashville, TN.*

**Background:** Chemical exchange saturation transfer (CEST) and magnetization transfer (MT) MR imaging enables the assessments of metabolites with exchangeable protons and macromolecules in tissues, respectively. Since these techniques are poorly applied to kidney disease, we evaluated its ability in sensing kidney injury/progression using mice with unilateral ureteral obstruction (UUO).

**Methods:** MRI protocols were optimized on Agilent 7T MRI. MT ratio (MTR) was measured based on a 2D RF-spoiled gradient echo sequence. Off-resonant RF irradiation was accomplished using Gaussian RF pulses (6000 Hz, 12ms), and a control scan was performed without MT pulses. CEST was performed using a continuous wave CEST sequence (a 8.0 s irradiation pulse followed by a multishot SE-EPI readout). A control scan was performed by setting the RF offset to 20000 Hz. $MTR_{asym}$ was acquired using asymmetric analysis. The CEST and MTR maps were generated for UUO and sham-operated mice at day 1, 3, and 6 after surgery.

**Results:** 1. MTR was significantly reduced in inner medulla (IM) and papilla (P) in UUO day 3 kidneys, while the changes in cortex (C) and outer medulla (OM) were minimum. At day 6, the IM and P of UUO kidneys showed remarkably reduced MTR, perhaps due to severe cell injury or death. At this stage, MTR was also slightly decreased in C and OM in UUO kidneys. 2. CEST: The z-spectrum of IM and P in day 3 and 6 UUO kidneys was highly asymmetric and the CEST contrasts were broadly and largely increased from ~1.2 to 3.5 ppm in $MTR_{asym}$ curve. The CEST contrasts at 2 and 3.5 ppm offsets in the OM of UUO kidneys and at 1.2 ppm in contralateral kidneys increased from day 3 to day 6. The MTR and $MTR_{asym}$ maps in UUO kidneys showed focal variations on the disease progression.

**Conclusions:** The CEST and MTR techniques can be used for the assessment of UUO injury and progression. The MTR may be used for assessing severe renal cell injury that decreases tissue macromolecular components, while CEST may provide the changes in metabolites including glucose, amine and amide.

*Funding:* NIDDK Support

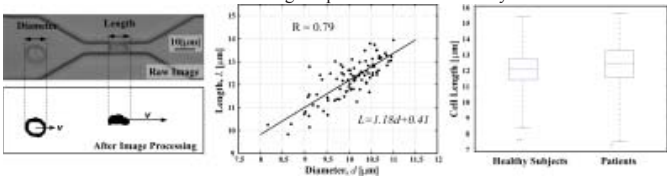
## TH-PO112

**Image-Based Screening for Erythrocyte Characteristics of Patients Receiving Dialysis Service** Chia-Hung Dylan Tsai,[1] Shuhei Yoshikawa,[1] Shinya Sakuma,[1] Fumihito Arai,[2] Makoto Kaneko.[1] *[1]Osaka Univ, Suita, Osaka; [2]Nagoya Univ.*

**Background:** Dialysis process includes massive blood exchange between a patient and a dialysis device. It is known that erythrocytes of a patient receiving dialysis tend to have shorter life cycles. This work is aimed to understand how the dialysis process changes cell characteristics in terms of cell size and cell deformability.

**Methods:** An image-based screening for erythrocyte condition is introduced. The system is constructed of three parts: a high-speed camera, a PDMS microchannel and an image-processing algorithm. The frame rate of the camera can be up to 10,000 fps, which provides the capability of high-throughput screening. The microchannel with width of 4.5μm forces erythrocytes through the channel one after another. Image-based algorithm is implemented for processing the images captured from the camera, and erythrocyte conditions, such as size and deformability, are evaluated.

**Results:** Erythrocytes from 4 patients receiving dialysis service and 4 healthy subjects were tested. The cell length inside a microchannel is found highly correlated with cell diameter, which shows that the cell length is a valid index for cell size. The results indicate that the patients have greater variation in the size of their erythrocytes and, furthermore, distribution of cell size is wider among the patients than the healthy ones.




**Conclusions:** The greatest advantage of the proposed system is that erythrocyte can be evaluated one-by-one, and we can visually check the cell from the captured frames, if necessary. We confirmed that the patients receiving dialysis services tend to have bigger erythrocytes and wider distribution. This could be a reason causing shorter life cycle of patients' erythrocytes. For the future work, cell motion inside the channel will be analyzed for evaluating cell deformability.

*Funding:* Government Support - Non-U.S.

## TH-PO113

**Regional Renal Perfusion Assessment with Contrast Enhanced Ultrasound in Ischemia-Reperfusion Injury** Krisztina Fischer,[1,2] Can Meral,[1] Ferenc A. Jolesz,[1] Takaharu Ichimura,[2] Joseph V. Bonventre.[2] *[1]Radiology, Brigham and Women's Hospital; [2]Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Alterations in renal microperfusion play an important role in the development of acute kidney injury. Currently, however, there is no reliable technique that provides assessment of the renal perfusion at the bedside. Contrast enhanced ultrasound (CEUS) is an ultrasound imaging technique which uses gas microbubbles administered into the bloodstream.

**Methods:** We developed a renal perfusion estimation method, using Vevo 2100 ultrasound equipment and MicroMarker contrast agent (VisualSonics) with our own analysis software. CEUS with destruction-refilling sequences was performed in a 28-min bilateral ischemia-reperfusion (IR) model in mice (n=6) at pre-IR, 15, 30, 45, 60 min and 24 hr post-I. Plateau image intensity was used to estimate renal perfusion. High resolution, pixel-by-pixel, analysis was performed on each imaging clip, resulting in parametric maps of the kidney, representing relative blood volume in each pixel.

**Results:** Region analysis showed that the perfusion decreased the most in the outer medulla (OM) by 63% (p=0.004) 60 min post-I and remained 38% (p=0.002) less than pre-IR at 24 hr post-I.

| Average Plateau Image Intensity N=6 | Pre-IR | 15 min post-I | 30 min post-I | 45 min post-I | 60 min post-I | 24 hr post-I |
|---|---|---|---|---|---|---|
| C (SD) | 39(7) | 36(12) | 31(13) | 25(14) | 18(14) | 34(7) |
| OM (SD) | 36(7) | 30(12) | 27(11) | 20(11) | 13(8) | 22(2) |
| IM (SD) | 37(7) | 38(12) | 35(12) | 27(10) | 16(8) | 30(8) |

Parametric perfusion maps confirmed that the outer medullary perfusion decreased disproportionately to the reduction in the cortical © and inner medullary (IM) perfusion.

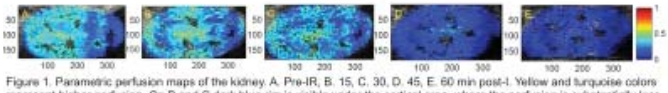


Figure 1. Parametric perfusion maps of the kidney. A. Pre-IR, B. 15, C. 30, D. 45, E. 60 min post-I. Yellow and turquoise colors represent higher perfusion. On B and C dark blue rim is visible under the cortical area, where the perfusion is substantially less than the other parts of the kidney.

**Conclusions:** CEUS can detect and monitor renal microvascular perfusion changes in space and time in IR injury in mice and represents a technique that can be translated to use in man.

*Funding:* NIDDK Support

## TH-PO114

**Immunomodulation with a Selective Cytopheretic Device (SCD) Reduces Myocardial Infarct Size in a Canine Model of Ischemia-Reperfusion Injury (IRI)** H.D. Humes,[1,2,3] D. Buffington,[1] A. Westover,[1] P. Smith,[1] Hani N. Sabbah.[4] *[1]Innovative BioTherapies; [2]Univ of MI; [3]CytoPherx; [4]Henry Ford HS.*

**Background:** IRI, characterized by a vigorous inflammatory response immediately post reperfusion via molecular signals generated by injured endothelium and cardiomyocytes, results in increased injury from leukocyte (LE) infiltration into the peri-infarct zone. This response becomes important in healing necessary to reestablish cardiac performance, but is excessive and maladaptive. The SCD, a novel biomimetic device, has shown efficacy in preclinical models of acute/chronic diseases for which inflammation is an exacerbating factor. When placed in an extracorporeal blood circuit (EBC) with regional citrate anticoagulation (RCA), the low ionized calcium environment provides a synchronized effect to both sequester and alter the activity of the transiently bound LE. The safety/efficacy of the SCD was demonstrated in clinical studies of ICU ARF/MOF patients and is currently being evaluated in a US multicenter, randomized pivotal IDE trial. Pilot studies were initiated to determine SCD effects on the acute inflammatory cascade indicative to IRI.

**Methods:** SCD was evaluated in a canine model of IRI, based on left circumflex coronary artery occlusion. Myocardial Infarct (MI) was induced for 3h and SCD therapy established using an EBC with RCA, 30min prior to reperfusion and continued for up to an additional 3h post. Systemic inflammation was monitored by CD11b expression of LE populations using flow cytometry and LE infiltration was evaluated by histology. Systemic cytokine and cardiac injury marker troponin-I (cTn-I) levels were assayed. Left ventricular (LV) function, MI size and edema were evaluated in treated (n=3) and compared to control (n=4) animals.

**Results:** SCD therapy resulted in 50% reduction of MI size (19.2±2.7 vs. 10.2±4.5% of LV volume respectively, p<0.05), less edema (LV wall thickening) and 10x lower cTn-I levels. LE infiltration was prominent in the peri-infarct zone of controls but negligible in the SCD group.

**Conclusions:** Pilot studies demonstrate that immunomodulation with the SCD represents a novel therapy with the potential to improve outcomes associated with myocardial IRI.

*Funding:* Other U.S. Government Support

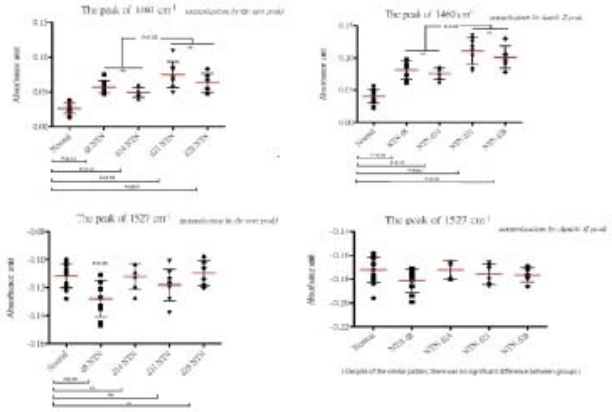
## TH-PO115

**Application of Fourier Transform Infrared Spectroscopy to Plasma Biomarker Detection in Experimental Glomerulonephritis** Mei-Ching Yu,[1] Frederick W.K. Tam,[1] Robert J. Unwin,[2] Peter R. Rich,[3] Liberty Foreman.[3] *[1]Kidney & Transplant Institute, Imperial College London; [2]UCL Centre for Nephrology, Univ College London Medical School, Royal Free Hospital; [3]Structural and Molecular Biology, UCL, United Kingdom.*

**Background:** FTIR has been used in medicines for studying molecular composition and structures in cells and tissues. However, the application to biofluids, particularly blood is limited. Our previous work showed specific characteristic bands of urine spectra in the experimental glomerulonephrits (GN),and the changes were accordance with the progression of renal injury (ASN 2012). Although plasma creatinine ($P_{Cr}$) is a common clinical test to assess renal impairment, it is insensitive at the early stage of renal disease. The aim of this work is to employ FTIR to analyse plasma from nephrotoxic nephritis (NTN) for exploring potentially biomarkers of early renal injury.

**Methods:** Plasma from NTN Wistar Kyoto rats on day 8 (9), day 14 (6), day 21 (8) and day 28 (6) were compared to normal (15). 5µl plasma was required and dried for FTIR. Sample spectrum intensity normalized by the intensity of the 1640 $cm^{-1}$ peak of urea was compared to by the 1542 $cm^{-1}$ peak of Amide II. The subsequent analytic procedures were the same as our previous work (ASN 2012).

**Results:** The change in the band at 1460 $cm^{-1}$ showed the same pattern after normalization either with urea or Amide II peaks, of the progression of the experimental GN. Additionally,significant differences were validated.



In this model, $P_{Cr}$ remains normal until at least day 14. To detect this novel marker is more sensitive than measuring $P_{Cr}$.

**Conclusions:** Several renal biomarkers relevant to progression of experimental GN have been identified from IR spectra of plasma and urine samples. Conclusively, FTIR is becoming an emerging non-invasive diagnostics of rapidly screening patients at risk of progressive kidney disease.

*Funding:* Private Foundation Support

## TH-PO116

**Sensitive and Specific Measurement of Urine Neutrophil Gelatinase-Associated Lipocalin by Localized Surface Plasmon Resonance Using Artificial Antibodies on Gold Nanocages** Jeremiah J. Morrissey,[1,3] Limei Tian,[2] Keng-Ku Liu,[2] Naveen Gandra,[2] Evan D. Kharasch,[1,3,4] Srikanth Singamaneni.[2,3] *[1]Anesthesiology, Washington Univ in St. Louis; [2]Mechanical Engineering and Materials Science, Washington Univ in St. Louis; [3]Siteman Cancer Center, Washington Univ in St. Louis; [4]Biochemistry and Molecular Biophysics, Washington Univ in St. Louis, St. Louis, MO.*

**Background:** Biosensing platforms based on localized surface plasmon resonance (LSPR) provide highly sensitive, cost-effective, and point-of-care diagnostic tools. Similar to ELISAs, current plasmonic biosensors use expensive and labile natural antibodies. Macromolecular imprinting aims to overcome storage, stability and variability problems of natural antibodies by synthesizing specific artificial antibodies with high stability, reusability and cost-efficiency.

**Methods:** We used organo-siloxane monomers to provide amine, hydroxyl and methyl functional groups mimicking a natural antibody and when polymerized around absorbed recombinant human neutrophil gelatinase-associated lipocalin (NGAL), a biomarker of acute and chronic kidney injury, molded reversible recognition cavities specific for NGAL detected as a shift in LSPR wavelength.

**Results:** The artificial antibodies, cast on cuboidal gold nanocages (about 60nm/side) reliably detected 25 ng/ml of NGAL and each sensor pad, after stripping, may be recycled at least 5 times. The LSPR signal from NGAL was stable over a pH range from 5.0 to 8.5 and specific gravity from 1.005 to 1.030. Other human urinary proteins with lipocalin-like domains such as FABP1, FABP3 or orosomucoid; and myoglobin or hemopexin (1-10 ug/ml); and hemoglobin or serum albumin (up to 500 ug/ml) interfered less than 20% with the LSPR signal of NGAL.

**Conclusions:** Molecular imprinting of nanocages with surface synthetic antibodies opens up a novel class of plasmonic nanostructures with sensitive and specific biorecognition capability. These technical and conceptual developments open up new possibilities in biomedical applications of plasmonic nanostructures particularly in label-free and antibody-free diagnostic tools relevant to disease diagnosis, toxicology testing, and biotechnology.

*Funding:* Other NIH Support - 1R01CA141521, Clinical Revenue Support

## TH-PO117

**Development of a Microphysiological Organ-on-a-Chip Incorporating Primary Human Renal Epithelial Cell Cultures** Edward J. Kelly,[1] Zhican Wang,[1] Jenna L. Voellinger,[1] Jeremy Stuart Duffield,[2] Thomas Neumann,[3] Anna Tourovskaia,[3] Gregory Kramer,[3] Jonathan Himmelfarb.[2] *[1]Pharmaceutics, Univ WA, Seattle, WA; [2]Medicine, Univ WA, Seattle, WA; [3]Nortis Inc., Seattle, WA.*

**Background:** Kidney disease is a public health problem that affects more than 20 million in the USA, yet little is understood about the impact of kidney disease on drug disposition. Consequently there is a critical need to model the human kidney to improve our understanding of drug efficacy, safety, and toxicity, especially during drug development. The proximal tubule (PT) plays a central role in the elimination of endogenous toxins and xenobiotics, but cultured PT cells bear little resemblance to their in vivo counterparts. In addition, the role of peri-tubular capillaries in tubule secretion is poorly understood. Finally, rodent modeling of xenobiotic handling in vivo has proven a poor predictor of human handling. To study human PT functions we have developed a microphysiological system (MPS) to recapitulate the PT.

**Methods:** We have developed and optimized methods for purifying, expanding, storing human Kidney PT cells. We developed single lumen 3D microphysiological devices with flow in which a kidney PT can be recapitulated.

**Results:** In optimized culture conditions in 2D human PT cells uniformly express E-cadherin, aquaporin-2, KIM-1 and form tight junctions as demonstrated by ZO-1 localization. PT cells proliferate with a doubling time of ~ 30hrs and remain viable following freeze/thaw cycles. When seeded into the MPS, they are able to form a confluent and patent tubule under conditions of continuous flow. PT cells cultured under these conditions retain expression of the epithelial marker E-cadherin and form tight junctions, exhibiting >90% cell viability for >21 days ex vivo. These tubules are currently being evaluated functionally, the results of which will be presented.

**Conclusions:** It is possible to recapitulate a functioning PT ex vivo in a MPS for several weeks. This system more accurately reflects human physiology, being able to predict renal handling of xenobiotics and potentially assess mechanisms and biological response relating to kidney injury.

*Funding:* Other NIH Support - UH2TR000504

## TH-PO118

**RNA-Seq of Microdissected Renal Tubules Identifies Segment-Specific Transcription Factors** Jae Wook Lee, Chung-Lin Chou, Fahad Saeed, Mark A. Knepper. *NIH.*

**Background:** We carried out RNA-seq in microdissected rat renal tubule segments to identify the transcriptomes of the 12 major renal tubule segments and to identify transcription factors that could be involved in cell type-specific gene expression.

**Methods:** Renal tubules were manually microdissected using a high resolution microscopy system capable of discriminating cell types. After reverse transcription and amplification, cDNA libraries were sequenced using an Illumina HiSeq 2000 sequencer. Sequences were aligned to the rat reference genome (rn4) and expression levels were calculated in 'reads per kilobase exons per million mapped reads' (RPKM). The accuracy of tubule identification was successfully confirmed by plotting distributions of well established markers for each tubule segment. Expression of a gene was considered region- or tubule-specific if the RPKM in a region or tubule was larger than 90% of the sum of RPKMs across all tubule segments. Multiple samples were analyzed for each renal tubule segment.

**Results:** Individual tubule segments had between 6888 and 9300 transcripts with RPKM > 0. Several homeobox family transcription factors were found to be segment-specific, such as *Pbx4* in proximal tubule segments; *Irx1* and *Irx2* in the cortical thick ascending limb; *Hmx2* and *Pbx3* in the cortical collecting duct; and *Hoxb5* and *Pbx1* in the inner medullary collecting duct. Interestingly, *Hoxb7* (thought to be collecting duct-specific) was found not only in collecting ducts but also thick ascending limbs. Transcription-factor mediators of TGF and Wnt signaling such as *Smad2*, *Tcf4,* and *Tcf12* were found only in collecting duct segments. Other examples of region-specific transcription factors include: *Hnf4a* (proximal segments); *Rarg* (thick ascending limbs); *Mafb* (cortical collecting duct); and *Pparg* and *Elf5* (inner medullary collecting duct).

**Conclusions:** These data provide baseline information needed to model cell type-specific gene expression along the nephron and collecting duct.

*Funding:* Other NIH Support - NHLBI

## TH-PO119

**PPARα Promotes $Na^+/H^+$ Exchanger NHE1 Expression to Inhibit Renal Tubule Cells Apoptosis through Recruiting Ezrin/Radixin/Moesin Proteins and Phosphoinositide 3-Kinase** Yung-Ho Hsu,[1] Tso Hsiao Chen,[2] Cheng-Hsien Chen.[1,2] *[1]Div of Nephrology, Dept of Internal Medicine, Shuang Ho Hospital, Taipei Medical Univ, Taipei, Taiwan; [2]Div of Nephrology, Dept of Internal Medicine, Wan Fang Hospital, Taipei Medical Univ, Taipei, Taiwan.*

**Background:** Peroxisome proliferator-activated receptor-alpha (PPARα) is a transcription factor and has been reported to inhibit gentamicin-mediated proximal tubule cell apoptosis. However, the antiapoptotic mechanism of PPAR-α is still unknown.

**Methods:** In this study, we investigated the mechanism of antiapoptotic effect of PPARα by overexpressing PPARα in NRK-52E cells.

**Results:** We found that PPAR-α overexpression induced the expression of $Na^+/H^+$ exchanger NHE1 in NRK-52E cells. Three potential PPAR response elements in NHE1 promoter region were identified by chromatin immunoprecipitation. The $Na^+/H^+$ exchanger activity was also increased in PPARα-transfected cells. Flow cytometry showed that PPARα overexpression augmented the resistance to apoptosis-induced shrinkage in NRK-52E cells. A potent $Na^+/H^+$ exchanger inhibitor, 5-(N-Ethyl-N-Isopropyl) Amiloride (EIPA), inhibited the antiapoptotic effect of PPARα in gentamicin-treated cells. NHE1 siRNA transfection also inhibited PPARα-induced $Na^+/H^+$ exchanger activity and the antiapoptotic effect. In immunoprecipitation, NHE1 physically associated with phosphorylated ezrin/radixin/moesin (ERM) and phosphoinositide 3-kinase (PI3K) in PPARα-transfected cells. We also found that PPARα induced activation of the pro-survival kinase, Akt. PPARα-induced Akt activity was inhibited by EIPA and NHE1 siRNA transfection. Wortmannin, a phosphoinositide 3-kinase (PI3K) inhibitor, inhibited PPARα-induced Akt activity and the antiapoptotic effect of PPARα. But wortmannin did not influence the association between NHE1 and phospho-ERM.

**Conclusions:** We conclude that PPARα promotes NHE1 expression, and then recruits ERM proteins and PI3K to mediate Akt-dependent cell survival.

## TH-PO120

**Elucidating the Mechanism of Tenofovir-Induced Kidney Injury** Laura Chappell-Campbell, Jeremy S. Leventhal, Pengfei Gong, Michael J. Ross. *Icahn Sch of Med at Mt Sinai.*

**Background:** Tenofovir (TFV) is the most commonly-used antiretroviral drug for treatment of HIV-1 infection. 2% of patients treated with TFV develop AKI and 30% have subclinical signs of proximal tubular epithelial cell (PTEC) dysfunction. Though studies suggest mitochondrial injury is an important component of TFV nephrotoxicity, little is known about the mechanism by which TFV injures mitochondria.

**Methods:** We created an novel *in vitro* model of TFV toxicity using human PTEC. To increase PTEC susceptibility to TFV toxicity, studies were performed using PTEC stably transfected with *OAT1*, which mediates basolateral TFV uptake. Protein expression was analyzed for markers of mitochondrial fission and fusion, autophagy-associated protein LC3, and the cellular energy sensor AMPK. Subcellular localization of mitochondria and LC3 was visualized via confocal microscopy. Electron microscopy (EM) was used to analyze mitochondrial morphology and to identify autophagosomes.

**Results:** *OAT1* transfection markedly increased the toxicity of TFV. TFV treatment increased expression and activation of AMPK in *OAT1*-overexpressing PTEC. Since AMPK activation can reflect cellular energy deprivation, we studied whether TFV also increased autophagy in PTEC. TFV incubation markedly increased LC3-I levels, with more modest effects on LC3-II. Confocal imaging of TFV-treated PTEC demonstrated colocalization of mitochondria with LC3, suggesting autophagosomal localization of mitochondria (mitophagy). EM studies corroborated these findings, showing increased autophagosome accumulation and mitophagy in TFV-treated cells. No significant effect of TFV on mitochondrial fission and fusion was observed.

**Conclusions:** These data demonstrate that TFV induces activation of AMPK, an important regulator of autophagy. Mitophagy was also induced by TFV and our finding that TFV increased LC3-I levels without changes in LC3-II suggests either a defect in autophagosome formation or increased autophagosome turnover. In conclusion, TFV induces activation of cellular energy deprivation response pathways. Future studies are needed to determine whether this response is due to aberrant mitophagy leading to loss of mitochondria or to direct mitochondrial injury.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO121

**HIV-Induced Downregulation of Deptor Contributes to the Activation of mTOR Pathway in HIV-Associated Nephropathy** Partab Rai,[1] Kavithalakshmi Sataranatarajan,[2] Viki Kumar,[1] Rivka Lederman,[1] Ashwani Malhotra,[1] Balakuntalam S. Kasinath,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Medicine, Univ of Texas Health Science Center, San Antonio, NY.*

**Background:** mTOR pathway has been shown to play a pathogenic role in the development of HIV-associated nephropathy (HIVAN). However, the role of deptor, a negative regulator of mTOR has not been studied to date. We hypothesized that HIV may be activating mTOR pathway in HIVAN via down regulation of kidney cell deptor expression.

**Methods:** Kidneys were harvested from age and sex matched control and Tg26 mice (n=6).Renal cortical sections were immunolabeled for deptor, phospho-mTOR, and total mTOR. Protein blots from renal tissues of control and Tg26 mice were probed for deptor,

phospho-mTOR, phospho-p70S6K, phospho-Akt, total mTOR, and actin. To determine the effect of HIV on tubular cell deptor expression and mTOR activation, mouse proximal tubular cell (MTC) were transduced with either empty vector (EV/MTC) or NL4-3 (HIV/MTC). Protein blots of EV/MTCs and HIV/MTCs were probed for deptor and phospho-p70S6K expression. To establish a causal relationship between deptor down regulation and HIV-induced tubular cell protein synthesis, MTC were transfected with deptor plasmid and then evaluated for the effect of HIV milieu (deptor/HIV/MTC) on mTOR activation and tubular cell protein synthesis.

**Results:** Renal cortical sections in Tg26 mice displayed attenuated kidney cell expression of deptor but enhanced expression of phospho-mTOR. Renal tissue lysates of Tg26 mice also exhibited attenuated expression of deptor and enhanced expression of phsopho-p70S6K, an effector molecule of mTOR activation. In *in vitro* studies, deptor/HIV/MTC displayed enhanced expression of deptor but attenuated expression of phospho-mTOR and phospho-p70S6K. Additionally deptor/HIV/MTC displayed attenuated tubular cell protein synthesis when compared to HIV/MTC.

**Conclusions:** These findings indicate that HIV-induced down regulation in renal cell deptor expression contributed to the activation of the mTOR pathway in HIVAN.

*Funding:* NIDDK Support

## TH-PO122

**Quality Control of Mitochondria by Autophagy in the Kidney Has an Essential Role for the Adaptation to Metabolic Acidosis**

Tomoko Namba,[1] Tomonori Kimura,[1] Yoshitsugu Takabatake,[1] Atsushi Takahashi,[1] Takeshi Yamamoto,[1] Jun Matsuda,[1] Fumio Niimura,[2] Taiji Matsusaka,[3] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Pediatrics, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan; [3]Internal Medicine, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan.*

**Background:** It has been shown that autophagy plays a protective role in kidney. Metabolic acidosis (MA), a common acid-base disorder, causes various cellular adaptations including ammoniagenesis. We aimed to prove a hypothesis that autophagy promotes cellular adaptation of kidney proximal tubular cells under MA through mitochondrial homeostasis.

**Methods:** MA was induced in proximal tubule-specific autophagy-deficient mice and GFP-LC3 transgenic mice by treating with NH4Cl in drinking water for 4 weeks. We also used autophagy-deficient proximal tubular cells and examined the role of autophagy in adaptations for MA with pH conditioned medium.

**Results:** We demonstrated that autophagy is induced in proximal tubules by acid *in vivo* and *in vitro* as assessed by the GFP-positive dot formation in the kidney of acid-loaded GFP-LC3 transgenic mice and degradation of p62 using kidney proximal tubular cells when cultured in an acidic medium. Next, acid-loaded autophagy-deficient mice demonstrated severe MA with significantly reduced ammonium production in kidney compared with those of wild type mice, which was confirmed by *in vitro* analysis. To prove our hypothesis, we analyzed the mitochondrial function of autophagy deficient condition under MA. COX and SDH stains of kidney indicated the reduced activity of mitochondrial respiratory chain in acid-loaded autophagy-deficient mice. Mitochondrial membrane potential was significantly reduced in Atg5-deficient kidney proximal tubular cells compared with that of the control when cultured in both normal and acidic medium, respectively.

**Conclusions:** Our results indicate that MA evokes mitochondrial dysfunction and quality control of mitochondria by autophagy has an essential role for the adaptation to MA.

## TH-PO123

**Klotho Protects against Mouse Renal Fibrosis by Regulation of Autophagy**

Hye Eun Yoon, Sung Jun Kim, Su Jin Choi, Sungjin Chung, Seok Joon Shin. *Internal Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

**Background:** Autophagy is a cellular process of degradation of damaged components in the cytoplasm and regulates cell death. Klotho is known to have a renoprotective effect. In this study, we investigated whether Klotho protein protects against renal fibrosis by regulating autophagy in an obstructive kidney model.

**Methods:** Mice received unilateral ureteral obstruction (UUO) surgery with or without intraperitoneal administration of secreted Klotho protein. We evaluated renal fibrosis and apoptosis and expressions of superoxide dismutase (SOD), heat-shock protein 70 (Hsp70), beclin-1, LC3-II/LC3-I, Akt, FoxO3a, Bax, Bcl-2, Bim, cleaved PARP, and alpha-smooth muscle actin (αSMA), in the obstructed kidneys on days 1, 3 and 7.

**Results:** Secreted Klotho protein significantly decreased collagen deposition and fibrotic areas in obstructed kidneys compared with those of controls. Secreted Klotho protein significantly increased the expressions of antioxidant enzymes, SOD (SOD1 and SOD2) and Hsp70. Administration of secreted Klotho protein significantly induced beclin-1, an autophagy gene, and increased the LC3-II/LC3-I ratio in obstructed kidneys compared with controls. The numbers of apoptotic cells and the expression of Bax/Bcl-2, Bim and cleaved PARP were significantly decreased in obstructed kidneys of Klotho-treated mice compared with controls. Secreted Klotho protein significantly attenuated expression of α-SMA, a marker of epithelial mesenchymal transition, in obstructed kidneys compared with controls. In Klotho protein-treated mice, the expression of phosphorylated Akt did not show significant changes in obstructed kidneys, but the expression of phosphorylated FoxO3a was significantly decreased compared with controls.

**Conclusions:** We speculate that Klotho protects against renal fibrosis in an obstructed kidney model via direct modulation of FoxO3a signaling, without regulation of Akt, and subsequent attenuation of oxidative stress and induction of autophagy.

*Funding:* Government Support - Non-U.S.

## TH-PO124

**The Crosstalk between NF-kB and c-mip Regulates Podocyte Survival**

André Pawlak, Virginie Ory, Melanie Mangier, Mario Ollero, Djillali Sahali. *INSERM, U 955, Equipe 21, Univ Paris-Est Creteil Val-de-Marne, Créteil, France.*

**Background:** We have shown upregulated c-mip expression in nephrotic proteinuria patients concomitantly with downregulation of the antiapoptotic factor Rel-A, a master member of NF-kB. In contrast, in inflammatory glomerular diseases such us proliferative lupus nephritis, RelA is upregulated in podocytes while c-mip is decreased at transcript and protein levels. In light of the divergent expression of these two proteins, we hypothesized that c-mip is transcriptionally repressed by NF-kB.

**Methods:** We identified within the human c-mip promoter a palindromic sequence corresponding to a consensus NF-kB response element (kB-RE). To assess whether NF-kB binds to this sequence in vivo, we performed chromatin immunoprecipitation in mouse podocytes and electrophoretic mobility shift assay (EMSA) using nuclear extracts from HEK cells overexpressing RelA and a radiolabeled c-mip kB-RE oligonucleotide. The full-length sequence containing the entire c-mip proximal promoter was ligated upstream of luciferase gene and cotransfected to HEK cells with NF-kB p50, p65/RelA or both, as well as with the empty vector. C-mip expression was analyzed in wild-type and RelA-deficient mouse embryonic fibroblasts (MEF).

**Results:** The short region flanking the kB-RE was precipitated by the anti-RelA antibody, but not by the rabbit IgG control. Incubation of nuclear extracts from HEK cells overexpressing RelA with the radiolabeled c-mip kB-RE produced only one band shift in EMSA. This shift appeared to be specific as it was abolished by co-incubation with a mutated probe and shifted upwards in the presence of an anti-RelA antibody. In transfected HEK, luciferase activity driven by c-mip promoter was strongly reduced in the presence of RelA, but not by NF-kBp50. Moreover, in RelA-deficient MEF, c-mip was dramatically increased, while it was barely detected in wild-type.

**Conclusions:** These results suggest that RelA binds to the c-mip promoter and exerts a powerful inhibitory effect on c-mip transcription. Conversely, c-mip promotes RelA downregulation.Thus, a tightly regulated crosstalk between these two proteins seems to play a key role in the regulation of podocyte survival.

## TH-PO125

**DNA Damage Response with Cell Cycle Restriction Has Implications for Tissue Repair in Cisplatinum-Induced Nephrotoxicity**

Samriti Dogra,[1] Preeti Viswanathan,[2] Sriram Bandi,[3] Sanjeev Gupta.[3] *[1]Pediatric Nephrology, Montefiore Medical Center, Bronx, NY; [2]Pediatric Gastroenterology, Montefiore Medical Center, Bronx, NY; [3]Medicine, Albert Einstein College of Medicine, Bronx, NY.*

**Background:** Molecular mechanisms of chemotherapy-induced nephrotoxicity will help improve clinical outcomes. We defined whether cisplatinum (CP)-induced nephrotoxicity involved DNA Damage Response (DDR) and recruitment of master switches activated by ATM and related pathways.

**Methods:** We used HK-2 and MCT proximal tubular epithelial cells and HuH-7 hepatocytes. After exposing cells to CP, we performed MTT assays for cell viability, qRT-PCR arrays including 84 relevant genes for DDR, Comet assays for double-stranded DNA breaks, cytostaining or western for expression of ATM-related genes, ATM promoter expression assays with TdT reporter after lentiviral transduction, and FACS for cell cycling.

**Results:** In response to CP in IC50 doses, HuH-7, HK-2 and MCT cells showed losses of viability. In CP-treated HuH-7 cells, typical evidences of DDR were obvious, including differences in expression of multiple genes at the mRNA level, γH2AX expression, Comet formation, induction of ATM promoter activity with TdT reporter, along with rapid loss of cells in S or G2/M, more cells in G0/G1, and greater p53 expression. After CP, MCT cells showed similar evidences of DDR, although cell cycle restriction was less pronounced, and p53 expression was unchanged, possibly due to underlying transformation with SV40 antigen. In HK-2 cells, which were transformed by HPV E6/7 oncogenes, p53 expression conversely declined, Comets were absent, ATM promoter was not regulated, and cell cycling was only minimally affected after CP.

**Conclusions:** Renal tubular cells were susceptible to CP toxicity and MCT cells exhibited characteristic ATM-dependent DDR. In HK-2 cells, ATM-dependent DDR and cell cycle arrest were altered via p53 counter-regulation. Therefore, CP-induced DDR in MCT cells offers suitable systems to dissect molecular mechanisms driving DDR and restricting cell cycle progression, and also to develop exciting new ways for either preventing renal damage or promoting tissue repair in drug-induced nephrotoxicity.

*Funding:* NIDDK Support

## TH-PO126

**Localization of Bcl-xL and Bax Interactions in Kidney Cells**

Nang San Hti Lar Seng,[1] Judit Megyesi,[1,2] Adel Tarcsafalvi,[1] Peter M. Price.[1,2] *[1]Univ AR Med Sci; [2]VA Med Ctr.*

**Background:** Cisplatin is a widely-used chemotherapeutic agent in various solid tumors. However, in addition to its cytotoxic effect on cancer cells, cisplatin also causes kidney cell death especially in the S3 segment of proximal tubules, which is associated with acute kidney injury (AKI). This side effect compromises the usefulness of cisplatin as an anti-cancer agent. The molecular mechanisms of how cisplatin kills kidney cells is still unknown. We reported that Cdk2, a cell cycle protein, is involved in cisplatin-induced kidney cell death and y inhibiting Cdk2, we protected kidney cells from apoptosis. Here we report Bcl-xL, an anti-apoptotic protein belonging to the Bcl-2 family, is a novel substrate of Cdk2.

**Methods:** Using MS/MS, we identified the localization of Cdk2 phosphorylation sites on Bcl-xL and generated phosphomimetic Bcl-xL and phosphodefective Bcl-xL. We performed apoptosis assays to investigate the downstream effects of these Bcl-xL modifications. Bcl-xL was shown to elicit its pro-survival function by interacting with pro-death proteins, such as Bax. When Bcl-xL is prevented from interacting with Bax, Bax oligomerizes, and permeabilizes mitochondria to induce apoptosis. A GFP fragment reassembly system was utilized in kidney cell lines to determine Bcl-xL/Bax interaction and localization. GFP was divided into N-terminal and C-terminal fragments, and Bcl-xL and Bax were fused with different fragments of GFP individually. If Bcl-xL and Bax interact, the two GFP fragments are brought close enough together to allow reassembly and emit fluorescence. Here we report the interaction and the localization of wild type and modified Bcl-xL's with Bax in kidney cells.

**Results:** We found that in normal kidney cells, Bcl-xL and Bax interact at the mitochondrial membrane, which supports the "embedded together" and "retrotranslocation" models for Bcl-xL/Bax interaction. These models propose that Bcl-xL and Bax interact at the mitochondrial membrane and that Bcl-xL retrotranslocates Bax into the cytoplasm, preventing its conformational activation.

**Conclusions:** We hypothesize that Cdk2 serves as a catalyst to disrupt Bax and Bcl-xL interaction and/or localization, resulting in cell death after cisplatin exposure.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO127

**Aldosterone Promotes Autophagy in Podocytes** Junming Fan,[1,2] Nan Mao,[1] Ji Wen,[1] Zi Li.[1] *[1]Dept of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China; [2]Div of Nephrology, Dept of Internal Chinese Medicine, Luzhou Medical College, Luzhou, Sichuan, China.*

**Background:** The concentration of aldosterone was inappropriately higher in many disease, such as hypertension and chronic kidney disease. Previous reports have showed that aldosterone could induce podocyte injury by reactive oxygen species (ROS). When podocytes were induced with cytokines, some oxidized proteins and damaged organelles may be accumulated, then autophagy may be triggered.Our study is toinvestigate that effection of aldosterone on the autophagy and the ROSsignaling pathway in podocyte.

**Methods:** Podocytes were incubated with aldosterone ($10^{-8}$ M), Nacetylcysteine (NAC, 50 mM), 3-Methyladenine (3-MA, 4 mM) and rapamycin (1ng/ml), respectively. Morphological changes were observed by transmission electron microscopy and fluorescence microscope. Examination of autophagic vacuoles with monodansylcadaverine or acidine orange was under confocal microscope. Flow cytometry was performed to detect the generation of intracellular ROS and cell apoptosis. The expression of the LC3B, Beclin-1, Nephrin, $SOD_2$ or Catalase was detected by western blot.

**Results:** Aldosterone promotes autophagy in podocytes. It was observed that the number of autophagosomes was increased in aldosterone and rapamycin treatment groups, whereas rapamycin further enhanced aldosterone-induced autophagosome formation by transmission electron microscopy, immunofluorescence, MDC and AO staining examinations. The expressions of LC3-II, beclin-1, $SOD_2$ and catalase were increased in aldosterone and $H_2O_2$ treatment groups. Flow cytometry showed that the generation of intracellular ROS was increased in aldosterone and $H_2O_2$ treatment groups. But all these results were reversed by NAC pretreatment. The inhibition of autophagy by 3-MA promoted aldosterone-induced podocyte apoptosis and decreased the expression of nephrin in podocytes.

**Conclusions:** Aldosterone promotes the podocyte autophagy which was inhibited by antioxidants. These results suggested that aldosterone promoted podocyte autophagy through the ROS pathway.

*Funding:* Government Support - Non-U.S.

## TH-PO128

**DNases Mediate Pyknosis in Kidney Tubular Epithelial Cells** Tariq Fahmi,[1] Kanika Topiwala,[1] Anna G. Stewart,[1] Sudhir V. Shah,[1,2] Alexei G. Basnakian.[1,2] *[1]Pharmacology and Toxicology, Univ of Arkansas for Medical Sciences, Little Rock, AR; [2]Central Arkansas Veterans Healthcare System, John L. McClellan Memorial Veterans Hospital, Little Rock, AR.*

**Background:** Apoptosis in kidney cells is commonly associated with pyknosis, an irreversible nucleus/chromatin condensation. The mechanism of pyknosis, which could be used as a target for kidney protection against injury, is unknown. The aim of this study was to determine the role of apoptotic endonucleases as possible mechanisms of pyknosis in rat kidney tubular epithelial NRK-52E cells in response to serum deprivation.

**Methods:** We utilized a new near infrared (NIRF) florescent probe to measure DNase activity in live cells. DNA fragmentation in fixed cells was measured by TUNEL assay. Expression of apoptotic endonucleases, chaperones and markers of cellular compartments was assessed by immunocytochemistry.

**Results:** We demonstrated that during pyknosis, DNase activity asymmetrically concentrates on one side of the nucleus (near Golgi) and it is strongly colocalized with DNase 2α, caspase-activated DNase (CAD) and DNase I while EndoG and DNase I like2 showed very low colocalization with the activity. Confocal 3D microscopy showed that nuclear membrane stained for lamin B was not broken. siRNA silencing of DNase 2α, CAD, DNase I and EndoG showed suppression of pyknosis and TUNEL staining by all endonucleases except EndoG. Overexpression of all the endonucleases except EndoG showed induction of pyknosis and TUNEL. Among studied chaperones, which included HSP60, HSP70 and HSP90, only HSP60 was highly colocalized with DNase activity. siRNA knockdown of HSP60 resulted in the inhibition of both pyknosis and TUNEL.

**Conclusions:** These data identify endonucleases DNase 2α, CAD and DNase I and chaperone HSP60 as mediators of pyknosis in the kidney cells.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO129

**GLCCI1 Executes Glucocorticoid Action in Thymocyte Apoptosis** Zentaro Kiuchi, Yukino Nishibori, Yugo Ito, Kunimasa Yan. *Pediatrics, Kyorin Univ School of Medicine, Tokyo, Japan.*

**Background:** Glucocorticoid (GC)-induced apoptosis contributes to a variety of cellular phenomena including programmed-death of thymocytes and cell-death of lymphoid malignancy; however, what molecules are the actual executioners remains largely obscure. GC induced transcript 1 (GLCCI1) is known as an upregulated gene by synthetic GC; therefore, we aimed to study the protein function of GLCCI1 possibly involved in apoptotic pathway.

**Methods:** Full-length human GLCCI1 cDNA was cloned and used for the generation of recombinant protein, anti-GLCCI1 specific antibody, and stably expressing cell-line (HEK-GLC). Thymocyte cell line (WEHI) was treated with dexamethasone (DEX) in the absence or presence of glucocorticoid receptor (GR) antagonist, subjected to RT-PCR and Western blot study. Immunofluorescence was studied to determine the cellular localization of GLCCI1 in HEK-GLC, WEHI. Mice were injected by DEX and their thymi were subjected to RT-PCR and Western blot study. Mass spectrometry was studied to identify phosphorylation sites in HEK-GLC. Yeast-two hybrid screen followed by co-immunoprecipitaion and in vitro phosphorylation assay was studied to identify functional ligand of GLCCI1.

**Results:** GLCCI1 was a tubulin-associated and highly phosphorylated-protein, possessing serine/threonine phosphorylation sites more than 50. GLCCI1 was bound to both dynein light chain LC8-type 1 (LC8) and its functional kinase p21-protein activated kinase 1 (PAK1), thereby inhibiting kinase activity of PAK1 on LC8 phosphorylation. Synthetic GC upregulated GLCCI1 in the presence of GR, associated with the activation of caspase-3 and -9 and Bim. $CD4^+CD8^+$ double-positive thymocytes endogenously expressed far higher amount of GLCCI1 compared with non-double positive thymocytes and splenic T cells, which was further upregulated by synthetic GC, associated with upregulation of caspase-3 and -9 and Bim.

**Conclusions:** These findings uncover a novel intrinsic pathway that controls thymocyte apoptosis and further suggests that GLCCI1 may function as a direct executioner of synthetic GC's action against many immunological disorders and lymphoid malignancy through interacting with a number of kinases including PAK1.

## TH-PO130

**Angiogenin Is Secreted by the Stressed Renal Epithelium and Induces the Production of tiRNA** Nicolas Bouvier,[1] Alexandre Karras,[2] Eric Thervet,[2] Nicolas Pallet,[1,2] Iadh Mami.[1] *[1]INSERM U775, Paris, France; [2]Nephrology, HEGP, Paris, France.*

**Background:** Angiogenin (ANG) is a stress-activated ribonuclease that cleaves tRNA, producing stress-induced tRNA fragments (tiRNA) can directly inhibit protein synthesis by a novel mechanism of RNA interference**.**

The objective of our study is to test whether ER stress induces ANG production, and to delineate the mechanisms and biological functions of ANG synthesis and secretion by kidney epithelial cells during ER stress.

**Results:** ER stress induces the production and secretion of ANG by human renal epithelial cells in culture. Using siRNA, we have demonstrated that the UPR transducers IRE1 and ATF6, but not PERK, regulate ANG production at the transcripts and protein levels. ANG expression is also increased in rat kidneys challenged with tunicamycin, cyclosporine, and ischemic stress. Recombinant human ANG reduces UPR-induced apoptotic cell death, UPR-induced autophagy, and the intensity of ER stress. During ER stress, ANG is translocated from the nucleus to the cytoplasm, where it colocalizes within stress granules. ANG promotes the formation of tiRNA during ER stress which interfere with the initiation of translation. Therefore, ANG-mediated translation inhibition may involve tiRNA production, to decrease protein load, thereby promoting ER stress relief. Our results also indicate that inhibition of ANG expression by RNA interference reduces the intensity of UPR-induced N-kB signaling, as well as proinflammatory cytokines secretion, suggesting that ANG promotes tubular inflammation. Finally, we measured the concentration of ANG in urines of 100 patients with kidney disease by ELISA. Urinary ANG/creatinine ratio were positively correlated with estimated glomerular filtration rate. By immunohistochemistry analysis of human kidney biopsies we show that ANG is expressed in ischemic kidney and during cyclosporine nephrotoxicity, but not in normal kidneys.

**Conclusions:** ANG is directly and specifically be secreted by the stressed kidney epithelium during the UPR, promotes tiRNA production and protects against UPR-induced apoptosis. ANG is a potential non invasive diagnostic biomarker of tissue injury.

## TH-PO131

**Tubular DNA Damage Response and Cell Cycle Arrest Mediate Progression of Diabetic Nephropathy** Jae-Hyung Chang, Takaharu Ichimura, Joseph V. Bonventre. *Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Diabetic nephropathy (DN) has been traditionally considered as a primary glomerular disease. Increasing evidence, however, suggests that the kidney tubule plays a central role, and that tubulointerstitial injury may contribute to glomerular changes in diabetes. Recently, our laboratory has shown that in ischemic, toxic and obstructive mouse models, injured proximal tubular epithelial cells (PTEC) undergo cell cycle arrest in G2/M and assume a pro-fibrotic secretory phenotype as a consequence of maladaptive repair. This process involves activation of DNA damage response (DDR) proteins and results in interstitial fibrosis and glomerulosclerosis.

**Methods:** Using a mouse model of type 1 diabetes and porcine tubular cell lines, we determined if the diabetic environment facilitates DNA damage in PTEC, activates DDR signaling and promotes senescent cell cycle arrest, ultimately leading to tubulointerstitial fibrosis and glomerulosclerosis.

**Results:** Hyperglycemic treatment of KIM-1-PK1 cells, a porcine tubular cell line which stably expresses human kidney injury molecule-1 (KIM-1), results in enhanced expression of markers of nuclear DNA damage and DDR activation (pATM and pH2AX), as well as induction of p16, a marker of senescent cell cycle arrest. Also, expression of TGF-β was up-regulated in these cells. In streptozotocin (STZ)-induced diabetic mice, 15 weeks after induction of diabetes, kidneys had interstitial fibrosis, tubular atrophy and glomerulosclerosis, together with significantly enhanced expression of nuclear pH2AX and apical KIM-1 in PTEC when compared to control non-diabetic animals. At this timepoint, there were significantly fewer Ki67-positive proliferating PTEC in diabetic kidneys compared to the control kidney. Also, cortical tubular expression of p16 and the number of phosphohistone H3 positive tubular cells were increased in diabetic mice, indicating an increase in the proportion of PTEC in cell cycle arrest in diabetes.

**Conclusions:** These data suggest that tubular DDR signaling and premature senescent cell cycle arrest may lead to accelerated fibrosis and may represent novel pharmacological targets for the treatment of DN.

*Funding:* NIDDK Support

## TH-PO132

**URAT 1 Inhibition Protects Humans Proximal Tubule Cells from Apoptotic Damage Induced by Uric Acid** Emanuele L. Parodi, Daniela Verzola, Francesca Viazzi, Debora Garneri, Annalisa Gonnella, Roberto Pontremoli, Giacomo Garibotto. *Dept ofInternal Medicine and Cardionephrology, Univ of Genoa, Genoa, Italy.*

**Background:** Serum uric acid (UA) has been shown to predict the development of chronic kidney disease (CKD) and has been associated with signs of renal damage such as albuminuria, intrarenal vascular stiffness and tubular atrophy in several conditions. The mechanisms underlying the effects of UA on renal proximal tubular epithelial cells (PTCs) have not been elucidated. In CKD, increased apoptosis contributes to cell loss and structural damage. We investigated the effect of UA on apoptosis in human PTCs line (HK-2) and the underlying mechanisms.

**Methods:** Apoptotic damage was induced by serum deprivation and adding UA at different concentrations (7,5 -12 mg/dl) for 48 hours. Cell viability was evaluated by MTT test and apoptosis by positivity for cleaved caspase-3 (p17 fragment). Pro- and anti-apoptotic proteins were studied by Western Blot. The pathways involved in UA apoptosis were investigated by Caspase inhibitors, NADPH Oxidase inhibitor (DPI), urate transporter antagonists (losartan and probenecid).

**Results:** Highest UA concentration decreased tubule cell viability (-30%, p=0.015) and increased significantly apoptotic cells (14%±0,35 vs 2%±0,87 untreated cells p=0.0001). UA up-regulated Bax ( +60% p<0.05) and down regulated X-linked inhibitor of apoptosis protein (XIAP) (-30% p<0.05).Caspase-9 inhibitor blunted apoptosis, while caspase-8 inhibitor had no effects. UA induced changes in the mitochondrial membrane as shown by mitocapture apoptosis assay. DPI had inhibitory effects on apoptosis (-70%, p=0.0001). Incubation with probenecid 20 μM and losartan 10 μM inhibited apoptosis induced by 12 mg/dl UA exposition (p<0.01 and <0.0001, respectively).

**Conclusions:** These results indicate that mildly elevated UA levels promote apoptosis in PTCs by triggering intrinsic caspase activation, thus contributing to mechanisms of cell loss which have been already shown to be activated in CKD. This mitochondrial pathway seems to be attenuated by losartan and probenecid, probably through their binding to URAT-1. These findings support a role for UA in promoting renal injury in humans.

## TH-PO133

**Effect of the F11R Receptor Antagonist P4D on Human Renal Mesangial Cells (HRMCs)** Anna Babinska, Albert Sohn, Sabeen Khan, Isabelle Ayoub, Moro O. Salifu. *Div of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

**Background:** We recently tested and demonstrated the expression of F11R (a receptor involved in atherosclerosis), on mesangial cells. The presence of F11R on mesangial cells and other cells of mesenchymal origin (endothelial and smooth muscle cells) raises the question as to whether F11R may be involved in mesangial sclerosis associated with TGF beta such as seen in diabetic nephropathy. In this study, we tested the hypothesis as to whether the administration of specific F11R inhibitor peptide 4D (P4D) could inhibit glucose induced mesangial cell proliferation (formazan production) and TGF beta secretion.

**Methods:** Cultured HRMCs were treated with 500μM of Peptide 4D in the present of 10mM of glucose for 24h and 48 h and mitochondrial activity (Formazan production) using MTT Assay (Sigma) was measured, as a marker of proliferation. TGF beta secretion was measured using ELISA method. HRMCs with 10mM glucose plus P4D was Group A while HRMCs with 10mM glucose alone was Group B.

**Results:** There was a dose a dose dependent (100, 250 and 500 μM) inhibitory effect of P4D on proliferation of HRMCs. At 24 hours, there was a 12.7% inhibition of proliferation (formazan production) in HRMCs in Group A compared to Group B (100% vs. 87.3 ±1.3%, p=0.04). At 48 hours, there was 16.7% inhibition of proliferation in Group A compared to Group B (100% vs. 83.3±2.9%, p=0.01). The P4D induced inhibition of proliferation increased at 48 hours. With respect to TGF beta release, at 24 hours, there was no significant inhibition of TGF beta release in Group A (normalized at 10,000 cells) compared to Group B (12.1± 5.3 vs. 18.5± 8.8 pg/ml, p=ns). However at 48 hours, there was significant inhibition of TGF beta release in Group A compared to Group B (47.8±10.5 vs. 76.9±9.1 pg/ml, p=0.02). The P4D induced inhibition of TGF beta release increased at 48 hours.

**Conclusions:** We observed a dose dependent inhibitory effect of P4D on proliferation of HRMCs. Inhibitory effect of P4D on mesangial cell proliferation and TGF beta secretion in the present of glucose was also observed. Further studies are needed to determine the role of P4D in diabetic nephropathy.

## TH-PO134

**Senescent Renal Tubular Epithelial Cells Promote an Epithelial-Mesenchymal Transition State through the Paracrine Secretion of TGF-β1** Guangyan Cai, Jingchao Wang, Xiang-Mei Chen. *Dept of Nephrology, State Key Laboratory of Kidney Disease, Chinese PLA General Hospital, Beijing, Beijing, China.*

**Background:** Kidney aging is often associated with renal fibrosis, which leads to renal function decline. The relationship between senescence and fibrosis in the aging kidney was still unknown.This study is to investigate whether epithelial-mesenchymal-transition (EMT) develops in senescent renal tubular epithelial cells and the potential mechanisms.

**Methods:** We detected both senescent and EMT markers in aged kidney tissue from 24-month-old F344 rats, and in high glucose induced premature senescent primary proximal tubular cells (PTC). The conditional medium from senescent PTC was collected to co-culture with primary PTC for 48h to investigate its role in mediating EMT. p53 siRNA was introduced to inhibit PTC senescence. TGF-β1 protein level in the supernatant of senescent PTCs were evaluated by ELISA. TGF-β1 receptor I inhibitor was used to investigate the role of TGF-β1 signaling in mediating EMT during PTC senescence.

**Results:** In the aged kidney,SA-β-gal positive staining, decreased expression of E-cadherin and increased expression of α-SMA were observed in renal tubulo-interstitial fibrosis region. SA-β-gal positive staining,p53 and p21 expressions were significantly increased in PTC induced by high glucose for 24h.While decreased expression of E-cadherin and increased expression of vimentin and α-SMA,were found in PTC after high glucose treatment for 48h, which suggested that senescence developed earlier than EMT in PTC.Conditional medium from senescent PTC down-regulated E-cadherin but up-regulated vimentin and α-SMA expressions of primary PTC. Silenced p53 inhibited the PTC senescence and further inhibited EMT state through paracrine effect.Conditioned medium from senescent PTC contained high level of TGF-β1 by ELISA. TGF-β1 receptor I inhibitor inhibited activation of Smad3 and Smad4, and blocked pro-EMT effect of the conditioned medium from senescent PTC.

**Conclusions:** Senescent PTCs promote an EMT state through the paracrine secretion of TGF-β1, which might play an import role in renal fibrosis during kidney aging.

*Funding:* Government Support - Non-U.S.

## TH-PO135

**Uremia Triggers Differentiation of Proatherogenic $CD14^{++}CD16^{+}$ Monocytes** Adam M. Zawada, Jenny Sarah Schneider, Kyrill S. Rogacev, Danilo Fliser, Gunnar H. Heine. *Internal Medicine IV - Nephrology and Hypertension, Saarland Univ Medical Center, Homburg, Saarland, Germany.*

**Background:** Monocytes, the central drivers of vascular inflammation, are a heterogenous cell population. Three monocyte subsets are characterized flow-cytometrically: classical CD14++CD16-, intermediate CD14++CD16+ and non-classical CD14+CD16++ monocytes. Recently we provided evidence that CD14++CD16+ monocytes are independent predictors of cardiovascular outcome in patients with chronic kidney disease (CKD). However, until now it remains enigmatic which mechanisms are responsible for the increase of CD14++CD16+ monocytes in CKD.

**Methods:** In order to analyze whether uremia *per se* induces shifts in monocyte subsets we developed a method for *in vitro* monocyte subset differentiation. Hematopoetic stem cells were isolated (CD34 Microbead Kit, Miltenyi) and cultivated in the presence of TPO, SCF, Flt-3L and IL-3 to generate monocyte subsets. In addition, media were supplemented with 10 % uremic or control sera. Expression of signature markers of CD14++CD16+ monocytes (CCR5, ACE, TLR2, TLR4, CD74, HLADR, Tie2 and ENG) as well as their functional characteristics (ROS production and phagocytosis) were also determined by flow-cytometry.

**Results:** Uremia induced differentiation of CD14++CD16+ monocytes (percentages of CD14++CD16+ monocytes at day 6 of differentiation; control: 9.8 ± 1.4 %, uremia: 14.5 ± 2.5 %; $p < 0.05$). Moreover, expression of TLR2 on CD14++CD16+ monocytes was significantly increased under uremic conditions, whereas expression of other surface markers was not altered. Finally, CD14++CD16+ monocytes differentiated in presence of uremia produced significantly more reactive oxygen species ($p < 0.05$) and showed higher phagocytosis capacity ($p < 0.05$).

**Conclusions:** We show for the first time that uremia *per se* induces differentiation of CD14++CD16+ monocytes. These results may contribute to a better understanding of monocyte dysregulation in CKD patients. Further studies will have to analyze which uremic factors are responsible for the increase in CD14++CD16+ monocytes in CKD.

## TH-PO136

**Transfection of sirt-1 Gene Decreases the Apoptosis of Podocyte under the Diabetic Condition** Eun Kyoung Lee,[1] Kyung Sun Park,[2] Jai Won Chang,[3] Hyun woo Kim.[4] *[1]Internal Medicine, Dankook Univ Hospital, Cheonan, Republic of Korea; [2]Internal Medicine, Samsung Medical Center, Sungkyunkwan Univ School of Medicine, Seoul, Republic of Korea; [3]Internal Medicine, Asan Medical Center, Univ of Ulsan College of Medicine, Seoul, Republic of Korea; [4]Internal Medicine, Jeju National Univ Hospital, Jeju, Republic of Korea.*

**Background:** Podocyte injury plays a role in the pathogenesis of diabetic nephropathy, leading cause of end-stage renal disease. The human sirt-1 gene, the mammalian ortholog of yeast Sir2, is associated with prolonged life span.

**Methods:** We performed this study as the first step to investigate whether the prevention of apoptosis of podocyte under the diabetic condition can ameliorate decline of renal function. The human nephrin promoter was inserted into the plasmid including human sirt-1 gene. We used lipofectamin for the transfection of final plasmid into the mouse podocytes. We compared the degrees of apoptosis between transfected and non-transfected podocytes under the mixture of 30 mM of glucose, 200 μM of linoleic acid and 1 μM of angiotensin II.

**Results:** Human sirt-1 gene tranfected podocytes decreased the degree of apoptosis of them in dose-dependent manner, confirmed by reduction of caspase-3 via western blot ($p < 0.05$). The plasmid containing only nephrin promoter did not show the anti-apoptotic effect on podocytes under the same condition (p = NS). These results were determined by the protein produced by sirt-1 gene which was confirmed by polyclonal antibody to sirt-1 derived protein ($p < 0.05$). The addition of 2 μM of resveratrol, sirt-1 activator, potentiated anti-apoptotic effect of sirt-1 gene in the podocyte confirmed by further decreasing the concentration of caspase-3 ($p < 0.05$). In contrast, podocyte culture with 10 mM of nicotinamide, sirt-1 antagonist, completely reverse previously mentioned anti-apoptotic effect of sirt-1 confirmed by further increasing caspase-3 ($p < 0.05$).

**Conclusions:** Current findings suggest that the activation of human sirt-1 gene in the podocyte reduce the apoptosis of them under the diabetic milieu.

*Funding:* Private Foundation Support

## TH-PO137

**The Basal Expression of Key Autophagy Genes Is Reduced in the Hearts of Uremic Rats** William Edward White, Julius Edward Kieswich, Andrew Duncan Stewart Findlay, Steven Michael Harwood, Magdi Yaqoob. *William Harvey Research Institute, Barts & The London, Queen Mary School of Medicine & Dentistry, London, United Kingdom.*

**Background:** End stage renal disease (ESRD) is characterised by an accelerated aging phenotype. Normal and pathological aging is associated with a decrease in autophagy, the process by which worn out and dysfunctional cellular components are delivered to lysosomes for degradation. A reduction in basal autophagy may thus be implicated in accelerated aging seen in ESRD. We have analysed the expression of autophagy genes in cardiac tissues in a rat model of uremia to determine whether this differs from that in healthy animals.

**Methods:** Rats were rendered uremic using an adenine diet. RNA was purified from cardiac tissues from these and age and sex matched controls and reverse transcribed. Real-time PCR was performed using a Qiagen autophagy-dedicated microarray. Fold change in gene expression between the control and uremic group was calculated using 2 (-Δ Δ Ct) method using the manufacturer's data analysis software.

**Results:** The expression of 21 of 84 autophagy-related genes tested was ≥ 2-fold down-regulated in the uremic compared to the control group (n=3). Key genes involved in autophagosome formation *Ambra1*, *Atg4b*, *Atg7*, *Atg9a*, *Atg101* and *ULK1*, autophagy regulating genes *Arsa*, *Bak1*, *Bid*, *Cxcr4*, *Dapk1*, *Dram2*, *Mapk14*, *Nfkb1*, *Pink1*, *Park2*, *Rgs19*, *Tgfb1*, *and Tm9sf*, and lysosome-related genes *Cln3* and *Ctsd* were all down-regulated. The genes encoding traditionally investigated autophagy-related proteins LC3 and Atg5 were also down-regulated, but less than 2-fold.

**Conclusions:** Although limited, these preliminary investigations suggest a reduced expression of several key autophagy genes in the hearts of uremic rats. Whilst this needs to be corroborated with translational evidence of impaired autophagy, previous studies have demonstrated corresponding changes in mRNA levels of autophagy genes associated with changes in autophagy activity, thus these results suggest that basal autophagy is impaired. Given that impaired autophagy is associated with ageing, it may have a role in pathological aging in ESRD, and may be a target for pharmacological interventions to correct this.

## TH-PO138

**Identification of Small Molecule Inhibitors of Kidney Podocyte Injury Using High Content Imaging** Kenneth Young,[1] Insa Winzenborg,[2] Uwe Andag.[3] *[1]In Vitro Pharmacology, Evotec AG; [2]Screening Operations, Evotec AG, Hamburg, Germany; [3]Diabetic Complications, Evotec AG, Goettingen, Germany.*

**Background:** Podocytes are essential constituents of the glomerular filtration barrier. Diabetic nephropathy, the leading diagnosis in end-stage renal disease, is recognized to involve injury or loss of podocytes with the resultant onset of proteinuria. High content imaging using cellular screening assays with predictive relevance to human disease states provides a mechanism for identifying novel drug targets. As part of a greater CureNephron initiative, Evotec has screened for small molecule inhibitors of kidney podocyte injury using treatment with palmitate combined with high glucose to mimic diabetic conditions. The primary screen measured apoptosis in human conditionally immortalized podocytes, while secondary assays measured changes in the podocyte actin cytoskeleton.

**Methods:** In total, 50,000 maximally diverse compounds were screened from Evotec's drug discovery library. Apoptosis was quatified using a caspase 3/7 detection reagent (Essen BioSciences) measured on an Opera automated confocal imaging platform. In conjunction, a small number compounds from a commercially available annotated library were also screened. Amongst these, compounds with known podocyte protective qualities were identified thereby providing proof of concept for the assay set-up.

**Results:** With the set threshold the screen identified 48 novel hits capable of reducing palmitate/high glucose-induced podocyte apoptosis. Of these, 7 compounds were also able to reduce podocyte apoptosis in response to addition of puromycin aminonucleoside. Further characterization of selected hits indicated that all compounds were able to reduce palmitate/high glucose-induced changes in the podocyte actin cytoskeleton. Key compounds (demonstrating 40–80% protection, and 0.4–1.8 μM potency) were then selected for hit expansion.

**Conclusions:** Evotec have identified novel compounds suitable for small molecule derived target deconvolution. These compounds may enable the identifation of novel druggable targets capable of reducing podocyte injury and hence having therapeutic potential against diabetic nephropathy.

*Funding:* Pharmaceutical Company Support - Evotec AG

## TH-PO139

**Role of Adapter Protein Nck in Actin Polymerization Mediated by Dynamin** Valentina A. Shchedrina, Sanja Sever. *Dept of Medicine, Nephrology Div, Massachusetts General Hospital, Charlestown, MA.*

**Background:** Nck is an adaptor protein that links receptor and receptor-associated tyrosine kinases with proteins that directly or indirectly regulate reorganization of actin cytoskeleton. For example, Nck binds to plasma membrane protein, nephrin, a key structural and signaling component of the slit diaphragm in podocytes, and to downstream effectors that regulate actin cytoskeleton. Based on our hypothesis SH3 domains of Nck1 interact with proline-rich domain of dynamin (dyn) and facilitate dynamin oligomerization that causes actin polymerization at membrane vicinity.

Thus, we investigated whether SH3 domains of Nck1 can regulate dynamin oligomerization state and subsequent actin remodeling in podocytes.

**Methods:** To study a possibility of the Nck-driven dynamin oligomerization *in vitro*, two methods were applied. First, the GTPase activity of human dyn isoform 1 (dyn1) was measured in the presence of SH3 domains of human Nck1. Second, dynamin oligomerization state was detected using fluorescence-lifetime imaging microscopy (FLIM) of ECFP/EYFP-tagged dyn1 in the presence of SH3 domains of Nck1. To study a possibility of Nck-driven actin reorganization *in vivo*, podocytes overexpressing SH3 domains of Nck1 were stained with actin antibodies and changes in actin cytoskeleton were detected by confocal microscopy.

**Results:** Binding SH3 domains of Nck1 to dyn1 to promotes dynamin oligomerization and increases the rate of GTP hydrolysis. Nck1-SH3 domains increase the rate of GTP hydrolysis in concentration-dependent manner that reflects specificity of this interaction. In addition, FLIM analysis detected dynamin oligomerization upon interaction with SH3 domains of Nck1.

**Conclusions:** Our data suggest that Nck-driven dynamin oligomerization could contribute in actin cytoskeleton regulation and that in this case dynamin is one of the downstream effector of the nephrin-Nck signaling pathway.

*Funding:* NIDDK Support

## TH-PO140

**miR-34c Ameliorates Renal Fibrosis via Down-Regulation of Jagged1** Ryuji Morizane, Toshiaki Monkawa, Shizuka Fujii, Hiroshi Itoh. *Internal Medicine, Keio Univ School of Medicine, Tokyo, Japan.*

**Background:** micro RNAs (miRNAs) are small non-coding RNAs that act as posttranscriptional repressors by binding to the target mRNAs. On the other hand, mesenchymal-epithelial transition (EMT) and renal fibrosis are pathological process of chronic kidney disease (CKD), and the relationship to miRNAs is becoming recognized as a potential target for CKD therapies.

**Methods:** We examined miRNA expression in three experimental models; unilateral ureteral obstruction (UUO) of mice, TGF-β on a mouse renal tubular cell line (MCT) as EMT models, and Activin induced renal epithelialization of mouse embryonic stem cells as an epithelialization model. We performed microarray of miRNA using these three experimental models. We found three miRNAs involved in these three models, and analyzed functions of one of three miRNAs using TGF-β and UUO.

**Results:** We identified three miRNAs presumably involved in EMT using microarray analysis, and focused on one of three miRNAs, miR-34c. Over-expression of miR-34c under TGF-β stimulation in MCT significantly attenuated up-regulation a mesenchymal gene, Snail1, and retained a epithelial gene, KSP-cadherin. To confirm these effects of miR-34c, we performed over-expression of miR-34c in mice with UUO. Mice were injected with miR-34c precursor from the tail vein, and the unilateral ureter was subsequently ligated. On day 7 of UUO, we harvested kidneys, and analyzed EMT and renal fibrosis using real-time PCR and α-SMA staining. miR-34c injected mice showed significantly less fibrosis than sham injected mice, and Snail1 and Vimentin were significantly suppressed by miR-34c. To find the target mRNA of miR-34c, we have examined the expression of genes that were known to involve in TGF-β pathway and have a complementary sequence to miR-34c. PCR and Western blot showed miR-34c significantly suppressed Jag1 while TGF-β up-regulated Jag1.

**Conclusions:** We have identified a new miRNA involved in EMT and renal fibrosis. miR-34c attenuated EMT and renal fibrosis in UUO mice via down-regulation of Jag1, and our study suggested a new therapeutic approach for renal fibrosis.

*Funding:* Government Support - Non-U.S.

## TH-PO141

**Collagen I Downregulates the Inner Medulla Tubular Cells AQP2 Expression and Traffic through Integrin Linked Kinase (ILK)** Jose Luis Cano-Peñalver,[1] Mercedes Griera,[1] Alicia Luengo,[1] Andrea Garcia-Jerez,[1] Nuria Troyano,[1] Diego Rodriguez-Puyol,[2] Sergio De Frutos Garcia,[1] Manuel Rodriguez-Puyol.[1] *[1]Biología de Sistemas, Unidad Fisiología, Universidad de Alcala, Alcala de Henares, Madrid, Spain; [2]Fundacion de Investigacion Biomedica, Hospital Universitario Principe de Asturias, Alcala de Henares, Madrid, Spain.*

**Background:** Progressive interstitial fibrosis produces structural changes in the kidney, where abnormal extracellular matrix (ECM) may lead to functional changes.

The collecting tubule AQP2 modifies the tubular water reabsorption through transcriptional and postraslational regulations of its content as well as its quick cytoplasm-to-apical membrane trafficking. ECM messenger ILK (integrin linked kinase) modulates different transcription factors as well as cytoskeleton function.

**Methods:** We studied the ILK implication in the AQP2-dependent tubular absorption by using an in vitro model of cultured inner medulla collecting duct cells line (mIMCD3) over collagen I (COL I) or inactive gelatine as control. We depleted ILK expression in mIMCD3 by transfection with specific siRNAs.

**Results:** We previously shown (ASN kidney week 2012) that in vivo model of conditional ILK-deletion in adult mice, basal poliuria and decreased urine osmolality was due to cortical and medullar AQP2 expression and cytoplasm-to-apical traffic downregulation. LK-depleted mIMCD3 have decreased expression and cytoplasm-to-membrane traffic of AQP2, both in basal conditions or under activation with desmopressin. ILK-depleted cells, where AQP2 activity was diminished, got a lower decrease in intracellular osmolality under hypotonic stress. Confirming that changes in ECM components may modify AQP2, COL I-cultured cells have diminished ILK expression, AQP2 expression and traffic, as the intracellular osmolality change under hypotonic stress compared with gelatine-cultured control cells.

**Conclusions:** ILK levels decreased by ECM component COL I or by siRNA transfection in mIMCD3, reducing the water reabsorption capability, due to impaired ILK-dependent AQP2 expression and traffic.

*Funding:* Government Support - Non-U.S.

## TH-PO142

**Cellular Inhibitor of Apoptosis Protein 1 (cIAP1) Represses Albumin-Induced Chemokines and Adhesion Molecules Synthesis by Kidney Tubular Epithelial Cells** Joseph C.K. Leung,[1] Ai Ing Lim,[1] Loretta Y.Y. Chan,[1] Wai Han Yiu,[1] Kar Neng Lai,[2] Sydney C.W. Tang.[1] *[1]Dept of Medicine, The Univ of Hong Kong, Hong Kong; [2]Dept of Health Assessment, Hong Kong Sanatorium and Hospital, Hong Kong.*

**Background:** Protein overload activates proximal tubule epithelial cells (PTEC) to release chemokines and stimulates inflammatory response in the interstitium. Bone morphogenic protein-7 (BMP-7) has been shown to reduce infiltrating cells and kidney damage in acute and chronic renal failure. The present study examines the inhibitory effects and the relevant molecular mechanism of BMP-7 on chemokines and adhesion molecules synthesis by PTEC activated with human serum albumin (HSA).

**Methods:** PCR array was used to screen the expression profiles of chemokines and adhesion molecules in cultured human PTEC. The molecules that were upregulated by HSA and can be significantly reduced by BMP-7 were identified. The kinetic of their expression in PTEC was determined by RT-PCR and ELISA. The activation of NF-κB and the relevant molecules of the signaling pathways were examined by RT-PCR, western blotting, immunofluorescence microscopy and transcriptional factors ELISA.

**Results:** Expression of CXCL1, CXCL2 and VCAM-1 by PTEC was upregulated by HSA, and the expression was significantly reduced by BMP-7. Further experiments confirm that BMP-7 reduced the HSA up-regulated gene and protein expression of CXCL1, CXCL2 and VCAM-1 dose and time dependently. HSA activated both the canonical and noncanonical NF-κB pathways in PTEC, as shown by the reduced expression of IκB, the nuclear translocation of p50 and p52, and data from the transcriptional factor ELISA. The NF-κB activation was repressed by BMP-7. Interestingly, the HSA-upregulated expression of cIAP1 and TRAF3 by PTEC, was further increased by BMP-7. To the contrary, the expression of NIK in PTEC was increased by HSA but down-regulated by BMP-7.

**Conclusions:** Our data suggest that HSA up-regulates CXCL1, CXCL2 and VCAM-1 *via* both the canonical and noncanonical NF-κB pathways in PTEC. BMP-7 suppresses these events through increased production of cIAP-1 and TRAF3, which inhibits the accumulation of NIK and the subsequent activation of NF-κB pathways.

*Funding:* Other NIH Support - Supported by Hong Kong General Research Fund

## TH-PO143

**The Protective Effect of Adrenomedullin on Liver Injury Induced by Bilateral Nephrectomy or Renal Ischemia-Reperfusion in Mice** Tatsuhiko Tabata,[1] Saki Takahashi,[1] Hiroko Sonoda,[1] Yu Miyahara,[1] Mari Isomi,[1] Johji Kato,[2] Kazuo Kitamura,[3] Masahiro Ikeda.[1] *[1]Veterinary Pharmacology, Univ of Miyazaki, Miyazaki, Japan; [2]Frontier Science Research Center, Univ of Miyazaki, Miyazaki, Japan; [3]Internal Medicine, Univ of Miyazaki, Miyazaki, Japan.*

**Background:** Experimental studies have shown that acute kidney injury (AKI) induces distant organ failure including hepatic dysfunction. However the mechanism of AKI-induced hepatic dysfunction remains unclear. Recent studies have suggested that uremic toxin causes tissue injury, which is mediated by a mechanism related to hypoxia.

**Methods:** In this study, therefore we examined whether hypoxia-related mechanisms are involved in AKI-induced hepatic injury using two mice models of bilateral nephrectomy (BNx) and renal ischemia-reperfusion (I/R).

**Results:** Plasma urea nitrogen, creatinine, aspartate transaminase, and alanine transaminase were significantly increased 6 and 24 h after induction of either BNx or I/R, compared with those of sham-operated animals. PCR array analyses showed that BNx and I/R up-regulated 20 genes associated with hypoxia, including adrenomedullin (AM), and down-regulated 6 genes. As AM has been reported to have protective effect against tissue injury, we then analyzed the expression level of AM-related genes, including AM binding protein-1 and AM receptor components such as calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein (RAMP) 2 and 3. Real-time PCR analyses showed that BNx and I/R up-regulated RAMP2 and RAMP3 within 32 h after either operation. Finally, we examined the effect of AM on BNx- or I/R-induced liver injury in vivo. AM (10 mg/kg) was intraperitoneally administered to mice 30 min before and just after each operation. AM significantly lessened the severity of liver injury 24 h after each AKI-operation, without improving renal function, in comparison with controls.

**Conclusions:** These results suggest that the hypoxia-related mechanism is involved in AKI-induced hepatic injury, and that AM may have the potential to protect against AKI-induced liver injury.

## TH-PO144

**Study of NOV/CCN3 Expression and Effects in Different In Vivo Models of Chronic Kidney Disease** Pierre-Olivier Marchal,[1] Chantal Kazazian,[1] C. Chatziantoniou,[2] Christos E. Chadjichristos,[2] Cécile Martinerie.[1] *[1]INSERM UMRS_938/UPMC, Paris, France; [2]INSERM UMR_702/UPMC, Paris, France.*

**Background:** Independently of its origin chronic kidney disease (CKD) results in high inflammation and fibrosis. Current treatments are partially effective and consequently new therapeutic targets are required. NOV/CCN3 was reported to be antifibrotic with pro-angiogenic and anti-mesangioproliferative effects in anti-Thy1.1 model and to inhibit profibrotic CTGF/CCN2 in mesangial cells. NOV is a secreted multifunctional protein belonging to the CCN family involved in different physiological and pathological processes (angiogenesis, inflammation, cancers). To further explore NOV involvement in CKD we analyzed its expression in other *in vivo* models and tested its ability to modulate expression of profibrotic and inflammatory molecules.

**Methods:** NOV expression was analyzed at various times in the following models: renin transgenic mice (RenTg) at early stages (9-12-21w) and late stages (53w), Angiotensin II (AngII) perfused mice (1-2-4w) and unilateral ureteral obstruction (UUO, 3-7-15d). In these models expression of NOV, profibrotic and inflammatory molecules were assessed in cortex by RT-qPCR and Western blot. NOV *in vitro* effects were studied on rat aortic vascular smooth muscle cells (VSMC).

**Results:** In RenTg mice we observed two phases: one early in which NOV expression was increased by AngII (3.5 folds, 12w), especially in VSMC, then returned at basal level (21w) and one late in which NOV was correlated with the increase of TNFα (53w). In VSMC NOV downregulated profibrotic AT1R (90%) and CTGF (40%) whereas it stimulated *MCP-1 (23 folds) and was increased by TNFα (3.5 folds). In AngII-perfused mice and UUO we also observed a correlation between* NOV, TNFα and MCP-1. Moreover, NOV injection stimulated MCP-1 in kidney of healthy mice.

**Conclusions:** Our results suggests that NOV could have a dual role: protective at early stage but participates to inflammation at late stage of CKD. Ongoing experiments on NOV KO mice should bring us more evidences to this hypothesis.

## TH-PO145

**$P2Y_{12}$ Receptor Protein Is Co-Localized with AQP2 in the Collecting Duct Principal Cells of Rat and Mouse** Bellamkonda K. Kishore,[1] Janos Peti-Peterdi,[2] Karie G. Villanueva,[2] David L. Strasburg,[1] Noel G. Carlson,[1] Donald E. Kohan,[1] Yue Zhang.[1] *[1]VA Medical Center & Univ of Utah, Salt Lake City, UT; [2]Univ of Southern California, Los Angeles, CA.*

**Background:** $P2Y_{12}$ receptor (R) is an ADP-activated G protein-coupled receptor that inhibits adenylyl cyclase activity, thus potentially reducing cellular cAMP levels. We observed that administration of clopidogrel (Plavix®), which irreversibly blocks $P2Y_{12}$-R, significantly increased urinary concentration and protein abundance of AQP2 in the medulla, and ameliorated lithium-induced polyuria in rats. Immunoblots using a commercial antibody confirmed the expression of $P2Y_{12}$-R protein in the kidney, however, the precise cellular localization of it in the kidney has not yet been established.

**Methods:** We generated and characterized a peptide-derived rabbit polyclonal antibody specific for a 16-amino acid C-terminal sequence of mouse $P2Y_{12}$-R, which differs from rat sequence by only one amino acid residue. This peptide has no sequence identity with $P2Y_{13}$, $P2Y_{14}$, GPR34, GPR82, GPR87, or GPR171. The affinity purified antibody was used in immunoperoxidase and confocal immunofluorescence microscopy to localize $P2Y_{12}$-R protein expression in normal healthy rat and mouse kidneys and to compare its distribution to that of AQP2.

**Results:** In both rat and mouse renal cortex, strong labeling for $P2Y_{12}$-R was seen on the brush border of the proximal tubules (PT). Moderate labeling was seen in the vascular smooth muscle cells (VSMC) of large and small arterioles. Weaker labeling was found at the apical membrane of the collecting ducts (CD) in the cortex and medulla, co-localizing with AQP2. $P2Y_{12}$-R labeling in CD was present exclusively in AQP2-positive cells suggesting expression in principal, but not intercalated cells.

**Conclusions:** The observed expression pattern of $P2Y_{12}$-R and AQP2 in vasopressin-responsive CD principal cells is consistent with the in vivo effects of pharmacological blockade of $P2Y_{12}$-R in normal and lithium-treated animals. The functional significance of the expression of $P2Y_{12}$-R on the brush border of PT cells and in the cortical arterioles needs to be elucidated.

*Funding:* Veterans Affairs Support, Private Foundation Support

## TH-PO146

### Elevated Soluble Tumor Necrosis Factor Alpha Receptor 1 and 2 Concentrations in Hemodialysis Patients Are Not Reflected by Alterations in Membrane Expression

Nathalie Neirynck, Griet Lrl Glorieux, Eva Schepers, Annemieke Dhondt, Raymond C. Vanholder. *Nephrology Div, Ghent Univ Hospital, Belgium.*

**Background:** Elevated serum concentration of soluble TNFα receptor 1 (sTNFR1) and 2 (sTNFR2) and TNFα in hemodialysis (HD) patients are at least in part attributed to decreased renal clearance. It is not known whether the membrane expression of these receptors is altered in HD and as a consequence also contributes to the differences in serum concentration and possibly to the altered leukocyte function in HD patients, since membrane expression of TNFR1 and 2 is important for TNFα signal transduction. In this study, the soluble and membrane expression of TNFR1, 2 and TNFα were determined in parallel in order to unravel the link between both.

**Methods:** Whole blood samples of healthy controls (C) and predialysis samples of HD patients were labelled with fluorescent anti-membrane (m)TNFR1, anti-mTNFR2 and anti-mTNFα and analyzed by flow cytometry (n= 8). The mean fluorescence intensity (MFI) was measured when all leukocytes were gated together (tMFI), as well as the MFI of the monocytes, granulocytes and lymphocytes when gated separately. Soluble concentrations were measured by ELISA.

**Results:** The soluble concentrations were markedly higher in HD for sTNFR1 (C: 993 vs HD: 9558 pg/ml, $p < 0.001$), sTNFR2 (C:2245 vs HD: 16344 pg/ml, $p < 0.001$) and TNFα (C: 2.2 vs 9.9 pg/ml, $p < 0.001$), while the total leukocyte membrane expression did not differ for mTNFR1 (tMFI: C: 682 vs HD: 693, p = NS), mTNFR2 (tMFI: C: 762 vs HD: 852, p = NS) and mTNFα (tMFI: C: 616 vs HD 670, p = NS). Furthermore, evaluating the different leukocyte subtypes separately, there was also no difference in receptor expression on monocytes, granulocytes and lymphocytes, except for the expression of mTNFR2, which was decreased on lymphocytes in HD compared to C (C: 648 vs HD: 521, $p < 0.05$).

**Conclusions:** This study suggests that the elevated concentrations sTNFR1 and sTNFR2 in hemodialysis patients compared to healthy controls were rather due to a decreased renal clearance than an altered cellular expression.

*Funding:* Government Support - Non-U.S.

## TH-PO147

### Blockage of IL-8 Receptor Signaling Inhibits Cyst Development in ADPKD

Jong Hoon Park, Eunji Lee, Je Yeong Ko. *Biological Science, Sookmyung Women's Univ, Seoul, Korea.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is one of the most highly prevalent inherited disorders and results in the progressive development of thousands of cysts in both kidneys. There are several signaling pathways, which are already known or continuously identifying as well, to initiate and lead to the progression of ADPKD. Some cytokines and growth factors secreted by the cyst-lining epithelia were defined to initiate and promote cyst growth. In our previous study, interleukin (IL)-8 increased significantly in cells following knockdown of Mxi1, an antagonist for c-myc, which is overexpressed in ADPKD.

**Methods:** We used WT9-7 and WT9-12 as human PKD cell lines and the HRCE cell line as the control. Interlukin-8 was measured by enzyme-linked immunosorbent assay and the expression of IL-8 receptor or other signaling molecules were checked by western blot. IL-8 signaling was inhibited either by IL-8 receptor antagonist treatment or gene siliencing of receptor. Inhibiting effect on IL-8 signaling and cell proliferation was tested by western blot and XTT assay, respectively. Finally, 3D culture of MDCK cells under antagonist treatment or knockdown of IL-8 receptor was performed to identify the effect on kidney cystogenesis *in vitro.*

**Results:** In this study, we focused on whether IL-8 signaling is associated with renal cyst formation, and tested the possibility of IL-8 as a new therapeutic target for PKD. First, IL-8 secretion and expression of its receptor increased remarkably in human PKD cells. Furthermore, receptors specific for IL-8 was easily detected particularly at the cyst-lining epithelial cells of the kidney in human patients with PKD. IL-8 secretion decreased under treatment with an IL-8 receptor antagonist, and activation of the signaling molecules diminished as well. A three-dimensional culture was performed to understand how IL-8 affected cyst formation, and resulted in alleviation of cystogenesis after blocking the IL-8 receptor signals.

**Conclusions:** These results suggest that IL-8 and its mediating signals might be a factor stimulating cystogenesis in ADPKD and have potential as a new biomarker and therapeutic target for the disease.

## TH-PO148

### Prostaglandin E2 Inhibits Growth and Stimulates Sodium Transporters through EP4, but Increases Fibronectin and ROS Production through EP1 in Mouse Proximal Tubule Cells

Rania Nasrallah, Andrew J. Karam, Richard L. Hebert. *Kidney Research Centre, Cellular and Molecular Medicine, Univ of Ottawa, Ottawa, Canada.*

**Background:** Proximal tubule (PT) growth promotes PT hyper-reabsorption in chronic kidney disease, which in turn contributes to hyperfiltration. Prostaglandin (PG) $E_2$ is a key regulator of renal growth and tubular transport, but its role in the PT is unknown.

**Methods:** Transforming growth factor β (TGFβ) is a main regulator of PT in kidney disease, thus we compared the effects of $PGE_2$ to TGFβ. Mouse PT cells (MCT) were stimulated for 24 hours with $PGE_2$, in the presence or absence of TGFβ.

**Results:** By Realtime PCR we showed that $PGE_2$ $EP_1$ receptors are increased 2-3 fold by $PGE_2$ and TGFβ, but $EP_4$ is unchanged. $EP_1$ was inhibited with the $EP_1$ receptor antagonist ONO-8711. Both TGFβ and $PGE_2$ attenuated DNA and protein synthesis, with 50-80 % reductions in $H^3$-thymidine and $H^3$-leucine incorporation, the inhibition was additive upon co-stimulation. In MCT lysates assayed by Western blotting, the cyclin-dependent kinase inhibitor p27 is increased 2-fold by $PGE_2$; TGFβ attenuated the $PGE_2$ response upon co-stimulation but ONO-8711 had no effect. In contrast TGFβ but not $PGE_2$ increased p21 by 4-fold. While TGFβ increases cyclin D1 up to 3-fold, $PGE_2$ alone had no effect, but it attenuated TGFβ-mediated cyclin D1. Na-K-ATPase α1 (NaK) was increased 2.5-fold by $PGE_2$. TGFβ had no effect on NaK, but attenuated $PGE_2$-stimulated NaK to 0.6-fold of control. Consistently, TGFβ also reduced $PGE_2$-stimulated sodium hydrogen exchanger NHE1 by 50% and NHE3 by 70%, but neither was affected by ONO-8711. $PGE_2$ and TGFβ also increased fibronectin levels in MCT, by 2-fold and 6-fold respectively. Finally, $PGE_2$ increased ROS generation (lucigenin activity) by 2-fold. ONO-8711 blocked $PGE_2$-mediated fibronectin and ROS.

**Conclusions:** In summary, $PGE_2$ and TGFβ are acting via different mechanisms to inhibit the cell cycle, and have opposite effects on sodium transporters. Accordingly, $PGE_2$ stimulates fibronectin and ROS generation via $EP_1$ receptors, but affects growth and sodium transporters via $EP_4$ pathways in the proximal tubule.

*Funding:* Government Support - Non-U.S.

## TH-PO149

### Autophagy Is Impaired in Unilateral Ureteral Obstruction (UUO) Model of Renal Fibrosis

Yang Ou,[1] Djamali Muhoza,[1] Christian Herzog,[1] Randy S. Haun,[2,3] Gur P. Kaushal.[1,3] *[1]Internal Medicine, Univ of Arkansas for Medical Sciences; [2]Pharmaceutical Sciences, Univ of Arkansas for Medical Sciences; [3]Central Arkansas Veterans Healthcare System, Little Rock, AR.*

**Background:** Autophagy is a highly conserved protein degradation system in eukaryotes. Very little is known about the role of autophagy during progressive renal fibrosis. We examined whether renal fibrosis results from defective autophagy using UUO as a model of renal fibrosis.

**Methods:** Renal fibrosis was induced in mice using the UUO model. Autophagy was inhibited by autophagy flux inhibitor chloroquine and upregulated by autophagy enhancing drug Torin-1. We used Beclin-1 transgenic (Beclin-1 Tg) mice under the control of the androgen-inducible KAP2 (kidney androgen-regulated protein) promoter and obtained overexpression of Beclin-1 in the proximal tubules.

**Results:** The autophagy-related proteins Beclin-1, Atg5, Atg7, and Atg12, and the conversion of LC3-I to LC3-II, were increased at 1d, 3d, 5d, and 9d after UUO. Production of matrix proteins including α-SMA, fibronectin, and collagen-I was elevated following UUO. Accumulation of autophagy-specific substrate p62, Keap1, ubiquitinated proteins and LC3-I/LC3-II conversion were observed in obstructed kidneys. The level of p62 and LC3-I/LC3-II conversion were not further increased in UUO mice administrated with chloroquine, indicating that autophagy is impaired during the progression of renal fibrosis. Concomitant accumulation of p62, Keap1, ubiquitinated proteins supports the notion that autophagy is defective in the UUO model since Keap1 is degraded in a p62-dependent manner and p62 plays a central role for targeting ubiquitinated proteins to autophagosomes. Accumulation of p62 and collagen-I was slightly reduced in Beclin-1 Tg mice and in mice receiving Torin-1, suggesting that impaired autophagy status following UUO injury could not be efficiently recovered by upregulation of autophagy.

**Conclusions:** Accumulation of renal fibrosis is accompanied by defects in autophagy in the UUO model. Overexpression of Beclin-1 in transgenic mice and administration of Torin-1 were unable to completely overcome the autophagy deficiency.

*Funding:* Veterans Affairs Support

## TH-PO150

**Endostatin – A Contributor to Fibrosis of Aging Kidney** Chi Hua Sarah Lin, Jun Chen, Michael S. Goligorsky. *New York Medical College, Valhalla, NY.*

**Background:** Aging is associated with progressive organ fibrosis. Microvascular rarefaction is a permanent companion of fibrotic process occurring with a relative synchrony in all organs, kidney included, partly due to impaired angiogenesis in the elderly. Therefore, we hypothesize that a common denominator - an anti-antigenic substance - might exist to explain it.

**Methods:** Endostatin, a key anti-angiogenic factor, is a 20-22 kDa c-terminal fragment of collagen XVIII, a ubiquitous component of sub-endothelial membranes especially in vessels with fenestrated endothelium, like those in renal glomeruli and peritubular capillaries. Therefore, we sought to investigate the level of endostatin in kidney and plasma of aging mice by immunoblotting and ELISA as well as cellular effect of endostatin.

**Results:** In aged mice (22 mo-old) kidney, the expression of p16 gene and the ratio of short/long (S/L) endoglin, markers of senescence, were increased as examined by RT-PCR. Examination of aged kidney showed decreased microvascular density. The protein level of endostatin in the plasma and kidney samples from aged mice was 2 and 3 fold increased. To understand cellular effects of Endostatin, we isolated primary endothelial cells (ECs) from kidney of control mice by enzymatic digestion followed by double selection on CD31-coated magnetic beads. The isolated ECs (CD31+, VE-cadherin+ and Endoglin+) were treated with mouse Endostatin at concentrations 25 μg/ml for 5.5 days. Cells showed signs of impaired angiogenesis, as evidenced by decreased formation of cord-like structures. Endostatin treatment also decreased VE-cadherin expression with a concomitant increase in the expression of α-smooth muscle actin in primary EC, indicative of a phenotypic switch toward endothelial-mesenchymal transition.

**Conclusions:** These data document that 1) Endostatin levels are increased in the aged kidney and plasma and 2) Endostatin causes primary ECs transdifferentiation into mesenchymal-like cells. This mechanism of endostatin action may account for microvascular rarefaction of kidney aging.

*Funding:* NIDDK Support

## TH-PO151

**RNA-Seq Mapping of G Protein-Coupled Receptor Expression along the Nephron and Collecting Duct** Jae Wook Lee, Fahad Saeed, Chung-Lin Chou, Mark A. Knepper. *NIH.*

**Background:** G protein-coupled receptors (GPCRs) are important regulators of physiological function in renal tubule epithelia. Here we describe the expression profile of all known GPCRs along the renal tubule using RNA-seq.

**Methods:** We manually microdissected each renal tubule segment from collagenase-treated rat kidneys, using a high resolution microscopy system capable of discriminating cell types. After reverse transcription and amplification, cDNA libraries were sequenced using an Illumina HiSeq 2000 sequencer. Sequences were mapped to the rat reference genome (rn4) and expression levels were calculated in 'reads per kilobase exons per million mapped reads' (RPKM). GPCRs were identified from the list of all expressed genes (PANTHER search term "G protein-coupled receptor").

**Results:** Of 853 annotated rat GPCRs, 60 GPCRs had RPKM > 1.0 in at least one renal tubule segment. Expression patterns of vasopressin receptors (*Avpr1a* and *Avpr2*), calcium-sensing receptor (*Casr*), and other receptors (*Pth1r*, *Gcgr*, *Calcr*, and *Ptger3*) were consistent with the literature. Based on rule-based clustering algorithm, we identified segment-specific expression patterns for additional GPCRs. These include: growth hormone-releasing hormone receptor (*Ghrhr*) in the thin descending and ascending limbs (median RPKM 647.0 and 17.5); taste receptor type 1, member 1 (*Tas1r1*) in the medullary and cortical thick ascending limbs (6.1 and 3.1); frizzled proteins in the connecting tubule, cortical collecting duct, and outer medullary collecting duct (*Fzd1*, 8.9, 31.8, 11.5 and *Fzd4*, 44.1, 27.4, 14.7); soluble frizzled-related protein 2 (*Sfrp2*) in the cortical collecting duct and distal convoluted tubule (50.5 and 60.8); and purinergic receptors in the collecting duct (*P2ry2* in the cortical collecting duct, 6.6; *P2ry14* in the outer medullary collecting duct, 166.7). Eleven orphan GPCRs were found: *Gpr56* and *Gprc5c* showed widespread expression along the renal tubule.

**Conclusions:** Further studies are needed to assign physiological functions to the GPCRs not previously known to be expressed in the kidney. Additional analysis of these RNA-seq data (in addition to GPCR genes) will provide further information on segment-specific signaling pathways.

*Funding:* Other NIH Support - NHLBI

## TH-PO152

**APOL1 Risk Variants Cause a Greater Increase in Autophagy, Apoptosis and Necrosis in Podocytes, Accompanied by Decreased Podocyte Viability Compared to Wild-Type APOL1** Xiqian Lan, Kang Cheng, Partab Rai, Tejinder Singh, Nirupama Chandel, Rivka Lederman, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical Center, Great Neck, NY.*

**Background:** Compared with European Americans, African Americans develop much higher rates of nephropathy including hypertension-associated end-stage renal disease (ESRD), focal segmental glomerulosclerosis (FSGS), and HIV-associated nephropathy (HIVAN). Recently, emerging reports show that this major health disparity is strongly associated with two coding sequence variants in the *APOL1* gene encoding Apolipoprotein L1 (ApoL1). However, the cellular function of Apol1 and the role of these variants in the risk for nephropathy are still unknown.

**Methods:** Lentiviruses for over expression of ApoL1 wild type and variant proteins were constructed, using constructs kindly provided by the laboratory of Karl Skorecki at the Technion in Haifa. Human podocytes were infected with these lentiviruses. Western blots, MTT assay, and staining assays with acridine orange, Annexin V, or 7-AAD were performed to compare the effects of wild type and variant Apol1 on human podocytes.

**Results:** Western blots results showed that ApoL1 wild type and variant proteins were successfully over expressed in human podocytes. Staining assays revealed that over expression of wild type ApoL1 increased autophagy, apoptosis, and necrosis; while all of these effects were further increased by overexpression of the Apol1 risk variants. MTT assay indicated that cell viability was dramatically decreased by Apol1 risk variants.

**Conclusions:** Apol1 risk variants displayed cytotoxicity to podocytes through enhancing autophagy, apoptosis, and necrosis. Since loss of podocytes is an initiating step in the development of FSGS, the present study supports a pathogenic role for podocyte expression of APOL1 risk variants in the development of kidney lesions in in susceptible subjects.

*Funding:* NIDDK Support

## TH-PO153

**Effect of Uremic Toxins on Leukocytes: The Enigma of Organic Solute Transport Finally Unraveled** Eva Schepers,[1] Henricus A.M. Mutsaers,[2] Annemieke Dhondt,[1] Nathalie Neirynck,[1] Rosalinde Masereeuw,[2] Raymond C. Vanholder,[1] Griet Lrl Glorieux.[1] *[1]Internal Medicine, Nephrology Divsion, Ghent Univ Hospital, Ghent, Belgium; [2]Pharmacology and Toxicology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands.*

**Background:** Several uremic toxins (UT), both protein bound and not, have been shown to cause leukocyte dysfunction. As a consequence of the increased levels of UT in the circulation in chronic kidney disease, induction of cytotoxicity must be linked to increased cytoplasmic levels. However, until now, the expression of organic uptake transporters, as abundantly expressed on e.g. proximal tubular cells, has never been demonstrated on leukocytes.

**Methods:** Monocytes were isolated by a positive selection using MACS after Ficoll density gradient centrifugation from healthy controls (C) and hemodialysis patients (HD). Gene expression of the uptake transporters organic anion transporter 1 (OAT1), OAT3, organic cation transporter 2 (OCT2), OATpolypeptide4C1 (OATP4C1) was evaluated using RT-pPCR. In addition, expression at protein level of OATP4C1 [mean fluorescence intensity (MFI)] was evaluated by flow cytometry.

**Results:** Only the uptake transporter, OATP4C1, was expressed in monocytes. No difference in OATP4C1mRNA levels could be observed in monocytes from HD (n=9) versus C (n=10) (ΔCt: 7.9±0.4 vs. 8.1±0.6,NS). Protein expression of OATP4C1 on monocytes was confirmed by flow cytometry also revealing expression on lymphocytes and granulocytes. However, no significant difference in the protein expression level in HD (n=8) versus C (n=4) was observed (470±139 vs. 479±155; 282±102 vs. 272±120; 532±146 vs.692±181, respectively, NS). OATP4C1 was significantly more expressed on monocytes and granulocytes compared to lymphocytes ($P<0.05$).

**Conclusions:** These data show for the first time the expression of an organic uptake transporter protein, OATP4C1, on leukocytes. This transporter has been suggested to play a role in the uptake of the known UT. Thus, diffferent UT could enter leukocytes via OATP4C1 contributing to many of the known cytotoxic effects. The functionality of the transporter in HD remains to be evaluated but this protein could be a target for preventing cytotoxicity in CKD.

## TH-PO154

**Nanoscale Protein Architecture of the Mouse and Human Kidney Glomerular Basement Membrane** Hani Suleiman,[1] Lei Zhang,[1] Jeffrey H. Miner,[2] Andrey S. Shaw,[1] Adish Dani.[1] *[1]Pathology & Immunology, Washington Univ, Saint Louis, MO; [2]Renal Div, Washington Univ, Saint Louis, MO.*

**Background:** In multicellular organisms, proteins of the extracellular matrix play structural and functional roles in a variety of organs, so understanding ECM protein organization is an important goal. The kidney glomerular basement membrane (GBM) is a unique, thick BM that is an integral part of the glomerular filtration barrier and participates in filtering large volumes of plasma to form the ultrafiltrate. The GBM is thought to result from fusion of two distinct basement membranes secreted by two different cells, podocytes and endothelial cells.

**Methods:** We used well characterized epitope-specific antibodies to carry out sub-diffraction resolution stochastic optical reconstruction microscopy (STORM) and deep-etch electron microscopy correlation to resolve the in situ molecular organization of proteins within the mouse and human GBM.

**Results:** We constructed a molecular reference frame that revealed a laminar organization of ECM proteins within the GBM. Agrin, a large heparan sulfate proteoglycan, was localized in two parallel leaflets with a peak distance between each other of 138 nm. With agrin as a reference, we mapped the position of a panel of molecules in the GBM, including the integrin b1 ectodomain. The laminin α5 C-terminus co-localized with podocyte and endothelial cell integrin b1, whereas the more N-terminal LM-521 epitopes were found near the center of the GBM, revealing a definitive orientation. The human GBM possesses a dense layer of collagen α3α4α5(IV) covering more than 50% of the GBM's thickness, but it is excluded from the GBM's edges where integrin b1 penetrated.

**Conclusions:** Separate analyses of domains near the N- and C-termini of agrin, laminin, and collagen IV in mouse and human revealed a highly organized arrangement within the GBM, visualized *in situ*. The results are consistent with separate basement membrane layers contributed by podocytes and endothelium. Integrin b1's major ligands are likely the laminin a5 C-terminus and agrin.

*Funding:* NIDDK Support

## TH-PO155

**Indoxyl Sulfate Signals for Rapid mRNA Stabilization of Cbp/p300-Interacting Transactivator with Glu/Asp-Rich Carboxy-Terminal Domain 2 (CITED2) and Suppresses the Expression of Hypoxia-Inducible Genes in Experimental CKD and Uremia**

Tetsuhiro Tanaka, Junna Yamaguchi, Yoshiki Higashijima, Masaomi Nangaku. *Div of Nephrology and Endocrinology, Univ of Tokyo School of Medicine, Tokyo, Japan.*

**Background:** Tubulointerstitial hypoxia serves as a final common pathway in progressive renal disease. Circumstantial evidence suggests that the function of hypoxia-inducible factor (HIF)-1 may be suppressed in a CKD milieu. In this study, we investigated the effect of indoxyl sulfate (IS) on the expression of hypoxia-inducible genes in vitro and in vivo.

**Methods:** In vitro, the expression of HIF-1α, CITED2 and HIF-1 target genes was quantified by immunoblotting and real-time PCR. Binding of HIF-1 to the target gene promoters was evaluated by chromatin immunoprecipitation (ChIP). mRNA stability of CITED2 was measured by actinomycin D treatment. The role of MAP kinase pathways was evaluated by using specific inhibitors. In vivo, the effect of IS on HIF-1 target genes was investigated in the rat remnant kidney, with or without the oral adsorbent, AST-120. Additionally, angiogenic, HIF-1 target genes was quantified in the isoproterenol-induced heart failure (HF) model in rats treated with or without Indole.

**Results:** In vitro, IS reduced the hypoxic induction of HIF-1-target genes, which was not associated with quantitative changes in HIF-1α, but with the functional impairment of the HIF-1α C-terminal transactivator domain (CTAD). Among candidate suppressors of the HIF-1αCTAD, CITED2 was significantly upregulated by IS. The induction of CITED2 was mediated by posttranscriptional mRNA stabilization through the ERK1/2 signaling. In a CKD model in vivo, tubular expression of several HIF-1-target genes, such as vascular endothelial growth factor (VEGF) and heme oxygenase-1 (HO-1) was markedly restored by treatment with AST-120. Furthermore, Indole significantly suppressed the induction of hypoxia-inducible, angiogenic genes in rats with heart failure.

**Conclusions:** Results of these studies reveal a novel role of IS in modulating the transcriptional response of HIF-1 and provide insight into the molecular mechanisms underlying progressive nephropathies as well as cardiorenal anemia syndrome.

*Funding:* Government Support - Non-U.S.

## TH-PO156

**Pharmacological Blockade of ADP-Activated $P2Y_{12}$ Receptor in Rat IMCD Potentiates dDAVP-Induced AQP2, AQP3 and V2 Receptor Expression**

Yue Zhang,[1] Younis Baqi,[2] Noel G. Carlson,[1] Donald E. Kohan,[1] Christa E. Müller,[2] Bellamkonda K. Kishore.[1] *[1]VA Medical Center & Univ of Utah, Salt Lake City; [2]Univ of Bonn, Bonn, Germany.*

**Background:** $P2Y_{12}$ receptor (R) is an ADP-activated G protein-coupled receptor that inhibits adenylyl cyclase activity. We found that $P2Y_{12}$-R protein is expressed in rat renal medullary collecting ducts (IMCD), and its irreversible blockade by the administration of clopidogrel (Plavix®) significantly increased urinary concentration and protein abundance of AQP2 in the medulla. These effects were not seen in Brattleboro rats, which genetically lack arginine vasopressin (AVP). So, in order to understand the exact relationship between AVP and $P2Y_{12}$-R here we used PSB-0739, a potent non-nucleotide antagonist of $P2Y_{12}$-R in primary cultures of rat IMCD cells, and examined the interaction between the $P2Y_{12}$-R and AVP, in relation to AQP2 expression.

**Methods:** PSB-0739 was synthesized and characterized by the methods established in our laboratory. Primary cultures of rat IMCD cells grown in Transwell® inserts were incubated with no agents (control) or dDAVP (50 nM) or PSB-0739 (100 nM) or a combination of these agents for 24 h (n = 6 inserts/condition). Cells were harvested, mRNA extracted, reverse transcribed and real-time RT-PCR was performed for AQP2, AQP3 and V2-R. Expression levels of target genes were normalized to that of β-actin.

**Results:** PSB-0739 alone did not affect AQP2 expression, but dDAVP alone increased AQP2 expression by 9.5-fold. Combination of PSB-0739 and dDAVP further increased AQP2 expression by 3.4-fold (i.e., 32-fold vs. controls). PSB-0739 also enhanced dDAVP-induced modest expression of AQP3 and V2-R, making them significantly higher than the control group ($P < 0.05$).

**Conclusions:** These data suggest that blockade of $P2Y_{12}$-R in IMCD in the absence of dDAVP has little or no effect, but it markedly potentiates the effect of dDAVP on AQP2, AQP3 and V2-R expression. This is the first example showing that pharmacological blockade of a purinergic receptor in IMCD can markedly potentiate the effect of AVP on AQP2, AQP3 or V2-R expression, thus emphasizing the significance of autocrine/paracrine regulation by extracellular nucleotides.

*Funding:* Veterans Affairs Support, Private Foundation Support

## TH-PO157

**Targeting PHD2 with Antisense ASOs Can Increase Erythropoietin Production for the Treatment of Anemia Associated with Chronic Kidney Disease**

Sue Murray, Raechel Peralta, Melanie Katz, Andy Watt, Shuling Guo, Gene Hung. *Antisense Drug Discover, ISIS Pharmaceuticals, Carlsbad, CA.*

**Background:** Anemia is a prevalent and premature comorbidity in chronic kidney disease (CKD). It is associated with multiple adverse clinical consequences including increased mortality. Today Erythropoiesis-stimulating agents (ESAs), together with iron supplementation, are the cornerstones of therapy for correcting anemia in CKD patients. Prolyl hydroxylase domain protein 2 (PHD2) is an oxygen/redox-sensitive enzyme that induces cellular adaptations to stress conditions. Inactivation of Phd2 enhances a hypoxia-inducible transcription factor (HIF)-mediated detoxification program in healthy organs, which prevents oxidative damage, organ failure, and tissue demise. In the present study, we examined the effects of reducing PHD2 mRNA expression in liver and kidney in balb/c mice, using an optimized antisense oligonucleotide (ASO) designed specifically for PHD2.

**Methods:** ASO was administered subcutaneously at a dose of 25mg/kg, twice a week for 3 weeks.

**Results:** ASO treatment caused ~80% and 65% reduction in PHD2 mRNA expression in liver and kidney respectively. A 7-fold increase in spleen weight was observed as compared to saline treated group, similar to what was observed in PHD2 conditional knockout mice (BLOOD, 15 March 2008, Vol 11 (6) 3229-3235). In addition, PHD2 ASO treated kidney EPO mRNA levels were increased 600 fold compared to the saline treated group. Further analysis of the kidney by in-situ EPO RNA staining confirmed the increase and showed it to be localized to the cells in the interstitial space. As a result, serum EPO levels were also elevated after PHD2 ASO treatment, ~5000 ± 128 (pg/ml) in PHD2 treatment group compared to saline control group, which is ~60 ± 13 (pg/ml).

**Conclusions:** In conclusion, specific reduction of PHD2 with ASO treatment in mice increased EPO production and this approach may have therapeutic implications for treatment of anemia associated CKD.

*Funding:* Pharmaceutical Company Support - ISIS Pharmaceuticals

## TH-PO158

**Four-Week Safety, Efficacy and Pharmacodynamic Study of Prolyl Hydroxylase Inhibitor GSK1278863 in Anemic Non-Dialysis Subjects Not Receiving Recombinant Human Erythropoietin**

Louis Holdstock, Amy M. Meadowcroft, Rayma Maier, Delyth Jones, Brendan Johnson, Alexander Ralph Cobitz, John J. Lepore. *GlaxoSmithKline.*

**Background:** Prolyl hydroxylase inhibitors (PHIs), such as GSK1278863, are an emerging therapeutic class of oral agents for the treatment of anemia. PHIs inhibit hypoxia inducible factor (HIF)-prolyl hydroxylases, resulting in accumulation of stable HIFα transcription factors, thus stimulating pathways activated during hypoxia, including erythropoiesis and iron mobilization. PHIs raise hemoglobin (Hgb) concentrations with lower circulating erythropoietin (EPO) levels than recombinant human erythropoietins (rhEPOs; Provenzano, 2011), therefore potentially reducing the increased cardiovascular risk that has been associated with rhEPO therapy (Pfeffer, 2009).

**Methods:** This randomized, placebo-controlled, double-blind study explored the relationship between GSK1278863 dose and Hgb response in 73 subjects with anemia secondary to chronic kidney disease (CKD; stage 3-5) who were naïve to rhEPO and non dialysis dependent (NDD). The study also explored the effects of GSK1278863 on circulating EPO, hepcidin and vascular endothelial growth factor (VEGF) levels. Subjects with a stable baseline Hgb of 8.5-11.0g/dL were randomized to receive 0.5, 2 or 5mg GSK1278863 or placebo for 4 weeks.

**Results:** GSK1278863 produced a dose-dependent change in mean Hgb from baseline at Week 4 (placebo: -0.26g/dL; 0.5mg: 0.12g/dL; 2mg: 0.32g/dL; 5mg: 0.95g/dL). Hepcidin levels also decreased in a dose-dependent manner. The increases in Hgb occurred with small, dose-dependent increases in EPO within or close to the normal physiologic range and no changes in VEGF. GSK1278863 was generally safe and well tolerated.

**Conclusions:** These data inform the dose-response relationship of GSK1278863 for Hgb elevation in anemic NDD CKD subjects not receiving rhEPO, and they suggest that GSK1278863 can raise Hgb within a clinically relevant range with small elevations in EPO and without increasing VEGF.

*Funding:* Pharmaceutical Company Support - GlaxoSmithKline

## TH-PO159

**Hepcidin in Chronic Kidney Disease after Iron Infusion and Its Relationship to Haemoglobin Increment**

Sourabh Chand,[1] Douglas G. Ward,[2] Zhi-Yan Valerie Ng,[1] Mark Trehane Drayson,[2] Richard Borrows.[1] *[1]Queen Elizabeth Hospital Birmingham, United Kingdom; [2]Univ of Birmingham.*

**Background:** There is a drive to use less EPO and more iron infusions for CKD anaemia management but this still comes at a financial and potentially toxic cost. Hepcidin is an iron regulator which promotes iron retention in the reticulo-endothelial system. We investigated hepcidin and traditional clinical iron markers to their predictive utility to haemoglobin (Hb) increment post iron infusion and what influences hepcidin levels in CKD.

**Methods:** Over a 6 month period, 129 consecutive non-dialysis CKD patients attended our bolus Ferinject® service as part of their anaemia management. Hepcidin levels as determined by mass spectrometry, transferrin saturation (TSAT), ferritin, eGFR, liver function tests, Hb and high sensitive CRP levels (hsCRP) were measured pre iron infusion and 6 weeks later.

**Results:** Ferinject® mean dose was 12.6mg/Kg. Ferritin and TSAT were highly correlated. In a multivariate model (adjusted for ferritin, hsCRP, eGFR, age, iron dose/weight, gender, functioning renal transplant, access, use of:ESA, anti-proliferative, ACE I/ARB, low B12 and a low folate) only ferritin was significantly associated with hepcidin levels (CI 0.594 (0.473-0.715) p<0.001). eGFR was the only predictor of pre-infusion Hb level in a multivariate model adjusted for the same variables (CI 1.42 (0.23-2.61) p=0.02). In the multivariate model with the addition of pre-infusion Hb adjustment, hepcidin was an independent predictor of Hb increment post Ferinject® infusion (CI -0.84 (-1.38 to -0.31) p=0.002). Ferritin, TSAT and hepcidin had similar predictive utility for a 1g/dL Hb increase post Ferinject® infusion (c-statistics: 0.68, 0.70, 0.69). Alkaline phosphotase was significantly increased post Ferinject® infusion (p<0.001).

**Conclusions:** Hepcidin is an iron storage marker which is moderately predictive for Hb increment following iv Ferinject® infusion. Although displaying similar predictive performance to ferritin and TSAT, hepcidin may also play a mechanistic role. This data suggests hepcidin antagonists may be of use for patients with higher hepcidin levels to increase iron utilisation following intravenous infusion.

## TH-PO160

### Atherosclerotic Plaque Characterization and Heme Oxygenase-1 Expression in Chronic Kidney Disease: The 'Vulnerable Plaque Phenotype'

Kristien El Daenen,[1] Inge Fourneau,[2] Eric Verbeken,[3] Bert Bammens.[1] *[1]Laboratory of Nephrology, KU Leuven, Belgium; [2]Vascular Surgery, KU Leuven, Belgium; [3]Dept of Imaging and Pathology, KU Leuven, Belgium.*

**Background:** CKD is characterized by accelerated atherosclerosis as compared to the general population. Heme Oxygenase-1 (HO-1), an inducible heme degrading enzyme, is protective against atherosclerosis and has a major role in plaque stabilization a.o. through inhibition of monocyte migration and differentiation.

**Methods:** Patients planned for peripheral artery or abdominal aorta aneurysm surgery were included to retrieve an arterial biopsy during surgery (n=82). For the purpose of this study, the biopsies of 23 patients with CKD stages 3-5D were compared with those of 36 patients without CKD. Atherosclerotic plaques were scored blindly for lesion type [intimal thickening or xanthoma (IT or IX), (thin) fibrous cap atheroma ((T)FCA) or fibrocalcific plaque (FCP), according to an adapted scoring system based on the AHA classification by Virmani et al], presence of inflammation and/or plaque complications (erosion, rupture, hemorrhage, not specified). HO-1 expression was judged semi-quantatively by immunostaining of the paraffin sections.

**Results:** CKD and non-CKD patients were well-matched for Framingham risk factors, cardiovascular event history and lipidlowering therapy. There was an equal distribution of lesion types in the two groups. Plaques of CKD patients showed higher inflammatory activity by higher number of foam cells, independent of statin use (p 0.035). There were more complicated plaques in the CKD group (p0.03). There was clear HO-1 expression in (T)FCA and FCP, but the semi-quantitative scoring system didn't reveal any significant differences between the CKD and non-CKD patients.

**Conclusions:** Atherosclerotic plaques of CKD patients have higher inflammatory activity and higher rates of complications as compared to non-CKD patients. These findings are supportive for a 'vulnerable plaque phenotype' of atherosclerosis in CKD. Expression of HO-1, although known to play a role in plaque stabilisation, is not different between the two groups. Whether this finding represents a relative lack of HO-1 effectivity in CKD needs further investigation.

## TH-PO161

### LDL Receptor Deficient Mice Are a Suitable Model of Aggravated Atherosclerosis in Renal Impairment and the Impact of Bone Marrow Derived Cytokines

Sibylle Von Vietinghoff, Shuwang Ge, Barbara Hertel, Inga Soerensen, Hermann G. Haller. *Internal Medicine, Hannover Medical School, Germany.*

**Background:** Chronic kidney disease significantly elevates the risk of atherosclerosis. Inflammatory leukocyte infiltration of the vascular wall is mediated by specific cytokines in normal kidney function. To date, the experimental study of atherosclerosis in renal failure has used the Apolipoprotein E deficient mouse that is limited by (a) excessive hyperlipidemia and (b) the fact that ApoE is expressed in diverse cell types including bone marrow cells. This study evaluated LDL receptor deficient (LDLr-/-) mice regarding renal function and the suitability of bone marrow transplantation for ablation of a specific cytokine.

**Methods:** LDLr-/- mice underwent unilateral nephrectomy or sham surgery and were maintained on high-fat diet for six weeks. Glomerular filtration rate was assessed by FITC-inulin clearance. Bone marrow transplantation was conducted after lethal irradiation two weeks before renal surgery, efficacy was assessed by PCR and flow cytometry. Aortic lesion size was quantified histologically and leukocyte infiltration determined by confocal microscopy and flow cytometry.

**Results:** Unilateral nephrectomy did not significantly alter serum creatinine but reduced measured glomerular filtration rate by 30% six weeks after surgery. This significantly increased aortic root lesion size. Similar effects were observed in male and female mice. Circulating leukocyte, erythrocyte and thrombocyte counts, total cholesterol and triglycerides were not significantly altered, however, in renal impairment there was a trend towards increased LDL and vLDL assessed by lipid chromatography. Renal impairment enhanced aortic leukocyte accumulation and proliferation. Bone marrow transplantation depleted specific T cell cytokine expression and decreased the effect of renal impairment on atherosclerotic lesion size.

**Conclusions:** Unilateral nephrectomy significantly decreases GFR and increases atherosclerotic lesion size in LDLr-/- mice. Reconstitution with specific cytokine deficient bone marrow in this model is a new approach for the study of the impact of kidney function on inflammation in atherosclerosis.

*Funding:* Government Support - Non-U.S.

## TH-PO162

### Possible Involvement of microRNAs in Vascular Damage in Experimental CKD

Fatiha Taibi,[1] Valérie Metzinger-Le Meuth,[2] Eleonore Ourouda Mbaya,[1] Laurent Metzinger,[1] Ziad Massy.[3] *[1]Univ Picardie Jules Verne, Amiens, France; [2]Univ Paris 13 Nord, Paris, France; [3]Chief, Div of Nephrology, Paris-Ile-de-France-Ouest Univ (UVSQ), Paris, France.*

**Background:** Chronic kidney disease (CKD) is associated with vascular calcifications and atherosclerosis. There is a need to discover novel biomarkers in order to achieve earlier diagnosis of these disorders, predict illness progression and response to adjuvant therapy. We focused on microRNAs since they are implicated in a variety of cellular functions in cardiovascularphysiopathology.

**Methods:** We examined changes over time in microRNAs in aortas of CKD and non-CKD wild type mice and of CKD and non-CKD apolipoprotein E knock-out mice. We evaluated the expression of miRNAs known to be implicated in vascular biology (miR-126, -143, -145, -221, -222 and -223), in order to assess their prognostic significance.

**Results:** The vascular smooth muscle-specific miR-143 and miR-145 were lowered in the pathological states and the level of protein target Myocardin was accordingly increased. The inflammatory miR-223 was increased at the later stages of CKD, and specific protein targets NFI-A and GLUT-4 were dramatically decreased. Expression of miR-126 was severely increased and expression of protein targets VCAM-1 and SDF-1 was altered during the course of CKD. Finally, we detected marginal changes in miR-221 and miR-222 expression. The phosphate binding drug sevelamer alleviates miRNA deregulations suggesting a direct link between the miRNA alterations we found and vascular disorders.

**Conclusions:** In conclusion, miR-126, -143, -145 and -223 are deregulated during the course of aortic vascular calcification and atherosclerosis in our CKD and atherosclerotic models, and are thus potential biomarkers of these diseases.

*Funding:* Government Support - Non-U.S.

## TH-PO163

### Apolipoprotein B/A1 Ratio, as a Risk Predictor of Cardiovascular Disease in Patients with Chronic Kidney Disease

Soo Bong Lee, Il Young Kim, Dong Won Lee, Min Ji Shin, Byeong Yun Yang, Harin Rhee, Eun Young Seong, Ihm Soo Kwak. *Dept of Internal Medicine, Pusan National Univ School of Medicine, Republic of Korea.*

**Background:** The apolipoprotein B/A1 ratio (apoB/A1) was known to be risk predictor of cardiovascular disease (CVD). CVD is the leading cause of death in chronic kidney disease (CKD) patients. Measuring carotid artery intimal-medial thickness (CIMT) is non-invasive modality used to evaluate subclinical atherosclerosis and to predict future CVD. In this study, we hypothesized that apo B/A1 could be associated with CVD in CKD patients and investigated the association between apo B/A1 and CIMT.

**Methods:** We retrospectively reviewed the 293 patients who had visited the health screening and promotion center in our university hospital in Korea. The patients were divided into 2 group which are CKD group (n=99, estimated glomerular filtration rate (eGFR): 15-59 mL/min and non-CKD group (n=194, eGFR $\geq$ 60 mL/min). Information on demographics and clinical data were obtained in each patient at the time of measuring CIMT. CIMT was measured by high-resolution B-mode ultrasonography. To investigate which factors are associated with CIMT in each 2 groups, simple and multiple linear regression analysis between variables in baseline characteristics and CIMT were performed.

**Results:** In simple linear regression analysis, non-CKD group showed age, presence of diabetes and presence of hypertension correlated with CIMT. However, in CKD group, not only age, presence of diabetes and presence of hypertension but also apo B/A1(r=0.521, P<0.001) correlated with CIMT. In multiple linear regression analysis, non-CKD group showed age, presence of diabetes, and presence of hypertension were independently associated with CIMT. In CKD group, apo B/A1 in addition to age , presence of diabetes, and presence of hypertension was independently associated with CIMT.

**Conclusions:** This study showed serum apo B/A1 was independently associated with CIMT only in CKD group, not in non-CKD group. Because CIMT is a strong predictor of CVD, the result of this study demonstrates serum apo B/A1 could be included in cardiovascular risk stratification in CKD patients.

## TH-PO164

### Increased Inducibility of Ventricular Arrhythmia in a Rat Model of Chronic Kidney Disease

Chia-Hsiang Hsueh,[1] Neal X. Chen,[2] Peng-Sheng Chen,[1] Shien F. Lin,[1] Sharon M. Moe.[2] *[1]Div of Cardiology, Indiana Univ, Indpls, IN; [2]Div of Cardiology, Indiana Univ, Indpls, IN.*

**Background:** Patients with chronic kidney disease (CKD) suffer from higher incidence of cardiovascular mortality than those without CKD and over 25% of patients on dialysis die of sudden cardiac death (SCD). The mechanisms remain unclear. We used a rat model to study mechanisms of sudden cardiac death in CKD.

**Methods:** We studied 7 normal rats (NL) and 9 Cy/+ Han:SPRD rat with CKD (CKD) at 35-45 weeks old, which we have observed to have unexpected sudden death with progressive CKD. Hearts were removed for Langendorff perfusion and optical mapping.

We determined ventricular fibrillation (VF) inducibility, action potential duration (APD), calcium transient duration (CaT), dominant frequency during VF and used real time PCR to gauge gene expression.

**Results:** CKD rats had higher blood urea nitrogen (BUN) than control (52.4±6.9 vs. 23.8±1.5 mg/dL, p<0.01). Echocardiography and histology documented hypertrophic myocardium in CKD rats, but the LV ejection fraction of CKD rats was comparable to that of normal rat (69±3 vs. 72±4 %). CKD rats had longer RR interval (237±11 ms) than normal (206±9 ms, p<0.05). APD at the 80% of repolarization (APD80) in CKD rats (78±5) was longer than normal (61±4 ms, p<0.05), but CaT80 was unchanged (98±4 vs. 91±4 ms). VF was induced in 7 of 9 CKD rats compared to 1 of 7 normal rats (p<0.05). Dominant frequency during VF was higher in CKD rats (26.5±1.2 Hz) than in NL rats (19.8±2.0 Hz, p<0.05). There was up-regulation of calcium transporters (TRCP6 and NCX1), angiotensin 1 receptor, and TGFβ1 mRNA (p<0.05) suggesting altered micro-environment in CKD rats that are known to predispose to arrhythmias.

**Conclusions:** CKD rats with advanced CKD, compared to age matched normal rats, had bradycardia, hypertrophic myocardium and longer APD than control. The hearts exhibited increased VF inducibility with slower activation rate (lower dominant frequency). These data demonstrate that structural and/or cellular level changes in CKD predispose to ventricular arrhythmias and are a likely etiology of the increased risk of ventricular arrhythmias and sudden cardiac death in CKD.

## TH-PO165

**Glyoxalase I Prevents Age-Related Endothelial Dysfunction through Modulation of eNOS Phosphorylation** Airi Jo,[1] Takamoto Ohse,[1] Hiroaki Nishimatsu,[2] Masao Takahashi,[3] Satoshi Unuma,[1] Takehiko Wada,[1] Yoichiro Ikeda,[1] Toshio Miyata,[4] Yasunobu Hirata,[3] Reiko Inagi,[1] Masaomi Nangaku.[1] *[1]Div of Nephrology and Endocrinology, The Univ of Tokyo, Tokyo, Japan; [2]Dept of Surgical Sciences / Urology, The Univ of Tokyo, Tokyo, Japan; [3]Dept of Cardiovascular Medicine, The Univ of Tokyo, Tokyo, Japan; [4]United Centers for Advanced Research and Translational Medicine, Tohoku Univ, Sendai, Japan.*

**Background:** Development of chronic kidney disease (CKD) is closely related to aging. Both glycative stress and endothelial dysfunction are linked to aging-associated changes including CKD. To elucidate the mechanisms for CKD progression in elderly patients, we investigated how glycative stress alters endothelial function with aging, using experimental anti-glycation model rats with systemic overexpression of glyoxalase I (GLO1), which detoxifies a representative glycation precursor methylglyoxal (MG).

**Methods:** Four groups of rats were examined, namely young (13-week-old) and mid-age (53-week-old) wild type (WT) and GLO1 transgenic (Tg) rats. Endothelial glycation level was evaluated by immunohistochemistry for MG-modified proteins. Age-related changes of endothelium-dependent and -independent vasorelaxation were assessed by vascular functional studies. To investigate the mechanism on the change of endothelial function, we studied nitrotyrosine formation, protein expression and post-translational modification of endothelial nitric oxide synthase (eNOS).

**Results:** Accelerated MG-modification of proteins by aging was significantly attenuated in Tg endothelium. Age-related impairment of endothelium-dependent vasorelaxation was attenuated in Tg rats, whereas vasorelaxation independent of endothelium was identical between WT and Tg rats. While total and dimeric form of eNOS did not differ between WT and Tg rats, inhibitory eNOS phosphorylation on Thr495, was increased with aging and was decreased in mid-age Tg rats compared with WT rats.

**Conclusions:** GLO1 attenuated age-related glycative stress and endothelial dysfunction in endothelium, with decreased eNOS phosphorylation on Thr495. Our study strongly suggests that regulation of glycative stress present a promising strategy for the prevention of aging and CKD.

*Funding:* Government Support - Non-U.S.

## TH-PO166

**Impact of Vascular Calcifications Assessed by Simple Radiography in the Prognosis of Non-Dialysis Chronic Kidney Disease Patients: Results of the 3-Year Observational OSERCE-II Study** Jose L. Gorriz, Pablo Molina, Jordi Bover, Javier Nieto, Alberto M. Martinez-Castelao, Angel Luis M. De Francisco, Guillermina Barril, Maria angeles Guerrero-Riscos, Luis Miguel Molinero. *OSERCE-II Study Investigators, Spain.*

**Background:** Whereas the poor prognosis effect of the presence of vascular calcifications (VC) has been clearly defined in dialysis patients, their impact in the earlier stages of chronic kidney disease (CKD) is not well established. The present study evaluated the prevalence of the VC in non-dialysis CKD patients, its correlation with other cardiovascular risk factors, and its value for the prediction of death, hospitalizations and renal and cardiovascular events.

**Methods:** OSERCE-II is a multicenter, observational, prospective, study which enrolled 722 non-dialysis CKD subjects (3-stage: 40%; 4-stage: 46%; 5-stage:14%; mean age:66±13 years; women:35%; diabetes mellitus:37%) attending 39 hospitals in Spain from 2009 to 2012. At baseline, VC were assessed by the Adragao score (AS;X-ray pelvis and hands) and the Kauppila score (KS;X-ray lateral lumbar spine). Ankle-brachial index, blood pressure and biochemical parameters were measured. Cardiovascular and renal events and hospitalization episodes were assessed.

**Results:** VC were present in 79% of the patients, being prominent in 47% (AS ≥3:30%; KS>6:31%). During an observational period of 3 years there were 74 deaths(10%), 174 patients(24%) needed hospitalizations and 154 patients(21%) started dialysis treatment. Cardiovascular was the most common cause of death (42%). By multivariate analysis, the factors independently associated with mortality were age [OR:1.067(1.033-1.101); p<0.001], diabetes [OR:1.738(1.003-3.012); p=0.049] and AS ≥3 [OR:2.130(1.222-3.712); p=0.008]. Diabetes [OR:1.880(1.319-2.681);p<0.001], low albumin levels [OR:0.661(0.434-0.861); p=0.005] and KS>6 [OR:1.524 (1.056-2.199); p=0.024] were independent predictors of hospitalization. Patients with VC didn't showed higher risk for need to start dialysis.

**Conclusions:** VC detected by plain radiography is highly prevalent in CKD patient. AS and KS represent a reliable and inexpensive tool for the assessment of death and hospitalization risks in these patients.

## TH-PO167

**Histopathology of Peripheral Arterial Disease in Chronic Kidney Disease** W. Charles O'Neill,[1] Randolph A. Hennigar.[2] *[1]Renal Div, Emory Univ, Atlanta, GA; [2]Dept of Pathology, Emory Univ, Atlanta, GA.*

**Background:** Peripheral arterial disease is a serious problem in patients with end-stage renal disease (ESRD) and chronic kidney disease (CKD) but the underlying pathology, its relationship to vascular calcification, and whether it differs from that in patients without kidney disease is unclear.

**Methods:** A total of 175 arteries (45 above the knee and 130 below the knee) in lower limb amputations performed for critical limb ischemia in 60 patients were examined retrospectively by reviewing sections stained with hematoxylin and eosin.

**Results:** ESRD was present in 21 patients, CKD in 21, and no CKD (defined as a serum creatinine < 1.0) in 18. Mean age was 65.0 +/- 1.9 years and 58% had diabetes, which did not differ between groups. The serum creatinine was 1.6 +/- 0.1 and 0.80 +/- 0.03 in patients with and without CKD. Of the 15 patients using warfarin, 7 had ESRD and 6 had CKD. Intimal thickening was present in 92% of arteries but lipid accumulation and inflammation were each apparent in only a third of these. This did not differ between patients with ESRD, CKD, or no CKD. The prevalence of medial calcification was similar in CKD and non-CKD arteries (63%) but increased to 86% in ESRD arteries (p <0.01). The prevalence of intimal calcification increased from 29% to 43% to 52% in non-CKD, CKD, and ESRD respectively, but this was not significant. Both medial and intimal calcification were present in 35% of arteries and 46% of calcified arteries but the intimal calcification was almost always less extensive than the medial calcification. Arterial pathology was similar in diabetic vs. non-diabetic arteries, and warfarin use did not alter the prevalence or severity of either medial or intimal calcification.

**Conclusions:** The principal arterial lesions in ESRD and CKD patients with critical limb ischemia are non-atherosclerotic intimal thickening and medial calcification. Except for a greater prevalence of medial calcification in ESRD arteries, the histopathology did not differ from that in patients without CKD and was unaffected by diabetes or warfarin use. Most of the vascular calcification in lower extremities is medial rather than atherosclerotic.

## TH-PO168

**High Ankle Brachial Index Is Associated with Vascular Calcification in Chronic Kidney Disease** Jing Chen,[1] Matthew Jay Budoff,[2] Jiang He,[1] Faheemuddin A. Ahmed,[1] Yanxi Liu,[1] Chung-Shiuan Chen,[1] Heather LaGuardia,[1] Damodar R. Kumbala,[1] Arnold B. Alper,[1] Vecihi Batuman,[1] L. Lee Hamm.[1] *[1]Tulane Univ; [2]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center in Torrance.*

**Background:** The ankle brachial index (ABI) is a noninvasive measure of subclinical CVD and atherosclerosis of the lower extremities. Recently, data suggested that high ABI was associated with cardiovascular mortality and vascular calcification in dialysis patients. However, the association of the spectrum of vascular calcification and high ABI is not well studied in pre-dialysis patients.

**Methods:** We investigated the association of vascular calcification with risk of high ABI (>1.4) in 243 patients with chronic kidney disease (CKD). Vascular calcification was assessed using electron-beam computed tomography (EBCT) and coronary artery calcification (CAC) Agatston score was calculated.

**Results:** CAC was classified as none (0), moderate (>0-100) or severe (>100) according to Agatston scores. Compared to those without CAC, the patients with moderate and severe CAC had increased risk of having high ABI. For example, the odds ratio (95% confidence interval) associated with moderate and severe CAC was 7.5 (1.0, 58.3) and 18.3 (2.6, 128.4) for ABI ≥ 1.4, respectively, after adjustment for age, gender, race, high-school education, physical activity, current cigarette smoking, weekly alcohol drinking, body mass index, LDL-cholesterol, plasma glucose, mean arterial pressure, estimated glomerular filtration rate, and history of cardiovascular disease.

**Conclusions:** These data indicate that CAC may be associated with risk of high ABI in patients with CKD independent of the risk factors for arthrosclerosis.

*Funding:* Other NIH Support - the National Center for Research Resources, National; Institutes of Health, Bethesda, MD.

## TH-PO169

**Calcification Is a Risk Factor for Intraplaque Hemorrhage in Coronary Atherosclerosis of Patients with Chronic Kidney Disease: The Hisayama Study** Toshiaki Nakano,[1,2] Toshiharu Ninomiya,[1] Kazuhiko Tsuruya,[1] Yutaka Kiyohara,[3] Takanari Kitazono.[1] *[1]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [2]Pathophysiological and Experimental Pathology, Kyushu Univ, Fukuka, Japan; [3]Dept of Environmental Medicine, Kyushu Univ, Fukuoka, Japan.*

**Background:** People with chronic kidney disease (CKD) are at the increased risk of coronary heart disease. We previously reported that CKD was associated significantly with severity of atherosclerosis and frequencies of intraplaque hemorrhages in coronary arteries(Am J Kidney Dis 2010:55:21-30, Kidney Int 2013:doi:10.1038/ki.2013.111). The aim of this study is to investigate the risk factors for intraplaque hemorrhage in coronary atherosclerosis among patients with CKD.

**Methods:** We randomly selected 126 subjects from 844 consecutive autopsy samples of residents of the town of Hisayama, Japan, and examined the frequencies of calcified lesions and intraplaque hemorrhages in coronary arteies. The three main coronary arteries were evaluated in each subject. The subjects were classified into four categories based on estimated glomerular filtration rate (eGFR): ≥ 60, 45-59, 30-44, and <30 ml/min/1.73 $m^2$.

**Results:** The frequencies of intraplaque hemorrhages increased gradually with lower eGFR levels (p for trend =0.01), and were higher in subjects with coronary calcification than those without it for each eGFR level (with calcification: 7.7%, 12.5%, 12.8%, 18.8%, without calcification: 1.47%, 0%, 1.69%, 9.5% for eGFR ≥ 60, 45-59, 30-44, and <30 ml/min/1.73 $m^2$, P=0.037, $\chi^2$ test). The likelihood of intraplaque hemorrhages increased with lower eGFR (OR 1.29 [95% CI, 1.03-1.62]), lower HDL cholesterol (OR 1.45 [1.09-1.92]), and the presence of coronary calcification (OR 7.68[2.57-22.95])) in univariate analysis. In multivariate analysis, coronary calcification was significantly associated with a greater risk of the presence of intaplaque hemorrhages (OR 8.78 [2.71-28.44]).

**Conclusions:** Our findings suggest that coronarycalcification is a risk factor for the presence of intraplaque hemorrhages in coronary atherosclerosis. Patients with CKD have frequent coronary calcification, which possibly lead to vulnerable plaques.

## TH-PO170

**"Middle Molecules" Are Key Mediators of Uremia Associated Osteoblastic Transformation of Mesenchymal Stromal Cells** Bjoern Hegner,[1] Theres Schaub,[1] Daniel Zickler,[1] Claudia Lange,[2] Joachim Jankowski,[1] Ralf Schindler,[1] Duska Dragun.[1] *[1]Charité Universitätsmedizin Berlin, Germany; [2]Univ Medical Center Hamburg, Germany.*

**Background:** Severe vascular calcification resembling heterotopic osteogoenesis remains unsolved problem in uremic patients. Cells of mesenchymal origin including endothelial and vascular smooth muscle cells are prime targets of uremic solutes. Mesenchymal stromal cells (MSCs) as common progenitors of both are suitable model to identify mechanisms by which uremic milieu may disturb vascular health. We studied effects of 64 individual uremic retention solutes (URS) on osteoblastic transformation of MSCs in order to identify therapeutic strategies feasible for targeting of vascular calcification.

**Methods:** Bone marrow derived MSCs were separately treated with 64 individual URS at uremic concentrations in osteoblastic induction medium. Osteoblastic differentiation was measured by alkaline phosphatase activity, western blot, immunocytochemistry, and calcium deposition. Co-incubation with specific blockers of peptidic URS in uremic serum exposed MSCs served to determine their relative importance for calcification. In an additional translational approach, osteoblastic potential of serum obtained upon high cut-off (HCO) dialyzer treatment was compared to that obtained upon conventional dialysis.

**Results:** Pro-inflammatory cytokines (IL-1β, TNF-α) followed by 3 other "middle molecules": FGF-2, FGF-23, and PTH were the strongest inducers of a calcifying osteoblastic MSC phenotype among 64 URS in a dose dependent manner. Blocking IL-1β, TNF-α, and FGFs alone, or in combination effectively obviated osteoblastic properties of uremic serum. Moreover, substance removal with HCO dialyzers had similar favorable effects attenuating osteoblastic differentiation and calcium deposition by MSCs.

**Conclusions:** Our findings emphasize importance of "middle molecules" as key mediators of uremic calcifying MSC phenotype. Since conventional dialysis strategies fail to effectively remove this group of URS, either targeted dialysis modalities or specific pharmacologic interventions may be instrumental for reduction of unsolved problem of vascular calcification in chronic kidney diseases.

*Funding:* Government Support - Non-U.S.

## TH-PO171

**Parathyroid Hormone-Mediated Chondrocyte Transition of Endothelial Eells Promotes Media Calcification in Experimental Secondary Hyperparathyroidism** Min Wu, Rining Tang, Hong Liu, Dan Liu, Bi-Cheng Liu. *Institute of Nephrology, Southeast Univ, Nanjing, China.*

**Background:** Secondary hyperparathyroidism (SHPT) is closely associated with development of media calcification in patients with chronic kidney disease. Studies have demonstrated that heterotopic chondrogenesis contributes to AMC. And recent investigations suggest the potential of endothelial cells to transdifferentiate into chondrocytes. Thus, the purpose of this study is to investigate whether elevated PTH could induce transition of endothelial cells into chondrocyte-like cells in experimental SHPT.

**Methods:** Uremia-related SHPT was induced by feeding rats an adenine diet (0.75%) for 4 weeks followed by high phosphorus diet (1.03%) until they were euthanized. Cultured human aortic endothelial cells (HAECs) were treated with PTH. Endothelial, mesenchymal and chondrocyte markers were examined by immunohistochemistry, immunofluorescent staining, real-time PCR and western blot.

**Results:** After 4 weeks of an adenine diet, serum creatine , BUN and PTH concentrations significantly increased compared with control rats ($p$<0.05), reflecting the installation of uremia-related SHPT. At 8 weeks after the establishment of uremia, von Kossa staining showed severe media calcification in the aortas. Western blot analysis showed that downregulation of endothelial marker CD31 and the upregulation of mesenchymal markers (a-SMA and FSP1) and chondrocyte markers (SOX9 and COL2A1). Confocal microscopy further revealed the co-localization of CD31 and SOX9 in the aortic media of SHPT group. Besides, exposure of cultured HAECs to PTH upregulated the expressions of FSP1 and α-SMA and downregulated the levels of CD31 in concentration-dependent and time-dependent manners ($P$<0.05). And a western blot analysis indicated that expressions of SOX9were significantly increased in PTH-treated cells compared with the control.

**Conclusions:** Elevated PTH could induce the transition of HAECs into chondrocyte-like cells via EndMT, which might be involved in media calcification in uremia-related SHPT.

## TH-PO172

**Plasma Hydrogen Sulfide ($H_2S$) Levels in CKD Patients** Stephen G. John,[1] Michael J. Garle,[2] Paul J. Owen,[1] Vera Ralevic,[2] Saoirse O'Sullivan,[2] Chris W. McIntyre.[1,2] *[1]Royal Derby Hospital, United Kingdom; [2]Univ of Nottingham, United Kingdom.*

**Background:** Animal models of chronic kidney disease (CKD) and CKD patients have reduced plasma hydrogen sulfide ($H_2S$) concentrations. $H_2S$ inhibits phosphate-induced calcification and osteoblastic differentiation of vascular smooth muscle cells, which suggests a role in arterial calcification. We aimed to investigate the role of $H_2S$ in vascular calcification and arterial stiffness in CKD.

**Methods:** We recruited 54 hypertensive non-diabetic patients ( ≥70 yrs) (CKD 3/4 (n=29), non-CKD controls (n=25)). Pulse wave velocity (PWV) and analysis were assessed by applanation tonometry, central hemodynamics by continuous digital pulse wave analysis, vascular calcification (VC) by superficial femoral artery CT and routine bloods were collected. Plasma $H_2S$ was assayed following conversion to methylene blue using the zinc-dimethyl phenylenediamine trapping method. Patients were assessed at baseline (after antihypertensive (AHT) withdrawal), after AHT reintroduction to BP 130/80mmHg, and after 12 months follow-up. Data are presented as median±IQR or Spearman correlations.

**Results:** Baseline $H_2S$ level (2.25±2.42μM) was independent of CKD status, gender and age but lower in smokers (1.77±1.64, 2.43±3.07μM, p=0.037). In those with significant VC (50%), $H_2S$ inversely correlated with VC score (r=-0.427, p=0.026) and arterial stiffness (AIx r=-0.463, p=0.015). Protein-corrected $H_2S$ inversely correlated with carotid-femoral PWV (r=-0.408, p=0.039). $H_2S$ inversely correlated with PTH (r=-0.297, p=0.038) and, in CKD, with serum calcium (r=0.470, p=0.010). No correlations were seen with vitamin D, phosphate or central hemodynamics.

AHT reintroduction was associated with a significant rise in $H_2S$ (2.25±2.42, 4.82±6.15μM, p<0.001), with a trend to a blunted improvement with beta-blockade (1.15±4.49, 4.14±6.03μM, p=0.089).

Over 12 months $H_2S$ fell (4.82±6.15, 3.74±1.92μM, p=0.005) and VC change inversely correlated with $H_2S$ change (r=-0.343, p=0.023).

**Conclusions:** These data support a central role for $H_2S$ in both cardiovascular risk and the progression of VC. $H_2S$ modulation by AHT therapy suggests a further non-traditional role for both AHT therapy and potentially the choice of agents used.

*Funding:* Private Foundation Support

## TH-PO173

**Chronic Kidney Disease Is an Independent Factor for Atheromatosis Disease** Angels Betriu,[1,4] Montserrat Martinez-Alonso,[2,4] M. Vittoria Arcidiacono,[3,4] Merce Borras,[1,4] Jose M. Valdivielso,[3,4] Elvira Fernandez.[1,3,4] *[1]Nephrology, Univ Hospital Arnau de Vilanova, Lleida, Spain; [2]Biostatistics Unit, IRBLleida, Lleida, Spain; [3]Experimental Nephrology, IRBLleida, Lleida, Spain; [4]NEFRONA Group.*

**Background:** Although cardiovascular (CV) mortality in patients with chronic kidney disease (CKD) is well known, it is still questioned if CKD per se is a promoting factor of the atheromatosis process. Therefore, the aim of the study was to study the impact of CKD on atheromatosis from early to late stages: 3 (E3); 4-5 (E4-5) and 5D (E5D) in comparison with a control population not affected by CKD (C) taking into account age, gender and diabetes, three well known atherogenic factors.

**Methods:** We analyzed 2445 CKD patients (CKD3: 937; CKD4-5: 820; CKD5D: 688) and 559 controls, 18 to 75 years old without previous cardiovascular events (NEFRONA study). CV risk was measured by SCORE charts. Carotid (common, bulb, internal and external) and femoral (common and superficial) arteries were explored with B-mode ultrasound to detect atheromatous plaques. The estimation of atheromatous plaques presence was calculated by a multivariate logistic regression model by gender, age and diabetes; plaque prevalence was stratified by the same variables.

**Results:** CKD patients were 61.7% men, 57.9 years old, and 25.7% of them were diabetic. While control subjects were 53.3% men, 54.6 years old and 10.7% of them were diabetic. CKD patients presented a higher prevalence of plaque compared to that observed in control subjects (70% vs 51%; p<0.001). Moreover, plaque presence was higher as the age of patients increased, in men and diabetic subjects. Importantly, plaque prevalence increased in parallel with CKD severity with the highest value at 5D stage. This trend was

observed in both genders, for all the age ranges and presence or absence of diabetes. In contrast, SCORE values were higher in E3 and decreased with CKD severity and plaque presence (C:1.4, E3:2.6; E4-5:2.2; E5D:1.3).

**Conclusions:** CKD per se is a cause of atheromatosis and the process is exacerbated by the severity of the disease, age and diabetes. Importantly, B-mode ultrasound of the carotid and femoral arteries is a better predictor of CV disease than SCORE charts.

*Funding:* Pharmaceutical Company Support - ABBVIE

## TH-PO174

**Chronic Kidney Disease Promotes Carotid Adventitial Neovascularization**

M. Vittoria Arcidiacono,[1] Merce Borras,[2] Ana Vilar,[2] M. Luisa Martin-Conde,[2] Belart Montserrat,[3] Lourdes Craver,[2] Angels Betriu,[2] Elvira Fernandez.[1,2] *[1]Experimental Nephrology, IRBLleida; [2]Nephrology, Univ Hospital Arnau de Vilanova; [3]Sistemes Renals, Lleida, Spain.*

**Background:** Plaque vulnerability is strongly associated with the presence of Vasa Vasorum (VV) coming from the adventitial layer. Detection of the increases of carotid adventitial VV could be a good tool for early detection of the atheromatosis. Knowing that chronic kidney disease (CKD) patients are prone to develop atheromatosis, we studied the adventitial VV in CKD and healthy patients, and its association with causes of neoangiogenesis. Moreover, the right common carotid artery is less subject to hemodynamic factors, therefore we studied separately the left and right carotid arteries.

**Methods:** Forty-two healthy patients with none of the classical atheromatous risk factors were compared with 89 CKD patients (3-4: 44, 5D:45), age<75 years and without previous cardiovascular events. Anthropometric, blood pressure, and analytical data (at fasting: lipid profile, C-reactive protein, glucose, calcium, phosphorous, PTH and Vascular Endothelial Growth Factor (VEGF)) were collected. Contrast-enhanced ultrasound (CEUS) was performed to evaluate adventitial VV in plaque-free common arteries, and B-mode ultrasound was performed to evaluate the intima-media thickness (IMT) and plaque presence.

**Results:** Control and CKD patients did not differ in age and gender. CKD patients showed a higher right adventitial VV and a higher right IMT than those observed in control patients (p=0.01; p=0.006), while the left carotid did not differ. Moreover, the right adventitial VV only correlates with the relative IMT (p=0.04) and with total cholesterol (p=0.03). Besides higher levels in CKD patients than in control patients (p=0.005), there was no correlation between VEGF levels and adventitial VV.

**Conclusions:** The differences between the left and right carotid arteries in VV increases, suggest that is mandatory to study separately the two arteries. Moreover, the correlation with IMT and cholesterol levels, a well know cardiovascular risk factor, suggest that CEUS imaging is a good tools in exploring the etiopatogenesis of atheromatosis in CKD patients.

## TH-PO175

**ADAM17 Inhibition by the Calcimimetic R568 and Its Combination with Vitamin D Attenuates Systemic Inflammation, Renal Damage and Motor-Neuron Dysfunction in Experimental Kidney Disease**

M. Vittoria Arcidiacono,[1] Sabrina Degaspari,[1] Petya Valcheva,[1] Sandra De la Fuente,[1] Elvira Fernandez,[1,2] Adriana S. Dusso.[1] *[1]Experimental Nephrology, IRBLleida; [2]Nephrology, HUAV, Lleida, Spain.*

**Background:** ADAM17 causes systemic inflammation, renal and cardiovascular damage through the cleavage and release to the circulation of the inflammatory cytokines TNFα, ICAM-1 and VCAM-1, and impairs motor-neuron function through the cleavage of pentraxin, essential for neuronal synapsis. Vitamin D inhibition of ADAM17 and induction of klotho contribute to renal and cardiovascular protection. Because high dietary calcium prevents the parathyroid hyperplasia caused by enhanced ADAM17-TGFα/EGFR signals, this study examined the efficacy of the calcimimetic R568 and its synergy with vitamin D in attenuating renal, cardiovascular and motor-neuron dysfunction in mouse kidney disease.

**Methods:** Two months after 75% nephron reduction, mice were treated with either R568 (s.c. 60 μg/g body weight), 25-hydroxyvitamin D (i.p. 80 ng weekly) + paricalcitol (i.p. 16 ng thrice weekly) or the combination for 6 weeks.

**Results:** Treatment with R568, at doses ineffective to suppress PTH, prevented the progression of renal damage (ΔBUN=0; p<0.01 ), while the R568-Vitamin D combination improved renal function (ΔBUN<0; p<0.05). The reversal of renal damage could be partially accounted for by vitamin D prevention of reductions in renal klotho, the longevity gene also cleaved by ADAM17. R568 alone or its combination with vitamin D elicited similar efficacy in preventing the loss of motor-neuron function assessed in Ladder rung walking and Rota-Rod tests, an effect associated to parallel reductions in monocyte ADAM17 expression (p<0.05), a marker of systemic inflammation. Despite the absence of vascular calcification in these uremic mice, serum VCAM-1, a marker of vascular damage, correlated directly with the degree of renal damage.

**Conclusions:** Thus, the efficacy of R568 or its combination with vitamin D to prevent the impairment of motor-neuron function in CKD parallels their ability to inhibit monocyte and/or renal ADAM17 and to attenuate renal klotho loss in the course of CKD.

*Funding:* Pharmaceutical Company Support - Amgen

## TH-PO176

**Evaluation of Cardiac Troponin I in Patients with Chronic Kidney Disease**

Om Parkash Kalra, Nithyananthan Peramanathan, Sunil Agarwal, Ashok K. Tripathi, Anil Kumar Yadav. *Univ College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.*

**Background:** Elevated levels of cardiac troponin I (cTnI) have been reported in patients with chronic kidney disease (CKD) even in the absence of acute coronary syndrome (ACS). Elevated levels of cTnI may predict future development of cardiovascular events. In this study, we assessed the cTnI levels in patients with CKD and correlated them with the structural and functional abnormalities of left ventricle (LV).

**Methods:** This cross-sectional study involved 90 subjects in the age group 31 to 60 years, divided into three groups (30 in each). Group I: Healthy controls (HC), Group II: Patients with CKD stages 3 – 5 not on hemodialysis, Group III: Patients with acute myocardial infarction (AMI) without CKD. cTnI was measured using a highly sensitive ELISA test in all groups and 2D echocardiography was done in patients with CKD to assess the following parameters: LV mass (LVM), LV mass index (LVMI) and LV systolic as well as diastolic functions.

**Results:** The mean cTnI levels in patients with CKD (142.83 pg/mL) were significantly higher (p<0.001) than healthy controls (75.22 pg/mL) and significantly lower (p<0.001) when compared with patients having AMI (456.16 pg/mL). The prevalence of elevated cTnI levels (>122. 21 pg/mL i.e., >99$^{th}$ percentile value of cTnI in healthy controls) in patients with CKD was found to be 43.3%. In patients with CKD, there was significant positive correlation (p<0.01) between cTnI and various parameters, such as LVM, LVMI and E/E' (r=0.470, 0.500 and 0.461 respectively); however, correlation between cTnI and LV ejection fraction was not significant (r= -0.276, p=0.141).

**Conclusions:** In patients with CKD, cTnI levels were elevated even in the absence of AMI. The prevalence of elevated cTnI levels in patients with CKD stages 3-5 not on hemodialysis was found to be 43.3%. The elevated levels of cTnI were associated with the presence of LV hypertrophy and diastolic dysfunction in patients with CKD. Elevated cTnI levels may help in identifying patients with CKD at increased risk for future cardiovascular events, so that these patients may be subjected to more aggressive and targeted intervention.

*Funding:* Government Support - Non-U.S.

## TH-PO177

**Renalase Is Removed by Kidneys and during Dialysis – Excess Related to CKD Complications?**

Jolanta Malyszko, Ewa Koc-Zorawska, Jacek S. Malyszko. *Nephrol. Dept, Med Univ, Bialystok, Poland.*

**Background:** Hypertension and cardiovascular complications are very common in CKD. Renalase could be synthesized not only by the kidney but also by cardiomyocytes, liver, and adipose tissue. Renalase deficiency was claimed to be present and reponsible for hypertension and cardiovascular complication in CKD. Data on renalase levels in serum were conflicting with low activity and high levels reported in patients with hypertension and CKD. We aimed to assess the renalase levels in patients after unilateral (CKD) and bilateral nephrectomy (HD), in HDF as well as in urine, ultrafiltrate in HD patients. We also assessed the effect on HD session on renalase levels.

**Methods:** Renalase (plasma, ultrafiltrate, urine) in 100 HD patients were studied using commercially available assays. Renalase was also assessed in 17 HDF patients and 24 healthy controls. We also studied renalase presence using Western Blot analysis.

**Results:** Renalase was found in ultrafiltrate in HD patients and was not affected by type of dialysers (high-flux vs low-flux). Urine renalase in HD patients (n=60) was lower than in healthy volunteers (n=24). Patients with residual renal function had lower renalase relative to anuric patients (p<0.001). In univariate analysis, plasma renalase correlated with urinary renalase (r=-0.28, p<0.05) and renalase in the ultrafiltrate (r=0.31, p<0.05) in HD patients and with urinary renalase in healthy volunteers (r=0.61, p<0.01). Urinary renalase correlated with residual renal function. HD session lower slightly renalase and type of heparin has no effect. HDF patients has significantly lower renalase than HD patients. In Western blot we found that patients after bilateral nephrectomy had the highest renalase, followed by unilateral nephrectomy.

1-control, 2- renalase commercial, 3, 4 HD, 5-7 after bilateral nephrectomy, 8, 9- after unilateral nephrectomy

**Conclusions:** Renalase is cleared by kidneys and it seems that renalase excess not deficiency might contribute to the observed cardiovascular complications in CKD.

*Funding:* Government Support - Non-U.S.

## TH-PO178

**In Chronic Kidney Disease Signal Regulatory Protein-a Not Only Impairs Insulin Signaling in Skeletal Muscle but also Contributes to Fibrosis**
Sandhya S. Thomas,[1] Liping Zhang,[1] William E. Mitch.[1] *[1]Medicine, Baylor College of Medicine, Houston, TX; [2]Medicine, Baylor College of Medicine, Houston, TX; [3]Medicine, Baylor College of Medicine, Houston, TX.*

**Background:** Insulin resistance in chronic kidney disease (CKD) begins as early as a serum creatinine 1.0 mg/dL impairing insulin signaling with loss of muscle mass. We have demonstrated for the first time that signal regulatory protein alpha (SIRPα) is upregulated in CKD and adversely influences insulin signaling by forming an immunocomplex with the insulin receptor and insulin receptor substrate-1. This results in tyrosine dephosphorylation of the insulin receptor and IRS-1 downregulating p-Akt causing muscle protein loss.

**Methods:** Western blots analysis was performed based on C2C12 cell lysates and CKD vs. control human skeletal muscles. C2C12 myoblasts were treated to overexpress SIRP-a or GFP, with silencing RNA (SiRNA) SIRP-α or scrambled SiRNA (control) and finally infected with adenovirus dominant negative AKT (Ad-AKT-AAA) vs. GFP control.

**Results:** There is a 3.8-fold increase of SIRPα in skeletal muscle samples of patients with advanced CKD vs. aged matched controls. Overexpression of SIRPα in muscle cells stimulates expression of p-Smad3, a transcription factor initiating activin and TGFβ signaling, and expression of the fibrosis marker alpha smooth muscle actin (α-SMA). Silencing SIRPα in muscle cells treated with a cytokine mixture similar to that which is found in CKD patients, caused a decrease in expression of p-Smad3 reducing expression of α-SMA. To determine if SIRPα influences p-Smad3 via a PI3/Akt dependent pathway, we silenced SIRPα in skeletal muscle cells that were infected with Ad-AKT-AAA. In SIRPα-silenced muscle cells that had been infected with Ad-AKT-AAA, p-Akt was upregulated and p-Smad3 was downregulated when compared to results in control muscle cells transfected with a scrambled SiRNA (and infected with Ad-AKT-AAA), which revealed a decrease in p-Akt and upregulation of p-Smad3.

**Conclusions:** These results imply that SIRPα increases p-Smad3 by a mechanism that is dependent on the PI3/Akt pathway, suggesting that SIRPα influences muscle fibrosis via a new pathway in chronic kidney disease.

*Funding:* Other NIH Support - T32 Training Grant

## TH-PO179

**Circulating dp-ucMGP: Modifiable Risk Marker in Chronic Kidney Disease**
Elke Theuwissen,[1] Elke Magdeleyns,[1] Heather Pham,[2] Cees Vermeer.[1] *[1]Cardiovascular Research Institute, Maastricht Univ, VitaK, Maastricht, Limburg, Netherlands; [2]Immunodiagnostic Systems, Boldon, United Kingdom.*

**Background:** Vascular calcification is a major burden in chronic kidney disease (CKD), associated with increased morbidity and mortality. Matrix Gla-protein (MGP) is primarily synthesized by vascular smooth muscle cells and acts as an inhibitor of vascular calcification; its activity depends on vitamin K-dependent γ-glutamate carboxylation. Vascular vitamin K insufficiency leads to the formation of inactive undercarboxylated MGP. Circulating inactive desphospho-uncarboxylated MGP (dp-ucMGP) is a recognized marker for vascular vitamin K status.

**Methods:** Circulating dp-ucMGP was measured with our lab-developed sandwich dual-antibody ELISA. The capture antibody was directed against the non-phosphorylated MGP sequence [3–15] and the detecting antibody directed against the uncarboxylated MGP sequence [35–49].

**Results:** Circulating dp-ucMGP levels varied between 1000 and 8000 pmol/L in CKD patients (normal range: 100 and 600 pmol/L), which is consistent with a strongly increased vascular calcification risk and which demonstrates the pronounced vascular vitamin K insufficiency in these patients. Circulating dp-ucMGP was shown to augment progressively with CKD stage. In CKD patients, increased dp-ucMGP levels were associated with aortic calcification and overall mortality. As for healthy subjects, increased vitamin K intake resulted in a dose-dependent decrease of circulating dp-ucMGP in CKD patients.

**Conclusions:** Our data suggest that in all stages of kidney disease/failure, vitamin K insufficiency is a strong risk factor for the progression of the disease and overall mortality. Further, these data form a strong argument to investigate potential clinical benefits of extra vitamin K intake for kidney disease patients.

## TH-PO180

**Normalization of AMPK Activity Corrects Renal Insulin Resistance in Non-Diabetic CKD**
Aihua Deng, Roland C. Blantz. *Medicine, UCSD&VASDHS, San Diego, CA.*

**Background:** Our last study observed that the ablation infarction (A/I) kidney, a typical non-diabetic CKD model, develops renal insulin resistance, defined as reduced effects of insulin on renal metabolism & function, and that such insulin resistance is associated with an early alteration of AMPK pathway. This study is to further test renal insulin functional resistance and to test treatments that can correct such insulin resistance.

**Methods:** A/I was created by removing right kidney and ligating two branches of left renal artery. Treatments by metformin or ANG II blockade with captopril and losartan (C&L) began on day 1. GFR and RBF were measured before and after acute arginine or insulin administrations; expressions of AMPK and its target ACC detected by Western blot; blood glucose measured after over night fasting.

**Results:** A/I kidney lost responses to arginine and insulin along with reductions in phosphorylations of AMPK and its target ACC. Both treatments which increased AMPK activity recovered kidney functional response to arginine or insulin.

| | Normal | 1wk A/I | 1wk A/I + Metformin | 1wk A/I + C&L |
|---|---|---|---|---|
| | Basal/Arginine | Basal/Arginine | Basal/Arginine | Basal/Arginine |
| GFR(ml/min) | 1.19±0.08/1.64±0.07* | 0.65±0.02/0.64±0.06 | 0.94±0.01/1.03±0.01* | 1.18±0.08/1.39±0.13* |
| RBF(ml/min) | 7.80±0.66/9.75±0.65* | 3.93±0.19/3.73±0.19 | 6.05±0.31/7.25±0.31* | 7.29±0.4/8.46±0.7* |
| | Basal/Insulin | Basal/Insulin | Basal/Insulin | Basal/Insulin |
| GFR(ml/min) | 1.16±0.07/1.40±0.09* | 0.71±0.08/0.61±0.11 | 0.98±0.06/1.26±0.05* | 1.09±0.14/1.36±0.07* |
| RBF(ml/min) | 7.90±0.12/9.43±0.36* | 4.44±0.7/4.36±0.7 | 6.05±0.31/7.25±0.31* | 6.88±0.6/8.33±0.6 |
| | | Normal/1wkAI | Normal/1wkAI+ Metformin | Normal/1wkAI+C&L |
| Fasting Blood Glucose(mg%) | | 78±4/101±6* | 89±7/86±2 | 70±3/53±5 |

**Conclusions:** A reduction of AMPK activity in the early stage of non-diabetic CKD is associated with the development of renal insulin resistance and normalization of renal AMPK activity can correct such insulin resistance.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO181

**Blockade of KCa3.1 Attenuates Renal Inflammation in Diabetic Mice**
Chunling Huang, Xinming Chen, Carol A. Pollock. *Renal Lab, Kolling Institute of Medical Research, Univ of Sydney, Sydney, NSW, Australia.*

**Background:** Inflammation plays a key role in the development and progression of diabetic nephropathy. KCa3.1, a potassium channel protein, is associated with vascular inflammation, atherogenesis, and proliferation of endothelial cells, macrophages, and fibroblasts. We previously demonstrated that blockade of KCa3.1 ameliorated renal fibrosis in diabetic nephropathy through inhibition of TGF-β1 pathway. The present study aimed to identify the role of KCa3.1 in the inflammatory responses of diabetic nephropathy.

**Methods:** Two animal models of diabetes induced with streptozotocin were used in this study: (1) wild type verse KCa3.1-/- mice, and (2) diabetic eNOS-/- mice treated with or without a selective inhibitor of KCa3.1 (TRAM34). After mice were sacrificed, the expression of proinflammatory cytokines Chemokine (C-C motif) ligand 20 (CCl20), IL-6 and TNF-α were examined by real time PCR and immunohistochemistry staining. The activity of NF-kB and markers of inflammation (CD68 and CD45) were measured by immunohistochemistry staining.

**Results:** Both mRNA and protein levels of CCL20, IL-6 and TNF-α significantly decreased in kidneys of diabetic KCa3.1-/- mice compared to diabetic wild type mice. Similarly, TRAM34 reduced the expression of inflammatory markers described above in diabetic eNOS-/- mice compared to diabetic vehicle groups. Furthermore, blocking the KCa3.1 channel in both animal models led to the reduction of phosphorylation of NF-kB.

**Conclusions:** KCa3.1 mediated renal inflammation under diabetic condition through the NF-kB pathway.

*Funding:* Government Support - Non-U.S.

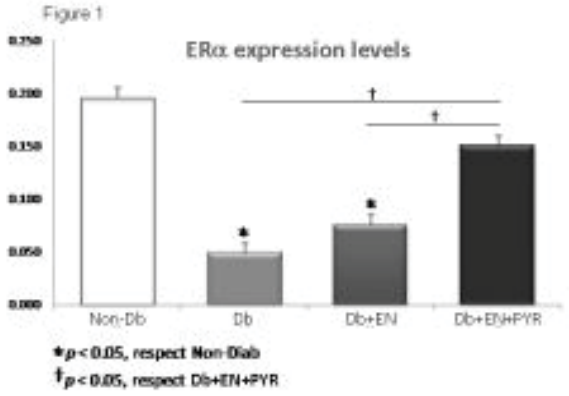
## TH-PO182

**Restoration of Repressed Estrogen Receptor α in Post-Menopausal Women and Female Mice with Diabetic Kidney Disease**
Elena M. Yubero-Serrano,[1] Shobha M. Swamy,[1] Jaime Uribarri,[1] Mark Woodward,[2] Helen Vlassara,[1] Gary E. Striker.[1] *[1]Mount Sinai School of Medicine, New York; [2]George Institute for Global Health, Sidney, Australia.*

**Background:** ERα expression is reduced in diabetic kidney disease (DKD) and post-menopausal (PMP) females by high oxidative stress (OS). Since advanced glycation endproducts (AGEs) increase OS in DKD and sevelamer carbonate (SevCarb) blocks absorption of food AGEs, reduces OS and restores cellular anti-OS defenses in DKD, we asked if SevCarb restores ERα expression in peripheral monocytes in T2DM/DKD PMP women. The mechanism was explored using pyridoxamine (PYR), a B6 vitamer that inhibits intracellular AGE formation, decreases OS and reduces DKD in mice. Thus, we asked if PYR increases ERα expression in the kidneys of female sclerosis-prone (ROP/Os) diabetic mice.

**Methods:** PMP women (n=125)/T2DM (HbA1c>6.5)/DKD (eGFR 25-80ml/min/1.73 $m^2$, AER>300mg/day) were randomly assigned to either SevCarb (4800mg/day) or calcium carbonate ($CaCO_3$,1950mg/day) for 3 months in an intention-to-treat, semi-blinded trial. Female ROP Os/+ diabetic mice (Db) (8-12 weeks old) were randomized to received enalapril (EN) and EN+PYR for 5 months.

**Results:** SevCarb treated T2DM/DKD women showed a robust increase in ERα levels after 3 months (baseline:0.96; 3 months:2.91; p=0.012). ERα levels also increased in men (strong trend). SevCarb reduced HbA1c, AGEs, Nrf2 and total cholesterol. These changes were absent after $CaCO_3$ treatment. PYR+EN treatment increased ERα levels compared to Db or Db+EN mice.




**Conclusions:** Sevelamer carbonate, but not $CaCO_3$, reduced AGE levels in PMP women with DKD and restored ERα levels, improved anti-oxidant defenses and decreased selected CVD risk factors. A longer and larger clinical trial is necessary to determine if these changes affect clinical endpoints. Pyridoxamine restored ERα levels in sclerosis-prone mice with DKD.

*Funding:* Pharmaceutical Company Support - Sanofi Pharmaceuticals

## TH-PO183

**Atrasentan Does Not Significantly Impact Thoracic Bioimpedance in Patients with Type 2 Diabetes and Nephropathy**

Dick de Zeeuw,[1] Blai Coll,[2] Hans-Henrik Parving,[3] The Radar Steering Committee.[4] *[1]Univ of Groningen, Groningen, Netherlands; [2]AbbVie, N. Chicago; [3]Univ Hospital of Copenhagen, Copenhagen, Denmark; [4]Multiple Affiliations.*

**Background:** Atrasentan, a selective endothelin receptor A antagonist, has been shown to reduce residual albuminuria in patients with type 2 diabetes and proteinuria. The potential long term renoprotective properties could be hampered by fluid retention, a common side effect of endothelin antagonists. Fluid retention was assessed by measuring thoracic bioimpedance following atrasentan treatment in subjects who are receiving maximum tolerated labeled doses of RASi.

**Methods:** 48 subjects with type 2 diabetes, macroalbuminuria (UACR ≥300 and ≤3500 mg/g) and eGFR between 30-75 ml/min/1.73 $m^2$, were enrolled in this multicenter, double-blind, placebo-controlled study. Subjects (n=16 per group) were randomized to atrasentan 0.5 mg/day, 1.25 mg/day, or placebo once daily for 8 wks. Thoracic bioimpedance was measured using the ZOE® Fluid Status Monitor. The changes from baseline to each postbaseline visit up to wk 8 were tested for significance by repeated measures analysis of variance.

**Results:** Mean baseline thoracic bioimpedance was 23±4, 22±4, and 24±5 Ohms for the placebo, 0.5 mg, and 1.25 mg dose groups. Thoracic bioimpedance did not change significantly over 8 wks for the 0.5 mg (-0.4 Ohms, p=0.718) and 1.25 mg (0.2 Ohms, p=0.836) groups, compared to placebo. No significant differences were observed in peripheral edema (44%, 31% and 50%, respectively), and there was only one case of congestive heart failure in the 1.25 mg group. Body weight increased by 1.5 kg in the 0.5 mg (p=0.053) and 1.2 kg in the 1.25 mg (p=0.125) groups, compared to placebo. Forty subjects completed the study at wk 8: placebo (n=15), 0.5 mg dose (n=14), and 0.75 mg dose (n=11). After stopping atrasentan for 30 days, bioimpedance measurements (placebo, n=14; 0.5 mg dose, n=11; and 1.25 mg dose, n=11) and body weight (placebo, n=14; 0.5 mg dose, n=12; and 1.25 mg dose, n=11) remained stable.

**Conclusions:** Administration of atrasentan in doses of 0.5 mg or 1.25 mg once daily for 8 weeks does not show any significant changes on thoracic bioimpedance.

*Funding:* Pharmaceutical Company Support - AbbVie

## TH-PO184

**Blockade of Angiotensin II Type I Receptor (AT1) and CC Chemokine Receptor 2 (CCR2) Heteromers in 5/6 Renal Ablation Model**

Yuan Zhang,[1] Robyn S. Kelly,[1] Alison Joy Cox,[1] James H. Williams,[2] Elizabeth Ann McCall,[2] Kevin Pfleger,[3] Darren J. Kelly.[1] *[1]The Univ of Melbourne; [2]Dimerix Bioscience; [3]The Univ of Western Australia.*

**Background:** Progressive chronic kidney disease (CKD) is associated with pathological fibrosis and podocyte loss, characteristic features that correlate closely with declining renal function and development of proteinuria. Both AT1 and CCR2 receptors have been consistently implicated in this disease progression. The combination of AT1/CCR2 blockade has been shown to inhibit the heteromer of these receptors and potentially increase the therapeutic effects in CKD. This study sought to test the hypothesis that blockade of the AT1-CCR2 heteromer with a combination of Irbesartan and Propagermanium (CCR2 pathway inhibitor) would have an additive benefit on progressive renal injury in 5/6 renal ablated rats.

**Methods:** 5/6 renal ablated rats (n=12) were randomly assigned to receive daily either Irbesartan (Irb; 10mg/kg) or a combination of Irb (10mg/kg) and Propagermanium (PPG; 30mg/kg) or vehicle (V) for 12 weeks. In addition to renal function and histopathology examination, podocyte loss and the concentration of urinary MCP-1 were also measured.

**Results:** Without affecting body weight and blood pressure, the combination of Irb and PPG therapy in 5/6 renal ablated rats was associated with significantly less proteinuria (V: 372±52, Irb: 287±32, Irb+PPG: 136±16 mg/day), urinary MCP-1 (V: 4048±587, Irb: 2533±409, Irb+PPG: 2105±538 pg/ml) and podocyte loss (V: 9±0.8, Irb: 10±0.6, Irb+PPG: 13±0.7 podocytes/glomerulus) when compared to vehicle or Irbesartan treatment. Both mono and combination therapy attenuated tubulointerstitial fibrosis (V: 5.69±0.8, Irb: 3.72±0.41, Irb+PPG: 4.10±0.7 %/area) and glomerulosclerosis (V: 1.37±0.13, Irb: 0.52±0.08, Irb+PPG: 0.66±0.13), along with improved glomerular infiltration rate (V: 0.43±0.13, Irb: 0.91±0.12, Irb+PPG: 0.90±0.16 ml/min/kg) when compared with vehicle treated in 5/6 renal ablated rats.

**Conclusions:** Blockade of the AT1-CCR2 heteromer with Irbesartan and Propagermanium was superior to Irbesartan alone in reducing proteinuria and podocyte loss in the 5/6 renal ablation rat model of CKD.

*Funding:* Pharmaceutical Company Support - Dimerix Bioscience, Perth, Australia

## TH-PO185

**Chronic Nicotine (Ch-NIC) Induced Exacerbation of Subpressor Angiotensin-II (SP-AngII) Induced Renal Dysfunction and Chronic Kidney Disease (CKD) Is Partially Mediated through the Endothelin-A (ET-A) Receptor**

Kiran B. Chandrashekar,[1] Istvan Arany,[3] Arnaldo F. Lopez-Ruiz,[1] Andrea P. Soljancic,[1] Ruisheng Liu,[1,2] Luis A. Juncos.[1,2] *[1]Nephrology, Univ of Miss Medical Center; [2]Physiology & Biophysics, Univ of Miss Medical Center; [3]Pediatrics, Univ of Miss Medical Center.*

**Background:** Previously we reported that Ch-NIC worsens SP-AngII induced renal dysfunction despite insubstantial blood pressure (BP) changes and also that it incites renal vascular remodeling which partly contributes to SP-AngII induced CKD. Smoking (Ch-NIC) increases plasma endothelins which damage microcirculation by regulating vascular growth–promoting factors and inducing microvascular remodeling.We hypothesized that ET-A receptor blockade protects against Ch-NIC induced renal dysfunction in SP-AngII induced CKD.

**Methods:** SD rats received saccharine or nicotine with or without SP-AngII. Separate groups were pretreated with ABT-627 (selective ET-A antagonist).

**Results:**

| | Systolic BP(mmHg) | RVR(ml/min/mmHg) | CRVR(TPU/mmHg) | MRVR(TPU/mmHg) | NGAL(UI/mg creat) | TGF(ng/µg protein) |
|---|---|---|---|---|---|---|
| **Control** | 110±3 | 16±3 | 3±0.3 | 4±0.4 | 0.3±0.1 | 14±2 |
| **Ch-NIC** | 114±6 | 15±1 | 3±0.1 | 4±0.1 | 0.7±0.1* | 20±1* |
| **Ch-NIC+ABT-627** | 107±4 | 16±1 | 3±0.2 | 4±0.1 | 0.7±0.1 | 10±1# |
| **SP-AngII** | 164±6 *#† | 24±2 #† | 4±0.4 #† | 6±0.4 #† | 6±0.5 #† | 28±1 #† |
| **SP-AngII+ Ch-NIC** | 171±3 | 39±2 ψ | 4±0.1 ψ | 10±1 ψ | 8±0.1 ψ | 40±2 ψ |
| **SP-AngII+ Ch-NIC+ABT-627** | 142±3 ψπ | 20±1 ψπ | 3±0.1 ψπ | 5±0.2 ψπ | 4±0.6 ψπ | 24±2 ψπ |

Data:Mean ± SEM;P< 0.05;*vs. CT;# vs. SP-AngII;† vs. Ch-NIC+ABT-627;Ψ vs. SP-AngII;π vs. SP-AngII+Ch-NIC.

**Conclusions:** ET-A blockade prevents Ch-NIC-induced worsening of SP-AngII induced changes in renal hemodynamics, injury, fibrosis and vascular remodeling, albeit attenuating SP-AngII-induced hypertension. This protective effect was independent of heme oxygenase system (data not shown). Our data suggests that Ch-NIC induced exacerbation of SP-AngII induced renal dysfunction and CKD is partially mediated through activation of the ET-A receptor.

*Funding:* Other NIH Support - NIH DK073401

## TH-PO186

**Protective Effects of Enalapril, Atrasentan, and Paricalcitol on Glomerulosclerosis, Proteinuria, and Cardiac Oxidative Stress in Uremic Rats**

Eduardo Slatopolsky,[1] Cynthia S. Ritter,[1] Jane L. Finch,[1] Helen Liapis,[1] Edu Suarez,[2] Leon Ferder,[2] James A. Delmez,[1] Sarah Zhang.[1] *[1]Washington Univ School of Medicine, St. Louis; [2]Ponce School of Medicine.*

**Background:** The cardio-renal involvement in CKD patients accelerates the progression of renal insufficiency. Activation of RAAS, the endothelin A receptor (ETaR), and oxidative stress play a critical role in the deterioration of renal function.

**Methods:** We compared the action of enalapril, atrasentan (an ETaR antagonist) and paricalcitol in the kidney using the 5/6$^{th}$ nephrectomized rat model, in the following groups: Normal control (NC), Uremic (U), U+enalapril (UE), U+atrasentan (UA), U+paricalcitol (UP), and a combination of the 3 drugs together (UEAP). The rats were treated for 3 month.

**Results:** The combination treatment had the greatest protective effect on all parameters examined. Uremia resulted in a marked rise in 24hrs protein excretion, 4.4±1.8 (NC) *vs* 360.6±45.4 mg/24 hrs (U); the UEAP group had a marked reduction to 134.7±24.1 mg/24 hrs ($p<0.001$). Serum creatinine increased from 0.48±0.02 (NC) to 1.22±0.11 in the U group, and decreased to 0.87±0.05 mg/dl in the UEAP group. The systolic BP increased to 169±7mmHg in the U group and remained normal in the UEAP group. The U rats showed significant glomerulosclerosis, interstitial sclerosis and inflammation; this was decreased significantly in the UEAP group. The monocyte chemotactic protein MCP-1 significantly increased 12-fold in the U group, but only 3.5-fold in the UEAP group (ns *vs* control). Similarly, ED-1, a marker of macrophage infiltration in the kidney, significantly increased 18-fold in the U group, but only increased 3-fold in the UEAP group (ns *vs* control). The mortality in the U group was 33.3% and zero in UEAP group. Hypertrophy of the left ventricle (LV) was prevented in the UEAP group; this group had the lowest level of lipid peroxidation, and the anti-oxidant enzyme glutathione peroxidase was significantly increased.

**Conclusions:** In conclusion, the combination of these 3 drugs greatly improved renal function and histology, proteinuria, LV hypertrophy and cardiac oxidative stress in the uremic rat.

*Funding:* Pharmaceutical Company Support - Abbott Pharmaceutical

## TH-PO187

**Inverse Correlation of Epidermal Growth Factor and Lipocalin-2 in Patients with Chronic Kidney Disease** Shahaan Smith,[1] Viji Nair,[1] Felix H. Eichinger,[1] Ivan Formentini,[2] Maria Bobadilla,[2] Maria Chiara Magnone,[2] Keith A. Bellovich,[3] Susan P. Steigerwalt,[3] Matthias Kretzler,[1] Wenjun Ju.[1] *[1]Univ of Michigan; [2]Hoffmann-La Roche; [3]Renaissance Renal Research Institute, for the Michigan O'Brien Renal Center (P30) & ERCB Consortium.*

**Background:** Epidermal growth factor (EGF) and neutrophil gelatinase-associated lipocalin (LCN2) are potential urinary biomarkers for CKD. Therefore, it is important to understand the mechanism linking these two tubular markers to CKD. A study using LCN2 deficient mice demonstrated a critical role of LCN2 in renal failure, and their *in vitro* data suggested that EGF might activate LCN2 expression. However, if LCN2 is a down-stream target that links EGF to CKD progression in humans remains unknown. This prompted us to study the relationship between EGF and LCN2 in patients with CKD in cohort studies from Europe (European Renal cDNA Bank, ERCB) and North-America (Michigan O'Brien Renal Center P30, CPROBE).

**Methods:** Renal RNA levels were derived from gene expression profiling performed on tubulointerstitial compartment of micro-dissected renal biopsy tissue from two independent cohorts: 164 ERCB subjects and 42 CPROBE subjects. Pearson correlation was used to correlate marker levels with kidney function, assessed by estimated glomerular filtration rate (eGFR) using the MDRD equation. Urinary EGF and LCN2 were measured by ELISA.

**Results:** Our study shows that intra-renal RNA levels of EGF and LCN2 in tubulointerstitial compartment are significantly correlated with eGFR-MDRD in ERCB (r=0.66, and r=-0.48, respectively), and in CPROBE (r=0.42, and r=-0.63, respectively). A significant inverse correlation was observed between EGF and LCN2 RNA level in both study cohorts (r=-0.54, and r= -0.61, respectively). The inverse correlation is retained at the urine marker protein level in CPROBE patients (r=-0.46, p=0.007).

**Conclusions:** The consistent association between reduced EGF level and increased LCN2 expression in CKD patients supports that LCN2 may be one of the paths that mediate the role of EGF in CKD progression. Future studies will focus on determining the signaling mediators between EGF and LCN2 in human tubular cells.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Hoffmann-La Roche

## TH-PO188

**Uric Acid and Risk of Chronic Kidney Disease Progression in a United Kingdom Cohort** Matthew Denker, Jason Roy, Harold I. Feldman. *Medicine, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** Uric acid (UA) is a potential risk factor for CKD progression, supported by some but not all observational studies. We aimed to identify patients with CKD with varying degrees of kidney dysfunction in order to test whether the association between UA and CKD progression differs by baseline CKD severity.

**Methods:** We conducted a retrospective cohort study using UK's The Health Improvement Network (THIN) database to identify adult patients with CKD who had a UA recorded after meeting CKD criteria, a corresponding serum creatinine (Cr), and at least 1 Cr subsequent to both UA and latter Cr. Those with ESRD prior to UA or with <1 year follow-up were excluded. Linear mixed effects models were fit to model slope of ΔeGFR over time, adjusted for baseline covariates including age, sex, BMI, BP, eGFR, hemoglobin, albuminuria, RAAS-blockers, diuretics, and hypouricemic agents. Censoring occurred at the earliest of dialysis initiation, kidney transplant, death, or January 2012.

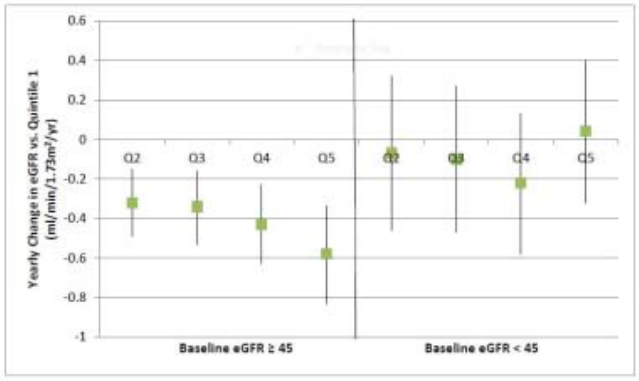
**Results:** We identified 21,478 patients: 47% men, median age 73.9 yrs, mean baseline eGFR (beGFR) 53 ml/min/1.73m². Median follow-up was 3.7 yrs.

Table 1. Baseline characteristics by uric acid quintile.

| Baseline characteristic | Q1 | Q2 | Q3 | Q4 | Q5 |
|---|---|---|---|---|---|
| Number of patients | 4410 | 4294 | 4142 | 4488 | 4144 |
| Median uric acid (mg/dl) | 4.4 | 5.7 | 6.8 | 8.0 | 9.7 |
| Mean SBP (mmHg) | 139.0 | 140.7 | 140.3 | 139.6 | 136.8 |
| Gout (%) | 15.4 | 16.4 | 23.5 | 37.6 | 46.3 |
| Mean eGFR (ml/min/1.73m²) | 61.3 | 56.5 | 53.4 | 49.7 | 43.1 |

The association between UA and CKD progression differed by beGFR (test for interaction p<0.001). In patients with more preserved kidney function, higher UA was associated with faster loss of eGFR (p<0.001). UA was not associated with progression in those with beGFR<45.

Figure 1. Adjusted yearly change in eGFR associated with each quintile of uric acid when compared to quintile 1.




**Conclusions:** UA was associated with more rapid loss of eGFR in those with mild-moderate CKD. Since the UK population is 3% black, generalizability of findings may be limited to white patients in US.

*Funding:* NIDDK Support

## TH-PO189

**Effects of Febuxostat versus Allopurinol in Reducing Serum Urate in Subjects with Hyperuricemia and CKD Stage 3-5: One Year, Randomized Trial** Nobuhito Hirawa,[1] Keisuke Yatsu,[2] Mari Katsumata,[1] Akira Fujiwara,[2] Sanae Saka,[1] Yoshiyuki Toya,[2] Gen Yasuda,[1] Satoshi Umemura.[2] *[1]Dept of Nephrology and Hypertension, Yokohama City Univ Medical Center, Yokohama, Kanagawa, Japan; [2]Dept of Medical Science and Cardiorenal Medicine, Yokohama City Univ Graduate School of Medicine, Yokohama, Kanagawa, Japan.*

**Background:** Febuxostat (Feb), an orally administered non-purine selective inhibitor of xanthine oxidase, was developed recently. Compared to allopurinol (Allo), Feb directly inhibits xanthine oxidase and is metabolized by liver, partly. Thus, Feb may be easy to use in patients with renal impairment. In this study, to clarify the urate-lowering efficacy and safety of Feb, we compare the effectiveness of Feb and Allo in subjects with hyperuricemia and CKD stage 3-5 for one year.

**Methods:** Forty patients with CKD stage 3-5 and hyperuricemia (serum urate levels > 8mg/dl) were randomized to receive once-daily Feb (10mg) or Allo (50mg) for four weeks. After that, Feb and Allo were permitted to increase the dose until 40mg/day (Feb) in all participants, and 100 mg (Allo) in CKD stage 3. The 1st endpoint was the UA values at one year. Furthermore, we examined the effects on renal function and investigated the safety of Feb and Allo treatments in CKD stage 3-5 patients.

**Results:** The average age and eGFR of the participants were 63 years old and 22 ml/min/1.73m2. There were no differences in BP, Cr, UA values between Feb group and Allo group in the control period. Feb and Allo significantly decreased the serum UA levels at 2 weeks after treatments. The UA level of Feb group was significantly lower than Allo group in 12 weeks, 24 weeks and one-year of treatments (p<0.01). There was no difference in serum creatinine and eGFR by two treatments. The changes of the slope of 1/Cr were negatively correlated with the alterations of the serum UA (P<0.05). Using the multivariate analysis, the UA values of one-year treatment were associated with Feb use, baseline UA value, but not with baseline eGFR. Obvious side effects were not recognized in Feb treatments.

**Conclusions:** Febuxostat is effective and safe in patients with hyperuricemia and CKD stage 3-5 for one-year treatment.

## TH-PO190

**Interaction between Hyperuricemia and Smoking on Renal Arteriolopathy in Chronic Kidney Disease Patients** Kentaro Kohagura,[1] Tsuyoshi Miyagi,[1] Masako Kochi,[1] Yusuke Ohya,[1] Kunitoshi Iseki.[2] *[1]Cardiovascular Medicine, Nephrology and Neurology, Univ of the Ryukyus, Nishihara-Cho, Okinawa, Japan; [2]Dialysis Unit, Univ of the Ryukyus, Nishihara-Cho, Okinawa, Japan.*

**Background:** We have recently reported that hyperuricemia (HU) was associated with renal arteriolopathy in chronic kidney disease (CKD) patients. Smoking (SMK) is also potential risk factor for renal arteriolopathy. However, the effect of combination SMK and HU on renal arteriopathy is unknown.

**Methods:** We examined the cross-sectional association between HU and renal arteriolopathy with or without SMK (current or past) using renal biopsy specimen. Arteriolar hyalinosis and wall thickening were assessed by semi quantitative grading for arterioles among 167 patients with CKD (mean age, 43.4 yrs; 88 men and 81 women).

**Results:** Subgroup analysis showed that HU+/SMK+ group had highest grade of arteriolopathy followed by HU+/SMK-, HU-/SMK+. Interaction between HU and SMK on index of renal arteriolopathy was significant (p=0.003). That is, effect of HU on renal arteriolopathy was significantly augmented by coexistence of SMK. Multiple logistic analysis adjusted for age hypertension and diabetes mellitus showed that HU+/ SMK+ was significantly associated with higher risk for the presence of higher-grade renal arteriolar

hyalinosis and wall thickening compared with HU-/SMK- as a reference, but HU+/SMK- was not. The adjusted odds ratios (95% CI) of hyalinosis and wall thickening were 4.2 (1.1 to 15.4) and 3.6 (1.0-12.7), respectively.

**Conclusions:** In conclusion, significant interaction between HU and SMK on renal arteriolopathy was observed in CKD patients. Further prospective study is needed to determine whether CKD patients in hyperuricemia who have smoking habit show rapid decline in eGFR.

## TH-PO191

**Effect of Cholecalciferol on Proteinuria in Patients with Chronic Kidney Disease** Subir K. Paul, Shejuti Paul, Rajesh Boorgu, Narasimha R. Boorgu, Jamie N. Cockrell. *Shoals Kidney and Hypertension Center, Florence, AL.*

**Background:** Proteinuria is well-known to be associated with adverse cardiovascular outcome. When angiotensin converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) are contraindicated, poorly tolerated, or have inadequate effects, the therapeutic choice for proteinuria in chronic kidney disease (CKD) patients becomes mostly limited to optimum blood pressure control. Recently, Vitamin D receptor activation has shown to reduce proteinuria in diabetic nephropathy. The effect of nutritional Vitamin D on proteinuria in CKD is unknown. This study examines the effects of cholecalciferol (CHO) on proteinuria in patients with CKD.

**Methods:** 61 patients with stage 3 and early stage 4 CKD with urine protein creatinine ratio (UPCR) of 0.5 or more and 25 OH Vitamin D (25OHD) level of less than 32 ng/ml were studied. 31 patients were treated with an average dose of 2050 mg of CHO daily for 3 to 9 months. 30 patients did not receive CHO treatment. Patient characteristics including age, race, gender, estimated GFR, presence of diabetes, hypertension, glomerulopathy, number of patients on ACEI or ARB, control of hypertension and diabetes were similar in both groups. Comprehensive metabolic profile, UPCR, and 25OHD levels were measured at baseline and during 4 to 9 months.

**Results:** Baseline 25OHD level for treated and untreated patients was 20.03 ng/mL and 21.73 ng/mL respectively (P=0.32). 25OHD level increased to 36.4 ng/mL (P<0.0001) following therapy in treated group and remained similar at 21.8 ng/mL (P=0.96) in untreated group. Baseline UPCR in treated and untreated group was 3.91 and 3.55 (P= 0.74) respectively. While in treated group, UPCR decreased from 3.91 to 1.64 (P=0.003), UPCR mildly increased from 3.55 to 4.07 (P=0.69) in untreated group. Baseline serum albumin was 3.71 g/dL and 3.71 g/dL (P= 0.98) in treated and untreated patients respectively. Serum albumin increased from 3.71 g/dL to 3.97 g/dL (P=0.01) in treated group but was unchanged from 3.71 g/dL to 3.72 g/dL (P=0.91) in untreated group.

**Conclusions:** We conclude that CHO therapy may ameliorate proteinuria in patients with CKD. Large randomized trials are warranted.

## TH-PO192

**Endothelial Glycocalyx Perturbation Accompanies Renal Failure** Martijn Dane,[1,3] Meriem Khairoun,[1] Bernard Van Den Berg,[1] Dae Hyun Lee,[1] Margien G.S. Boels,[1] Angelique Rops,[2] Johan Van der Vlag,[2] Hans Vink,[3] Anton Jan Van Zonneveld,[1] Marlies Reinders,[1] Ton J. Rabelink.[1] *[1]Nephrology, LUMC, Leiden, Netherlands; [2]Nephrology, RUNMC, Nijmegen, Netherlands; [3]Physiology, MUMC, Maastricht, Netherlands.*

**Background:** End-stage renal disease (ESRD) is accompanied by endothelial dysfunction. Since the endothelial glycocalyx (endothelial surface layer, ESL) governs interactions between flowing blood and the vessel wall, perturbation could influence disease progression. In this study we used non-invasive sidestream darkfield (SDF) imaging to investigate whether renal failure is associated with perturbation of the ESL. To this end we measured the accessibility of red blood cells to the endothelial surface in the microcirculation (perfusion boundary region, PBR).

**Methods:** The PBR was measured in patients with ESRD (n=23), after a successful living donor kidney transplantation (n=12),in patients who developed interstitial fibrosis / tubular atrophy after kidney transplantation (IFTA, n=10) and healthy controls (HC) (n=10). In addition, the endothelial activation marker Angiopoietin-2 (Ang2) and the shed ESL components syndecan-1 and soluble thrombomodulin (sTM) were measured using ELISA. All differences shown have a P-value of <0.05.

**Results:** ESRD patients showed an increased PBR compared to controls (2.05 ± 0.25 vs. 1.82 ± 0.16 µm), which was accompanied by increased circulating levels of sTM (19.9 ± 8.2 ng/mL vs. HC 7.1 ± 1.2 ng/mL) and syndecan-1 (107.5 ± 51.1 ng/mL vs. HC 49.8 ± 17.4 ng/mL). Patients with IFTA also revealed an increased PBR (2.18 ± 0.20 µm), which coincided with elevated sTM levels (41.9 ± 24.7 ng/ml, vs. HC) which were even higher than in ESRD. Interestingly, after successful transplantation, patients had a PBR of 1.84 ± 0.2 µm, indistinguishable from healthy controls, without elevated plasma levels of sTM (13.0 ± 3.0 ng/mL) or syndecan-1 (54.8 ± 32.6 ng/mL). In addition, a significant correlation between PBR, Ang2 and eGFR, (Spearman's rho = 0.306 and -0.342 resp.) was observed.

**Conclusions:** Loss of renal function is strongly associated with perturbation of the endothelial glycocalyx. Successful kidney transplantation, however, results in recovery of the endothelial glycocalyx.

*Funding:* Private Foundation Support

## TH-PO193

**Activation of Coagulation during Chronic Kidney Disease Is Counterbalanced by Defective Platelets Adhesion: An Intravital Videomicroscopy Analysis** Stephane Burtey, Stéphane Poitevin, Roxane Darbousset, Bertrand Gondouin, Christophe Dubois. *UMR_S 1076, Aix-Marseille Univ, Marseille, France.*

**Background:** Patients with chronic kidney disease (CKD) have an increased risk of thrombosis and an increased risk of hemorrhage. The physiopathology of this counterintuitive association is unknown. We identified indolic solutes as critical in the induction of tissue factor (TF) expression and procoagulant activity. We hypothesize that TF could play an important role in vivo to promote clot formation.

**Methods:** To confirm it we used a well described in vivo live imaging of thrombi formation dependant of TF. Endothelial cells of the cremaster arterioles in anesthetized mouse were activated by laser pulse. Platelets aggregation and fibrin formation at the lesion site were imaging by intravital videomicroscopy. The fibrin and the platelets were revealed by labels antibodies. The kinetics of thrombi was analyzed in more than 30 thrombi in five C57/bl6 mice with renal failure induced by 5/6 nephrectomy (Nx) and four control mice. The integrative fluorescence activity is expressed in median of arbitrary units (AU).

**Results:** In mice with 5/6 Nx the fibrin formation began earlier. The same mean fluorescence intensity (5AU, 10 AU and 20 AU) was observed 50+/-10 seconds earlier in mice with renal failure than in control mice. At time 300s, the fibrin formation at the lesion site is more important in 5/6 Nx mice (30 AU) than in control mice (20 AU) (p<0.0001). In contrast, we observed less fluorescence for platelets staining at all the time in 5/6 Nx mice compared to control mice. We confirmed this defect in platelets aggregation by performing bleeding time experiment. The median bleeding time of 5/6 Nx mice was significantly increased compared to control.

**Conclusions:** We confirmed in vivo the association of activated coagulation and defective platelets adhesion in a mouse model of renal failure. The coagulation activation is dependant of TF. This association could explain the increased risk of thrombosis observed in human combined with increased bleeding risk. The intravital videomicroscopy give us the opportunity to identify the mechanisms leading to the paradoxal hemostatic state observed in CKD.

## TH-PO194

**Vascular Injury Promotes Glomerulotubular Junction Abnormalities (GTJA) which May Progress Chronic Kidney Disease (CKD): Three-Dimensional (3D) Inspection of Vasculo-Glomerular Alterations** Noriko Uesugi,[1] Yoshihito Shimazu,[2] Takaaki Aoba,[2] Michio Nagata.[1] *[1]Pathol., Tsukuba Univ, Tsukuba, Japan; [2]Pathol., Nippon Dental Univ, Tokyo, Japan.*

**Background:** GTJA including atubular glomeruli (AG) has been identified as a cause of nephron loss in number of glomerular diseases. GTJA is 3D event and was likely underappreciated because of difficulties of morphologic recognition.To verify the hypothesis that vascular injury by hypertension(HT) concern occurrence of GTJA promoting nephron loss in CKD, we investigated three-dimensional vasculo-glomerular architectures of GTJA in human kidney with CKD stage 1-3.

**Methods:** Kidney tissue was obtained from 23 samples of surgically removed non-tumor parts of renal carcinoma in Japanease (Male 18, Female 5; age: 45-86.; without proteinuria, 13 with HT). The prevalence of GTJA in CKD was first surveyed on 500 serial sections in 3mm$^2$ size with double staining by PAS and Elastica. On that basis, we selected 7 cases for 3D reconstruction, in which 120 serial sections were double or triple-stained in combination with CD10 (anti-proximal tubular marker), CD34 (anti-endothelial marker), and smooth muscle actin (SMA; anti-vascular medial marker). Individual immunostained images of a whole histological section were acquired with virtual slide system and 3D reconstruction were conducted using Image-J software.

**Results:** High resolution 3D reconstruction images from interlobular arteries to glomeruli with proximal tubular image was successfully obtained. GTJA was noticed in 4.3±5.2% in HT cases and 0.7±0.3% in non–HT cases (p=0.09). Severe GTJA including AG showed complete disconnection of vascular pole and afferent arterioles (AA) associated with severe interstitial fibrosis. Occlusion of near interlobular arteries (AA) was found in some cases. Other GTJA showed marked narrowing of AA by intimal fibrosis and long distance convoluted running of AA with opened lumina. Loss of CD10 expression was often noticed in atrophic tubules.

**Conclusions:** In the pathogenesis of CKD, arteriolosclerosis caused by HT likely promote development of GTJA , resulting in nephron loss. Anomalous vasculo-glomerular architecture in CKD kidney can be monitored in 3D reconstruction.

*Funding:* Government Support - Non-U.S.

## TH-PO195

**Chronic Kidney Disease and Fracture Risk in the Action to Control Cardiovascular Risk in Diabetes Trial** Tamara Isakova,[1] Timothy Craven,[2] Julia J. Scialla,[1] Huiliang Xie,[1] Thomas L. Nickolas,[3] Adrian Schnall,[4] Joshua Barzilay,[5] Ann Schwartz.[6] *[1]Univ of Miami Miller School of Medicine; [2]Wake Forest School of Medicine; [3]Columbia Univ Medical Center; [4]CWRU School of Medicine; [5]Emory Univ School of Medicine; [6]UCSF School of Medicine.*

**Background:** Large epidemiological studies demonstrate that type 2 diabetes independently increases fracture risk. One mechanism that could explain this increased risk is the higher prevalence and incidence of chronic kidney disease (CKD) that is associated with type 2 diabetes.

**Methods:** In secondary analysis of 6661 participants included in an ancillary study of fractures in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, we used logistic regression to examine relationships between different definitions of CKD and centrally adjudicated non-vertebral fracture rates over a median follow up time of 4.9 years.

**Results:** We found that definitions relying on baseline estimated glomerular filtration rate (eGFR) and/or albuminuria were not associated with fracture risk.

| CKD Definition | Incidence Densities† (95% CI) | Adjusted‡ Odds Ratio (95% CI) |
|---|---|---|
| Reference | 1.53 (1.39 – 1.68) | 1 |
| Baseline eGFR < 60 ml/min/1.73 m² | 1.87 (1.55 – 2.27) | 1.13 (0.81 – 1.60) |
| Reference | 1.59 (1.43 – 1.76) | 1 |
| Baseline UACR ≥ 30 mg albumin/g creatinine | 1.58 (1.35 – 1.85) | 1.03 (0.82 – 1.28) |
| Reference | 1.54 (1.38 – 1.73) | 1 |
| Baseline eGFR <60 ml/min/1.73 m² and baseline UACR >30 mg albumin/g creatinine | 1.93 (1.43 – 2.61) | 1.38 (0.82 – 2.33) |
| Reference | 1.42 (1.23 – 1.62) | 1 |
| Incident CKD* | 1.97 (1.65 – 2.34) | 1.47 (1.14 – 1.89) |

*Incident CKD defined as last eGFR <60 ml/min/1.73 m² and 20% drop during follow up, or UACR ≥ 30 mg albumin/g creatinine during follow up
†Per 100 person-years at risk
‡Adjusted for age, gender, socioeconomic status, living situation, smoking, glycemic control intervention arm, BP, HbA1C, baseline eGFR and albuminuria, and use of hormone replacement therapy, steroids, diuretics, statins and anti-psychotic medications.

However, incident CKD, defined as last eGFR <60 ml/min/1.73 m² and 20% drop in eGFR during follow-up or development of urinary albumin to creatinine ratio ≥30 mg albumin/g creatinine during follow-up, was significantly associated with more fractures. Compared to participants who did not develop incident CKD, those who did had significantly increased odds of non-vertebral fracture, independent of other covariates (odd ratio, 1.47; 95% confidence intervals, 1.14 – 1.89).

**Conclusions:** These data suggest that longitudinal measures of kidney function are more sensitive predictors of fracture risk than baseline CKD status. The findings also justify further investigation of abnormalities in mineral metabolism that develop early in the course of CKD as potential mediators of bone fragility in this population.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

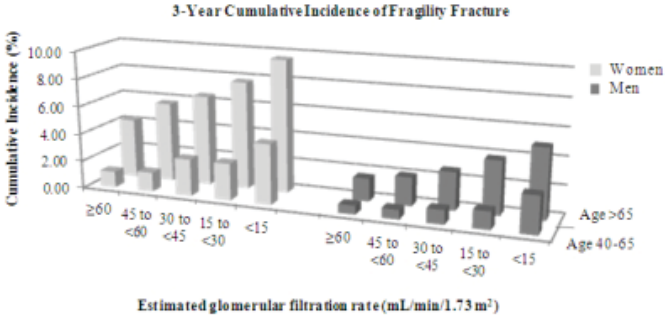
## TH-PO196

**3-Year Incidence of Fragility Fracture in Chronic Kidney Disease** Kyla Lynn Naylor,[1,2] Eric McArthur,[3] Amit X. Garg.[1,2,3] *[1]Nephrology, Western Univ, London, Canada; [2]Epidemiology and Biostatistics, Western Univ, London, Canada; [3]Institute for Clinical Evaluative Sciences, London, Canada.*

**Background:** There is much clinical value in simply knowing how likely a person is to fracture according to their level of kidney function, sex and age.

**Methods:** Using large healthcare databases from Ontario, Canada we conducted a retrospective cohort study of adults aged ≥ 40 years (n = 679,114, mean age 62 years) stratified at cohort entry by their estimated glomerular filtration rate (eGFR ≥60 mL/min/1.73 m², 45 to <60, 30 to <45, 15 to <30, <15 or chronic dialysis), sex and age (40-65 versus >65 years). The primary outcome was the 3-year cumulative incidence of fragility fracture (proportion of adults who fractured at least once within 3-years of follow-up). Fractures of the hip, forearm, pelvis or humerus were counted in the primary outcome. In additional analyses the following were examined: fracture incidence per 1000 person-years; hip fracture alone; stratification by prior history of fracture (yes *vs* no); and stratification by both baseline eGFR and proteinuria.

**Results:** The 3-year cumulative incidence of fracture increased in a graded manner in groups of adults with a lower level of eGFR for both men and women for both age groups. For example, the 3-year cumulative incidence of fracture in women aged > 65 years across the five eGFR groups were 4.3%, 5.8%, 6.5%, 7.8% and 9.6%. The corresponding estimates for men aged > 65 were 1.6%, 2.0%, 2.7%, 3.8% and 5.0%. Similar graded relationships were observed in all additional analyses.




**Conclusions:** Many men and women with chronic kidney disease will fracture. These results can be used for simple bedside prognostication and to guide sample size requirements for future fracture prevention trials.

*Funding:* Government Support - Non-U.S.

## TH-PO197

**Toll Like Receptor 2 / 4, MYD88, TNF-α and IL-6 Expression in Leukocytes of Hemodialysis and Pre-Dialysis Patients** Caren Cristina Grabulosa,[1] Edgar Ferreira Cruz,[1] Jose Tarcisio Carvalho,[1] Aline Trevisan Peres,[1] Beata Marie Redublo Quinto,[1] Lilian Cuppari,[1] Maria Dalboni.[1] *[1]Universidade Federal de Sao Paulo; [2]Tufts-New England Medical Center.*

**Background:** Toll-like receptors (TLR) are involved in mechanisms of immunity response. There are few studies that describe TLRs expression in leukocytes from chronic kidney disease patients. However, to evaluate TLR-2, TLR-4, MyD88, TNF-α and IL-6 expression in neutrophils (PMN), monocytes (MN) and lymphocytes (LYM) from HD and PD patients.

**Methods:** Blood samples from 38 hemodialysis (HD) patients, 33 pre-dialysis (PD) and 43 age-and gender-matched healthy volunteers (CONT) were analyzed for TLR2, TLR4, MYD88, TNF-α and IL-6 expression on PMN, MN and LYM. Besides, we studied PMN and MN from PD patients unstimulated and stimulated by lipopolysaccharide (LPS) and peptidoglycan (PG), by flow cytometry.

**Results:** PMN from PD patients without stimulus exhibited significant upregulation of TLR2 (4446±2426 vs 285±79; p=<0.001), TLR4 (4479±2189 vs 317±293; p=<0.001) and MYD88 (1018±670 vs 374±44; p= <0.001) expression compared to CONT and HD groups. HD patients showed upregulation only for PMN TLR2+MyD88+ expression compared to CONT and PD groups. We observed in PMN TLR2+ and PMN TLR4+ a positive correlation with TNF-α, IL-6 and MyD88 expression stimulated by LPS and PG in all groups ( r = 0.40; p < 0.05 and r = 0.37; p < 0.05, respectively). PD neutrophils stimulated by LPS and PG showed a higher expression of TLR2 and TLR4 compared to unstimulated PD neutrophils: TLR2 (unstimulated: 4446±2426; LPS: 5751±1445 and PG: 5706±1419; p <0.01) and TLR4 (unstimulated: 4786±2558; LPS: 6934±3141 and PG: 5779±2402; p <0.01). In contrast, PD stimulated by LPS and PG were significantly lower than unstimulated monocytes (unstimulated: 170±161; LPS: 111±35 and PG: 109±30; p < 0.05).

**Conclusions:** It is possible that the activation of PMN from PD patients (more TLR2 and TLR4 expression) probably reflected the effect of high serum levels concentration of uremic toxins. Moreover, the positive correlations between TLR2/4 and MyD88 with cytokines from leukocytes unstimulated and stimulated confirm the role of this mechanism in CKD inflammatory response.

*Funding:* Government Support - Non-U.S.

## TH-PO198

**Kidney-Brain Crosstalk during Sepsis** Misako Asada, Motoko Yanagita. *Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan.*

**Background:** Patients with chronic kidney disease (CKD) have a higher prevalence, severity, and mortality of sepsis. However, the mechanism that CKD influences the outcome of sepsis remains unclear. The main cause of death in septic patients is multi-organ failure, and increasing evidences support the presence of crosstalk between kidney and other distant organs via soluble and cellular inflammatory mediators. Here we investigated the influences of CKD on kidney-brain crosstalk in the context of systemic inflammation.

**Methods:** We divided C57BL/6J male mice (8~9week) into 4 groups: sham-operated mice injected with vehicle (sham/vehicle mice), sham mice injected with lipopolysaccharides (LPS, 2.5mg/kg BW)(sham/LPS mice), mice operated with unilateral ureter obstruction (UUO)(UUO mice), and mice operated with UUO and injected with LPS (UUO/LPS mice). Mice were sacrificed 5 days after the operation, and organs were subjected to histological analysis and quantitative reverse transcription polymerase chain reaction (qPCR).

**Results:** The expression of IL-6, TNF-a and MCP1 was significantly up-regulated in both kidneys of UUO/LPS mice compared to that of UUO and sham/LPS mice. The expression of kidney tubular damage markers increased in both kidneys in UUO/LPS mice compared to UUO mice. We evaluated the damages in the brain and demonstrated that the expression of IL-6, IL-6R and GFAP, a marker for activated glial cells during brain inflammation, as well as cleaved caspase 3, a marker for apoptosis, was significantly up-regulated in UUO/LPS mice compared to other 3 groups. Induction of GFAP was further confirmed by immunostaining. To analyze the molecular mechanism for kidney-brain crosstalk, we evaluated the expression of neuroprotective factors and found that EGF was significantly decreased in both kidneys in UUO/LPS mice compared to other 3 groups. Furthermore, we confirmed the EGFR phosphorylation in the brain of UUO/LPS mice was decreased significantly.

**Conclusions:** Existence of fibrotic kidneys during sepsis aggravates brain injury, possibly due to the reduced expression of EGF in the kidneys. EGFR mediated signaling in brain may be important to maintain the brain condition.

## TH-PO199

**Race and Severity of Kidney Disease Modify the Associations between Hyponatremia and Death** Sankar D. Navaneethan,[1] Jesse D. Schold,[2] Jonathan J. Taliercio,[1] Susana Arrigain,[2] Stacey Jolly,[3] James F. Simon,[1] Joseph V. Nally.[1] *[1]Nephrology; [2]Quantitative Health Sciences; [3]Medicine, Cleveland Clinic.*

**Background:** Hyponatremia is associated with falls and higher risk for death in elderly population. We studied the associations between hyponatremia and death among chronic kidney disease (CKD) patients.

**Methods:** The risk for development of hyponatremia and association with death was examined using logistic regression and Cox-proportional hazard models. We tested 2-way interactions between serum sodium and the following covariates in the adjusted model: age, gender, race, diabetes, heart failure and eGFR.

**Results:** Out of 45,021 CKD patients, 3.6% (n=1645) had hyponatremia (sodium <135 mg/dl in men and <132 mg/dl in women). Male gender, presence of diabetes and malignancy were associated with higher risk for hyponatremia while higher age, eGFR and BMI were associated with lower risk for hyponatremia. After covariate adjustment that included heart failure, kidney and liver function, hyponatremia was associated with a 47% increased risk (95% CI 1.36, 1.60) for death. Caucasians but not blacks had higher risk for death with hyponatremia. The association between hyponatremia and death was pronounced among those with stage 3 CKD and younger patients.

| Variable | Hazard ratio | 95% CI |
|---|---|---|
| Age | | |
| 18-64.3 years | 1.47 | 1.26, 1.72 |
| 64.3-73.0 years | 1.87 | 1.57, 2.22 |
| 73.0-80.6 years | 1.65 | 1.39, 1.96 |
| >80.6 years | 1.17 | 1.01, 1.36 |
| Race | | |
| Caucasians | 1.52 | 1.39, 1.66 |
| African Americans | 1.20 | 0.97, 1.48 |
| Stage of CKD | | |
| Stage 3a (eGFR 45-59) | 1.54 | 1.37, 1.73 |
| Stage 3b (eGFR 30-44) | 1.49 | 1.30, 1.71 |
| Stage 4 (eGFR 15-29) | 1.27 | 1.05, 1.53 |

Similar results were noted in a subset of patients (n=8,205) when adjusted for LVEF (instead of clinical diagnosis of heart failure).

**Conclusions:** Hyponatremia is associated with death among those with CKD. Caucasians and those with stage 3 CKD sustain higher risks for death than African Americans and those with advanced kidney disease. Future studies should explore the reasons for the modified effects noted in our study.

## TH-PO200

**Association of Anemia and Mineral and Bone Disorders with Health-Related Quality of Life in Predialysis Patients** Priscilla P. How,[1] Jun Jie Benjamin Seng,[1] Jia Jia Lee,[1] Hwee-Lin Wee,[1] Pallavi Tyagi,[2] Vathsala Anantharaman.[2] *[1]Pharmacy, National Univ of Singapore, Singapore, Singapore; [2]Medicine (Div of Nephrology), National Univ Hospital, Singapore, Singapore.*

**Background:** Chronic kidney disease (CKD) patients have poor health-related quality of life (HRQoL), which has been associated with increased morbidity and mortality. Compared with dialysis patients, the impact of CKD-related complications such as anemia and, mineral and bone disorders (MBD), on HRQoL in predialysis patients is less well-studied. This study aimed to examine the association of anemia and MBD with HRQoL in predialysis patients.

**Methods:** A cross-sectional study involving predialysis patients from the National University Hospital, Singapore, outpatient renal clinic was conducted. Patients' HRQoL were assessed using the Kidney Disease Quality of Life Short Form (KDQOL-SF™) and EuroQol 5 Dimensions–3 levels (EQ5D-3L). Psychosocial variables were evaluated using the Medical Outcomes Family Functioning Measure questionnaire. Separate hierarchical multiple linear regressions were performed using HRQoL scales as dependent variables, with adjustment for socio-demographic, clinical and psychosocial variables, to determine associations.

**Results:** Among 246 patients who completed the study, 227 and 225 patients possessed the relevant data for the anemia and MBD analyses, respectively. Eighty-four (37.0%) patients had anemia while 96 (42.7%) patients had MBD. After adjusting for potential confounders, anemia was associated with poorer physical component summary (β=-3.95, p=0.006) and mental component summary scores (β=-4.50, p=0.003), but better EQ5D-3L visual analogue scale scores (β=6.64, p=0.021). Anemia was not associated with EQ5D-3L utility scores. Although no association was found between MBD and the four summary HRQoL scales, it was associated with poorer scores in other disease-specific HRQoL domains such as symptoms (β=-22.30, p<0.001).

**Conclusions:** Anemia and MBD were associated with poorer HRQoL in predialysis patients. Future research should explore the impact of optimal anemia and MBD treatment on improving patients' HRQOL.

## TH-PO201

**Daily Patient Reported Outcome Reporting Is Practical and Demonstrates Differential Patient Experience** Zoe C.L. Pittman,[1] Stephen G. John,[1] Chris W. McIntyre.[1,2] *[1]Royal Derby Hospital, United Kingdom; [2]Univ of Nottingham, United Kingdom.*

**Background:** Patient reported outcomes (PRO) are increasingly recognised as a critical metric in chronic disease. Whilst a variety of generic and disease specific PRO measures (PROMs) have been used in CKD, studies are mainly cross-sectional with limited longitudinal data. None of the available PROMs are designed for iterative application. We designed and implemented a CKD specific online dynamic PROM for daily use.

**Methods:** PRO selection was informed by published data on symptom burden and a patient survey. 6 separate domains (general well being (GWB), pain, sleep, breathing, energy and appetite) were included. A custom website was built to capture PROs daily utilising visual analogue scales (free selection of status from 0(worst) to 100(best)). Additional clinical information was collected from the patient record.

**Results:** 43 patients submitted at least one set of data via the website with a median follow up at analysis of 247 days (range 10 – 312 days), 64% male, mean age 62±12 yrs. 80% (17 Dialysis and 17 CKD) submitted data for > 30 days. 65% continued to submit data at 90 days. Dialysis patients had significantly lower median [IQR] scores for sleep (51[32–83], 96[71–98], p=0.001), appetite (69[52–95], 96[78–99], p=0.014), energy (50[40–81], 83[67–95], p=0.013) and GWB (60[47–84], 87[71–97] p=0.010). There was no statistical difference in scores for pain or breathing. Individual patients demonstrate a variety of mainly stable bandwidths of response. Analysis of individual patient response patterns revealed detectable score changes associated with specific events (admission, fistula formation, dialysis start). In individual dialysis patients there were changes related to fluid status, admissions and differing scores dependent on dialysis or non-dialysis day.

**Conclusions:** We have successfully introduced a web based PROM for frequent use which is acceptable to patients and has the ability to discriminate symptom burden cross-sectionally and longitudinally. Further work will look to prospectively examine the predictive power of changes in PRO and make formal comparison with current measures of health related quality of life and symptom scores.

## TH-PO202

**Depression in the Chronic Kidney Disease in Children (CKiD) Cohort Study** Amy Kogon, Matthew Matheson, Joseph T. Flynn, Bradley A. Warady, Susan L. Furth, Stephen R. Hooper. *CKiD Study Group.*

**Background:** No large studies exist regarding the prevalence of depression in pediatric chronic kidney disease (CKD). Using CKiD data, we assessed the factors associated with depression.

**Methods:** Depression was defined as a score ≥ one standard deviation above the mean on the Children's Depression Inventory (CDI) or a history of depression concurrent with active treatment. Associated factors were determined by multivariate linear and logistic regression analyses. Separate models assessed clinical/demographic factors and cognitive/psychosocial factors.

**Results:** 344 children (median age 13 years [IQR 10, 15]) completed the CDI; 18 had elevated depression scores and 7 had normal scores and were undergoing treatment for depression. Higher maternal education and higher height z-score were protective for depression. There was a trend towards more depression in patients with congenital CKD. Depressed subjects had lower academic achievement scores (WIAT-II) (p=0.06) and lower quality of life scores (PedsQL) (p<.0001).

| Factor | OR for depression [95% CI] |
|---|---|
| Age | 1.08 [0.89,1.30] |
| Male | 1.59 [0.56,4.47] |
| Race | |
| Caucasian | 1.00 |
| AA | 0.90 [0.19,4.30] |
| Hispanic | 1.64 [0.40,6.68] |
| Other | 1.48 [0.29,7.69] |
| Maternal Education | |
| High School or less | 1.00 |
| Some college | 0.86 [0.28,2.65] |
| College or more | 0.09 [0.01,0.77] |
| Duration of CKD | 0.95 [0.83,1.08] |
| Etiology of CKD | |
| Glomerular | 3.37 [0.31,36.64] |
| Non-glomerular, congenital | 8.07 [0.94,69.33] |
| Non-glomerular other | 1.00 |
| GFR (per 10 ml/min/1.73m$^2$) | 1.12 [0.79,1.60] |
| Height z-score | 0.58 [0.35,0.96] |
| BMI z-score | 0.87 [0.55,1.36] |

**Conclusions:** The prevalence of depression in this pediatric CKD cohort was lower than in other reports. Few clinical factors predicted depression. The association with congenital CKD without an association with duration of disease suggests that there may be a common pathway linking congenital CKD to the occurrence of depression. Depression may be a modifiable factor contributing to academic achievement and quality of life in these patients.

*Funding:* NIDDK Support

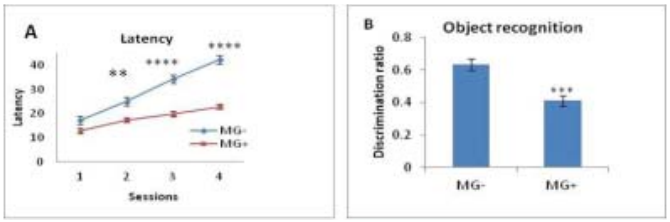
## TH-PO203

**Advanced Glycation End Products (AGEs) Increase the Risk for Cognitive Decline in Aging Mice with Chronic Kidney Disease (CKD)** Shobha M. Swamy, Elena M. Yubero-Serrano, Jaime Uribarri, Helen Vlassara, Gary E. Striker. *Mount Sinai School of Medicine, New York, NY.*

**Background:** Cognitive decline in CKD has been associated with elevated inflammation and reactive oxygen species (ROS) and decreased SIRT1. Accumulation of glycotoxins (AGEs) has been implicated in the increased inflammation/ROS, leading to the complications of aging, including cognition and CKD.We recently reported that high levels of circulating MG-derivatives (very reactive AGEs) were associated with higher brain and cerebrospinal fluid (CSF) AGE levels and a significantly faster rate of cognitive decline in T2D and in non-diabetic elderly subjects. Here we asked if there was an effect of methylglyoxal on motor co-ordination, learning and memory in aged mice with CKD fed with an AGE-enriched diet, compared to isocaloric pair-fed controls.

**Methods:** C57BL6 mice (18-19 month old) fed a Low AGEs diet supplemented with MG (MG+) diet for 10 generations developed insulin resistance and CKD, whereas mice pair-fed a Low AGEs (MG-) were unaffected. AGE-induced cognitive changes were assessed by Rotorod and object recognition tests.

**Results:** Despite repeated learning sessions MG+ mice showed a significant decrease in the latency (time to fall from the Rotorod) (Fig. A) at lower speed and shorter distance, compared to MG- mice. MG+ mice showed poor recognition ability with significantly lower discriminatory capacity between novel and a familiar object, compared to MG- mice (Fig. B).




**Conclusions:** As in aging humans, the motor and memory dysfunction in the current study of C57Bl6 mice with CKD appear to be due to increased ROS and inflammation secondary to the accumulation of AGEs in the brain. Littermates pair-fed a low AGE diet did not show these changes. Clinical studies will need to be conducted to determine if reduction of dietary AGEs may protect against cognitive decline in CKD patients.

## TH-PO204

**Effect of Ferumoxytol and Iron Sucrose on Serum Phosphorus Levels** Naomi V. Dahl, Gloria Lau, Zhu Li, Pallabi De, William Strauss. *AMAG Pharmaceuticals.*

**Background:** Administration of certain IV iron preparations (ferric carboxymaltose [FCM], saccharated ferric oxide, and iron polymaltose) has been associated with hypophosphatemia (Wolf et al., Bone Min Res, 2013; Schouten, J Clin Endoc Metab, 2010), with an incidence of serum phosphate reductions to <2 mg/dL occurring in up to 70% of patients with abnormal uterine bleeding (AUB) around 14 days after treatment with FCM (Van Wyck, Transplant 2009). Recent studies have demonstrated an acute, FGF23-mediated phosphate wasting in response to FCM but not iron dextran, suggesting that the specific carbohydrate moiety might be involved in the FGF23 response to IV iron. Ferumoxytol (FER) is an IV iron approved for the treatment of iron deficiency anemia (IDA) in adult patients with chronic kidney disease (CKD). It is also being evaluated as a treatment for IDA patients without CKD who have a history of unsatisfactory oral iron therapy or in whom oral iron cannot be used.

**Methods:** To explore the effect of FER on serum phosphate (P) levels in women with AUB, we pooled data from 2 phase-3, randomized controlled studies in 1413 patients with IDA and eGFR ≥30mL/min/1.73m$^2$. In these studies, patients were randomized to FER (2 doses, 510mg, 3-8 days apart) or comparator: placebo (0.9% saline) or iron sucrose (IS), 1g (5 doses, 200mg, over 14 days).

**Results:** This subgroup analysis includes the 605 women with AUB (65% white, 22% African American) and available baseline and follow-up P values (432 FER, 91 IS, 82 placebo), mean age 40.2±8.5. Mean baseline P for all 3 groups was 3.4±0.5mg/dL. There was no decrease in mean P at any week, except for IS at week 3 (-0.3 ±0.7 mg/dL). No FER or placebo patients experienced any P reduction below 2 mg/dL during the 5-week study period. The decreases in the IS group mainly occurred at weeks 2 and 3.

| Patients Experiencing Post-baseline Decrease in Phosphorus from ≥2.5 mg/dL, n(%) | Ferumoxytol (N=432) | Iron Sucrose (N=91) | Placebo (N=82) |
|---|---|---|---|
| to <2.5 – 2.0 mg/dL | 8 (1.9) | 10 (11.0) | 5 (6.1) |
| to <2.0 – 1.0 mg/dL | 0 | 3 (3.3) | 0 |
| to <1.0 mg/dL | 0 | 0 | 0 |

**Conclusions:** In this study, ferumoxytol did not appear to decrease serum P levels in an at-risk population.

*Funding:* Pharmaceutical Company Support - AMAG Pharmaceuticals

## TH-PO205

**Predictor for Bleeding-Related Upper Gastrointestinal Lesions in Anemic Patients with Chronic Kidney Disease** Youn Mi Song,[1] Sangju Lee,[2] Hye Eun Yoon,[2] Yoon-Kyung Chang,[2] Chul Woo Yang,[2] Suk Young Kim,[2] Hyeon Seok Hwang.[2] *[1]Div of Gastroenterology, Depts of Internal Medicine College of Medicine, The Catholic Univ of Korea, Daejeon, Korea; [2]Div of Nephrology, Depts of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Daejeon, Korea.*

**Background:** Anemia and iron deficiency are common complication in patients with chronic kidney disease (CKD). However, information about the diagnostic indicators for bleeding-related upper gastrointestinal (GI) lesions is scarce and few studies have investigated anemic upper GI lesions.

**Methods:** We included 165 anemic patients with nondialysis-dependent CKD stages 3–5 (44 patients at stage 3, 52 patients at stage 4, and 69 patients at stage 5). Transferrin saturation (TSAT), serum ferritin, mean corpuscular volume (MCV), and corrected reticulocyte count data were collected to evaluate diagnostic utility for bleeding-related upper GI lesions, which were identified by esophagogastroduodenoscopy.

**Results:** Bleeding-related GI lesions were found in 57 (34.5%) patients. The areas under the receiver operating characteristic curves used to predict bleeding-related lesions was 0.63 for TSAT ($p = 0.007$) and the best cutoff value was 19.7% (sensitivity, 0.53, specificity, 0.76). The combination of cutoff values of TSAT < 20% or serum ferritin level < 100 ng/mL provided the 17% increment of sensitivity, compared to TSAT <20% alone. The corrected reticulocyte levels and MCV had no significant diagnostic utility. In patients with CKD stage 5, the sensitivity for TSAT or its combination with serum ferritin was significantly lower than in patients with CKD stage 3 (all $p < 0.05$).

**Conclusions:** TSAT is a significant predictor of anemic lesions in the upper-GI tract and serum ferritin can increase the sensitivity of TSAT. However, these indicators should be used with caution in patients with CKD stage 5 because their sensitivity is poor in this context.

## TH-PO206

**Glycosuria under Normoglycemia May Be an Unrecognized Characteristic of Chronic Kidney Disease** Takahito Ito, Yoko Shima, Naoko Morikage, Ryuta Fujimura, Chisako Nakano, Akira Wada, Masafumi Yamato. *Div of Nephrology, Osaka National Hospital, Osaka, Japan.*

**Background:** Normal nephrons do not lose glucose in the urine under normoglycemia. We studied the relation between chronic kidney disease and glycosuria.

**Methods:** From April/1/2010 to October/31/2011, we had 3786 datasets in each of which urine dipstick glucose level, serum glucose concentration, serum creatinine concentration, and HbA1c were measured at the same time. If a subject was tested repeatedly during the period, the oldest data set was adopted. Finally, we used 1629 datasets of the different subjects (64.1±13.5 y, 1049 males) in the following analysis. None took phenazopyridine, timiperone or procaine which might result in a false-positive glycosuria.

**Results:** Glucose concentration and HbA1c were 113.0 mg/dL [IQR: 97.0-155.0] and HbA1c 5.70% [IQR: 5.00-6.60], respectively. When the presence of glucose in the urine (1-plus or more) was defined as glycosuria, the numbers of subjects with and without glycosuria were 523 (Group G) and 1106 (Group N), respectively. There was no statistical difference of age or estimated glomerular filtration rate (eGFR) between Groups G and N. Using 596 subjects whose serum glucose and HbA1c were lower than their median values (glucose ≤112 and HbA1c≤5.6, respectively), we performed two sub-analyses. (1) Subjects with and without glucosuria were re-classified into Group GG and NN, respectively. eGFR of Group GG (N=29) was significantly lower than that of Group NN (N=567)(50.9±35.7 vs. 68.9±27.4, P=0.0061). Age was not significant. (2) We defined the subjects with eGFR lower than 60 as "CKD" (N=199) and compared them with "non-CKD" (N=397). The non-adjusted and age-adjusted odd ratios for glycosuria in "CKD" were 2.230 [95% CI: 1.050-4.767] and 2.256 [1.013-5.075], respectively. Even when a trace level of glucose in the urine was included in the definition of glycosuria, we obtained similar results.

**Conclusions:** Glycosuria under normoglycemia is more often in CKD. This phenomenon may come from abnormal glucose transport, saturated glucose transport in the reduced number of nephrons, and/or delayed tubular fluid flow leading to the dissociation between blood and urinary glucose levels.

*Funding:* Clinical Revenue Support

## TH-PO207

**Modeled eGFR, eGFR Slope, and Mortality Risk among Adults with Moderate and Advanced CKD** Xiaoqin Tang,[1] Robert M. Perkins,[2] Ion D. Bucaloiu,[1] H. Lester Kirchner.[1] *[1]Geisinger Medical Center, Danville, PA; [2]Bassett Medical Center, Cooperstown, NY.*

**Background:** Single time-point GFR estimates are used for risk prognostication among those with CKD. Various applications of longitudinal eGFR using linear models—to include time-independent slope and variability—have been shown to be independent predictors of mortality. We wished to extend these applications by modeling both longitudinal eGFR history--forming estimates of current value of eGFR and time-dependent slopes at any time point--and then test the independent association of these variables with mortality risk in an adjusted, joint survival model.

**Methods:** We retrospectively assembled a cohort of Geisinger primary care patients with stage G3 or G4 CKD (1/1/2001 to 6/30/2012) who had a minimum of 4 outpatient eGFR results and 6-months follow-up. Patients were excluded for prior history of dialysis or renal transplantation. Patients were followed through 12/31/2012 for death. EGFR was modeled using a random intercept and slope model with a flexible spline transformation of time to capture patient-specific, non-linear patterns. Time to death was modeled with Cox Proportional Hazard regression.

**Results:** A total of 16,737 patients met cohort entry criteria. The sample had mean age 69.4 years, 28% had diabetes, and mean baseline eGFR was 51 mL/min. During 98,075 person-years of follow up, 3709 (22%) patients died. In fully adjusted models, each 5 mL/min/1.73m$^2$ decrease in current eGFR value was associated with a mortality hazard ratio(HR) of 1.05 (95% CI 1.03, 1.09). For the time-dependent slope of the eGFR trend, each 2 mL/min/1.73m$^2$/year decrement was associated with a HR of 1.09 (95% CI 1.05, 1.12). Both diabetes and gender modified these associations, such that the mortality risk was significantly greater among non-diabetics and females.

**Conclusions:** Modeled longitudinal eGFR and time-dependent eGFR slope each independently associates with mortality risk among patients with moderate and advanced CKD. Comparing results to baseline eGFR (HR=1.07 (95% CI: 1.05, 1.07)), the joint model may provide more prognostic value to predicting mortality.

## TH-PO208

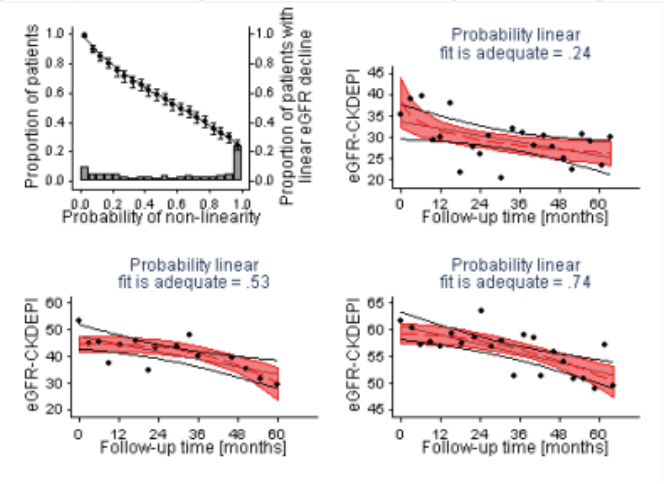
**Mathematical Models for the Evaluation of eGFR Decline in CKD Patients: Linear versus Non-Linear** Jan A.J.G. van den Brand,[1] Tjeerd Maarten-Hein Dijkstra,[3] Arjan D. Van Zuilen,[2] Peter J. Blankestijn,[2] Jack F. Wetzels,[1] Tom Heskes.[3] *[1]Nephrology, Radboud Univ Medical Centre, Nijmegen, Netherlands; [2]Nephrology, Univ Medical Centre Utrecht, Netherlands; [3]Institute for Computing and Information Sciences, Radboud Univ, Nijmegen, Netherlands.*

**Background:** Li et al. recently questioned the concept that eGFR decline is linear in CKD patients (Li et al. AJKD 2012). We aimed to evaluate what proportion of CKD patients showed possible non-linear eGFR decline with a simpler technique.

**Methods:** We analysed data of patient that participated in MASTERPLAN (Van Zuilen et al. Kidney Int. 2012). Kidney transplant patients were excluded. GFR was estimated using the eGFR-CKDEPI equation for serum creatinine. Ordinary least squares regression was used to obtain a linear model of eGFR over time. An optimal first order model was

selected from possible powers $x^{-3}$, $x^{-2}$, $x^{-1}$, $x^{-1/2}$, ln(x), □x, $x^2$ and $x^3$ using STATA's fractional polynomials (Statacorp, TX). The fit for both models was compared using Akaike's Information Criterion (AIC). The probability that a linear fit was acceptable was calculated as probability = exp[ ($AIC_{fp}$-$AIC_{lin}$) / 2].

**Results:** In total, 640 patients were included, 69% were male and mean age was 60 ± 12.6 years, baseline eGFR was 37 ± 15 ml/min per 1.73m$^2$. The mean number of serum creatinine values per patient was 14.6 ± 5.2 over the course of 4.2 ± 1.2 years. The histogram shows the proportion of patients by the probability of linearity (top left panel); the line plot shows the total proportion of patients with a linear eGFR decline by acceptance threshold probability for linearity (top left) and eGFR course for three patients as an example.




**Conclusions:** In most CKD patients eGFR decline is linear. However, even when accepting a low threshold (5%), eGFR decline is non-linear in 10% of patients. Our findings confirm previous work by Li et al. using a simpler technique.

*Funding:* Private Foundation Support

## TH-PO209

### Predictors of Progression in European CKD Patients: The Tangri Model and Beyond

Mieke J. Peeters,[1] Arjan D. Van Zuilen,[2] Jan A.J.G. van den Brand,[1] Michiel Bots,[2] Peter J. Blankestijn,[2] Jack F. Wetzels.[1] *[1]Radboud Univ Nijmegen Medical Centre, Nijmegen; [2]Univ Medical Center Utrecht, Utrecht, Netherlands.*

**Background:** Patients with CKD may progress to ESRD and benefit from early referral to nephrological care. However, treatment adds costs and may also have adverse effects. Therefore, adequate prediction of CKD progression is important. We validated the predictive models developed by Tangri *et al* [Peeters *et al* NDT 2013]. We performed analyses to identify additional predictors of CKD progression.

**Methods:** MASTERPLAN is a controlled trial in patients with CKD. CKD progression was defined as ESRD (initiation of chronic dialysis or kidney transplantation) or a 50% increase in serum creatinine concentration. Patients who died before reaching an endpoint were censored. Clinical parameters and laboratory data at MASTERPLAN baseline were assessed as predicting factors for CKD progression, using univariate and multivariate Cox regression.

**Results:** We evaluated all 788 patients (67% male, mean age 59 years, mean eGFR 36 ml/min), 330 patients showed CKD progression. In univariate analysis treatment center, cause of kidney disease, diabetes mellitus, age, BMI, systolic blood pressure, current smoking, activity score, eGFR, protein creatinine ratio, and serum levels of hemoglobin, PTH, calcium, phosphate, albumin, bicarbonate, potassium, and uric acid were associated with CKD progression. Predictors of CKD progression in multivariate analysis are shown in the table.

| | HR (95% CI) |
|---|---|
| Congenital kidney disease (mainly ADPKD) | 3.04 (2.16-4.27) |
| Systolic blood pressure (per 10 mmHg) | 1.13 (1.06-1.21) |
| Current smoking | 1.48 (1.10-1.99) |
| eGFR (per 5 ml/min/1.73m$^2$) | 0.86 (0.80-0.92) |
| Protein creatinine ratio (per 10 mg/g) | 1.003 (1.002-1.004) |
| Hemoglobin (per 1 g/dl) | 0.88 (0.81-0.97) |
| Calcium (per 1 mg/dl) | 0.67 (0.52-0.87) |
| Uric acid (per 1 mg/dl) | 1.05 (1.02-1.08) |

**Conclusions:** We identified independent predictors of CKD progression in our MASTERPLAN cohort. We confirmed variables that were included in models by Tangri *et al*. The cause of kidney disease, blood pressure, current smoking, hemoglobin, and uric acid provided added value.

*Funding:* Pharmaceutical Company Support - Dutch Kidney Foundation (Nierstichting Nederland), Netherlands Heart Foundation (Nederlandse Hartstichting), Amgen, Genzyme, Pfizer, Sanofi-Aventis

## TH-PO210

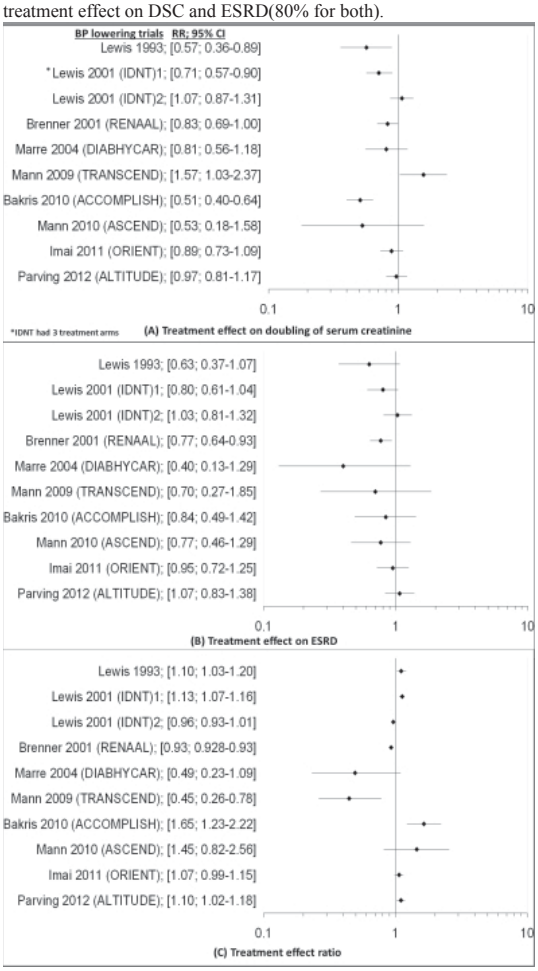
### The Validity of Doubling of Serum Creatinine as a Surrogate Marker for ESRD: A Systematic Review of Randomized Trials

Min Jun,[1,2] Tanvir Chowdhury Turin,[1] Hiddo Jan Lambers Heerspink,[3] Mark Woodward,[2] Braden J. Manns,[1] Marcello Tonelli,[4] Vlado Perkovic,[2] Brenda Hemmelgarn.[1] *[1]U/Calgary, Canada; [2]George Institute for Global Health, Australia; [3]U/Groningen, Netherlands; [4]U/Alberta, Canada.*

**Background:** This systematic review assessed the validity of doubling of serum creatinine(DSC) as a surrogate for endstage renal disease (ESRD) in trials assessing the effects of blood pressure(BP) lowering in high-risk adults with diabetes, CKD stages 1-4 and/or renal transplantation.

**Methods:** MEDLINE, EMBASE and the Cochrane Library were searched (1946-Jun 2012) for randomized trials reporting the effects of BP lowering on ESRD and DSC. Abstract review and data extraction was done independently by 2 reviewers. The association between treatment effects on DSC and ESRD was expressed as the "treatment effect ratio(TER)" (effect on ESRD/effect on DSC; ideal surrogate would have a TER close to 1). This test is a key element of Prentice's operational criteria for validating surrogate markers(treatment has a significant impact on surrogate and the true outcome[TO];surrogate has a significant impact on the TO; the full effect of treatment on the TO is captured by the surrogate).

**Results:** The systematic review yielded 7531 abstracts of which 9 BP lowering trials in 36500 patients with diabetes, hypertension and/or CKD 1-4 met the inclusion criteria. 1 trial had 3 arms and was treated as 2 trials. Majority of the trials showed a beneficial treatment effect on DSC and ESRD(80% for both).




TERs varied(0.45-1.65) although 6 studies were within ±13% of the null.

**Conclusions:** These results show that in trials assessing the effects of BP lowering drugs in high-risk patients, DSC is a relatively good surrogate for ESRD. We assessed the validity of DSC according to only 1 of 3 elements of Prentice's criteria for validation of a surrogate marker. Future studies will need to consider for the remaining criteria where possible.

**TH-PO211**

**Validity and Statistical Power of Alternative eGFR-Based Endpoints: A Report from an NKF FDA Workshop** Tom Greene,[1] Chia-Chen Teng,[1] Jian Ying,[1] Andrew M. Redd,[1] Mark Woodward,[2] Lesley Inker,[3] Josef Coresh,[2] Andrew S. Levey.[3] *[1]Univ of Utah; [2]Johns Hopkins Univ; [3]Tufts Univ.*

**Background:** Because the follow-up period required to observe the FDA approved doubling of serum creatinine (SCR) endpoint can be prohibitive, we investigated the performance of alternative endpoints defined by ESRD or confirmed reductions in eGFR of either 57% (approximate doubling of SCR), 40% or 30% on the validity and efficiency of CKD RCTs.

**Methods:** We used statistical simulation to determine the efficiency (defined as the required sample size [N] to achieve 90% power) and validity (defined as the Type 1 error for the eGFR-based endpoint when there is no treatment effect on time to ESRD) of alternative eGFR-based endpoints under different scenarios. Simulations were calibrated with data from 11,982 patients in 41 CKD RCTs.

**Results:** We simulated 800 repetitions for each endpoint over more than 800 scenarios. In the base case scenario, use of a 30% eGFR decline endpoint reduced the required Ns for 3 year RCTs by 19% to 54% compared to the required Ns for a 57% eGFR decline endpoint in trials with a moderate to high baseline eGFR for treatments with no acute effect. However, use of a 30% eGFR decline endpoint increased the required N and led to inflated Type 1 error for treatments with an acute effect of -1.5 ml/min/1.73m$^2$ in the opposite direction of the long term effect.

| % Differences in Required N for 30% and 40% eGFR Decline vs. Required N for 57% eGFR Decline | | | | |
|---|---|---|---|---|
| Alternative Outcome, ESRD or: | Acute Effect (ml/min/1.73m$^2$) | Median Baseline eGFR (ml/min/1.73m$^2$) | | |
| | | 67.5 | 42.5 | 27.5 |
| 30% eGFR Decline | 0 | -54% | -19% | +13% |
| | -1.5 | +18%* | +46%* | +48% |
| 40% eGFR Decline | 0 | -45% | -17% | -7% |
| | -1.5 | -14% | -1% | +12% |
| * Actual type 1 error exceeded 10% when the targeted type 1 error was 5% | | | | |

**Conclusions:** A moderate acute effect in the opposite direction from the long term treatment effect can lead to increased Type 1 error and/or increased required N when using smaller eGFR event thresholds. However, the endpoint of ESRD with 30% or 40% decline in eGFR may reduce the required sample size and thereby increase the efficiency of trials with moderate to high baseline eGFR when an acute effect is not expected.

*Funding:* Private Foundation Support

**TH-PO212**

**Longitudinal eGFR, eGFR Slope, and Incident Cardiovascular Events among Adults with CKD** H. Lester Kirchner,[1] Robert M. Perkins,[2] Ion D. Bucaloiu,[1] Xiaoqin Tang.[1] *[1]Geisinger Medical Center, Danville, PA; [2]Basset Medical Center, Cooperstown, NY.*

**Background:** Single time-point GFR estimates are used for risk prognostication among those with CKD. Various applications of longitudinal eGFR using linear models—to include time-independent slope and variability—have been shown to independently predict cardiovascular events. We wished to extend these applications by modeling both longitudinal eGFR—forming estimates of current eGFR value and time-dependent eGFR slope at any time point—and then test the independent association with incident cardiovascular event risk in an adjusted, joint model.

**Methods:** We retrospectively assembled a cohort of Geisinger primary care patients with stage G3-G4 CKD(1/1/2001 to 6/30/2012) who had a minimum of 4 outpatient eGFR results and 6-months follow-up. Exclusion criteria included a history of dialysis, renal transplantation, myocardial infarction (MI), heart failure(HF), or TIA/stroke. Patients were followed through 12/31/2012 for the first occurrence of MI, HF, or stroke (incident CVD), or were censored at ESRD, death or end of study. EGFR was modeled using a random intercept and slope model with a flexible spline transformation of time to capture non-linear trends. Time to incident CVD was modeled using Cox Proportional Hazard regression.

**Results:** 16,304 patients met cohort entry criteria (mean age 68.4 y, 37% male, 23% with diabetes, mean baseline eGFR 51 mL/min) and contributed 89,682 person-years of follow up. 1997 (12%) patients developed incident CVD. In fully adjusted models, each 5 mL/min/1.73m$^2$ decrease in current eGFR was associated with a hazard ratio(HR) of 1.07 (95% CI 1.04, 1.09). For the time-dependent slope of eGFR trend, each 2 mL/min/1.73m$^2$/year decrement was associated with a HR of 1.22 (95% CI 1.15, 1.30). Both diabetes and gender modified the eGFR slope associations, such that the associations were significantly greater among non-diabetics and females.

**Conclusions:** Longitudinally modeled eGFR and time-dependent eGFR slope each independently associates with incident CVD risk among patients with moderate and advanced CKD. The relative prognostic value of static, single time point eGFR vs. joint modeling of eGFR warrants investigation.

**TH-PO213**

**Impact of the Number of Albuminuria Measurements on Sample Size Requirements for Clinical Drug Trials** Tobias Felix Kröpelin,[1] Hiddo Jan Lambers Heerspink,[1] Dennis L. Andress,[2] Dick de Zeeuw.[1] *[1]Univ Medical Center Groningen; [2]AbbVie Laboratories.*

**Background:** Change in albuminuria is used as a surrogate endpoint in clinical trials of CKD progression. Yet, the number of urine samples (albumin measurements) needed to precisely quantify an anti-albuminuric drug effect is unknown. We assessed the impact of the number of urine samples on the precision of anti-albuminuric drug effects and sample size requirements for clinical trials.

**Methods:** Data from the Selective vitamin D receptor activation for albuminuria lowering (VITAL) trial were used. Urinary albumin:creatinine ratio (UACR) was measured at randomization and every 4 weeks during 24-weeks of treatment. At each visit three consecutive first morning void (FMV) urines were collected. The effect of paricalcitol 2 µg/day versus placebo, in subjects that submitted at each visit three first morning voids (N=137), was assessed as the geometric mean UACR change from baseline to week 24 and secondly as the geometric mean change considering all follow up visits. In both analyses, UACR change was calculated based on 1, 2, or 3 consecutive FMV UACR measurements.

**Results:** The mean UACR reduction slightly varied according to the number of UACR measurements and was somewhat smaller when all UACR data over time were considered (Table 1). However, the precision of the treatment effect increased (decreased standard error) with increasing number of UACR measurements. Thus, a post-hoc power calculation showed that the number of patients required to obtain a significant treatment effect decreased with more UACR measurements and markedly decreased when all UACR measurements over time were considered.

| | FMV UACR measurements | | | FMV UACR | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| | Albuminuria change % (SE) | | | Sample size | | |
| Δ UACR Baseline to week 24 | -17% (11.6) | -15% (10.4) | -17% (10.2) | 268 | 244 | 188 |
| Δ UACR over time (including all visits) | -13% (5.0) | -14% (4.8) | -16% (4.6) | 109 | 85 | 61 |

**Conclusions:** Clinical trials can be more efficiently designed if they take into account all albuminuria measurements collected over time rather than considering a change in albuminuria between two pre-determined time-points.

**TH-PO214**

**Variation in GFR Estimates and Prevalence of CKD among the Veterans with Use of Two Creatinine Values** Nilang G. Patel,[3] Neha Nainani,[2] Rajiv Ranjan,[1,2] Nida Arabi,[2] James W. Lohr,[1,2] Pradeep Arora.[1,2] *[1]Medicine, VAMC, Buffalo, NY; [2]Medicine, Univ at Buffalo, Buffalo, NY; [3]Medicine, VAMC and VCU, Richmond, VA.*

**Background:** A GFR of less than 60ml/min/1.73 m$^2$ for at least 3 months duration is considered to be CKD. Most of the epidemiological studies used only one serum creatinine value to estimate GFR. However, this would likely overestimate the true prevalence of CKD. The purpose of this study was to assess the prevalence of CKD in the veteran population using 2 creatinine values.

**Methods:** A retrospective cohort study of 97,451 veterans seen in primary care clinic in the VISN 2 network over 7 years. GFR estimation and CKD prevalence were determined using the MDRD study equation and the CKD-EPI study equation based on one (MDRD-1, CKD-EPI-1) and two serum creatinine values (MDRD-2, CKD-EPI-2) at least 3 months apart.

**Results:** Out of 97451 patients, 95% were male, 91% were white and 56% were above the age of 60 years. For any given age,the average eGFR was higher when estimated by CKD-EPI equation than by MDRD equation except in very elderly patients (> 80 yrs of age) where eGFR was lower by CKD-EPI than MDRD equation. With increase in age, the prevalence of CKD also increased after 60 years of age. Prevalence of CKD by the different equations is shown in table.

| | MDRD-1 | MDRD-2 | CKD-EPI-1 | CKD-EPI-2 |
|---|---|---|---|---|
| CKD Prevalence | 51.6% | 31.9% | 47.2% | 29.7% |
| Net decrease in Prevalence | 38% | | 37% | |

By using 2 values of serum creatinine and the MDRD equation the prevalence of CKD decreased from 51.6% to 31.9%, a net decrease in the prevalence by 38%. The decrease in prevalence was seen primarily in stage 3a CKD. Similarly using 2 values of serum creatinine and CKD-EPI equation the prevalence of CKD decreased from 47.2% to 29.7% a net decrease in the prevalence by 37%, mostly due to overestimation of CKD stage 3a. The use of 2 serum creatinine values did not add any additional value for those who had an index GFR of less than 45 ml/min/1.73 m$^2$.

**Conclusions:** Veterans have a higher prevalence of CKD than the general population. CKD prevalence is more accurately classified using 2 serum creatinine values.

**TH-PO215**

**Effect of Renin Angiotensin System Inhibitor Therapy on Renal and Cardiovascular Outcomes in Patients with Chronic Kidney Disease: A Systematic Review and Meta-Analysis** Xinfang Xie,[1] Youxia Liu,[1] Jicheng Lv,[1] Xiangling Li,[2] Wanyin Hou,[1] Hong Zhang.[1] *[1]Renal Div, Peking Univ First Hospital, Beijing, China; [2]Hospital of Weifang Medical College.*

**Background:** We undertook a systematic review and meta-analysis to evaluate the effect of ACE inhibitors or angiotensin receptor blockers (ARBs) on the cardiovascular and renal outcomes in people with CKD.

**Methods:** We systematically searched MEDLINE Embase and Cochrane library for trials published between 1970 and November 2012. We included prospective randomised controlled trials assessing the effects of ACE inhibitors or ARBs on renal or cardiovascular outcomes in people with CKD. Summary estimates of relative risk(RR) used a random effects model.

**Results:** Overall 109 trials were included, providing data for 50,740 patients with CKD. As compared to placebo, ACE inhibitors reduced kidney failure (defined as doubling serum creatinine or end-stage kidney disease) by 38% (RR 0.62, 95% CI 0.53-0.72) and ARBs by 11% (0.89, 0.82-0.97). There's significant difference on the kidney protection between ACE inhibitors and ARBs (p<0.001). Compared to other blood pressure lowering agents, both ACE inhibitors (0.78, 0.67-0.91) and ARBs (0.77, 0.61-0.97) produced more kidney protection without difference (p =0.88). ACE inhibitors and ARBs reduced the risk of major cardiovascular events in patients with CKD as compared to either placebo (RR 0.86, 95% CI 0.80-0.93) or antihypertensive agents (0.88, 0.79-0.97) without heterogeneity (p=0.22). ACE inhibitors also reduced cardiovascular death (0.87, 0.77-0.99) or all death (0.89, 0.79-0.99) while ARB didn't. Adverse events were significantly increased by ACE inhibitors or ARBs, including hyperkalemia and cough compared to placebo. ARBs therapy was associated with greater risk of hyperkalemia than ACE inhibitors (p for heterogeneity =0.004).

**Conclusions:** ACE inhibitors or ARBs reduce the risk of kidney failure or cardiovascular events in the patients with CKD as compared to placebo or other blood pressure agents. ACE inhibitors showed more kidney protection ascompared to ARBs and also reduced the death thus could be of the first choice in this population.

## TH-PO216

**Effects of Spironolactone in Combination with ACE Inhibitor or Angiotensin Receptor Blockers in Aldosterone Escape and Non-Escape Group of Patients with Proteinuria** Ha Yeon Kim, Yong Un Kang, Chang Seong Kim, Joon Seok Choi, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Dept of Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea.*

**Background:** The present study was aimed to investigate whether add-on aldosterone blockade therapy has beneficial antiproteinuric effects in aldosterone escape and non-escape group, and to compare changes in proteinuria in patients according to estimated GFR (eGFR) in association with changes of vitamin D level.

**Methods:** In this prospective intervention trial, we studied the effects of spironolactone (25mg/day) in 219 patients who showed persistent proteinuria after ACE inhibitor or ARB medications for more than 3 months. Plasma aldosterone, potassium, eGFR and urine protein to creatinine ratio (UPCR) were determined at baseline and 6 months after spironolactone add-on treatment. Patients were divided according to their aldoterone level during ACE inhibitor or ARB treatment in an escape group (plasma aldosterone > 80 pg/ml, n=69, 31.5%) and a non-escape group (n=150, 68.5%).

**Results:** There was not a decrease in UPCR in both groups (p=0.763 in aldosterone escape group, p= 0.528 in non-escape group). UPCR was significantly decreased with treatment of spironolactone in patients with eGFR ≥ 60mL/min/1.73 $m^2$ (1.36±1.269 to 1.19±1.333 g/g Cr, p=0.042), not in eGFR < 60mL/min/1.73 $m^2$ (1.54±1.250 to 1.59±1.581 g/g Cr, p=0.712). Hyperkalemia (K ≥5.5mEq/L) was developed in 30 of the 103 patients with eGFR < 60 mL/min/1.73 $m^2$ (29.8 %) and in 8 of the 120 patients with eGFR ≥ 60 mL/min/1.73 $m^2$ (7.1 %). In all patients, 25-OH vitamin D was increased after adding spironolactone (p=0.004 in aldosterone escape group, p <0.001 in non-escape group, p=0.003 in patients with eGFR ≥ 60mL/min/1.73 $m^2$, p <0.001 in patients with eGFR < 60 mL/min/1.73 $m^2$). UPCR was correlated with 25-OH Vitamin D negatively (R=-0.222 P = 0.008).

**Conclusions:** Change of proteinuria was not different between aldosteron escape and non-escape group after add-on spironolactone treatment. Proteinuria was significantly decreased by add-on spironolactone treatment in patients with eGFR ≥ 60mL/min/1.73 $m^2$but not in patients with eGFR < 60mL/min/1.73 $m^2$.

## TH-PO217

**Renal Protctive Effects by Rosvastatin through the Amelioration of Intra-Renal Vascular Resistance** Keiko Fujimura, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Dept of Internal Medicine, School of Medicine, Keio Univ, Tokyo, Japan.*

**Background:** Nephrosclerosis has been one of the common causes of renal insufficiency. One of the major clinical characteristics of nephrosclerosis is the increased intra-renal vessel resistance and the decreased renal blood flow. The effects of these renal anatomical changes were not clinically fully evaluated. A recent trial has shown that statin have renal protective effects in patients with CKD. By using Ultrasound pulse-doppler analysis (Doppler), we aimed to assess the effects of rosuvastatin treatment on the progression of CKD with nephrosclerosis.

**Methods:** We used our CKD cohort composed of the patients with nephrosclerosis with CKD whose eGFR levels were less than 60 ml/min/1.73$m^2$. The patients with diabetes and biopsy-proven nephritis were excluded. The relationship between various parameters of Doppler and biochemical parameters were examined. Among these subjects, we recruited 33 patients, treated them with rosuvastatin (Ros) at the dose of 2.5-5mg until the LDL-cholesterol (LDL-C) levels reached under 100mg/dl and observed for a year. We compared the changes of various parameters including those of Doppler before and after Ros treatment.

**Results:** In regression analysis, resistance index (RI) and maximum velocity of renal blood flow(Vmax) were corrlated with eGFR.Ros significantly lowered LDL-C (before vs. after; -5.5±4.5% vs. -17.7±6.7%,p<0.05). Although Ros did not supressed the increase in proteinurea, albuminuria,and the excreation of renal tubular marker, it blunted the decline of eGFR (before vs. after; -4.2±2.0% vs. 4.2±2.4%,p<0.05).In Doppler analysis, Ros significantly supressed the increase of RI (before vs. after;4.2±3.7% vs. -4.6±2.1%,p<0.05), although it had no effects on the increasing rate in Vmax andVmin, that are supposed to reflect the stenotic changes of main renal artery.The changes in RI and eGFR by Ros were not associated with those in LDL-C levels.

**Conclusions:** Ros had renal protective effects with the improvement of renal vessel resistance and that effects were independent of LDL-C lowering effects.

## TH-PO218

**Metformin Is Safe and Effective in Stage 3 and 4 Diabetic Chronic Kidney Disease Patients – A Randomized Trial** Sanjib Kumar Sharma, Denis Piyyush, Anup Ghimire, Bhawesh Koirala, Madhab Lamsal. *B P Koirala Institute of Health Sciences, Dharan, Nepal.*

**Background:** In absence of evidence against use of metformin in CKD 3-4, we conducted clinical trial to study the use of metformin in diabetic CKD in stage 3 and 4. The aim of the study was to evaluate the efficacy of metformin in terms of glycemic control, incidence of lactic acidosis, episodes of hypoglycemia in patient with diabetic stage 3 and 4 CKD.

**Methods:** In a randomized open labeled clinical trial, a total of 218 patients were randomized by computer-generated table of random number in two groups - Insulin group (109) and Metformin group (109). Baseline characteristics of the patients in both groups were similar. During initial wash out period all the existing anti-diabetic medication were stopped. After the wash out phase, the patients were allocated to one of the two groups as per their randomization number. The OHA and Insulin were initiated and increased stepwise as per protocol. Patients were followed at 1 week, 2 weeks, 1 month and then monthly for six month. Blood analysis for acidosis and bicarbonate, serum creatinine, electrolyte, glucose profile, was measured in each visit. HbA1c was measured at 3 and 6 month. When targeted blood sugar was not achieved in metformin group sulfonylureas was added.

**Results:** Four patients from Metformin group and 5 patients from Insulin did not complete the study. Two patients on metformin were switched over to insulin due to intolerance to metformin.

The glycosylated hemoglobin decreased by 1.2% and 1.4% percent at 6 month in metformin and Insulin group respectively. There was no case of lactic acidosis in either group. One patient in metformin group showed increase serum creatinine that return to baseline on switching over to Insulin. There was no case of sevre hypoglycemia in either group, though minor episodes hypoglycemia therapy was recorded in 3 and 7 patients in Metformin and Insulin group respectively.

**Conclusions:** Metformin is a safe and effective oral hypoglycemic agent in diabetic stage 3 and 4 CKD. Its use in this group of patients is not associated with lactic acidosis.

## TH-PO219

**Renoprotective Effect of Combining Pentoxiphylline with Angiotensin Converting Enzyme Inhibitor or Angiotensin II Receptor Blocker in Moderate to Severe Kidney Disease** Ping-Min Chen,[1] Ping Yu Chen,[1] Tai-Shuan Lai,[2] Wen-Chih Chiang,[1] Shuei-Liong Lin.[1] *[1]Dept of Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ Hospital, Bei-Hu Branch, Taipei, Taiwan.*

**Background:** Several studies showed that pentoxifylline (PTX), a phosphodiesterase inhibitor, had beneficial effect on proteinuria and chronic kidney disease (CKD). This study was to examine whether there was an add-on effect of combining PTX with angiotensin converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB).

**Methods:** A single-center retrospective study was conducted, enrolling total of 697 patients with CKD stage of 3B-5 (age 18-80). All patients were documented long-term use of ACEI or ARB. Patients were stratified into combination treatment group (combined PTX with ether ACEI or ARB) and single medication group (ACEI or ARB use only). The primary outcome was long-term renal replacement therapy (RRT). Survival analysis was compared between the two groups. Multivariate cox regression analysis was used to evaluate the effect of individual risk factor. Subgroup analysis was also performed by dividing the patients into lower and higher proteinuria groups (baseline urine protein/creatinine ratio < 1 g/g or ≥ 1 g/g).

**Results:** Addition of PTX with either ACEI or ARB showed a better renal outcome (p = 0.044). There were no between group differences regarding all-cause mortality or cardiovascular events. Subgroup analysis revealed that the protective effect of add-on PTX was demonstrated in the higher proteinuria group (p = 0.009), but not in the lower proteinuria group. Multivariate cox regression model confirmed combination therapy had less chance of RRT again (HR 0.705, 95% CI 0.505 – 0.985, $P$ = 0.040), especially in higher proteinuria group (HR 0.606, 95% CI 0.420 – 0.873, $P$ = 0.007).

**Conclusions:** Combination treatment of PTX with ACEI or ARB showed a better renal outcome than single use of ACEI or ARB, especially in higher proteinuria patients. Large randomized control trials are needed to provide more evidence of the add-on effect of PTX in CKD patients.

**TH-PO220**

**NT-proBNP, Troponin T, and Risk of Incident Heart Failure in Chronic Kidney Disease: The CRIC Study** Nisha Bansal,[1] Amanda Hyre Anderson,[2] Wei Yang,[2] Myles S. Wolf,[3] John W. Kusek,[3] Christopher deFilippi,[3] Alan S. Go,[3] Robert Christenson,[3] Rajat Deo,[2] James P. Lash,[3] Dominic S. Raj,[3] Sylvia E. Rosas,[2] Michael Shlipak,[1] Harold I. Feldman.[2] *[1]UCSF; [2]UPenn; [3]CRIC Study.*

**Background:** Heart failure (HF) is a major complication of chronic kidney disease (CKD). NT-proBNP and high sensitivity troponin T are associated with HF in the general population, but these associations remain unclear in the setting of CKD.

**Methods:** We studied 3,483 participants of the Chronic Renal Insufficiency Cohort (CRIC) Study (mean age: 58 yrs; 48% diabetic; 42% black; mean eGFR: 45 mL/min) who did not report HF at baseline. Incident HF hospitalizations were adjudicated using standard criteria. Associations of each biomarker with incident HF were assessed using Cox models after multivariable adjustment, and then additionally adjusted for both biomarkers.

**Results:** Median (IQR) NT-proBNP and troponin T was 135.3 (58.6-335.6) and 11.19 (5.4-21.8) pg/mL, respectively. Over a median of 4.7 years, 284 incident HF events occurred. Those in the highest (versus lowest) quintile of NT-proBNP had an 11-fold higher adjusted rate of incident HF (Table). Compared to those with undetectable troponin T, those in the highest quartile of detectable troponin T had a 4-fold higher adjusted rate of incident HF. Results did not change after adjustment for the alternative biomarker.

| | | HR* (95% CI) | P-value |
|---|---|---|---|
| NTproBNP (ng/mL) | Ref: ≤47.6 | | |
| | >47.6-95.8 | 3.3 (1.4-8.1) | <0.001 |
| | >95.8-188.8 | 3.9 (1.6-9.5) | |
| | >188.8-433 | 6.3 (2.6-15.0) | |
| | >433 | 11.4 (4.7-27.5) | |
| Troponin T (ng/mL) | Ref: undetectable | | |
| | >3-7.8 | 1.4 (0.6-3.4) | <0.001 |
| | >7.8-13.6 | 2.3 (1.0-5.3) | |
| | >13.6-24.4 | 2.6 (1.2-6.0) | |
| | >24.4 | 4.1 (1.8-9.4) | |

* Adjusted for demographics, clinical center, diabetes, Hx of CVD, smoking, alcohol, log(24-hr urine protein), eGFR, SBP, BMI, LDL, hemoglobin, medications, serum phosphorus, log(PTH), and log(FGF23)

**Conclusions:** Troponin T and NT-proBNP, possibly reflecting subclinical myocardial stress and dysfunction, were significantly and independently associated with incident HF among a diverse cohort of CKD patients.

*Funding:* NIDDK Support

**TH-PO221**

**Risk of Nephropathy Associated with Choice of Add-On Therapy for Diabetes Treatment after Metformin** Adriana Hung,[1,2] Christianne Roumie,[1,2] Robert Greevy,[1,2] Xulei Liu,[1,2] Carlos Grijalva,[1,2] Harvey J. Murff,[1,2] T. Alp Ikizler,[1,2] Marie Griffin.[1,2] *[1]Veterans Affairs, Nashville, TN; [2]Vanderbilt Univ, Nashville, TN.*

**Background:** Glycemic control reduces the risk of chronic kidney disease (CKD). The addition of a second agent is often needed when diabetes is sub-optimally controlled with metformin monotherapy. We compared the effectiveness of adding either insulin or sulfonylurea to metformin in preventing kidney function decline or death.

**Methods:** We assembled a national retrospective cohort of veterans who initiated diabetes treatment with metformin monotherapy between October 2001 and September 2008 and added either insulin or a sulfonylurea after at least 3 months of monotherapy through September 2010.The primary renal composite outcome was a GFR event, defined by both a decline in GFR of 25% and reaching a GFR<60 ml/min or dialysis. The secondary outcome was a composite of a GFR event, dialysis or death. Marginal structural Cox proportional hazard models compared the hazard of the outcomes between exposure groups, after adjusting for baseline and time-varying covariates. Sensitivity analyses and subgroup analysis by HbA1c at the time of add-on therapy were also performed.

**Results:** There were 29505 patients (15%) that added a second drug to metformin during the specified time frame and were included in this analysis; 1,310 (4%) insulin and 28195 (96%) sulfonylurea. Patients who added insulin were younger (59 vs. 61 years), more often African Americans (21% vs. 12%) and had higher HbA1c median (8.5% vs. 7.7%). There was no difference in the rates of the primary composite outcome of eGFR events or dialysis between those who added insulin versus sulfonylureas, (31 versus 23 per 1000 person-years, respectively ([aHR] 0.91, 95% Confidence Intervals [CI] 0.62, 1.31)). The rates for the secondary outcome of eGFR event, dialysis or death were 58 and 37 per 1000 person-years, respectively (aHR 1.11, 95% CI 0.85, 1.45). The risk for the primary outcome did not differ by HbA1c subgroups at the time of add-on therapy.

**Conclusions:** The risk of renal events or death was similar among patients who added insulin to metformin monotherapy and patients who added a sulfonylurea.

*Funding:* Other NIH Support - AHRQ

**TH-PO222**

**Comparative Effectiveness of Insulin versus Combination Insulin and Sulfonylurea in Preventing Renal Outcomes** Adriana Hung,[1,2] Christianne Roumie,[1,2] Robert Greevy,[1,2] Xulei Liu,[1,2] Carlos Grijalva,[1,2] Harvey J. Murff,[1,2] Marie Griffin,[1,2] T. Alp Ikizler.[1,2] *[1]Dept of Veterans Affairs, Nashville, TN; [2]Vanderbilt Univ, Nashville, TN.*

**Background:** Evidence based recommendations for the choice of diabetic regimen for prevention of renal outcomes among those with low GFR are lacking. We compared renal outcomes among diabetes patients who intensified their sulfonylurea monotherapy regimen by adding insulin versus those who switched to insulin monotherapy.

**Methods:** This retrospective cohort included veterans who initiated treatment with a sulfonylurea between 2001 and 2008 and then added or switched to insulin therapy through 12/2010. The primary outcome was a renal composite of a sustained decline of GFR of 35% or ESRD; the secondary outcome was a composite of an GFR event, ESRD or death. Follow-up started at the time of addition or switching to insulin regimen and continued until an outcome, loss to follow-up, death or end of the study. The risk for the outcomes was compared between regimens using marginal structural Cox models. Time-varying covariates were: HbA1c, blood pressure, BMI, co-morbidities and medications. Stratified analyses included subgroups: by GFR ( ≥ or <60 ml/min) and by HbA1c (<7, 7-9 and >9%).

**Results:** Among sulfonylurea monotherapy initiators, we identified 1857 patients that added insulin to sulfonylurea and 1581 that switched to insulin monotherapy. Patients' characteristics were similar between groups, median age was 64 years and 98% were men. There were 4.9 versus 5.2 renal composite events per 100 person-years among sulfonylurea + insulin versus insulin monotherapy users respectively ( [*aRH*] 0.91 95% CI 0.73, 1.13). For the secondary outcome of an eGFR event, ESRD or death, the respective rates were 11.1 versus 13.1 renal composite events per 100 person-years ( [*aRH*] 1.07 95% CI 0.93, 1.24). Results were similar by eGFR and HbA1c subgroups, and in analyses that required persistence on the intensifying regimen.

**Conclusions:** There were no differences in the risk of a sustained decline in eGFR, ESRD or death between patients who intensified their sulfonylurea regimens by adding insulin versus those that switched to insulin monotherapy.

*Funding:* Other NIH Support - AHRQ

**TH-PO223**

**Low Dose Aspirin for Prevention of Cardiovascular Events in Patients with Chronic Kidney Disease: A Propensity-Matched Study** Ji Yong Jung,[1] Han Ro,[1] Chungsik Lee,[2] Sun Moon Kim,[3] Ae Jin Kim,[1] Hyung Soo Kim,[1] Jae Hyun Chang,[1] Hyun Hee Lee,[1] Wookyung Chung.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Gachon Univ of Gil Medical Center, Incheon, Korea; [2]Div of Nephrology, Dept of Internal Medicine, Cheju Halla General Hospital, Jeju, Korea; [3]Div of Nephrology, Dept of Internal Medicine, Chungbuk National Univ Hospital, Cheongju, Korea.*

**Background:** Chronic kidney disease (CKD) is a powerful risk factor for development of cardiovascular disease (CVD). Previous trials have investigated the effect of low dose aspirin on prevention of CVD in patients with diabetes but not in CKD patients. In addition, the role of aspirin in diabetics is controversial and the available literature is contradictory. Therefore, we studied whether low dose aspirin would be beneficial in patients with chronic kidney disease (CKD) as high risk group for CVD.

**Methods:** Using propensity score matching, 191 low dose aspirin (100mg/day) recipients and 382 non-recipients were 1:2 paired for analysis from 2,624 patients with CKD. The primary endpoint was atherosclerotic CVD including coronary arterial disease, stroke, and peripheral arterial disease. Secondary endpoints included death from any cause, bleeding events, and time to serum creatinine doubling and renal death.

**Results:** Low dose aspirin was prescribed for 8.9% of patients with CKD. The incidence of primary endpoint of any atherosclerotic CVD was not significantly different in the aspirin users than in the non-aspirin users after PS matching (hazard ratio = 1.025; 95% confidence interval = 0.642 – 1.637; P = 0.917). Secondary endpoints including all-cause mortality, time to serum creatinine doubling and renal death, hemorrhagic stroke and significant gastrointestinal bleeding events were not significantly different between the aspirin and non-aspirin users.

**Conclusions:** These results suggest that use of low dose aspirin did not reduce the risk of atherosclerotic CVD in CKD patients. Further randomized clinical trials are warranted to confirm the effect of low dose aspirin therapy on the development of CVD in these patients.

**TH-PO224**

**Warfarin Treatment Associates with Better Outcomes in Myocardial Infarction Patients with Atrial Fibrillation Regardless of the Severity of Concurrent Renal Disease** Juan Jesus Carrero,[1] Marie Evans,[1] Karolina Szummer,[3] Jonas Spaak,[2] Robert Edfors,[3] Lars Lindhagen,[3] Stefan H. Jacobson,[2] Peter Stenvinkel,[1] Tomas Jernberg.[3] *[1]Div of Renal Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Clinical Sciences, Danderyd Univ Hospital, Stockholm, Sweden; [3]Div of Cardiology, Karolinska Institutet, Stockholm, Sweden.*

**Background:** Recent cohort studies show that warfarin treatment in patients with advanced chronic kidney disease (CKD) and atrial fibrillation (AFib) is associated with increased mortality and increased incidence of ischemic stroke. We assessed outcomes associated to warfarin treatment in patients with established cardiovascular disease and AFib in relation to kidney function.

**Methods:** Prospective cohort study from the 2003-2010 SWEDEHEART registry including consecutive survivors of a MI with AFib and known serum creatinine (n=24317). Patients were classified into CKD stages based on their eGFR. Primary outcome was a composite of death, readmission due to MI, ischemic stroke and major bleeding within 1 year from discharge. Secondary outcome was the composite of death, MI and ischemic stroke. Multivariate adjustment included age, sex, comorbidities, presentation at admission, hospital course and discharge medication.

**Results:** A total of 5292 (21.7%) patients were treated with warfarin at discharge, and as many as 53.5% of included patients had manifest CKD (eGFR<60 ml/min/1.73 $m^2$). As compared with those who did not receive warfarin treatment, warfarin was associated with a lower risk of the primary outcome in each CKD strata: eGFR>60, Hazard ratio (HR) 0.77, 95% confidence interval (CI) 0.69-0.85; eGFR 60-30, 0.75 (0.68-0.82); eGFR 29-15, 0.83 (0.69-0.99); and eGFR<15 ml/min/1.73 $m^2$, 0.58 (0.39-0.58). The same was observed for the secondary outcome. Major bleeding events were neither increased nor reached statistical significance in warfarin-treated versus non-treated patients across dysfunctional CKD stages.

**Conclusions:** Warfarin treatment was associated with a decreased 1-year risk of death, readmission for MI, stroke and bleeding in consecutive MI patients with AFib. This association was not influenced by the severity of concurrent renal disease.

*Funding:* Government Support - Non-U.S.

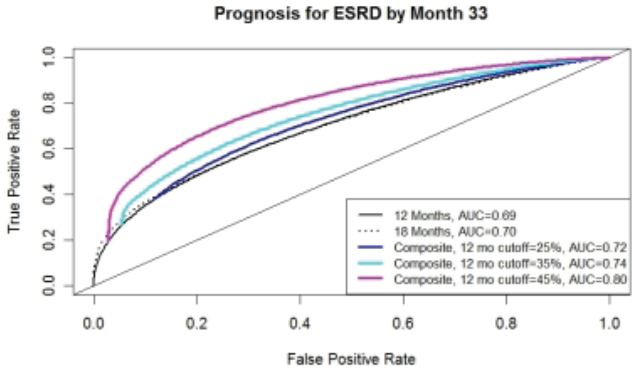
## TH-PO225

**Diabetic Kidney Disease: Results from the Irbesartan Diabetic Nephropathy Trial (IDNT)** Jamie P. Dwyer,[1] Julia Lewis,[1] Tom Greene,[2] Nan Hu,[2] Edmund J. Lewis,[3] The Collaborative Study Group.[3] *[1]Vanderbilt Univ; [2]Univ of Utah; [3]Rush Univ.*

**Background:** The composite endpoint of time to of SCR (dSCr) or ESRD is accepted by the FDA and traditionally used to determine treatment effects on progression of chronic kidney disease (CKD) in contemporary clinical trials. However, the follow-up time required for these events can be substantial.

**Methods:** We assessed the accuracy of endpoints defined by smaller confirmed increases in SCR at either 12 months or 18 months for prediction of subsequent occurrences of ESRD using analyses of time dependent receiver operating characteristic (ROC) curves in the Irbesartan Diabetic Nephropathy Trial (IDNT) data base.

**Results:** Shown are time-dependent ROC curves with associated areas under the curve (AUC) for alternative early SCR endpoints relative to occurrence of ESRD by 33 months. We considered the following early SCR endpoints: a) Confirmed increases in SCR at 12 months; b) Confirmed increases in SCR at 18 months, and c) Three additional composite endpoints defined by confirmed increases in SCR by varying levels at 18 months OR a confirmed increase in SCR at 12 months by either 25%, 35% or 45%.




Sensitivities at thresholds defining 90% specificity were 34.9% for a 27.7% confirmed increase in SCR at 12 months, 36.4% for a confirmed increase of 36.3% at 18 months, and 51.1% for a composite defined by a 45% confirmed increase at 12 months OR a 29.3% confirmed increase at 18 months.

**Conclusions:** Endpoints defined by confirmed increases in SCR during the first 12 to 18 months of follow-up are predictive of occurrence of ESRD. Composite endpoints incorporating designated increases at either 12 or 18 months may further improve prognostic accuracy compared to SCR endpoints defined at a single follow-up time.

*Funding:* Pharmaceutical Company Support - Nephrogenix, Private Foundation Support

## TH-PO226

**Urinary Albumin:Creatinine and Protein:Creatinine Are Prognostically Equivalent for CKD Progression: Results from CANPREDDICT** Claudio Rigatto,[1] Adeera Levin,[2] Catherine M. Clase,[3] Brendan J. Barrett,[5] Francois Madore,[4] Norman Muirhead,[6] Navdeep Tangri,[1] Mila Tang,[7] Ognjenka Djurdjev.[8] *[1]Univ of Manitoba; [2]Univ of British Columbia; [3]McMaster Univ; [4]Univ de Montréal; [5]Memorial Univ of Newfoundland; [6]London Health Science Center; [7]St. Paul's Hospital; [8]British Columbia Renal Provincial Agency.*

**Background:** Proteinuria is an important prognostic variable in patients with chronic kidney disease (CKD). Both albumin-to-creatinine ratio (ACR) and protein-to-creatinine ratio (PCR) are widely used to estimate proteinuria. No study has directly compared the ability of these measures to predict progression to end-stage renal disease (ESRD).

**Methods:** We examined the ability of baseline measures of ACR and PCR to predict progression to ESRD over one year in 2544 patients participating in CanPREDDICT, a prospective cohort study of adult patients with advanced CKD from multiple causes. ACR and PCR were log transformed for analysis. We created a base Cox multivariable model (BCM) for prediction of RRT using standard clinical variables, and then compared differences in the performance (discrimination [c statistic, IDI] and reclassification [NRI]) of enriched Cox models (ECM) created by addition of either logACR or logPCR.

**Results:** Both ACR and PCR independently predicted need for RRT at one year, and each remained highly significant after adjustment for the BCM variables. Both log ACR and log PCR improved BCM performance significantly and to the same degree.

**Table 1:** Multivariate performance PCR vs. ACR in predicting kidney failure in patients with stage 3-4 CKD

| Variable | BCM + logPCR | BCM + logACR | P value (ACR vs PCR) |
|---|---|---|---|
| C-statistic | 0.87 [0.83,0.89] | 0.87 [0.83,0.89] | NS |
| Δ C-statistic (vs. BCM alone) | 0.02 [0.01,0.04] | 0.03 [0.01, 0.04] | NS |
| NRI | 0.57 [0.38, 0.72] | 0.59 [0.36, 0.69] | NS |
| IDI | 0.04 [0.02, 0.06] | 0.04 [0.02,0.06] | NS |

Sensitivity analyses using alternate choices for BCM variables did not change these results.

**Conclusions:** ACR and PCR are equally and interchangeably valid predictors of progression to RRT at 1 year. Clinicians can choose whichever test is most appropriate for the specific renal diagnosis, without loss of general prognostic information applicable to CKD.

## TH-PO227

**Microalbuminuria and CKD Modify the Association of Arterial Stiffness with Death** Javier A. Neyra,[1] Ian McCoy,[1] Nishank Jain,[1] Robert D. Toto,[1] Susan Hedayati.[1,2] *[1]Univ of Texas Southwestern, Dallas; [2]Veterans Affairs North Texas.*

**Background:** Pulse pressure (PP), a marker of arterial stiffness, is associated with mortality and is increased in advanced CKD. Few studies investigated the effect modification of CKD on this association of PP with death. We hypothesized that the independent association between higher PP and death would be greater among patients with CKD and microalbuminuria compared to those without.

**Methods:** Logistic regression was used to explore the association of PP with mortality among 2,859 participants of the probability-sampled, population-based, multi-ethnic Dallas Heart Study followed for a median of 8 years. Odds ratios were expressed for each 5-unit increase in PP. Models were adjusted for age, race, gender, eGFR and urinary albumin-to-creatinine ratio (ACR). Interactions between PP X eGFR and PP X ACR were tested. Analyses were stratified by CKD presence, defined as an eGFR of <60 mL/min/1.73 $m^2$ or presence of microalbuminuria ( ≥ 17 mg/g creatinine in men and ≥25 in women).

**Results:** Mean (SD) age was 45 (10) years, eGFR 100 (24), and ACR 19 (138). 50% were African American, 31% Caucasian, 17% Hispanic, and 2% of other races. Eight percent (242/2,859) had CKD. Those with CKD had a higher PP at 55.0 mmHg, 95% CI (53.0, 57.0) vs. those without, 48.0 (47.6, 48.4) mmHg, p<.0001. There were 155 total deaths with a higher number in the CKD group (48 deaths or 20% vs. 107 deaths or 4% in non-CKD), p<.0001. Every 5 unit increase in PP was associated with a 23% increase in death, OR 1.23, 95% CI (1.18, 1.32), which remained significant after adjustment, OR 1.08 (1.01, 1.16). There was a significant interaction between PP and both eGFR and ACR, p = 0.02 and 0.04. In stratified adjusted models, PP was associated with death in CKD, OR 1.11 (1.003, 1.23) but not in non-CKD participants, OR 1.04 (0.96, 1.13). This difference was highly significant, p<.0001.

**Conclusions:** Among a multi-ethnic community-dwelling sample, arterial stiffness, estimated by PP, was independently associated with death more so in CKD compared to non-CKD participants. This data suggests that arterial stiffness as estimated by increased PP may be a way to risk-stratify patients with early CKD.

*Funding:* NIDDK Support, Other NIH Support - USPHS GCRC grant #M01-RR00633 from NIH/NCRR-CR. Supported in part by grant UL1TR000451 from the National Center for Advancing Translational Sciences, National Institutes of Health., Veterans Affairs Support, Private Foundation Support

**TH-PO228**

**Chronic Kidney Injury and Hypertension Associate with Increased Urinary Succinate Levels** Peter M.T. Deen,[1] Joris Hubertus Robben,[1] Juliette Hadchouel,[5] Gerjan Navis,[3] Ewout J. Hoorn,[4] Jack F. Wetzels.[2] *[1]Physiology, Radboud Univ Nijmegen Med. Centre, Nijmegen, Netherlands; [2]Nephrology, Radboud Univ Nijmegen Med. Centre, Nijmegen, Netherlands; [3]Nephrology, Univ Med. Centre Groningen, Groningen, Netherlands; [4]Nephrology, Erasmus Univ Rotterdam, Rotterdam, Netherlands; [5]INSERM U970, Paris Cardiovascular Research Center, Paris, France.*

**Background:** The renal succinate receptor SUCNR1 senses the krebs cycle intermediate succinate, which is secreted from cells in response to (pseudo)oxidative stress. As it recently emerged as an activator of RAAS and pro-fibrotic events in DM1 and hypertension, we measured urinary succinate excretion in renal diseases to identify disorders in which SUCNR1 may play a role.

**Methods:** Urinary succinate was measured with an optimized low-volume enzymatic assay. Measured succinate concentrations were corrected for urinary creatinine levels. P-values <0.05 were considered significant.

**Results:** Urinary succinate levels were: 2.7 fold higher in CKD stage 2-4 patients than of healthy controls; 4.2 fold higher in hypertensive CKD patients versus controls; 1.4 fold higher in primary aldosteronism patients versus controls; 1.7 fold higher in a mouse model of Familial Hyperkalemic Hypertension (FHHt) with *WNK1* mutations vs. wild-type littermates. In hypertensive CKD patients treated with ACE inhibitors, urinary succinate decreased 3.1 fold following use of Angiotensin receptor blockers (ARBs) and 2.7 fold when subjected to a low-sodium diet. In contrast, subjecting healthy individuals to a high/low sodium diet, or 1-5 days aldosterone infusion in adrenalectomized rats did not change urinary succinate levels.

**Conclusions:** Renal succinate release is increased in all animal models and patients with affected renal function associated with chronic oxidative stress. With hypertensive CKD patients, urinary succinate levels correlated with the extent of sodium intake, hypertension and proteinuria. As urinary succinate activates SUCNR1, these data indicate to a role for SUCNR1 in activation of the intrarenal RAAS system and/or renal fibrosis as are commonly observed in these chronic renal disorders.

*Funding:* Government Support - Non-U.S.

**TH-PO229**

**Microalbuminuria and Risk of Cancer** Ali Abbasi,[1,2] Ron T. Gansevoort,[2] Rudolf A. De Boer,[3] Eva Corpeleijn,[1] Rijk O.B. Gans,[2] Hans L. Hillege,[1] Pim Van der Harst,[3] Ronald P. Stolk,[1] Gerjan Navis,[2] Stephan J.L. Bakker.[2] *[1]Epidemiology, Univ Medical Center Groningen, Univ of Groningen, Groningen, Netherlands; [2]Internal Medicine, Univ Medical Center Groningen, Univ of Groningen, Groningen, Netherlands; [3]Cardiology, Univ Medical Center Groningen, Univ of Groningen, Groningen, Netherlands.*

**Background:** Information on the potential association of microalbuminuria (MA) with cancer risk is sparse. We aimed to investigate whether MA is associated with overall and specific cancer risk in the general population.

**Methods:** We acquired data of 40,584 participants (aged 28-75 years) from the PREVEND study, an observational cohort study (Groningen, the Netherlands), that started in 1996-97. All incident cancers requiring hospitalisation were ascertained via the Dutch national hospital registry up to 2009.

**Results:** During a median follow-up of 12.7 years, 4156 (10.2%) subjects developed cancer. The most common cancer sites were respiratory (4.0%), oropharynx (2.4%), genitourinary (2.3%) and digestive system (2.2%). In an age-and sex-adjusted Cox regression model, the HR (95%CI) for all cancer was 1.26 (1.14-1.40) in subjects with MA (urine albumin concentration (UAC)>20mg/L) as compared to normoalbuminuria (UAC<10mg/L) (P=0.001). In a multivariable model adjusted for age, sex, smoking and diabetes, the significant association of MA remained, corresponding with a 23% increased risk of cancer. HRs for MA versus normoalbuminuria for specific cancers were for respiratory 0.93 (0.78-1.12), oropharynx 1.29 (1.04-1.61), genitourinary 1.66 (1.36-2.02) digestive system 1.18 (0.94-1.49), breast 1.17 (0.88 -1.56) and prostate 1.29 (0.93-1.80). In a subcohort of 8,592 subjects, who were screened in detail at baseline, the association of MA (>30mg/24hr) with cancer was corroborated adjusted for the aforementioned variables, even when additionally adjusted for history of cancer, hsCRP, leukocyturia and erythrocyturia (HR 1.29 (1.05-1.53)). No significant independent association was found with eGFR (or impaired eGFR).

**Conclusions:** MA is independently associated with an increased risk of incident cancer in the general population, particularly genitourinary cancers.

**TH-PO230**

**Overall Prevalence of Proteinuria Is Low but the Relative Prevalence of Non-Albumin Proteinuria Is High in a Community Cohort of Patients with Chronic Kidney Disease: Results from the Triple A Kidney Project** Shona Methven,[1] Alan G. Jardine,[2] Mark S. MacGregor.[3] *[1]School of Clinical Sciences, Univ of Bristol, United Kingdom; [2]Institute of Cardiovascular and Medical Sciences, Univ of Glasgow, United Kingdom; [3]Renal Unit, Univ Hospital, Crosshouse, Kilmarnock, United Kingdom.*

**Background:** Measurement of proteinuria with albumin: creatinine ratio (ACR) alone does not take account of non-albumin proteinuria (NAP). We describe the prevalence of NAP in a community cohort of patients with chronic kidney disease (CKD).

**Methods:** Participants with a diagnosis of CKD Stage 3 were recruited from 7 primary care facilities across Ayrshire, Scotland in the Triple A Kidney Project. Detailed baseline clinical and laboratory assessment was undertaken, including measurement of early morning ACR and protein:creatinine ratio (PCR) in all participants. Albumin:protein ratio (APR) was calculated and compared with that of a nephrology clinic population (n=1874).

**Results:** Four hundred and eleven participants were recruited, mean age 70.6±9.6 years, 59% female, 99.5% white, 20% diabetic and median eGFR 54 (IQR 44 – 61)mL/min/1.73m$^2$. Only 2.7% of the cohort had documented glomerular disease and 69% were taking renin-angiotensin blocking drugs. Twenty six per cent (n=107) had detectable proteinuria measured by PCR ( ≥15mg/mmol, [ ≥133mg/g equivalent]), 17% by ACR ( ≥3mg/mmol, [ ≥2.65mg/g equivalent]) and 14% had elevated PCR and ACR. Of those with PCR 15-50mg/mmol (133-442mg/g) (n=91), only 50% had detectable albuminuria and 70% had an APR<0.4 (ie predominantly NAP). Of those with PCR>50mg/mmol (>442mg/g), 44% had APR<0.4. In the nephrology clinic population, 55% of those with PCR 15-50mg/mmol had an APR <0.4 and 23% of those with PCR>50mg/mmol had an APR <0.4.

**Conclusions:** Overall prevalence of proteinuria was low in this community cohort but the relative prevalence of NAP was high, and notably higher than a nephrology clinic population, especially in those with <0.5g/day equivalent total proteinuria. This would not have been detected using ACR alone. The presence of albuminuria and NAP may reflect different underlying disease processes and the outcomes of this discordant group will be assessed.

*Funding:* Pharmaceutical Company Support - unrestricted educational grant from Bristol-Myers Squibb

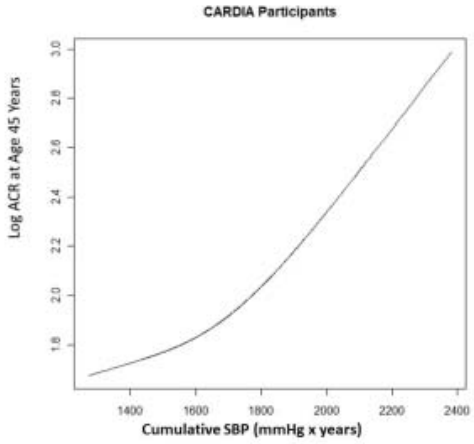
**TH-PO231**

**Urine Albumin/Creatinine Ratio (ACR) and Cumulative Systolic Blood Pressure (CumSBP) in the Coronary Artery Disease in Young Adults (CARDIA) Study** Holly J. Kramer,[1] David R. Jacobs,[2] Cora E. Lewis,[5] Kirsten Bibbins-Domingo,[3] Alex Chang,[4] Carmen A. Peralta,[3] Paul Muntner,[5] David Siscovick,[6] Mark J. Pletcher,[3] Kiang Liu.[7] *[1]Loyola Univ Chicago; [2]Univ of Minnesota; [3]Univ of California San Francisco; [4]Johns Hopkins Univ; [5]Univ of Alabama; [6]Univ of Washington; [7]Northwestern Feinberg School of Medicine.*

**Background:** We hypothesized that an association exists between CumSBP exposure from age 30 through 45 years and ACR at age 45 among black and white adults without diabetes at age 30 (n=1895).

**Methods:** SBP values were averaged between consective visits (years 0, 2 5, 7, 10, 15, 20 and 25) and then multiplied by number of years between visits and summed to determine CumSBP (mmHg x years) during ages 30-45 years. Log ACR at age 45 vs. cumulative SBP was plotted using an additive model after adjustment for race, sex, smoking, waist circumference, education, incident diabetes and blood pressure medication use. Multinomial logistic regression models were used to examine the association between cumSBP exposure and presence of high normal ACR (sex and race adjusted ACR 13-24.9 mg/g) at age 45 or albuminuria (sex and race adjusted ACR => 25 mg/g) at age 45 vs. normal ACR (sex and race adjusted ACR < 13 mg/g) at age 45 after adjustment for covariates.

**Results:** After adjustment for covariates including use of anti-hypertensive medications during age 30-45 years, each 75 mmHg x year increase (5 mmHg per year from age 30-45 years) in CumSBP was associated with a 11% (95% CI 1.02, 1.21) higher odds of high normal ACR at age 45 and a 24% higher odds of albuminuria at age 45 vs. normal ACR at age 45 (95% CI 1.13, 1.36). Figure:




log ACR at age 45 vs. CumSBP after adjustment for covariates.

**Conclusions:** Cumulative SBP during young adulthood is associated with increased ACR at age 45 years.

*Funding:* Other NIH Support - NHLBI

## TH-PO232

**Obesity, Chronic Kidney Disease (CKD) and End-Stage Renal Disease (ESRD) among Individuals in the REGARDS Study** Holly J. Kramer,[1] Suzanne E. Judd,[2] David A. Shoham,[1] Paul Muntner,[2] David G. Warnock,[2] Orlando M. Gutierrez,[2] Rikki M. Tanner,[2] William M. McClellan.[3] *[1]Loyola Univ Chicago; [2]Univ of Alabama; [3]Emory Univ.*

**Background:** The association between obesity and chronic kidney disease (CKD) is poorly understood. This study examines the independent associations between BMI and waist circumference (WC) with prevalent albuminuria, reduced estimated glomerular filtration rate (eGFR) and incident end stage renal disease (ESRD).

**Methods:** Participants in the REasons for Geographic and Racial Differences in Stroke (REGARDS) study (n=27, 085) with BMI, WC, spot urine albumin-to-creatinine ratio (ACR), and estimated glomerular filtration rate (eGFR) measured at baseline were linked to the United States Renal Data System to identify incident cases of ESRD.

**Results:** Prevalence of albuminuria (ACR ≥30 mg/g) and CKD (eGFR < 60 ml/min/1.73m$^2$), increased across World Health Organization defined obesity categories for BMI and gender-specific WC. After multivariable adjustment there was a significant trend toward lower prevalence for albuminuria and CKD in the BMI groups ≥ 25 kg/m$^2$ vs. BMI < 25 kg/m$^2$ but higher prevalence in the WC groups ≥ 80 and ≥ 94 cm compared to WC referent group (< 80 and < 94 cm in women and men, respectively). A total of 160 individuals developed ESRD (0.6%); increased BMI was associated with a reduced risk of incident ESRD after adjustment for WC and all covariates while higher WC was associated with heightened risk of ESRD. Individuals in the highest WC category ( ≥ 108 and ≥ 122 cm in women and men respectively) had a 5.05-fold (95% CI 2.23, 11.2 ) higher hazard ratio for ESRD compared to WC referent group after adjustment for BMI but this association was no longer significant after adjustment for all covariates (HR 2.03; 95% CI 0.90, 4.55).

**Conclusions:** Higher WC is associated with an increased risk of ESRD after adjustment for BMI while higher BMI is associated with decreased ESRD risk after adjustment for WC. The association between higher WC and ESRD risk support the possibility that increased abdominal adiposity may influence kidney disease progression.

*Funding:* Other NIH Support - U01 NS041588 from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Department of Health and Human Services, Pharmaceutical Company Support - Additional funding was provided by an investigator-initiated grant-in-aid from Amgen Corporation

## TH-PO233

**A Cross Sectional Study on the Relationship of Retinal Vascular Diameter, Hypertension and Albuminuia** Wen Huang,[1] Ning Ding,[1] Liping Jiang,[1] Guojuan Zhang,[1] Ningli Wang,[2] Yuan Bo Liang.[2] *[1]Nephrology, TongRen Hospital, Beijing, China; [2]Eye Center, TongRen Hospital, Beijing, China.*

**Background:** Microvascular abnormalities are common findings in patients with chronic kidney disease.Recent studies have reported an association between retinal vessel diameter and chronic kidney disease.But there are still some controversies and researches based on Chinese population are scarce.Hence we did a cross-sectional study to test the hypothesis that the retinal vessel diameter may correlated with renal microvascular abnormalities.

**Methods:** This was a population-based cross-sectional study based on the data of Handan eye study(HES).HES recruited 7577 participants,of whom 5541 had complete information on retinal vessel diameter,albuminuria and blood pressure.Retinal arteriolar and venular diameters were measured and summarized as central retinal arterial equivalent(CRAE) and central venular equivalent(CRVE).The presence of microalbuminuria was defined as urinary albumin:creatinine ratios ≥17 mg/g for men and ≥25 mg/g for women. Hypertension was defined as either systolic blood pressure ≥140mmHg or diastolic blood pressure ≥90mmHg or use of antihypertensive medication.

**Results:** Among the 5541 patients,male45.9%,age(51.83±11.63),hypertension (48.2%),DM(6.1%).After adjustment for age,gender and other confounders,the narrowest retinal arterial diameter correlated with an increased risk of albuminuria (multivariable OR: 1.24, 95%CI 1.02-1.51) compared with the widest. Retinal venular diameter had no correlation with albuminuria.Hypertension correlated with an increased risk of albuminuria (multivariable OR: 1.41, 95%CI 1.21-1.64) compared with absence of hypertension. Exposure to both hypertension and retinal arterial narrowing obviously increased the risk of albuminuria (multivariable OR: 1.69, 95% CI 1.31-2.19).

**Conclusions:** We concluded that retinal arterial diameter was independently correlated with albuminuria,while no such association was found in retinal venular diameter.In hypertensive patients,retinal arterial narrowing was significantly correlated with albuminmuia.

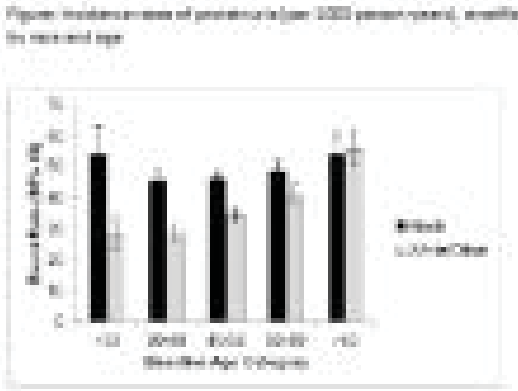
## TH-PO234

**Risk Factors for Proteinuria in a National Cohort of HIV-Infected Veterans** Tanushree Banerjee,[1] Rebecca Scherzer,[1] Neil R. Powe,[1] Diane Steffick,[2] Vahakn B. Shahinian,[2] Meda E. Pavkov,[3] Sharon Saydah,[3] Michael Shlipak.[1] *[1]UCSF; [2]UM; [3]CDC.*

**Background:** As patients with HIV disease live longer, kidney disease has become more common, manifested by proteinuria and rapid progression to ESRD. Few studies have evaluated risk factors associated with proteinuria in HIV-infected individuals. We examined non-HIV and HIV-related factors associated with incident proteinuria in a national registry of HIV-infected veterans.

**Methods:** HIV-infected veterans without pre-existing kidney disease receiving health care in the Veterans' Health Administration medical system between 1996 and 2011 were included. Race (black versus non-black) was self-reported. Proteinuria was defined by consecutive dipstick measures of 1+ or higher. We computed incidence rates stratified by age and race. A Fine-Gray competing risk model (accounting for death) adjusted for HTN, DM, CVD, CD4 lymphocyte count, viral load (>100k copies/mL), hepatitis C virus (HCV) co-infection, and eGFR was used to estimate independent associations with incident proteinuria.

**Results:** During follow-up (median=5.2 years; range 0.1-14.1 years), of the 21,346 participants, 7,362 developed proteinuria. Compared with non-blacks, blacks had 1.48-fold higher risk of proteinuria (95% CI 1.41-1.55), but the association varied by age.



Both HIV and non-HIV related factors were associated with new proteinuria: elevated viral load (HR=1.14, 95% CI 1.05-1.25), CD4 lymphocyte count ≤200 cells/mm$^3$ (HR=1.12, 95% CI 1.06-1.19), HCV infection (HR=1.21, 95% CI 1.15-1.27]); HTN (HR=1.26, 95% CI 1.19-1.33), and DM (HR=1.64, 95% CI 1.53-1.75) were also independently associated with incident proteinuria.

**Conclusions:** Risk of proteinuria was overall higher in blacks compared with non-blacks and the association of age with proteinuria differed by race. Several HIV and kidney related risk factors were associated with higher proteinuria risk.

*Funding:* Other U.S. Government Support

## TH-PO235

**High Dietary Acid Load Is Associated with ESRD in a Longitudinal Cohort of U.S. CKD Adults** Tanushree Banerjee,[1] Deidra C. Crews,[2] Donald E. Wesson,[3] Anca Tilea,[4] Rajiv Saran,[4] Desmond Williams,[5] Nilka Rios Burrows,[5] Neil R. Powe.[1] *[1]UCSF; [2]JHU; [3]Texas A&M College of Medicine; [4]UM; [5]CDC.*

**Background:** Previous research in adults with CKD has shown an improvement in kidney injury and slowed GFR decline due to the reduction in dietary acid. We sought to examine the association between high dietary acid load, quantified by dietary net acid excretion (NAE), and progression to ESRD, in a nationally representative sample of US adults.

**Methods:** Among 1,486 CKD (15<eGFR<60) adults aged ≥20 years who participated in the National Health and Nutrition Examination Survey III between 1988 till 1994, NAE was determined by the dietary recall questionnaire (24 hrs) using a model by Remer and Manz. The development of ESRD was ascertained over an average of 13.2 years of follow-up via linkage with the Medicare ESRD Registry. We used the Fine-Gray competing risks model (accounting for competing risk of mortality) to estimate the hazard ratio [HR] for ESRD associated with high versus low dietary acid load after adjusting for demographics, socio-economic position, diabetes and hypertension, eGFR and urinary albumin:creatinine, and daily caloric intake.

**Results:** The median value of NAE was 47.24 mEq/d. Persons with higher NAE were younger (p<0.0001). Persons with higher dietary acid load were more likely to be males (58.9%), non-Hispanic black (31.6%), have diabetes (36.9%), or lower eGFR (30.2%) compared to their counterparts (all p<0.05). Poverty, level of education, and hypertension, did not differ across tertiles of NAE. Adults in the highest and middle versus lowest tertile of dietary acid were more likely to develop ESRD (HR [95% CI]: 6.16 [6.12-6.20] and 3.48 [3.46–3.50], respectively] after adjustment even when the competing risk of death before developing ESRD was accounted for. On studying the association of early ESRD events (within 6 years) with dietary acid, the HRs [95% CI] of the highest and middle tertile were 9.93 [9.35-10.54] and 3.79 [3.66-3.92] respectively, compared with the lowest tertile.

**Conclusions:** Dietary acid load in persons with CKD is independently and strongly associated with increased risk of ESRD over time in a graded fashion.

*Funding:* Other U.S. Government Support

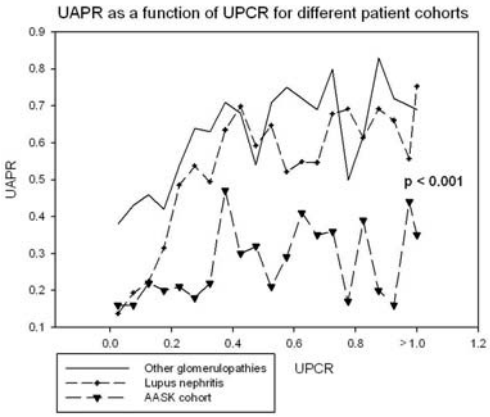
**TH-PO236**

**The Nephropathy of the Patients in the African American Study of Kidney Disease and Hypertension Manifests Low Albumin/Protein Ratios Typical of a Tubulo-Interstitial Nephropathy: Clinical and Pathophysiologic Implications** Udayan Y. Bhatt,[1] Robert D. Toto,[2] Shona Methven,[3] Stephan J.L. Bakker,[4] Ron T. Gansevoort,[4] Mahboob Rahman,[5] Daniel J. Birmingham,[1] Cynthia A. Kendrick,[6] Jennifer J. Gassman,[6] Christopher J. Deighan,[7] Tom Greene,[8] Brad H. Rovin,[1] Lee A. Hebert.[1] *[1]Internal Medicine, Ohio State Univ Wexner Med Ctr, Columbus, OH; [2]Univ of Texas Southwestern; [3]Univ of Bristol, United Kingdom; [4]Univ Medical Center Groningen, Netherlands; [5]Case Western Univ; [6]Cleveland Clinic; [7]Western Infirmary Glasgow, United Kingdom; [8]Univ of Utah.*

**Background:** The pathogenesis of the nephropathy (N) studied in African American Study of Kidney Disease and Hypertension (AASK) is unclear. AASK-N is believed to be from hypertension, but in the AASK trial better BP control did not slow GFR decline. Also, biopsy in AASK-N shows pervasive global glomerulosclerosis, suggesting a glomerulopathy, but AASK-N progresses at proteinuria levels far below that of traditional glomerulopathies. Another paradox is that AASK-N albumin/protein ratios (APR) are low, typical of tubulo-interstitial (T-I)-N not glomerulopathy. The present work addresses this paradox.

**Methods:** We analyzed APR and PCR (protein/creatinine ratio) from published works (# of pts): AASK cohort (691), Glasgow glomerular disease and T-I-N (5,586), Groningen Univ transplant-N (606), Ohio SLE Study lupus N (127).

**Results:**




At any given PCR, APR of AASK-N is below that of glomerulopathies ($p < 0.001$ by ANOVA repeated measures) but not T-I-N or transplant-N (data not shown). Neither preservation artifact or African ancestry explain these differences (data not shown).

**Conclusions:** AASK-N manifests as a tubulopathy. So, in AASK-N non-albumin proteinuria may predict progression better than albuminuria.

*Funding:* NIDDK Support

**TH-PO237**

**The Effect of Normalizing Spot Albumin to Creatinine Ratio Normalized for Creatinine Excretion Rate on Accuracy of Albuminuria Estimation: The Prevention of REnal and Vascular ENd-Stage Disease (PREVEND) Study** Joseph A. Abdelmalek,[1] Ron T. Gansevoort,[2] Hiddo Jan Lambers Heerspink,[2] Joachim H. Ix,[1,3] Dena E. Rifkin.[1,3] *[1]Div of Nephrology, UCSD, San Diego, CA; [2]Univ Medical Centre Groningen, Groningen, Netherlands; [3]Dept of Med, VA San Diego, San Diego, CA.*

**Background:** The urine albumin-to-creatinine ratio (ACR) is recommended instead of 24-hour urine collections for estimation of albuminuria. In those with extremes of muscle mass, however, variations in the spot urine creatinine value may lead to false under- or over-estimation of albuminuria. Correcting this value for estimated 24-hour creatinine excretion rate (CER), as a proxy of muscle mass, may improve the accuracy of the ACR for estimating albuminuria.

**Methods:** We evaluated 2711 community-living individuals with spot ACR and two 24 hour urine collections for albuminuria. We created a normalized ACR (ACR*eCER) by multiplying the ACR and estimated 24-hour CER using a previously validated equation. The average of the two 24-hour urine albumin excretion (UAE) measurements served as the gold standard for comparison. We compared the accuracy of the ACR and ACR*eCER by comparing the percentage of participants falling within 20% (1-P20) and 30% (1-P30) of the gold standard among all participants, and subgroups stratified by weight, age, and gender. We hypothesized that the biggest improvements in accuracy might be in those at extremes of body weight.

**Results:** The mean age was 49 years, 46% were male, mean eGFR was 84 ± 15 ml/min/1.73m$^2$ and the median 24 hour UAE was 7.2 mg/day (interquartile range 5.4-11). In the full cohort, ACR*eCER improved accuracy (1-P20: 40.3 vs 59.5, 1-P30: 62 vs 76.5, $p<0.001$). In subgroup analysis, the greatest improvement was noted among subgroups with higher rates of creatinine excretion. Improvement was seen in the middle and highest weight tertiles, in all tertiles of age (particularly the lowest and middle tertiles), and in both genders (particularly males).

**Conclusions:** In a large community-dwelling cohort, adjusting ACR for estimated CER modestly improved the accuracy of the ACR for estimation of 24 hour UAE, particularly among individuals with higher rates of creatinine excretion.

*Funding:* NIDDK Support

**TH-PO238**

**Exercise Ameliorates Incidence of Proteinuria in a Large Japanese General Population Sample** Yasuyuki Nagasawa,[1,2] Ryohei Yamamoto,[2] Maki Shinzawa,[2] Yukiko Hasuike,[1] Takahiro Kuragano,[1] Hiromi Rakugi,[2] Yoshitaka Isaka,[2] Takeshi Nakanishi,[1] Kunitoshi Iseki,[3] Kunihiro Yamagata,[3] Kazuhiko Tsuruya,[3] Hideaki Yoshida,[3] Shouichi Fujimoto,[3] Koichi Asahi,[3] Tsuyoshi Watanabe,[3] Toshiki Moriyama.[2,3] *[1]Dept of Internal Medicine, Div of Kidney and Dialysis, Hyogo Medical College, Nishinomiya, Hyogo, Japan; [2]Div of Nephrology and Geriatric Medicine, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [3]Steering Committee for the "Research on the Positioning of Chronic Kidney Disease in Specific Health Check and Guidance in Japan", Tokyo, Japan.*

**Background:** Exercise habitant is well known to have favorable effect upon metabolic syndrome. And metabolic syndrome might cause proteinuria and CKD. But it remains unknown that exercise habitant have favorable effect upon proteinuria. Aim of this study is to reveal the effect of exercise habitant upon proteinuria.

**Methods:** This study is cross-sectional cohort study. Subjects were 290213 persons who received the Specific Health Check and Guidance in Japan in Okinawa, Ibaraki, Miyagi, Niigata, Tokyo, Osaka, Fukushima, Fukuoka prefectures, without missing-data.The exercise score (ES) was defined as total of three exercise habitats; more than once a week half hour or over exercise with slight sweat, more than one hour daily walk or physical activity, faster walking than average of same generation. Outcome was defined as proteinuria. Explanatory variables included sex, age, BMI, mean BP, HbA1c, TG, HDL-C, UA, eGFR, smoking, previous cardiac or stroke or kidney diseases.

**Results:** In whole subjects, ES significantly ameliorated the incidence of proteinuria (ES1 0.9 [ 0.86 - 0.94 ],P<0.001; ES2 0.85 [ 0.81 - 0.9 ] ,P<0.001; ES3 0.77 [ 0.73 - 0.81 ] HR0.9, P<0.001 ). There was significant interaction between ES and BMI in male. After the male subjects were divided into quintile as following BMI, in more than third groups (22.9<BMI<24.1), there was no significant ES effect upon incidence of proteinuria. In female, ES improves incidence of proteinuria, regardless of BMI. Reporting bias might cause this phenomenon in male.

**Conclusions:** Exercise habitant might ameliorate the incidence of proteinuria.

*Funding:* Government Support - Non-U.S.

**TH-PO239**

**Hyperuricemia Is Associated with Cardiovascular Mortality in Chronic Kidney Disease** Diana Chiu, James Ritchie, Darren Green, Smeeta Sinha, Philip A. Kalra. *Salford Vascular Research Group, Univ of Manchester, MAHSC, Manchester, United Kingdom.*

**Background:** Hyperuricemia is a common finding in patients with Chronic Kidney Disease (CKD). There are few long-term outcomes studies examining the association of hyperuricemia with cardiovascular deaths in CKD.

**Methods:** Baseline bloods were analyzed from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS), a prospective observational study of outcome in patients with all-cause CKD stages 3-5, managed in secondary care. Hyperuricemia was defined as serum uric acid >0.43mmol/l. Predictors of cardiovascular deaths, data from Office for National Statistics, were selected using forward stepwise cox regression (included variables with p<0.05 on univariate analysis). As Allopurinol affects uric acid levels, only patients not on this medication were included in this particular analysis.

**Results:** From 883 patients, with available baseline uric acid results and not taking allopurinol, mean eGFR was 35±16.2 ml/min/1.73m$^2$, 542 (61.4%) were males, 293 (33.2%) were diabetics, 171 (19.4%) had history of ischemic heart disease (IHD) and 596 (67.5%) were current/ex-smokers. Median follow-up was 41 months (interquartile range 25-57months), and there were 225 (25.5%) deaths with 60 (6.8%) cardiovascular deaths. In the final cox regression model hyperuricemia was an independent predictor of cardiovascular death.

| Cox Regression Model | | | |
|---|---|---|---|
| Variable | Hazard Ratio | 95% CI | P |
| Hyperuricemia | 2.22 | 1.10-4.47 | 0.03 |
| Age | 1.05 | 1.02-1.08 | <0.01 |
| Diabetes Mellitus | 2.40 | 1.37-4.19 | <0.01 |
| History of IHD | 2.76 | 1.59-4.79 | <0.01 |
| Albumin | 0.90 | 0.84-0.97 | <0.01 |
| eGFR | 0.96 | 0.93-0.98 | <0.01 |
| Predictors included: Age, gender, smoker, diabetes mellitus, history of ischemic heart disease, diuretic use, ACE inhibitor use, albumin, hemoglobin, C-reactive protein, eGFR | | | |

There was no association between hyperuricemia with all-cause mortality in a cox regression model with the same predictors (p=0.325).

**Conclusions:** Hyperuricemia is an independent predictor of cardiovascular deaths in this non-dialysis CKD cohort. Further studies are required to explore causality in this relationship.

**TH-PO240**

**The Association between Serum Uric Acid and Change of Renal Function in a Community-Based Population: A Longitudinal Survey of a Nationwide Cohort in Japan** Keita Kamei,[1] Kazuko Suzuki,[1] Kazunobu Ichikawa,[1] Tsuneo Konta,[1] Shouichi Fujimoto,[2] Kunitoshi Iseki,[2] Toshiki Moriyama,[2] Kunihiro Yamagata,[2] Kazuhiko Tsuruya,[2] Kenjiro Kimura,[2] Ichiei Narita,[2] Koichi Asahi,[2] Tsuyoshi Watanabe.[2] *[1]Dept of Cardiology, Pulmonology and Nephrology, Yamagata Univ School of Medicine, Yamagata, Japan; [2]Steering Committee of Research on Design of the Comprehensive Health Care System for Chronic Kidney Disease (CKD) Based on the Individual Risk Assessment by Specific Health Checkup.*

**Background:** Hyperuricemia is a risk for adverse renal outcomes in chronic kidney disease. This study investigated the effect of uric acid on renal function in a large community-based population.

**Methods:** We used a nationwide database of 110,616 subjects (aged 29-74, male 39%), participated in an annual health check, "The Specific Health Check and Guidance in Japan" between 2008-2010, and examined the relationship between serum uric acid level at baseline and 2-year change of estimated GFR obtained by the Japanese equation.

**Results:** The analysis of variance (ANOVA) showed the decline of eGFR was faster in subjects with low uric acid. However, after the adjustment with gender, age, baseline eGFR, and other possible confounders, the eGFR change was inversely correlated with uric acid and the mean value of the 2-year change of eGFR (mL/min/1.73m$^2$) was +0.43, +0.24, +0.09, -0.20 and -0.72 in subjects with baseline uric acid <4.0, 4.0-4.9, 5.0-5.9, 6.0-6.9 and >7.0 mg/dL, respectively (P <0.001). Multiple linear regression analysis showed that baseline uric acid was independently associated with eGFR change and the regression coefficient of per 1 mg/dL increase of uric acid was -0.24 (95% confidence interval [-0.30, -0.18], P <0.001) in total population. In sensitivity analysis, the effect of serum uric acid on eGFR change was significant especially in elderly, female, and subjects without obesity, hypertension, diabetes, and alcohol consumption.

**Conclusions:** This study showed that an increase of serum uric acid is independently associated with faster renal decline in the Japanese general population, especially elderly, female and subjects without comorbidities.

*Funding:* Government Support - Non-U.S.

**TH-PO241**

**Association of Serum Uric Acid Genetic Risk Score with Chronic Kidney Disease in Mexican American, American Indian and Alaska Native Populations** V. Saroja Voruganti,[1] Sandra L. Laston,[1] Karin Haack,[1] Shelley A. Cole,[1] Jack W. Kent,[1] Sven O.E. Ebbesson,[2] Jean W. Maccluer,[1] John Blangero,[1] P. Zager,[3] Jason G. Umans,[4] Anthony Comuzzie.[1] *[1]Genetics, Texas Biomedical Research Institute, San Antonio, TX; [2]Norton Sound Health Corporation, Nome, AK; [3]Univ of New Mexico, Albuquerque, NM; [4]Medstar Health Research Institute, Hyattsville, MD.*

**Background:** Serum uric acid (SUA) has been associated with prevalent and incident CKD. We previously reported that SUA independently predicted incident CKD stage 3 in American Indians of the Strong Heart Family Study (SHFS). Additionally, we have identified genetic linkages and associations of polymorphisms (SNPs) in renal urate transporters in SHFS, Mexican Americans of the San Antonio Family Heart (SAFHS), Zuni Indians of the Genetics of Kidney Disease in Zuni Indians (GKDZI) and Western Alaska Natives from Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) studies.

**Methods:** We applied the principle of Mendelian randomization to elucidate causality between SUA and CKD using a genetic risk score by summing six risk alleles of *SLC2A9* SNPs (rs10805346, rs16890979, rs6832439, rs737267, rs13131257, and rs6449213), each weighted by its association with SUA.

**Results:** The risk score was associated with eGFR ($p < 4 \times 10^{-3}$) in SHFS and with urinary albumin/creatinine ratio (UACR) ($p < 7 \times 10^{-4}$) in SAFHS. These associations were attenuated by adjustment for SUA to $p > 0.54$ and $p > 0.35$, respectively. The risk score was not associated with CKD in the GKDZI or GOCADAN populations.

**Conclusions:** Genetic approaches in two distinct populations support epidemiologic evidence for a causal role of SUA in CKD.

*Funding:* NIDDK Support, Other NIH Support - PO1 HL045522 -NHLBI; MH059490 - NIMH; C06 RR013556 and C06 RR017515 - Research Facilities Improvement Program; S10RR029392 -NCRR; HL65520, HL41642, HL41652, HL41654 and HL65521, and TR000101 -NHLBI; U01 HL64244 -NHLBI; HL082458 - Norton Sound Health Corporation grant; R01DK092238 - NIDDK; DK066660-03 and DK57300-05 -NIDDK; P30 ES-012072 - NIEHS

**TH-PO242**

**Genetic Variants of SLC22A12 Are Associated in a Population-Specific Manner with Serum Uric Acid and CKD Phenotypes** V. Saroja Voruganti,[1] Benjamin Umans,[1] Karin Haack,[1] Sandra L. Laston,[1] Sven O.E. Ebbesson,[2] Jean W. Maccluer,[1] Jason G. Umans,[3] Anthony Comuzzie,[1] Shelley A. Cole.[1] *[1]Texas Biomedical Research Institute, San Antonio, TX; [2]Norton Sound Health Corporation, Nome, AK; [3]Medstar Health Research Institute, Hyattsville, MD.*

**Background:** Renal transporters play a key role in the regulation of serum uric acid (SUA) levels. The solute carrier family 22, member 12 (SLC22A12) gene encodes a urate/anion exchanger localized primarily to the apical membrane of renal proximal tubular epithelial cells. In a previous genome-wide linkage scan of SUA in American Indian participants of the Strong Heart Family Study, we identified a locus on chromosome 11 whose confidence interval contains the candidate gene SLC22A12.

**Methods:** We conducted a follow-up fine mapping by genotyping 20 single nucleotide polymorphisms (SNPs) in SLC22A12 to identify potential functional variants that might affect SUA. We used measured genotype analysis, implemented in SOLAR, to investigate the association between SLC22A12 and SUA and CKD phenotypes.

**Results:** We found significant association of rs17146104 (p = 0.0006) and nominal association with rs12221796 (p = 0.005) with lower SUA levels (minor allele frequency (MAF)= 1- 41%). As a replication step we genotyped these same SNPs in Western Alaska Natives from the Genetics of Coronary Artery Disease in Alaska Natives study. While these two SNPs were not associated with SUA; seven other SNPs (rs2360872, rs538737, rs552307, rs11602903, rs12802649, rs10897518 and rs12786214) from SLC22A12 (MAF =1- 42%) were significantly ($p < 0.004$) associated with lower SUA, possibly reflecting differences in linkage disequilibrium patterns and/or functional alleles between these populations. Interestingly, these seven SNPs were associated with increased estimated glomerular filtration rate ($p < 0.002$) in American Indians but not in Western Alaska Natives. In contrast, albumin-creatinine ratio was not associated with this gene in either population.

**Conclusions:** Our results highlight the value of studies in unique non-European populations to define physiologically-relevant variants contributing to renal phenotypes and outcomes.

*Funding:* Other NIH Support - C06 RR013556 and C06 RR017515- Research Facilities Improvment Program; MH59490-NIMH; U01 HL082490- NHLBI; U01-HL65520, U01-HL41642, U01-HL41652, U01-HL41654 and U01-HL65521 -NHLBI

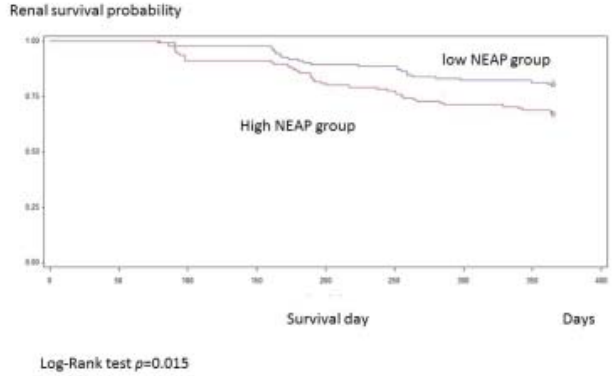
**TH-PO243**

**Dietary Acid Load Is Associated with Chronic Kidney Disease Progression in Elderly Patients** Eiichiro Kanda,[1] Masumi Ai,[2] Renjiro Kuriyama,[3] Masayuki Yoshida,[2] Tatsuo Shiigai.[4] *[1]Tokyo Kyosai Hospital; [2]Tokyo Medical and Dental Univ; [3]Kokubunji Minamiguchi Clinic; [4]Shiigai Clinic.*

**Background:** Nonvolatile acid is produced from the metabolism of organic sulfur in dietary protein. The acid load loeads to increased acid excretion, and promotes kidney damage. However, the evidence for this is not sufficient. In this retrospective cohort study, we investigated the role of dietary acid load in chronic kidney disease (CKD) progression.

**Methods:** 249 CKD patients (male 76.3%) were enrolled in this retrospective cohort study in Japan. The primary endpoint was renal death;a 25% decline in estimated glomerular filtration rate (eGFR) or starting dialysis. Their net endogenous acid production (NEAP) was measured as an alternative marker of dietary acid load using protein intake and potassium excretion per day calculated from 24 hour urine samples. We used time-dependent Cox proportional hazard models adjusted for demographics to examine the association between NEAP level and the outcome.

**Results:** Average age (SD) was 70.6 (7.0) years; eGFR, 22.2 (14.1) ml/min/1.73 m$^2$; NEAP level, 81.4 (41.5) mEq/day. Patients with NEAP levels in the upper half of the range [109.7 (41.1) mEq/day] showed a high risk of CKD progression compared with patients with NEAP levels in the lower half of the range [52.9(12.0) mEq/day]: hazard ratio (HR), 2.013 (95% CI, 1.214, 3.338); adjusted HR, 1.928 (95% CI, 1.143, 3.251).




A longitudinal relationship between the increase in NEAP level and GFR decline was shown by adjusted generalized estimating equation [beta=-0.1076 (0.0080), p=0.029].

**Conclusions:** In elderly CKD patients, our findings suggest that a high NEAP level is independently associated with CKD progression and that an increase in NEAP level is associated with eGFR decline. Monitoring and control of dietary acid load are necessary for the prevention of CKD progression.

**TH-PO244**

**Higher Intake of Vegetable Protein Is Associated with Lower All-Cause Mortality in Chronic Kidney Disease** Xiaorui Chen, G. Wei, R. Filipowicz, Tom Greene, A. N. Habib, T. S. Bjordahl, Srinivasan Beddhu. *Dept of Med, U of Utah, SLC, UT.*

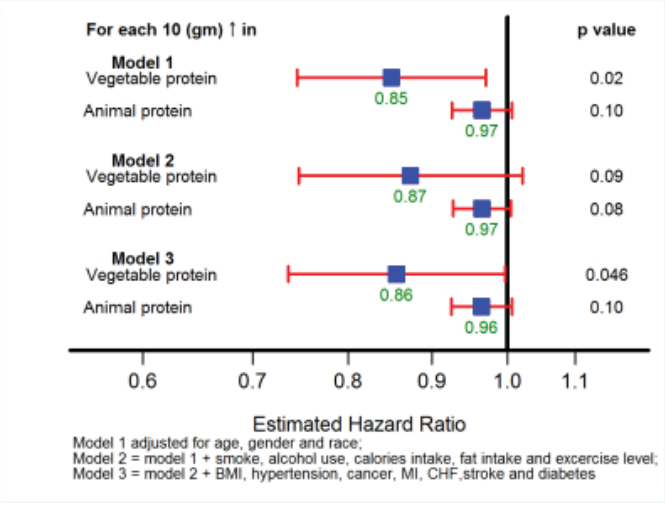
**Background:** Compared to animal protein (AP), vegetable protein (VP) intake is associated with lower production of uremic toxins and lower serum phosphorus levels. It is unclear whether VP has a mortality benefit in CKD.

**Methods:** The study population consisted of 1,104 National Health and Nutrition Examination Survey (NHANES) III participants aged 20 yrs or older with eGFR<60 ml/min/1.73 $m^2$ and non-missing data for VP, AP and mortality. VP and AP intake was estimated from 24h dietary recalls. Mortality was based upon the results from a probabilistic linkage with vital statistic records through 12/31/2006. Cox regression models were used to relate each 10g in VP or AP with all-cause mortality (ACM).

**Results:** The clinical characteristics of the study population by VP groups (defined by median VP intake) are summarized in Table1.

| | VP≤17.8 g/d | VP>17.8 g/d |
|---|---|---|
| VP(g/d) | 12.4 ± 4.2 | 27.3 ± 9.4 |
| Age(yr) | 69 ± 15 | 69 ± 13 |
| Male(%)* | 24 | 48 |
| AA Race(%)* | 10 | 4 |
| MI(%) | 14 | 16 |
| CHF(%) | 13 | 12 |
| Stroke(%) | 8 | 10 |
| Diabetes(%) | 21 | 21 |
| Cancer(%) | 11 | 11 |
| Smoking(%) | 14 | 12 |
| Alcohol use(%) | 25 | 33 |
| Calories intake* | 1245 ± 523 | 1983 ± 758 |
| Fat intake* | 47.0 ± 27.2 | 74.0 ± 40.6 |
| AP(g/d)* | 37.8 ± 26.2 | 48.3 ± 32.0 |
| eGFR(ml/min/1.73$m^2$) | 48.8 ± 10.5 | 49.7 ± 9.6 |
| BMI | 27.6 ± 6.0 | 27.7 ± 5.7 |
| Cancer(%) | 11 | 11 |
| * P value≤0.001 | | |

During the follow-up period, 591 deaths (43.0%) occurred. The association of VP and AP with ACM is displayed in Figure 1.




**Conclusions:** We conclude that VP intake might be associated with lower ACM in CKD population. Interventional trials are needed to establish whether increasing VP will decrease mortality in CKD population.

*Funding:* NIDDK Support

## TH-PO245

**Prevalence and Correlates of Sleep Disorders in Hispanics with Chronic Kidney Disease in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL)** Ana C. Ricardo, Michael F. Flessner, Gerardo N.M.N. Heiss, Leopoldo Raij, Alberto R. Ramos, Susan Redline, Sylvia E. Rosas, Phyllis C. Zee, Daniela Sotres-Alvarez, Martha L. Daviglus, James P. Lash. *On behalf of the HCHS/SOL Investigators.*

**Background:** Hispanics/Latinos are disproportionally affected by chronic kidney disease (CKD). In non-Hispanics, sleep disorders are more common in individuals with CKD than in those without CKD. However, few data exist on measures of sleep in Hispanics with CKD.

**Methods:** HCHS/SOL, a population based study, enrolled 16,415 adults in 4 US communities. We estimated prevalence and associations of sleep disorders with albuminuria (urine albumin-to-creatinine ratio ≥30mg/g) and low estimated glomerular filtration rate (CKD-EPI eGFR <60ml/min/1.73$m^2$) in 11,873 participants with complete data. Sleep apnea was defined as apnea-hypopnea index (AHI, 3% desaturation) ≥15. Sleep symptoms were based on questionnaires.

**Results:** Mean age (SD) was 41 (0.3) yrs and 52% were women. Prevalence of albuminuria and low eGFR were 8.7% (95% CI: 8.0,9.5), and 2.7 % (95% CI: 2.2,3.1), respectively. Prevalence of sleep disorders are shown in Table.

| Prevalence | Albuminuria, % (95% CI) | | p | Low eGFR, % (95% CI) | | p |
|---|---|---|---|---|---|---|
| | Yes | No | | Yes | No | |
| AHI ≥15 | 21.1 (17.5,24.8) | 8.7 (7.9,9.5) | <0.001 | 26.3 (19.3,33.2) | 9.3 (8.5,10.2) | <0.001 |
| ESS>11 | 12.2 (9.5,14.9) | 11.6 (10.7,12.5) | 0.682 | 13.3 (7.9,18.7) | 11.6 (10.8,12.5) | 0.529 |
| Insomnia | 9.9 (7.6,12.2) | 7.1 (6.4,7.9) | 0.006 | 16.1 (7.4,24.8) | 7.1 (6.4,7.8) | 0.004 |
| Restless legs | 1.8 (0.8,2.8) | 1.3 (0.9,1.7) | 0.219 | 8.0 (0.0,16.7) | 1.2 (0.9,1.5) | <0.001 |

In multivariable analyses, albuminuria was associated with a 47% increased odds of AHI ≥15 (odds ratio 1.5, 95% CI: 1.1,2.0), but not independently associated with Epworth Sleepiness Scale (ESS) >11. No association was seen between low eGFR and sleep disorders. In participants with albuminuria, risk factors significantly associated with AHI ≥15 included older age, male, and higher body mass index.

**Conclusions:** In a large cohort of Hispanic/Latinos, prevalence of sleep apnea and self-reported sleep disorders was higher in persons with CKD compared with those without CKD. In addition, albuminuria was independently associated with a higher risk of sleep apnea.

*Funding:* NIDDK Support, Other NIH Support - National Heart, Lung, and Blood Institute (NHLBI)

## TH-PO246

**The Sleep and Nephrology Outcomes Research (SNORE) Study: Design and Preliminary Findings** Muna T. Canales,[1,2] Nicole Kay,[1] Areef Ishani,[3] I. David Weiner,[1,2] Richard Berry,[1,2] Rebecca Beyth.[1,2] *[1]Malcom-Randall VAMC; [2]Univ of Florida, Gainesville, FL; [3]Minneapolis VAMC, Minneapolis, MN.*

**Background:** Sleep apnea (SA) is common and deleterious to the cardiovascular system. Through similar mechanisms SA may cause renal injury in patients with moderate to severe CKD. We report the design and preliminary findings of an ongoing 3-year prospective study of 250 veterans with CKD to: 1) determine the impact of SA on CKD progression, QOL and 2) validate and refine a SA risk assessment tool, the Berlin Questionnaire (BQ), for CKD.

**Methods:** We identified veterans age 18-99 in NF/SGVHS with ≥2 MDRD egfr 15-44, ≥3 months apart. We excluded those with dialysis or kidney transplant, PAP or $O_2$ therapy, inability to consent, active cancer, or solid organ transplant. Veterans were randomly invited in batches of 30-100 letter mailings every 1-2 weeks to participate with follow up phone call for non-response. At baseline, participants undergo full sleep study, KDQOL-SF, Epworth Sleepiness Scale (ESS) and BQ and renal function measurement. Renal function and KDQOL-SF will be assessed annually for 3y. Assuming prevalence of SA (apnea-hyponea index or AHI>5) 54%, ESRD rate 5%/y, 10% doubling SCr over 3y, 2y accrual period with 3y follow-up, 10% lost to follow-up, we can detect HR 1.5 for the composite outcome (doubling SCr or ESRD) with n=250 (80% power, $\alpha$=0.05).

**Results:** 34 baseline visits have occurred through 6/4/13. Mean age of enrollees is 76±9.5y; 100% male, 88% white, 35% DM, 85% HTN. Mean BMI 30±4; mean MDRD eGFR 33±7; median UACR 44 mg/gCr (IQR 12-190). Median ESS score 7.5 (IQR 4-10), 25% had ESS>10. 44% were high risk for SA by BQ score ≥2; preliminary sleep study scoring found 65% with AHI ≥5 and 30% with AHI ≥15. 38% reported excellent/very good health. From the KDQOL-SF, mean physical component summary score was 61±25 and mental component summary score was 74±23. Burden of kidney disease and sleep (pre-specified key kidney-specific QOL domains) were 82±22 & 67±17, respectively.

**Conclusions:** The SNORE Study will provide valuable insight into the impact of SA on CKD progression and QOL. Validation and refinement of a SA risk assessment tool could improve risk assessment in CKD.

*Funding:* Veterans Affairs Support

## TH-PO247

**Low Serum Magnesium Is Associated with the Development of End-Stage Renal Disease (ESRD) in the Atherosclerosis Risk in Communities (ARIC) Study** Adrienne Tin,[1] M. Grams,[1] Nisa M. Maruthur,[1] Brad C. Astor,[2] David J. Couper,[4] Tom Mosley,[3] Josef Coresh,[1] Wen Hong Linda Kao.[1] *[1]Johns Hopkins Univ; [2]Univ of Wisconsin; [3]Univ of Mississippi Medical Center; [4]Univ of North Carolina.*

**Background:** Magnesium (Mg) is important in many enzymatic reactions and the regulation of mitochondrial function and vascular tone. Low serum Mg levels have been associated with incident hypertension and diabetes; both are important risk factors of ESRD. Whether serum Mg might independently associate with incident ESRD is unknown.

**Methods:** ARIC participants (10986 EA, 3697 AA) with baseline eGFR >15 were followed from 1987-2010. We analyzed the association between ESRD and serum Mg levels by quartiles using Cox regression adjusted for self-reported race, age, gender, baseline eGFR, diabetes, systolic blood pressure, smoking status, prevalent coronary heart disease, HDL-C, triglycerides, hypertension medication, BMI, serum albumin, and LDL-C. We further tested for this association excluded diuretics users.

**Results:** AA had significantly lower Mg than EA (mean of 0.79 mmol/L in AA vs 0.83 mmol/L in EA, p=1.1x$10^{-140}$). We observed a total of 276 ESRD events (141 in AA and 135 in EA). Individuals in lower quartiles of Mg levels were more likely to develop ESRD compared with individuals in the higher quartiles (Table 1). The association of quartile 1 vs. quartile 4 remain significant after excluded diuretics users (event/n=183/12048). Table 1. Hazard ratio (HR) and 95% confidence interval (CI) for ESRD by quartiles of serum Mg.

| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | P Trend |
|---|---|---|---|---|---|
| Range (mmol/L) | 0.25-0.7 | 0.75 | 0.8 | 0.85-1.15 | |
| HR (CI) adjusted for self-reported race only | 4.4 (3.2, 6.1) | 1.8 (1.3, 2.6) | 1.3 (0.9, 1.8) | 1.0 | <0.0001 |
| HR (CI) adjusted for all covariates | 2.4 (1.7, 3.3) | 1.6 (1.1, 2.2) | 1.2 (0.9, 1.7) | 1.0 | <0.0001 |
| HR (CI) adjusted for all covariates, excluded diuretics users | 2.4 (1.5, 3.7) | 1.5 (1.0, 2.3) | 1.4 (0.9, 2.1) | 1.0 | 0.0002 |

**Conclusions:** In a large sample of middle-aged adults, low serum Mg conferred an increased risk of ESRD. This finding raises the possibility of whether alteration of Mg may be a novel therapeutic target in chronic kidney disease.

*Funding:* NIDDK Support

## TH-PO248

**Serum Phosphorus and Outcomes in an African-American CKD Population**
Shailesh Basani,[1] Praveen Kandula,[2] Ranjani N. Moorthi,[1] Deming Mi,[1] He Xia,[1] Marc Rosenman,[1] Sharon M. Moe,[1] Jonathan W. Bazeley.[1] *[1]Indiana Univ, Indpolis, IN; [2]Massachusetts General Hospital, Boston, MA.*

**Background:** Serum phosphorus ($PO_4$) levels, even within the normal range, have been positively associated with adverse outcomes in chronic kidney disease (CKD) populations. However, subjects in the previous studies were mainly white and racial differences have not been previously examined despite differences in vitamin D and PTH homeostasis in African-Americans (AA). We tested the hypothesis that race alters the relationship between $PO_4$ level and mortality and/or time to dialysis initiation.

**Methods:** A retrospective cohort of adult patients with CKD (eGFR < 60ml/min at baseline) visiting the nephrology clinic at a large urban county hospital from 2007-10 was studied. Excluding subjects with no $PO_4$ measurement at index date (first clinic visit in 2007-2010a total of 996 subjects were identified and were followed until death or May 2012. A Cox proportional hazard model adjusted for baseline demographics, comorbidities, medications, and laboratory values was used to study the association of $PO_4$ and race on the composite end point of time to death or dialysis initiation (days to event from index date).

**Results:** Of 996 patients, 53% were female, 60% were AA and 56% had diabetes mellitus. The mean age was 58.5 ± 13.8 years (mean ± SD), mean eGFR was 33.4 ± 13.3 ml/min/1.73m$^2$. The median $PO_4$ level was (3.8mg/dl; interquartile range 3.3-4.4). During a median follow up of 1125 days, 331 (33.2 %) participants either died or initiated on dialysis. In univariate analysis higher $PO_4$ was associated with increased mortality, time to dialysis or both in the whole cohort and in AA only. However, in the multivariable model $PO_4$ was not a significant factor in predicting time to death or dialysis initiation. The adjusted hazard ratio was 1.13 (CI 0.98, 1.29) for every 1mg/dl increase in serum phosphorus in the whole cohort and it was similar 1.07 ( CI 0.89, 1.3) in the AA cohort.

**Conclusions:** Phosphorus was not associated with mortality or dialysis initiation in an urban population of CKD patients that was predominantly AA, raising questions on the generalizability of previous research studies to the clinic setting.

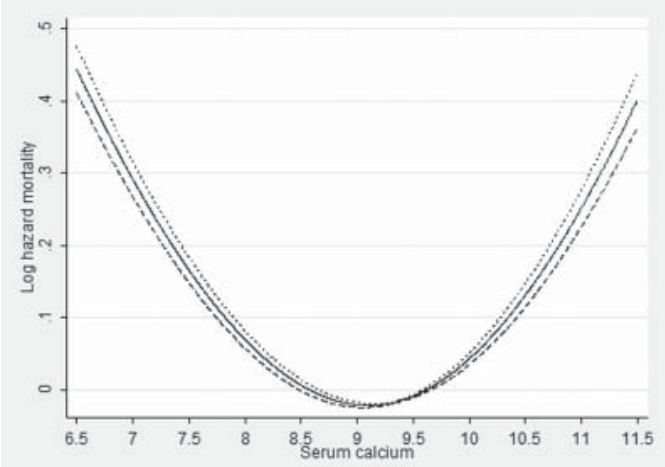
## TH-PO249

**Association of Serum Calcium Concentration with Mortality in a Nationally Representative Cohort of U.S. Veterans with Non-Dialysis Dependent Chronic Kidney Disease**
Barry M. Wall,[1,2] Kamyar Kalantar-Zadeh,[3] Leigh Darryl Quarles,[1] Jun Ling Lu,[1] Csaba P. Kovesdy.[1,2] *[1]Univ of Tennessee Health Science Center, Memphis, TN; [2]VA Medical Center, Memphis, TN; [3]Univ of California, Irvine, CA.*

**Background:** Epidemiologic studies have shown an association between higher serum calcium concentration and increased mortality in both dialysis dependent and non- dialysis dependent CKD patients. Data concerning the association of lower calcium with mortality have been conflicting.

**Methods:** We evaluated the association of serum calcium concentration with all-cause mortality in a national cohort of 528,388 US veterans with stable non-dialysis dependent CKD stages 1-5. The association of calcium concentration with all-cause mortality was examined in time-dependent Cox models. The analyses were adjusted for age, gender, race, comorbidities (DM, CVD, CHF and Charlson index), estimated GFR, serum albumin and bicarbonate concentration, and administration of antihypertensive medications. Uncorrected calcium concentration was used for all analyses.

**Results:** The mean age of the cohort was 74 ± 9 yr and 98% were male. Over a median follow-up of 4.6 years, 177,502 patients died (mortality rate: 78.3/1000 patient-years (95% confidence interval [CI] 77.9 - 78.7). Compared to patients with serum calcium 8.4 to<10.2 mg/dL, those with serum calcium <8.4 and > 10.2 mg/dL had adjusted mortality hazard ratios (95% CI) of 1.34 (1.34-1.37) and 1.47 (1.43-1.52), respectively. Calcium concentration displayed a U-shaped association with mortality.




**Conclusions:** We conclude that both low and high calcium concentrations are associated with increased mortality in patients with non-dialysis dependent CKD. Calcium concentrations of 9.0 - 9.2 mg/dL were associated with the lowest mortality in this population.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO250

**Serum Calcium and Renal Outcomes in Patients with Chronic Kidney Disease**
Lee-Moay Lim,[1] Hung-Chun Chen.[1,2] *[1]Div of Nephrology, Dept of Internal Medicine, Kaohsiung Medical Univ Hospital, Kaohsiung, Taiwan; [2]Faculty of Renal Care, College of Medicine, Kaohsiung Medical Univ, Kaohsiung, Taiwan.*

**Background:** Mineral disorders especially hyperphosphatemia are associated with adverse renal outcome in chronic kidney disease (CKD) patients. Hypercalcemia and hypocalcemia have been associated with mortality in some studies but the association with renal outcome is not well characterized. Whether adding calcium besides phosphorus or in the form of calcium x phosphorus into the predictive model could improve discrimination of renal outcome is not known.

**Methods:** We examined whether serum calcium is associated with renal outcomes in a prospective cohort study of 3303 CKD stage 3-5 outpatients. Cox proportional hazard models and logistic regression were performed by quartiles of calcium.

**Results:** The mean age of our cohort was 63.5 ± 13.5 years, mean eGFR 24.7 ± 15.1 ml/min per 1.73m$^2$ and mean calcium level 9.1 ± 0.8 mg/dL. In the baseline data, cardiovascular disease, hyperphosphatemia, high PTH and hypoalbuminemia were associated with hypocalcemia but eGFR did not show the association. Low serum calcium (<8.7 mg/dL) was associated with increased risk for renal replacement therapy (RRT) [adjusted hazard ratio (HR) 1.34(1.11-1.61), P<0.01] and rapid renal function progression (eGFR slope < -4ml/min/1.73m2/yr) [adjusted odd ratio (OR) 1.28(1.01-1.63), P<0.01] compare those with normal serum calcium (9.1-9.6mg/dL). The result remained significant after stratification for serum phosphors and iPTH. When combination of serum calcium and phosphorus were modeled, high serum phosphorus (>4.2 mg/dL) in conjunction with low serum calcium (<9.1 mg/dL) had highest risk for RRT and rapid renal function progression [HR 1.69 (1.32-2.18), P<0.01].

**Conclusions:** In advanced CKD patients, low serum calcium was associated with increased risk of RRT and rapid renal function progression. Integration of serum calcium besides phosphorus should be considered in predicting renal outcomes.

## TH-PO251

**Multivariate Risk Factors for Progression of CKD from Stage 4 to Stage 5**
Mark D. Faber, Hassan Fehmi, Naima Ogletree, Denise White Perkins. *Div of Nephrology, Depts of Int Med, Fam Med & Public Health Sciences, Henry Ford Hospital, Detroit, MI.*

**Background:** The incidence of ESRD in African-Americans (AA) far exceeds that of non-AA patients. There is much less data or agreement about the effect of race and other variables on rates of progression from stage 4 CKD (CKD-4, the likely "last chance" to prevent or delay ESRD) to stage 5 (CKD-5).

**Methods:** Administrative databases within Henry Ford Health System identified persons with CKD-4 (6 variable MDRD eGFR 15-29 ml/min/1.73 m$^2$) during 2010. The first eGFR defined the index date. Subsequent eGFRs and health care services were assessed for a 2 year follow-up period. Patients dying within 30 days or not returning for eGFR were excluded. Progression to CKD-5 was defined as 2 or more eGFRs <15 ml/min/1.73 m$^2$ at least 30 days apart. Analyses were conducted using SPSS v19.0.

**Results:** Of 5,780 patients with CKD-4, 868 had no follow-up eGFR and 56 died within 30 days. The remaining population of 4,586 was 39% male and 30% AA with mean age 72.8 yrs. 23.7% had a subsequent eGFR< 15, confirmed in 512 (10.5%) with a second eGFR <15. 2-year mortality was 4.1%. 40.7% had a nephrology visit during follow-up but only 7.9% had it in CKD-4. Further analysis was limited to the 512 with confirmed CKD-5. Progression was associated with male gender (12.9% v 9.0%, p <.001), AA race (12.3% v 9.8%, p <.01), younger age (66.6 v 73.5 years, p <.001) and diabetes (DM, 13.8% v 8.8%, p <.001). In a multivariable logistic regression model, DM, male gender and younger age were still risk factors for progression but the effect of race disappeared. During follow-up, patients with CKD-5 utilized more hospital stays (mean 1.3 v .89, p <.001), inpatient days (mean 8.7 v 5.8, p <.001) and ER visits (mean .73 v .49, p <.001).

**Conclusions:** The risk of progression from CKD-4 to CKD-5 over two-years was significant. Progression was associated with greater health care utilization. The infrequency of early nephrology evaluation, which may reduce risk of progression to ESRD, is alarming. There is a need to further clarify the relationship between CKD progression, which did not appear to be affected by race, and the widely disparate rates of incident and prevalent ESRD.

## TH-PO252

**Impact of Febuxostat on Renal Function in Gout Subjects with Moderate-to-Severe Renal Impairment** K. Saag,[1] A. Whelton,[2] M. Becker,[3] P. MacDonald,[4] Y. Zhou,[4] L. Gunawardhana.[4] *[1]Birmingham VA Medical Center, AL; [2]UCRC Inc and Johns Hopkins Univ, MD; [3]Univ of Chicago Pritzker School of Medicine, IL; [4]Takeda, IL.*

**Background:** Up to 20% of patients with hyperuricemia (serum urate [sUA] >6.8 mg/dL) and gout have moderate-to-severe renal impairment. Higher sUA (>8.5 mg/dL) further increases this risk. This 12-m pilot study evaluated the efficacy of urate-lowering with febuxostat (FEB) on the progression of renal impairment in gout subjects.

**Methods:** In this randomized controlled trial, subjects received 1:1:1 FEB 30mg BID, FEB 40/80mg QD (40 to 80mg to achieve sUA <6.0 mg/dL) or placebo (PLB) daily. Eligible subjects fulfilled ARA criteria for gout, were without tophi, had an sUA >7.0 mg/dL and estimated glomerular filtration rate (eGFR; MDRD) ≥15 to ≤50 mL/min (severe, 15 to <30 mL/min; moderate, 30 to ≤50 mL/min). Assessments included change from baseline (CFB) in serum creatinine (sCr) and eGFR, and percentage of subjects with sUA <6.0 mg/dL.

**Results:** Of 96 subjects enrolled, 80% were men with baseline mean (SD) sUA 10.5 (1.70) mg/dL; 95 qualified for the primary efficacy analysis. Titration from FEB 40mg QD to 80mg QD occurred for 20/32 subjects. CFB in sCR and eGFR was small (**Table**). At m 12, 69%, 45% and 0% of subjects in the FEB 30mg BID, 40/80mg QD and PLB groups, respectively, achieved target sUA <6.0 mg/dL ($p$<0.001). Overall, ≥1 adverse event was reported by 78%, 88% and 78% of subjects in the FEB 30mg BID, FEB 40/80mg QD and PLB groups, respectively. One PLB subject had treatment-related renal impairment.

**Conclusions:** Our data show a small favorable trend in renal function in subjects receiving FEB compared to PLB. Differences in sCr and eGFR were not statistically different. In this prospective study, reduction of sUA to target level was safe and effective in this selected study population with renal impairment.

| LS Mean (SE) | FEB 30 BID N=32 | FEB 40/80 QD N=31 | PLACEBO N=32 |
|---|---|---|---|
| **Serum creatinine (mg/dL)** | | | |
| Baseline | 2.10 (0.13) | 2.22 (0.13) | 2.52 (0.13) |
| CFB | 0.09 (0.09) | 0.23 (0.09) | 0.19 (0.09) |
| **Estimated glomerular filtration rate (mL/min)** | | | |
| Baseline | 34.14 (1.46) | 34.08 (1.48) | 29.31 (1.46) |
| CFB | 0.33 (1.17) | -0.86 (1.19) | -2.05 (1.20) |
| LS=least squares; SE=standard error; FEB=febuxostat. | | | |

*Funding:* Pharmaceutical Company Support - Takeda Global Research & Development Center, Inc.

## TH-PO253

**Short-Term Aerobic Training Reduces Endothelin-1 but Not Arterial Stiffness in Stage 3 CKD** Sam A. Headley, Michael J. Germain, Richard J. Wood, Anthony E. Poindexter, Jyovani W. Joubert, Charles M. Milch, Elizabeth E. Evans, Allen Cornelius, Britton W. Brewer, Beth Parker. *ESSS, Springfield College, Springfield, MA.*

**Background:** Short-term aerobic training has been shown to lead to a reduction in arterial stiffness. The current study was designed to examine the effect of 16 weeks of moderate intensity aerobic training (MIT) on the stiffness of central arteries, as assessed by pulse wave velocity (PWV) and the augmentation index (Aix), in a sample of stage 3 CKD patients. Also examined was the effect of MIT on some key biomarkers of vascular function.

**Methods:** Forty-six CKD patients (mean age = 57 ± 8.2 yrs) were randomly assigned to either the treatment group (T, n= 25) or to the control group (C, n= 21). A graded exercise test was performed at baseline (BL) and resting Aix and PWV were measured prior to an acute bout of 40 mins of aerobic exercise or a seated non-exercise control session. Fasting blood samples were also taken. Subjects in T then performed 16 weeks of supervised aerobic training 3x /week at 50-60% of their measured aerobic capacity ($VO_{2peak}$). Individuals randomized to C were told to follow their doctor's recommendations but not to start an exercise program. Identical measures as described above were repeated after 16 weeks. Blood samples were analyzed for endothelin-1, FGF23, and HsCRP. Data were analyzed by ANCOVA with BL and age as covariates.

**Results:** All data are presented as means ± SD.The 16 week intervention was associated with a significant increase in $VO_{2peak}$ and a decrease in endothelin-1 in T compared to C. There were no significant changes in any other variables.

| Variable | Pre | Post | p-value |
|---|---|---|---|
| $VO_2$ peak (ml/kg/min) | 19.6 ± 6.7 (T)<br>18.0 ± 6.0 (C) | 21.2 ±7.7(T)<br>17.5 ± 5.7(C) | p<0.05 |
| PWV (m/s) | 10.7 ± 2.9 (T)<br>11.0 ± 2.2 (C) | 10.8 ± 2.9(T)<br>10.9 ± 2.4 (C) | p=0.75 |
| Endothelin-1 (fmol/ml) | 1.60 ± 1.0 (T)<br>1.68 ± 1.3 (C) | 1.27 ± 0.7 (T)<br>2.19 ± 2.2 (C) | p =0.027 |

**Conclusions:** Short-term aerobic training that is associated with an increase in aerobic capacity does not change central arterial stiffness in stage 3 CKD but seems to reduce endothelin-1 levels.

*Funding:* Other NIH Support - NHLBI (1R15HL096097-01)

## TH-PO254

**The Association between Change in Weight and Change in GFR: Does Body Surface Area Adjustment Matter?** Alex Chang,[1] Xuelei Wang,[4] Cynthia A. Kendrick,[7] Lawrence J. Appel,[6] Holly J. Kramer,[3] Jackson T. Wright,[4] Brad C. Astor,[5] Tom Greene.[2] *[1]Nephrology, Johns Hopkins Univ; [2]Epidemiology, Univ of Utah; [3]Nephrology, Loyola Univ Medical Center; [4]Nephrology, Case Western Reserve Univ; [5]Nephrology, Univ of Wisconsin; [6]Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Univ; [7]Cleveland Clinic; [8]Statistical Sciences Core, Case Western Reserve Univ.*

**Background:** Controversy exists over whether glomerular filtration rate (GFR) should be indexed to body surface area (BSA). Higher body mass index is associated with higher absolute GFR but not GFR indexed to BSA in cross-sectional studies. Previous longitudinal studies have indexed GFR to baseline rather than concurrently measured BSA. Little is known about whether longitudinal changes in weight track with changes in GFR, and whether this relationship exists after adjusting for concurrently-measured BSA.

**Methods:** Participants in the African-American Study of Kidney Disease and Hypertension underwent measurements of weight and GFR at baseline, 3, 6, and every 6 months during the trial. Using linear mixed effects models adjusted for treatment assignment, we examined the association between change in weight from baseline and change in iothalamate-measured GFR indexed to baseline BSA and then indexed to concurrently-measured BSA.

**Results:** Every 5kg increase in weight was associated with a 1.04ml/min/1.73m$^2$ (95% CI: 0.82 to 1.27; p<0.001) increase in GFR indexed to baseline BSA, but not when adjusted for current BSA (0.14ml/min1.73m$^2$; 95% CI: -0.08 to 0.37; p=0.2).

**Relationship between Weight Change Categories and GFR**

| Weight Change Category (kg) | iGFR/baseline BSA (ml/min/1.73m$^2$) Estimate (95%CI) | iGFR/current BSA (ml/min/1.73m$^2$) Estimate (95%CI) |
|---|---|---|
| ≤ -13.43 | -4.21 (-5.99 to -2.43) | -1.56 (-3.54 to 0.43) |
| -13.42 to -8.95 | -4.53 (-6.87 to -2.19) | -1.05 (-3.53 to 1.42) |
| -8.94 to -4.48 | -5.36 (-7.74 to -2.97) | -2.40 (-4.85 to 0.06) |
| -4.47 to 4.48 | Ref | Ref |
| 4.49 to 8.95 | 4.20 (1.41 to 6.99) | -0.38 (-3.14 to 2.38) |
| 8.96 to 13.43 | 10.88 (5.67 to 16.10) | 0.95 (-4.10 to 6.00) |
| ≥13.44 | 20.26 (11.69 to 28.83) | 2.07 (-6.23 to 10.36) |

**Conclusions:** Weight change is directly associated with changes in measured GFR; this association essentially disappeared after adjusting for concurrently-measured BSA. Future studies need to be done to understand how weight-related changes in GFR may affect long-term kidney outcomes.

*Funding:* NIDDK Support

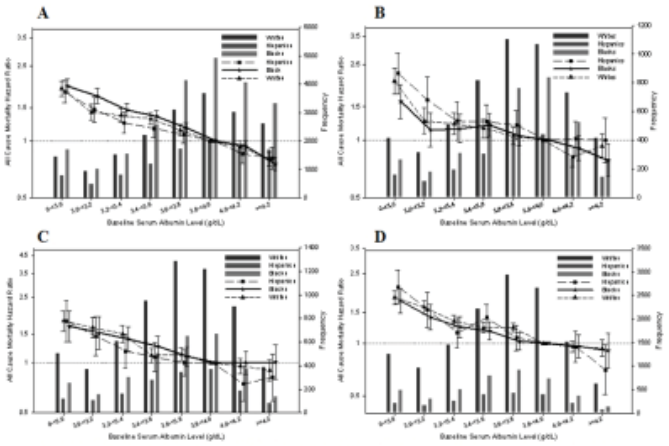
## TH-PO255

**Combined Effect of Age and Race on Mortality Predictability of Serum Albumin Level in Hemodialysis Patients** Alla Victoroff,[1] Vanessa A. Ravel,[1] Elani Streja,[1] Jongha Park,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Univ of Ulsan College of Medicine, Ulsan, Korea; [3]Memphis VA Medical Center, Memphis, VA.*

**Background:** Serum albumin level is a well-known marker for morbidity and mortality in end stage renal disease patients and is one of several markers of nutritional status. Although serum albumin is positively associated with better survival overall, the association could be modified by race/ethnicity as well as age.

**Methods:** Over a 5-year period (7/2001-6/2006), we identified 105,523 maintenance hemodialysis (MHD) patients who had a baseline serium albumin. We used Cox proportional hazard model to compare the mortality among non-Hispanic Whites, Hispanics, and African Americans in 4 different age groups.

**Results:** After full adjustment, Whites and Hispanics in the younger age group (<65 y) maintained a slight survival advantage over Black MHD patients across the lowest albumin levels (<3.0-3.4 g/dL). In patients aged 65 – <70 years, Whites and Hispanics with serum albumin <3.6 g/dL reported increased mortality, but Blacks with serum albumin as low as 3.0-3.4 g/dL failed to show significant mortality increase. In patients aged over 75 years, associations between lower serum albumin levels (<3.6 g/dL) and increased mortality are seen similarly across racial/ethnic groups. However, higher serum albumin levels ( ≥4.0 g/dL) were less frequently observed in this age group compared to patients <65 years old, and did not demonstrate a significant association with greater survival.




(A) <65 y. (B) 65-<70 y. (C) 70-<75 y. (D) ≥75 y. Reference group: hemodialysis patients with serum albumin level 3.8 – <4.0 g/dL.

**Conclusions:** Although baseline serum albumin levels were associated with survival over all, the association could be modified by race/ethnicity as well as age. Younger Black MHD patients may be more tolerant of lower serum albumin levels compared to their counterparts. However, elderly MHD patients seemed to be more vulnerable to protein-energy wasting.

*Funding:* NIDDK Support, Private Foundation Support

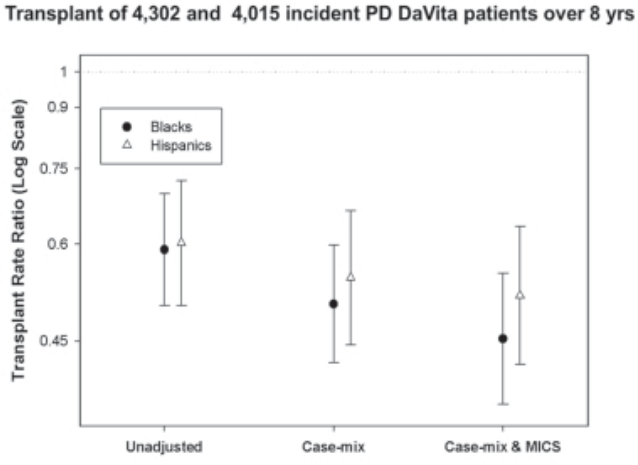
## TH-PO256

**Association of Race-Ethnicity with Transplant Rates in Incident Peritoneal Dialysis Patients** Elani Streja,[1] Chunyang Li,[1] Miklos Zsolt Molnar,[1] Wei Ling Lau,[1] Connie Rhee,[1] Csaba P. Kovesdy,[2] Kamyar Kalantar-Zadeh,[1] Rajnish Mehrotra.[3] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Harborview Medical Center, Univ of Washington, Seattle, WA; [3]Memphis Veterans Affairs Medical Center, Memphis, VA.*

**Background:** Racial disparities in dialysis patients receiving kidney transplants have been previously reported, particularly for African Americans receiving hemodialysis. However, racial disparities for receipt of kidney transplant among peritoneal dialysis patients is less well known.

**Methods:** We examined 8-year (7/2001-6/2009) rates of transplant in a cohort of 5,067 DaVita incident (< 90 days dialysis vintage days at the time of entry into cohort) PD patients including 1,052 African-Americans, 765 Hispanic, and 3,250 Whites using Cox proportional hazard regression models with adjustment for case mix and surrogates of the malnutrition-inflammation complex syndrome (MICS) and censoring for death.

**Results:** Among African-Americans, Hispanics, and Whites the mean age (mean + SD) was 52+14, 53+16, and 59+15 yrs old and included 55%, 48% and 42% women; and 46%, 57% and 48% diabetics, respectively. Over 8 years, 22% of Whites, 17% of African-Americans and 18% of Hispanics had a kidney transplant. Compared to Whites, African-Americans and Hispanics had a decreased likelihood of transplant: HRs (95%CI) 0.45 (0.37-0.55) and 0.52 (0.42- 0.63), respectively. Compared to African-Americans, Hispanics had a slightly higher likelihood of transplantation.




**Conclusions:** We corroborate previous findings that African-American and Hispanic PD patients are less likely to undergo kidney transplantation in comparison to white PD patients.

*Funding:* NIDDK Support, Private Foundation Support

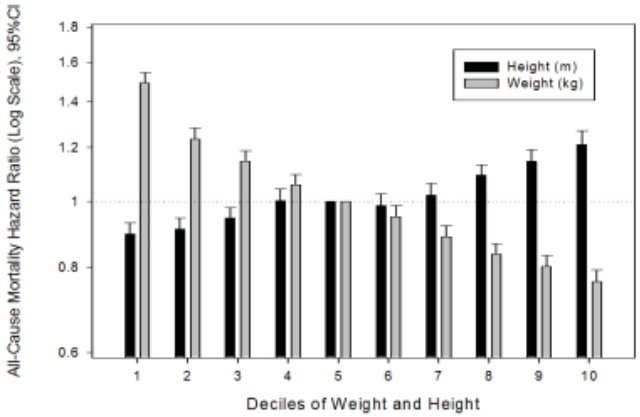
## TH-PO257

**Major Contribution of Height to Altered Effect of Body Mass Index on Mortality Risk in ESRD Patients** Elani Streja,[1] Vanessa A. Ravel,[1] Bryan B. Shapiro,[1] Kamyar Kalantar-Zadeh,[1] Joel D. Kopple.[2] *[1]Harold Simmons Cntr, UCI, Orange, CA; [2]Harbor-UCLA Med Cntr, UCLA, Torrance, CA.*

**Background:** It is well established that in maintenance dialysis (MD) patients, the hazard ratio for mortality (HrM) is related to body mass index (BMI) in a pattern that is essentially opposite of that of the general population. This phenomenon is thought to reflect the effect of body weight on mortality. However, BMI values are also determined by the square of body height. We therefore examined the contribution of height to the BMI relation to HrM.

**Methods:** We compared all-cause mortality of deciles of weight and height in 117,644 MD patients who were followed for up to 6 years (2001 through 2007), using the fifth decile as reference. Mortality risks were estimated with Cox proportional hazards models adjusted for case-mix and MICS markers. Weight analyses were adjusted for height and vice-versa.

**Results:** Patients were 62 ± SD15 years old and included 45% women and 57% diabetics. For a given weight decile, there was an inverse relationship between height and HrM, which is the reverse of what has been reported in the general population (A Kemkes-Grottenthaler Am J Phys Anthropol 2005;128:340). Whereas both weight and height exhibited incremental and strictly linear associations with death risk, weight deciles showed a wider range of HrMs (0.76 to 1.49), whereas the height decile HrM range was 0.90 to 1.21, suggesting a stronger association of weight than height with mortality in these patients.




**Conclusions:** In contrast to the general population, body height is inversely related to HrM in MD patients and contributes importantly to the inverse relation between HrM and BMI in these individuals. Nonetheless, the dramatically altered HrM vs. BMI in MD patients vs. normals is driven more by body weight than height. The cause of the altered relationship between HrM and height in ESRD needs investigation.

*Funding:* NIDDK Support

## TH-PO258

**A Target Organ Damage Based Scoring System (ABCK) Combined with a Cardiovascular Risk Score Improves Prediction of Overall Mortality and Events in a Low Risk Population Cohort** Branko Braam,[1] Lutgarde Thijs,[2] Tatiana Kouznetsova,[2] Jasjeet K. Minhas Sandhu,[3] Dean Eurich,[3] Jan A. Staessen,[2] Carlo A. Gaillard.[4] *[1]Medicine/Nephrology, Univ of Alberta, Edmonton, Canada; [2]Studies Coordinating Centre, Div of Hypertension and Cardiovascular Rehabilitation, Dept of Cardiovascular Diseases, Univ of Leuven, Belgium; [3]Public Health Sciences, Univ of Alberta, Edmonton, Canada; [4]Medicine/Nephrology, Univ Groningen, Netherlands.*

**Background:** Current cardiovascular (CV) prediction models rely on risk factors, but not on target organ damage (TOD) in an individual. The current study tested whether a newly developed multi-organ TOD classification improved prediction of death and CV events.

**Methods:** A population-based prospective cohort (n=1970) was evaluated for traditional CV risk factors. In addition, TOD was staged in arteries, brain, cardiac and kidneys (ABCK classification). Using baseline SCORE, Framingham and ABCK scores, cox proportional hazard models were developed to asses independent effects of each system in predicting 10 year mortality and CV events.

**Results:** Over 10 years, 163 subjects died, 49 (2%) had an AMI, 45 (2%) had a stroke, 46 (2%) developed end-stage renal disease and 14 (0.7%) developed severe peripheral artery disease. Using patients with a very low TOD burden as reference, increasing ABCK scores were associated with increased 10 year risk of overall mortality and CV events: low TOD group hazard ratio (HR) 2.10 (95%CI 0.15-2.88); medium TOD group risk HR 5.97 (95%CI 4.15-8.58), high TOD group risk HR 27.7 (95%CI 14.7-52.6). When related to the SCORE and Framingham scores, the ABCK score was still independently associated with death and CV events in medium and high-risk individuals. Moreover, when combined with Framingham or SCORE, ABCK substantially increased the HR for mortality (HR ranged from 2 in low risk and TOD groups to 60 in highest risk and TOD groups).

**Conclusions:** Addition of a relatively simple-to-use TOD score can improve the prediction of CV events in a population cohort.

## TH-PO259

**Arterial Stiffness Is Associated with Vascular Calcification in Chronic Kidney Disease** Jing Chen,[1] Raymond R. Townsend,[2] Matthew Jay Budoff,[3] Faheemuddin A. Ahmed,[1] Chung-Shiuan Chen,[1] Yanxi Liu,[1] Maria Waight,[1] Heather LaGuardia,[1] Damodar R. Kumbala,[1] Fred E. Husserl,[4] Eric E. Simon,[1] L. Lee Hamm,[1] Jiang He.[1] *[1]Tulane Univ, New Orleans, LA; [2]Univ of Pennsylvania School of Medicine, Philadelphia, PA; [3]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center in Torrance, Los Angeles, CA; [4]Ochsner Health System, New Orleans, LA.*

**Background:** Arterial stiffness is very common and associated with increased risk of cardiovascular disease in patients with chronic kidney disease (CKD). The underlying etiology for increased arterial stiffness is not fully understood.

**Methods:** We investigated the association of vascular calcification with risk of arterial stiffness in 235 patients with chronic kidney disease (CKD). Arterial stiffness was measured by aortic pulse wave velocity (PWV) and defined as PWV greater or equal to 12 m/sec. Vascular calcification was assessed using electron-beam computed tomography (EBCT) and coronary artery calcification (CAC) Agatston score was calculated.

**Results:** The prevalence of arterial stiffness was 37% in this study population. In the linear regression model, log-transformed coronary arterial calcification (CAC) was significantly and positively associated with log-transformed pulse wave velocity. For example, one standard deviation higher log-transformed CAC Agatston score (2.8) was associated with a 0.03 m/sec (95% confidence interval: 0.02, 0.05, p<0.0001) higher log-transformed pulse wave velocity after adjustment for age, gender, race, high-school education, physical activity, current cigarette smoking, weekly alcohol drinking, body mass index, LDL-cholesterol, plasma glucose, mean arterial pressure, estimated glomerular filtration rate (eGFR), and history of cardiovascular disease.

**Conclusions:** These data indicate that CAC may be associated with increased arterial stiffness in patients with CKD independent of the risk factors for arthrosclerosis.

*Funding:* Other NIH Support - the National Center for Research Resources, National; Institutes of Health, Bethesda, MD.

## TH-PO260

**Varied Relationships between Urinary Symptoms and CKD in the U.S. Population** Paul L. Kimmel,[1] Chyng-Wen Fwu,[2] Kevin C. Abbott,[3] Ziya Kirkali,[1] Tamara G. Bavendam,[1] Paul W. Eggers.[1] *[1]Div of Kidney, Urologic and Hematologic Diseases, NIDDK, NIH, Bethesda, MD; [2]Social & Scientific Systems, Inc., Silver Spring, MD; [3]Walter Reed National Military Medical Center, Bethesda, MD.*

**Background:** The relationship between urinary symptoms, genitourinary disease and CKD has been unclear. Conventional wisdom suggests nocturia is an early sign of CKD, without supporting evidence.

**Methods:** We used the US National Health and Nutrition Examination Survey (NHANES) 2005-2010 to assess the subjective report of nocturia ≥ 2 times per night and NHANES 2001-2010 to identify self-reported cases of urinary incontinence (UI) with markers of CKD (defined as urinary albumin-creatinine ratio ≥ 30 mg/g or eGFR < 60, by CKD-EPI equation). We limited our study sample to participants ≥ 40 years old because CKD, nocturia, and UI are not prevalent among younger populations. Logistic regression odds ratios (OR) were calculated for nocturia and UI associated with CKD after adjusting for age, race or ethnicity, diabetes mellitus, hypertension, BMI, parity (for models in women), diuretics and renin-angiotensin inhibitors. Because of gender differences in occurrence of nocturia and UI, separate analyses were conducted in men and women.

**Results:** 8,960 participants were included in nocturia analyses and 14,187 participants were included in UI analyses.The multivariate-adjusted OR of nocturia in CKD compared to non-CKD was 1.44 (95% confidence interval, 1.19-1.74), significant, among men, and 1.26 (1.07-1.47), significant, among women. The adjusted OR of UI was 1.19 (0.98-1.44) and 1.14 (0.97-1.33) among men and women, respectively.

**Conclusions:** Although the association of CKD and UI could not be detected after adjustment for known risk factors, a robust relationship between nocturia and CKD was found. We conclude possible relationships between UI and CKD are mediated by other factors, but nocturia is a potential diagnostic symptom independently associated with CKD. Absence of nocturia makes a diagnosis of CKD less likely.

*Funding:* NIDDK Support, Other U.S. Government Support

## TH-PO261

**Persistent Asthma Increases the Risk of Chronic Kidney Disease: A Retrospective Cohort Study in China** Zhangsuo Liu.[1,2] *[1]Nephrology, The First Affiliated Hospital of Zhengzhou Univ, Zhengzhou, Henan, China; [2]Key-Disciplines Laboratory Clinical-Medicine Henan, Zhengzhou, Henan, China.*

**Background:** Chronic kidney disease (CKD) is a growing public health problem with well-established risk factors. Other contributing factors, however, remain to be identified. Systemic inflammation in asthma plays a significant role in the development of other diseases. We therefore initiated a study to assess whether the growing prevalence of asthma is associated with an increase in the risk of CKD.

**Methods:** This retrospective study used data from 3015 patients with asthma at the Department of Respiratory Medicine from 2005 to 2011. History, asthma control test (ACT), and asthma stage were used to assess the traits of asthma. CKD was defined as estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 $m^2$ in twoconsecutive follow-up surveys. Odds ratios (OR) were obtained by logistic regression.

**Results:** A total of 2354 subjects with complete data were recruited for this study. After 6 years of follow-up, 72 (3.1%) participants developed CKD. Persistent asthma was associated with a significantly increased risk for CKD (OR, 2.87; 95% CI, 1.41-5.03) in unadjusted analyses. After adjustment for demographic factors and additional adjustment for hypertension, diabetes, BMI, and personal smoking habits, the increased risk for CKD (OR, 2.07; 95% CI, 1.34-4.42) remained significant. Compared to patients with no asthma traits, there was a significant risk for those who met all three criteria (OR, 3.39; 95% CI, 1.36-8.73) after adjusting for potential confounding factors.

**Conclusions:** We found that persistent asthma was associated with an increased risk of CKD in a study group from China.

*Funding:* Government Support - Non-U.S.

## TH-PO262

**Oral Disease in People with Chronic Kidney Disease: A Systematic Review and Meta-Analysis of Cohort Studies** Giovanni F.M. Strippoli,[1,2,3,4] Suetonia Palmer,[5] Marinella Ruospo,[1,2] Jonathan C. Craig,[4] Giorgio Gentile,[6] David W. Johnson,[7] Pauline J. Ford,[7] Marcello Tonelli,[8] Michele De Benedittis,[3] Massimo Petruzzi.[3] *[1]Diaverum; [2]Mario Negri Sud Consortium; [3]Univ of Bari; [4]Univ of Sydney; [5]Univ of Otago; [6]Univ of Perugia; [7]Univ of Queensland; [8]Univ of Alberta.*

**Background:** Oral disease includes a wide spectrum of clinical abnormalities affecting the mouth including mucosa, teeth, periodontal tissue and salivary function. While observational data for these diseases are available in people with chronic kidney disease (CKD), existing published information has not yet been systematically evaluated. We aimed to summarize the overall prevalence of oral diseases in people with CKD and explore associations between oral disease and mortality.

**Methods:** We conducted a systematic review and meta-analysis of observational studies reporting prevalence or clinical outcomes of oral disease in people with CKD. English-language studies were identified from systematic searching MEDLINE through April 2010. Multiple reviewers extracted participant characteristics. Estimates of prevalence, mean score, or risk of mortality were summarized using random-effects meta-analysis and expressed as rates or means and 95% confidence intervals (CI). Effects of severity of CKD on estimates were analyzed using subgroup analysis.

**Results:** 112 studies (150 cohorts) including 18 339 people with CKD and 16 310 controls were analyzed. 103 cohorts were in people on dialysis, 22 cohorts were in earlier stages of CKD and 25 cohorts were in kidney transplant recipients (15.6%). The mean decay/missing/filled teeth (DMFT) index in people with CKD was 13.7 and number of teeth was 19.4. Nearly 40% of people with CKD had enamel hypoplasia and over half had periodontitis. Overall, the mean plaque index was 1.62 and periodontal pocket depth (PPD) was 2.30 mm. Approximately 25% of people with CKD reported never brushing.

**Conclusions:** Data evaluating the prevalence and severity of oral disease in people with CKD are sparse and incomplete. Large longitudinal studies of the prevalence and clinical associations with oral disease in CKD are now needed.

## TH-PO263

**Reasons Patients Might Not Pursue Kidney Transplant: A Survey of Nephrologists' Perceptions** Jeffrey P. Yourshaw, Ankita Tandon, Kevin C. Roe, Nasrollah Ghahramani. *Internal Medicine, Penn State College of Medicine, Hershey, PA.*

**Background:** Unfavorable views about kidney transplant (KT) have been cited as potential reasons for reluctance by some patients to actively pursue KT. We examined nephrologists' perceptions of their patients' reasons for not choosing KT and analyzed the association between these perceptions and respondent demographics, practice characteristics, and previous training.

**Methods:** Invitations were sent to 3180 nephrologists in the eastern US. 822 expressed interest, and 250 were randomly invited to complete a questionnaire about demographics, practice characteristics, and their perceptions of reasons why patients might not choose KT. A total of 216 surveys with complete responses were analyzed. Chi-square and stepwise logistic regression were performed.

**Results:** The 3 most common factors perceived by nephrologists as important reasons their patients might not choose KT included "inadequate understanding of the process" (84% of respondents), "inadequate financial resources" (76%), and "cumbersome pre-transplant testing" (70%). In multivariate analysis, nephrologists whose fellowship training had a transplant program were more likely to consider inadequate understanding (OR: 4.0; p=0.01) and inadequate financial resources (OR: 5.01; p=0.002) as important reasons patients might not pursue KT. Nephrologists who attended at least one transplant-related CME per year considered inadequate financial resources as an important reason patients might not pursue KT (OR 2.61; p=0.01). In practices where less than half of the respondent's patients completed high school, cumbersome testing was considered an important reason patients would not pursue KT (OR:2.75; p=0.01).

**Conclusions:** Nephrologists believe that inadequate understanding, insufficient financial resources and cumbersome testing are regarded by patients as the most important reasons they might not pursue KT. Nephrologists' perceptions are influenced by their training and ongoing medical education, as well as the characteristics of their patient population. It is possible that nephrologists' perceptions of the reasons for patient reluctance might, in turn, impact their encouragement of pursuing KT.

*Funding:* NIDDK Support

## TH-PO264

**MDRD versus CKD-EPI Equation to Estimate Glomerular Filtration Rate in Obese Patients** Pierre Delanaye,[1] Emmanuelle Vidal-Petiot,[2] Antoine Bouquegneau,[1] Francois Vrtovsnik,[2] Etienne Cavalier,[3] Jean-Marie H. Krzesinski,[1] Martin Flamant.[2] *[1]Nephrology-Dialysis-Transplantation, Univ of Liege, CHU Sart Tilman, Liege, Belgium; [2]Dept of Renal Physiology and Nephrology, AP-HP and Denis Diderot Univ, Paris, France; [3]Clinical Chemistry, Univ of Liege, CHU Sart Tilman, Liege, Belgium.*

**Background:** Obesity is a recognized risk factor both for the development and progression of chronic kidney disease (CKD). Accurate estimation of glomerular filtration rate (GFR) is thus important in these patients. We tested the performances of two creatinine-based GFR estimates, the MDRD and CKD-EPI equations, in an obese population.

**Methods:** Patients with body mass index (BMI) higher than 30 kg/m² were included. The reference method for measured GFR (mGFR) was $^{51}$Cr-EDTA (single injection method, two blood samples at 120 and 240 minutes). Both indexed and non-indexed results were considered. Serum creatinine was measured using the IDMS-traceable compensated Jaffe method. Mean bias (eGFR-mGFR), precision (SD around the bias) and accuracy within 30% (percentage of estimations within 30% of mGFR) were calculated for both equations.

**Results:** The population included 366 patients (185 women) from two different areas. Mean age was 55 ± 14 years and mean BMI was 36 ± 7 kg/m². Mean mGFR was 56 ± 26 mL/min/1.73 m² (71 ± 35 mL/min without indexation). In the total population, mean bias was +1.9 ± 14.3 and +4.6 ± 14.7 mL/min/1.73 m² ($p<0.05$), and accuracy 30% was 80 and 76% for the MDRD and CKD-EPI equations ($p<0.05$), respectively. In patients with mGFR>60 mL/min/1.73 m², mean bias was +4.6 ± 18.4 and +9.3 ± 17.2 mL/min/1.73 m² ($p<0.05$), and accuracy 30% was 81 and 79% (NS) for the MDRD and CKD-EPI equations, respectively.

**Conclusions:** The CKD-EPI equation did not outperform the MDRD study equation in this population of obese patients. The unexpected positive bias of the MDRD study equation in patients with a mGFR above 60 mL/min/1.73 m² questions the physiological relevance of BSA indexation which may lead to underestimating renal function in obese patients.

## TH-PO265

**Determinants of the Creatinine Clearance to Glomerular Filtration Rate Ratio in Patients with Chronic Kidney Disease** Yen Chung Lin,[1,2] Nisha Bansal,[2] Eric Vittinghoff,[3] Alan S. Go,[4] Chi-Yuan Hsu.[2] *[1]Div of Nephrology, Dept of Internal Medicine, Taipei Medical Univ Hospital; Dept of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical Univ, Taipei, Taiwan; [2]Div of Nephrology, School of Medicine, Univ of California-San Francisco, San Francisco, CA; [3]Dept of Epidemiology and Biostatistics, School of Medicine, Univ of California-San Francisco, CA; [4]Div of Research, Kaiser Permanente Northern California, Oakland, CA.*

**Background:** Creatinine secretion, as quantified by the ratio of creatinine clearance (CrCl) to glomerular filtration rate (GFR), may introduce another source of error when using serum creatinine concentration to estimate GFR. We studied in a large modern cohort of patients with chronic kidney disease (CKD) factors which influence CrCl/iGFR ratio. We specifically wanted to examine whether higher levels of albuminuria would be associated with higher, and black race with lower CrCl/GFR ratio, as suggested by prior literature.

**Methods:** We included 1342 Chronic Renal Insufficiency Cohort (CRIC) participants who had baseline measure of iothalamate GFR (iGFR) and 24-hour urine collections. We cross-sectionally analyzed the relationship between albuminuria (categorized as: <30, 30-299, 300-2999 and ≥3000 mg), and race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic) and CrCl/iGFR ratio by multivariate-adjusted regression model.

**Results:** Mean iGFR was 48.0 ± 19.9 ml/min/1.73m², median albuminuria was 84 mg per day, and 36.8% of the study participants were black. Mean CrCl/iGFR ratio was 1.19 ± 0.48. There was no association between the CrCl/iGFR ratio and urine albuminuria (coefficient 0.11 [95% CI -0.01 - 0.22] for higest verus lowest levels of albuminuria, p= 0.07). There was also no association between race and CrCl/iGFR ratio (coefficient for blacks was -0.03 [95% CI-0.09 - 0.03] compared with whites, p=0.38).

**Conclusions:** Contrary to what had been suggested by prior smaller studies, CrCl/GFR ratio does not vary with degree of proteinuria or race/ethnicity. The ratio is also closer to 1.0 than reported by several frequently cited reports in the literature.

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## TH-PO266

**Assessment of Cystatin C Glomerular Filtration Rate in Patients with Liver Cirrhosis** Kriengsak Vareesangthip,[1] Sukit Raksasuk,[1] Supot Nimanong,[1] Wimolphan Artsom,[1] Sunanta Chiewvit,[2] Kanit Reesukumal.[3] *[1]Dept of Medicine, Siriraj Hosp., Mahidol U.; [2]Dept of Radiology, Siriraj Hosp., Mahidol U.; [3]Dept of Clinical Pathology, Siriraj Hosp., Mahidol U., Bangkok, Thailand.*

**Background:** In cirrhotic patients have lower serum creatinine (SCr) due to a decrease production of SCr in liver and due to an increase in the volume distribution cause by retention of water. Moreover, in cirrhosis patients have hypoalbuminemia from malnutrition making a decrease in muscle mass. Thus, the cirrhotic patients have a lower baseline of SCr level less than the general population and have found that SCr have been slowly to change. Therefore, SCr in cirrhotic patients were not correlated with actual glomerular filtration rate (GFR). There is evidence that GFR assessed by cystatin C might be showed positive correlation with actual GFR. This study aimed to assessment of GFR between to SCr-GFR (c-GFR), DTPA-scan GFR and cystatin C GFR methods in cirrhotic patients.

**Methods:** Twenty-two cirrhotic patients were recruited and were monitored for GFR in all three methods (c-GFR, DTPA-scan GFR and cystatin C GFR). Clinical and laboratory parameters were prospectively collected.

**Results:** Among 22 cirrhotic patients (male = 14, female = 8) were enrolled to study (mean age 58.64 years). The causes of cirrhosis were chronic hepatitis B (32%), chronic hepatitis C (36%), alcohol (14%), autoimmune hepatitis (14%) and miscellaneous (4%). The Child Pugh classification A was 86% and B 14%. Three patients (13.6%) were known case of diabetes. Eight patients (36.4%) were diagnosed of hypertension. Two patients (9%) were used spironolactone. The agreement analysis between Tc99-DTPA scan and c-GFR and between Tc99-DTPA scan and cystatin C were calculated according to Altman-Bland plot. The results demonstrated no significant correlation between Tc99-DTPA scan and SCr and between Tc99-DTPA scan and cystatin C estimating to GFR in subgroup analysis including, gender, Child Pugh classification, HbA1C levels and diuretic used.

**Conclusions:** In this study using the GFR assessment by Tc99-DTPA scan as a gold standard, cystatin C GFR has no advantage over c-GFR in order to estimate GFR in patients with liver cirrhosis.

*Funding:* Government Support - Non-U.S.

## TH-PO267

**Comparing Cystatin C and Creatinine for Estimating Measured GFR and CKD Prevalence in a Community-Based Sample of the Elderly** Lesley Inker,[1] Li Fan,[1] Aghogho A. Okparavero,[1] Vilmundur Gudnason,[2] Gudny Eiriksdottir,[2] Margret B. Andresdottir,[3] Hrefna Gudmundsdottir,[3] Olafur S. Indridason,[3] Runolfur Palsson,[3] Lenore J. Launer,[4] Tamara Harris,[4] Gary F. Mitchell,[5] Andrew S. Levey.[1] *[1]Tufts MC; [2]Icelandic Heart Association; [3]National Univ Hospital Iceland; [4]NIA; [5]CV Engineering.*

**Background:** There is controversy about the accuracy of GFR estimating equations compared to measured GFR (mGFR) in the elderly.

**Methods:** We measured GFR using plasma clearance of iohexol in 805 older adults enrolled in the community-based Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. We estimated GFR using the CKD-EPI equations based on creatinine (eGFRcr), cystatin C (eGFRcys) and both (eGFRcr-cys), and determined prevalence of CKD Stage 3-5 (GFR<60 ml/min/1.73m²). Creatinine and cystatin C assays are traceable to reference materials.

**Results:** Mean age was 79.4±3.8 years. The table compares level of mGFR and eGFR, equation performance and CKD prevalence.

**Table Comparison the performance of eGFRcr, eGFRcys and eGFRcr-cys**

| | Median GFR ($25^{th}$,$75^{th}$) | Bias Median Difference (95%CI) | Precison IQR(95%CI) | Accuracy P30(95%CI) | CKD Stages 3-5(%) (95%CI) |
|---|---|---|---|---|---|
| mGFR | 64(52,73) | NA | NA | NA | 39.0(35.8,42.5) |
| eGFRcr | 68(54,80) | -2.7(-3.5,-2.2) | 12.1(11.2,13.5) | 91.4(89.3,93.4) | 34.8(31.4,38.0) |
| eGFRcys | 61(48,74) | 1.9(1.2,2.6) | 11.5(10.7,12.5) | 93.5(91.8,95.2) | 48.0(44.5,51.4) |
| eGFRcr-cys | 65(51,76) | -0.6(-1.2,0.0) | 10.0(9.0,11.0) | 95.9(94.5,97.1) | 38.3(35.5,42.2) |

Bias=mGFR-eGFR. $P_{30=\%}$ eGFR within 30% of mGFR. Units for GFR, bias and IQR= ml/min/1.73 m².

eGFRcys had a higher rate of false positive diagnosis of CKD Stage 3-5 [29%(24-33)] compared to eGFRcr [14%(10-19)] and eGFRcr-cys [18%(14-22)].

**Conclusions:** eGFRcr and eGFRcys have similar accuracy but opposite direction of bias. In contrast, eGFRcr-cys is unbiased in estimating GFR, leading to a closer approximation of mGFR and CKD prevalence than either eGFRcr or eGFRcys. We suggest using eGFRcr-cys when accurate estimates of mGFR or CKD prevalence in the elderly are required.

## TH-PO268

**Estimation of Glomerular Filtration Rate in Persons Aged 70 Years or Older** Yanni Wang,[1] Chenggang Shi,[1] Xilian Qiu,[2] Jianhua Huang,[3] Cailian Cheng,[1] Xun Liu.[1] *[1]Div of Nephrology, Dept of Internal Medicine, The 3rd Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China; [2]Dept of Laboratory Medicine, The First Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China; [3]Dept of Laboratory Medicine, The 3rd Affiliated Hospital of Sun Yat-sen Univ, Guanghzou, China.*

**Background:** GFR estimating equations need proper validation in the elderly population because of the age related change in renal function and the progressive loss of muscle mass with age.

**Methods:** A total of 345 Chinese participants aged 70 years or older who had undergone technetium-99m diethylenetriaminepentaacetic acid($^{99m}$Tc-DTPA) renal dynamic imaging were enrolled. The two-level race CKD-EPI equation, the four-level race CKD-EPI equation and the BIS1 equation were compared.

**Results:** Although bias was similar among the two-level race CKD-EPI equation and the BIS equation (median difference, -0.2 ml/min/1.73m²for the two-level race CKD-EPI equation vs.0.3 ml/min/1.73m²for the BIS equation[P = 0.6]), precision was improved with the BIS equation (IQR for the difference, 14.1 ml/min/1.73m²), as compared with the two-level race CKD-EPI equation and with the four-level race CKD-EPI equation (IQR for the difference, 19.3 and 20.4 ml/min/1.73m²[P < 0.001 for bothcomparisons]), leading to an improvement in accuracy (15% accuracy, 40.3 vs. 29.9 and 28.4 %; 30% accuracy, 66.1 vs.

53.0 and 51.0 %; 50% accuracy, 85.8 vs. 75.1 and 74.8 %; P < 0.001 for allcomparisons]), as was GFR category classification (GFR category misclassification rate, 38.8 vs. 44.9 and 47.5 %, respectively[P = 0.007 and P = 0.001].

**Conclusions:** The BIS1 equation may be the optimal one for elderly Chinese persons aged 70 years or older.

*Funding:* Government Support - Non-U.S.

## TH-PO269

**Extended Iohexol Clearance Protocol in Older Adults with Reduced Kidney Function** Natalie Ebert,[1] Amina Loesment,[1] Peter Martus,[2] Markus van der Giet,[3] Elke Schaeffner.[1] *[1]Charité Campus Virchow, Nephrology, Berlin, Germany; [2]Biostatistics and Clin Epidemiology, Eberhard Karls Univ, Tübingen, Germany; [3]Charité Campus Benjamin Franklin, Nehrology, Berlin, Germany.*

**Background:** Performing iohexol clearance in people with considerably reduced kidney function is a logistic challenge as it requires a 24h measurement. As an additional project of the Berlin Initiative Study (BIS) we measured iohexol clearance including a 24h-measurement in 61 individuals with chronic kidney disease and compared measured GFR (mGFR) including the 24h measurement to mGFR results without the 24h measurement.

**Methods:** In a sample of 61 subjects mGFR was determined by using iohexol measurements at 120 min, 180 min, 240 min, 300 min (=5h), and 1440 min (= 24h). mGFR was determined using measurements until 300 min ($mGFR_{300}$) and measurements until 1440min ($mGFR_{1440}$). For both approaches the slow component according to the method of Schwartz was determined and the bodysurface area was adjusted for by the Dubois formula. Absolute and percentage differences were calculated.

**Results:** Patients were 79.4 years old (mean, range 70 to 94 yrs), 23% were female. $mGFR_{300}$ and $mGFR_{1400}$ were highly correlated ($R^2 = 0.90$) but $mGFR_{1400}$ (25.0 ± 7.44 ml/min/$m^2$) was significantly smaller than $mGFR_{300}$ (31.0 ± 8.26 ml/min/$m^2$). The raw difference $mGFR_{300}$ - $mGFR_{1400}$ was 6.0 ± 2.65 ml/min/$m^2$ and the percentage difference was 19.8 ± 8.2%. Moreover, in 60 of 61 subjects (98%) $mGFR_{1400}$ was smaller than $mGFR_{300}$ (p < 0.001). Applying the correction formula $mGFR_{1400}$ = -1.486 + 0.854*$mGFR_{300}$ led to an error of ±4.76 ml/min/$m^2$ (two-fold standard error of estimate). Inclusion of a quadratic term did not improve the prediction of $mGFR_{1400}$.

**Conclusions:** The reason for the decline of $mGFR_{1400}$ compared to $mGFR_{300}$ was the fact that the actual iohexol measurement values at 24h were considerably larger as predicted by extrapolation of the slow component from measurements at 5 h. We conclude that the exponential decay does not fit the longterm filtration of iohexol. A linear correction formula for $mGFR_{300}$ leads to an $R^2$ of 0.90 which should be sufficient for applications.

*Funding:* Private Foundation Support

## TH-PO270

**GFR Estimating Equations in HIV-Infected Patients: One Patient, One GFR Estimating Equation** Amandine Gagneux-Brunon,[1] Pierre Delanaye,[2] Christopher R. Mariat,[1] Olivier Moranne.[3] *[1]Universitary Hospital of Saint-Etienne, Saint-Etienne, France; [2]CHU de Liege, Belgium; [3]CHU de Nice, France.*

**Background:** An accurate Glomerular Filtration Rate estimation is needed in HIV-infected (HIV+) patients. CKD-EPI equations based on serum creatinine only or with serum cystatin C were described as accurate in this population. Yet, they are biased; Our aim was to validate GFR estimating Cockcroft and Gault (CG), MDRD, CKD-EPI equations, and to identify factors affecting performance of eGFR in European HIV+ patients with measured GFR greater than 60 mL/min/1.73$m^2$.

**Methods:** GFR was measured by iohexol plasma clearance, serum creatinine (Scr) and cystatin C (Scys) were measured by traceable and standardized methods. We evaluated CG, MDRD, and CKD-EPI equations based on Scr and/or Scys.

**Results:** One hundred eighty five (93% of whites, 33 women, 152 men,) HIV+ patients have been included from februar 2011 to june 2012. Ninety three percent were receiving highly active antiretroviral therapy. Mean (±SD) age was 49 years (±10). Mean mGFR was 99 (±20) ml/min/1.73$m^2$. Mean absolute bias (± SD) were respectively for CG, MDRD, CKD-EPI Scr, CKD-EPI Scys, CKD-EPI combined 1.1 (± 24.6), -3.3 (± 23.5), 0.8 (±19.6), 0 (±21), 0 (±20) mL/min/1.73$m^2$. Accuracy-30 % for CG, MDRD, CKD-EPI Scr, CKD-EPI Scys, CKD-EPI combined were respectively 77, 79, 88, 83, 87. CKD-EPI Scr and combined were significantly more accurate than CG and MDRD. Absolute bias of CG was in multivariate analysis significantly associated with age, body mass index and measured GFR while bias of CKD-EPI Scys was associated with diabetes mellitus, HIV-Viral load, ethnicity and measured GFR. HIV-viral load was not associated with bias of CKD-EPI combined equation. Bias of MDRD was associated with mGFR and CKD-EPI with mGFR level and age.

**Conclusions:** In European HIV+ patients, CKD-EPI Scr was the one of the most accurate GFR equations. mGFR level was significantly associated with the bias of all GFR estimating equations. Clinicians should be aware that factors as GFR level diabetes mellitus, HIV-Viral load, age, ethnicity and body mass index may influence the performance of GFR estimating equations in HIV+patients.

## TH-PO271

**Demographic and Biochemical Determinants of Glomerular Filtration Rate in Chinese Children with Chronic Kidney Disease** Mengchun Gong, Yan Qin, Xuemei Li. *Nephrology, Peing Union Medical College Hospital, Beijing, China.*

**Background:** There is no research on Chinese children's renal function evaluation using the plasma clearance of an exogenous substance, the golden standard method of GFR measurement, and thus no GFR-estimating tools based on data from this vast population. The objectives of this study include measurement of the GFR, analysis of the determinants of measured GFR and selection of the best parameters in estimating GFR, which can be used to develop a GFR-estimating equation for the Chinese children.

**Methods:** Plasma clearance of $^{99m}$Tc-DTPA was measured using the radioactive counts of the plasma sampled at two different time spots (2 hours and 4 hours) after the peripheral bolus injection of measured dose of $^{99m}$Tc-DTPA and calculated as measured GFR (mGFR) . mGFR was normalized by body surface area (BSA), calculated by the Haycock equation. Univariate linear regression was used to determine the capacity of demographic markers, including gender, age, body weight, height and BSA, and the biochemical markers, including Jaffe-Creatinine (JCr), enzymatic creatinine (ECr), urea and cystatin C (CysC), in explaining the inter-individual variance of mGFR and nGFR.

**Results:** Eighty-nine children were included with 49% male. The average level of mGFR and normalized GFR (nGFR) was 67.5±2.8mL/min and 93.4±23.2mL/min×1.73$m^2$, respectively. Univariate analysis showed that the inter-individual variance of mGFR can be explained mainly by demographic markers and the $R^2$ value for height, body weight, age and BSA were 0.313, 0.365, 0.296 and 0.367 (all with p<0.01). However, the inter-individual variance of nGFR can be mainly explained by renal function-related biochemical markers, with $R^2$ value for H/ECr 0.620, H/JCr 0.472, 1/Ecr 0.582, 1/JCr 0.506, 1/CysC 0.588 and 1/BUN 0.372 (all with p<0.001), and can not be explained by demographic markers, with all the $R^2$ value for height, body weight, age and BSA lower than 0.005 and p value >0.05.

**Conclusions:** The inter-individual variance of the BSA-normalized GFR can be mainly explained by the renal function-related biochemical markers. Height/ECr and 1/CysC are two parameters selected for developing the GFR-estimating equations for the Chinese children.

*Funding:* Government Support - Non-U.S.

## TH-PO272

**Comparison of Creatinine-Based and Cystatin C-Based Glomerular Filtration Rate-Estimating Equations in Children with Lupus Nephritis** Mengchun Gong, Yan Qin, Xuemei Li. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

**Background:** There is no data about glomerular filtration rate (GFR), measured through plasma clearance of the exogenous markers, and its estimation tools in children with lupus nephritis (LN).

**Methods:** We determined GFR with plasma clearance of $^{99m}$Tc-DTPA in 21 hospitalized children with clinically (N=10) or pathologically (N=11) proved LN and compared the performance of ten different equations, biochemically based on serum Creatinine (4), serum Cystatin C (3) or their combination (3), in estimating GFR, with parameters of correlation, precision and accuracy.

**Results:** The Schwartz1976, Schwartz2009 and CKiD equations, the most widely accepted tools for GFR estimation in children, significantly overestimate GFR in LN children (bias 38.6, 43.6, 11.5 mL/min×1.73$m^2$, respectively, all p<0.01 in the paired t-test). Compared with the other nine equations, the Filler (eGFR=91.62×(1/sCysC)^1.123)and the Zappitelli-CysC equation (eGFR=75.94×(1/sCysC)^1.17×[1.384]^Tx) produced eGFR with better correlation with nGFR, stronger explanation capacity of variance in nGFR, smaller and non-significant bias, higher intra-class correlation coefficients, higher ratio of eGFR within nGFR±10% and ±30%.

| Equations | BiasΦ | γ¶ | R²†(%) | ICC‡ | nGFR±10% (%) | nGFR±30% (%) |
|---|---|---|---|---|---|---|
| Filler | 7.5 | 0.85 | 72 | 0.87 | 29 | 86 |
| Zappitelli-CysC | -8.4 | 0.85 | 72 | 0.87 | 43 | 86 |
| Grubb | 16.5* | 0.83 | 69 | 0.68 | 27 | 57 |
| Schwartz1976 | 38.6* | 0.73 | 53 | 0.45 | 19 | 38 |
| Schwartz2009 | 43.6* | 0.86 | 74 | 0.57 | 19 | 38 |
| Counahan | 8.5 | 0.77 | 60 | 0.68 | 24 | 81 |
| Leger | 8.3 | 0.64 | 41 | 0.59 | 14 | 57 |
| CKiD | 11.5* | 0.88 | 77 | 0.81 | 33 | 75 |
| Bouvet | -7.6 | 0.70 | 49 | 0.71 | 38 | 62 |
| Zappitelli-Combi | 37.6* | 0.90 | 80 | 0.65 | 0 | 29 |

Φ mL/min×1.73$m^2$; * p<0.01;¶ linear correlation factor; † linear regression; ‡ ICC: intraclass correlation factor.

**Conclusions:** Equations biochemically based on serum creatinine significantly overestimate GFR in children with LN while those based on serum Cystatin C provide eGFR with satisfactory precision and accuracy.

*Funding:* Government Support - Non-U.S.

## TH-PO273

**Development of a Bedside Glomerular Filtration Rate-Estimating Equation for Chinese Children with Chronic Kidney Disease** Mengchun Gong, Xuemei Li, Yan Qin. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

**Background:** It has been proved that American and European equations to calculate estimated glomerular filtration rate (eGFR) significantly overestimate the value in Chinese children and adults. No equation had been developed, based on the measurement of GFR

with the exogenous-marker clearance method and the enzymatic creatinine, for the Chinese children with Chronic Kidney Disease (CKD).

**Methods:** Plasma clearance of $^{99m}$Tc-DTPA was measured using the radioactive counts of the plasma sampled at two different time spots (2 hours and 4 hours) after the peripheral bolus injection of measured dose of 99mTc-DTPA and calculated as measured GFR (mGFR) . mGFR was normalized by body surface area (BSA), calculated by the Haycock equation. which was calculated using the Haycock equation, and nGFR was used for further analysis. Height to Creatinine ratio (H/Cr) was used as the base for coefficient adjustment according to linear regression study and literature. To assess the properties of the estimating equations, we calculated the root mean square error (RMSE) and $R^2$ in linear regression, the correlation coefficient and the percentage of eGFR within 30 and 15% of nGFR.

**Results:** 89 children were included with 49 percent male. The average level of nGFR was 93.4±23.2mL/min×1.73m$^2$. Univariate analysis showed that the inter-individual variance of nGFR can be mainly explained by H/Cr, with the $R^2$ of 0.620 (p<0.001). For its bedside use, the exponent of H/Cr as set to be 1 and the equation becomes eGFR=27.6×Height (cm)/Cr (mmol/L), named as the PUMCH Equation. In the training dataset, the average eGFR was 96.3±38.2mL/min×1.73m$^2$ and the bias is 2.9mL/min×1.73m$^2$ (P=0.27). The correlation coefficient between eGFR and GFR is 0.84 (P<0.001). Linear regression showed $R^2$ of 0.711 and root mean square error as 0.090. Accuracy analysis showed 38% and 77% of eGFR were within the range of GFR±15% and ±30%, respectively.

**Conclusions:** The newly-developed PUMCH equation for GFR-estimation produced satisfactory precision and accuracy in Chinese children with CKD with its simple structure for bedside use. Further internal and external validation study is necessary to approve its wide-spread application.

*Funding:* Government Support - Non-U.S.

## TH-PO274

### Can Estimation of GFR Be Improved by Using Bioimpedance Techniques?

Fansan Zhu,[1] Mathew Hotta,[2] Christina M. Wyatt,[2] Lesley Inker,[3] Li Liu,[1] Nathan W. Levin.[1] *[1]Renal Research Institute, New York, NY; [2]Mount Sinai Medical School, New York, NY; [3]Tufts Univ School of Medicine, Boston, MA.*

**Background:** The current method to estimate glomerular filtration rate (GFR) from creatinine (GFR CKD-EPI) uses serum creatinine (Scr) or cystatin (CysC) and surrogates for muscle mass, particularly race and gender. The aim of this study was to investigate whether estimation of GFR can be improved by adding bioimpedance parameters which incorporates assessment of extracellular fluid (ECV), muscle mass (MM) and calf normalized resistivity (CNR) to models with Scr or CysC or both together to compare with the gold standard GFR (mGFR).

**Methods:** 39 HIV patients (13 females) were studied. Plasma clearance of iohexol was used as mGFR, CysC and sCr were measured. Bioimpedance techniques (BI) were performed to provide ECV, MM and CNR (Zhu et al., ASN abstract 2009). A linear mixed regression model (LMRM) using parameter of sex, race, age, height and weight in the basic model with sCr or CysC or both, and combining ECV, MM and CNR as other models were established according to mGFR.

**Results:** Using correlation with mGFR the basic LMRM with CysC ($R^2$=0.72) is better than with Scr ($R^2$=0.69) but the best result was when were included both CysC and Scr ($R^2$=0.76) (Table 1). Correlation and standard error of estimation (SEE) with mGFR was better in females than in males in all models. Correlations and SEE with mGFR were improved for model with Scr ($R^2$=0.73-0.77), CysC ($R^2$=0.74-0.79) or Scr and CysC ($R^2$=0.79-0.83) when the model included BI parameters.

**Conclusions:** The accuracy of GFR estimation can be improved by use of a BI model which considers muscular mass, fluid volume and degree of hydration, regardless of filtration marker used and the models using both markers is better than using either alone. This model could be helpful to improve GFR estimation.

Table1

| | With Scr | | | With CysC | | | With Scr and CysC | | |
|---|---|---|---|---|---|---|---|---|---|
| R^2 | All | Males | Females | All | Males | Females | All | Males | Females |
| Basic model | 0.69 | 0.651 | 0.816 | 0.72 | 0.712 | 0.692 | 0.755 | 0.733 | 0.7 |
| Basic+ECV | 0.73 | 0.706 | 0.843 | 0.74 | 0.769 | 0.699 | 0.787 | 0.79 | 0.89 |
| Basic+ECV+MM | 0.75 | 0.743 | 0.858 | 0.74 | 0.769 | 0.823 | 0.793 | 0.79 | 0.89 |
| Basic+ECV+MM+CNR | 0.77 | 0.756 | 0.903 | 0.79 | 0.817 | 0.754 | 0.827 | 0.82 | 0.94 |
| SEE (ml/min/1.73m$^2$) | All | Males | Females | All | Males | Females | All | Males | Females |
| Basic model | 18.3 | 20.33 | 11.9 | 16.8 | 18.49 | 15.39 | 16.43 | 18.3 | 14.5 |
| Basic+ECV | 17.2 | 19.16 | 11.66 | 16.3 | 16.98 | 16.16 | 15.6 | 16.8 | 11.2 |
| Basic+ECV+MM | 16.9 | 18.39 | 11.87 | 16.6 | 17.44 | 13.25 | 15.6 | 17.1 | 12.2 |
| Basic+ECV+MM+CNR | 16.4 | 18.42 | 10.58 | 15.2 | 15.96 | 16.85 | 14.5 | 16.3 | 10.3 |

## TH-PO275

### Association of Traumatic Amputation with Concentrations of Serum Creatinine and Cystatin C in Male Soldiers: A Case Control Study

John Stephen Thurlow, Kevin C. Abbott, Dustin J. Little, Stephen W. Olson. *Nephrology, Walter Reed National Military Medical Center, Bethesda, MD.*

**Background:** Whether concentrations of serum creatinine, or Cystatin C, are significantly altered after traumatic amputation has not been systematically studied.

**Methods:** Healthy male soldiers who (n=33) were identified in the Armed Forces Amputee Care Program Data Base as having undergone traumatic amputations. Matched controls selected for age, duration of pre and post sample intervals, and comparable health screening were also selected (n=12). **Predictors:** Presence or absence of traumatic amputation, further categorized as small, medium, and large subgroups by Osterkamp estimations using DEXA scan measurements. **Outcomes:** serum creatinine and Cystatin C concentrations. **Measurements:** The Department of Defense Serum Repository was used to compare pre and post traumatic amputation levels (minimum interval of 3 months after amputation) of mean serum creatinine (mg/dl), Cystatin C (mg/L), serum albumin, and white blood cell count. Paired Student's t-test was used for comparisons.

**Results:** Mean serum creatinine was significantly lower after amputation overall (1.05±0.18 pre vs. 0.84±0.14 post; p<0.001), in the medium amputation subgroup (1.10±0.17 vs. 0.84±0.12; p<0.001), and in the large amputation subgroup (0.97±0.17 vs. 0.76±0.11; p<0.001). The difference was not statistically significant in the small amputation subgroup (1.08±0.23 vs. 0.94±0.13; p=0.15). Cystatin C remained unchanged overall (0.80±0.10 vs. 0.79±0.08, p=0.55), as well as in the small amputation (0.75±0.10 vs. 0.79±0.09; p=0.60), medium amputation (0.81±0.09 vs. 0.80±0.07; p=0.92), and large amputation (0.84±0.08 vs. 0.79±0.11; p=0.21) subgroups. In the control group, interval serum creatinine and Cystatin C were not significantly different.

**Conclusions:** Concentrations of mean serum creatinine, but not Cystatin C, are significantly lower after traumatic amputation, changes not observed in matched controls. Reliance on current creatinine based estimates of renal function in patients after amputation could limit identification and management of kidney disease.

*Funding:* Other U.S. Government Support

## TH-PO276

### Plasma Cystatin C Is a Valid Marker of Glomerular Filtration Rate in Mice

Marie Buleon,[1,2] Acil Jaafar,[1] Nicolas Mayeur,[1,2] Marion Vallet,[1] Francoise Praddaude,[1,2] Ivan A. Tack.[1,2] *[1]Service Explorations Fonctionnelles Physiologiques, Laboratoire Physiologie, CHU Toulouse, Toulouse, France; [2]INSERM U1048, I2MC, Univ P Sabatier, Toulouse, France.*

**Background:** Determination of the glomerular filtration rate (GFR) is ideally performed by measuring the renal clearance of exogenous markers such as inulin and $^{Cr51}$EDTA. In mice, this technic present numerous shortcomings. Thus, for years, the follow up of renal function has been based on the dosage of blood markers, such as creatinine and urea. However, various works have underlined the limitations of these markers and a reliable and easy to use endogenous marker to estimate GFR is still lacking in mice. Cystatin C is a valid marker of GFR in human but its relevance, in mice, is unknown.

**Methods:** We prospectively compared cystatin C (human PETIA and mouse ELISA), creatinine (Jaffé and enzymatic assays) and urea to GFR (inulin clearance: mGFR) in male or female C67Bl6 mice, aged between 16 and 40 weeks, healthy or subjected to chronic renal failure (partial nephrectomy or following acute kidney injury).

**Results:** In a first group (n=80), urea, enzymatic creatinine and ELISA cystatin C were significantly correlated to mGFR. Neither PETIA Cystatin C, nor Jaffe creatininemia were correlated with mGFR. We therefore focused on ELISA cystatin C, enzymatic creatinine and urea using complementary groups. Urea was significantly but weakly correlated to mGFR (r Spearman -0.28, p<0.05). ELISA Cystatin C and creatinine were significantly correlated although cystatin C correlation was tighter (r Spearman -0.76 and -0.62, respectively). When applied to a population splitted by mGFR level (<120, 120 to 240, >240 µL/min, n=15 in each group), ELISA cystatin C was the only marker able to significantly distinguish groups from each other (Kruskall-Wallis test, p<0.001). Finally, a Cystatin C-based equation to estimate GFR in mice was developed and validated using Bland & Altman procedure.

**Conclusions:** Our results indicate that mELISA cystatin C, but not hPETIA, is better correlated to GFR in mice than enzymatic creatininemia and could be an interesting and highly discriminative GFR marker. Conversely, the use of Jaffé creatinine and urea to estimate GFR is not relevant.

*Funding:* Government Support - Non-U.S.

## TH-PO277

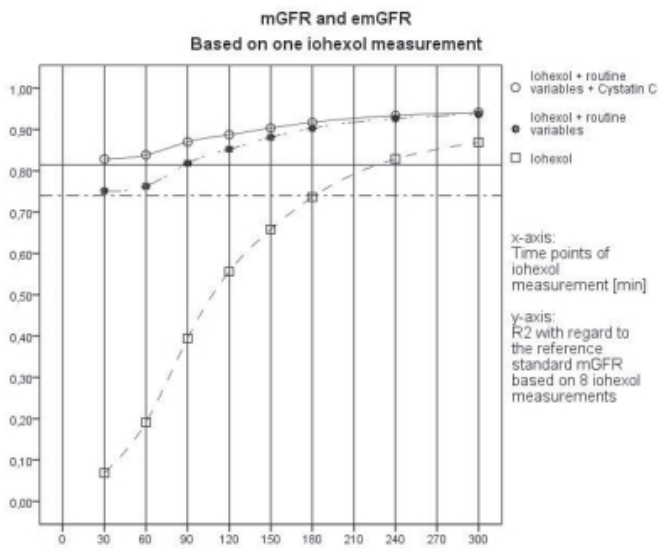
### Efficient Approaches for GFR Assessment in Older Adults

Peter Martus,[1] Natalie Ebert,[2] Markus van der Giet,[2] Olga Jakob,[3] Elke Schaeffner.[2] *[1]Inst Clinical Epidemiology and Med. Biostatistics, Eberhard Karls Univ, Tübingen, Germany; [2]Dept. of Nephrology, Charité, Berlin, Germany; [3]Inst of Biostatistics and Clin Epidemiology, Charité, Berlin, Germany.*

**Background:** Assessment of glomerular filtration rate (GFR) is crucial as the GFR value determines the stage of chronic kidney disease (CKD) and is associated with mortality and cardiovascular morbidities. Equations to estimate GFR have limitations.

**Methods:** We combined variables for the estimation of GFR (eGFR) with a reduced set of measurements of the marker iohexol (mGFR). In a population based sample of 570 subjects (70 ys +) from the Berlin Initiative Study (BIS) we investigated 3 types of GFR equations: (1) the BIS1 and BIS2 equations based on age, gender, serum creatinine, and serum cystatin C, (2) equations based on one or two iohexol measurements, and (3) equations based on the combination of BIS1 or BIS2 with additional iohexol measurements. Thus, our approaches combine estimation and (reduced) measurement of GFR (labeled "em GFR"). We investigated strategies in which only a subset undergoes iohexol measurements. Reference standard was based on eight iohexol measurements. The cut off of 60 mL/min per 1.73 m$^2$ was chosen to assess accuracy. Equations were constructed in a learning sample (n = 285) and validated in an independent sample (n = 285).

**Results:** Misclassification rates were 17.2% (BIS1), 11.6% (BIS2), 12.6% (iohexol$_{240}$, iohexol measurement 240 min), 10.5% (iohexol$_{300}$), 4.9% (iohexol$_{300}$ combined with BIS1), and 5.3% (iohexol$_{300}$ combined with BIS2). Using iohexol only in subjects with eGFR$_{BIS1}$ between 50 and 70 mL/min per 1.73 m$^2$ (52%) led to a misclassification rate of 5.6%.




**Conclusions:** Existing eGFR equations may benefit from the addition of a single iohexol measurement. Strategies for the determination of GFR lead to a relevant increase of diagnostic validity.

*Funding:* Private Foundation Support

## TH-PO278

**Assessment of Estimated Glomerular Filtration Rate Using Creatinine and Cystatin C in Patients with Rheumatoid Arthritis**

Mitsuhiro Kawano,[1] Ryo Inoue,[2] Yukiko Tani,[2] Yasushi Kakuchi,[2] Hiroshi Fujii,[1] Kazunori Yamada.[1] *[1]Div of Rheumatology, Kanazawa Univ Hospital, Kanazawa, Japan; [2]Div of Internal Medicine and Rheumatology, Ishikawa Prefectural Saiseikai Kanazawa Hospital, Kanazawa, Japan.*

**Background:** The estimated glomerular filtration rate (eGFR) using creatinine (Cr) may be overestimated in patients with rheumatoid arthritis (RA) due to their loss of muscle mass. Recently, Cystatin C (CysC) has been focused on as a useful marker of renal function. This prompted us to clarify the factors causing discrepancies between eGFR using Cr (eGFRcr) and that using CysC (eGFRcys).

**Methods:** 245 RA patients were enrolled in this study. We calculated eGFRcr/eGFRcys as a marker of the discrepancy of measurement of renal function. We divided patients into two groups: group A with a ratio<0.8, n=39, and group B with a ratio ≥0.8, n=206. We searched for associated factors, such as age, body weight, disease duration, and serum albumin.

**Results:** In no patients was a significant difference noted between eGFRcr and eGFRcys. The average age (years), body weight (kg), disease duration (month), serum albumin (mg/dl), and SDAI of the patients in group A were 70.6, 51.3, 190.3, 3.9, and 10.7 respectively, while those in group B were 61.0, 57.0, 110.0, 4.1, and 6.1, respectively, showing significant differences between the two groups.

**Conclusions:** The renal function of the patients with high age, low body weight, long disease duration, malnutrition and high disease activity was overestimated by eGFRcr. This should be kept in mind when assessing renal function in RA patients.

## TH-PO279

**Comparison of CKD-EPI Creatinine and Cystatin C-Based Formula and Simple Cystatin C Formula in Patients with Moderate Decreased GFR**

Nina Hojs,[1] Sebastjan Bevc,[1] Radovan Hojs,[1] Robert Ekart,[2] Maksimiljan Gorenjak,[3] Ludvik Puklavec.[4] *[1]Dept of Nephrology, Univ Clinical Centre, Maribor, Slovenia; [2]Dept of Dialysis, Univ Clinical Centre, Maribor, Slovenia; [3]Dept of Clinical Chemistry, Univ Clinical Centre, Maribor, Slovenia; [4]Dept of Nuclear Medicine, Univ Clinical Centre, Maribor, Slovenia.*

**Background:** New KDIGO guidelines suggest subdividing of stage 3 CKD (GFR=30-59 ml/min/1.73m$^2$) into stages 3A and 3B because a marked increase of all-cause and cardiovascular mortality is seen at GFR <45 ml/min/1.73m$^2$. Recently, serum cystatin C ($S_{cys}$)-based formulas and the creatinine & cystatin C-based formula (CKD-EPI creatinine & cystatin formula) were proposed. The aim of our study was to compare CKD-EPI creatinine & cystatin formula and simple $S_{cys}$ formula (100/$S_{cys}$) against 51CrEDTA clearance in patients with stage 3 CKD.

**Methods:** 151 adult Caucasians (75 women, 76 men; mean age 60.6 years) were included. In each patient 51CrEDTA clearance, serum creatinine ($S_{crea}$; IDMS traceable method) and $S_{cys}$ (immunonephelometric method) were determined. GFR was calculated using the CKD-EPI creatinine & cystatin formula and simple $S_{cys}$ formula.

**Results:** Mean 51CrEDTA clearance was 42.7±8.7 ml/min/1.73m$^2$, mean $S_{crea}$ 1.9±0.7 mg/dl, mean $S_{cys}$ 2.1±0.7 mg/l. Correlations between 51CrEDTA clearance and both formulas were found (P<0.0001). In ROC analysis (cut-off for GFR 45 ml/min/1.73m$^2$) no significant difference of diagnostic accuracy between CKD-EPI creatinine & cystatin formula and simple $S_{cys}$ formula was found (P=0.453). Bland and Altman analysis for the same cut-off showed that CKD-EPI creatinine & cystatin formula underestimated (bias: -10.1 ml/min/1.73m$^2$) and simple $S_{cys}$ formula (bias: 11.8 ml/min/1.73m$^2$) overestimated measured GFR. Both equations lacked precision. Analysis of ability to correctly predict patient's GFR below or above 45 ml/min/1.73m$^2$ showed similar ability for simple $S_{cys}$ formula (66.2%) and CKD-EPI creatinine & cystatin formula (66.9%).

**Conclusions:** Our results indicate that simple $S_{cys}$ formula is a reliable marker of GFR in stage 3 CKD patients and is comparable to CKD-EPI creatinine & cystatin formula.

## TH-PO280

**The Correlation and Comparison among Three Methods for GFR Measurement: Renal Inulin Clearance, Systemic Inulin Clearance, and 51Cr-EDTA Plasma Clearance**

Ran-Hui Cha,[1] Hajeong Lee,[2] Dong Ki Kim,[2] Yon Su Kim.[2] *[1]National Medical Center, Seoul, Korea; [2]Seoul National Univ Hospital, Seoul, Korea.*

**Background:** Renal inulin clearance with continuous infusion method has been recognized as the gold standard for the measurement of glomerular filtration rate (GFR). However, inconvenience and complications associated with the method led to the development of alternative methods. We aimed to explore the relationships among three different methods for GFR measurement.

**Methods:** Three different tests including renal inulin clearance with continuous infusion method (gold standard), systemic inulin clearance with a single injection method (standard protocol over 4 hours and extended protocol over 24 hours), and plasma $^{51}$Cr-EDTA clearance over 5 hours were performed in each subject to measure GFR. Total of 114 participants had all of three tests.

**Results:** There was a strong correlation between systemic inulin clearance and renal inulin clearance with a single injection method ($\gamma$=0.89, P<0.001), but renal inulin clearance overestimated systemic inulin clearance in overall. There was a very strong correlation between systemic inulin clearance measured by standard protocol and extended protocol ($\gamma$=0.99, P<0.001), and the difference between them was very small. The strong correlation between gold standard method and systemic inulin clearance with single injection method (standard protocol) was observed ($\gamma$=0.89, P<0.001). The systemic inulin clearance (standard protocol) underestimated renal inulin clearance, in general (correlation coefficient:0.89; slope:0.64). Plasma $^{51}$Cr-EDTA clearance also had a very strong correlation with gold standard method ($\gamma$=0.92, P<0.001), and overestimated the renal inulin clearance on the whole (correlation coefficient:0.92; slope:0.81).

**Conclusions:** Renal inulin clearance overestimated systemic inulin clearance using a single injection method in overall. Alternative methods for GFR measurement such as systemic inulin clearance with a single injection method and plasma $^{51}$Cr-EDTA clearance correlated well with gold standard, even though those alternatives somewhat underestimated and overestimated gold standard, respectively.

## TH-PO281

**Correlation between MDRD-4 and CKD-EPI Formulas to Estimate Glomerular Filtration Rate in Hypertensive and Type 2 Diabetic Patients**

Victor Omar Frías-Navarro, Laura Cortes-Sanabria, Alfonso M. Cueto-Manzano, Héctor R. Martínez Ramírez, Erika Fabiola Gómez-García, Blanca Liliana Maldonado-Ruiz, Roberto A. Atilano Maciel. *Medical Research Unit in Kidney Diseases, Mexican Institute of Social Security, Guadalajara, Jalisco, Mexico.*

**Background:** The simplified equation of the MDRD-4 is one of the most used formula to estimate GFR. More recently, CKD-EPI has been developed suggesting a better performance especially in the case of normal kidney function. Notwithstanding, few data are available regarding the correlation of such formulas in diabetic and hypertensive hispanic populations.

**Methods:** Cross sectional study performed in 3 out of 24 randomly selected primary healt-care units. From these units, patients with DM2 and/or hypertension (diagnosed and treated by family physicians) were randomly selected and invited to participate. Serum creatinine was measured by Jaffe method, and GFR was estimated with the simplified MDRD and CKD-EPI formulas.

**Results:** 1480 patients, mean age 60±11 years, were included. There were 936 (63%) women, and 519 (35%) patients with DM2, 615 (42%) with DM2+H, and 346 (23%) hypertensives only. The prevalence of CKD by the MDRD-4 formula was 13.6% and 13.9% with the CKD-EPI formula. Correlation between MDRD-4 and CKD-EPI in the whole sample was r= 0.886 (CI95% 0.72-0.92), whereas it was r=0.94.8 (CI95% 0.92-0.97) in DM2; r=0.912 (CI95% 0.85-0.94) in DM2+hypertension, and r=0.939 (CI 95% 0.93-0.99) in hypertensives only.

| Characteristics | DM2 (n=519) | DM2/H (n=615) | Hypertension (n=346) |
|---|---|---|---|
| Age, years | 57 ± 12 | 63 ± 10* | 60 ± 11* † |
| Female, % | 57 ± 12 | 411 (67)* | 248 (72) † |
| Evolution, years | | | |
| DM2 | 8 (3-13) | 10 (4-15) | - |
| Hypertension | - | 7 (3-13) | 8 (3-13) |
| SBP, mmHg | 124 ± 18 | 137 ± 20* | 135 ± 19* |
| DBP, mmHg | 74 ± 10 | 80 ± 11* | 80 ± 10* |
| BMI, kg/m$^2$ | 28 ± 4 | 31 ± 6* | 31 ± 5* |

**Conclusions:** The prevalence of CKD was similar when calculated by the MDRD-4 or the CKD-EPI formulas. There is a strong correlation between these two formulas in hypertensives patients without DM2, and in diabetic patients with or without hypertension. Any of these formulas could be used interchangeably to estimate GFR in hispanic populations with these diseases.

## TH-PO282

**Performance of Japanese and CKD-EPI GFR Equations in Japanese Subjects** Yoshinari Yasuda, Imai Junko, Shoichi Maruyama, Seiichi Matsuo. *Nephrol & CKD Initiatives, Nagoya Univ, Nagoya, Japan.*

**Background:** Japanese GFR equations based on serum creatinine (Scr) (Eqcr), serum cystatin C (Scys) (Eqcys) and average value of eGFRcr and eGFRcys (Eqave) were developed in 413 Japanese and validated in 350 Japanese participants, however they have not well evaluated in another validation set. Thus, their performances were analyzed in comparison with CKD-EPI equations based on Scr (CKD-EPIcr), Scys (CKD-EPIcys) and Scys in combination with Scr (CKD-EPIcr-cys) in this study.

**Methods:** Study subjects were 291 consecutive Japanese patients examined GFR by inulin renal clearance in Nagoya University Hospital. Standardized Scr was measured by enzymatic method. Standardized Scys was measured by colloidal gold immunoassay. Performance of Japanese equations (Eqcr, Eqcys and Eqave) were compared with CKD-EPI equations (CKD-EPIcr, CKD-EPIcys and CKD-EPIcr-cys), respectively.

**Results:** Age (mean +- SD, years old) and gender distributions (male %) were 59.7 +- 13.5, 67% in 183 CKD, 64.0 +- 13.5, 80% in 86 malignancy before uni-nephrectomy and 61.0 +- 7.9, 40% in 22 renal transplant donor. GFR (mean +- SD, ml/min/1.73 m2) were 47.6 +- 24.7 in CKD, 61.3 +- 25.1 in malignancy and 74.2 +- 18.1 in donor. 15% and 30% accuracy, correlation and root mean square error (RMSE) were shown in Table.

| | 15% accuracy | 30% accuracy | correlation | RMSE |
|---|---|---|---|---|
| Eqcr | 50.1 | 74.6 | 0.862 | 13.82 |
| Eqcys | 48.1 | 75.6 | 0.876 | 13.43 |
| Eqave | 55.0 | 77.0 | 0.892 | 12.32 |
| CKD-EPIcr | 19.9 | 47.1 | 0.881 | 22.31 |
| CKD-EPIcys | 47.4 | 74.9 | 0.882 | 14.44 |
| CKD-EPIcr-cys | 29.6 | 46.0 | 0.902 | 22.57 |

15% and 30% accuracy were better in Eqave compared to other equations. And CKD-EPIcr and CKD-EPIcr-cys equations overestimated GFR.

**Conclusions:** Japanese and CKD-EPIcys GFR equations performed well among Japanese patients.

*Funding:* Government Support - Non-U.S.

## TH-PO283

**Regional Differences in Chronic Kidney Disease Prevalence in Japan: A Japanese Nationwide Health-Check Study** Yoshinari Yasuda,[1] Kiyoshi Shibata,[1] Kunitoshi Iseki,[2] Toshiki Moriyama,[2] Kunihiro Yamagata,[2] Kazuhiko Tsuruya,[2] Hideaki Yoshida,[2] Shouichi Fujimoto,[2] Koichi Asahi,[2] Tsuyoshi Watanabe,[2] Seiichi Matsuo.[1] *[1]Nephrology/CKD Initiatives, Nagoya Univ, Nagoya, Japan; [2]Research on the Positioning of CKD in Specific Health, MHWL, Japan.*

**Background:** Regional variations in the increasing rate of End Stage Kidney Diseases (ESKD) was reported in Japan, however, factors associating these regional differences have not been fully elucidated. In this study, prevalence of Chronic Kidney Disease (CKD) and its risk factors were analyzed in a Japanese nationwide database with a focus on the regional differences.

**Methods:** Study subjects were 386,517 (163,454 male) participants in a Japanese nationwide health-check including 13 prefectures. Prevalence of CKD and risk factors, including hypertension (HTN), diabetes mellitus (DM), dyslipidemia (DL) and obesity (OB), were analyzed in 4 regions divided by the increasing rate of ESKD as follows; the highest (H), 2 middle (M1 and M2) and the lowest (L) areas. CKD was defined as an estimated glomerular filtration rate less than 60 mL/min/1.73 m$^2$ and/or proteinuria greater than 1+ by a dipstick method. Odds ratios for CKD were analyzed in 4 areas. Regional differences in optimal treatment rate in HTN, DM and DL were assessed according to each guideline.

**Results:** CKD prevalence in H, M1, M2 and L areas were 21.4%, 25.5%, 20.9% and 18.5% in male and 18.6%, 15.7%, 16.4% and 11.4% in female, in good agreement with the increasing rate of ESKD. Odds ratios for CKD were significantly high in HTN, DM and OB in all 4 regions. Prevalence of HTN was significantly high in L area, however, the rate of under treatment in HTN and good blood pressure control rate were significantly high in L area. In H area, the rate of no treatment was the highest among 4 areas in HTN, DM and DL.

**Conclusions:** Association between regional variations in CKD prevalence and those in the increasing rate of ESKD was demonstrated. Although HTN, DM and OB were risk factors for CKD in all 4 areas, the rate of under treatment and good control rate in HTN and DM may affect regional differences.

*Funding:* Government Support - Non-U.S.

## TH-PO284

**Validation of Korean Coefficient for GFR Estimation by the Modification of IDMS MDRD Equations** Yun Jung Oh,[1] Ran-Hui Cha,[2] Chungsik Lee,[1] Hajeong Lee,[3] Dong Ki Kim,[3] Yon Su Kim.[3] *[1]Internal Medicine, Cheju Halla General Hospital, Jeju, Korea; [2]Internal Medicine, National Medical Center, Seoul, Korea; [3]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea.*

**Background:** Race and ethnicity are important determinants of estimating glomerular filtration rate(GFR). Korean coefficients for the isotope dilution mass spectrometry(IDMS) Modification of Diet in Renal Disease(MDRD) equations were developed in 2010, however, those coefficients have not been validated thoroughly yet. We validated performance of Korean coefficients for IDMS MDRD equations from previous study, and derived new Korean coefficients using both development and validation study data.

**Methods:** Equation development and validation were performed in separate groups(development group; N=147 from 2008 to 2009, validation group; N=125 from 2010 to 2012). We compared the performance of IDMS MDRD equations and modified equations with Korean coefficients. And the performance was assessed by correlation coefficient, bias, and accuracy between estimated GFR(eGFR) and reference GFR(rGFR; systemic inulin clearance with a single injection method).

**Results:** We found strong correlation between eGFR calculated by modified equations with Korean coefficients and rGFR($\gamma$=0.89 and 0.89 for modified 4 and 6 variable IDMS MDRD equation, P<0.001, P<0.001, respectively), but it was not different from the correlation coefficients between eGFR calculated by IDMS MDRD equations and rGFR. Despite the modified equations performed with less bias than original IDMS MDRD equations, overall performance was not significantly different from each other showing a similar accuracy. New Korean coefficients for 4 and 6 variable IDMS MDRD equations were 1.020 and 0.973, respectively. The overall performance of new Korean IDMS MDRD equations was not different from original IDMS MDRD equations, either.

**Conclusions:** Korean coefficients for IDMS MDRD equations developed before showed good performance in the validation study group. However, there was no significant difference between original and modified IMDS MDRD equation with new Korean coefficient in terms of performance of GFR estimation after all.

*Funding:* Government Support - Non-U.S.

## TH-PO285

**Prevalence of Chronic Kidney Disease in Europe** Katharina Brueck,[1] Vianda S. Stel,[1] Christoph Wanner,[2] Wim Van Biesen,[3] Charles Tomson,[4] Giovanni Tripepi,[5] Carmine Zoccali,[5] Kitty J. Jager.[1] *[1]ERA-EDTA Registry, Netherlands; [2]Univ Hospital Würzburg, Germany; [3]Ghent Univ Hospital, Belgium; [4]Southmead Hospital, United Kingdom; [5]CNR-IBIM, Italy; [6]On behalf of the European CKD Burden Consortium.*

**Background:** The wide variation in definitions for prevalence of chronic kidney disease (CKD) complicates attempts to compare the burden of CKD between countries. The aim of our study was to examine the prevalence of CKD in all stages in subjects not on renal replacement therapy across European countries, using standardized definitions.

**Methods:** A literature review was performed to identify all European studies that could provide data on CKD prevalence. The representatives of eligible studies received a questionnaire on the availability of data and health care system characteristics. Based on available data and the new KDIGO practice guideline, standardized definitions for CKD prevalence were defined as presence of albuminuria >= 30mg/g and/or eGFR by CKD-EPI formula <60 ml/min/1.73m² without the chronicity criterion (as only 4 studies had these data). These definitions were used to extract the prevalence of CKD for various subgroups (e.g. age group, gender and diabetic status). All prevalence data were age and sex standardized to the EU27 population.

**Results:** Out of 71 studies, we so far received data on prevalence from 16 studies from 11 countries. The age and sex standardized prevalence of CKD stage 3-5 in the age group 20-74 years ranged from 1% in central Italy to 5.4% in Portugal. In this age group, the CKD stage 3-5 prevalence ranged from 1.3-6.4% in females and 0.8-4.2% in males. In the age group 65-74 years, this prevalence was 4.8% in central Italy, 11.4% in Finland, 13.3% in Poland, 19.7-21.5% in Germany, 22.3% in the Netherlands and 24.3% in Portugal.

**Conclusions:** This is the first study on international differences in the prevalence of CKD using standardized definitions. The prevalence of CKD varied hugely across European countries. Data on co-morbidities and health care system characteristics will be used to explain the international differences in CKD prevalence. Together they will provide a complete overview of the burden of CKD in Europe.

## TH-PO286

**Estimated Glomerular Filtration Rate and Urine Albumin Creatinine Ratio Category-Specific Prevalence and Descriptions of People with Type 2 Diabetes in the U.S.** Robert A. Bailey,[1] Yiting Wang,[2] Vivienne J. Zhu,[2] Marcia Rupnow.[1] *[1]Janssen Scientific Affairs, LLC, Raritan, NJ; [2]Janssen Research and Development, LLC, Titusville, NJ.*

**Background:** Kidney Disease: Improving Global Outcomes (KDIGO) updated classification of chronic kidney disease (CKD) in 2013, using both estimated glomerular filtration rate (eGFR) and urine albumin creatinine ratio (ACR). However, little is known about eGFR and/or ACR category-specific prevalence among people with type 2 diabetes (T2D) using this new classification.

**Methods:** We used the continuous National Health and Nutrition Examination Surveys (NHANES), 1999-2010, and identified participants with T2D using a definition similar to previous studies. eGFR was calculated using the CKD Epidemiology Collaboration (CKD-EPI) equation. eGFR and ACR categories were defined according to KDIGO, and prevalence was age-adjusted to the 2010 diabetes population according to the National Health Interview Survey (NHIS). We used SAS®(v9.2) survey procedures to account for the stratified, clustered sampling with unequal sampling weights in NHANES.

**Results:** The final analysis included 2,413 participants with T2D; mean age was 61.4;diabetes duration was 9.8 years. Mean (standard error) of eGFR (ml/min/1.73m$^2$) and ACR(mg/g) were 79.6 (0.7) and 107.8(1.9), respectively. The weighted eGFR and ACR category-specific prevalence is shown in Table 1.

| Weighted %(unweighted N) | ACR (mg/g) Categories | | | Row |
|---|---|---|---|---|
| eGFR (ml/min/1.73 m$^2$) Categories | A1 (<30) | A2 (30-300) | A3 (>300) | |
| G1 (≥ 90) | 27.8% (582) | 9.5% (230) | 1.5% (48) | 38.8% (860) |
| G2 (60-89) | 27.8% (637) | 8.8% (239) | 2.6% (81) | 39.2% (957) |
| G3a (45-59) | 7.7% (197) | 3.6% (104) | 1.5% (44) | 12.8% (345) |
| G3b (44-30) | 2.9% (72) | 2.1% (59) | 0.9% (29) | 5.9% (160) |
| G4 (15-29) | 0.7% (19) | 1.2% (27) | 0.8% (28) | 2.7% (74) |
| G5 (<15) | 0.05% (2) | 0.0% (1) | 0.33% (14) | 0.4% (17) |
| Column | 66.9% (1,509) | 25.2% (660) | 7.9% (244) | 100% (2413) |

**Conclusions:** Our study is the first to provide estimates of eGFR and ACR category-specific prevalence for T2D based on the KDIGO classification in a nationally representative sample.

*Funding:* Pharmaceutical Company Support - Janssen Scientific Affairs, LLC

## TH-PO287

### Detection of Subclinical Chronic Kidney Disease (CKD), in Relatives of Dialysis Patients and Cardiovascular Disease Patients in Chile

Alvaro A. Castillo,[1,2] Mauricio J. Castillo,[2] Domingo Lancelloti,[2] Alejandra Lagos.[2] *[1]Dialysis Unit, Hosp. La Serena, La Serena, Chile; [2]Salud Publica, Faculty Medicine, U. Catolica Norte, Coquimbo, Chile.*

**Background:** CKD is a serious public health problem. It can be prevented with early detection and treatment. Our objective is to determine the prevalence of sub-clinical CKD in different risk groups in Chile.

**Methods:** Performed in 3 regions of Chile (Coquimbo, Santiago, Valdivia). Groups analyzed: 1) Primary Health Care (PHC) consultants without cardiovascular risk factors (CON), 2) Patients from Cardiovascular (CV) program of PHC (PHC-CV) 3) Family ≥ 18 y. (parent-brother-children) of dialysis patients (non genetic etiology) (FAM). Aproved by Ethical Committee. 1,143 subjects signed informed consent, from 12 health centers. Survey applied based on: Chilean CKD Guideline Questionnaire, blood pressure measurement, weight, height, lab tests: Creatinine, MicroAlbuminuria/Creatininuria Ratio (ACR), Urine dipstick. eGFR using MDRD-4 equation. Classification of CKD and ACR according to NKF-KDOQI.

**Results:**

| RESULTS | CON n | CON% | PHC-CV n | PHC-CV% | FAM n | FAM% | p value |
|---|---|---|---|---|---|---|---|
| Number | 324 | | 356 | | 463 | | |
| Female | 198 | 61 | 249 | 70 | 311 | 67 | |
| Mean Age (SD) | 40.2(13) | | 58.8(14.1) | | 41.2(14.6) | | <0.0001 |
| Declared Diabetes | 1 | 0.3 | 115 | 32.3 | 45 | 9.7 | <0.001 |
| Declared Hypertension | 4 | 1.2 | 298 | 83.7 | 120 | 25.9 | <0.001 |
| Measured Hypertension | 17 | 5.3 | 93 | 26.1 | 103 | 22.2 | <0.001 |
| CKD stage 3 | 4 | 1.4 | 23 | 7.1 | 8 | 1.8 | |
| CKD stage 4 | 0 | | 4 | 1.2 | 1 | 0.2 | |
| CKD stage 5 | 0 | | 0 | | 0 | | |
| ACR 30 - 300 | 10 | 3.3 | 41 | 12.9 | 42 | 9.3 | |
| ACR > 300 | 3 | 1 | 14 | 4.4 | 9 | 2 | |

**Conclusions:** PHC-CV and FAM showed high prevalence of CKD and abnormal ACR, significantly higher than CON. Early detection of CKD should focus on these groups. FAM is easy to access through the relative on dialysis. In Chile 18,000 patients are on dialysis, data allows estimating that 8,000 FAM would suffer CKD, currently undetected. FAM presents high prevalence of hypertension, ACR and CKD, despite being significantly younger than PHC-CV.

Funded by FONIS-CONICYT (SA10I20040).

*Funding:* Government Support - Non-U.S.

## TH-PO288

### Prevalence of Chronic Kidney Disease and Metabolic Syndrome in an Adult Korean: Result from the Korean National Health Screening

Yong Un Kang, Joon Seok Choi, Chang Seong Kim, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Dept of Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea.*

**Background:** Chronic kidney disease (CKD) and metabolic syndrome (MS) are increasing public health problems. However, there has been limited data on CKD and MS prevalence, and its relationship in large based-population. This study was aimed to examine the prevalence of CKD and MS, and the association between MS and its components with CKD in Korea.

**Methods:** We performed a cross-sectional study in non-institutionalized civilians using the general health screening data of 10,549,230 (47.2±13.9 years, men 56.3%) participants aged more than 20 years from the Korean National Health Screening 2011. CKD was defined as dipstick proteinuria or an estimated GFR (eGFR) of <60 ml/min per 1.73 m$^2$.

**Results:** The prevalence of CKD (n=688,182) was 6.52% (5.75% in men, 7.52 in women). The prevalence estimates of CKD stage, respectively, were 0.86% for stage 1; 0.95% for stage 2; 3.74% for stage 3; 0.1% for stage 4; 0.86% for stage 5. 23.5% of the study population (n=2,477,247) had MS (12.1% for central obesity; 12.5% for high triglyceride; 5.9% for low HDL cholesterol; 15.5% for high blood pressure; 5.9% for high fasting glucose). 41.1% of the study population with CKD (n=282,551) had MS (11.4% for central obesity; 8.2% for high triglyceride; 5.1% for low HDL cholesterol; 17.3% for high blood pressure; 4.4% for high fasting glucose). In multivariate-adjusted analysis, presence of MS was associated with an increased risk for CKD (odds ratio=1.458, 95% confidence interval 1.450–1.467, $P < 0.0001$). Also, the individual components were associated with increased risk for CKD.

**Conclusions:** MS and the individual components predict the risk of prevalent CKD.

## TH-PO289

### The Relationship between eGFR and Fasting Blood Sugar in a Multiethnic Adolescent Population: National Health and Nutrition Examination Survey Data 2001-2003

Gangadarshni Chandramohan,[1] Kamyar Kalantar-Zadeh,[3] Magda Shaheen.[2] *[1]Pediatrics, Horbor-UCLA Medical Center, Torrance, CA; [2]Internal Medicine, Charles Drew Univ Research Center, Los Angeles, CA; [3]Internal Medican & Pediatrics, Univ of California: Irvine, Irvine, CA.*

**Background:** Prevalence of type II diabetes (DM), is high among minority children. In type II DM, the early sigh of renal injury is increase in eGFR due to hyperfiltation.

**Methods:** A retrospective cross-sectional analysis of children between the ages of 13-17 years from the National Health and Nutrition Survey (NHANES) obtained from 2004-2005, was performed. Abnormal fasting blood sugar (FBS) was defined using the standard guidelines. HIgh eGFR was defined as the 4th quartile sGFR of the study population. Statistical analysis were done using SUDAAN and SAS software program.

**Results:** There were 1149 children, high FBS 10%. Among Whites, AA and Hispanics, high eGFR noted in 25%, 18% and 13% (p=0.009), respectively. High FBS found in 10, 11, 10% in White, AA and Hispanics respectively. The mean eGFR differed significantly between the ethnic groups. Odds ratio to have high eGFR was high among Whites and Blacks with high FBS. Table 1: The odds ratio for having 4th quartile eGFR Based on FBS among the Total population and by Ethnicity.

| Variables | Odds Ratio (95% CI) | p-Value |
|---|---|---|
| Total population with Abnl. FBS vs. nl FBS | 3.1 (2.2 - 4.3) | |
| Whites with abnl FBS vs. nl FBS | 3.5 (2.2 - 5.7) | 0.0001 |
| AA with abnl FBS vs. nl FBS | 2.9 (1.5 - 5.5) | 0.001 |
| Hispanic with abnl FBS vs. nl FBS | 2.1 (1.0 - 4.5) | 0.05 |

**Conclusions:** Prevalaence of high eGFR was significantly high among Hispanic than other ethnic groups. Mean eGFR was significantly higher among Hispanic than AA and Whites. Analyzing FBS independently in each ethnic groups revealed an interesting finding among adolescent children, excibiting an association with high eGFR. Therefore, in determing eGFR in children with abnoraml FBS may shed light on future management in regards to the aggressiveness of the intervention, with a potential role for ACE inhibition, even before the development of microalbuminuria.

*Funding:* Other NIH Support - National Institutes of Health: NIMHD grants P20MD000182 and U54MD007598 (formerly U54RR26138)

## TH-PO290

### Epidemiology of Autosomal Dominant Polycystic Kidney Disease in the United States

Cynthia J. Willey,[1] Frank S. Czerwiec,[2] Holly Krasa,[2] Robert D. McQuade,[2] Robert W. Schrier,[3] Vicente E. Torres.[4] *[1]Pharmacy Practice, Univ of RI, Kingston, RI; [2]Otsuka Pharmaceutical, Rockville, MD; [3]Univ of Colorado, Aurora, CO; [4]Mayo Clinic, Rochester, MN.*

**Background:** Most studies of PKD in the U.S. focus upon small cohorts of patients and have limited generalizability. Only one population-based study examined US incidence of ADPKD using data from Olmsted County, Minnesota from 1935 to 1980. Our study reviewed current literature and analyzed national data to estimate total and diagnosed prevalence of APDKD in the U.S.

**Methods:** Diagnosed and total prevalence were derived using incidence rates from Iglesias et al based on symptomatic or screened cases compared with the addition of cases diagnosed at autopsy. Diagnosis rates were then increased by 25% to reflect recent improvements in diagnostic imaging technology. Data from the U.S. Renal Data System (USRDS) were used to estimate average survival time. Validation analyses were performed using the National Ambulatory Medical Care Survey (NAMCS) to examine consistency between expected patients in the U.S. with ADPKD and the annual number of ambulatory visits for patients with ADPKD.

**Results:** USRDS data suggest an average duration of disease of approximately 61.8 years for adult patients with ADPKD. Taken with incidence rates from the Rochester Epidemiology Project, a total prevalence rate of 0.46 per 1,000 was calculated with an estimate that 95% of those are currently diagnosed. Validation analysis using data from the NAMCS focused on probable ADPKD and included patients with autosomal dominant or unspecified polycystic kidney disease (ICD9 CM: 753.12-753.13). Results were consistent with 97,667 diagnosed cases estimated from the NAMCS and 116,228 estimated using data from the Rochester Epidemiology project.

**Conclusions:** Although total prevalence (including undiagnosed cases) will be slightly higher, these data support a diagnosed prevalence of approximately 1/2000 and yield an estimate of less than 200,000 diagnosed ADPKD cases in the U.S.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical

## TH-PO291

### Switching from Modification of Diet in Renal Disease to Chronic Kidney Disease Epidemiology Collaboration Formula Has Negligible Impact on Staging in an Established Chronic Kidney Disease Population

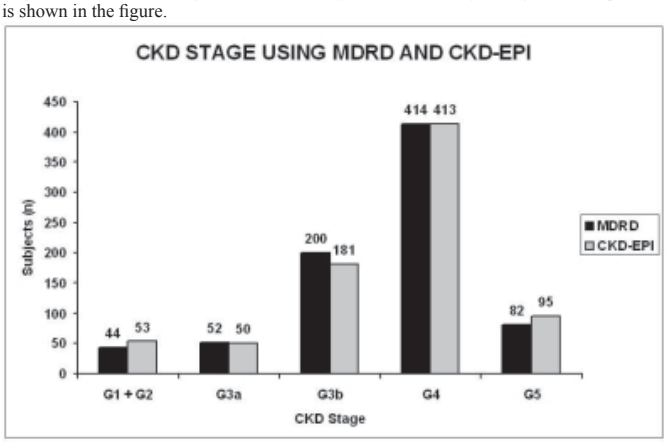
Eduard A. Iliescu,[1,2] Sandra Belanger,[2] M. Khaled Shamseddin,[1,2] Sarah Aloudat,[1,2] Christine A. White.[1,2] *[1]Queen's Univ; [2]Kingston General Hospital, Kingston, Canada.*

**Background:** The 2012 Kidney Disease Improving Global Outcomes (KDIGO) Guidelines recommend the CKD-EPI formula to calculate eGFR from serum creatinine (1B). The aim of this study is to examine the practical impact of switching from MDRD

to CKD-EPI formula on the staging of chronic kidney disease (CKD) in the region of South-Eastern Ontario (pop. 485,000).

**Methods:** This is a cross-sectional study of prevalent CKD patients followed at Kingston General Hospital. This is the only CKD clinic in the region and manages all referred cases of CKD with few exceptions. The primary variables were serum creatinine, and eGFR calculated using the CKD-EPI and MDRD formulae (ml/min/1.73m$^2$). The number of patients in each KDIGO CKD stage with the two equations was compared (G1 > 90, G2 = 60 – 89, G3a = 45 - 59, G3b = 30 - 44, G4 = 15 – 29, and G5 < 15 ml/min).

**Results:** There were 792 subjects, 42 % female, 0 % African Canadian, mean age 71 years. Looking at individual subject classification, compared to MDRD, CKD-EPI classified subjects to the same stage in 91 %, a less severe stage in 4 %, and a more severe stage in 5 %. The resulting distribution of subjects by CKD stage using the two equations is shown in the figure.




**Conclusions:** The results of this study suggest that, in an established CKD population, the impact of the CKD-EPI vs. MDRD formula in the staging of CKD is negligible. As expected there was a net increase in the number of subjects classified to G1 + G2, but there was also a net increase subjects in Stage G5 which could impact CKD clinic resource planning. The strength of this study is the regional perspective reducing selection bias. Generalizability is limited by lack of racial diversity.

## TH-PO292

**Prevalence of CKD and Associated Risk Factors in Italy: The CARHES Study** Luca De Nicola,[1] Chiara Donfrancesco,[2] Roberto Minutolo,[1] Cinzia Lo Noce,[2] Luigi Palmieri,[2] Amalia De Curtis,[3] Licia Iacoviello,[3] Giuseppe Conte,[1] Diego Vanuzzo,[4] Simona Giampaoli.[2] *[1]Italian Society Nephrology; [2]National Institute Health; [3]Catholic Univ Campobasso; [4]National Association of Cardiogists-ANMCO, Italy.*

**Background:** Identification of CKD and associated cardiovascular (CV) comorbidities is a key priority for public health worldwide. No study however has addressed this issue in a nationally representative survey in Europe.

**Methods:** The CArdiovascular risk in Renal patients of the 2008-12 national Health Examination Survey (CARHES) examined random samples of Italian general population aged 35-79 years, stratified by age and gender, in all regions (220 persons/1,5 million of residents; response rate 56%). CKD was defined as urinary albumin:creatinine ratio ≥30 mg/g (stage 1-2) or estimated GFR (CKD-EPI equation-enzymatic assay of serum creatinine) <60 mL/min/1.73m$^2$ (stage 3-5). Biochemical tests were centralized.

**Results:** 3848 men and 3704 women were examined. Table shows CKD prevalence overall and by stage, % (95%CI).

| | Total | Men | Women |
|---|---|---|---|
| **CKD all** | 7.1(6.5-7.7) | 7.5(6.7-8.4) | 6.5(5.8-7.4) |
| **1** | 2.6(2.3-3.0) | 2.7(2.2-3.2) | 2.6(2.1-3.2) |
| **2** | 1.5(1.3-1.8) | 2.1(1.7-2.6) | 0.9(0.6-1.3) |
| **3a** | 2.1(1.8-2.5) | 2.0(1.5-2.4) | 2.3(1.8-2.8) |
| **3b** | 0.5(0.4-0.7) | 0.5(0.3-0.7) | 0.5(0.3-0.8) |
| **4** | 0.2(0.1-0.3) | 0.2(0.1-0.4) | 0.1(0.0-0.3) |
| **5** | 0.1(0.0-0.2) | 0.1(0.0-0.3) | 0.1(0.0-0.3) |
| **Estimated CKD population (N)** | 2,194,572 | 1,090,124 | 1,104,448 |

Stage 1-2 constituted 58% of CKD cohort. CKD prevalence increased with ageing (age 35-49: 2.7%; 50-59: 3.4%; 60-69: 8.7%; 70-79: 17.0%). As compared to No CKD, CKD subjects were older (66±11 vs 56±12 y), had higher albuminuria, fasting glucose, triglycerides, systolic BP, BMI and waist circumference while total and HDL cholesterol were lower. CKD was also associated with higher prevalence of hypertension (75 vs 48%), diabetes (29 vs 10%), obesity (39 vs 25%) and CV disease (7.1 vs 1.8%).

**Conclusions:** In Italy CKD is characterized by lower prevalence as compared to other countries, predominance of early stages and higher CV risk profile vs No CKD due, at least in part, to the older age.

## TH-PO293

**Thyroid Function and Chronic Kidney Disease in a Large Canadian Survey** Alexis Payette, Jacobien Verhave, Josee Bouchard, Barbara Duda, Francois Madore, Stephan Troyanov. *Div of Nephrology and Endocrinology, Hôpital du Sacré-Cœur, Montreal, Canada.*

**Background:** Hypothyroidism is common in patients with CKD and TSH levels are negatively correlated with eGFR in the general population. However, the effect of levothyroxine (LT) treatment on this association is uncertain.

**Methods:** We analyzed biological and clinical data from a random cohort of 20,004 participants aged 40 to 69 years from the general population. This cross-sectional study is part of the CARTaGENE data bank, a research infrastructure contributing to diagnosis, treatment and prevention of chronic diseases.

**Results:** Among 19,212 subjects with measured serum TSH and creatinine, we observed a negative correlation between TSH and eGFR in both LT treated and untreated individuals. In addition, at equal TSH, the eGFR was on average 4 mL/min/1.73 m$^2$ lower in subjects under LT:

| | Normal TSH no LT (n=16884) | Elevated TSH no LT (n=380) | Normal TSH under LT (n=1232) | Elevated TSH under LT (n=146) | Low TSH no LT (n=324) | P |
|---|---|---|---|---|---|---|
| eGFR | 88 ± 14 | 86 ± 15 | 84 ± 15 | 82 ± 15 | 91 ± 14 | <0.01 |
| eGFR<60(%) | 3.4 | 5.5 | 7.1 | 8.2 | 2.8 | <0.01 |
| Women(%) | 49 | 49 | 76 | 68 | 49 | <0.01 |
| Age | 54 ± 8 | 54 ± 8 | 57 ± 8 | 55 ± 8 | 53 ± 8 | <0.01 |
| BMI | 27 ± 5 | 28 ± 5 | 29 ± 6 | 29 ± 6 | 27 ± 5 | <0.01 |
| Diabetes(%) | 7 | 4 | 11 | 14 | 5 | <0.01 |
| Dyslipidemia(%) | 28 | 25 | 35 | 32 | 25 | <0.01 |

In reference to a normal TSH without LT, the odds ratio (95% CI) for CKD (eGFR<60 mL/min/1.73m<sup style="text-indent: 36pt;">>2[/sup]) was 1.96 (1.24-3.11) for an elevated TSH without LT, 2.2 (1.8-2.8) for a normal TSH under LT and 2.23 (1.20-4.14) for an elevated TSH under LT, after adjustment for gender, age, BMI, diabetes and dyslipidemia.

**Conclusions:** In conclusion, the negative correlation between eGFR and TSH exists in both treated and untreated subjects. In addition, at equal TSH, patients under LT therapy have a lower eGFR than untreated subjects, even after adjusting for confounders. These results suggest an association between thyroid disease and CKD regardless of current thyroid function status and therapy.

## TH-PO294

**Prevalence of Chronic Kidney Disease in the SardiNIA Study Cohort and Its Relationship to eGFR-Related Genetic Loci and Clinical Risk Factors** Antonello Pani,[1] Jennifer Bragg-Gresham,[2] Marco Masala,[3] Doloretta Piras,[1] Alice Atzeni,[1] Maria G. Pilia,[3] Liana Ferreli,[3] Lenuta Balaci,[3] Nicolò Curreli,[3] Alessandro Delitala,[3] Francesco Loi,[3] David Schlessinger,[4] Francesco Cucca.[3] *[1]Nefrologia e Dialisi, Ospedale Brotzu, Cagliari, Italy; [2]Center for Statistical Genetics, Univ of Michigan, Ann Arbor, MI; [3]IRGB, CNR, Monserrato e Lanusei, Italy; [4]Laboratory of Genetics, NIA, Baltimore, MD.*

**Background:** An increase in leading CKD risk factors explains only part of the differential prevalence seen worldwide. We aimed to measure CKD prevalence, eGFR, and their relationship with traditional and additional risk factors in a Sardinian founder population.

**Methods:** Calibration of creatinine was done to correctly estimate eGFR, that was calculated by CKD-Epi and MDRD 175 equations. A new genetic score based on 13 previously reported eGFR- and CKD-related loci was used. We performed: a cross sectional analysis to establish CKD prevalence among 4,842 individuals, and a longitudinal analysis of renal function in 4,074 of these individuals who underwent 3 visits along 10 years. Uni- and multivariable analyses were done to establish the correlation between clinical, ultrasound, and genetic variables with changes in eGFR (linear mixed models), CKD and fast vs slow eGFR decline (logistic regression analysis).

**Results:** CKD prevalence was 14.5%. Micro- and macroalbuminuria prevalence was 9.5% and 3.4%, respectively. The average decline in eGFR during the follow-up was 1 ml/min/year. Genetic risk score, old age, female gender, and hyperuricemia were independently correlated with all the outcomes. Diabetes and abnormal kidney length were associated with CKD; hypertension was correlated to fast eGFR decline. Diabetes, hypertension, and high baseline eGFR were significantly predictive of future decline of eGFR.

**Conclusions:** CKD prevalence was similar to observations in China, higher than in Norway, and lower than in the USA. Genetic risk score contributes independently of traditional CKD risk factors. Along with differences in health practice and in traditional risk factors, it may provide an entrée to further explication of differential CKD prevalence worldwide.

*Funding:* Other NIH Support - NIA

**TH-PO295**

**Prevalence of Renal Impairment (RI) among Cancer Patients with Bone Metastases in the United States** Vahakn B. Shahinian,[1] Kristina Chen,[2] Rohini Hernandez,[2] Jane Quigley,[3] Melissa Pirolli,[3] David Quach,[3] Alexander Liede,[2] Jorge Arellano.[2] *[1]Univ of Michigan Medical School, Ann Arbor, MI; [2]Amgen, Inc, Thousand Oaks, CA; [3]IMS Health, Plymouth Meeting, PA.*

**Background:** Pts diagnosed with cancer and bone mets are at risk of serious bone complications. Intravenous bisphosphonates (IVBPs) are approved for prevention of skeletal-related events, but are known to be nephrotoxic and may require dose adjustment or discontinuation in pts with RI which can result in higher rates of complications (Hatoum et al 2008). This study aimed to measure the extent of RI in cancer pts with bone mets in a real-world setting.

**Methods:** The study identified pts treated in oncology clinics across the US using the Oncology Services Comprehensive Electronic Records (OSCER), a large electronic medical record database. Pts (age ≥18) with solid tumors and bone mets diagnosed in 2008–2012 were identified, excluding pts with an ICD-9 diagnosis(dx) code for AKI, ESRD, or multiple myeloma. RI was defined by a single value of eGFR <60 mL/min/1.73m$^2$ (CKD-EPI); CKD was defined by ≥2 eGFR values <60, at least 90 days apart. Percent of pts treated with an IV BP was also examined by eGFR category.

**Results:** The cohort included 15,623 pts (mean age 67.1 ± 12 years, weight 76.5 ± 18 kg; female 56%; breast, prostate or lung cancer 83%), of whom, 49.2% had RI during a mean 23.7 months of follow-up after bone mets dx (9.7% with eGFR <30).

| Lowest eGFR | All | < 15 | < 30 | < 60 | < 90 |
|---|---|---|---|---|---|
| N (%) | 15623 | 216 (1.4) | 1516 (9.7) | 7686 (49.2) | 13779 (88.2) |
| Mean eGFR | 61.1 | 10.1 | 22.1 | 41.7 | 55.8 |
| Pt Receiving IV BP within 12 months | 65% | 35% | 51% | 62% | 65% |

Among pts with RI and at least one eGFR after 90 days, 80.7% (4,686/5,810) had CKD and 62% received an IVBP within 12 months from eGFR<60. Similarly, among pts with evidence of severe RI (eGFR <30), 51% received IVBP therapy.

**Conclusions:** These data from pts treated in community oncology clinics across the US demonstrate that nearly half of pts with bone mets had evidence of RI during the course of their disease. Despite RI, a large proportion of pts were treated with an IVBP. Careful consideration of treatment options and close monitoring of renal function in this population is imperative to achieve optimal therapeutic outcomes.

*Funding:* Pharmaceutical Company Support - Amgen, Inc

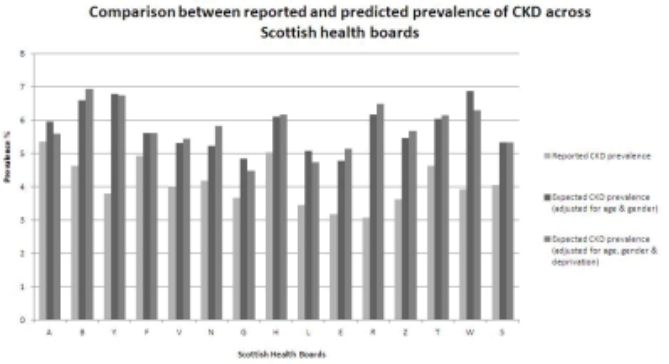
**TH-PO296**

**Understanding Variation in CKD Prevalence amongst Scottish Health Boards** Beng Hock So,[1,2] Mario D. Hair,[1] Shona Methven,[3] Alan G. Jardine,[2] Mark S. MacGregor.[1] *[1]Univ Hospital Crosshouse, Scotland; [2]Univ of Glasgow, Scotland; [3]Univ of Bristol, England.*

**Background:** Almost all people in Scotland are registered with primary care practices, which maintain registers of chronic kidney disease (CKD) stages 3-5. Registry CKD prevalence is 4.1% nationally. However, there is marked variation between the 14 Health Boards (HB) from 3.1 to 5.4%. We explored the reasons for this variation.

**Methods:** CKD prevalence, age and gender distribution for each HB was obtained from NHS Scotland. We derived age and gender standardised CKD (AGCKD) rates: all CKD stages 3-5 were identified from NHS Ayrshire & Arran's (A&A) laboratory database ( ≥18 population 313,503) with two or more eGFRs <60 mL/min/1.73m$^2$ ≥90 days apart. We applied AGCKD to the population structures of the other HBs to derive expected rates. Based on A&A data we derived a regression co-efficient to adjust CKD rates for deprivation.

**Results:** In A&A, 18,291 adults had CKD (5.83% total, 7.34% women, 4.24% men). The reported prevalence is 5.4%; the gap represents failure of ascertainment. Applying AGCKD to the population structure of each HB reduced variation. The gap between observed and expected prevalence varied from 0.6% to 3.1% and was significant (t(13)=7.53, p<0.001). At primary care practice level, AGCKD was associated with deprivation (r=0.66, p<0.001). After adjusting HB prevalence for deprivation the gap between observed and expected rates varied from 0.2% to 3.4%.




**Conclusions:** There is considerable unexplained variation in CKD rates amongst HBs. By deriving age and gender specific rates of CKD relevant to our population then adjusting for deprivation, we derived expected CKD rates for each HB, allowing benchmarking. The gap between observed and expected CKD rates may represent failure of ascertainment, or true prevalence differences due to unexplained factors in those populations and requires further exploration.

**TH-PO297**

**Chronic Kidney Disease in Pakistani Population: A Community Based Cross-Sectional Study** Saleem Jessani,[1] Muhammad Islam,[1] Rasool Bux,[1] Tazeen H. Jafar.[1,2] *[1]Aga Khan Univ, Karachi, Pakistan; [2]Duke-NUS Graduate Medical School, Singapore.*

**Background:** Chronic Kidney Dsease (CKD) is increasing being recognized as a global public health problem. However, there is dearth of information on the prevalence and determinants of CKD from low and middle income countries. The objectives of the study were to determine the prevalence and determinants of CKD and to explore the existing management of such patients with regards to blood pressure (BP) control and use of anti-hypertensive medications.

**Methods:** Glomerular filtration rate was estimated (eGFR) using the CKD-EPI equation with a correction factor (0.686 × eGFR CKD-EPI$^{1.059}$) for South Asians. CKD was defined as eGFR < 60 ml/min per 1.73m$^2$ (reduced eGFR) or urinary albumin to creatinine ratio (UACR) ≥30 mg/g (albuminuria) based on spot urine sample. Multivariable models were built for the primary outcome of CKD using logistic regression analysis. The candidate socio-demographic predictors were age, sex, education, tobacco use, physical activity, and clinical predictors were BMI, hypertension, diabetes, systolic and diastolic BP, fasting plasma glucose, serum triglycerides, low density lipoproteins, high density lipoproteins, total cholesterol, history of coronary artery disease and history of stroke.

**Results:** A total of 2873 adults (48% men) were screened from 12 randomly selected communities in Karachi. The prevalence (95%CI) of reduced eGFR, albuminuria and CKD was 5.3% (4.5 – 6.2), 9.4% (8.4 – 10.5) and 12.5% (11.3 – 13.8) respectively. The factors independently associated with CKD were older age, hypertension, diabetes, history of stroke, raised systolic BP, raised fasting glucose and raised triglycerides. About 51% CKD patients with hypertension were on anti-hypertensive medications and only 6.7% had their BP controlled to <130/80 mm Hg.

**Conclusions:** CKD is common among adults in Pakistan, especially among older individuals with co-morbidities. Blood Pressure control and use of anti-hypertensive medications among CKD patients is sub-optimal. Efforts to prevent to CKD in Pakistan must target high risk population and be integrated with major non-communicable diseases care in Pakistan and neighboring South Asian countries.

*Funding:* Private Foundation Support

**TH-PO298**

**Short Term Risk versus Lifetime Risk in Stage 3 Chronic Kidney Disease** Candace D. Grant, Shayan Shirazian, Pardeep Kumar, Milind K. Bhagat, Joseph Mattana. *Dept of Medicine, Winthrop Univ Hospital, Mineola, NY.*

**Background:** While there are proven therapies that slow CKD progression, these therapies can at times be harmful and costly. The ability to accurately predict the risk of CKD progression and overall lifetime risk would be extremely valuable. The short term versus lifetime risk of CKD progression should be taken into account when making clinical decisions based on risk assessments. In a representative CKD practice, we compared the short term and lifetime risk assessment in our stage 3 CKD patients to determine whether decisions based on a short term risk assessment would underestimate the lifetime risk of CKD progression.

**Methods:** The electronic charts of 120 CKD stage 3 patients seen in our nephrology practice were reviewed. Eligible patients had at least one documented eGFR within the past year. Additional variables collected for risk assessment were urine albumin to creatinine ratio, urine protein to creatinine ratio, calcium, phosphorus, albumin, and bicarbonate. Patients were risk stratified using a validated 2 year ESRD risk calculator and a lifetime ESRD risk estimator. Patients were considered to be at a low risk for progression to ESRD if their 2 year calculated risk was <5%.

**Results:** Of the 120 patient charts reviewed, 44 patients met the study criteria and had complete data sets. The average age was 61.4±13.2, 58% were men, 77% were white, and the mean GFR was 40 ml/min/1.73m$^2$. The average 2 year calculated ESRD risk was 3.5% and the average lifetime risk was 13.5%. When stratified by age, there was no significant discrepancy in the 2 year risk and lifetime risk for patients 70 years and older (2.3% and 4.9% respectively). Comparison of the 2 year ESRD risk (4.2%) to the lifetime risk (18.5%) resulted in an underestimation of risk in 22 out of 27 (82%) patients below 70 years of age.

**Conclusions:** For younger patients, short term risk calculators should be used in conjunction with lifetime risk calculators to get a more accurate sense of the patients overall prognosis. This will ensure that despite the low short term risk, the high long term risk of progression would necessitate aggressive risk attenuation therapy.

**TH-PO299**

**Renoprotective Effect of Pentoxifylline in Childhood Chronic Kidney Disease** Yo Han Ahn, Jiwon M. Lee, Hee Gyung Kang, Hae Il Cheong, IL-Soo Ha. *Dept of Pediatrics, Seoul National Univ Children's Hospital.*

**Background:** Pentoxifylline (PTX) attenuated renal fibrosis in animal studies. It was also reported to offer beneficial effects on renal function and proteinuria in diabetic kidney disease and membranous nephropathy in adults. Previously our group presented a preliminary data of PTX in pediatric CKD patients, and here we report our follow-up result.

**Methods:** From 2004 to 2013, 52 children with stage 2 to 4 CKD (estimated glomerular filtration rates (eGFR) 15~90 mL/min/1.73m$^2$) were treated with PTX of 700 mg/m$^2$/day

for 25±28 months. All of them were also on either angiotensin-converting enzyme inhibitor or angiotensin receptor blocker to attenuate progression of CKD. Among them, 29 patients (M:F 21:8, age 14.0±4.4 years) had taken PTX for more than 12 months, so their eGFR before and after PTX administration were analyzed to assess renoprotective effect of PTX.

**Results:** During the 12-month period before administration of PTX, eGFR of those patients decreased significantly, from 43.1±17.4 at 12 months before PTX treatment (-12mo) to 38.2±14.6 mL/m/1.73m$^2$ at the beginning of PTX (0mo, *P*=0.009). However after administration of PTX, there was no significant decline of eGFR during the next 12 months (38.0±17.2 mL/m/1.73m$^2$ at 12 months after PTX treatment (+12mo, P=0.863). When analyzed the change of eGFR (DGFR) before and after PTX treatment, PTX showed reno-protective effect in patients with lower grade CKD, stage 2&3a, -4.2±11.1 before vs. 1.5±0.5 after treatment, P=0.019) while those with CKD stage 3b&4 showed no significance (0.4±4.2 vs. 1.5±0.5, P=0.710). Compare to patients with underlying glomerulopathy (GN) against those with non-GN, PTX was more beneficial for those with non-GN (P = 0.037). On the other hand, significant number of patients (n=23, 44.2%) experienced side effects of PTX, such as nausea or vomiting and drug fever, and had to discontinue PTX.

**Conclusions:** PTX showed renoprotective effect in childhood CKD, especially in early stage CKD and in those with non-GN cause of CKD. However, significant side effect was observed at the same time, thus randomized controlled trial is necessary to validate the benefit of this anti-fibrotic medication.

## TH-PO300

### Is Glomerular Filtration Rate Related to Vascular Lesions in HIV Patients?

Corinne Isnard-Bagnis,[1] Philippe Rouvier,[2] Sophie Tezenas du Montcel,[3] Paulo Francisco Fernandes,[6] Macroui Sonikian,[5] Jerome Tourret,[1] Rachid Agher,[4] Gilbert Deray,[1] Marc Antoine Valantin,[4] Roland Tubiana,[4] Christine Katlama.[4] *[1]Nephrology, Assistance Publique Hôpitaux de Paris, Paris, France; [2]Pathology Dept, Assistance Publique Hôpitaux de Paris, Paris, France; [3]Clinical Research Unit, Assistance Publique Hôpitaux de Paris, Paris, France; [4]Infectious Disease Dept, Assistance Publique Hôpitaux de Paris, Paris, France; [5]Nephrology, A Fleming General Hospital, Athens, Greece; [6]Nephrology, Hopital Santa Maria, Lisboa, Portugal.*

**Background:** Renal diseases in HIV patients are secondary to the viruses (HIV or HIV/HCV co-infection), their treatments or to metabolic complications. As the causes of chronic kidney disease evolve in HIV population, it is critical to describe what histopathological lesions correlate with glomerular filtration rate (GFR).

**Methods:** 196 biopsies performed in a single center over 10 years in HIV patients were retrospectively analyzed. For 82 biopsies, corresponding clinical and biological data were available both at the time of biopsy. GFR was estimated as sMDRD.

**Results:** Among the 196 biopsies, the main renal histopathological diagnosis included: Acute tubular necrosis (n=34, 17.4%), HIVAN (n=29, 14.8%), nephroangiosclerosis (NAS) (n=28, 14.3%), FSGS (n=25, 12.8%), interstitial fibrosis, tubular atrophy (IFTA) (n=11, 5.61%) and diabetic glomerulosclerosis (n=10, 5.1%). IFTA was present in 25% of HIV-related diagnosis (FSGS+HIVAN) and 36% in non HIV-related (all others) diagnosis (p=0.11). NAS was present in 25% of HIV-related diagnosis (FSGS+HIVAN) and 44% in non HIV-related (all others) diagnosis (p=0.058). IFTA did not influence GFR in any diagnosis. When NAS lesions were present in HIV-related diagnosis, GFR was significantly lower (p=0.032).

**Conclusions:** Nephroangiosclerosis lesions are more frequent in non HIV-related histopathological diagnosis (patients with metabolic complications) and significantly influence glomerular filtration rate in HIV patients. Accelerated ageing and endothelial vascular lesions secondary to HIV infection and HAART may explain this correlation.

*Funding:* Private Foundation Support

## TH-PO301

### Chronic Kidney Disease in Patients with Sickle Cell Anemia

Elvira Gosmanova,[1] Sahar Zaidi,[1] Jim Y. Wan,[2] Patricia Adams-Graves.[1] *[1]Medicine, UTHSC, Memphis, TN; [2]Preventive Medicine, UTHSC, Memphis, TN.*

**Background:** Little is known about the prevalence of different stages of chronic kidney disease (CKD) in patients with sickle cell anemia (SCA). We evaluated the prevalence and the changes of CKD stages during a 5-year period in a cohort of SCA patients aged >18 years who were followed at the Diggs-Kraus Sickle Cell Clinic, Memphis, TN.

**Methods:** We studied 98 patients with SCA [mean age 31.6 years, 43% males, 56% with Hemoglobin SS disease, mean serum creatinine (SCr) 0.79 mg/dL, mean blood pressure (BP) 120.2/74.0 mmHg]. CKD stages 1 through 5 were defined using estimated glomerular filtration rate (eGFR), and albuminuria grades were defined based on spot urine protein to creatinine ratio (UPCR) (normal or A1- UPCR<0.15mg/g, A2 or microalbuminuria-UPCR 0.15-0.5mg/g, A3 or macroalbuminuria- UPCR>0.5mg/g) according to the latest KDIGO recommendations. In patients with eGFR>60ml/min/1.73m$^2$, CKD diagnosis was made if grade A2 or A3 albuminuria was present. CKD progression was defined as an increase in CKD stage with an additional >25% eGFR reduction from baseline.

**Results:** At baseline, 28.6% of patients had CKD. After a mean follow up of 5.0+0.9 years, 17 patients developed new CKD (mainly stage 1 based on A2 or A3 albuminuria) and the overall CKD prevalence increased to 41.8%. In addition, 8 patients had worsening of preexisting CKD stage. In univariate analysis, older age (p=0.02) and higher baseline systolic BP (SBP) (p=0.03) were associated with the development of new CKD; whereas, lower baseline eGFR (p=0.001), A3 albuminuira (p=0.005), and lower serum hemoglobin (p=0.04) predicted CKD progression. In multivariate analysis, each 1mmHg increase in SBP was associated with increased risk of new CKD (adjusted OR 1.06, p=0.005), and baseline A3 albuminuria strongly predicted the progression of CKD (adjusted OR 17.0, p=0.006).

**Conclusions:** CKD is common in patients with SCA, and its prevalence increases with increasing age. Several baseline modifiable and non-modifiable factors were associated with the development and progression of CKD in SCA patients. Strategies targeting control of blood pressure and proteinuria may be beneficial for individuals with SCA.

## TH-PO302

### Renal Pathology in West Side of Chicago

Esho Georges, Harsha C. Gondi, Rafath Ullah, Mana Dissadee, Andres Serrano. *Nephrology Div, Mount Sinai Hospital Medical Center, Chicago, IL.*

**Background:** The purpose of our study is to review all renal pathologies done at our institution between 2008-2012, trying to identify the renal diseases that affect our population which composed mainly of Hispanics and African-Americans.

**Methods:** We reviewed medical records of our (100) cases retrospectively looking for renal pathology, gender, ethnicity, age group, labs and other data done at time of diagnosis.

**Results:** The mean age of the group was 45.7 yr with 55% females and 45% males. Hispanics account for 52% and African-Americans for 44%. At the time of the biopsy, 67% of patients have hypertension and 34% have diabetes mellitus. The mean serum creatinine is 2.8 mg/dl and mean urine protein/creatinine is 5.1 gm/gm.

Diabetic nephropathy (DN) and focal segmental glomerulosclerosis (FSGS) are the most common pathology (each accounts for 19%), followed by advanced global glomerulosclerosis (AGGS) (17%), IgA nephropathy (IgAN) (12%) and membranous nephropathy (MN) (12%). Interestingly, almost close to one fifth of the patients have end-stage renal disease, which likely indicates lack of medical evaluation in this group of patients!

Among Hispanics, FSGS was the most common diagnosis (23%), followed by IgAN (21.1%), DN (19.2%), AGGS (19.2%) and MN (9.6%). Among African-Americans, the most common diagnosis was DN (20.4%), followed by MN (15.9%), AGGS (13.6%), and FSGS (11.3%). It is interesting that MN was more frequent among African-Americans than FSGS, which traditionally has been described to be more prevalent in this population, and that FSGS was the most common diagnosis among the Hispanics.

Among diabetics, DN was the most common diagnosis (55.8%). However, (44.2%) of the diabetic patients have a different diagnosis.

**Conclusions:** In conclusion, DN and FSGS are the most common cause of renal disease at our institute. However, almost close to one fifth of cases have AGGS, which likely indicates lack of medical evaluation and unawareness of their renal disease. Among African-Americans, DM and MN were more frequent than FSGS. Finally, more than 40% of patient with diabetes mellitus, have a diagnosis that was not DN.

## TH-PO303

### A Panel of Novel Biomarkers of Kidney Injury Improves Prediction of Glomerular Filtration Rate Declines in Chronic Kidney Disease

Boon Wee Teo,[1] Peh Joo Ho,[2] Qi Chun Toh,[1] Hwee Min Loh,[3] Martin B. Lee,[1] Evan J.C. Lee.[1] *[1]Medicine, Yong Loo Lin School of Medicine, National Univ of Singapore, Singapore; [2]Statistics and Applied Probability, Faculty of Science, National Univ of Singapore, Singapore; [3]Medicine, National Univ Hospital, Singapore.*

**Background:** The association of a panel of novel kidney injury or disease biomarkers in spot urine (KIM1, kidney injury molecule 1; LFABP, liver-type fatty acid-binding protein; NGAL, neutrophil gelatinase-associated lipocalin; αGST and πGST, gluthathione s-transferase; collagen IV) with progression of chronic kidney disease (CKD) in stable patients is unknown. We hypothesize that a panel of these biomarkers can provide additional predictive value in estimated glomerular filtration rate (eGFR) decline beyond traditional predictors including proteinuria. We assess a panel of biomarkers with eGFR decline in a multi-ethnic Asian population of CKD.

**Methods:** We retrieved stored urine samples from the Asian Kidney Disease Study patients with CKD (n=81, 49.4% male). We examined traditional and novel biomarkers against eGFR decline using univariate and multivariate analysis. Variables of interest were natural log-transformed where appropriate. We used exhaustive stepwise selection of variables in multiple linear modeling to determine the best predictive models and Akaike Information Criterion (AIC) to select the best. We used cross-validation to validate and rank the models by prediction error.

**Results:** Population means: age 58.7±12.4 years, 53.1% diabetes history, serum creatinine 2.03±1.17 mg/dL , serum total protein 70.67±5.78 g/L, urine creatinine 6.9±4.6 mmol/L baseline eGFR 45.2±28.6 mL/min/1.73m$^2$, Kim-1 0.7±0.6 ug/L, LFABP 42.2±50.0 ug/L, NGAL 29.8±52.1 ug/L, αGST 6.5±23.8 ug/L, πGST 6.9±11.1 ug/L, Collagen IV 5.7±6.0 ug/L. Urine albumin to creatinine ratio (UACR) is rendered insignificant in a model including diabetes history, serum protein, αGST, NGAL and L-FABP but is the best model with the smallest AIC value (202.6) and smallest prediction error.

**Conclusions:** A panel of novel biomarkers of kidney injury offers additional prediction of CKD progression in a model including albuminuria.

*Funding:* Government Support - Non-U.S.

**TH-PO304**

**Uric Acid Transporter ATP-Binding Cassette Transporter G2 (ABCG2) Is Increased in the Intestine of the Rat 5/6 Nephrectomy Model of Chronic Kidney Disease** Hirofumi Yano, Yoshifuru Tamura, Kana Kobayashi, Shunya Uchida. *Nephrology, Teikyo Univ School of Medicine, Tokyo, Japan.*

**Background:** Uric acid (UA) remains to be a risk factor of chronic kidney disease (CKD). Therefore, it is important to clarify the mechanism of UA excretion in CKD. The specific mechanisms of intestinal excretion in extrarenal excretion are unknown. We evaluated the expression of UA transporter, intestinal tract of the ATP-binding cassette transporter G2 (ABCG2), in a rat 5/6 nephrectomy model of CKD.

**Methods:** Male Wistar rats (6 week old) were randomly assigned to the 5/6 nephrectomized (Nx) group or the sham-operated control group. Urine and blood samples were collected every 4 weeks. All the rats were sacrificed at 8 weeks to obtain liver, duodenum, jejunum, ileum, and transverse colon tissues. Uricase activity was measured in the liver. Expression of ABCG2 in intestinal mucosa was measured with a real time PCR.

**Results:** Nx group showed significantly decreased urine UA excretion/body weight and UA clearance compared to the control group at 4 and 8 weeks after nephrectomy. In contrast, serum UA and uricase activity were not significant. In Nx group, the expression of ABCG2 in the ileum showed significant increase upregulation. While other intestines revealed no changes.

**Conclusions:** Nx rats exhibited lower excretion of urine UA and over-expression of ABCG2 in the ileum. The fact that serum UA did not increase despite the decrease in UA excretion suggests that other excretory pathway, probably intestine, beside kidney may operate as a complementary role that corroborates the increase in ABCG2 expression in the ileum.

**TH-PO305**

**Visceral Fat Area (VFA) Assessed by Bioelectric Impedance Analysis (BIA) Is Associated with Renal Function in Healthy Subjects** Soo Bong Lee, Il Young Kim, Dong Won Lee, Min Ji Shin, Harin Rhee, Byeong Yun Yang, Eun Young Seong, Ihm Soo Kwak. *Dept of Internal Medicine, Pusan National Univ School of Medicine, Yangsan, Republic of Korea.*

**Background:** The bioelectric impedance analysis (BIA) can be used in many clinical settings and easily give us information about body composition of each person including muscle mass, percentage of total body fat and visceral fat area (VFA).Though some studies have been reported regarding the adverse effect of abdominal obesity on residual renal function in chronic kidney disease (CKD) patients, that effect in healthy population is less well known. Therefore, we evaluated the effects of VFA on glomerular filtration rate (GFR) in healthy subjects.

**Methods:** We searched healthy subjects over 18 years old who underwent BIA and routine laboratory tests through health screening and promotion center in our university hospital in 2012. All of them have no underlying illness and subjects with proteinuria or hematuria were excluded. GFR was estimated by the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease EPIdemiology (CKD-EPI) equations. Multiple linear regression analysis between eGFR and body composition measured by BIA was done. Because the eGFR was affected by body mass, subjects were divided two groups according to BMI and compared their eGFR.

**Results:** Total 500 healthy subjects were included. Percentage of total body fat, obesity index and waist hip ratio did not show meaningful relationship with eGFR. Age, body weight, skeletal muscle, VFA were statistically significant predicting factors of eGFR by multiple linear regression analysis ($p<0.001$). Interestingly, the association of increased VFA with decreased renal function is more prominent in non obese group with low BMI below 25 than others ($p=0.001$ vs. $p=0.295$, respectively).

**Conclusions:** VFA is a significant determinant of eGFR in company with Age, body weight, skeletal muscle especially in non obese group in healthy subjects. Although many studies focused on association of general obesity and renal function, visceral fat reflecting abdominal obesity seems to have important role on renal function and should not be ignored even in healthy subjects.

**TH-PO306**

**Lower Serum Albumin Level Is Associated with Higher Fractional Excretion of Creatinine** Masaru Horio,[1] Enyu Imai,[2] Yoshinari Yasuda,[3] Tsuyoshi Watanabe,[4] Seiichi Matsuo.[3] *[1]Functional Diagnostic Science, Osaka Univ Graduate School of Medicine, Osaka, Japan; [2]Nakayamadera Imai Clinic, Takarazuka, Japan; [3]Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan; [4]Third Dept of Medicine, Fukushima Medical Univ, Fukushima, Japan.*

**Background:** Creatinine clearance (Ccr) overestimates glomerular filtration rate (GFR) due to the tubular secretion of creatinine. Fractional excretion of creatinine (FE-Cr, ratio of Ccr to GFR ) increases with decreasing GFR. Association of serum albumin level with the tubular secretion of creatinine was also reported previously. Alteration of FE-Cr may affect the performance of GFR estimating equations based on serum creatinine. We analyzed the factors influencing FE-Cr and compared the performance of GFR equations in subjects stratified by serum albumin levels.

**Methods:** 757 Japanese subjects were included. GFR was measured by inulin renal clearance. GFR and Ccr were measured simultaneously. Multivariate analysis was used to evaluate the factors influencing FE-Cr. Age, gender, GFR, body mass index (BMI), body weight, height and serum albumin level were analyzed. Estimated GFR was calculated by Japanese GFR equations based on serum creatinine (Eq-cr), serum cystatin C (Eq-cys) and 5 variables including serum albumin (Eq-5var).

**Results:** FE-Cr in subjects with serum albumin <3.0, 3.0-3.9 and >4.0g/dl were 1.63±0.48, 1.53±0.55, and 1.40±0.36, respectively. Multivariate analysis showed that GFR ($p<0.0001$) and serum albumin level ($p=0.004$) were independent parameters affecting FE-Cr. Biases of Eq-cr, Eq-cys and Eq-5var in subjects with serum albumin <3.0 g/dl were -9.5±17.5, -0.7±17.1 and -0.6±14.8 ml/min/1.73m$^2$, respectively. Eq-cr significantly overestimated GFR compared with Eq-cys or Eq-5var. Biases in subjects with serum albumin >4.0 g/dl were 6.4±18.8, 2.0±18.1 and 3.0±18.3 ml/min/1.73m$^2$, respectively. Eq-cr significantly underestimated GFR compared with Eq-cys or Eq-5var.

**Conclusions:** GFR and serum albumin level were independent parameters affecting FE-Cr. Alteration of FE-Cr according to the serum albumin levels may be one of the reasons of the bias of GFR equation based on serum creatinine.

*Funding:* Government Support - Non-U.S.

**TH-PO307**

**Effects of C-Reactive Protein (CRP) on the Risk of Developing Chronic Kidney Disease in Rheumatoid Arthritis** Masako Kochi,[1] Kentaro Kohagura,[1] Kunitoshi Iseki,[2] Yusuke Ohya.[1] *[1]Cardiovascular Medicine, Nephrology and Neurology, Univ of the Ryukyus, Nishihara-Cho, Japan; [2]Dialysis Unit, Univ of the Ryukyus, Nishihara-Cho, Japan.*

**Background:** Chronic inflammation is common in Rheumatoid arthritis (RA) and related to the risk of cardiovascular, liver and pulmonary disease in RA patients. However, the effects of inflammation on chronic kidney disease (CKD) progression are unknown in RA.

**Methods:** We retrospectively investigated the effects of baseline C-reactive protein (CRP) on the incidence of CKD defined as positive dipstick testing for proteinuria and/or an eGFR (estimated glomerular filtration rate) of ≤60 ml/minute/1.73 m$^2$. We studied 361 outpatients, 61 men and 300 women, and the mean age and eGFR were 56.9 years and 89.2 ml/minute/1.73 m$^2$, respectively.

**Results:** Over a median follow-up of 6.5 years, 42 (12%) patients developed CKD. Elevated CRP were independently associated with the development of CKD. After adjustment for age, gender, traditional risk factors, anti-RA drug use, and the baseline eGFR, the adjusted hazard ratio of CRP was 1.37 (95% confidence interval: 1.19–1.55; $p < 0.0001$).

**Conclusions:** CRP had an independent association with the development of CKD in RA. The impact of anti-inflammation therapy on CKD remains to be studied further.

**TH-PO308**

**Markers of Inflammation and Oxidative Stress in Patients with Predialyis Chronic Kidney Disease** Luis H.B.C. Sette,[1] Edmundo Pessoa Lopes,[1] João Geraldo Carvalho Fernandes,[1] André Martins Galvão.[2] *[1]Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil; [2]Laboratório de Imunobiologia Keizo Asami LIKA, Recife, Pernambuco, Brazil.*

**Background:** Nontraditional cardiovascular risk factors such as inflammation, malnutrition and oxidative stress (OS) promote and accelerate atherosclerosis in CKD patients and can be acessed by measuring the inflammation markers: serum ferritin, C-reactive protein (CRP) and albumin and the OS markers: gamma glutamyl transferase(GGT), TBARS, thiol, carbonyl and catalase.

**Methods:** Between September 2011 and December 2012 were evaluated patients in the clinic of Nephrology at the Hospital of Clinics of the Federal University of Pernambuco (HC-UFPE). Glomerular filtration rate (GFR) was estimated by the Cockroft-Gault formula and the patients were classified into five stages of CKD. Were measured in serum: markers of inflammation: ferritin, CRP, albumin, GGT, and markers of EO: TBARS, thiol, carbonyl and catalase.

**Results:** Were included 140 patients with a mean age of 64.4 years and 51.4% male. Mean GFR was 28.8 mL/min/1,73m2 and patients showed the following distribution according to the stages of CKD: 16 (11.4%) stage 5 patients, 69 (49.2%) stage 4, 55 stage 3 patients (39.2%). Among the markers of inflammation assessed it was observed an increase in serum ferritin and reduction of albumin in accordance to the GFR and the progression of CKD. There was no association between serum GGT, CRP and markers of EO with the GFR or the stages of CKD.

| **Biomarkers*** | **GLOMERULAR FILTRATION RATE** | |
|---|---|---|
| | Coeficient | p-value |
| GGT | 0.063 | 0.460 |
| **Ferritin** | **-0.236** | **0.005** |
| RCP | 0.041 | 0.620 |
| TBARS | -0.060 | 0.468 |
| Catalase | 0.046 | 0.576 |
| Carbonyl | -0.012 | 0.885 |
| Thiol | -0.146 | 0.076 |
| **Albumin** | **0.265** | **0.001** |
| * Spearman correlation | | |

**Conclusions:** Among the markers of inflammation and oxidative stress evaluated, there was an increase in serum ferritin and reduction of albumin as the fall in GFR and progression of CKD.

**TH-PO309**

**The Time Is Now to Implement Novel Urinary Biomarkers to Identify Cardiorenal Syndrome in Children with Dilated Cardiomyopathy**
Ahmad Kaddourah, Stuart Goldstein, John L. Jefferies, Michael R. Bennett. *Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

**Background:** Type II cardio-renal syndrome (CRS) describes renal dysfunction in the presence of chronic heart disease. Traditionally, elevated serum creatinine (Cr) and proteinuria have been used as markers for renal dysfunction. The role of the novel urinary biomarkers (NUB) has not been investigated in children with dilated cardiomyopathy (DCM). We aim to evaluate the changes in traditional kidney function markers in DCM children, and determine the diagnostic value of NUB in DCM children.

**Methods:** This is a cross-sectional study in children with DCM and left ventricular dysfunction defined as ejection fraction < 55%. Urine and blood samples were collected to run mesure serum Cr, urinary protein / Cr ratio, and four NUB: KIM-1, NGAL, IL-18, and L-FABP. eGFR was calculated using modified Schwartz formula. The NUB concentrations were adjusted for each mg of urinary Cr. NUB concentrations were compared to 22 control urine samples taken from 22 healthy children.

**Results:** 40 subjects between 2 and 21 year-old have been enrolled. The mean (SD) eGFR was 116 (62.1) ml/min/m$^2$. The median urinary protein / Cr ratios was 0.15 (0.09-0.24) mg/mg (normal is < 0.2). Both NGAL and IL-18 concentrations were significantly higher in DCM children than controls. Similarly, FABP concentration was higher in DCM children but the difference was not statistically significant. KIM-1 concentration showed no difference between the two groups.

| NUB/Cr | DCM children Median (IQR) | Controls Median (IQR) | P |
|---|---|---|---|
| NGAL/Cr | 13 (6-49) | 8(3-21) | 0.03 |
| IL-18 / Cr | 58 (30-116) | 26 (16-41) | 0.01 |
| FABP /Cr | 6 (3-15) | 3 (2-5) | 0.05 |
| KIM/Cr | 412 (270-672) | 379 (277–553) | 0.50 |

**Conclusions:** Our data suggests that the majority of children with DCM have normal eGFR and urinary Protein/Cr ratio; however, both NGAL and IL-18 were significantly higher in DCM children than in controls indicating subclinical ongoing kidney injury that cannot be detected by currently available renal function markers. Our data demonstrates the importance of using more sensitive markers to diagnose CRS in children with DCM.

**TH-PO310**

**Estimated GFR Differs from Measured GFR in Its Associations with Retinal Vasculopathy in the General Population**
Bjorn Odvar Eriksen,[1,3] Geir Bertelsen,[1,3] Therese Von Hanno,[1,2] Ulla Dorte Mathisen,[3] Toralf Melsom,[3] Ellisiv B. Mathiesen.[1,3] *[1]Univ of Tromsø, Tromsø, Norway; [2]Nordland Hospital, Bodø, Norway; [3]Univ Hospital of North Norway, Tromsø, Norway.*

**Background:** Estimated GFR (eGFR) from creatinine and/or cystatin C is used extensively in epidemiological research. Validation of eGFR against measured GFR (mGFR) has demonstrated reasonably good performance. However, both creatinine and cystatin C depend on non-GFR factors which can confound associations with pathological conditions compared to mGFR. Few studies have investigated the extent of this problem in epidemiological research. We compared the associations between eGFR and mGFR and retinal vasculopathy, a marker of systemic microvasculopathy.

**Methods:** In the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6), we measured GFR as iohexol clearance and performed retinal photography in a representative sample of middle-aged persons from the general population without cardiovascular disease. A total of 1521 persons without chronic kidney disease, diabetes, or micro- or macroalbuminuria were examined. Three eGFR equations based on creatinine and/or cystatin C from the CKD-EPI-Collaboration were used. Cystatin C was calibrated to the international reference ERM-DA471/IFCC.

**Results:** mGFR in the lowest quartile was associated with an increased multivariable-adjusted odds ratio of having retinopathy (odds ratio 1.8, 95% CI 1.1-2.6) in logistic regression analyzes, but the associations with all three eGFR were weaker and not statistically significant. Retinal venular diameter had statistically significant but opposite multivariable-adjusted associations with eGFRcrea (-1.5µm per 10 mL/min/1.73 m$^2$ reduction, 95% CI -2.8 to -0.2) compared to eGFRcys (+1.3µm per 10 mL/min/1.73 m$^2$ reduction, 95% CI 0.3 to 2.3), but not with eGFRcyscrea or mGFR in multiple linear regression anlyzes. The results for arteriolar diameters were similar, but the association with eGFRcys was not statistically significant (P=0.09).

**Conclusions:** eGFR based on creatinine and/or cystatin C differ from mGFR in their associations with retinal vasculopathy. This is a potential source of bias in epidemiological studies.

*Funding:* Government Support - Non-U.S.

**TH-PO311**

**Late Dialysis Initiation Is Associated with Elevated Mortality among Patient Subgroups in the United States**
Austin G. Stack,[1,2] Ailish Hannigan,[2] Liam F. Casserly,[1,2] Hoang Thanh Nguyen.[2] *[1]Nephrology and Internal Medicine, Univ Hospital Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Ireland.*

**Background:** Recent studies have demonstrated an inverse relationship between the timing of dialysis initiation, based on estimated glomerular filtration rate (eGFR) and mortality. The extent to which this association is influenced other markers of kidney function (urea nitrogen and creatinine concentrations) is not clearly understood. We explored relationships of GFR with mortality at dialysis initiation in the context of varying concentrations of urea and creatinine.

**Methods:** Mortality risks were compared among 'early start' (eGFR: 10-15, and > 15ml/min/1.73 m$^2$) and 'late start' patients (eGFR: < 5 and 5-10) in 1, 220, 000 incident US patients who started dialysis between 1995-2008 and stratified by quintiles of urea (Q1 < 61.1, Q2 61.1-77.0, Q3 77.0-93.0, Q4 93.0-114.0 and Q5> 114.0 mg/dL) and serum creatinine. The association of eGFR with 2-year mortality was explored using Cox regression with adjustment for demographic, clinical and laboratory characteristics with eGFR 5-10 ml/min as the referent and adjusted Hazard ratios (HR) were determined. The Interaction terms (GFR*urea and GFR*creatinine) were significant in the full model.

**Results:** The association of GFR with mortality varied significantly by concentrations of urea nitrogen. For levels > 93 mg/dL, the association of GFR with mortality was u-shaped, while for levels > 93 mg/dL, the pattern was more direct. A similar mortality risk pattern emerged when stratification was made by serum creatinine concentrations.

| | Hazard Ratio for Death by eGFR at Dialysis Initiation (ml/min/1.73m$^2$) | | | |
|---|---|---|---|---|
| Urea Nitrogen (mg/dL) | < 5 | 5 to <10 | 10 to <15 | > 15 |
| < 61.1 | 1.26** | 1.00 | 1.03** | 1.08** |
| 61.1 to <77.0 | 1.16** | 1.00 | 1.07** | 1.12** |
| 77.0 to < 93.0 | 1.03** | 1.00 | 1.08** | 1.14** |
| 93.0 to < 114.0 | 0.94** | 1.00 | 1.10** | 1.14** |
| > 114.0 | 0.84** | 1.00 | 1.09** | 1.14** |

**P<0.001

**Conclusions:** The association of GFR estimated at dialysis initiation with mortality varies significantly and substantially according to measured concentrations of urea and creatinine. Late dialysis initiation is associated with elevated mortality risks in selected patient subgroups.

*Funding:* Private Foundation Support

**TH-PO312**

**Clinical Value of Pathologic Examination of Non-Neoplastic Kidney in Patients with Upper Urinary Tract Malignancies**
Hyunjin Noh,[1] Jee wan Wee,[1] Soon Hyo Kwon,[1] Jin Seok Jeon,[1] Dong-Cheol Han,[1] So-Young Jin.[2] *[1]Internal Medicine, Soon Chun Hyang Univ College of Medicine, Seoul, Republic of Korea; [2]Pathology, Soon Chun Hyang Univ College of Medicine, Seoul, Republic of Korea.*

**Background:** Despite surgical resection remains the standard of care in the treatment of upper urinary tract malignancies, nephrectomy is a recognized a risk factor for developing chronic kidney disease (CKD). Since a solitary kidney has been considered an absolute contraindication to percutaneous biopsy, histologic diagnoses in the patients received nephrectomy would be an obstacle. In this study, we investigated the clinical value of pathologic examination of non-neoplastic tissue from the resected kidney to determine unrecognized kidney disease.

**Methods:** Between January 2010 and July 2012, 32 patients with renal cell carcinoma and 2 patients with transitional cell carcinoma received uninephrectomy or uninephroureterectomy. The routine pathologic evaluation of non-neoplastic tissues was performed.

**Results:** Out of 31 patients, only 9 patients (29%) had normal kidney. Thirty-four cases of pathologic diagnoses were obtained because 3 patients had dual diagnoses. Of the 34 cases, 10 (29.4%) showed vascular diseases (7 hypertensive nephrosclerosis, 3 ischemic nephropathy), 8 (23.5%) showed diabetic nephropathy, 6 (17.6%) showed glomerulonephritis (2 IgA nephropathy, 4 C1q nephropathy). and 1 case (3%) showed reflux nephropathy. During the median follow-up period of 9 months, 14 patients (46.7%) experienced post-operative acute kidney injury (AKI) and 10 patients (32.3%) progressed to CKD. Patients who experienced AKI had higher rates of developing CKD (p = 0.002, odds ratio 16.3). None of the clinical variables was associated with the pathologic abnormality.

**Conclusions:** Our study indicates that a routine pathologic examination of nephrectomized, non-neoplastic tissue will provide valuable information on the remnant kidney. Further studies based on a larger patient population will be needed to determine the influence of pathologic diagnoses on the long-term renal outcome.

**TH-PO313**

**Early Detection of Kidney Disease in Children and Adolescents in Mexico: A Pilot Study**
Mara Medeiros,[1] Nadia Olvera,[2] Rebeca Reyes,[2] Luis Velasquez-Jones,[1] Georgina Toussaint,[1] Gregorio T. Obrador.[2] *[1]Hospital Infantil de Mexico Federico Gomez, Mexico, DF, Mexico; [2]Fundacion Mexicana del Riñon, Mexico, DF, Mexico.*

**Background:** Early detection of chronic kidney disease (CKD) is important to treat patients and prevent the progression to end stage kidney disease. The etiology of CKD is different in children and adolescents than in adult population, congenital anomalies (A/Hypo/dysplastic kidney, obstructive uropathy and reflux uropathy) and glomerulonephritis are the leading cause in pediatric patients, in contrast to adults where diabetes and hypertension are the main causes.

**Methods:** A pilot study was performed in Mexico City. Children and adolescents with any of the following risk factors were invited to participate: history of prematurity, history of urinary tract infection, history of acute renal injury, hypertension, obesity, weight or

height below 10th percentile for age and gender, ≥ 5 years with diabetes, familiar history of CKD (first grade). Patients with previous diagnosis of chronic kidney disease were excluded. Informed consent was obtained in all cases. A questionnaire was applied. Blood pressure, weight, height, abdominal circumference were measured. A urine sample was obtained for urinalysis and microalbumin/creatinine ratio. Blood sample was obtained for serum creatinine, blood gasometry, glucose, sodium, potassium, chloride, calcium, BUN, hemoglobin, hematocrit. Glomerular filtration rate was estimated with Schwartz formula.

**Results:** Eighty-eight participants were included, median age 10 years (range 1 month-18 years), 45 (52%) were female. The most common risk factors were family history of CKD in 33 (37.5%), prematurity in 18 (20.4%), obesity 14 (15.9%). Twenty-nine participants (33%) had an abnormal renal evaluation including one or more of the following: 12 (13.6%) had microalbuminuria, 8 had anemia (9%), 7 had hematuria (7.9%), 10 had hypertension (11.3%), 3 leukocyturia (3.4%), 1 renal glycosuria (1.1%), only two patients (2.2%) had eGFR<60ml/min/1.73m$^2$ (CKD stage 3). None had metabolic acidosis.

**Conclusions:** The prevalence of kidney disease was 33% in children with known risk factors. Early detection programs are useful to diagnose and prevent CKD progression in this population.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO314

**Implementing Surveillance/Education Programs to Facilitate Timely Renal Referral in Chronic Kidney Disease** Nicole Piero,[1] Loretta Simbartl,[1] Charuhas V. Thakar.[2] *[1]Nephrology, VA Medical Center, Cincinnati, OH; [2]Nephrology, Univ of Cincinnati, Cincinnati, OH.*

**Background:** Referral to a renal subspecialty in patients with Stage IV chronic kidney disease (CKD)[glomerular filtration rate (GFR) < 30 ml/min/1.73m2] allows optimal management of its complications including end stage renal disease (ESRD) planning. We examined the impact of development and implementation of a surveillance/education program to facilitate timely renal referral followed by a shared care approach to manage CKD complications.

**Methods:** Within our Veterans Affairs healthcare system, starting Nov 2012, a customized informatics program compiles a monthly report of patients who have had 1 outpatient lab revealing a GFR < 30; we also identify demographics, comorbidities [e.g. hypertension, including home blood pressure (BP) monitoring], and renal evaluation in the prior 24 months. Concurrently, an educational program, led by nephrology and pharmacy is offered to primary care providers to raise awareness of CKD evaluation, and management of its complications. We compared (Chi-square) a historical cohort (2006 – 2010) with the 6-month implementation period (11/2012-04/2013) to evaluate improvement in renal referral in those with Stage IV CKD.

**Results:** In the historical cohort (42,942 veterans within our system) 79% patients were without CKD 19% with CKD Stage III, and 2% had CKD Stage IV/V. Compared to those without CKD, proportion of patients with renal referral in Stage III and Stage IV/V CKD was 35.7% and 47.6% respectively ($p < 0.0001$). Since the beginning of the surveillance program in Nov 2012 there were 348 patients with Stage IV CKD seeking care in our system, with 63% completing renal referrals. 97.4% of them had a diagnosis of hypertension, and 8% were enrolled in the program for home BP monitoring. After accounting for differences in demographics and comorbid conditions, renal referral during the implementation period was higher than historical period (63% vs 47.6%, $p < 0.0001$).

**Conclusions:** A combination of system-wide surveillance and education facilitates timely renal referral in patients with Stage IV CKD. This allows for increased opportunities for co-morbidity management and proper ESRD planning.

*Funding:* Veterans Affairs Support, Private Foundation Support

## TH-PO315

**The Cost Effectiveness of Primary Screening for Chronic Kidney Disease: A Systematic Review** Thomas W. Ferguson,[1] Paul Komenda,[2] Kerry Macdonald,[4] Claudio Rigatto,[2] Manish M. Sood,[3] Navdeep Tangri.[2] *[1]Community Health Sciences, Univ of Manitoba, Winnipeg, Canada; [2]Nephrology, Seven Oaks General Hospital, Winnipeg, Canada; [3]St. Boniface General Hospital, Winnipeg, Canada; [4]Library Services, Univ of Manitoba, Winnipeg, Canada.*

**Background:** Chronic Kidney Disease (CKD) is a major health problem with a rising incidence and prevalence worldwide. Data on the cost-effectiveness of CKD screening in the general population have been conflicting. Our objective was to evaluate key factors influencing the cost-effectiveness of screening for CKD by proteinuria or eGFR.

**Methods:** To achieve our objective we conducted a systematic review of studies from PubMed, Scopus, EMBASE, and Cochrane Database of Systematic Reviews from the date of their establishment until June 2012. Studies reporting incremental cost-effectiveness ratios (ICER) of screening for CKD by either protenuria or estimated glomerular filtration rate (eGFR) in comparison with no screening or usual care were selected. Articles were assessed for quality of reporting and risk of bias based on validated criteria.

**Results:** We screened 1,462 abstracts of which 161 were selected for a full-text review. Nine studies met criteria for inclusion. Eight studies evaluated the cost-effectiveness of proteinuria screening and two evaluated screening with eGFR. For proteinuria based screening, the reported cost per quality-adjusted life year (QALY) ranged from $14,063.18 to $160,018.03 in the general population, $5,297.59 to $54,943.12 in the diabetic population, and from $23,028.37 to $73,939.36 in the hypertensive population. For eGFR screening, one study reported a cost per QALY of $23,679.71 in the diabetic population and the range across the two studies was $100,252.88 to $109,911.56 in the general population. The incidence of CKD, rate of progression, and the effectiveness of drug therapy were major drivers of cost-effectiveness.

**Conclusions:** Screening for CKD is suggested to be cost-effective in patients with diabetes and hypertension. CKD screening may be cost-effective in populations with higher incidence of CKD, rapid rates of progression, and more effective drug therapy.

## TH-PO316

**Exploration of Candidate Biomarkers for the Rapid Screening of Glomerular Disease and Its Application Research** Ye Tao, Jin Qiaoling. *Dept of Nephrology, West China Hospital, Chengdu, Sichuan, China.*

**Background:** So far, GD is the leading cause of CKD in China .The national CKD survey was completed recently in china, it shows that the prevalence rate of CKD was10.8% for adults above the age of 18, which has become a serious public health problem. The early screening of GD will help clinicians to take corresponding treatment scheme, to delay the development of renal disease, improve the prognosis of the patients. Estimation of renal lesion severity is an important part of clinical treatment, Besides the clinical manifestation, pathological diagnosis of renal biopsy is the most commonly used and effective means, but it is not suitable for repeated examination because of trauma. So, researchers have been working to find strong evidence in blood and urine of patients, in order to reflect the injury and severity of renal.Therefore, search for a quick, accurate and non-invasive examination method for early diagnosis of glomerular disease is very necessary.

**Methods:** A protein array which can screen 274 cytokines simultaneously was used to screen urine specimen collected from the GD patients and healthy subjects. Enzyme linked immunosorbent (ELISA) was used to quantify those potentially useful cytokines. ROC curve (relative operating characteristic curve) assess the values for clinical application.

**Results:** We obtained four cytokines through the protein array, monocyte chemotactic factor -1(MCP-1), angiogenin, adiponectin ( Acrp30), dipeptide peptidase IV (DPPIV/CD26). Researchers have reported the clinicalsignificance of MCP-1 in glomerular disease, and there is no urinary Acrp30 detection kit for sale. Therefore, only the cytokine CD26 was quantified by ELISA and analyzed by ROC curve. The area under the curve of CD26 was 0.898±0.022, the sensitivity was 86.3% and the specificity was 81.9%.

**Conclusions:** In this study we found that the CD26 can be used for clinical screening of GD, but can't be used in the identification of glomerular disease. Otherwise, the study found CD26 was related to urinary protein, which suggested that CD26 can be used to noninvasive monitoring treatment response of GD.

*Funding:* Clinical Revenue Support

## TH-PO317

**Decrease of Glomerular Filtration Rate at 30-Day Readmission after Percutaneous Coronary Intervention** Claudine T. Jurkovitz, Paul Kolm, Daniel Elliott, James R. Bowen, William S. Weintraub. *Value Institute, Christiana Care Health System, Newark, DE.*

**Background:** Chronic kidney disease (CKD) is a risk factor for 30-day readmission in patients who undergo Percutaneous Coronary intervention (PCI). Whether patients with CKD have a decline in kidney function between the first and the repeat admissions is unknown.

**Methods:** We conducted a retrospective analysis of all patients admitted in our hospital for PCI in 2010-2012. Glomerular filtration rate (GFR) was estimated using CKD-Epi. CKD was defined as GFR<60 ml/min/1.72m$^2$. A significant decrease in kidney function was defined as a decline in GFR ≥30% between the first (index visit) and 30-day admissions. We used Paired T-test and McNemar's test to compare results between index visit and readmission and logistic regression to calculate the probability of significant decline at each CKD stage, adjusted for demographics, diabetes, hypertension, congestive heart failure (CHF), angiotensin converting enzyme inhibitor (ACEI) or receptor blocker (ARB) at index visit.

**Results:** A total of 4,432 patients had PCI of whom 616 (14%) were readmitted within 30 days. Of those, 69% were male, 49% were 65 or older, 83% were white, 15% black, 13% had diabetes, 80% hypertension, 26% CHF, 25% CKD and 63% were taking ACEI/ARB at index visit. Average GFR was 75.6±26.9 at index visit and 72.6±28.5 at readmission ($p<0.001$); Of those without CKD at index visit, 6% had a significant decline in GFR between index visit and readmission, whereas it raised to 11% among those with CKD ($p=0.02$). CHF was the only significant predictor of GFR decline (Odds ratio (OR)= 3.1; 95%CI=1.5-6.2). Although index visit GFR was not an independent predictor (OR=1.1/10 ml decrease, 95%CI=1.0-1.3, $p=0.09$) there was an apparent trend in the probability of kidney function decline. Probability was 0.05 for those without CKD at index visit, 0.11, 0.14, 0.16 for those with GFR 30-59, 15-29 and <15 respectively.

**Conclusions:** The decline in kidney function between the 2 hospital admissions is mostly seen in patients with CKD and seems to be related essentially to CHF. Monitoring these patients at discharge is key to prevent further decline of kidney function and end stage renal disease.

*Funding:* Other U.S. Government Support

## TH-PO318

**Impact of Reimbusement Change of ESAs on Management of Anemia in Patients with Non-Dialytic Chronic Kidney Disease** Sun Moon Kim, Hye-Young Kim. *Internal Medicine, Chungbuk National Univ Hospital.*

**Background:** Anemia is common in patients with chronic kidney disease (CKD). The correction of anemia with erythropoiesis-stimulating agents (ESAs) is of particular financial interest. Recently, use of ESAs in non-dialytic patients was reimbursed by Korean

National Health Insurance System and it enabled physicians to use ESAs in non-dialytic CKD patients at half the previous cost. In this study, we investigated the prescription rate of ESAs and the status of anemia control before and after reimbursement.

**Methods:** Medical records of patients with non-dialytic CKD stage 4, 5 (eGFR <30ml/min/1.73m$^2$) were reviewed in one tertiary medical center. The prescription of ESAs, Hb, estimated GFR, history of transfusion, and iron supplementation at 6 months before, 6 months and 12 months after the change of reimbursement criteria.

**Results:** A total of 607 medical records were analyzed. The mean age of patients was 59.2 ± 14.5, and mean eGFR was 19.10 ± 6.80 ml/min/1.73m$^2$. The proportion of patients received ESAs was 20.6% before reimbursement while the proportion was increased to 37.5% at 6 months and 40.4% at 12 months after reimbursement (p <0.001). Especially, 36.9% of patients with Hb <10g/dl were prescribed ESAs before reimbursement, whereas 57.7%, and 64.0% of those patients at 6 months and 12 months after reimbursement (p <0.001). However, neither mean Hb nor the proportion of patients with Hb < 10g/dl was changed. Mean Hb was 9.60 ± 1.97 g/dl before reimbursement, 9.34 ± 1.83 g/dl, and 9.71 ± 1.78 g/dl and at 6 months and 12 months after reimbursement (p=0.195). The proportion of patients with Hb <10 g/dl was 51.0% before reimbursement, 59.9%, and 56.8% at 6 months and 12 months after reimbursement (p=0.458). The requirement of red blood cell transfusion was 0.46 pint/person-year before reimbursement, 0.32 pint/person-year, and 0.15 pint/person-year at 6 months and at 12 months after reimbursement (p=0.001).

**Conclusions:** With reimbursement of ESAs in patients with nondialytic CKD, the prescription rate of ESAs in the population was increased. Although mean hemoglobin levels did not change in the population, the requirement of red blood cell transfusion was significantly decreased.

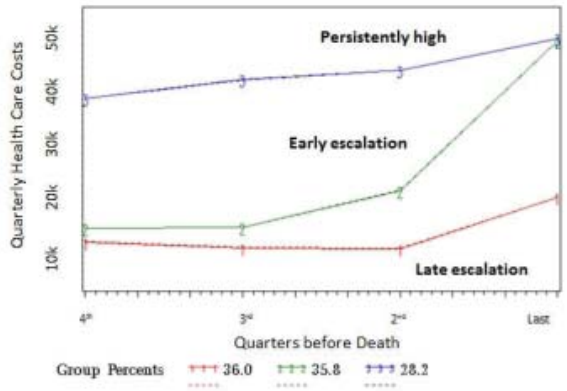
## TH-PO319

**End-of-Life Cost Trajectories among Medicare Beneficiaries with End-Stage Renal Disease** Ann M. O'Hare,[1] William Kreuter,[2] Paul Louis Hebert,[3] Kenn B. Daratha.[4] *[1]Depts of Medicine and Health Services, VAPSHCS and Univ of Washington, Seattle, WA; [2]School of Nursing, Washington State Univ, Spokane, WA.*

**Background:** Little is known about trajectories of healthcare intensity before death among patients with ESRD.

**Methods:** We used group based trajectory modeling to describe cost trajectories during the year before death among adults who entered the USRDS registry between January 1, 1995 and December 31, 2007, had Medicare as primary payer for ESRD during the year before death and died before January 1, 2009 (n=199,120).

**Results:** We identified three groups of patients with distinct cost trajectories: those with late cost escalation, those with early cost escalation and and those with persistently high costs (Figure).




Across these respective groups, median costs during the last year of life ranged from $57,513 to $157,476, median hospital days ranged from 7 to 44, median ICU days ranged from 0 to 7, and the percentage of patients that died in the hospital ranged from 33.8 to 52.6%. Compared with the group with late cost escalation, those with early cost escalation and persistently high costs were younger, had a shorter duration of ESRD, and included a higher proportion of women and black patients, a higher proportion with Medicaid eligibility, and a higher proportion with diabetes as the cause of ESRD. As compared with patients who died between 1995 and 2000, those who died from 2005-2008 were 41% (95% confidence interval (CI) 36-47%) more likely to have early cost escalation and 63% (95% CI 56-69%) more likely to have persistently high costs during the last year of life after adjustment for other patient characteristics.

**Conclusions:** Patients who died in more recent years, younger patients, black patients, women, and those with diabetes as a cause of ESRD were more likely to have experienced a prolonged period of intensive healthcare utilization before death.

*Funding:* Private Foundation Support

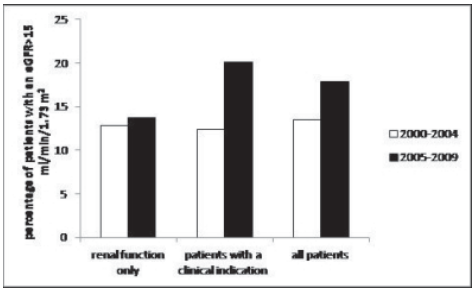
## TH-PO320

**Trends in Timing and Indications for Chronic Dialysis within a Large U.S. Health System (2000-2009)** Ann M. O'Hare,[1,2,4] Bruce Wynar,[1] Mark Perkins,[1] Chuan-fen Liu,[1,3] Jaclyn Lemon,[1] Paul Louis Hebert.[1,3] *[1]Dept of Veterans Affairs, VA Health Services Research & Development Center of Excellence, Seattle, WA; [2]Dept of Medicine, Univ of Washington, Seattle, WA; [3]Dept of Health Services, Univ of Washington, Seattle, WA; [4]Group Health Research Institute, Seattle, WA.*

**Background:** Reasons for an upward trend in estimated glomerular filtration rate (eGFR) at the time of dialysis initiation are not known. Prior studies have been limited by an absence of information on clinical indications for dialysis.

**Methods:** To evaluate whether trends in eGFR at dialysis initiation reflect changes in indications for dialysis and/or timing of initiation, we reviewed the electronic medical records of a national random sample of patients who initiated chronic dialysis in the Department of Veterans Affairs (VA) in fiscal years (FY) 2000-09 (n=1,735).

**Results:** Between FY2000-04 and 2005-09, the percentage of patients with an eGFR>15ml/min/1.73 m$^2$ at initiation increased from 13.5 to 17.9% (P=0.012).




In both time periods, types of clinical indications were similar, as was the proportion of patients in whom the decision to initiate dialysis was based only on level of renal function (27.6% vs. 29.1%, P=0.48). The adjusted odds ratio for initiation at an eGFR>15ml/min/1.73$^2$ in FY2005-09 vs. 2000-04 was 1.31 (95% confidence interval (CI) 0.99, 1.71) for the overall cohort, 0.84 (95% CI 0.48, 1.47) for those in whom the decision to initiate dialysis was based only on level of renal function, and 1.49 (95% CI 1.09, 2.05) for those with one or more clinical indications.

**Conclusions:** Recent trends in eGFR at initiation within the VA were driven by a selective tendency toward earlier initiation of dialysis among patients with clinical signs and symptoms, and did not reflect changing indications for dialysis or earlier initiation in asymptomatic patients.

*Funding:* Veterans Affairs Support

## TH-PO321

**Variability in Management of Hemodialysis Patients and Associated Healthcare Utilization** Bjoerg Thorsteinsdottir,[1] Priya Ramar,[2] Megan Reinalda,[2] Robert C. Albright,[1] Amy W. Williams,[1] Nilay D. Shah.[2] *[1]Medicine, Mayo Clinic, Rochester, MN; [2]Health Care Policy and Research, Mayo Clinic, Rochester, MN.*

**Background:** With new efforts to define care models, it is essential to understand the impact of different providers on quality and resource utilization. We studied the role of nephrologists (Neph) and primary care physicians (PCP) in the care of hemodialysis(HD) patients in a health system serving a population of 395.000. Nephrologist presence varies between units, offering a natural experiment in the management of HD patients between PCP and Neph.

**Methods:** Patients initiating HD from 2001-2010 were linked to United States Renal Database System Medicare claims. Patients with <90 days follow up were excluded as were services after a transplant. Outpatient (OP) visits were identified using CPT-4 codes and specialty. Mid-Level providers were grouped with Neph as most worked in HD. The proportion of PCP visits per person-year (ppy) was grouped into quartiles. We estimated the association between the quartile of PCP visits with hospitalizations, emergency department (ED) and other specialty visits.

**Results:**

| | Groups* | | |
|---|---|---|---|
| | <25$^{th}$ percentile(N=939) | 25$^{th}$-75$^{th}$ percentile (N=1961) | >75$^{th}$ percentile (N=926) |
| Follow-up(py) | 2877.2 | 7767.0 | 2987.9 |
| Mean age(years) | 56.0 | 61.0** | 63.2** |
| Males(%) | 64.1 | 58.6** | 57.0** |
| Died(%) | 28.0 | 40.6** | 57.6** |
| Transplant(%) | 45.7 | 34.0** | 8.3** |
| Median survival (years) | 4.7 | 4.2 | 3.6** |
| Charlson score first year of HD | 1.9 | 3.2** | 3.6** |
| PCP visits ppy | 2 | 3.5** | 7.3** |
| Nephrology visits ppy | 5.6 | 6.3** | 1.5** |
| Other specialty ppy | 4.1 | 7.8** | 8.2** |
| Hospitalizations ppy | 1.0 | 1.6** | 2.2** |
| ED visits ppy | 0.7 | 1.3** | 1.6** |

*Groups based on proportion of primary care E&M visits out of total E&M visits to primary care, mid-level/nephrology providers. **Denotes p-value < 0.05 when compared to <25$^{th}$ percentile group.

**Conclusions:** Patients who get most of their care from primary care providers are older, sicker, utilize more resources, and have worse outcomes than those managed primarily by nephrologists. Further studies are needed to better understand the differences in these patient groups.

## TH-PO322

**Role of Meditation in Reducing Sympathetic Hyperactivity and Quality of Life in Lupus Nephritis Patients with Chronic Kidney Disease**
Sirawit Bantornwan, Nuttasith Larpparisuth, Tanyarat Teerapornlertratt, Kriengsak Vareesangthip. *Dept of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol Univ, Bangkoknoi, Bangkok, Thailand.*

**Background:** Lupus nephritis is an important leading cause of chronic kidney disease (CKD) among the young population. Some physiological studies reveal that meditation may reduce sympathetic hyperactivity which is commonly found in SLE. We hypothesized that meditation could be beneficial in correcting autonomic dysfunction and improving quality of life in lupus nephritis patients with CKD.

**Methods:** We prospectively enrolled thirty lupus nephritis patients and categorized into meditation group and control group. Method of meditation was instructed by an expert in Buddhist studies every month. Participants in the intervention group were advised to meditate every day for 24 weeks. To evaluate change in sympathetic activity, normetanephrine level was measured at beginning and the end of study compared between both groups. Quality of life was determined by SF-36. Heart rate variability was also measured in meditation group at baseline and after practice.

**Results:** After meditation for 6 months, serum normetanephrine level decreased in both groups, but without statistically significant ($p$=0.28). In aspect of quality of life, score of physical and mental components improved significantly. In meditation group, physical score increased from 21.4 (5.0-50.2) to 62.2 (51.8-88.4) points ($p$<0.01) and mental score increased from 16.9 (4.4-46.0) to 72.4 (45.1-81.6) points ($p$<0.01). Although, physical and mental score in control group also increased, from 19.4 (10.4-49.2) to 55.4 (36.4-83.4) points ($p$<0.01) and 13.9 (7.7-44.2) to 45.0 (29.8-77.6) points ($p$<0.01), respectively, but quality of life score in meditation group increased more significantly (p<0.01). The parameter of heart rate variability in time and frequency domain also improved in meditation group.

**Conclusions:** In lupus nephritis patients with CKD, meditation reduced normetanephrine, improved quality of life and heart rate variability. Our results support the important role of meditation as a valuable adjunctive treatment of CKD from lupus nephritis.

*Funding:* Government Support - Non-U.S.

## TH-PO323

**Patient Awareness of Chronic Kidney Disease Is Associated with Blood Pressure Control**
Jillian Molli Berkman,[1] H. Omer Ikizler,[1] Julie A. Wright Nunes,[2] T. Alp Ikizler,[1] Kerri L. Cavanaugh.[1] *[1]Vanderbilt Univ; [2]Univ of Michigan.*

**Background:** Patient awareness of kidney disease is limited and variably relates to clinical outcomes. Different methods to characterize awareness may contribute these inconsistent findings. Furthermore, little is known about the impact of a patient's understanding of the severity of their chronic kidney disease (CKD).

**Methods:** In a cross-sectional study from May 2012 to July 2013, adults with CKD (eGFR<60 ml/min) seen in nephrology clinic were asked questions regarding their CKD diagnosis and its severity. Blood pressure (BP) readings were abstracted from the medical record.

**Results:** Among 252 patients the mean age was 57.9 (12) years, 42% were male, 17.3 % were Black, and 13.2% had limited health literacy. Patients answered three awareness questions: "Do you have a problem with your kidneys?" (96% correct), "Do you have chronic kidney disease?" (80 % correct), and "Do you have decreased kidney function?" (84% correct). Thirty-six percent (n=91) correctly identified their CKD stage; 12% (n=30) reported no CKD. Compared to those aware of the CKD stage, those unaware were more likely to be older (59 vs. 55 yrs; p=0.01), African American (22% vs. 9%; p=0.01), of lower income (38% vs. 24%; p=0.02), and were less likely to have participated in a CKD education class (25% vs. 38%; p=0.03). Unaware patients also had significantly lower kidney knowledge (63% vs. 71%; p=0.01), and less self-care participation (26.0 vs 27.9; p=0.03). Aware patients were more likely to have a controlled blood pressure (<130/80 mmhg) (52% vs. 34%; p=0.007), and lower systolic blood pressure (132 vs 139; p=0.02). These findings persisted in regression analyses adjusted for age, gender, race, income, education, diabetes, and previous CKD-education [OR 2.49 (95% CI 1.31-4.76); p=0.006], and [B (95%CI): -5.8 (-12.2 - 0.57)); p=0.07] respectively.

**Conclusions:** Awareness of CKD remains poor. Those aware are more likely to attain recommend blood pressure goals. Assessing patient awareness of CKD severity may identify those at risk for poor outcomes including less optimal BP control.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO324

**The Optimal Time to Measure Blood Pressure as a Representative Value of 24-Hour Mean Blood Pressure**
Jiwon Ryu,[1] Ran-Hui Cha,[2] Sunae Yoon,[3] Dong-Ryeol Ryu,[4] Jieun Oh,[5] Sejoong Kim,[1] Sang Youb Han,[6] Eun-Young Lee,[7] Yon Su Kim.[8,9] *[1]Internal Medicine, Seoul National Univ Bundang Hospital, Republic of Korea; [2]Internal Medicine, National Medical Center; [3]Internal Medicine, Catholic Univ College of Medicine; [4]Internal Medicine, Ewha Womans Univ College of Medicine; [5]Internal Medicine, Hanlym Univ College of Medicine; [6]Internal Medicine, Inje Univ College of Medicine; [7]Internal Medicine, Soon Chun Hyang Univ College of Medicine; [8]Internal Medicine, Seoul National Univ College of Medicine; [9]Kidney Research Institute, Seoul National Univ Medical Research Center.*

**Background:** Hypertension is important treatment target in chronic kidney disease(CKD). 24 hours blood pressure(BP) is gold standard to measure high BP, but, which is quite discomfortable. However representative time for BP as a 24-hrs BP in CKD patients is still unknown.

**Methods:** We evaluated whether there may be proper time to measure BP for predicting the average of 24 hrs BP in CKD patients(stage 2-4) from the multicenter, cross-sectional study (n, 1317; mean age, 57 years; men, 62.9%).

**Results:** Regression analysis showed that systolic BP(SBP) at the 7AM and 9:30PM are best agreed with the 24-hr mean SBP(mSBP)(r=0.677, r=0.656, respectively). The proportion of patients within 30% of 24-hr mSBP was the most at 7AM(95.6%) and then 9:30PM(95.3%). Among corresponding values of BP to 135mmHg by calculation with regression, 7AM was the closest(136.74mmHg), 9:30PM was the next(138.78mmHg), office mSBP was the last(142.61mmHg). The agreement between hypertensive groups classified by 135mmHg of 24-hrs mSBP and calculated values mentioned above showed the group of 7AM was most agreed with 24-hr mSBP(70.8%). The 9:30PM was lesser agreed(68.1%) and the office mSBP was least agreed(60.7%). By ROC curve, in evaluation of diagnostic values for hypertension, its standard is 135mmHg, 7am had the best(AUC: 0.843) and office mSBP had least(AUC: 0.774).

**Conclusions:** These datas suggest that optimal time to measure BP in CKD patients may be 7AM in the morning, and 9:30PM in the evening. This shows that office mean SBP is less useful than our time to measure BP.

*Funding:* Pharmaceutical Company Support - sanofi-aventis Korea Company

## TH-PO325

**Seroconversion Rates following Intradermal and Intramuscular Hepatitis B Vaccine in Hemodialysis Patients Not Responding to Primary Vaccination Protocol – A Review of Prospective Trials**
Aliya Saeed, Farhanah Yousaf, Haroon Rashid, Josephine E. Kulogowski, Marilyn Galler, Bruce S. Spinowitz, Chaim Charytan. *New York Hospital Queens, Flushing, NY.*

**Background:** The response to Hepatitis B vaccine in dialysis population is reduced. Alternative route of vaccine administration may elicit a response via a distinct immunologic pathway that may help achieve seroconversion.

**Methods:** We performed a literature search using Medline with Mesh terms including "hepatitis B," "hepatitis B vaccines," and "injections, intradermal." The inclusion criteria was any prospective trial comparing seroconversion rates defined as antibody titer of > 10 IU/L immediately (1-3 months), 6 months, or at 12 months after the completion of intradermal (ID) and intramuscular (IM) administration of a fixed hepatitis B vaccination protocol in previously non-responding dialysis patients.

**Results:** Four of 158 studies met inclusion criteria. A total of 204 patients were studied, 120 of whom received ID hepatitis B vaccine while 84 received IM. At 1-3 months, 6 months, and 12 months, the mean seroconversion rate in ID group was 85%, 70%, and 61% versus mean seroconversion rates of 64%, 61%, and 28% in IM group, respectively.

| Author (Year of Publication) | Sample Size (ID:IM) | Vaccine | Vaccine Dose Protocol ID IM | Seroconversion [1-3 months after completion] (ID%:IM%) | Seroconversion [6 months after completion] (ID%:IM%) | Seroconversion [12 months after completion] (ID%:IM%) |
|---|---|---|---|---|---|---|
| Barraclough et al (2009) | 59(29:30) | Engerix B (GlaxoSmith Kline Pharmaceuticals) | 5 mcg 2 x/wk x 8 wks 40 mcg at wk 1 and 8 | 79:40 | 28:50 | 38:0 |
| Radziszewski et al (2007) | 62(49:13) | Engerix B (Smith Kline Beecham) | 10 mcg qwk x 12 10 mcg qwk x 12 | 82:85 | 88:100 | 88:69 |
| Micozkadioglu et al (2007) | 33(17:16) | Genhevac B, Pasteur | 10 mcg qwk x 8 40 mcg at 0, 1, 2, 6 months | - | 94:50 | - |
| Fabrizi et al (1997) | 50(25:25) | Engerix B (Smith Kline & Beecham) | 5 mcg qwk x 16 40 mcg at 0 and 1 month | 94:67 | 69:43 | 57:14 |

**Conclusions:** ID vaccination may achieve higher seroconversion rate and the effect may be maintained longer versus IM vaccination. Additional trials are needed to elaborate the role of ID vaccination in previous non-responding hemodialysis patients.

## TH-PO326

**Volume-Outcome Relationship in Percutaneous Native Renal Biopsy** Hiroyuki Yamamoto, Hideki Hashimoto, Mitsuhiro Nakamura, Hideo Yasunaga. *Univ of Tokyo School of Public Health, Tokyo, Japan.*

**Background:** Hemorrhagic complication remains the major complication of percutaneous renal biopsy. Our aims in this study are to identify individual-level risk factors relating to hemorrhagic complications of the procedure, and to investigate a volume-outcome relationship after adjusting for these risk factors.

**Methods:** Using a large claim-based data called Diagnosis Procedure Combination (DPC) database in Japan, we identified inpatients with renal disorders who underwent percutaneous biopsy within four days after admission during July to December in years of 2007 to 2010 (total 24 months). Patients were classified by age, sex, clinical syndromes (chronic nephritic syndrome, nephrotic syndrome, rapidly progressive nephritic syndrome, recurrent or persistent hematuria, acute nephritic syndrome and other disorders), and quintiles of hospital volume of the biopsy. Generalized estimation equation was used to account for clustering of patients at institutional levels.

**Results:** A total of 15191 patients from 942 hospitals were enrolled to this study. Overall hemorrhagic complication incidence was 2.1%, including hemorrhagic events on ICD codes (1.6%); red blood cell transfusion (0.5%); required angiography or endovascular procedure (0.1%); surgical interventions (0%). In-hospital deaths with hemorrhagic complications were observed in 0.06% of the patients. Significantly high complication incidence was observed in patients group younger than 30 (adjusted OR 1.97, 95% CI 1.39-2.80), and those aged 60 and over (adjusted OR 1.64, 95% CI 1.15-2.36) with reference to middle-aged patients group. Patients who had rapidly progressive nephritic syndrome (adjusted OR 3.41, 95% CI 2.22-5.25) had significantly higher hemorrhagic complication incidence with reference to patients who had chronic nephritic syndrome. No significant difference was observed across the hospital volume groups after adjustment of patient-level variables.

**Conclusions:** This study finds no significant volume-outcome relationships in hemorrhagic complication.

*Funding:* Government Support - Non-U.S.

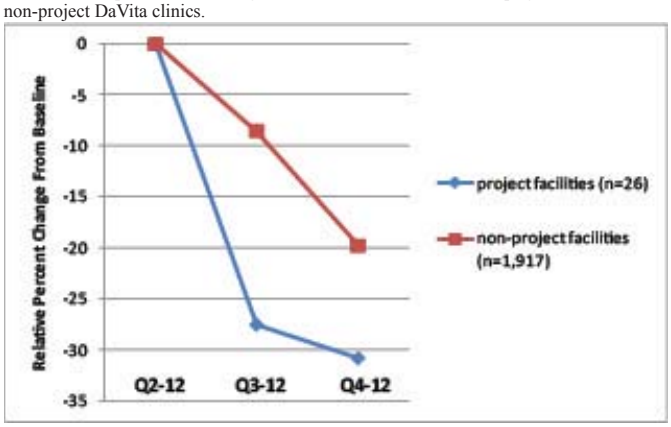
## TH-PO327

**A Pilot Quality Improvement Program to Minimize Catheter-Related Bloodstream Infection in an Outpatient Hemodialysis Setting** David B. Van Wyck,[1] Nancy Culkin,[1] Peter J. Pronovost,[2] Christine A. Goeschel,[2] Mahesh Krishnan,[3] Allen R. Nissenson.[1] *[1]DaVita Healthcare Partners, Denver, CO; [2]Johns Hopkins Univ Armstrong Institute for Patient Safety and Quality, Baltimore, MD; [3]DaVita Clinical Research, Minneapolis, MN.*

**Background:** Technical and adaptive approaches from the Johns Hopkins University Armstrong Institute have reduced catheter-related bloodstream infection (CRBSI) in hospitals.

**Methods:** To evaluate whether similar approaches could prevent CRBSI in chronic dialysis patients, we conducted a collaborative, multifaceted, quality improvement program in 26 Maryland-area DaVita hemodialysis facilities. We used the Armstrong Comprehensive Unit-based Safety Program (CUSP) to survey elements of culture and practice in patient safety, adapted tools and interventions for the dialysis setting, and monitored CRBSI rates.

**Results:** The survey was completed by 431/497 teammates (TMs; employees). We found areas for systematic improvement in pre-, intra-, and post-dialysis central venous catheter (CVC) care from the medical literature. We created a procedural kit with checklist, antimicrobial swabs for skin prep, triple-antibiotic ointment for exit site application, alcohol swabs to facilitate hub scrub, and exit site dressing. Another kit/procedural checklist was created for CVC dialysis initiation and termination. To enhance TM engagement, clinics implemented daily team briefings to collectively identify and plan for high-risk patients. TMs charted each new CRBSI episode utilizing a calendar to monitor progress. During the evaluation period, we saw a greater decline in CRBSI rates in project clinics than in non-project DaVita clinics.




**Conclusions:** Safety assessment tools and interventional approaches that reduce CRBSI in hospitals can be successfully applied to reduce CRBSI in chronic dialysis facilities.

## TH-PO328

**Reduction in Catheter-Related Bacteraemia in a Renal Network Using Quality Improvement Methodology** Sajeda Youssouf, Azri Nache, Janet Hegarty. *Salford Royal NHS Foundation Trust, United Kingdom.*

**Background:** Haemodialysis catheter related bacteraemia remains a significant safety issue in HD care. Despite initiatives to increase definitive access, 30% of patients in the UK still use catheters for haemodialysis. In two of our dialysis units we had 36 catheter-related bacteraemias in 12 months at baseline, resulting in 24 admissions, 296 bed days and 3 transfers to level 3 care, despite evidence-based interventions such as antimicrobial line locks to reduce infections.

**Methods:** We sought to improve our catheter-related bacteraemia rate using multifaceted interventions learned from best available evidence and learning from best performing units as part of a quality improvement collaborative over a two year period. We used plan-do-study-act (PDSA) cycles to rapidly introduce small scale changes and implement them fully if successful. This method allowed frontline staff to learn from each other and share their experience of improvement. Successful changes were compiled into a change package for use elsewhere in our network. Data was collected prospectively for a formal evaluation of our work.

**Results:** Our units tested and implemented mutliple changes including: dressing care, exit site and bacteraemia database, traffic light system and an algorithm for high-risk HD catheters, nurse-led anticoagulation protocols and patient information leaflets.

The total number of catheter-related bacteraemias decreased by 83% (36 to 5). There was a significant reduction in admissions (24 to 5) and bed days (296 to 108). No patient required level 3 care. Our bacteraemia rate decreased by 78% from 1.8 to 0.4 per 1000 catheter days ($p<0.01$) over the 2-year cycle. This has been sustained for 7 months since the end of the project, at 0.57 per 1000 catheter days.

**Conclusions:** We have demonstrated a significant improvement in our catheter-related bacteraemia rate. This was achieved by staff being given the responsibility and power to change practice within an improvement framework. Critically, we have also begun to achieve a change in our culture and attitude to preventable harm from bacteraemia. Results obtained with this method are comparable to those achieved in randomised controlled trials of antimicrobial interventions.

## TH-PO329

**Preparation for Renal Replacement Therapy (RRT): Perspectives of Nephrology and Primary Care Providers** Raquel Greer,[1] Jessica M. Ameling,[1] Carrie E. Andrews,[1] Patti Ephraim,[1] Bernard G. Jaar,[1] Kerri L. Cavanaugh,[2] Vanessa Grubbs,[3] L. Ebony Boulware.[1] *[1]Johns Hopkins Univ; [2]Vanderbilt; [3]UCSF.*

**Background:** Early shared decision-making (SDM) and preparation for RRT is recommended for patients with advanced chronic kidney disease (CKD), but many patients continue to initiate RRT urgently with temporary vascular access.

**Methods:** We conducted directed telephone interviews of nephrology providers (n=10, nephrologists, physician assistants, and nurses) and primary care physicians (PCPs, n=4) to identify modifiable challenges to optimal RRT preparation for future interventions. Providers were affiliated with nephrology practices in San Francisco, CA and Baltimore, MD. Using open-ended questions, we assessed providers' perceived: 1) challenges to patients' RRT preparation and 2) role of PCPs and nephrologist-PCP collaboration in patients' RRT preparation. Two independent abstractors coded transcribed audio-recorded interviews and identified major themes.

**Results:** Nephrology providers' most commonly identified challenges focused on patients' difficulty "fully appreciating" the health impact of CKD and the need to prepare for RRT, including patients': 1) denial of CKD severity, 2) fear of dialysis, 3) lack of CKD knowledge, 4) preference for alternative CKD treatment approaches, and 5) low perceived urgency for RRT preparation. Many nephrology providers also cited late referrals from PCPs limited adequate time to build trusting relationships with patients to improve their preparation. PCPs' desired more involvement in preparation to ensure RRT transitions could be as "smooth as possible", but cited several challenges including their: 1) uncertainty of optimal time of nephrology referral and 2) limited RRT knowledge. PCPs desired role was to provide patients with emotional support, help patients weigh RRT options, and affirm nephrologist recommendations. Nephrologists and PCPs desired improved collaboration, including better information exchange and delineation of roles during RRT preparation.

**Conclusions:** Interventions facilitating patients' acceptance of CKD diagnoses and engagement in SDM, as well as nephrology-PCP collaboration may improve patients' RRT initiation outcomes.

*Funding:* NIDDK Support

## TH-PO330

**Development and Evaluation of a Preventive Program for Contrast-Induced Nephropathy: A Quality Improvement Report** Keita Hirano,[1] Kumiko Shimasaki,[1] Kenichiro Koitabashi,[2] Fumika Taki,[1] Masahiko Nagahama,[1] Yasuhiro Komatsu.[1] *[1]Div of Nephrology, Dept of Internal Medicine, St. Luke's International Hospital, Japan; [2]Div of Nephrology and Hypertension, Dept of Internal Medicine, St. Marianna Univ School of Medicine, Japan.*

**Background:** A prophylactic program for contrast-induced nephropathy (CIN) can prevent acute kidney injury while providing appropriate imaging studies that involve radio-contrast media. We report the effectiveness of a hospital-wide electronic medical record-based CIN prevention program.

**Methods:** We conducted a prospective cohort study including a total of 720 consecutive chronic kidney disease patients being seen on an outpatient basis at the St. Luke's International Hospital (Tokyo, Japan) between April 2012 and March 2013. Admitted patients receiving a CT or those undergoing hemodialysis or emergency CT were excluded (n=507). Our CIN prevention program consists of (1) an automatic alerting system when a physician orders enhanced CT for patients with GFR less than 45 mL/min/1.73m$^2$, (2) an automatic nephrologist referral to order prophylactic hydration and follow-up, and (3) monthly quality indicator (QI) measurements and feedback to physicians. The percentage of patients with eGFR≤45mL/min/1.73m$^2$ who received appropriate prophylactic saline hydration before and after CT and nephrologists' follow-up were selected as quality indicators and were reported monthly.

**Results:** The mean age of the study participants was 73.8±9.6 years and comprised of 65.3% males and an average eGFR of 35.6±10.4 ml/min/1.73m$^2$. Implementation rates of the protocol improved from 0.0% to 40.7%, and the hydration performed rates improved from 20.0% on April 2012 to 74.1% on March 2013. Two patients developed CIN (1.8%). The rate was relatively lower than that of previous studies.

**Conclusions:** To close the evidence-practice gap of contrast-induced nephropathy, hospital-wide prevention programs and monthly QI measurements and reporting can enhance evidence-based practice, reduce the incidence of CIN, and increase the appropriate use of enhanced CT.

## TH-PO331

**Chronic Kidney Disease in Primary Care: Assessing the Role for Sub-Specialty Co-Management** John Andrew Updike, George P. Bayliss. *Medicine, Rhode Island Hospital, Providence, RI.*

**Background:** This study sought to determine whether nephrology sub-specialty co-management of chronic kidney disease (CKD) positively affects adherence to the Kidney Disease Outcomes and Quality Initiative practice guidelines and whether co-management affects screening and treatment independent of CKD severity or identification of disease by the primary care provider as evidenced by a designated ICD-9 code.

**Methods:** A retroactive cohort study of 160 patients with at least one provider visit at the Rhode Island Hospital Medical Primary Care Unit resident trainee clinic between Feb. 1, 2012, and Feb. 1, 2013, with an eGFR<60 ml/min/1.73m$^2$ on two lab draws 3 months apart. Independent variables included age; gender; severity of renal impairment; nephrology sub-specialty referral; co-management correspondence; ICD-9 coding; KDOQI recommended annual assessment of anemia, nutrition, bone health, electrolytes, kidney disease progression; treatment with Angiotensin coverting enzyme inhibitor or receptor blocker.

**Results:** 90% of patients had stage III CKD. 26% were co-managed with nephrology sub-specialists. Documented correspondence was present in only 53% of these patients. Only 54% of patients had ICD-9 codes documenting CKD. Serum creatinine level and the presence of Stage IV CKD demonstrated significant logistic fit between nephrology referrals ($p<0.0001$, $p<0.0001$), co-management ($p<0.0001$, $p<0.0015$) and ICD-9 documentation ($p<0.0003$, $p<0.0018$). There were significant one-way comparisons between ICD-9 documentation and nephrology referrals ($p<0.0001$) and co-management ($p<0.0001$). Multivariate analysis showed nephrology co-management to be an independent predictor of screening for bone health (serum phosphorus, $p<0.037$; serum PTH, $p<0.0088$) and anemia (hemoglobin, $p<0.012$).

**Conclusions:** There are significant correlations between adherence to screening recommendations and identification, documentation and sub-specialist CKD co-management in the primary care setting. Nephrology co-management independently affects bone health and anemia screening. Further work should be directed at assisting medical residents identify patients with stage III CKD and increase the percentage of successful nephrology referrals.

*Funding:* Clinical Revenue Support

## TH-PO332

**Multidisciplinary Intervention Model Is Better Than Conventional Health-Care and Nurse-Coordinated Models in the Management of Early CKD in Type 2 Diabetes Mellitus** Héctor R. Martínez Ramírez, Laura Cortes-Sanabria, Erika Fabiola Gómez-García, Alfonso M. Cueto-Manzano. *Unidad de Investigacion Medica en Enfermedades Renales, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico.*

**Background:** Multidisciplinary approach could optimize care in early CKD. ***Aim:*** to compare the effect of a multiple intervention model (MIM); conventional health-care model (CHCM);nurse-coordinated model (NCM) on lifestyle and renal function of DM2 patients and early CKD.

**Methods:** MIM was carried out in a Family Medicine Unit with patients receiving an educational intervention, by multidisciplinary team: social worker, dietitian, physical trainer and family physician (FP) for 4 wks, and supported by self-help groups; in other unit, NCM were seen by a nurse, focused on individuals' needs; CHCM were managed by FP. Patients had lifestyle, clinical, biochemical evaluations and follow up by 12 months. Max. score of lifestyle questionnaire is 100. All FP had course in early CKD.

**Results:** Comparison of delta results

| Lifestyle Questionnaire | MIM | NCM | CHCM |
|---|---|---|---|
| Knowledge of disease | 2.05 ± 3.5*†£ | 0.17 ± 3.5 | 0.84 ± 3.4 |
| Adherence to treatment | 2.25 ± 4.4* | 1.9 ± 5.0* | 0.52 ± 3.6 |
| Emotion management | 1.5 ± 4.5* | 1.4 ± 4.0* | 1.0 ± 3.6 |
| Exercise | 1.18 ± 4.5 | 0.23 ± 4.1 | 1.1 ± 4.9 |
| Tobacco Consumption | 0.36 ± 1.5 | 0.17 ± 1.0 | 0.0 ± 1.9 |
| Alcohol Consumption | 1.2 ± 2.4* | 0.3 ± 2.1 | 0.6 ± 2,6 |
| Diet | 4.3 ± 5.4* | 2.0± 5.8 | 2.1 ± 6.5 |
| Total score | 12. 9 ± 12.3*† | 5.2 ±15* | 4.9 ±14.2* |
| **Clinical and Biochemical Variables** | | | |
| SBP (mmHg) | -18 ± 19* | - 8 ± 13* | -21± 20* |
| DBP (mmHg) | - 5 ± 13* | 0.3 ± 11 | -5 ± 9* |
| BMI (Kg/m$^2$) | - 0.71 ± 1.6*£ | 0.50 ± 2.5 | -0.49 ± 1.6 |
| Hb $A_{1c}$ (%) | - 1.8 ± 2.0* | 0.3 ± 1.9 | - 0.3 ± 2.0 |
| Estimated GFR (ml/min/1.73m$^2$) | - 4.7 ± 15 | - 2.9 ± 22 | - 5.7 ± 16 |
| Albuminuria (mg/day) | - 28.1 ± 98.5* | 21.2 ± 139 | 31.9 ± 132 |

*$p$ <0.05 *vs* baseline same group; †$p$<0.05 *vs* same evaluation CHCM; ≤ $p$<0.05 *vs* same evaluation NCM.

**Conclusions:** MIM could improve the lifestyle habits of patients with DM2 and early CKD and preserver better renal function than NCM and CHCM.

## TH-PO333

**PRIMED for Patient Care: Multi-Disciplinary Simulation-Based Team Training Develops Staff Confidence and Skills in Managing Dialysis Emergencies** Laura E.A. Harrison, Katie Fielding, Richard J. Fluck, Lindsay J. Chesterton. *Renal Medicine, Royal Derby Hospital, Derby, United Kingdom.*

**Background:** Emergency situations can occur frequently during haemodialysis (HD) treatments and present a number of specific challenges to ensuring best patient outcomes. Realistic relevant simulation training can improve clinical knowledge and technical skills and strengthen communication and team behaviour, with a resulting improvement in patient safety.

**Methods:** We designed a simulation-based team education programme, PRactical Interdisciplinary Management of Emergencies on Dialysis (PRIMED) training, which has been delivered to over 100 renal staff (53% nurses, 16% doctors, 31% HD support workers). Clinical emergency scenarios take place in the dialysis unit with a Laerdal SimMan® 3G, standard HD machine and a dummy HD treatment. Session format includes facilitated debrief and feedback, with the opportunity for further guided, hands-on practice. Impact on staff confidence and skills was assessed using Likert-based evaluation forms, coupled with audit of weekly emergency buzzer calls.

**Results:** More staff felt confident in their management of emergencies during HD after PRIMED training (70% pre, 89% post). 95% of staff felt the training had a beneficial effect on their clinical practice. Composite confidence score was 44/55 pre-session, 48/55 post-session and 50/55 at follow-up, p=0.04. Staff confidence increased collectively in 2-3 specific areas after individual sessions, dependent on clinical and human factors highlighted in the session, including information handover, management of the HD machine, leadership and decision-making. There was a significant decrease in emergency buzzer use from ≥5/day to 0-3/week.

**Conclusions:** PRIMED is an innovative workplace based simulation programme that aims to equip all renal team members with a structured approach to the recognition, assessment and management of the acutely unwell patient on HD. Implementation has improved confidence across the spectrum of staff involved in dialysis. Frequency of emergency calls has fallen, potentially related to earlier recognition and treatment of unwell patients. Further work is ongoing to assess the impact on patient care and clinical practice.

## TH-PO334

**Podocalyxin Regulates Pronephric Glomerular Development in Zebrafish** Tomoko Obara. *Cell Biology, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK.*

**Background:** Vertebrate glomerular podocytes possess a highly sialylated transmembrane glycoprotein, Podocalyxin. In mammals, the sialic acid of Podocalyxin plays a crucial role in the formation of the characteristic podocyte architecture required for glomerular filtration.

**Methods:** To explore the possibility, we examined the function of Podocalyxin in the developing zebrafish pronephros by disrupting the expression of *podocalyxin* through the use of morpholino antisense oligonucleotides.

**Results:** Podocalyxin was localized at the apical membrane of podocytes throughout pronephric glomerular development in zebrafish. Translational blocking of *podocalyxin* expression resulted in pericardial edema and a hypoplastic glomerulus. Whereas regular foot processes with a slit diaphragm covered 66.7 ± 7.8% of the urinary surface of glomerular basement membrane, only 14.4 ± 7.5% of this area was covered with regular foot processes in the translationally blocked morphants. Splice blocking of *podocalyxin* exon 2, which partially encodes the bulky mucin domain with extensive sialic acid-containing sugar chains, resulted in the deletion of 53% of mucin domain-coding sequence from *podocalyxin* mRNA. Approximately 40% of these splice-blocked morphants had mild pericardial edema. Although the pronephric glomerulus in the splice-blocked morphants exhibited almost normal appearance with developed glomerular capillaries and mesangium, they had only 36.3 ± 6.9% of the area covered with regular foot processes.

**Conclusions:** Podocalyxin is predominantly expressed in the podocytes and plays a distinct role in the formation of the podocyte foot processes with a slit diaphragm during zebrafish pronephric development.

## TH-PO335

**Zebrafish Pronephros Tubulogenesis Is Reliant on the Polarity Proteins Prkc Iota and Zeta** Gary F. Gerlach, Rebecca A. Wingert. *Dept of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

**Background:** The zebrafish pronephros provides an excellent in vivo renal system to study the mechanisms of vertebrate nephrogenesis. When and how renal progenitors undergo tubulogenesis to form nephrons is poorly understood, but is known to involve a mesenchymal to epithelial transition (MET) and the acquisition of polarity.

**Methods:** Here, we determined the precise timing of these critical events during pronephros tubulogenesis, and document that lumen formation is preceded by the apical and basolateral segregation of proteins. As the ternary polarity complex is an essential regulator of epithelial cell polarity across tissues, we performed gene knockdown studies to assess the roles of the related factors protein kinase C iota and zeta (prkci, prkcz), which exhibited tight localization to the nephron epithelium apical surface prior to lumen formation and maintained this localization within the fully developed nephron.

**Results:** Nephron epithelial cells in prkci/z morphants had disrupted polarity, with aberrant localization of phospho-ERM and $Na^+/K^+$ ATPase to their respective apical and basolateral domains, and the actin cytoskeleton displayed a disordered arrangement. In addition, prkci/z morphants had dramatic defects in nephron tubule morphogenesis, with the proximal tubule failing to undergo normal convolutions. prkci/z morphants showed several deficiencies in nephron functionality, including abrogated renal clearance and proximal tubule dextran uptake, a measure of endocytosis. Surprisingly, tubule cells in prkci/z morphants displayed ectopic expression of podocyte-specific genes, including wt1a, wt1b, and podx1-like.

**Conclusions:** These data suggest a model in which the redundant activities of prkci and prkcz are essential to establish polarity and also maintain proper epithelial cell-type identity in the nephron tubule. Our studies provide a useful foundation for further analysis of MET during nephrogenesis, and have implications for understanding the pathways that affect nephron epithelial cells during kidney disease and regeneration.

*Funding:* NIDDK Support, Other NIH Support - NIH Director's Innovator

## TH-PO336

**mecom Is Essential for Proximodistal Segmentation and Epithelial Cell Fate Choice in the Zebrafish Pronephros** Yue Li, Christina N. Cheng, Valerie Verdun, Rebecca A. Wingert. *Dept of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

**Background:** The zebrafish pronephros provides a conserved developmental model to study nephrogenesis, in particular to delineate the poorly understood processes of how nephron segment pattern and cell type choice are established. Zebrafish nephrons are segmented into distinct epithelial regions that include a series of proximal and distal tubules, comprised of intercalated transporting epithelial cells and multiciliated cells (MCC).

**Methods:** The transcription factor MDS1 and EVI1 complex locus (mecom) is broadly expressed in renal progenitors and later restricted to the distal tubule. Here, we show that mecom is necessary to form the distal tubule, and to restrict both proximal tubule formation and MCC fate choice.

**Results:** Previous studies have shown that RA regionalizes the renal progenitor field into proximal and distal domains and that Notch signaling later represses MCC differentiation. We found that mecom and RA have opposing roles in patterning discrete proximal and distal segments. Further, we discovered that RA is required for MCC formation, and that one mechanism by which RA promotes MCC fate choice is to inhibit mecom. Next we determined the epistatic relationship between mecom and Notch signaling, which works by lateral inhibition to limit MCC fate choice. Inhibition of Notch signaling with the γ-secretase inhibitor DAPT revealed that Notch and mecom do not have additive effects in blocking MCC formation, suggesting they function in the same pathway. Ectopic expression of the Notch signaling effector, Notch Intracellular Domain (NICD) rescued the expansion of MCCs in mecom morphants, indicating that mecom activity promotes Notch signaling.

**Conclusions:** Our findings suggest a model in which mecom and RA arbitrate proximo-distal segment patterning, and that MCC fate choice is modulated by a complex interplay in which RA inhibition of mecom, and mecom promotion of Notch, titrates MCC number. Taken together, our studies have revealed several essential and novel roles for mecom during nephrogenesis.

*Funding:* NIDDK Support

## TH-PO337

**Single-Minded Homologue 1a (sim1a) Mediates Renal Progenitor Patterning during Zebrafish Nephrogenesis** Christina N. Cheng, Rebecca A. Wingert. *Dept of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

**Background:** During zebrafish development, a functional embryonic kidney known as the pronephros arises from renal progenitors in the intermediate mesoderm. Initial patterning of the renal progenitor field is characterized by the formation of rostral and caudal domains. Once the pronephros is established, it consists of two nephron tubules that have proximal and distal segments mostly similar to mammals like humans—including the proximal convoluted tubule (PCT), proximal straight tubule (PST), distal early (DE), Corpuscle of Stannius (CS), distal late (DL), and a duct. Thus, the zebrafish is a simplified yet conserved model for the study of vertebrate nephrogenesis.

**Methods:** To date, the mechanisms that establish nephron segmentation pattern remain poorly understood. Using whole mount *in situ* hybridization (WISH) to profile renal progenitor gene expression in zebrafish, we discovered that the transcription factor, *single-minded homologue 1a* (*sim1a*), is the initial marker of the renal progenitor caudal domain. *sim1a* expression is dynamic during nephrogenesis and ultimately maintained in both the proximal tubule segments (PCT, PST) along with the cluster of CS progenitors.

**Results:** These findings suggested that sim1a might have roles in nephron segment patterning and CS formation. We performed functional analysis of sim1a using morpholino knockdowns. sim1a deficiency caused an expansion of the PCT and an abrogation of the PST and CS populations. However, the domains of both the DE and DL segments were unchanged. Our results suggest that sim1a activity is necessary to pattern the PST and CS, andmay negotiate the PCT/PST boundary, possibly by inhibiting PCT fate choice.

**Conclusions:** Taken together, our study has revealed novel roles for sim1a during nephrogenesis. These findings provide useful insights into the genetic pathways that direct nephron development, and may have implications for understanding the causes of renal birth defects in humans.

*Funding:* NIDDK Support, Other NIH Support - NIH Director's Innovator

## TH-PO338

**Development of Intercalated Cells Requires Transcription Factor CP2L1** Max Werth,[1] Kai M. Schmidt-Ott,[2] Andong Qiu,[3] Jonathan M. Barasch.[1] *[1]Medicine, Columbia Univ, New York, NY; [2]Max-Delbrueck Center for Molecular Medicine, Berlin, Germany; [3]Tongji Univ, Shanghai, China.*

**Background:** Kidney epithelia derive from metanephric mesenchyme by a process that includes epithelial conversion followed by specification of different epithelial cell types. Little is known about how multiple cell types come to occupy the collecting ducts. Here we investigated a transcription factor that was induced during tubulogenesis.

**Methods:** To identify genes that regulate specific cell types, we created an *in vitro* model of nephrogenesis. We incubated rat metanephric mesenchyme with growth factors (LIF and FGF), and observed the time course of epithelial induction using microscopy and gene expression profiling. We identified 70 transcription factors that were regulated during epithelial conversion. We next over-expressed each of these factors in the metanephric mesenchyme using adeno-viral mediated gene transfer and found that only one transcription factor induced conversion, the Grainyhead family member, Cp2l1. We then generated kidney specific knockout of Cp2l1.

**Results:** During development Cp2l1 was expressed in distal parts of s-shaped bodies and in the ureteric bud. In the adult Cp2l1 was observed in the loop of henle, distal convoluted tubule and collecting ducts. In the conditional knockout we observed the complete absence of alpha- and beta-Intercalated Cells and their markers and instead we observed a monotonous pattern of principal cell-like epithelia. To further investigate Cp2l1 function we compared ChIP-seq with gene expression data from Cp2l1 knockouts. The comparison yielded 82 genes that were significantly downregulated in the knockouts and contained Cp2l1 binding sites within 10KB of the transcriptional start site. Many of these genes were components of mature intercalated cells, confirming a direct role for Cp2l1 in the regulating intercalated cells differentiation. Moreover, both gene expression and ChIP-seq data suggested that Cp2l1 regulates the patterning of Notch signaling throughout the collecting duct.

**Conclusions:** Taking together, this data implicate that Cp2l1 plays a continuing role in the establishment and maintenance of the kidney intercalated cells.

*Funding:* NIDDK Support

## TH-PO339

**Ureteropelvic Junction Obstructions in Mice with Conditional Inactivation of Exocyst Sec10 in Kidney and Upper Urinary Tract Epithelium** Noemi Polgar,[1] Vanessa H. Lui,[1] Amanda J. Lee,[1] Xiaofeng Zuo,[2] Joshua H. Lipschutz,[2] Ben Fogelgren.[1] *[1]Anatomy, Biochemistry, and Physiology, Univ of Hawaii; [2]Medicine, Univ of Pennsylvania.*

**Background:** Congenital obstructive nephropathy, the most common cause of pediatric chronic kidney disease and ESRD, is caused by obstruction of the urinary tract during fetal development. The most common cause of congenital obstructive nephropathy is ureteropelvic junction obstruction (UPJ obstruction), when the stenosis is localized to the upper urinary tract where the renal pelvis transitions into the ureter. Despite the high prevalence and medical burden of UPJ obstructions, we have a poor understanding of its molecular and genetic basis, with a scarcity of non-surgical genetic animal models.

**Methods:** In previous studies using cell culture models, we have shown the eight-protein exocyst trafficking complex to be critical in maintaining aspects of polarized epithelial cells. To further *in vivo* studies of polarized exocytosis in renal development and disease, we have generated a novel transgenic mouse to conditionally knockout Sec10, a central component of the exocyst. This is the first conditional mouse strain for any exocyst subunit, and should be valuable in studying the exocyst's role in various tissues and diseases.

**Results:** We crossed this floxed-Sec10 strain with the Ksp-Cre mouse strain to induce a knockout in Sec10 specifically in ureteric bud-derived epithelial cells during development. Surprisingly, 90% of the $Sec10^{FL/FL}$;Ksp-Cre conditional knockout mice died quickly after birth, displaying severe bilateral hydronephrosis due to congenital obstructions of the upper urinary tract. From histological analysis, these blockages were due to an overgrowth of the surrounding smooth muscle cells at the UPJ, with complete disappearance of the ureter lumen.

**Conclusions:** This novel transgenic mouse model of UPJ obstruction may be valuable for further studies of the causes and consequences of human congenital obstructive nephropathy.

*Funding:* NIDDK Support, Private Foundation Support

**TH-PO340**

**Bmp7 Maintains Undifferentiated Kidney Progenitor Population and Determines Nephron Numbers at Birth** Motoko Yanagita,[1] Mayumi Tomita,[1] Misako Asada,[1] Nariaki Asada,[1] Jin Nakamura,[1] Akiko Oguchi,[1] Atsuko Y. Higashi,[1] Shuichiro Endo,[1] Aris N. Economides.[2] *[1]Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [2]Regeneron Pharmaceuticals, Inc., NY.*

**Background:** The number of nephrons, the functional units of the kidney, varies among individuals. A low nephron number at birth is associated with a risk of hypertension and the progression of renal insufficiency. The molecular mechanisms determining nephron number during embryogenesis have not yet been clarified. Germline knockout of bone morphogenetic protein 7 (Bmp7) results in massive apoptosis of the kidney progenitor cells and defects in early stages of nephrogenesis. This phenotype has precluded analysis of Bmp7 function in the later stage of nephrogenesis.

**Methods:** We utilized conditional-null allele of Bmp7 in combination with systemic inducible Cre deleter mice in order to analyze Bmp7 function at desired time points during kidney development. We also employed kidney explants culture system and colony-forming assays to investigate the inhibitory effect of Bmp7 on epithelialization of the kidney progenitor cells.

**Results:** Systemic knockout of Bmp7 in vivo after the initiation of kidney development resulted in the precocious differentiation of the kidney progenitor cells to nephron (glomeruli and tubules), in addition to the prominent apoptosis and the reduction of progenitor cells. Quantitative assessment also confirmed that knocking out of Bmp7 in kidney explants culture resulted in accelerated differentiation of progenitor population, and the same effect could be imparted by inhibiting Smad signaling in wild-type kidney explants. Finally colony-forming assays demonstrated that Bmp7 inhibited epithelialization of the progenitor cells.

**Conclusions:** During embryonic development, Bmp7 acts on progenitor cells as an anti-apoptosis and anti-differentiation factor, and thereby coordinately maintains the renal progenitor pool in an undifferentiated status, and determines the nephron number at birth.

*Funding:* Government Support - Non-U.S.

**TH-PO341**

**Preterm Birth: Kidney Size and Function in Early Adulthood** Megan R. Sutherland, Catherine Fallaha, Anne-Laure Lapeyraque, Fanny Huyard, Mariane Bertagnolli, Anik Cloutier, Thuy Mai Luu, Anne Monique Nuyt. *Dept of Pediatrics, Sainte-Justine Univ Hospital and Research Center and the Faculty of Medicine, Univ of Montreal, Montreal, Canada.*

**Background:** Infants born preterm at <29 weeks gestation (term=37 completed weeks) are delivered at a time of active nephrogenesis; postnatally, ongoing renal development is likely impaired. The aim of this study was to determine the long-term impact of preterm birth on renal size and function in early adulthood.

**Methods:** Young adults born preterm at <29 weeks gestation (n=9 male, 11 female), between 1987-1993 (Montreal, Quebec), were compared to age-matched controls born at term (n=7 male, 13 female). Morning blood and urine samples were assessed for creatinine levels and microalbuminuria; GFR was estimated using the MDRD equation. Ultrasonography was performed to measure kidney size, and to determine the resistivity index (RI) of the renal arteries.

**Results:** Subjects born preterm had a significantly greater GFR compared to the term-born controls, particularly the females (males: control 93.8 ± 8.4, preterm 104.8 ± 5.3 ml/min/1.73m$^2$; females: control 107.5 ± 4.8, preterm 140.3 ± 12.2 ml/min/1.73m$^2$). There was no significant difference in the albumin:creatinine ratio between groups (control: 0.70 ± 0.07 mg/mmol; preterm: 0.67 ± 0.09 mg/mmol). Significant reductions in both absolute right (males: control 172.8 ± 18.1, preterm 140.3 ± 5.0 ml; females: control 145.7 ± 9.5, preterm 115.0 ± 8.3 ml) and left kidney volumes were observed in the preterm group. There was also a trend (p=0.08) for decreased relative volume of the right kidney in the preterm group compared to the controls, but no difference in the relative volume of the left kidney. There was no difference between groups in the RI of the renal arteries.

**Conclusions:** In early adulthood, both renal size (significantly reduced) and function (possible hyperfiltration) were affected by preterm birth. In the long-term, these changes have the potential to predispose to renal disease.

*Funding:* Government Support - Non-U.S.

**TH-PO342**

**The Super Kidney: Putting the Foundation for the Future** Ashraf El-Meanawy, Sterling E. Udom, Adil Jadoon. *Medical College of Wisconsin.*

**Background: Nephron loss** is the quintessence of progressive renal disease. Subjects **born** with **low nephron number** are at a disadvantage and have higher risk for kidney disease and hypertension. Prematurity and low birth weight (LBW) are the leading causes for low nephron endowment. The mechanism of low nephron number associate with LBW and prematurity is not known and no interventions exist to increase nephron number. In this work, we show that we identified potential molecular target and we can increase the number of nephrons in mice.

**Methods:** 1)Cells expressing HHIP were exposed to Dimethyloxallyl Glycine (DMOG) or vivo-morpholino and subjected to western blots to evaluate HHIP expression. 2)e12.5 mouse embryonic kidney cultures were exposed to DMOG, room air oxygen, or at 6,9, or 12% oxygen concentration. Cultured kidneys were then stained for ureteric bud (UB) marker calbindin D28K. UB branches were examined using confocal microscopy. 3) Time-pregnant CD1 mice were injected with a vivo-mopholino oligonucleotide against HHIP mRNA or DMOG 1 day before expected delivery. The pops also received the same treatment daily for 2 days after birth. Control mice received saline injections. Mice were sacrificed at 28-32 days of age and nephrons were counted.

**Results:** DMOG and vivo-morpholino knocked down HHIP protein level in cell cultures. Kidney cultures showed increase in ureteric bud branching when cultured under lower oxygen tension or in presence of DMOG. Mice treated with vivo-morpholino oligo specific to HHIP or DMOG showed statistically significant increase in nephron number compared to control animals.

**Conclusions:** Mice are born before termination of nephrogenesis and thus similar to extreme premature humans with regard to kidney development. Extreme premature infants present a future health care challenge as well as therapeutic opportunity. Prematurity causes early termination of nephrogenesis which lead to reduction and nephron number. We provide experimental evidence for potential target and ability to increase number of nephrons in live animals. The kidney with more nephrons might maintain function for longer time against nephron loss stressors and could represent the SUPERKINEY.

*Funding:* Clinical Revenue Support

**TH-PO343**

**Modulation of Maternal Light Exposure Affects Circadian Gene Expression in Fetal Kidneys** Krisztina Meszaros,[1] Linda Pruess,[1] Matthias Gondan,[2] Eberhard Ritz,[3] Franz S. Schaefer.[1] *[1]Center for Pediatrics and Adolescent Medicine, Heidelberg Univ; [2]Institute of Medical Biometry and Informatics; [3]Ruperto Carola Univ of Heidelberg, Germany.*

**Background:** Homeostatic kidney functions oscillate in a circadian manner, based on the oscillatory expression of a 'clock' gene network that controls numerous kidney specific genes regulating key kidney functions such as electrolyte and water excretion *(e.g. αENaC, NHE3, BSC1, AVPR2 and SGK1)*. Whereas alterations of circadian light exposure are known to cause a variety of adverse effects on body functions in adults, little is known about a potential impact on fetal organ development and function. We sought to explore the effect of altered maternal circadian light exposure on the development of oscillatory clock- and clock-controlled gene expression in the fetal kidney.

**Methods:** Pregnant SD rats were randomly allocated to different light patterns (12h:12h (LD), constant light (LL) and constant darkness (DD)) with free access to food and water at constant temperature. Mothers (6-10 per group) with their offspring were sacrificed at 4-h intervals one day before the expected delivery. Intrarenal circadian gene expression patterns were profiled by RT-PCR for the canonical clock genes Clock, Per1, Cry1, Cry2 and the clock-controlled genes αENaC, NHE3, BSC1, AVPR2 and SGK1.

**Results:** Pups from mothers kept at LD displayed circadian expression of the core clock gene ***Clock*** *(p<0.01)* and the clock-controlled genes ***αENaC*** *(p<0.001)* and ***NHE3*** *(p<0.001)*. Kidneys of fetuses from mothers exposed to LL exhibited circadian expression of ***Clock*** *(p<0.01)*, ***Per1*** *(p<0.05)*, ***Cry2*** *(p<0.05)*, ***aENaC*** *(p<0.001)*, ***SGK1*** *(p<0.001)*, ***NHE3*** *(p<0.001)*, ***BSC1*** *(p<0.01)* and ***AVPR2*** *(p<0.05)*. In contrast, fetal kidneys with maternal DD exposure completely lacked circadian fluctuations of intrarenal gene expression.

**Conclusions:** Circadian expression of clock and clock controlled genes is present even before birth in the developing kidney and differentially affected by modulation of light exposure. Maternal exposure to constant light enhances, whereas constant darkness suppresses the development of circadian gene expression in the fetal kidney.

**TH-PO344**

**Abstract Withdrawn**

**TH-PO345**

**The Histone H3K79 Methyltransferase, Dot1l, Regulates the Fate of Ureteric Bud Tip Cells** Fenglin Wang, Xiao Yao, Zubaida R. Saifudeen, Samir S. El-Dahr. *Pediatrics, Tulane Univ School of Medicine, New Orleans, LA.*

**Background:** Ureteric bud tip cells (UB-TC) act as progenitors during branching morphogenesis by generating daughter tip and trunk cells. Current models suggest that UB epithelial progenitors give rise to plastic principal cells, which have the potential to give rise to intercalated cells. This process is restrained epigenetically by the histone H3K79 methlytransferase, Dot1l. The role of Dot1l in UB-TC fate is unknown.

**Methods:** 1. Developmental expression of Dot1l and H3K79me2 was determined by QPCR, immunofluorescence and Western blots; 2. The role of Dot1l in the UB lineage was examined by crossing Hoxb7-Cre:GFP and Dot1$^{(f/f)}$ mice and progeny was examined at E12.5-P1; 3. The renal phenotype was analyzed by section IF and ISH.

**Results:** 1. p63, a master regulator of epithelial cell differentiation in skin and prostate, marks a subset of Ret$^+$ UB-TC. p63$^+$ cells display two distinctive features: transient expression from E17.5-P5 and lack of phospho-H3, a marker of mitosis. P63$^+$ cells are not normally seen in the UB trunk and may represent a progenitor UB-TC population.

2. *Dot1l* and H3K79me2 are developmentally up-regulated and are highly enriched in chromatin of differentiated epithelial cells of the distal nephron.

3. Loss of H3K79me2 in UB lineage increases the number of p63$^+$ UB-TCs; this effect is preceded by premature onset of p63 expression at E12.5, expansion of the *Ret$^+$/Wnt11$^+$* domain, and increased number of dividing (pH3$^+$) cells in terminal ampulla. UB branch number and overall kidney size are not affected by *Dot1l* deletion.

4. At P1, elongating collecting ducts lacking H3K79me2 exhibit ectopic presence of p63$^+$ cells, a lower intercalated cell/collecting duct ratio, and decreased expression of the intercalated cell master regulator, Foxi1.

**Conclusions:** 1. Chromatin-based mechanisms mediated via Dot1l/H3K79 methylation control the terminal differentiation fate of UB-TC; 2. In the absence of Dot1l, $p63^+$ UB-TC proliferate and later populate elongating collecting ducts; 3. Dot1l deletion disrupts the balance between p63 vs Foxi1, which may determine the fate of collecting duct precursor cell types.

*Funding:* NIDDK Support

## TH-PO346

**Ureteric Bud Branching Is Suppressed by the Loss of Trps1 due to the Activation of TGF-β Signaling** Ting Gui,[1] Yasuteru Muragaki,[2] Gengyin Zhou.[1] *[1]Pathology Dept, The Medical School of Shandong Univ, Jinan, Shandong, China; [2]The First Dept of Pathology, Wakayama Medical Univ, Wakayama, Japan.*

**Background:** We previously found that ureteric bud branching is suppressed in the embryonic kidneys of Trps1-deficient (KO) mice (published in JASN, 2011). However, how Trps1 is involved in UB branching remains unknown.

**Methods:** E11.5 ureteric bud and E12.5 whole kidney culturing exposure with smad3 inhibitor and TGF-b; Trps1 KO mice; DNA array.

**Results:** In the present study, we unveil the molecular mechanisms by which the loss of Trps1 suppresses UB branching. When we compared gene expression patterns via DNA microarray analysis using cultured ureteric buds isolated from E11.5 kidneys of WT and KO embryos, we found aberrant expression of genes associated with the transforming growth factor (TGF)-b/Smad3 signaling pathway in the KO UBs. Western blot and immunohistochemistry analyses showed increased levels of Rb1cc1, Arkadia1, and phosphorylated Smad3 and decreased levels of Smurf2, Smad7, and c-Ski in the KO embryonic kidneys. In addition, TUNEL staining and immunohistochemical detection of PCNA revealed that the apoptosis of UB cells was upregulated and, conversely, that cell proliferation was suppressed. Finally, we demonstrated that the suppression of UB branching in the KO UBs was restored by the exogenous addition of the Smad3 inhibitor SIS3, whereas the addition of TGF-b1 accelerated the suppression of UB branching in organ cultures of both isolated UBs and whole embryonic kidneys.

**Conclusions:** Considering these results, we conclude that UB branching is suppressed through increased activation of the TGF-b/Smad3 signaling pathway when Trps1 is lost.

*Funding:* Government Support - Non-U.S.

## TH-PO347

**Unraveling the Molecular Mechanisms Regulating Principal Cell Differentiation** Kameswaran Surendran,[1,2] Justin J. Grassmeyer,[1] Malini Mukherjee.[1] *[1]Children's Health Research Center, Sanford Research/USD, Sioux Falls, SD; [2]Dept of Pediatrics, Sanford School of Medicine, Sioux Falls, SD.*

**Background:** The mammalian collecting ducts are composed of principal and intercalated cell types that arise from a common precursor. Studies in mice identified *Mind bomb-1* (*Mib1*), a mediator of Notch and Wnt signaling, to be critical for principal cell differentiation. Mice with *Mib1* deficient collecting ducts develop symptoms similar to patients with mutations in *Aquaporin-2* (*Aqp2*) and *arginine-vasopressin receptor 2* (*Avpr2*) who suffer from nephrogenic diabetes insipidus (NDI). Considering defective principal cell differentiation can result in NDI, our goal is to identify genes regulating principal cell differentiation. For this purpose we analyzed mice in which *RBPJ* and hence Notch signaling is inactivated in developing collecting ducts.

**Methods:** We compared the following between wild-type littermates and mice with RBPJ-deficient collecting ducts: number of principal and intercalated cells by immunohistochemistry and quantitative(q) RT-PCR, urine concentrating capacity,gene expression profiles using Illumina mouse bead chips, and expression pattern of Elf5 using $Elf5^{+/LacZ}$ mice. We ectopically activated Notch1 in the developing collecting ducts of mice and measured *Elf5* mRNA levels by qRT-PCR.

**Results:** Collecting duct inactivation of *RBPJ* increased intercalated and, diminished principal cell number, and reduced urine-concentrating capacity. Comparison of gene expression profiles of wild-type developing kidneys and those with Notch signaling deficient collecting ducts revealed a total of 180 differentially expressed genes by at least 1.25-fold in wild type versus mutant kidneys with $p<0.05$. One of the down-regulated genes is Elf5, which we observe is expressed in principal cell lineage prior to Aqp2. Ectopic Notch activation in developing collecting ducts results in precocious Elf5 expression.

**Conclusions:** Notch/RBPJ signaling regulates principal cell fate selection to ensure proper water homeostasis.Elf5 is an early principal cell lineage marker activated in a Notch signaling dependent manner and is a candidate regulator of principal cell differentiation.

*Funding:* Private Foundation Support

## TH-PO348

**Dicer Ureteric Bud Mutant Analysis: New Insights in Mammalian Kidney Development** Vidya Nagalakshmi Kusma Harinathan, Jing Yu. *Dept of Cell Biology, Univ of Virginia, Charlottesville, VA.*

**Background:** In order to understand the ureteric bud (UB)-derived microRNAs (miRNAs) in mammalian kidney development, we have generated a conditional *Dicer* UB mutant with a *HoxB7Cre* driver line. Our earlier mutant phenotype analyses imply a critical role for miRNAs in UB branching and tubule size control. The present study aims at identifying the target genes up-regulated in *Dicer* UB mutants and analyzing the UB- expression pattern of up-regulated transcripts during various stages of embryonic kidney development.

**Methods:** Our combined approach involved, transcriptional profiling, *in situ* hybridization, and bioinformatics analyses on some of the potential miRNA target genes up-regulated in *Dicer* UB mutants.

**Results:** We have observed that some of the potential miRNA target genes up-regulated in *Dicer* mutants are spatially and temporally restricted during kidney development. We identified, for the first time, temporally regulated genes in the wild-type UB epithelium whose expression is present in the UB epithelia during early stages and absent in later stages of kidney development. Moreover, this temporal regulation is subjected to miRNA regulation, as in *Dicer* mutants expression of these genes persists into later stages. Further analysis on mechanistic roles of these temporal genes in kidney development is underway and this will help us to understand the intricate developmental processes occurring at cellular level in UB epithelial morphogenesis and the importance of miRNAs in their regulation during kidney development.

**Conclusions:** In summary, our study demonstrated the requirement of UB-derived, *Dicer*-dependent miRNAs in mammalian kidney development and revealed a previously unrecognized developmental phenomenon which implicates miRNAs in temporal control of mammalian UB development.

*Funding:* Private Foundation Support

## TH-PO349

**A Grainyhead-Like 2/Ovo-Like 2 Pathway Regulates a Transcriptional Network That Controls Collecting Duct Lumen Size** Janett Ruffert,[1,2] Annekatrin Aue,[1,2] Christian Hinze,[1,2] Katharina Walentin,[1,2] Max Werth,[1,2] Jonathan M. Barasch,[3] Andong Qiu,[3] Kai M. Schmidt-Ott.[1,2] *[1]Max Delbrück Center for Molecular Medicine, Berlin, Germany; [2]Dept of Nephrology, Charité-Universitätsmedizin Berlin; [3]Dept of Medicine, Columbia Univ College of Physicians and Surgeons, New York.*

**Background:** The molecular and cellular mechanisms that govern tubular lumen size regulation may provide critical insights into the pathogenesis of kidney diseases. The transcription factor grainyhead-like 2 (Grhl2) is expressed in epithelial cells of the ureteric bud and the distal nephron.

**Methods:** To investigate the role of Grhl2 in the renal collecting duct (CD) we bred Hoxb7/Cre; $Grhl2^{flox/-}$ mice, which exhibit a deletion of functional Grhl2 protein in most cells of the CD. For further analysis of Grhl2 function in CD epithelia, we generated inner medullary collecting duct (IMCD3) cells with a lentiviral knockdown of Grhl2.

**Results:** A closer examination of the CD in Grhl2-deficient mice revealed that the papillary CD lumen area was markedly reduced at 4 weeks of age. In three-dimensional culture, IMCD3 cells formed cysts of polarized epithelial cells enclosing a central lumen. However, in Grhl2 knockdown cells lumen formation was perturbed resulting in cysts with a markedly reduced lumen area. Genome-wide screening for Grhl2 targets by microarray analyses of Grhl2-deficient IMCD3 cells and chromatin immunoprecipitation with next generation sequencing in IMCD3 cells and kidneys revealed that Grhl2 regulates multiple genes involved in epithelial junction formation and polarity, as well as other transcription factors including the gene encoding the zinc-finger transcription factor ovo-like 2 (Ovol2). Ovol2 expression was markedly reduced in IMCD3 cells following Grhl2 knockdown and in Hoxb7/Cre; $Grhl2^{flox/-}$ kidneys. Overexpression of Ovol2 in Grhl2 knockdown cells rescued cystic lumen formation. Microarray analyses of Grhl2 knockdown cells overexpressing Ovol2 showed reconstitution of the transcription levels for the majority of Grhl2-dependent genes.

**Conclusions:** Our data thus indicate a novel Grhl2/Ovol2 pathway that controls a transcriptional network governing collecting duct lumen size regulation.

## TH-PO350

**Tissue Specific Knockout of Dragon in Collecting Duct Leads to Urinary Concentrating Defect** Wenjie Chen,[1] Richard Bouley,[1] Dennis Brown,[1] Yin Xia,[2] Herbert Y. Lin.[1] *[1]Program in Membrane Biology, Div of Nephrology, Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; [2]Program in Reproduction, Development and Endocrinology, The Chinese Univ of Hong Kong, Hong Kong, China.*

**Background:** Dragon, encoded by the *RGMB* gene, acts as a bone morphogenetic protein (BMP) coreceptor that enhances BMP signaling. The Dragon global knockout mouse dies 2-3 weeks after birth (Ma et al. 2011). In a previous study, we showed that Dragon localized predominantly to the apical surfaces of tubular epithelial cells in the thick ascending limbs, distal convoluted tubules, and collecting ducts of mice, and may play a role in maintaining tight junction integrity in these cells (Xia et al. 2010).

**Methods:** To further investigate the role of Dragon in kidney function, we generated the Dragon-Floxed mouse and crossed this line with the HoxB7-Cre mice, to generate the collecting duct conditional knockout mice Dragon (cd/cd) with a C57BL6/CD-1 mixed genetic background.

**Results:** The 6-week old Dragon (cd/cd) mice had increased volume of urine by 54 percent and water intake by 18 percent, and had decreased urine osmolarity by 28 percent compared to control mice. Interestingly, real-time PCR analysis showed that AQP2 mRNA levels were decreased by 20 percent in Dragon (cd/cd) mice. In addition, in some Dragon (cd/cd) mice the papilla was grossly hypomorphic.

**Conclusions:** We hypothesize that Dragon regulates the development of the collecting duct and the expression of AQP2 and urine concentration in the kidney.

*Funding:* NIDDK Support

## TH-PO351

**The Ureteric Bud Epithelium-Derived Cdc42 Regulates Fzd4/8 Expression and Ureteric Bud Branching Morphogenesis during Mammalian Kidney Development** Qun Ren, Jing Yu. *Cell Biology, Univ of Virginia, Charlottesville, VA.*

**Background:** Cdc42 is a ubiquitously expressed small GTPase belonging to the Rho family. Active Cdc42 regulates a wide variety of cellular function and affect multiple cellular processes such as establishing and maintain cell polarity and regulate epithelial morphogenesis. However, its role in the ureteric bud epithelium during kidney development has not been examined.

**Methods:** To address this issue, we specifically ablated Cdc42 function from the ureteric bud epithelium with Hoxb7cre (referred to as Cdc42 mutants).

**Results:** This genetic manipulation led to neonatal lethality within 48 hours post-partum. The mutant kidneys were hypoplastic and exhibited cystic dilatation, and were defective in principal and intercalated cell differentiation. Ureteric bud branching morphogenesis was compromised as early as E12.5, and expression of Fzd4 and Fzd8 in the ureteric bud epithelium was greatly reduced. Interestingly, apico-basal polarity of the ureteric bud epithelium was unaffected, nor was the apical localization or phosphorylation of aPKC , indicating that Cdc42 does not regulate apico-basal polarity of the ureteric bud epithelium and does not employ aPKC as its effector in the ureteric bud epithelium. Instead, PAK1 phosphorylation was reduced significantly in the ureteric bud epithelium. Consistent with a role of PAK in mediating Cdc42 function in the ureteric bud epithelium, inhibition of the PAK activity disrupted ureteric bud branching morphogenesis.

**Conclusions:** Our work demonstrates that Cdc42 regulates the expression of Fzd4/8 in the ureteric bud epithelium and is required for kidney branching morphogenisis,most likely through its effector PAK1.

*Funding:* NIDDK Support

## TH-PO352

**Ureteric Bud (UB) Prorenin Receptor (PRR) Is Essential for Normal Collecting Duct (CD) Development and Function** Renfang Song, Graeme James Preston, Ihor V. Yosypiv. *Pediatrics, Tulane Univ School of Medicine, New Orleans, LA.*

**Background:** We tested the hypothesis that targeted inactivation of the *PRR in the UB epithelia using Hoxb7$^{Cre}$ (PRR$^{UB-/-}$) is essential for normal CD development and function.*

**Methods:** UB-specific knockout of PRR was confirmed by qRT-PCR analysis which revealed an 80% decrease in PRR mRNA levels in E11.5 intact UBs (iUBs) isolated from PRR$^{UB-/-}$ compared with PRR$^{UB+/+}$ mice (0.19±0.03 vs. 1.0±0, p<0.001).

**Results:** In situ hybridization and immunohistochemistry demonstrated reduced expression of Aqp2, Foxi1, $H^+$-ATPase α4 and AE1 in mutant CDs on E18.5. On P1, kidney weight (15.6±0.45 vs. 24.5±0.43 mg, p<0.001) and kidney-to-body weight ratio (3.77±0.09 vs. 5.90±0.26 mg/g, p<0.01) was lower in mutants than in controls. Histological examination demonstrated the presence of cysts in collecting ducts, dilation of proximal tubules and decreased glomerular number in mutants. On P30, kidney weight (130±5.8 vs. 280±20 mg, p<0.001) and kidney-to-body weight ratio (7.9±0.23 vs. 16.2±0.6 mg/g, p<0.01) was lower in mutants than in controls. On P30, dilated CDs with thin epithelial layer were observed in PRR$^{UB-/-}$ kidneys. To test the functional consequences of the deficiency of the collecting duct PRR, we examined renal function, urinary volume, osmolality and pH on P30. Mutant mice had increased levels of serum creatinine (237±0.13 vs. 59±14 μmol/l, p<0.001). Despite similar water intake (2.5±0.37 vs. 2.2±0.36 ml, p=0.52), 24-hour urine volumes were higher (3.2±0.32 vs. 2.2±0.24 ml, p<0.05) and urine osmolalities were lower (830±39 vs. 1645±160 mg, p<0.001) in mutants compared with controls. Baseline urine pH did not differ between PRR$^{UB-/-}$ and PRR$^{UB+/+}$ mice (6.40±0.05 vs. 6.52±0.17, p=0.51). Acidic loading with $NH_4Cl$ resulted in a decrease in urine pH in control (6.40±0.05 vs. 6.02±0.12, p<0.05) and no change in urine pH in mutant (6.52±0.17 vs. 6.50±0.13, p=0.91) mice.

**Conclusions:** We conclude that UB/collecting duct PRR is essential for kidney morphogenesis, differentiation and acquisition of function by collecting duct cells involved in water and acid-base homeostasis.

*Funding:* NIDDK Support

## TH-PO353

**Mutations in Renin-Angiotensin System (RAS) Genes Are Associated with Isolated Multicystic Dysplastic Kidney (MCDK) in Children** Renfang Song, Graeme James Preston, Ihor V. Yosypiv. *Pediatrics, Tulane Univ, New Orleans, LA.*

**Background:** To determine whether MCDK is linked to mutations in RAS genes, we amplified the coding exons and the adjacent intronic sequences of the RAS genes by PCR: renin (9 exons), angiotensinogen (AGT, 5 exons), ACE (26 exons) and angiotensin 1 receptor (AT1R) (1 exon).

**Methods:** DNA was extracted from peripheral blood of 10 unrelated patients with MCDK diagnosed by renal ultrasonography (US) (mean age 8.5±1.1 years) and pooled healthy race-, age- and sex-matched controls according to standard procedures. 6 patients with MCDK were females and 4- males. Left kidney was affected in 6 cases. Renal function in all affected individuals was normal. Absence of MCDK or other renal anomalies in controls was confirmed by US. Both strands of PCR products were sequenced using dideoxy chain termination method on a 373A DNA sequencer (Applied Biosystems). The effect of mutation on protein function was predicted with PolyPhen-2 software.

**Results:** 9 novel mutations in *RAS* genes were found in 6 patients with MCDK. 3 novel and known heterozygous *AGT* mutations were found. 1 variation was probably damaging, 1 was possibly damaging and one was benign. 7 heterozygous *renin* mutations were found. 3 were probably damaging and 4 were benign. 6 heterozygous ACE mutations were found. 3 mutations were probably damaging and 3-benign. 3 heterozygous *AT1R* mutations were found. 2 variations were possibly damaging and one was benign.

**Conclusions:** In summary, we report novel associations of mutations in the genes encoding renin, AGT, ACE or AT1R with isolated MCDK in children in the United States. These findings highlight the crucial role of the RAS in the pathogenesis of MCDK in children.

*Funding:* NIDDK Support

## TH-PO354

**Dual Loss of Par-1a/b Proteins Leads to Altered Proximal Tubular Epithelial Cells Polarity in Murine Kidneys** Oleh M. Akchurin,[1] Nadira Ramkellawan,[1] Zhongfang Du,[1] James M. Pullman,[1] Anne Muesch,[1] Katalin Susztak,[2] Kimberly J. Reidy.[1] *[1]Albert Einstein College of Medicine; [2]Univ of Pennsylvania.*

**Background:** Par-1 is a member of the "partitioning defective" family of proteins, which are required to establish columnar epithelial cell polarity in vitro, but their role in kidney epithelial polarity in vivo has not been described. Par-1 has four mammalian homologues (Par-1a, -1b, -1c, and -1d), and complete loss of both Par-1a and -1b is early embryonic lethal. We have previously shown that all 4 homologues of Par-1 are expressed in the developing kidney, and block of Par-1 signaling inhibited growth of metanephric mesenchymes in vitro. We **hypothesized** that Par-1a/b are required for establishing renal epithelial polarity and normal kidney development in vivo.

**Methods:** Double-heterozygous Par-1a/b mutant mice were bred and offspring were sacrificed on day 1 of life. Par-1a-/-Par-1b+/- (Par1a/b KH) and Par-1a+/-Par-1b-/- (Par1a/b HK) genotypes were compared to the wild type (WT).

**Results:** Par-1a/b HK kidneys appear hypoplastic with a reduced kidney weight to body weight ratio (3.3±0.5 mg/g HK vs. 5.9±0.3 mg/g WT; p<0.002). A dilated renal pelvis was observed in 20% of the Par-1a/b KH mice, suggestive of ureteric bud branching defects. Grossly visible cysts were identified in a subset of Par-1a/b KH mice. On light microscopy, Par-1a/b KH mouse kidneys had dilated cystic tubules. Immunofluorescence (IF) labeling demonstrated that dilated tubular structures were LTL-positive and DBA-negative, suggesting that they originate from the proximal tubule. We also observed a decreased megalin expression in Par-1a/b KH proximal tubules and drastically reduced surface expression of E-cadherin. Preliminary results of IF labeling with acetylated tubulin suggest absence of the primary cilia in cysts and shorter primary cilia in Par-1a/b KH mice compared with a WT.

**Conclusions:** Par-1a/b are required for proper apical-basal polarity in vivo and contribute to normal kidney development. Par-1a/b gene deletion leads to renal hypoplasia and cystic dilatation of the proximal tubules. Ongoing studies are examining the mechanisms by which Par-1a/b contribute to cystogenesis and kidney development.

*Funding:* NIDDK Support

## TH-PO355

**Hypoxia Inducible Factor-1a Regulates Branching Morphogenesis in Kidney Development without Ureteric Bud Cell Proliferation** Kenji Tsuji, Shinji Kitamura, Hitoshi Sugiyama, Hirofumi Makino. *Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Okayama, Japan.*

**Background:** Embryos are exposed under hypoxia ($O_2$ 2-9%) in developing stage. During kidney development, GDNF signaling from metanephric mesenchyme through Ret and GFR-a1 is the common pathway involved in ureteric bud (UB) branching which is related to GFR associated nephron numbers. It has been reported that hypoxia-inducible factor (HIF)-1a makes an important role for development. However there are few reports about the relationship between HIF-1a and UB branching. Here we examined whether HIF-1a regulates the UB branching on renal development or not.

**Methods:** We harvested embryonic 13 days kidneys (E13Ks) from pregnant rats and cultured under normoxia (20% $O_2$ / 5% $CO_2$) or hypoxia (5% $O_2$ / 5% $CO_2$). We evaluated the kidneys on the point of morphology and gene expression. We also examined UB cell proliferation under normoxia or hypoxia using electric cell-substrate impedance sensing (ECIS) method.

**Results:** The number of ureteric end buds of cultured E13Ks under hypoxia significantly increased compared to under normoxia (the numbers of end buds under hypoxia v.s. normoxia; 57.7±12.5 v.s. 40.6±6.6/ kidney, p<0.01). Quantitative RT-PCR revealed increased GDNF, GFR-a1, RET and FGF10 mRNA level in cultured E13Ks under hypoxia compared to under normoxia. When we cultured E13Ks under hypoxia with HIF-1a inhibitor (digoxin), we could not observe increased UB branching (the numbers of end buds under hypoxia v.s. hypoxia with digoxin; 57.7±12.5 v.s. 41.5±7.5/ kidney, p<0.01). Direct inhibition of HIF-1a using siRNA decreased UB branching during E13Ks culture under hypoxia (the numbers of end buds under hypoxia v.s. hypoxia with HIF-1a siRNA; 57.7±12.5 v.s. 39.0±4.76/ kidney, p<0.01) and GDNF, GFR-a1 mRNA level significantly. In addition, UB cells under hypoxia proliferated significantly less than under normoxia in vitro.

**Conclusions:** HIF-1a regulates branching morphogenesis via GDNF/RET and/or FGF10 signal pathway without UB cell proliferation.

## TH-PO356

**Whole Exome Profiling Identifies Biological Processes Modified by Ebf1 during Podocyte Maturation** Jackie A. Fretz. *Orthopaedics and Rehabilition, Yale School of Medicine, New Haven, CT.*

**Background:** We previously identified Early B Cell Factor 1 (Ebf1) as a gene up-regulated in podocytes during the final stages of postnatal murine nephrogenesis. Deficiency of this transcription factor results in a unique phenotype of abrogated glomerular development that disproportionately effects peripheral glomerular development. Although this coincides with the late expression profile of Ebf1 in podocytes, it raised intriguing questions regarding the function of Ebf1 in the development of peripheral vs. juxtamedullary glomeruli.

**Methods:** To address these questions we performed RNA-seq to compare expression profiles of Ebf1-replete and Ebf1-deficient podocytes at three separate postnatal times: P2, 14, and 21. Samples were compared between genotypes and across time for multivariate analysis.

**Results:** Several podocyte-related markers including Pod1/Tcf21 and Lmx1b, were identified showing the specificity for the selected cells. The most similar populations were P0 podocytes from Ebf1-replete and –deficient cells. P14 and P21 Ebf1-replete podocytes were the next most closely related populations, and also more like the P0 podocytes than deficient cells at P14 or P21.

**Conclusions:** Analysis of the biological processes affected by Ebf1-deficiency identified modification in extracellular matrix formation, cell adhesion and locomotion, Wnt signaling, and neuronal development. Many of these processes have been shown previously to be essential for proper podocyte differentiation. Additionally, enrichment of processes involved in skeletal development and lipid metabolism were identified. These correlate to other known functions of Ebf1 in disparate cell lineages, and may identify conserved functions of this transcription factor in multiple cell types.

*Funding:* NIDDK Support, Other NIH Support - NIAMS

## TH-PO357

**The Role of miR-222 in Glomerular Development by Targeting NPM1** Shiying Cui,[1] Yanfang Ding,[1] Jinyao Zhao,[1] Xian Wu,[1] Guang Wang,[1] Mingyi Cui,[2] Tianbai Li.[1] *[1]Dalian Medical Univ, Dalian, China; [2]The Fourth People's Hospital, Liaoyang, China.*

**Background:** MicroRNA (miRNA) has been implicated in the regulation of organic development processes through repression of specific mRNAs at the posttranscriptional stage. The purpose of this study is to define a miRNA expression signature in glomerular development, and verify the function of miRNA-target in developing and developed glomeruli.

**Methods:** To reach this goal, mouse kidneys at the developed (P28) and developing (E18.5) stages, and podocytes (MPC-5) at undifferentiated (33'C) and differentiated (37'C) status were used in the study. Total RNA and protein were purified from glomeruli that were isolated from the kidneys at both developing and developed stages. MicroRNA microarray, Western Blotting, quantitative Real-Time Polymerase Chain Reaction (qRT-PCR), and immunohistochemistry were further performed.

**Results:** Of the 224 mouse miRNAs analyzed in the microarray, 62 were significantly expressed, and 39 with at least 2-fold difference in the glomerulus at P28 compared to E18.5. Target prediction from three bioinformatics softwares showed that the 803 targets of all key miRNAs had functions being relevant to cellular development. Of which, the reciprocal expression of miR-222- NPM1 (Nucleophosmin 1) , a pair of miRNA-target predicted, was validated by qRT_PCR, immunohistochemistry and western blotting in vivo, and further confirmed by miR-222 overexpression and luciferase reporter assays in vitro. The function of miR-222 by targeting NPM1 in podocyte proliferation and migration was finally verified in MPC-5 cells.

**Conclusions:** This study may advance the current level of understanding of miRNAs in glomerular development.

*Funding:* Government Support - Non-U.S.

## TH-PO358

**MicroRNAs Are Critical Regulators of FOXD1 Progenitors and Kidney Stroma during Nephrogenesis** Naoki Nakagawa,[1] Allie M. Roach,[1] Ivan G. Gomez,[1] Akio Kobayashi,[2] Jeremy Stuart Duffield.[1] *[1]Renal Div, Kidney Research Institute, Univ of Washington, Seattle, WA; [2]Harvard Stem Cell Institute, Boston, MA.*

**Background:** Recent studies have identified important roles for post-transcriptional regulators including microRNA in nephron development and in adult kidney diseases but the importance of microRNA in regulating stromal cell functions has not been explored and stromal functions in nephrogenesis remain ill-defined.

**Methods:** In order to study the function of kidney stromal cells, and the importance of post-transcriptional gene regulation in kidney stroma, during nephrogenesis, we mutated the microRNA activating enzyme *Dicer-1*, specifically in stromal cells that derive from *Foxd1* expressing kidney progenitors of the nephrogenic interstitium.

**Results:** *Dicer-1* mutants were born at less than expected Mendelian ratios, and surviving pups died shortly after birth. There was a striking reduction in kidney volume and absence of urine production. Histological examination revealed highly disorganized tubular and microvascular compartments as well as glomerular abnormalities. Elongation and differentiation of the loop of Henle was attenuated resulting in absence of inner medulla and papilla, and polarization of proximal tubules was impaired with loss of brush border. Peritubular capillary formation was disorganized with large highly branched capillaries, indicating that mesenchymal cells are necessary for correct blood vessel formation. These phenotypic changes were associated with evidence of reduced activation of stromal cells and lack of segmentation of the stroma. In addition Foxd1 is activated after nephrogenesis in podocyte progenitor epithelium, where Foxd1 and dicer activity in podocyte progenitors are necessary for differentiation into mature podocytes.

**Conclusions:** We conclude that microRNA post-transcriptional gene regulation is critical in *Foxd1* progenitor derived kidney stroma functions in nephrogenesis. These studies highlight the strategic importance of kidney stroma in organogenesis.

## TH-PO359

**Soluble Form of Angiotensin-Converting Enzyme (ACE) Is Enough for Kidney Development, but Not to Maintain Blood Pressure** Michifumi Yamashita,[1,2,3] Saurabh Chattopadhyay,[3] Ganes C. Sen.[2,3] *[1]Pathology, Univ Hospitals Case Medical Center, Cleveland, OH; [2]Pathology, Case Western Reserve Univ, Cleveland, OH; [3]Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.*

**Background:** ACE, a central component of Renin-Angiotensin System (RAS), is essential for kidney development and blood pressure (BP) regulation. ACE-/- mice show glomerular and tubular hypoplasia, and low BP. There are two forms of ACE: membrane-bound form and soluble form. ACE is expressed on the cell surface as type I ectoprotein, with enzymatically active ectodomain, transmembraine domain, and short cytoplasmic domain. The ectodomain can be cleaved to generate the soluble form. These two forms are equally enzymatically active. We reported (Kessler SP, et al. JBC 2003) membrane-bound ACE in the vascular endothelial cells is enough for kidney development as well as normal blood pressure. Here we investigate the roles of the soluble ACE in kidney development and renal functions.

**Methods:** We generated transgenic (TG) mice with Tie-1 promoter-driven expression of the soluble ACE in the background of ACE-/- mice, and analyzed the mice morphologically and physiologically, as well as at molecular level.

**Results:** The expressed ACE protein lacked the transmembrane and the cytoplasmic domains, and was constitutively secreted into the blood stream. The TG mice showed normal kidney structure grossly and microscopically. The urine output, water intake, and urine osmolarity were normal. However, TG mice showed still low BP compared to Ace+/+ wild type (WT) mice. The local RAS activity in kidney from TG mice was similar to WT mice: Renin and Angiotensin II in the kidney tissue from TG mice were comparable to WT mice, although TG mice did not show any ACE expression in kidney tissue. The systemic RAS activity was also similar between two groups: plasma renin activity, serum ACE activity, and plasma Angiotensin II level did not show significant difference between the two groups.

**Conclusions:** Soluble ACE is enough for kidney development, but not to maintain BP. The cytoplasmic domain of ACE in vascular endothelial cells, and its signaling might be important for BP regulation.

*Funding:* Other NIH Support - National Heart, Lung, and Blood Institute, R01HL048258

## TH-PO360

**The bHLH Transcription Factor Tcf21 Is Required for Differentiation and Maintenance of Podocytes in Development, Health and Disease** Yoshiro Maezawa,[1] Tuncer Onay,[1,5] Rizaldy P. Scott,[1] Lindsay S. Keir,[2] Henrik Dimke,[1] Chengjin Li,[1,5] Vera Eremina,[1] Asish Ghosh,[5] Jeffrey H. Miner,[3] Seppo Vainio,[4] Susan E. Quaggin.[1,5] *[1]SLRI, Toronto, Canada; [2]Uniersity of Bristol, United Kingdom; [3]Washington Uniersity, St. Louis; [4]Biocenter Oulu, Finland; [5]Feinberg Cardiovascular Research Institute and Div of Nephrology, Northwestern Univ, Chicago.*

**Background:** Tcf21 is a bHLH transcription factor essential for embryonic development. Global Tcf21 knockout (gl-KO) mice die minutes after birth with hypoplastic lungs and tetralogy of Fallot. In kidneys of glo-KO mice, mesenchyme-to-epithelial transformation, branching morphogenesis and nephrogenesis are arrested.

**Methods:** In addition to mesenchymal expression, Tcf21 is expressed in embryonic and adult podocytes. To examine the role of Tcf21 in podocytes, we created a conditional allele for Tcf21 and crossed them to PodocinCre (pod-KO) and Wnt4-Cre mice resulting in deletion of Tcf21 from differentiated and progenitor podocyte populations, respectively.

**Results:** Pod-KO mice don't exhibit overt defects in podocyte differentiation, but the glomerular structure is greatly simplified with decreased endothelial and mesangial cells. By 4 weeks of age, 30-40% of Pod-KO mice develop FSGS-like lesions and massive proteinuria, while 60% of mice never develop proteinuria. Microarray analysis of glomeruli from Pod-KO mice revealed candidate downstream targets, including Vegf, Pgf and Wif1. ShRNA knockdown of Tcf21 in human podocytes results in altered cell shape and migration. Earlier deletion of Tcf21 from podocyte precursors (Wnt4-Cre) leads to columnar shaped podocytes, aberrant distribution of Podocin and defects of mesangial migration, suggesting more profound defect of podocyte differentiation. Strikingly, induction of diabetes in the non-proteinuric Pod-KO mice results in increased proteinuria, suggesting a protective role for Tcf21.

**Conclusions:** Our results demonstrate a critical role for Tcf21 in the differentiation and maintenance of podocytes in both developing and mature kidneys. Identification of direct targets of this transcription factor may provide new therapeutic targets for proteinuric renal disease including diabetic nephropathy.

*Funding:* Government Support - Non-U.S.

## TH-PO361

**Maternal Diabetes Impairs Nephrogenesis via Augmented Hedgehog Interacting Protein (Hhip) Gene Expression** Xin-Ping Zhao,[1] Min-Chun Liao,[1] Shiao-Ying Chang,[1] Shaaban Abdo,[1] Yessoufou Aliou,[1] Isabelle Chenier,[1] Julie R. Ingelfinger,[2] Shao-Ling Zhang.[1] *[1]CRCHUM-Hotel Dieu, Univ of Montreal, Montreal, Canada; [2]Pediatric Nephrology Unit, Massachusetts General Hospital, Boston, MA.*

**Background:** We hypothesized that maternal diabetes impairs nephrogenesis via augmented hedgehog interacting protein (Hhip) gene expression, given screening gene arrays (Affymetrix GeneChip Mouse Gene 1.0ST array platform) we had performed had indicated that Hhip was significantly modulated by maternal diabetes.

**Methods:** We created an in vivo murine model using kidneys from the suckling pups of non-diabetic, diabetic, and insulin-treated diabetic dams. MK4 (an embryonic late metanephric mesenchymal (MM) cell line) and ureteric bud (UB) cell lines, were used for *in vitro* studies.

**Results:** Kidneys of the offspring of diabetic dams as compared to the control offspring displayed features suggesting retarded nephrogenesis, such as small kidneys and less UB branching morphogenesis. Augmented Hhip expression was observed on the offspring of diabetic dams, initially localized to the differentiated MM and UB epithelium, and then in maturing glomerular and tubulointerstitial cells. We also observed heightened Hhip active transforming growth factor beta 1 (TGFβ1) signaling, associated with dysmorphogenesis in these offspring. The *in vitro* studies demonstrated that Hhip overexpression decreased sonic hedgehog (Shh) and paired box gene 2 (Pax2) expression and increased transcriptional nuclear factor-kappa B (NF-kB, p50/p65), phosphorylation of p53 and TGFβ1 gene expression. In contrast, Pax2 gene overexpression inhibited Hhip and NF-kB and activated Shh, N-myc and $p27^{Kip1}$ gene expression. Moreover, high glucose stimulated Hhip gene expression, and then targeted TGFβ1 signaling. Thus, Pax2 via a negative autocrine feedback mechanism attenuated the stimulatory effect of high glucose on Hhip gene expression.

**Conclusions:** We conclude that impaired nephrogenesis induced by maternal diabetes is mediated, at least in part, via augmented Hhip gene expression.

*Funding:* Government Support - Non-U.S.

## TH-PO362

**Wilms' Tumor 1 Initiates the Differentiation Cascade by Suppressing Enhancer of Zeste Homolog 2-Mediated Silencing of Key Genes** Murielle M. Akpa,[1] Lee Lee Chu,[2] Diana Iglesias,[2] Paul R. Goodyer.[1,2] *[1]Human Genetics, McGill Univ, Montreal, Canada; [2]Pediatrics, McGill Univ Health Center Research Institute, Montreal, Canada.*

**Background:** During nephrogenesis, stem cells from the metanephric mesenchyme are induced to form nephrons upon receipt of an inductive signal from the ureteric bud. Wilms tumor 1 (WT1) is believed to play a key role in triggering the differentiation cascade. Stem cell state is maintained by repressive epigenetic marks in key differentiation genes catalyzed by EZH2, a member of Polycomb Repressor Complex 2 (PRC2).

We hypothesize that WT1 is crucial to the derepression of differentiation genes through modulation of EZH2 expression.

**Methods:** We studied the effect of WT1 on EZH2 expression in human amniotic fluid-derived mesenchymal stem cells (amMSCs) and its mechanism of action. We transfected and used immunoblots, luciferase-reporter gene assay and quantitative PCR as analysis tools. The active WT1 binding site on the EZH2 promoter was defined by EMSA. The effect of WT1 on DNA and histone methylation was determine by bisulfite analysis and ChIP-qPCR.

**Results:** An ontogeny of EZH2 in the developing mouse kidney showed high levels of EZH2 during early kidney development, decreasing drastically after birth and disappearing by adulthood. We showed a downregulation of the endogenous expression of EZH2 in amMSCs due to WT1. *in silico* analysis of the EZH2 promoter sequence identified three putative WT1 binding sites. We show WT1 exerting a repressive effect on EZH2 through site S1. We observed increase in CTNNB1 expression and amMSCs showed increased responsiveness to WNT9B in the presence of WT1. Expression of nephron differentiation genes, such as *PAX8*, *WNT4* and *RAR-alpha*, was also enhanced. DNA and histone methylation patterns of the CTNNB1 promoter showed that the derepression of CTNNB1 was mediated by epigenetic modifications at the histone level.

**Conclusions:** We show that WT1 plays a key role in the differentiation process, achieved in part through direct transcriptional repression of EZH2. This repression leads to de-repression of key differentiation genes through epigenetic modifications at the histone level.

*Funding:* Government Support - Non-U.S.

## TH-PO363

**COUP-TFII Heterozygous Mutant Mice as an Animal Model for Nephron Insufficiency and Chronic Kdney Disease** Chengtai Yu,[1] Seymour Rosen,[2] Akio Kobayashi,[3] Sophia Y. Tsai,[1] Ming-Jer Tsai.[1] *[1]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; [2]Dept of Pathology, Harvard Medical School, Boston, MA; [3]Dept of Medicine, Harvard Medical School, Boston, MA.*

**Background:** The nephron is the basic structural and functional unit of the kidney. Nephron formation is regulated by the cross talk between the ureteric bud and the metanephric mesenchyme, but in humans this initial nephron population is highly variable. It has been suggested that more limited nephronogenesis is related to chronic renal injury. Currently, there is no good animal model available to address this hypothesis.

**Results:** Herein, we demonstrate that the orphan nuclear receptor, chicken ovalbumin upstream promoter transcription factor II (COUP-TFII), is required for kidney progenitor cell renewal and nephrogenesis. Using $Six2^{Cre/+}$ to delete one copy of *COUP-TFII* in kidney progenitor cells, we observed hypoplastic kidneys at the postnatal day (P0). The mutant kidney showed a decreased subcapsular nephrogenic zone, reduced nephron mass and proximal tubular hypertrophy. These mutant mice survived into adulthood and had hypoplastic kidneys with marked tubular hypertrophic changes and glomerulosclerosis. There was renal failure and high grade proteinuria in these COUP-TFII heterozygous mice. Mechanistically, COUP-TFII directly regulates Six2 expression to maintain the size of progenitor cell pool, which subsequently determines the number of nephrons in the kidney. Our data suggest losing one copy of *COUP-TFII* reduces the kidney progenitor cell pool, impairs nephrogenesis, and eventually leads to nephron deficiency. Moreover, COUP-TFII conditional heterozygotes exhibit reduced expression of WT1 and its downstream targets podocalyxin and nephrin in the podocyte, causing development of glomerulosclerosis, which may have a component of hypertrophic injury as well. In summary, we present COUP-TFII heterozygous mice as a genetic animal model of nephron insufficiency and it can serve as a platform to develop future therapeutic interventions.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

## TH-PO364

**New Epigenetic Marker, H4K20ac Related to Transcriptional Suppression Is Induced in Postneonatal Kidney Maturation and Organ Hypertrophy** Jun-Ya Kaimori,[1] Kazumitsu Maehara,[2] Yasuyuki Ohkawa,[2] Hiroshi Kimura,[3] Yoshitsugu Obi,[4] Masaki Hatanaka,[4] Hidetoshi Tsuda,[1] Shiro Takahara,[1] Hiromi Rakugi,[4] Yoshitaka Isaka.[4] *[1]Advanced Technology of Transplantation, Osaka Univ, Suita, Osaka, Japan; [2]Dept of Advanced Initiative Medicine, Kyushu Univ, Fukuoka, Kita-Kyushu, Japan; [3]Graduate School of Frontier Biosciences, Osaka Univ, Suita, Osaka, Japan; [4]Dept of Geriatrics & Nephrology, Osaka Univ, Suita, Osaka, Japan.*

**Background:** Post-neonatal kidney maturation was characterized by brake in cell proreferation, drastic gene expression changes and also change in suseptibility to genetic dease after gene deleation in mouse model. In order to elucidate this developmental change from epigenetic point of view, we set the complehensive epigenetic screen by using multiple antibodies angainst different kinds histone modifications. We discovered that new histone modification H4K20ac was induced in this period and also induced by various stimuli related to organ hypertrophy.

**Methods:** We made chip-seq analyses by H4K20ac antibody and next generation sequencer. And we conducted MACS analysis for peak localization and motief search analysis for transcription factors binding to H4K20ac.

**Results:** In peak localizatin analysis, H4K20ac was precipitated in the promoter regions of silent genes, which was totally opposite to the conventional views of histone acetylations. And we also found this modification did not localise on Lamina Associated Domain in which lots of permanent silent genes are located, suggesting that H4K20ac is related to regulated silent genes expressions. In the motif search, we found that lots of transcriptional activators, AP-2, STAT3, and Myc could not bind to H4K20ac precipitating promoters, whereas only transcriptional suppressor, NRSF coud bind to this region. In the comparison with ENCODE data base, H4K20ac also was localised on the future open chromatin regions, suggestiong that H4K20ac can predict the future chromatin changes.

**Conclusions:** H4K20ac is totally different and new kind of histone acetylation and may be related to organ hypertrophy by suppressing regulated genes by repulsing transcriptional activators form promoter resion.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO365

**Hypoxia-Inducible Factor-1α Is a Negative Regulator of Nephrogenesis** Bjoern Buchholz,[1] Gunnar Schley,[1] Carsten Willam,[1] Nicolai Burzlaff,[2] Holger Scholz,[3] Kai-Uwe Eckardt.[1] *[1]Dept of Nephrology and Hypertension, Univ of Erlangen-Nuremberg, Erlangen, Germany; [2]Dept of Chemistry and Pharmacy, Univ of Erlangen-Nuremberg, Erlangen, Germany; [3]Dept of Physiology, Charité - Universitaetsmedizin Berlin, Berlin, Germany.*

**Background:** Outgrowth and branching of the ureteric bud is essential to generate the renal collecting system which concurrently induces nephrogenesis, thereby determining the definitive number of nephrons. Limited nephron number due to intrauterine growth restriction (UGR) predisposes to arterial hypertension and chronic kidney disease. IUGR is associated with increased renal expression of the Hypoxia-Inducible Factor-1α (HIF-1α). The functional relevance of HIF-1α within this context remains elusive. Therefore, we analysed the effects of HIF-1α on nephrogenesis.

**Methods:** Metanephric mouse kidneys at E12.0-12.5 and E13.0-E13.5 were dissected and cultured ex vivo. One kidney served as control whereas the contralateral kidney was cultured either in the presence of 1% $O_2$, 5% $O_2$, the HIF-stabilizers dimethyloxalylglycine (DMOG; 1mM) or 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido) acetate (ICA; 10 and 100μM) or the HIF-1α inhibitor chetomin (100nM). In addition, metanephric kidneys with a tubule-specific overexpression of HIF-1α (THP-Cre; $VHL^{lox;lox}$) were compared with corresponding controls. After 72h, kidneys were photographed and z-stacks were generated providing omnifcoal photos of the kidneys. Then, kidney sizes (optical area in $mm^2$), number of branches (Dolichos biflorus Agglutinin positive tubules) and number of glomeruli (WT1 positive structures) were analysed.

**Results:** 1% $O_2$, 5% $O_2$,DMOG, ICA (100μM) as well as tubule-specific overexpression of HIF-1α signficantly inhibited kidney growth at both time-points which was accompanied

by reduced branching and reduced number of glomeruli. In contrast, chetomin increased kidney sizes and the number of branches in preliminary experiments.

**Conclusions:** HIF-1α is a negative regulator of nephrogenesis. Therefore, HIF-1α could be a mediator of IUGR-dependent reduction in nephron number.

### TH-PO366

**Planar Cell Polarity Effectors, Daam1 and WGEF, Are Required for Cilia Formation and Nephric Tubulogenesis** Rachel Katherine Miller,[1] Malgorzata Kloc,[2] Le Huang,[3,4] Qianghua Hu,[3] Sharada Mokkapati,[3] Vicki Huff,[3,4] Pierre D. McCrea.[3,4] *[1]Biochemistry and Molecular Biology, The Univ of Texas MD Anderson Cancer Center, Houston, TX; [2]Immuno-Biology Laboratory, The Methodist Hospital Research Institute, Houston, TX; [3]Genetics, The Univ of Texas MD Anderson Cancer Center, Houston, TX; [4]Program in Genes & Development, The Univ of Texas Graduate School of Biomedical Sciences, Houston, TX.*

**Background:** Recent studies from our group and others indicate that canonical (beta-catenin-mediated) Wnt signaling is essential for induction of nephron development. Additionally, studies suggest that primary cilia are required for a switch from canonical to non-canonical Wnt signaling, and that defects in primary cilia formation or signaling lead to cystogenesis. Deficiencies in non-canonical Wnt signaling, which contributes to planar cell polarity (PCP), underlie a number of developmental or later defects, such as polycystic kidney disease and nephronophthisis.

**Methods:** Using *Xenopus laevis* (frog) embryos, supplemented with *Danio rerio* (zebrafish) and more recently the mouse Wilms tumor model, we assess the roles of PCP components in kidney tubule morphogenesis utilizeing RT-PCR, in situ hybridization, knockdown, immunostaining, electron microscopic and biochemical techniques.

**Results:** We find that Daam1 and WGEF are expressed in the *Xenopus* embryonic kidney. Daam1 knockdown reduces the expression of pronephric differentiation/ development markers, while having little impact upon determination and patterning. Additionally, knockdown of Daam1 and WGEF result in reduced tubulogenesis. Daam1 and WGEF are important for forming cilia in both the skin of embryos and in the kidney, which have functional associations with morphogenesis. We are beginning investigate renal tumors to determine if cilia or PCP are altered in these tissues. Thus far, we have preliminary data indicating that ciliogenesis may be increased in a mouse model of Wilms tumor.

**Conclusions:** Our data together suggest that the Daam1/ WGEF/ Rho PCP trajectory is necessary for tubulogenesis and possibly ciliogenesis within the developing kidney and may be relevant to human pediatric kidney diseases.

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### TH-PO367

**Role of the Primary Cilium in Early Steps of Nephrogenesis** Evelyne Fischer,[1] Filippo Massa,[2] Armelle Jm Christophorou,[1] Cecile Madaras,[1] Serge Garbay,[1] Marco Pontoglio.[1] *[1]Cochin Institute, INSERM/CNRS/Paris Descartes Univ, Paris, France; [2]Institute of Biology Valrose, Nice, France.*

**Background:** Renal morphogenesis is a highly coordinated process that requires reciprocal inductive interactions between the ureteric bud and the metanephric blastema. Nephron formation starts with the condensation of mesenchymal cells around a ureteric bud tip that ultimately give rise to a mature nephron formed by a glomerulus and a long tubular structure connected to the collecting system. Several studies have highlighted the important role of a subcellular organelle, the primary cilium, in the maintenance of tubular diameter. During nephrogenesis, disruption of this organelle in already formed, but still elongating, tubules leads to tubular cysts formation.

**Methods:** However, the role of the primary cilium during the initial steps of nephrogenesis is still elusive. To identify this potential role, we have inactivated Kif3a, a kinesin gene necessary for ciliary assembly, specifically in the metanephric mesenchyme and not in the ureteric bud (Six2-GFP::Cre).

**Results:** Mutant pups were born with a normal mendelian ratio but died within a few days after birth. At birth, mutant kidneys were enlarged and filled with cysts. Histological analyses showed that mesenchymal to epithelial transition was not affected by the lack of primary cilium. Nephron progenitors were formed but they were abnormally shaped and gave rise to cystic tubules and glomerular cysts. In addition, the total number of nephrons was significantly reduced.

**Conclusions:** Our results suggest that, in addition to its known roles, the primay cilium plays an important role in nephron morphogenesis and is involved in the regulation of nephron number.

*Funding:* Government Support - Non-U.S.

### TH-PO368

**p53 Promotes Adhesion of Six2+ Cells within the Nephron Progenitor Niche** Yuwen Li,[1] Jiao Liu,[1,2] Marilyn Li,[3] Samir S. El-Dahr,[1,2] Zubaida R. Saifudeen.[1,2] *[1]Pediatrics, Tulane Univ, New Orleans, LA; [2]Hypertension and Renal Centers of Excellence, Tulane Univ; [3]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.*

**Background:** Retention of nephron progenitors cells (NPC) within the niche is necessary to allow NPC-niche interactions and timely activation of transcriptional programs that drive differentiation. Six2+ cap mesenchyme (CM) devoid of transcription factor p53 ($Six2^{p53-/-}$) is disorganized, with a greatly diminished Cited1+ self-renewing population. We hypothesize that p53 regulates transcriptional programs to retain progenitor cells within a structured niche.

**Methods:** To test our hypothesis we compared transcription profiles by PolyA-selected paired-end RNA-Seq of $Six2^{p53+/+}$ and $Six2^{p53-/-}$ FACS-isolated cells from E15.5 kidneys. Cell aggregation assessment of isolated mutant cap cells was done.

**Results:** Differential expression of ~3400 genes (Audic Claverie p-value<0.05, FC 1.5) include 283 lincRNA and 21 premiRNAs. Purity of isolated CM was confirmed by high read density of CM genes (Six2, GDNF, Crym, Cited1), but not RV (Cdh1, Fgf8) or UB (Wnt9b, cRet) genes.Expression of floxed-p53 gene exons 2-10 was nearly abolished in $Six2^{p53-/-}$ cells. Accordingly p53 target genes p21, PUMA and NOXA are downregulated. Cited1 expression was decreased, but not Six2. Highest enrichment of differentially expressed genes was observed for genes in cell adhesion (>5-fold, Itga6, Netrin, Col1A1), migration (regulatory proteins for Rho/Rac/Cdc42 - ARHGAP9, 24, 30 and Cdc42ep5), development/morphogenesis (>1.8-fold, PDGFRA/B, Hnf1b, Wnt5a, HNF4, Gas6) and calcium signaling - S100A4 (-8.5 fold) and S100A6 (-3.8 fold). IF staining showed decreased NCAM and Ncad in caps of $Six2^{p53-/-}$ kidneys. $Six2^{p53-/-}$ cells showed decreased aggregation at isolated ureteric tips in hanging drop assays, compared to $Six2^{p53+/+}$ cells.

**Conclusions:** p53 loss from the Six2+ cells resulted in loss of niche architecture and a smaller CM with diminished Cited1+ population. In support, RNA-Seq identified deregulated expression of genes involved in cell adhesion, migration, development and signaling. Our data support a role for p53 as a regulator of transcriptional programs that maintain niche integrity.

*Funding:* NIDDK Support, Other NIH Support - NIGMS

### TH-PO369

**Podocyte-Specific Autophagy Deficiency Exacerbates High-Fat Diet-Induced Glomerular Injury** Kosuke Yamahara,[1] Mako Yasuda,[1] Atsuko Tagawa,[1] Shinji Kume,[1] Yuki Tanaka,[1] Shin-ichi Araki,[1] Daisuke Koya,[2] Masakazu Haneda,[3] Takashi Uzu,[1] Hiroshi Maegawa.[1] *[1]Dept of Medicine, Shiga Univ of Medical Science, Otsu, Shiga, Japan; [2]Div of Diabetology & Endocrinology, Kanazawa Medical Univ, Kahoku, Ishikawa, Japan; [3]Dept of Medicine, Asahikawa Medical Univ, Asahikawa, Japan.*

**Background:** Diabetic nephropathy is a leading cause of end-stage renal disease worldwide. Methods for reducing proteinuria in diabetic nephropathy patients are still required. Podocytes are terminally differentiated and are unable to proliferate. Disruption of cell homeostasis in podocytes therefore results in impairment to glomerular filtration barrier function, leading to proteinuria in diabetic nephropathy. Intracellular degradation systems are essential for maintaining cell homeostasis. One of these systems, autophagy, is evolutionary conserved machinery for bulk degradation of cytoplasmic components. Alterations in autophagy have recently been found to be the pathogenesis for some metabolic diseases. This study examined the role of podocyte autophagy in diabetic nephropathy.

**Methods:** We generated podocyte-specific autophagy-deficient (Podo-$Atg5^{-/-}$) mice by podocyte-specific *Atg5* gene deletion. Eight-week-old control ($Atg5^{f/f}$) and Podo-$Atg5^{-/-}$ mice were fed with either a standard diet or a high-fat diet for 32 weeks.

**Results:** At the end of the experimental period, both $Atg5^{f/f}$ and Podo-$Atg5^{-/-}$ mice developed obesity and hyperinsulinemic hyperglycemia resembling type 2 diabetes mellitus. In Podo-$Atg5^{-/-}$ mice, high-fat diet-induced increases in urinary albumin excretion were significantly higher compared with those of $Atg5^{-/-}$, although high-fat diet-induced glomerular histological changes were almost the same in both groups. Fibrosis and infiltration of inflammatory cells in tubulointerstitial lesions and proximal tubular cell apoptosis were significantly exacerbated in Podo-$Atg5^{-/-}$ mice fed a high-fat diet.

**Conclusions:** The results suggest that autophagy is essential to protect podocytes from diabetes-related cellular toxicity. Although further study is required, autophagy appears to be a possible new therapeutic target for reducing proteinuria in diabetic nephropathy.

*Funding:* Government Support - Non-U.S.

### TH-PO370

**Enhanced Glomerular Toll-Like Receptor 4 Expression and Signalling in Patients with Type 2 Diabetic Nephropathy and Microalbuminuria** Giacomo Garibotto,[1] Laura Cappuccino,[1] Barbara Villaggio,[1] Fabio Enzo Gianiorio,[1] Mariano Mij,[2] Francesca Viazzi,[1] Gennaro Salvidio,[1] Daniela Verzola.[1] *[1]Div of Nephrology, Dialysis and Transplantation, Genoa Univ and AOU San Martino-IST, Genoa, Italy; [2]Div of Nephrology and Dialysis, General Hospital, Imperia, Italy.*

**Background:** Toll-like receptors 4 (TLR4) can be activated by endogenous ligands in diabetes and promote inflammation in the kidney tubulointerstitial compartment at a clinical stage of diabetic nephropathy . However, there is no information on molecular events taking place in diabetic microalbuminuria, when renal damage is most likely to be reversible.

**Methods:** We studied TLR4 gene and protein expression and TLR4 downward signaling in kidney biopsies of type 2 diabetic patients with microalbuminuria(n=12, age 60±12 yr, 6M/6F, eGFR= 99± 4, AER 158±15 mg/min), in patients with overt nephropathy (n=11,age 63±3 yr, 5M/6F, eGFR 32±3, proteinuria 3.7±1.0 g/day) and in control kidney (5M/4F, age 60±4 yrs,eGFR= 102± 3). As a second step, to identify specific transcriptional pathways that underlie the pathogenesis of disease we studied selected TLR-4 downward gene expression profiles from laser-capture microdissected glomeruli and tubulointerstitium.

**Results:** In microalbuminuric patients TLR4 mRNA was markedly overexpressed in the glomeruli and in tubulointerstitium. Within the glomerular tuft, TLR4 was localized in podocytes. NF-kB signalling was ~ 4 fold higher in the glomeruli as compared to normal kidney. TNF-α, IL6, CCL-5 and CCR-5 -mRNAs were markedly (about 3-5 fold, p<0.01)

upregulated in microdissected glomeruli, while TNFα and TNFR1 were upregulated both in glomeruli and in the tubulointerstitial compartment ($p<0.02$). Surprisingly, both CCL2-mRNA and CD68 were not expressed in microalbuminuria, while they were both upregulated in the tubulointerstitial compartment of clinical diabetic nephropathy.

**Conclusions:** Our data demonstrate the activation of innate immunity in the glomeruli of patients with type 2 diabetes and early nephropathy and suggest that enhanced TLR4 signaling contribute to the progression that occurs after the microalbuminuric form of nephropathy evolves to overt disease.

*Funding:* Government Support - Non-U.S.

## TH-PO371

**RTN1 Is a Novel Risk Gene for Kidney Disease** Ying Fan,[1,2] Jason A. Bonomo,[3] Peter Y. Chuang,[1] Wenzhen Xiao,[1,2] Sandeep K. Mallipattu,[1] Nicholette D. Palmer,[3] Donald W. Bowden,[3] Barry I. Freedman,[3] Niansong Wang,[2] John C. He.[1] *[1]Medicine, Mount Sinai School of Medicine, NY; [2]Nephrology, Shanghai Six Municipal Hospital, Shanghai Jiaotong Univ, Shanghai, China; [3]Section on Nephrology and Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, NC.*

**Background:** Factors contributing to the progression of kidney disease remain unclear.

**Methods:** We profiled gene expression in kidneys from a murine model of HIV-associated nephropathy (HIVAN) and identified an association between the expression of reticulon-1 (Rtn1), which encodes for an endoplasmic reticulum (ER)-associated protein, and the severity of kidney disease. We validated RTN1 expression in both mouse and human diseased kidneys by real-time PCR, western blot, and immunostaining. We determined the role of RTN1 in ER stress.We performed genetic association studies of RTN1 SNPs and ESRD.

**Results:** Upregulation of protein and mRNA expressions of Rtn1 was confirmed in diseased kidneys of murine models for HIVAN, diabetic kidney disease (DKD), and unilateral ureteral obstruction. Renal RTN1 mRNA level was higher in patients with DKD and correlated with estimated glomerular filtration rate (eGFR). Of the three known RTN1 isoforms, only RTN1-A's protein expression was elevated in kidneys of mice and human with HIVAN and DKD. Protein expression of RTN1-A in the human kidneys, as shown by immunostaining, correlated inversely with eGFR in patients with DKD. In HK2 cells, RTN1 overexpression induced ER stress and apoptosis whereas RTN1 knockdown attenuated tunicamycin-, hyperglycemia-, and albumin-induced ER stress and apoptosis, indicating that RTN1 is a key molecule mediating ER stress in kidney cells. We identified three single nucleotide polymorphisms of RTN1 (rs1952034, rs12434215, and rs12431381) in African Americans that significantly associated with type 2 diabetes (T2D)-associated ESRD, non-diabetic ESRD, and all-cause ESRD, but not T2D without nephropathy, with and without adjustment for age, gender, APOL1 G1/G2 allele status, and ancestry.

**Conclusions:** Our data suggest that RTN1 is a novel risk gene for kidney disease and may contribute to kidney injury through ER stress.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO372

**Establishment of a Novel Inflamed Animal Model of Diabetic Nephropathy** Kun Ling Ma, Yang Zhang, Jing Liu, Wu Yu, Jie Ni, Bi-Cheng Liu. *Institute of Nephrology, Southeast Univ School of Medicine, Nanjing, China.*

**Background:** It has been demonstrated that diabetic nephrology (DN) is a chronic inflammatory disease. Inflammatory stress plays crucial roles in the progression of DN. Therefore, an inflamed model of DN would be helpful to clarify the pathogenesis of DN. However, an adequate reliable inflamed model of DN is currently lacking. This study aimed to establish a novel inflamed animal model of DN and evaluate its significance in DN.

**Methods:** Twenty male db/db mice were randomly divided into two groups for eight weeks (n=10, each group): mice with daily distilled water injection (Control) or mice with every one day 10% casein injection (inflamed group). Body weight and 24-hour urinary protein were measured every week. The plasma levels of serum amyloid A (SAA) and tumor necrotic factor-a (TNF-a) were checked by enzyme-linked immunosorbent assay. The morphological changes of renal pathology and ultra-microstructures were checked by pathological staining and electron microscope. Immunofluorescent staining and Western blotting were used to check the protein expression of podocyte specific molecules and inflammatory cytokines in kidneys.

**Results:** The 24-hour urinary protein, plasma levels of SAA and TNF-α, as well as the protein expression of inflammatory cytokines in kidneys were significantly increased in inflamed group compared to the control. Moreover, there were more significant mesangial expansion, collagen accumulation, and foot process effacement in inflamed group compared to the control. Further analysis showed that inflammation markedly decreased the expression of wilims tumor-1 and nephrin, which were specific biomarkers of podocyte, suggesting accelerating injuries of podocyte induced by inflammation.

**Conclusions:** An inflamed animal model of DN was successfully established.This inflamed model may provide a useful tool for investigating the pathogenesis of DN under inflammatory stress.

## TH-PO373

**Semaphorin3a Role in Diabetic Nephropathy** Alda Tufro,[1] Pardeep Kumar Aggarwal,[1] David B. Thomas.[2] *[1]Pediatrics/ Nephrology, Yale Univ, New Haven, CT; [2]Pathology, Univ of Miami, Miami, FL.*

**Background:** Accumulating evidence supports crucial pathogenic roles of vascular endothelial growth factor–a, nitric oxide and semaphorin3a (sema3a) disregulation in diabetic nephropathy (DN). Sema3a is a guidance protein secreted by podocytes and essential for normal kidney development. We recently showed that excess podocyte sema3a causes renal disease via direct plexin$A_1$-nephrin interaction and integrin disregulation, and identified increased sema3a expression in Db/Db and STZ-induced diabetic kidneys.

**Methods:** Renal biopsies from T1D and T2D patients were examined by IHC. Sema3a was measured by ELISA and immunoblotting. Proteinuria was assessed by SDS-PAGE-Coomassie staining and albumin ELISA. Diabetes was induced with streptozotocin using the ADMCC protocol. Mouse renal phenotype was examined by LM, IF and EM.

**Results:** Here we report that podocyte sema3a is increased in human diabetic nephropathy, as detected by immunohistochemistry in renal biopsies (DN Class III–IV), whereas sema3a was barely expressed in non-diabetic control biopsies. Moreover, podocyte sema3a was increased in T1D diabetic mice, as assessed by immunoblotting and IHC. Using a sema3a ELISA assay we find that sema3a plasma level and urine excretion are significantly higher in diabetic mice than in non-diabetic controls, and urine sema3a excretion is higher in diabetic mice with advanced DN than in those with mild DN. Next, we examined the role of sema3a in DN using a doxycycline inducible, podocyte-specific Sema3a overexpression mouse model (podocin-rtTA:tet-O-Sema3a, herein called Sema3a$^+$). Sema3a$^+$ mice were made diabetic with streptozotocin (DM-Sema3a$^+$). Induced diabetic Sema3a$^+$ overexpressing mice (DM-Sema3a$^+$ + dox) developed massive proteinuria, decreased renal function and extensive nodular glomerulosclerosis consistent with advanced DN, while uninduced diabetic mice (DM-Sema3a$^+$ – dox) with identical genotype had a mild DN phenotype.

**Conclusions:** Collectively, our data indicate that excess sema3a plays a pathogenic role in DN and worsens the progression of the disease.

*Funding:* NIDDK Support

## TH-PO374

**Renal Failure but Hypercalciuria in Mice with Deletion of the Gene Encoding the Transient Receptor Potential Canonical 1 (TRPC1) Channel: Model of Metabolic Syndrome with Chronic Kidney Disease (CKD)** Bonnie Eby,[1] Alexander Lau,[1] Chris Skaggs,[1] Lindsay J. Barron,[1] Leonidas Tsiokas,[2] Kai Lau.[1] *[1]Renal, Univ of Oklahoma, Oklahoma City, OK; [2]Cell Biology, Univ of Oklahoma, Oklahoma City, OK.*

**Background:** TRPC1 gene on chromosome 3q22–24 is in the linkage region for diabetic nephropathy. TRPC1 expression was reduced in diabetic rodents & patients, but a causal relationship was unclear. Since null mice display features of metabolic syndrome, we tested the hypothesis of renal phenotypes induced by TRPC1 deficiency. In parathyroid cells, TRPC1 protein complexes with STIM1 to mediate store-operated Ca entry. Activating STIM1 mutations produce hypoparathyoidism. So we asked if TRPC1 deficiency produces hyperparthyroidism.

**Methods:** From 1st to 20th months (mon), standard metabolic, ultrasonic (US) & clearance (Cl) studies were done in male littermates of TRPC1 +/+, +/- & -/- born to heterozygotic parents. Creatinine (Cr) was measured by HPLC.

**Results:** Through the 1st year, null mice were uniformly ~10% heavier than +/- & +/+. At 2 mon, their livers were heavier & density from 7-20 mon increased, indicative of hepatosteatosis. At 1 year, they had hypertriglyceridemia, confirming metabolic syndrome. In null mice, serum creatinine (Cr) (0.132 vs. 0.087 mg%) was elevated & Cr clearance (Cl) (1.6 vs. 3.1 ml/miin) reduced. CrCl remained ~50% lower even factored for body (48 vs. 90 µl/min/g) & kidney weight (3.2 vs. 6.4 ml/min/g). Urine albumin (12 vs.7 µg/d; 23 vs.14 µg/mg Cr) was elevated & hematocrit reduced in null mice. Renal US from 7-20 mon uniformly showed >20% reduced volume. There was no difference in serum Ca [9.6 vs. 9.1 mg%], urine Ca (1 vs. 0.99 mg/d), or Ca Cl (12.3 vs. 12.9 µl/min), but fractional Ca excretion was elevated in null mice (0.9 vs. 0.5%). +/- mice had similar renal phenotypes.

**Conclusions:** 1. TRPC1 null mice have 50% □ in GFR, reduced kidney volume & increased proteinuria, offering an excellent model to study obesity- & metabolic syndrome-related CKD. 2. They show no signs of hyperparathyroidism but display hypercalciuria, suggesting a role of TRPC1 channels in renal Ca homeostasis. 3. Haploid deficiency seems sufficient to induce the renal phenotypes.

*Funding:* NIDDK Support, Private Foundation Support, Clinical Revenue Support

## TH-PO375

**Reducing Leukocyte Infiltration with a Novel Integrin Agonist Prevents Diabetic Nephropathy** Saravana Kumar Kanagavelu, Mohd Hafeez Faridi, Tristan Hays, Vineet Gupta. *Dept of Internal Medicine, Rush Medical Univ, Chicago, IL.*

**Background:** Diabetic nephropathy(DN) is a major complication of chronic kidney disease and the leading cause of end stage renal disease in humans. DN leads to a number of complex pathologic changes in human kidneys, including basement membrane thickening, glomerular mesangial expansion, glomerulosclerosis and a significant loss of podocytes. Additionally, inflammation plays a significant role in the development and progression of DN and a high influx of leukocytes in the tissue biopsies. This suggests that reducing leukocyte activation, recruitment and influx may be a beneficial strategy for developing therapeutics

against DN. We recently identified novel compounds (leukadherins) for reducing the influx of inflammatory leukocytes in the tissue. This abstract describes our results with our novel compounds in a murine model of DN.

**Methods:** We used a newly established murine model(BTBR ob/ob) of DN, where the disease closely resembles human DN. Starting at the onset of DN in 4 wks old animals, the animals were administered our novel compound(LA1) daily for eight weeks. Animals were monitored for blood glucose level, body weight, renal function and for glomerular defects using histopathology at different time points.Daily administration of LA1 significantly reduced the number of infiltrating leukocytes and preserved kidney function in fully diabetic animals. We saw no effect of LA1 interventions on glycemia, as expected. Histo analyses showed a significant reduction in glomerular mesangial sclerosis in treated animals.

**Results:** Daily administration of LA1 significantly reduced the number of infiltrating leukocytes in the kidney and preserved kidney function in fully diabetic animals. We saw no effect of our interventions on glycemia, as expected. Histopathological analyses showed a significant reduction in glomerular mesangial sclerosis in treated animals.

**Conclusions:** Our research suggests that LA1 significantly protects the kidney function in DNand provides a novel approach to the treatment of DN. It validates CD11b/CD18 as a novel therapeutic target for DN and integrin CD11b/CD18 activation as a mechanistic strategy for developing novel therapeutics.

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## TH-PO376

**Cathepsin L Is Essential for the Development of Proteinuria in Diabetic Mice**

Marjolein Garsen,[1] Angelique Rops,[1] Toin Van Kuppevelt,[2] Henry Dijkman,[3] Thomas Reinheckel,[4] Jo H.M. Berden,[1] Johan Van der Vlag.[1] *[1]Nephrology, Radboud Univ Medical Center; [2]Biochemistry, Radboud Univ Medical Center; [3]Pathology, Radboud Univ Medical Center, Nijmegen, Netherlands; [4]Molecular Medicine, Albert-Ludwigs-Univ Freiburg, Freiburg, Germany.*

**Background:** Proteinuria in diabetic nephropathy (DN) is caused by damage to the glomerular filtration barrier (GFB). Heparan sulfate (HS) is a negatively charged polysaccharide and abundantly expressed in the GFB. HS expression is reduced in proteinuric patients, which is associated with an increased expression of the HS-degrading enzyme heparanase (HPSE). HPSE is essential for the development of proteinuria in DN. Cathepsin L (CTSL) is a lysosomal cysteine protease that cleaves pro-HPSE, thereby giving HPSE its biologic activity. In addition, CTSL degrades the actin-associated protein synaptopodin in podocytes. Both mechanisms may contribute to the development of proteinuria.

**Methods:** To precise the exact role of CTSL in the development of proteinuria, we induced type 1 diabetes mellitus by streptozotocin in wildtype (wt) and CTSL knockout (ko) mice. Mice were sacrificed after 4 weeks and urine, blood and kidneys were collected.

**Results:** Diabetic wt mice developed proteinuria, whereas diabetic CTSL ko mice failed to develop proteinuria. Serum creatinine was increased in diabetic wt mice. HPSE mRNA expression was increased in both diabetic wt and CTSL ko mice. CTSL mRNA expression remained unaltered by induction of diabetes in wt mice. HPSE protein expression and activity were increased in diabetic wt mice, whereas the glomerular HS expression was decreased. HPSE protein expression and activity were low in both non-diabetic and diabetic CTSL ko mice, which was associated with an preserved glomerular HS expression. The protein expression of synaptopodin was comparable between all mice. Furthermore, no differences in glomerular ultrastructure were observed by transmission electron microscopy in these mice.

**Conclusions:** Our data show that CTSL is causally involved in the pathogenesis of proteinuria in DN. Most likely CTSL-deficiency caused loss of HPSE activity, which prevents the loss of glomerular HS expression.

## TH-PO377

**microRNA-214 (miR-214) Regulates Mesangial Cell (MC) Hypertrophy and Matrix Expansion through PTEN**

Amit Bera,[1] Falguni Das,[1] Meenalakshmi M. Mariappan,[1] Nandini Ghosh-Choudhury,[2] Balakuntalam S. Kasinath,[1] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA.*

**Background:** Our recent report demonstrates that decreased expression of the tumor suppressor protein PTEN contributes to mesangial cell (MC) hypertrophy and matrix protein expression in response to high glucose (HG). The mechanism of PTEN repression by HG is not clear. MicroRNAs strategically silence mRNA translation.

**Methods:** Analysis of 3'UTR of PTEN mRNA revealed the presence of miRNA recognition element (MRE) for miR-214.

**Results:** HG significantly enhanced the expression of miR-214 in MC. To determine the link between miR-214 and PTEN, in vitro we used a sensor plasmid containing the PTEN 3'UTR fused to luciferase cDNA. Cotransfection of this reporter plasmid with miR-214 expression vector significantly inhibited the luciferase activity similar to HG in MCs. Expression of miR-214 in MCs markedly inhibited PTEN protein level analogous to its downregulation by HG, resulting in increased phosphorylation of Akt and its endogenous substrate GSK3beta. Furthermore, miR-214 significantly increased fibronectin expression similar to HG. Conversely, anti-miR-214 markedly inhibited HG-induced fibronectin expression. Moreover, expression of miR-214 increased inactivating phosphorylation of PRAS40, resulting in activation of mTOR and MC hypertrophy analogous to HG treatment. Anti-miR-214 significantly attenuated HG-stimulated mTOR and hypertrophy of MC. Finally, in the diabetic renal cortices of type 1 OVE26 transgenic mice, we found increased expression of miR-214, which correlated with renal hypertrophy, decreased PTEN and, increased activation of Akt, mTOR and fibronectin expression.

**Conclusions:** Together, our results for the first time demonstrate a significant functional role of miR-214 in targeting PTEN to regulate MC hypertrophy and fibronectin expression, two features of diabetic renal complications.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO378

**Urinary Microvesicles Detect Podocyte Injury in Diabetic Nephropathy**

Ladan Zand, Muthuvel Jayachandran, Stephen T. Turner, M. Regina Castro, Joseph P. Grande, Vesna D. Garovic. *Mayo Clinic, Rochester, MN.*

**Background:** Podocyte injury plays a major role in the pathogenesis of diabetic nephropathy. Previous studies have suggested that this injury may occur in the early phases of glomerular disease prior to development of proteinuria. We hypothesized that podocyte injury in diabetic nephropathy could be identified by urinary microvesicles (MVs) containing podocyte-specific markers & is present prior to proteinuria. MVs are small membrane-enclosed sacs that are shed from injured cells & contribute to a variety of pathophysiological processes.

**Methods:** Digital flow cytometry was used to identify urinary MVs derived from podocytes. Forty one patients (male=24, female=17) were recruited, 31 had type 2 diabetes mellitus (DM) & 10 were healthy controls (median age 42 years). DM patients were divided into 3 groups: Group I (<30 mg/L of albumin, n=13, median age 61 yrs), Group II (albumin 30-300 mg/L, n=10, median age 61 yrs), and Group III (albumin >300 mg/L, n = 8, median age 64.5 yrs). Data were analyzed by non-parametric Kruskal-Wallis test. Results are shown as median. Units for urinary MVs are expressed as MVs/μL of urine.

**Results:** There was no difference in GFR between healthy controls and Group I (88 vs. 82ml/min/1.73m$^2$), but GFR was significantly lower in patients with proteinuria (62ml/min/1.73m$^2$). Group I patients compared to the healthy controls had significantly higher number of urinary MVs expressing podocyte markers such as synaptopodin (1074 vs. 33, P<0.0001), nephrin (567 vs. 22, P=0.0002), podocin (1454 vs. 37, P=0.0007), and podocalyxin (838 vs. 11, P<0.0001). There were also significantly greater number of parietal cell specific markers, claudin-1 plus CK-8 positive MVs (479 vs. 3, P=0.0007). There was a trend towards fewer MVs expressing podocyte markers in patients with proteinuria compared to those without proteinuria in DM groups (data not shown).

**Conclusions:** Podocyte injury is present in type 2 diabetic nephropathy & predates proteinuria. Characterization of podocyte-derived MVs in the urine could be used as a novel biomarker for podocyte injury in type 2 DM patients.

*Funding:* Other NIH Support - NICHD

## TH-PO379

**SREBP Inhibition Modulates Diabetic Nephropathy in db/db Mice with Type 2 Diabetes**

Xiaoxin Wang, Moshe Levi. *Medicine, Univ of Colorado, Aurora, CO.*

**Background:** Sterol Regulatory Element Binding Proteins (SREBPs) are master transcriptional regulators of fatty acid and cholesterol synthesis. In view of the importance of SREBPs in a diverse number of target organ complications in obesity and diabetes, including atherosclerosis and nonalcoholic fatty liver disease, there has been a concentrated effort to develop inhibitors of SREBP activation. Recently, Betulin [lup-20(29)-ene-3β, 28-diol] has been shown to specifically inhibit the activation of SREBP.

**Methods:** In this study we examined the effects of Betulin induced SREBP inhibition on diabetic nephropathy in db/db mice with type 2 diabetes. We treated db/db mice on C57 BKS background with 30mg/kg body weight/day of Betulin mixed with chow for 12 weeks.

**Results:** We have found increased renal lipid accumulation in the kidneys of db/db mice mediated by increased expression of nuclear SREBP-1 and SREBP-2 protein. Treatment with Betulin prevented the upregulation of SREBPs in the kidney resulting in a correspondent reduction of kidney triglyceride and cholesterol accumulation. SREBP inhibition prevented the increases in urine albumin excretion (db/db: 59±13 μg/24hr; db/db-betulin: 21±3 μg/24hr; p<0.05) and mesangial matrix expansion as shown by PAS staining of the kidneys of db/db mice (mesangial matrix index: db/db, 2.25±0.08; db/db-betulin, 1.60±0.11; p<0.05). The treatment also prevented podocyte loss determined by staining with p57 and WT-1. Furthermore, improvement in oxidative stress and inflammation was also observed in the treatment groups (urinary TBARS: db/db, 181±27 mmol/day vs. db/db-betulin, 103±24 mmol/day, p<0.05; urinary MCP-1: db/db, 146±22 mmol/day vs. db/db-betulin, 83±15 mmol/day, p<0.05). To determine if Betulin has direct effects in kidney podocytes, we cultured human podocytes in high glucose conditions. Betulin prevented the high glucose induced increased the expression of SREBP-1 and SREBP-2 and lipid accumulation in human podocytes.

**Conclusions:** Our study therefore indicates a renoprotective role for SREBP inhibition in diabetic nephropathy by preventing lipid accumulation and modulating oxidative stress and inflammation.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO380

**SGLT2 Inhibition Modulates Renal Lipid Metabolism and Inflammation and Prevents the Development of Nephropathy in Diabetic Mice**

Xiaoxin Wang, Moshe Levi. *Medicine, Univ of Colorado, Aurora, CO.*

**Background:** Inhibition of the renal sodium gradient dependent glucose transporter SGLT2 results in lowering of blood glucose in diabetic humans and rodent models but the effects on progression of diabetic renal disease has not been fully established.

**Methods:** We determined the effects of the inhibition of SGLT2 via a selective SGLT2 inhibitor on diabetic nephropathy in db/db mice.

**Results:** After 12 weeks treatment, we found that SGLT2 inhibition caused marked decreases in urinary albumin (745±36 mg/g in db/db vs. 207±5 mg/g in treated db/db, $p<0.001$) and urinary TBARS (thiobarbituric acid-reacting substances), (1.09±0.13 mmol/g in db/db vs. 0.48±0.10 mmol/g in treated db/db, $p<0.01$) an indicator of oxidative stress. SGLT2 inhibition prevented renal lipid accumulation via inhibition of LPK, SCD-1 and DGAT1, key enzymes that mediate fatty acid and triglyceride synthesis. SGLT2 inhibition also prevented inflammation via inhibition of CD68 macrophage accumulation, and expression of p65, TLR4, MCP-1 and OPN. These effects were associated with prevention of mesangial expansion, accumulation of extracellular matrix proteins as determined by fibronectin and type IV collagen quantitative immunofluorescence microscopy, and podocyte loss as determined by WT1 and synaptopodin quantitative immunofluorescence microscopy (all $p<0.05$).

**Conclusions:** In summary, our study showed that SGLT2 inhibition modulates renal lipid metabolism and inflammation and prevents the development of nephropathy in db/db mice.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Johnson and Johnson

## TH-PO381

**Effects of Bile Acid Sequestrants on Kidney Disease in Mice with Diet Induced Obesity, Insulin and Leptin Resistance** Xiaoxin Wang, Moshe Levi. *Medicine, Univ of Colorado, Aurora, CO.*

**Background:** Bile acid sequestrants (BAS) including colesevelam are orally administered nonabsorbable polymers that decrease serum cholesterol and serum glucose in patients and animal models with type 2 diabetes mellitus. The effects of BAS on renal dysfunction in obesity and insulin resistance or type 2 diabetes mellitus have not been studied. Sevelamer, an effective phosphate binder for treating ESRD, has been shown to bind bile acids, advanced glycation end products and uremic toxics, which would be of benefit to treating diabetic patients with chronic kidney disease. Our goal was to evaluate the effect of sevelamer on metabolic and renal parameters in an obese animal model with insulin and leptin resistance and to determine underlying mechanisms.

**Methods:** Our studies were performed in C57BL/6J male mice fed a control low fat (LF) diet (10 kcal% fat) or high fat (HF) diet (60 kcal% fat). The diets were supplemented with a) no addition or b) 2% sevelamer.

**Results:** BAS prevented the increase in urine albumin excretion (HF: 47.7±6.66 mg/g; HF/sevelamer: 24.8±3.17 mg/g, $p<0.05$) and decreased the PAS staining in the kidney of HF mice. Treatment with BAS decreased the renal lipid accumulation as shown by oil red O staining. HF diet induced upregulation of profibrotic genes (TGF-β, CTGF, and PAI-1) and proinflammatory genes (MCP-1, TLR2, and TLR4) in the kidney which were decreased by treatment with BAS. In addition BAS induced downregulation of extracellular matrix protein fibronectin and macrophage marker CD68 expression in the kidney. The beneficial effects of BAS were also associated with decreases in renal gluconeogenic gene expression (PEPCK and G6Pase) and renal glucose transporter (SGLT2). Treatment with BAS also and most notably increased renal expression of Nrf1, SIRT1, PGC-1a,SIRT3, ERRa, that mediate mitochondrial biogenesis, MCAD and LCAD, that mediate fatty acid oxidation, and Nrf2, HO-1, and SOD, that mediate anti-oxidative stress.

**Conclusions:** In summary, our study showed a novel renoprotective role for bile acid sequestrant treatment in mice with diet induced obesity, insulin and leptin resistance.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Genzyme

## TH-PO382

**Loss of IFT Complex A Protein, THM1, Causes Hyperphagia-Induced Obesity with Fatty Liver Disease, Prediabetes, and Hypertension** Damon T. Jacobs,[1] Michael P. Schonfeld,[1] Luciane M. Silva,[1] Anindita Chatterjee,[1] George Talbott,[2] David Beier,[2,3] Pamela Vivian Tran.[1] *[1]Anatomy and Cell Biology, Univ of Kansas Medical Center, Kansas City, KS; [2]Genetics Div, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [3]Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA.*

**Background:** Primary cilia are antenna-like structures present on most vertebrate cells that mediate signaling pathways. Cilia defects cause ciliopathies, which manifest common clinical features, such as obesity. Obesity is a costly, worldwide epidemic and treatments are invasive and largely ineffective. The IFT complex A gene, *Thm1*, negatively regulates Hedgehog (Hh) signaling and is the most commonly mutated gene in ciliopathies.

**Methods:** We examined a role for murine *Thm1* in obesity using a *Thm1* conditional knock-out (cko) allele together with a ubiquitous, tamoxifen-inducible Cre recombinase.

**Results:** While *Thm1* deletion during early embryogenesis resulted in cystic kidney disease, *Thm1* deletion during adulthood caused a two-fold weight gain relative to wild-type mice over a 13-week period. In adult *Thm1* cko mice, adipose depots were significantly larger than in wild-type, and *Thm1 cko* livers had numerous lipid droplets, indicative of fatty liver disease. *Thm1* cko serum glucose, leptin and insulin levels were elevated, suggesting a prediabetic state. Further, *Thm1* cko mice showed higher blood pressure. To determine if the 15% increase in food intake observed in *Thm1* cko mice caused obesity, *Thm1* cko mice were pair-fed for 13 weeks following *Thm1* deletion. Indeed, body weights and fat depots of pair-fed *Thm1* cko mice were similar to wild-type, suggesting hyperphagia is a primary cause of the obese phenotype. In the hypothalamus, *Thm1* cko primary cilia appear stunted. We are examining signaling pathways that are differentially regulated in the *Thm1* cko hypothalamus and may play a role in hyperphagia.

**Conclusions:** The *Thm1* cko mouse is a novel model of obesity. Since THM1 negatively regulates Hh signaling, increased Hh activity may contribute to cilia-mediated hyperphagia and may reveal a novel therapeutic target for obesity.

*Funding:* NIDDK Support, Other NIH Support - COBRE

## TH-PO383

**SGLT2 Inhibitor Empagliflozin Attenuates Hyperglycemia, Kidney Growth, and Albuminuria and Prevents Glomerular Hyperfiltration in Diabetic Akita Mice** Volker Vallon,[1] Maria Gerasimova,[1] Michael A. Rose,[1] Takahiro Masuda,[1] Eric Mayoux,[3] Hermann Koepsell,[2] Scott C. Thomson,[1] Timo M. Rieg.[1] *[1]Div of Nephrology, UC San Diego & VA San Diego Healthcare System, La Jolla, CA; [2]Univ of Wurzburg, Germany; [3]Boehringer-Ingelheim, Biberach, Germany.*

**Background:** Inhibitors of the sodium-glucose cotransporter SGLT2 are new antidiabetic drugs that enhance urinary glucose excretion. We determined whether the SGLT2 inhibitor empagliflozin (EMPA) alters blood glucose levels (BGL) and early kidney changes in Akita mice ($Ins2^{+/C96Y}$), a non-obese insulin-dependent model of type 1 diabetes.

**Methods:** Akita were compared with littermate $Ins2^{+/+}$ controls (WT). At 4 weeks of age, mice received a diet containing EMPA (300mg/kg diet) or repelleted control diet (veh) for 15 weeks (n=10-13/group). GFR was measured after 14 weeks in conscious mice by plasma kinetics of FITC-Sinistrin. * $P<0.05$ vs veh.

**Results:** EMPA, Akita, and EMPA/Akita increased SGLT2 expression in renal membrane fraction vs veh-treated WT (+47±11*, +38±10* and +56±9*%, respectively). EMPA lowered BGL in non-fasted Akita vs veh (means of 187-261* vs 517-535mg/dl (100-140mg/dl in WT). In WT, EMPA modestly reduced GFR vs veh (250±12* vs 306±23µl/min) and tended to increase kidney weight (KW:312±5 vs 299±5mg). EMPA prevented the diabetes-induced GFR increase in Akita (255±14* vs 397±34µl/min) and attenuated the rise in KW (340±7* vs 410±9mg) and urinary albumin/creatinine ratio (151±18* vs 315±73µg/mg). EMPA increased urinary glucose/creatinine ratios in WT (2110±153* vs 7±1µmol/mg) but not in Akita (7099±430 vs 6909±1165µmol/mg); i.e., in Akita, EMPA-induced inhibition of glucose reabsorption was counterbalanced by reduced filtered glucose. EMPA attenuated/prevented the modest upregulation of systolic blood pressure and markers of kidney injury/inflammation in Akita (incl. mRNA for NFkB, CCL2, CD14, TIMP2).

**Conclusions:** Inhibition of SGLT2 with empagliflozin in diabetic Akita mice lowers hyperglycemia and attenuates kidney growth and albuminuria in proportion. Prevention of diabetes-induced rise in GFR by EMPA is consistent with a role of SGLT2 in proximal hyperreabsorption and the tubulocentric concept of hyperfiltration.

*Funding:* NIDDK Support, Veterans Affairs Support, Pharmaceutical Company Support - Boehringer Ingelheim Pharma GmbH & Co. KG

## TH-PO384

**Reduction of Renal Mitochondria in Type 1 Diabetes Is Associated with Increased Mitophagy** Joseph Satriano, Kumar Sharma. *Nephrology, UC San Diego & VASDHS, San Diego, CA.*

**Background:** Autophagy has been implicated in diabetic kidney disease however, the role of specific types of autophagy have not been identified and its functional role remain unclear.

**Methods:** Autophagy was evaluated in type 1 diabetic F1 Akita mice. Renal histology, albumin/creatinine ratio and mitochondrial protein and complex activity was measured. Autophagy protein expressions were evaluated by immunoblotting. All values are relative densitometry units±SEM normalized against alpha-Tubulin.

**Results:** In F1 Akita mice the kidneys underwent hypertrophy, mesangial matrix expansion and albuminuria. There were also a significant decreases in kidney mitochondrial density and Complex I and IV activities. As expected mTORC1 activity markedly increased (via p-p70S6K Wt 10549±1202, Ak 16008±608) and AMPK activity markedly decreased (p-AMPK T172 Wt 3968±1049, Ak 1047±487). In accord with these changes we observed a pronounced decrease in autophagy (LC3-II/LC3-I ratio; Wt 0.237±0.027, Ak 0.101±0.024), and this was verified by the accumulation of the autophagy adapter or linker protein, p62/Sequestosome-1 (p62; Wt 6998±724, Ak 15945±7734). A number of specific AuTophaGy (Atg) genes and proteins are induced in response to autophagic stimulation. There are 2 conjugation pathways involved in the developing autophagosomal membrane. Atg3, utilized only in the pathway specific to LC3 lipidation, was decreased in accord with LC3-II levels (Atg3 Wt 11493±1380, Ak 6703±1157). However, Atg5 and Atg7, required for the Atg12-conjugation pathway, and Beclin-1, required for initiation of phagophore nucleation, were not changed (Atg5 13650±2110, Ak 14551±1340; Atg7 Wt 12565±2078, Ak 12101±1232; Beclin-1 Wt 8008±579, Ak 6610±505) Mitophagy selective adapter proteins, PINK1 (Wt 1975±463, Ak 4580±1744) and Bnip3 (Wt 2446±811, Ak 10430±2464), were increased in Akita mice kidneys. Studies are ongoing with AMPK activation and in AMPK KO mice.

**Conclusions:** A shift from general autophagy to selective mitophagy may lead to a progressive reduction of mitochondria and homeostatic function in diabetic mice.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO385

**The Lin28-Let7 Pathway Modulates TGF-β-Induced Collagen Expression in Glomerular Mesangial Cells under Diabetic Conditions** Jung Tak Park, Mitsuo Kato, Nancy E. Castro, Rama Natarajan. *Beckman Research Institute, City of Hope, Duarte, CA.*

**Background:** Accumulation of mesangial extracellular matrix (ECM) proteins such as collagen type 1-α2 (col1a2) and type 4-α1 (col4a1) is a key feature of diabetic nephropathy (DN). Although transforming growth factor-β1 (TGF-β) plays an important role in ECM regulation, the mechanisms are not fully understood. Here we examined the role of microRNAs (miRNAs).

**Methods:** miRNAs, mRNA and protein levels were examined by RT-qPCR or Western blotting in TGF-β-treated mouse mesangial cells (MMC) with or without let7-b mimic or inhibitor oligonucleotide transfection. Promoter and 3'-UTR constructs were cloned for reporter assays.

**Results:** RNA deep sequencing showed down-regulation of the miRNA let-7 family members (let-7b/c/d/g/i) in TGF-β-treated MMC. qPCR confirmed this down-regulation along with the up-regulation of col1a2 and col4a1. Ectopic expression of let-7b in TGF-β-treated MMCs attenuated the col1a2 and col4a1 up-regulation. Interestingly, let7-b inhibitors increased col1a2 and col4a1 levels, suggesting let7-b mediates TGF-β induced col1a2 and col4a1. Co-transfection of MMCs with mouse col1a2 or col4a1 3'-UTR luciferase constructs and let-7b inhibitors increased luciferase activity. TGF-β induced col 3'UTR luciferase activity was attenuated by Let-7b mimic, suggesting col1a2 and col4a1 are direct targets of let-7b. In addition, Lin28b, a negative regulator of let-7, was up-regulated in TGF-β-treated MMCs. To test if TGF-β induces Lin28b through Smad-response elements, the promoter region of Lin28b was cloned into the pGL4P vector. Luciferase assays showed that promoter constructs with potential Smad sites responded to TGF-β, which was abolished in constructs without the Smad binding site, suggesting that Lin28b is transcriptionally increased through the Smad site. Furthermore, let-7b levels were decreased, while Lin28b, col1a2 and col4a1 levels were increased in glomeruli of diabetic mice compared to nondiabetic controls, demonstrating in vivo relevance of this Lin28/let-7/collagen axis.

**Conclusions:** These results demonstrate a novel new role for the Lin28/let-7 pathway in controlling TGF-β-induced collagen accumulation in DN.

*Funding:* Other NIH Support - DK081705 and DK058191

## TH-PO386

**Neutralization of TGF-Alpha Attenuates Progression of Experimental Kidney Disease** Josef G. Heuer, Catherine Beidler, Elaine Conner, Jeffrey Boyles, Dianna L. Jaqua, Derek D. Yang, Derrick R. Witcher, Matthew D. Breyer. *Biotherapeutic Discovery Research, Eli Lilly and Company, Indianapolis, IN.*

**Background:** Previous studies have demonstrated a role for transforming growth factor-alpha (TGFa) in the progression of chronic kidney disease in subtotal nephrectomized FVB/N mice (Laouari et al., (2012) EMBO Mol Med 4, 1–15). To determine if TGFa plays a role in the progression of renal disease in other experimental models of progressive renal injury, a TGFa neutralizing monoclonal antibody (Mab41) was generated and used in models of subtotal nephrectomized 129S6 mice and uninephrectomized (uniNx) db/db mice.

**Methods:** Mab41 or a control IgG was dosed once weekly subcutaneously for 4 months to subtotal nephrectomized male 129/S6 mice beginning at 2 weeks post surgery or uninephrectomized (UniNx) female db/db mice beginning at 8 and 16 weeks of age. Animals were assessed for serum clinical chemistry markers, cytokines, albuminuria, urine TGFa, renal pathology and urine NGAL (UniNx db/db only).

**Results:** Albuminuria, serum creatinine, inflammatory markers and renal fibrosis were all significantly elevated in subtotal nephrectomized 129/S6 mice compared to sham animals and 16 weeks of treatment with Mab41 significantly improved all these parameters. Similarly, diabetic UniNx db/db mice exhibited significant increases in albuminuria, mesangial matrix expansion, inflammatory markers, urine TGFa and urine NGAL relative to lean db/m mice. Neutralization of TGFa with Mab41 for 16 weeks significantly reduced all these parameters relative to a control antibody.

**Conclusions:** We conclude that TGFa plays an important role in progression of chronic kidney disease in diverse experimental models of renal injury.

*Funding:* Pharmaceutical Company Support - Eli Lilly and Company

## TH-PO387

**The NMDA Antagonist Memantine Reduces Albuminuria and Podocyte Foot Process Effacement in the Akita Mouse Model of Type 1 Diabetes Mellitus** Stuart E. Dryer,[1,2] Hila Roshanravan.[1] *[1]Biology and Biochemistry, Univ of Houston, Houston, TX; [2]Div of Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** We have previously characterized NMDA receptors in primary rat podocytes and in mouse and human podocyte cell lines. These receptors are activated by L-homocysteic acid (HCA), the primary oxidation product of L-homocysteine. NMDA receptors are ligand-gated ion channels (ionotropic receptors) that are highly permeable to $Ca^{2+}$ ions. Activation of podocyte NMDA receptors evokes $Ca^{2+}$ influx, and increased generation of reactive oxygen species via activation of NADPH oxidases. Hyperhomocysteinemia is common in diabetes, and there is evidence that genes regulating homocysteine metabolism comprise susceptibility loci for diabetic nephropathy. Therefore we hypothesized that hyperactivation of NMDA receptors drives a portion of the progression of diabetic nephropathy.

**Methods:** We implanted 7-week old male Akita mice (B2.B6-Ins2$^{Akita/Mathj}$) with subcutaneous Alzet minipumps filled with either memantine or saline. Memantine is a weak non-competitive NMDA antagonist used for treatment of Alzheimer's dementia, and the daily dose delivered in this study was similar to those used clinically. Similar minipumps were implanted into control DBA/2J mice. Renal function was estimated after 28 days.

**Results:** Akita mice had substantial albuminuria by 11 weeks of age. However we observed that 28 days of memantine treatment markedly reduced 24-hr albumin excretion in Akita mice compared to saline-treated controls. Memantine had no effect on albumin excretion in DBA/2J mice. The effects of genotype, the interaction between memantine and genotype are both significant by two-way ANOVA ($P < 0.05$). Using transmission EM we saw a reduction in podocyte foot process effacement in memantine-treated Akita mice. Memantine had no effect on foot process morphology in DBA/2J mice.

**Conclusions:** These data suggest that sustained hyperactivation of NMDA receptors in vivo may contribute to development of diabetic nephropathy, and suggests a therapeutic strategy based on a drug already approved by FDA for pharmacotherapy of dementias.

*Funding:* Private Foundation Support

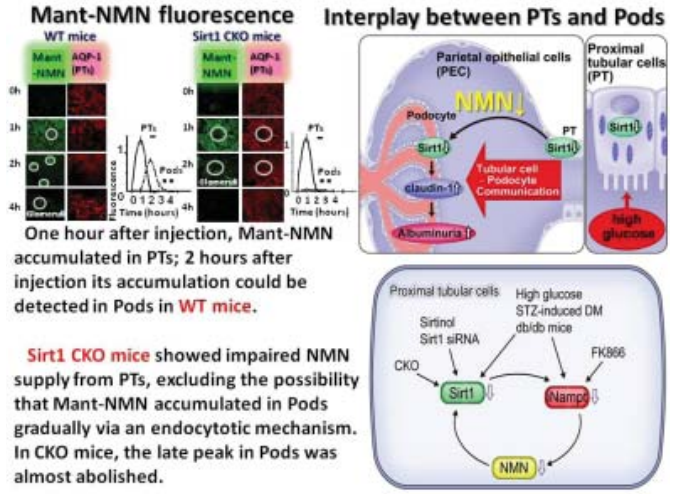
## TH-PO388

**Interaction of Proximal Tubular Cells and Podocytes through a Mediator of Nicotinamide Mononucleotide in Diabetic Nephropathy** Kazuhiro Hasegawa, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Dept of Nephrology, Keio Univ, Tokyo, Japan.*

**Background:** We previously showed that a decrease in Sirt1 expression in proximal tubules (PT) reduces Sirt1 levels and increases claudin-1 levels in podocytes (Pods), leading to the initiation of albuminuria (Alb) in diabetic nephropathy (DN) mice (STZ and db/db). PT-specific Sirt1 transgenic (**TG**) and conditional knockout (**CKO**) mice showed prevention and aggravation of Alb, respectively (oral sessions at ASN 2011, 12). We also showed a role for nicotinamide mononucleotide (**NMN**) as a candidate mediator of the interplay between PTs and Pods using conditioned medium from PTs. In this study, we investigated the retrograde interplay from PTs to Pods in an *in vivo*.

**Methods:** We synthesized **Mant (N-methylanthraniloyl)-NMN** as a fluorescence-labeled nucleotide. Saline (Sal) or Mant-NMN was administered by arterial injection and kidneys were examined by multiphoton microscopy.

**Results:** We observed a PT–Pods flow of NMN.



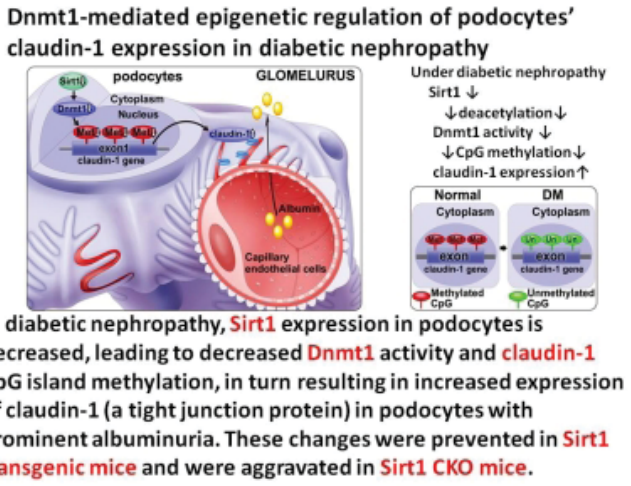

NMN in **WT+Sal** mice was detected mainly in PTs because of the high expression levels of the NMN-producing enzyme iNampt there. **NMN** and iNampt levels were decreased in **WT+DN**, but these decreases were prevented in **TG+DN**. Similarly, **NMN** and iNampt levels were decreased in **CKO+Sal**, and were further reduced in **CKO+DN**. These results suggested that iNampt levels are affected, at least in part, by glucose and/or the Sirt1-determined **NMN** levels around PTs, which regulate Sirt1 and claudin-1 expression levels in Pods. To confirm a role for **NMN** in the progression of DN, we examined the effects of FK866, an inhibitor of iNampt. FK866 (30 mg/kg) decreased **NMN** levels leading to increased claudin-1 expression and Alb, which were rescued in **TG+FK866** and the **NMN injection+FK866** condition, and worsened in **CKO+FK866**.

**Conclusions:** Sirt1 in PTs protects against Alb in DN by maintaining **NMN** levels around Pods and controlling Pods function.

## TH-PO389

**In-Vivo Effect of Podocyte DNA Methyltransferase 1 on Suppressing Diabetic Albuminuria through Epigenetic CpG Methylation of Claudin-1** Kazuhiro Hasegawa, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Dept of Nephrology, Keio Univ, Tokyo, Japan.*

**Background:** We previously created kidney-specific **Sirt1** transgenic (**TG**) and conditional knockout (**CKO**) mice (oral sessions at ASN 2011, 12).




Although we found that DNA methyltransferase 1 (**Dnmt1**) mediates Sirt1-induced CpG methylation, there is a lack of evidence showing whether **Dnmt1** in podocytes (Pods) reduces albuminuria (Alb).

**Methods:** To elucidate the role of Dnmt1, we used gene delivery of NPHS2-Dnmt1 cDNA vector (**Dnmt1 cDNA**), **Dnmt1 siRNA**, and control (**Cont**) plasmid using the HVJE vector by tail vein injection. Efficiency of transfer was tested by immunostaining and immunoelectron microscopy. Mice were injected with saline (**Sal**) or streptozotocin (**STZ**) to induce diabetic nephropathy (DN).

**Results:** We found that Sirt1 directly activates **Dnmt1** via its deacetylation using cultured and laser micro-dissected Pods.In **Cont+Sal**, endogenous Dnmt1 was mainly detected in Pods. Gold particles labeling Dnmt1 were characteristically located on the bottom and sides of foot processes. In **Dnmt1 cDNA+Sal**, Dnmt1 upregulation was detectable in a Pods-specific way owing to the NPHS2 promoter. The **Dnmt1 siRNA** successfully silenced Dnmt1 in kidneys. In **Cont+STZ**, Dnmt1 activity was decreased resulting in increased claudin-1 expression and Alb, which were prevented in **Dnmt1 cDNA+STZ** and worsened in **Dnmt1 siRNA+STZ**. In **Cont+Sirt1 CKO**, reduced Dnmt1 activity was accompanied by increased claudin-1 levels and Alb, which were blocked in **Dnmt1 cDNA+Sirt1 CKO**. Alb was dramatically enhanced by the **Dnmt1 siRNA** but not by a scrambled siRNA. These changes were blocked in **Dnmt1 siRNA+Sirt1 TG**. These phenomena were also observed in obese-type DN (**db/db**) and **Dnmt1 hetero-KO** (Dnmt1 +/-) mice, but not in 5/6 nephrectomized mice.

**Conclusions: Dnmt1** epigenetically protects against Alb and is beneficial for the maintenance of Pods function in DN.

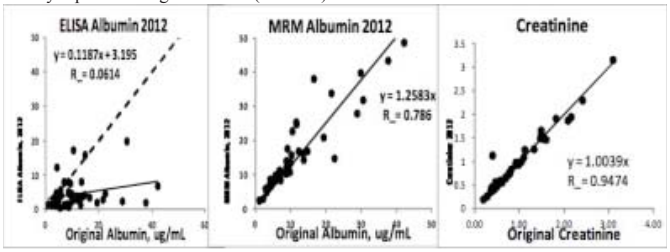
## TH-PO390

**Analysis of Urinary Albumin by Peptide MRM Circumvents Partial Protein Degradation and Loss of Antibody Recognition after Long-Term Storage**
Yun Jiang,[1] Susan E. Kupcho,[1] Blanche M. Chavers,[1] Gregory P. Rynders,[1] John H. Eckfeldt,[1] Michael Mauer,[1] Gary L. Nelsestuen,[1] Theodore E. Mifflin,[2] Dawei Xie,[2] Harold I. Feldman,[2] Vasan S. Ramachandran,[3] Paul L. Kimmel,[4] William Knowler,[4] Robert G. Nelson.[4] *[1]Univ of Minnesota; [2]Univ of Pennsylvania; [3]Boston Univ; [4]NIDDK/NIH.*

**Background:** Many potentially valuable biological samples have been stored for extended time under sub-optimal conditions such as -20° C. Unfortunately, proteins can aggregate or denature at -20° and become refractory to standard methods of protein analysis. Peptide-level analysis may overcome these effects, thereby permitting the use of these samples in clinical research. For the CKD Biomarkers Consortium.

**Methods:** Urine samples were collected in 1982-91 from 49 Pima Indians with type 2 diabetes. Creatinine and albumin were measured (original) and the remaining sample was stored at -20° C. Albumin and creatinine were assayed again in 2012 after storage at -20°C. Albumin was measured by two antibody-based methods and by a newly developed multiple reaction monitoring (MRM) method with peptide-based mass spectrometry. MRM assay of fresh samples compared favorably with 3 established antibody-based assays. Proper sampling for MRM required prior homogenization of particulate material.

**Results:** Antibody-based assays (in 2012) detected lower albumin concentration than the original antibody-based assay. In contrast, analysis of albumin by MRM recapitulated the original measure ($R^2$=0.78). New measurement of creatinine by chemical assay also closely replicated original values ($R^2$=0.95).




**Conclusions:** Long term sample storage under sub-optimal conditions need not impede all uses of valuable sample collections. Analysis of albumin and potentially other proteins by peptide MRM can overcome protein degradation that occurs at -20°. This may prove valuable to many sample collections.

*Funding:* NIDDK Support

## TH-PO391

**Rapamycin Fails to Ameliorate Established Renal Injury and Augments Mortality in Diabetic Mice**
Kavithalakshmi Sataranatarajan, Alex F. Bokov, Denis Feliers, Hak Joo Lee, Meenalakshmi M. Mariappan, Goutam Ghosh-Choudhury, Jeffrey L. Barnes, Arlan Gilbert Richardson, Balakuntalam S. Kasinath. *Univ of Texas Hlth Sc Ctr, San Antonio, TX.*

**Background:** Rapamycin (Rapa) ameliorates kidney injury in young diabetic mice; its efficacy in aging diabetic mice with established kidney injury is not known.

**Methods:** We performed 2 studies in type 2 diabetic mice. **Reversal Study**: Male and female 7 month-old db/m (non-diabetic) and db/db (diabetic) mice received control diet or rapa (2.5mg/kg/day) diet for 4 mos (n=20, each group) and sacrificed at 11 mos of age. **B. Life span Study**: 4 month-old male and female db/db mice were treated with control diet or rapa diet (n=40, each group) till natural death; necropsy was done.

**Results: A. Reversal Study**: At 11 mos of age, diabetic mice showed increase in glomerular area, mesangial matrix area, albuminuria vs. non-diabetic mice; creatinine clearance was unchanged. Rapa did not change any of these parameters. Glomerular hypertrophy, PAS+ fractional mesangial area, and, increase in renal cortical type IV collagen (male) and fibronectin seen in diabetic mice was not reversed by rapa. Signaling assays did not show evidence of mTORC1, Akt or Erk activation in the kidney of diabetic mice and rapa did not affect these parameters. Whole blood rapa levels by HPLC were 1.25-3ng/ml. **B. Life span Study**: Including 120 days when diet study started, the median life span of male and female diabetic mice on control diet was 349 and 487 days, respectively. After 210 days on diet, rapa fed diabetic mice had greater mortality than control diet mice (p=0.02 female, NS for male) (log rank). Cox proportional hazard analysis showed mortality risk was lower (60%) for female vs. male in control diet fed diabetic mice. Adjusting for gender difference, rapa increased mortality risk by 1.7-fold for both male and female diabetic mice (p=0.05). On necropsy, suppurative inflammation in several organs was the main cause of death; this was augmented by rapa. About 30% of deaths were due to neoplasms, mostly hepatocellular carcinoma; rapa reduced these neoplasms.

**Conclusions:** Rapa not only fails to ameliorate established renal injury but worsens mortality in type 2 diabetic mice.

*Funding:* NIDDK Support, Other NIH Support - NIA, Veterans Affairs Support

## TH-PO392

**Protective Effects of Endogenous Vasohibin-1 on Podocytes in Diabetic Nephropathy**
Norikazu Hinamoto,[1] Yohei Maeshima,[1] Hiroyuki Watatani,[1] Haruyo Ujike,[1] Katsuyuki Tanabe,[1] Kana Masuda,[1] Hitoshi Sugiyama,[1] Yasufumi Sato,[2] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Vascular Biology, Development, Aging, and Cancer, Tohoku Univ, Sendai, Miyagi, Japan.*

**Background:** We recently demonstrated the protective role of Vasohibin-1 (VASH1),a negative feedback regulator of angiogenesis, in mouse models of diabetic nephropathy. Regulatory roles of VASH1 on albuminuria, renal inflammatory/fibrotic alterations, angiogenic factors and slit-proteins were observed. In endothelial cells, VASH1 mediates cell survival under oxidative stress via inducing Sirt1 and SOD2 and Ezh2 mediates down-regulation of VASH1. Here, we aimed to elucidate the mechanism involved in the protective role of endogenous VASH1 in diabetic nephropathy.

**Methods:** Type 1 diabetes was induced in male VASH1+/- or wild-type littermates by injections of low-dose streptozotocin. Mice were sacrificed after 16 weeks, kidneys were obtained and subjected to real-time PCR and immunoblot analysis. Conditionally immortalized mouse podocytes were cultured under high glucose condition (HG) with VASH1 siRNA (24 hrs). Then, cells were harvested and subjected to immunoblot or real-time PCR analysis.

**Results:** Renal mRNA levels for Sirt1 were reduced in the diabetic wild-type mice, but was partly recovered in the diabetic VASH1+/- mice.Renal mRNA levels of Ezh2, SOD1, SOD2 were not significantly different between diabetic wild-type and VASH1+/- mice. In cultured podocytes, mRNA levels of nephrin were reduced under HG, and treatment with VASH1 siRNA resulted in further reduction. VASH1 siRNA, augmented the expression of VEGF-A, attenuated the expression of Ang-1 and ZO-1 (immunoblot). Treatment with VASH1 siRNA increased mRNA levels of Ezh2, and tended to suppress mRNA levels of SOD1, but did not alter mRNA levels of SOD2 under HG.

**Conclusions:** These results suggest that endogenous VASH1 may exert the direct protective effects on podocytes through regulating slit-proteins and stress response in association with the amelioration of albuminuria, thus implicating its potential use in treating diabetic nephropathy.

**TH-PO393**

**SGLT2 Inhibition Only Excretes 50-60% of Filtered Glucose in Euglycemic Mice Because of Compensation by SGLT1** Takahiro Masuda,[1] Timo M. Rieg,[1] Maria Gerasimova,[1] Eric Mayoux,[2] Hermann Koepsell,[3] Volker Vallon.[1] *[1]Div of Nephrology, UC San Diego & VA San Diego Healthcare System, San Diego, CA; [2]Boehringer-Ingelheim, Biberach, Germany; [3]Anatomy and Cell Biology, Univ of Wurzburg, Germany.*

**Background:** The sodium-glucose cotransporter SGLT2 reabsorbs most of the filtered glucose in the kidney. Inhibitors of SGLT2 enhance urinary glucose excretion (UGE) and are new antidiabetic drugs but they only excrete 50-60% of the filtered glucose under euglycemia. We aimed to define the role of SGLT1 under these conditions which accounts for ~3% of renal glucose reabsorption in euglycemic mice (Gorboulev 2012).

**Methods:** A. Wild-type (WT) and Sglt1-deficient mice (Sglt1-/-) were treated by oral gavage with vehicle (veh) or SGLT2 inhibitor, empagliflozin (EMPA; 0.1-10mg/kg) followed by quantitative urine collection in metabolic cages for 3 hrs. B. WT and Sglt1-/- were treated with EMPA (300mg/kg of diet) for 3 weeks. Urine was collected weekly followed by renal $^3$H-inulin clearance studies under terminal anesthesia. * P<0.001 vs WT.

**Results:** A. EMPA dose-dependently increased UGE in WT: 0.1±0.1 (veh) to maximum of 30±5nmol/min/g ($ED_{50}$ ~1.3mg/kg); compared with WT, the EMPA-induced UGE was doubled in Sglt1-/-: 1.2±0.2* (veh) to 63±3*nmol/min/g; $ED_{50}$ of ~0.3mg/kg (n=6). B. Within 24h, EMPA (in diet) increased urine glucose/creatinine ratio from 8±1 (basal) to 2283±519µmol/mg in WT and from 95±10* to 3868±450*µmol/mg in Sglt1-/- (n=10-11); doubling of the ratio in Sglt1-/- vs WT was maintained at 1-3 weeks. EMPA-induced reduction in blood glucose (BG) was enhanced in Sglt1-/- vs WT after 24h (-33±5* vs -11±5%); BG and food intake were similar between groups at 1-3 weeks. Subsequent clearance studies in chronic EMPA-treated mice revealed fractional renal glucose excretion (FGE) of 36±2% in WT and 83±2%* in Sglt1-/- (n=9-10). Additional application of EMPA (10mg/kg ip; 1hr prior to study) increased FGE to 56±3% in WT (mimicking values of FGE in Sglt2-/- mice - Vallon 2011) and to 101±3%* in Sglt1-/- (n=4-6).

**Conclusions:** SGLT2 and SGLT1 account for renal glucose reabsorption. SGLT1 reabsorbs 40-50% of filtered glucose during SGLT2 inhibition in euglycemic mice.

*Funding:* NIDDK Support, Veterans Affairs Support, Pharmaceutical Company Support - Boehringer Ingelheim Pharma GmbH & Co. KG

**TH-PO394**

**Relevance of Myo-Inositol Oxigenase (MIOX) in Renal Tubular Injury in Obesity/Metabolic Syndrome** Tatsuya Tominaga, Ming Zhan, Yashpal S. Kanwar. *Pathology, Northwestern Univ, Chicago, IL.*

**Background:** MIOX, a renal tubular enzyme, channels myo-inositol into xylulose-pentose pathway. Previous studies indicate that its expression increases in diabetic state and its transcription is modulated by high glucose ambience, osmotic agents and oxidant stress. Overexpression of MIOX also increases the synthesis of ECM fibronectin, analogus to tubulo-interstitial fibrosis seen in diabetic nephropathy. Its modulation in obesity or glycolipid disorder, such as, metabolic syndrome is unknown. We examined its transcription/translational regulation under the influence of high fat diet (HFD) in vivo and free fatty acid (FFA) in vitro.

**Methods:** Mice were fed with HFD for 2 -8 weeks, and their kidneys harvested for various studies. Similarly, HK2 cells were subjected to FFA (palmitic acid) treatment, and processed for MIOX expression, Chromatin Immuno-Precipitation (ChIP) and MIOX promoter analysis following the binding to the consensus sequence of Sterol Regulatory Element-Binding Protein 1 (SREBP1).

**Results:** MIOX expression increased in renal tubules in mice receiving HFD compared with those receiving normal diet. In addition, increased expression of SREBP1 and Bax was detected in the renal cortices. MIOX expression also increased following FFA treatment in a dose-dependent (0.01-0.3 mM) manner in HK2 cells. Simultaneously, FFA treatment increased p53 and Bax expression. MIOX promoter included multiple segments with consensus binding sequence for sterol regulatory element-binding site (-2460 to -1887, -1032 to -458, -523 to -22). ChIP assay confirmed that SREBP1 binds to MIOX promoter. Furthermore, we cloned the MIOX promoter (-1463 to +33) in pGL3 vector. MIOX promoter activity was increased by 100% with FFA treatment in HK2 cells.

**Conclusions:** These preliminary findings indicate that the MIOX transcription, besides being regulated by hyperglycemic, osmotic and oxidant stresses, is also modulated by FFA and HFD, suggesting that this enzyme may be responsive to the adverse effects of obesity and is likely to lead to tubulointerstitial disease, as seen in other metabolic disorders.

*Funding:* NIDDK Support

**TH-PO395**

**A Novel Dipeptidyl Peptidase IV Inhibitor Ameliorates Diabetic Nephropathy Independent of Metabolic Effects in db/db Mice** Mi Jin Lee,[1] Young Sun Kang,[1] Jin Joo Cha,[1] Young Youl Hyun,[2] Ji Eun Lee,[3] Hyunwook Kim,[3] Mihwa Lee,[1] Jung Eun Kim,[1] Hye Kyoung Song,[1] Jee-Young Han,[4] Dae R. Cha.[1] *[1]Nephrology, Korea Univ Ansan Hospital, Ansan, Kyunggido, Republic of Korea; [2]Nephrology, Sungkyunkwan Univ Kangbuk Samsung Hospital, Seoul, Republic of Korea; [3]Nephrology, Wonkwang Univ Sanbon Hospital, Republic of Korea; [4]Pathology, Inha Univ Hospital, Incheon.*

**Background:** Although DPP IV inhibitor has been widely used for management of hyperglycemia in type 2 diabetic patients, its role on renal function is not clear yet. The aim of present study is to investigate the effects of DA-1229, a newly developed DPP IV inhibitor, on glucose and lipid metabolism and renal injury in db/db mice.

**Methods:** Experimental groups were divided into three groups; non-diabetic db/m mice (n=8), untreated db/db mice (n=8), and db/db mice treated with DA-1229 (300mg/kg/d) for 12 weeks (n=8).

**Results:** DPP-4 activities in heart, liver, and adipose tissue did not significantly different between db/m and db/db mice. However, DPP-4 activity in kidney showed significantly higher in diabetic kidney, and DA-1229 treatment significantly suppressed DPP-4 activity in the kidney. Although DA-1229 treated db/db mice showed an improvement in dyslipidemia, DA-1229 did not induce significant improvement in insulin resistance determined by HOMA-IR and insulin tolerance test, glycemic control and HbA1c levels. Additionally, DA-1229showed a little effect on body weight and systolic blood pressure. However, DA-1229 treatment markedly decreased urinary albumin excretion, kidney/body weight, serum creatinine levels and improved pro-fibrotic molecule synthesis including TGFβ1, PAI-1 and type IV collagen. Furthermore, DA-1229 treatment improved renal lipid metabolism. Interestingly, DA-1229 treatment significantly protected urinary loss of nephrin and restored renal nephrin expression in diabetic glomeruli. In addition, DA-1229 also markedly suppressed renal expression of HMGB-1 protein expression observed in diabetic kidney.

**Conclusions:** These results suggest that a novel DPP IV inhibitor, DA-1229 provide renal protective effects independent of its hypoglycemic metabolic effects.

**TH-PO396**

**Aggravation of Diabetic Nephropathy in Bis-Haploinsufficient Mice along with Impaired Induction of SOD Activity** Keun Suk Yang, Ji Hee Lim, Min Young Kim, Sungjin Chung, Seok Joon Shin, Hyung Wook Kim, Yong-Soo Kim, Cheol Whee Park. *Div of Nephrology, Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea.*

**Background:** Bcl-2 interacting cell death suppressor (Bis), known as anti-stress and anti-apoptotic protein, has been reported to modulate susceptibility to oxidative stress. In this study, we investigated the potential role of Bis as an antioxidant protein in diabetic nephropathy (DN).

**Methods:** DN was induced in Bis-heterozygote mice (Bis-HT) via streptozotocin injections and the resulting phenotypes were compared to those of wild type (Bis-WT) mice over the course of 20 weeks after diabetes induction.

**Results:** Renal injuries, represented by increased plasma creatinine levels and increased albuminuria, were greater in diabetic Bis-HT (Bis-HT DM) mice than in diabetic Bis-WT (Bis-WT DM) mice, accompanied by a significant increase in oxidative stress markers. Moreover, glomerular matrix expansion, TGF-b1 and HIF-1a expression, tubulointerstitial fibrosis and proportion of apoptotic cells were significantly higher in Bis-HT DM mice than in Bis-WT DM mice with the same degree of hyperglycemia. These severe outcomes of DN were all restored by tempol treatment. The expression of SOD1 and SOD2 increased on the induction of DM in Bis-WT mice, correlating with the total SOD activity, but was not observed in Bis-HT and Bis-HT DM mice. An in vitro study showed that the knockdown of Bis expression also resulted in the impaired induction of SOD activity as well as SOD expression in HK-2 and NMS cells.

**Conclusions:** Our results suggest that decreased antioxidant capacity of Bis may aggravate DN in Bis-HT DM mice, which could possibly result from disruption in the regulation of SOD1 or SOD2 protein quality upon oxidative stress.

**TH-PO397**

**Fenofibrate Ameliorates Diabetic Nephropathy through the Activation of AMPK-PGC-1α-ERR-1α Signaling in *db/db* Mice** Keun Suk Yang, Yul Hee Cho, Min Young Kim, Ji Hee Lim, Hoon Suk Park, Sun Ryoung Choi, Seok Joon Shin, Hyung Wook Kim, Yong-Soo Kim, Cheol Whee Park. *Div of Nephrology, Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea.*

**Background:** The peroxisome proliferative-activate receptor-α is a lipid-sensing transcriptional factor that has a role in gluco-oxidative stress and lipotoxicity. Here, we evaluated whether fenofibrate has renoprotective effects through the change of AMPK-PGC-1α-ERR-1α and their downstream PI3K-Akt pathway in *db/db* mice.

**Methods:** Male C57 BLKS *db/db* mice and *db/m* controls at 8 weeks of age were divided to receive either a regular diet chow or a diet containing fenofibrate (0.2% wt/wt, n=6, respectively). The treated *db/m* mice and *db/db* mice were administrated fenofibrate for 12 weeks and were evaluated about renal functional and pathologic phenotypes and the AMPK-PGC-1α-ERR-1α pathway.

**Results:** The mesangial area expansion, inflammatory cell infiltration, and the accumulation of intra-renal lipids were observed in *db/db* mice and this was related to a downregulation of PPARα suppressed AMPK-PGC-1α-ERR-1α expressions and increased

PI3K-Akt-phosphorylation of FoxO3a, which led to oxidative stress and decreases fatty acid oxidation. Fenofibrate significantly decreased albuminuria and reversed all of the renal phenotypes by increasing the PPARα expression with concomitant activation of AMPK-PGC-1α-ERR-1α and inactivation of PI3K-Akt pathway, dephosphorylation of FoxO3a signaling in *db/db* mice. Fenofibrate ameliorated high glucose induced oxidative stress and apoptotic cell death in cultured mesangial cells through the same pathways.

**Conclusions:** Our results suggest that PPARα agonists are associated with improvement of lipotoxicity and oxidative stress through the activation of AMPK-PGC-1α-ERR-1α signaling in *db/db* mice, which may be a potentially therapeutic modality to treat diabetic nephropathy.

## TH-PO398

**Inhibition of Factor Xa with Rivaroxaban Ameliorates Proteinuria and Reduces Inflammatory Cytokines with Diminished Loss of Glycocalyx in Animal Models of Diabetic Nephropathy** Jan Menne, Nelli Shushakova, Joon-Keun Park, Torsten Kirsch, Anna Bertram, Putri Andina Agustian, Hermann G. Haller. *Clinic of Hypertension, Hannover Medical School, Hannover, Germany.*

**Background:** Activated factor Xa(FXa) plays an important role in the coagulation cascade. In addition, FXa binds to protease-activated receptors PAR-1 and PAR-2 and may exert cellular function in the endothelium. We have recently shown that the endothelial glycocalyx surface layer (ESL) is important for the initiation of proteinuria and contribute to the early inflammatory changes in diabetic nephropathy. We therefore tested the hypothesis that (1) treatment with a FXa inhibitor prevents proteinuria and inflammatory changes in diabetic and ATII-infused mice and (2) that this effect is mediated via the endothelium.

**Methods:** STZ (*n*=8), db/db mice (*n*=8) and control animals received sham or rivaroxaban RIVA (50 mg/kg/day) before or during hyperglycemia. After 2 and 8 weeks of hyperglycemia, the animals were analyzed. Immunohistochemistry was performed on cryostat/paraffin sections. EM analysis of ESL was carried out after Alcian blue perfusion. Gene and protein expression was analyzed by rt-qPCR and western blot analysis. Signal transduction of FXa was assessed by measuring ERK phosphorylation pERK.

**Results:** Treatment with RIVA reduced the hyperglycemia–induced increase in proteinuria in both STZ and db/db mice significantly. Decrease of heparansulfates and endothelial glycocalyx density was prevented. The increase in inflammatory cytokines and adhesion molecules in diabetic animals was also reduced by RIVA treatment. Macrophage infiltration in the diabetic kidney was reduced by RIVA treatment. The increase in pERK in diabetic glomeruli was diminished by RIVA treatment. All these changes were not observed in AngII-treated animals.

**Conclusions:** Inhibition of factor Xa signalling with rivaroxaban in glomerular endothelial cells reduces proteinuria in diabetic animals. The amelioration of proteinuria is associated with reduced breakdown of endothelial glycocalyx and less inflammation. Rivaroxaban may have beneficial effects on endothelial glycocalyx thereby improving proteinuria and inflammation in diabetic nephropathy.

## TH-PO399

**Increased Tubulointerstitial Damage, Macrophage Infiltration, and Nuclear Factor-κB Activity in the Kidneys of Diabetic STAT5 Knockout Mice** Karen T. Coschigano,[1,2] Erich P. Heine,[3] Ramiro Malgor.[1,2] *[1]Dept of Biomedical Sciences, Ohio Univ College of Osteopathic Medicine, Athens, OH; [2]The Diabetes Institute of Ohio Univ, Athens, OH; [3]Ohio Univ College of Osteopathic Medicine, Athens, OH.*

**Background:** Diabetic nephropathy is currently the most common cause of chronic renal failure in the United States. Mounting evidence indicates that inflammatory mechanisms are involved in the development of diabetic nephropathy. Our prior analysis of nondiabetic and streptozotocin-induced diabetic wildtype and Signal Transducer and Activator of Transcription 5 (STAT5) knockout mice revealed that RNA expression of many inflammation-related genes is dramatically upregulated in kidneys of diabetic STAT5 knockout mice. We hypothesized that Nuclear Factor κB (NF-κB), a major inflammatory transcriptional activator, would have increased activity in diabetic STAT5 knockout mice that would coincide with this increased gene expression, as well as with increased macrophage infiltration and kidney damage.

**Methods:** We used immuno- and histochemical methods to assess lymphocytic infiltration and kidney morphology and a DNA binding assay to measure NF-κB activity of kidneys isolated from nondiabetic and streptozotocin-induced diabetic wildtype and STAT5 knockout mice.

**Results:** We demonstrated that diabetic STAT5 knockout mice had a greater number of macrophages and lymphocytic aggregates, increased fibronectin expression, and tubulointersititial damage when compared to the other nondiabetic and diabetic mice. In addition, we showed that NF-κB DNA binding activity was significantly greater in diabetic STAT5 knockout mice when compared to their counterparts.

**Conclusions:** The data suggest that the STAT5 knockout mice have lost some inherent protection from inflammatory responses that wildtype mouse groups retain, indicating that the STAT5 proteins may serve a protective role in diabetic nephropathy.

## TH-PO400

**CYP2C-Derived EETs Contribute to Insulin Sensitivity in Mice and in Humans** James M. Luther, Mahesha Gangadhariah, Jorge H. Capdevila, Ambra Pozzi. *Medicine, Vanderbilt Univ, Nashville, TN.*

**Background:** The P450 arachidonic acid epoxygenases (CYP2C, CYP2J) modulate blood pressure via renal and vascular effects, and interventions which prevent degradation of their epoxyeicosatrienoic acid products (EETs) via epoxide hydrolase inhibition may favorably alter glucose homeostasis.

**Methods:** We tested the hypothesis that endogenous CYP2C-derived epoxyeicosatrienoic acids alter insulin sensitivity in vivo using *Cyp2c44*-deficient mice during 16 weeks of high fat or regular chow diet. We assessed whole organism insulin sensitivity using hyperinsulinemic clamps and tissue insulin sensitivity using 2-deoxyglucose uptake. To examine this association in humans, we assessed insulin sensitivity and plasma EETs during controlled dietary sodium intake in healthy and metabolic syndrome subjects.

**Results:** Despite similar body weight and adiposity, insulin sensitivity decreased in *Cyp2c44(-/-)* mice compared to wild-type (WT) controls (40.4±2.7 vs 57.8±3.8 mg/kg/min; *P*=0.003). High fat-fed WT and *Cyp2c44(-/-)* mice were both significantly insulin resistant compared to WT mice, but were similar between genotype. Hepatic glucose production during regular chow diet was similar at baseline, but was incompletely suppressed in *Cyp2c44(-/-)* mice during insulin-glucose infusion (-1.2±1.8 vs 6.4±0.6). Muscle and adipose tissue $^{14}$C 2-deoxyglucose uptake in vivo was diminished in *Cyp2c44(-/-)* mice. However, insulin-stimulated 2-deoxyglucose uptake was similar in isolated muscle *ex vivo*, suggesting that tissue perfusion may contribute to the defect. In human subjects with metabolic syndrome, plasma EETs were reduced compared to healthy controls (*P*=0.014) and were positively associated with insulin sensitivity assessed during intravenous glucose tolerance tests ($R^2$=0.62, *P*<0.001).

**Conclusions:** These studies demonstrate that CYP2C-derived EETs contribute to insulin sensitivity in mice and in humans. Interventions which increase circulating EETs in humans could provide a novel approach to improve insulin sensitivity and treat hypertension.

*Funding:* NIDDK Support, Other NIH Support - UL1 RR024975 and UL1 TR000445

## TH-PO401

**Urinary Podocalyxin Reflects Podocyte Injury in Hypoxia-Exacerbated Diabetic Glomerulosclerosis** Naoki Takahashi,[1] Kenji Kasuno,[1] Kazuko Kamiyama,[1] Tomomi Kurose,[2] Hiroyuki Kurosawa,[3] Yoshiaki Hirayama,[3] Seiji Yokoi,[1] Yoshinari Yokoyama,[1] Daisuke Mikami,[1] Hideki Kimura,[2] Haruyoshi Yoshida,[4] Masanori Hara,[5] Masayuki Iwano.[1] *[1]Nephrology, Fukui Univ; [2]Clinical Laboratories, Fukui Univ Hosp.; [3]Reagent R&D Dept, Denka Seiken Co.; [4]Internal Medicine, Obama Hosp.; [5]Pediatrics, Yoshida Hosp.*

**Background:** We have previously reported that chronic hypoxia exacerbated diabetic glomerulosclerosis (GS) in db/db mice exhibiting microaneurysms with podocyte loss after 16 weeks of hypoxic breeding, and that urinary albumin (U-Alb) levels were increased at 2 weeks in hypoxia. In this study, we examined urinary podocalyxin (U-PCX) in the early stage of the hypoxia-exacerbated GS in db/db mice, because U-PCX has been reported as a useful biomarker for early podocyte injury in diabetic patients (Diabetologia 55: 2913, 2012).

**Methods:** Eight w.o. male db/db mice were bred in a normobaric hypoxic chamber (12% $O_2$) for 4 weeks (n=6) (H-group) and the control mice were bred in room air (n=6) (N-group). U-PCX and U-Alb were weekly measured by ELISA using 24h urine samples. Urine samples were centrifuged at a low speed to detect cellular PCX in sediments or the resulting supernatants were further ultracentrifuged for analyzing cytoplasmic PCX, by immunofluorescence and western blotting. The kidney specimens obtained at 12 w.o. were immunoenzymatically stained for WT-1 positive podocytes.

**Results:** The average podocyte number per glomerulus did not differ between the two groups, however, the number per glomerular area was significantly decreased in H-group associated with glomerular swelling. Both U-PCX and U-Alb levels were significantly increased in H-group as compared with N-group at 1 week in hypoxia and continued to be elevated. U-PCX and U-Alb showed a positive correlation ($p<0.01$). In both group, PCX-positive cells were not found in urinary sediments, however, PCX-positive granular particles were detectable in the bottom fraction after the ultracentrifugation.

**Conclusions:** U-PCX may be a novel biomarker for podocyte injury characterized as cytoplasmic shedding and U-Alb may be attributable to such podocyte injury in the early stage of diabetic GS in this mouse model.

## TH-PO402

**Effect of Febuxostat (FBX) on the Development of Nephropathy in Obese Zucker Rats (ZO) with Type 2 Diabetes (DM2)** Radko Komers,[1] Bei Xu,[1] Terry T. Oyama,[1] Robert Jackson,[2] Robert N. Palmer.[2] *[1]Div of Nephrology and Hypertension, Oregon Health and Science Univ, Portland, OR; [2]Global Medical Affairs, Takeda Pharmaceuticals, Deerfield, IL.*

**Background:** Xanthine oxidase (XO) inhibitors have beneficial effects in the diabetic kidney. However, the long-term protective potential of XO inhibition in advanced diabetic renal lesions remains to be determined.

**Methods:** We tested the effects XO inhibitor FBX on the development of nephropathy in ZO in vivo, and in vitro in renal tubular cells (RTC) stimulated with a RAGE agonist s100B and with TGF beta (TGFb).10-week old ZO were divided in groups treated with FBX (ZO-FBX, 5mg/kg), enalapril (E) (ZO-E, 10mg/kg) and a combination of both agents (ZO-FBX+E) for 18 weeks and compared to vehicle-treated ZO (ZO-V) and lean controls (ZL).

The late intervention with FBX initiated at 20 weeks of age was also studied (ZO-FBXlate).

**Results:** ZO-V developed severe glomerulosclerosis (GS) and tubulointerstitial fibrosis (TIF), albuminuria, and mild elevations of BP as compared to ZL. ZO-FBX and ZO-FBXlate rats demonstrated significant decreases in GS score (27% and 25%, resp., $p<0.05$) and in proportion of obsolete glomeruli, compared with ZO-V. The TIF score was also reduced by FBX (35%, $p<0.05$ vs. ZO-V), albeit not after delayed treatment. Treatment with E, or combination of both agents resulted in resp. 44% and 55%, reduction in GS and resp. 70% and 80% reduction in TIF scores (<0.001 vs. ZO-V, $p<0.05$ vs. ZO-FBX). In ZO-FBX+E, the GS score was no more different from ZL, suggesting additive effect of FBX+E. Significant reductions in BP and albuminuria were observed in ZO-E and ZO-FBX+E. The uric acid (UA) levels, elevated in ZO rats, were only slightly reduced in FBX-treated rats. On the molecular level, FBX reduced expression of collagen 4 (Col4), fibronectin (FN) and CTGF to a similar extent as in ZO-E., and attenuated s100B- or TGFb-induced increases in Col4 and FN production in RTC in vitro.

**Conclusions:** FBX slowed down the development of GS and TIF in experimental DM2, although to a lesser degree than E. These effects were BP-independent and not entirely attributable to UA lowering. Moreover, there were additive effects of FBX+E.

*Funding:* Pharmaceutical Company Support - Takeda Pharmaceuticals

## TH-PO403

**Genome-Wide Profiling of Inflammation Related Genes in High Glucose Stimulated Mesangial Cells and Tubular Epithelial Cells**

Yasunori Iwata,[1] Kengo Furuichi,[1] Haruka Yasuda,[2] Norihiko Sakai,[1] Shinji Kitajima,[1] Tadashi Toyama,[1] Yasuyuki Shinozaki,[1,2] Akihiro Sagara,[1] Takashi Wada.[1,2] *[1]Nephrology, Kanazawa Univ, Kanazawa, Ishikawa, Japan; [2]Laboratory Medicine, Kanazawa Univ, Kanazawa, Ishikawa, Japan; [3]Disease Contorol and Homeostasis, Kanazawa Univ, Kanazawa, Ishikawa, Japan.*

**Background:** Diabetic nephropathy (DN) is a major cause of end stage kidney disease and a strong risk factor for cardiovascular diseases. Growing data show chronic inflammation plays an important role for the progression of DN. As for the immune cells, anti- inflammatory leukocytes as well as inflammatory leukocytes play an important role in DN. In addition to leukocytes, renal resident cells contribute to the inflammatory process of DN. However, precise functions, phenotypes and immune balance of renal resident cells remain to be explored. Therefore, we hypothesized that the aberrant immune balance of renal resident cells contributes to the pathogenesis of DN.

**Methods:** To explore this, we performed genome-wide transcriptome profiling in mesangial cells and tubular epithelial cells (TECs), which were stimulated by high glucose (HG) and detected the expression of inflammation associated genes.

**Results:** High glucose (HG) increased the mRNA expression of oxidative stress, inflammasome and mammalian target of rapamycin (mTOR) related genes in mesangial cells. Pro-inflammatory/Th1 gene expression was upregulated, but Th2 related gene expression was downregulated in mesangial cells. In TECs, HG stimulation increased pro-inflammatory/Th1/Th2 gene expression. Phosphorylation of signaling proteins shifted towards pro-inflammatory phenotype with suppressed phosphorylation of Th2 related signaling in TECs.

**Conclusions:** The data taken together indicate that HG shifts the immune balance toward pro-inflammaotry/Th1 phenotype in mesangial cells and TECs, which might initiate and/or prolong inflammation, thereby possibly resulting in diabetic nephropathy.

*Funding:* Government Support - Non-U.S.

## TH-PO404

**Febuxostat Attenuates Diabetic Renal Injury via an Anti-Inflammatory and Anti-Oxidative Mechanism**

Hong Joo Lee,[1] Jungkook Wi,[2] Ju Young Moon,[3] Sang Ho Lee,[3] Chun-Gyoo Ihm,[3] Tae-Won Lee,[3] Kyung Hwan Jeong.[3] *[1]Div of Nephrology, Gachon Univ Dongincheon Gil Hospital; [2]ISU Medical Center; [3]Div of Nephrology, School of Medicine, Kyung Hee Univ.*

**Background:** Oxidative stress and inflammation are known to play a central role in the development of diabetic nephropathy. Febuxostat (Fx) is a novel nonpurine xanthine oxidase (XO)-specific inhibitor for treating hyperuricemia.The reduction in oxidative stress by administering the XO inhibitor has been shown to slow the progression of renal dysfunction. In this study, we investigated whether Fx could attenuate diabetic kidney injury and impart renoprotective effects, including anti-oxidative stress and anti-inflammatory mechanisms.

**Methods:** Male Sprague–Dawley rats were divided into three groups: normal, vehicle-treated diabetes (DM), and febuxostat-treated diabetes (DM+Fx). We administered 5mg/kg of Fx to experimental rats for 7 weeks.

**Results:** Diabetic rats (DM and DM+Fx groups) had higher blood glucose, higher kidney weight relative to body weight, and increased urine output compared to normal rats. Urinary albuminuria was only significantly reduced in Fx-treated diabetic rats. Quantitative analysis showed that hepatic XO and XDH activity was increased in the DM group but reduced after treatment with Fx. Urine 8-OHdG concentrations and renal cortical nitrothyrosine also indicated reduced oxidative stress in the DM+Fx group. We observed no differences in renal histopathology between the three groups. However, we observed a greater number of ED-1 stained cells in the glomerulus and tubule of diabetic renal tissue compared to normal; after administration of Fx, ED-1 stained cell count decreased. Finally, diabetic rats showed increased mRNA expression of inflammatorygenes (E-selectin and VCAM-1), inflammation inducible enzymes (COX-2), and inflammatory mediators (ED-1 and NF-kB): after administration of Fx, these showed decreased significantly.

**Conclusions:** Febuxostat ameliorates the diabetic renal injury such as albuminuria. Renoprotective effects of Fx may attenuate the inflammatory and oxidative stress mechanisms of renal damage in diabetes by inhibiting XO and XDH activity.

## TH-PO405

**Regulation of Fatty Acid Oxidation Affects the Susceptibility of Podocytes to Palmitic Acid: Critical Role of Acetyl-CoA Carboxylase 1 and 2**

Kapil Dev Kampe,[1] Jonas Sieber,[2] Jana Orellana,[1] Peter H. Mundel,[2] Andreas Werner Jehle.[1,3] *[1]Dept of Biomedicine, Molecular Nephrology, Univ Hospital, Basel, Basel Stadt, Switzerland; [2]Div of Nephrology, Massachusetts General Hospital, Boston; [3]Dept of Internal Medicine, Transplantation Immunology & Nephrology, Univ Hospital, Basel, Basel Stadt, Switzerland.*

**Background:** Type 2 diabetes (T2D) is characterized by dyslipidemia with elevated free fatty acids (FFAs). FFAs and renal FFA-oxidation (FAO) potentially play a direct role in the pathogenesis of diabetic nephropathy (DN), and a SNP in acetyl-CoA carboxylase (ACC) 2 is associated with proteinuria in T2D. SNP results in higher expression of ACC2 which likely inhibits FAO by producing higher malonyl-CoA, an inhibitor of carnitine palmitoyltransferase I (CPT1), the rate-limiting enzyme of FAO. Here, we addressed the role of FAO in palmitic acid induced podocyte death, and further explored the role of ACCs in this process.

**Methods:** Conditionally immortalized murine podocytes differentiated for at least 11 days were used. 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (Aicar) and etomoxir (CPT1 inhibitor) were employed to alter FAO. $^3$H palmitic acid was used to determine FAO by measuring the release of $^3H_20$. ACC 1 and 2 were silenced using lentiviral system.

**Results:** Aicar decreased palmitic acid induced apoptosis and necrosis by 50.5 ± 1.5% ($p < 0.01$) and 42.5 ± 6.1% ($p < 0.05$). Contrariwise etomoxir exacerbated apoptosis and necrosis by 184.3 ± 6.0% ($p < 0.001$) and 185.1 ± 16.3% ($p < 0.01$) respectively. Aicar phosphorylated AMPK and ACC. Aicar increased oxidation of palmitic acid by 146.6 ± 22.0% ($p < 0.05$) and co-treatment with etomoxir reversed this effect. Only knocking down of both ACC 1 and 2 reduced palmitic acid induced apoptosis 59.6 ± 4.5% ($p < 0.01$) and necrosis 64.4 ± 6.4% ($p < 0.01$). Aicar did not cause added protection in ACC depleted podocytes.

**Conclusions:** Regulation of FAO in podocytes profoundly affects their susceptibility to palmitic acid cytotoxicity. Our data may explain the risk for proteinuria in T2D patients with SNP in ACC2 as reported in previous studies. AMPK-ACC-CPT1 pathway is a potential target to prevent and treat DN.

*Funding:* Government Support - Non-U.S.

## TH-PO406

**Role of the TNF Pathway in the Progression of Diabetic Nephropathy**

Tomohito Gohda, Keisuke Omote, Yasuhiko Tomino. *Div of Nephrology, Dept of Internal Medicine, Juntendo Univ Faculty of Medicine, Tokyo, Japan.*

**Background:** Chronic inflammation promotes the progression of diabetic nephropathy (DN). However, the role of TNFα remains unclear. The objectives of the present study are 1) to examine whether TNFα inhibition with a soluble TNF receptor 2 (TNFR2) fusion protein, Etanercept (ETN) improves DN in type 2 diabetic model of KK-$A^y$ mouse, and 2) to also investigate which TNF pathway, TNFR1 or TNFR2, involves predominantly in the progression of DN.

**Methods:** ETN was injected intraperitoneally to the diabetic mice for 8 weeks. Urinary and serum samples were collected at the beginning and end of the experiment. Renal damage was evaluated by immunohistochemistry and/or real time PCR. In vitro, mouse tubular proximal (mProx) cells were stimulated by TNFα and/or high glucose, and treated by ETN. Their supernatants and mRNA were collected.

**Results:** ETN treatments dramatically reduced the levels of not only urinary albumin but also casual blood glucose and HbA1c. However, they did not affect the levels of body weight and blood pressure. Renal mRNA and/or protein expression levels of TNFR2, but TNFα and TNFR1, in the ETN treated diabetic mice (treated mice) were significantly decreased compared with the non-treated diabetic mice (non-treated mice). The mRNA expression levels of ICAM-1, VCAM-1 and MCP-1, and the number of F4/80 positive cells were all dramatically decreased after the treatment. The numbers of cleaved caspase 3 and tunel positive cells in non-treated mice were very few, and did not different from the treated mice. In vitro, TNFα or high glucose markedly increased both TNFRs (TNFR1 and TNFR2) mRNA expression levels unlike in the case of in vivo. While, ETN treatment partly recovered TNFα induced both TNFRs mRNA expressions, but did not affect high glucose induced those expressions.

**Conclusions:** It appears that ETN may improve the progression of DN through predominantly anti-inflammatory action of TNFα-TNFR2 pathway.

## TH-PO407

**Characterization of Angiotensin-Converting Enzyme 2 Shedding from Proximal Tubular Cells**

Fengxia Xiao,[1] Joe A. Zimpelmann,[1] Susan B. Gurley,[2] Lawrence Puente,[1] Kevin D. Burns.[1] *[1]Div of Nephrology, Dept of Medicine, Kidney Research Centre, Ottawa Hospital Research Institute, Univ of Ottawa, Ottawa, Canada; [2]Div of Nephrology, Dept of Medicine, Duke Univ Medical Centre, Durham, NC.*

**Background:** Angiotensin-converting enzyme 2 (ACE2) is expressed in the proximal tubule (PT), where it converts angiotensin (Ang) II to Ang-(1-7). Since urinary ACE2 levels increase in diabetes, the aims of this study were to determine if ACE2 is shed from PT cells, to characterize ACE2 fragments, and to study pathways for shedding.

**Methods:** Studies involved primary cultures of mouse PT cells, and urines from mice with streptozotocin (STZ)-induced diabetes. ACE2 activity was measured with a fluorogenic substrate, and ACE2 fragments were analyzed by immunoblots and mass spectrometry (MS).

**Results:** In media from unstimulated mouse PT cells, a time-dependent increase in ACE2 activity was observed. ACE2 activity also increased in media from cells from ACE2 knockout mice transfected with a human ACE2 vector. In culture media, mouse ACE2 was detected as two bands at ~90 and ~70 kDa on immunoblots. Deglycosylation reduced fragment sizes to ~75 and ~60 kDa. MS of the two fragments identified peptides matching mouse ACE2 at positions 18-706 and 18-577, respectively. The amino acid $Met^{706}$ was identified as a candidate cleavage site for ACE2 shedding. Incubation of PT cells in high D-glucose for 48-72 h significantly increased ACE2 activity in the media ($p<0.001$), an effect blocked by a disintegrin and metalloproteinase (ADAM)-17 antagonist, TAPI-1 ($p<0.001$). High D-glucose significantly increased ADAM-17 activity in cell lysates ($p<0.05$). Wild type STZ-diabetic mice showed a time-dependent increase in urinary ACE2 activity, compared to non-diabetic mice. On immunoblots from diabetic mouse urines, ACE2 appeared as two bands of ~100 and ~75 kDa. MS of these fragments confirmed their origin from ACE2.

**Conclusions:** These data indicate that two glycosylated ACE2 fragments are shed from mouse PT cells. ACE2 shedding is stimulated by high D-glucose via an ADAM-17-mediated pathway. Our results also suggest that diabetes stimulates shedding of ACE2 fragments into the urine.

*Funding:* Government Support - Non-U.S.

## TH-PO408

**Lipoxins Attenuate High-Fat-Diet Induced Chronic Kidney Disease**

Emma Borgeson,[1] Catherine Godson,[2] Kumar Sharma.[1] *[1]Center for Renal Translational Medicine, Div of Nephrology-Hypertension, Dept of Medicine, Institute for Metabolomic Medicine, San Diego, CA; [2]Diabetes Research Centre, Conway Institute, School of Medicine, Dublin, Ireland.*

**Background:** Obesity is an independent risk factor for CKD, even when excluding variables such as diabetes.Visceral obesity is considered a driving force of obesity-related complications and the adiponectin/AMPK pathway has been linked to CKD. Lipid-related metabolites may have disease-promoting and modifying effects, e.g. Lipoxin$A_4$ ($LXA_4$) which displays potent anti-inflammatory and anti-fibrotic actions.

**Methods:** We investigated the therapeutic potential of LXs in a 12wk model of high-fat-diet (HFD) induced CKD in C57BL/6 mice, and the potential underlying role of adiponectin. $LXA_4$ (5ng/g) and benzo$LXA_4$analogue (1.7 ng/g) were given as interventional therapeutics 3 times weekly, between wk 5-12 of standard (10% fat) or HFD (60% fat) regime.

**Results:** HFD increased albuminuria and $H_2O_2$, a marker of ROS and renal injury. LXs significantly attenuated urine $H_2O_2$ (-41% $p<0.05$) and glomerular expansion (-78% $p<0.01$), although albuminuria was not reduced. Interestingly, LXs attenuated adipose inflammation and switched adipose macrophage (MΦs) phenotype from M1 ($CD11c^+$) to M2 ($CD206^+$). LXs did not affect renal MΦ infiltration in this model, suggesting that the LX-mediated reno-protective effect occurred via attenuation of adipose inflammation. We further evaluated the role of the reno-protective adipokine adiponectin, by comparing LX-mediated effects in HFD-WT and HFD-Adiponectin-KO mice. As predicted, KO mice presented with augmented renal injury (increased $H_2O_2$) compared to WT. Surprisingly, LX-induced reduction of renal $H_2O_2$ was augmented in the KO strain (-69 % $p<0.01$). LXs restored insulin-stimulated glucose uptake in the KO strain, although not in the WT animals, suggesting that adiponectin may mask some of the protective LX effect. Importantly, LXs did not alter weight gain, ensuring that the protective phenotype was not due to altered caloric intake.

**Conclusions:** Collectively these data suggests that LXs, through adiponectin-independent reduction of inflammation and MΦ phenotype, may have therapeutic potential in obesity-induced CKD.

*Funding:* NIDDK Support, Veterans Affairs Support, Government Support - Non-U.S.

## TH-PO409

**Association of Serum Amyloid A with Diabetic Kidney Disease in People and Mice**

Katherine R. Tuttle,[1,2] Sheryl K. Cooney,[1] Robert J. Anderberg,[1] Kelly L. Hudkins,[2] Renee C. Leboeuf,[2] Charles E. Alpers,[2] Rick L. Meek.[1] *[1]Providence Sacred Heart Medical Center, Spokane; [2]Pathology; Metabolism, Endocrinology, Nutrition; Nephrology, Univ of Washington School of Medicine, Seattle, WA.*

**Background:** Serum amyloid A (SAA) is a powerful pro-inflammatory mediator that we detected in the glomeruli and tubulointerstitium of kidneys in mouse models of early/type 1 diabetic kidney disease (DKD) and advanced/type 2 DKD. SAA also directly induced podocyte apoptosis. The study objectives were to assess: 1) SAA in human kidneys; 2) associations of plasma SAA levels with DKD in people and mice; 3) effects of SAA on apoptosis in kidney cells other than podocytes.

**Methods:** SAA immunostaining was performed on kidneys from people with DKD and controls (normal; non-diabetic glomerular disease). Plasma SAA (ELISA) was measured in diabetic people and mice (early/type 1 DKD: streptozotocin C57BL/6; advanced/type 2 DKD: BTBR ob/ob). Mesangial cells and tubular epithelial cells were cultured with SAA to assess early and late-phase apoptosis (caspase 3/7 activity and TUNEL staining).

**Results:** Kidneys from people with DKD (n=3) showed greater amounts of SAA in the glomeruli and tubulointerstitium in a pattern similar to the mouse models. Plasma SAA levels in people with type 2 diabetes and macroalbuminuria were higher than in those with diabetes and normoalbuminuria or non-diabetic controls (0.75+/-0.54, 0.52+/-0.38, 0.28+/-0.17 ug/ml, mean+/-SD, n=10-11 per group, p=0.018). Plasma SAA inversely correlated with estimated glomerular filtration rate (CKD-EPI, r=-0.38, p=0.029). SAA was higher in plasma of diabetic compared to non-diabetic mice: early/type 1 DKD (0.49±0.19 vs 0.10±0.08 ug/ml, n=6-9, p=0.010); advanced/type 2 DKD (0.53±0.38 vs 0.04±0.03 ug/ml, n=5-6, p=0.034). After SAA exposure, mesangial cells and tubular cells exhibited increased caspase 3/7 activity (3-5-fold, n=6-7) and TUNEL staining (2-3-fold, n=8).

**Conclusions:** In people and mice with DKD, SAA localized at sites of glomerular and tubulointerstitial injury and was significantly elevated in plasma. As in podocytes, SAA induced death of mesangial cells and tubular cells, which may contribute to widespread kidney damage in DKD.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Astra Zeneca

## TH-PO410

**Enhanced Store-Operated $Ca^{2+}$ Entry in Glomerular Mesangial Cells in Diabetes Suppresses Extracellular Matrix Protein Expression**

Rong Ma,[1] Sarika Chaudhari,[1] Peiwen Wu,[2] Yanxia Wang.[1] *[1]Integrative Physiology, Univ of North Texas Health Science Center, Fort Worth, TX; [2]Endocrinology, The First Affiliated Hospital of Fujian Medical Univ, Fuzhou, Fujian, China.*

**Background:** The ubiquitous store operated $Ca^{2+}$ entry (SOCE) has a diverse array of function, including promoting cell growth and proliferation. Diabetic nephropathy is characterized with glomerular/mesangial cell (MC) hypertrophy and extracellular matrix expansion. The aim of the present study was to determine if SOCE was involved in the glomerular pathology in diabetes.

**Methods:** Western blot was used to detect the expression levels of STIM1/Orai1 proteins which are molecular components of SOCE, as well as fibronectin and collagen IV proteins, which are the major extracellular matrix proteins in glomerular mesangium. Fura-2 fluorescence ratiometry was utilized to assess SOCE. Whole-cell patch clamp was employed to measure store-operated currents.

**Results:** In cultured human MCs, high glucose (HG) treatment (25 mM for 7 days) significantly increased STIM1/Orai1 protein expressions in a dose dependent manner. The abundance of STIM1/Orai1 proteins was also increased in glomeruli and the renal cortex isolated from diabetic rats induced by streptozotocin and high fat diet, respectively. $Ca^{2+}$ imaging showed that the cyclopiazoic acid (25 μM)-stimulated $Ca^{2+}$ entry was significantly enhanced in human MCs with HG treatment and this enhancement was nearly abolished by GSK-7975A (10 μM), a selective inhibitor of SOCE. Similarly, the whole cell currents induced by the intracellular $Ca^{2+}$ store depletion were significantly augmented in the human MCs with HG treatment. To our surprise, activation of SOCE by thapsigargin (1 μM) dramatically suppressed HG-induced fibronectin and collagen IV protein expressions. In agreement with those findings, inhibition of SOCE by 2-APB (50 μM) or by siRNA against STIM1 increased fibronectin expression.

**Conclusions:** Our results indicate that diabetic hyperglycemia enhances SOCE in MCs by upregulating STIM1/Orai1 proteins, and this enhanced $Ca^{2+}$ signals may protect kidney from diabetic insults by inhibiting synthesis of extracellular matrix proteins.

*Funding:* NIDDK Support

## TH-PO411

**Genetic Deletion of $P2Y_2$ Receptor Confers Significant Resistance to Development of Diet-Induced Obesity and Improves Glucose Tolerance**

Yue Zhang,[1] Carolyn M. Ecelbarger,[2] Bellamkonda K. Kishore.[1] *[1]VA Medical Center & Univ of Utah, Salt Lake City, UT; [2]Georgetown Univ, Washington, DC.*

**Background:** Experimental, clinical and epidemiological data link obesity to the development of diabetes mellitus, metabolic syndrome and chronic kidney disease. Extracellular ATP modulates insulin secretion, and P2 receptors have a role in insulin-stimulated letpin production and lipolysis in white adipocytes. We hypothesized that mice with whole-body knockout (KO) of $P2Y_2$ receptor (R) may have altered response to high-fat diet (HFD)-induced obesity and glucose tolerance.

**Methods:** Groups of age-matched adult wild type (WT) and KO mice were fed regular diet (10% calories as fat; n = 6) or high-fat diet (HFD; 60% calories as fat; n = 14) with free access to food and water for 16 weeks, and euthanized. Feces samples were collected periodically, and glucose tolerance (GT) test was performed during the $14^{th}$ week.

**Results:** Adjusted for body weights (bw), KO mice consumed modestly, but significantly more HFD vs. WT mice, and excreted well-formed feces with no taint of fat or oil. Starting from the $2^{nd}$ week the HFD KO mice displayed significantly lower bw, with terminal mean difference of 20% vs. HFD WT mice. Terminal weights of total white or brown fats were significantly lower in the HFD KO vs. HFD WT mice. Serum insulin, leptin and adiponectin levels were significantly elevated in the HFD WT mice, but not in the HFD KO mice. HFD KO mice had significantly lower levels of blood glucose vs. HFD WT mice at all time points of a GT test, including time 0. Real-time RT-PCR analysis of white fat showed significantly higher expression of PPAR-α, associated with significantly lower expression of angiopoietin-like 4 protein (Angptl4) and IL6 in HFD KO mice vs. HFD WT mice.

**Conclusions:** Since the KO mice consumed modestly higher quantities of the HFD and did not show signs of steatorrhea, these data suggest a higher metabolic rate in the KO mice. Thus, $P2Y_2$-R may have a role in energy metabolism, and can be a potential target for the treatment of diet-induced obesity and/or metabolic syndrome. Future studies should establish the pathways involved in $P2Y_2$-R regulation of energy metabolism.

*Funding:* Veterans Affairs Support, Private Foundation Support

**TH-PO412**

**Renoprotective Effects of Dipeptidyl Peptidase-IV Inhibition in High Fat Diet-Induced Obese Mice** Hyunwook Kim,[1] Ji Eun Lee,[1] Jung Eun Kim,[2] Mihwa Lee,[2] Hye Kyoung Song,[2] Jin Joo Cha,[2] Mi Jin Lee,[2] Sang Youb Han,[3] Young Youl Hyun,[4] Jee-Young Han,[5] Young Sun Kang,[2] Dae R. Cha.[2] *[1]Wonkwang Univ College of Medicine, Korea; [2]Korea Univ College of Medicine Ansan Hospital, Korea; [3]Inje Univ College of Medicine, Korea; [4]Kangbuk Samsung Hospital, Korea; [5]Inha Univ College of Medicine, Incheon, Korea.*

**Background:** Dipeptidyl peptidase-IV (DPP-IV) inhibition is currently being regarded as a promising strategy for diabetes management. However, its direct anti-enzymatic effect on kidney and its consequences are largely unknown.

**Methods:** Normal chow-fed control mice, vehicle-treated high fat diet-induced obese mice, and DPP-IV inhibitor (LC15-0444)-treated high fat diet-induced obese mice were assessed for changes in various metabolic parameters, DPP-IV activity in target organs including kidney, and the parameters for renal damages.

**Results:** After 3 months of treatment, the high fat-induced obese mice administered LC15-0444 at the dose of 3 mg/kg showed no differences in the levels of blood glucose, blood pressure, insulin resistance, and oxidative stress parameters both in kidney and adipose tissue compared with control obese mice. However, treatment with LC15-0444 significantly decreased levels of DPP-IV activity in serum, kidney, fat, and liver of the experimental mice. Furthermore, the mice treated with LC15-0444 showed significantly reduced albuminuria and the tendency to preserve creatinine clearance. In addition, renal histologic examinations showed that the mice treated with LC15-0444 also showed a significantly decrease in glomerulosclerosis and expression of pro-fibrotic markers. In support for these results, a subsequent in vitro study showed that LC15-0444 ameliorated obesity-induced increases in protein expressions of HMGB1, NF-κB, and P-ERK1/2 in renal cortical tissues.

**Conclusions:** In conclusion, Administration of LC15-0444 reduced obesity-induced Increases in enzymatic activity of DPP-IV in the kidney as well as in plasma, and ameliorated renal damages independently of glucose-lowering effect. This renoprotection might be at least partly mediated by anti-inflammatory and anti-proliferative properties.

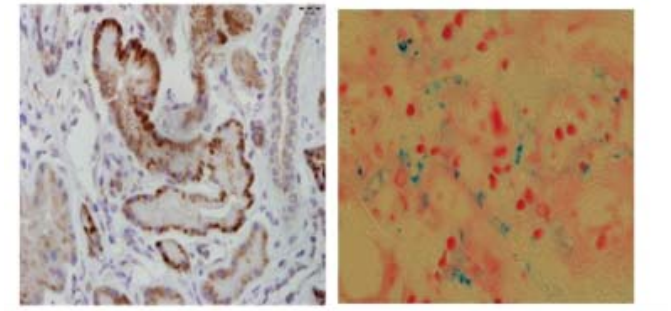
**TH-PO413**

**Increased Expression of Oxidation-Stress and Apoptosis Are Associated with Haptoglobin Genotype in Diabetes Mellitus Patients** Farid M. Nakhoul,[1,2,3] Inbal Mezrahi.[1] *[1]Nephrology Div Baruch Padeh Poriya Medical Center; [2]Diabetic Nephropathy Lab; [3]Faculty of Medicine Bar Ilan Univ, Israel.*

**Background:** We had demonstrated that in diabetic mice with different haptoglobin genotype ( 1-1 , 2-2) there is different susceptibility to diabetic nephropathy (DN). Hp 1-1 DM mice appears to be more protective against diabetic nephropathy than DM mice with Hp 2-2. We found increased iron deposition and oxidative stress in kidney proximal convolute tubules (PCT) of Hp 2-2 vs Hp 1-1, Impaired Hb clearance and increased iron release, induces oxidative stress leading to apoptosis and kidney damage.

**Methods:** 1. We used slides from kidney biopsies of diabetes mellitus patients with different Hp genotype for iron deposition and oxidative stress markers. 2. We will define whether increased renal iron deposits correlates with increased Expression of Oxidation-Specific Epitopes (OSEs) and apoptotic cells in the glomerular and proximal tubules. 3. Quantification of OSEs will perform by immunostaining using an EO6 and IK17 antibodies. The percentage of apoptosis-positive cells will quantify by different apoptotic assays such as Tunel assay, Immunohistochemistry of cleaved caspase 3.

**Results:** 1.Increased iron deposition in PCT of kidney biopsies from patients with Hp 2-2 than in kidney biopsies of patients with Hp 1-1.



Incrased caspase-3 expression H0 2-2 | Increased iron deposition in PCT Hp 2-2

2. Increased expression of Caspase-3 in kidney biopsies of Hp 2-2 patients than in patients with Hp 1-1 genotype.

**Conclusions:** These findings provide insights into genetic predisposition to oxidative stress in Hp 2-2 DM patients. The relationship between increased iron deposition in Renal PCT and macrophage apoptosis that may explain advanced kidney damage and sclerosis in human Hp2-2 kidney biopsies.

*Funding:* Government Support - Non-U.S.

**TH-PO414**

**The SGLT2 Inhibitor Empagliflozin Ameliorates Albuminuria in BTBR ob/ob Type 2 Diabetic Mice with and without Hypertension** Florian Gembardt,[1] Natalia Jarzebska,[1] Christoph Bartaun,[1] Eric Mayoux,[2] Vladimir T. Todorov,[1] Bernd Hohenstein,[1] Christian Hugo.[1] *[1]Div Nephrology, Dept Internal Medicine III, Univ Hospital CGC, Dresden, Germany; [2]Div Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riss, Germany.*

**Background:** Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in man in the western world. Recent development of sodium-glucose co-transporter (SGLT) inhibitors offers a new anti-diabetic therapy via enhanced glucose excretion. Whether this strategy exerts beneficial effects on developing type 2 DN is unclear. We investigated the effects of the SGLT 2 inhibitor empagliflozin (EMPA; Boehringer Ingelheim) in BTBR ob/ob mice, which spontaneously develop type 2 DN.

**Methods:** First, 25 eight week old female BTBR ob/ob mice either received a 12 week diet containing 300ppm EMPA or equicaloric placebo chow. Second, 16 female BTBR ob/ob mice received osmotic minipumps releasing 1µg/kg BW/d angiotensin (Ang) II and were separated in the same 2 diet groups for 6 weeks. Urine, blood, and tissues were harvested and blood pressure was monitored by tail cuff measurements.

**Results:** In both experiments, EMPA treatment enhanced glucosuria vs vehicle (1191±127 vs 384±118 mg/mg-creatinine, $P<0.001$; Ang II: 1887±96 vs. 1113±250 mg/mg-creatinine, $P<0.05$), thereby lowering blood glucose (204±16 vs 402±27mg/dl $P<0.001$; Ang II: 170±20 vs 368±40mg/dl $P<0.01$). While Ang II infusion induced profound hypertension (146±4 vs 84±1mmHg, $P<0.0001$), EMPA treatment had no influence on blood pressure in normotensive or hypertensive mice (81±1 vs 84±1mmHg; 139±6 vs 146±4mmHg). In both experiments, EMPA reduced albuminuria in diabetic mice (0.5±0.1 vs 1.3±0.4mg/mg-creatinine, $P<0.05$; Ang II: 1.5±0.2 vs 4.8±1.8mg/mg-creatinine $P<0.05$). Immunohistochemical analysis assessed glomerular hypertrophy, matrix expansion, inflammation and proliferation. In contrast to albuminuria findings, histology did not demonstrate any differences.

**Conclusions:** Empagliflozin demonstrated significant anti-hyperglycemic effects ameliorating the development of albuminuria in female BTBR ob/ob mice with and without hypertension while no effects on histology were observed.

*Funding:* Pharmaceutical Company Support - Boehringer Ingelheim Pharma GmbH & Co. KG

**TH-PO415**

**Angiotensin Converting Enzyme-2 (ACE2) Deficiency Accelerates Maternal Diabetes-Induced Perinatal Programming** Shiao-Ying Chang,[1] Min-Chun Liao,[1] Xin-Ping Zhao,[1] Yun-Wen Chen,[2] Yessoufou Aliou,[1] Isabelle Chenier,[1] Julie R. Ingelfinger,[3] Shao-Ling Zhang.[1] *[1]CRCHUM-Hotel Dieu, Univ of Montreal, Montreal, Canada; [2]Pharmacology, National Cheng Kung Univ, Tainan, Taiwan; [3]Pediatr Nephrol Unit, MassGeneral Hosp. for Children, Boston, MA.*

**Background:** Increasing evidence suggests that alterations in the ACE2/ACE ratio within the kidney may participate in perinatal programming of hypertension. We aimed to determine whether ACE2 knock-out ($ACE2^{-/-}$ mice on C57BL/6 background) increases the risk of programmed hypertension and kidney injury in the offspring of dams with gestational diabetes and examined the potential underlying mechanisms *in vivo*.

**Methods:** The 20-week-old offspring of non-diabetic and diabetic dams ($ACE2^{-/-}$, $ACE2^{-/+}$, and $ACE2^{+/+}$) were studied. Systolic blood pressure (SBP), glomerular filtration rate (GFR), renal morphology and reactive oxygen species (ROS) generation, as well as protein expression of Wilms tumor-1 (WT-1), transforming growth factor-beta 1 (TGF-β1) and components of the intrarenal renin-angiotensin system (RAS)--e.g., angiotensinogen (Agt), angiotensin converting enzyme (ACE) and angiotensin II receptor 1 and 2 (AT1R and AT2R), were assessed by immunohistochemistry staining (IHC).

**Results:** As compared with the control littermates, $ACE2^{-/-}$ mice with decreased AT2R in the kidney had a small increase in SBP and age-dependent development of glomerulosclerosis. Offspring of diabetic $ACE2^{+/+}$ dams developed hypertension, renal hyperfiltration, podocyte loss and kidney injury. Renal ROS generation and TGF-β1, Agt, AT1R/AT2R and ACE protein were markedly upregulated in kidneys of hypertensive offspring of diabetic $ACE2^{+/+}$ dams. These changes were more pronounced, but, without affecting AT2R expression in the offspring of diabetic $ACE2^{-/-}$ dams.

**Conclusions:** Our data suggest that ACE2 deficiency is pivotal for the development of the adverse perinatal programming induced by maternal diabetes.

*Funding:* Government Support - Non-U.S.

**TH-PO416**

**Urinary miR-29a Content Correlates with Proteinuria and Small Vascular Fibrosis in Type 2 Diabetes Patients** Hui Peng, Meirong Zhong, Wenbo Zhao, Cheng Wang, Jun Zhang, Yuanqing Li, Xun Liu, Tan-Qi Lou. *Div of Nephrology, Dept of Medicine, The Third Affiliated Hospital of Sun Yat-Sen Univ, Guangzhou, Guangdong, China.*

**Background:** Cell-free microRNAs are small non-coding RNAs which stably exist in urine, plasma and other body fluids. Emerging evidence has shown that content of cell-free microRNAs may correlate with particular pathophysiologic conditions and might serve as diagnostic biomarkers. MiR-29 family was reportedly down-regulated in renal fibrosis; therefore, we investigated the possibility of urinary miR-29s as biomarker for diabetic nephropathy in patients with type 2 diabetes (T2DM).

**Methods:** 83 patients with T2DM were divided into 2 groups: with proteinuria (n=42, age 60.62±12.00yrs) and without proteinuria (n=41, age 58.54±14.40yrs). There were no significant differences in age and gender between groups. The content of miR-29a, miR-29b and miR-29c in urine supernatant was determined by TaqMan qRT-PCR, and a synthetic cel-miR-39 was added to the urine as a spike-in control. The clinical laboratory parameters were collected, while retinopathy serves as non-invasive method to assess vascular fibrosis.

**Results:** There was no significant difference in HA1C levels and duration of diabetes between two groups, while the diabetes with proteinuria group had higher comorbidity of diabetes retinopathy and decline in renal function compared with the results of diabetes without proteinuria group. Patients with proteinuria group showed significantly higher level of miR-29a in urine (p=0.035), and urinary albumin excretion rate were significantly correlated with urinary miR-29a level (r = 0.286, p = 0.016). However, there was no significant difference in urinary miR-29b (p=0.148) or miR-29c level (p=0.321) between groups. Similar, no correlation was found between miR29b/c and each of clinical parameters.

**Conclusions:** Urinary miR-29a level rather than miR-29b or miR-29c correlated with proteinuria and vascular fibrosis in patients with T2DM and could serve as alternative biomarker for diabetic nephropathy in T2DM.

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*Funding:* Government Support - Non-U.S.

## TH-PO417

**Puerarin Attenuated Diabetic Kidney Injury through Down-Regulation of Matrix Metalloproteinase 9 in Streptozotocin-Induced Diabetic Rats**

Yifei Zhong,[1] Yueyi Deng,[2] Yiping Chen.[3] *[1]Nephropathy, Longhua Hospital, Shanghai Univ of Traditional Chinese Medicine, Shanghai, China; [2]Inst#1; [3]Inst#1.*

**Background:** Radix puerariae, a traditional Chinese herbal medication, has been used successfully to treat patients with early stage of diabetic nephropathy (DN). However, the underlined mechanism of its renal protective activity has yet to be investigated. Oxidative stress is one of the key mechanisms in DN. Over-production of ROS induces several changes in the kidneys, which could be detrimental to podocyte during DN.

**Methods:** STZ induced diabetic rat model was successfully generated. Treatment of STZ diabetic rats with puerarin and/or an angiotensin II receptor blocker (ARB) was compared with vehicle control. Kidney morphology and podocyte slit diaphragm were examined by PAS staining and electron microscope respectively. Expression of MMP9, nephrin and podocin were examined by both realtime PCR and immunostaining or western blot. Oxidative stress was determined by measuring 8-hydroxy-2'-deoxy-guanosine (8-OHdG) in the kidney cortex by ELISA.

**Results:** Both puerarin and losartan significantly attenuated proteinuria and podocyte foot process effacement in STZ rats compared with vehicle control.In addition, both puerarin and losartan increased expression of podocyte slit diaphragm proteins such as nephrin and podocin in STZ-induced diabetic rats. However, puerarin appeared to have more potent effect on reduction of oxidative stress and MMP-9 level in diabetic kidneys than ARB.

**Conclusions:** These data suggest that puerarin could prevent podocyte injury in diabetic nephropathy through a mechanism that is different from ARB. Therefore, puerarin could be used with ARB for early stage of diabetic nephropathy.

## TH-PO418

**LP-925219, a Dual SGLT1/SGLT2 Inhibitor, Markedly Increases Urinary Glucose Excretion in Mice Lacking SGLT2**

David R. Powell, Melinda G. Smith, Deon D. Doree, Angela L. Harris, Wendy W. Xiong, Faika Mseeh, Brian Zambrowicz, Zhi-Ming Ding. *Lexicon Pharmaceuticals, Inc, The Woodlands, TX.*

**Background:** Inhibiting sodium glucose cotransporter 2 (S2) to increase urinary glucose excretion (UGE) improves glycemic control in diabetic patients, but clinical trials show that inhibitors highly selective for S2 over SGLT1 (S1) block glucose (G) reabsorption by only 30%. Our past work explained this by using S1 knockout (KO), S2 KO and S1/S2 double KO (DKO) mice to show that S1 reabsorbs 70% of filtered G in the absence of S2. This suggests that dual S1/S2 inhibitors will increase UGE to much more than 30% of maximal values in S2 KO mice and in wild type (WT) mice, a hypothesis tested here.

**Methods:** We developed LP-925219 (LP219) and tested its' ability to inhibit G transport by HEK293 cells expressing mouse S1 or S2. S1 KO, S1 KO/S2 heterozygous (Het), S2 KO, S2 KO/S1 Het, DKO and WT mice were bred, fed G-free diet, and kept in metabolic cages to measure 24-hr UGE after pretreatment with either vehicle or 60 mg/kg LP219 delivered by oral gavage. GFR was measured using FITC-inulin. G was measured by Cobas Integra 400.

**Results:** LP219 IC50=22 nM vs mouse S1, =0.5 nM vs mouse S2; GFR (10-12/group): WT=345±82, S2 KO=426±99, DKO=359±59 uL/min; Blood G (10-12/group): WT=130±15, S2 KO=117±12, DKO=113±10 mg/dL; DKO (10/group): predicted G filtered=586±126 mg/day; measured UGE=648±166 mg/day; UGE data are in Table 1:

Table 1. 24-hr UGE as % of Maximal (DKO) Value

Study 1. Effect of Genotype on 24-hr UGE (n = 12 for each group)

| WT | S1 KO | S1 KO/S2 Het | S2 KO | S2 KO/S1 Het | DKO |
|---|---|---|---|---|---|
| 0 ± 0 | 2 ± 1 | 2 ± 1 | 28 ± 21 | 58 ± 21 | 100 ± 26 |

Study 2. Effect of LP219 on 24-hr UGE (n = 4-5 for each group)

| WT | WT + LP219 | S2 KO | S2 KO + LP219 | DKO |
|---|---|---|---|---|
| 0 ± 0 | 61 ± 11 | 26 ± 18 | 82 ± 22 | 100 ± 38 |

**Conclusions:** 1) UGE differences reflect differences in G reabsorption, not differences in GFR or blood G; 2) in DKO, predicted G filtered over 24-hr is similar to measured UGE, suggesting DKO UGE is maximal; 3) the amount of renal S2 >> S1; 4) dual S1/S2 inhibitor LP219 increases UGE to > 60% of maximal (DKO) in WT and S2 KO mice, suggesting that combined inhibition of renal S1 and S2 can significantly increase UGE over selective inhibition of renal S2 alone.

## TH-PO419

**Profiling of Urinary Proteases in Patients with Diabetic Nephropathy**

Luca Musante,[1] Dongfeng Gu,[1,2] Dorota Ewa Tataruch,[1] Carol Forsblom,[3,4] Per-Henrik Groop,[3,4] Harry B. Holthofer.[1] *[1]Centre for Bioanalytical Sciences, Dublin City Univ, Ireland; [2]The Third Affiliated Hospital of Southern Medical Univ, Guangzhou, China; [3]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Helsinki, Finland; [4]Dept of Medicine, Div of Nephrology, Helsinki Univ Central Hospital, Helsinki, Finland.*

**Background:** Urinary extracellular vesicles originate from all nephron segments and epithelial cell lining the urinary tract. They are released by direct budding of the plasma membrane or through established endosomal secretory pathways. Interestingly they contain a wide spectrum of different molecules including proteins, DNA and various RNA classes. Accordingly, urinary vesicles can be a source of disease biomarkers.

**Methods:** We have deviced a new and simple method to enrich vesicles from urine and employed it successfully in a pilot study of diabetic nephropathy (DN) to detect the variety of proteases associated with the vesicles. Here we wanted to study whether specific proteases similarly characterize DN progression. For this purpose, urinary vesicle from 16 healthy volunteers and a total of 36 patients with type 2 diabetes mellitus (T2DM) were divided into three groups based on the level of albuminuria were concentrated and screened for relative changes of 34 different proteases. Protease profiles were established from the pixel average of fluorescent density changes of spots using an infrared Odyssey scanner and plotted with its image analysis software.

**Results:** The results showed a progressive increase of cathepsins C, D, and X/Z/P in the samples with macroalbuminuria while no changes were observed in the array of kallikreins. The array recapitulates the altered expression of metalloproteases MMP2, -8 and -9. Similarly dysregulation of gelatinase activity were visualized by gel based zymography. Distinct protease activities, including DDP-IV were verified employing specific chromogenic substrates.

**Conclusions:** This study shows for the first time characteristic alterations in protease profiles associated with urinary vesicles in DN and suggests that the underlying mechanisms may reveal important mechanistic, prognostic and diagnostic features in advancing kidney damage.

## TH-PO420

**Akita-DBA2J Mouse: A Model of Diabetic Nephropathy**

Cynthia C. Nast,[1,2] Ying Wang,[2] Janine A. La Page,[2] Sharon G. Adler.[2,3,4] *[1]Cedars-Sinai Medical Center, Los Angeles; [2]LA Biomedical Sciences Inst; [3]Harbor-UCLA Medical Center, Torrance, CA; [4]DaVita Clinical Research.*

**Background:** Better animal models of diabetic nephropathy (DN) are needed. The Akita ($Ins2^{-/-}$) mouse (Ak) develops some DN features. The DBA2J (hematopoietic growth factor-inducible neurokinin-1 (HGFIN) KO) mouse is susceptible to and develops DN features after streptozotocin-induced DM. Ak backcrossed with DBA2J (Ak/DBA2J) mice are reported to have albuminuria, but have not been characterized morphologically.

**Methods:** Ak, DBA2J, and Ak/DBA2J mice had urine ACR, plasma glucose (Glu) and BUN measured. Animals were weighed and kidneys collected at 6 months (mos), stained with PAS and Jones for light microscopy, processed for EM, and examined using semiquantitative scores for features of DN as recommended by the Animal Models of Diabetic Complications Consortium (AMDCC).

**Results:** Glu was normal in DBA2J and increased similarly in Ak and Ak/DBA2J (NS). BUN was numerically, but not statistically, higher in Ak/DBAJ. At 6 mos ACR was increased in non-diabetic DBA2J ($p<.05$ vs 3 mos DBA2J). At 6 mos ACR in Ak/DBA2J (711) trended higher vs DBA2J (290) and was significantly higher vs Ak (180, $p<.02$). Weights were not different. Ak/DBA2J had significantly more diffuse and nodular mesangial matrix expansion (p=0.002 vs DBA2J and vs Ak). Focally, Ak/DBA2J nodules had increased cells, sometimes peripheral. All strains had similar arteriolar hyalinization. Ak/DBA2J (201 nm) and Ak (209 nm) had similarly thickened glomerular basement membranes (GBM) (NS). DBA2J had extensive tubular cell vacuoles, less frequent in Ak/DBA2J and absent in Ak; ultrastructurally these had outer double membranes consistent with autophagic vacuoles.

**Conclusions:** With similar Glu levels, Ak/DBA2J have more albuminuria and matrix expansion with nodules, and similar hyalinization and GBM thickening compared with Ak at 6 mos. Ak/DBA2J and DBA2J have tubular cell vacuolar injury consistent with autophagosomes. The Ak/DBA2J mouse is a potentially useful DN model, meeting several AMDCC criteria. This may be mediated, in part, by arrested autophagosome maturation due to HGFIN reduction from the DBA2J strain, providing a potential therapeutic target in DN.

*Funding:* Pharmaceutical Company Support - DaVita Clinical Research

## TH-PO421

**Evolution of Glomerular Nodular Lesions in Diabetic Pigs Carrying a Dominant-Negative Mutant Hepatocyte Nuclear Factor 1-Alpha** Satoshi Hara,[1] Kazuhiro Umeyama,[2] Takashi Yokoo,[3] Hiroshi Nagashima,[2] Michio Nagata.[1] *[1]Kidney and Vascular Pathology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Meiji Univ International Institute for Bio-Resource Research, Kawasaki, Kanagawa, Japan; [3]Internal Medicine, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Glomerular nodular lesion is characteristic pathology in human diabetes, however its morphogenesis is still unknown, partly because of lacking good animal model to have nodular sclerosis. We created diabetic pigs carrying a dominant-negative mutant hepatocyte nuclear factor 1-alpha P291fsinsC and analyzed the process of diabetic nodular formation in these diabetic pigs.

**Methods:** Biochemistry and renal pathology between diabetic pigs and wild-type were analyzed with age of one to nine months. Immunostaining using collagen fibers (type I, III, IV, V, VI), advanced glycation end-products (AGE), and carboxymethyl lysine (CML) was performed to see the content of the lesion. In addition, transmission electron microscopy (TEM) for detecting nodular components and glomerular basement membrane (GBM) thickness were estimated.

**Results:** In diabetic pigs, the blood glucose levels have elevated around 600 mg/dl from 2 weeks old. Proteinuria and renal dysfunction were absent. Glomerular nodular lesions were formed as early as 4 weeks old. The nodules increased and enlarged at 19 weeks old. They distributed deep cortex superior to superficial cortex. Immunohistochemically, the nodules consisted of collagen fibers (type I, III, IV, V, VI). AGE and CML were also deposited in the nodules. TEM showed that the main components of the nodules were interstitial type form of fibril collagens which were located in mesangial area. GBM thickness was not significant between diabetic and wild-type pigs. Moreover, these diabetic pigs did not show any other characteristic features in human diabetic nephropathy.

**Conclusions:** Glomerular nodules in this model of diabetes were characterized by juxta-medullary predominant growth with various types of collagens as well as AGE and CML deposition, without having associated lesion in humans. Thus persistent hyperglycemia and hemodynamic factor can be associated with glomerular nodular formation in diabetic pigs.

## TH-PO422

**Effects of Recombinant Adenoviral Vector-Mediated PC Gene Transfection on Diabetic Nephropathy Atherosclerosis Rats** Li Wang. *Renal Dept, Sichuan Provincial People's Hospital, China.*

**Background:** To observe the expression of plasma APC after recombinant adenovirus gene transfection. And to explore the possible mechanism between atherosclerosis as measured by apoptosis, inflammation, and oxidative stress and APC generation in diabetic nephropathy (DN).

**Methods:** DN with atherosclerosis model rats were randomly divided into three groups: NS group,Ad-GFP group ,Ad-FLT1/PC group. Normal saline, Recombinant adenoviral carrying green fluorescent protein (GFP) gene and recombinant adenoviral carrying GFP gene and FLT-1/PC gene capable of rendering endothelial-specific transgene expression were respectively injected to caudalvein. They were then randomly sacrificed to observe fluorescence distribution in vessel wall. The expression of TNF-a and ICAM-1 and SOD and MDA in the plasma were measured. Cell apoptosis were measured by TUNEL.

**Results:** 3 days after injection of Ad-FLT-1/PC, the green fluorescent was observed in vascular endothelium and PCmRNA was observed. IHC showed the protein C locating in the endothelial cells in Ad-FLT1/PC group was higher.The plasma APC in Ad-FLT1/PC group was significantly higher than GFP or NS group at four time points ($P<0.05$). Detection of TNF-a,ICAM-1 in the vessel wall: Ad-FLT-1/PC transfection significantly decreased the expression of TNF-a, ICAM-1 at 7 days and 14 days ($P<0.05$.GFP group and NS group had no difference at any time ($P>0.05$). Ad-FLT1/PC group, GFP group and NS group rats were showed the phenomenon of cell apoptosis in the vessel wall. The incidence of the cell apoptosis was no statistical difference at each time point ($P > 0.05$).14 days after transfection, Ad-FLT-1/PC group rats had higher concentration of plasma SOD and lower concentration of plasma MDA than Ad-GFP group rats and NS group rats had ($P < 0.05$).

**Conclusions:** The recombinant adenovirus Ad-FLT1/PC can targetly transfect endothelial cell of diabetic nephropathy associated with atherosclerosis rats. The PC can effectively express and be activated into APC in vivo. The APC protects the vessel wall by inhibiting inflammatory cytokines expression and oxidative stress. These dates demonstrate APC may be used as a new therapeutic target to prevent the development of atherosclerosis in diabetic nephropathy.

## TH-PO423

**Augmenting Podocyte Injury Promotes Advanced Diabetic Kidney Disease in Akita Mice** Liming Wang,[1,2] William Eisner,[1,2] Robert F. Spurney.[1,2] *[1]Medicine, Duke Univ Medical Center, Durham, NC; [2]Medicine, Durham VA Medical Center, Durham, NC.*

**Background:** To determine if augmenting podocyte injury promotes the development of advanced diabetic kidney disease, we created mice that expressed the yeast enzyme cytosine deaminase (CD) specifically in podocytes of diabetic Akita mice (Akita-CD mice). In these mice, treatment with the prodrug 5-flucytosine causes podocyte injury as a result of conversion to toxic the metabolite 5-fluorouracil (5-FU) in podocytes.

**Methods:** Akita-CD mice were treated with 5-FC for 5 days at the onset of hyperglycemia in 4-5 week old animals.

**Results:** Treatment with 5-FC significantly enhanced albuminuria at 16 and 20 weeks of age compared to 5-FC treated Akita controls, which do not express the CD transgene (Akita CTL mice). By 20 weeks of age, there was a significant increase in mesangial expansion in Akita-CD mice compared to Akita CTLs, which was associated with interstitial fibrosis in some animals. Podocyte number was reduced in both Akita-CD mice and Akita CTLs at 20 weeks of age and was inversely correlated with the degree of mesangial expansion. Serum creatinine levels were mildly and similarly elevated in Akita-CD and Akita CTLs compared to 5-FC treated wild type controls (CTL mice). Blood glucose levels were similar in both groups of Akita mice.

**Conclusions:** Enhancing podocyte injury at the onset of hyperglycemia promotes the development of advanced features of diabetic kidney disease later in the disease process. Taken together, these data provide additional evidence that glomerular podocytes play a key role in the pathogenesis diabetic nephropathy.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO424

**Improved Renal Function after Gastrectomy in Patients without Severe Obesity** Jeong Eun Kim,[1] Gheun-Ho Kim,[1] Chang Hwa Lee.[1] *[1]Internal Medicine, Hanyang Univ College of Medicine, Seoul, Korea; [2]General Surgery, Hanyang Univ College of Medicine, Souul, Korea.*

**Background:** Obesity is a risk factor for developing chronic kidney disease (CKD) that may be improved with bariatric weight reduction. The benefitof renal function after bariatric surgery is generally interpreted as a result of weight loss. The effect of weight loss caused bygastrectomy on glomerular filtration rate (GFR) in patients without severe obesity is unclear. We analyzed the changes in renal function after gastrectomy in patients without severe obesity.

**Methods:** Serum from 99 patients were tested before and 6, 24 months after gastrectomy. The estimation of the glomerular filtration rate (GFR) was addressed by the CKD-EPI creatinine equation.

**Results:** The subjects of this study were 99 patients who did not receive chemotherapy after gastrectomy patients without severe obesity and more than 60 ml/min/1.73 $m^2$ body surface of CKD-EPI eGFR. The patients completed the 6 months follow-up in 99 patients and 2 years follow-up in 55 patients. Of these, ( )% were female. The mean age was 62.5 ± 12.93 years and the mean body mass index was 23.7 ± 3.35 kg/$m^2$, decreasing to 21.3 ± 2.99 kg/$m^2$ at 6 months and 21.4 ± 2.59 kg/$m^2$ at 2 years after gastrectomy. Before surgery, the estimated GFR was 87.2 ± 12.29 mL/min. At the 6 months follow-up, favorable changes in the GFR (90.74 ± 12.13 mL/min, $p<0.001$) not related BMI status were observed and it was continued until 2 years follow up (91.03± 12.94 mL/min, $p<0.01$).

**Conclusions:** A favorable change of gastrectomy in patients without severe obesity was not related BMI change.

## TH-PO425

**Inflammation and Endothelial Function Biomarkers in Adolescents with Obesity and Type 1 Diabetes Mellitus** Salim Aljabari, Sudha P. Chennasamudram, Ruchi Singh, Christopher Sheehan, Grant C. Weinheimer, Tetyana L. Vasylyeva. *Pediatrics, Texas Tech Univ Health Science Center, Amarillo, TX.*

**Background:** Obesity and diabetes have a common pathway in the pathogenesis of atherosclerosis and microvascular damage as both conditions are characterized by chronic low grade inflammatory state which leads to endothelial dysfunction(ED). The aim of the study was to evaluate urinary biomarkers of inflammation and ED in adolescents with obesity(OA) and diabetes(DA). Our ultimate goal is to provide a basis for the development of non-invasive biomarkers that improve diagnostic tests for cardio-vascular health monitoring.

**Methods:** Research subjects were recruited from TTUHSC clinic. An EndoPat 2000 device was used to measure the reactive hyperemia index (RHI). Urine samples were tested for interleukin 6 (IL-6), endothelin 1(ET-1), alpha-1-acid glycoprotein(AGP), and tumor necrosis factor (TNF-α). Statistical significance was assessed using analysis of variance followed by t-tests. Pearson's correlation was calculated between various study parameters. All the markers were normalized with urinary creatinine.

**Results:** 87 adolescents with an average age of 14 years were enrolled. 21 teenagers had diabetes, 30 were obese, 13 were overweight (OW), and 20 had normal weight (NA) (control group). The urinary concentration of TNF-α was significantly higher in the OA (3.01±0.41), OW (2.15±0.35) and DA (2.11±0.22) compared to the NA (1.37±0.08) ($P <0.01$). DA had significantly higher ET-1 (0.68±0.35) vs. NA (0.37±0.20) ($P= 0.03$), and OA and OW showed a higher trend of ET-1 compared to NA (0.63±0.75 $P = 0.13$, and 0.55±0.43; $P=0.11$). There were no significant differences of RHI among different groups.

Levels of IL-6 and AGP were lower in the urine of DA (0.56±0.07 units; P<0.01; 57.3 ±5.8 units; P<0.01) compared to NA (1.28±0.21, 141.7±11.9 Units) and highly correlated (P<0.001) with each other.

**Conclusions:** Urinary levels of TNF-α, IL-6, ET-1, and AGP are significantly different in adolescents with obesity and diabetes compared to their healthy peers. TNF-α seems to be the most sensitive factor. Noninvasive urinary biomarkers are useful in evaluating the degree of inflammation and endothelial function.

## TH-PO426

**Elevations in Fibroblast Growth Factor-23 in Obese, Normotensive Adolescents Are Associated with Adverse Cardiac Structure** Farah N. Ali,[1] Bonita E. Falkner,[2] Heather E. Price,[1] Craig B. Langman.[1] *[1]Pediatrics, Div of Kidney Diseases, Northwestern Univ, Chicago, IL; [2]Div of Nephrology, Thomas Jefferson Univ, Philadelphia, PA.*

**Background:** Fibroblast Growth Factor-23 (FGF23) is a new biomarker for cardiovascular disease in populations with and without chronic kidney disease. Animal models suggest that adipocyte-derived leptin may modulate FGF23 production. Our objectives were: to determine whether obese African American (AA) adolescents without CKD have elevated FGF23 levels compared to non-obese AA adolescents; to determine if FGF23 is related to markers of insulin resistance; to determine if FGF23 is associated with cardiac structure.

**Methods:** Cross-sectional data were obtained from a cohort of 146 normotensive, AAs aged 13-18 yrs with normal kidney function and absent proteinuria; 87 were obese and 59 were non-obese. Plasma C-terminal FGF23, fasting glucose, insulin, adiponectin, and hsCRP were measured; M-mode echocardiography was performed.

**Results:** FGF23 levels were higher in obese versus non-obese subjects (43 vs 22 RU/mL, p<0.001). FGF23 directly correlated with multiple measures of adiposity, including BMI (p<0.001), BMI z-score (p<0.001), and waist circumference (p=0.001). FGF23 directly correlated with fasting insulin (p<0.0005) and HOMA scores (p=0.001). FGF23 directly correlated with hsCRP (p=0.044) and inversely correlated with adiponectin (p=0.040). Median LVM and LVMI increased with increasing quartiles of FGF23, including models adjusted for age, sex, and hsCRP. Left atrial dimension indexed to height increased with increasing FGF23 quartiles. In the obese, FGF23 values above the group median value of 40 RU/mL were associated with larger diastolic time interval ratio. Subjects with eccentric and concentric left ventricular hypertrophy (LVH) had higher FGF23 values than those without LVH (p=0.003).

**Conclusions:** In this homogeneous cohort only differing by BMI, FGF23 was associated with adverse cardiac structure. We postulate that FGF23, a novel CV risk factor in CKD, may be operative in otherwise healthy obese AA adolescents and that the adipocyte may be a critical determinant for the cardiovascular risk in obesity, mediated by FGF23.

*Funding:* NIDDK Support, Other NIH Support - 1RO1HL092030, Private Foundation Support

## TH-PO427

**A Study of Pattern of Emphysematous and Non Emphysematous Pyelonephritis amongst Hospitalized Type 2 Diabetes Mellitus Patients** Krishan L. Gupta, Raja Ramachandran, Harbir Singh Kohli, Vivekanand Jha, Vinay Sakhuja. *Nephrology, PGIMER, Chandigarh, India.*

**Background:** Diabetes Mellitus is a common cause of pyelonephritis both emphysematous (EPN) and, non-emphysematous pyelonephritis (NEPN) associated with poor outcome. This study aimed at finding the incidence and outcome of these complications in diabetic patients in a tertiary care centre.

**Methods:** A total of 105 diabetic patients with pyelonephritis were admitted from July 2009 to June 201and diagnosis of EPN was based on Huang classification. Patients were treated with appropriate antibiotics and percutaneous drainage (PCD) as and when indicated. Nephrectomy was carried out in patients of EPN who were refractory to conservative measures for 2 weeks. Outcome was termed as poor if there was a need of nephrectomy or the patient died.

**Results:** NEPN and EPN were seen in 79 (75.2) and 26 (24.7) patients respectively. E.coli was the commonest organism. Stone disease (n=13) and poor glycemic control (n=76) were important predisposing factors. Pyelonephritis was associated with renal abscess and papillary necrosis in 13 (12.4) and 4 (3.8) patients. Worsening of renal functions were seen in 92 and 93% of patients with EPN and NEPN respectively. Class 1 EPN was seen in 2(7.7%), class II in 8(30.7%), IIIa in 7(27%), IIIb in 5(19.3) and IV in 4(15.4%) patients. Antibiotics alone were sufficient in 38.5% of EPN vs 62% in NEPN, additional PCD was required in 42.3% in EPN & 21.4% in NEPN. Nephrectomy was required in 5(19.2%) EPN patients with class IIIB or IV. A total of 13 patients (12.4%) expired, 4 (15.4%) in EPN and 9 (11.4%) in NEPN group. Patients with EPN had higher incidence of shock (6 vs. 0; p<0.05) & poorly controlled blood sugar (26 vs. 50; p<0.05). Presence of shock and altered sensorium were associated with poorer outcome in patients with EPN.

**Conclusions:** There is no difference in treatment outcome in both EPN and NEPN group other than a greater need for nephrectomy in EPN patients. Presence of shock and altered sensorium at presentation were poor prognostic factors in EPN.

## TH-PO428

**Urinary Free Light Chain Excretion in Obesity and Diabetes** Tina Kaur Thethi,[1,2] Bonnie Katalenich,[1] Shuqian Liu,[3] Radha Pasala,[1,2] Vivian A. Fonseca,[1,2] Vecihi Batuman.[1,2] *[1]Dept of Medicine, Tulane Univ Health Sciences Center, New Orleans, LA; [2]Dept of Medicine, Southeast Louisiana Veterans Health Care System, New Orleans, LA; [3]Global Health Systems and Development, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.*

**Background:** Obesity is a risk factor for type 2 diabetes (DM). DM is a leading cause of chronic kidney disease (CKD). Urinary free light chains (uFLC) excretion is elevated in early diabetic nephropathy before GFR decline. Purpose was to investigate uFLC, Kappa (k) and Lambda (λ) light chains in obese subjects with and without DM.

**Methods:** Cross sectional, subjects aged 18 to 70 with body mass index(BMI) <27 or >30. uFLC was measured on $SPA_{PLUS}$ analyzer (The Binding Site, Inc., San Diego, CA) using Binding Site Freelite® immunoassays.

**Results:** Table 1 shows comparison between obese subjects with DM and hypertension [HTN](DM+/HTN+) and those without DM and HTN(DM-/HTN-). Comparison between obese(n=442) vs non-obese(n=195) group shows significant differences in urinary(U) k(43.6+81.8,29.4+51.4, $p$<0.01) and Uλ (5.2+17.1,3.0+8.9,$p$=0.04). Multiple regression showed significant positive relationship of urinary microalbumin creatinine[Cr] ratio(UMACR) with λ/UCr($p$<0.001) not Uk/UCr after adjusting for DM, obesity, HTN, gender, age, glucose, BMI, diastolic blood pressure, serum(S)Cr, GFR. UMACR is expected to increase by 178 mg/g for each 1 unit increase in λ /UCr.

**Conclusions:** Excretion of k and λ uFLC is significantly increased in obese subjects with DM and HTN compared to those without DM and HTN. Longitudinal study is needed to assess if uFLC excretion predicts CKD onset earlier than UMACR.

| | Obese DM+/HTN+ | Obese DM-/HTN- | *P* Value |
|---|---|---|---|
| BMI | 37.0 ± 7.1 | 32.3 ± 8.7 | <0.001 |
| S. Cr (mg/dL) | 0.9 ± 0.4 | 1.1 ± 0.6 | <0.001 |
| UMACR (mg/g) | 12.2 ± 42.1 | 2.5 ± 10.9 | <0.05 |
| Urine κ (mg/L) | 61.0 ± 115.0 | 34.4 ± 60.3 | <0.05 |
| Urine κ/Cr (mg/L) | 0.4 ± 0.6 | 0.2 ± 0.3 | <0.05 |
| Urine λ (mg/L) | 8.9 ± 2.0 | 3.5 ± 1.3 | <0.05 |
| Urine κ+λ/Cr (mg/L_ | 0.42 ± 0.7 | 0.2 ± 0.3 | <0.05 |
| Glucose (mg/dL) | 131.0 ± 55.3 | 83.5 ± 21.0 | <0.001 |

*Funding:* Private Foundation Support

## TH-PO429

**Elevated Serum Free Light Chains Predict Poor Cardiovascular Outcomes in Type 2 Diabetes** Srikanth Bellary,[1] Jeffrey Faint,[2] Lakhvir Assi,[2] Stephen Harding,[2] Anthony H. Barnett.[1] *[1]Heart of England NHSFT, Birmingham, United Kingdom; [2]The Binding Site Group Ltd., Birmingham, United Kingdom.*

**Background:** Several risk factors including glycated hemoglobin, proteinuria and hypertension are associated with increased risk of cardiovascular disease (CVD) in diabetes; however, their ability to predict the risk of CVD events is limited. Elevated polyclonal combined serum free light chains (cFLC) are associated with adverse outcomes in chronic kidney disease and other inflammatory conditions, reflecting both the degree of renal impairment and the severity of inflammation. Here we investigate the role of cFLC in predicting CVD related events in chronic diabetes.

**Methods:** Serum cFLC and hsCRP were assessed in 352 South Asian type 2 diabetic patients and compared with other standard clinical measurements, treatment and prior history of CVD. Each variable was assessed for utility in predicting CVD related deaths or events, over 2 years, using logistic regression analysis.

**Results:** 29/352 (8%) patients suffered an adverse CVD outcome and cFLC was significantly elevated in these patients, compared to patients with no events (50.7vs 42.8mg/L p=0.004); hsCRP did not show a significant difference (4.5vs3.6mg/L p=0.389). cFLC>57.2mg/L (odds ratio (OR) 3.3,95%CI 1.3-8.2, p=0.012), triglycerides>6.7mmol/L (OR 18.3, 95%CI 5.1-65.5, p<0.001), and SBP>155mmHg (OR 3.6, 95%CI 1.5-8.7, p=0.004) were independently associated with CVD outcomes and remained significantly associated after adjusting for age, ACR, diabetes duration or treatment. ACR and prior history of CVD were not independently associated with outcome. Individually, elevated cFLC, SBP or triglycerides identified 45%, 41% and 21% of patients with adverse CVD outcomes, respectively. A simple risk stratification model including these 3 markers identified patients with 0, 1 (OR 5.7, 95%CI 2.1-15.0 p=0.001) or 2 (OR 15.4, 95%CI 4.8-49.3 p<0.001) risk factors. 79% of patients who suffered an adverse CVD event were abnormal for ≥1 risk factor.

**Conclusions:** Elevated cFLC concentrations independently predicted CVD outcomes in diabetic patients. Further studies are required to assess the utility of cFLC measurements in this setting.

*Funding:* Pharmaceutical Company Support - The Binding Site Group Ltd

## TH-PO430

**Renal Amyloidosis in Patients with Type 2 Diabetes Mellitus** Ramón A. Díez,[1] Gerardo Gamba,[3] Virgilia Soto,[1,2] Juan Soriano,[2] Magdalena Madero.[1] *[1]National Heart Institute Mexico; [2]Hospital General de Mexico; [3]INCMNS.*

**Background:** Type 2 Diabetes Mellitus (T2DM) is the leading cause of chronic kidney disease (CKD) and a major cause of cardiovascular disease (CVD) mortality. Inflammation is closely involved in the pathogenesis of T2DM and in the development of

chronic vascular complications. Reactive amyloidosis (AAA) is a condition that occurs in the presence of chronic inflammation. We hypothesized that patients with T2DM may have a higher prevalence of Renal Amyloidosis (RAAA) and that this could contribute to worse kidney and vascular disease.

**Methods:** This was an observational cross-sectional study that included autopsy kidneys from patients with previous diagnosis of T2DM. Vascular tissue damage (chronic ischemic cardiomyopathy, myocardial infarction, aortic, coronary and intrarenal atherosclerosis) and RAAA were the variable outcomes of interest. The kidney tissue was evaluated by two different pathologists in order to determine the presence of diabetic nephropathy, RAAA (defined as the positivity of immunohystochemistry through indirect immunoperoxidase with the primary AA antibody) and the severity of vascular tissue damage.

**Results:** 330 autopsy cases of T2DM were included in the study. The mean age of the population was 61± 13 years and 47% were female. Eighty percent of the population had diabetic nephropathy. RAAA was detected in 9% of our population and was associated with an increased risk [OR (95% CI)], [11 (2.04 to 59.16)] for nodular sclerosis, [4.59 (2.02 to 10.42)] for chronic ischemic cardiomyopathy, [3.41 (1.52 to 7.64)] for myocardial infarction and [4.75 (1.09 to 20.69)], [3.22 (1.47 to 7.04)] and [3.84 (1.46 to 10.09)] for aortic, coronary and intrarenal atherosclerosis, respectively. The presence of RAAA was confirmed by electronic microscopy in some randomly selected cases.

**Conclusions:** RAAA is prevalent amongst T2DM and is associated with worse cardiovascular, aortic and renal disease. RAAA is a marker of severe chronic inflammation and this likely explains our results. Studies replicating our findings in other populations are warranted in order to confirm these associations.

*Funding:* Government Support - Non-U.S.

## TH-PO431

**Pulse Pressure Is Not a Predictor of Outcome in Type 2 Diabetes Patients with Chronic Kidney Disease and Anemia–The TREAT TRIAL**

Simone Theilade,[1] Brian Claggett,[2] Jerome A. Rossert,[3] Tine Hansen,[1] Hicham Skali,[2] Eldrin F. Lewis,[2] Scott D. Solomon,[2] Hans-Henrik Parving,[4,5] Marc A. Pfeffer,[2] John J. Mcmurray,[6] Peter Rossing.[1,4] *[1]Steno Diabetes Center, Denmark; [2]Harvard Medical School, Brigham and Women's Hospital; [3]Amgen; [4]Univ of Copenhagen, Denmark; [5]Rigshospitalet, Denmark; [6]Univ of Glasgow, United Kingdom.*

**Background:** After more than 2 decades of research pulse pressure (PP) remains an elusive cardiovascular (CV) risk factor with findings being inconsistent. We clarified the prognostic value in patients with the triad of type 2 diabetes, chronic kidney disease (CKD), and anemia in the Trial to Reduce cardiovascular Events with Aranesp (darbepoetin-alfa) Therapy (TREAT).

**Methods:** In TREAT, 4038 patients were randomized to darbepoetin alfa or placebo. Using Cox proportional hazards models we calculated multivariable-adjusted (including age, gender, kidney function, CV disease, $HbA_{1c}$, hemoglobin and other conventional risk factors) hazard ratios (HRs) to assess risk related to PP at randomization.

**Results:** Median (IQR) age was 68(60-75) years, 57.3 % women, diabetes duration was 15.4(8.3-21.7) years, eGFR was 34(26-43) ml/min/1.73m$^2$. SBP, DBP and PP was 136(122-148), 71(64-80) and 60(50-74) mmHg.

Over 29.1 months (median), the number of patients experiencing the composite of CV death, nonfatal myocardial infarction (MI), stroke, hospitalization for myocardial ischemia, or heart failure, that is, the primary end point excluding non-CV deaths was: 1010, MI:253, stroke 154 or end stage renal disease (ESRD) 668.

Unadjusted analyses showed that higher quartiles of PP was associated with increased event rates per 100 years of follow up ($p\leq0.04$), except for stroke ($p=0.52$). However, adjusted HRs (95% confidence interval) per 10mmHg increase in PP were 1.00(0.96-1.04) for fatal and nonfatal combined CV events, 1.06(0.98-1.14) for MI, 0.96(0.87-1.06) for stroke, and 1.02(0.98-1.07) for ESRD.

**Conclusions:** In patients with type 2 diabetes, CKD, and anemia, PP did not predict CV events or ESRD. This may reflect the relatively narrow range of PP, partly because of extensive antihypertensive treatment in this specific population.

## TH-PO432

**Central Hemodynamics Is Associated with Diabetic Complications in Type 1 Diabetes**

Simone Theilade,[1] Tine Hansen,[1] Peter Rossing.[1,2,3] *[1]Steno Diabetes Center, Denmark; [2]Aarhus Univ, Denmark; [3]Univ of Copenhagen, Denmark.*

**Background:** To investigate the associations between central hemodynamics and diabetic complications in type 1 diabetes.

**Methods:** Cross-sectional study, 676 type 1 diabetes patients, mean±SD age 55±13, 375(56%) male. Arterial stiffness was measured by pulse wave analyses (PWA), as central aortic systolic pressure (CASP), central diastolic pressure (CADP), central pulse pressure (CPP) and subendocardial viability ratio (SEVR) (an index of myocardial oxygen supply and demand). All measurements were obtained with the SphygmoCor device (Atcor Medical, Australia), and standardized values of hemodynamic measures used in adjusted analyses. Complications were presence of albuminuria ( ≥30mg/24-hour), cardiovascular disease (CVD), retinopathy or autonomic dysfunction (heart rate variability <11 beats/minute).

**Results:** PWAs were available in 636 patients. Mean±SD CASP: 118±17 mmHg, CADP: 75±10 mmHg, CPP: 43±14mmHg, and SEVR: 150±32. Albuminuria, CVD, retinopathy and autonomic dysfunction was present in 52, 21, 78 and 59% of patients. CVD and autonomic dysfunction were associated with: CASP (per 1 SD increase): odds ratios (OR)=3.7(2.0-6.6) and 4.7(2.6-8.4); CADP: OR=0.3(0.2-0.6) and 0.3(0.2-0.6); CPP: OR=2.0(1.5-2.8) and 2.2(1.6-3.0); and SEVR: OR=0.6(0.4-0.9) and 0.4(0.3-0.6) (adjusted for gender, diabetes duration, mean arterial pressure, heart rate, height, urinary albumin excretion rate (UAER), eGFR, $HbA_{1c}$, cholesterol, antihypertensive medication and smoking). None of the hemodynamic variables were associated with albuminuria or retinopathy ($p\geq0.14$). However, when UAER was analysed as a continuous variable in adjusted regressions, all hemodynamic variables were significantly assocatiated with the level of UAER ($p\leq0.001$).

**Conclusions:** In patients with type 1 diabetes, measures of central hemodynamics are independently associated with CVD, autonomic dysfunction and level of UAER, but not with albuminuria or retinopathy. Future studies are needed to determine if targeting central hemodynamics improve outcome.

## TH-PO433

**Long-Term Intraindividual Variability in Albumin Excretion Rate with Type II Diabetes**

Amanda Leong,[1] Elif I. Ekinci,[2,4,5] George Jerums,[2] Richard J. MacIsaac.[3] *[1]The Univ of Melbourne, Australia; [2]Dept of Endocrinology, Austin Health, Australia; [3]Dept of Endocrinology & Diabetes, St. Vincent's Health, Australia; [4]Senior Research Officer Menzies School of Health Research; [5]Senior Research Officer The Univ of Melbourne.*

**Background:** There is paucity of data on the long-term variability of Albumin Excretion Rate (AER) in patients with type II diabetes. This study aimed to determine the variability of AER and factors that influence it, in type II diabetic patients.

**Methods:** Consecutive AER measurements from 1999-2012 of 497 type II diabetic patients were recorded in a cohort study. Coefficient of variation (CV) was used as a measure of intraindividual AER variability. First three AER measurements were used to classify individuals into normo-, micro- and macroalbuminuria groups at baseline. Linear regression examined the effects of baseline demographic variables on the AER CV: AER group, HbA1c, age, gender, duration of diabetes, total cholesterol, HDL, systolic BP, BMI, ACEi/ARB use at baseline and smoking. Logarithmic transformation was applied to CV prior to analyses.

**Results:** The intraindividual variability in AER was higher in those treated with ACEi/ARB use in 2000 (n=312) than those untreated (n=185) patients (p=0.003). There was no evidence of a relationship between the AER CV and baseline characteristics. Linear regression of log CV in treated and untreated groups and AER groups was used. After adjustment for AER groups, the difference in mean CV between the treated and untreated groups remained significant (p=0.013). After adjustment for ACEi/ARB use, the mean CV in the microalbuminuria group was 1.27 times that of the normoalbuminuria group, 1.15 times greater in macroalbuminuria compared to the normoalbuminuria, with no difference for micro- versus macroalbuminuria groups. Overall, p<0.001.

**Conclusions:** Patients with type II diabetes and micro- or macroalbuminuria have a greater AER CV than those with normoalbuminuria at baseline. There was a greater intraindividual CV in those treated with ACEi/ARB indicating greater changes in AER over time in these patients. These results emphasize the need for serial AER measurements in assessing AER trajectory.

## TH-PO434

**The Effect of Insulin Sensitivity on the Magnitude of Albuminuria**

Amy K. Mottl, Jasmin Divers, Dana Dabelea, David M. Maahs, Santica M. Marcovina, David J. Pettitt, Lawrence M. Dolan, Michael Mauer, Elizabeth Mayer-Davis. *The SEARCH for Diabetes in Youth Study Group.*

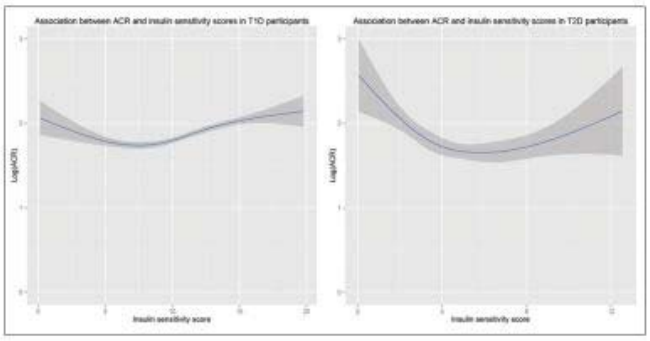
**Background:** We sought to identify a threshold of insulin sensitivity (IS) below which urine albumin excretion increases, and whether this threshold differs according to diabetes type.

**Methods:** SEARCH is an observational study of children with newly diagnosed DM. IS was estimated using a validated equation. Urinary albumin to creatinine ratio (UACR) was calculated from a random urine specimen. Within each diabetes type, segmented regressions were fitted to identify the threshold at which the slope of the function relating insulin sensitivity to UACR changes. After estimating the threshold, multivariate regression analyses were performed using the generalized linear model, stratifying according to the estimated IS threshold.

**Results:** Baseline characteristics stratified by diabetes type are shown below.

| Characteristics | Type 1 n=2551 mean (SD) | Type 2 n=399 mean (SD) |
|---|---|---|
| Age, yrs | 11 (4) | 15 (3) |
| Male, n(%) | 1352 (53) | 160 (40) |
| Non-Hispanic White, n(%) | 76 (4) | 24 (42) |
| IS score | 10.5 (3.3) | 4.4 (2.2) |
| Microalbuminuria, n(%) | 10 (30) | 17 (38) |

The IS threshold below which UACR was statistically associated with IS was 4.7 for type 2 and 6.7 for type 1 diabetic participants (Figure 1). For both DM types, analyses stratified by the IS threshold showed an association with UACR only in the stratum below the threshold. The beta coefficient in the model adjusted for age, gender and race/ethnicity was -0.17 ($p=4.6\times10^{-6}$) in type 1 participants and -0.27 ($p=2.1\times10^{-4}$) for type 2 participants.




**Conclusions:** In youth with diabetes, there is a threshold of IS, below which UACR is negatively associated with IS. In children with type 2 versus type 1 DM, the effect is stronger, but the threshold is lower.

*Funding:* NIDDK Support, Other NIH Support - CDC

## TH-PO435

**Pathologic Classification of Diabetic Nephropathy in Predicting Renal Death**

Amy K. Mottl, A. Gasim, Fernanda Payan Schober, Yichun Hu, Caroline J. Poulton, Susan L. Hogan, J. Charles Jennette. *Univ of North Carolina Kidney Center.*

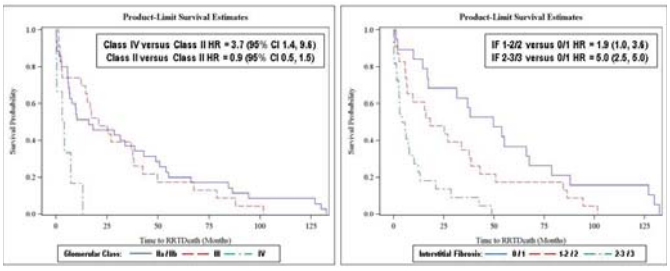
**Background:** A novel nephropathological classification scheme for diabetic nephropathy was devised by the Renal Pathology Society (RPS) in 2010. The utility of this system in predicting subsequent end-stage renal disease (ESRD) has not been determined across populations of broad ethnic background.

**Methods:** Specimen from clinical kidney biopsies performed between 1998-2006 and containing diabetic glomerulosclerosis in the final diagnoses, were reanalyzed according to the RPS classification system for diabetic nephropathy. Tubular atrophy was categorized between 0 and 4. Clinical data was extracted from medical records from the time of biopsy until the onset of renal replacement therapy (RRT). Kaplan–Meier estimates calculated the percentage of participants who required RRT during follow-up, stratified by glomerular class or severity of fibrosis. Univariate and multivariate Cox proportional-hazards regression analyses were run, adjusting for ethnicity, baseline creatinine and urine protein:creatinine ratio.

**Results:** Demographic and clinical data from the date of biopsy are displayed in the following table.

| Characteristics | Mean (95% CI))<br>N=70 |
|---|---|
| Age, years | 51 (47,55) |
| Female gender, % | 35 (50) |
| NonHispanic Black, % | 34 (46) |
| HbA1c, % | 8.2 (7.4,9.1) |
| Diabetes duration, yrs | 13.0 (11.1,14.1) |
| Body mass index | 54 (45,62) |
| Serum creatinine, mg/dl | 3.8 (3.2,4.4) |
| Urine protein:creatinine | 6.5 (4.9,8.0) |
| Glomerular class:<br>IIa/IIb<br>III<br>IV | <br>38 (54%)<br>25 (36%)<br>7 (10%) |
| Interstitial fibrosis:<br>0/1<br>1-2/2<br>2-3/3 | <br>19 (27%)<br>25 (36%)<br>26 (37%) |

Survival analysis according to glomerular class and interstitial fibrosis and unadjusted hazard ratios are displayed in the following figures. Multivariate adjustment did not significantly change the results.




**Conclusions:** The severity of glomerular sclerosis and interstitial fibrosis are highly predictive of impending need for RRT.

## TH-PO436

**A New Cutoff for Abnormal Proteinuria in Diabetes Mellitus Patients: Relationship to Albuminuria**

Arie Erman,[1] Orit Erman,[1] Alina Vodonos,[3] Uzi Gafter,[1,2] David Jonathan Van Dijk.[1,2] *[1]Nephrology and Hypertension, Rabin Medical Center, Petah Tikva, Israel; [2]Sackler School of Medicine, Tel Aviv Univ, Tel Aviv, Israel; [3]Public Health, Faculty of Health Sciences, Ben-Gurion Univ of the Negev, Beer Sheva, Israel.*

**Background:** New and more sensitive methods have been developed to detect low levels of proteinuria, which could not be detected in the past. The main goal of this study was to evaluate the association between proteinuria below 300mg/24 hours and albuminuria, as well as a possible association with renal function in patients with diabetes mellitus (DM).

**Methods:** Medical files of patients with type 1 and type 2 DM who presented with proteinuria below 300 mg/24h at three different visits to the Diabetic Nephropathy Clinic, spaced with a minimal three months interval, were screened. The 245 patient files that were screened, included 723 visits. The data collected included demographics; protein, albumin and creatinine levels in urine collections; blood biochemistry; clinical and treatment data. The data were analyzed using SPSS 18.

**Results:** A moderate association was found between proteinuria in the range below 300mg/24hr and albuminuria. The strongest association was found in type 2 diabetic patients (r=0.624, P<0.001). Proteinuria cutoff, based on albuminuria cutoff of 30mg/24hr, was found to be 160.5mg/24hr. BMI was the sole independent predictor of proteinuria above 160.5mg/24hr. The other variables examined such as creatinine clearance, age, hypertension, duration of diabetes, HbA1c, ACE inhibitors and angiotensin II blockers, were not predictive. Changes in proteinuria, but not albuminuria, were not associated with changes in creatinine clearance.

**Conclusions:** A new cutoff value of 160.5mg/hr was set empirically, for the first time, for abnormal proteinuria in diabetic patients. As there was no association between proteinuria and creatinine clearance, it appears that proteinuria below 300mg/24hours is not sufficient as a sole prognostic factor for kidney injury.

## TH-PO437

**Non-Invasive Assessment of Electrochemical Skin Conductance Associates with Kidney Function in African Americans (AA) with Type 2 Diabetes (T2D)**

Barry I. Freedman, Jasmin Divers, Susan Carrie Smith, Jianzhao Xu, Donald W. Bowden. *Wake Forest Univ Health Sciences.*

**Background:** SUDOSCAN® (Impeto Medical, Paris France) is a patented non-invasive measure of sweat gland dysfunction employing electrochemical skin conductance (reverse iontophoresis and chronoamperometry) and is useful for assessing peripheral small fiber and autonomic nerve function.

**Methods:** We tested whether SUDOSCAN® measures differed between AA and European Americans (EA) and for associations with parameters of diabetic kidney disease (estimated glomerular filtration rate [eGFR] and urine albumin:creatinine ratio [UACR]) within each population. A cross-sectional study of renal parameters and SUDOSCAN® skin conductance was performed in 390 cases with T2D (206 AA; 184 EA) and 168 non-diabetic controls (93 AA; 73 EA). Linear models were fitted stratified by population ancestry and T2D status, adjusted for co-variates.

**Results:** Relative to EA, AA had markedly lower mean (hand + foot) skin conductance (AA T2D vs. EA T2D p<0.0001; AA controls vs. EA controls p<0.0001). As expected, mean (hand + foot) skin conductance was also lower in T2D cases vs. non-diabetic controls within each population ancestry (p<0.0001 for both AA and EA). Adjusting for age, gender, body mass index, and HbA1c, significant positive associations were detected between mean (hand + foot) skin conductance and eGFR in AA cases with T2D (parameter estimate 15.4, standard error 5.7; p=0.0073); but not in EA with T2D (p=0.44). Significant associations between UACR and mean skin conductance were not observed in AA or EA with T2D.

**Conclusions:** Non-invasive measures of skin conductance previously associated with diabetes mellitus and neuropathy, strongly associate with eGFR in AA. This replicates results in a Hong King Chinese population. In contrast, skin conductance was not associated with measures of kidney disease in EAs. SUDOSCAN® may prove useful as a low cost and non-invasive screening tool in African ancestry populations with T2D allowing for the detection of undiagnosed kidney disease. Differences in skin conductance between EA and AA with T2D and non-diabetic controls is an interesting finding that warrants further study.

*Funding:* Pharmaceutical Company Support - Impeto Medical, Paris France

## TH-PO438

**Vitamin D Levels and Renal Progression in Type 2 Diabetic Patients with Blockade of the Renin Angiotensin System**

Jose Luno,[1] Fernández Gema Maria,[2] Soledad Garcia de Vinuesa,[1] Marian Goicoechea,[1] Jesus Oliva Dominguez,[3] Luisa Casas Losada,[2] Vicente Lahera.[4] *[1]Hospital General Universitario Gregorio Marañon, Madrid, Spain; [2]Hospital Fundación Alcorcon, Madrid, Spain; [3]IS Carlos III, Madrid, Spain; [4]Universidad Complutense, Madrid, Spain; [5]On behalf of the PRONEDI Study Group.*

**Background:** Experimental studies show that vitamin D is a suppressor of renin biosynthesis and that vitamin D deficiency has been associated with the progression of chronic kidney disease (CKD). Patients with type 2 diabetes and CKD have an exceptionally high rate of severe vitamin D deficiency, but it is not known whether this deficiency is a risk factor for progression of diabetic nephropathy (DN).

**Methods:** We measured 25 (OH) vitamin D levels in the patients included in a multicenter randomized controlled trial to compare the efficacy of combining the

angiotensin-converting enzyme inhibitor lisinopril and the angiotensin receptor blocker irbesartan with the efficacy of each drug in monotherapy to slow progression of established type 2 DN. The primary composite outcome was a >50% increase in baseline serum creatinine or end-stage renal disease or death. Samples from 103 patients were obtained to determine levels of 25 (OH) vitamin D at baseline and at 4 and 12 months.

**Results:** Fifty-three (51.5%) patients had 25 (OH) vitamin D deficiency (<15 ng/mL). After a median follow-up of 32 (18-48) months, the endpoint was reached by 23 patients with deficiency (43.4%) and 8 patients without (16%). Cox regression analysis showed that 25 (OH) vitamin D deficiency was associated with primary outcome (HR, 2.88; 95% CI, 1.84 -7.67; p=0.04) and renal outcome (HR, 3.79; 95% CI, 1.20-12.02; p=0.02). The effect of 25 (OH) vitamin D did not change with age, gender, weight, treatment with RAS blockers (monotherapy or combined treatment) or levels of aldosterone, PTH, albumin, and phosphorus.

**Conclusions:** Our results show that 25 (OH) vitamin D deficiency is an independent risk factor for renal progression of type 2 DN.

*Funding:* Pharmaceutical Company Support - Bristol-Myers Squibb of Spain; Fondo de Investigaciones Sanitarias (Spanish Ministry of Science and Innovation); Spanish Society of Nephrology, Private Foundation Support, Government Support - Non-U.S.

## TH-PO439

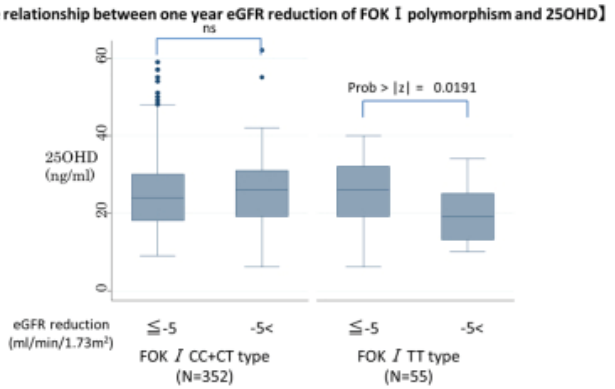
**Interaction between Vitamin D Receptor Polymorphisms and 25OHD Levels on Reduction of eGFR in Type 2 Diabetes Mellitus** Akio Nakashima,[1] Keitaro Yokoyama,[1] Ichiro Ohkido,[1] Mitsuyoshi Urashima,[2] Takashi Yokoo.[1] *[1]Div of Kidney and Hypertension, Dept of Internal Medicine, Jikei Univ School of Medicine, Tokyo, Japan; [2]Div of Molecular Epidemiology, Jikei Univ School of Medicine, Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** FokI polymorphisms of vitamin D receptor (VDR) gene were shown to be different in patients with type 2 diabetes mellitus from controls. Furthermore, deficiency of 25-hydroxyvitaminD (25OHD) was reported to decrease insulin production, increase insulin resistance and diabetic nephropathy. However there are few reports to research interactions between 25OHD and VDR polymorphisms in diabetic patients.Therefore, we aimed to investigate the relationship between one year-reduction of eGFR and serum 25OHD levels stratified by VDR FokI polymorphisms in patients with diabetes.

**Methods:** The prospective cohort study was carried out at Jikei University hospital. A total of 407 type 2 diabetic outpatients agreed to participate in this study. We used polymerase chain reaction and direct sequencing analyze VDR FokI polymorphisms.

**Results:** Median age was 63 years, mean eGFR was 66.3±23.0 ml/min/1.73/m$^2$ and average disease duration of diabetes was 12.4±8.8 years. Genotype frequencies were FokI: CC:180 CT:172 TT:55. Mean reduction of eGFR during one year was -2.2 ml/min/1.73m$^2$. Primary outcome was defined as -5ml/min/1.73m$^2$ reduction in eGFR during one year in this study. Lower 25OHD levels significantly increased the risk of the primary outcome in patients with FokITT polymorphism (P=0.0191), whereas 25OHD levels had no significant associations with the primary outcome in those with either FokICC or CT polymorphisms.

**Conclusions:** These results suggest that eGFR may rapidly decrease in diabetic patients with lower 25OHD levels and FokITT polymorphism.




## TH-PO440

**Effect of Aldosterone Blockade on Galectin 3 in Patients with Diabetic Nephropathy** Morten Lindhardt,[1] Maria Lajer,[1] Hiddo Jan Lambers Heerspink,[2] Peter Rossing,[1,3,4] Rudolf A. de Boer.[2] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Univ Medical Centre, Univ of Groningen, Netherlands; [3]Univ of Aarhus, Denmark; [4]Univ of Copenhagen, Denmark.*

**Background:** Fibrosis and expansion of extra cellular matrix in the kidney is part of the pathogenesis of diabetic nephropathy. Plasma galectin-3 (p-gal3) is linked to fibrogenesis in the heart and kidney, and treatment with spironolactone has beneficial effect in patients with heart failure. We hypothesize, that a potential beneficial effect of spironolactone on fibrosis in diabetic nephropathy is mediated through a reduction in p-gal3.

**Methods:** A post-hoc analysis of three clinical controlled double masked intervention trials all with randomisation to either spironolactone or placebo for 8 weeks in a cross-over design. The first trial consist of 25 patients with 1 DM and macroalbuminuria, the second consist of 23 patients with type 2 DM and macroalbuminuria and the third consist of 21 patients with type 1 DM and microalbminuria. Mean(SD) age of 53 years (10.8) and a mean duration of DM of 28 years (14.6).

**Results:** As previously reported albuminuria was reduced with 30, 33 and 60% in the three trials. P-gal3 was associated with GFR in the placebo period ($R^2$=0.42 p<0.0001). Mean(95% CI) level of plasma p-gal3 after treatment with spironolactone was 16.0(14.7-17.4) and after placebo 15.5(14.3-16.7). In an unadjusted mixed model, the effect of treatment insignificant increased p-gal3 by 1.03 ng/ml (1.02-1.05) (p=0.074). However, when adjusted for after treatment values of mean 24h systolic blood pressure, 24h urine albumin excretion, $^{51}$Cr GFR, $Hba_{1c}$ and cholesterol, the treatment effect on p-gal3 was attenuated (p=0.69). Patients with p-gal3 below the median in the placebo period had a greater reduction in albuminuria 31.3%(25.1-37.6) vs. those above 8.8%(-2.9-20.4), p=0.021.

**Conclusions:** Galectin 3 was associated with GFR. Spironolactone for two months reduced albuminuria but did not change p-gal3 levels. This suggests an initial effect mediated by hemodynamic changes, whereas an effect on fibrosis may require a longer treatment period or p-gal3 is not affected by spironolactone. Low level of p-gal3 was associated with greater reduction in albuminuria.

## TH-PO441

**A Panel of Novel Biomarkers to Predict Renal Function Decline in Type 2 Diabetes** Michelle Pena,[1] Sara S. Roscioni,[1] Stephan J.L. Bakker,[2] Paul Perco,[3] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink.[1] *[1]Dept of Clinical Pharmacology, Univ Medical Center Groningen; [2]Div of Nephrology, Univ Medical Center Groningen; [3]Emergentec Biodevelopment GmbH.*

**Background:** We aimed to explore a panel of novel biomarkers representing different disease pathways for their potential association with accelerated renal function decline in type-2 diabetes.

**Methods:** A systematic data integration approach was used to identify promising biomarkers for this study. The following biomarkers were selected and tested in 82 patients with type-2 diabetes recruited from an outpatient diabetes center in The Netherlands: amino-terminal propeptide of CNP (NTproCNP), tumor necrosis factor receptor-2 (TNFR2), sclerostin, fibroblast growth factor-23 (FGF23), endostatin, alpha-2-glycoprotein-1 zinc-binding (AZGP1), nephrin, neuropilin, podocin, and galectin-3. Follow-up for renal function decline was 3.7 ± 1.1 years. Associations between concentrations of individual biomarkers and annual eGFR decline were assessed by univariable regression and multivariable regression adjusted for conventional risk markers age, gender, baseline eGFR, and baseline UACR.

**Results:** The average rate of renal function decline was -2.0 ± 4.4 mL/min/1.73m$^2$/year. Serum NTproCNP and podocin, and serum and urine endostatin and TNFR2 were significantly associated with annual eGFR decline in univariable analysis. Serum TNFR2 and urine endostatin remained significant in multivariable analysis (Table). Finally, serum TNFR2 and urine endostatin were combined as a panel that significantly improved prediction of annual renal function decline on top of conventional risk markers ($R^2$ increase from 0.41 to 0.49; p<0.001).

| Biomarker | | Univariable | | | Multivariable | |
|---|---|---|---|---|---|---|
| | | β | p-value | | β | p-value |
| Serum | Endostatin | -3.2 | 0.02 | | -2.2 | 0.24 |
| | NTproCNP | -2.1 | 0.01 | | -1.6 | 0.07 |
| | Podocin | -2.1 | <0.01 | | -0.9 | 0.24 |
| | TNFR2 | -4.5 | <0.01 | | -4.1 | 0.02 |
| Urine | Endostatin | -1.3 | <0.01 | | -1.0 | 0.05 |
| | TNFR2 | -1.3 | 0.04 | | -0.6 | 0.30 |

**Conclusions:** A panel of novel biomarkers representing different disease pathways can improve prediction of accelerated renal function decline on top of conventional risk markers in type-2 diabetes. These results need to be confirmed in a large prospective cohort.

## TH-PO442

**Novel Urinary Exosome Isolation as a Platform to Study Diabetic Nephropathy (DN)** Daniel Armando Flores,[1,2] Yu Liu,[2] Belinda Bun Jim,[3] Payal B. Mittal,[2] Weijia Zhang,[2] Ruijie Liu,[2] Sandeep K. Mallipattu,[2] John C. He,[1,2] Rajeev Rohatgi.[1,2] *[1]Medicine, James J. Peters VAMC, Bronx, NY; [2]Medicine, Icahn School of Medicine, New York, NY; [3]Medicine, Jacobi Medical Center, Bronx, NY.*

**Background:** DN remains a major cause of end-stage renal disease (ESRD) worldwide. Discovery of early and reliable biomarkers of disease progression promises to help physicians develop personalized care plans for patients based on their risk of ESRD. Exosomes are plasma membrane bound vesicles secreted by all cells which contain protein, mRNA and miRNA, representative of the cells of their origin. The goal of these studies is to test the hypothesis that urinary exosomal miRNAs, which originate from cells lining the genitourinary tract, are an ideal source of biomarkers for DN.

**Methods:** Urinary exosomes were isolated from patients without diabetes (DM; controls), early DN, and moderate DN. RNA was extracted and microRNAs assayed by qRT-PCR array (Exiqon).

**Results:** 742 miRNAs were assayed in 4 control (no DM, normal kidney function, no proteinuria), 3 early DN (normal kidney function, with microalbuminuria), and 4 later stage DN (serum creatinine>1.3) subjects. Because this novel technique is unvalidated, we tested whether the miRNAs known to play a role in research models of DN (miRNA-192, 21, 29a, 216a, 217, 377, 200b, 93, and 29C) were expressed in urinary exosomes of DN

patients. Of these miRNAs, miR-192, 21, 29a, 200b, 93, and 29C were up-regulated in DN patients compared to controls, recapitulating the prior studies. Finally, of the 742 miRNAs evaluated 89 (12%) were dysregulated in exosomes derived from DN patients compared to controls. Of these 89 dysregulated miRs, miR-200b, miR-99b, and Let-7b were upregulated in DN patients and have been implicated in TGF-β-dependent fibrosis, TGF-β-dependent epithelial–mesenchymal transition, and podocyte-specific laminin downregulation, respectively.

**Conclusions:** Six of nine miRNA dysregulated in models of DN were also dysregulated in urinary exosomes of DN patients, suggesting that miRNAs associated with DN are expressed in exosomes. Evidence from our array also suggests potentially novel miRNAs are associated with DN.

*Funding:* Veterans Affairs Support, Pharmaceutical Company Support - Exiqon Grant Program

## TH-PO443

**Urinary Type IV Collagen: Can It Be Used as an Indicator for the Progression of Diabetic Nephropathy?** Salah S. Naga,[1] Iman Ezzat Elgohary,[2] Doaa I. Hashad,[3] Marwa Abdelahad Elaty.[4] *[1]Internal Medicine, Faculty of Medicine, Alexandria, Egypt; [2]Internal Medicine, Faculty of Medicine, Alexandria, Egypt; [3]Clinical Pathology, Faculty of Medicine, Alexandria, Egypt; [4]Internal Medicine, Faculty of Medicine, Alexandria, Egypt.*

**Background:** Diabetic nephropathy is a complication seen in long standing diabetes mellitus where progressing impairment of kidney function leads to end-stage renal disease. In the advanced diabetic nephropathy, immunoreactive type IV collagen was detected in glomerular basement membrane (GBM), tubular basement membrane and Bowman's capsule much more than that in the normal kidney. However, there is little information on whether the increase in type IV collagen excretion in urine is a predictor of progression of diabetic nephropathy or deterioration of renal function in type 2 diabetic patients. We conducted this work with the aim to determine whether the urinary levels of type IV collagen can predict the progression of diabetic kidney disease in Type 2 DM.

**Methods:** This study included 100 adult patients; who were classified into: 20 diabetic normoalbuminuric patients (group1), 20 diabetic microalbuminuric patients (group2), 20 diabetic macroalbuminuric (group3), 20 diabetic CKD patients with GFR: 60-90ml/min (group4), 20 diabetic CKD patients with GFR: 30-59ml/min (group5), and compared with 20 controls (group 6). All included subjects were subjected to renal function test and estimated creatinine clearance by Modification of Diet in Renal Disease (MDRD) formula. Measurements of urine albumin/creatinine ratio. Fasting blood glucose and hemoglobin $A_{1C}$. The urinary concentrations of type IV collagen were measured using a highly sensitive one-step sandwich enzyme immunoassay kit.

**Results:** Urinary type IV collagen was significantly higher in normoalbuminuric patients than the control group, in microalbuminuric patients than both control and normoalbuminuric, in macroalbuminuric than normo and micro albuminuric and control, in CKD1, CKD2 groups it was significantly higher than all other groups and highest in CKD2.

**Conclusions:** Urinary type IV collagen can be used as an indicator for the progression of kidney disease in diabetic patients.

*Funding:* Private Foundation Support

## TH-PO444

**Global Metabolomic Profile in Type 2 Diabetes and Subsequent Progression to ESRD** Monika A. Niewczas,[1] Tammy L. Sirich,[2] Anna V. Mathew,[3] Jan Skupien,[1] Adam Smiles,[1] Edward D. Karoly,[5] Joseph V. Bonventre,[4] Subramaniam Pennathur,[3] Timothy W. Meyer,[2] James Warram,[1] Andrzej S. Krolewski.[1] *[1]Genetics and Epidemiology, Joslin Diabetes Center, Harvard Medical School; [2]Dept of Medicine, Stanford School of Medicine; [3]Dept of Internal Medicine, Univ of Michigan; [4]Renal Div, Brigham and Women's Hospital, Harvard Medical School; [5]Metabolon Inc.*

**Background:** We studied plasma metabolomic profiles as determinants of progression to ESRD in Type 2 diabetes (T2D).

**Methods:** This case-control study used Joslin Kidney Study participants with T2D with well-preserved renal function at baseline: 40 cases who progressed to ESRD and 40 controls who remained alive without ESRD during a 10 year follow-up. Controls were matched with cases regarding baseline clinical characteristics. Plasma metabolites at baseline were measured by mass spectrometry-based global profiling.

**Results:** Of the named metabolites in the library, 274 were detected in at least two-thirds of the study subjects. Among the 78 uremic solutes recognized by the platform, 12 were elevated in baseline plasma of cases. Other uremic solutes were either not different or not commonly detectable. Essential amino acids and their derivatives were depleted in the cases, whereas related acylcarnitines were increased. All our findings remained highly significant after adjustment for multiple comparisons and controlling for clinical covariates.

| **metabolite** | **univariable** OR (95%CI) | **eGFR adjusted** OR (95%CI) |
|---|---|---|
| p-cresol sulfate | 4.0 (1.7,9.6) | 3.5 (1.4, 8.8) |
| pseudouridine | 21 (6.0, 77) | 44 (9, 219) |
| leucine | 0.3 (0.1, 0.7) | 0.4 (0.2, 0.8) |
| 2-hydroxyisocaproate | 0.2 (0.1, 0.4) | 0.2 (0.1, 0.5) |
| methylglutarylcarnitine | 3.7 (1.6, 8.6) | 3.3 (1.4, 7.8) |

**Conclusions:** Certain metabolomic signatures (increased plasma concentrations of the putative uremic solutes, decreased levels of essential amino acids accompanied by increased levels of acylcarnitines) are associated with subsequent progression to ESRD in T2D and those changes in plasma levels are already present at an early stage of diabetic nephropathy.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO445

**Glycemic Control and the Risk of Mortality among Hemodialysis Patients in Japan** Noriko Kaneyama,[1] Hirotaka Komaba,[1] Hajjime Suzuki,[2] Takatoshi Kakuta,[1] Takao Suga,[3] Masafumi Fukagawa.[1] *[1]Div of Nephrology, Endocrinology and Metabolism, Tokai Univ School of Medicine, Isehara, Japan; [2]Div of Nephrology and Diabetes, Tokai Univ Oiso Hospital, Oiso, Japan; [3]Medical Corporation Showakai, Japan.*

**Background:** Diabetes mellitus is the most common cause of end-stage renal disease in Japan. While tight glycemic control decreases the risk of diabetic complications, it remains to be determined whether better glycemic control reduces the risk of death in diabetic patients undergoing dialysis.

**Methods:** We retrospectively collected data on maintenance hemodialysis patients (n = 2,292) beginning in December 2008. The diagnosis of diabetes was made based on the medical records. We analyzed the association between presence or absence of diabetes, glycemic control, and the risk of death over three years, using Cox proportional hazards models. We also performed time-dependent Cox models to account for changes in glycemic control over time. Data were collected at quarterly intervals.

**Results:** A total of 1,038 patients had diabetes. Overall, diabetes is well controlled and the mean HgbA1c values were <7.0% in 86%. HgbA1c values showed a significant correlation with random glucose values (r = 0.58; P <0.001). Patients with diabetes had a significantly increased risk of death compared to those without (HR, 0.62; 95% CI, 0.49 to 0.77). No significant association was observed between HbA1c values and the risk of death. These results were unaltered in time-dependent Cox models.

**Conclusions:** More studies are needed to define the optimal management of diabetes for patient receiving dialysis.

*Funding:* Government Support - Non-U.S.

## TH-PO446

**Effect of Dapagliflozin on Blood Pressure and Body Weight in Patients with Type 2 Diabetes and Cardiovascular Disease** Tjerk de Bruin,[1] Shamik Parikh,[1] Jennifer Sugg,[1] Shoba Ravichandran.[2] *[1]AstraZeneca, Wilmington, DE; [2]Bristol-Myers Squibb, Princeton, NJ.*

**Background:** Patients with type 2 diabetes (T2D) often have hypertension (HTN) and cardiovascular disease (CVD). Selective SGLT2 inhibitor dapagliflozin (DAPA) reduces hyperglycemia by promoting renal glucose excretion and is associated with osmotic diuresis and caloric loss that provide additional benefits. Our analysis assessed the effect of DAPA vs placebo (PBO) on blood pressure (BP) and body weight (BW) in patients with T2D and established CVD treated for 52 weeks in 2 clinical trials (Study 18, NCT01031680, N=922; Study 19; NCT01042977, N=965).

**Methods:** Patients with glycated hemoglobin ≥7% and ≤10% were randomized to double-blind PBO or DAPA 10 mg/d, added to pre-existing anti-hyperglycemic (including insulin), antihypertensive (ACEI/ARBs, diuretics), antiplatelet, and lipid-lowering drugs. A medical history of HTN was an inclusion criterion for Study 18 and was present in 93% of Study 19 patients. Adjusted mean changes from baseline to week 52 were analyzed by longitudinal repeated measures.

**Results:** Compared with PBO, DAPA reduced systolic BP and BW **(Table)**. Small decreases in diastolic BP were also observed. Hematocrit increased, and urine albumin/creatinine decreased with DAPA. There were small decreases in eGFR across treatment groups. Frequency of volume-related adverse events was low and inconsistent across studies (Study 18, DAPA 2.8% vs PBO 0.4%; Study 19, DAPA 1.5% vs PBO 2.7%). Urinary tract infections in 1 study (Study 18, 5.9% vs 5.8%; Study 19, 11.0% vs 5.8%) and genital infections in both studies (Study 18, 6.1% vs 0.9%; Study 19, 7.5% vs 0.4%) were more common with DAPA vs PBO.

**Conclusions:** In patients with T2D and CVD, DAPA added to usual care reduced BW. BP was also reduced, potentially through a diuretic-like mechanism, without compromising renal function.

| | Adjusted Mean Change (SE) From Baseline to Week 52 | | | | | |
|---|---|---|---|---|---|---|
| | Study 18 | | | Study 19 | | |
| | PBO | DAPA | Difference (95% CI) | PBO | DAPA | Difference (95% CI) |
| Seated Systolic BP, mm Hg | 0.2 (0.6) | −3.4 (0.6) | −3.6 (−5.4, −1.8) | −0.9 (0.6) | −3.6 (0.6) | −2.7 (−4.3, −1.0) |
| Seated Diastolic BP, mm Hg | −0.2 (0.4) | −1.7 (0.4) | −1.5 (−2.7, −0.4) | −0.6 (0.4) | −2.4 (0.4) | −1.7 (−2.8, −0.7) |
| Body weight, kg | −0.2 (0.2) | −2.7 (0.2) | −2.5 (−3.0, −2.0) | −1.0 (0.2) | −2.9 (0.2) | −1.9 (−2.4, −1.4) |
| | Mean Change (SE) From Baseline to Week 52 | | | | | |
| | PBO | | DAPA | PBO | | DAPA |
| Hematocrit, % | −1.0 (0.1) | | 1.7 (0.1) | −1.3 (0.1) | | 1.7 (0.1) |
| eGFR, mL/min/1.73m² | −2.1 (0.6) | | −2.8 (0.6) | −2.2 (0.6) | | −2.3 (0.6) |
| Urine albumin/ creatinine ratio, mg/g | 10.4 (10.2) | | −13.6 (6.7) | 8.6 (7.3) | | −6.2 (6.5) |

*Funding:* Pharmaceutical Company Support - Supported by AstraZeneca and Bristol-Myers Squibb

## TH-PO447

**Effect of Atorvastatin on Renal NO Availability and Tubular Function in Patients with Stage II-III Chronic Kidney Disease and Type 2 Diabetes**

Frank H. Mose,[1,2] Thomas Larsen,[1,2] Janni Majgaard Jensen,[1,2] Jesper N. Bech,[1,2] Erling B. Pedersen.[1,2] *[1]Dept of Medical Research, Holstebro Hsopital, Holstebro, Denmark; [2]Univ of Aarhus, Aarhus, Denmark.*

**Background:** Statins have beneficial effects on cardiovascular morbidity and mortality independently of reduction of plasma cholesterol.

**Methods:** In patient with type 2 diabetes and nephropathy, chronic kidney disease stage II-III, we tested the hypothesis that atorvastatin increased systemic and renal nitric oxide (NO) availability using L-NMMA as an inhibitor of NO production. We performed a randomized, placebo-controlled, crossover study, using atorvastatin/placebo treatment for five days with a standardized diet and fluid intake. We measured brachial BP (bBP), central BP (cBP), GFR, urinary output (OU), free water clearance ($C_{H2O}$), fractional excretion of sodium ($FE_{Na}$), urinary excretion of albumin (UAER and UACR), AQP2 (u-AQP2) and ENaC (u-ENaC$_\gamma$) and plasma concentrations of vasoactive hormones: renin, angiotensin II, aldosterone, arginine vasopressin, endothelin-1 and brain natriuretic peptide.

**Results:** During L-NMMA infusion, atorvastatin and placebo changed the effect variables significantly to the same extent, i. e. an increase in bBP and cBP, and a decrease in GFR, OU, $C_{H2O}$, $FE_{Na}$, u-AQP2 and u-ENaC$_\gamma$. In addition, renin and angiotensin II was reduced, aldosterone increased, and vasopressin, endothelin-1 and brain natriuretic hormone unchanged.

**Conclusions:** During inhibition of nitric oxide synthesis, atorvastatin and placebo induced the same response in brachial and central blood pressure, GFR, renal tubular function and vasoactive hormones. Thus, atorvastatin did not change nitric oxide availability in type 2 diabetic nephropathy.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO448

**Proteinuria Reduction with Probucol and Telmisartan in Patients with Type 2 Diabetic Nephropathy**

Xiang-Mei Chen, Hanyu Zhu, Guangyan Cai, Ying Zheng. *Dept of Nephrology, State Key Laboratory of Kidney Disease, General Hospital of Chinese People's Liberation Army, Beijing, China.*

**Background:** Combination therapy with telmisartan and probucol in the treatment of diabetic nephropathy has not been proved with sufficient clinical evidence. This study examined the clinical efficacy of telmisartan and probucol in proteinuria reduction of type 2 diabetic nephropathy.

**Methods:** A total of 160 type 2 diabetic nephropathy patients with proteinuria (0.5-3.0 g/24h) were enrolled in a randomized, double-blind, placebo-controlled, multicenter study. The patients were randomly divided into two groups. Telmisartan group (n=80) was administered with telmisartan (80 mg/day) and probucol placebo (1000 mg/day for 24 weeks, and then reduced to 500 mg/day for next 24 weeks). Probucol group (n=80) was administered with telmisartan (80mg/day) and probucol (1000 mg/day for 24 weeks, and then reduced to 500 mg/day for next 24 weeks). All patients were followed throughout the 48-week period, and the percentage change of urinary protein from baseline to 48 weeks was assessed.

**Results:** The baseline characteristics of the two groups were similar, as well as the BP and HbA1c profile over the study period. There was a significant reduction (27.02%) in urinary protein level in the probucol group from baseline to 48 weeks. However, urinary protein level was increased in the telmisartan group (1.45%). For patient with lower urinary protein (<1.0g/24h at baseline), the reduction in urinary protein level from baseline is significantly greater in probucol group than in telmisartan group (36.54% vs. 8.55%, P=0.013). For the patient with higher urinary protein ( ≥ 1.0g/24h at baseline), there was a significantly reduction of 15.38% in urinary protein level in probucol group from baseline. However, urinary protein was increased in telmisartan group, with an average increase of 10.96% ( P=0.011 for the comparison with probucol group).

**Conclusions:** The combination therapy of probucol and telmisartan lowers urinary protein more effectively than telmisartan alone.

*Funding:* Government Support - Non-U.S.

## TH-PO449

**In Type 1 Diabetic Patients with Albuminuria Insulin Pump Treatment Is Associated with Reduced Arterial Stiffness**

Signe Rosenlund,[1] Simone Theilade,[1] Tine Hansen,[1] Peter Rossing.[1,2,3] *[1]Steno Diabetes Center, Denmark; [2]Univ of Copenhagen, Denmark; [3]Aarhus Univ.*

**Background:** Insulin pump treatment is often associated with reduced glucose variability and in improvements in glycemic control, which could reduce development of vascular complications. We investigated the relationship between arterial stiffness, evaluated by pulse wave velocity (PWV), and treatment with insulin pump in patients with type 1 diabetes, and examined if this association was dependent of glucose control.

**Methods:** Cross-sectional study, from 2009-2011, including 639 Caucasian patients with type 1 diabetes. PWV measurements (SphygmoCor, AtCorMedical, Australia) were available in 59 patients with insulin pump (35 with albuminuria ≥ 30mg/d) and 580 (296 with albuminuria ≥30mg/d) treated with multiple daily insulin injections ((MDI) ≥2 injections). ANCOVA compared groups and adjusted multiple linear regression analyses investigated the association between insulin pump treatment and arterial stiffness.

**Results:** In the albuminuric group insulin pump *vs.* MDI treated patients were 46% *vs.* 60% men, 50±12 *vs.* 56±11 years of age, 36±10 *vs.* 36±13 years diabetes duration and $HbA_{1c}$ 65±11 *vs.* 67±14mmol/mol ($p$>0.45 for all except age ($p$=0.01)). PWV was lower in patients with insulin pump (9.3±2.5 *vs.* 11.3±3.4m/s; $p$<0.001). This difference remained significant ($p$=0.002) after adjustment for gender, diabetes duration, eGFR, albuminuria, $HbA_{1c}$, total-cholesterol, smoking, office MAP, heart rate and BMI. For all patients, PWV was also lower in the insulin pump group (9.3±2.8 *vs.* 10.4±3.4 m/s, (adj. $p$=0.002)). In multiple regression analysis, pump treatment was significantly associated with lower PWV, while $HbA_{1c}$ was not associated with PWV in neither patients treated with MDI or insulin pump.

**Conclusions:** Insulin pump treatment was independently associated with reduced arterial stiffness, while $HbA_{1c}$ was not. Although glucose variability was not assessed, our results suggest that glucose variability and not $HbA_{1c}$-level may modify arterial stiffness. This needs confirmation in randomised prospective studies.

## TH-PO450

**Risk Stratification with P-NT-proBNP and Coronary Calcium Score Predicts All-Cause Mortality in Microalbuminuric Type 2 Diabetic Patients**

Bernt Johan Illum von Scholten,[1] Henrik Reinhard,[1] Peter Godsk Jørgensen,[2] Simone Theilade,[1] Peter R. Hansen,[2] Niels Wiinberg,[3] Andreas Kjaer,[4] Hans-Henrik Parving,[4] Jan Skov Jensen,[2] Peter Karl Jacobsen,[4] Peter Rossing.[1] *[1]Steno Diabetes Center, Denmark; [2]Gentofte Hospital; [3]Frederiksberg Hospital; [4]Rigshospitalet.*

**Background:** The burden of CAD is significantly increased in type 2 diabetes and associated with mortality. An effective screening tool for subclinical CAD is needed to predict and prevent cardiovascular mortality in these patients.

**Methods:** NT-proBNP, coronary calcium score (CCS) and echocardiography (echo) were performed in 200 asymptomatic type 2 diabetic patients with elevated UAER (>30mg/24h) and without prior history of CAD. NT-proBNP >45.2 ng/L and/or CCS ≥400 stratified patients as high risk for CAD and were further examined for significant CAD by MPI and/or CT-angiography and/or CAG. Following imaging, patients were stratified into low risk (n=67), high risk without CAD (n=63) and high risk with CAD (n=70). After 5.3 years of follow-up, vital status was assessed, and echo re-performed in available patients (n=130).

**Results:** At baseline, patients were 59±9 years, 152(76%) male with eGFR: 96±26 ml/min/1.73m$^2$. Of 130 patients with follow-up echo-data, 117 had normal ( ≥50%) LVEF at baseline. Of these, 2(5%) and 9(12%) patients with low *vs.* high risk had reduced LVEF (<50%) at follow-up ($p$=0.324). During follow-up, 22(11%) patients died, of which 1(1%) low risk, 9(14%) high risk without CAD and 12(17%) high risk with CAD (log rank p=0.012). In Cox regression analysis high risk no CAD patients *vs.* low risk had higher mortality ($p$=0.041), as was the case for high risk CAD patients *vs.* low risk ($p$=0.017). All high risk *vs.* low risk had higher mortality (HR: 8.7, $p$=0.047, adjusted for gender, age, HbA1c, cholesterol, SBP, smoking and creatinine). Mortality was similar in the high risk groups ($p$=0.5).

**Conclusions:** Risk stratification with p-NT-proBNP and CCS predicts all-cause mortality in asymptomatic type 2 diabetes patients with microalbuminuria and normal kidney function. Additional cardiovascular evaluation did not improve risk prediction. Deterioration from normal to impaired LVEF was not different between groups.

## TH-PO451

**Lowering of Albuminuria Reduces Cardio-Renal Events: Insights from ALTITUDE**

Hiddo Jan Lambers Heerspink,[1] Frederik I. Persson,[2] Toshiharu Ninomiya,[1] Barry M. Brenner,[2] Nish Chaturvedi,[2] Scott D. Solomon,[2] Marc A. Pfeffer,[2] Hans-Henrik Parving,[2] Dick de Zeeuw.[1] *[1]Clinical Pharmacology, UMC Groningen, Netherlands; [2]ALTITUDE Steering Committee.*

**Background:** Direct renin inhibition with aliskiren on top of ACEi/ARB therapy decreases albuminuria but did not decrease renal or cardiovascular (CV) events in the ALTITUDE (The Aliskiren Trial in Type 2 Diabetes Using Cardiorenal Endpoints) trial. Could the overall reduction in albuminuria (14%) have been too small to afford protection? We investigated post-hoc whether in ALTITUDE albuminuria predicts renal/CV outcome, and whether the degree of albuminuria reduction is related to renal/CV risk reduction.

**Methods:** In a randomized controlled double blind trial, 8561 subjects with type 2 diabetes and CKD, CV disease, or both were assigned to aliskiren 300 mg daily or placebo on top of ACEi or ARB therapy. Renal and CV events were collected during a median follow-up of 2.8 years.

**Results:** After adjusting for multiple risk markers, patients with high albuminuria ( ≥1000 mg/g creatinine; N=1903) at baseline showed a 9-fold renal (p<0.001) and 2-fold CV (p<0.001) risk increase compared to the low albuminuria group (<100 mg/g; N=2678). The change in albuminuria in the first 6 months of aliskiren therapy varied widely (IQR -49 to +42%). Subjects with larger reductions in albuminuria in the first 6 months (>30%; N=2738) showed a risk reduction of 58% for renal (95% CI: 69 to 45%; p=0.01) and 22% for CV (95% CI: 35 to 5%; p<0.01) events compared to subjects with an albuminuria increase between 0 and 30% (N=1251). Similar results were obtained when the aliskiren or placebo groups were separately analyzed. Residual albuminuria at month 6 showed an almost identical association with renal/CV outcome as baseline albuminuria.

**Conclusions:** Change in albuminuria is associated with a proportional effect on renal and CV outcome: the greater the reduction in albuminuria the greater the risk reduction. Baseline as well as residual albuminuria is directly related to renal and CV risk: the higher the albuminuria the more risk. These data imply that albuminuria should be monitored and targeted to the lowest achievable level in an attempt to reduce renal and CV risk.

*Funding:* Pharmaceutical Company Support - The ALTITUDE trial was sponsored by Novartis

## TH-PO452

**No Increase in Bone Fractures with Empagliflozin (EMPA) in a Pooled Analysis of More Than 11,000 Patients with Type 2 Diabetes (T2DM)**

Christoph Wanner,[1] Robert D. Toto,[2] John Gerich,[3] Thomas Hach,[4] Afshin Salsali,[4] Gabriel Kim,[4] Stefan Hantel,[5] Hans-Juergen Woerle,[4] Uli Christian Broedl.[4] *[1]Univ of Würzburg, Germany; [2]Univ of Texas Southwestern Medical Center, Dallas; [3]Univ of Rochester School of Medicine, Rochester, NY; [4]Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; [5]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.*

**Background:** The SGLT2 inhibitor EMPA is in development for the treatment of T2DM. SGLT2 inhibitors may alter the renal reabsorption of calcium and phosphate, and may potentially have effects on bone metabolism.

**Methods:** Using pooled data from Phase I, II and III trials, adverse events (AEs) consistent with bone fracture were evaluated through a search of investigator-reported AEs in patients with T2DM treated with placebo (PBO; n=3522), EMPA 10 mg (n=3630) or EMPA 25 mg (n=4602) in sub-groups of gender, age (<50, ≥50–<65, ≥65–<75, ≥75 yrs), BMI (<25, ≥25–<30, ≥30–<35, ≥35 kg/m²) and eGFR ( ≥90, ≥60–<90, ≥30–<60, <30 mL/min/1.73m²). Mean (SD) baseline age was 59.6 (10.0) yrs, BMI 30.0 (5.5) kg/m², eGFR 80.1 (22.1) mL/min/1.73m² and 63% of patients were male.

**Results:** The percentage of patients with fractures was similar with PBO (55/3522 [1.6%]) and EMPA 10 mg (59/3630 [1.6%]), but slightly lower with EMPA 25 mg (51/4602 [1.1%]). A greater proportion of female than male patients reported fractures in all groups (females: 1.2–2.5%; males: 1.0–1.3%) with comparable rates between PBO and EMPA. The percentage of patients with fractures was generally similar between PBO and EMPA in subgroups defined by age, BMI or eGFR. No changes in serum calcium or phosphate were observed. Small changes from baseline were observed in iPTH (mean [SD] changes -3.0 [15.7], 0.4 [15.0], -2.1 [31.1] ng/L with PBO, EMPA 10 mg, EMPA 25 mg, respectively), 25-OH vitamin D (mean [SD] changes 2.6 [40.0], 3.1 [34.8], 8.4 [36.2] nmol/L, respectively), and N-telopeptide (NTx)/creatinine ratio (mean [SD] changes -2 [17], 3 [19], 6 [23] nM/mM Cre, respectively).

**Conclusions:** In a pooled analysis of data from >11000 patients with T2DM, EMPA was not associated with an increased frequency of bone fractures versus PBO.

*Funding:* Pharmaceutical Company Support - Boehringer Ingelheim

## TH-PO453

**Is Peritoneal Dialysis Associated with New-Onset Diabetes Mellitus?**

Chih-Chiang Chien. *Dept of Nephrology, Chi-Mei Medical Center, Taiwan.*

**Background:** Few published studies have focused on post-dialysis new-onset DM.This study investigates whether there is an association between dialysis modality and new-onset DM.

**Methods:** In the Taiwan National Health Insurance Research Database, we examined records of ESRD patients who initiated dialysis between 1999 and 2005. Patients were followed until death, transplant, dialysis withdrawal, or 31 December 2008. Predictors of new-onset DM were calculated using Cox models.

**Results:** A total 51,487 incident dialysis patients were examined in this study, including 25,321 patients with pre-existing DM, 3,346 with new-onset DM, and 22,820 without DM at any time. Patients' age (mean ± SD) was 61.8 ± 11.5, 61.6 ± 13.7 and 56.5 ± 16.6 years in pre-existing, new-onset DM and without DM groups, respectively. The cumulative incidence rate of new-onset DM was 4% at one year and 21% at nine years. Being female, being older, and having baseline comorbidities were independent risk factors for new-onset DM in dialysis patients. Dialysis modality was not a risk factor for new-onset DM (HD to PD hazard ratio of new onset DM = 0.94 (95% CI: 0.83-1.06). The mean duration between new-onset DM diagnosed and death was 6.10 ± 1.01 years. Pre-existing DM was associated with 80% higher death risk (HR: 1.81, 95% CI: 1.75-1.87), whereas the new-onset DM was associated with 10% increased death risk (HR: 1.10, 95% CI: 1.03-1.17).

**Conclusions:** Whereas dialysis modality does not appear to associate with new-onset DM, both pre-existing and new-onset DM are related to higher long-term mortality in maintenance dialysis patients.

## TH-PO454

**Diabetic Foot Ulcers and Mortality in Chronic Hemodialysis Patients**

Eduardo K. Lacson, Weiling Wang, Shu-Fang Lin, Franklin W. Maddux, Jeffrey L. Hymes. *Fresenius Medical Care, North America, Waltham, MA.*

**Background:** Foot ulcers are associated with poor outcome in diabetic hemodialysis (HD) patients. We evaluated the development of foot ulcers detected by a national monthly foot check program at Fresenius Medical Care, North America facilities.

**Methods:** All adult diabetic HD patients with ≥1 foot check documented electronically during 7/1/09-6/30/12 in 329 facilities with ECube clinical systems were included. Foot ulcer status and deaths were tracked until 12/31/12. Patients developing ulcers on follow-up were "cases", others only "at-risk". As of the 1st foot check date, age, gender, race, & incident patient status (vintage ≤120 days) were recorded. Logistic regression defined odds ratio (OR) for death between cases and patients at-risk.

**Results:** 764 (5.4%) of 14,103 patients had foot ulcers at 1st recorded exam. Among 13,339 ulcer-free patients, 1,769 (13.3%) developed ulcers (cases). The mean age was 63.5±12.6 years, with more males (58.4% vs. 52.8%) and whites (71.0% vs. 63.0%) in cases (all p<0.0001). Cases had longer median follow-up time at 462 vs. 352 days. The nested median time to first ulcer was ~5 months (Table); the nested median time to death from the 1st foot ulcer was ~3.5-4.0 months. Unadjusted OR of death was 1.45 compared to patients at-risk, and remained OR=1.51 after adjusting for case-mix (both p<0.0001). Mortality risk was not significantly different in incident patients.

| (Excludes 5.4% with pre-existing ulcer) | All Cases | Subset: Incident Cases |
|---|---|---|
| N (%) | 1,769 (13.3) | 377 of 1,769 (21.3) |
| Days of follow-up, Mean (SD) | 462 (235) | 421 (219) |
| Median (IQR) | 428 (269, 645) | 388 (253, 577) |
| Days to 1st Ulcer, Mean (SD) | 211 (174) | 183 (151) |
| Median (IQR) | 166 (72, 302) | 151 (58, 267) |
| Days to Death after 1st Ulcer, Mean (SD) | 180 (165) | 160 (147) |
| Median (IQR) | 124 (56, 259) | 109.5 (47, 257) |

**Conclusions:** About 1 in 5 diabetic HD patients had or developed foot ulcer over a median follow-up of ~15 months. De novo ulcers associated with ~50% greater risk of death. Routine foot checks may allow for early diagnosis of foot ulcers, neuropathy and peripheral circulatory compromise. Further clinical trials should be considered to identify these issues so that care strategies can be implemented to optimize long term outcomes in this population.

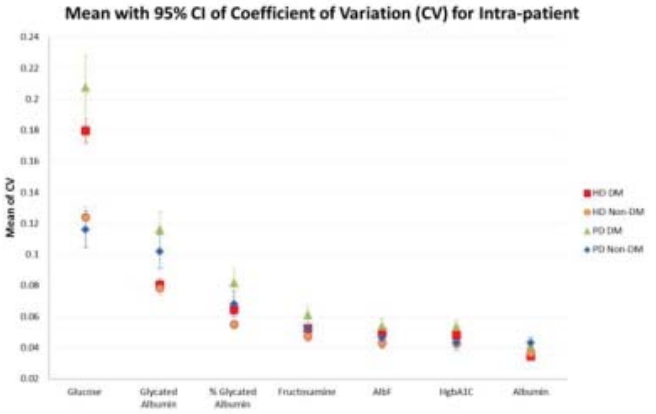
## TH-PO455

**Monthly Variation in Glycemic Indices in Patients Maintained with Dialysis**

Neal Mittman,[1] Lin Ma,[2] Mark E. Williams,[3] Julia I. Brennan,[4] Chinu M. Jani,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[2] Eduardo K. Lacson.[2] *[1]Long Island College Hospital, Brooklyn, NY; [2]Fresenius Medical Care, North America, Waltham, MA; [3]Joslin Diabetes Center, Boston, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

**Background:** Glycemic control is conventionally monitored utilizing quarterly measurement of glycosylated hemoglobin (A1c). Because A1c may not be an ideal marker for dialysis patients, other glycemic indices have been sought. Furthermore, little is known about the comparative variability of these indices which reflect different durations of glycemia.

**Methods:** Simultaneous measurement of pre-dialysis serum fructosamine (SF), glycated albumin (GA), A1c and casual glucose (BG), from residual blood (of routine monthly draws) was obtained from a national sample of 1977 DM and 1454 nondiabetic (NDM) dialysis patients from 26 FMCNA facilities. Mean intra-patient variability was compared by coefficient of variation (CV) for January-March 2013. SF was albumin-corrected (AlbF) and GA was expressed as percent glycated (%GA) as recommended.




**Results:** BG exhibited the highest intra-patient CV in diabetics on PD, followed by DM HD, while NDM HD and PD pts had lesser CV. A1c and AlbF were the most stable for all groups. GA exhibited greater variability than SF or A1c. PD pts (both DM and NDM) had greater CV with GA than HD pts. CV with GA was lower when expressed as %GA, the method recommended by the manufacturer.

**Conclusions:** Monthly intra-patient variability is modest for A1c, SF, AlbF, and %GA, a necessary requirement for a periodic monitoring tool. A1c reflects a longer period of glycemia (2-3 months) and should be the most stable relative to protein-based indices (2-3 weeks of glycemia).These concurrent tests provide baseline information as we prospectively evaluate their utility and association with outcomes.

## TH-PO456

**Correlates of Glycosylated Hemoglobin Level in Diabetic Patients Maintained with Dialysis**

Neal Mittman,[1] Lin Ma,[2] Mark E. Williams,[3] Julia I. Brennan,[4] Chinu M. Jani,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[2] Eduardo K. Lacson.[2] *[1]Long Island College Hospital, Brooklyn, NY; [2]Fresenius Medical Care, North America, Waltham, MA; [3]Joslin Diabetes Center, Boston, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

**Background:** Serial measures of glucose and Hemoglobin A1c is used to monitor glycemic control in diabetic dialysis patients (pts) and in the general population. We explored the distribution of A1c in a national sample of 1979 DM patients from 26 FMCNA facilities across the US.

**Methods:** Residual blood specimens were used to obtain mean monthly chemistries for the period January-March 2013. Pts were stratified by A1c as <=5.6% (n=553), >5.6-7.0% (n=861), and >7.0% (n=565). Demographic and biochemical correlates of glycemia by A1c category were evaluated.

**Results:** As expected, serum glucose increased by A1c group accompanied by a decreasing percentage of pts with casual glucose <120mg%. Higher A1c was associated with younger age, shorter dialysis vintage and peritoneal dialysis modality (all p<0.001). There were no differences by gender, race, ethnicity, HD catheter usage, cardiovascular comorbidities (CVA, CHF, PAD, AMI), mean eKtv and albumin between groups. Interestingly, pts with A1c<5.6%, had lower mean Hgb while receiving higher mean EPO doses to maintain these Hgb levels. WBC count increased in the opposite direction of ESA dose, with no differences in neutrophil to lymphocyte ratios between groups.

| | DM w HgbA1c<=5.6% | DM w HgbA1c:>5.6-7.0% | DM w HgbA1c>7.0% |
|---|---|---|---|
| N | 553 | 861 | 565 |
| *Glucose(Mean±SD) | 119.4±31.6 | 152.3±46.2 | 215.4±78.9 |
| Glucose<120 (%) | 59.0 | 25.0 | 7.2 |
| eKtv (Mean±SD) | 1.5±0.3 | 1.5±0.3 | 1.5±0.4 |
| *Hemoglobin (Mean±SD) | 10.6±1.0 | 10.7±1.0 | 10.9±1.1 |
| Albumin (Mean±SD) | 3.8±0.4 | 3.8±0.4 | 3.8±0.4 |
| Albumin < 3.5 (%) | 19.7 | 19.3 | 21.8 |
| Phosphorus (Mean±SD) | 5.2±1.4 | 5.2±1.3 | 5.3±1.4 |
| Phosphorus <3.5 (%) | 8.5 | 6.8 | 5.8 |
| *WBC (Mean±SD) | 6.5±2.2 | 7.1±2.2 | 7.4±2.4 |
| *Weekly EPO dose (Mean±SD) | 11988.9±14180.6 | 9038.0 ±11805.9 | 8384.6±10568.9 |

**Conclusions:** While A1c tracks with glucose levels, it may be further affected by high ESA dose requirements. Although a high ESA dose may be accompanied by a high red cell turnover that leads to lower A1c, it is also possible that existing factors predisposing to low A1c may likewise induce ESA resistance. Factors unrelated to glycemic control need to be considered in interpreting A1c values in diabetic patients on dialysis.

## TH-PO457

**Glycemic Control Impacts Cardiovascular Outcome in Incident Dialysis Patients with Diabetes** Mi Jung Lee,[1] Shin-Wook Kang,[1,2,3] Chan Ho Kim,[1] Ji Sun Paeng,[2] Hyung Jung Oh,[1] Tae-Hyun Yoo.[1,2] *[1]Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea; [2]Brain Korea 21, Yonsei Univ, Seoul, Korea; [3]On behalf of Clinical Research Center for End-Stage Renal Disease Investigators.*

**Background:** Most current guidelines recommend that target hemoglobin A1C levels (A1C) for patients with diabetes mellitus (DM) should be <7.0% irrespective of the presence of chronic kidney disease. However, there has been much debate whether such recommendation can be generalized to end-stage renal disease (ESRD) population.

**Methods:** A prospective cohort of 907 incident dialysis patients with type 1 or type 2 DM from 36 dialysis centers of the Clinical Research Center for ESRD in Korea was selected for this study. Based on the baseline A1C levels, patients were divided into 5 groups: <6.0%, ≥9.0%, and every 1.0% increment in between. Cox proportional hazard analysis was performed to determine the prognostic value of A1C for primary outcome, a composite of cardiovascular death or non-fatal major cardiovascular events.

**Results:** The mean baseline A1C was 6.6±1.4%. During a mean follow-up duration of 15.0 months, 39 patients died of cardiovascular diseases and 78 non-fatal major cardiovascular events occurred. Compared to patients with A1C of 7.0-7.9%, the adjusted hazard ratios (HRs) of A1C <6.0%, 6.0-6.9%, 8.0-8.9%, and ≥9.0% groups were 1.02 [95% confidence interval (CI), 0.55-1.86; P=0.12], 1.59 (95% CI, 0.89-2.82; P=0.9), 2.04 (95% CI, 0.92-4.52; P=0.08), and 2.17 (95% CI, 1.02-4.62; P=0.04), respectively. Sensitivity analysis revealed that A1C ≥9.0% was significantly associated with an increased risk of reaching the composite outcome in patients with body mass index ≥24 kg/m$^2$ (HR, 2.51; 95% CI, 1.09-5.82; P=0.03) and patients with serum albumin levels ≥3.3 g/dL (HR, 3.93, 95% CI, 1.51-10.25; P=0.01).

**Conclusions:** Poor glycemic control (A1C ≥9.0%) was significantly associated with adverse cardiovascular outcome in incident dialysis patients with DM, especially in patients with a good nutritional status. A further long-term observational study is needed to determine the optimal levels of A1C in these patients.

*Funding:* Government Support - Non-U.S.

## TH-PO458

**Initial Application and Effects of Basic Carbohydrate Counting for Diabetics Undergoing Hemodialysis in Countries in which the Entity of Carbohydrate Counting Has Not Been Introduced** Toru Hyodo,[1,2] Noriko Mikami,[1] Daisuke Ishii,[2] Yasuhisa Kurata,[1] Miho Hida,[1] Kazunari Yoshida,[2] Masatsugu Iwamura,[2] Junko Kawakami.[3] *[1]Dialysis Center, Eijin Clinic and Kurata Hospital, Hiratsuka, Kanagawa, Japan; [2]Dept of Urology, Kitasato Univ, School of Medicine, Sagamihara, Kanagawa, Japan; [3]Dept of Clinical Nutrition, Sagami Women's Univ, Sagamihara, Kanagawa, Japan.*

**Background:** In Japan, carbohydrate counting, which is based on the fact that only carbohydrates change into blood sugar, has not been announced to citizens. In Europe and the United States, this method has been commonly established as diet therapy for diabetics. In this study, we introduced basic carbohydrate counting, which makes the range of blood sugar changes constant by establishing an equivalent intake at every meal based on the carbohydrate contents of foods, in diabetic HD patients, and examined its effects.

**Methods:** The subjects were 19 HD patients. They consisted of 10 males and 9 females, with a mean age of 61.7±10.3 years. The dialysis period was 3.5±4.0 years. Fifteen patients had and 4 had not received insulin. During the observation period, the doses of insulin, oral diabetic and lipids drugs were not changed. Physicians and dietitians explained the dietary method with basic carbhydrate counting. Changes in HbA1c, the dry weight (DW), T-Cho and protein catabolic rate (PCR, = protein intake) were investigated for 6.4± 3.1 months (1 to 9 months).

**Results:** Only the HbA1c value significantly decreased, but DW, T-Cho and PCR did not show significant change.

| Months | 0 | 1 | 3 | 6 | 9 |
|---|---|---|---|---|---|
| Pt. No. | 19 | 19 | 15 | 13 | 10 |
| HbA1c(%) | 7.5±1.23 | 7.16±1.03* | 6.7±0.81* | 6.49±0.54* | 6.64.75 |
| PCR(g/kg/day) | 0.85±0.14 | 0.81±0.12 | 0.81±0.12 | 0.82±0.18 | 0.76±0.15 |
| T-Cho(mg/dL) | 159.7±32.2 | 159.7±34.3 | 160.3±24.7 | 163.4±25.7 | 159.6±35.0 |
| DW(kg) | 61.9±15.9 | 62.0±15.8 | 59.5±14.7 | 54.9±10.7 | 55.0±10.8 |

[ * : $P < 0.05$].

**Conclusions:** Basic carbohydrate counting is simple and beneficial for diabetic HD patients.

## TH-PO459

**Is Albuminuria a Sensitive Biomarker for Early Detection of Kidney Endpoints in Type 2 Diabetes? A Systematic Review** Veeda O. Landeras,[1] Ivy Hsieh,[2] Abdulah Alrifai,[1] Marian Simonson,[3] Donald E. Hricik,[1] Michael S. Simonson.[1] *[1]Dept of Medicine, Div of Nephrology, Univ Hospital Case Medical Center, Cleveland, OH; [2]Physiology & Biophysics, Case Western Reserve Univ, Cleveland, OH; [3]Alumni Library, Cleveland Clinic, Cleveland, OH.*

**Background:** Albuminuria predicts progression to chronic kidney disease and end-stage renal disease in type 2 diabetes. The objective of this systematic review was to synthesize best available evidence regarding performance of albuminuria for early detection of renal endpoints.

**Methods:** We searched PubMed, EMBASE and SCOPUS for longitudinal studies of type 2 diabetes with renal endpoints. Studies were selected if participants had baseline eGFR > 60 ml/min/1.73 m$^2$, measurements of albuminuria at baseline and predefined renal endpoints of chronic kidney disease (eGFR < 60 ml/min/1.73 m$^2$), eGFR decline > 4.0 ml/year or end-stage renal disease (ESRD). Studies with fewer than 500 participants, and therefore few renal events, were excluded. Performance of albuminuria for predicting renal endpoints was assessed as sensitivity and specificity.

**Results:** Eight studies that included 36,947 participants with 2,410 renal endpoints met inclusion criteria. Albuminuria (micro-and macroalbuminuria) was specific for predicting all kidney endpoints: 0.73, or 73% (95% CI, 0.72 – 0.74). However, the sensitivity for predicting different endpoints varied considerably: ESRD, 0.69, (0.63 – 0.75); eGFR < 60 ml/min/1.73m$^2$, 0.44 (0.41-0.46); eGFR decline > 4.0 ml/year 0.24 (0.20-0.28). Summary estimates for sensitivity in a fixed-effects model were not affected by baseline eGFR, patient age, and length of follow-up or method of albumin testing.

**Conclusions:** In patients with type 2 diabetes and eGFR > 60 ml/min/1.73m$^2$, albuminuria is a specific prognostic marker (73%) for kidney endpoints. However, albuminuria may be insensitive for predicting eGFR < 60 ml/min/1.73 m2 and annual eGFR decline > 4.0 ml/year even though it is a reasonably accurate predictor of ESRD.

*Funding:* Other NIH Support - NIH RO1 DK096549

## TH-PO460

**Onset and Progression of Diabetes in Kidney Transplant Patients Receiving Everolimus or Cyclosporine: Post Hoc Analysis of 2 Open-Label, Multicenter, Randomized Trials** Claudia Sommerer,[1] Klemens Budde,[1] F. Lehner,[1] Wolfgang Arns,[1] Petra Reinke,[1] Ute Eisenberger,[1] Rudolf P. Wuthrich,[1] Martina Porstner,[2] Christoph May,[2] Eva-Maria Paulus,[2] Anja Susanne Muhlfeld,[1] Heiner H. Wolters,[1] Katharina M. Pressmar,[1] Rolf A. Stahl,[1] Oliver Witzke,[1] Hans-Hellmut Neumayer,[1] Markus Guba,[1] Johannes Jacobi,[1] Volker Kliem,[1] Ingeborg A. Hauser,[1] Martin G. Zeier.[1] *[1]ZEUS/HERAKLES Study Group, Germany; [2]Novartis Pharma Nuremberg, Germany.*

**Background:** Analysis of NODAT(new-onset diabetes mellitus(DM) post transplantation(Tx)) or progression of pre-existingDM in *de novo* KTx patients (pts) after conversion to everolimus(EVR)/Enteric-Coated Mycophenolate Sodium(EC-MPS) regimen and withdrawal or reduction of cyclosporine (CsA) combined with EVR.

**Methods:** *Post hoc* analysis from ZEUS &HERAKLES, 12months(Mo),prospective, open-label, multicenter, randomized(RDZ) trials. De novo KTx pts received standard CsA/EC-MPS +steroids after Tx and were RDZ to either continue CsA or convert to EVR at Mo4.5 post Tx(ZEUS) or at Mo3 post Tx(HERAKLES) with 3rd RDZ arm CNI-low. *Post hoc* categorization: according to either NODAT development or progression of DM.

**Results:** ZEUS: 8%(25/300) of pts had DM at Tx. NODAT at Mo12: 8%(22/275) of nonDM pts(Fig1). 7%(20/275) of these already developed at RDZ (13/142 EVR vs 7/133 CsA).NODAT after RDZ was similar(p=0.97). Mean blood glucose change from RDZ to Mo12 was similar in EVR vs CsA within NODAT(p=0.10) and DM(p=0.52) subpop. eGFR was similar at RDZ,significantly higher at Mo12 in EVR vs CsA pts in all subpop(Fig1). HERAKLES: 7%(30/438) of pts developed NODAT to Mo12, but no difference between the 3 regimen(p=0.62)(Fig1).

| Fig.1 | ZEUS | | HERAKLES | | |
|---|---|---|---|---|---|
| | EVR | CsA | EVR (CNI-free) | CsA | CNI-low |
| Incidence of NODAT N(%) from Tx to Mo12 | 14/142 (10%) | 8/133 (6%) | 10/149 (7%) | 11/133 (8%) | 9/143 (6%) |
| eGFR change (SD) from RDZ to Mo12 (Nankivell [mL/min/1.73m2]) | NODAT subgroup: +14.0 (11.4) Pre-exist DM subgroup: +5.5 (5.9) | NODAT subgroup: -9.2 (15.9) Pre-exist DM subgroup: +0.7 (10.0) | NODAT subgroup: +4.9 (16.7) Pre-exist DM subgroup: +5.3 (12.9) | NODAT subgroup: -1.9 (15.8) Pre-exist DM subgroup: +0.1 (11.7) | NODAT subgroup: -2.8 (9.6) Pre-exist DM subgroup: +2.8 (11.8) |
| BPAR in NODAT subgroup N(%) | 2 (14%) | 1 (13%) | 1 (10%) | 2 (18%) | 1 (11.1%) |

**Conclusions:** Within 12Mo post Tx no difference in NODAT or DM progression after CNI withdrawal and EVR conversion were found *post hoc*. However, benefit on renal function from early conversion to EVR translated into NODAT subpop.

*Funding:* Pharmaceutical Company Support - Novartis Pharma Germany

## TH-PO461

### Influence of Normal and Bifurcated Cephalic Arch Anatomy and Hemodynamics on Cephalic Arch Stenosis Onset

Mary S. Hammes,[1] Michael Boghosian,[2] Kevin Cassel,[2] Jane E. Hines.[1] *[1]Univ of Chicago; [2]Illinois Institute of Technology.*

**Background:** A primary site of failure for patients with brachiocephalic fistula (BCF) is in the curved cephalic arch (CA). Extreme hemodynamic conditions as a result of fistula creation are responsible for locally low wall shear stresses (WSS), intimal hyperplasia and resultant cephalic arch stenosis (CAS). We compare anatomy, geometry, and hemodynamic parameters (HDP) at 3 and 12 months using computational fluid dynamic modeling (CFD).

**Methods:** Twelve subjects were referred for hemodialysis for permanent vascular access with a decision to place a BCF. A venogram, Doppler, and whole blood viscosity was performed at 3 and 12 months post-fistula. Geometric measurements and HDP were obtained using CFD at these stated time intervals.

**Results:** Of the twelve subjects, six had normal CA anatomy, while six had multiple vessels (bifids) in the CA at baseline. At 12 months, six of the bifids and three of the normal anatomy arches had no CAS. All three patients with CAS evident at 12 months had extensive regions of low WSS at 3 months. Three patients with normal CA anatomy without CAS at 12 months, had smaller average venous diameters (0.44 vs. 0.64 cm) and blood velocities (82.7 vs. 189.2 cm/s) than the three patients that developed CAS.

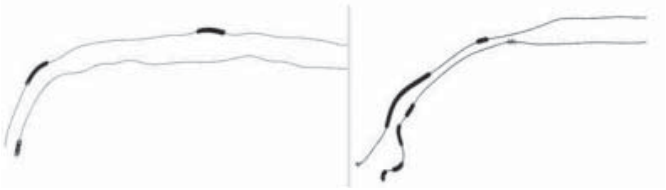


**Figure 1.** An example of a CFD of the CA at 3 months (left) and 12 months (right). Highlighted portions of the boundaries indicate WSS below the threshold for IH (0.076 Pa).

**Conclusions:** The anatomic variant of a bifid CA is common with a lower incidence of CAS at one year. Alternative flow paths in bifid arches mitigate high flow resulting in less extreme hemodynamics. Patients having more extensive regions of low WSS in the CA at 3 months are the most susceptible to CAS at 12 months. These findings support our hypothesis that failure of BCF arises from altered hemodynamics in the CA after fistula creation.

*Funding:* NIDDK Support, Other NIH Support - This study was funded by RO1DK090769

## TH-PO462

### The Negative Hemodynamic Consequence of Acute Angulation by Three Months in Brachiocephalic Fistula Access

Mary S. Hammes,[1] Michael Boghosian,[2] Kevin Cassel,[2] Jane E. Hines.[1] *[1]Univ of Chicago; [2]Illinois Institute of Technology.*

**Background:** Cephalic arch stenosis is a common problem leading to failure in patients with a brachiocephalic fistula (BCF). A contribution to fistula failure is geometrical changes occurring in the cephalic arch resulting from post-fistula creation hemodynamics. From our measurements we find that the cephalic arch angle decreases, becoming more acute, over a 3-month time period beginning at fistula creation. The consequences of this acute angle reduction on subsequent hemodynamic factors are elucidated.

**Methods:** ESRD patients receiving hemodialysis were referred for permanent vascular access with a decision to place an upper arm BCF. Enrollment was by written consent with a venogram, Doppler, and whole blood viscosity performed pre-fistula and at three months. Geometric measurements including arch angle and vein diameter were obtained. Hemodynamic parameters including wall shear stress (WSS) and modeled pressure were calculated using computational fluid dynamic modeling (CFD) at stated time intervals on reconstructions of patient-specific geometries.

**Results:** Eight subjects were included over a three-month time period. The average decrease in arch angles was -16.6±15.5° from the pre-fistula geometry to that at 3 months ($p = 0.02$). The venous pressure and velocity increased by 2.3 ± 3.1 Pa and 41.2 ± 32.1 cm/s, respectively. The minimum WSS decreased by -1.2 ± 2.0 Pa, and the maximum WSS increased by 6.7 ± 10.1 Pa from 0 to 3 months. Thus, the pressure and velocity are found to increase by one order of magnitude, while the range of WSS between its minimum and maximum widens. The arch angle is relatively constant from 3 to 12 months; however, the wall shear stress ranges further diverge.

**Conclusions:** Based on the measured acute arch angle reductions and corresponding CFD simulations, a postulated failure scenario for the BCF is as follows. Increased blood pressure in the vein is responsible for the acute arch angle decrease at 3 months after fistula creation. The resulting geometry changes in the cephalic arch are shown via CFD simulations to contribute to the non-physiological hemodynamics and clinical consequences only 3 months after fistula creation.

*Funding:* NIDDK Support, Other NIH Support - This study was funded by RO1DK090769

## TH-PO463

### Small Artery Elasticity Index (SAE) Strongly Correlates with Arterial Micro-Calcification and Increased Arterial Media Thickness in Patients Undergoing Arteriovenous Fistula (AVF) Creation

Christopher Heaton,[2] William D. Paulson,[1] Daniel T. Kleven,[2] Mufaddal F. Kheda,[1] James J. Wynn,[3] John Jason White.[1] *[1]Medicine, Georgia Regents Univ, Augusta, GA; [2]Pathology, Georgia Regents Univ, Augusta, GA; [3]Surgery, Georgia Regents Univ, Augusta, GA.*

**Background:** The high maturation failure rate of AVFs is a major unsolved problem, and preoperative methods of accurately predicting failure are urgently needed. We have previously shown that SAE predicts AVF maturation failure, and have hypothesized that this failure is due to impaired dilatation of the inflow artery and outflow vein. In this study, we hypothesized that low SAE correlates with changes in arterial histopathology that indicate calcification and increased arterial media thickness.

**Methods:** Eighteen patients underwent measurement of SAE (ml/mmHg x100) preoperatively on the day of AVF creation. SAE was measured with the HDI/PulseWave CR-2000 System; arterial pulse pressure was measured as an indicator of arterial stiffness. Arterial samples were obtained at the arteriovenous anastomosis during AVF creation. Samples were paraffin embedded and cross-sections were stained with hematoxylin-eosin, trichrome, and von Kossa (for calcium). Arterial intima and media thickness were measured, and calcification was graded on a semi-quantitative scale, by two pathologists who were blinded to all other measurements (0 = none; 1 = mild; 2 = moderate; 3 = severe).

**Results:** Three arteries were excluded from analysis due to processing problems. Multiple regression analysis in the remaining 15 patients indicates that the dependent variable SAE correlated with the independent variables micro-calcification score, arterial media thickness, and pulse pressure (all $P \leq 0.02$). The correlation of SAE with these 3 variables explained two-thirds of patient-to-patient variation in SAE ($R^2 = 0.677$).

**Conclusions:** Pre-operative measurement of SAE appears to be a strong indicator of inflow artery calcification, media thickness, and stiffness in patients undergoing AVF creation. This result suggests that SAE may be a useful tool in planning AVF creation.

## TH-PO464

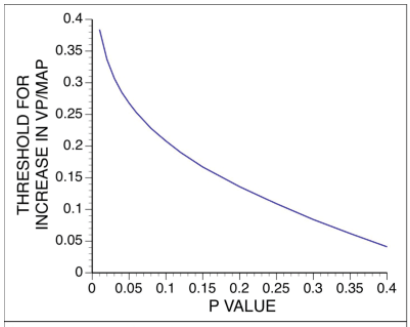
### Thresholds for Significant Increase in Dialysis Venous Pressure (VP): Criteria That Help Decide Whether to Refer for Intervention

Eduard R. Fatakhov,[1] John Jason White,[1] Anatole Besarab,[2] William D. Paulson.[1] *[1]Charlie Norwood VAMC & Georgia Regents Univ, Augusta, GA; [2]Henry Ford Hospital, Detroit, MI.*

**Background:** Vascular access failure is a major cause of morbidity and mortality in hemodialysis. Access failure is usually caused by stenosis, which causes thrombosis and access abandonment. An increase in static VP is an important method of detecting stenosis so that it can be corrected before thrombosis. However, criteria for determining when an increase in VP is statistically significant have not been established.

**Methods:** Fifty-five patients who were dialyzed with synthetic grafts or arteriovenous fistulas had 9 static VP measurements within 2 wks. VP was adjusted for mean arterial pressure (VP/MAP). Thirty-six differences between 2 VP/MAP measurements were computed from each patient's 9 measurements. The standard deviation of all these differences was taken to indicate background variation in VP/MAP that was not influenced by increases in stenosis. After confirming VP's were normally distributed, we used the student t distribution to determine thresholds for a significant increase in VP.

**Results:** The standard deviation for the difference between 2 VP/MAP measurements was 0.160. We computed thresholds for a significant increase in VP/MAP for a range of P values (figure). At $P < 0.05$, the increase must be >0.27 to be significant, whereas at $P < 0.20$, the increase must be >0.14. If avoiding unnecessary procedures is a priority, then a low P value is optimal, whereas if detecting an increase in stenosis is a priority, then a larger P value is optimal.




Thresholds for determining whether increase in static VP/MAP is significant for a range of P values.

**Conclusions:** This study defines thresholds for a significant increase in static VP/MAP that can help determine whether access referral for correction of stenosis is indicated. Selection of a referral threshold should consider the relative importance of avoiding thrombosis vs. avoiding unnecessary invasive procedures.

*Funding:* Clinical Revenue Support

## TH-PO465

**Analysis of Vascular Access Interventional Therapy: 9-Year Follow Up Survey in 3,000 Cases with Vascular Access Failure** Teruhiko Maeba,[1] Shigeru Owada.[2] *[1]Internal Medicine, Asao Kidney Clinic, Kawsaki, Kanagawa, Japan; [2]Dialysis Center, Asao Clinic, Kawasaki, Kanagawa, Japan.*

**Background:** Keeping a functional vascular access (VA) is one of the most important factors in the maintenance of an HD modality. The application of vascular access interventional therapy (VAIVT) for VA trouble is increasing recently but the effectiveness of VAIVT has not been entirely satisfactory because of the relatively high rate of re-stenosis.

**Methods:** We have experienced 3,000 cases of VA trouble treated with VAIVT over the last 9 years in which primary assisted and secondary assisted rates were analyzed.

**Results:** 1. We have used a noncompliant type of balloon catheter for 97.7% of cases and primary assisted patency rates were 98.9%. 2. Secondary assisted patency rates were 79.6% at two years, 67.5% at 4 years, 59.5% at 6 years and 46.6% at 8years in all patients. 3.In diabetic patients, assisted patency rate was 40.3% at 8 years and it was50.0% in non-diabetic patients (p=0.09, Log rank). There were no significant differences found between the arteriovenous fistulas (AVF) and the arteriovenous grafts (AVG) ; 49.3% and 39.4% at 8 years, respectively. 4. Failure of secondary assisted patency was observed in 271 patients. In these, a new AVF was made on the same side in 39.9% of the patients, a contralateral side AVF was made in 5.5% and an AVG in 45.4%, superficialized reposition of brachial artery in 1.8% and death occurred in 7.4%.

**Conclusions:** 1. It was possible to reduce the economic burden for patients using a noncompliant type of balloon catheter. 2. The results in primary and secondary assisted patency rates were excellent. 3. Long-term maintenance of AVG was possible by the application of VAIVT.

## TH-PO466

**Management of Dysfunctional Vascular Access by Repeated Surgery or Angioplasty. Results on a Series of 264 Procedures at 24 Months** Paola De Rango, Basso Parente, Beatrice Fiorucci, Luca Farchioni, Gianbattista Parlani, Giuseppe Giordano. *Vascular and Endovascular Surgery; Hospital S.M. Misericordia; Univ of Perugia, Perugia, Italy.*

**Background:** Dysfunctional vascular access is a common problem that remains unsolved for patients with end stage renal disease (ESRD) requiring dialysis. Object of this study was to evaluate 24 months outcomes of angioplasty compared to repeated open surgery in patients with dysfunctional vascular access.

**Methods:** Consecutive dysfunctional vascular accesses repaired during 2006-2013 were reviewed. Endovascular repair was performed with angioplasty alone and selective stent. Repeated open surgery was performed with multiple techniques. Patients were followed for 24 months. Primary outcome was functional access patency at 6, 12 and 24 months assessed by Kaplan Meier with log rank test. Secondary outcomes included complications, reinterventions and costs. Costs were derived from procedure hospital fees according to regional codes.

**Results:** A total of 264 dysfunctional vascular accesses (163 males;mean age71.7y) were treated during the study period; 96 underwent angioplasty and 168 open reintervention. Kaplan Meier estimates of primary patency at 6, 12, and 24 months were 75.4%, 68.5% and 46.2% in the angioplasty group and 92.6%, 80.7% and 68.4% in the open group (P<.0001). Access related complications occurred in 5.2% patients in the angioplasty group and 6.8% patients in the open group. A total of 82 reinterventions were required during follow-up ranging from 1 to 5 per patient; rates were 60% in the angioplasty group and 40% in the open group. Patients after angioplasty required a mean 0.6+1.1 and those after open surgery a mean 0.4+0.97 reinterventions after the first procedure. Mean costs per patient (including first procedure and additional reinterventions) ranged from €6900 to €7012,9 in the angioplasty group and from €7114 to €7200 in the open surgical group.

**Conclusions:** Angioplasty can be used as an alternative procedure for the maintenance of vascular access with 46.2% patency rate at 24 months. Costs may be contained when no routine stent is applied.

## TH-PO467

**Fistula Patency Rate after Venous Neointimal Hyperplasia Stripping/ Fistula Reconstruction in Patients with Late Autologous Arteriovenous Fistula Stenosis** Fangping Lu,[1] Xuemei Li.[2] *[1]Nephrology, First Hospital of Tsinghua Univ, Beijing, China; [2]Nephrology, Peking Union Medical College Hospital, Beijing, China.*

**Background:** Although autologous arteriovenous fistula(AVF) is still the best permanent hemodialysis access, but mature AVF can occur venous neointimal hyperplasia ( VNH), which lead to venous stenosis with or without thrombosis, that is the primary cause of vascular access failure. Peri-anastomotic stenosis is the main place. At present treatment of VNH is very limited.We adopt a surgical method( stripping VNH and fistula reconstruction) to repair AVF.Here we report the effect of venous neointimal hyperplasia(VNH) stripping / fistula reconstruction on the patency rate in patients with late autologous arteriovenous fistula(AVF) stenosis.

**Methods:** We retrospectively collected clinical data of the hemodialysis patients receiving AVF operation in our hospital from January 2007 to December 2011. There were a total of 305 patients, including 76 cases of VNH stripping/ fistula reconstruction(see Fig.). We observed the fistula patency rate at 3, 6, 12 months after operation, and compared with 128 non VNH stripping/ fistula reconstruction patients and 101 newly AVF patients during the same period.

**Results:** We found that VNH stripping/ fistula reconstruction had a good fistula patency rate(93.4%, 89.5%, 84.2% at 3,6,12 month respectively).

| | 3 month | 6 month | 12 month |
|---|---|---|---|
| VNH stripping/fistula reconstruction | 71(93.4%) | 68(89.5%) | 64(84.2%) |
| Non-VNH stripping/ fistula reconstruction | 118(92.2%) | 115(89.8%) | 110(85.9%) |
| newly AVF | 93(92.1%) | 89(88.1%) | 82(81.2%) |
| *P* value | 0.935 | 0.913 | 0.622 |
| $x^2$value | 0.135 | 0.183 | 0.95 |

Compared with other two kinds of fistula , there was no significant difference in the patency rate.

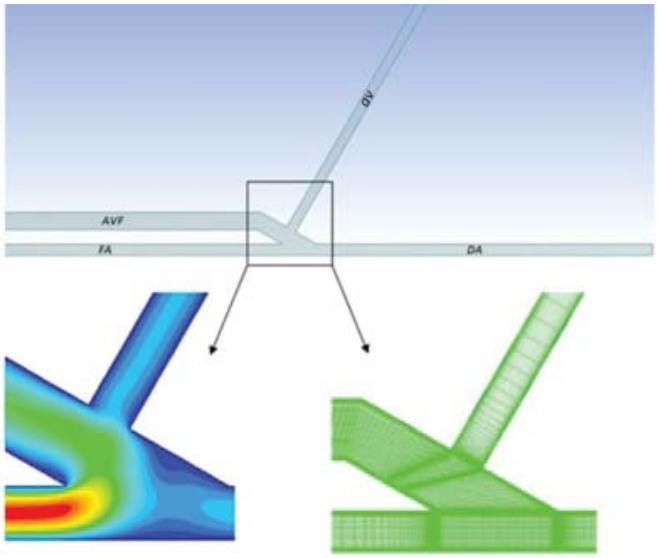
**Conclusions:** VNH stripping / fistula reconstruction operation was an effective method to relieve late stenosis of AVF and improve utilization rate of fistula.

## TH-PO468

**Criteria for Accessory Vein Obliteration. A Scientific Approach towards a Complex Paradigm** Naveed U. Haq, Mamdouh N. Albaqumi. *Nephrology, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.*

**Background:** Native arteriovenous fistula (AVF) is the preferred vascular access for hemodialysis. AVF Stenosis and/or accessory vein (*av*) are the most common causes of early fistula failure. While treatment of stenosis is better defined, there are no clear criteria for obliteration of *av*. The purpose of this study was to establish scientific criteria for the management of *av*.

**Methods:** Various Computational Fluid Dynamics (CFD) simulations were performed to analyze blood flow in AVF. The flow was assumed to be incompressible, laminar, Newtonian and transient. 2D model geometries were constructed by ANSYS Design Modeler. The meshing of all AVF models was carried out in ICEM CFD mesh. A finer, high quality mesh was defined at the level of anastamosis, the delicate region (figure). *av* of different diameters, angles, and locations were added in the AVF, and comparison of simulation results was carried out. *av* stealing >20% flow from the AVF was considered significant.




AVF Geometry with pressure, wall shear stress changes and mesh at the anastomosis.
FA: Feeding artery, DA: Distal artery, AVF: Arteriovenous fistula, *av:* Accessory vein

**Results:** *av* diameter was the major determinant of flow while location and angle were insignificant. *av* of at least 60% diameter of the main AVF was significant. If 2 *av*'s were present, effect was additive. *av* presence caused wall shear stress disturbances, while early branching of *av* was also important. This information was combined with known clinical facts to establish the criteria (table 1).

| Table 1: HAQ criteria for *av* obliteration |
|---|
| 1. 60% or greater diameter of the main AVF |
| 2. 50% diameter of AVF with at least one more *av* ≥40% in diameter. |
| 3. 50% in diameter and divides into branches of same size. |
| 4. *av* likely to interfere with cannulation on physical examination. |
| 5. >30% in diameter and associated with stenosis at site of origin. |

**Conclusions:** Our study provides the first standardized and objective criteria for *av* obliteration. This needs to be validated and tested in randomized clinical trials.

## TH-PO469

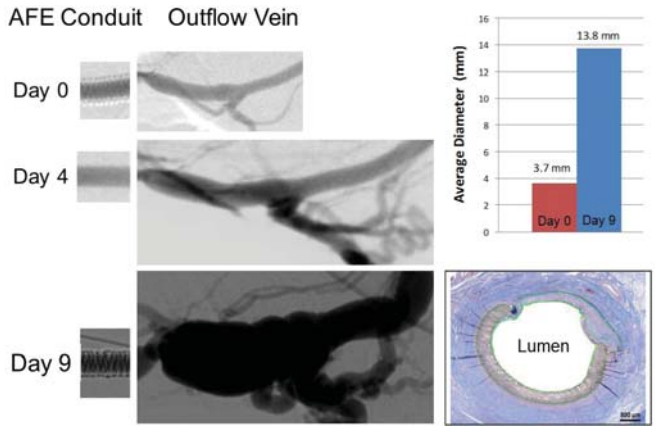
### Rapid Dilation of Porcine Peripheral Vein with Rotary Blood Pump System

Nicholas Franano,[1] Howard M. Loree,[2] Mark R. Cunningham,[3] Dale M. Groth,[3] Lesley A. Szenay,[3] Robert D. Ainsworth,[4] Barrett S. Hutto,[4] James Lee,[4] Steve P. Woodard,[4] Geoff D. Tansley.[5] *[1]Novita Therapeutics LLC, Olathe, KS; [2]Flow Forward Medical LLC, Lowell, MA; [3]Surpass Inc., Osceola, WI; [4]CIRTEC Medical Systems LLC, Los Gatos, CA; [5]School of Engineering, Griffith Univ, Gold Coast, QLD, Australia.*

**Background:** The arteriovenous fistula (AVF) is the preferred form of vascular access for hemodialysis. The Arteriovenous Fistula Eligibility (AFE) System™ is designed to dilate peripheral veins prior to AVF surgery by increasing vein wall shear stress (WSS) and stimulating vascular remodeling, thereby increasing AVF eligibility and improving AVF maturation.

**Methods:** The pre-clinical prototype device comprises a small extracorporeal centrifugal blood pump, cuffed and heparin-coated inflow and outflow conduits, and a benchtop power unit. In a 28 kg anticoagulated pig, the device was implanted in a left external jugular vein to left saphenous vein (SV) configuration and the SV was treated for 9 days with a WSS dose of 4 Pa.

**Results:** Pump flow increased from 270 mL/min on Day 1 to 947 mL/min on Day 9, and the outflow segment of SV dilated from 3.7 mm to 13.8 mm without angiographic evidence of stenosis. Necropsy on Day 9 showed emboli in the lungs and thrombus around the inflow conduit, consistent with known hypercoagulability in the pig model. The dilated SV was easily mobilized. Histology in the treated vein demonstrated remodeling and very minimal intimal hyperplasia, particularly at the graft anastomosis.




**Conclusions:** This pilot study shows the feasibility of the AFE System in a porcine model, demonstrating a 10 mm increase in vein diameter over 9 days of treatment and easily exceeding the 6 mm vein diameter threshold for successful AVF maturation. Ongoing work includes a larger nonclinical study and development of mobile electronics.

*Funding:* Pharmaceutical Company Support - Novita Therapeutics is a medical device technology incubator funded by Open Prairie Ventures II LP, Kansas Bioscience Authority, River Valley Investors, Wichita Technology Corporation, Mid-America Angels, Women's Capital Connection, and individual angel investors.

## TH-PO470

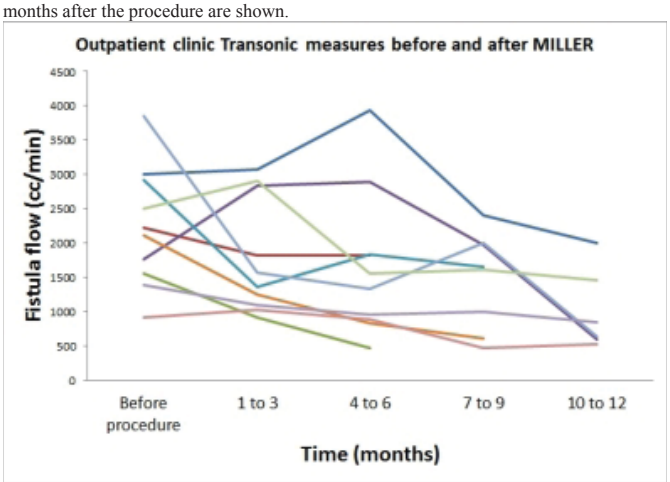
### Hemodialysis Fistula Flow Rates before and after MILLER Procedure: Intra-Operative Flows Are Lower Than Clinic Flows

Jackson Wang,[1] Andrew I. Chin.[1,2] *[1]Div of Nephrology, Univ of California, Davis School of Medicine, Sacramento, CA; [2]Div of Nephrology, VA Health Care, Northern California, Mather, CA.*

**Background:** The **M**inimally **I**nvasive **L**imited **L**igation **E**ndoluminal-assisted **R**evision (MILLER) procedure is a method of decreasing arterio-venous fistula (AVF) diameter to decrease flow rate in hemodialysis (HD) patients who have steal or excessive AVF flow rates. We sought to compare intra-operative AVF flow rates, before and after MILLER, with outpatient clinic measured flow rates.

**Methods:** We included HD patients who had recent MILLER procedures. Intra-operative and outpatient HD clinic flow measures (when available) were analyzed. Paired t-test was used to compare before and after values.

**Results:** 15 patients had MILLER procedures. Baseline factors: mean age 50±11 years; 40% men; 40% diabetic; 14 brachial artery AVFs, 1 femoral AVF. Intra-op AVF flow tests showed significant reduction after the MILLER (1666 ± 953 cc/min vs 849 ± 218 cc/min, p=0.003) with mean flow reduction of 41 ± 17%. All patients had decrease in symptoms. Outpatient clinic measured flow rates (n=10, Figure) before MILLER and for up to 12 months after the procedure are shown.




Outpatient flow rates were: pre-MILLER = 1965 ± 665; 1-3 Mos = 1733 ± 821; 4-6 Mos = 1573 ± 1073; 7-9 Mos = 1148 ± 659; 10-12 Mos = 1086 ± 562. Flow rates were not statistically lower until the 7-9 Mos time period after MILLER. Pre-MILLER outpatient flow rates were higher than intra-operative flow rates (p=0.04).

**Conclusions:** Intra-operative AVF flow rates were significantly lower than outpatient flow rates in patients undergoing MILLER. This may be due to hemodynamic effects of anesthesia, and/or difference in measurement technique. After MILLER, outpatient measured mean flow rates did decrease over time compared with pre-MILLER flow, but did not become statistically lower until 7-9 months later.

*Funding:* Clinical Revenue Support

## TH-PO471

**Arterial Micro-Calcification Is Associated with Coronary Artery Calcium Score in Hemodialysis Patients** Su Jin Choi, Young Soo Kim, Sunae Yoon, Young ok Kim. *Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Uijeongbu-City, Kyunggi-do, Korea.*

**Background:** We have reported that arterial micro-calcification (AMC) of vascular access has a negative impact on access patency and cardiovascular mortality in hemodialysis (HD) patients. Reasons behind increased cardiovascular mortality in AMC are not fully understood, but it is believed that aortic stiffness is a major contributing factor. Whereas, coronary artery calcification (CAC) is quite common in HD patients and it is known as predictor of future cardiovascular events and all cause mortality in HD patients. The aim of this study was to explore the relationship between AMC and CAC in HD patients.

**Methods:** Sixty-four HD patients who received vascular access operation were included in this study. The AMC was diagnosed by pathologic examination of arterial specimen by von Kossa stain, which was acquired during the operation. All patients underwent a multi-detector computed tomography (MDCT) imaging procedure and coronary artery calcium score (CACS) was calculated. Patients were classified into two groups, according to the CACS, as low (<100), in 28 patients, and high ( ≥100), in 36 patients. We compared CACS between the patients with and without AMC.

**Results:** Mean age was 65.8 ± 12.5 years and the male gender was 37 (57.8%). The incidence of AMC was 62.5% (n=40). The mean CACS was 439.3 ± 901.1 (0-5674.1), and the median value was 128.4. Patients with the positive AMC group showed a significantly older age (68.6 ± 10.2 vs 61.2 ± 14.7, p=0.036) and a higher prevalence of diabetes (85.0% vs 45.8%, p=0.001). Positive AMC group showed high incidence of high CACS compared to negative AMC group (77.5% vs 20.8%, p=0.000). By binary logistic regression, high CACS was independently associated with positive AMC (OR 8.894, 95% CI 1.174-46.154, p=0.008).

**Conclusions:** The present study suggests that AMC is closely associated with CACS in HD patients.

## TH-PO472

**Resistance to Erythropoiesis-Stimulating Agents May Be Associated with Arterial Micro-Calcification in Hemodialysis Patients** Su Jin Choi, Hye Sung Won, Young Soo Kim, Sunae Yoon, Young ok Kim. *Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea.*

**Background:** Vascular calcification, which is independent risk factor of cardiovascular mortality, and anemia are very common in hemodialysis (HD) patients. Some uremic milieu such as inflammation, oxidative stress, and mineral bone disturbance may contribute to these conditions. The aim of this study was to evaluate the relationship between arterial micro-calcification (AMC) and erythropoiesis-stimulating agent (ESA) hypo-responsiveness in HD patients.

**Methods:** 84 patients received with ESAs for anemia without iron deficiency were evaluated. We assessed ESA hypo-responsiveness of patients using ESA hypo-responsiveness index (EHRI), defined as the weekly ESA dose per kilogram of body weight divided by the hemoglobin level. The AMC was diagnosed by pathologic examination of arterial specimen by von Kossa stain, which was acquired during the vascular access surgery.

**Results:** AMC was detected in 35 (41.7%) patients. There were no significant differences between patients with and without AMC with respect to clinical characteristics except for age and the presence of diabetes. Among the 35 patients with AMC, 28 (80.0%) patients had diabetes compared with 16 (32.7%) of 49 patients without AMC (p=0.001). The serum levels of albumin and total cholesterol were higher in patients without AMC than in patients with AMC (p=0.048 and 0.014). The serum levels of phosphate, intact parathyroid hormone and calcium-phosphate product were lower in patients with AMC than in patients without AMC (p=0.010, 0.013, and 0.053). The mean EHRI value was higher in patients with AMC than in patients without AMC (p=0.043). The patients were divided into tertiles according to their EHRI values. 6 (21.4%) patients in T1 group, 12 (42.9%) patients in T2 group, and 17 (60.7%) patients in T3 group showed arterial micro-calcification, respectively (p=0.012). In the multivariate logistic regression analysis, diabetes and ESA hypo-responsiveness showed a significant association with arterial micro-calcification.

**Conclusions:** ESA hypo-responsiveness as well as diabetes may be clinically relevant parameters related to AMC in HD patients.

## TH-PO473

**Biocompatibility of Decellularized Bovine Artery and Expanded Polytetrafluorethylene Arteriovenous Grafts in a Sheep Model** Marcos Alexandre Vieira,[1,2] João Gabriel Roderjan,[1] Eduardo Discher Vieira,[1] Francisco D.A. Costa,[1] Miguel C. Riella.[1] *[1]Laboratory of the Center for Cardiovascular Grafts of Tissue Engineering and Cell Culture, Catholic Univ of Parana, Brazil; [2]Nephrology, Pró Rim Foundation, Brazil.*

**Background:** Early dysfunction is a major problem in expanded polytetrafluorethylene arteriovenous(ePTFE) grafts utilized for hemodialysis vascular access. The objective of this study was to evaluate the histological and functional results of decellularized heterografts,and to compare it with that of ePTFE.

**Methods:** ePTFE was implanted in 11 sheep between the carotid artery and the jugular vein and in 10 sheep with a internal thoracic bovine artery(DBA),decellularized following the Brazilian patent PI0800603-2 utilizing sodium dodecyl sulfate.Doppler was conducted prior to graft explantation.Stain:hematoxylin-eosin, alizarin red to assess calcification and Russel Movat's to study the extracellular matrix.Cellular in-growth, re-endothelialization, thrombosis, neovascularization, calcification, neointimal hyperplasia, inflammatory infiltration were studied.Immunohistochemistry:Antibody anti-CD34 [EP373Y](RabMAbs®).

**Results:** Decellularization was confirmed.The DBA extracellular matrix was preserved and repopulated by local tissue.Endothelial cells were expressed by CD34+cells.The DBA was more organized,with intima,media,and adventitia.Both groups exhibited neointimal hyperplasia.Five animals in the ePTFE group had thrombosis versus none in the DBA(p=0,036).A larger internal diameter of the DBA was identified by Doppler(p=0,024).

**Conclusions:** Experimental studies with decellularized grafts described good patency, as well as repopulation of the graft with host cells.The endothelial and hematopoietic progenitor cells was confirmed by staining for CD34, supporting the histologic findings of re-endothelialization, cell growth, migration, and tissue replacement.It is possible that improvement of the blood-graft interface could decrease the rate of thrombosis in the DBA group.The larger internal graft diameter in the DBA group may be related to a mechanism of distensibility similar to what occurs in an arteriovenous fistula.The results suggest a better biological behavior associated with DBA in a sheep model.

*Funding:* Private Foundation Support

## TH-PO474

**In Vivo Tissue Engineered Blood Vessel as Vascular Graft in a Porcine Model** Carolien Rothuizen,[1] Febriyani Damanik,[2] Michel Visser,[4] Tom Lavrijsen,[3] ChunYu Wong,[1] Martijn Cox,[3] Ton J. Rabelink,[1] Lorenzo Moroni,[2] Joris I. Rotmans.[1] *[1]Dept of Nephrology, Leiden Univ Medical Center, Netherlands; [2]Dept of Tissue Regeneration, Univ Twente, Netherlands; [3]Xeltis BV, Netherlands; [4]Dept of Surgery, Leiden Univ Medical Center.*

**Background:** ePTFE grafts are still commonly used as arteriovenous(AV) graft for hemodialysis vascular access, despite their risk of infection and thrombosis. Tailor made tissue engineered blood vessels (TEBVs) that resemble a native vessel may be a suitable alternative. We developed an autologous TEBV generated in the body itself and evaluated its efficacy as vascular graft in a porcine model. Our in vivo tissue engineering method utilizes the foreign body response directed to implanted synthetic material. Implantation of a cylindrical shaped polymer rod evokes an inflammatory response culminating in the encapsulation of the rod by a tube shaped fibrocellular tissue capsule. After extrusion of the rod and grafting the tissue capsule in the vasculature, it can further differentiate into an adequate vascular access conduit.

**Methods:** Per pig, 4 polymer rods were implanted subcutaneously. After 4 weeks, rods with tissue capsules formed around it were harvested and analysed by histology and mechanical tests. Per pig, 1 tissue capsule was implanted as AV-graft in between the carotid artery and jugular vein for 4 weeks and analyzed on wall remodeling.

**Results:** Before grafting, tissue capsules were mainly composed of circumferentially aligned myofibroblasts and few leukocytes. Extracellular matrix consisted mainly of glycosaminoglycans and circular aligned collagen. Tissue capsules exhibited burst pressures of ca. 2000 mmHg and suture strengths of >3N, both exceeding the mechanical properties of native vessels. After 4 weeks of grafting, tissue capsule wall was remodelled, with a 2-fold increase in collagen and 4.3-fold increase in myofibroblast content, while the leukocyte content was reduced to 1%.

**Conclusions:** Using an in vivo tissue engineering approach, a completely biological, autologous TEBV was created with sufficient mechanical strength to enable AV-grafting. Currently we are evaluating patency of these TEBV as vascular graft.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO475

**Novel Antithrombotic Fusion Protein to Inhibit Arteriovenous Graft Stenosis** Christi M. Terry,[1] Huan Li,[1] Ilya S. Zhuplatov,[1] Tze-Chein Wun,[2] Alfred K. Cheung.[3,1] *[1]Univ of UT, SLC, UT; [2]EVAS Therapeutics, Ballwin, MO; [3]VASLHCS, SLC, UT.*

**Background:** Coagulation generates activated platelets, thrombin, fibrin and mural thrombi that induce cell proliferation, migration, and matrix deposition that lead to neointimal hyperplasia (NH) and stenosis of arteriovenous grafts (AVG). Yet current antiplatelet and anticoagulant drugs, that yield systemic anti-coagulation, do not substantially prevent AVG stenosis when administered in clinically safe doses. A novel antithrombotic protein has been developed to *locally* inhibit thrombosis: A6L15 consists of an annexin V domain that targets the protein to thrombogenic cell membranes, fused to a Kunitz protease inhibitor domain that inhibits the tissue factor/VIIa complex. A6L15 has potential for safely inhibiting thrombus-induced NH formation and its administration safety was examined in a pig model of AVG stenosis.

**Methods:** Pigs received an i.v. bolus of A6L15 protein (300 μg). A segment of the carotid artery and the ipsilateral jugular vein were isolated by clamping and irrigated with 5, 20 or 100 μg/mL of A6L15 for 5 min. The lumen of a polytetrafluoroethylene graft was filled with A6L15 at the same concentration as the native vessels, then placed between the artery and vein segments. Control pigs had AVG placed but received systemic heparin (n=4) or no anticoagulants (n=1).

**Results:** No bleeding episodes were observed in any animal at any time during the 4-week follow-up. The AVG of the untreated pig clotted at the time of surgery, while the AVG of 1 of 9 pigs treated with high-dose A6L15 clotted at 4 wks. ACT and aPTT at 4 wks were unchanged with either A6L15 or heparin, compared to pre-operative values. In contrast, PT were marginally prolonged after heparin treatment (10.8±0.5 vs. 10.3±0.4 sec; p=0.05 paired t-test; n=4) but not after the highest dose A6L15 treatment (11.0±0.45 vs. 10.8±0.4 sec; p=0.22; n=9). Less fibrosis was noted around the AVG in the 6L15-treated pigs, compared to controls.

**Conclusions:** The A6L15 fusion protein appears safe and well-tolerated. Efficacy studies are ongoing to determine if these fusion proteins inhibit NH associated with AVGs. Preliminary results appear to be promising.

*Funding:* Other NIH Support - NHLBI 2R44HL093848

## TH-PO476

**Differences in Coagulation Protein Concentrations, Platelet Function, and Viscoelasticity among Clot-Forming, and Non-Clot-Forming Hemodialysis Patients with Arteriovenous Grafts**

Chinedu P. Nweke,[1] Daniel E. Carl,[1] Erika Martin,[1] Todd W. Gehr,[1] Donald F. Brophy.[2] *[1]Virginia Commonwealth Univ; [2]Virginia Commonwealth Univ School of Pharmacy.*

**Background:** Arteriovenous Graft thrombosis is a frequent cause of graft failure. We evaluated thrombogenic factors in 20 HD patients with AVGs. The goal was to determine whether significant differences in protein concentrations, platelet function and viscoelasticity factors exist among dialysis patients requiring frequent AVG declot procedures vs those who do not.

**Methods:** 20 HD patients were enrolled: 10 frequent clotters (>3 declots in the previous year) and 10 were non-clotters. Patients on anti-platelets or chronic anticoagulation were excluded. Coagulation protein concentrations including tissue factor (TF), tissue factor pathway inhibitor (TFPI), prothrombin fragment 1+2 (F1+2), von Willebrand's factor (vWf), and fibrinogen were assayed. The time to clot onset was measured by Force Onset Time (FOT) and the Reaction time (R). K represents the time from clot initiation until final clot firmness. Platelet contractile force (PCF) measured the force produced by platelets during clot retraction, while clot rigidity was measured as clot elastic modulus (CEM). FOT, CEM and PCF were measured by Hemodyne; R and K were measured by TEG.

**Results:** There were no significant differences in coagulation protein concentrations and platelet function; however most viscoelasticity factors were statistically significantly different in clotters compared to non-clotters.

| | NORMAL | CLOTTERS | NON-CLOTTERS | P Value |
|---|---|---|---|---|
| PCF | 4.0-9.5 Kdyne | 10.4±4.4 | 6.0±0.7 | 0.003 |
| CEM | 14.0-35.0 kdyn/cm$^2$ | 37.0±14.8 | 21.9±6.4 | 0.008 |
| FOT | 3.0-8.0 min | 6.0±1.9 | 8.1±2.3 | <0.05 |
| R | 3.0-8.0 min | 7.0±1.7 | 8.7±1.7 | 0.04 |
| K | 1.0-3.0 min | 1.4±0.4 | 1.7±0.2 | NS |

**Conclusions:** Our study suggests that HD patients with recurrent AVG thrombotic events form more rapid clots with higher tensile strength compared to HD patients without recurrent graft thrombosis. Identifying effective therapies in modulating these properties that confer higher tensile strength and viscoelasticity as observed among clot-formers may be worth investigating.

## TH-PO477

**Buttonhole versus Rope-Ladder Cannulation for Hemodialysis – A Systemic Review**

Ben C. Wong,[1] Maliha Muneer,[2] Dale Storie,[3] Sabin C. Shurraw,[1] Alexa L. Grudzinski,[4] Gihad E. Nesrallah,[5] Neesh I. Pannu,[1] Robert P. Pauly.[1] *[1]Div of Nephrology and Transplant Immunology, Univ of Alberta, Edmonton, Canada; [2]Alberta Health Services, Edmonton, Canada; [3]Univ of Alberta, Edmonton, Canada; [4]Humber River Regional Hospital; [5]Univ of Western Ontario, London, Canada.*

**Background:** The buttonhole (BH) technique has commonly been a method of arteriovenous fistula (AVF) cannulation in hemodialysis (HD) patients, particularly among patients performing independent home HD. However, an association between BH and a higher incidence of access-related infections has been increasingly noted. We sought to systematically review the documented risks and benefits of BH compared to standard rope-ladder (RL) among HD patients with AVFs.

**Methods:** An extensive search of medical and nursing databases was conducted to identify relevant studies. The results were manually reviewed to identify studies that compare the BH with RL on the following outcomes: cannulation pain, access failure and intervention, infectious outcomes, hemostasis time, and risks of hematoma and aneurysm formation. We excluded the following studies: abstracts, case reports, review articles or editorials without original data, non-English publications, and those that did not include relevant outcomes.

**Results:** Of the 1145 citations, 17 studies were selected for inclusion. Substantial heterogeneity among the studies precluded meta-analysis. BH, as compared to RL, appeared to be associated with an increased risk of local and systemic infections, while conferring a potential benefit in access survival, and reduced risks of hematoma formation and aneurysm formation. There appeared to be equivocal evidence with respect to cannulation pain, the need for access intervention, and hemostasis time.

**Conclusions:** Evidence does not support the preferential use of BH over RL cannulation in either facility-based conventional HD or home intensive HD. This does not preclude BH cannulation as being appropriate for some patients with difficult-to-access AVF.

## TH-PO478

**Utility of Preoperative Vascular Mapping to Select Patients at High Risk for Early Thigh Hemodialysis Graft Failure**

Mark Little,[1] Michael Allon,[2] Michelle McNamara,[1] Song Ching Ong,[2] Mark E. Lockhart,[1] Carlton Young,[3] Michelle L. Robbin.[1] *[1]Dept of Radiology, Univ of Alabama at Birmingham; [2]Dept of Medicine, Univ of Alabama at Birmingham; [3]Dept of Surgery, Univ of Alabama at Birmingham.*

**Background:** To determine whether noninvasive preoperative evaluation of vascular diameters and calcification can identify patients in whom arteriovenous thigh graft survival is likely to be impaired.

**Methods:** IRB approval was obtained and informed consent was waived. Retrospective analysis, including a qualitative assessment of calcification burden, was performed on 143 hemodialysis patients who received ultrasound vascular mapping prior to thigh graft placement. Severity of pelvic arterial calcification was scored in 80 patients who received peri-operative CT, using a semi-quantitative 5-point scale. Patient characteristics and graft outcomes were examined.

**Results:** Preoperative ultrasound screening identified no or mild arterial calcification in 113 of 143 patients (79%) and moderate to severe calcification in 30 of 143 patients (21%). Patients with moderate to severe calcification had significantly increased technical graft failure (hazard ratio=6.59; 95% CI, 2.06-21.05; p=0.002) and decreased cumulative graft survival (hazard ratio=2.32; 95% CI, 1.48-6.69, p= 0.003) compared to patients with no or mild disease. Cumulative graft survival was not associated with venous (HR 1.06; 95% CI, 0.63-1.80, p=0.82) or arterial diameters (HR 1.19; 95% CI, 0.70-2.03, p=0.43). Low CT calcification score was seen in 74 of 80 patients (93%). Primary technical failure occurred in 3 of 6 patients (50%) with high, versus 5 of 74 patients (6.8%) with low calcification score (hazard ratio=7.4; 95% CI, 2.31-23.72, p=0.01). US was more sensitive (64% versus 38%) but less specific (83% versus 96%) than CT in predicting immediate technical graft failure. Positive predictive value of CT and US was 50% and 23%, respectively.

**Conclusions:** Preoperative sonographic assessment of thigh vessel diameter and calcification can select patients who need further CT assessment of pelvic calcifications to identify those at higher risk for primary technical graft failure and decreased cumulative graft survival.

## TH-PO479

**Ultrasonographic Characteristics of Vessels and Survival of AV Fistulas in Octogenarian Population**

Vanja Persic, Rafael Ponikvar, Miha Arnol, Jadranka Buturovic-Ponikvar. *Dept of Nephrology, Univ Medical Center, Ljubljana, Slovenia.*

**Background:** Due to the limited life expectancy, decreased functional status and comorbidities that damage the vessels (e.g. diabetes) native arteriovenous (AVF) placement remains questionable in elderly end-stage renal disease (ESRD) population. We aimed to compare the ultrasonographic (US) characteristics of vessels and the outcome of created AVFs in octogenarians versus younger old patients.

**Methods:** 56 patients aged ≥80 years (G ≥80) and 158 aged between 65 and 80 years (G<80), with the first attempt of AVF placement participated in this retrospective study. Internal diameters (ID) of arteries and veins of both arms and forearms were evaluated by preoperative duplex US examination, and presence of atherosclerotic changes and calcifications was assessed. Position of AVF was recorded after the AVF construction and estimation of patency rates was performed using Kaplan-Meier survival analysis. Cox proportional hazard model was used to determine the effect of age on the primary and secondary AVF patency rate.

**Results:** IDs of cephalic veins and cubital arteries were similar between the two groups, but IDs of radial arteries (RA) were smaller in G ≥80 (right RA 2.1±0,4 vs 2.3±0.5 mm, P=0.04; left RA 2.1±0,5 vs 2.3±0.5 mm, P= 0.06). There was no significant difference in frequency of atherosclerotic changes and calcifications between G ≥80 and G<80 (63% vs 61%, P=0.88 and 54% vs 45%, P=0.27, respectively). AVF was placed in 38 (68%) patients in G ≥80 and in 121 (77%) in G<80 (P=0.2). Patients in G<80 more often received forearm AVF than patients in G ≥80 (82% vs 66%, P=0.04). Primary failure was 17% in both groups. No significant differences between the groups were observed in one-year primary and secondary patency rates (G ≥80: 67% and 77 %; G<80: 68% and 76%; P=0.20 and P=0.22, respectively). Patient age ≥80 years was not an independent predictor of AVF loss (HR 1.47, 95% CI 0.76-2.81; P=0.25).

**Conclusions:** Our results demonstrate that even in patients ≥80 years of age, native AVF can be successfully created with good cumulative patency rate at one year. In our opinion, any patient with ESRD, regardless of age, should be evaluated for AVF placement.

## TH-PO480

**Pre-Operative Fistula Assessment with Ultrasound: Accuracy and Association with Outcome**

Damien Ashby, Jeremy Crane. *Imperial College Kidney and Transplant Centre, Imperial College London, London, United Kingdom.*

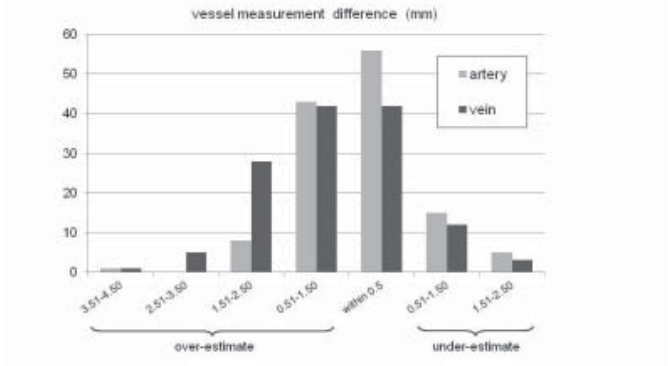
**Background:** The success of arteriovenous fistula formation depends on the quality of artery and vein. The accuracy of pre-operative vessel measurement and relevance to surgical outcome are unclear.

**Methods:** In all patients undergoing elective upper-limb fistula creation during an 18 month period, pre-operative ultrasound vessel measurements were compared with direct intra-operative measurements, and clinical outcomes recorded.

**Results:** In 141 patients (aged 18-85, 71.9% male), 146 fistulae were formed, comprising 18 radiocephalic, 111 brachiocephalic and 17 brachiobasilic.

Pre-operative measurements were closely correlated with intra-operative findings for both arteries (R=0.44, p<0.001) and veins (R=0.35, p<0.001) but biased towards over-estimation for both.

Arterial assessment was reasonably reliable with arteries being within 0.5mm of expected size in 33.6%, and smaller than expected in 40.6%. For vein diameter the pre-operative measurement was less reliable, with veins being within 0.5mm of expected size in 31.6%, and smaller than expected in 57.1%. Gross over-estimation of vein size was common with 25.6% being more than 1.5mm smaller than expected.




The measurements taken intra-operatively appeared to be better predictors of outcome, with primary failure occurring in only 5.7% of cases with optimal vessels at surgery (both artery and vein over 2mm) versus 13.3% of cases where vessels were deemed optimal pre-operatively (p=0.10). If vessel measurements had been more accurately known up to 53% of surgical failures could have been avoided.

**Conclusions:** Pre-operative ultrasound provides reasonable prediction of vessel sizes prior to fistula surgery, but size over-estimation is common, particularly with veins. Measured intra-operatively, both artery and vein size are important predictors of outcome – further refinement of venous assessment could therefore improve access planning.

*Funding:* Clinical Revenue Support

## TH-PO481

### Haemodialysis Access: Patient Reasoning and Fistula Refusal

Damien Ashby, Lina Johansson, Neill D. Duncan, Jeremy Crane. *Imperial College Kidney and Transplant Centre, Imperial College London, London, United Kingdom.*

**Background:** Compared to other forms of access, arteriovenous fistulae are associated with improved outcomes in haemodialysis patients. Guidelines and incentives have been developed to increase fistula prevalence but some patients are reluctant to undergo fistula formation.

**Methods:** Patient reasoning, knowledge and influences were explored using questionnaires and brief structured interviews, in a group of prevalent haemodialysis patients declining fistula access.

**Results:** Fifty-eight patients (aged 34–87, 58% male) currently dialysing on a tunnelled line were identified. All completed questionnaires and 16 patients went on to give structured interviews.

Most reasons for declining fistula formation were common to a number of patients, including pain during dialysis (cited by 71%), appearance (67%), pain at home (57%), permanence (24%) and fear of bleeding (21%). The risk of surgical failure was often cited (eg. "my veins are really difficult") but other individual-specific reasons were also often given (eg. "I work in a prison"; "I wouldn't be able to use crutches") which were considered by the patient to outweigh other considerations.

All patients understood what a fistula is and were able to describe key features. Most patients reported being told that a fistula is a safer form of access, but in structured interviews, all patients clearly thought that this is not the case.

Other patients were far more influential than professionals in decision making (cited by 87% and 26% respectively, p<0.001). Some patients expressed mistrust of professional advice (eg. "it was told very one-sidedly") often associated with perceived pressure (eg. "they tried to convince me"; "because its more cost-effective"), and all patients felt strongly that access is a personal choice.

**Conclusions:** Patient-specific components to reasoning are not uncommon - many have individual and rational reasons which outweigh generic advice. Decision-making is more influenced by other patients than by professionals, with negative observations leaving a lasting impression. Better understanding of patient reasoning could improve concordance between patient and professional and facilitate informed choice.

*Funding:* Clinical Revenue Support

## TH-PO482

### Ultrasound-Guided Percutaneous Thrombolysis in Patients with Thrombosed Hemodialysis Grafts

Sara M. Vigano,[1] Giuliana Loizzo,[2] Giuseppe Bacchini,[1] Francesco Locatelli.[1] *[1]Nephrology and Dialysis, A. Manzoni Hospital, Lecco, Italy; [2]Nephrology, Dialysis and Transplantation, Univ of Bari, Bari, Italy.*

**Background:** The purpose of this study is to assess the safety and efficacy of the Arrow-Trerotola percutaneous thrombolysis device (PTD) using ultrasound-guided technique in the treatment of thrombosed vascular access grafts in dialysis patients and to evaluate the immediate and long term outcome in this cohort of patients.

**Methods:** Fifty-nine patients with graft occlusion underwent mechanical thrombolysis with PTD during 107 months. To maintain the patency during the procedure we used saline solution (0.9 %) with 5000 UI of Sodic Heparin. All patients had large clot burden. Technical success, complications, primary and secondary patency rates were noted. Kaplan-Meier analyses were performed.

**Results:** The mean follow-up period was 29.2 months. Whereas patients with a graft vascular access are subjected to a regular Doppler follow-up, the technical success rate was 100%, while the clinical success rate was 91.7%. There were no major complications. Now a day the grafts are still functional in 6 patients. In 47 patients, additional procedures were needed for patency. The 3-month primary and secondary patency rates were 42.2 % and 73.3 % respectively. In fourteen patients the procedure was performed twice, in eight patients three times, in two other patients four and five times respectively.

**Conclusions:** Ultrasound-guided percutaneous mechanical thrombolysis with the Arrow-Trerotola device is a safety and less invasive method for the treatment of thrombosed grafts; it potentially allows to remove clots with minimal risk of wall damage requiring the administration of small amounts of thrombolytics.Moreover it is an out-patients procedure,that not imply patients' hospitalizationand it is performed in a sterile room. Furthermorepatients are not exposed to radiation and to iodinated contrast agents. Thus this procedure could be a valid alternative to the surgical thrombectomy.

## TH-PO483

### Uremia Induces a Proliferative Response through SERCA2a and Related Genes Dysregulation: A Novel Theory for Artero-Venous Fistula Failure

Bertrand N. Mukete,[1,2] Georges Khoueiry,[1] Suchita J. Mehta,[1] Lahouaria Hadri,[2] Jasvinder Singh,[1] Jared M. Radbel,[1] Chadi Saifan,[1] Elie El-Charabaty,[1] Frank M. Rosell,[1] Marianne Smith,[1] Roger Hajjar,[2] Suzanne E. El Sayegh.[1] *[1]Staten Island Univ Hospital, NY; [2]Mount Sinai School of Medicine, NY.*

**Background:** Sacoendoplasmic reticulum calcium ATPase-2a (SERCA2a) has been shown to be reduced in proliferating vascular smooth muscle cells while SERCA2b is upregulated. Arterio-venous fistula (AVF) closure is a major cause of morbidity in patients with end stage renal disease (ESRD). The molecular basis of AVF closure has not been fully elucidated and we hypothesize that the expression of SERCA2a and related genes are affected in subjects with ESRD.

**Methods:** In this case control study, we enrolled 18 patients, nine with ESRD going for AVF insertion and nine with normal renal function (NRF) going for coronary artery bypass surgery. Brachial and saphenous veins from ESRD and NRF patients respectively were isolated. Gene expression for SERCA2a, SERCA2b, phospholamban (PLN), endothelial nitric oxide synthase (eNOS) and CD31 were measured using real time quantitative polymerase chain reaction. Hematoxylin/eosin staining was used to analyze the integrity of the vessels and immunoflouroscense microscopy (IFM) was used to visually quantify genes of interest. Two-tailed t-test was used to calculate the difference between means and 95% confidence interval (CI) between the two groups.

**Results:** The difference in mean and 95% CI for gene expression in NRF versus ESRD was +1.9 (0.74 to 3.1), p < 0.01 for SERCA2a, -1.3 (-2.5 to -0.16), p = 0. 03 for SERCA2b, +1.3 (0.59 to 2.0), p < 0.01 for PLN, -4.4 (-9.6 to 0.83), p = 0.09 for eNOS and -1.1 (-3.8 to 1.5), p = 0.37 for CD31. Protein expression was confirmed using IFM.

**Conclusions:** SERCA2a was significantly decreased while SERCA2b was increased in the brachial vein of ESRD subjects when compared to corresponding genes in the saphenous veins of subjects with NRF. The level of eNOS expression was statistically unchanged in both groups. Our findings suggest a pro-proliferative response of ESRD based on SERCA2 expression, which may exacerbate or accelerate AVF shunt failure in ESRD subjects.

*Funding:* Other U.S. Government Support

## TH-PO484

### Bone Morphogenetic Protein-7 (BMP-7) Inhibits Oxidative Stress-Induced Monocyte Chemoattractant Protein-1 (MCP-1) Expression in Vascular Endothelial Cells

Yong-Soo Kim, Jeong-Sun Han. *Div of Nephrology, The Catholic Univ of Korea College of Medicine, Seoul, Korea.*

**Background:** Oxidative stress and activation of chemokines including MCP-1 are known to be downstream mechanisms responsible for the neointimal hyperplasia after creation of hemodialysis vascular access.

**Methods:** After stimulating the human umbilical vein endothelial cells with $H_2O_2$, cell viability, MCP-1 mRNA expression (by quantitative real-time PCR), MAPK activity and transcription factors activities including NF-kB and AP-1 (by Western blot) were measured. Oxidative stress was analyzed by confocal microscopy using 2',7'-dichlorofluorescin diacetate (DCF-DA) and by measuring 8-isoprostane in cell culture medium.

**Results:** Cell viability was over 90% when the cells were incubated with 0.5 mM of $H_2O_2$ for 4 hr. 0.5 mM $H_2O_2$ stimulated MCP-1 mRNA expression in a dose- and time-

dependent manner with a peak at 4 hr. $H_2O_2$ stimulated phosphorylation of p38 and JNK with a peak at 30 min, respectively. In addition, $H_2O_2$ stimulated phosphorylation of NF-kB subunit, p65, and degradation of cytoplasmic IkBa, and also stimulated phosphorylation of AP-1 subunits, c-Jun and c-Fos. When the cells were pre-incubated with SB203580 (P38 inhibitor), SP600125 (JNK inhibitor), pyrrolidine dithiocarbamate (NFkB inhibitor), or curcumin (AP-1 inhibitor), $H_2O_2$-induced MCP-1 mRNA expression was significantly inhibited. BMP-7 significantly inhibited $H_2O_2$-induced MCP-1 mRNA expression in a dose-dependent manner. BMP-7 significantly inhibited $H_2O_2$-induced intracellular ROS production and 8-isoprostane levels in cell culture medium. BMP-7 significantly inhibited $H_2O_2$-induced phosphorylation of JNK, c-Jun and c-Fos, but did not inhibit $H_2O_2$-induced phosphorylation of p38 and p65.

**Conclusions:** These data suggest that oxidative stress directly stimulates MCP-1 expression in vascular endothelial cells and BMP-7 inhibits it through the anti-oxidant effect and inhibition of JNK-AP-1 pathway. This study provides a new insight into the potential of BMP-7 as an anti-oxidant to prevent neointimal hyperplasia in vascular access.

## TH-PO485

**Association of Intravenous Vitamin D Receptor Activator and Sevelamer with Vascular Access-Related Hospitalization: Japan-DOPPS**
Masatomo Taniguchi,[1] Hirotaka Komaba,[2] Takayuki Hamano,[3] Masafumi Fukagawa.[2] *[1]Depts of Medicine & Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Div of Nephrology, Endocrinology and Metabolism, Tokai Univ School of Medicine, Isehara, Japan; [3]Dept of Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Osaka, Japan.*

**Background:** Only a few studies have investigated the association between mineral and bone disorders (MBD) and the risk of hospitalization.

**Methods:** The subjects were 2,215 Japanese hemodialysis patients registered in the Japan-DOPPS Phase III. Main exposure is the medication for MBD, administration of vitamin D receptor activator (VDRA) and phosphate binder (sevelamer, calcium carbonate). Serum phosphorus (P), adjusted calcium (Ca) and parathyroid hormone (PTH) concentrations was entered into the model as covariates. Main outcome was association hospitalization due to cardiovascular disease [CVD], infection and vascular access. Using counts model including the Poisson and negative binomial regression, the association between the drugs and outcome was investigated by the "inverse probability of treatment weighting" method.

**Results:** The total number of hospitalizations was 1,098. Serum P, Ca, and PTH concentrations showed no significant association with each hospitalization. Despite association no statistically significant relationship of VDRA with the hospitalization for CVD or infection, a significant decrease in vascular access-related hospitalization was observed in VDRA users (rate ratio [RR] = 0.67: 95% CI = 0.47-0.95). The risk of vascular access-related hospitalization was also significantly decreased in sevelamer user (RR = 0.71: 95% CI = 0.52-0.98), but not in calcium carbonate user (RR = 0.93: 95% CI = 0.70-1.24). In stratified analyses, both VDRA and sevelamer was associated with markedly decreased risk of vascular access-related hospitalization in male patients.

**Conclusions:** Administration of VDRA or sevelamer was associated with reduced risk of vascular access-related hospitalization, suggesting that practice patterns for MBD are involved in vascular patency.

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## TH-PO486

**Clinicopathologic Correlations of Explanted Fistulas and Synthetic Grafts**
Laura Minhui Kim, Pooja Gupta, Rohan John, Jagdish Butany, Charmaine E. Lok. *Toronto General Hospital, Toronto, Canada.*

**Background:** In hemodialysis patients, both fistulas (AVF) and grafts are associated with complications which may require their excision. In contrast, kidney transplanted patients may have arteriovenous accesses that are no longer required and may be excised if they become problematic. We aimed to describe the histopathologic changes associated with excised fistulas and grafts to determine if there is any association with their clinical reason for removal.

**Methods:** All explanted AVF and grafts from 2001-2012 were identified from the Surgical Pathology database of Toronto General Hospital, and detailed histopathologic evaluation performed. Differences in pathologic findings versus access type were compared by Chi-Square (SAS, v9.2).

**Results:** Among 110 explanted specimens, there were 64 AVF and 46 grafts. Patients with AVF were on average 47 years old with 69% males; and those with grafts were 59 years old with 39% males. Patients with grafts were more likely to be hypertensive and have coronary artery disease. Glomerulonephritis was the most common cause of ESRD in both groups. Successful transplant with problematic (e.g. aneurysmal or symptomatic) access was the most common reason for fistula removal whereas aneurysm and infection were the most likely reasons for grafts. There was significantly more adventitial chronic inflammation ($p<0.0001$), medial acute inflammation ($p=0.01$), medial chronic inflammation ($p=0.03$) and intimal acute inflammation ($p=0.004$) in explanted grafts compared to fistulas. The media of synthetic grafts was also more prone to calcification (<0.0001). Neointimal proliferation was commonly seen in both fistulas and synthetic grafts (95% and 93% of specimens in each group).

**Conclusions:** Histopathologically, synthetic grafts were more commonly inflamed, likely corresponding to infection as a more common reason for graft removal compared to AVF. Significant vessel inflammation existed in AVF that would have otherwise been functional if not for transplantation, indicating that inflammation itself cannot be a sole pathologic criteria distinguishing between infection and other causes of inflammation. Gram staining and developing a classification system may be helpful in this regard.

*Funding:* Clinical Revenue Support

## TH-PO487

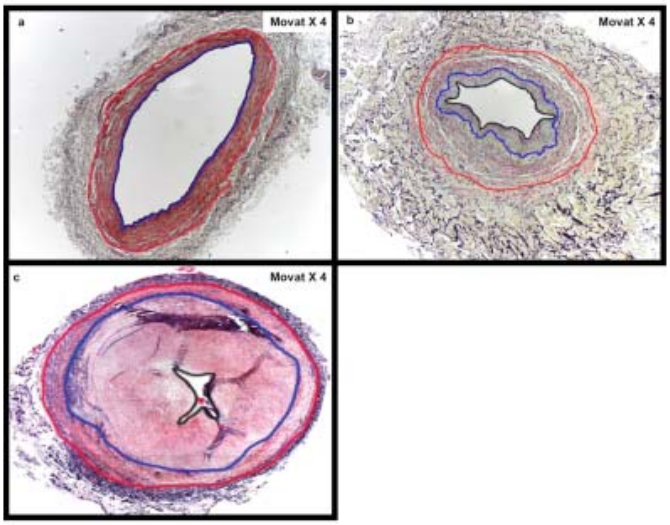
**Natural History of Venous Morphologic Changes in Dialysis Access Stenosis**
Timmy C. Lee,[1,2] Maheshika Srimali Somarathna,[1] Begoña Campos,[1] Lois J. Arend,[3] Prabir Roy-Chaudhury.[1] *[1]Dept of Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Dept of Medicine, Univ of Alabama at Birmingham, Birmingham, AL; [3]Dept of Pathology, Johns Hopkins Univ, Baltimore, MD; [4]Dept of Pathology, Univ of Kentucky, Lexington, KY.*

**Background:** The natural history of arteriovenous fistula (AVF) non-maturation is likely influenced by vascular insults to the vein prior to and after AVF creation and involves different responses to vascular injury from uremia independently, and uremia in combination with hemodynamics. The objective of this study was to perform a morphometric analysis from vein specimens from subjects without chronic kidney disease (CKD), collected at time of new surgery, and from failed stenotic AVFs.

**Methods:** Vein samples from 11 non-kidney disease subjects from deceased donors, 29 CKD subjects at time of new access creation, and 20 subjects with stenotic AVF were collected and morphometric analysis performed.

**Results:** The mean values of average intimal/media thickness and maximal intimal/media thickness±S.E. for veins from subjects with non-CKD, at time of new access creation, and stenotic AVFs were 0.16±0.02 and 0.49±0.10, 0.43±0.07 and 0.86±0.14, and 3.84±0.55 and 7.78±0.88, respectively. Using ANOVA, the mean values were significantly different between the average and maximal intimal/medial thickness analyses, $p<0.0001$. Among non-CKD samples diabetes ($p=0.0007$) was associated with increased average intima/media thickness.

**Conclusions:** Our results show significantly progressive increases in neointimal hyperplasia from non-CKD to stenotic vein samples (see figure). These changes suggest that vascular injuries from uremia and hemodynamics may play a key role in these progressive vascular changes and natural history of vascular access dysfunction.




**Histology of Vein Specimens.** Representative sample from veins collected from (a) non-CKD donor vein sample, (b) vein from advanced CKD subject at time of access surgery, and (c) vein sample from an early AVF failure. Note the progressive increase in amount of neointimal hyperplasia from non-CKD period to early failure.

*Funding:* NIDDK Support

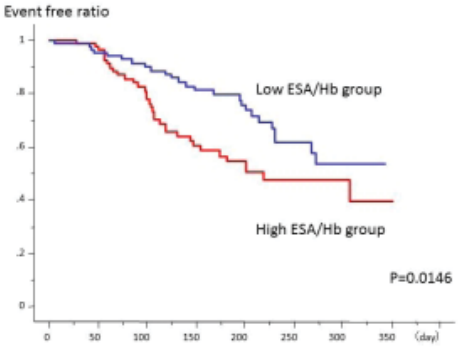
## TH-PO488

**Erythropoiesis Stimulating Agents (ESA)-Hyporesponsiveness Is Associated with Second Patency Rates of Vascular Access in Patients on Maintenance Hemodialysis (MHD)**
Yukiko Hasuike,[1] Naoto Kakita,[2] Motohiro Kamimura,[2] Shoji Kaibe,[1] Soshi Yorifuji,[1] Takeshi Nakanishi.[1] *[1]Dep. Internal Medicine, Div Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]Vascular Access Center, Tanaka-Kitanoda Hospital, Sakai, Osaka, Japan.*

**Background:** Malfunction of the vascular access (VA) is an important cause of poor prognosis in MHD. The purpose of this study was to evaluate the factors affecting VA patency after percutaneous-transluminal angioplasty (PTA).

**Methods:** A total 246 HD patients who were treated with PTA were enrolled. The end points were the re-vascularization of VA during the observational period. ESA/hemoglobin ratio (ESA/Hb) was calculated as an index of ESA-responsiveness. The blood flow volume and resistance index of VA were evaluated by Doppler ultrasonography, and tibial- and ankle-brachial index (ABI) were measured by ABI-form.

**Results:** Dialysis vintage of participants was 69.8±10.4 months, and 134 patients (54.5%) were female, 176 (71.5%) have native arteriovenous fistula, 169 (68.7%) were administered ESA. During follow-up period, re-vascularization was performed in 146 patients (59.3%). Graft use, oral anticoagulant administration, higher ESA/Hb, and lower ABI were associated with patency rates. Based on the median value of ESA/Hb, all the patients were divided into 2 groups: the High and Low ESA/Hb group. The Kaplan-Meier analysis showed High ESA/Hb was significantly associated with VA failure event (p=0.0146).




Cox regression analysis revealed that not only AV graft (hazard ratio 2.267, p=0.0013) but also High ESA/Hb (hazard ratio 1.773, p=0.0303) were related to VA failure event.

**Conclusions:** The present research indicates that ESA-hyporesponsiveness is associated with the risk of VA failure. ESA-hyporesponsiveness could influence on the patency of VA. Further evidence is required to validate the maintenance of long-term patency of VA in MHD.

## TH-PO489

### Effect of Arteriovenous Hemodialysis Shunt Location on Cardiac Events in Patients Having Coronary Artery Bypass Graft Using an Internal Thoracic Artery

Leonid Feldman,[1,2] Shai Efrati,[1,2] Ilia Beberashvili,[1,2] Zhan Averbukh.[1,2]
*[1]Nephrology Dept, Assaf Harofeh Medical Center, Zerifin, Israel; [2]Sackler School of Medicine, Tel Aviv Univ, Tel Aviv, Israel.*

**Background:** The possibility of developing coronary steal in patients having coronary artery bypass graft (CABG) using internal thoracic artery (ITA) and ipsilateral upper extremity arteriovenous (AV) hemodialysis shunt has been reported. The impact of this phenomenon on clinical outcomes is uncertain. The aim of this study was to investigate an association between the AV dialysis shunt location regarding the side of the ITA CABG and clinical outcomes.

**Methods:** This is a retrospective cohort study based on data recorded in the patient files. Inclusion criteria were defined as ages between 18 and 85, availability of clinical data and presence of a patent upper extremity AV shunt (native fistula or synthetic graft) as a vascular access to dialysis. Patients with a catheter or with lower extremity AV shunt as dialysis access and patients having CABG without using an ITAwere excluded from the study. The eligible patients were divided into 2 groups: those with ipsilateral and those with contralateral location of ITA CABG and AV shunt. The outcomes were: death from any cause, cardiac death and a first cardiac event.

**Results:** In a group of 112 chronic hemodialysis patients having CABG, 32 had an ipsilateral and 25 had a contralateral location of ITA CABG and an upper extremity AV shunt. Mean follow up period was 39.0±24.7 months. Significantly more cardiac events occurred in the group with an ipsilateral compared to a contralateral location of coronary ITA CABGs and dialysis AV shunts (hazard ratio, 2.16 [95% CI, 1.11 to 4.19], p=0.023). There was no difference between the groups in the all cause mortality risk (hazard ratio, 1.005 [95% CI, 0.43 to 2.37], p=0.990) and in the risk of cardiac death (hazard ratio, 2.43 [95% CI, 0.64 to 9.17], p=0.191).

**Conclusions:** The ipsilateral location of a CABG with the use of ITA and upper extremity AV dialysis shunt may be associated with increased risk of cardiac events. Placing an upper extremity AV hemodialysis shunt in patient with functioning ITA CABG would be better performed on a contralateral side.

## TH-PO490

### Influence of Dialysis on the Cerebrovascular Supply – Role of Arteriovenous Fistula (Pilot Study)

Ivan Rychlik,[1] Tomas Zahradnicek,[1] Tomas Peisker.[2]
*[1]Dialysis Unit, FMC, Prague 10; [2]Dept. Neurology, 3rd Fac. Med, Prague 10, Czech Republic.*

**Background:** Part of patients(pts) on maintenance hemodialysis(HD) therapy complains of neurological symptoms like vertigo,headache,during HD.No data are available concerning influence of blood flow of the arteriovenous fistula(AVF) on vertebrobasilary(VB) vascular supply.We tested whether AVF flow rate had negative impact on blood supply in VB arteries during HD session.

**Methods:** Pts were defined as stable on HD >1y,with native brachio- or radiocephalic AVF,normotensive,normovolemic according to Fresenius Body Composition Monitor. Using dopplersonography(dUSG),following parameters were measured:i/peak-systolic velocity(PSV),end-diastolic velocity (EDV),calculated mean flow velocity(MFV) before and during HD session in vertebral artery(segm.$VA_2$ and $VA_4$)and basilar artery(BA);ii/ a.brachialis blood flow.Furthermore,AVF-blood flow was measured by Fresenius blood temperature monitor(BTM).We compared the blood flow in VB supply before and during HD session in connection with AVF blood flow.

**Results:** 8 pts(mean age 75y,M/F 5/3, 25% diabetics,50% CHD) were enrolled.During HD session,mean BP=132/72mmHg,mean UF 2530 ml,mean Qb (blood pump) 383 ml/ min.Blood flow data are given in Tab.1:

| No. | dUSG vascular pathology | blood flow AVF (ml/min) | | ipsilateral artery bood flow (cm/s) | | | |
|---|---|---|---|---|---|---|---|
| | | | | BA | | VA4 | |
| | | dUSG-BA | BTM | pre-HD | in-HD | pre-HD | in-HD |
| 1 | + | 850 | 650 | 58 | 31 | 26 | 22 |
| 2 | + | 800 | 620 | 49 | 50 | 30 | 16 |
| 3 | - | 1200 | 1200 | 29 | 24 | 49 | 24 |
| 4 | - | 850 | 643 | 44 | 51 | 46 | 50 |
| 5 | - | 900 | 711 | 27 | 28 | 25 | 22 |
| 6 | - | 800 | 556 | 21 | 16 | 19 | 16 |
| 7 | - | 900 | 643 | 35 | 34 | 29 | 31 |
| 8 | - | 540 | 598 | 57 | 45 | 42 | 37 |

Significant decrease(>25%) of blood flow in distal part of VB arteries($VA_4$ and BA) with VB clinical symptoms was disclosed in 3 pts(37%)and dUSG vascular pathology(>50% stenosis or occlusion)on VB arteries was proven in 2 of them.The influence of inappropriate hydration, intradialysis hypo/hypertension,ion-dysbalances,etc., during dialysis sessions were excluded.

**Conclusions:** According to our results,cerebral hypoperfusion due to the pre-existing vascular pathology on VB arteries seems to be impacted by hemodynamic effect of AVF blood flow. A dUSG-screening of during-dialy sis-symptomatic pts could be useful to prevent cerebral ischemic impairment.

*(supported by PRVOUK 34)*

## TH-PO491

### Frequent Pre-Dialysis Nephrology Care and Later Dialysis Initiation Are Associated with Arteriovenous Access Use at Dialysis Start: A DOPPS Study

Brian Bieber,[1] Yun Li,[1,2] Ronald L. Pisoni,[1] Christi Priya Dhayanandhan,[2] Christian Combe,[3] Joan Fort,[4] David C. Mendelssohn,[5] Hideki Kawanishi,[6] Friedrich K. Port,[1,2] Panduranga S. Rao,[2] Bruce M. Robinson.[1,2]
*[1]Arbor Research; [2]U. Michigan; [3]CHU de Bordeaux; [4]Hosp. Vall d'Hebron; [5]Humber River Regional Hosp.; [6]Tsuchiya General Hosp.*

**Background:** Catheter use at dialysis start remains exceptionally high in the US and likely contributes to high early dialysis mortality rates. The average estimated glomerular filtration rate (eGFR) at dialysis start is higher in the US than in Europe and Japan and has risen over the past decade. We hypothesized that arteriovenous fistula or graft (AV access) use at study start would be associated with (a) more frequent pre-nephrology care and (b) later dialysis start.

**Methods:** Data were from phase 4 of the Dialysis Outcomes and Practice Patterns Study (DOPPS, 2009-2011). Among patients on dialysis <120 days at enrollment and with data on eGFR at dialysis start and frequency of pre-dialysis care (n=835), logistic regression was used to estimate odds of an AV access at dialysis start, accounting for facility clustering and listed covariates. AV access at DOPPS enrollment was used when access at dialysis start was missing.

**Results:** 28% of US patients used an AV access at HD start, compared to 51% in Europe and 87% in Japan. In the adjusted model, patients who saw a nephrologist more often in the year before dialysis were more likely to have an AV access(Table). Patients with a lower eGFR at start of dialysis were also more likely to have an AV access.

**Table:** Associations with use of AV access at dialysis start

| Variable | Unadjusted Odds Ratio (95% CI) | Adjusted[a] Odds Ratio (95% CI) |
|---|---|---|
| eGFR at dialysis start (per 2.5 mL/min lower) | 1.15(1.06,1.24)* | 1.13(1.00,1.27)* |
| Frequency of pre-HD care[b] | | |
| 0 visits | 0.68(0.37,1.27) | 0.38(0.16,0.87)* |
| 1 visit | 1.00(ref) | 1.00(ref) |
| 2-4 visits | 2.57(1.52,4.37)* | 2.73(1.40,5.33)* |
| 5+ visits | 4.19(2.35,7.46)* | 4.58(2.26,9.31)* |

a. Adjusted for listed variables plus age, vintage, country and US-black race, 12 summary comorbid conditions, and facility clustering
b. Number of visits in the year before starting dialysis
* p < 0.05

**Conclusions:** AV access use at start of dialysis is much lower in US than Europe or Japan. Emphasis on frequent pre-dialysis nephrology care and avoiding unnecessary early dialysis starts in order to establish an AV access before starting dialysis may improve outcomes during the dialysis transition period.

*Funding:* Pharmaceutical Company Support - The DOPPS is supported by research grants from Amgen (founding sponsor, since 1996), Kyowa Hakko Kirin (since 1999, in Japan), AbbVie (since 2009), Sanofi Renal (since 2009), Baxter Healthcare (since 2011), and Vifor Fresenius Medical Care Renal Pharma (since 2012), with additional country-specific support provided in Canada by Amgen-Canada, Janssen, BHC Medical, Takeda and Kidney Foundation of Canada, and in Germany by Hexal and WiNe Institute. Support from the DOPPS sponsors is provided without restrictions on publications.

**TH-PO492**

**Local Anesthesia in Arteriovenous Fistula Placement: Patient Perception of Pain and Early Patency Outcomes** Rick E. Mishler,[1] Amanda M. Valliant,[2] Alexander S. Yevzlin.[2] *[1]Arizona Kidney Disease and Hypertension Centers, Phoenix, AZ; [2]Univ of Wisconsin Hospitals and Clinics, Madison, WI.*

**Background:** US end stage renal disease (ESRD) vascular access outcomes, though improving, continue to lag behind those of Europe. As a result of this persistent trend, US Nephrologists have recently started to create arteriovenous (AV) access. This retrospective study involving 170 patients presenting for AV fistula creation to US Nephrologists explores several aspects of fistula creation using monitored anesthesia care (MAC) rather than brachial plexus nerve blockade, including adequacy of analgesia and early post-operative access patency as well as intraoperative complications.

**Methods:** Patients undergoing AV fistula placement using only MAC from September 2011 to January 2012 were surveyed post-operatively and asked to rate their intraprocedural pain (IPP) using a predefined pain scoring system. In total, 170 patients were included in this retrospective review with 50 patients providing pain score assessments. Additional data was collected at the time of fistula placement and in the subsequent two weeks, including incidence of common medical comorbidities and 2-week post-operative complication rates.

**Results:** Amongst the 170 patients, 60.6% were diabetic, 96.5% were hypertensive,and 37.7% had ESRD (defined as stage 5 CKD) at the time of AV fistula placement. A subset of 50 patients consented to an assessment of pain scores immediately following the fistula placement under local anesthesia; pain scoring system is described elsewhere. Of the 50 patients, 96% reported an(IPP) score of 1, consistent with no pain or minimal pain. Four percent of patients reported an IPP of 2, corresponding to mild to moderate pain. When evaluating the success of fistula placement, 95.88% of the patients underwent a successful procedure with no complications noted and adequately maturing access at 2 weeks.

**Conclusions:** The placement of AV fistulas using MAC can be easily achieved with adequate analgesia. In addition, the success rates of these procedures is high and the rate of early fistula failure in this study significantly lower than the average fistula failure rates demonstrated in the literature.

**TH-PO493**

**Intravenous Ferric Carboxymaltose (Ferinject®) Reduced the Need for the Erythropoietin-Stimulating Agent Aranesp® in Chronic Hemodialysis Patients** Marijke Dekker,[1] Catharina H.M. Kerskes,[2] Constantijn Konings.[1] *[1]Internal Medicine, Catharina Hospital, Eindhoven, Netherlands; [2]Clinical Pharmacology, Catharina Hospital, Eindhoven.*

**Background:** The treatment of iron deficiency and renal anemia in chronic hemodialysis patients is expensive due to frequent iron infusions and, especially, due to prescription of an erythropoietin-stimulating agent (ESA). In our hemodialysis population, we investigated if the frequency and dosage of iron infusions and ESA administration could be reduced, without compromising ferritine and hemoglobin (Hb) levels, by switching from intravenous LMW iron dextran (LMW-ID) (CosmoFer®) to intravenous ferric carboxymaltose (FCM) (Ferinject®).

**Methods:** In this retrospective study, we analyzed chronic hemodialysis patients [HD73; HDF 5], six months before switching from intravenous LMW-ID to intravenous FCM and once a month for six months after the switch. Anemia was treated according to the 2012 KDIGO guidelines; Aranesp® was administered as ESA when Hb levels were below 9g/dl in all patients. We compared: Hb, ferritine, transferrine and CRP values, plus the FCM, LMW-ID and ESA dosages.

**Results:** In total, we analyzed 78 patients, mean age 70±12.9yrs. We found a significant reduction in the weekly ESA dosage from 45.75µg to 39.69µg (p=0.017) between the first and sixth month after introducing FCM. This equals an ESA reduction in 30.8% (N=24) of the patients, and the ESA could even be stopped in 3 patients. Besides, the used FCM dosage could also be significantly reduced in the first 6 months, from 39.64mg to 34.08mg a week (p=0.049), without compromising ferritine levels. Six 6 months after the switch, over 90% of the patients had Hb levels over 9 g/dl as required by the 2012 KDIGO anemia guideline.

**Conclusions:** Using ferric carboxymaltose (Ferinject®) instead of LMW-ID significantly reduced the need for the erythropoietin-stimulating agent (Aranesp®), without compromising Hb levels.

**TH-PO494**

**Soluble Ferric Pyrophosphate (SFP) Administered via Dialysate Reduces ESA and IV Iron Requirements while Maintaining Hemoglobin in CKD-HD Patients** Vivian H. Lin, Ajay Gupta, Tasha M. Farmer, Carrie D. Guss, Raymond D. Pratt. *Rockwell Medical Inc.*

**Background:** The effects of SFP, a soluble form of iron delivered via the dialysate, on ESA, IV iron requirements and hemoglobin, were examined in the PRIME study, a 36 week, double-blind RCT.

**Methods:** Iron-replete CKD-HD patients on stable ESA doses (N=103) were randomized to SFP (2µM or 110µg iron/L) or placebo (standard dialysate without SFP). At baseline, groups did not differ significantly in any study parameter. ESA prescriptions were managed by a centralized anemia management center to facilitate consistent adherence to the protocol-specified hemoglobin target range of 95 to 115 g/L. Per protocol, investigators gave IV iron for ferritin <200 µg/L and/or TSAT <15%.

**Results:** Adverse events did not differ significantly between study groups. From baseline to end of treatment (EoT: last 2 weeks on study), the change in prescribed ESA in the SFP group (388 U/wk) was 35% less (p=0.045) than in the placebo group (3,423 U/wk), the primary endpoint of the trial. At EoT, the SFP group had required 48% less IV iron than the placebo group (24 vs 45 mg/wk; p=0.028). Despite receiving less ESA and IV iron, the hemoglobin in the SFP group was maintained from baseline to EoT (110 to 106 g/L) and did not differ (p=0.833) from that in the placebo group (111 to 104 g/L). In patients who received study drug, no supplemental iron was needed by subgroups of 40 (77%) in the SFP group and of 31 (60%) in the placebo group (p=0.09). At EoT, the prescribed ESA dose was reduced from baseline by 5% in the SFP subgroup and increased by 33% in the placebo subgroup, a difference of 38% (p=0.058). In the SFP subgroup, reticulocyte hemoglobin (CHr) remained stable, but declined in the placebo subgroup by a mean of 1.61 pg (p=0.008) at EoT.

**Conclusions:** SFP, delivered via dialysate, requires 35% less ESA and 48% less IV iron compared to placebo, while maintaining hemoglobin concentrations. SFP reduced ESA requirements by 38% among patients who did not require IV iron, while maintaining iron availability for erythropoiesis, as evaluated by CHr. SFP is a promising means of providing iron to CKD-HD patients.

*Funding:* Pharmaceutical Company Support - Rockwell Medical Inc.

**TH-PO495**

**Initial Change of Iron Metabolic Flux prior to Differentiation of Erythroid Progenitors after Epoetin Beta Pegol (C.E.R.A.) Treatment** Yusuke Sasaki, Mariko Noguchi-Sasaki, Yukari Matsuo, Junko Fukumura, Fumi Sato-Inomata, Keigo Yorozu, Yasushi Shimonaka. *Chugai Pharmaceutical Co., Ltd., Japan.*

**Background:** Epoetin beta pegol (C.E.R.A.) is a novel long-acting erythropoiesis stimulating agent. C.E.R.A. has been reported to promote mobilization of iron storage through intensive suppression of serum hepcidin levels. The aim of this study is to analyze the mechanism underlying suppression of hepcidin and initial change of iron metabolic flux after C.E.R.A. treatment.

**Methods:** Serum hepcidin and iron levels were analyzed in C57BL/6N (B6) mice intravenously treated with 10 µg/kg of C.E.R.A., carbamylated C.E.R.A. (C-C.E.R.A.) which has erythropoiesis effect silenced by modification of lysines by carbamylation, or vehicle. Initial transitions of hematological and iron parameters, as well as the maturation status of bone marrow erythroblast stained with Ter119 and transferrin receptor (CD71) were analyzed in B6 mice after intravenous treatment with 10 µg/kg of C.E.R.A. or vehicle.

**Results:** C.E.R.A. significantly suppressed serum hepcidin levels at 48 hours after treatment, while C-C.E.R.A. has no effect on them. There were no changes in serum iron levels by C.E.R.A. or C-C.E.R.A. treatment at 48 hours. C.E.R.A. suppressed serum hepcidin levels from 9 to 24 hours after treatment, while serum iron levels were significantly decreased at 9 hours followed by recovery to the control levels at 24 hours. Ter119(+) CD71(high) immature erythroblast was decreased and CD71 expression levels on the same cells were increased at 9 hours after treatment.

**Conclusions:** These results indicate that erythropoietic activity is essential for suppression of hepcidin after C.E.R.A. treatment. Suppression of serum hepcidin levels and transient decrease in serum iron levels in early phase after C.E.R.A. treatment, prior to differentiation of erythroid progenitors suggest that sensing initial change of iron metabolic flux leads to suppression of hepcidin after C.E.R.A. treatment. It is possible that this flux occurs by the accelerated maturation of immature erythroblast which has ability to absorb iron after C.E.R.A. treatment, but further investigation is needed.

**TH-PO496**

**Relative Safety of Peginesatide versus Epoetin Alfa** Eric D. Weinhandl,[1] David T. Gilbertson,[1] Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Univ of Minnesota, Minneapolis, MN.*

**Background:** Omontys (peginesatide) was recalled in February 2013, after reports of serious hypersensitivity reactions and deaths. We used Medicare Quarterly Standard Analytical Files (QSAFs) to compare adverse event rates in patients who received a first dose of peginesatide (PEG) between July 1 and November 30, 2012, and matched controls who received a dose of epoetin alfa (EPO) during the same era.

**Methods:** PEG and EPO exposure were ascertained from outpatient facility claims. Patients with recent exposure to ferumoxytol or iron dextran or residing in states without PEG use during the study era were excluded. For each PEG user, we identified 2 matched controls with identical dialysis provider affiliation, dialytic modality, and same-day IV iron exposure; and similar propensity score (PS) of PEG initiation. The PS was a function of demographic factors; state of residence; hemoglobin; recent exposure to EPO, darbepoetin alfa, IV iron, and IV antibiotics; and comorbidity. We searched mortality records and institutional claims for the occurrence of 3 outcomes on either the day of or the day after ESA administration: the composite event of death or hospitalization due to cardiovascular disease (CVD), all-cause hospitalization, and emergency department care.

**Results:** We identified 8,693 PEG users (number of composite events, 29) and 17,386 matched controls (21). The hazard ratio (HR) of the composite event for PEG versus EPO was 2.8 (95% confidence interval, 1.6-4.8); among hemodialysis (HD) patients without same-day IV iron exposure, the HR was 3.6 (1.9-6.9). For all-cause hospitalization, the HR was 1.4 (1.1-1.9); among HD patients without same-day IV iron exposure, the HR was 1.5 (1.1-2.1). For emergency department care, the HR was 1.1 (0.9-1.4); among HD patients without same-day IV iron exposure, the HR was 1.3 (1.0-1.7).

**Conclusions:** Relative to treatment with epoetin alfa, initial exposure to peginesatide was associated with increased risk of death or hospitalization due to cardiovascular disease; increased risk persisted in patients without concurrent intravenous iron use. These data illustrate the potential utility of Medicare QSAFs for timely pharmacosurveillance.

*Funding:* NIDDK Support

## TH-PO497

**A Randomized Controlled Trial of Costs Associated with Anemia Therapy in Hemodialysis Patients Treated with Intravenous Darbepoetin alfa versus Intravenous Epoetin alfa** Andrea L. Woodland,[1] Sean W. Murphy,[2] Brendan J. Barrett,[2] Bryan M. Curtis.[2] *[1]Pharmacy Dept, Eastern Health, St.John's, Canada; [2]Dept of Medicine, Memorial Univ, St.John's, Canada.*

**Background:** Anemia of Chronic Kidney Disease is associated with adverse outcomes and a reduced quality of life. Erythropoiesis stimulating agents (ESAs) have improved anemia management and two agents are available in Canada, epoetin alfa(EPO) and darbepoetin alfa(DA). EPO and DA are considered to be equally effective in achieving target hemoglobin in dialysis patients but it is not clear if there is a cost difference. There have been few head-to-head comparisons of the two; most published data is from observational switch studies.

**Methods:** An open label randomized controlled trial of intravenous (IV) DA versus EPO was conducted in 50 hemodialysis patients. A dose stabilization run-in phase was followed by a 12 month active phase. ESAs and iron were dosed using an algorithm to maintain hemoglobin(Hb) within 100-120g/L. The primary outcome was ESA cost (Canadian $) per patient over 12 months. Secondary outcomes included deviation from target ranges in anemia indices, iron dose and cost, time and number of dose changes required for dose stabilization, number of dose changes in the active phase and the dose conversion ratio.

**Results:** The median cost over 12 months was $4179(IQR $2416-5955) for EPO and $2303(IQR $1178-4219) for DA with a difference of $1876 (p=0.017). The median weekly iron dose was 40.4mg for EPO and 41.7mg for DA (p=0.992). There were no significant differences in Hb: 108.0g/L EPO and 109.8g/L DA (p=0.336); serum ferritin: 848µg/L EPO and 726µg/L DA (p=0.202); TSAT: 26.7% EPO and 28.6% DA (p=0.472).The number of dose changes and the time to hemoglobin stability were similar for both groups. The dose conversion ratio was 280:1 (95% CI 197-362:1) at the end of the run-in phase, 360:1 (95% CI 262-457:1) at the 3 month point of the active phase and 382:1 (95% CI 235-529:1) at the 6 month point of the active phase.

**Conclusions:** In this study of hemodialysis patients with comparable anemia management IV darbepoetin cost $1876 less per year per patient than IV epoetin.

## TH-PO498

**Optimal Hemoglobin Target Might Be Different between Elderly Hemodialysis Patients and Non-Elderly Patients** Norio Hanafusa,[1,4] Takanobu Nomura,[2] Takeshi Hasegawa,[3,4] Masaomi Nangaku.[1,4] *[1]Dept of Nephrology and Endocrinology, The Univ of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan; [2]Kyowa Hakko Kirin, Co. Ltd., Chiyoda-ku, Tokyo, Japan; [3]Div of Nephrology, Showa Univ School of Medicine, Yokohama, Kanagawa, Japan; [4]Japanese Dialysis Outcomes and Practice Patterns Study for Anemia Working Group, Tokyo, Japan.*

**Background:** Elderly population is growing in their numbers among hemodialysis population. However, there is no evidence about the relationship between hemoglobin (Hb) level and survival by age. We investigated the effect of age on the relationship between Hb and survival in Japan DOPPS (JDOPPS) cohort.

**Methods:** We included the entire JDOPPS phase 3 and 4 population into the study. The population was divided at the age of 70 years old to make the elderly and the non-elderly population. We used Cox's proportional hazard model to investigate survival with Hb values incorporated as time dependent variable. Interaction between Hb and age was investigated. Inverse propensity treatment weight (IPTW) method was used to adjust propensity for erythropoietin use.

**Results:** The study population included 3,359 patients with 568 deaths during median follow up of 2.66 years. Interaction between age groups and Hb values was significant ($p<0.001$). Non-elderly population experienced poorer survival for Hb <9g/dl compared to elderly population (HR 4.90: 95%CI 3.46 – 6.95 vs HR 1.79: 95% CI 1.30 – 2.48, $p<0.001$ for interaction; with Hb 9 – 10g/dl made as reference group). Hb values between 9 and 10 g/dl among non-elderly population and Hb ≥ 12 g/dl among elderly population were both related to poorer survival after IPTW adjustment (HR 1.37: 95% CI 1.06 – 1.77; HR 1.40: 95%CI 1.04 – 1.88, respectively), although the interaction did not reach its significance ($p=0.089$, 0.364).

**Conclusions:** Older population might be tolerant for lower Hb values, while higher Hb levels might relate to poorer prognosis among the population. Therefore, optimal Hb ranges might be different for the elderly population compared to the non-elderly population. These results render evidences to the detailed consideration about anemia management by patients' characteristics and about its individualization.

*Funding:* Pharmaceutical Company Support - Kyowa Hakkko Kirin Co., Ltd.

## TH-PO499

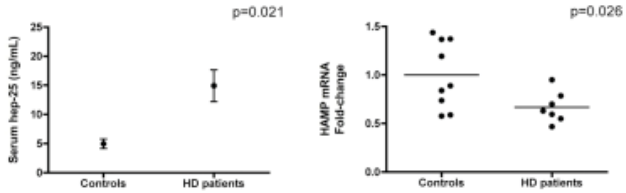
**Is Hepcidin-25 Production Increased in Patients with Chronic Kidney Disease?** Adam Rumjon, Iain C. Macdougall. *Dept of Renal Medicine, King's College Hospital, London, United Kingdom.*

**Background:** Hepcidin-25 (hep-25) plays a critical role in iron metabolism, and the levels of this peptide are greatly increased in end-stage renal disease. It remains unclear whether this is due to reduced clearance, or increased production. The aim of this study was to explore hep-25 production *ex vivo* in haemodialysis patients.

**Methods:** Inclusion criteria for this study were; HD for >3 months, Hb >10 g/dL, CRP <20 mg/L, HD via an AVF/PTFE graft, stable IV iron/EPO dose for >1 month, and no hospitalisations for >1 month. A pre-dialysis blood sample (10ml) was separated by Ficoll Hypaque centrifugation. Serum hep-25 levels were measured using liquid chromatography mass spectrometry. Peripheral blood mononuclear cells (PBMCs) were processed immediately (5-6 x$10^6$ cells/mL); qRT-PCR was performed using the Applied Biosystems 7900HT Fast Real-Time system. Amplification reactions were performed (triplicate) using Taqman HAMP and GAPDH primers.

| Parameter | Mean (SD) |
|---|---|
| Age, years | 55.5 (19.5) |
| Haemoglobin, g/dL | 11.3 (0.8) |
| CRP, mg/L | 10.4 (5.0) |
| Albumin, g/L | 39.7 (1.8) |
| Ferritin, ng/mL | 367 (90) |

**Results:** To date, 7 patients (5 males, 2 females) have been studied. Median IV iron dose was 100mg/week, and median EPO dose was 46.2 [IQR 18.9-114.6] iu/kg/wk. Serum hep-25 levels were significantly elevated in the HD population (14.9±2.7 vs 4.9±0.8, $p=0.021$). Interestingly, HAMP mRNA levels were significantly lower than in healthy controls (mean fold-change 0.667±0.06 in HD compared to controls ($p=0.026$)).




**Conclusions:** Despite higher circulating levels of hep-25, significantly lower transcription of HAMP mRNA was unexpectedly observed in HD patients. This counter-intuitive result, which has not been described previously, requires confirmation in a larger cohort of patients. Elucidation of this effect is also required; it is possible that negative feedback mechanisms may be in operation in HD patients to reduce hep-25 levels.

## TH-PO500

**ESA Hyporesponsiveness Is Associated with Adverse Event in Maintenance Hemodialysis (MHD) Patients, but Not Iron Storage** Takahiro Kuragano, Takeshi Nakanishi. *Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

**Background:** Recently several studies revealed the strong association between hyporesponsiveness to erythropoiesis-stimulating agent (ESA) and adverse events in the MHD patients. An iron deficiency has been cited as the most common cause of hyporesponsiveness in MHD. In this study, we evaluated relationship among iron storage, responsiveness to ESA, and adverse events on the MHD patients.

**Methods:** In 1086 MHD patients, we measured serum ferritin, Hb, and transferrin saturation (TSAT) levels every 3 month during 2 years. The weekly dose of ESA /Hb (ESA/Hb) value was also calculated as an index of ESA responsiveness index. The associations between ESA/Hb and several adverse events were investigated with the cox proportional hazards model for time-dependent variables.

**Results:** At the beginning of this study, patients were stratified into 4 groups according to serum ferritin levels (< 50, 50 ≤ ferritin < 100, 100 ≤ ferritin < 300, ≥ 300 ng/mL). There was no significant difference in ESA/Hb and Hb levels among 4 groups. On the other hand, a significant correlation ($p<0.001$, $R=0.89$) between ferritin and Hb was found only in the patients with ferritin levels under 50 ng/mL. In the multiple regression analysis, female ($p<0.001$, $\beta=0.34$) and prealbumin ($p<0.015$, $\beta=-0.21$) were selected as the significant predictors of ESA/Hb. Compared to the patients with lower ESA/Hb (≤ 280), patients with higher ESA/Hb level (> 280) were associated with the significant higher risk of cerebro-cardio-vascular disease (CCVD) (HR:1.91, $p=0.03$), infection disease (HR:1.22, $p=0.046$), and hospitalization (HR:1.39, $p=0.021$).

**Conclusions:** In patients with ferritin < 50 ng/mL, Hb was dependent on ferritin, but not in those with ferritin ≥ 50 ng/mL. Although the patients with the hypo-responsiveness to ESA had higher risk for CCVD and hospitalization, ESA/Hb were not related to the iron storage. From these results, we concluded that excessive iron storage dose not necessarily lead to the improvement of the responsiveness to ESA in MHD patients.

## TH-PO501

**Continuous Erythropoietin Receptor Activator Improves Chronic Kidney Disease-Associated Anemia via Correction of Iron Availability and Suppression of Plasma Hepcidin-25 Levels in a Rat Model of Chronic Kidney Disease** Michinori Hirata, Yoshihito Tashiro, Kenji Yogo, Koichi Endo. *Chugai Pharmaceutical Co., Ltd.*

**Background:** Resistance to erythropoiesis-stimulating agents (ESAs) is often observed in patients with chronic kidney disease (CKD). A major cause of ESA resistance is the reduction of iron availability due to increased synthesis of hepcidin following stimulation by pro-inflammatory cytokines. Continuous Erythropoiesis Receptor Activator (C.E.R.A.) is widely used to treat anemia in CKD and dialysis patients, but mechanisms of iron metabolism on treatment with C.E.R.A. in CKD-associated anemia have not been fully elucidated. In this study we investigated iron metabolism under conditions of anemia corrected by C.E.R.A. in a rat model of CKD.

**Methods:** C.E.R.A. (0.6 µg/kg, every 2 weeks) was subcutaneously administered from 1 week after 5/6-nephrectomy (Nx). After the start of treatment, we evaluated changes over time in hemoglobin (Hb) level, reticulocyte (Ret) number, plasma iron, and hepcidin-25 (Hep-25) concentration. At Week 9 we measured plasma creatinine, urea nitrogen, ferritin, and interleukin-6 (IL-6).

**Results:** Compared with Hb levels in normal rats, levels in Nx rats gradually and significantly decreased as kidney disease progressed. In contrast, C.E.R.A. significantly inhibited the decreased Hb levels (Hb: Nx, 12.5±0.5 g/dL; C.E.R.A., 14.7±0.6 [p<0.05]; Normal, 15.6±0.3; n=7–9), and increased Ret numbers to around normal range. Whereas iron availability was lowered in Nx rats regarding the significant elevation of plasma Hep-25 and ferritin levels as CKD progressed, C.E.R.A. treatment improved these parameters to within normal range (Hep-25: Nx, 17.1±1.9 ng/mL; C.E.R.A., 9.8±1.6 [p<0.05]; Normal, 9.0±1.5; Ferritin: Nx, 609.8±118.8 ng/mL; C.E.R.A., 322.3±140.1 [p<0.05]; Normal, 298.7±74.3) with improving CKD-associated anemia. There was no significant change in plasma IL-6 levels among these groups.

**Conclusions:** C.E.R.A. protected against gradually decreasing Hb levels in anemia model rats possibly by correcting iron availability in addition to erythropoiesis as reported.

## TH-PO502

**Anemia Management Trends in Hospital-Based Dialysis Centers: 2010-2013** George N. Coritsidis,[1] Anjali Acharya,[1] Chun-Lan Chang,[2] Jerrold W. Hill,[2] Gregory A. Maglinte,[3] Anjali B. Saxena,[4] Mark Stephens,[5] Richard A. Lafayette.[4] *[1]NYC Hospital Corp; [2]IMS Health; [3]Amgen; [4]Stanford; [5]PHA.*

**Background:** In response to changes in the prescribing information of erythropoiesis stimulating agents (ESAs) and dialysis reimbursement, anemia management practices in dialysis have changed in the past three years. Hospital-based dialysis centers (HBDCs) differ from free-standing dialysis centers and may warrant special analysis. This study describes trends and regional variation in anemia management practices in HBDCs from January 2010 to March 2013.

**Methods:** Electronic medical records of 5404 hemodialysis patients in 50 US-based HBDCs were analyzed retrospectively. Patients were at least 18 years old and had at least one hemoglobin (Hb) measurement and one ESA dose between January 2010 and March 2013. Descriptive statistics were collected monthly for Hb and iron biomarkers (serum ferritin and transferrin saturation), and every 4 weeks for darbepoetin alfa, epoetin alfa and IV iron doses.

**Results:** Mean(standard deviation) monthly Hb declined from 11.4(1.2) to 10.7(1.2) g/dL, the percent of patients with mean monthly Hb <10 g/dL rose from 11.3% to 24.4%, and the percent of patients with mean monthly Hb >12 g/dL declined from 30.1% to 11.2%. Most patients received darbepoetin alfa. The darbepoetin alfa median (interquartile range) cumulative 4-week dose declined 41% from 170(100,340) to 100(50,200) mcg, and the median cumulative 4-week IV iron dose increased 50% from 250 to 375 mg. Regional differences were observed in ESA and iron dosing levels, while average Hb differed only slightly by region. Mean darbepoetin alfa doses were 5% lower while IV iron doses were 51% higher in HBDCs in the South and West than in the Northeast/Midwest.

**Conclusions:** Consistent with reported national trends, HBDCs have modified anemia management practices since 2010, with continuing declines in ESA doses, higher doses of IV iron and falling Hb. The relatively small variation in regional ESA utilization, but relatively large variation in IV iron doses, did not have an observable impact on the Hb level achieved, which may be due to demographic differences or other factors not observed in this study.

*Funding:* Pharmaceutical Company Support - Amgen Inc.

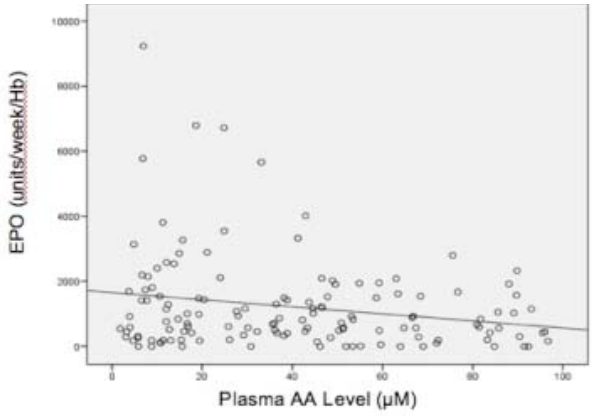
## TH-PO503

**Correlation of Plasma Ascorbic Acid Level with Erythropoietin Resistance** William D. Sirover,[1] Yuguan Liu,[2] Swomya Bal,[1] Krystal Hunter,[1] Amanda Logan,[1] Lawrence S. Weisberg,[1] Garry J. Handelman.[2] *[1]Cooper Medical School of Rowan Univ; [2]Univ of Massachusetts.*

**Background:** In end stage renal disease (ESRD), one cause of erythropoietin (EPO) resistance is functional iron deficiency. Iron stores are normal, but erythropoiesis is less effective due to decreased iron delivery to the bone marrow. Ascorbic acid (AA) may improve iron availability. The relation of plasma AA level with EPO resistance in a contemporary, prevalent hemodialysis (HD) population is not known. We hypothesize that plasma AA level correlates inversely with EPO resistance.

**Methods:** Prevalent HD patients in 2011 were recruited for enrollment. Inclusion criteria were age >18 years and vintage >3 months. Plasma samples collected in the outpatient setting were preserved with metaphosphoric acid and placed on dry ice or stored at -80 $^0$F until assayed for AA by HPLC. Hematologic data on a subset of 179 HD patients with ferritin >100 ng/ml was analyzed. Individual EPO dose was calculated as mean weekly dose over a 3-month period. Pearson correlation was used to assess the relationship between plasma AA level and EPO resistance (units/week/Hb), transferrin saturation (TSAT,%) and ferritin.

**Results:** Over the entire plasma AA range (1.78-409.3 µM), AA did not correlate with EPO resistance. However, in 148 patients with plasma AA levels in the physiologic range (0-100 µM), plasma AA inversely correlated with EPO resistance (r= -0.211,p= 0.01), directly correlated with TSAT (r= 0.162,p= 0.05), and did not correlate with ferritin.




**Conclusions:** AA at physiologic plasma levels is associated with less EPO resistance and increased TSAT. In patients with low plasma AA levels, supplemental AA may treat functional iron deficiency and EPO resistance by increasing iron availability. These results support proceeding with a randomized controlled trial.

*Funding:* Private Foundation Support

## TH-PO504

**Implementation of Anemia Protocol and Changes in Quality of Life for Chronic Hemodialysis Patients** Eduardo K. Lacson, Nien-Chen Li, Cindy Allegretti, Norma J. Ofsthun, Franklin W. Maddux, Jeffrey L. Hymes. *Fresenius Medical Care, North America, Waltham, MA.*

**Background:** A national Medical Advisory Board endorsed anemia management protocol for hemodialysis (HD) patients, consistent with the FDA black box warning on erythropoietin (ESA) use, was incrementally adopted at FMCNA beginning April 2011. We evaluated the impact on Kidney Disease Quality of Life-36 (KDQOL) scores from the first 815 participating facilities.

**Methods:** All 11,307 chronic HD patients with paired KDQOL scores (PCS, MCS, Symptom Problems, Burden, and Effects domains) up to 12 months before and within 3-15 months after protocol implementation were evaluated. The last available hemoglobin (Hgb) and ESA dose/treatment prior to the KDQOL surveys were recorded. Subgroups with large changes in hemoglobin (drop of >1 g/dL) and ESA dose/treatment (drop of >5,000 units) were also evaluated.

**Results:** The cohort: mean age 61.4 ±14.2 years; 54.6% male; 54.4% white (42.2% black); and 53.3% diabetics. The median time from KDQOL to initiating the anemia protocol was 3.3 months (range <1 to 11.9), and from then to the 2nd KDQOL was 9 months (range 3.0 to 14.9, last survey: 4/26/13). Mean scores for PCS (37.7 to 37.6) and Symptoms (79.4 to 79.6) were unchanged while MCS (51.0 to 51.2), Burden (53.1 to 54.6), and Effects (73.9 to 74.9) increased (p<0.05). Mean/median Hgb pre-baseline KDQOL were both 11.5 g/dL and pre-2$^{nd}$ KDQOL was both 11.2 g/dL (p<0.001). Patients with >1 g/dL drop in Hgb (N=2497, 28.8%, ff-up mean Hgb 10.3) had a drop in PCS (-0.7), Symptoms (-0.7), and increase in Burden (+1.3); a >5,000 units decline in ESA dose (N=1934, 22.1%) increased PCS (+0.45), Burden (+1.7), and Effects (+1.9) with all p<0.05. The clinical relevance of these statistically significant score changes is unknown.

**Conclusions:** Implementation of an anemia protocol that was consistent with FDA guidelines significantly decreased our HD patients' mean Hgb and ESA dose over 3-15months. Overall, there was no decline in KDQOL scores in the same patients treated using the protocol over time.

## TH-PO505

**Predicting Hemoglobin Response to Erythropoietin, Effect of Dose and Hemoglobin Concentration** Michael E. Brier, Jenny Feng, Adam E. Gaweda. *Univ of Louisville.*

**Background:** Several factors moderate the response to exogenous erythropoietin (EPO) in hemodialysis dependent end-stage renal failure patients. The determination of starting doses and dosage adjustment schedules were based on data obtained when subjects exhibited a different dose response curve than they do currently. We performed a linear mixed model regression analysis of hemoglobin (Hb) response in individual patients for the years 2004 to 2012 to test the hypothesis that Hb response is a function of the change in 4 months of prior EPO, current Hb, and current EPO dose.

**Methods:** The data consisted of 5580 EPO and Hb combinations from 153 patients. We calculated the mean weekly EPO dose per month, the change in EPO dose from the prior month, change in Hb from the prior month, and the target Hb for the year in which the data were collected. Hb target ranged from about 12.5 in 2004 to 10.5 in 2012 and was estimated as the mean achieved Hb for all Hb measurements for the year. We divided EPO dose in to 5 groups < 3000, 3001-6000, 6001-9000, 9001-15,000, and > 15,000. The independent variable was the change in Hb and we investigated the following factors: Dose, difference in current Hb from target, past 3 months change in Hb, past 4 months change in EPO, and current Hb.

**Results:** The results of the statistical analysis are shown in the following table which represent the change in Hb concentration in response to a increase in dose of 1,000 units.

| | | | | | Dose Group | | |
|---|---|---|---|---|---|---|---|
| | Hb | Dose (1/1000) | 0-3,000 | 3,001 to 6,000 | 6,001 to 9,000 | 9,001 to 15,000 | > 15,000 |
| Parameter Estimate | -0.013 | 0.017 | 0.24 | 0.23 | 0.17 | 0.13 | 0.07 |

The current change in Hb is influenced by up to 4 months of past EPO doses, the prior change in Hb over the last 3 months, current Hb, Dose, and difference between the current Hb and the target Hb.

**Conclusions:** In the range of observed Hb concentrations (10.5 to 12.5 g/dL), the response to a change in EPO dose demonstrates a negative relationship as Hb increases, the response decreases. Also, as dose increases response is decreased by as much 70%. One must take into account up to 4 months prior EPO and Hb in order to predict the response to a EPO dose change.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO506

### Therapeutic Effects of Renal Anemia Treated with Two Different Erythropoiesis Stimulating Agents: Darbepoetin Alpha and Continuous Erythropoietin Receptor Activator in Predialysis Patients with Chronic Renal Failure

Takashi Yokoyama. *Nephrology and Dialysis, Sapporo Higashi-Tokushukai Hospital, Sapporo, Hokkaido, Japan.*

**Background:** A study was conducted to clarify the therapeutic effects of Darbepoetin Alpha (DA) and Continuous Erythropoietin Receptor Activator (C.E.R.A.) in predialysis patients with renal anemia.

**Methods:** 36 predialysis patients were observed for six months. They were divided into two groups according to Erythropoiesis Stimulating Agents (ESAs) : Group A (n=19, age=76.47±10.46 years old) was treated with DA and Group B (17, 68.41±12.62) with C.E.R.A. Percutaneous administration of DA (10~180 μg) or C.E.R.A. (75~250 μg) at 21~35 day intervals was performed in order to maintain the target hemoglobin (Hb) level between 10.0 and 13.0 g/dL. The weekly average values of Hb, FRN, high sensitive C-reactive protein (hsCRP), albumin (Alb) and estimated GFR (eGFR) were measured at the same intervals and the serial changes of these clinical markers in both groups were observed.

**Results:** 1) At the baseline, no marked differences were observed between the two groups according to Hb (A : 9.28±1.17 g/dL vs B : 9.37±0.50), eGFR (17.16±7.66 mL/min/1.73$^2$ vs 15.50±10.30), Alb (3.79±0.52 g/dL vs 3.64±0.61) and hs CRP (0.16±0.37 mg/dL vs 0.13±0.17). 2) Hb levels from the second day measured to the sixth day measured in the two groups were as follows : second day (A : 9.97±1.05 g/dL vs B : 9.64±0.53), third (10.36±1.20 vs 10.27±0.86), fourth (10.16±1.36 vs 10.79±1.28), fifth (10.15±1.34 vs 10.44±1.34) and sixth (10.39±1.13 vs 10.13±0.85). No marked differences were found between the two groups during the six months. Administration volumes of ESAs for the two groups were as follows: baseline (A : 68.3±33.3 μg vs B : 141.2±19.6), third month (82.0±50.1 vs 152.9±27.8), six month (104.0±35.8 vs 171.4±56.7). The average rate between DA and C.E.R.A. over the six months was 1:1.74. 3) No marked differences were detected in FRN, hsCRP, Alb and eGFR during this period.

**Conclusions:** The evidences of smaller amounts of administration volume at 21~35 day intervals and equal therapeutic effects of renal anemia demonstrate DA's superiority to C.E.R.A. in predialysis treatment of anemia.

## TH-PO507

### Hemoglobin Variability of Japanese Hemodialysis Patients with Long Acting Erythropoiesis Stimulating Agent Treatment

Kiyohito Kawashima,[1] Hirotake Kasuga,[1] Chieko Matsubara,[1] Keiko Kimura,[1] Ryo Takahashi,[1] Yasuhiko Ito.[2] *[1]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan; [2]Nephrology, Nagoya Graduated School of Medicine, Nagoya, Japan.*

**Background:** Anemia is one of the most important complications in HD patients. Erythropoiesis stimulating agents (ESA) are standard therapy for renal anemia, and these therapies improved not only Hb levels of HD patients, but also their prognosis. In addition, some investigators presented that Hb variability also affects their prognosis. Recently, long acting ESA, epoetin beta pegol (C.E.R.A.), have been used for renal anemia treatment in Japan. In this study, we investigated the Hb variability and its influence for HD patients' prognosis.

**Methods:** 591 HD patients were enrolled. ESA therapy switched from epoetin beta (EPO) to C.E.R.A., and they were followed up for 6 months. According to Hb levels during this period, patients were classified into 6 category groups reported by Ebben; constant target (T, Hb levels of every month within Hb target, from10g/dL to 12g/dL), constant high (H, Hb levels constantly over target), constant low (L, Hb levels constantly under target), high amplitude (HA, Hb levels over, under and within target), low amplitude high (LAH, Hb levels over and within target), and low amplitude low (LAL, Hb levels under and within target). We checked patients' hospitalizations and deaths for next 6months, and examined the influence of every category for these events. We compared these data with our previous data under EPO treatment.

**Results:** Mean Hb level before usage of C.E.R.A. was 10.9±0.8g/dL. Hb levels of every month showed from 10.6±0.9g/dL to 11.0±0.9g/dL during 6 months. Rates of every Hb category under C.E.R.A treatment were 17% (T), 0% (H), 1% (L), 17% (HA), 20% (LAH) and 45% (LAL), and those under epoetin beta treatment were 14.9% (T), 1.1% (H), 5.6% (L), 16.5% (HA), 14.1%(LAH) and 47.9% (LAL). Hospitalization rate were 4.4% (T), 22.2% (L), 14.9% (HA), 11.4% (LAH) and 9.0% (LAL). Hb variability and its effects for prognosis were similar with C.E.R.A. and EPO.

**Conclusions:** C.E.R.A. was useful for renal anemia treatment of Japanese HD patients. Hb variability of C.E.R.A. and its effect for prognosis was similar with that of EPO.

## TH-PO508

### Soluble Ferric Pyrophosphate (SFP) Administered via Dialysate Shows No Acute Safety Signals

Carrie D. Guss, Tasha M. Farmer, Ajay Gupta, Vivian H. Lin, Raymond D. Pratt. *Rockwell Medical Inc.*

**Background:** SFP is the first parenteral iron product that is non-colloidal and free of carbohydrate or polyethylene glycol shell. Ferric iron compounds have been associated with multiple acute toxicities, including anaphylactoid reactions, hypotension and shock. This study examined the acute safety profile of SFP administered to a large cohort of CKD-HD patients compared to placebo.

**Methods:** This safety study was a randomized, double blind, placebo controlled crossover study in CKD-HD patients. Subjects were iron replete and on stable ESA doses. Subjects received either SFP or placebo for 2 weeks followed by a one week washout then a second 2 week period with the alternate treatment. The final SFP concentration in dialysate was 110 μg/L (2 μM). Premixed liquid bicarbonate concentrates with SFP or placebo maintained the blind. Safety assessments included adverse events, assessments of intra-dialytic hypotension (IDH), cardiac events and acute hypersensitivity-like reactions.

**Results:** 718 patients were randomized. 92.8% of subjects completed the blinded treatment. There was an intradialytic increase in serum iron (186%) with a concomitant decrease in unbound iron binding capacity (57%). The most common reasons for discontinuation were adverse events (7 SFP/6 placebo), and withdrawal of consent (9 SFP/8 placebo). There were 2 deaths during the placebo phase and none during the SFP phase. Procedural hypotension was the most common AE reported in 4.6% SFP and 4.7% placebo. There were no differences between treatment groups in intradialytic hypotension, composite CV events, thromboses or infections. No hypersensitivity events were attributed to SFP. There were no differences in other clinical laboratory parameters including serum phosphate and liver function tests between SFP and placebo.

**Conclusions:** The safety profile of SFP delivered via dialysate during dialysis in this large cohort was similar to placebo. No acute first use events, anaphylactoid reactions or hypotensive episodes were associated with SFP. Acute administration of SFP via the hemodialysate in CKD-HD patients is well tolerated and is expected to replace the iron lost during HD treatments.

*Funding:* Pharmaceutical Company Support - Rockwell Medical Inc.

## TH-PO509

### High-Density Lipoprotein Cholesterol and Matrix Gla Protein Are Implicated in the Progression of Aortic Calcification through Bone Morphogenetic Protein 4- or Activin-Like Kinase 1-Induced Smad1 Activation in Patients Undergoing Maintenance Hemodialysis

Kazuhiro Yoshikawa,[1] Hideharu Abe,[1] Motokazu Matsuura,[1] Kojiro Nagai,[1] Akira Mima,[1] Eriko Shibata,[1] Jun Minakuchi,[2] Toshio Doi.[1] *[1]The Dept of Nephrology, Institute of Health Biosciences, Univ of Tokushima Graduate School, Tokushima, Japan; [2]The Dept of Kidney Disease, Dialysis and Kidney Transplantation, Kawashima Hospital, Tokushima, Japan.*

**Background:** Aortic calcification (AC) is a common finding in patients undergoing maintenance hemodialysis (MHD). High-density lipoprotein (HDL) cholesterol and matrix Gla protein (MGP) are known as inhibitors of AC. In addition, HDL cholesterol progressively enhances expression of MGP mediated by activin-like kinase (ALK) in vitro. However, the exact mechanisms for accelerated AC in MHD patients have not been fully determined. In this study, we explored the role of MGP and HDL cholesterol for the progression speed of AC in MHD patients and investigated the underlying molecular mechanisms of the function of MGP in the bone morphogenetic protein (BMP) 4- or ALK1-induced Smad1 singnals.

**Methods:** First, a retrospective cohort study of MHD patients whose AC could be followed in multi-detector computed tomography (MDCT) examinations was conducted. Serum HDL cholesterol and MGP levels were measured. The progression speed of AC was examined by plotting the abdominal aortic calcium volume scores. Using cultured perivascular cells, molecular mechanisms were investigated.

**Results:** The HDL cholesterol levels were affected by MGP genotype. The progression speed of AC of patients with the MGP -138CC genotype was significantly slower than that of patients with other genotypes. Multiple regression analysis and cell-based analyses revealed that the HDL cholesterol levels and MGP were associated with progression of AC and that MGP was involved in the intracellular negative regulation of activation of Smad1 by BMP4 or ALK1 signaling pathways.

**Conclusions:** Serum HDL cholesterol levels and MGP genotype may be associated with slower progression of AC in MHD patients. In this setting, HDL cholesterol and MGP might be implicated in the molecular regulation of Smad1 in vascular cells.

*Funding:* Government Support - Non-U.S.

## TH-PO510

**Effect of Reduced CoQ10 on Anti-Oxidative Status in Hemodialysis Patients** Shigeru Owada,[1] Teruhiko Maeba,[1] Aki Hirayama,[2] Atsushi Ueda,[3] Sohji Nagase.[4] *[1]Dialysis Center, Asao Clinc, Kawasaki, Kanagawa, Japan; [2]Center for Integrative Medicine, Tsukuba Univ of Technology, Tsukuba, Ibaraki, Japan; [3]Nephrology, Tsukuba Univ Hospital Hitachi Medical Education and Research Center, Hitachi, Ibaraki, Japan; [4]Internal Medicine, Nagase Clinic, Moriya, Ibaraki, Japan.*

**Background:** Reduced CoQ10 (CoQH) has a principal role of mitochondrial function and also has an antioxidative properties. Serum CoQH level is lower in dialysis patients. Then we investigated the effects of CoQH supplementation on antioxidative capacities in hemodialysis patients (HD).

**Methods:** 200mg of CoQH /day was administered by orally to 30 HD patients (67±8 years ) for 3 months after written consent obtained. 1. Serum CoQH concentration, 2. soluble lectin-like oxidized low-density lipoprotein receptor-1 (s-LOX-1) and LOX-1 ligand containing Apo B (LAB) were measured and calculate LOX-index (s-LOX-1 x LAB) as a predictive marker for CVD (Clinical Chemistry, 2010. 56:550). 3. Ratio of triglyceride content and negative charge value of LDL (LDL-CMF), 4. hydroxyl radical scavenging activity (OHSA) were measured.

**Results:** Each parameter of 3 months after taking CoQH were as follows: 1. Total and reduced CoQ10 (μg/ml) levels were significantly increased (CoQ10; 0.61±0.19 → 3.36±1.70, CoQH; 0.57±0.18 → 3.20±1.43). 2. LOX-index was significantly decreased (3225±2010 → 2190±1488). 3. Tg ratio in LDL was not different (0.33±0.07 → 0.31±0.07), but LDL-CMF was significantly decreased (2.8±5.8 →-1.0±4.7). 4. Serum OHSA (mM GSH equivalent ) was significantly increased (7.5±1.5 → 8.4±1.7).

5. There were positive correlations were found between LOX-index and LDL Tg ratio (r=0.585), between LOX-index and LDL-CMF (r=0.371), between LDL-CMF and LDL Tg ratio (r=0.419).

**Conclusions:** We investigated the relationship between LOX-index and lipoprotein profile and effects of CoQH on the anti-oxidative activities in HD patients. Before supplementation of CoQH, serum total CoQ10 and CoQH levels were significantly lower compared to normal subjects and these were increased after supplementation. LOX-index and LDL-CMF were decreased and OHSA was increased. These results suggest that oxidative stress in HD patients was improved by CoQH supplementation.

## TH-PO511

**Low Selenium Levels in Diabetic Hemodialysis Patients Correlate with Increased Marker for Oxidative Stress** Anna J. Bryland,[1,2] Ola Carlsson.[1,2] *[1]Therapeutic Fluid Research, Gambro Lundia AB, Lund, Sweden; [2]Dept of Nephrology, Lund Univ, Lund, Sweden.*

**Background:** Hemodialysis (HD) patients are at elevated risk for oxidative stress and inflammation. For diabetic HD patients the risks are further elevated due to the effects of the disease. Deficiencies of selenium could lead to impaired inflammatory defence and oxidative stress. The objectives of this study were to investigate markers for advanced glycation end-products (AGEs), inflammation and oxidative stress in HD patients with or without diabetes compared to a healthy control group. We also wanted to investigate if the markers could be correlated to selenium concentrations.

**Methods:** Forty-seven HD patients (30 non-diabetic and 17 diabetic) were recruited to the study and plasma samples were taken pre- and post-dialysis of one dialysis treatment. Pentosidine levels were analyzed by HPLC; pentraxin-3 (PTX-3) and 8-hydroxydeoxyguanosine (8-OHdG) levels were analyzed by ELISA. Pre- and post-dialysis plasma samples were analyzed for selenium and compared to plasma samples from 51 healthy control subjects by ICP-MS.

**Results:** All HD patients had increased pre-dialysis plasma levels of pentosidine, PTX-3 and 8-OHdG compared to controls; the levels of pentosidine, PTX-3 and 8-OHdG were 10.2-, 2.6-, and 4.2-fold higher, respectively, in HD patients. The HD patients had 35% and 32% lower concentrations of selenium pre- and post- dialysis compared to controls. Selenium concentrations were lowest in the diabetic HD group. Furthermore, there was a correlation between decreased selenium and increased 8-OHdG in the diabetic HD group, figure 1a-b.

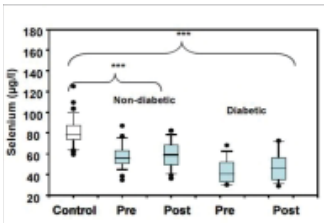



**Figure 1a** Plasma selenium concentrations in healthy controls and pre- and post-dialysis plasma samples from non-diabetic and diabetic HD-patients

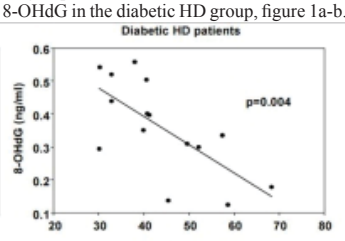



**Figure 1b** Significant correlation between decreased selenium concentrations and increased marker for oxidative stress in diabetic HD patients

**Conclusions:** The correlation between selenium deficiency and oxidative stress in diabetic HD patients supports further investigations on selenium and its effect on the inflammatory and oxidative stress status in HD patients.

*Funding:* Pharmaceutical Company Support - Gambro Lundia AB, Government Support - Non-U.S.

## TH-PO512

**Mechanisms Linking Atherosclerosis and End-Stage Renal Disease: Alterations in Lymphocyte Activation Molecules in Dialysis Patients with Atherosclerosis** Miguel Hueso,[1] Mariona Mestre,[2] Estanis Navarro,[3] Juan Torras,[1] Inés Rama,[1] Alberto M. Martinez-Castelao,[1] Jose M. Grino.[1] *[1]Nephrology, Hospital Bellvitge, L'Hospitalet, Barcelona, Spain; [2]Immunology, Hospital Bellvitge, L'Hospitalet, Barcelona, Spain; [3]Laboratori d'Oncologia Molecular, IDIBELL, L'Hospitalet, Barcelona, Spain.*

**Background:** The mechanisms linking Chronic Kidney Disease (CKD) and atherosclerosis (ATS) are not well known. T lymphocytes display a key role in ATS and L-selectin is important in lymphocyte-endothelial cell interaction. Thus, the aim of this study is to investigate if patients on dialysis with atherosclerosis displayed alterations in the expression of L-selectin or of other lymphocyte activation molecules.

**Methods:** Study of cases (dialysis patient with ATS) and controls (dialysis patient without ATS) based in dialysis patients waiting for their first kidney transplantation. Atherosclerosis was diagnosed by angioTAC or arteriography. Whole blood samples were obtained before dialysis and were co-stained with the following mAbs: anti-CD62L$^{PE}$ (L-selectin), anti-CD44$^{PE}$, anti-CD25$^{PE}$, anti-HLA-DR$^{FITC}$ and anti-CD134$^{PE}$ (OX40) with lymphocytes markers CD3$^{FITC/PerCP}$, CD4$^{FITC}$ or CD8$^{PE/PerCP}$ prior to be studied by citometry.

**Results:** 64 dialysis patients (26 with ATS and 38 without ATS) were studied. Patients with ATS displayed a higher proportion of CD4$^+$ lymphocytes (801±360 cells/mm$^3$ in patients without ATS vs 1073±548 cells/mm$^3$ in ATS; p=0.032), CD8hi$^+$ (323±172 cells/mm$^3$ in patients without ATS vs 455±254 cells/mm$^3$ in ATS; p=0.026), CD3$^+$CD25$^+$ (393±196 cells/mm$^3$ in patients without ATS vs 603±336 cells/mm$^3$ in ATS; p=0.008), CD3$^+$CD25$^+$HLADR$^+$ (33±18 cell/mm$^3$ in patients without ATS vs 48±33 cell/mm$^3$ in ATS; p=0.035), CD3$^+$CD25$^{hi+}$(16±10 cel/mm$^3$ in patients without ATS vs 24±16 cel/mm$^3$ in ATS; p=0.030) and less Mean Fluorescence Intensity (MFI) of L-selectin expressed by lymphocytes T (118±73 in patients without ATS vs 84±39 in ATS; p=0.027).

**Conclusions:** Dialysis patients with ATS displayed a down-regulation of L-selectin and the enhanced expression of lymphocyte T activation markers (CD25$^+$ and HLADR$^+$), suggesting the presence of a chronic inflammatory background that is lacking in dialysis patients not suffering ATS.

*Funding:* Government Support - Non-U.S.

## TH-PO513

**Malnutrition Inflammation and Survival in End Stage Renal Disease: Association with Variants of Appetite (Ghrelin, Leptin) and Energy Regulation (UCP2) Genes** Raj K. Sharma, Richa Sharma, Anita Saxena, Suraksha Agarwal. *Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, India.*

**Background:** Malnutrition inflammation syndrome (MIS) is common among ESRD patients. In the present study we have evaluated role of genetic markers associated with appetite and energy regulation with Malnutrition inflammation syndrome in end stage renal disease (ESRD) patients.

**Methods:** 257 patients on maintenance hemodialysis (MHD) and 200 normal healthy controls were included in the study. Nutritional assessment was done by Subjective Global Assessment Scores (SGA). Genotyping of leptin-2548 G/A (rs7799039), ghrelin Leu72Met (rs696217-408 C/A), Arg51Gln (rs34911341-346 G/A), Uncoupling protein 2 (UCP2) 45 bp Insertion deletion (ID) was done using PCR-RFLP. Levels of leptin and acyl ghrelin were assessed using ELISA.

**Results:** Leptin-2548 AA genotype was associated with 2 fold higher risk of disease susceptibility while UCP2 insertion deletion heterozygote's showed protective effect. Ghrelin Gln51gln and met72met genotype were associated with 3.4 and 2.5 fold higher disease susceptibility. The met72 and gln51 allele showed 3.3 and 2.1 fold higher susceptibility to malnutrition in severe SGA group. Further the levels of acyl ghrelin were significantly less in severe category of malnutrition and in poor appetite group. On combined analysis the group-2 (presence of 3-4 risk alleles) showed 1.5 and 2 fold higher susceptibility to disease and malnutrition respectively. On docking analysis it was observed that higher receptor binding energy was associated with the mutant form of ghrelin (gln51). Moderate and severe SGA were associated with 2.2 and 4.1 fold higher death hazard.

**Conclusions:** Our study suggests that ghrelin may be major marker contributing to susceptibility to MIS among ESRD patients.

## TH-PO514

**Monocyte and Plasma Expression of TAM Ligand and Receptor in Renal Failure: Links to Unregulated Immunity and Chronic Inflammation?** Iris J. Lee, Brendan A. Hilliard, Jean Lee, Crystal A. Gadegbeku, Philip L. Cohen. *Dept of Nephrology and Rheumatology, Temple Univ School of Medicine, Philadelphia, PA.*

**Background:** The TAM ligand, Growth Arrest Specific Gene 6 Protein (Gas6) and tyrosine kinase family TAM receptors Axl and Mer are important regulators of proinflammatory monocyte cytokine production. Mer expression in particular is increased upon macrophage differentiation into immunoregulatory M2c macrophages. Upon monocyte activation, TAM receptors are cleaved from the cell surface. We examined plasma and monocyte expression of gas6, Axl and Mer in hemodialysis (HD) and chronic kidney disease (CKD) patients.

**Methods:** Blood samples were from controls, HD and CKD. Plasma and monocytes were studied ex vivo or after culture with normal and uremic serum in the presence of M-CSF and dexamethasone. Gas6, Axl and Mer were evaluated by real time PCR, ELISA and FACS.

**Results:** Monocytes from HD had decreased gene expression of Mer and Axl and increased expression of TNF-α and IL-1b. Mer expression was significantly decreased in circulating CD14++CD16+ monocytes from HD, a specific subset linked to adverse vascular events. Significantly increased levels of soluble receptor sAxl and sMer were found in CKD and HD plasma, consistent with ongoing monocyte/macrophage activation. Furthermore, Gas6 levels, known to be increased in systemic inflammation, were significantly elevated in CKD and HD and associated with increased sMer and sAxl levels. Addition of uremic sera to monocytes cultured with M-CSF and dexamethesone, suppressed the expression of Mer, CD163 and CD206, receptors associated with M2c polarization.

**Conclusions:** TAM receptors have known immunoregulatory function and decrease proinflammatory cytokine secretion. We show increased TAM ligand and circulating soluble TAM receptor in the plasma and decreased TAM receptor expression on monocytes from HD and CKD patients. These data support the hypothesis that there is a deficiency of TAM receptor mediated regulation of inflammation in patients with chronic renal failure.

*Funding:* Private Foundation Support

## TH-PO515

**Modifications of Levels of 1, 25 $(OH)_2$ D Vitamin due to Administration of a Single, Large Oral Dose of 25-Hydroxycholecalciferol in Hemodialysis Patients**

Jose Luis Merino,[1] Jose Luis Teruel,[2] Milagros Fernandez-Lucas,[2] Blanca Bueno,[1] Juan Jose Villafruela,[2] Antonio Gomis,[2] Vicente Paraiso,[1] Carlos Quereda.[2] *[1]Nephrology, Hospital U del Henares, Coslada, Madrid, Spain; [2]Nephrology, Hospital U Ramon y Cajal, Madrid, Spain.*

**Background:** Low levels of Vitamin D have been associated with increased cardiovascular risk, and mortality. The deficiency is common in patients with chronic kidney disease and dialysis. We have evaluated the modifications of serum levels of D vitamin and mineral metabolism markers after the administration of a high and single, oral dose of 25-OHcholecalciferol (3 mg, Hidroferol®, 180,000 UI).

**Methods:** Chronic hemodialysis patients with 25(OH)VitD < 30ng/ml were included. Serum calcium> 10 mg/dl or PTH > 800 pg/ml were excluded. The patients were randomized in treated and controlled group. Time follow-up: 16 weeks. The usual treatment for controlling Ca/P levels neither the dialysis bath (calcium: 2.5 mEq/L) were modified. 86 patients ended the study, 42 patients in treated group.

**Results:** A higher levels of 1,25 and 25(OH)VitD were observed in the treated group and was maintained for 16 weeks. The levels of 1,25 and 25(OH)VitD were even bigger in the treated group than in the controlled one. This fact was associated with a significant decrease of PTH levels in the 8 post-treatment weeks. There were no difference due to technique (HDF-OL group, $1,25(OH)_2D$ level: 29,1+/-22 vs HD group, $1,25(OH)_2D$ level: 27,1 +/- 20) neither due to residual renal function (2 HD/week group: 30,3+/-25 vs 3 HD/ week group: 27,2+/-18). Serum calcium was >10 mg/dl in 16 of 252 (6%) performed samples in the treated group. Only two cases showed serum calcium > 10.5 mg/dl. There were no differences between both groups neither in phosphorous level nor in number of samples with serum phosphorous > 5.5 mg/dl.

**Conclusions:** A higher level of $1,25(OH)_2D$ has been observed due to an isolated dose of 3 mg of 25-OHcholecalciferol, this dose keeps enough levels of 25(OH)Vit D with a decreased level of PTH for three months. This data supports an extrarenal conversion of 25(OH)VitD. The correction of 1,25 and 25(OH)VitD levels and their potentially beneficial effects require long-term follow-up studies.

## TH-PO516

**The Impact of Transferrin Saturation on the Clinical Outcome in Incident Dialysis Patients**

Hyang Mo Koo,[1] Fa Mee Doh,[1] Hye-Young Kang,[2] Hyung Jung Oh,[1] Shin-Wook Kang.[1,2] *[1]Dept of Internal Medicine, Yonsei Univ College of Medicine; [2]Severance Biomedical Science Institute, Brain Korea 21, Yonsei Univ, Seoul, Korea.*

**Background:** Transferrin saturation (TSAT) is a marker reflecting the availability of iron for erythropoiesis and is closely associated with hemoglobin (Hb) levels. Although anemia is an independent risk factor for cardiovascular (CV) events and mortality in patients with end-stage renal disease (ESRD), the impact of TSAT on the clinical outcome has never been explored in dialysis patients.

**Methods:** A total of 879 anemic incident dialysis patients were recruited from 36 dialysis centers of the Clinical Research Center for ESRD in Korea and were divided into 3 groups according to baseline TSAT of ≤ 20%, 20-40%, and > 40%. Cox regression analysis was performed to determine the independent prognostic values of TSAT for a composite of CV mortality or hospitalization and all-cause mortality. The relationships between TSAT and echocardiographic findings and inflammatory/cardiac biomarkers were also evaluated using logistic regression analysis.

**Results:** There were no differences in Hb levels and the proportion of patients on erythropoiesis-stimulating agents or iron supplements between the 3 groups. During a mean follow-up duration of 19.3 mo, 51 (5.8%) patients died. CV composite (11.71 vs. 5.55 events/100 patient-years, P < 0.001) and all-cause mortality rates (5.38 vs. 2.31 events/100 patient-years, P = 0.004) were significantly higher in patients with TSAT ≤ 20% compared to those with TSTA 20-40% (reference group). Cox regression analysis revealed that patients with TSAT ≤ 20% had 1.62- and 2.19-fold higher risks for CV composite outcome (P = 0.046) and all-cause mortality (P = 0.030). Moreover, TSAT ≤ 20% was an independent predictor of left ventricular hypertrophy [odds ratio (OR), 1.46], high-sensitivity C-reactive protein ≥ 3 mg/dL (OR, 2.09), N-terminal pro B-type natriuretic peptide ≥ 10000 pg/mL (OR, 2.04), and troponin-T ≥ 0.1 ng/mL (OR, 2.02).

**Conclusions:** Low TSAT was a significant independent risk factor for adverse clinical outcomes in incident dialysis patients with anemia, which may be partly attributed to cardiac dysfunction and inflammation.

## TH-PO517

**Evil Communications between Scavenger Receptor Expression and Highly Elevated Macrophage Stimulating Factor in Hemodialysis Patients**

Miki Nishida,[1] Minoru Ando,[2] Yusuke Iwamoto,[3] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Dept Medicine, Kidney Center, Tokyo Women's Medical Univ; [2]Tokyo Metropolitan Komagome Hospital; [3]Saitou Memorial Hospital.*

**Background:** Macrophage-colony stimulating factor (M-CSF) may develop atherosclerosis by increasing macrophage viability/differentiation and the expression of the class A macrophage scavenger receptor (SR-A). SR-A plays a pivotal role promoting foam cell formation by binding and internalizing oxidized low-density lipoprotein (LDL).

**Methods:** Transcriptional levels of SR-A, M-CSF, and c-fms (M-CSF receptor) were simultaneously measured in peripheral monocytes by quantitative real-time RT-PCR, using the comparative threshold (Ct) method. Relative quantity of the gene was calculated as $2^{-\Delta\Delta Ct}$. Peripheral monocytes were isolated using magnetically-labeled Whole Blood $CD14^+$ MicroBeads (Miltenyi Biotec). Plasma level of M-CSF was measured with a M-CSF ELISA kit. Subjects included 39 chronic HD patients and 14 healthy controls.

**Results:** The SR-A gene expression was significantly higher in monocytes from the HD patients than in that from the controls (mean [95% CI of the mean]; 3.14 [2.58-3.69] versus 1.09 [0.80-1.39], P <.0001). Moreover, the gene expression was significantly higher in HD patients who had cardiovascular disease (CVD) than in those who did not (3.56 [2.89-4.22] versus 2.19 [1.29-3.10], P=0.0137). Plasma concentration of M-CSF was 6-fold higher in HD patients than in controls (995 ± 252 versus 157 ± 44.2 pg/ml, P <.0001). It was significantly correlated with the gene expression of SR-A ($r^2 = 0.244$, P = 0.0002), and inversely with that of c-fms ($r^2 = 0.134$, P = 0.0071); however, not with that of M-CSF.

**Conclusions:** Plasma M-CSF concentration is extremely increased in HD patients, which is not due to up-regulation of M-CSF gene, but likely due to excessive suppression of c-fms gene. SR-A expression is constitutively enhanced in the HD patients, and increased with increasing plasma M-CSF concentrations. This vicious collaboration could be one of the hidden mechanisms for atherosclerosis in HD patients.

## TH-PO518

**Relationship between Immune System and Morbidity and Mortality in Hemodialysis Patients**

Maria Molina, Enrique Morales, Luis Allende, Manuel Praga. *Nephrology, Hospital 12 de Octubre, Madrid, Spain.*

**Background:** Last stages of chronic kidney disease are associated with increased of morbidity and mortality, mainly for cardiovascular disease (CVD) and infections. Uremia is linked with a dysfunction of immune system and it could be associated with the most important causes of morbidity and mortality in hemodialysis (HD). The aim of this study was to analyse the relationship between morbidity and mortality with cuantitative changes of immunological system in HD patients.

**Methods:** We began a single centre prospective cohort study from April 2011 to April 2013 in HD patients. We analysed complement (C3 and C4), immunoglobulins (IgA, IgG, IgM) and lymphocytes (CD4 helper T lymphocyte and CD8 citolytic T lymphocyte; B lymphocytes; and natural killer (NK)) levels. We considered low range as lower than usually normal range used in our laboratory department. We followed patients just they died, received a kidney transplant or they were admitted to a hospital for CVD or infection disease.

**Results:** We included 103 patients (52% males) with mean age 64,4±15,1 years. Charlson comorbidity index medium (interquartile range: IQR) was 3 (1-4), 33% of patients had a previous kidney transplantation, 29% were diabetic and 22% had a positive hepatitis C virus antibodies. Meidum time with renal replacement therapy was 36 (9-84) months and 43% received online haediafiltration therapy. The rate of death was 23% with a medium follow of 8 (5-14) months. Low range of NK lymphocytes (OR:3,5; 1,1-10,8, p=0,02) and higher CRP (OR: 2,5; 1,1-6,2, p = 0,03) were associated with higher risk of death. The incidence of infection and CVD hospitalization were 24% and 14%, respectively. Both cases, B lymphopenia was linked with higher risk of develop a event, (OR: 3,8; 1,3-11,1; p=0,01) and (OR: 9,6; 1,2-73,5; p=0,02), respectively. Higher CRP was associated with more risk of infection hospitalization (OR:2,5; 1,1-5,8 p=0,03).

**Conclusions:** NK lymphopenia and higher CRP is associated with higher risk of death and B lymphopenia is linked with higher incidence of hospitalization (CVD and infection causes). These findings suggest quantitative immunological change could be new markers of morbidity and mortality in HD patients.

## TH-PO519

**The Impact of Inflammatory/Anti-Inflammatory Macrophages/Monocytes for the Immune Response to Influenza Vaccination in Hemodialysis Patients**

Haruka Yasuda, Yasunori Iwata, Kengo Furuichi, Takashi Wada. *Nephrology, Kanazawa Univ.*

**Background:** The infection of influenza is a high risk for the progression to pneumonia and can be a life-threatening in the hemodialysis patients. However, the effectiveness and mechanisms of the immune response to influenza vaccine are not clear in the hemodialysis patients. Moreover, resent data show that the balance of inflammatory/anti-inflammatory cells play an important role for the immune status in inflammatory diseases. Therefore, we hypothesize that the balance of inflammatory/anti-inflammatory cells are involved in the immune response to influenza vaccine in the hemodialysis patients.

**Methods:** Thirty five hemodialysis patients and 20 healthy controls were vaccinated. Influenza hemagglutination inhibition (HI) assay was performed before and 1 month after the influenza vaccination. At the same time, flow cytometory analysis of peripheral blood leukocyte was conducted to detect inflammatory (M1) macrophage/monocyte (Mf) ($CD14^+$ $CD16^+$), anti-inflammatory (M2) Mf ($CD14^+$ $CD206^+$), myeloid derived suppressor cell (MDSC) ($CD14^+$ $HLA\text{-}DR^-$) and regulatory T cell ($CD4^+$ $CD25^+$ $Foxp3^+$). Responder wad defined as ≥4 fold increase in HI titer.

**Results:** There was no difference in the number of responder after vaccination between the hemodialysis patients and healthy controls. The number of responder was lower in the hemodialysis patients with diabetes as compared to those without diabetes. Responder showed the decreased frequency of M1 Mf before vaccination. The frequency of MDSC was also decreased but not significant. The balance of inflammatory/anti-inflammatory cells was not different between responder and non-responder in the hemodialysis patients.

**Conclusions:** The frequency of M1 Mf might have the impact for the immune response of influenza vaccination in the hemodialysis patients, even though the balance of inflammatory/anti-inflammatory cells did not differ.

## TH-PO520

**Cinacalcet Treatment Decreases Serum Free Testosterone Concentration in Male Hemodialysed Patients with Chronic Kidney Disease and Secondary Hyperparathyroidism** Andrzej Wiecek, Piotr Kuczera, Marcin Adamczak. *Dept of Nephrology, Endocrinology and Metabolic Diseases, Medical Univ of Silesia, Katowice, Poland.*

**Background:** Calcium Receptor is expressed, among others in testis. Cinacalcet is used in the treatment of secondary hyperparathyroidism (sHPT) in hemodialysed patients with chronic kidney disease (HDP). In most of male HDP, serum free testosterone concentrations are low. The aim of this study was to assess the influence of six-month treatment with cinacalcet on the serum free testosterone concentration.

**Methods:** In 40 male HDPwith sHPT (PTH>300pg/ml), enrolled in this prospective, open-label, single arm study, plasma PTH and serum free testosterone concentrations were assessed before the first dose of cinacalcet and then after 3 and 6 months of treatment. Wilcoxon matched pairs test and Spearman test were performed. Results are shown as means with 95% confidence index.

**Results:** In the 35 patients who completed the study cinacalcet treatment caused significant decrease of serum PTH from 1106pg/ml (805-1407pg/ml) at the baseline, to 781pg/ml (475-1086pg/ml) after 3 month of treatment (p=0.002), and to 605pg/ml (298-912pg/ml; p<0.0001) after 6 months of treatment and also led to decrease of serum free testosterone concentration from 6.96pg/ml (5.61-8.30)pg/ml to 5.87pg/ml (4.99-6.95)pg/ml; p<0.05 and to 5.94pg/ml (4.93-6.95)pg/ml; p=0.03, respectively. There were no significant correlation between changes of serum free testosterone concentration and changes of plasma PTH concentration and cinacalcet dose, respectively.

**Conclusions:** 1. Treatment with cinacalcet decreases serum free testosterone concentration in male HDP with sHPT. 2. Such a decrease is not related to the changes of plasma PTH concentration.

*Funding:* Government Support - Non-U.S.

## TH-PO521

**Ferric Citrate as a Phosphate Binder Reduces IV Iron and Erythropoietin Stimulating Agent (ESA) Use** Kausik Umanath,[1] Samuel S. Blumenthal,[3] Mohammed Sika,[2] Barbara A. Greco,[5] Diana I. Jalal,[4] Efrain Reisin,[6] John Manley,[7] Steven Zeig,[8] Dana G. Negoi,[9] Anand N. Hiremath,[10] Jamie P. Dwyer,[2] The Collaborative Study Group.[11] *[1]Henry Ford Hosp; [2]Vanderbilt Univ; [3]Med Coll Wis/VA; [4]Univ of Colorado; [5]W New Engl Renal & Tplt Assoc. & Baystate Med Ctr; [6]Louisiana State Univ; [7]Mt Kidney & HTN Assoc.; [8]Pines Clin Res; [9]Fletcher Allen Health Care; [10]Neph HTN Clin; [11]CMM.*

**Background:** Ferric Citrate (FC), a novel phosphate binder, has potential for iron absoprtion.

**Methods:** We report the IV iron and ESA use in a multi-center, randomized trial in 441 dialysis subjects treated with FC or active control (AC), sevelamer carbonate and/or calcium acetate. After a 2 week washout period, subjects were randomized 2:1 to FC or AC, and followed during a 52-week safety assessment period (SAP). IV iron use was at the discretion of the site as long as the serum ferritin was ≤ 1000ng/mL and the transferrin saturation (TSAT) was ≤ 30%. Oral iron and vitamin C were prohibited. Hemoglobin (Hgb) levels, IV iron and ESA use were collected and analyzed.

**Results:** 292 subjects were on FC and 149 subjects were on AC. FC subjects received less IV elemental iron (1.88 mg/day) than AC (3.84 mg/day; p<0.001) in the SAP. More subjects on FC did not require IV iron compared to AC, Table 1. At week 52 Hgb was 11.4 g/dL in FC and 11.1g/dL in AC (treatment difference, p=0.01). FC subjects received less erythropoietin units (U) per day (756 U/day) than AC subjects (993 U/day, p<0.05).

| Time in SAP (Weeks) | % FC Subjects Not Receiving IV Iron | % AC Subjects Not Receiving IV Iron | P Value |
|---|---|---|---|
| 0 - 12 | 31 | 19 | 0.014 |
| 12 – 24 | 53 | 26 | <0.0001 |
| 24 - 36 | 67 | 39 | <0.0001 |
| 36 - 52 | 67 | 37 | <0.0001 |

**Conclusions:** Use of FC as a phosphate binder both reduced the total amount of IV iron and ESA subjects received on dialysis as well as the frequency with which they received IV iron. These results suggest some ferric iron absorption in this patient population. Thus, FC, an efficacious phosphate binder, also reduced the need for IV iron and ESA in dialysis patients.

*Funding:* Pharmaceutical Company Support - Keryx Biopharmaceuticals

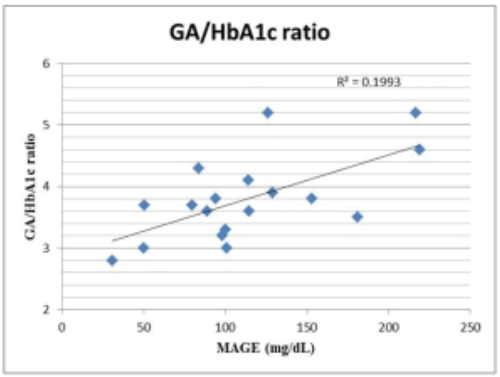
## TH-PO522

**Underestimation of Glycated Hemoglobin (HbA1c) Relative to Glycated Albumin (GA) Caused by Glycemic Variability in Diabetic Hemodialysis (HD) Patients: Assessment Using Continuous Glucose Monitoring (CGM)** Yoshiaki Lee,[1] Satoshi Funakoshi,[1] Kenichi Miyazaki,[1] Jyunichiro Hashiguchi,[1] Kenji Sawase,[1] Takashi Harada,[1] Tomoya Nishino,[2] Yoko Obata,[2] Shigeru Kohno,[2] Kazunori Utsunomiya.[3] *[1]Div of Blood Purification, Nagasaki Renal Center, Nagasaki, Japan; [2]Dept of Internal Medicine, Nagasaki Univ Graduate School of Medicine, Nagasaki, Japan; [3]Dept of Diabetology, Jikei Univ, Tokyo, Japan.*

**Background:** Several reports indicate that GA is a better glycemic indicator than HbA1c in HD patients with diabetes due to altered erythrocyte survival and erythropoiesis-stimulating agents (ESA) therapy, and HbA1c significantly underestimates glycemic control relative to GA. We herein investigated the significance of glucose variability in GA and HbA1c in diabetic HD patients using a CGM system.

**Methods:** We recruited 17 relatively well-controlled patients with type 2 diabetes under maintenance HD with monthly GA HbA1c values recorded for 3 months, and GA / HbA1c ratio in each patient was calculated over the period. Administrated dose of ESA stayed the same. To measure glycemic variability, we calculated mean amplitude of glucose excursion (MAGE) as described in detail elsewhere.

**Results:** Mean ± SD diabetes duration was 14.4 ± 7.8 years and dialysis vintage 2.7 ± 2.1 years, and mean HbA1c 6.4 ± 2.5, GA 24.5 ± 7.7, respectively. As shown in Figure 1], significant positive correlations were found for GA / HbA1c ratio and MAGE ($R^2 = 0.1993$), indicating HbA1c is underestimated relative to GA as daily glycemic variability increase.




**Conclusions:** Glycemic control measured by HbA1c was dissociated from GA in diabetic HD patients, with the degree of underestimation getting worse as glycemic variation increases.

*Funding:* Private Foundation Support

## TH-PO523

**Gastrointestinal Microbiota and Inflammation in Dialysis Patients** Teena Cherian, Madhumathi Rao. *Medicine/Nephrology, Tufts Medical Center, Boston, MA.*

**Background:** Alterations in gastrointestinal (GI) microflora could potentially mediate inflammation in dialysis patients. We examined the relationship between GI microbiota, markers of bacterial translocation and immune activation among hemodialysis (HD), and peritoneal dialysis (PD) patients and healthy controls (HC).

**Methods:** Blood and stool samples were collected from 22 HD and 13 PD patients and 9 age-matched HC. Recent antibiotic use, dialysis catheter, GI pathology or surgery were excluded. Stool microbiota was studied by pyrosequencing the 16S rRNA genes. Serum concentrations of Interleukin-6 (IL-6), C-reactive protein (CRP), soluble CD14 and LPS binding protein were measured; bacterial translocation from the GI tract was measured using quantitative PCR of the 16S rDNA genes in spent dialysate and plasma.

**Results:** Table 1 compares clinical and laboratory parameters among HD, PD and HC; CRP, IL-6, sCD14 and LPS binding protein concentrations were significantly higher among dialysis patients. Profiles of GI microbiota showed differences in diversity and equitability indices and the numbers of operational taxonomic units in dialysis patients and in the Firmicutes and Bacteroidetes phyla compared to healthy controls. Finally spent dialysate tested positive for LPS in 6 of 22 HD patients suggesting bacterial translocation during dialysis.

**Conclusions:** Dialysis patients show measurable alterations in GI microbiota in the setting of elevated immune activation markers. This could predispose to colonization by pathogens, impair barrier function and immune response in GI epithelium.

| Clinical Characteristics | HD (N=22) | PD (N=13) | HC (N=9) |
|---|---|---|---|
| Age (yrs) | 59±12 | 59±15 | 55±8 |
| Male (%) | 73% | 69% | 44% |
| Caucasian | 23% | 54% | 78% |
| Median duration on dialysis (months) | 56 | 23 | - |
| GI symptoms (bloating, bowel disturbance, anorexia) | 18%-68% | 23%-62% | - |
| **Immune marker assays** | | | |
| hsCRP (mcg/mL) | 7.9±12.0 | 3.6±2.5 | 1.5±1.2 |
| IL-6 (pg/ml) | 1.2±0.6 | 1.5±0.7 | 0.6±1.0 |
| sCD14 (ng/ml) | 2200±496 | 2309±612 | 1429±159 |
| LPS binding protein (ug/ml) | 14.3±4.4 | 15.5±4.2 | 9.1±1.8 |

*Funding:* Private Foundation Support

## TH-PO524

### The Relation between Serum Hepcidin-25 (HPC) Level and Other Iron Metabolism Markers during Intravenous Iron Administration in Hemodialysis (HD) Patients

Noriko Saito,[1] Shigeru Miyazaki,[1] Kazuhide Saito,[2] Hiroki Takimoto,[1] Masaaki Shimotori,[1] Yutaka Tsubata,[1] Kozo Ikarashi,[1] Tetsuo Morioka,[1] Hisaki Shimada.[1] *[1]Shinraku-en Hospital; [2]Niigata Univ.*

**Background:** HPC is a crucial player of iron metabolism. Iron-overload and inflammation stimulate HPC production, whereas anemia, iron depletion inhibit HPC production. It is not elucidated that the sequential changes of the relation between HPC level and other iron parameters during intravenous iron administration in HD patients.

**Methods:** 21 anemic HD patients with low serum ferritin level ( 23±16 ng/ml ) were administered saccharated ferric oxide (Fe 40mg) intravenously for 11 consecutive HD sessions. We evaluated the following markers at 0 to 16 weeks after starting the therapy: HPC, GDF15, soluble transfferin receptor(sTfR) andstandard hematological parameters including high sensitive CRP (hs-CRP). Serum HPC level was determined by LS-MS/MS. GDF 15 and sTfR levels were measured by ELISA. Serum samples of 20 normal volunteers (N) were served as controls.

**Results:** HD patients with low serum ferritin level showed lower Fe(μg/ml) and HPC(ng/ml), higher GDF15(ng/ml), sTfR(nmol/ml) and hs-CRP(ng/ml) than N at 0W ( 43±31 vs 110±52**, 5.8±9.4 vs 11.1±11.9*, 7.4±2.4 vs 0.6±0.2**, 30.8±11.7 vs 16.3±6.5** and 1454 ± 2100 vs 237±290**, respectively). HPC level was increased at 4W(30.0± 33.1) and then decreased to 0W level at 10W(5.6±9.3). Hb (g/dl) was increased from 8.8±1.1 at 0W to 10.9±1.0 ** at 10W. In stepwise multiple regression analyses, UIBC and Fe were independently associated with HPC before the therapy, and at 4W, ferritin, GDF15 and hs-CRP were significant variables influencing HPC level which were the same as seen in N. At 6W and thereafter, ferritn was the only significant predictor of HPC. *:p<0.05, **:p<0.01.

**Conclusions:** Predictors of HPC level were quite different before and after intravenous iron administration. HPC level were significantly influenced by ferritin concentration after iron administration therapy in HD patients.

## TH-PO525

### Plasma Pentraxin-3 Levels Are Associated with Risk of Nutritional Status in Hemodialysis Patients

Tetsu Miyamoto,[1] Mika Matsumoto,[2] Hiroshi Tanaka,[3] Emi Hasegawa,[1] Akihiro Kuma,[1] Yoko Fujimoto,[1] Kenichiro Bando,[1] Ryota Serino,[1] Narutoshi Kabashima,[1] Yutaka Otsuji,[1] Masahito Tamura.[1] *[1]Second Dept of Internal Medicine, Univ of Occupational and Environmental Health School of Medicine, Kitakyushu, Japan; [2]Yukuhashi Clinic; [3]Iseigaoka Clinic.*

**Background:** Persistent inflammation is a major driving force of the uremic phenotype including protein energy wasting (PEW). Regular monitoring of inflammatory biomarkers in dialysis patients may help clinicians to estimate patient nutritional risk. Development of new inflammatory markers which are closely and specifically linked to nutritional status is thus needed in the dialysis population. Pentraxin 3 (PTX3) is a multifunctional soluble pattern recognition receptor which modulates immunoinflammatory response.

**Methods:** Plasma PTX3 levels (n=382) and one-year changes in PTX3 levels (ΔPTX3, n=160) were analyzed in relation to nutritional risk assessed by geriatric nutritional risk index (GNRI) in maintenance HD-patients.

**Results:** PTX3 [5.2 (2.6–10.5) ng/mL] were positively correlated with duration of dialysis (P<0.001) and HDL-cholesterol (P<0.001), and negatively with GNRI (P<0.001), BMI (P<0.001), and triglyceride (P<0.001). In a multivariate logistic regression model, both PTX3 (OR 1.12, 95%CI 1.05-1.29, p<0.01) and CRP (OR 1.22, 1.02-1.50, p<0.05) were independently associated with nutritional risk defined by GNRI < 91.2. While CRP levels were positively associated with Body weight/Ideal body weight (BW/IBW, P<0.001) but negatively with albumin (P<0.001), PTX3 had a negative association with BW/IBW (P<0.001), but not with albumin. ΔPTX3 levels were inversely correlated with ΔGNRI (p=0.02) in patients with lower PTX3 levels.

**Conclusions:** Both PTX3 and CRP predict nutritional risk in HD-patients. The association between CRP and nutritional risk calculated on the basis of GNRI depends on albumin levels, yet PTX3 had a strong association with anthropometric parameters. An increase of PTX3 levels has an association with nutritional risk marker. PTX3 may be a candidate for emerging inflammatory markers for the assessment of PEW because of the unique correlation with nutritional status in the dialysis population.

## TH-PO526

### Mild Nutritional Impairment Is Associated with Autonomic Dysfunction and Decreased Survival in Hemodialysis Patients

Dan Sapoznikov, Rebecca Backenroth, Yosef S. Haviv, Dvora Rubinger. *Nephrology and Hypertension Services, Hadassah Univ Medical Center, Jerusalem, Israel.*

**Background:** Severe malnutrition is associated with poor prognosis in end stage renal disease. The predictive value of mild nutritional impairment in chronic hemodialysis (HD) patients is not well established.

**Methods:** Multivariate analysis was performed to assess the role of nutritional risk (NR) as predictor of mortality and cardiovascular morbidity in HD. NR was evaluated using the NRS2002 screening method (*Clin Nutr 2003, 22:321-336*).The study population (n=112) was divided according to NR score into 4 categories: 0 (normal nutritional status), 1 (mild), 2 (moderate) and 3 (severe) impairment. Clinical and biochemical markers, CRP and the variability in the low frequency range of systolic blood pressure (LF SBP) and of interbeat intervals (LF IBI) rate, representative of autonomic function, were also analyzed in all patients. Primary (death) and secondary (fatal and non-fatal cardiovascular events) outcomes were recorded during a follow-up period of 60 months (range 1-60).

**Results:** Plasma albumin, LF SBP and LF IBI progressively decreased from NR1 to NR3, while CRP increased in NR2 and NR3. Kaplan-Meier analysis of primary outcome (5 y mortality) showed gradually decreasing survival rates for patients with increasing NR score from NR1 to NR3 (p 0.004, 0.001 and 0.001). Cox regression analysis with age, clinical and biochemical data, CRP and autonomic markers in the model identified NR score and age as powerful predictors of all cause mortality.The probability of the secondary outcome was markedly increased in NR2 and NR3 as compared to NR0 and NR1.Ischemic heart disease and intradialytic hemodynamic instability were significant predictors of cardiovascular events (p 0.015 and 0.001).

**Conclusions:** In HD, even mild nutritional impairment is associated with autonomic dysfunction and increased mortality. Moderate and severe nutritional impairment are associated with both increased mortality and cardiovascular morbidity, especially in patients with cardiac disease. Malnutrition in concert with autonomic dysfunction and inflammation are associated with increased mortality and morbidity in elderly HD patients.

## TH-PO527

### The Longitudinal Change of Corrected Mid-Arm Muscle Area Predicts Mortality in Hemodialysis Patients

Sumi Hidaka, Kunihiro Ishioka, Hidekazu Moriya, Takayasu Ohtake, Shuzo Kobayashi. *Dept of Nephrology, Immunology and Vascular Medicine, Shonan Kamakura General Hospital, Kamakura, Japan.*

**Background:** Protein-energy wasting is associated with increased morbidity and mortality in maintenance hemodialysis (HD) patients. Therefore, nutritional status needs to be regularly assessed and to detect malnutrition earlier. The aim of this study was to investigate the predictive value of anthropometric parameters on mortality.

**Methods:** This study included a cohort of 52 maintenance HD patients (36 men, mean age 63.3 ± 4.2 years). Anthropometric measurements were made in 2001, 2003, and 2005 and patients were followed up until 2012. Anthropometric measurements included body mass index (BMI), mid-arm circumference (MAC), triceps skinfold (TSF), mid-arm muscle circumference (MAMC) and corrected mid-arm muscle area (cMAMA). We investigated the predictors for all-cause death using Kaplan-Meier analysis and Cox proportional hazards analyses.

**Results:** During the mean follow-up of 117.8 months, 27 patients died. The deceased group had a significantly higher age at the enrolment in the study (p=0.001), but there was no significant difference in HD duration between the surviving and the deceased groups. Basal serum albumin level in deceased group was lower and serum β2-microglobulin level was higher than those in the surviving group (p=0.012 and p=0.042, respectively). Basal BMI, AC, and cMAMA did not differ significantly between the two groups, but the 4-year changes (Δ) of each value were significantly increased in the surviving group. Kaplan-Meier analysis revealed that the patients with decreased Δ cMAMA (N=15) had a significantly lower survival rate compared with those with increased Δ cMAMA (N=37, p=0.003). Multivariate Cox proportional hazards analyses demonstrated that the Δ cMAMA was a significant predictor of all-cause mortality after adjustments for confounding factors, such as age, serum albumin, serum β2-microglobulin, and Δ BMI. An increase in Δ cMAMA of 1 $cm^2$ reduced mortality by 6.0%.

**Conclusions:** These results demonstrate that the decrease in cMAMA is a significant predictor for mortality in HD patients. The interventions to increase Δ cMAMA are well worth trials.

## TH-PO528

### Relationship of Nutritional Intake and Body Composition for Undergoing Stable Hemodialysis

Yukie Kitajima,[1] Taeko Takahashi,[2] Yuzuru Sato.[2] *[1]Tokyo Healthcare Univ, Tokyo, Japan; [2]Sato Junkanki Hospital, Matsuyama, Japan.*

**Background:** It is important to maintain activities of daily living (ADL) in elderly dialysis patients. Further, cardiovascular disease (CVD) is a major cause of morbidity and mortality. Assessment of body composition is necessary for evaluating the nutritional status of patients with hemodialysis patients. We investigated the factors of for undergoing stable hemodialysis by examining the relationship of nutritional intake and changes of body composition, especially grip strength for six years in hemodialysis patients.

**Methods:** The studied subjects were 40 stable outpatients who were undergoing hemodialysis between 2007 and 2013. Grip strength measurement was measured before dialysis. Body composition was measured after completion of dialysis on the last day of the week. Patients were divided into two groups according to rate of grip strength; those

with rate of change (decrease) of grip strength ≥ - 15% (Group A) and with < - 15% (Group B). The method of evaluating for nutritional intakeused the meal recording method.

**Results:** Average of grip strength of hemodialysis patients was lower than average value of a healthy person (60.5%), their grip strength was significantly reduced for six years (-17.0%). Grip strength correlated with lean body mass (r=0.552, p<0.002). In nutritional intake, their energy intake (p<0.05) were significantly higher, protein catabolic rate (PCR) did not show significantly change. N-3 fatty acid intake (p<0.001) were significantly higher. % grip strength (46.8%, p<0.001) and Alb (3.5mg/dL, p<0.0002) of Group A were significantly lower than % grip strength (73.2%) and Alb (3.9mg/dL) of Group B. Group A was hospitalized with CVD and fracture (p<0.01) than Group B.

**Conclusions:** For six years, the more rate of decrease of grip strength is large, the more patient's malnutrition and amount of muscles decreases, hospitalization including CVD and fracture increase. However, it is necessary for hemodialysis patients to maintain energy intake and activity for stable survival and ADL. Therefore, to prevent thedecrease inmuscle mass, to intake enough energy is important factor for undergoing stable hemodialysis.

*Funding:* Government Support - Non-U.S.

## TH-PO529

**The Effects of Parenteral Amino Acid Therapy on Protein Carbamylation in End Stage Kidney Disease** Sahir Kalim,[1] Anders H. Berg,[2] Caitlin A. Trottier,[1] Hector Tamez,[1] Julia Beth Wenger,[1] Joseph James Deferio,[1] S. Ananth Karumanchi,[3] Ravi I. Thadhani.[1] *[1]Nephrology Div, Massachusetts General Hospital, Boston, MA; [2]Div of Clinical Chemistry, Beth Israel Deaconess Medical Center, Boston, MA; [3]Nephrology Div and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center, Boston, MA.*

**Background:** Carbamylation is a spontaneous chemical modification of proteins and amino acids by reactive cyanate, a byproduct of urea. Protein carbamylation is associated with adverse outcomes in ESRD. Proteins and free amino acids can compete with each other for binding reactive cyanate and our recent work in *Science Translational Medicine* demonstrates that increasing amino acid concentrations in vitro and in animals can attenuate the carbamylation reaction. Thus, amino acid therapy in humans may reduce uremic complications if targeted to reducing protein carbamylation.

**Methods:** 5 long-term hemodialysis subjects have undergone 6 weeks of post dialysis infusions of the amino acid supplement NephrAmine 5.4% (2 subjects receiving 125cc infusions and 3 subjects receiving 250cc infusions; 18 infusions total per subject). Carbamylated albumin levels (a surrogate for overall carbamylation burden) measured at baseline and 6 weeks were compared.

**Results:** No metabolic, hemodynamic, or other adverse events were observed. Baseline carbamylated albumin levels (C-Alb, mmol C-Alb/mol albumin) for the 5 subjects were 9.0, 7.8, 5.1, 6.2, and 8.0. Respective percent changes in C-Alb at the end of the 6 week study period were: -23.6%, -17.0% +12.9%, -4.8%, and -4.6%. Notably, the only subject experiencing an increase in C-Alb level had the lowest baseline level.

**Conclusions:** Amino acid therapy is safe and may be effective in reducing C-Alb levels in ESRD patients, especially those with high baseline carbamylation levels. Results from several additional subjects enrolled in this ongoing pilot study will be available at Kidney Week 2013 and will be useful in better characterizing the effects of amino acid therapy on protein carbamylation in ESRD.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO530

**Effects of Branched-Chain Amino Acid on Elderly Dialysis Patients Suffering from Malnutrition** Taeko Takahashi,[1] Yuzuru Sato,[1] Yukie Kitajima,[2] Ayako Naka,[1] Yoshiko Miyazaki.[1] *[1]Sato Junkanki Hospital, Matuyama, Ehime, Japan; [2]Tokyo Healthcare Univ, Tokyo, Japan.*

**Background:** The present study investigated whether elderly dialysis patients suffering from malnutrition accompanied by decrease in both body protein and skeletal muscle experienced improvement in nutrition as a result of ingesting branched-chain amino acid (BCAA).

**Methods:** 29 hemodialysis patients (13 males, 16 females, average age 78.5 ± 7.0 years, average dialysis duration 6.7 ± 4.8 years, diabetes mellitus 48.3%) were chosen from a total of 61 people subjected to dietary management in our hospital and nursing home. Patients receiving tube feeding or with ALB of ≥3.5 g/dl were excluded. The patients consumed galette-like food (4,000 mg containing BCAA ). Results of amino acid well as ALB, CRP, BUN analyses and appetite survey conducted for 3 months before and after BCAA ingestion were compared.

**Results:** The average daily amount of food containing BCAA was 3,318.8 mg. BUN significantly increased from 63.6 ± 12.8 mg/dl to 70.7 ± 16.8 mg/dl. A comparison of median values for essential amino acids showed that the following 6 of 9 items increased significantly: valin from 186.3 ± 47.6 to 246.7 ± 76.3 nmoL/mL, methionine from 23.0 ± 4.7 to 25.9 ± 7.7 nmoL/mL, isoleucine from 63.5 ± 21.4 to 86.2 ± 43.5 nmoL/mL, leucine from 97.1 ± 28.8 to 153.7 ± 78.4 nmoL/mL, phenylanine from 65.0 ± 14.6 to 71.5 ± 14.5 nmoL/mL, and histidine from 83.2 ± 14.2 to 89.1 ± 15.4 nmoL/mL. In addition, BCAA levels increased significantly from 346.8 ± 93.4 to 499.8 ± 188.0 nmoL/mL and the Fisher ratio increased significantly from 3.96 ± 4.7 to 4.04 ± 1.6 nmoL/mL. No significant changes were observed for TP, Alb, CRP levels or the appetite surveys.

**Conclusions:** It is difficult for hemodialysis patients suffering from malnutrition to supplement the BCAA intake with diet alone. Therefore, the consumption of food containing BCAA by dialysis patients who are able to take food orally has the potential to improve their nutritional status. Continuous ingestion results in increase in both BCAA levels and the Fisher ratio; thus, it is also effective in the regulation of the balance of the amino acids necessary for protein synthesis within the body.

## TH-PO531

**Salt and Sodium: HD Patients Do Not Know the Difference** Camila Machado de Barros,[1] Carolina Quintella,[1] Thalita Lima Melho,[2] Camila Morales,[2] Felipe Linhares Arruda,[2] Letícia Alberto,[2] Edeli Simioni Abreu,[2] Rosana Farah,[2] Cláudia Gimenes,[1] Bárbara Margareth Menardi Biavo,[1] Jacqueline Santos,[1] Carmen B. Tzanno-Martins.[1] *[1]Grupo CHR; [2]Universidade Presbiteriana Mackenzie.*

**Background:** The control of dietary sodium is a challenge in the treatment of patients with chronic kidney disease (CKD) on hemodialysis. However, the use of educational strategies may contribute to a better adherence to the treatment of these patients. **Objective**: To evaluate the knowledge of salt and sodium in patients with CKD and effectiveness of nutritional approach for content assimilation.

**Methods:** The study was described as pre and post-test, where one group was its own control. 448 patients undergoing hemodialysis therapy (57% males, 57.5 ± 14.5 years) participated in the intervention for a period of 3 weeks. The educational material used included a questionnaire assessing knowledge, a lecture of 50 minutes and the presentation of food labels. In the pre-test was assessed knowledge about the topic and the post-test was evaluated assimilation of the content.

**Results:** In the pre and post-test: 66.8% knew and 85.5% assimilated what was salt, 38% knew and 65.8% assimilated what was sodium, 14% knew and 58% assimilated the difference between salt and sodium, 30% knew and 61.5% assimilate which the recommendation salt grams/day (p <0.05), 8.8% knew and 25% assimilate which equivalence in mg of sodium (p <0.05 ), 63.2% knew and 72% assimilated in household measures as they could consume salt/meal (p <0.05) and 44.4% knew and 66% assimilated to identify sodium on food labels (p <0.05), respectively.

**Conclusions:** The increase in knowledge after the lectures presented reinforces the need for intervention programs in sectors of hemodialysis as a prevention of clinical complications and consequent improvement in quality of life.

*Funding:* Private Foundation Support

## TH-PO532

**Nutrition Education: New Tool for Guidance of Hemodialysis Patients with Focus on Humidity, Phosphorus and Potassium** Camila Machado de Barros,[1] Bruna Benedetti,[2] Juliana Salgado Azeg,[2] Maria Flávia Sgavioli,[2] Edeli Simioni Abreu,[2] Rosana Farah,[2] Bárbara Margareth Menardi Biavo,[1] Jacqueline Santos,[1] Elzo Ribeiro Júnior,[1] Carmen B. Tzanno-Martins.[1] *[1]Nutrition, Grupo CHR, São Paulo, Brazil; [2]Nutrition, Universidade Presbiteriana Mackenzie, São Paulo, Brazil.*

**Background:** The objective of this study was to determine humidity, potassium and phosphorus of foods (fruits, leaf vegetables and non-leaf vegetables) in order to evaluate the best foods to be consumed by CKD patients and develop an educational material for them.

**Methods:** We prepared an educational material to be presented to patients. Three comparative tables were created, one for each of three food groups: fruits, leaf vegetables and non-leaf vegetables.The food selection was made according to the nutritional guidelines for patients with CKD. The survey data for the elaboration of the project was carried out by three different sources: TACO - Brazilian Table of Food Composition (UNICAMP, 2000); UNIFESP Nutritional Table; and Food Composition Table of the United States Department of Agriculture (USDA, 2007).

**Results:** We've confirmed that fruits considered 'dry' obviously contain less water in its composition (69% moisture). However, phosphorus (30 mg / 100 g) and potassium (321 mg / 100g) levels are concentrated. So they have great increase of its percentage. The three fruits with higher humidity content were pyriform orange (*Citrus aurantium pyriforme*) (89.6%), Key lime (*Citrus aurantifolia*) (87.4%) and strawberry (91.5%). And the ones with the lowest rates, excluding 'dry' fruits, were avocado (83.8%), tangerine (83.7%) and guava (80.8%). It was found that all plant foods should be cooked, because they have large amount of potassium. Although we have examined the phosphorus content in these foods, it is known that these are not the usual sources for this mineral, but they can influence phosphorus' increase in the patient's body when consumed in large quantities.

**Conclusions:** It was evident the lack of literature related to the amount of water contained in food. Therefore, it is necessary to develop more studies in order to analyze the influence of foods in daily intake of liquid by CKD patient.

*Funding:* Private Foundation Support

## TH-PO533

**Effects of Carnitine on Oxidative Stress and Inflammatory Responses to Intravenous Iron Administration to Patients with CKD** Zaher Armaly,[1] Amir Abd Elkadir,[1] Adel Rafik Jabbour,[1] Bishara Shafik Bisharat,[1] Abdalla Bowirrat,[1] Hashem J. Bishara,[1] Zaid Abassi.[2] *[1]Nazareth Hospital-EMMS, Galilee Faculty of Medicine - Bar Ilan Univ, Zafed, Israel; [2]Physiology, Faculty of Medicine, Technion, Israel.*

**Background:** Anemia is a common problem in CKD patients. It is attributed to decreased erythropoietin (EPO) and iron stores. Therefore, therapy includes EPO and iron replenishment. However, the latter induces oxidative stress. Randomized studies suggested that L-carnitine supplementation might have positive effects on the response to EPO in long term HD patients. Unfortunately, there is no evidence whether this approach is also beneficial in earlier-stage of CKD .

**Methods:** This study included 26 anemic CKD patients (stages 2-4) that were given a weekly IVIR (Sodium ferric gluconate, 125 mg/100 ml) for 8 weeks, and during weeks 5-8

also received carnitine (20mg/kg, IV) prior to IVIR administration. Weekly blood samples were drawn before and after each IVIR for advanced oxidative protein products (AOPP), neutrophil gelatinase-associated lipocalin (NGAL), in addition to routine complete blood count and biochemical analyses.

**Results:** IVIR for 4 weeks did not increase hemoglobin levels, yet worsened the oxidative burden as was evident by elevated plasma AOPP from 229.1±27.3 to 318.1±39.3 and 367.8±41.8μM ($p<0.05$) after 1 and 4 weeks, respectively. Plasma NGAL levels were not significantly affected by IVIR (265.4±48.6 vs. 265.1±44.7 and 205.7±39.4 ng/ml, after 1 and 4 weeks, respectively). Simultaneous administration of carnitine with IVIR resulted in a mild hemoglobin increase. Interestingly, carnitine therapy abolished the IVIR-induced oxidative stress, where AOPP levels were 237.4±31.85 and 271.8±23.8 μM after 1 and 4 weeks, respectively,P=NS). Furthermore, carnitine therapy decreased NGAL levels to 201.3±36.63 ($p=0.02$), and 239±48.55 ng/ml ($p=0.05$). No changes in CRP, SCr, BUN, albumin or WBC were observed following IVIR alone or combined with carnitine.

**Conclusions:** This study demonstrates that IVIR in CKD patients provokes oxidative stress, as evident by elevation of AOPP. The antioxidative effects of carnitine, suggests a role for carnitine therapy also in earlier-stage CKD patients.

*Funding:* Government Support - Non-U.S.

## TH-PO534

### The Plasma Concentration of Free Carnitine Should Be Increased above Normal in Order to Reduce the Darbepoetin Alfa Dose in HD Patients Administered Levocarnitine

Masaki Aono, Yuzuru Sato. *Sato Junkankikanaika, Matuyama, Ehime, Japan.*

**Background:** Plasma concentration of free carinitine decreases significantly in hemodialysis patients, and the free carnitine depletion impairs the response to Darbepoetin Alfa (DA) in renal anemia patients on HD. Levocarnitine can be administered to improve DA-resistance anemia, but the adequate dose for improvement has not yet been determined.

**Methods:** Over a three-month period, a daily dose of levocarnitine chloride (1800mg/day) was administered orally to 134 patients on HD who were diagnosed with carnitine deficiency (plasma concentration of free carnitine < 30μmol/L). At the end of the three months, the patients were divided into 3 groups according to the concentration of plasma free carnitinne (FC) as follows: group1: FC<30umol/L, N=22, group2: 30<FC<72, N=13, group3: FC>72, N=99. DA dosage and the erythropoietin resistance index (ERI: DA dose (μg/week)/Hb (g/dL)/body weight (Kg) x 100) were compared among the patients in the three groups before and after the treatment.

**Results:** In group 2, the plasma concentration of free carnitine increased from a mean ± SD of 22.5±4.7μmol/L to 45.8±11.3μmol/L after administration of levovcarnitine (paired t-test $P<0.01$);in group 3, it increased from 20.4±4.6μmol/L to 275.5±136.6μmol/L (paired t-test $P<0.01$). But in the group 1, it did not increase significantly (from 23.4±3.1 to 25.0 ± 4.6 mol / L, n.s). The dosage of DA and ERI decreased significantly only in group 3 (DA: from 23.8±19.4 to 18.4±15.6μg/week,$p<0.01$,ERI:4.7±4.2 to 3.5±3.1,$P<0.01$)), but remained unchanged in the other two groups (in group 1, DA : from 23.3±18.2 to 20.3±15.4, $P=0.43$.ERI:from 4.4±3.9 to 3.9±2.8, $P=0.46$);in group 2, DA: from 11.4±11.4 to 10.2±13.7, $P=0.87$. ERI: from 3.4±4.7 to 2.0±2.9, $P=0.36$).

**Conclusions:** This study suggested that levocartine can reduce the dose of DA and improve the resistance to DA in renal anemia patients on HD. But it appears to be necessary to increase the level of plasma carnitine above normal to obtain a sufficient effect of carnitine in HD patients. The monitoring of plasma concentration of free carnitine is important to assess the effect of levocarnitine to improve erythropoietin resistance.

## TH-PO535

### Predictors of Habitual Physical Activity in Hemodialysis Patients

Sivakumar Sridharan,[1] Jocelyn Berdeprado,[1] Kirsten L. Rennie,[2] Neil Ashman,[3] Andrew Davenport,[4] Michael K. Almond,[5] Anindya Banerjee,[6] Enric Vilar,[1,2] Justin Roberts,[2] Ken Farrington.[1,2] *[1]Lister Hospital; [2]Univ of Hertfordshire; [3]Royal London Hospital; [4]Royal Free Hospital; [5]Southend Hospital; [6]Arrowe Park Hospital.*

**Background:** Hemodialysis (HD) patients have reduced levels of physical activity (PA). It is vital to understand factors influencing PA to develop interventions to encourage PA in this patient population.

**Methods:** Each study subject was administered the validated Recent Physical Activity Questionnaire (RPAQ). RPAQ enquires about time spent on activities at home, at work and during various recreational activities in the preceding 4 weeks. We assigned MET (metabolic equivalent of task) values for each activity. Resting energy expenditure (REE) was estimated using a novel predictive equation validated in renal failure. Total energy expenditure (TEE) was estimated from MET and REE. TEE estimation assumed 6-8 hours sleep and that the remaining unreported hours from RPAQ were spent in light activity. Energy Expenditure from physical activity (PAEE) was calculated as TEE – REE.

**Results:** 1500 HD patients (910 males) completed the RPAQ. Mean age was 62.9(±15.5) years. There were 682 Whites, 418 Asians and 400 Blacks. 790 patients were on Hemodiafiltration(HDF). Mean REE was 1545 kcal/day, mean TEE 1840 kcal/day and mean PAEE 295 kcal/day. Male patients, those aged < 65 and those who were employed, had significantly higher REE, PAEE and TEE ($p<0.001$). No significant difference was noted in PAEE among various ethnic patients. PAEE was significantly less in those with heart disease, diabetes and arthritis and higher in those receiving HDF. In multivariate regression analysis, the independent predictors of PAEE were age, sex, employment status ($p<0.001$ for all), HDF therapy ($p=0.01$) and dialysis vintage ($p=0.02$) with these factors accounting for 50% of the variance in PAEE.

**Conclusions:** Mean daily MET values of the study subjects indicate a sedentary lifestyle for most HD patients. Age, sex, employment status, HDF therapy and dialysis vintage are the principal determinants of energy expenditure from physical activity in HD patients.

## TH-PO536

### Effects of Kibow Probiotic Supplementation Renadyl™ on Uremic Toxins in Hemodialysis Patients

Subodh J. Saggi,[1] Mary C. Mallappallil,[1] Usha N. Vyas,[2] Griet Lrl Glorieux,[3] Peter Liang,[1] Pari Ranganathan,[2] Bohdan Pechenyak,[2] Gary R. Briefel,[1] Lorraine L.A. Thomas,[1] Raymond C. Vanholder,[3] Natarajan Ranganathan,[2] Eli A. Friedman.[1] *[1]SUNY Downstate Medical Center, Brooklyn, NY; [2]Kibow Biotech, Inc, Newton Square, PA; [3]Ghent Univ Hospital, Ghent, Belgium.*

**Background:** Our prior studies in patients with CKD 3-4 (n=31) given Renadyl™, a safe proprietary probiotic dietary supplement that metabolizes nitrogenous wastes in the bowel, at a dose of 90-270 B CFU per day, over a 4 month period showed that BUN, creatinine and $K^+$ levels declined.

**Methods:** We now conducted a prospective double blind cross over trial with placebo and Renadyl™ in 26 stable CKD patients on hemodialysis. Dosage administered was 180 B CFU per day, given in 3 divided doses. Our primary aim was a 20% reduction in BUN levels over an 8 week period. Patients' dialysis prescriptions were unchanged. Our secondary aim was to see if there would be changes in WBC count, C-reactive protein (CRP), total and/or free serum concentrations of indoxyl sulfate, indole acetic acid, p-cresyl sulfate, hippuric acid, serum pentosidine, 3-carboxyl-4-methyl-5-propyl-2-furan-propanoic acid (CMPF), uric acid and beta-2 microglobulin. Solutes were measured by HPLC and ELISA. QoL changes were assessed by a modified SF-36 questionnaire. Patient adherence was assessed by pill count and stool culture to verify probiotic growth during study and absence during placebo period. Data were analyzed using ANOVA for a crossover study with a mixed model methodology in SAS to account for treatment, period and sequence effects.

**Results:** Administration of probiotics was safe and showed a decline in WBC counts ($6.02x10^3$/uL to $5.51x10^3$/uL, $p=0.05$) and total indoxyl glucuronide (0.76mg/dL to 0.65mg/dL, $p= 0.05$) and a trend towards reduction in CRP (13.72mg/dL to 5.11mg/dL, $p= 0.07$). Other chemicals and QoL were unchanged.

**Conclusions:** Administration of Kibow Probiotic Renadyl™ in ESRD patients is safe and showed a protective effect by the trend to reduce markers of inflammation. Further investigation in a larger population or at a higher dose might yield mechanistic insights into the probiotic effects on the inflammatory cascade of uremia.

*Funding:* Pharmaceutical Company Support - Kibow Biotech, Inc.

## TH-PO537

### Effect of Convection Therapy on Advance Glycation End Products

Arkom Nongnuch,[1,2] Andrew Davenport.[2] *[1]Renal Unit, Dept of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol Univ, Bangkok, Thailand; [2]Centre for Nephrology, Royal Free Hospital, Univ College London, London, United Kingdom.*

**Background:** Cardiovascular disease (CVD) is a major cause of death in the dialysis population. Advance Glycation End Products (AGEs) have been strongly association with CVD. AGEs are formed by the Maillard reaction and are predominantly renally excreted. Thus both serum AGEs and AGEs deposited in tissues are increased in dialysis patients. Previous studies have shown that tissue AGEs had greater association with CVD than serum AGEs, and tissue AGE deposition can be measured by skin autofluorescence (sAF). As AGEs are middle molecules we determined whether patients treated by HDF with increased convective clearance had lower tissue AGEs compared to those treated by standard highflux HD.

**Methods:** We measured tissue AGEs using skin autofluorescence (sAF) (DiagnOptics, Groningen, Netherland) on the non-fistula arm of 332 chronic hemodialysis patients, 169 on HD and 163 on HDF.

**Results:** The results are shown in the table (figure).

| Parameters | Hemodialysis | Hemodiafiltration | P value |
|---|---|---|---|
| Skin autofluorescence AU (mean ± SD) | 3.18 ± 0.83 | 3.35 ± 1.06 | NS |
| Age years | 64.1 ±14.7 | 66.4 ± 15.4 | NS |
| Diabetes (%) | 72 (42.6) | 67 (41.1) | NS |
| Hypertension (%) | 105 (62.1) | 100 (61.3) | NS |
| Smoking (%) | 69 (40.8) | 54 (33.1) | NS |
| B2 Microglobulin (median, IQR) mg/dL | 28.5 (12-57) | 26 (7-164) | NS |
| History of CVD | 43 (25.4) | 64 (39.2) | 0.001 |
| History of PVD | 24 (14.2) | 29 (17.1) | 0.01 |
| Dialysis vintage (median, IQR) months | 24 (1-350) | 55 (2-413) | 0.01 |

Previous studies have reported that sAF increases with renal failure, age, diabetes and history of CVD. Our groups were comparable for age, diabetes and hypertension. Despite substantially longer dialysis vintage, and greater history of cardiovascular and peripheral vascular disease the sAF measurements were not higher in the HDF cohort. B2 microglobulin levels, a marker of middle molecule clearance and residual renal function were also similar between the groups despite the differences in dialysis vintage.

**Conclusions:** The addition of convective clearance with HDF appears to reduce the accumulation of tissue AGEs, by improving middle molecule clearances. Prospective studies are required to determine whether HDF reduces the risk of cardiovascular death in long term hemodialysis patient.

*Funding:* Private Foundation Support

## TH-PO538

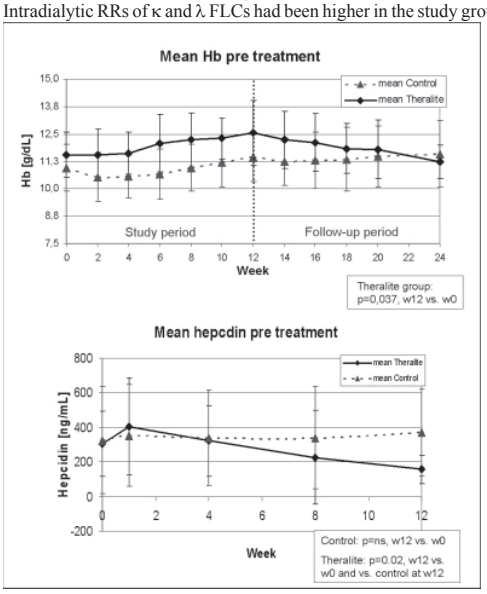
**Treatment by High Cut-Off Hemodialysis Leads to Higher Hemoglobin and Decreased Hepcidin in HD Patients** Ugo Teatini,[1] Werner Beck,[2] Ariane Liebchen,[2] Giorgio Romei Longhena.[1] *[1]Bollate Hospital; AO Garbagnate M.se, Milan, Italy; [2]Gambro Dialysatoren GmbH, Hechingen, Germany.*

**Background:** Multiple inflammatory stimuli are linked to clinical signs and symptoms and CV mortality in HD patients. Inflamed HD patients also show impaired responses to erythropoiesis-stimulating agents (ESAs).

Aim of this pilot study was to investigate if HD treatment with a membrane having a high molecular permeability in a broad MW range improves ESA responsiveness (ESA resistance index, ERI) and decreases inflammatory markers.

**Methods:** 24 ESRD patients (6 w, 65±16 years, 70±11 kg) on 3x/week high-flux HD/HDF ( ≥3 months) with adequate iron status (TSAT >20%, ferritin >100 ng/mL) were randomized into a study group (Theralite high cut-off membrane (Gambro) alternating with routine high-flux HD/HDF) or control group (routine HD/HDF) and treated for 12 weeks. ESA dose was adapted acc. to guidelines to maintain Hb levels in between 10-12 g/dL. Hepcidin, κ- and λ-FLCs, ESA dose, Hb and albumin were analyzed. (NCT01526798)

**Results:** 5 patients dropped out during the study period. One patient was excluded due to unusual high ferritin. In both groups a reduction of ERI has been observed. Only in the study group a significant increase in Hb levels and a decrease of hepcidin values to a significant lower level was detectable during the study period (fig. 1). An albumin drop was detectable in the study group and values stabilized after ~2 weeks at the lower level. Intradialytic RRs of κ and λ FLCs had been higher in the study group compared to controls.




**Conclusions:** High cut-off dialysis allows a significant better removal of large uremic toxins (λ FLCs) and improves ESA responsiveness (indicated by rise in Hb and fall in hepcidin). Larger studies are needed to confirm if regular treatment with high permeability membranes leads to an improvement in anemia of chronic inflammation in HD patients.

*Funding:* Pharmaceutical Company Support - Gambro Lundia AB

## TH-PO539

**Depressive Affect in Incident Hemodialysis Patients: Prevalence and Risk Factors** John W. Larkin, Kathryn A. McDougall, Len A. Usvyat, Rebecca L. Wingard, Eduardo K. Lacson, Franklin W. Maddux. *Fresenius Medical Care North America (FMCNA), Waltham, MA.*

**Background:** Depression is common among patients with end-stage renal disease, yet is not well studied in incident hemodialysis (iHD) patients in their first month of chronic outpatient dialysis. This study investigated the prevalence of and risk factors for depressive affect (DA) in the FMCNA RightStart Program.

**Methods:** From a random sample of 108 dialysis centers, 429 iHD patients in their first month of dialysis at FMCNA during Jan-Mar 2013 were contacted by telephone for up to three tries for depression screening utilizing the Patient Health Questionnaire 2 (PHQ2). The PHQ2 consists of two questions that determine presence of depressed mood and anhedonia during the previous two weeks. Scores range from 0-6; a positive DA score was defined as ≥3. Clinical and lab parameters were collected up to the first 120 days of dialysis and associations between DA were assessed using multivariate logistic regression.

**Results:** Of 429 patients, 172 (40.1%) were successfully screened with the PHQ2, 192 (44.8%) could not be reached, 6 (1.4%) refused and 59 (13.8%) did not have a valid telephone number. Responders were: 41.3% females; mean age of 65.0±14.6 years; 64.5% had diabetes; and 67.4% utilized dialysis catheters. The PHQ2 was positive in 23.3% of patients. Logistic regression identified male iHD patients had significantly lower risk of DA (OR=0.32, p=0.02), while higher log of creatinine (Cr) trended towards greater risk for DA (OR=3.48, p=0.08). There were no associations between DA and age, diabetes, access type, ethnicity, race, body mass index, residual renal function, number of comorbidities, albumin, interdialytic weight gain, or urea reduction ratio.

**Conclusions:** The prevalence of DA by PHQ2 was 23.3% in this iHD patient cohort, consistent with prior reports in the literature. A decreased risk for DA was observed in males. Creatinine levels may play a role as a determinant of DA, but further studies are required to better understand these associations.

*Funding:* Pharmaceutical Company Support - Fresenius Medical Care North America

## TH-PO540

**Reduced Renal Fibrosis after Unilateral Ureteral Obstruction in Mice Lacking $\alpha_{1B}$ Subunit of N-Type Calcium Channels** Akito Maeshima, Keiichiro Mishima, Masao Nakasatomi, Noriyuki Sakurai, Hidekazu Ikeuchi, Toru Sakairi, Keiju Hiromura, Yoshihisa Nojima. *Medicine and Clinical Science, Gunma Univ Graduate School of Medicine, Maebashi, Japan.*

**Background:** Renal tubules are innervated by sympathetic nerves in which N-type $Ca^{2+}$ channels are densely distributed. It has been reported that sympathetic nerve activity was increased in patients with chronic renal diseases. We recently reported the increased expression of N-type $Ca^{2+}$ channel in the kidneys after unilateral ureteral obstruction (UUO) and the reduction of renal fibrosis by L/N-type $Ca^{2+}$ channel blocker in rats (AJP Renal Physiol 304: F665-73, 2013). However,the role of N-type $Ca^{2+}$ channel in renal fibrosis is not totally understood.

**Methods:** To address this issue, we induced UUO in male mice lacking the $\alpha_{1B}$ subunit of N-type $Ca^{2+}$ channel (Cav2.2) and wild type (WT) littermates and analyzed several renal fibrotic parameters in this study.

**Results:** In C57BL/6N mice, the expression of Cav2.2 was absent in normal, contralateral, and sham-operated kidney, while Cav2.2 became detectable in the interstitium of the kidney after UUO. In UUO kidneys, Cav2.2 was expressed in the interstitial cells positive for a-SMA, a marker for myofibroblasts, but not in T-lymphocytes, Macrophages, and endothelial cells. At baseline as well as after UUO, there was no significant difference in mean blood pressure, heart rate, and renal function (serum creatinine and blood urea nitrogen levels) between WT mice and Cav2.2 mutant mice. The expression level of a-SMA in the UUO kidneys of Cav2.2 mutant mice was significantly decreased compared to that in WT mice. Cav2.2 deficiency reduced the production of fibronectin, but not type I or type III collagen in the kidney after UUO. Sirius red-positive area was significantly reduced in Cav2.2 mutant kidney compared to that in WT kidney after UUO (1.97% vs. 3.57%, P<0.001).

**Conclusions:** Our data suggest that Cav2.2 is implicated in myofibroblast activation and the production of extracellular matrix during renal fibrosis. Cav2.2 might be a novel therapeutic target for the treatment of fibrotic kidney disease.

## TH-PO541

**Effects of Tissue-Specific, Targeted Expression of Baculovirus p35 Pan-Caspase Inhibitor on Renal Fibrogenesis following Ureter Obstruction** Tsutomu Inoue, Hiromichi Suzuki, Hirokazu Okada. *Nephrology, Saitama Medical Univ, Irumagun, Saitama, Japan.*

**Background:** The caspase family of enzymes participates in cell apoptotic and proinflammatory reactions. Although caspase activation was known to be involved in renal fibrogenesis following unilateral ureter obstruction (UUO), in such an obstructive kidney where and how caspase activation plays a role remain to be determined.

**Methods:** Three lines of transgenic (tg) mice were used. p35 tg mice that bear the pan-caspase inhibitor protein p35 gene separated from a universal CAG promoter by a floxed STOP sequence were crossed with γGT.Cre and FSP.Cre tg mice that express Cre recombinase in tubular epithelial cells and fibroblasts, respectively. Two double-tg mice (γGT.Cre;p35 and FSP.Cre;p35) and wild-type mice were then challenged with UUO. Cell apoptosis was defined by in situ end labeling and the point-counting method. Fibrosis-related parameters in the kidneys were determined by histopathology and qPCR.

**Results:** Matrix deposition by Masson's trichrome staining in the peritubular interstitium and mRNA levels of fibronectin(FN)-EIIIA were significantly increased in the UUO kidneys of the wild-type and FSP.Cre;p35 tg mice, but not in those of the γGT.Cre;p35 mice, possibly due to tubular epithelium-specific induction of p35 gene product, compared to the native kidneys (8.1+1.9, 7.9+3.2, 3.8+1.2, vs. 0.9+0.1 [% blue area]; 9.4+3.1, 10.3+3.6, 5.7+1.5, vs. 1.0+0.5 [FN-EIIIA mRNA/GAPDH mRNA]). Additionally, compared to the wild-type mice, the number of apoptotic cells and mRNA levels of NLRP3, a caspase-1-dependent, central component of inflammasome, in the UUO kidneys were significantly lower in the γGT.Cre;p35 mice while those were not significantly altered in the FSP.Cre;p35 tg mice.

**Conclusions:** Tissue-specific, targeted expression of p35 pan-caspase inhibitor provides evidence of the critical role of caspase activation, i.e., induction of apoptosis and inflammasome, in tubular epithelium, but not in fibroblasts, in renal fibrogenesis following ureter obstruction.

*Funding:* Government Support - Non-U.S.

## TH-PO542

**Induction of Renal Fibrosis by a Cell Cycle Regulator** Judit Megyesi,[1,2] Adel Tarcsafalvi,[1] Nang San Hti Lar Seng,[1] Shenyang Li,[1,2] Didier Portilla,[1,2] Peter M. Price.[1,2] *[1]Univ AR Med Sci; [2]VA Med Ctr.*

**Background:** Expression of a cell cycle regulatory protein, $p21^{WAF1/Cip1}$, in cells after partial nephrectomy is associated with fibrotic changes and progression. The p21 protein is an inhibitor of cyclin-dependent kinases-1 and -2 (Cdk1, Cdk2), but the mechanism of how its expression contributes to fibrosis is unclear.

**Methods:** Cultured mouse proximal tubule cells were transduced by adenoviruses including those expressing full-length p21, N- and C-terminal p21, and DN-Cdk2. The Cdk-inhibitory drug purvalanol was used at 9 μM. Also, several mouse models of renal fibrosis were used, either unilateral ischemia/reperfusion, ureteral obstruction, or partial nephrectomy.

**Results:** We reported previously that p21 KO mice were protected from renal fibrosis using 5/6 nephrectomy, UUO/release, or unilateral ischemia/reperfusion and that after AKI, proximal tubules were the major sites of p21 mRNA induction. We now show that in a p21 KO background, induction of transgenic p21 specifically in proximal tubules resulted in expression of fibrotic markers after unilateral ischemia or 14 days following UUO that was released after 3 days, showing that proximal tubular cells are a significant source of the signal for induction of fibrosis. We also show that p21 over-expression in cultured kidney cells increased TGFβ mRNA 5.5 to 8.2 times control levels. This increase was not by transcriptional activation, but rather by stabilization of the mRNA.

The TGFβ protein was detected in wild-type mouse kidney after UUO in thick ascending limbs and in the S1 and S2 segments of convoluted proximal tubules. TGFβ is reported to play a major role in stimulating extracellular matrix production after UUO and release of active TGFβ by injured epithelial cells is implicated in the pathogenesis of epithelial cell injury and interstitial fibrosis in the kidney.

**Conclusions:** Based on these findings, we propose that induction of p21 following kidney injury, subsequent stabilization and accumulation of TGFβ mRNA and release of TGFβ protein by proximal tubule cells is a major causative mechanism for development of kidney fibrosis *in vivo*.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO543

**Sonic Hedgehog Is an Inducible, Tubule-Derived Growth Factor for Interstitial Fibroblasts after Kidney Injury** Dong Zhou,[1] Yingjian Li,[1] Li Li Zhou,[1] Roderick J. Tan,[2] Youhua Liu.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** Tubular epithelium constitutes the major part of renal parenchyma, and is the primary target of various kidney injuries. However, how the injured tubules drive fibroblast proliferation and activation in the interstitium remains elusive. Here we show that sonic Hedgehog (Shh), an evolutionarily conserved, secreted and extracellular signaling protein, is an inducible, tubule-derived growth factor that plays an important role in promoting fibroblast proliferation and expansion.

**Methods:** Various animal models of CKD as well as human kidney biopsies were used. Shh signaling was inhibited by cyclopamine (CPN) in vitro and in vivo.

**Results:** We found that Shh was specifically induced in renal tubular epithelia in various models of CKD induced by adriamycin (ADR), ischemia/reperfusion injury (IRI) and 5/6 nephrectomy, respectively. Shh was also markedly induced in renal tubules of human kidney biopsies from CKD patients with different etiologies. In vitro, recombinant Shh promoted rat kidney fibroblasts (NRK-49F) proliferation, as assessed by cell counting, MTT assay, and BrdU incorporation assay, which was accompanied by induction of numerous proliferation-related genes, including c-fos, c-myc, proliferative cell nuclear antigen, and cyclin D1. However, Shh had no appreciable effect on the proliferation of other major kidney cells, such as podocytes, proximal tubular epithelial cells (HKC-8) and inner medullar collecting ducts cells (mIMCD-3). To investigate the role of Shh in fibroblast proliferation in vivo, we administered CPN, a small molecule Smo inhibitor, at 3 days after IRI. At 10 days after IRI, we found that CPN markedly inhibited fibroblast proliferation, reduced myofibroblast population, and attenuated fibrotic lesions in vivo.

**Conclusions:** Collectively, these studies identify Shh as a potent tubule-derived growth factor that specifically promotes interstitial fibroblast proliferation. Our data also indicate that blockade of Shh signaling is a novel strategy for therapeutic intervention of renal fibrosis.

*Funding:* NIDDK Support

## TH-PO544

**Matrix Metalloproteinase-7 Is a Biomarker and Pathogenic Mediator of Kidney Fibrosis** Dong Zhou,[1] Li Li Zhou,[1] Roderick J. Tan,[2] Fan Fan Hou,[3] Youhua Liu.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA; [3]Renal Div, Nanfang Hospital, Southern Medical Univ, Guangzhou, Guangdong, China.*

**Background:** Matrix metalloproteinase-7 (MMP-7), a secreted, zinc and calcium dependent endopeptidase that degrades a broad range of extracellular matrix (ECM) and other substrates, is a transcriptional target of canonical Wnt/β-cateninn signaling. We previously demonstrated that urinary MMP-7 is surrogate biomarker for renal Wnt/β-catenin activity. However, Whether MMP-7 plays a role in renal fibrogenesis is completely unknown.

**Methods:** Urinary MMP-7 from normal subjects and CKD patients was measured by ELISA. MMP-7 knockout and wild-type control mice were subjected to UUO. Cultured human proximal tubular cells were treated with recombinant MMP-7.

**Results:** Urinary MMP-7 level was markedly increased in CKD patients, comparing to normal subjects. Similarly, increased MMP-7 was observed in renal tubules of human kidney biopsies from CKD patients. MMP-7 mRNA and protein expression were also induced in animal models of renal fibrosis such as UUO. To study the role of MMP-7 in renal fibrogenesis, MMP-7-/- mice and their WT controls were subjected to obstructive injury for 7 days. Compared with WT controls, MMP-7-/- kidneys displayed reduced fibrotic lesions, characterized by decreased expression of Snail1, α-smooth muscle actin, vimentin, Fsp1, PAI-1, fibronectin, type I and type III collagen. Ablation of MMP-7 in vivo also preserved E-cadherin primarily by inhibiting its extracellular shedding, with no effect on E-cadherin mRNA abundance. In vitro, MMP-7 incubation reduced E-cadherin staining in plasma membrane of proximal tubular cells (HKC-8), which resulted in increased β-catenin nuclear translocation. Co-immoprecipitation revealed a diminished E-cadherin/β-catenin complex formation, with concomitant increase of b-catenin/LEF1 binding, after MMP-7 treatment in HKC-8 cells.

**Conclusions:** These observations suggest that MMP-7 not only is a biomarker, but also plays a crucial role in mediating tubular injury and interstitial fibrosis by promotes E-cadherin shedding and β-catenin activation.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## TH-PO545

**Slit2 Inhibits Renal Fibrosis after Ischemia Reperfusion Injury** Darren A. Yuen,[1] Yi-Wei Huang,[1] Guang-Ying Liu,[1] Sajedabanu Patel,[1] Rohan John,[2] Lisa Robinson.[1] *[1]Hospital for Sick Children, Canada; [2]Univ Health Network, Canada.*

**Background:** Fibrosis is a critical contributor to CKD progression for which no specific therapies exist. Acting on its cognate Robo receptors, Slit2 is a secreted protein that modulates the actin cytoskeleton of multiple cells. As fibroblast activation is dependent upon cytoskeletal rearrangements, we hypothesized that Slit2 may exhibit anti-fibrotic activity and prevent renal fibrosis after AKI.

**Methods:** Renal fibroblasts were examined for Robo1 expression using qRT-PCR, immunoblotting, and immunostaining. The effects of Slit2 alone or in combination with its decoy receptor RoboN on TGF-β-induced fibroblast collagen production were tested using a [$^3$H]-proline incorporation assay. To assess the effects of Slit2 *in vivo*, mice undergoing left renal ischemia-reperfusion injury (IRI) received 3X weekly i.p. injections of N-Slit2 (a bioactive Slit2 fragment), C-Slit2 (an inactive Slit2 fragment) or vehicle. Thirteen days post-IRI, mice underwent right nephrectomy and 24 h later, renal function and structure were analyzed.

**Results:** Renal fibroblasts expressed Robo1 mRNA and protein. Slit2 dose-dependently reduced TGF-β-induced [$^3$H]-proline incorporation, whereas Slit2 incubation with its decoy receptor blocked its anti-fibrotic activity. In the mouse model of renal IRI, administration of inactive C-Slit2 had no effect, but bioactive N-Slit2 improved serum creatinine and urea. N-Slit2 also reduced α-SMA$^+$ activated fibroblast number and collagen deposition in the injured kidney.

**Conclusions:** These data demonstrate that Slit2 exerts previously undescribed anti-fibrotic effects by inhibiting TGF-β-induced fibroblast activation. Slit2 may represent a novel therapy targeting renal fibrosis.

| | Sham | IRI-Vehicle | IRI-N-Slit2 |
|---|---|---|---|
| Plasma urea (mM) | 7.2 ± 0.3 | 63.1 ± 1.2 * | 47.7 ± 4.7 * † |
| Plasma creatinine (μM) | 24 ± 1 | 180 ± 4 * | 141 ± 20 * † |
| Renal collagen (PSR staining, AU) | 5.0 ± 0.5 | 34.6 ± 1.0 * | 28.0 ± 2.0 * † |
| Renal α-SMA staining (AU) | 3.2 ± 0.6 | 30.2 ± 2.8 * | 19.2 ± 1.5 * † |

* $p < 0.05$ vs. sham. † $p < 0.05$ vs. IRI-Vehicle.

*Funding:* Government Support - Non-U.S.

## TH-PO546

**Reduced Renal DDAH1 Activity Protects against Progressive Kidney Damage** James Alexander Tomlinson,[1] Ben Caplin,[2] Dirk Dormann,[1] Peter Allen Faull,[1] Sanjay Khadayate,[1] Jill T. Norman,[2] David C. Wheeler,[2] James M. Leiper.[1] *[1]Medical Research Council Clinical Sciences Centre, Imperial College, London, United Kingdom; [2]Centre for Nephrology, UCL Medical School Royal Free, London, United Kingdom.*

**Background:** Asymmetric dimethylarginine (ADMA) competitively inhibits nitric oxide (NO) synthesis whilst dimethylarginine dimethylaminohydrolase 1 (DDAH1) metabolises ADMA; representing an alternative pathway for NO regulation. Although an association between elevated circulating ADMA and poor cardiovascular and renal outcomes has been widely reported, a causal link is unresolved. We recently published evidence of a DDAH1 gene variant that leads to lower plasma ADMA but counter-intuitively, associates with a *steeper* rate of eGFR decline.

**Methods:** The principal site of renal DDAH1 expression is within the renal proximal tubule (PT). We tested the hypothesis that reduced kidney DDAH1 activity slows the progression of kidney function decline by; (**A**) generating a novel PT-specific DDAH1 gene knock-out (PTD1KO) mouse; (**B**) subjecting it to a folate model of CKD and (**C**) confirmed gene expression associations observed in the KO mouse within human renal allografts.

**Results:** (**A**) KO mice had elevated PT cell ADMA (60%, $p<0.05$), with a reduction in NO synthesis (60%, $p<0.05$) and no effect observed in other organs, urine, plasma or systemic BP. Urinary proteomic analysis revealed an 8-fold reduction in uromodulin (UMOD; $p<0.001$) with PTD1 deletion. (**B**) At 12 weeks post folate injury, WT mice had a >2-fold rise in fibrotic gene expression (Col12α and TGFβ; $p<0.05$), whereas KO mice

were protected. Renal collagen deposition was reduced in KO mice (WT vs KO; 7.2 vs 4.5%, $p<0.01$). (**C**) A significant correlation between UMOD and DDAH1 gene expression was confirmed in human renal allograft protocol biopsies ($p<0.05$).

**Conclusions:** Renal DDAH1 activity contributes to the progression of kidney function decline following injury. Our work highlights the importance of NO-ADMA imbalance at a tissue level and suggests that circulating ADMA may be misrepresentative of organ-specific disease. Our animal and human data demonstrate an association between UMOD and DDAH1 gene expression and suggest a plausible mechanistic role of uromodulin in kidney injury.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

### TH-PO547

**A Simple Method for Detection of Epithelial-Mesenchymal Transition during Renal Fibrosis In Vivo** Masao Nakasatomi, Akito Maeshima, Noriyuki Sakurai, Hidekazu Ikeuchi, Toru Sakairi, Keiju Hiromura, Yoshihisa Nojima. *Dept of Medicine and Clinical Science, Gunma Univ Graduate School of Medicine, Maebashi, Gunma, Japan.*

**Background:** Epithelial-mesenchymal transition (EMT) in renal fibrosis is generally defined by the loss of epithelial markers and the acquisition of mesenchymal phenotypes by damaged tubules. However, structural details of this process have not been clarified. Using bromodeoxyuridine (BrdU) labeling method, we previously reported that renal progenitor-like tubular cells, also called as label-retaining cells, migrated into the interstitium after unilateral ureteral obstruction (UUO) (JASN 16: 2044-51, 2005). By modifying this method, we examined in this study whether EMT process could be detected and quantified in vivo.

**Methods:** Using osmotic pump, BrdU (20mg/kg/day) was continuously given into 7-week-old Wistar rats for 1, 2, 3 and 4 weeks. UUO was induced in these rats and the kidneys were removed at 4, 6, 8, 10 days after UUO. Localization, phenotype, and number of BrdU+ cells were examined by immunostaining. The number of BrdU+ bone marrow cells was also measured.

**Results:** The number of BrdU+ cells was positively associated with labeling period. BrdU+ cells were detectable in AQP1-positive proximal tubules, but not in the interstitium of normal rat kidneys. Most proximal tubular cells became BrdU+ after 4-week labeling. After UUO, some of BrdU+ tubular cells were protruded from the basement membrane and were migrated into the interstitium. Interstitial BrdU+ cells were co-localized with alpha-SMA, fibroblast-specific protein 1, and type I collagen. The number of interstitial BrdU+ cells significantly increased and reached the maximum at 8 days after UUO. Few BrdU+ cells were observed in the interstitium of normal and sham-operated kidneys. UUO treatment did not significantly change the number of bone marrow BrdU+ cells.

**Conclusions:** Long-term BrdU treatment labels most proximal tubular cells with BrdU and enabled us to detect and quantify EMT in vivo. This technique will be useful for the search of novel EMT inhibitor(s) for the treatment of renal fibrosis.

### TH-PO548

**Sphingosine Kinase 2 Deficiency Attenuates Kidney Fibrosis in Mice** Amandeep Bajwa, Piotr Chroscicki, Liping Huang, Hong Ye, Mark D. Okusa. *Dept of Med./CIIR, Univ of Virginia.*

**Background:** S1P and the sphingosine kinases(SphK1/SphK2) responsible for its formation are important in acute and CKD. In the current study we examined the role of SphK2 in kidney fibrosis.

**Methods:** Fibrosis was induced in WT, SphK1$^{-/-}$ and SphK2$^{-/-}$ mice by folic acid (FA; 250mg/kg) or unilateral ischemia-reperfusion(IRI) and mice were followed for 14d and RT-PCR and IHC were performed.

**Results:** SphK2$^{-/-}$ mice had less fibrosis (trichrome staining) compared to WT or SphK1$^{-/-}$ mice 14d after FA or unilateral IRI. Compared to kidneys of FA-treated SphK1$^{-/-}$ mice, SphK2$^{-/-}$ mice expressed lower mRNA encoding TGF-β (15.79%, $p<0.001$), α-SMA (30.8%, $p<0.01$) and fibronectin (36.9%, $p<0.01$) and had lower expression of collagen and fibronectin immunoreactivity in kidney. Kidneys of FA-treated SphK2$^{-/-}$ mice had reduced infiltration of macs (45.1%, $p<0.05$)) neuts (27.9%, $p<0.01$) and T cells (55.8%, $p<0.05$) compared to SphK1$^{-/-}$ mice. SphK2$^{-/-}$ CD4$^{+}$ T cells are hyperproliferative and produce significantly higher amounts of IFN-γ compared to WT or SphK1$^{-/-}$ T cells. In chronic studies IFN-γ is anti-fibrotic; therefore, we examined the role of SphK2$^{-/-}$ CD4$^{+}$ T cells in fibrosis. Transfer of CD4$^{+}$ T cells from SphK2$^{-/-}$ to WT or SphK1$^{-/-}$ mice attenuated the progression of fibrosis (less trichrome staining 14d after FA) in the recipients. Treatment of SphK2$^{-/-}$ mice with blocking IFN-γ Ab resulted in more FA-induced fibrosis compared to control IgG-treated mice. Additionally, IFN-γ$^{-/-}$ or SphK2$^{-/-}$/IFN-γ$^{-/-}$ double KO mice had more fibrosis compared to WT and SphK1$^{-/-}$ mice after FA and/or unilateral IRI. Treatment of WT mice with a SphK2 inhibitor (SKX002411, 3mg/kg; SphynKx Therapeutics) 2d after FA lowered fibrosis compared to vehicle- treated mice.

**Conclusions:** In summary 1) susceptibility to fibrosis is similar in WT and SphK1$^{-/-}$ mice but SphK2 deficiency or SK2 inhibitor attenuates fibrosis, and 2) the protective phenotype in SphK2$^{-/-}$ mice is dependent on IFN-γ. Understanding the function of SphK2 may contribute further to our understanding of the pathogenesis of fibrosis. Development of selective SphK2 inhibitors may lead to a new therapeutic strategy to impede or reverse the progression of kidney injury to ESRD.

*Funding:* NIDDK Support

### TH-PO549

**Different Roles for the Three AKT Isoforms in TGF-β Induced Fibrogenesis** Benaya Rozen-zvi, H. William Schnaper, Tomoko Hayashida. *Pediatrics, Northwestern Univ Feinberg School of Medicine, Chicago, IL.*

**Background:** The transforming growth factor (TGF)-β pathway is known to activate phosphoinositide 3-kinase (PI3K) and its main target, AKT. We have reported that this activity has complex regulatory effects on TGF-β-induced fibrogenesis. Since the three isoforms of AKT may have different and sometimes conflicting biological actions, here we evaluated the role of specific isoforms in TGF-β-induced collagen expression.

**Methods:** Collagen expression was evaluated in TGF-β-treated human mesangial cells (HMC) in which the three isoforms were silenced transiently or stably by shRNA. We also treated HMC with a pan-AKT inhibitor (AKT inhibitor X) and a more selective inhibitor that acts only on AKT1 and AKT2 (AKT inhibitor VIII). In addition, we evaluated mouse embryonic fibroblast (MEF) from mice null for AKT1 or for both AKT1 and AKT2. Collagen expression was evaluated by reporter assay and western blot. Smad3 and GSK3-β phosphorylation were evaluated by western blot.

**Results:** TGF-β-induced collagen expression was increased by stably or transiently silencing AKT2. In contrast, silencing AKT3 and, to a lesser degree, AKT1, inhibited collagen expression. Treatment with the pan-AKT inhibitor prevented TGF-β-induced collagen expression, whereas treatment with the AKT1/2-specific inhibitor was stimulatory. In similar fashion, collagen expression was increased in AKT1/2-null MEF but was reduced in AKT1 null MEF. As Smad3 phosphorylation and nuclear translocation were not changed in any of the conditions tested, the effect of the different AKT isoforms on TGF-β-induced collagen induction appears to be Smad3 independent. Inactivating phosphorylation of glycogen synthase kinase 3 (GSK3)-β was reduced by silencing AKT3 but not by silencing AKT1 or AKT2.

**Conclusions:** AKT3 activity is essential for TGF-β-induced collagen expression while AKT2 has an inhibitory effect. AKT1 inhibition reduces collagen expression, possibly by increasing AKT2 activity. Since GSK3-β activity is known to be antifibrotic, the antifibrotic effect of AKT3 inhibition may occur through enhanced GSK3-β activity. Selective inhibition of AKT3 is a potential target for anti-fibrotic therapy.

*Funding:* NIDDK Support

### TH-PO550

**A Splice Site Mutation in Mouse *Col4a4*: Detection of Abnormal Collagen IV Protomers, and Effects of Genetic Background on the Alport Syndrome Phenotype** Jeffrey H. Miner,[1] Christina R. Caputo,[2] Susan A. Cook,[2] Roderick T. Bronson,[2] Muriel T. Davisson,[2] Ron Korstanje.[2] *[1]Renal Div, Washington Univ, St. Louis, MO; [2]The Jackson Laboratory, Bar Harbor, ME.*

**Background:** A spontaneous autosomal recessive mutation termed bilateral wasting kidneys (*bwk*) was identified in a colony of NONcNZO recombinant inbred mice. Mutants exhibit a rapid increase of urinary albumin at an early age associated with glomerulosclerosis, interstitial nephritis, and tubular atrophy. The mutation was hypothesized to affect a gene important for kidney function, so we set out to map and identify the mutation.

**Methods:** Mapping of the mutation in an intercross with CAST/EiJ mice identified a location on Chromosome 1 containing the *Col4a3* and *Col4a4* genes, for which mutations in the human orthologs cause the hereditary glomerulonephritis Alport syndrome. *Col4a3* was ruled out as the affected gene because animals carrying both *bwk* and *Col4a3* null mutations were normal. The *Col4a4* gene was therefore sequenced.

**Results:** We identified a G to A mutation in the conserved GT splice donor of *Col4a4* intron 30, resulting in skipping of exon 30 but maintenance of the reading frame. Protein and histological analyses showed that mutant collagen α3α4α5(IV) protomers were secreted and formed NC1 hexamers in the GBM, but levels were low, and Alport GBM lesions were still observed. Moving the mutation into the more renal damage-prone DBA/2J and 129S1/SvImJ genetic backgrounds showed differences in albuminuria and its rate of increase, suggesting an interaction between the *Col4a4* mutation and modifier genes.

**Conclusions:** This novel mouse model of Alport syndrome is the only one shown to accumulate abnormal collagen α3α4α5(IV) in the GBM, a phenomenon observed in a subset of Alport patients. These mice will serve as a valuable new tool for testing potential therapies, for understanding abnormal collagen IV structure and assembly, as well as for gaining better insights into the mechanisms leading to Alport syndrome and to the great variability in the age of onset and associated phenotypes.

*Funding:* NIDDK Support, Other NIH Support - NCI, NCRR, Private Foundation Support

### TH-PO551

**Selective Delivery of Interferon Gamma to Renal Interstitial Myofibroblasts Reduces Renal Fibrosis** Fariba Poosti,[1] Ruchi Bansal,[3] Jai Prakash,[3] Eduard Post,[2] Catharina Reker-Smit,[2] Pieter A. Klok,[1] Geert Harms,[1] Marian L.C. Bulthuis–van der Horst,[1] Klaas Poelstra,[2] Jan-Luuk Hillebrands.[1] *[1]Pathology, Univ Medical Center Groningen, Groningen, Netherlands; [2]Pharmacokinetics, Toxicology and Targeting, Univ of Groningen; [3]MIRA Institute, Univ of Twente, Enschede, Netherlands.*

**Background:** Although mechanisms underlying the pathogenesis of renal fibrosis have been uncovered, yet no therapies are available that specifically target fibrosis. IFNγ is a potent antifibrotic cytokine to halt & inhibit fibrosis. However, administration of IFNγ may cause systemic side effects. Interstitial myofibroblasts are key effector cells in renal

fibrogenesis. We here tested the hypothesis that cell-specific delivery of IFNγ to platelet-derived growth factor receptor beta (PDGFRβ)-expressing myofibroblasts attenuates renal fibrosis & reduces systemic side effects.

**Methods:** PDGFRβ expression was determined in fibrotic mouse and human kidney by specific staining. IFNγ conjugate (IFNγ-PEG-PPB) was synthesized by coupling of PDGFRβ-specific peptide (PPB) to IFNγ via a PEG linker. The biological activity & anti-fibrotic effects were analyzed *in vitro*. Anti-fibrotic effects of targeted (IFNγ-PEG-PPB) and free IFNγ were investigated in the unilateral ureteral obstruction (UUO) model in C57BL/6 mice at 3 & 7 days.

**Results:** PDGFRβ expression was highly upregulated in mouse & human fibrotic kidneys. PDGFRβ expression co-localized with αSMA-positive myofibroblasts. In vitro, IFNγ-PEG-PPB induced NO release in RAW cells (indicative of biological activity) & inhibited expression of col1a1, col1a2 and αSMA in fibrotic NIH 3T3 fibroblasts. In vivo, IFNγ-PEG-PPB specifically accumulated in the PDGFRβ-positive myofibroblasts. IFNγ-PEG-PPB treatment induced a significant reduction of renal collagen I, fibronectin and αSMA expression (both at mRNA & protein level), and was more effective than non-targeted IFNγ. In contrast to non-targeted IFNγ, IFNγ-PEG-PPB did not induce IFNγ–related side effects.

**Conclusions:** Specific targeting of IFNγ to PDGFRβ-expressing myofibroblasts attenuates renal fibrosis without causing adverse effects in an experimental for renal fibrosis in mice.

## TH-PO552

**Contribution of Hydrogen Peroxide-Inducible Clone-5 to the Regulation of Mesangial Cell Proliferation in Mesangioproliferative Glomerulonephritis** Ariunbold Jamba,[1] Shuji Kondo,[1] Maki Urushihara,[1] Takashi Nagai,[1] Toshiaki Tamaki,[2] Shoji Kagami.[1] *[1]Dept of Pediatrics, Institute of Health Bioscience, Univ of Tokushima, Tokushima, Japan; [2]Dept of Pharmacology, Univ of Tokushima, Tokushima, Japan.*

**Background:** Hydrogen peroxide-inducible clone-5 (Hic-5) is a transforming growth factor (TGF)-β1-inducible focal adhesion protein homologous to paxillin. We have previously demonstrated that Hic-5 was localized in mesangial cells (MC) and its expression has been associated with glomerular cell proliferation and matrix expansion in rat and human glomerulonephritis (GN) (Nephron Exp Nephrol 120: e59-68, 2012).

**Methods:** The potential role of Hic-5 was investigated in cell proliferation and extracellular matrix (ECM) expression in mesangioproliferative GN produced by injection of Habu venom (4 mg/kg) into heminephrectomized wild type (Hic-5+/+) and Hic-5 deficient (Hic-5-/-) mice. In addition, we examined how Hic-5 is involved in MC proliferation using isolated cultured MC from Hic-5+/+ and Hic-5-/- mice.

**Results:** Habu venom-induced GN in Hic-5+/+ mice demonstrated glomerular cell proliferation at day 7. Surprisingly, glomerular cell number was significantly increased in Hic-5-/- GN mice compared to Hic-5+/+ GN mice ($P<0.01$). Ki-67 positive cells as well as expressions of fibronectin and alpha-smooth muscle actin were also increased in Hic-5-/- GN mice compared to Hic-5+/+ GN mice ($P<0.01$). In vitro experiments about MC proliferation by cell counting and WST-8 assay showed that Hic-5-/- MC significantly proliferated compared to Hic-5+/+ MC. Interestingly, TGF-β1 induced proliferation in Hic-5-/- MC but did not in Hic-5+/+ MC. In contrast, PDGF-BB, another growth factor, increased both Hic-5+/+ and Hic-5-/- MC in the same degree. These data suggest that Hic-5 might be a specific downstream molecule of TGF-β1 to control MC proliferation in glomerular injury. Finally, Hic-5-/- MC showed increased level of phospho-paxillin$^{118}$, which has mitogenic potential, suggesting competitive role of Hic-5 against paxillin signaling for MC growth.

**Conclusions:** In conclusion, Hic-5 might regulate MC proliferation under TGF-β1 stimulation in the development of mesangioproliferative GN.

*Funding:* Government Support - Non-U.S.

## TH-PO553

**AMP-Kinase Activation by AICAR Inhibits Renal Fibrosis and Inhibits Transforming Growth Factor-β1 Induced Activation of Kidney Myofibroblasts** Kuan-hsing Chen, Hsiang-Hao Hsu, Ming-Yang Chang, Cheng-chieh Hung. *Nephrology, Chang Gung Memorial Hospital, Taoyuan, Taiwan.*

**Background:** Activation of interstitial myofibroblasts and excessive production of extracellular matrix proteins are final common pathways contributing to chronic kidney disease. In a number of tissues, AMPK (AMP-kinase) activation has been shown to inhibit fibrosis *in vitro*. Here, we examined the effect of an AMPK activator, AICAR, on inhibiting the progression of renal fibrosis *in vivo* and TGF-β1 activation of renal interstitial fibroblasts *in vitro.*

**Methods:** Urinary unilateral obstruction (UUO) model was induced in adult male BALB/c mice. Mice with UUO were given with intra-peritoneal AICAR (500 mg/Kg body weight/day) or saline daily, one day prior the UUO surgery. Both obstructed and contralateral kidneys were harvested 7 days after surgery. Kidney tissues were prepared for further pathological and molecular biological analysis. Cultured rat renal interstitial fibroblasts (NRK-49F) were stimulated with recombinant human TGF-β1 1ng/ml. Alternatively, NRK-49F cells were pre-incubated with AICAR or specific inhibitors for 30 mins before TGF-β1 treatment. Total RNA was extracted for RT-PCR or real-time PCR and total cell lysates were extracted for Western Blot analysis.

**Results:** In a mouse model of renal interstitial fibrosis induced by unilateral ureteral obstruction (UUO), administration of AICAR attenuated the extracellular matrix protein deposition following injury. AICAR suppressed the expression α-smooth muscle actin (α-SMA), type I collagen and fibronectin in UUO kidney. Treatment of cultured rat renal interstitial fibroblasts with AICAR inhibited their activation by TGF-β1, as evidenced by dose-dependent blockade of α-SMA and collagen I expression. Moreover, the effects of AICAR in inhibition of kidney myofibroblast activation by TGF-β1 were associated with down-regulation of ERK 1/2 MAPK pathways.

**Conclusions:** Thus, our results suggest that AICAR reduces the tubulointerstitial fibrosis in UUO mice and inhibits TGF-β1 induced kidney myofibroblast activation. The AMPK activation by AICAR may hold therapeutic potential in the treatment of renal tubulointerstitial fibrosis.

*Funding:* Government Support - Non-U.S.

## TH-PO554

**The Cannabinoid Receptor 1 Is Involved in Renal Fibrosis** L. Lecru,[1] J. Giron-Michel,[1] C. Desterke,[4] S. Ferlicot,[1] C. Ledent,[3] S. Vandermeersch,[2] B. Charpentier,[1] C. Chatziantoniou,[2] A. Durrbach,[1] H. Francois.[1] *[1]INSERM U1014, Villejuif; [2]INSERM U702, Paris; [3]Univ Libre de Bruxelles, Brussels; [4]INSERM U972, Villejuif.*

**Methods:** To explore new pathways involved in renal fibrogenesis, we performed a microarray analysis during the Unilateral Ureteral Obstruction (UUO) experimental model of renal fibrosis. Unexpectedly, the Cnr1 gene expression, coding for the cannabinoid 1 receptor (CB1R), was found higher during UUO ($p<0.01$, n=6). We decided to determine the role of CB1 during renal fibrogenesis in both mice and humans.

**Results:** The CB1R expression is increased during various nephropathies in human kidney biopsies (Acute Interstitial Nephritis, IgA and diabetic nephropathies) (n=6 compared to normal kidneys, $p<0.05$). Similarly, in the UUO model in mice, using both genetic disruption of Cnr1 (KO mice) and a specific CB1R pharmacological blockade (rimonabant-treated mice, R), we found a 33 to 65% reduction in the collagen accumulation (12.90±0.94% versus 8.50±0.81% in WT versus KO, $p<0.01$, n=6-11; 9.62±1.82% in vehicle mice compared with 4.25±0.61% in R, $p<0.01$, n=7), with no modulation of TGFb1 expression. Mesenchymal markers such as vimentin were reduced in both KO (n=6-11, $p<0.05$) and R mice (n=7, $p<0.05$). Interestingly, obstructed kidneys of R but not KO presented a reduction of F4/80$^+$ cells infiltrating the kidney cortex with a significantly reduced expression of M1 markers compared to controls (n=7, $p<0.05$ and n=7, $p<0.05$). Conversely, KO mice presented an increase of M2 markers in kidneys (n=6-11, $p<0.05$) suggesting that CB1 genetic invalidation promoted a macrophage type M2 polarization. Also, as CB1 and CB2 may exert opposite effects, we investigated the role of CB2 during UUO as its expression is also increased ($p<0.01$, n=11). However, both CB2 agonist and antagonist treatment did not modify fibrogenesis during UUO when associated with CB1 pharmacological blockade ($p<0.05$, n=7) suggesting that the anti-fibrotic effect of CB1 blockade is independent of the CB2 pathway.

**Conclusions:** Overall, CB1 blockade represents a new anti-fibrotic strategy during renal disease and may act in part through resident macrophages signaling.

*Funding:* Pharmaceutical Company Support - partial funding by Sanofi-Aventis

## TH-PO555

**Ets-1 Targeted by MicroRNA-221 Regulates Angiotensin II-Induced Renal Fibroblast Activation** Jia Di, Chunsun Dai, Junwei Yang. *2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

**Background:** Fibroblast activation is one of the most important non-hemodynamic effects of Angiotensin II (Ang II) on the progression of renal fibrosis. Transcription factor Ets-1 is a transcription factor up-regulated in fibrosis. However, the mechanisms of Ets-1 activation in fibrotic kidney are not fully understood.

**Methods:** Mice were subcutaneously administered with Ang II at a dose of 1.4mg/kg body weight daily via osmotic mini-pumps. At day 28, mice were killed with their kidney harvested. Cultured normal rat kidney interstitial fibroblast (NRK-49F) was incubated with Ang II at different doses for various time periods.

**Results:** Ang II administration resulted in renal interstitial fibrosis at day 28. Ets-1 protein expression was up-regulated in fibrotic kidney, which is located mainly in interstitial spaces. Similarly, Ang II activated cultured NRK-49F as demonstrated by up-regulated α-SMA and fibronectin expression and increased cell migration. Ang II induced Ets-1 expression in cultured NRK-49F in a dose and time dependent manner. Knock-down of Ets-1 by RNA interference attenuated Ang II-induced activation of NRK-49F. Ets-1 was previously reported as a target of microRNA-221 (miR-221). In Ang II-induced fibrotic kidney, miR-221 was markedly down-regulated. Similar results were observed in Ang II treated NRK-49F. Ectopic expression of miR-221 attenuated the up-regulation of Ets-1 by Ang II in cultured NRK-49F, which further prevented the activation of NRK-49F. However, Knock-down of miR-221 aggravated Ang II induced Ets-1 expression and NRK-49F activation.

**Conclusions:** Ets-1 mediates Ang II-induced renal fibroblast activation, whose up-regulation is probably resulted from the derepression after the down-regulation of miR-221 in activated fibroblast.

*Funding:* Government Support - Non-U.S.

## TH-PO556

**Loss of Endothelial Nitric Oxide Augments Renal Fibrosis via Promoting Local Fibroblast Proliferation** Jinhua Li,[1] Yu Bo Yang Sun,[1] Xinli Qu,[1] David J. Nikolic-Paterson.[2] *[1]Dept of Anatomy and Developmental Biology, Monash Univ, Australia; [2]Dept of Medicine, Monash Univ, Australia.*

**Background:** Deficiency in nitric oxide synthase 3 (NOS3/eNOS) exacerbates renal injury in the remnant kidney and diabetic nephropathy. Nitric oxide (NO) is a potent vasodilator released by the endothelium which suppresses vascular smooth muscle cell growth and inhibits platelet aggregation. This study investigated whether the loss of endothelial-derived NO contributes to renal interstitial fibrosis through promoting local fibroblast proliferation.

**Methods:** Unilateral ureteral obstruction (UUO) and STZ-induced diabetic nephropathy were performed in wild type (WT) and NOS3-/- C57BL/6J mice.

**Results:** Within 6hr of UUO surgery in WT mice, Western blotting showed a marked reduction in total NOS3 levels. Interstitial fibroblast proliferation was first seen at 48hr in WT UUO, indicating that endothelial injury proceeds interstitial fibroblast proliferation. Compared to WT mice, UUO in NOS3-/- mice had significantly increased accumulation of α-smooth muscle actin (α-SMA)+ myofibroblasts and a higher level of myofibroblast proliferation (Ki67+α-SMA+ cells), which was associated with increased deposition of collagen I and fibronectin. Similarly, STZ-induced diabetes resulted in greater accumulation of α-SMA+ myofibroblasts and higher levels of myofibroblast proliferation compared to WT mice. In vitro, the supernatant from mouse microvascular endothelial cells (MMECs) in which NO production was blocked by L-NAME, significantly increased the proliferation of renal fibroblasts (NRK49F) compared to normal media. Also, fibroblasts stimulated by hypoxia (5% oxygen) or PDGF-BB showed greater proliferation in the presence of L-NAME-treated supernatant. Finally, the supernatant from MMECs overexpressing NOS3 significantly inhibited PDGF-BB-induced proliferation of NRK49F cells. Since the half-life of NO is less than 5 seconds, this effect is likely through an indirect pathway.

**Conclusions:** In summary, our studies show that endothelial NO production is a powerful regulator of fibroblast proliferation and renal fibrosis.

*Funding:* Government Support - Non-U.S.

## TH-PO557

**Role of Interactions between the Slit Diaphragm and Glomerular Basement Membrane in Alport Syndrome** Diana Rubel, Jenny Kruegel, Rainer Girgert, Gerhard A. Mueller, Oliver Gross. *Nephrology & Rheumatology, Univ Medicine Goettingen, Goettingen, Germany.*

**Background:** Heterozygous Podocin-mutations and -polymorphisms can aggravate the phenotype of heterozygous carriers of type IV collagen mutations (COL4A3/4/5) with an unusual early onset of renal failure. This points toward an interaction between the slit diaphragm and glomerular basement membrane (GBM), which appears essential for podocytes´ structure and function. In the present study, we report about COL4A3-, integrin α2β1 (ITGA2)- and DDR1- single (sKO), double (dKO) and triple knockout (tKO) mice, respectively, and its effect on slit diaphragm proteins.

**Methods:** COL4A3$^{-/-}$, ITGA2$^{-/-}$, DDR1$^{-/-}$ and their respective dKO, as well as tKO and wildtype (WT) mice were investigated at different ages using real-time PCR, light and electron microscopy and immunogoldhistochemistry.

**Results:** In sKO mice, the relative expression of nephrin and podocin was strongly reduced compared to WT. Their expression in COL4A3/ITGA2 or COL4A3/DDR1 dKO and tKO, was comparable to WT till 4.5 weeks of age and decreased by 7.5 weeks. These results were consistent with a longer survival, later progress of kidney fibrosis and maintenance of podocyte footprocesses. In immunogoldhistochemistry, quantity of nephrin and podocin in 4.5 week COL4A3$^{-/-}$ was comparable to WT, but podocin accumulated in the areas of podocyte effacement in an age-dependent manner.The same accumulation was found in tKO at a later stage of disease. Nephrin increased in tKO in areas of still preserved footprocesses.

**Conclusions:** In conclusion, the impaired GBM in Alport-mice has an impact on podocyte function and development of fibrosis. Nephrin and podocin expression is strongly regulated by impaired GBM composition as well as altered signaling to the collagen IV receptors integrin α2β1 and DDR1. The knockout of these collagen receptors in Alport-mice results in preserved expression of nephrin and podocin proteins. Knowledge about the regulatory mechanisms of these interactions could lead to a better understanding of the podocyte behavior in pathological processes, the impact of GBM maturation on podocytes and direct to new therapeutic targets for glomerular diseases.

## TH-PO558

**MMP-9-Dependent Notch Signalling Contributes to Endothelial-Mesenchymal Transition in Kidney Endothelial Cells** Ye Zhao,[1] Yun Zhang,[3] Jianlin Zhang,[2] Guoping Zheng,[1] David C. Harris.[1] *[1]The Univ of Sydney; [2]Shanxi Medical Univ; [3]First Teaching Hospital of Shanxi Medical Univ; [4]Children's Hospital at Westmead, Sydney.*

**Background:** Endothelial-mesenchymal transition (EndoMT) has been shown to be a major source of myofibroblast formation in kidney fibrosis. Previously we showed that MMP-9 induced EndoMTin glomerular endothelial cells. This study investigated whether Notch signaling plays a role MMP-9-induced EndoMT.

**Methods:** Mouse renal peritubular endothelial cells (MRPEC) were isolated by magnetic microbead separation using anti-CD146 Ab. MRPECs were co-cultured with tubular epithelial cells over a polyester insert to maintain their phenotype. Human renal glomerular endothelial cells (HRGEC) and MRPECs were treated with TGF-β1 to induce EndoMT. EndoMT was assessed by morphological changes, immunofluorescence staining and Western blot (WB) of endothelial (CD31 and VE-cadherin) and mesenchymal markers (α-SMA and vimentin). Snail expression and Notch signaling were examined by RT-PCR and WB. MMP-9 expression was examined by zymography.

**Results:** TGF-β1 (10 ng/ml) and recombinant MMP-9 (2 μg/ml) induced EndoMT in HRGEC and MRPEC as evidenced by significant downregulation of VE-cadherin & CD31 and upregulation of α-SMA & vimentin. Recombinant MMP-9 also induced EndoMT in both HRGECs and MRPECs with upregulation of Notch signaling evidenced by an increase of Notch intracellular domain (NICD) accompanied by a decrease of Notch 1. Inhibition of MMP-9 or Notch signaling demonstrated a dose-dependent response in preventing TGF-β1-induced α-SMA and NICD in HRGECs. MMP-9 deficiency also led to a significant reduction in TGF-β1-induced NICD and α-SMA proteins in MRPECs of MMP-9 KO mice.

**Conclusions:** MMP-9-dependent Notch signaling plays an important role in TGF-β1-induced EndoMT in mouse renal endothelial cells.

*Funding:* Government Support - Non-U.S.

## TH-PO559

**ASK1-p38/JNK Signalling Promotes Renal Fibrosis and Apoptosis in the Obstructed Mouse Kidney** David J. Nikolic-Paterson,[1,2] Frank Yuanfang Ma.[1,2] *[1]Dept of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia; [2]Dept of Medicine, Monash Univ, Clayton, Victoria, Australia.*

**Background:** Apoptosis signal-regulating kinase 1 (ASK1) is a member of the large mitogen-activated protein kinase kinase kinase (MAP3K) family. ASK1 is activated in response to oxidative stress and then can activate the downstream stress-activated protein kinases, p38 and JNK, which have been implicated in promoting renal fibrosis. The aim of this study was to determine whether ASK1 plays a role in p38/JNK signalling and interstitial fibrosis in experimental kidney disease.

**Methods:** Unilateral ureteric obstruction (UUO) was induced in groups of 6 wild type (WT) and ASK1 gene deficient (Ask1-/-) and mice. Mice were killed on day 7 after UUO.

**Results:** Western blotting of WT UUO kidney identified a 3-5 fold increase in the levels of phosphorylation of ASK1, p38, and JNK compared to the non-obstructed kidney. The WT UUO kidney exhibited marked accumulation of α-SMA+ myofibroblasts and macrophages together with increased deposition of collagen IV, up-regulation of mRNA levels for pro-fibrotic (Col I, Col IV, α-SMA, PAI-1, TGF-β1) and pro-inflammatory molecules (CCL2), and increased tubular and interstitial cell apoptosis. In contrast, increased activation of p38 and JNK signalling was prevented in Ask1-/- UUO mice (P<0.001 vs WT UUO). Ask1-/- UUO mice were protected from renal fibrosis on the basis of: □29% in α-SMA+ myofibroblasts (P<0.001 vs WT UUO); □53% in macrophage accumulation (P<0.001); □43% in collagen IV deposition (P<0.001), and; □40-51% mRNA levels of pro-fibrotic and pro-inflammatory molecules (all P<0.05). In addition, apoptosis of tubular epithelial cells and interstitial cells was significantly reduced in Ask1-/- UUO (□37% and □41%, respectively; both P<0.05). However, Ask1-/- UUO kidney showed no protection from tubular injury based on KIM-1 mRNA levels.

**Conclusions:** This study defines ASK1 as an important upstream activator of p38 and JNK signalling in the obstructed kidney, and identifies ASK1 as a potential therapeutic target in renal fibrosis.

*Funding:* Government Support - Non-U.S.

## TH-PO560

**Kruppel-Like Factor 15 Modulates Renal Interstitial Fibrosis by ERK/MAPK and JNK/MAPK Pathways Regulation** Xiang Gao, Changlin Mei, Guiqun Wu. *Kidney Institute of PLA, Changzheng Hospital, Second Military Medical Univ, Shanghai, China.*

**Background:** Renal interstitial fibrosis is a hallmark of progressive chronic kidney disease (CKD). Previous studies reported that kruppel-like factor 15 (KLF15) is an important regulator of cardiac fibrosis and could reduce the expression of extracellular matrix in mesangial cells. However, the role of this transcription factor in renal interstitial fibrosis has not been reported.

**Methods:** In this study, we examined KLF15 expression in the remnant kidney of 5/6 nephrectomized rats 12 or 24 weeks after operation. In vitro we examined the effect of altered KLF15 expression on the production of extracellular matrix and the pro-fibrotic factor CTGF in rat renal fibroblasts (NRK-49F), and further explored the related mechanisms.

**Results:** The level of KLF15 was drastically decreased in the renal interstitium of 5/6 nephrectomized rats with progressive interstitial fibrosis, especially at 24 weeks. Our in vitro evidence showed that overexpression of KLF15 repressed basal and TGF-β1-induced extracellular matrix and CTGF in NRK-49F cells. In addition, transforming growth factor-β1 (TGF-β1)-mediated activation of extracellular-regulated kinase (ERK) / mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK) /MAPK downregulated KLF15 expression and increased the level of extracellular matrix and CTGF, and all these effects were completely abolished by ERK1/2 inhibitor and JNK inhibitor in NRK-49F cells.

**Conclusions:** Our findings implicate that KLF15 plays an important role and may prove to be an antifibrotic factor in renal interstitial fibrosis through regulation of ERK/MAPK and JNK/MAPK signaling pathways.

*Funding:* Government Support - Non-U.S.

## TH-PO561

**The Interstitial Compartment in Kidney Disease: Toxin and Fluid-Rich, with Biological Significance?** Leonard Ebah, Shiv Bhutani, Paul E. Brenchley, Sandip Mitra. *Renal Medicine and Renal Research, Manchester Royal Infirmary, Manchester, United Kingdom.*

**Background:** Fluid and (uremic) toxins are retained in chronic kidney disease (CKD), with their accumulation in plasma (P) and its impact extensively studied. Little attention has been paid to interstitial fluid (IF) and toxin accumulation and their potential physiological impact. We studied IF biochemical composition and mechanical properties in CKD patients.

**Methods:** Microdialysis (MD), reverse iontophoresis (RI), subcutaneous cannulae and microneedles were used to sample IF in CKD patients.IF pressure was measured by the wick-in-needle technique and volume by bioimpedance. Comparisons were made with plasma and with healthy controls.

**Results:** IF volume was significantly expanded in CKD patients with edema (mean difference with controls 9L; p=0.0009), with plasma and intracellular volumes being similar. IF in CKD patients was a high pressure compartment; mean 4.6±1mmHg (-0.9mmHg in controls; p=0.0005). A novel parameter, the edema refill time (ERT) correlated strongly with IF pressure and volume . Small uremic toxins seemed to circulate relatively freely between IF and plasma, with high IF:P correlations for urea, creatinine, urate and phosphate in "steady states" (r=0.74-0.98). Hemodialysis (HD) induced a difference in the IF and plasma decay curves, especially for phosphate where IF decay seemed to lag behind that of plasma (p=0.04). Paired metabolomic (LCMS/MS) analysis identified at least 36 "small" uremic toxins up to 444Da with similar IF:P ratios. Proteomics revealed a clearly different large molecule expression between P and IF, with IF accumulation of several molecules including known large uremic toxins such as beta-2 microglobulin, complement factor D, IgG kappa light chain, cystatin C and retinol binding protein (IF: P normalized relative abundance 1.5 -10.2).

**Conclusions:** Volume accumulation in CKD is mainly in IF, a high pressure compartment with mechanical properties potentially defined by the ERT. IF is toxin-rich, with small toxins circulating freely between plasma and IF although IF toxin decay on HD may lag behind, with potential implications for dialytic efficacy. Large uremic toxins may be "sequestered" in IF in spite of deceptively "normal" plasma levels.

## TH-PO562

**Oral Treatment with PBI-4050 Reduces Kidney Fibrosis** Lyne Gagnon, Brigitte Grouix, Kathy Hince, Lilianne Geerts, Liette Gervais, Mikaël Tremblay, François Sarra-Bournet, Alexandra Felton, Shaun Abbott, Jean-Simon Duceppe, Martin Leduc, Boulos Zacharie, Christopher Penney, Pierre Laurin. *ProMetic BioSciences Inc., Laval, Canada.*

**Background:** PBI-4050 is a first-in-class novel orally active compound which displays anti-inflammatory/antifibrotic activities via a novel mechanism of action. PBI-4050 has demonstrated strong anti-fibrotic activities in different kidney fibrosis animal models. PBI-4050 plays a key role in inflammation/fibrosis regulation by reducing pro-fibrotic cytokines, fibrocyte differentiation, myofibroblast activation and epithelial–mesenchymal transition (EMT) resulting in improvement of organ function.

**Methods:** PBI-4050 was tested in the following animal models: 5/6-nephrectomized rats (end-stage renal failure), doxorubicin-induced nephrotoxicity and renal ischemia (acute kidney injury), and db/db mice (diabetic kidney disease).

**Results:** PBI-4050 reduces fibrosis via the regulation of macrophages, T cells, fibrocytes/fibroblasts/myofibroblasts and epithelial cells. In a pro-fibrotic phase, PBI-4050 plays a regulation role by promoting a Type 1, anti-fibrotic cytokine production phenotype in macrophages and T cells, resulting in a reduction of the over-expression or over-production of TGF-β, MCP-1, CTGF, IL-4, IL-13 and IL-23. Interestingly, MCP-1 is also an important inducer of fibrocyte precursor migration in tissue. Fibrocyte differentiation, resident fibroblast activation and EMT are the key sources of activated myofibroblasts and successive accumulation of extracellular matrix protein deposition and fibrosis. PBI-4050 inhibits fibrocyte differentiation, fibroblast activation and EMT as demonstrated by a reduction in alpha smooth muscle actin (α-SMA), collagen I and fibronectin mRNA expression. PBI-4050 also plays a role in tissue remodeling by regulating MMP and TIMP expression.

**Conclusions:** Taken together, these preclinical results suggest that PBI-4050 offers the potential as a novel therapy for the treatment of kidney fibrosis.

## TH-PO563

**The RNA-Binding Protein QUAKING Is a Potent Modulator of the Fibrogenic Response to Kidney Injury** Ruben de Bruin,[1] Eric P. Van der Veer,[1] Hetty C. de Boer,[1] Janine van Gils,[1] Roel Bijkerk,[1] Jacques Duijs,[1] Erik Biessen,[2] Ton J. Rabelink,[1] Anton Jan Van Zonneveld.[1] *[1]Nephrology, LUMC; [2]Pathology, MUMC.*

**Background:** Chronic progressive kidney diseases are characterized by tubule-interstitial deposition of extracellular matrix, tubular atrophy and dilation. Recruitment of circulating monocytes to sites of tissue injury leads to the local generation of cytokines and growth factors that promote fibroblast activation, proliferation and production of extracellular matrix. We have identified Quaking (QKI) as a novel post-transcriptional regulator of both monocyte and fibroblast activation and differentiation, implicating a central position in the pathogenesis of renal fibrosis.

**Results:** Following FACS sorting of human $CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{+}$ monocyte subpopulations, we identified that QKI mRNA is moderately expressed in $CD14^{++}CD16^{-}$ monocytes, while QKI is potently induced in the activated $CD16^{+}$ monocytes (n=3, p<0.05). Importantly, the differentiation of monocytes to macrophages by GM-CSF was coupled with a further increase in QKI expression (n=6, p<0.05). Abrogation of QKI in THP-1 and U937 monocytes using lentiviral shRNA reduced cellular adhesion in a cell perfusion assay that simulates endothelial denudation (p<0.05,n=6). In parallel, we investigated the consequences of reduced QKI protein levels in human renal fibroblasts (TK173), which yielded marked attenuation of cellular proliferation, and a perturbed capacity to acquire the myofibroblast phenotype in response to TGF-β1, as evidenced by decreased SM-α-actin expression as compared to control cells (n=3, p<0.05). Finally, we performed unilateral urethral obstruction on mice hypomorphic for QKI (quaking viable). These studies revealed a two-fold reduction in macrophage (F4/80 and CD115) and fibroblast (collagen, FSP-1 and SM-α-actin) marker expression as compared to wild-type mice (n=6, p<0.05).

**Conclusions:** Collectively, we identified that QKI serves as a double-edged sword in renal interstitial fibrosis, as it possesses the capacity to drive critical events in both myeloid and mesenchymal cells.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO564

**Glomerular Fibrosis Revisited: Podocyte Injury Shuts Down *Col1a2* mRNA, Leading to Abnormal Collagen Accumulation** Masahiro Koizumi, Masafumi Fukagawa, Taiji Matsusaka. *Tokai Univ School of Medicine, Japan.*

**Background:** Type I collagen (ColI) accumulates in sclerotic glomeruli, which is often ascribed to transcriptional upregulation of the *Col1* genes. On the other hand, our microarray analysis indicated that normal mouse glomeruli abundantly express *Col1a2* mRNA, which is markedly decreased after podocyte injury. Normal ColI is composed of two a1(I)and one a2(I) chains. It has been shown that *Col1a2* mutant mice abnormally accumulate $a1(I)_3$ homotrimer, forming glomerulosclerosis. We, therefore, hypothesized that podocyte injury downregulates *Col1a2* mRNA, which leads to accumulation of ColI.

**Methods:** Glomerular *Col1a2* mRNA was quantified by RT-PCR in immunotoxin-inducible podocyte injury mice (NEP25, n=9). *Col1a2* transcriptional activity was also monitored, utilizing *Col1a2-EGFP* mice (n=12).

**Results:** Glomerular *Col1a2* mRNA was decreased to 24% 7 days after induction of podocyte injury, while *Col1a1* mRNA did not change. In non-injured *Col1a2-EGFP* mice, EGFP was intensely expressed in podocytes and mesangial cells. Unlike previous reports, immunostaining detected intense ColI in normal glomeruli. We next induced various degrees of podocyte injury in *NEP25/Col1a2-EGFP*. Two weeks after high dose toxin, most glomeruli were severely injured with podocyte loss, mesangial expansion and mesangiolysis. EGFP almost disappeared in them. ColI staining also declined in the lesion of mesangiolysis. Two weeks after moderate dose toxin, EGFP declined in both podocytes and mesangial cells in glomeruli containing injured podocytes, with EGFP area being 15.9±7.7%, but not in normal glomeruli (30.5±9.8%, p<0.05). Eight weeks after mild podocyte injury, FSGS was established, where EGFP disappeared in sclerotic glomeruli, in which ColI was intensely stained.

**Conclusions:** Collectively, podocyte injury suppresses *Col1a2* mRNA in both podocytes and mesangial cells. The latter, in severe case, causes mesangiolysis. Our study also suggests that prolonged suppression of *Col1a2* may induce accumulation of MMP-resistant $a1(I)_3$ homotrimer, thereby leading to the characteristic phenotype of glomerulosclerosis.

## TH-PO565

**A Selective JAK3 Inhibitor, CP690,550, Suppresses Bone Marrow-Derived Fibroblast Activation and Renal Fibrosis** Jingyin Yan, William E. Mitch, Yanlin Wang. *Dept of Medicine, Div of Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** Renal fibrosis is the final common manifestation of chronic kidney disease resulting in progressive loss of kidney parenchyma and renal function. We have recently demonstrated that bone marrow-derived fibroblast precursors contribute significantly to the pathogenesis of renal fibrosis. However, the signaling mechanisms underlying the activation of bone marrow-derived fibroblast precursors in the kidney are not fully understood. In this study, we investigated the role of JAK3/STAT6 signaling pathway in the activation of bone marrow-derived fibroblasts using a selective JAK3 inhibitor, CP690,550.

**Methods:** We examined the role of JAK3/STAT6 signaling in monocyte-to-fibroblast transition in vitro and myeloid fibroblast activation and renal fibrosis induced by unilateral ureteral obstruction.

**Results:** In cultured mouse monocytes, profibrotic cytokines IL-4 and IL-13 activated STAT6 and induced expression of α-smooth muscle actin and extracellular matrix proteins (fibronectin and type I collagen), which was blocked by CP690,550. Obstructive injury led to activation of STAT6 in the interstitial cells of the kidney, which was abolished by treatment with CP690,550. Furthermore, mice treated with CP690,550 accumulated fewer bone marrow-derived fibroblasts in the kidneys after the obstructive injury compared with vehicle-treated mice. Finally, treatment with CP690,550 significantly reduced myofibroblast activation and suppressed expression of fibronectin and type I collagen in response to the obstructive injury.

**Conclusions:** Our results demonstrate that JAK3/STAT6 signaling plays an important role in the activation of bone marrow-derived fibroblasts during the development of renal fibrosis. CP690,550 may serve as a novel therapeutic agent for the treatment of chronic kidney disease.

*Funding:* NIDDK Support

## TH-PO566

**Matrix Metalloproteinase 12 Directly Act on the Proliferation and Extracellular Matrix Production of Renal Fibroblasts** Beáta Szebeni,[1,2] Leonora Himer,[1,2] Erna Szikszs,[1,2] Domonkos Pap,[2] Anna Ónody,[2] Andrea Fekete,[3] Yoichiro Iwakura,[4] Gyorgy S. Reusz,[2] Tivadar Tulassay,[1,2] Adam Vannay.[1,2] *[1]Research Laboratory for Paediatrics and Nephrology, SE-HAS, Budapest, Hungary; [2]First Dept of Paediatrics, SE, Budapest, Hungary; [3]SE-HAS, Budapest, Hungary; [4]Center for Experimental Medicine, Institute of Medical Science, Univ of Tokyo, Tokyo, Japan.*

**Background:** Chronic kidney diseases (CKDs) affect millions and are leading cause of morbidity and mortality in the western word. Renal fibrosis is an uncontrolled wound-healing process defined by excessive deposition of the extracellular matrix (ECM). Recently the determinative role of interleukin (IL)-17 has been suggested in matrix metalloproteinases (MMP) dependent ECM remodeling. However, the role of MMP12 in progressive kidney diseases is still unknown.

**Methods:** Unilateral ureteral obstruction induced (UUO) mice model of renal fibrosis on C57Bl/6J (WT) and IL17 KO mice and HK2 proximal tubular epithelial (PTEC) and NRK49F renal fibroblasts were used in our experiments.

**Results:** 5 days after the onset of UUO the number of IL17+ T cells was elevated in the kidney. Simultaneously the number of MMP12 positive PTECs increased in WT but not in IL17 KO mice. IL17 treatment of the HK2 cells resulted in a JNK dependent increase in the number of MMP12+ cells. rMMP12 treatment of NRK49F cells increased the proliferation and collagen production of the fibroblasts. Specific MMP12 inhibitor treatment of WT animals decreased the level of renal αSMA and collagen1.

**Conclusions:** Our data suggest that PTECs are responsible for the production of MMP12 in the fibrotic kidney. According to our observations MMP12 may be a link between inflammation and the severity of renal fibrosis. Moreover, beside the previoulsy hypothetised role of MMP12 on ECM degradation we suggest that MMP12 may directly act on renal fibrosis by inducing proliferation and ECM production of renal fibroblasts.

*Funding:* Government Support - Non-U.S.

## TH-PO567

**Proximal Tubule PPARalpha Attenuates Renal Fibrosis and Inflammation Caused by Unilateral Ureteral Obstruction (UUO)** Shenyang Li,[1,2] Nithya Mariappan,[1,2] Judit Megyesi,[1,2] Brian B. Shank,[1,2] Sue Theus,[2] Peter M. Price,[1,2] Jeremy Stuart Duffield,[3] Didier Portilla.[1,2] *[1]Medicine/ Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR; [2]Medicine, Central Arkansas Veterans Healthcare Systems, Little Rock, AR; [3]Medicine, Univ of Washington, Seattle, WA.*

**Background:** Previous studies suggest that activation of proximal tubule PPARα reduces inflammation and ameliorates tissue fibrosis but the mechanisms involved are not clear.

**Methods:** We compared the effects of UUO on wild type and proximal tubule PPARα Tg mice and also examined the effects of increased PPARα expression using adenovirus transduction in cultured mouse proximal tubules exposed to aristolochic acid.

**Results:** After 5 days of UUO PPARα expression was significantly reduced in kidney tissue of wild type mice but this downregulation was attenuated in PPARα Tg mice. When compared with wild type mice subjected to UUO, PPARα Tg mice had reduced mRNA and protein expression of proximal tubule TGFβ1, with reduced production of extracellular matrix proteins including collagen 1, fibronectin, α-SMA, and reduced tubulo-interstitial fibrosis. UUO-mediated increased expression of microRNA 21 in kidney tissue was also reduced in PPARα Tg mice. Over-expression of PPARα in cultured proximal tubular cells by adenoviral transduction reduced aristolochic acid(AA)-mediated increased production of TGFβ, collagen4, and laminin B demonstrating PPARα prevented AA-induced TBM degradation. Flow cytometry studies of dissociated whole kidneys demonstrated reduced macrophage infiltration to kidney tissue in PPARα Tg mice after UUO. Increased expression of pro-inflammatory cytokines including IL1-β, IL-6, and TNF-α in wild type mice was also significantly reduced in kidney tissue of PPARα Tg mice. In contrast, the expression of anti-inflammatory cytokines IL-10 and Arginase-1 was significantly increased in kidney tissue of PPARα Tg mice when compared with wild type mice subjected to UUO.

**Conclusions:** Our studies demonstrate several mechanisms by which preserved expression of proximal tubule PPARα reduces tubulo-interstitial fibrosis and inflammation associated with obstructive uropathy.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO568

**Myeloid Cells-Derived Tissue-Type Plasminogen Activator Promotes Renal Fibrosis and Inflammation** Ling Lin, Kebin Hu. *Dept of Medicine, Penn State Univ College of Medicine, Hershey, PA.*

**Background:** Tissue-type plasminogen activator (tPA), a serine protease up-regulated during chronic kidney disease, has been shown to promote renal fibrosis and inflammation by activating various signaling cascades. However, the origin and the responsible cells of the activated tPA signaling remain unknown.

**Methods:** We created chimeric mice that lack tPA in either their myeloid cells or renal parenchyma by bone marrow transplantation between tPA wild-type (WT) and knockout (KO) mice using the following donor/recipient combinations: WT/WT, WT/KO, KO/WT, and KO/KO, and subjected these mice to unilateral ureteral obstruction for 7 days, followed by evaluation of renal damages, fibrosis, and inflammation.

**Results:** It was found that WT/WT and WT/KO mice displayed similar level of tubulointerstitial injuries, fibrosis, and inflammation as demonstrated by HE staining, Western blot or immune fluorescence staining for α-SMA and collagen1, as well as quantitative PCR for chemokines. However, KO/WT and KO/KO mice showed lower level of damages and decreased fibrosis and inflammation than that from WT/WT and WT/Ko mice, suggesting that mice that lack of tPA in the myeloid cells were protected from fibrotic and inflammatory injuries.

**Conclusions:** Thus, it is persumable that myeloid cells contribute to tPA signaling in response to chronic kidney injury.

*Funding:* Private Foundation Support

## TH-PO569

**Fibrogenic Pathways Are Activated by the Macrophage Phagocytic Receptor CD36 with Apoptotic Cell Clearance** Daryl M. Okamura,[1] Lixia Zeng,[2] Ikuyo Yamaguchi,[1] Subramaniam Pennathur.[2] *[1]Dept of Pediatrics, Div of Nephrology, Seattle Childrens Research Institute, Seattle, WA; [2]Dept of Internal Medicine, Div of Nephrology, Univ of Michigan, Ann Arbor, MI.*

**Background:** The progression of chronic kidney disease is due to a maladaptive wound healing response where tubular apoptosis and macrophage activation are two important processes. The removal of apoptotic cells is an innate function of tissue macrophages (mphi), however, its role in disease progression is unclear.

**Methods:** The present study was designed to investigate the role of mphi CD36, a recognized receptor of apoptotic cells, by unilateral ureteral obstruction (UUO). In order to differentiate the mphi CD36-specific effects in vivo, we generated CD36 chimeric mice by bone marrow transplantation and performed UUO surgery: CD36 ko/wt (donor/recipient) with chimeric controls.

**Results:** CD36 expression is up-regulated with M2 activation. After UUO, CD36 mphi comprise 30-50% of the phagocytic subpopulation. Following phagocytosis of apoptotic cells, there was 65-75% reduction in TNF-a and TGF-b mRNA levels in CD36-/- compared to CD36+/+ mphi. Fibrosis severity was decreased by 41% and fibronectin and procollagen mRNA levels were decreased by 40-50% in CD36 ko/wt mice at day 14. There was a 30% decrease in NF-kB activation in CD36 ko/wt mice at day 14 after UUO compared to CD36 wt/wt mice. There was a 60-70% reduction in kidney TGF-b and TNF-a mRNA levels in CD36 ko/wt mice after UUO. Since oxidized phospholipids/lipids are the major ligands for CD36, we measured the level of apoptotic cells by TUNEL staining. We found that there was a 56% increase in TUNEL+ cells in CD36 ko/wt at day 14 despite a reduction in fibrosis severity. Furthermore, mphi isolated from UUO kidneys demonstrated that there was 40-50% reduction in lipid hydroperoxides and a 60-70% reduction in F2 isoprostane levels in CD36-/- mice compared to CD36+/+ mice at day 14.

**Conclusions:** These data suggest that mphi CD36 is a critical regulator of fibrogenic signaling and extracellular matrix expansion during chronic kidney injury and suggests that oxidative mechanisms may play a key role in mediating these pathways.

*Funding:* NIDDK Support

## TH-PO570

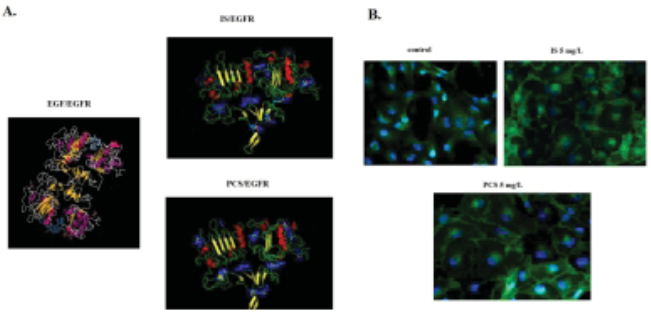
**Tissue Remodeling Induced by Indoxyl Sulfate and *p*-Cresol Sulfate: Roles of Epidermal Growth Factor Receptor Activation** Chiao-Yin Sun. *Nephrology, Chang Gung Memorial Hospital, Keelung, Taiwan.*

**Background:** Uremic toxins have determinant roles in the progression of chronic kidney disease clinically. Indoxyl sulfate (IS) and p-cresol sulfate (PCS) could cause renal fibrosis by activating RAAS. Epidermal Growth Factor (EGF) is known for its role in promoting cell cause proliferation and matrix deposition. It is hypothesized that EGFR activated by IS and PCS might cause renal tissue remodeling and fibrosis.

**Methods:** Cultured renal tubular cells (HK2) and B-6 mice with ½-nephrectomy were used for this study. EGFR activation was analyzed by dimerlization and phosphorylation of EGFR. The downstream signal proteins of EGF pathway and the expression of MMP2 and MMP9 were quantified *in vitro* and *in vivo*.

**Results:** Docking models for the putative interaction between IS/PCS and EGFR were made based on the x-ray structure of extra-cellular domains of EGFR. The results showed that IS/PCS interacted with extracellular domains of EGFR near the EGF binding site by the hydrophobic force. EGFR diamerlization and phosphrylation increased by IS and PCS *in vitro*. IS and PCS activated EGFR downstream signaling proteins (Jak1, and Stat1/3) *in vitro.* Increased cell membrane localization of EGFR was noted *in vitro*.

**Figure 1.**



IS and PCS increased MMP2 and MMP9 expression *in vitro,* which was antagonized by N-acetylcystein and EFGR inhibitor. *In vivo* study with 1/2 nephrectomy mice showed that IS and PCS significantly increased the serum EGF concentration and renal EGFR phosphorylation. IS and PCS also increased renal MMP2 and MMP9 expression significantly *in vivo*.

**Conclusions:** It was suggested that IS and PCS could activate EGFR via a ligand independent and dependant pathway by direct EGFR biding and increasing EGF level. The EGFR activation might induce renal tissue remodeling by increasing the expression of MMPs.

## TH-PO571

**Participation of WNT Protein in Acute Kidney Injury** Akihiro Kuma, Tetsu Miyamoto, Ryota Serino, Narutoshi Kabashima, Masahito Tamura, Yutaka Otsuji. *The Second Dept of Internal Medicine, School of Medicine, Univ of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

**Background:** Acute kidney injury (AKI) can recover or progress to chronic kidney disease (CKD) or end-stage renal disease. Furthermore, kidney fibrosis which is not effectively treated can lead to renal failure. WNT family proteins have been implicated in organ fibrosis. Although previous studies revealed the expression of WNT10A in fibroblasts, the mechanisms of such expression have been elusive. We focused on kidney fibrosis following interstitial fibrosis in AKI and investigated the function of WNT10A protein in kidney fibroblasts.

**Methods:** Expressions of WNT10A were confirmed by immunohistochemical staining in kidney biopsy tissue from 25 male AKI patients (age, ≥60 years) who were received biopsy to diagnose cause of AKI in the past 6 years. *In vitro,* we used COS1(kidney fibroblasts of African green monkey) transfection of WNT10A plasmid (COS1-W10). The COS1-W10 was investigated cell survival ability and proliferation against oxidative stress and high glucose stress.

**Results:** 10 Patients with WNT10A expression had a significantly lower estimated glomerular filtration rate than 15 WNT10A-negative patients (15.2 ± 8.5 vs 46.5 ± 36.9 mL/min per 1.73m$^2$, p = 0.015). COS1-W10 exhibited cell survival ability against oxidative stress induced hydrogen oxide ($p < 0.05$), and the COS1-vector reduced the proliferation rate in high-glucose medium (11mM and 22mM) compared with low-glucose medium (5.5mM). However, COS1-W10 maintained proliferation ability in high-glucose medium ($p < 0.05$).

**Conclusions:** WNT10A protein protects fibroblasts from oxidative stress and high glucose stress. Taken together, expression of WNT10A protein in kidney fibroblasts in AKI might lead to CKD. Therefore, inhibition of WNT10A protein can improve kidney function in AKI patients and prevent progression to kidney dysfunction.

## TH-PO572

**Hyaluronan Exacerbates Renal Fibrosis in NZB/W Mice through Increased Monocyte Chemoattractant Protein-1 and Transforming Growth Factor-β1 Secretion by Mesangial Cells** Susan Yung, Wan Wai Tse, Mel Chau, Daniel Tak Mao Chan. *Dept of Medicine, The Univ of Hong Kong, Hong Kong.*

**Background:** Lupus nephritis is characterized by the production of anti-dsDNA antibodies and immune-mediated renal injury leading to glomerular and tubulointerstitial fibrosis. We have previously demonstrated that circulating hyaluronan (HA) level and glomerular HA expression are increased in patients and lupus-prone mice with active lupus nephritis, and are associated with increased glomerular matrix protein accumulation. This study investigates the role of HA on disease manifestations and renal fibrogenesis in a murine model of lupus nephritis.

**Methods:** Pre-disease female NZB/W mice were randomized to receive sterile PBS or high molecular weight HA by tail-vein injection (1mg/ml, 200µl) once weekly for periods up to 24 weeks, after which the mice were sacrificed, blood and urine collected, and kidneys harvested to assess renal histology and expression of fibrosis mediators. Mesangial cells were isolated from the renal cortex of NZB/W mice to investigate the mechanisms that mediate increased HA synthesis.

**Results:** Treatment of mice with HA had no effect on survival, proteinuria or anti-dsDNA antibody level compared to control mice, but was associated with increased glomerular IgG and C3 deposition, significantly increased glomerular and tubulointerstitial expression of HA receptor CD44, MCP-1, TGF-β1, fibronectin and collagen type I from 18 to 24 weeks. Exogenous HA for 24h had no effect on mesangial cell proliferation, but significantly increased CD44, HA synthase III, fibronectin, laminin and collagen synthesis, in part through the induction of MCP-1 and TGF-β1 secretion ($P<0.01$ for both). Stimulation of mesangial cells with exogenous MCP-1 and TGF-β1 significantly increased cell associated and secreted HA levels respectively ($P<0.05$ for both).

**Conclusions:** These results suggest that HA plays a significant role in renal fibrosis of lupus nephritis by inducing MCP-1 and TGF-β1 secretion, which in turn increase HA synthesis resulting in a positive feedback loop that amplifies the fibrotic process.

*Funding:* Government Support - Non-U.S.

## TH-PO573

**Periostin Staining as Novel Biomarker in Progressive Glomerular Injury** Bancha Satirapoj,[1] Wiwat Charungkiattikul,[1] Peepattra Wantanasiri,[2] Prajej Ruangkanchanasetr,[1] Naowanit Nata,[1] Ouppatham Supasyndh,[1] Panbubpa Choovichian.[1] *[1]Div of Nephrology, Dept of Medicine, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand; [2]Dept of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Bangkok, Thailand.*

**Background:** Glomerulonephritis (GN) is a progressive kidney injury and is a common form of kidney disease. Periostin, a recently characterized matricellular protein participating in the tissue remodeling, is markedly induced in renal injury. There is limited information about periostin expression in GN.

**Methods:** In this study involving 49 patients with IgA nephropathy (IgAN) (n=12), lupus nephritis (LN) (n=33) and minimal change disease (MCD) (n=4), periostin was stained by immunohistology in renal biopsies. Perisotin expression and intensity was studied with other kidney injury markers. Progression kidney injury, assessed as declining of glomerular filtration rate (GFR), was evaluated during follow-up.

**Results:** Periostin expression was predominantly located in the injured regions and the sites of fibrosis including periglomerular and tubulointerstitial areas. Periostin staining had significantly increased patients with LN (14.97±8.41, p=0.02) and IgAN (18.50±9.69, p=0.01) when compared to MCD patients (5.80±4.76). Periositn staining correlated positively with renal activity index in GN patients. It also correlated positively with renal chronicity index in GN and LN patients. By multivariate analysis, renal periostin were inversely related to GFR. After a median follow-up of 34 weeks, a trend for higher declining of GFR was found in patients with higher periostin scores (-0.19 (IQR -1.21 to 0.46) vs -0.05 (IQR -0.54 to 4.36) mL/min/1.73 m$^2$/month, p=0.239), and higher periostin scores were not predictor of end stage renal disease.

**Conclusions:** Periostin is markedly induced in glomerular disease and the levels correlated positively with the severity of renal lesions. Periostin staining may serve as a marker of progressive glomerular injury.

## TH-PO574

**Antisense Oligonucleotides Targeting Kirsten Ras Reduce Interstitial Fibrosis and Protect Kidney Function in the Chronic Folic Acid Nephropathy Model** Lucy Jade Newbury,[1] Gene Hung,[2] Bruce M. Hendry,[1] Claire C. Sharpe.[1] *[1]Dept of Renal Sciences, Kings College London, London, United Kingdom; [2]Isis Pharmaceuticals, Carlsbad, CA.*

**Background:** Previously we have demonstrated the importance of Kirsten Ras (Kras) in the pathogenesis of renal fibrosis secondary to ureteric obstruction. In this study we have characterised a novel mouse model of chronic folic acid nephropathy (CFAN) and have used antisense oligonucleotides (ASO) to silence Kras expression within this model. Here we report the effects of these ASOs on interstitial fibrosis and renal function.

**Methods:** CFAN model: Male CD1 mice were given Folic acid (FA) 125mg/kg IV in $NaHCO_3$ on day 0 (d0) and d21. Shams received $NaHC0_3$. CFAN and Shams were sacrificed at d35, d56 and d85 for model characterisation. Therapeutic study: CFAN groups received saline (vehicle) or ASO (20mg/kg) SC 3X per week from d35 reducing to 2X per week from d49 and were sacrificed at d85.

**Results:** CFAN model: Induction of fibrosis was seen at d56 and d85 with a 3.4 fold mean increase in collagen staining and a 2 fold increase in collagen mRNA by D85 associated with a doubling of Kras mRNA (p= 0.0016, n=11). An induction of α-SMA expression was seen at d56 and d85 of 2.2 and 1.6 fold respectively (n=3); IHC showed diffuse α-SMA expression in fibrotic areas, co-localising with collagen deposition. Therapeutic study: ASO resulted in a knockdown of Kras of 43% compared to saline treated (p= 0.0185, n=7). IHC for ASO showed marked accumulation of the ASO in the proximal tubules and interstitium. Total area of fibrosis, as measured by collagen deposition, was reduced by 37% on PMT staining and 27% on PSR staining in ASO-treated groups compared with saline mice (n=7). ASO-treatment was associated with a 70% reduction in serum creatinine and 40% reduction in Blood Urea Nitrogen at d85 compared with saline. (p=0.053/p=0.0693, n=7).

**Conclusions:** In this study we have demonstrated that Kras inhibition inhibits renal fibrosis in a new model of CFAN. We have also shown that this is associated with an improvement in renal function. We conclude that targeting Kras may prove to be a useful therapeutic tool for treating human chronic kidney disease.

## TH-PO575

**Modulating Alternative Splicing of Fibronectin with Antisense Oligonucleotides** Felicia Heidebrecht,[1] Vaishnavi Gnanananthan,[1] Benjaman To,[1] Frank Rigo,[2] Susan M. Freier,[2] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]Isis Pharmaceuticals, Carlsbad, CA.*

**Background:** The EDA+ splice variant of fibronectin(Fn) is an important component of fibrotic extracellular matrix facilitating fibroblast activation and collagen deposition. Directing the alternative splicing away from EDA inclusion could provide a therapy to limit fibrosis. The aim of this study was to test the possibility of modulating the TGFβ-induced Fn splicing using RNase-H independent antisense oligonucleotides(ASO).

**Methods:** In a pilot study 20 ASOs targeting overlapping sequences within both exon-intron junctions were assessed for their ability to inhibit EDA+ protein and mRNA in primary tubule epithelial cells. Selected ASOs were administered pre and post TGFβ1

challenge as a model of therapeutic intervention. Expanding the study we analysed further 42 ASOs covering the whole exon, including splicing enhancer and silencer sites, and assessed their effects on EDA+ and EDA- splice variants.

**Results:** From the pilot study an ASO designated ASO5 reproducibly inhibited EDA+ mRNA and protein expression. Furthermore, treatment with ASO5 post TGFβ1 inhibited a number of TGFβ-induced fibrotic markers including αSMA & MMP9. Many ASOs that showed a switch in the splicing (reducing EDA+ > 3 fold and inducing EDA- 2 to 3.5 fold) did so in the presence of TGFβ stimulation or the effect in shifting the balance away from EDA+ was stronger with TGFβ. Thus, this is an attractive therapeutic approach as it alters the ratio EDA+/EDA- when TGFβ is present. The best sites for ASO-mediated splicing modulation were not predicted a priori. This might reflect the role of the 3-D structure of the mRNA on the accessibility of a target sequence and efficacy in splicing inhibition.

**Conclusions:** These observations together with striking differences between various ASOs including ASOs with a high degree of overlap demonstrate the importance of complete screening for finding the most effective sequence. Detailed analysis of this screening could highlight unknown regulatory sequences. Altering Fn splicing using ASO may provide an effective therapy by inhibiting EDA+ production and limiting subsequent fibrogenesis.

*Funding:* Private Foundation Support

## TH-PO576

**Gremlin1 Displays Differential Affinities for Bone Morphogenetic Proteins – Implications for Diabetic Nephropathy** Derek P. Brazil,[1] Rachel Church,[1] Arjun Krishnakumar,[1] Stefan Geschwinder,[2] Barbro Basta,[2] Maria Stromstedt,[2] Finian Martin.[3] *[1]Centre for Vision and Vascular Science, Queen's Univ, Belfast, United Kingdom; [2]UCD Conway Institute, Univ College, Dublin, Ireland; [3]AstraZeneca, Gothenburg, Sweden.*

**Background:** Gremlin1 is a secreted glycoprotein that limits the action of bone morphogenetic proteins (BMP) in multiple cell types in the body. Grem1 dimers bind directly to BMP dimers preventing binding to their membrane receptors. Our group has shown that levels of Grem1 increase in the kidneys of patients with DN, and correlate well with decreased renal function. We also demonstrated that mice lacking one copy of the *grem1* gene are partially protected from early DN-like changes.

**Methods:** Therefore, the aim of this project was to determine the relative affinities of Grem1 for various pro- and anti-fibrotic BMPs in vitro and in vivo.

**Results:** Surface plasmon resonance (Biacore) kinetic and affinity analysis allowed a rank order of affinity for Grem1 to be determined with BMP-2>2/7>4 >4/7 >6>7. In HK-2 cells, the addition of 25ng/ml rhGrem1 caused complete inhibition of pSmad1/5/8 phosphorylation, whereas cells treated with rhBMP-4 required 200ng/ml rhGrem1 for maximal inhibition. Consistently, higher concentrations of Grem1 were required to inhibit BMP4-mediated Id1 and Smad6 gene expression in HK2 cells compared to BMP2, with little or no inhibition of BMP7 responses seen. Overexpression of Grem1 inhibited BMP2 and 4, but not BMP7-mediated Smad1/5/8 phosphorylation. Analysis of *grem1*-/- mice on a mixed genetic background showed that these mice survive post-natally, are smaller, and develop only one kidney that is larger than wild-type controls and display tubular damage.

**Conclusions:** Our data suggest that in the extracellular milieu of the diabetic kidney, secreted Grem1 is more likely to bind BMP2 than BMP4. In addition, *grem1*-/- mice are viable and will be further analysed to determine which BMPs are involved during the critical stages of BMP-driven kidney development. These mice will also be useful tools to determine if the absence of Grem1 in the kidney can protect against DN, further validating current efforts to target Grem1 protein in human kidney disease.

## TH-PO577

**Automated Quantitation of Interstitial Fibrosis by Sirius Red Staining/ Polarization Microscopy Is More Objective Than Masson Trichrome** Jonathan Street, Alejandro Alvarez-Prats, Taro Horino, Xuzhen Hu, Robert A. Star, Peter S.T. Yuen. *NIDDK, NIH, Bethesda, MD.*

**Background:** Histological measurement of interstitial fibrosis by Masson Trichrome staining is only *semi*-quantitative because of subjective scoring by an ordinal scale, which limits statistical power. Sirius Red staining enhances the birefringence of type I and III collagen fibrils which appear bright against a dark background with polarization contrast microscopy. This striking contrast is more amenable to automated analysis than the subtle color differences of Masson Trichrome. An automated technique to measure the fibrotic area might also reduce operator-dependent error and reduce variability.

**Methods:** Paraffin-embedded kidney sections from a folic acid injury model (21 mice) were stained with Masson Trichrome and scored by two independent observers. Adjacent sections were stained with Sirius Red and then imaged at 100x magnification using polarization contrast microscopy. Images were collated by Leica software, and a Fiji/ ImageJ script was developed to set thresholds, based on a histogram-shape based analysis, and quantitate fibrosis within the region of interest.

**Results:** The intra-observer correlation for Sirius Red was r = 0.919 (n = 16) compared to r = 0.607 (n = 42) for Masson Trichrome. The correlation for Sirius Red scores between the left and right kidney for a) 20 high power fields or b) the whole kidney sections were r = 0.960 and r = 0.971 (n = 7), respectively. For Masson Trichrome the correlation between left and right kidneys for 20 high power fields was r = 0.866 (n = 7). The correlation for Masson Trichrome measured on 20 high power fields and Sirius Red measured on 20 high power fields or whole kidney sections were r = 0.789 and 0.478 (n = 7) respectively.

**Conclusions:** Sirius Red staining is an objective and quantitative technique with superior reproducibility compared to Masson Trichrome. Sirius Red staining is reproducible on both entire kidney sections as well as 20 high power fields. Analysis of entire kidney sections is more easily automated and would support high-throughput preclinical screening of anti-fibrotic therapeutics and validation of non-invasive biomarkers for interstitial fibrosis.

*Funding:* NIDDK Support

## TH-PO578

**Comprehensive Analysis of Hypoxia-Regulated Gene Transcripts in Chronic Kidney Disease and Renal Cells** Natallia Shved,[1] Maja Lindenmeyer,[1] Simone Brandt,[2] Matthias Kretzler,[3] Peter Wild,[2] Clemens D. Cohen.[1] *[1]Univ of Zurich, Zurich, Switzerland; [2]Univ Hospital Zurich, Zurich, Switzerland; [3]Univ of Michigan, Ann Arbor.*

**Background:** Most chronic kidney diseases (CKD) are initiated as glomerular damage with loss of glomerular capillaries. The best morphologic indicator of disease progression and development of end-stage renal disease, however, is interstitial fibrosis accompanied by capillary rarefaction. As hypoxia has been associated with fibrosis the question arises whether renal cells indeed face hypoxia in CKD and respond with a transcriptional program which could lead to progression of renal disease.

**Methods:** Proximal tubular cells (HK-2 cells) and podocytes with stable hypoxia-induced transcription factors (HIF1a and/or HIF2a) suppression were generated. Gene expression profiles from cell lines and more than 160 renal biopsies from patients with different CKD stages were obtained using Affymetrix arrays.

**Results:** Expression of hypoxia-associated genes in genome-wide expression profiles revealed correlation of HIF-target genes with eGFR in the cortical tubulointerstitium and glomerular samples. These correlations were both positive and negative and in part compartment-specific. To study the celltype-specific response to hypoxia and the relevance of given HIFs we investigated gene expression profile of HK-2 cells and podocytes with stable HIF1α and/or HIF2α suppression under hypoxic conditions. In HK-2 cells microarray analysis revealed 163 (86% HIF1α and/or HIF2α dependent) significantly upregulated and 31 (81%) downregulated hypoxia target genes. In podocytes, 416 (47% HIF1α and/or HIF2α dependent) genes were significantly up- and 318 (95%) significantly downregulated. To validate the results on protein level immunohistochemistry of HIF-target genes in human biopsies with different eGFRs was established and showed a similar pattern to correlation analysis.

**Conclusions:** Our gene expression studies do not indicate an over-all hypoxic milieu in acquired kidney diseases. However, the data clearly point to compartment- and celltype-specific dysregulation of hypoxia-associated gene transcripts.

*Funding:* Government Support - Non-U.S.

## TH-PO579

**Transcriptome Analysis of Human Hypertensive Chronic Kidney Disease Highlights the Role of Peroxisome Proliferator-Activated Receptor (PPAR) Pathway in Tubulointerstitial Fibrosis** Hyun Mi Kang,[1] Peter Choi,[1] Jianling Tao,[1] Sang Youb Han,[2] Ae Seo Deok Park,[1] Yi-An Ko,[1] Katalin Susztak.[1] *[1]Renal Electrolyte and Hypertension Div, Univ of Pennsylvania, Philadelphia; [2]Dept of Medicine, Ilsan Paik Hospital, Korea.*

**Background:** Hypertensive chronic kidney disease (H-CKD) is the second most common cause of end stage renal disease in the United States. Very little is known about the pathomechanism of H-CKD. Here we perform gene expression analysis on tubule samples obtained from control, hypertensince and H-CKD patients to catalogue differentially expressed genes and pathways. The results were compared mouse models of kidney fibrosis.

**Methods:** Human kidney tissues (n=92) were obtained from elective nephrectomies and they were grouped based on the clinical and histological parameters. Tubular epithelial cells were isolated by manual microdissection. Affymetrix GeneChip expression arrays (U133A 2.0) were used to analyze transcript levels. We also cultured renal epithelial primary cells from control and H-CKD samples. The results were compared mouse models of non-diabetic kidney fibrosis induced by Notch overexpression or folic acid administration.

**Results:** We identified 1,792 differentially expressed probesets in CKD tubules, with 1372 unique gene transcripts. Among them, the probesets with the highest fold change were albumin, EGF, and uromodulin known to play a role in CKD. Pathway analysis highlighted differences in PPAR alpha and gamma signaling. Lipid oxidation, mitochondrial biogenesis and lipid synthesis pathways were also differentially expressed. We also found that these pathways are also regulated in mouse models of fibrosis induced by folic acid administration or Notch1 overexpression. PPARa seems to have a direct role in fibrosis development as cells treated with PPARa agonist showed significantly reduced the expression of fibronectin, collagen1, SMAD7 and aSMA following TGFb1 treatment.

**Conclusions:** This is the first genome wide transcriptome analysis of tubular epithelial cells in human hypertensive nephropathy. These results demonstrate that PPAR pathways are regulated in the H-CKD and PPAR agonists could present a novel treatment approach in H-CKD patients.

## TH-PO580

**Thrombin Activated Fibrinolysis Inhibitor (TAFI) Inhibition Increases Survival and Halts the Progression of Renal Fibrosis in the Rat 5/6th Subtotal Nephrectomy Model** John M. Atkinson,[1] Nick Pullen,[2] Timothy Scott Johnson.[1] *[1]Sheffield Kidney Institute, United Kingdom; [2]Pfizer PGRD.*

**Background:** Previously we reported that application of the TAFI inhibitor UK-396082 increased plasmin activity & reduced ECM levels Induced by high glucose treatment of NRK52E cells.

**Methods:** To test whether TAFI inhibition could inhibit renal fibrosis and improve renal function, we tested UK-396082 in the 5/6th sub-total nephrectomy (SNx) rat in preventative & remission treatment arms. Rats were fed normal rat chow or chow containing UK-396082 (60mg/kg/d) from day (d)14 post SNx (prevention, p) or 35 (remission, r).

**Results:** Serum TAFI activity increased & plasmin activity decreased in untreated SNx animals. UK-396082 reduced TAFI activity (-45%/-30%, p/r) & increased plasmin activity (+52%/+52%). 16% of untreated SNx rats survived to d60 c.f. 80% treated from d14. 75% of SNx rats receiving UK-396082 from d35 survived to d60 c.f 27% left untreated. Creatinine clearance increased (+35%, p), while serum creatinine (-35%/-29% p/r)), proteinuria (-45%/-26%) & urea fell (-65% /-32%). Tubular damage biomarkers NGAL (-31%/-24%, p/r) & KIM-1 (-68.3%/-31.3%) were lower in urine. Scarring was ameliorated as measured by Masson's Trichrome staining (-77%/-45%, tubules/glomeruli) due to decreases in collagens I (-60%/-52%), IV (-57%/-52%) and III (-55%). Collagen reduction was confirmed by total kidney hydroxyproline (-65%). ECM proteins fibronectin (-72%/-46%) and laminin (-11%/-33%) were also lower. UK-396082 had no effect on blood pressure. Supporting the importance of this axis in patients with CKD, TAFI serum activity was also measured. In a mixed cohort of CKD patients, serum TAFI activity was 1.5x elevated c.f normal individuals serum and TAFI activity showed a strong correlation with the extent of renal function impairment.

**Conclusions:** In conclusion, we demonstrate here that TAFI inhibition with UK-396082 is highly effective in the rat SNx model. Collagen reduction suggests some benefit is by protecting plasmin activation of matrix metalloproteinases as collagens are poor plasmin substrates. These data support the hypothesis that TAFI is a potential therapeutic target for CKD.

*Funding:* Pharmaceutical Company Support - Pfizer

## TH-PO581

**Biglycan Fragmentation Is Associated to Survival in Patients Undergoing Hemodialysis** Federica Genovese,[1] Diana Julie Leeming,[1] Morten Asser Karsdal,[1] Alexandra Scholze,[3] Martin Tepel.[3] *[1]Biomarkers and Research, Nordic Bioscience, Herlev, Denmark; [2]Nephrology, Institute for Molecular Medicine, Cardiovascular and Renal Research, Odense, Denmark.*

**Background:** Biglycan is a small leucine-rich proteoglycan which is found in the extracellular matrix (ECM) of many organs, including kidneys. It is a key regulator of lateral assembly of collagen fibers and by binding to TGF-β1, biglycan is able to inhibit its activity. MMP mediated tissue turnover has clearly been described to be associated with many connective tissue diseases. BGM is a neo-epitope of biglycan generated by matrix metalloproteinase-9 (MMP-9) degradation and has previously been associated with ECM remodeling in fibrosis.The objective of the study was to investigate the association of plasma BGM levels with survival among hemodialysis patients.

**Methods:** BGM was measured by means of a specific enzyme-linked immunosorbent assay (ELISA) in an observational cohort study of 371 hemodialysis patients followed-up for 5 years. Survival was analyzed in an adjusted Cox model.

**Results:** Hemodialysis patients with a plasma BGM concentration higher than the median of 7.87 ng/mL had increased risk of death (hazard ratio, 1.35; 95% confidence interval, 1.01 to 1.81), with a survival of 388 days compared to 744 days. Multivariable-adjusted Cox regression showed increased odds for death with higher age and higher BGM concentrations.

**Conclusions:** Biglycan fragmentation can lead to TGF- β1 release and activation, with a consequent worsening of the fibrotic process and it is highly deposited in sites affected by fibrosis. This is the first time an association among levels of circulating biglycan fragments and mortality in kidney failure has been observed.

## TH-PO582

**Tamoxifen Ameliorates UUO-Induced Tubulointerstitial Fibrosis by Modulation of Renal TGF-β1/Smad Pathway in Mice** Kyung Pyo Kang, Dal Kim, Aesin Lee, Yujin Jung, Sik Lee, Won Kim. *Internal Medicine, Chonbuk National Univ Medical School, Jeonju, Korea.*

**Background:** After insult to the kidney, renal fibrotic process is initiated with sustained inflammation, activation of matrix-producing fibroblast and accumulation of extracellular matrix, which is part of repair process. Tamoxifen, known as selective estrogen receptor modulator, has been used anti–estrogen for the prevention and treatment of the breast cancer and also has antifibrotic properties. Therefore, we investigated the effect of tamoxifen on unilateral ureteral obstruction (UUO)-induced renal tubulointerstitial fibrosis and their mechanism.

**Methods:** Renal fibrosis was induced by UUO in the six-week-old C57BL/6 mice for 14 days. Tamoxifen (50 mg/kg) was treated by oral for 5 days before induction of renal fibrosis. Histologic examination and Western blot analysis for α-SMA, vimentin, fibronectin and ICAM-1 were performed. We also evaluated TGF-β1/Smad signaling pathway after ureteral obstruction.

**Results:** Renal tubular injury and fibrosis was increased after ureteral obstruction. The number of FSP-1 (+) fibroblast and expression of α-SMA (+) myofibroblast were increased in UUO kidney. Tamoxifen treatment significantly decreased UUO-induced renal tubular injury and fibrosis and also decreased UUO-induced increase of FSP1 (+) fibroblast and α-SMA (+) myofibroblast infiltration. The deposition of extracellular matrix such as type I collagen and fibronectin was increased after ureteral obstruction. Tamoxifen ameliorated UUO-induced increase of extracellular matrix deposition. For evaluation of renal inflammation, ER-HR3 (+) macrophage infiltration and ICAM-1 expression were determined after ureteral obstruction. Tamoxifen treatment significantly decreased UUO-induced increase of ER-HR3 (+) macrophage infiltration and ICAM-1 expression. Finally, tamoxifen was effectively suppressed UUO-induced activation of TGF-β1/Smad signaling pathway.

**Conclusions:** These results suggest that tamoxifen has a beneficial effect on UUO-induced tubulointerstitial fibrosis and inflammation by modulation of renal TGF-β1/Smad pathway.

*Funding:* Government Support - Non-U.S.

## TH-PO583

**Silencing of Pericyte MicroRNA-132 Reduces Renal Fibrosis and Myofibroblast Proliferation and Is Associated with Altered Sirt1 and Cox2 Expression** Roel Bijkerk,[1,2] Jacques Duijs,[1] Ton J. Rabelink,[1] Benjamin D. Humphreys,[2] Anton Jan Van Zonneveld.[1] *[1]Nephrology and Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Netherlands; [2]Renal Div, Dept of Medicine, Brigham and Women's Hospital and Harvard Medical School.*

**Background:** Lineage analysis has shown that during nephrogenesis, FoxD1-positive mesenchymal cells give rise to adult interstitial pericytes. These FoxD1-derivative interstitial (FDI) cells expand and differentiate into smooth muscle actin (α-SMA) positive myofibroblasts during renal fibrosis, accounting for a large majority of myofibroblasts. MicroRNAs (miRNAs) involved in this differentiation could serve as a target to decrease myofibroblast formation in fibrotic kidney disease.

**Methods:** Fibrosis was induced in FoxD1-GC;Z/Red mice by unilateral ureteric obstruction (UUO) and FDI cells (dsRed positive) were isolated using FACS sorting. To identify differentially expressed miRNAs we profiled these cells in UUO and healthy controls. To investigate the role of miRNA-132 in vivo in renal fibrosis we injected antagomirs i.v. to silence its function. Mice were sacrificed both 5 and 10 days after surgery.

**Results:** miR-132 was amongst the most highly upregulated miRNAs in the FDI cells in the fibrotic kidney. In vitro we demonstrated that silencing miR-132 results in reduction of myofibroblast marker α-SMA, reduced proliferation and increased levels of its established target Sirt1. In vivo silencing of miR-132 in the UUO model resulted in a 35% decrease in collagen deposition and decreased tubular apoptosis after 10 days as compared to scramblemir controls, while no difference was observed yet after 5 days. IHC analysis demonstrates that the number of interstitial α-SMA positive cells is similarly decreased, which is confirmed by both western blot and qRT-PCR analyses. No difference is observed in capillary density. Surprisingly, silencing miR-132 is associated with reduced levels of Sirt1. Furthermore, we demonstrated that miR-132 silencing decreases the number of proliferating interstitial cells.

**Conclusions:** miR-132 is a new microRNA that regulates pericyte and fibroblast activation during renal fibrosis.

## TH-PO584

**Human Anti-dsDNA Antibodies Induce Amplification Loop of Transforming Growth Factor-β1, Fibronectin and Collagen Type I in Proximal Renal Tubular Epithelial Cells** Daniel Tak Mao Chan, Shirli S.K. Ho, Claudia Ng, Kwok Fan Cheung, Susan Yung. *Dept of Medicine, The Univ of Hong Kong, Hong Kong.*

**Background:** Nephritis affects up to 60% of patients with systemic lupus erythematosus and is characterized by the production of anti-dsDNA antibodies and immune-mediated renal injury. Severity of tubulointerstitial fibrosis is a strong predictor of reduced renal survival. Fibronectin (FN) is amongst the matrix components showing early deposition during the process of tubulointerstitial fibrosis. This study investigates the mechanisms through which FN is induced in renal proximal tubular epithelial cells (PTEC) following stimulation with human anti-dsDNA antibodies, and its role in tubulointerstitial fibrosis of lupus nephritis.

**Methods:** Confluent growth-arrested PTEC were incubated with serum free medium (SFM), control IgG or human polyclonal anti-dsDNA antibodies (10μg/ml) for 24h in the presence or absence of Gö6976 or TGF-neutralizing antibody, and the synthesis of FN assessed. In separate studies, PTEC were stimulated with soluble FN to determine its effect on fibrotic processes.

**Results:** Control IgG had no effect on FN synthesis compared to SFM. Anti-dsDNA antibodies significantly increased cell-associated and soluble FN compared to SFM and control IgG (6.4- and 5.2-fold respectively for cell-associated FN; 1.85- and 1.62-fold respectively for soluble FN, $P<0.01$ for all). This was accompanied by significantly increased TGF-β1 secretion ($P<0.05$), and increased PKC-α ($P<0.05$) and PKC-βII ($P<0.01$) but not PKC-βI phosphorylation. Pre-treatment of PTEC with Gö6976 (20μM) and TGF-neutralizing antibody (100μg/ml) significantly suppressed anti-dsDNA antibody-induced FN ($P<0.01$ for both cell-associated and soluble FN). Incubation of PTEC with soluble FN significantly increased TGF-β1 secretion and collagen type I synthesis in a dose-dependent manner ($P<0.05$ and $P<0.01$ respectively for 10μg/ml soluble FN).

**Conclusions:** These results suggest that anti-dsDNA antibodies induce an amplification loop comprising TGF-β1, FN and collagen type I in PTEC through PKC activation.

*Funding:* Government Support - Non-U.S.

## TH-PO585

**Fibroblast-Specific PAI-1 Depletion Ameliorates Renal Interstitial Fibrosis in Mice after Unilateral Ureteral Obstruction (UUO)** Lan Yao, Laura S. Peterson, Amir Khan, Haichun Yang, Agnes B. Fogo. *Pathology, Microbiology, and Immunology, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** Plasminogen activator inhibitor-1 (PAI-1), a member of the serine proteinase inhibitor family,inhibits matrix breakdown and regulates cell migration and proliferation. Systemic PAI-1 knockout mice have decreased interstitial fibrosis after UUO, but worse injury in a crescentic anti-GBM model. Multiplerenal cells express PAI-1 after injury. We explored impact of fibroblast-specific PAI-1 depletion on interstitial fibrosis in the UUO model.

**Methods:** We bred floxed PAI-1 mice with tenascin C Cre mice,in which PAI-1 in fibroblasts can be knocked down after tamoxifenadministration. UUO was performed in *PAI-1$^{loxP/loxP}$*/Cre after tamoxifen treatment (Cre/lox, n=11) and control *PAI-1$^{loxP/loxP}$* mice (Cont*, n=8)*, and kidneys were harvested and injury assessed after 10 days.

**Results:** *PAI-1$^{loxP/loxP}$*/Cre mice showed no PAI-1 immunostaining in fibroblasts and cortex PAI-1 mRNA was decreased 41% in Cre/lox *vs.* control mice. Sirius red staining, a marker of interstitial fibrosis,was decreased in Cre/lox mice (0.88±0.10 *vs.* Cont 1.34 ± 0.16, P<0.05). In parallel, collagen I mRNA was decreased in Cre/lox *vs.* control(0.59± 0.32 *vs.* 1.23± 0.82, P<0.05). The number of fibroblasts in obstructed kidneys, measured by FSP-1 immunostaining, was decreased in Cre/lox mice (21.33 ± 1.7 *vs*. Cont 31.8 ± 1.1/ HPF, P<0.05). Less fibroblast proliferation was found inCre/lox *vs.* control mice, detected by PCNA/α-SMA double staining (Cre/lox 253.4±34.3 *vs.* Cont 405.4 ±53.4/mm2, P<0.05). E-cadherin mRNA level in obstructed kidney cortex, was preserved in Cre/lox mice (Cre/lox 1.55 ± 0.41 *vs*. Cont 1.09 ± 0.5, P<0.05). Surprisingly, F4/80 positive cells were increased in Cre/lox (32.9 ± 1.1 *vs.* Cont 24.1 ± 2.1/HPF, P<0.05).

**Conclusions:** Deletion of PAI-1 in fibroblasts ameliorates interstitial fibrosis, in part by reducing fibroblast proliferation, despite increased macrophage F4/80 infiltration. Whether these cells' phenotype is altered in paracrine fashion by fibroblast PAI-1 knockdown awaits further study.

*Funding:* NIDDK Support

## TH-PO586

**Fibrosis of Kidneys and of Other Solid Organs: Towards a Unifying Classifier across Species** Hans-Peter Marti,[1] James C. Fuscoe,[2] Joshua C. Kwekel,[2] Aikaterini Anagnostopoulou,[3] Andreas Scherer.[4] *[1]Dept of Clinical Medicine, Univ of Bergen, Bergen, Norway; [2]Div of Systems Biology, National Center for Toxicological Research, FDA, Jefferson, AR; [3]Institute of Anatomy, Univ of Bern, Bern, Switzerland; [4]Spheromics, Kontiolahti, Finland.*

**Background:** Fibrosis causes solid organ failure. We described a transcriptomic classifier consisting of metzincins and related genes (MARGS) discriminating renal allograft biopsies with/without fibrosis and extended analyses to non-transplant solid organs (AJT,9:2009; Virchows Arch,458:2011). We have also used our classifier in experimental, lithium-induced nephropathy (Nephrology,17,S2:2012). We now apply our MARGS-based algorithm to a rat model of age-induced interstitial renal fibrosis.

**Methods:** Untreated Fisher 344 rats (n=76) were sacrificed from 2 to 104 weeks of age. For gene expression studies we used single color (Cy3) Agilent Whole Rat Genome 4x44k microarrays; males: n=4 at weeks 2, 5, 6, and 8; n=5 at weeks 15, 21, 78, and 104; females: n =5 at weeks 2, 5, 6, 8, 15, 21, 78 and 104. Intensity data were subjected to variance stabilization including log2 transformation (www.Partek.com). Data were analyzed with ANOVA using gender/age as factors and with Pearson correlation.

**Results:** Fibrosis severity increased with age. Across age groups 60 MARGS were differentially expressed. PCA visualized segregation of age groups by gender from week 6. More MARGS were differentially expressed in older males than in older females. Expression level of MMP-7 correlated best with fibrosis grade. Expression values of 15/19 genes of the original classifier present on the Agilent array, in conjunction with linear discriminant analysis, were sufficient to correctly classify a set of 20 samples - for which gene expression data and fibrosis scores were available - into non-fibrosis (n=4) and fibrosis (n=16). Immunofluorescence confirmed up-regulation of MMP-2, CD44 and osteopontin in fibrosis.

**Conclusions:** Our MARGS classifier represents a cross-organ and cross-species classifier of fibrosis irrespective of etiology. This finding provides evidence for a common pathway leading to fibrosis and helps to design a PCR-based clinical test.

*Funding:* Government Support - Non-U.S.

## TH-PO587

**Apelin Receptor Blockade Exaggerates Unilateral Ureteral Obstruction Induced Tubular Injury in Rats** Gurdal Birdal,[1] Zarife Ozdemir,[2] Naziye Ozkan,[3] Dilek Ozbeyli,[2] Aysin Tulunay,[4] Sule Cetinel,[3] Berrak Yegen,[2] Mehmet Koc.[1] *[1]Div of Nephrology, Marmara Univ; [2]Dept of Physiology, Marmara Univ; [3]Dept of Histology, Marmara Univ; [4]Dept of Immunology, Marmara Univ, Medical Faculty, Turkey.*

**Background:** Unilateral ureteral obstruction (UUO) is a well-characterized fibrosis model exhibiting tubular injury and interstitial inflammation in the obstructed kidney. Apelin (Ap), a peptide identified as the endogenous ligand of the human orphan G-protein-coupled receptor, is reported to have anti-fibrotic and anti-inflammatory actions in the cardiovascular system, while the blockade of Ap receptor was shown to be effective in reducing hepatic fibrosis and angiogenesis. However, the role of Ap in the development of UUO model has not yet been elucidated.

**Methods:** In male Wistar albino rats under ketamine anesthesia (100 mg/kg; intraperitoneally, ip) UUO was performed by ligating left ureters. The rats were injected ip with either saline (n=8) or the Ap-receptor blocker Ala-Ap (75 mg/kg/day, n=8) for 7 days. On the 7th day, obstructed and non-obstructed kidneys were removed for the isolation of leukocytes by flow cytometry and for the assessment of histopathological changes.

**Results:** Tubular injury scores, which were calculated by grading interstitial edema, tubular dilatation, hyalinization and atrophic/necrotic tubular cells, were similar in non-obstructed and saline-treated obstructed kidneys. On the other hand, in the obstructed kidneys with Ala-Ap-treatment, injury score was significantly elevated (p<0.05), but the interstitial fibrosis score determined by Masson-trichrome staining was not increased. The percentages of $CD3^+$ T-lymphocytes and $CD3^+/CD8^+$ T-lymphocytes infiltrating obstructed kidneys were increased in the Ala-Ap-treated non-obstructed and Ala-Ap-treated obstructed-kidneys (p<0.001) as compared to corresponding saline-treated groups.

**Conclusions:** The present data demonstrate that pharmacological blockade of Ap exaggerates UUO-induced tubular injury that appears to involve the infiltration of lymphocytes. These data suggest that Ap may have a regulatory role in protecting against obstruction-induced tubular injury.

## TH-PO588

**Reversal of Epithelial to Mesenchymal Transition following Relief of Unilateral Ureteral Obstruction in the Rat** Nan Shen, Hong Li Lin, Wang Da Peng. *Dept of Nephrology, The 1st Affiliated Hospital of DaLian Medical Univ, Da Lian, Liao Ning Province, China.*

**Background:** Renal fibrosis begins with renal tubular epithelial mesenchymal transition (EMT); the progression thereafter depends upon a number of fibrotic factors. Unilateral ureteral obstruction (UUO) is a well-described model of EMT. We used an improved reversible unilateral ureteral obstruction (RUUO) model to investigate whether a progressive renal injury model of EMT could be reversed into the opposite direction, into mesenchymal-epithelial transition (MET) after relief of UUO in rats.

**Methods:** Rats were subjected to UUO or sham operation and the obstruction was removed five days later (or was left in place). Rats developed EMT after reversal of 2 or 4 weeks of ureteral obstruction as assessed by the expressions of fibrotic factors, EMT and MET markers in this post-obstructive model.

**Results:** We found a significant decrease in the kidney weight and renal cortical thickness in the UUO group compared with the sham groups. This rise in the RUUO group was significantly reduced. The elevated level of TGF-β1, TGF-β receptors and core fucosylationin the UUO group was significantly reduced in the RUUO groups. The EMT markers staining showed results parallel to those of TGF-β1 expression levels. In addition, UUO rats exhibited pronounced inflammatory and intrinsic proliferative cellular responses, and ultimately fibrosis. By comparison, RUUO mice had more controlled and measured extrinsic and intrinsic responses to EMT with a return to MET within several weeks after release of ureteral obstruction.

**Conclusions:** Our findings provide a model that allows investigation of the fibrotic factors during reversal of EMT that contribute to the development of fibrosis. EMT of the progressive renal injury could be actively reversed into MET and renal architecture is better maintained throughout injury and recovery from injury after relief of UUO in rats.

*Funding:* Private Foundation Support

## TH-PO589

**Long-Term Amiloride Therapy Attenuates Lithium-Induced Kidney Interstitial Fibrosis** Priyakshi Kalita,[1] Andrew Bahn,[1] Jennifer J. Bedford,[2] John P. Leader,[2] Robert J. Walker.[2] *[1]Physiology, Univ of Otago, Dunedin, New Zealand; [2]Medicine, Univ of Otago, Dunedin, New Zealand.*

**Background:** Long-term lithium therapy may produce chronic interstitial fibrosis. Short term amiloride attenuates lithium-induced NDI. However, the effect of amiloride on renal interstitial fibrosis induced by long-term lithium treatment, is unknown. This study investigated the effects of long-term amiloride on lithium-induced interstitial kidney fibrosis.

**Methods:** Male Wistar rats were divided into control, lithium alone, and lithium + amiloride groups (N=6 each group). Lithium was provided in rat chow (60 mmol $kg^{-1}$ food) for 6 months. Amiloride was introduced in drinking water (0.2 mmol $l^{-1}$) after 1 month of lithium treatment and continued for 5 months. At 6 months, rats were euthanized and kidneys were processed for histology, immunohistochemistry and Western blotting.

**Results:** In rats given lithium alone, histology revealed extensive dilatations of the cortical collecting ducts and focal areas of cortical fibrosis (0.5±0.04% area) vs controls (0.1± 0.003% .area), p<0.001. Rats given lithium for 1 month, followed by lithium + amiloride for a further 5 months showed significantly less fibrosis (0.1%± 0.01. area), p<0.001. Tubular morpholgy was unchanged

Western blotting (%) demonstrated greater expression of fibrotic markers in the rats treated with lithium alone compared with the rats given lithium + amiloride, and both groups showed greater expression than in the controls.

| *p<0.05, **p<0.01 | Controll | Lithium alone | Lithium and amiloride |
|---|---|---|---|
| alpha SMA | 100 ± 14 | 206 ± 29 | 114 ± 14 * |
| TGFβ | 100 ± 20 | 235 ± 15 | 114 ± 14 * |
| CTGF | 100 ± 13 | 375 ± 50 | 125 ± 25 ** |
| collagen III | 100 ± 35 | 350 ± 40 | 150 ± 40 ** |

**Conclusions:** This study reveals that amiloride appears to limit the fibrosis induced by lithium, although the abnormal morphology remains. Decreasing intracellular lithium concentrations may alter cAMP regulation of key pro-fibrotic pathways. Additional studies are underway to examine these possibilities.

*Funding:* Government Support - Non-U.S.

### TH-PO590

**Therapeutic Efficacy of DNA-Demethylation Therapy Initiated during Progressive Renal Fibrosis** Nathalie Le Clef, Patrick C. D'Haese, Benjamin Arthur Vervaet. *Biomedicine, Univ Antwerp, Belgium.*

**Background:** Acute kidney injury (AKI) can lead to chronic kidney disease (CKD). Research in animal models demonstrated that a DNA-demethylating treatment, when started immediately after induction of AKI, is able to attenuate development of chronic renal fibrosis, the hallmark of CKD. In patients diagnosed with CKD, however, fibrosis often has already developed to a substantial degree. Therefore, the efficacy of such a treatment, when initiated late in the progression of fibrosis, was tested in a mouse model of ischemia/reperfusion (I/R)-induced renal fibrosis.

**Methods:** Male C57BL/6J mice underwent left renal I/R (30 min, 37°C) and were treated with (a pre-optimized dose of) 0.25 mg/kg/day decitabine (AdC) or 0.9% NaCl, injected s.c. for 10 days. Treatment started either at 6 (early chronic phase) or 9 weeks (late chronic phase) after I/R. Sham operated mice were included as controls. Animals were killed either 1.5 week after end of treatment or 12 weeks after I/R. Kidney fibrosis was evaluated through gene expression (qPCR) analysis of TGFβ and collagen I. In addition, gene expression of DNA-methyltransferase (Dnmt) 1, 3a and 3b was examined.

**Results:** In vehicle treated animals, I/R led to a stable 32.1±10.7 (p=0.010) and 6.2±0.9 (p=0.004) fold upregulation of TGFβ and col I (vs. sham) from 6 weeks after I/R on, respectively. AdC treatment, irrespective of start of intervention was able to suppress median TGFβ upregulation by ca. 39%, however this was not significant (p>0.05 vs. vehicle). No effect was noted on col I expression. Likewise, the impact of AdC on gene expression of the various Dnmt's is limited, although all Dnmt's showed a (non-significant; resp. ca. 13% Dnmt 1, 28% Dnmt 3a and 37% Dnmt 3b) decrease when treatment was started at 6 weeks post I/R.

**Conclusions:** In this model of rather severe AKI induced renal fibrosis, a demethylating therapy is not that effective in attenuating progression of fibrosis. Whether AdC treatment as anti-fibrotic therapy might be effective only when administered during AKI or in a milder model of kidney damage remains to be determined.

*Funding:* Government Support - Non-U.S.

### TH-PO591

**AqF026 Is a Pharmacologic Agonist of the Water Channel Aquaporin-1** Olivier Devuyst,[1] Johann Morelle,[2] Andrea J. Yool.[3] *[1]UZH, Zurich, Switzerland; [2]UCL, Brussels, Belgium; [3]Univ Adelaide, Adelaide, Australia.*

**Background:** Aquaporin-1 (AQP1) facilitates the osmotic transport of water across the capillary endothelium, among other cell types, and thereby has a substantial role in water removal during peritoneal dialysis. At present, pharmacologic agents that enhance AQP1-mediated water transport, which would be expected to increase the efficiency of peritoneal dialysis, are not available.

**Methods:** In the present study, we tested the capacity of the novel agent AqF026 (Aq: aquaporin ligand; F: furosemide scaffold) to potentiate the water channel activity of AQP1 *in vitro* and *in vivo*.

**Results:** In the *Xenopus laevis* oocyte system, extracellular AqF026 potentiated the channel activity of human AQP1 by >20% but had no effect on channel activity of AQP4. *In silico* modelling suggested the binding of AqF026 to the intracellular loop D domain, a region associated with channel gating. Site-directed mutagenesis of intracellular residues in the human AQP1 loop D domain showed that the agonist effect of AqF026 could be reversed by mutations of Thr157 or Arg159. In a mouse model of peritoneal dialysis, AqF026 enhanced the osmotic transport of water across the peritoneal membrane but did not affect the osmotic gradient, the transport of small solutes, or the localization and expression of AQP1 on the plasma membrane. Furthermore, AqF026 did not potentiate water transport in *Aqp1*-null mice, suggesting that indirect mechanisms involving other channels or transporters were unlikely. Last, in a mouse gastric antrum preparation, AqF026 did not affect the Na-K-Cl cotransporter NKCC1.

**Conclusions:** These results are the first to define a pharmacological ligand that potentiates AQP1 water channel activity, to show that it is effective *in vitro* and *in vivo*, and to identify a candidate molecular site of action. The data are consistent with direct binding of the arylsulfonamide compound AqF026 at a site involving the intracellular regulatory domain loop D in AQP1. AqF026 directly and specifically potentiates AQP1-mediated water transport, suggesting a potential interest for peritoneal dialysis and clinical situations associated with defective water handling.

*Funding:* Pharmaceutical Company Support - Baxter Extramural Grant, Private Foundation Support, Government Support - Non-U.S.

### TH-PO592

**Ser-261 Phosphorylation Is an Endocytotic Signal of Aquaporin-2 Water Channel** Naofumi Yui, Shinichi Uchida, Sei Sasaki. *Dept of Nephrology, Graduate School of Medcine, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** Recently, several new vasopressin (VP)-sensitive phosphorylation sites other than Ser-256 (S256) were identified in the C-terminus of aquaporin-2 (AQP2). S261 is one of these newly identified phosphorylation sites, which is significantly decreased after VP stimulation. The importance of S261 phosphorylation on AQP2 ubiquitination was reported recently, however, its importance on AQP2 intracellular trafficking remains unclear.

**Methods:** In this study, we investigated intracellular trafficking of pS261-AQP2 in rat AQP2-stably expressing MDCK (AQP2-MDCK) cells using specific pS261-AQP2 antibody.

**Results:** Under control condition, AQP2 was mainly located in the perinuclear region (PNR), and the plasma membrane accumulation of AQP2 was clearly increased after FK (20 μM, 10 min) treatment. However, pS261-AQP2 was located in PNR, and was not detected in the plasma membranes with or without FK treatment. These results suggested two possibilities: one is that pS261-AQP2 is not transported and stably located in the intracellular site, and the other is that pS261-AQP2 is continuously targeted to the plasma membranes, however, immediately endocytosed. Therefore, we further investigated plasma membrane targeting of pS261-AQP2 using cold shock (4°C, 15 min). We previously reported that clathrin function is instantly arrested during cold shock. As a result, rapidly occurring plasma membrane targeting and subsequent internalization of proteins, which is usually undetectable under normal condition, can be visualized. Surprisingly, pS261-AQP2 was strongly accumulated in the plasma membrane after cold shock.

**Conclusions:** These observations clearly indicated that pS261-AQP2 is continuously targeted to the plasma membranes, then, instantly retrieved into intracellular vesicles. Thus, the phosphorylation of AQP2 at S261 might be a potent endocytotic signal. Further investigations to identify agents to modify S261 phosphorylation might contribute to the development of novel therapy for water balance disorders.

*Funding:* Other U.S. Government Support

### TH-PO593

**Intrarenal Mechanisms for Cyclophsophamide-Induced Water Retention in Rat Kidney** Gheun-Ho Kim,[1] Sua Kim,[1] Chor Ho Jo,[1] Joon-Sung Park,[1] Hyo-Jung Choi,[2] Tae-Hwan Kwon.[2] *[1]Dept of Internal Medicine, Hanyang Univ College of Medicine, Seoul, Korea; [2]Dept of Biochemistry and Cell Biology, Kyungpook National Univ School of Medicine, Taegu, Korea.*

**Background:** Cyclophosphamide can produce hyponatremia when hypotonic fluid intake is not limited. Because cyclophosphamide-induced hyponatremia was reported to occur in central diabetes insipidus, we hypothesized that cyclophosphamide or its active metabolite 4-hydroperoxycyclophosphamide (4-HC) may directly dysregulate the expression of water channels or sodium transporters in the kidney.

**Methods:** After a single intraperitoneal cyclophosphamide (25 mg/kg BW) administration to male Sprague-Dawley rats, kidneys were harvested for immunoblotting, immunohistochemistry, and real-time PCR. Primary cultures enriched ininner medullary collecting duct (IMCD) cells prepared from pathogen-free male Sprague-Dawley rats were treated with 10 and 30 μM of 4-HC. Laser scanning confocal miscroscopy was used for AQP2 localization.

**Results:** Cyclophosphamide-treated rat kidneys showed increases in the expression of aquaporin-2 (AQP2) protein (137 ± 10% vs. 100 ± 6%, $P < 0.05$), Na-K-2Cl cotransporter type 2 (NKCC2) protein (205 ± 32% vs. 100 ± 11%, $P < 0.05$) and $V_2R$ (vasopressin receptor type 2) mRNA (169 ± 18% vs. 100 ± 13%, $P < 0.05$) compared with vehicle-treated controls. Immunohistochemistry for AQP2 and NKCC2 revealed compatible results with immunoblot analyses. In primarily cultured IMCD cells, AQP2 protein abundance was increased by 10 μM 4-HC treatment (118 ± 6% vs. 100 ± 6%, $P < 0.05$). The expressions of AQP2 mRNA (185 ± 18% vs. 100 ± 29%, $P < 0.05$) and $V_2R$ mRNA (221 ± 49% vs. 100 ± 5%, $P < 0.05$) were also increased by treatment with 10 μM of 4-HC. Moreover, AQP2 targeting to the apical plasma membrane was remarkably increased when primary cultured IMCD cells were treated with 4-HC in the absence of vasopressin stimulation.

**Conclusions:** In rat kidneys, cyclophosphamide may induce upregulation of AQP2 and NKCC2 via activation of $V_2R$ and increasing AQP2 trafficking in the absence of vasopressin stimulation. These intrarenal mechanisms suggest a possibility of drug-induced nephrogenic syndrome of inappropriate antidiuresis.

*Funding:* Government Support - Non-U.S.

### TH-PO594

**Characterization of the Putative Phosphorylation Sites of Aquaporin-2 C-Terminus and Their Role in Aquaporin-2 Trafficking in LLC-PK1 Cells** Julian R. Arthur,[1,2] Jianmin Huang,[1,2] Dennis Brown,[1,2] Hua Ann Jenny Lu.[1,2] *[1]Center for Systems Biology, Program in Membrane Biology, Div of Nephrology, Massachusetts General Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA.*

**Background:** Phosphorylation dependent apical accumulation of water channel Aquaporin-2 (AQP2) mediates water reabsorption in the principal cells of kidney collecting ducts in response to vasopressin (VP), contributing to water homeostasis in mammals. Using an unbiased mutational analysis of all the potential phosphorylation sites in AQP2 C-terminus, we were able to study the role of each potential site in regulated trafficking and constitutive recycling of AQP2.

**Methods:** We generated stable LLC-PK1 cell lines with point mutations of alanine (A) or glycine (G) to replace all seven potential C-terminal phosphorylation sites in AQP2. We then introduced phosphorylation mimics (D, aspartic acid) to each individual site in the S all A/G background. For Western blots, membranes were incubated with Goat anti-AQP2 and Mouse anti-β-actin. For chemical treatments, cells were treated with 10 nM VP, 10 μM Forskolin (FK), or 10 mM methyl-β-cyclodextrin (MβCD) for 30 min. For cold block, cells were pre-treated with cyclohexamide for 60 min, then incubated at 20°C for various timepoints to visualize the formation of AQP2 "perinuclear patches." Fixed cells were visualized using the Nikon 80i.

**Results:** S all A/G AQP2 showed no membrane accumulation in response to VP/FK, but accumulated in response to MβCD, which disrupts endocytosis. S all A/G AQP2 with either S226D, S229D, T244D, S256S, S261D, S264D, or S269D did not exhibit membrane accumulation under basal conditions. These mutant AQP2 accumulated at the plasma membrane after MβCD treatment and in the trans Golgi network after cold block. S all A/G AQP2-S256D showed membrane accumulation in the absence of VP/FK, and was largely resistant to cold block as previously reported. S all A/G AQP2-S256S responded to VP/FK similarly to AQP2-WT.

**Conclusions:** Our data suggest for the first time that constitutive recycling of AQP2 does not require any phosphorylation in its C-terminus. Phosphorylation of S256 alone is sufficient to cause membrane accumulation of AQP2.

*Funding:* NIDDK Support

## TH-PO595

**Changes in the Excretion of Urinary Exosomal AQP1 and AQP2 in Rats with PAN-Induced Nephrotic Syndrome** Ayaha Kaito, Hiroko Sonoda, Saki Takahashi, Masahiro Ikeda. *Veterinary Pharmacology, Univ of Miyazaki, Miyazaki, Japan.*

**Background:** Nephrotic syndrome exhibits abnormal handling of renal water excretion and this abnormality is thought to be associated with the level of expression of renal aquaporin water channels (AQPs). Urinary exosomes are small vesicles secreted into urine from all renal epithelial cell types and known to contain proteins that are involved in renal secretion and reabsorption. Among these proteins, the level of urinary exosomal AQP1 or AQP2 excretion has been reported to be altered by renal insults such as renal ischemia/reperfusion and cisplatin, accompanied with the changes of their renal expression levels. However, it is unclear whether the urinary exosomal AQP1 and AQP2 excretion are affected by nephrotic syndrome.

**Methods:** In this study, we examined the levels of urinary exosomal AQP1 and AQP2 excretion in a well-characterized animal model viz. puromycin aminonucleoside (PAN) nephrosis. PAN (125 mg/kg) or saline was intravenously injected to rats. Urinary exosomes were isolated from the urine samples by differential ultracentrifugation.

**Results:** Plasma creatinine concentration and urinary protein level were significantly increased 96 h or later after the injection of PAN. Urine osmolarity was higher 96 h and lower 168 h after the injection, in comparison with controls. Urinary exosomal AQP1 was decreased 120 h or later, whereas reduced urinary exosomal AQP2 excretion was detected even 24 h after the injection. We then examined the abundance of AQP1 and AQP2 proteins in the renal cortex and medulla. Both proteins were not changed 48 h, but significantly decreased only in the cortex 120 h after the injection of PAN.

**Conclusions:** Together with these results, only urinary exosomal AQP2 excretion was decreased before proteinuria without being accompanied with renal protein level, whereas both urinary exosomal AQP1 and AQP2 excretion were reduced after proteinuria, associated with renal protein levels. These data suggest that urinary exosomal AQPs may be potential biomarkers for nephrotic syndrome.

## TH-PO596

**Kidney Stone Risk during Microgravity and Long-Term Bed Rest: Role of Hypercalciuria and Aquaporins** Grazia Tamma,[1] Annarita Di Mise,[1] Marianna Ranieri,[1] Maria Svelto,[1] Giancarlo Bilancio,[2] Massimo Cirillo,[2] Natale Gaspare De Santo,[3] Giovanna Valenti.[1] *[1]Dept Biosciences, Biotechnologies and Biopharmaceutics, Univ of Bari, Bari, Italy; [2]Dept of Medicine, Univ of Salerno, Salerno, Italy; [3]Dept of Medicine, Second Univ of Naples, Naples, Italy.*

**Background:** Exposure to microgravity results in alterations of renal function, fluid redistribution and bone loss which contributes to the potential risk of renal stone formation. Hypercalciuria is recognized as a condition predisposing to calcium nephrolitiasis and long-term space flights cause bone loss coupled to a rise of urinary calcium excretion.

**Methods:** AQP2 excretion was measured by ELISA in urines collected from healthy volunteers participating at the studies.

**Results:** We recently demonstrated that high calcium delivery to the collecting duct reduces local Aquaporin 2 (AQP2) mediated water reabsorption under vasopressin action, thus limiting the maximal urinary concentration and reducing calcium saturation. To analyze alteration of renal water handling during microgravity, we evaluated two ground-based analog of spaceflight, thermoneutral water immersion and bed rest. AQP2 excretion and diuresis were measured in two separated studies mimicking acute adaptation (6 hours water immersion) or chronic adaptation (35 days bed rest) to microgravity. Water immersion resulted in a significant increase in urinary output apparently not related to AQP2 alteration and manly due to reduced vasopressin secretion. On the other hand 35 days bed rest resulted in an increase in urinary calcium, which coincided with a significant decrease in AQP2 excretion (645±7.4 fmol/ml to 569±10.3 fmol/ml), which is expected to result in urine dilution reducing the risk of calcium saturation.

**Conclusions:** Our data indicate that calciuria and water balance have to be strictly controlled during microgravity and long-term bed rest as key elements for the risk of kidney stone formation.

*Funding:* Government Support - Non-U.S.

## TH-PO597

**Vasopressin Inhibits Apoptosis in Renal Collecting Duct Cells** Jason D. Hoffert, Pablo Sandoval, Lance Miller, Trairak Pisitkun, Mark A. Knepper. *Epithelial Systems Biology Laboratory, NIH, Bethesda, MD.*

**Background:** Arginine vasopressin (AVP) plays a critical role in regulating salt and water transport in the mammalian kidney. In addition to its central role in renal physiology, recent studies have demonstrated that AVP can promote cell survival in glomerular mesangial cells as well as in cultured hippocampal neurons through V1 receptors. The current study addresses whether AVP can inhibit apoptosis in kidney collecting duct cells via V2 receptors and also explores the downstream signaling pathways regulating this phenomenon.

**Methods:** The amount of cellular apoptosis was assessed in a cultured cortical collecting duct cell line (mpkCCD) by measuring the amount of DNA damage (i.e. TUNEL assay) as well as by measuring the relative amounts of cleaved caspase isoforms by immunoblotting.

**Results:** We found that an analog of AVP, 1-desamino-8-d-arginine vasopressin (dDAVP), inhibited staurosporine-induced apoptosis in mpkCCD cells. Incubation with dDAVP also inhibited apoptosis induced by the phosphatidylinositol 3-kinase (PI3K) pathway inhibitor LY294002, suggesting that the anti-apoptotic effects of dDAVP are largely independent of PI3K signaling in collecting duct cells. The V2 receptor antagonist SR121463 completely abolished the anti-apoptotic effects of dDAVP in mpkCCD cells. In addition, incubation of cells with 8-cpt-cAMP, a cell permeable analog of cyclic AMP, reproduced the anti-apoptotic effects of dDAVP. Both dDAVP and 8-cpt-cAMP increased phosphorylation of pro-apoptotic Bcl-2 family member proteins Bad and Bok. Two of these phosphorylation sites in Bad, Ser-112 and Ser-155, are known to inhibit pro-apoptotic activity. Pre-incubation of cells with H89 blocked dDAVP-induced phosphorylation of both Bad and Bok suggesting dependence on protein kinase A (PKA).

**Conclusions:** This study provides evidence that AVP can inhibit apoptosis through the V2 receptor and downstream cAMP-mediated pathways in mammalian kidney, a process that may be relevant in the context of renal proliferative disorders such as autosomal dominant polycystic kidney disease (ADPKD).

*Funding:* Other NIH Support - National Heart, Lung, and Blood Institute intramural budget (ZO1-HL001285)

## TH-PO598

**Cyclooxygenase-2 Mediates Induction of the Renal Stanniocalcin-1 Gene by Arginine Vasopressin** Graham F. Wagner,[1] Richard L. Hebert.[2] *[1]Physiology & Pharmacology, Western Univ, London, Canada; [2]Dept of Cellular and Molecular Medicine, Univ of Ottawa, Ottawa, Canada.*

**Background:** The hormone, stanniocalcin-1 (STC-1), is expressed in most nephron cells. But the gene is differentially induced in cortical and medullary segments in response to dehydration. The cortical gene is upregulated solely by hypertonicity, whereas that in medulla is induced by hypovolemia. In both cases gene induction is mediated by arginine vasopressin (AVP) acting via V2 receptors (V2R). V2R occupancy activates two pathways in response to dehydration; 1) antidiuresis mediated by direct V2R occupancy and 2) AVP counter-regulation mediated indirectly via COX-2 (cyclooxygenase-2). Because the role of renal STC-1 is still unknown, we sought to establish if V2R mediated gene upregulation occurred directly or indirectly, as a means of narrowing the scope of its possible actions.

**Methods:** Models of COX inhibition and COX gene deletion were employed to address the possible involvement of the COX pathway.

**Results:** Both general and specific inhibitors of COX-2 blocked STC-1 gene induction in response to dehydration. Gene induction in response to dehydration was also abolished in COX-2 null mice (cortex and medulla), but not in COX-1 null mice.

Gene induction in response to V2R occupancy was also abolished in COX-2 nulls (cortex and medulla), but not in COX-1 nulls.

In situ hybridization analysis revealed that gene upregulation took place in most nephron segments except for the thin loops of Henle.

**Conclusions:** The findings clearly showed that all V2R-mediated rises in renal STC-1 gene expression are wholly dependent on functional COX-2 activity. This implies that STC-1 is not part of the antidiuretic pathway and that it likely has actions that include AVP counter-regulation, renal cytoprotection and/or renal osmolyte synthesis.

## TH-PO599

**Transcriptional and Translational Heterogeneity of the SLC2A9 Gene Encoding the GLUT9 Urate Transporter** Asim Mandal,[1] David B. Mount.[1,2] *[1]Renal Div, Brigham and Women's Hospital, Boston, MA; [2]Renal Div, VA Boston Healthcare System, Boston, MA.*

**Background:** There are ~30 genes linked to serum uric acid (SUA) levels and gout, yet variation in the SLC2A9 gene encoding the urate transporter GLUT9 remains the major single genetic determinant; however, the causative variants are unknown. Two distinct N-terminal isoforms, GLUT9a and GLUT9b, are generated by alternative 5' ends and alternative promoters.

**Methods:** Novel exons, alternative splicing, and transcriptional start sites were identified by database mining, 5'-RACE PCR of kidney mRNA, and RT-PCR. GLUT9 expression constructs were generated in for $^{14}$C-urate uptake experiments in *Xenopus oocytes.*

**Results:** Five novel 5' UTR exons were identified, with substantial alternative splicing of these exons in human renal transcripts. RT-PCR did not link exon 1a and 1b, indicating that GLUT9a and GLUT9b are generated by transcriptional initiation at separate promoters. A transcriptional initiation site flanking exon 1a was identified by 5'-RACE; a second site was identified in GLUT9b transcripts, flanking a novel 5' UTR exon that is ~35 kb 5' of

exon 1b. Alternative splicing that deletes coding exons 3 +/- 4 was also identified; exon 3 is a cassette exon encoding most of transmembrane domain 1 (TM1) and part of the first, glycosylated extracellular loop. Surprisingly, GLUT9b constructs with deletion of exon 3 (GLUT9b-delta3) were functional, generating urate uptakes that were 10-fold higher than that of water-injected control Xenopus oocytes - versus 40-fold higher in oocytes injected with full-length GLUT9b cRNA. Western blotting indicated that GLUT9b-delta3 protein is not glycosylated, likely due to altered topology of the first extracellular loop.

**Conclusions:** We have identified substantial 5' heterogeneity of SLC2A9 transcripts, with five novel 5' UTR exons and at least two transcriptional initiation sites. There are thus at least two 5' promoters in SLC2A9, one some 35 kb 5' of exon 1b flanking a novel 5' UTR exon. Alternative splicing that removes exon 3 generates functional GLUT9 urate transporters, despite removal of TM1, absent N-glycosylation, and scrambled topology of the amino terminus of GLUT9 protein.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO600

**SGLT2 Inhibitors Act from the Extracellular Surface of the Cell Membrane** Chiara Ghezzi, Edurne Gorraitz, Erika Patino, Bruce A. Hirayama, Donald D.F. Loo, Ernest M. Wright. *Physiology Dept, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

**Background:** SGLT2 inhibitors are a new class of drugs that have been developed to treat diabetes. They lower glucose levels by blocking the renal Na/glucose cotransporter SGLT2, thereby increasing the amount of glucose excreted in the urine.

Questions have arisen about how these inhibitors reach SGLT2 in the brush border membrane of the S1 and S2 segments of the renal proximal tubule: are these drugs filtered by the glomerulus and act from within the tubule, or do they act from the blood?

**Methods:** Human SGLT2 was expressed in HEK293T cells and we determine the relative potency of SGLT2 inhibitors from the extra- and intracellular side of the plasma membrane We used the selective hSGLT2 inhibitor JNJ-30980924, that is similar to canagliflozin, and phlorizin that is a non selective SGLT2 inhibitor. SGLT2 activity ($Na^+$/glucose currents) was measured using the whole cell patch-clamp method that allowed us to control the composition of both the extracellular and intracellular solutions.

**Results:** Our result confirmed that JNJ-30980924 is a SGLT2 specific inhibitor and a potent extracellular inhibitor of the inward SGLT2 transport. We estimated a $IC_{50}$ of 10-40 nM that is 10 times higher than the one estimated for hSGLT1 (300 nM). To test if the inhibitor can act from the intracellular side we add the compound to the patch pipette solution and we measured the effect on the glucose inward current generated by SGLT2. Both phlorizin and JNJ-30980924 were ineffective from the intracellular compartment at both low (5 mM) and high (150 mM) intracellular NaCl concentrations.

**Conclusions:** We conclude that SGLT2 inhibitors only bind to SGLT2 from the extracellular side of the plasma membrane and this suggests that they act from the glomerular filtrate and not from the blood through the tubular epithelium.

*Funding:* Pharmaceutical Company Support - Janssen Pharmaceuticals

## TH-PO601

**14-3-3s Regulate Osmoprotective Gene Expression by Regulating NFAT5 Activity** Yuichiro Izumi, Maurice B. Burg, Joan D. Ferraris. *National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.*

**Background:** Nuclear Factor of Activated T-Cells 5 (NFAT5) is a transcription factor that induces transcription of osmoprotective genes in response to high NaCl. In the renal medulla NFAT5 protects cells from the effects of NaCl, which normally is present in concentrations high enough to perturb and even kill cells. When cells are exposed to high NaCl, NFAT5 accumulates in the nucleus where it binds to the Osmotic Response Elements (OREs) flanking target genes and increases their transcription. 14-3-3s are regulatory proteins that have numerous roles in protein interaction, phosphorylation, and enzymatic activity. There are multiple 14-3-3 isoforms that form homo- and hetero-dimers and play isoform-specific regulatory roles. We previously identified six isoforms of 14-3-3 in nuclei of HEK293 cells and found that their abundances in the nucleus decrease in response to high NaCl (Physiol. Genomics. 2012). The purpose of the present studies was to test whether 14-3-3s modulate the activity of NFAT5.

**Methods:** The effects of knocking down 14-3-3s on NFAT5 function were examined in HEK293 cells incubated at 300 and 500 mosmol/kg (NaCl added). Using small interfering RNAs, we knocked down 14-3-3 isoform β, ε, γ, η, θ, and ζ either individually or in combination. We used Luciferase reporters to measure NFAT5 transcriptional (ORE-X) and transactivating (TAD) activity. We quantified mRNA abundance of the NFAT5 target genes, aldose reductase (AR) and betaine/GABA transporter (BGT1), and of NFAT5, itself, by real time PCR. We also measured nuclear localization of NFAT5 by quantitative Western analysis of nuclear and cytoplasmic proteins.

**Results:** Knock down of 14-3-3-β or 14-3-3-ε significantly inhibited high NaCl-induced ORE-X and TAD activities, but knockdown of the other isoforms had no significant effect. Knockdown of β or ε also decreased mRNA abundances of AR and BGT1, but not of NFAT5. The effects of knocking down β and ε were not additive. Knock down of β and ε did not affect the nuclear localization of NFAT5.

**Conclusions:** 14-3-3 isoforms β and ε contribute to NFAT5 transcriptional activity by supporting high NaCl-induced increase of NFAT5 transactivating activity.

*Funding:* Other NIH Support - The Intramural Research Program of NHLBI, NIH

## TH-PO602

**Mechanisms of Erythropoietin Production by Aldosterone in the Intercalated Cells** Yuichiro Izumi,[1] Yukiko Yasuoka,[2] Takanori Nagai,[3] Kahori Hori,[3] Yushi Nakayama,[4] Masayoshi Nanami,[3] Takeshi Nakanishi,[3] Akito Tanoue,[5] Kimio Tomita,[4] Katsumasa Kawahara,[2] Hiroshi Nonoguchi.[6] *[1]National Heart, Lung, and Blood Institute, NIH, Bethesda, MD; [2]Physiology, Kitasato Univ, Sagamihara, Saitama, Japan; [3]Kideny and Dialysis, Hyogo Coll. of Med., Nishinomiya, Hyogo, Japan; [4]Nephrology, Kumamoto Univ, Kumamoto, Japan; [5]Pharmacology, National Res. Inst for Child Health and Develop., Setagaya-ku, Tokyo, Japan; [6]Int Med, Kitasato Univ Med. Cent., Kitamoto, Saitama, Japan.*

**Background:** Erythropoietin (Epo) production occurs in the peritubular cells, which experimentally requires severe anemia and hypoxia. Other site maintaining basal Epo production is speculated. We have shown that cultured intercalated cells of the collecting ducts (CD) (IN-IC cells) produce Epo by hypoxia and aldosterone and that vasopressin V1a receptor (V1aR) is required for the effect of aldosterone. In the present study, we investigated the Epo production in basal condition and the mechanisms of aldosterone-induced erythropoiesis in intercalated cells.

**Methods:** Mice in basal condition, blood-draw induced anemia with 4-hr hypoxia and fludrocortisone treatment were used for high sensitive in situ hybridization (ISH) of Epo mRNA and Immunohistochemistry (IHC) of PHD2. Double staining of anti AQP3 and AE1 was performed to distinguish intercalated cells of CD. V1aR-KO and wild type (WT) mice and the IN-IC cells were used for Western blot analysis of HIF1α,2α, and PHD1,2,3.

**Results:** Epo mRNA was detected in type B intercalated cells (IC-B) and slightly in proximal tubule (PT) but not in periubular cells in basal condition. Hypoxia increased Epo mRNA expression in IC-B and remarkably in peritubular cells. PHD2 was not detected on basal condition while it was stained in DCT, CCD and peritubualr cells by anemia and fludrocortisone. Fludrocortisone increased PHD2, HIF1α and 2α expressions in WT mice but not in V1aR-KO mice. Aldosterone increased PHD2, HIF1α and 2α expressions in IN-IC cells.

**Conclusions:** IC-B is the main site of Epo production in basal conditions. Aldosterone stimulates Epo production in IC-B through the activation of HIF1α, 2α and PHD2 pathway. V1aR is required for Epo production by aldosteorne.

*Funding:* Pharmaceutical Company Support - Dainippon Sumitomo Pharma Co., Government Support - Non-U.S.

## TH-PO603

**Urinary Citrate Excretion in NaDC1 Knockout Mice** Kathleen S. Hering-Smith,[1] Federico Teran,[1] L. Lee Hamm.[1,2] *[1]Medicine, Tulane Univ, New Orleans, LA; [2]Research, SLVHCS, New Orleans, LA.*

**Background:** Citrate is an important inhibitor of calcium stone formation. Citrate excretion in the urine has been thought to be predominantly regulated by NaDC1 (Na-dicarboxylate cotransporter 1) in the apical membrane of the proximal tubule. However, since we have recent evidence of another citrate reabsorptive process in the proximal tubule, we reexamined citrate excretion in NaDC1 knock out mice.

**Methods:** Citrate and other Krebs cycle intermediates were measured by LC/MS in plasma and spot urine collections from wild type (WT), KO and heterozygotes (Het) and are expressed in ug/mL.

**Results:** Renal function and urine concentration were not different in the three groups of animals as indicated by plasma and urine creatinines. Excretion of succinate, fumarate, and malate were significantly higher in KO animals compared to Het or WT: succinate 92 ± 29, 37 ± 11, 32 ± 6; fumarate 45 ± 11, 3 ± 0.7, 3.8 ± 0.9; malate 161 ± 47, 24 ± 3, 32 ± 11, respectively in KO, Het, and WT. However, citrate excretion was not higher in KO compared to WT and Het, 89 ± 15, 115 ± 42, 149 ± 24. Interestingly lactate excretion in urine was significantly lower in KO compared to WT or Het. Phosphoenolpyruvate, pyruvate, isocitrate, and alpha-ketoglutarate were not different in the three groups.

**Conclusions:** In sum, NaDC1 KO animals exhibit increased excretion of several Krebs cycle intermediates but not increased urinary citrate. This data is consistent with transporters other than NaDC1 having a potentially important role in regulation of urinary citrate excretion and hence in prevention of calcium stone formation.

*Funding:* NIDDK Support

## TH-PO604

**Effect of Direct Renin Inhibitor Aliskiren on the Urinary Concentrating Defect in Obstructive Nephropathy** Weidong Wang,[1] Renfei Luo,[1] Yu Lin,[1] Moshe Levi,[2] Tianxin Yang,[1] Chunling Li.[1] *[1]Institute of Hypertension and Kidney Research, Zhongshan School of Medicine, Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Div of Hypertension and Kidney Diseases, Univ of Colorado Denver, Aurora, CO.*

**Background:** Ureteral obstruction is associated with reduced expression of renal aquaporins (AQPs) and urinary concentrating defect, in which renin-angiotensin system (RAS) activation may play an important role. We evaluated whether RAS blockade by injecting renin inhibitor aliskiren in mice would prevent decreased protein expression and intracellular trafficking of AQPs in bilateral ureteral obstruction (BUO) and unilateral ureteral obstruction (UUO).

**Methods:** BUO was performed for 24 hours and UUO was performed for 3 days and 7 days. Protein, mRNA expression and staining of aquaporins were examined by western blots, RT-PCR, and immunohistochemistry.

**Results:** There were no significant changes in serum osmolality, sodium, and potassium concentrations between BUO/UUO mice and aliskiren-treatment groups. During BUO, aliskiren (BUO+Ali) prevented the reduction of AQP2 protein expression (1.06±0.07 in BUO+Ali vs. 0.73±0.05 in BUO, P<0.05), but not AQP1 and AQP3. Aliskiren reversed the weak intracellular and apical staining of AQP2 in the cortical and inner medullary collecting duct (CD) principal cells during 24 hr BUO. In UUO mice, at day 3 (3U) and day 7 (7U), aliskiren prevented downregulation of AQP2 protein (0.85±0.07 in 3U+A vs. 0.51±0.07 in 3U, P<0.05; 1.00±0.02 in 7U+A vs. 0.73±0.04 in 7U, P<0.001) and mRNA levels (1.54±0.32 in 3U+A vs. 0.60±0.11 in 3U, P<0.05; 0.64±0.18 in 7U+A vs. 0.14±0.03 in 7U, P<0.05), but not AQP1 and AQP3 protein expressions in the obstructed kidneys. There were no changes of AQP1, 2 and 3 expression in non-obstructed kidneys with or without aliskiren treatment. The reduced immunostaining of AQP2 in the cortical and medullary CD principal cells of obstructed kidneys at day 7 of UUO was also prevented by aliskiren.

**Conclusions:** RAS blockade with renin inhibitor was associated with increased AQP2 expression in the kidney and activation of renal RAS may play an important role in urinary concentrating defect seen in obstructive nephropathy.

*Funding:* Government Support - Non-U.S.

## TH-PO605

**Primary Cilia Modulate the Effects of TRPM3 and TRPV4 on Survival of Renal Epithelial Cells Exposed to Osmotic Stress** Bradley P. Dixon,[1] Brian J. Siroky,[1] Raven Gail Comer,[1] Nancy Kleene,[2] John J. Bissler.[1] *[1]Div of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Dept of Cancer and Cell Biology, Univ of Cincinnati College of Medicine, Cincinnati, OH.*

**Background:** Primary cilia detect osmolality, but the mechanism is unclear. Both TRPV4 and TRPM3 channel activities are inhibited by hyperosmolality. We investigated the role of primary cilia in response to acute hyperosmolal stress, and whether TRPV4 and TRPM3 mediate this response.

**Methods:** TRPV4 and TRPM3 expression in ciliated (176-5) and nonciliated (176-5Δ) renal epithelial cells was determined by qPCR. Cells were challenged with 16-24 hours of osmotic stress at 400, 500, or 600mOsm/kg with NaCl, or maintained under control conditions. This stress was applied either in the presence or absence of a TRPV4 agonist (GSK1016790A), or a TRPM3 agonist (pregnenolone). Cell number was measured by crystal violet DNA binding assay, and viability was determined by calcein uptake. Markers of apoptosis were measured by immunoblotting.

**Results:** Both ciliated and nonciliated renal epithelial cells expressed TRPV4 and TRPM3, and TRPV4 expression was several fold higher in nonciliated cells. Nonciliated cells had reduced cell number and viability at and above 400mOsm/kg in comparison to ciliated cells. Nonciliated cell survival following osmotic stress was reduced further in the presence of a TRPV4 agonist, suggesting that TRPV4 may be critical for osmosensation in the absence of cilia. Cleavage of caspase 3 and PARP was seen in nonciliated cells treated with TRPV4 agonist under hyperosmolal and, to a lesser extent, isoosmolal conditions. Cell viability was reduced in ciliated cells pretreated with TRPM3 agonist at 600mOsm/kg, suggesting the importance of this channel for osmotic response in ciliated cells.

**Conclusions:** These findings support a role for TRPV4 and TRPM3 in osmosensation by renal epithelial cells. In ciliated cells, TRPM3 may be the predominant osmosensor, and increased expression of TRPV4 in nonciliated cells may represent a compensatory adaptation to loss of ciliary osmosensation.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO606

**Na-K-ATPase and the Urine Concentrating Mechanism in the Rodent Medulla** Thomas Pannabecker, C. Michele Nawata, Tamara M. Armstrong, Kristen K. Evans, Mohammad Shahidullah. *Physiology, Univ of Arizona, Tucson, AZ.*

**Background:** Na reabsorption by medullary thick ascending limb and collecting duct is driven by basolateral Na-K-ATPase (NKA) with apical transport via NHE3/NKCC2 or ENaC. Reabsorption rates are influenced by regulated apical transport. By comparing NKA activity and expression in water-restricted Sprague-Dawley rats and in the kangaroo rat, a desert rodent that drinks no free water, we are investigating regulatory mechanisms that directly impact the active transport step.

**Methods:** Outer medullary tissue from control rats (*ad libitum* access to water) and water-restricted rats (water reduced to 40% of controls) was homogenized and a crude membrane fraction was prepared by centrifugation at 1100 x *g* (15 min) and at 150,000 x *g* (45 min). The membrane fraction was immunoblotted for NKA α-subunit (NKA-α) and ATP hydrolysis was determined by measuring release of $P_i$. NKA activity is the difference between ATP hydrolysis in the presence and absence of ouabain. Real-time PCR analysis was conducted on 1 μg total RNA from tissue homogenates.

**Results:** Urine osmolality (mOsm/kg $H_2O$) of Sprague-Dawley rats increased from 1185 (controls) to 2121 with 72 hr water restriction. Outer medullary NKA-α gene expression was unchanged with water restriction, whereas AQP2 gene expression increased 2-fold. Inner medullary NKA-α gene expression increased 2-fold. Prior studies have shown outer medullary NKA-α protein expression levels increase with restricted water intake. In the kangaroo rat (urine osmolality = 4159), outer medullary NKA-α protein expression levels are 2 to 3-fold higher than Sprague-Dawley control rats; however, NKA activity is no different.

**Conclusions:** Low correlation between outer medullary NKA-α1 gene expression and total NKA-α1 protein in water-restricted Sprague-Dawley rat suggests multiple regulatory mechanisms, possibly including lower protein degradation rate. Greater expression of outer medullary NKA-α1 protein but with equivalent NKA activity, in a species with sharply higher concentrating capacity could be consistent with greater fractional NKA-α1 expression in the plasma membrane. NIDDK DK083338, NSF IOS095285.

*Funding:* NIDDK Support, Other U.S. Government Support

## TH-PO607

**Missing Link between Hyponatremia and Impaired Thyroid Function** Christian Stefan Haas, Matthias J. Berndt, Birgit Harbeck, Ulrich Lindner. *Dept of Medicine I, Univ of Luebeck, Luebeck, Germany.*

**Background:** Hyponatremia (HN) is the most common electrolyte disorder in both out- and in-patients. Hypervolemic states (e.g. heart failure, liver cirrhosis), hypovolemia, and the syndrome of inappropriate ADH secretion account for most of the cases, while endocrine disorders are less frequently responsible. Despite insufficient data on a relationship between thyreotropin (TSH), free thyroxine (fT4) and serum sodium, hypothyroidism is often cited as a possible cause of HN. The study objective was to assess the association between impaired thyroid function and HN.

**Methods:** In a retrospective analysis, patients admitted to the emergency room (ER) or the intensive care unit (ICU) having manifest hypothyroidism (TSH>10 mU/L and fT4<2 ng/L) were identified. Serum sodium levels and distribution were determined, HN prevalence ($Na^+$<135 mmol/L) assessed and the results compared with an ER and ICU population presenting with a normal TSH level (0.1-4 mU/L). The desired statistical power to detect a difference of 1.5 mmol/L between both groups was ≥90%. Pearson correlation was used to determine dependence of TSH and sodium.

**Results:** A total of 132 patients with significant hypothyroidism were identified and compared with 1253 euthyroid individuals. The sodium level (mean±SD) in hypothyroid patients did not differ when compared to controls (137.8±5.1 vs. 137.4±5.3 mmol/L, n.s.). HN prevalence in the control group was 21% (n=259), with 87 patients (7%) having even moderate ($Na^+$<130 mmol/L) or severe HN ($Na^+$<120 mmol/L). In the hypothyroidism group, HN prevalence was comparable; of note, most patients had mild HN, while moderate or severe HN was virtually not present in patients with severe hypothyroidism (TSH>50 mU/L).

**Conclusions:** In summary, we showed that: (1) mean sodium in hypothyroid patients and controls is comparable; (2) HN prevalence in hypothyroidism is not higher than in control patients; and (3) there is no correlation between serum sodium and TSH levels. These data suggest that hypothyroidism is not likely to be a cause for the development of HN and that measurement of TSH may be waived in the differential diagnosis of the electrolyte disorder.

## TH-PO608

**Chronic Hyponatremia: A Novel Risk Factor for Bone Fractures in Chronic Kidney Disease** Sagar U. Nigwekar, Julia Beth Wenger, Ravi I. Thadhani, Ishir Bhan. *Massachusetts General Hospital.*

**Background:** Chronic hyponatremia is associated with reduced bone density and increased fracture risk in the general population. Given the high fracture risk and prevalence of chronic hyponatremia in chronic kidney disease (CKD), we evaluated whether chronic hyponatremia is associated with increased fracture risk in CKD.

**Methods:** Subjects for this matched case-control study were identified from the Partners Healthcare Research Patient Data Registry. CKD was defined as estimated glomerular filtration rate < 60 mL/min. Chronic hyponatremia was defined as serum sodium (sNa) < 135 mEq/L on at least 2 occasions (prior to fracture for cases). Cases comprised ambulatory elderly (> 60 y) CKD patients with femoral and/or vertebral fractures diagnosed between January 2009 and December 2011. Controls were ambulatory elderly CKD patients without femoral or vertebral fractures and were matched to cases by age, gender, and race. Associations between chronic hyponatremia and fractures were evaluated in univariate and multivariable logistic regression analyses.

**Results:** We identified 3,027 eligible subjects (cases=937; controls=2,090). Chronic hyponatremia was more common in cases than controls (13 vs. 9%; p=0.03). Amongst the hyponatremic subjects, sNa was between 130 and 134 mEq/L in 85%. Subjects with chronic hyponatremia had higher prevalence of lung disease (30 vs. 25%, p=0.04), diuretic use (61 vs. 51%, p<0.01), and cirrhosis (4 vs. 2%, p<0.01), but similar rates of congestive heart failure, hypothyroidism, psychiatric disorders, and antidepressants use compared to those without chronic hyponatremia. Chronic hyponatremia was associated with increased odds of fracture in univariate analysis (OR 1.32, 95% CI 1.03- 1.69). Additional significant associations with fracture included female gender, hyperparathyroidism, osteoporosis, lung disease, and absence of obesity. Adjusted for these covariates and demographic factors, chronic hyponatremia remained a significant predictor of fracture (OR 2.20, 95% CI 1.19-4.07).

**Conclusions:** Chronic hyponatremia is associated with increased fracture risk in CKD. Future studies are needed to examine whether correcting chronic hyponatremia can reduce fracture risk.

*Funding:* Private Foundation Support

## TH-PO609

**Collecting Duct Derived-Adenylyl Cyclase 3 Deficiency Does Not Affect Renal Sodium or Water Excretion** Wararat Kittikulsuth,[1] Deborah Stuart,[1] Elena V. Mironova,[2] James D. Stockand,[2] Donald E. Kohan.[1] *[1]Univ of Utah Health Sciences Center, Salt Lake City, UT; [2]Univ of Texas Health Sciences Center, San Antonio, TX.*

**Background:** The collecting duct (CD) is responsible for the final adjustment of renal water and Na excretion. Adenylyl cyclase (AC)-stimulated cAMP is a key mechanism for mediating these effects in the CD. At least two AC isoforms, AC3 and AC6, may be involved in vasopressin (AVP)-stimulated cAMP production in inner medullary CD (IMCD) cells. Recently, we showed that CD-derived AC6 is involved in regulation of CD water and Na transport. However, the role of CD-derived AC3 in regulating water and Na homeostasis is unknown.

**Methods:** We generated CD-specific knockout (KO) of AC3 mice using the Cre/loxP system. AC3 DNA recombination in microdissected nephron segments was determined by PCR and AVP-stimulated cAMP levels were determined in IM. KO mice and controls were placed under normal water intake, moderate and severe water restriction, or chronic water loading. These studies were done with a normal (0.3%Na) salt diet (NS). Mice were also fed a high (3.15%Na) salt diet (HS).

**Results:** IMCD from KO mice underwent 100% *Adcy3* gene recombination, while no DNA recombination occurred in proximal tubules of KO mice. AVP-enhanced cAMP production was reduced by 25% in the IM of KO mice. There were no detectable differences in water intake, food intake, urine volume or urine osmolarity between KO and controls during all water intake conditions. Plasma AVP levels tended to be higher in KO mice, but did not achieve statistical significance. Dietary Na intake and urinary Na excretion was similar between KO and controls on day 2 and day 3 of either an NS or HS diet. Arterial pressure was similar between both genotypes fed either an NS or HS diet. There were no differences in plasma renin concentration or urinary aldosterone excretion between KO and controls fed an NS diet. Finally, baseline or AVP-stimulated ENaC activity revealed no difference between KO and controls.

**Conclusions:** These data suggest that AC3 in the CD regulates AVP-stimulated cAMP accumulation, but this effect is modest and likely elicits changes that fully compensate for its absence.

*Funding:* Veterans Affairs Support

## TH-PO610

**The Rab-GAP Protein TBC1D4 (AS160) Regulates GLUT4 but Not ENaC and AQP2 in Mouse Kidneys** Marianna Di Chiara,[1,2] Dominique Loffing-Cueni,[1,] Bob Glaudemans,[3] Olivier Devuyst,[2,3] Nourdine Faresse,[1] Johannes Loffing.[1,2] *[1]Institute of Anatomy, Univ of Zurich, Zurich, Switzerland; [2]Zurich Center of Integrative Human Physiology (ZIHP), Univ of Zurich, Zurich, Switzerland; [3]Institute of Physiology, Univ of Zurich, Zurich, Switzerland.*

**Background:** The Rab-GTPase-activating protein TBC1D4 is implicated in GLUT4-mediated glucose uptake in adipo- and myocytes. We recently demonstrated that TBC1D4 is also expressed along the renal distal tubule, where it was proposed to control ENaC- and AQP2-mediated $Na^+$ and water reabsorption, respectively.

**Methods:** Here we used TBC1D4-deficient (KO) mice to characterize the role of TBC1D4 in the kidney in vivo. Functional phenotyping included analysis of mice on standard diet, low $Na^+$/high $K^+$ intake, and water restriction.

**Results:** Under these conditions, plasma ion concentrations, aldosterone levels, and urinary ion excretion and concentration ability did not differ between genotypes. Moreover, the abundance and subcellular localization of major renal sodium and water transport proteins (e.g. ENaC, AQP2, NCC, NKCC2) were similar in both genotypes. However, KO mice showed a prominent down-regulation of GLUT4 in the basolateral membrane of renal distal tubules.

**Conclusions:** TBC1D4-deficiency does not appear to perturb renal $Na^+$ and water transport, but TBC1D4 may play a role for GLUT4-mediated basolateral glucose uptake in the distal nephron. The latter may contribute to the known anaerobic glycolytic capacity of distal tubules.

*Funding:* Government Support - Non-U.S.

## TH-PO611

**Chronic Consumption of Fructose Increases Proximal Tubular Transport by Enhancing the Sensitivity to Angiotensin II** Agustin Gonzalez-Vicente, Nancy J. Hong, Pablo D. Cabral, Jeffrey L. Garvin. *Physiology & Biophysics, Case Western Reserve Univ, Cleveland, OH.*

**Background:** Salt-sensitive hypertension is a disease in which blood pressure increases with dietary salt. Consumption of high-fructose corn syrup as a sweetener has been implicated in the epidemic of diabetes, obesity, renal failure and hypertension. While high-fructose diets are able to increase blood pressure independently of salt intake, moderately-enriched fructose diets cause salt-sensitivity. The proximal tubule reabsorbs 60-70% of the filtered fluid, Na and $HCO_3$ via the Na/H exchanger 3 (NHE3). Salt reabsorption by this segment is regulated by angiotensin II (Ang II). We hypothesized that a moderately-enriched fructose diet increases Na reabsorption in proximal tubules by enhancing their sensitivity to Ang II.

**Methods:** To test this, rats were given 20% fructose in drinking water for 9 to 13 days. After treatment, the effects on transport of 1 pM Ang II were evaluated by oxygen consumption ($QO_2$) in proximal tubule suspensions from either fructose-fed rats or controls drinking tap water. NHE3 protein abundance was measured in cortical homogenates from either group. Components of the renin-angiotensin-system, angiotensinogen and AT1 receptor, were measured by mRNA expression.

**Results:** We found that 1 pM Ang II did not stimulate $QO_2$ in proximal tubule suspensions from control rats (-7±4 %; n=5). In contrast, it increased $QO_2$ in proximal tubules from fructose-fed rats by 18±8 % ($p<0.04$). To test whether this was due to increased NHE3, we measured protein abundance in cortical homogenates. We found that dietary fructose also increased NHE3 expression by 18±4 % ($p<0.03$; n=4) compared to the controls drinking tap water. We next tested whether components of the renin-angiotensin-system were elevated. AT1 receptor and angiotensinogen mRNA in cortical homogenates from the fructose-fed rats group were not different from controls.

**Conclusions:** We conclude that moderate fructose intake increases the sensitivity of the proximal tubule to Ang II and that enhanced NHE3 expression may contribute to this. AT1 receptor and angiotensinogen expression are not involved in this mechanism.

*Funding:* Other NIH Support - HL-90550

## TH-PO612

**Amiloride Inhibits uPA Activity and Plasminogen Activation in Urine from Rats with PAN-Induced Nephrotic Syndrome** Kristian B. Buhl,[1] Per Svenningsen,[1] Rikke M. Zachar,[1] Claus Bistrup,[2] Boye Jensen.[1] *[1]Dept of Cardiovascular and Renal Research, Institute of Molecular Medicine, Univ of Southern Denmark, Denmark; [2]Dept of Nephrology, Univ Hospital of Odense, Denmark.*

**Background:** Sodium retention in nephrotic syndrome (NS) is mediated by ENaC. The gamma subunit of ENaC is proteolytically activated in nephrotic syndrome. Aberrant filtration of serine protease zymogen plasminogen and conversion to active plasmin in the urinary space is a likely mediator. Urokinase-type plasminogen activator (uPA) is thought to mediate the activation in preurine. Amiloride is an inhibitor of uPA *in vitro*. We addressed the hypothesis that nephrotic syndrome results in aberrant glomerular filtration of uPA and urine uPA is inhibited by amiloride which prevents activation of plasminogen and ENaC proteolysis, sodium retention and edema *in vivo*.

**Methods:** Urine and kidney tissue uPA was determined in the PAN treated rat model of acute nephrotic syndrome and amiloride was tested for its ability to inhibit uPA. Rats were kept in metabolic cages and the effect of treatment was compared to vehicle treated nephrotic and healthy control rats.

**Results:** Development of proteinuria in the PAN rat model of nephrosis coincided with increased urinary serine protease activity as judged by gelatine zymography. PAN rats showed significant elevated urinary uPA activity. Immureactive uPA was detectable in urine samples of control and PAN treated rats. Amiloride significantly reduced uPA activity in nephrotic rat urine; it reduced urine gelatinolytic activity and concurrently increased the ratio plasminogen/plasmin and normalized 24 hour sodium excretion.

**Conclusions:** Urokinase–type plasminogen activator (uPA) is a target for amiloride in urine. Amiloride counteracts urinary plasminogen activation in proteinuric disease *in vivo*. ENaC is responsible for excess renal sodium retention in nephrotic syndrome.

*Funding:* Private Foundation Support

## TH-PO613

**Incidence and Factors of Post-Adrenalectomy Hyperkalemia in Patients with Aldosterone Producing Adenoma** Shih-Hua P. Lin, Sung-Sen Yang, Yu-Juei Hsu, Chih-Jen Cheng. *Div of Nephrology, Dept of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.*

**Background:** In patients with aldosterone-producing adenomas (APA), aldosterone secretion by the lesion severely suppresses the contralateral adrenal gland [8]. After adrenalectomy, patients with APA usually exhibit a state of hypoaldosteronism and are susceptible to developing hyperkalemia, a potentially serious complication. The aim of this study is to analyze the incidence and risk factors for hyperkalemia after adrenalectomy in patients with APA.

**Methods:** We retrospectively analyzed the records of 55 patients who underwent adrenalectomy for APA over the past 9 years. Demographic features, biochemical and hormonal profiles, imaging, and relevant medications were reviewed. Nadir serum $K^+$ was defined as the serum $K^+$ level when APA was first diagnosed. Hyperkalemia was defined as serum $K^+ > 5.0$ mmol/L and the exclusion of pseudohyperkalemia. Hyperkalemia that resolved during the first three months after adrenalectomy was defined as "transient;" those lasting more than three months requiring mineralocorticoid supplementation was defined as "persistent".

**Results:** Sixteen of 55 APA patients (29.1%) developed hyperkalemia (mean serum $K^+$ 5.6 ± 0.3 mmol/L) after adrenalectomy and three had persistent hyperkalemia requiring mineralocorticoid supplementation for more than nine months. Compared with normokalemic patients, hyperkalemic patients were characterized by male predominance, older age, longer duration of hypertension (12.8 ± 9.3 vs. 6.7 ± 5.0 years, $p < 0.05$), lower nadir serum $K^+$ ($p < 0.05$), higher preoperative serum creatinine ($p < 0.01$), and higher likelihood of residual hypertension. Using multivariate regression analysis, longer duration of hypertension and impaired renal function were the most important factors of post-adrenalectomy hyperkalemia.

**Conclusions:** Post-adrenalectomy hyperkalemia in patients with APA is not rare and associated with impaired renal function and longer duration of hypertension. Serum $K^+$ must be cautiously monitored in patients with long-term hypertension and kidney disease.

## TH-PO614

**Etiological and Therapeutic Analysis in Patients with Hypokalemic Paralysis due to Potassium Deficit** Chih-Chien Sung, Chih-Jen Cheng, Sung-Sen Yang, Shih-Hua P. Lin. *Div of Nephrology, Dept of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.*

**Background:** Non-hypokalemic periodic paralysis (non-HypoPP) represents a group of diverse causes of a large potassium ($K^+$) deficit. To rapidly diagnose its underlying causes with appropriate management is still challenging.

**Methods:** Fifty-eight patients (43 male and 15 female) with non-HypoPP and the exclusion of HypoPP were consecutively enrolled over an eight-year period. Blood and spot urine samples were collected for electrolytes, acid-base and biochemistry measurement on admission and during therapy. Intravenous potassium chloride (KCl) at a rate of 10-20 mmol/hour was administered until muscle strength recovered.

**Results:** The average $K^+$ concentration was 1.8 ± 0.3 mmol/L. Their etiology could be simplified by the urinary $K^+$ excretion rate and blood acid-base status. For a low urinary $K^+$ excretion, chronic alcoholism, anorexia/bulimia nervosa, and remote diuretics use were the most common caues. For a high urinary $K^+$ excretion with metabolic acidosis, renal tubular acidosis and chronic toluene abuse were common causes. Mineralocorticoid excess state with primary aldosteronism, Gitelman's or classic Bartter's syndrome, and diuretics use were common causes for metabolic alkalosis with high and normal blood pressure, respectively. Muscle strength was restored at serum $K^+$ concentration of 2.6 ± 0.2 mmol/L after administering approximately 245 mmol (3.8 ± 0.8 mmol/kg) KCl. Of note, non-HypoPP patients with high urinary $K^+$ excretion needed a higher KCl dose than those with low $K^+$ excretion (4.0 ± 0.8 vs 3.2 ± 0.5 mmol/kg, $p < 0.001$). KCl supplementation was higher in patients who developed a paradoxical fall in serum $K^+$ concentration (1.6 ± 0.2 mmol/L) than those without (4.1 ± 0.7 vs 3.4 ± 0.7 mmol/kg, $p < 0.001$). These patients often had typical volume depletion with higher plasma renin activity.

**Conclusions:** Understanding the common etiology of non-HypoPP may aid in early diagnosis. Non-HypoPP patients associated with renal $K^+$ wasting or hypovolemia that tend to develop paradoxical hypokalemia require a larger dose of KCl to prevent life-threatening complications.

## TH-PO615

**Revisiting the Effectiveness of Sodium Polystyrene Sulfonate in Treating Hyperkalemia** Baidaa Najeeb Mohammed,[1] Farhad Mohammed- Hasan,[1] Richard H. Sterns.[1,2] *[1]Medicine, Rochester General Hospital, Rochester, NY; [2]Medicine (Nephrology), Univ of Rochester School of Medicine and Dentistry, Rochester, NY.*

**Background:** Sodium polystyrene sulfonate (SPS) is a $K^+$ -binding resin commonly used to treat hyperkalemia. No studies in humans or animals show that SPS increases $K^+$ elimination [Sterns RH, J Am Soc Nephrol. 2010;21(5):733-5]. A recent study cited a dose- response relationship as evidence that SPS is effective [Kessler C, J Hosp Med. 2011;6(3):136-40 [4] .

**Methods:** We reviewed all records of inpatients who received SPS for hyperkalemia ( > 5.1 mEq/L) at Rochester General Hospital between November 1, 2011 and December 31, 2012. We excluded cases of SPS Rx with: chronic use; other Rx modalities for hyperkalemia (including dialysis) ; repeated doses < 6 hrs apart ; hemolysis and absence of follow up labs.

**Results:** 116 hyperkalemic subjects (mean pre-Rx serum $K^+$ = 5.64 ± 0.36 mEq/L) receiving oral SPS as sole Rx were studied. Mean serum $K^+$ dropped by 13% after a median of 8 hrs. A higher SPS dose (30 Gm, n = 49) was associated with a greater fall in serum $K^+$ (0.89 ± 0.49 mEq/L) than a lower dose (15 Gm , n = 67) (0.66 ± 0.53 mEq/L); $p = 0.02$ . However, a higher SPS dose was also associated with a higher pre- Rx serum $K^+$ level (5.76 ± 0.39 mEq/L in the 30 Gm subgroup vs. 5.56 ± 0.31 mEq/L in the 15 Gm subgroup; $p = 0.005$) and there was a statistically significant positive correlation between pre-Rx serum $K^+$ and the decrement in serum $K^+$ ($r = 0.51$, $p < 0.001$). Within the 30 Gm subgroup a similar correlation was found between pre-Rx serum K and decrement in $K^+$ ( $r= 0.3$; $p = 0.006$) but not in the 15 Gm subgroup. There was no correlation between the magnitude of the decrement in serum $K^+$ and the time to subsequent testing ($r = 0.01$, $p = 0.13$) , suggesting that serum $K^+$ may not fall progressively with time after SPS as would be expected for an oral drug that works in the distal colon.

**Conclusions:** A dose-response relationship between SPS and correction of hyperkalemia does not prove cause and effect. Placebo controlled trials of SPS are needed.

## TH-PO616

**Abstract Withdrawn**

## TH-PO617

**Relowering Sodium after Overcorrection of Hyponatremia: Save the Brain. Cases Series and Literature Review** Alain Soupart,[1,2] Guy Decaux,[2] Michel Coffernils,[1] Fabrice Gankam,[3] Frédéric Vandergheynst,[2] Bruno Couturier.[2] *[1]Internal Medicine, Jolimont/Tubize Hospital, Tubize, Belgium; [2]Internal Medicine, Erasmus Univ Hospital, Belgium; [3]Nephrology, Erasmus Univ Hospital, Belgium.*

**Background:** Overcorrection of severe chronic hyponatremia (HN) can lead to osmotic demyelination syndrome (ODS) with major brain damage.Too fast correction produces excessive brain dehydration, intracellular hyperionisation and demyelinating lesions. We showed in rats that relowering SNa after overcorrection dramatically reduced ODS and mortality (Kidney Int, 1994, J Neuropathol Exp Neurol 1996) and we report the first case in man (Clin Nephrol, 1999). Clinical experience of this therapy is however limited.

**Methods:** We report a series of 11 patients from our hospital with HN of various origins submitted to inadvertent overcorrection (DSNa > 10 mEq/l/24 h) in whom SNa was relowered using DDAVP and hypotonic fluids. After literature review, 7 cases with same inclusion criteria were found and added in series. Cases were separated whether they are asymptomatic (group I, 5 cases from our center) or present neurologic symptoms (group II, 6 cases from our center) prior relowering.

**Results:**

| Groups | Age | SNa T0 | ΔSNa/24hr | Relowering time from T0 | Final ΔSNa into next 24hr | Clinical Outcome |
|---|---|---|---|---|---|---|
| Group I n = 9 | 62±9 | 110±3 (106-117) | +17±3 (12-22) | Day 1-2 | +10±3 (3-16) | N (9/9) Brain MRI: N |
| Group II n = 9 | 64±11 | 108±6 (97-118) | +17±6 (12-26)* | Day 1-7 | +12±3 (8-19) | N (9/9) Brain MRI: N |

*Data means ± SD; SNa : mEq/l; *one case relowered after correction of 5 mEq/l/day during 3 successive days*

**Conclusions:** This largest reported series shows that SNa relowering in patients after overcorrection is safe and associated with a good clinical outcome and no ODS.

## TH-PO618

**Tritherapy (Saline, Urea, Desmopressin = SUD) as Initial Therapeutic Regimen for Sodium Rate Control in Patients with Non-Oedematous Severe Hyponatremia** Alain Soupart,[1,2] Guy Decaux,[2] Michel Coffernils,[1] Bruno Couturier,[2] Frédéric Vandergheynst.[2] *[1]Internal Medicine, Jolimont/Tubize Hospital, Tubize; [2]Internal Medicine, Erasmus Univ Hospital, Brussels.*

**Background:** There is no consensus about optimal treatment of severe hyponatremia (HN). Significant risks of inadvertent overcorrection and ODS exists with the usual methods of correction (normal or hypertonic saline ± furosemide). We evaluate the effect of a treatment combining normal saline with urea and DDAVP (SUD regimen) during the first 24hr as initial treatment in non-oedematous HN owing to control the rise (ΔSNa < 12 mEq/l/24hr) in SNa (DDAVP) and to protect brain against ODS (urea).

**Methods:** Ten consecutive non-oedematous patients with severe HN were included in the study. Patients with severe symptoms (coma, epilepsia) were excluded. Treatment consists in: [normal saline 1 l + oral urea 30 gr + DDAVP 4 µg s.c] every 12 hours during the first 24hr. Treatment was adapted at 12hr depending on the correction level (if ΔSNa ≥5 mEq/l, no second urea dose; ≤ 2 mEq/l, no second DDAVP dose; > 10 mEq/l at any time, active treatment stopped).

**Results:**

| N = 10 | T0 | T6hr | T12hr | T24hr | T48hr |
|---|---|---|---|---|---|
| SNa (mEq/l) | 113±3 | 117±3 | 118±3 | 121±3 | 126±2 |
| ΔSNa (mEq/l) Range | - - | 3.2±3 (0-12) | 4±3 (0-11) | 7.6±1.9 5-11) | 12.5±5 (3-21) |
| SUrea (mg/dl) | 18±8 | 70±26 | 66±29 | 61±27 | 39±19 |
| UNa (mEq/l) | 21±17 | 34±22 | 26±23 | 32±26 | 58±30 |
| UOsm (mOsm/Kg/$H_20$) | 353±175 | 455±113 | 579±146 | 575±141 | 515±180 |

*Data means ± SD*

The outcome was good in all the patients and brain CT or MRI were normal.

**Conclusions:** SUD regimen is associated with a good sodium rate control and a favourable outcome in patients with non-oedematous HN of various origins.

## TH-PO619

**Hyponatremia Is Associated with Increased Osteoporosis and Bone Fractures in a Large Health System Population** Laura Cowen,[1] Stephen Fernandez,[2] Nawar M. Shara,[1,2] Julianna Barsony,[1] Joseph G. Verbalis.[1] *[1]Endocrinology, Georgetown Univ, Washington, DC; [2]Medstar Health Research Institute, Washington, DC.*

**Background:** Four independent studies have shown increased bone fractures in patients with hyponatremia (HN). Likely major contributors include gait instability and increased falls in HN patients. Recent animal studies have also demonstrated HN-induced bone loss, and analysis of human subjects in NHANES III showed a significantly increased odds ratio for osteoporosis in HN subjects.

**Methods:** To assess the clinical significance of these findings, we analyzed the patient database of the MedStar Health system using the Explorys electronic health record (EHR) tool.

**Results:** A search of >2.8 million unique EHR records showed that 55,510 patients had osteoporosis by ICD-9 coding; of this group, 8,060 had HN by either ICD-9 coding or laboratory measurement (serum [$Na^+$] <135 mmol/L) and 47,450 did not. The prevalence of osteoporosis in the HN patients was 4.6% (8,060/177,100 total HN patients), compared to 1.8% in the non-HN patients (47,450/2,623,200 total non-HN patients). Because not all bone fractures are associated with osteoporosis, we also analyzed the occurrence of fractures in this database. A larger number of patients, 112,840, had bone fractures by ICD-9 coding; of this group, 16,820 had HN and 96,020 did not. The prevalence of fractures in the HN patients was 9.5% (16,820/177,100 total HN patients), compared to 3.7% in the non-HN patients (96,020/2,623,200 total non-HN patients). Demographic analysis of HN and non-HN patients with osteoporosis and fractures indicated a larger percentage of affected men in the HN group (17.5%) versus the non-HN group (14.2%). Age group predominance

was similar in both groups with the majority of affected patients aged 70-89. This analysis indicates that both osteoporosis and bone fractures are increased approximately 2.6-fold in HN compared with non-HN patients.

**Conclusions:** These results support the hypothesis that HN is a risk factor for both osteoporosis and bone fractures in patients; our results also demonstrate the power of focused analysis of the EHR to assess the clinical relevance of findings generated from translational science studies.

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### TH-PO620

**Hyponatremia Is a Surrogate Marker of Severity in Uremic Patients with Peritoneal Dialysis-Related Peritonitis** Min-Hua Tseng,[1] Shih-Hua P. Lin,[2] Sung-Sen Yang,[2] Chih-Jen Cheng.[2] *[1]Div of Nephrology, Dept of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan; [2]Div of Nephrology, Dept of Medicine, Tri-Service General Hospital, Taipei, Taiwan.*

**Background:** The association of hyponatremia with clinical outcome in dialysis patients is not well studied, especially in peritoneal dialysis-related peritonitis (PDRP). The aim of this study was to evaluate the impact of hyponatremia in patients with PDRP.

**Methods:** We retrospectively reviewed the medical records of the dialysis patients admitted with PDRP over the past eight years. Patients were divided into two groups: one with serum $Na^+$ <130 mEq/L (group I) and the other ≥130 mEq/L (group II). Primary endpoint was mortality during hospitalization. Secondary outcomes included length of hospital stay, removal of peritoneal dialysis (PD) catheter, peritonitis occurrence, and failure of PD.

**Results:** Of 99 patients with PDRP, group I had 27 and group II 72 patients. Gram-negative bacilli and gram-positive cocci accounted for the majority of PDRP in Group I and II, respectively. Despite no significant difference in age, duration of dialysis, PD catheter removal rate and technique failure between 2 groups, group I had a significantly higher serum CRP ($p < 0.001$), lower serum albumin ($p < 0.001$) and phosphate ($p < 0.05$). There was a positive correlation between serum $Na^+$ and albumin concentration ($p<0.001$) as well as serum phosphate level ($p<0.04$). Length of hospital stay ($p< 0.001$) and hospital mortality ($p < 0.001$) were also significantly higher in group I than II. In a multivariable logistic regression, hyponatremia on admission remained an independent predictor of hospital mortality (OR 76.89 95% CI 3.39-1741.67, $p< 0.05$) and independently associated with length of hospital stay (OR 5.37, 95%CI 1.58- 18.19, $p< 0.05$).

**Conclusions:** The degree of hyponatremia in uremic patients with PDRP reflects the severity of peritonitis and is associated with length of hospitalization and mortality.

*Funding:* Clinical Revenue Support

### TH-PO621

**Prognostic Impact of Hyponatremia Occurring at Various Time Points during Hospitalization on Long-Term Mortality in Patients with Acute Myocardial Infarction** Joon Seok Choi, Ha Yeon Kim, Yong Un Kang, Chang Seong Kim, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Dept of Internal Medicine, Div of Nephrology, Gwngju, Korea.*

**Background:** We investigated the incidence and prognostic impact of hyponatremia occurring at various time points during hospitalization on long-term mortality in patients surviving an episode of acute myocardial infarction (AMI).

**Methods:** We retrospectively studied 1,863 patients diagnosed with AMI. Baseline, nadir, discharge, and average sodium levels during hospitalization were recorded and statistically analyzed. Hyponatremia was defined as a serum sodium level <135 mEq/L.

**Results:** On the basis of baseline, nadir, discharge, and average sodium levels during hospitalization, hyponatremia was diagnosed in 309 (16.6%), 518 (27.8%), 147 (7.9%), and 140 (7.5%) patients, respectively. In a multivariate Cox-proportional regression analysis, discharge sodium level had the strongest significant relationship to long-term mortality (hazard ratio [HR] as continuous variable = 1.07, 95% confidence interval [CI]: 1.01-1.13, P = 0.014; HR as categorical variable = 1.75; 95% CI: 1.09-2.80; P = 0.021), but baseline sodium had no prognostic impact on long-term mortality after adjustment.

**Conclusions:** The serum sodium level and incidence of hyponatremia varied at different time points during hospitalization. In addition, the association between sodium level and long-term mortality differed at these various time points. The discharge sodium level appears to be the best predictor of long-term mortality in patients surviving an acute episode of AMI.

### TH-PO622

**The Physiological Role of Adaptor Protein 1B on the Surface Trafficking of Kidney Anion Exchanger 1** Ensaf Yousef Almomani, Emmanuelle Cordat. *Physiology, Univ of Alberta, Edmonton, Canada.*

**Background:** Distal Renal Tubular Acidosis (dRTA) is a disease characterized by metabolic acidosis, an inability to acidify urine, the development of kidney stones and renal failure if untreated. DRTA patients may carry a mutation on the SLC4A1 gene encoding the kidney anion exchanger 1 (kAE1), a $Cl^-/HCO_3^-$ exchanger basolaterally expressed in acid-secreting kidney intercalated cells. KAE1 binds to the epithelial-specific adaptor protein 1B (AP-1B) complex, which is essential to trafficking and recycling cargo proteins. The apically mistargeted kAE1-R901X that occurs naturally, and the engineered C-terminal Y904A/V907A mutants, both lack the interaction site with the mu subunit of AP-1B. We hypothesized that AP-1B is important for kAE1 stability at the cell surface of kidney epithelial cells and that the missing interaction of the kAE1-R901X mutant with AP-1B causes dRTA.

**Methods:** To study the role of AP-1B, we stably expressed kAE1 wild-type (WT) or mutants in porcine epithelial kidney cells (LLC-PK1) that are naturally devoid of endogenous AP-1B.

**Results:** Cell surface biotinylation and immunoblotting showed that the expression of AP-1B significantly increased the amount of total and cell surface kAE1-WT in LLC-PK1 cells treated with cycloheximide for 8 hours, but not that of kAE1-R901X or Y904A/V907A mutants. The AP-1B independent $Na^+/K^+$-ATPase did not show a significant difference of surface expression upon AP-1B expression, suggesting that the effect of AP-1B is specific to kAE1. We further examined the effect of AP-1B on kAE1 endocytosis and recycling to the cell surface.

**Conclusions:** This study highlights the importance of AP-1B for kAE1 stabiltity at the cell surface. Loss of mutated kAE1 / AP-1B interaction may explain the pathogenicity of dRTA.

*Funding:* Private Foundation Support

### TH-PO623

**Comparison of Arterial Blood Gas versus Laboratory Electrolyte Measurements in Critically Ill Patients at Cleveland Clinic Florida** Julianne M. Parente, Rute C. Paixao, Mauro Braun, Dianne T. Sandy. *Nephrology, Cleveland Clinic Florida, Weston, FL.*

**Background:** Electrolyte measurements are performed by Arterial Blood Gas (ABG) analyzers and laboratory analyzers. However, a significant time gap exists between availability of these results, with ABG giving faster results. The aim of the present study was to investigate weather ABG analyzers can provide an accurate measurement of electrolytes as compared to laboratory based analysis. ABG results may enable the critical care team to make faster clinical decisions, improve quality of care and decrease mortality rates. Data on sodium, potassium and chloride were examined.

**Methods:** We retrospectively studied 111 patients admitted to the Cleveland Clinic Florida (CCF) Intensive Care Unit (ICU) between February and June 2013. ABG electrolyte results were compared with results from that day's laboratory results when the samples were obtained within one hour of each other. All patients with any acute or chronic condition were considered possible subjects for the study. The research protocol was approved by the Institutional Review Board.

**Results:** A total of 111 blood samples were analyzed. The mean patient age was 66.7 years. 64.8% of the subjects were males. 57% of the patients were admitted to the Surgical ICU and the remainder to the Medical ICU. 66.6% of the patients had an arterial line in place at the time of the sample collection. The mean sodium concentration was 140.6 mmol/L (SD 5.74) using the serum and 137.7 mmol/L (SD 5.66) ($p=0.293$) using the ABG analyzer. Mean potassium level was 4.1 mmol/L (SD 0.63) using the serum and 3.96 mmol/L (SD 0.63) ($p=0.006$) using the ABG analyzer. Mean chloride values were 102.4 mmol/L (SD 7.58) and 104.8 mmol/L (SD 7.37) ($p=0.016$) using the laboratory and ABG analyzers, respectively.

**Conclusions:** Based on the above analysis, we found no significant difference between the sodium values measured by the blood gas machine and the laboratory analyzer. However, the differences between the measured potassium and chloride were found to be significant. We therefore conclude that critical decisions can be made by trusting only the sodium levels obtained from the blood gas analysis.

### TH-PO624

**Analysis of Chondroitin Sulfate Proteoglycans and Reactive Astrogliosis in a Rat Model of Osmotic Demyelination Syndrome** Bruno Couturier,[1] Fabrice Gankam,[2] Alain Soupart,[3] Guy Decaux.[1] *[1]Internal Medicine, Erasme Hospital Free Univ of Brussels, Brussels, Belgium; [2]Nephrology, Erasme Hospital Free Univ of Brussels, Brussels, Belgium; [3]Internal Medicine, Jolimont Hospital Free Univ of Brussels, Brussels, Belgium.*

**Background:** Osmotic demyelination syndrome (ODS) results from overly rapid correction of chronic hyponatremia (HypoNa) and its pathophysiology is not yet fully understood. Specifically it is not clear which factors might promote or impede remyelination and clinical recovery. Using a rat model, we studied several molecules known as markers of glial activation (nestin and vimentin) and inhibitors of axonal growth including chondroitin sulphate proteoglycans (CSPG's) in order to determine their role in CNS regeneration after osmotic insult.

**Methods:** Chronic hyponatremia was induced in rats by continuous infusion of ADH and corrected with hypertonic saline. At various times after the correction we used immunohistochemistry to assess expression of vimentin, nestin and several CSPG's. Immunofluorescence was used to identify which cells expressed these molecules.

**Results:** We confirmed the increase in glial fibrillary acidic protein at the borders of the ODS lesions after rapid correction of chronic hypoNa. Moreover, we showed an upregulation of Nestin and vimentin in astrocytes of corrected animals from day-to-day after the correction. We also confirm the expression of three CSPG's, CD44, neurocan and phosphacan KS in the direct vicinity of osmotic lesions following a peculiar pattern. CD44 and neurocan were distributed in the lesions penumbra whereas phosphacan KS was mainly found in the lesion core. CSPG's deposition only appeared 72h after correction and was more prominent 6 days after correction.

**Conclusions:** These results suggest a specific pattern of CSPG's and nestin/vimentin expression in brain after osmotic insult. Their role in myelin regeneration after osmotic insult remains to be determined.

*Funding:* Private Foundation Support

## TH-PO625

**Role of Tubular Luminal H(+)/Organic Cation Antiporter, MATE, in Natriuresis as a Dopamine Transporter** Moto Kajiwara, Tsuyoshi Ban, Kazuo Matsubara, Satohiro Masuda. *Clinical Pharmacology and Therapeutics, Kyoto Univ Hospital, Kyoto, Japan.*

**Background:** The intrarenal dopaminergic system is likely responsible for regulating over 50% of net renal salt and water excretion when salt intake increases. Although dopamine synthesis is localized at proximal tubular cells, the molecular mechanism underlying dopamine secretion into urine remains unknown. Multidrug and toxin extrusion (MATE, SLC47A) was identified in 2005; it is expressed in high levels in the brush border membrane of proximal tubular cells mediating the efflux of organic cations, such as metformin and cisplatin, from epithelial cells into urine. Therefore, we hypothesized that MATE mediates secretion of dopamine, a cationic catecholamine, into tubule lumen and consequently regulates natriuresis.

**Methods:** Sodium levels in the urine and whole blood of mice were respectively measured using an ion-selective electrode (cobas6000, Roche) and an iSTAT analyzer (Abott). Urinary dopamine concentration was measured with a liquid chromatography-tandem mass spectrometry.

**Results:** To determine whether dopamine is a substrate of MATE, we examined [$^3$H] dopamine uptake in HEK293 cells transiently expressing human (h) MATE1, hMATE2-K, and mouse (m) MATE1. Uptake of [$^3$H]dopamine by cells expressing hMATE1, hMATE2-K, and mMATE1 was higher than that of vector-expressing cells. *In vivo* acute saline-loading experiments using infusion pump were performed to examine MATE contribution on urinary dopamine transport in mice. Urinary dopamine was detected in wild-type mice (WT), but was hardly present in MATE1-knockout mice (KO). Another experiment showed that the amount of urinary sodium excretion in WT increased to 12.3-fold than that of the control group, although the amount in MATE1-KO increased to only 1.5-fold than that of the control group. In intact mice, average whole blood sodium levels were significantly higher ($n = 3$, mean ± SD, $P < 0.003$) in MATE1-KO (146.7 ± 0.58 mmol/L) than those in WT (143.7 ± 0.58 mmol/L).

**Conclusions:** In conclusion, our results indicated a critical role of MATE in dopamine secretion into tubular lumen, along with MATE's regulation of sodium excretion into urine.

*Funding:* Government Support - Non-U.S.

## TH-PO626

**Mapping of pH Sensor, GPR4, to Peritubular Capillaries** Doris P. Molina,[1] Xuming Sun,[1] Glen S. Marrs,[3] Thomas D. DuBose,[1,2] Snezana Petrovic.[1,2] *[1]Physiology and Pharmacology, Wake Forest Univ, Winston Salem, NC; [2]Internal Medicine Section of Nephrology, Wake Forest Univ, Winston Salem, NC; [3]Biology, Wake Forest Univ, Winston Salem, NC.*

**Background:** GPR4 is a proton receptor, a member of a unique family of G protein-coupled receptors that are activated by an acid pH within the physiological pH range. This family of receptors function as pH sensors in blood vessels, bone, kidney, airway smooth muscle, lung cancer, and possibly brain. GPR4 is the most abundant proton receptor in the kidney. We previously demonstrated that GPR4 mRNA is expressed in all three kidney zones and in kidney epithelial cells. Lack of specific GPR4 antibodies precluded more detailed studies of GPR4 distribution in the kidney.

**Methods:** Here, we employed an alternative strategy, mapping transgenic GFP expression driven by the GPR4 promoter. Namely, GPR4-/- mice (Mol Cell Biol 27: 1334-1347, 2007) were generated with a construct with a GFP insert, which was tagged with anti-GFP rabbit polyclonal antibody. As GFP expression is driven by the GPR4 promoter, GFP labeling was present selectively in GPR4-/- mice in cells which natively express GPR4, with GPR4+/+ mice serving as control.

**Results:** We found abundant expression of GPR4 in endothelial cells of the peritubular capillaries, consistent with reports of GPR4 expression in endothelial cell cultures. Double-labeling with isolectin IB4 confirmed the presence of GPR4 within endothelial cells and peritubular capillaries. GPR4 was expressed at lower levels in the glomerulus, localizing to mesangial cells (5' nucleotidase co-localization) as well as glomerular endothelial cells (IB4-co-localization). GPR4 did not co-localize with Wilms tumor antigen, indicating that GPR4 is absent from podocytes.

**Conclusions:** The expression of a pH sensor in the peritubular capillaries is an intriguing finding given the typically low pH of the kidney interstitium and its anticipated fluctuations with dietary acid loads. It is plausible that the response of GPR4 to a decline in interstitial pH may initiate a response by the peritubular capillaries, with potential implications for redox status, release of endothelin, activation of RAS, and renal perfusion.

*Funding:* Private Foundation Support

## TH-PO627

**Dietary Protein Restriction Induces an Integrated Response Involving Multiple Aspects of Renal Ammonia Metabolism** Hyun-Wook Lee,[1] Jesse M. Bishop,[1] Mary E. Handlogten,[1] Jill W. Verlander,[1] I. David Weiner.[1,2] *[1]Renal Div, Univ of Florida, Gainesville, FL; [2]Nephrology and Hypertension Section, GVAMC, Gainesville, FL.*

**Background:** Both low protein diets and systemic acid-base status have important effects on the progression of chronic kidney disease. Because ammonia metabolism is a major determinant of renal acid-base homeostasis, we examined the effect of dietary protein restriction on renal ammonia metabolism. Because we identified novel effects of protein restriction on expression of the ammonia transporter family member, Rhbg, we also determined the effect of collecting duct-specific Rhbg deletion (CD-Rhbg-KO) on the response to protein restriction.

**Methods:** C57Bl/6 mice were acclimated in metabolic cages and then randomly assigned to either low (6%) or control (20%) protein diets for two weeks. Daily 24 hr urine was collected. At the end of the study, tissue was obtained for immunoblot analysis and immunohistochemistry. CD-Rhbg-KO mice were generated using Cre-loxP techniques and were studied using an identical protocol.

**Results:** Low protein diet decreased urinary ammonia excretion by 85-90%. It also decreased by 40% renal cortical PEPCK, did not alter either renal cortical or outer medullary PDG expression, and increased renal cortical glutamine synthetase expression. Rhbg expression increased in the outer medulla, and immunohistochemistry demonstrated changes in expression were limited to intercalated cells in the outer medullary collecting duct (OMCD). However, CD-Rhbg-KO did not alter changes in urinary ammonia excretion in response to low protein diet, nor did it alter urine pH or low-protein induced changes in PEPCK, PDG or GS in renal homogenates.

**Conclusions:** We conclude: (1) Low protein diet decreases renal ammonia excretion through mechanisms involving both decreased ammoniagenesis involving PEPCK and increased ammonia assimilation involving glutamine synthetase; (2) low protein diet results in increased OMCD intercalated cell Rhbg expression which is not necessary for the reduced urinary ammonia excretion; and, (3) Rhbg may have previously unidentified roles in the renal response to low protein diet which are unrelated to ammonia excretion.

*Funding:* NIDDK Support, Other U.S. Government Support, Veterans Affairs Support

## TH-PO628

**Acid-Stimulated Pyk2 Regulates $H^+$,$K^+$-ATPase Activity via a p38 MAPK Signaling Pathway in Outer Medullary Collecting Duct Cells** Kimberly Fisher,[1,2] Thomas D. DuBose.[2] *[1]Medicine, Vanderbilt Univ, Nashville, TN; [2]Medicine, Wake Forest School of Medicine, Winston Salem, NC.*

**Background:** Proton secretion increases in the OMCD in response to an acid load that occurs as a product of the metabolism of protein derived from a typical Western diet to defend against metabolic acidosis. We have shown that proline-rich tyrosine kinase-2 (Pyk2), a putative a pH sensor, participates in the regulation of apical ATPase-mediated proton transport in the OMCD.

**Methods:** In the current study we used the $NH_4Cl$ pre-pulse technique to generate an acid intracellular pH ($pH_i \sim 6.7$) in mouse-derived inner medullary (mOMCD1) cells *in vitro* (control $pH_i = 7.4$). We then analyzed rates of $pH_i$ recovery after an $NH_4Cl$ pre-pulse using spectrofluorometry of BCECF-AM-loaded cells. Pyk2 inhibition was accomplished by two approaches: using an adenovirus expressing a truncated Pyk2 construct (AdCRNK), and inhibition of p38, using the inhibitor SB203580.

**Results:** Analysis of protein phosphorylation using immunoblot shows that Pyk2 and MAPK p38 are rapidly phosphorylated at acid $pH_i$ (3.4 and 3.8 fold increase, respectively, after 1 minute). $H^+$,$K^+$-ATPase-mediated $pH_i$ recovery was significantly inhibited after an $NH_4Cl$ pre-pulse (68% by the adenovirus construct and 69% by SB203580). We assume that the unaffected $pH_i$ recovery may be attributed to unaffected $H^+$-ATPase-mediated $pH_i$ recovery.

**Conclusions:** In summary, we show that Pyk2 is required for p38 phosphorylation and subsequent $H^+$,$K^+$-ATPase activation in response to acid pH in the outer medullary collecting duct. Taken together with our previous study (*Am J Physiol Renal Physiol* 303: F1353–F1362, 2012), these new findings indicate that in response to an acute decrease in $pH_i$, two distinct MAPK pathways (p38 and ERK1/2) regulate $H^+$-ATPase and $H^+$,$K^+$-ATPase activity, respectively, via the pH sensor, Pyk2.

*Funding:* Clinical Revenue Support

## TH-PO629

**Chronic Metabolic Acidosis Enhances SPAK-Dependent Phosphorylation of Thiazide-Sensitive Sodium Chloride Cotransporter** Yu-Wei Fang,[1,2] Sung-Sen Yang,[2,3] Chih-Jen Cheng,[3,] Shih-Hua P. Lin.[2,3] *[1]Div of Nephrology, Dept of Medicine, Shin Kong Wu Ho-Se Memorial Hospital; [2]Graduate Institute of Medical Sciences, National Defense Medical Center; [3]Div of Nephrology, Dept of Medicine, Tri-Service General Hospital.*

**Background:** Although chronic metabolic acidosis (MA) has been shown to increase the activity and abundance of renal Na-Cl cotransporters (NCC) in the distal convoluted tubule (DCT), its molecular mechanism remains unexplored. We evaluated whether SPAK–NCC signaling pathway is involved in chronic MA *in vivo or not.*

**Methods:** $Na^+/HCO3^-$ cotransporter 1 (NBC1) W516X/+ mice were used as spontaneous renal tubule MA model. Wild-type (WT) and SPAK null mice received water with $NH_4Cl$ (280mM) ad libitum for 5 days for inducing MA. Blood biochemistries were measured before and after $NH_4Cl$ treatment. The abundance of total and phosphorylated (p-) NCC and SPAK in kidney tissues was evaluated by immunobloting and immunoflurescence. NCC function was assessed by hydrochlorothiazide (HCTZ, 25mg/kg) inhibition test.

**Results:** Like the $NBC1^{W516X/+}$ mice (pH: 7.15 ±0.14 vs 7.42 ±0.18 in WT mice; $HCO^{3-}$: 21.5 ±0.3 vs 24.2 ±0.2 in WT, n=8, $p<0.01$), MA was induced in WT mice (pH: 7.29±0.03; $HCO^{3-}$: 22.8 ±0.5, n=6, $p<0.05$) and SPAK null (pH: 7.31±0.02; $HCO^{3-}$: 23.4 ±0.6, n=7, $p<0.05$) mice by oral $NH_4Cl$ intake. $NBC1^{W516X/+}$ mice and WT mice with $NH_4Cl$ both have significantly increased p-NCC (143.3 ±11% in $NBC1^{W516X/+}$ and 135 ±8% in WT with $NH_4Cl$) accompanied by the increased p-SPAK (137 ±12% in $NBC1^{W516X/+}$ mice and 122 ±6% in WT mice with $NH_4Cl$). These results were further confirmed by the immunoflurescence stain of the DCT. In addition, exaggerated response to HCTZ was observed in both $NBC1^{W516X/+}$ mice

(6.7±0.4% v.s 2.5±0.6% in WT, n=7, P<0.05) and WT mice with $NH_4Cl$ (FeNa 7.8±1.5% v.s 2.7±0.4% in WT without $NH_4Cl$, n=5, P<0.05). However, the NCC expression and activity was not increased in SPAK- null mice with $NH_4Cl$.

**Conclusions:** Chronic metabolic acidosis might alter NCC phosphorylation and function at least in part through SPAK-dependent signal cascade. Whether WNK1/4 kinase, the upstream stimulators of SPAK, is involved needs to be further investigation.

## TH-PO630

**A Mouse Model of Kidney Stone Caused by Double Deletion of Pendrin and the Na-Cl Cotransporter (NCC)** Sharon L. Barone,[1,2] Jie Xu,[1,2] Hassane Amlal,[1] Kamyar A. Zahedi,[1,2] Manoocher Soleimani.[1,2] [1]*Internal Medicine, Univ of Cincinnati, Cincinnati, OH;* [2]*Research Services, VA Administration, Cincinnati, OH.*

**Background:** Kidney stones are a *major cause of morbidity* due to associated infection and kidney failure. The $Cl^-/HCO_3^-$ exchanger pendrin and NCC are expressed on the apical membranes of distal nephrons and mediate salt absorption. In mice, solitary KO of NCC causes hypocalciuria whereas KO of pendrin in isolation causes mild hypercalciuria. Neither NCC- nor pendrin-KO mice have salt wasting under baseline conditions. Mice with simultaneous knockout of pendrin and NCC (dKO), on the other hand, show severe salt wasting and volume depletion.

We hypothesize that NCC deletion offsets the hypercalciuric effect of pendrin deletion in dKO mice.

**Results:** 24 hr urine analysis showed a 3-fold increase in calcium excretion in dKO mice. The histological analysis of kidneys demonstrated multiple calcium stones in the medullary collecting ducts in dKO mice but not in pendrin or NCC single KO mice. The stones did not polarize under microscope but stained with Von Kossa stain, consistent with calcium phosphate stones. Phosphate excretion increased by 2-fold and correlated with significant reduction in NaPi2a expression in proximal tubule in dKO mice. Serum calcium was mildly reduced in dKO mice but remained normal in single KO or WT mice. Blood levels for PTH, 1,25 Vitamin D and FGF23 in dKO mice were comparable to WT or single KO mice. Urine pH in dKO mice was comparable to WT animals (~6.2) even though it was reduced in pendrin KO mice (<5.0). Salt loading for 7 days in dKO mice rectified the volume depletion, increased the urine output and dissolved the kidney stones, but did not affect the hypercalciuria or hyperphosphaturia.

**Conclusions:** We were wrong about our prediction of calcium excretion in dKO mice. We conclude that the combined deletions of NCC and pendrin cause profound hypercalciuria and hyperphosphaturia and result in calcium phosphate kidney stones, which were resolved by salt repletion. Whether the combination of pendrin deletion, which down regulates TRPV5, and volume depletion predispose dKO mice to kidney stones remains to be determined.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO631

**Key Insights into the Regulation of Pendrin and AE1 Transporter Expression in Cortical Collecting Ducts by 3D Morphometric Analysis** Jeffrey M. Purkerson, George J. Schwartz. *Pediatrics, Univ of Rochester School of Medicine, Rochester, NY.*

**Background:** Previous studies utilizing kidney sections suggested altered distribution or trafficking of pendrin and AE1 as possible mechanisms for adaptation of the cortical collecting duct (CCD) to acid/base disturbances.

**Methods:** To better understand regulation of anion exchanger function in intercalated cells (ICs), we examined pendrin and AE1 morphology via 3D cell reconstruction of confocal images of microdissected CCDs. The α-IC cell shape was measured by calculating the width:depth (W:D) ratio of AE1 staining.

**Results:** The pendrin cap in normal rabbits (urine pH: 8.28±0.25, $HCO_3^-$:27.2± 4.1 mM) extended beyond the apical boundary defined by ZO-1 and overlapped with nuclear staining (DAPI). A subset of pendrin was contained within $EEA1^+$ endosomes. Acidosis (urine pH: 4.78±0.51, $HCO_3^-$ :17.6±4.3 mM) decreased the pendrin cap volume without shifting distribution. AE1 distribution in α-ICs during acidosis was more rectangular due to increased width and decreased depth (Normal W:D=1.3±0.05; Acidosis =2.4±0.2; p<0.001). Recovery from acidosis (rabbits administered $NH_4Cl$ for 3 days then abruptly transitioned to $NaHCO_3$ for 12-18 h; urine pH: 8.16±0.32, $HCO_3^-$ :29.4±5.6 mM) restored the W:D ratio to the normal range (W:D=1.4±0.05; Recovery: p<0.003). Acidosis also induced a radial redistribution of AE1 in α-ICs: AE1 staining intensity along the outer edge (O) versus staining overlapping the nuclear boundary (I) was measured. During acidosis, AE1 staining intensity increased the O:I ratio, reflecting an increase in AE1 expression on the outer edge (Normal= 1.1±0.03, Acidosis =1.6±0.09; p<0.0004). Upon recovery from acidosis, the ratio returned to near normal (1.2±0.04; vs. NL, p >0.5).

**Conclusions:** Pendrin trafficks through early endosomes, and adaptation to acidosis occurs at least in part via reduction in recycling pendrin. The observed changes in α-cell shape and AE1 distribution likely function to increase basolateral surface area and the supply of AE1 available for anion exchange.

*Funding:* NIDDK Support

## TH-PO632

**The Thick Ascending Limb Is an Important Site of Furosemide-Induced Urinary Acidification** Pauline I.A. de Bruijn,[1] Nina Himmerkus,[2] Markus Bleich,[2] Helle A. Praetorius,[1] Jens G. Leipziger.[1] [1]*Dept of Biomedicine-Physiology, Aarhus Univ, Aarhus, Denmark;* [2]*Institute of Physiology, Christian Albrechts Univ, Kiel, Germany.*

**Background:** Furosemide is a loop diuretic that inhibits NaCl reabsorption in the thick ascending limb (TAL). In addition, furosemide causes urinary acidification and eventually metabolic alkalosis. It is traditionally explained by an increased $Na^+$ load to the distal tubule, which is suggested to facilitate $H^+$ secretion via the apical $H^+$-ATPase in α-intercalated cells. The direct role of TAL on the urinary acidification, however, has never been investigated.

**Methods:** We measured $pH_i$ and $pH_{lumen}$ in single perfused mouse mTALs with BCECF-AM and BCECF acid, respectively.

**Results:** Interestingly, luminal furosemide (100 μM) caused a major, stable and reversible intracellular alkalinization both in HEPES (0.33 ± 0.04, n=7) and $CO_2/HCO_3^-$-buffered conditions (0.14 ± 0.03, n=5). This alkalinization likely indicates increased $H^+$ excretion from the mTAL cytosol. Intriguingly, the furosemide-induced alkalinization was completely blocked by 1 mM luminal amiloride that fully inhibits the apical NHE3 antiporter. This was confirmed with the NHE3 specific blocker #4167, which also was capable of fully inhibiting the alkalinization by furosemide. Blocking of the basolateral NHEs did not have any effect on the furosemide-induced alkalinization. Thus, furosemide likely causes a NHE3-dependent secretion of $H^+$ to the lumen. To investigate this, we measured the $pH_o$ of the tubular lumen with BCECF acid. Furosemide indeed caused a reversible luminal acidification from pH 6.92 ± 0.04 to 6.46 ± 0.03 (n=5) providing direct evidence for this suggested mechanism. In contrast, luminal amiloride alkalinized the lumen providing again direct evidence for an NHE-dependent mechanism of urinary acidification.

**Conclusions:** We show that furosemide causes an intracellular alkalinization in the mTAL that is mediated through activation of apical NHE3. This alkalinization is mirrored by an acidification of the tubular fluid, suggesting that furosemide-induced urinary acidification already takes place in the TAL. These results revise the mechanistic understanding of furosemide-induced urinary acidification.

## TH-PO633

**Acid-Induced Off-Response of PKD2L1 Channel in Xenopus Oocytes and Roles of Ca** Shaimaa Hussein, Chris Dyte, Wang Zheng, Jungwoo Yang, Xing-Zhen Chen. *Membrane Protein Disease Research Group, Dept of Physiology, Univ of Alberta, Edmonton, Canada.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is due to mutations in PKD1 or PKD2. PKD2L1 is a homologue of PKD2 but does not seem to be implicated in ADPKD. PKD2L1 is a non-selective cation channel that was reported to be implicated in sensing extracellular pH in the tongue as well as in the nervous system. PKD2L1 is activated by Ca and acid. Ca-induced activation was demonstrated when PKD2L1 alone was expressed in *Xenopus* oocytes while acid-induced activation was shown when PKD1L3, a homologue of PKD1, was co-expressed. Acid induces an off-response, ie; channel is not activated until the extracellular acid is removed. The mechanisms of, and relationship between, Ca- and acid-induced activations remain unclear.

**Methods:** Here, we showed by the two-microelectrode voltage clamp technique that PKD2L1 over-expressed alone in *Xenopus* oocytes exhibits acid-induced off-response current that was augmented with decreasing the extracellular pH than pH 4.5, in a dose-dependent manner.

**Results:** Neither acid application time nor extracellular trace amounts of Ca is essential for the off-response. Interestingly, addition of Ca to extracellular solution at pH 7.5 blocked the off-response current, with a Ki value ~150 μM, a phenomenon that we called 'Ca reset'. In addition, pre-injection of Ca chelator, EGTA, to PKD2L1-expressing oocytes abolished Ca-induced activation as reported but did not block the off-response. Further, EGTA pre-injection abolished the 'Ca reset', ie; extracellular Ca no longer blocked the off-response current, suggesting that the off-response is inhibited by intracellular Ca. Finally, we showed that PKD2L1 mutation D523N abolished Ca-induced activation and substantially reduced the off-response.

**Conclusions:** Taken together, our study showed that Ca- and acid-induced activations have distinct mechanisms and that Ca plays an important role in the off-response. PKD2L1 expressed alone in oocytes exhibits acid-induced off-response current that have similar characteristics as those previously reported for the PKD2L1/PKD1L3 channel complex. Supported by CIHR, AIHS (to XZC) and AITF (to SH).

*Funding:* Government Support - Non-U.S.

## TH-PO634

**The Role of Sevelamer Carbonate in Increasing Serum Bicarbonate in Hyperphosphatemic CKD Patients with Metabolic Acidosis** Sarah Bezzaoucha,[1] Vincent Pichette,[1] Jean-Philippe Lafrance,[1] Robert Zoël Bell,[1] Louis-Philippe Laurin,[2] Michel Vallee.[1] [1]*Nephrology, Hopital Maisonneuve-Rosemont, Montreal, Canada;* [2]*UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC.*

**Background:** Chronic metabolic acidosis and hyperphosphatemia are prevalent complications in patients with advanced chronic kidney disease. Sevelamer-HCL, a phosphate binder for which the hydrochloride acid is loaded in exchange for phosphate in the intestinal tract, has been previously shown to worsen metabolic acidosis in CKD-patients. However, the use of carbonate sevelamer, a buffered form of sevelamer-HCL in

which chloride is replaced by carbonate, is expected to increase serum bicarbonate and has been proposed as an alternative strategy for the treatment of CKD patients.

**Methods:** To further ascertain the role of sevelamer carbonate in increasing serum bicarbonate, we retrospectively studied the effects of sevelamer carbonate on serum bicarbonate level in 56 acidotic CKD patients not yet receiving maintenance dialysis and for whom sevelamer carbonate was started in order to control their hyperphosphatemia. Serum phosphorus and serum bicarbonate concentrations were assessed before and at monthly intervals (up to 4 months) after using sevelamer carbonate.

**Results:** Bicarbonate mean levels were compared using paired Student's t-tests and mixed-effects linear regression models. The mean bicarbonate level increased from 19.1 ± 2.5 mmol/L before sevelamer carbonate to 21.9 ± 2.9 (p=0.008), 22.2 ± 2.2 (p<0.001) and 22.9 ± 2.5 mmol/L (p<0.001) after the 2nd, 3rd and 4th months of sevelamer carbonate treatment, respectively. In addition, the use of sevelamer carbonate was associated with a statistically significant decrease in the mean serum phosphorus levels: from 1.62 ± 0.22 mmol/L to 1.38 ± 0.26 (p=0.006) and 1.42 ± 0.23 mmol/L (p=0.005) at the 3rd and 4th months, respectively.

**Conclusions:** Our study clearly shows that sevelamer carbonate is actually not only effective as a phosphate binder but also at increasing serum bicarbonate levels to normal values. This could be advantageous for patients who do not receive alkali benefits from dialysis and for whom sodium bicarbonate supplementation is problematic.

*Funding:* Government Support - Non-U.S.

## TH-PO635

### Regulation of the Vacuolar $H^+$-ATPase by 14-3-3 Proteins

Mohammad M. Al-Bataineh,[1] Fan Gong,[1] Hui Li,[1] Vivek Bhalla,[2] Kenneth R. Hallows,[1] Nuria M. Pastor-Soler.[1] *[1]Medicine, U. of Pittsburgh, Pittsburgh, PA; [2]Medicine, Stanford U., Stanford, CA.*

**Background:** The vacuolar $H^+$-ATPase (V-ATPase) mediates ATP-driven transport of $H^+$ across membranes against a gradient. The V-ATPase is expressed at the apical membrane of kidney collecting duct intercalated cells (ICs). Defects in the V-ATPase may cause renal tubular acidosis with kidney and systemic complications. We studied the regulation of V-ATPase in a cell line of IC origin, Clone C cells, and determined that the A subunit is phosphorylated directly by AMPK. In addition AMPK activators induced an acute cytoplasmic redistribution from the apical membrane in ICs. We have identified an AMPK phosphorylation site in the V-ATPase A subunit. We hypothesized that phosphorylation at this site modulates A subunit binding to 14-3-3 proteins, as it conforms to a 'mode 1' 14-3-3 binding sequence. Dimeric 14-3-3 proteins bind to phosphorylated sites on target proteins and thereby modulate their conformation, interactions with other proteins and trafficking.

**Methods:** We used Clone C cells of intercalated cell origin with either wild-type or mutant V-ATPase A subunit at the AMPK phosphorylation site and performed co-immunoprecipitation and immunoblot studies. Extracellular pH measurements, immunoflorescence labeling and confocal microscopy were also performed on polarized Clone C cells to examine V-ATPase activity and subcellular localization.

**Results:** We detected increased co-immunoprecipitation of 14-3-3 proteins to wild-type A subunit in Clone C cells treated with the AMPK activator AICAR as compared with untreated cells. Moreover, additional preliminary results show that the phosphorylation-deficient Ser-to-Ala A-subunit mutant has decreased binding to 14-3-3 proteins. Moreover, AMPK activation inhibits V-ATPase activity and expression at the apical membrane of ICs, an effect that was prevented with expression of this Ser-to-Ala mutant.

**Conclusions:** 14-3-3 proteins may directly bind the V-ATPase A subunit in an AMPK phosphorylation-dependent manner. We propose that this binding participates in the regulation of V-ATPase apical membrane expression and activity by AMPK during metabolic depletion in ICs.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Sanofi Fellowship

## TH-PO636

### Changes in Intercalated Cell Subtypes in Response to Chronic Furosemide Administration

Su-Youn Lee,[1] Ji-Eun Kim,[1] Sae Jin Lee,[1] I. David Weiner,[2,3] Ki-Hwan Han.[1] *[1]Dept of Anatomy, Ewha Womans Univ, Seoul, Korea; [2]Div of Nephrology, Hypertension, and Transplantation, Univ of Florida, Gainesville, FL; [3]Nephrology and Hypertension Section, North Florida/South Georgia Veterans Health System, Gainesville, FL.*

**Background:** Renal intercalated cells play a critical role in acid-base homeostasis and also contribute to the regulation of intravascular volume via $Cl^-$ transport. The diuretic furosemide causes intravascular volume depletion and stimulates proton secretion. The purpose of this study was to examine the effect of chronic furosemide administration on intercalated cell subtypes in the rat kidney.

**Methods:** Male Sprague-Dawley rats received furosemide (12 mg/kg/day) via an osmotic minipump for 7 days; control rats received vehicle.

**Results:** Furosemide administration increased urine volume and decreased urine pH significantly and simultaneously induced mild metabolic alkalosis. We determined furosemide's effect on different intercalated cell subtypes, using antibodies to AE1, pendrin, $H^+$-ATPase, Rhbg and AQP2, combined with quantitative morphometric analysis. In the CNT, furosemide increased the height and mean cellular pendrin and $H^+$-ATPase expression of pendrin-positive cells; the relative number of type B and non A-non B intercalated cells did not change. In the CCD, furosemide increased the number of non-A non B cells significantly, and increased the height and cellular $H^+$-ATPase expression of pendrin-positive cells. The number of principal cells decreased significantly. In the OMCD, furosemide increased the number of type B intercalated cells significantly. Furosemide did not alter significantly the number, height or cellular $H^+$-ATPase expression of AE1-positive cells in the CNT, CCD or OMCD.

**Conclusions:** We conclude that adaptive, multimodality changes in non-type A intercalated cells, including integrated changes in relative numbers, cell height and cellular pendrin and $H^+$-ATPase expression, occur in response to chronic furosemide administration.

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## TH-PO637

### Exogenous Acid Loads Increase ROS Production by the Kidney

Xuming Sun,[1] Julie Reisz,[3] Doris P. Molina,[1] Nebil Nuradin,[4] Rachel R. Tay,[5] Cristina M. Furdui,[3] Thomas D. DuBose,[1,2] Snezana Petrovic.[1,2] *[1]Physiology & Pharmacology; [2]Internal Medicine, Section on Nephrology; [3]Section on Molecular Medicine; [4]Hypertension & Vascular Research Center, Wake Forest School of Medicine; [5]Biology, Wake Forest Univ, NC.*

**Background:** Evidence from clinical trials indicate that high net endogenous acid production (NEAP) with or without frank metabolic acidosis is associated with enhanced progression of CKD. Activation of complement due to ammonium accumulation, ET-1, aldosterone, and AngII have been implicated in the pathogenesis of the adverse effect of acidosis on the kidney. Here, we hypothesized that the accumulation of ROS is associated with the adaptive response of the kidney to high exogenous acid loads. We based this hypothesis on our previous proteomics studies in mIMCD3 cells exposed to low pH, which revealed that changes in the redox state were associated with cellular response to low pH.

**Methods:** To test the hypothesis *in vivo*, we acid-loaded 6 mice with $NH_4Cl$ and determined 8-isoprostane in the urine as an index of kidney ROS levels. To detect oxidized proteins in tissue, we used two novel redox chemical probes, BP-1 and Alk-KE-585.

**Results:** Acid loading doubled urinary 8-isoprostane from 5156±1192 to 11157±2899 pg/mg creatinine after 7 d; p<0.05. 8-isoprostane remained slightly higher than baseline at 30 d (5881±1370 pg/mg creatinine, p=0.5 vs. baseline). We confirmed this with biotin-conjugated BP1 redox probe, visualized on kidney sections with DAB staining. At baseline, the staining was of moderate intensity, present predominantly in tubular epithelial cells in the outer medulla. Acid loading increased the intensity of the staining in both cortex and medulla, reflecting an increase in protein oxidation. The staining was very prominent in medullary rays, most intense in the collecting ducts, and moderate in the proximal tubules. The results were further validated in isolated tubules with fluorescent redox probe Alk-KE-585.

**Conclusions:** Our data suggest that high acid load increases ROS production by the kidney. We propose that the kidney response to high NEAP alters the redox state of kidney tissue, which, if maintained, may represent a mechanism by which high NEAP facilitates progression of CKD.

## TH-PO638

### Loss of 2Cl/H Exchange Function of CLC-5 Causes Dent's Disease

Nobuhiko Sato,[1] Hideomi Yamada,[1] Osamu Yamazaki,[1] Shoko Horita,[1] Masashi Suzuki,[1] Motonobu Nakamura,[1] Yoshitsugu Kaku,[2] Daisuke Yamamoto,[3] Akira Ashida,[3] Takashi Sekine,[4] George Seki.[1] *[1]Internal Medicine, Tokyo Univ, Japan; [2]Fukuoka Chidren's Hospital, Japan; [3]Osaka Medical College, Japan; [4]Toho Univ, Japan.*

**Background:** The molecular mechanism by which loss of CLC-5 functions induces defective endocytosis in renal proximal tubules remains speculative. In a typical Dent's disease patient we have identified a novel CLC-5 mutation E211Q, which may alter the properties of gatekeeping glutamate.

**Methods:** In Xenopus oocytes, the CLC-5 currents and changes in surface pH were measured by two-electrodes voltage-clamp method and pH-sensitive microelectrodes, respectively. In HEK293 cells, the ratiometric pH sensitive GFP mutant pHluorin fused to VAMP2 was used to measure endosome pH.

**Results:** In Xenopus oocytes, the wild-type (WT) CLC-5 showed strongly outward-rectifying currents at the positive voltages. However, E211Q, like an artificial mutant E211A, showed linear currents at the entire voltages. In oocytes injected with WT, the reduction in extracellular pH from 7.4 to 5.4 suppressed the currents by approximately 30%. Moreover, application of positive voltage pulse trains induced robust reduction in surface pH from 7.4 to less than 7.0, consistent with the operation of 2Cl/H exchange. In oocytes injected with E211Q or E211A, by contrast, the reduction in extracellular pH did not suppress the currents. Furthermore, positive voltage pulse trains did not induce any changes in surface pH, indicating that E211Q, like E211A, functions as a pure Cl channel. The conductance sequence (Cl > I) was unchanged in these mutants. The structural analysis based on the CLCe1 crystal structures also suggests that the E211Q mutation mimics the protonation of E211 side chain, thereby facilitating the Cl transport through the opened gate. In HEK293 cell, E211Q was properly directed to endosome but did not decrease endosome pH.

**Conclusions:** These data showed that, for the first time to our knowledge, the loss of 2Cl/H exchange function of CLC-5 causes Dent's disease. They further suggest that the 2Cl/H exchange function of CLC-5 may be required for the proper endosome acidification.

*Funding:* Government Support - Non-U.S.

## TH-PO639

**Association of Serum Bicarbonate with Incident Chronic Kidney Disease in Community Living Elders: The Health ABC Study** Leonard A. Goldenstein, Linda F. Fried, Todd Hayashida Driver, Dena E. Rifkin, Kushang V. Patel, Anne B. Newman, Mark J. Sarnak, Michael Shlipak, Joachim H. Ix. *Nephrology, UCSD, San Diego, CA.*

**Background:** In populations with prevalent chronic kidney disease (CKD), lower serum bicarbonate (HCO3) is associated with more rapid CKD progression, independent of baseline eGFR. Metabolic acidosis may also be a risk factor for development of CKD but that has not been studied. In addition, high bicarbonate may mark lung disease and smoking and may be associated with CKD.

**Methods:** We evaluated 1,076 well-functioning community-living elders who had HCO3 measured at baseline, creatinine and cystatin C at baseline and 7 years later, and did not have CKD (eGFR $\geq$ 60 ml/min/1.73m$^2$) at baseline. eGFR was measured using the combined cystatin C and creatinine equation. We evaluated the adjusted association of baseline HCO3 with incident CKD 7 years later, defined by an eGFR <60 at follow-up with >5 ml/min/1.73m$^2$ loss from baseline.

**Results:** At baseline, the mean age was 75±3 years, eGFR was 86±13 mL/min/1.73m$^2$, and HCO3 was 25.2±1.9 mmol/L. Factors associated with lower HCO3 included male gender, white race, and ace/arb use. Obstructive lung disease, smoking, and diuretic use were associated with higher HCO3. At follow-up, 270 (25%) had developed incident CKD. In models adjusted for baseline eGFR, demographics, and CKD risk factors, individuals with HCO3 <23mmol/L had a nearly 2-fold odds of incident CKD (Table). Higher levels were not associated with incident CKD.

**Conclusions:** Metabolic acidosis is a novel risk factor for development of incident CKD in community-living elders. If confirmed, HCO3 may give insights into kidney tubule health even among persons without CKD by standard clinical definitions.

Table. Association of HCO3 with Incident CKD in Community-Living Elders

| | HCO3 (mmol/L) | | |
|---|---|---|---|
| | <23.0 | 23.0-27.9 | ≥28.0 |
| | N=77 (7%) | N=896 (83%) | N=103 (10%) |
| Model 1*, OR (95% CI) | 1.88 (1.10-3.23) | 1.00 (ref) | 1.26 (0.75-2.13) |
| Model 2**, OR (95% CI) | 1.93 (1.11-3.35) | 1.00 (ref) | 1.03 (0.59-1.80) |

** Adjusted for age, race, gender, baseline eGFR*
*** Adjusted for Model 1 variables plus diabetes, systolic BP, obstructive lung disease, smoking, RAAS inhibitor use, diuretic use*

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## TH-PO640

**Lack of Interaction between *APOL1* Variants and Hypertension Treatment: The African American Study of Hypertension and Kidney Disease (AASK)** Wen Hong Linda Kao,[1] Brad C. Astor,[2] Man Li,[1] Rulan S. Parekh,[3] Tom Greene,[4] Jackson T. Wright,[5] Lawrence J. Appel,[1] Jeffrey B. Kopp,[7] Michael S. Lipkowitz.[6] *[1]Epidemiology and Medicine, Johns Hopkins Univ, Baltimore, MD; [2]Medicine and Population Health, Univ of Wisconsin, Madison, WI; [3]Nephrology, Hospital for Sick Children, Toronto, Canada; [4]Biostatistics, Univ of Utah, Salt Lake City, UT; [5]Medicine, Case Western Reserve Univ, Cleveland, OH; [6]Nephrology, Georgetown Univ, Washington, DC; [7]Kidney Disease Section, NIDDK, Bethesda, MD.*

**Background:** The apolipoprotein L1 (*APOL1*) G1 and G2 alleles are strongly associated with ESRD susceptibility in African Americans, but no study has examined the impact of these variants on the effect of hypertension treatments in a clinical trial setting.

**Methods:** We conducted a gene-by-treatment analysis in 693 participants of The AASK trial. Outcome was ESRD or doubling of serum creatinine. Cox regression was used to adjust for age, sex, ancestry, and baseline GFR.

**Results:** Overall, 288 (41.6%) reached the composite outcome. Of the 160 individuals with 2 copies of the *APOL1* high-risk haplotype, 58% progressed (adjusted relative hazard [$RH_{adj}$] of 2.03 compared to all others;p<0.001). There was no evidence for interaction between *APOL1* and baseline proteinuria or intervention assignment. The $RH_{adj}$ comparing the non-ACE inhibitor to ACE inhibitor was 1.36 (95% CI[0.91, 2.04]) for the low-risk *APOL1* group and 1.51 (95% CI[0.83, 2.73]) for the high-risk *APOL1* group ($p_{interaction}$=0.85). There was no effect of assignment to intensive vs. non-intensive blood pressure control in either the low-risk *APOL1* group ($RH_{adj=}$1.11, 95%CI[0.73, 1.65]) or the high-risk *APOL1* group ($RH_{adj=}$1.33, 95%CI[0.73, 2.40]) ($p_{interaction}$=0.85).

**Conclusions:** Almost 60% of these participants with hypertensive nephropathy and 2 copies of the *APOL1* high-risk haplotype progressed, a risk that was two-fold higher than their low-risk counterparts. Blood pressure targets or medications did not modify this association. Thus, it is important to identify other modifiable risk factors or treatment approaches that differentiate the progressors from non-progressors within the high-risk *APOL1* group.

*Funding:* NIDDK Support

## TH-PO642

**Association of the High Risk *APOL1* Genotype with Longitudinal GFR Is Modified by HIV** Michelle M. Estrella,[1] Man Li,[1] Rulan S. Parekh,[1] Alison G. Abraham,[1] Shehnaz K. Hussain,[2] Wen Hong Linda Kao.[1] *[1]Johns Hopkins Univ; [2]UCLA.*

**Background:** *APOL1* risk alleles are associated with kidney disease among African Americans. Whether HIV modifies this association is unknown.

**Methods:** We studied African American men infected with or at risk for HIV in the Multicenter AIDS Cohort Study from 2003 to 2012 (median follow-up 6.9y). Subjects were genotyped for the *APOL1* G1 and G2 risk alleles. We used mixed effects models to estimate the annual rate of eGFR (ml/min/1.73m$^2$) decline, comparing men with 2 vs. 0/1 allele and adjusting for traditional kidney disease and HIV risk factors.

**Results:** Of 272 HIV- and 343 HIV+ men, 37 and 53 had 2 risk alleles, respectively. Compared with men carrying 0/1 allele, those with 2 had a lower mean baseline eGFR (98.8 vs. 90.2, p<0.001) but were similar in age, diabetes and hypertension history, injection drug use as well as hepatitis C and HIV infection. Among HIV+ men, the mean CD4 count and proportion with undetectable HIV RNA were similar in those with 2 vs. 1/0 risk allele. The annual rate of eGFR decline among HIV- men was similar by *APOL1* genotype.

| Annual Change in eGFR (95% CI) Associated with the High Risk *APOL1* Genotype by HIV Status* | | |
|---|---|---|
| 2 vs. 0/1 *APOL1* allele | HIV- | HIV+ |
| Unadjusted | -0.44 (-4.28, 2.99) | -13.07 (-16.00, -9.27) |
| Adjusted for age, baseline diabetes and hypertension | -0.47 (-4.04, 2.77) | -12.88 (-15.72, -9.39) |
| Additionally adjusted for baseline hepatitis C infection and injection drug use | -0.49 (-3.92, 2.89) | -13.68 (-16.77, -10.32) |
| Additionally adjusted for baseline eGFR | 0.30 (-2.28, 3.23) | -4.64 (-7.07, -2.02) |
| Additionally adjusted for baseline HIV factors† | - | -5.03 (-7.34, -2.12) |

*P-int<0.001
†CD4 count, HIV RNA<50 copies/mL, AIDS and antiretroviral therapy

Conversely, HIV+ men with 2 risk alleles had a faster annual rate of eGFR decline compared to those with 0/1 allele (p-int<0.001). This remained significant after adjustment for HIV factors. Adjustment for ancestry (n=575) and exclusion of men with baseline eGFR<60 (n=26) yielded similar estimates.

**Conclusions:** Kidney function decline is augmented by HIV infection among carriers of the *APOL1* high risk alleles.

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## TH-PO643

**MYH9 Association with Glomerular Disease Independent of APOL1 Risk Factors** George W. Nelson,[1] Randall C. Johnson,[1] Sophie Limou,[2] Jeffrey B. Kopp,[3] Cheryl Ann Winkler.[2] *[1]BSP-CCR Genetics Core, SAIC-Frederick/FNLCR, Frederick, MD; [2]Basic Research Laboratory, SAIC-Frederick/FNLCR, Frederick, MD; [3]NIDDK-NIH, Bethesda, MD.*

**Background:** The increased risk of FSGS and HIV-associated glomerular disease (HIVAN) in African Americans (AA) prompted the use of admixture mapping which identified the profound association of Chr22 with these diseases. The initial association was centered on *MYH9*, previously associated with glomerular disease. Further screening identified more strongly associated factors, G1 and G2, in the adjacent gene *APOL1*. The associations with *MYH9* SNPs were then seen as resulting from linkage disequilibrium (LD) around G1 and G2, caused by selection for resistance against trypanosome infection. Several studies have reported *MYH9* genetic effects independent of *APOL1* variants. We looked for independent *MYH9* effects in our AA cohorts of FSGS and HIVAN cases and controls, which have shown some of the strongest reported Chr22 effects.

**Methods:** *APOL1* and *MYH9* variants were typed for FSGS on 218 FSGS cases and 386 controls, and 56 HIVAN cases and 243 HIV-positive controls. Due to complete negative LD, only 6 G1/G2 genotypes occur. We analyzed the association of the most strongly associated *MYH9* factor, rs2413396, in subsets of the subjects carrying each of these genotypes, and performed an analysis stratified for the 6 G1/G2 genotypes (CMH test).

**Results:** The stratified analysis shows an independent association for rs2413396 for combined HIVAN/FSGS: OR = 2.0, p = 0.0001. Five of the six subsets defined by G1/G2 genotype showed the same direction of association for rs2413396, which was significant in two subgroups: subjects carrying one copy of G1 and no G2: OR = 5.6, p = 0.006, and subjects carrying one copy each of G1 and G2: OR = 2.8, p = 0.04 (FET).

**Conclusions:** There is residual glomerular disease association of *MYH9* variants not attributable to *APOL1* G1/G2. The source of this association is unknown; rs2413396 is one of several *MYH9* SNPs with similar associations, none of which appear to be functional. It is possible that these SNPs are tracking an unknown factor, either one with a small effect, or a factor with a larger effect, tracked by both G1/G2 and the *MYH9* SNPs.

*Funding:* Other NIH Support - contract number HHSN261200800001E

## TH-PO644

**Factors Related to CKD Progression among African Americans with and without Apolipoprotein L1 (APOL1) Gene Variants** Teresa K. Chen,[1] Wen Hong Linda Kao,[1] Michael J. Choi,[1] Brad C. Astor,[1,2] Julia J. Scialla,[3] Lawrence J. Appel,[1] Deidra C. Crews.[1] *[1]Johns Hopkins U., Baltimore, MD; [2]U. of Wisconsin-Madison, Madison, WI; [3]U. of Miami, Miami, FL.*

**Background:** Common coding variants in *APOL1* are associated with increased risk of progressive CKD, however, not all with this high-risk genetic background experience progressive disease. We examined potentially modifiable factors associated with CKD

progression among African Americans with hypertension-attributed nephropathy by *APOL1* risk allele status.

**Methods:** We performed a retrospective cohort study including 693 African American Study of Kidney Disease and Hypertension (AASK) participants. Using multivariable Cox models, we determined the hazard ratio for CKD progression (defined as development of ESRD or doubling of serum creatinine during follow up) associated with each putative risk factor stratified by *APOL1* status (those with 2 copies of the high-risk variant vs. all others).

**Results:** Participant mean age was 54 years and 160 (23%) had *APOL1* high-risk allele status. A total of 288 (41.6%) participants experienced CKD progression during a mean follow up of 7.8 years. Our base model adjusted for age, gender, % European ancestry, baseline eGFR and randomized blood pressure and drug groups. We confirmed that the hazard ratio (HR) comparing high vs. low risk allele status was 1.87 (95% CI 1.45-2.41). When additional variables were added to the base model, none appeared to modify the association between *APOL1* high-risk allele status and CKD progression.

Table. Factors Associated with CKD Progression among AASK Participants, by *APOL1* Risk Allele Status

| Factors | APOL1 Low-Risk Status HR (95% CI) for CKD Progression | APOL1 High-Risk Status HR (95% CI) for CKD Progression | P interaction (from full Cox model) |
|---|---|---|---|
| **Base Model** | | | |
| Age, per 10 years older | 0.98 (0.97, 0.99) | 0.97 (0.95, 0.98) | 0.17 |
| Female vs. male | 0.98 (0.73, 1.31) | 0.86 (0.55, 1.33) | 0.57 |
| % European ancestry per natural log ↑ | 1.10 (0.93, 1.31) | 1.08 (0.82, 1.41) | 0.96 |
| Baseline eGFR, per 10 ml/min/1.73 m² ↓ | 1.63 (1.46, 1.82) | 1.87 (1.54, 2.27) | 0.21 |
| BP group, standard vs. low | 0.93 (0.69, 1.24) | 0.90 (0.59, 1.38) | 0.78 |
| Drug group | | | 0.68 |
| Ramipril | reference | reference | |
| Metoprolol | 1.13 (0.83, 1.56) | 1.12 (0.70, 1.79) | |
| Amlodipine | 1.19 (0.80, 1.76) | 1.68 (0.92, 3.07) | |
| **Additional Variables Added to Base Model** | | | |
| Current vs. never smoker | 1.15 (0.82, 1.62) | 1.07 (0.64, 1.79) | 0.50 |
| Family history of ESRD, yes vs. no | 0.90 (0.58, 1.40) | 0.80 (0.43, 1.48) | 0.83 |
| Annual income, mid-high vs. $0-14,999 | 0.95 (0.68, 1.33) | 0.84 (0.51,1.37) | 0.69 |
| No H.S. vs. at least H.S. education | 0.97 (0.70, 1.34) | 1.05 (0.63, 1.74) | 0.98 |
| Systolic BP, per 10 mmHg ↑ | 1.03 (0.98, 1.09) | 1.08 (0.99, 1.19) | 0.23 |
| BMI, per 1 kg/m² ↑ | 1.03 (1.01, 1.05) | 1.03 (0.99, 1.06) | 0.78 |
| Total cholesterol, per 10 mg/dL ↑ | 1.04 (1.01, 1.08) | 1.04 (0.99, 1.09) | 0.59 |
| HDL, per 10 mg/dL ↑ | 1.05 (0.95, 1.15) | 1.05 (0.90, 1.22) | 0.52 |
| Hematocrit, per 1% ↑ | 1.00 (0.96, 1.03) | 0.99 (0.95, 1.03) | 0.93 |
| Serum phosphorus, per 1 mg/dL ↑ | 1.18 (0.87, 1.59) | 1.10 (0.71, 1.71) | 0.88 |
| Serum calcium phosphorus product, per 1 unit ↑ | 1.01 (0.98, 1.04) | 1.00 (0.95, 1.05) | 0.87 |
| Serum uric acid, per 1 mg/dL ↑ | 1.03 (0.95, 1.12) | 0.92 (0.81, 1.05) | 0.36 |
| 24 hr urine protein, per 1 unit natural log ↑ | 1.88 (1.68, 2.11) | 1.59 (1.34, 1.88) | 0.37 |
| 24 hr urine sodium, per 1 gram ↑ | 1.05 (0.98, 1.13) | 1.08 (0.98, 1.19) | 0.74 |
| 24 hr urine potassium, per 1 gram ↑ | 1.15 (0.98, 1.35) | 0.96 (0.73, 1.27) | 0.36 |
| 24 hr urine urea nitrogen, per 1 gram ↑ | 1.07 (1.03, 1.12) | 1.01 (0.95, 1.09) | 0.20 |
| Net endogenous acid production, per 10 unit ↑ | 1.06 (1.01, 1.11) | 1.04 (0.98, 1.10) | 0.43 |

**Conclusions:** Among a cohort of African Americans with hypertension-attributed nephropathy, we found no factors modifying the risk of CKD progression associated with *APOL1* high-risk allele status. Further investigation in larger studies is warranted to determine whether other environmental risk factors for progressive kidney disease exist among individuals with high-risk *APOL1* alleles.

## TH-PO645

**APOL1 Genotype and Racial Disparity in Chronic Kidney Disease (CKD) Progression: The CRIC Study** Afshin Parsa,[1] Dawei Xie,[2] Chi-Yuan Hsu,[4] Harold I. Feldman,[3,2] John W. Kusek,[5] Lawrence J. Appel.[6,7] *[1]Dept of Medicine, Univ of Maryland; [2]Dept of Biostatistics and Epidemiology, Univ of Pennsylvania; [3]Dept of Medicine, Univ of Pennsylvania; [4]Dept of Medicine, UCSF; [5]NIDDK, NIH; [6]Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Univ; [7]CRIC Study Group.*

**Background:** African Americans (AAs) are at increased risk for end-stage renal disease (ESRD) compared to European Americans (EAs). *APOL1* risk variants, present in AAs but nearly absent in EAs, may partially account for this disparity. We evaluated *APOL1* genotype as a mediator of differences in CKD progression between AAs and EAs.

**Methods:** We studied 2955 participants of the Chronic Renal Insufficiency Cohort (CRIC) Study with CKD (48% AA, 46% with diabetes). We performed hierarchical multivariate analyses of CKD progression, stratified by diabetes and *APOL1* genotype. Primary outcomes were slope in estimated glomerular filtration rate (eGFR) and the composite renal event of ESRD or halving of eGFR.

**Results:** Among participants with and without diabetes, AAs without the *APOL1* risk genotype had a persistent, but attenuated elevation in the rate of renal events compared to EAs. Parallel comparisons of eGFR decline showed complete attenuation of AA-EA differences in the absence of *APOL1* risk genotype (Table). Table. Adjusted Differences in eGFR Slope and Rate of Renal Events.

| Comparison groups | | eGFR Slope difference (ml/min/1.73m²/yr) | | Hazard Ratio (HR) of Renal Events | |
|---|---|---|---|---|---|
| | | Estimate | P value | HR | P value |
| With Diabetes | AA with APOL1 Risk vs all EA | **-1.3** | <0.001 | **2.0** | <0.001 |
| | AA without APOL1 Risk vs all EA | **-0.4** | 0.093 | **1.4** | 0.006 |
| | | | | | |
| Without Diabetes | AA with APOL1 Risk vs all EA | **-1.1** | <0.0001 | **2.7** | <0.0001 |
| | AA without APOL1 Risk vs all EA | **0.1** | 0.648 | **1.6** | 0.010 |

**Conclusions:** *APOL1* genotype is significantly associated with CKD progression in Aas compared to Eas, regardless of diabetes status. Our results, in the context of prior studies, suggest that *APOL1* risk alleles increase CKD progression, irrespective of the etiology of CKD.

*Funding:* NIDDK Support

## TH-PO646

**p.E66Q Variant of α-Galactosidase A Does Not Affect the Progression of Chronic Kidney Disease** Hirofumi Watanabe,[1] Shin Goto,[1] Hiroki Maruyama,[2] Ichiei Narita.[1] *[1]Div of Clinical Nephrology and Rheumatology, Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Dept of Clinical Nephroscience, Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan.*

**Background:** The p.E66Q variant in the *α-galactosidase A (GLA)* is frequently found in the screening of Fabry disease in chronic dialysis patients in Japan. However, recent reports suggested that pathologically and biochemically, p.E66Q of *GLA* is not a disease-causing mutation but a functional polymorphism. To evaluate the role of p.E66Q in progression of renal diseases, we performed a genetic association study in patients with chronic kidney disease.

**Methods:** We extracted genomic DNA from the blood of 2691 patients who attended medical institutions in Niigata Prefecture, Japan and agreed to genome analysis. These patients included 1705 chronic hemodialysis patients and 986 non-dialysis patients. The p.E66Q was detected using TaqMan SNP genotyping assay. We compared the allele frequency of p.E66Q in chronic hemodialysis patients with that of non-dialysis patients and Japanese general population (Eur J Neurol 2013). Furthermore, in non-dialysis patients, we compared eGFR in the presence or absence of p.E66Q variant.

**Results:** Among 2401 alleles in the chronic hemodialysis patients, 21 alleles were found to harbor p.E66Q variant. Five alleles were hemizygotes from 1009 males, 14 were heterozygotes from 696 females, and one was homozygote from one female. The allele frequency in the chronic hemodialysis patients was 0.87%. Among 1505 alleles in non-dialysis patients, 8 were found to harbor p.E66Q variant, and allele frequency was 0.53%. There was no significant difference in the p.E66Q frequency of chronic hemodialysis patients compared with non-dialysis patients ($P$ = 0.22) and with Japanese general population (0.64%, $P$ =0.25). The eGFR of non-dialysis patients harboring p.E66Q was not significantly different compared with that of patients with wild-type allele (66.3 ± 11.4 vs. 62.9 ± 16.8 ml/min/1.73m², $P$ = 0.70).

**Conclusions:** The present genetic association study indicated that the p.E66Q variant of *GLA* does not affect the progression to end stage renal disease.

## TH-PO647

**Discovery of Novel Candidate Genetic Markers Associated with Pediatric CKD Progression in the PediGFR Study** Jayanta Gupta,[1] Peter A. Kanetsky,[1] Nandita Mitra,[1] Elke Wuehl,[2] Anna Kottgen,[3] Matthias Wuttke,[3] Susan L. Furth,[4] Bradley A. Warady,[5] Franz S. Schaefer,[2] Craig S. Wong.[6] *[1]Dept Biost/Epi, U Penn, Philadelphia, PA; [2]Ped Neph Div, U Heidelberg, Heidelberg, Germany; [3]Renal Div, U Frieburg, Frieburg, Germany; [4]Div Neph., Children's Hosp. of Philadelphia, Philadelphia, PA; [5]Sect Neph., Children's Mercy Hosp., Kansas City, MO; [6]Div Nephr, U New Mexico Children's Hosp., Albuquerque, NM.*

**Background:** Traditional risk factors for chronic kidney disease (CKD) only partially explain the varying rate of GFR decline in children with CKD. The PediGFR study is determining the genetic contribution linked to CKD progression via meta-analysis of the most promising SNP markers observed in the genome-wide scans from the pediatric CKD cohort studies:CKiD, ESCAPE, and 4C.

**Methods:** Each prospective study collected genetic samples and longitudinal data on GFR (measured or estimated). We genotyped DNA using the Illumina® Omni 2.5 BeadChip. For each study, we used linear regression models to determine associations between SNP markers and GFR slope adjusting for age, sex and population stratification. The 100 most significant markers from each of five subgroup populations [(CEU ancestry in CKiD (267), 4C (599) and ESCAPE (286); and Turkish ancestry in 4C (331) and ESCAPE (66)] were selected for meta-analysis. Using METAL, subgroup-specific effect estimates were combined by the inverse variance method.

**Results:** Analyses are currently ongoing. To date, 11 markers surpassed a threshold of $P < 1\times10^{-5}$ in combined results from at least 3 subgroup populations, although we noted heterogeneity of effect ($P < 0.05$ for Cochran's Q; $I^2 > 50\%$) at two of these markers. Of the remaining 9 markers, 8 localized to the same genomic region at 2q22.3, and one to 15q21.1.

**Conclusions:** The PediGFR will continue analyses to include association testing of SNP markers with ESRD outcomes and within subgroups defined by glomerular and non-glomerular disease. We will assess the putative biological importance of these markers using publicly available databases such as ENCODE. We will replicate these identified SNP markers in the adult Chronic Renal Insufficiency Cohort study.

*Funding:* NIDDK Support

## TH-PO648

**Genetic Markers Associated with Erythrocyte Traits in the Chronic Renal Insufficiency Cohort (CRIC) Study** Jayanta Gupta,[1] Meredith A. Atkinson,[2] Craig S. Wong,[3] Peter A. Kanetsky,[1] Susan L. Furth.[1] *[1]Univ of Pennsylvania; [2]Johns Hopkins Univ; [3]Univ of New Mexico.*

**Background:** Anemia is a prevalent comorbidity in CKD. However, across patients with CKD there is variability in the severity of anemia, likely due in part to genetic determinants of erythrocyte traits. Previous genome-wide association studies (GWAS) in healthy subjects have identified genetic variants associated with a variety of erythrocyte traits, but the associations in CKD patients has not been well studied.

**Methods:** In 1,450 African American (AA) and 1,560 European American (EA) subjects with CKD in the Chronic Renal Insufficiency Cohort Study (CRIC), we assessed

the association of baseline measures of 6 erythrocyte traits (Hgb, Hct, MCV, MCHC, MCH, and RBC) with previously identified single-nucleotide polymorphisms (SNPs). Linear regression models were adjusted for age, sex, baseline eGFR use of angiotensin converting enzyme inhibitors, erythropoietin stimulating agents, and population stratification (by principal components). We used Bonferroni correction to account for multiple comparisons.

**Results:** Among AA subjects a significant association between multiple SNPs on the 16p13.3 gene and MCH, MCHC, and MCV was found, consistent with previous analyses. A SNP within *MPP1* was associated with higher MCH ($p<0.0001$), higher MCV ($p<0.0001$), and higher RBC ($p=0.003$). None of the markers examined were associated with Hgb or Hct in this group. In EA subjects, a SNP within *SLC17A3* was associated with higher MCH ($p=1.6x10^{-5}$), higher MCHC ($p=0.003$), and higher MCV ($p=0.004$). A SNP within the *HBSIL/MYB* was associated with lower Hct ($p=0.001$). SNPs at *SLC17A3*, *HBSIL/MYB*, and *GCDH* were positively associated with MCH, and a SNP at the *TMPRSS6* locus was negatively associated with MCH. Further adjustment for use of iron supplementation did not alter interpretation of these findings.

**Conclusions:** We have confirmed the association of selected genetic variants with erythrocyte traits in a CKD cohort, including racial differences in specific loci. Further study will allow us to determine if specific SNPs are longitudinally associated with incident anemia.

*Funding:* NIDDK Support

## TH-PO649

**Association of IL6 -174G>C Polymorphism with Type 1 Diabetes and Diabetic Nephropathy** Marcela Ururahy,[1] Karla Souza,[1] Yonara Monique Oliveira,[1] Melina Bezerra Loureiro,[1] Heglayne P. Silva,[1] Thamara R. de Melo,[1] André D. Luchessi,[1] Rosario D.C. Hirata,[2] Maria Das Graças Almeida,[1] Ricardo Fernando Arrais,[3] Sonia Q. Doi,[4] Mario Hiroyuki Hirata,[2] Adriana Augusto de Rezende.[1] *[1]Clinical and Toxicological Analysis, UFRN, Natal, RN, Brazil; [2]Clinical and Toxicological Analysis, USP, São Paulo, SP, Brazil; [3]Pediatrics, UFRN, Natal, RN, Brazil; [4]Medicine, Uniformed Services Univ, Bethesda, MD.*

**Background:** Diabetic nephropathy (DN) is a major complication of type 1 diabetes (T1D). Studies have shown inflammation, and, mainly, pro-inflammatory cytokines, as key pathophysiological factors in T1D and DN development and progression. We aimed to investigate the association of *IL6* gene polymorphisms with T1D and markers of renal function.

**Methods:** *IL6* (-634C>G and -174G>C) polymorphisms were analyzed in 173 children and adolescents with T1D and 144 normoglycemic (NG) subjects. Glycemic control (glycated hemoglobin and fasting serum glucose), lipid metabolism (serum lipids), and kidney function markers (serum urea and creatinine, and urinary albumin-to-creatinine ratio – ACR) were evaluated.

**Results:** *IL6* -174G>C polymorphism was associated with susceptibility to T1D in a Log-additive model (OR=1.53, CI=1.01-2.31, $p<0.05$). Studying only T1D patients (Table 1), CC carriers for *IL6* -174G>C polymorphism showed higher concentrations of ACR, total cholesterol, and LDL-cholesterol ($p<0.05$). No association was found for *IL6* -634C>G polymorphism.

Table 1 - Relationship between *IL6* -174G>C genotypes and biochemical parameters of T1D patients

| Genotype | ACR mg/g of creatinine | Total cholesterol mg/dL | LDL-cholesterol mg/dL |
|---|---|---|---|
| GG+GC | 16.7±2.7 | 171.7±5.1 | 109.0±4.6 |
| CC | 65.6±41.4 | 243.2±43.6 | 172.2±42.9 |
| p-value | 0.001 | 0.002 | 0.002 |

Results are mean±SEM. Only the relationships with significant p-values are represented.

**Conclusions:** These results suggest that *IL6* -174G>C polymorphism is associated with T1D susceptibility, and, moreover, that T1D patients carrying CC genotype could be more susceptive to develop renal injury.

*Funding:* Government Support - Non-U.S.

## TH-PO650

**RREB1, a Modulator of the Renin-Angiotensin System (RAS), Is a Novel Nephropathy Gene in African Americans** Jason A. Bonomo,[1] Maggie Ng,[1] Barry I. Freedman,[2] Donald W. Bowden.[1] *[1]Ctr for Genomics & Personalized Med Res, Wake Forest School of Medicine, Winston Salem, NC; [2]Int. Med/Nephrology, WFSOM.*

**Background:** Genetic efforts to relate the renin-angiotensin (*RAS*) pathway to nephropathy have had mixed results. *RREB1* (ras-responsive element binding protein 1) is little-studied zinc-finger binding protein which represses the angiotensinogen gene (*AGT*).

**Methods:** Exome sequencing data from 500 African American (AA) cases with T2D-ESKD and 500 AA population-based controls from the T2D-GENES study was analyzed for association with T2D-ESKD. Variants from this discovery phase were genotyped in an independent cohort of 1,250 AA T2D-ESKD cases, 1,450 non-T2D ESKD cases, and 850 controls, analyzed, and adjusted for age, gender, admixture, and *APOL1* G1/G2. A locus-wide analysis was performed, compiling putatively functional low-frequency *RREB1* variants from 1000 Genomes, Exome Variant Server, and RegulomeDB, and were genotyped in the above cases and controls. Sequence kernal association testing (SKAT) was performed in non-T2D ESKD cases and controls.

**Results:** Exome-wide analysis identified two *RREB1* variants associated with T2D-ESKD (rs9379084 [N1171D] p=3.6E-4, OR=0.30 and rs41302867 [intron] p=7.5E-4, OR=0.23). Replication confirmed association (p=0.019-0.029; OR=0.54-0.56) with age, gender, admixture, and APOL1 G1/G2 adjustment. 22 *RREB1* variants were genotyped in the locus-wide analysis. A strong preference of *RREB1* association was noted in non-T2D ESKD cases; 6 variants were associated with non-T2D ESKD in single-SNP association testing (p=0.048-0.0075) with covariate adjustment. The most strongly associated variant was rs35147576 (M610I, OR=0.55). In unadjusted and adjusted SKAT model weighted for rare variants, association p-values of 5.6E-6 and 0.0041, resp., were computed.

**Conclusions:** *RREB1* offers a strong, plausible biological association to account for nephropathy risk through interactions with *AGT*. Variants studied were putatively functional, either resulting in missense mutations or functional intronic variants based on RegulomeDB score. These data suggest a novel role for *RREB1* in nephropathy and may provide a missing link between the RAS and nephropathy.

*Funding:* NIDDK Support

## TH-PO651

**Next Generation Sequencing Identifies Novel Genomic Variants as Candidates for African-American Familial FSGS** Siddharth A. Shah,[1] James Lyons-Weiler,[2] Abhay N. Vats.[1] *[1]Nephrology, Children's Hospital of Pittsburgh, Pittsburgh, PA; [2]Genomics and Proteomics Core Laboratories, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** In the past decade, various genes have been identified for FSGS. Previously, we described a four generation African American family with phenotype of hypertension and FSGS with a new linkage to 9q31-32 region. We performed whole exome sequencing (WES) to identify the candidate gene.

**Methods:** We identified new affected and non affected members of this family and the collected DNA was used for WES. Individual and pooled samples were used for this approach. Barcodes and final sequencing adapters were applied to each sample prior to pooling for emulsion PCR and sequencing. WES was performed using SOLiD platform with 50X coverage. The quality control, ambiguity analysis, and mapping was performed using various algorithms, databases and software tools including Lifescope V 2.5, Bamstats, Fast QC, Baconator, C-scoring and DbSNP.

**Results:** There were 101 members in the family with 37 affecteds. Unpooled analyses utilized DNA from 2 affected individuals while pooled samples utilized 13 affecteds in pool 1 and 10 non-affecteds in pool 2. DNA capture targeted human exons plus UTR totaling 71 Mb. Bamstats and Fast QC analysis revealed good quality data. The analyses of target 9q31-32 region (between bases 9108672867-114233187) identified 7648 variants in unpooled samples of which 632 were heterozygous (W-score:1.9-2). Further comparison analyses narrowed the focus to several interesting variants including rs114861534, rs 115259973 and rs138519256 in AKAP2, PTPN3 and LTB4DH genes respectively.

**Conclusions:** We were able to generate high quality data from exome sequencing performed on archived, individual as well as pooled samples. Several novel variants were identified as candidates for autosomal dominant FSGS in a large family belonging to the high risk African- American ethicity. 2 of the variants (rs114861534 and rs 115259973) are particularly interesting as they are highly expressed in kidneys and have very low allele frequency in target population. Further functional and population genetic studies will identify the role of identified variants in FSGS pathogenesis.

## TH-PO652

**Anillin-Mediated Dysregulation of CD2AP/Nephrin/AKT Signaling May Underlie Podocyte Dysfunction in Autosomal Dominant Focal Segmental Glomerulosclerosis** Gentzon Hall,[1,3] Alison Homstad,[3] Andrew F. Malone,[1,3] Guanghong Wu,[3] Thomas Lindsey,[3] Robert F. Spurney,[1] Nils Hanke,[4] Mario Schiffer,[4] Michelle P. Winn,[1,3] Rasheed A. Gbadegesin.[2,3] *[1]Internal Medicine, Duke Univ Medical Center, Durham, NC; [2]Pediatrics, Duke Univ Medical Center, Durham, NC; [3]Center for Human Genetics, Duke Univ Medical Center, Durham, NC; [4]Div of Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** A heterozygous missense mutation (Arg431Cys; R431C) within the F-actin binding domain of the cell cycle regulator anillin (ANLN) has been recently identified as a cause of autosomal dominant FSGS. In addition to its known roles in cytokinesis and the regulation of cytoskeletal dynamics, ANLN is recognized as a regulator of PI-3K signaling through interactions with the PI-3K regulatory subunit, p85, and the slit diaphragm proteins CD2AP and nephrin. Given these essential functions, we sought to characterize the role of ANLN in the modulation of PI-3K signaling and cell motility in podocytes.

**Methods:** Immunoprecipitation studies, immunoblot analyses, targeted gene knockdown (KD) studies and scratch wound healing assays were performed by standard methods in conditionally immortalized human podocytes (CIHPs). Immunoprecipitation studies were conducted in HEK 293 cells. Cell lines stably expressing tGFP-$ANLN_{WT}$ and tGFP-$ANLN_{R431C}$ were created via transduction of CIHPs with lentiviral vectors expressing *tGFP-$ANLN_{WT}$* and *tGFP-$ANLN_{R431C}$* driven by the CMV promoter.

**Results:** *ANLN* (KD) reduced CD2AP expression in CIHPs. $ANLN_{R431C}$ impaired CD2AP binding in HEK 293 cells relative to $ANLN_{WT}$-expressing cells. *ANLN* (KD) impaired basal motility and attenuated AKT phosphorylation at serine 473 (Ser473) in podocytes. Finally, overexpression of $ANLN_{R431C}$ enhanced basal podocyte motility.

**Conclusions:** ANLN appears to be a critical modulator of PI-3K signaling and podocyte motility possibly via a mechanism involving CD2AP/Nephrin/AKT-dependent signaling. Further study of the role of ANLN in the modulation of PI-3K-dependent podocyte motility signaling may uncover novel therapeutic targets for the treatment of FSGS.

*Funding:* NIDDK Support

## TH-PO653

**Discovery, Fine Mapping and Replication of Differential Methylation Associated with MicroRNAs and Chronic Kidney Disease** Laura Jane Smyth, Alexander P. Maxwell, A.J. McKnight. *Nephrology Research, Centre for Public Health, Queen's Univ of Belfast, Belfast, Co. Antrim, United Kingdom.*

**Background:** DNA methylation and miRNAs are associated with complex disease and are altered in uremic patients. This study investigated association between DNA methylation and chronic kidney disease (CKD) using a relatively large-scale, case-control approach.

**Methods:** The Infinium® HumanMethylation 450K BeadChip array (Illumina, Inc, USA) was used to analyse DNA methylation across the methylome in 255 CKD cases and 152 unaffected controls (MDRD eGFR>60 mL/min/1.73m$^2$). Following stringent quality control, methylation levels were analysed and results adjusted for multiple testing using the *BenjaminiHochberg* approach. Quantitative methylation values were obtained at single-CpG level for 2,249 sites associated with miRNA genes across the genome.

**Results:** Six miRNA genes on autosomes were selected for fine mapping and replication based on P<10^-5 in the discovery collection; *MIR550-2*, *MIR940*, *MIR34A*, *MIR429*, *MIR141*, and *MIR329-2*. Bidirectional Sanger sequencing of the genetic region immediately flanking CpG sites of interest in 23 cases and 23 controls for five of these miRNA genes revealed 37 methylated CpG sites; *MIR550-2* did not generate robust sequencing results. Sequencing of untreated genomic DNA revealed 13 known and two novel SNPs flanking the CpG site of interest. Replication using an independent cohort of cases with CKD compared to a general control population (n=400) on the sample 450K platform revealed additional support for methylation at these miRNA loci influencing CKD.

**Conclusions:** Epigenetic features such as DNA methylation and miRNAs may cause or accelerate renal injury. This pilot study has identified several miRNA genes with differential methylation profiles that are associated with CKD. Comparison to RNA-seq data in a subset of these individuals will help elicit the functional effect of the novel findings reported in this abstract on the pathogenesis or progression of CKD.

*Funding:* Private Foundation Support

## TH-PO654

**Nonfunction of the ECT2 Gene May Cause Renal Tubulointerstitial Injury Leading to Focal Segmental Glomerulosclerosis** Akane Izu, Keisuke Sugimoto, Tomoki Miyazawa, Hitomi Nishi, Mitsuru Okada, Tsukasa Takemura. *Pediatrics, Kinki Univ School of Medicine, Osakasayama, Osaka, Japan.*

**Background:** Secondary focal segmental glomerulosclerosis (FSGS) follows congenital or acquired tubulointerstitial alterations such as in Dent's disease, Lowe syndrome, and reflux nephropathy. Failure of adequate regeneration after tubulointerstitial injury, or abnormal tubulogenesis, can disturb intrarenal blood circulation, causing excessive glomerular filtration. The epithelial cell-transforming sequence 2 gene (*ECT2*) contributes to tight junction function in epithelial cells.

**Methods:** Gene expression was screened by the comparative genomic hybridization (CGH) in 15 FSGS patients under treatment at our department. In one patient, a-actinin 4, located on chromosome 19q.13, was deleted. In another, a 6p deletion-associated E2F3 gene aberration was found. Downregulation of *ECT2*, located at 3q26.1 to q26.2, was observed in both patients. To confirm *ECT2* deletions, PCR for *ECT2* and immunohistochemical staining for ECT2 protein in renal tissues was carried out.

**Results:** We encountered two patients with a nonfunctioning ECT2 genotype who later developed FSGS. Renal biopsy specimens showed marked tubulointerstitial nephritis at the onset of proteinuria, later progressing to FSGS consequent to tubulointerstitial injury. Both patients did not respond to corticosteroids and attained only incomplete remission upon cyclosporine A administration. One patient received a maternal renal transplant with good function and no rejection. Some clustered genes localized in chromosome 3q.26.1–3q.26.2 showed downregulation. Signal indicating the loss of copy number was recognized in the log4 zone, suggesting homozygous deletion of *ECT2* in both patients. In both patients, no amplification band was detected, confirming the CGH results. Immunohistological evaluation for ECT2 protein in these two patients revealed no expression of this molecule in renal tubular epithelium.

**Conclusions:** *ECT2* is important for tight junction function and maintenance of cell polarity. Nonfunction of this gene may cause renal tubulointerstitial injury, progressing to glomerular sclerosis.

## TH-PO655

**Whole Genome Sequencing Catalogue of Genetic Variants from 256 Nephrotic Syndrome Cases** Matthew G. Sampson,[1,3,4] Adrian Tan,[1] Crystal A. Gadegbeku,[2,3,4] John R. Sedor,[3,5] Matthias Kretzler,[1,3,4] Hyun Min Kang.[1] *[1]Univ of Michigan; [2]Temple Univ; [3]Nephrotic Syndrome Study Network (NEPTUNE); [4]Michigan O'Brien Renal Center; [5]Case Western Univ.*

**Background:** To identify known and novel genetic variants associated with intermediate and clinical phenotypes of primary nephrotic syndrome (NS), we are performing low-pass whole genome sequencing (WGS) and exome chip genotyping of 550 subjects with NS recruited into one of two prospective, observational cohorts. Here we present data on the first 256 patients.

**Methods:** Subjects were recruited at time of initial biopsy for suspected primary NS or had existing diagnoses of focal segmental glomerulosclerosis (FSGS) or membranous nephropathy (MN). All had Illumina Exome Chip genotyping and Illumina HiSeq WGS. WGS data was analyzed with GotCloud pipeline with linkage disequilbrium refinement and compared to Exome Chip genotypes. Variant frequencies were compared to the latest release of 1000 Genomes and Exome Sequencing Projects, stratified by ancestry.

**Results:** Diagnoses were 52% minimal change/FSGS, 25% MN, and 24% "other glomerulopathy". Ancestry was 49% European (EUR), 29% African (AFR), 15% Admixed American (AMR), and 7% Asian (ASN). Mean read depth was 4.2x. 20.9M SNPs were identified with transition/transversion ratio of 2.16. 330K (16%) SNPs were novel to dbSNP Build 135. Comparing to exome chip data, low-pass WGS had estimated 89% power to detect shared variants in cases and 30% power to detect singleton variants. We identified novel loss-of-function and essential splice-site variants shared in our cases in 55 and 64 genes, respectively. In AFR FSGS cases, the minor allele frequency (MAF) of the FSGS risk haplotype, G1, in *APOL1* was 31% (OR=1.6). In EUR MN cases, the allele frequencies of the known MN risk SNPs, rs2187668 and rs4664308, were 33% (OR=4.4) and 83% (OR=3.5), respectively.

**Conclusions:** With 47% of our cohort analyzed thus far, low-pass WGS has power to assess disease associations of coding and non-coding variants with a MAF >1%. We will integrate this data with molecular, histologic, and clinical data from the same subjects. This could clarify existing, and define new, associations between genetic variants and both endophenotypes and clinical outcomes.

*Funding:* NIDDK Support

## TH-PO656

**Adjustment for Genetic Determinants of Urinary KIM1 Does Not Alter the Relationship with All-Cause Mortality** Gearoid M. McMahon,[1] Shih-Jen Hwang,[1] Qiong Yang,[2] Caroline S. Fox.[1] *[1]NHLBI's Framingham Heart Study, Framingham, MA; [2]Biostatistics, Boston Univ, Boston, MA.*

**Background:** Kidney Injury Molecule-1 (KIM1) is a novel biomarker of kidney disease. We investigated if there were genetic determinants of KIM1 levels and if the addition of these variants improved the ability of KIM1 to predict clinical outcomes.

**Methods:** We did a genome-wide association (GWAS) of urinary KIM1/cr in 2640 participants from the Framingham Heart Study Offspring cohort (53% female, mean age 58 yrs). We constructed a logistic regression model to evaluate the association between urinary KIM1 and incident chronic kidney disease (CKD), albuminuria, cardiovascular disease (CVD) and all-cause mortality in up to 10yrs of follow-up. Finally, we constructed a model including variants identified in the GWAS to determine whether the addition of these variants altered the relationship between KIM1 and these outcomes.

**Results:** rs6555820, an intronic C□A substitution in the *HAVCR1* gene (MAF 0.43) explained 9% of the variance in KIM1. *HAVCR1* encodes the hepatitis A virus cellular receptor, also known as KIM1, which plays a key role in regulating immune cell activity. Per copy of the minor A allele, KIM1 levels were 0.4 pg/ml lower. In the multivariable-adjusted model, KIM1 was associated with all-cause mortality. There was no association of KIM1 with incident CVD, CKD or albuminuria. The presence of the minor allele was not associated with any outcome. The addition of the variant to the model did not change the relationship between urinary KIM1 and any primary outcome.

| | CKD | Albuminuria | CVD | Mortality |
|---|---|---|---|---|
| **Cases/total** | 178/1971 | 158/1668 | 195/2508 | 324/2635 |
| | **OR (95%CI)** | **OR (95%CI)** | **HR (95%CI)** | **HR (95%CI)** |
| **KIM1** | 1.15 (0.96-1.38) | 1.18 (0.99-1.42) | 1.08 (0.92-1.26) | 1.13 (1.00-1.28) |
| **rs6555820** | 0.92 (0.73-1.16) | 0.86 (0.68-1.10) | 0.98 (0.81-1.20) | 0.95 (0.81-1.11] |
| **Adjusted KIM1** | 1.14 (0.95-1.38) | 1.16 (0.96-1.41) | 1.08 (0.92-1.27) | 1.13 (1.00-1.29) |

**Conclusions:** A variant in the *HAVCR1* gene is associated with KIM1 levels. However, statistical adjustment for the presence of this variant did not alter the relationship between KIM1 and all-cause mortality.

*Funding:* Other NIH Support - NHLBI

## TH-PO657

**Genome-Wide Scan of Copy Number Variations (CNVs) Identifies a Variable Region at 3p21.1 Regulating the *TLR9* Expression in Familial IgA Nephropathy Patients** Fabio Sallustio,[1,2,3] Sharon N. Cox,[1] Grazia Serino,[1] Francesco Pesce,[4] Giuseppe De Palma,[2] Mario Falchi,[4] Francesco Paolo Schena.[1,2,5] *[1]Dept of Emergency and Organ Transplantation, Univ of Bari, Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy; [3]Dipartimento di Scienze e Tecnologie Biologie ed Ambientali (DiSTeBA, Università del Salento, Lecce, Italy; [4]Dept of Genomics of Common Disease, School of Public Health, Imperial College, London, United Kingdom; [5]Schena Foundation, Research Center of Kidney Diseases, Valenzano, Bari, Italy.*

**Background:** Immunoglobulin A Nephropathy (IgAN) is a multifactorial complex disease whose genetic bases remain unknown. In this context, an intriguing role could be ascribed to copy number variants (CNVs) that have been recognized as an important source of genetic variations in humans.

**Methods:** We performed a whole-genome screening of CNVs in familial IgAN patients, their healthy relatives and healthy subjects (HS). A total of 217 individuals consisting of 51 familial IgAN patients and 166 healthy relatives were included in the study. We used a statistical approach (MSA) that allowed us to identify aberrations that were concordant across multiple samples and that allowed to use a relatively little sample-size, simultaneously increasing the power of the analysis and the resolution.

**Results:** 178 IgAN-specific aberrations were identified, specifically 114 loss and 64 gain. Several CNVs overlapped with regions evidenced by our previous genome-wide genetic studies (AJHG, 2006). Moreover, we found that IgAN patients characterized by deteriorated renal function carried low copy numbers of a CNV in chromosome 3p21.1. This CNV contained the *TLR9* gene whose expression significantly correlated with the

loss aberration in patients with progressive renal damage. Data were validated by RT-PCR. Conversely, IgAN patients with normal renal function did not have this aberration and the *TLR9* mRNA was expressed at the same level as in HS.

**Conclusions:** We performed the first genome-wide CNV study in familial IgAN. We identified some structural variants specific to IgAN patients and provided a collection of new candidate genes and loci that could help to dissect the complex genomic pattern of the disease.

## TH-PO658

**Elevated Klotho Promoter Methylation Is Associated with Deterioration of Renal Function in IgA Nephropathy Patients** Xiaoyan Zhang, Jing Chen, Han Zhang, Yi Fang, Xiaoqiang Ding. *Nephrology, Zhongshan Hospital, Fudan Univ, Shanghai, China.*

**Background:** Recent studies prove Klotho (Kl) functions as a renoprotective factor and inhibition of Kl expression correlates with gene hypermethylation, which suggests epigenetic modification of Kl genes may be an important pathological mechanism of renal fibrosis. Our previous study demonstrates peripheral blood mononuclear cells (PBMC) level of Kl promoter methylation is positively correlated with intra-renal level of Kl promoter methylation and has high sensitivity and specificity at predicting intra-renal Kl promoter hypermethylation in chronic kidney disease patients. The aim of this study is to analyze the relationship of PBMC Kl promoter methylation level with eGFR in IgA nephropathy.

**Methods:** 70 Patients admitted to Shanghai Zhongshan hospital between 2012 April and 2013 April for renal biopsy and diagnosed as primary IgA nephropathy were included in the study. All patients provided informed consent. PBMC Kl methylation level was examined by pyrosequencing.

**Table 1 Primers and sequence for analysis**

| Primers | Sequence(5'→3') | Base pair |
|---|---|---|
| Primer forward | GTGGGAGAAAAGTGAGAGTAG | 21 |
| Primer reverse | AAACCCTCAAATTCATTCTCTTTACCTACC | 30 |
| Primer sequencing | AAGTGAGAGTAGGTG | 15 |
| Sequence for analysis | TTTTTTTAGYGGYGYGTTTYGTTAGGGTTYGGTAG | 51 |
| | GATTTYGTTTTTAAGT | |
| | (*After Bisulfite treatment*) | |

**Results:** The patients included 39 men and 31 women with a mean age of 37.89±11.81yr (range 16 to 66 yr). The eGFR ranged from 4.09~86.73 ml/min/1.73m2 (median 49.24 ml/min/1.73m2). The median of PBMC Kl promoter methylation in 70 IgAN patients was 4.08% (1.50~11.76%). eGFR inversely correlated with age ($\rho$=-0.307, P<0.01), hypertension($\rho$=-0.529, P<0.01), uric acid ($\rho$=-0.423, P<0.01), intact parathyroid hormone (iPTH) ($\rho$=-0.328, P<0.01), proteinuria ($\rho$=-0.427, P<0.01) and PBMC Kl promoter methylation level ($\rho$=-0.809, P<0.01). eGFR positively correlated with hemoglobin ($\rho$=0.246, P<0.05), serum calcium ($\rho$=0.293, P<0.05) and urinary calcium($\rho$=0.419, P<0.01) (table 2) (figure 2). Multiple regression analysis indicated older age (P<0.01), high iPTH (P<0.01), high proteinuria (P<0.05) and high PBMC Klotho promoter methylation level (P<0.01) correlated significantly with lower eGFR (table 3).

**Conclusions:** PBMC Klotho promoter methylation is an independent risk factor of deterioration of renal function in IgAN patients.

*Funding:* Government Support - Non-U.S.

## TH-PO659

**Systems Biology of Experimental Alport Syndrome: Computational Identification of Gene Expression Pathways** Xuewen Song,[1] Fei Fang,[1] Rohan John,[1] Ana Konvalinka,[1] Christoph Licht,[2] Paul S. Thorner,[3] James W. Scholey,[1] York P. Pei.[1] *[1]Div of Nephrology, Univ Health Network, Toronto, Canada; [2]Div of Nephrology, Hospital for Sick Children, Toronto, Canada; [3]Dept of Pathology, Hospital for Sick Children, Toronto, Canada.*

**Background:** Mutations of *COL4A-3*, -4 or -5 result in defective glomerular basement membrane assembly in Alport Syndrome (AS) but the downstream molecular events associated with renal disease progression are not well understood.

**Methods:** We sought to define the gene pathways associated with disease progression in Col4a3 $^{-/-}$ knock-out (KO) mice. Using Affymetrix Mouse Gene 2.0 ST arrays, we performed global gene profiling of renal cortical samples from male 129/Svj congenic KO and wild type (WT) mice at 4 and 7 weeks of age (n=8 each) in a 2x2 factorial design. We used Gene Set Enrichment Analysis (GSEA) and Significance Analysis of Microarrays (SAM) to identify overrepresented signaling pathways, biological processes, and genes associated with disease initiation and progression**.**

**Results:** Our data validated some of the known pathological features of AS, including down-regulation of Col4a3-5 and up-regulation of Col4a1-2; and up-regulation of laminins and matrix metalloproteases. At 4 weeks in the KO mice, we only found several genes (i.e. Rasl11A, Ctsl, Fstl, Cald1, Plod2, and R3hdml) that were differentially expressed and increased in expression during disease progression. Comparisons of KO mice between weeks 4 and 7 (minus any overlapping changes in WT at the same time points) identified multiple gene sets associated with tissue injuries and remodelling including collagen metabolism, immune/inflammatory responses (e.g. cytokine/ interleukin/chemokine, JAK-STAT, NF-kB, and complement signaling), TGF-β, ECM, and integrin signaling. By contrast, most down-regulated gene sets represent metabolic pathways.

**Conclusions:** Our study confirmed an arrest of the normal developmental switch from fetal α1-α1-α2 (IV) to mature α3-α4-α5 (IV) collagen network in AS and identified major molecular pathways which may mediate renal injuries and remodelling. Targeting key nodes of convergence of pathways of renal injuries may provide molecular targets for novel therapeutics.

*Funding:* Private Foundation Support

## TH-PO660

**Is There an Epigenetic Predisposition to New Onset Diabetes after Transplantation?** Jennifer A. McCaughan,[1,2] A.J. McKnight,[1] Alexander P. Maxwell.[1,2] *[1]Nephrology Research Group, Queen's Univ, Belfast, United Kingdom; [2]Regional Nephrology Unit, Belfast City Hospital, Belfast, United Kingdom.*

**Background:** New onset diabetes after transplantation (NODAT) is an increasingly common complication of renal transplantation. Recent evidence suggests that pancreatic beta cell toxicity, as a result of hyperglycemia in the peri-transplant period, may play a key role in NODAT pathogenesis. We recently demonstrated that single nucleotide polymorphisms in genes crucial to beta cell function predispose to NODAT in a White population. This study investigates the association between DNA methylation profiles prior to transplantation and the development of NODAT.

**Methods:** NODAT was defined as diabetes developing after transplantation and requiring pharmacotherapy. Epigenome wide methylation analysis was performed at 485,577 CpG sites using the Infinium HumanMethylation 450 BeadChip (Illumina) on blood-derived DNA from 348 renal transplant recipients from Northern Ireland. Quality control measures, including correction for dye bias, sample normalisation and evaluation of probe performance, were undertaken. Beta values were calculated and compared between NODAT cases and controls using ANOVA. Adjustment for multiple testing was performed.

**Results:** There were 46 (13%) cases of NODAT in the study cohort. All individuals were White. Following quality control analysis, methylation at 14 CpG sites was associated with NODAT with a p value < $10^{-5}$. After adjustment for multiple testing, these associations did not persist.

**Conclusions:** There is clinical evidence that a hyperglycemic insult within the peri-transplant period may contribute to the development of NODAT. Variation in genes influencing pancreatic beta cell function may also predispose renal transplant recipients to NODAT. This study did not provide evidence that DNA methylation profiles prior to transplantation are associated with NODAT. However, it is plausible that hyperglycemia alters DNA methylation in key genes following transplantation and may contribute to NODAT pathogenesis in this manner.

## TH-PO661

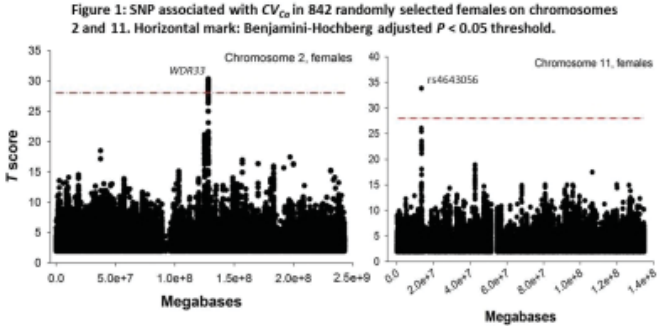
**SNP Associated with the Coefficient of Variation in 24-h Urinary Calcium Excretion Linked to Parathyroid Hormone and WD Repeat Domain 33** Guy M.L. Perry,[1] Martin R. Pollak,[2] Hakan R. Toka,[2] David B. Mount,[2] Gary C. Curhan,[3] Steven J. Scheinman.[4] *[1]SUNY Upstate Medical Univ, Syracuse, NY; [2]Harvard Medical School, Boston, MA; [3]Brigham and Women's Hospital, Boston, MA; [4]The Commonwealth College, Scranton, PA.*

**Background:** Residual variance (RV) is heritable in some systems. Our epidemiological and model work on this phenomenon suggests that residual variance in urinary calcium excretion is heritable in humans, and might have effects on conventional genetic analysis.

**Methods:** We performed a genome-wide association study on the coefficient of variation for paired measurements of 24-h urinary calcium ($CV_{Ca}$) in 1210 individuals with a history of confirmed stone disease and randomly-sampled unaffecteds (368 males, 842 females total).

**Results:** No single nucleotide polymorphism (SNP) was associated with $CV_{Ca}$ in the complete sample, or in males. A T/C polymorphism at rs4643056 (Chr 11, 13.5 MB) was significantly associated with $CV_{Ca}$ in females ($P$=6×10$^{-9}$). This region is syntenic with a chromosomal region (Chr 1, 170-227 MB) in rats carrying a quantitative trait locus for $CV_{Ca}$. Fourteen SNP in *WD repeat domain 33* (*WDR33*; Chr 2, 128.2-128.3 MB) were also associated with $CV_{Ca}$ in females ($P$=4×10$^{-8}$).

**Figure 1: SNP associated with $CV_{Ca}$ in 842 randomly selected females on chromosomes 2 and 11. Horizontal mark: Benjamini-Hochberg adjusted $P$ < 0.05 threshold.**




**Conclusions:** These results and our previous work indicate sex-limited genes affecting residual variance in urinary calcium. The nearest gene to rs4643056 is parathyroid hormone (*PTH*, 34 kB downstream); notably, a syntenic genomic region carrying *PTH* is also associated with $CV_{Ca}$ in rats. *PTH* has functions in acetylation, serum calcium regulation and bone mineralization, and WD repeat proteins with signal transduction, mRNA processing and methylation, which suggests mixed generalist/specialist genetic control

of this phenomenon. These SNP were not associated with mean urinary calcium; residual variance genes might dynamically control urinary solute excretion without detectable classical genetic effects.

*Funding:* NIDDK Support

## TH-PO662

**Exon-Skipping in Gitelman's Syndrome Families** Yoichi Takeuchi,[1] Hisato Shima,[1] Eikan Mishima,[1] Yasutoshi Akiyama,[1] Chitose Suzuki,[1] Takehiro Suzuki,[1] Tomohiro Nakayama,[2] Sadayoshi Ito,[1] Takaaki Abe.[1] *[1]Div of Nephrology, Endocrinology and Vascular Medicine, Tohoku Univ Graduate School of Medicine; [2]Nihon Univ School of Medicine.*

**Background:** More than 160 point mutations in SLC12A3 gene causing Gitelman's syndrome have been reported and missense mutations have been examined generally by single amino acid substitution.

However, there is sometimes no functional difference in expression experiment by the mutant sequence.

It is revealed a single mutation on exonic splicing enhancers (ESEs) weaken the exon recognition by spliceosome, which cause exon-skipping. The aim here is to find a single exonic mutation disrupting SLC12A3 exon sequence.

**Methods:** All 80 missense mutations in SLC12A3 were analyzed by ESEfinder to extract putative exonic mutations which generate exon-skipping. The exon sequence with flanking introns were PCR-amplified and subcloned into an exon-skipping detection vector. Resultant plasmids were transfected into HEK293 and evaluated whether the exon was skipped or not by RT-PCR. Total RNA from three Gitelman's syndrome patients (T180K/A588V, L858H/R861H and M672I/c.1926delC) was isolated from the blood and we examined the possibility of abnormal splicing.

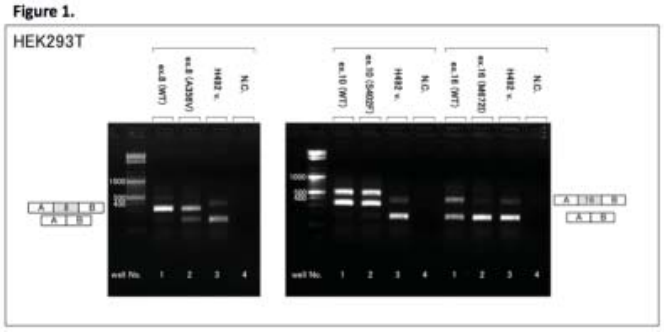
**Results:** ESEfinder detected three exonic mutations (A356V, S402F and M672I) as the best candidates of exon-skipping.

Whereas S402F mutation showed normal splicing by in-vitro assay, A356V and M672I created the skipped transcripts to produce newly aberrant proteins. All the bands were confirmed by sequencing.

Another missense mutation as R861C (frequent in European) showed normal splicing band by in-vitro assay.

All of the mutations of three patients remained normal transcripts.

**Figure 1.**




**Conclusions:** We found exonic mutations caused exon-skipping in SLC12A3. We need to examine not only single amino acids substitution by genomic mutations but also RNA transcripts and resultant loss-of-function.

## TH-PO663

**Do Single Nucleotide Polymorphisms Contribute to Urine pH Variation? A Genetic Association Study** Benjamin Canales,[1] Jennifer Smith,[2] Wei Zhao,[2] Gary C. Curhan,[3] I. David Weiner,[4] John C. Lieske.[5] *[1]Urology, Univ of Florida, Gainesville, FL; [2]Epidemiology, Univ of Michigan, Ann Arbor, MI; [3]Medicine, Renal Div, Brigham and Women's Hospital, Boston, MA; [4]Medicine, Renal Div, Univ of Florida, Malcom Randall VAMC, Gainesville, FL; [5]Medicine, Renal Div, Mayo Clinic, Rochester, MN.*

**Background:** Although a number of gene mutations have been implicated in rare urinary acid-base disorders, little is known regarding genetic control of urine pH in the general population.

**Methods:** 15 genes involved in renal acid-base homeostasis were identified. A genetic association study was performed in 2493 individuals of European descent across 4 cohorts (n=811, Minnesota cohort of the Genetic Epidemiology Network of Arteriopathy (GENOA); n=553, Health Professionals Follow-Up Study (HPS); n=494, Nurses' Health Study (NHS) I; n=635, NHS II). Single nucleotide polymorphism (SNP) dosages from 1000 Genomes imputed data were modeled for association with 24 hr urine pH using linear regression after adjustment for age, sex and body mass index. SNPs with minor allele frequency >0.01 were studied between 5 kb from a targeted gene start or endpoint. A fixed-effects meta-analysis was used with inverse variance weighting by standard error of the beta coefficient.

**Results:** 2906 SNPs met inclusion criteria. 17 SNPs across 5 genes (*ATP6V0A4*, *SLC9A3*, *RHCG*, *GLUD1*, and *OGDH*) had a meta-analysis p-value <0.01 in the joint analysis, plus a consistent direction of effect and at least marginal significance (p<0.1) in both GENOA and the combined sample of HPFS/NHS I/NHS II. The lowest meta-analysis p-value was observed for rs6955765 in the *ATP6V0A4* gene (p=0.0009). Maximal beta-coefficients for identified SNPs were 0.20 for *GLUD1*, 0.06 for *RHCG*, 0.06 for *SLC9A3*, 0.08 for *ATP6V0A4* and 0.20 for *OGDH*. Two blocks of SNPs on genes *ATP6V0A4* (n=9) and *SLC9A3* (n=4) were in strong linkage disequilibrium ($r^2$>0.8).

**Conclusions:** Common genetic variants of several genes involved in renal acid-base homeostasis correlate with basal urine pH. This study suggests that genetic variation in these genes could influence urine pH in the general population.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO664

**Whether Digenic Inheritance of NPHS1 "And" TRPC6 Polymorphisms Predisposes to Progression of Steroid-Resistant Nephrotic Syndrome in Children?** Larisa Prikhodina. *Pediatric Nephrology, Reasearch Institute of Pediatrics & Children' Surgery, Moscow, Russian Federation.*

**Background:** Mutations in NPHS1 and TRPC6 genes cause steroid-resistant nephritic syndrome (SRNS) in children and adults. The role of digenic inheritance of TRPC6 and NPHS1 single nucleotide polymorphisms (SNPs) in progression of SRNS in children is unknown.

**Methods:** We aim to investigate whether digenic inheritance of NPHS1/TRPC6 SNPs predispose to progression of idiopathic pediatric SRNS to chronic renal failure (CRF). 28 children (20F/8M) with primary non-familial SRNS, originating from Russia, were enrolled. Histological findings were FSGS in 60.7%, MPGN in 17.9%, mesangial proliferative GN in 10.7%, MN in 7.1%, MCD in 3.6% patients. The median age at onset of SRNS was 9.0 (IQR: 4.3; 10.9) years. The disease duration was 63.0 (38.4; 88.50) months. CRF defined as declining of eGFR <60 mL/min/1.73m$^2$. NPHS1 and TRPC6 mutation analysis was performed by Sanger sequencing of all 29 and 13 exons, respectively. Patients with congenital, infantile and secondary SRNS and with identified mutations in *NPHS1* or TRPC6 genes were excluded from the study.

**Results:** Digenic inheritance of NPHS1/ TRPC6 SNPs was identified in 8/28 (28.6%) children with SRNS, including two homo-/heterozygous NPHS1 SNPs: ?.349G>A (rs3814995), c.1223G>A (rs33950747) and three heterozygous TRPC6 SNPs: c.43C>T (rs3802829), c.1211C>T (rs36111323), c.1683T>C (rs12366144). Children with digenic inheritance of NPHS1/TRPC6 SNPs in comparison without the SNPs had no significant differences in frequency of FSGS: 87.5% vs. 100% (p=1.0), medium eGFR at the last follow up: 77.4 (52.0; 143.0)vs. 80.5 (37.1; 120.3) mL/min/1.73m$^2$ (p=0.39), frequency of CRF: 50% vs. 33.3% (p=0.72), annual slope of eGFR decline: 6.4 (-1.3; 20.7) vs. 0.1 (-2.8; 37.6) mL/min/1.73m$^2$ (p=0.46). The 5-year renal survival in patients with digenic inheritance of NPHS1/TRPC6 SNPs in comparison without SNPs had no significant differences: 72.9% vs. 66.7%; 10-year renal survival - 43.8% vs. 66.7%, respectively (p=0.88).

**Conclusions:** Our data indicate that digenic inheritance of TRPC6 and NPHS1 SNPs does not predispose to progression of idiopathic SRNS to CRF in children.

## TH-PO665

**Molecular Effects of Three Mutations Identified in Complement Component C3 in Japanese Patients with Atypical Hemolytic Uremic Syndrome** Akira Ashida,[1] Daisuke Yamamoto,[2] Yoko Yoshida,[3] Fan Xinping,[4] Masanori Matsumoto,[3] Toshiyuki Miyata,[4] Motoshi Hattori,[5] Yoshihiro Fujimura,[3] Hiroshi Tamai.[1] *[1]Pediatrics, Osaka Medical College, Takatsuki, Osaka, Japan; [2]Biomedical Computation Center, Osaka Medical College, Takatsuki, Osaka, Japan; [3]Blood Transfusion Medicine, Nara Medical Univ, Kashihara, Nara, Japan; [4]Molecular Pathogenesis, Research Institute National Cerebral and Cardiovascular Center, Suita, Osaka, Japan; [5]Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** Atypical hemolytic uremic syndrome (aHUS) has been associated with dysregulation of the alternative complement pathway. Mutations in complement factor H (CFH), factor I, factor B (CFB), thrombomodulin, C3, and MCP predispose development of aHUS. We identified 7 causative or potentially causative mutations of 6 candidate genes, including the C3 gene, in 8 out of 10 Japanese patients with aHUS. We analyzed the molecular effects of these C3 gene mutations on the alternative pathway of complement activation.

**Methods:** We examined three missense mutations in the C3 gene identified in 10 Japanese patients with aHUS. Each three-dimensional coordinate set was listed in the RCSB Protein Data Bank as 2I07 (C3b), 2WII (C3b-CFH), 2XWB (C3b-CFB/D), 2ICF (C3b-CR1G) and 3L5N (C3b-SCIn).

**Results:** We carried out the structural analysis of the R425, S562 and I1157 residues on the molecular surface, using the X-ray structures of C3b and each of its complexes with CFH, CFB, CR1g and SCIn (staphylococcal complement inhibitor). At the three mutation points, I1157 in the thioester-containing domain (TED) of C3b was located at the interface with CFH. The CR1g interface with C3b lay between R425 and S562, and mutations of these residues were considered to possibly reduce the interaction between C3b and CR1g. All of these mutation points were located at positions where they would have no direct effect on the interface of CFB and SCIn with C3b. From these findings, we postulate that the novel mutations R425 and S562 might cancel the inhibitory trigger of the C3b system by disturbing the interaction with CR1g.

**Conclusions:** We conclude that these mutations of C3 gene including mutations R425 and S562 are causative.

## TH-PO666

**Total Kidney Volume in Healthy Aging Adults** Carlos Kornhauser. *Medical Sciences, Guanajuato Univ, León, Guanajuato, Mexico.*

**Background:** Total kidney volume (TKV) and its relationship with pro-inflammatory factors such as TGF-β, VEGF and aldosterone has been poorly studied in aging. We evaluated the TKV by ultrasonography, and its relationship with eGFR, microalbuminuria, TGF-β, VEGF and aldosterone in healthy aging adults.

**Methods:** Renal ultrasonography was performed in 30 women <75, and 30 ≥75 years, 20 men <75 and 20 ≥75 years. eGFR was estimated according to the MDRD formula. Microalbuminuria, serum TGF-β, VEGF, and aldosterone levels were assessed in all subjects.

**Results:** TKV was significantly higher (p<0.0000) in the two groups of men, as compared to the two groups of women. TKV was significantly higher (p<0.0001) in women <75 y as compared to women >75 y. No difference was found in the TKV between men <75 y as compared to men >75 y. A negative correlation between TKV and age in men (r=.49, p=0.00004), and in women (r=38, p=0.001) was found. TKV and TGF-β (r=.47, p= 0.0001) showed a negative correlation only in men. The TKV and microalbuminuria showed a positive correlation only in men (r=.28, p=0.02). The eGFR did not show correlations with any of the variables. VEGF and aldosterone did not correlate with TKV.

| | Group 1 | Group 2 | Group 3 | Group 4 | p |
|---|---|---|---|---|---|
| | Women <75y | men <75 y | women >75y | men >75y | |
| | (n=30) | (n=20) | women >75y | (n=20) | |
| Total Kidney Volume ($cm^3$) | 102.9 ± 16.9 | 131.1 ± 32.9 | 87.2 ± 8.2 | 119.4 ± 6.6 | 0.000000 |

**Conclusions:** Men have bigger total kidney volumes than women at any age. The TKV in men does not significantly change with age. In women TKV gets significantly smaller with age. Microalbuminuria correlates positively with TKV in men, but not eGFR. TGF-β shows a negative correlation with TKV. In men, the smaller the TKV,the higher the TGF-β levels.

## TH-PO667

**Renal Function in Healthy Aging Adults. Age and Gender Differences** Carlos Kornhauser. *Medical Sciences, Guanajuato Univ, León, Guanajuato, Mexico.*

**Background:** Renal function is poorly studied in healthy aging adults. Role of TGF-β, VEGF and aldosterone in the aging process and its relationship with eGFR, and microalbumiuria needs to be more clearly established.

**Methods:** Two hundred normotensive subjects (100 women and 100 men) were included. Fifty women and fifty men <75 years, and 50 women and fifty men ≥75 years old. Serum glucose, uric acid, lipid profile, eGFR (MDRD), TGF-β, VEGF and aldosterone, and microalbuminuria were assessed.

**Results:** eGFR was significantly higher in men (85.3 ml/min/1.73$m^2$) as compared to women (66.5 ml/min/1.73$m^2$) at any age (p <0.00001). eGFR values were similar in both age groups of women, as well as in both age groups of men. Aldosterone was significantly higher in men <75 y as compared to women <75 y (p<0.0000), but higher in women >75 y as compared to men >75 y (p<0.0000). VEGF was higher in women >75 y as compared to men >75 y. Uric acid (p<0.01) and TGF-β (p<0.0000) were significantly different between each group of age. eGFR and microalbuminuria had a negative association (R= -.41 p=0.03) in men <75 y. Aldosterone did not show associations.

| | Group 1 | Group 2 | Group 3 | Group 4 | p |
|---|---|---|---|---|---|
| | Women <75y | Men <75y | Women >75y | Men >75y | |
| | (n=50) | (n=50) | (n=50) | (n=50) | |
| Uric Acid (mg/dl) | 3.5 ± .7 | 3.6 ± .7 | 3.2 ± .5 | 3.3 ± .6 | <0.01 |
| Cholesterol (mg/dl) | 170 ± 33 | 167 ± 22 | 170 ± 34 | 150 ± 34 | <0.003 |
| Cholesterol LDL (mg/dl) | 96 ± 30 | 88 ± 21 | 107 ± 33 | 77 ± 33 | <0.00001 |
| TGF-β1(pg/ml) | 6707±1729 | 7766 ±3849 | 12345±3317 | 13115±3732 | <0.0000 |
| Aldosterone (pg/ml) | 112 ± 61 | 195 ± 72 | 159 ±73 | 97 ±73 | <0.00000 |
| Microalbuminuria (mg/L) | 7.4 ± 4 | 10.4 ± 6 | 7.4 ± 4 | 9.6 ± 4 | <0.002 |
| eGFR (ml/min/1.73$m^2$) | 69 ± 12 | 88 ± 25 | 64 ±15 | 83 ± 20 | <0.00001 |

**Conclusions:** eGFR decreases in men and women with aging, but values were never under 60 ml/min/1.73$m^2$. Microalbuminuria absence define subjects as healthy. Uric acid, aldosterone, VEGF and TGF-β levels need to be clarified in these subjects without kidney disease.

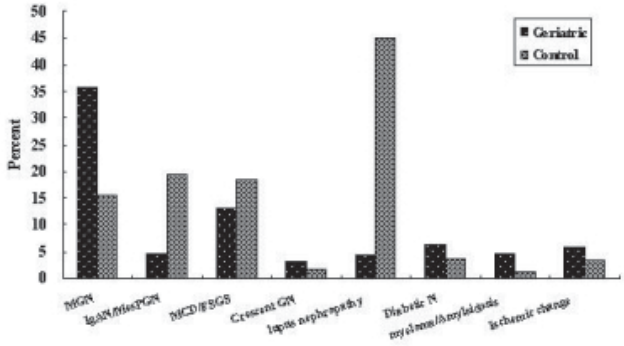
## TH-PO668

**Pattern of Biopsy-Proven Renal Diseases in Geriatric Patients from a Single Center in Taiwan** Cheng-Hsu Chen,[1,2,3,4] Ya-Wen Chuang.[1] *[1]Section of Nephrology, Dept of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; [2]School of Medicine, China Medical Univ, Taichung, Taiwan; [3]School of Medicine, Chung Shan Medical Univ, Taichung, Taiwan; [4]Dept of Life Science, Tunghai Univ, Taichung, Taiwan.*

**Background:** The elder population is growing fast as a major cause of chronic kidney disease (CKD) burden in Taiwan, however, the distribution of kidney diseases in elderly patients are not well known. The aim of this study was to compare the pattern of renal disease in elderly and control patients who underwent native renal biopsy.

**Methods:** Among 3622 renal biopsies, excluded 588 graft biopsies and 322 inadequate criteria biopsies, we did a single-center, retrospective study (1992-2008) of the biopsy-proven renal diseases between geriatric patients (age ≥65y/o; n=327) and control (18≤ age< 65; n=2385).

**Results:** The geriatric group with mean age was 71.8±4.5 (65.1-87.3 yrs), male gender (74%), age>70yr (n=200, 61%), the control group was 39.7±17.6(18-64.9yrs), male gender (41%). Membranous nephropathy (MN) was the most frequent diagnosis (35.8%), followed by minimal change disease/focal segmental glomerulosclerosis (MCD/FSGS) (13.1%), diabetic nephropathy (6.4%), hypertensive nephrosclerosis (5.8%), and IgA nephropathy (4.6%).




Comparison with the control group showed MN to be more frequent (P < 0.01), and lupus nephritis (P<0.001) and IgAN (P<0.001) less frequent in the elderly. However, ANCA-positive nephritis, and myeloma nephropathy and amyloid nephropathy were also more common in the elderly.

**Conclusions:** Renal biopsy in elderly patients is a valuable diagnostic tool that should be offered the potential to modify treatment with maximal potential benefit. Age should not be considered as a contraindication to renal biopsy.

## TH-PO669

**Renal Biopsy in Elderly Italian: A Single-Center Experience over 12 Years** Fausta Catapano, Lucia B. De Sanctis, Antonio Santoro. *Nephrology, Dialysis and Hypertension Unit, Policlinico S.Orsola Malpighi, Bologna, Italy.*

**Background:** The global increase in life expectancy has translated to more elderly patients affected from renal diseases. The role of renal biopsy (RB) in this subset of patients has never been explored; moreover the histological patterns of renal disease in elderly have rarely been described.

**Methods:** This is a retrospective study of all consecutive elderly patients (age ≥ 70 years) biopsied at S.Orsola-Malpighi Hospital of Bologna between 1st January 2000 and 31 December 2012. Data are extracted from renal biopsies database.

**Results:** No RB has been performed until 2005. 106 (M59, F47) elderly patients have been biopsied in the remnant study period (figure 1). The mean age was 76±4years without difference between sexes. In 8/108 (7.4%) RB was not adequate. Primary glomerulonephritis were seen in 39/100 patients and secondary glomerulonephritis in 25/100. Membranous glomerulonephritis (MGN) was the most common (21/100) and was significantly more frequent in males (M17: F4, p<0.05). Other common diagnoses included cast nephropathy (11/100) and amyloidosis (11/100). Nephrotic syndrome was the most common clinical presentation (43%) followed by acute renal failure (30%). After biopsy, 48% received immunosuppressive therapy.

**Figure 1. Histological diagnosis**

| Diagnosis | N. Patients |
|---|---|
| ***Primary glomerulonephritis*** | 39 |
| Membranous GN | 21 |
| Minimal change disease | 7 |
| Membranoproliferative GN | 8 |
| Mesangio-proliferative GN | 1 |
| Crescentic GN | 1 |
| Focal segmental glomerulosclerosis | 1 |
| ***Secondary glomerulonephritis*** | 25 |
| Amyloidosis | 11 |
| Vasculitis | 6 |
| Crioglobulinaemia | 5 |
| LCDD | 2 |
| Lupus nephritis | 1 |
| ***Non-inflammatory renal disease*** | 20 |
| Cast nephropathy | 11 |
| Diabetic glomerulopathy | 3 |
| Acute tubular necrosis | 6 |
| ***Vascular nephropathy*** | 5 |
| Nephrosclerosis | 4 |
| Atero-aembolic disease | 1 |
| ***Interstitial nephritis*** | 4 |
| ***Others*** | 5 |
| ESRD | 2 |
| CIN-nephrotoxicity | 2 |
| Lymphoproliferative tumor | 1 |

**Conclusions:** Renal biopsy, even in elderly patients, provides us with useful information for an accurate histological diagnosis and, interestingly, ESRD is not very common. In our study, GNM is the most common diagnosis followed by mieloma-related kidney diseases.

## TH-PO670

**Cognitive Impairment in Advanced Chronic Kidney Disease** Simon Richard Walker, Ranveer Singh Brar, Brett M. Hiebert, Frederick Eng, Paul Komenda, Claudio Rigatto, Manish M. Sood, Clara Bohm, Leroy J. Storsley, Rakesh C. Arora, Navdeep Tangri. *Medicine, Seven Oaks General Hospital, Univ of Manitoba, Winnipeg, Canada.*

**Background:** Chronic Kidney Disease (CKD) disproportionately affects the elderly and is associated with frailty and impaired quality of life. Patients with CKD suffer from multiple comorbid conditions putting them at risk of developing cognitive impairment. The purpose of this study was to describe the prevalence of cognitive impairment in patients with advanced CKD (eGFR < 30ml/min).

**Methods:** We approached all patients with advanced CKD who attended an interprofessional non-dialysis CKD clinic at a tertiary care center for enrollment in our study. We excluded patients who did not speak English or were unable to provide informed consent. We collected demographic variables, physical examination measurements, laboratory values, and performed an assessment of cognitive function using the Montreal Cognitive Assessment (MoCA).

**Results:** We studied 86 patients. Their mean age was 67.2 years (Standard Deviation [SD]+/- 12.8), and 34 patients were female (39.5%). The mean eGFR was 20.5 mL/min/1.73m$^2$ (SD +/- 8.1). Sixty seven patients (77.0%) scored < 26 on the MoCA and were thus defined as cognitively impaired. In particular, the recall, visual/executive and language domains were impaired in > 60 % of the participants. In contrast, naming and orientation were relatively well preserved (> 70 % achieving perfect score).

**Conclusions:** Patients with advanced CKD have a high burden of cognitive impairment. Further studies on the pathophysiology of cognitive decline and its impact on patient decision making and outcomes are needed.

## TH-PO671

**Marked Increased Risk of Prediabetes in Elderly ESRD Patients** Mark E. Williams,[1] Neal Mittman,[2] Lin Ma,[3] Julia I. Brennan,[4] Curtis D. Johnson,[4] Chinu M. Jani,[4] Franklin W. Maddux,[3] Eduardo K. Lacson.[3] *[1]Joslin Diabetes Center, Boston, MA; [2]Long Island College Hospital, Brooklyn, NY; [3]Fresenius Medical Care, North America, Waltham, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

**Background:** Prediabetes has become a major public health problem. Diagnosing prediabetes identifies individuals at high risk for diabetes and its complications. Increased age is known to be a significant risk factor for prediabetes in the general population. Because ESRD is also associated with impairments in glucose regulation, we sought to determine the association of aging with prediabetes in a cohort of nondiabetic dialysis patients, using ADA cutoffs of hemoglobin A1c at 5.7-6.4%.

**Methods:** Of 3,447 dialysis patients from 26 facilities with laboratory values obtained the first quarter of 2013, 1454 (with mean age 56.6 years; 78% on hemodialysis) were without known diabetes and had HgbA1c values. Age was categorized into decades (from <45 years to >=75 years) and the HgbA1c distribution was determined for each.

**Results:** 2% for those over age 65 years were found to be in the diabetic range (HgbA1c of >=6.5%). The per cent of patients with prediabetes by age category, using the lowest category as reference and analyzed by chi square, is shown:

| | Age (years) | | | | | |
|---|---|---|---|---|---|---|
| N/Col Pct | <45 | 45-55 | 55-65 | 65-75 | >=75 | Total |
| HgbA1c<=5.6 | 320 | 250 | 280 | 178 | 171 | 1199 |
| % | 87.67 | 81.97 | 82.6 | 77.39 | 79.53 | |
| HgbA1c: >5.6 - <6.5 | 44 | 45 | 51 | 48 | 42 | 230 |
| % | 12.05 | 14.75 | 15.04 | 20.87 | 19.53 | |
| HgbA1c>=6.5 | 1 | 10 | 8 | 4 | 2 | 25 |
| % | 0.27 | 3.28 | 2.36 | 1.74 | 0.93 | |
| Total | 365 | 305 | 339 | 230 | 215 | 1454 |

The prevalence of prediabetes increased dramatically over age 65 years in the nondiabetic ESRD population (both p<0.05).

**Conclusions:** Studies defining the natural history of prediabetes and the benefit of screening the ESRD elderly for preventive intervention are needed. Furthermore, as the population ages, the need for more studies focusing on patients with geriatric chronic kidney disease becomes increasingly apparent.

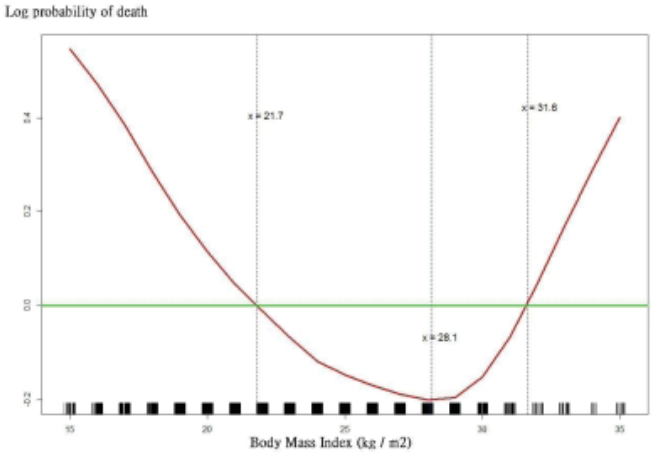
## TH-PO672

**Impact of Body Mass Index on Outcomes after Acute Kidney Injury in Geriatric Patients** Chia-Ter Chao, Hung-Bin Tsai. *Dept of Traumatology, National Taiwan Univ Hospital, Taipei, Taiwan.*

**Background:** Acute kidney injury (AKI) frequently occurs in critically ill patients and impairs both short-term and long-term outcomes. However, studies on outcome-modifying factors in geriatric patients with AKI are few, and the influence of body mass has not been determined previously.

**Methods:** We performed a multicenter prospective observational study and enrolled elderly patients ( ≥65 years) that developed AKI after major surgery in the intensive care units. We analyzed in-hospital mortality within each body mass index (BMI) category utilizing Cox proportional hazard regression analysis and generalized additive modeling (GAM).

**Results:** A total of 2015 postoperative elderly patients (age 75.2±6.4, men 57.8%) were studied. The survivors were significantly younger than the nonsurvivors (p=0.001) and had higher BMIs (23.2±3.9 kg/m$^2$ vs. 22.4±4.2 kg/m$^2$; p<0.001). GAM modeling showed that elderly AKI patients with a BMI between 21 and 31 (normal weight) had a lower mortality risk than those with a BMI < 21 (underweight) or ≥ 31 (obese). Both underweight and overweight individuals had a greater risk of hospital mortality compared with patients with normal BMI (underweight vs. normal, hazard ratio [HR] 1.60, 95% confidence interval [CI] 1.05-2.61; p=0.038; obese vs. normal, HR 1.22, 95%CI 1.01-1.49; p=0.042).




**Conclusions:** Our study is the first to demonstrate the effects of BMI on in-hospital mortality in geriatric patients sustaining AKI. The U-shaped association of BMI with hospital mortality in geriatrics is different from the association in other populations and therefore should alert physicians of factors potentially affecting the outcomes of this patient population.

## TH-PO673

**Association between Body Composition and Frailty among Prevalent Hemodialysis Patients: A USRDS Special Study** Kirsten L. Johansen,[1] Lorien S. Dalrymple,[2] Cynthia Delgado,[1] George A. Kaysen,[2] John Kornak,[1] Barbara A. Grimes,[1] Glenn M. Chertow.[3] *[1]Univ of California San Francisco; [2]UC Davis; [3]Stanford Univ School of Medicine.*

**Background:** Most studies of frailty among patients on hemodialysis have relied on definitions that substitute self-reported functioning for measures of physical performance and omit weight loss or substitute alternate criteria. Associations between body composition and frailty have not been carefully examined.

**Methods:** We examined the association between body composition and a definition of frailty that includes physical performance and weight loss in ACTIVE/ADIPOSE a cohort study of the USRDS Special Studies Centers. We included 638 patients receiving hemodialysis in a cross-sectional analysis. Frailty was defined as three of: weight loss, weakness, exhaustion, low physical activity, and slow gait speed. We performed logistic regression with body mass index (BMI) and estimates of intracellular water (ICW), fat mass, and extracellular water (ECW) by bioelectrical impedance spectroscopy (BIS) as the main predictors and age, sex, race, and comorbidity included as covariates. BMI and BIS data were then added to these covariates in separate models, and the areas under the ROC curves or (c-statistics) of the two resulting models were compared.

**Results:** Thirty percent of participants were frail. Older age (1.31 per 10 years, 95% CI 1.14-1.50) and diabetes (OR 1.65, 95% CI 1.13-2.40) were associated with higher odds of frailty. BMI was not associated with frailty (OR 1.02, 95% CI 0.99-1.05). Higher ICW was associated with lower odds of frailty (OR 0.80 per kg, 95% CI 0.73-0.87), and higher fat mass (OR 1.18, 95% CI 1.02-1.37) and ECW (OR 1.33, 95% CI 1.20-1.47) were associated with higher odds of frailty. Addition of BMI did not change the c-statistic (0.66 without BMI vs. 0.66 with BMI, p=0.71), but addition of BIS data did (0.72, p=0.0004).

**Conclusions:** Our data demonstrate the high prevalence of frailty in the hemodialysis population, and in particular, the poor performance of BMI as an indicator of frailty among patients on hemodialysis. We highlight the associations among variations in body composition and the odds of frailty.

**TH-PO674**

**Evaluation of Nutrition in Elderly Patients with Renal Failure** Rumeyza Kazancioglu,[1] Banu Buyukaydin,[1] Ahmet Turan Isik.[2] *[1]Nephrology, Bezmialem Vakif Univ, Turkey; [2]Geriatrics, Dokuz Eylul Univ.*

**Background:** Both malnutrition and chronic kidney diseases are clinical pathologies that increase morbidity and mortality incidence in elderly. We evaluated nutritional state and comorbidities in this group of patients.

**Methods:** 65 years and over 54 hospitalized patients were included in the study. The glomerular filtration rate (GFR) was calculated by using Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) formulas. All of the patients' calculated GFR was below 60 ml/min/1.73m$^2$. Nutritional status was evaluated with Mini Nutritional Assessment Short-Form (MNA-SF) and Geriatric Nutritional Risk Index (GNRI). Patients' waist circumference, hip circumference, waist-hip ratio and body mass indices (BMI) were measured and biochemical analysis was performed.

**Results:** Means of patients GNRI score was 97.9±13 (R = 66-128), and MNA-SF score was 7.9±2.9 (R= 1-14). In older patients mean MNA-SF was lower (R = 0.269, p=0.049). Between lower GNRI and higher creatinine level, there was detected significantcorrelation (R= 0.340, p= 0.012). In long-term hospitalized patients, GNRI and MNA-SF scores were lower (R = 0.455, p=0.001), (R = 0.356, p=0.008). Low GNRI correlated with low waist circumference, low hip circumference and low BMI, respectively (R = 0.436, p=0.001), (R = 0.554, p= 0.000), (R = 0.744, p= 0.000). And low MNA-SF correlated with low levels of same parameters also (R=0.377, p=0.005), (R=0.327, p=0.017), (R=0.440, p=0.001).

**Conclusions:** In elderly patients with chronic kidney disease, GNRI is a reliable nutritional test. Also waist circumference, hip circumference and BMI are usable measurements for nutritional evaluation in this group of patients.

**TH-PO675**

**Elderly Renal Replacement Therapy (RRT) Population an Emerging Problem: One Center Analysis** Ilaria Tantillo, Alessandra Brendolan, Monica Zanella, Federico Nalesso, Claudio Ronco. *Nephrology and IRRIV, San Bortolo Hospital, Italy.*

**Background:** The proportion of elderly people is rising throughout the world. Very elderly chronic kidney disease (CKD) patients (pts) are a growing group dialyzed and their morbility and outocomes have important implications for future Health policies. The aim of this study was to analyze the outcomes of incident RRT pts aged 75 years (yrs) or more between January 2008 and March 2013.

**Methods:** We analyzed retrospectively 97 pts (68% M), mean age (80.78±3.7 yrs), 57 hemodialysis (HD) and 40 peritoneal dialysis(PD), at least 2 months (mths) of RRT. The causes of CKD were hypertension (48.5%), glomerulopathy (13.4%), diabetes (DM) (10.3%), unknown (13.4%). In 20.6% of cases, the pts were late referral. Mean time of RRT was 17.04±12.04 mths. We defined 3 classes of age: 1) 51 pts (75-79 yrs); 2) 33 (80-84 yrs); 3) 13 ( ≥80 yrs). The parameters tested were survival between groups (tested by log-rank test and modeled in a multivariate fashion with Cox proportional hazards regression) and hospital admission rate (negative binomial regression, adjusted for exposure). Statistical analysis was performed using Stata 12.

**Results:** In a univariate perspective, a significant difference in survival was observed comparing pts above or below 80 yrs, with or without DM, and with ejection fraction above or below 50%. In a multivariate Cox regression (3 classes of age, DM, malignancy and major cardiovascular events at the beginning of RRT) the only significant 2 predictors of mortality were age class [HR (class 2 vs class 1): 2.80, p<0.01; HR(class 3 vs class 1): 3.25, p=0.048] and DM [HR: 2.48, p<0.01]. In the negative binomial regression of hospitalizations on RRT modality and age at beginning of RRT, we find that – adjusting for age and duration on the treatment – PD pts are hospitalized less frequently than HD pts [p<0.01].

**Conclusions:** This finding suggests that are no medical concernes to avoid RRT therapy in eldery CKD pts but life expectancy of pts who began RRT above 80 yrs is significantly shorter than class 1. DM is the only significative risk factor of poor outcome. The survival of HD than PD pts is the same, but the PD are hospitalized less frequently than HD.

**TH-PO676**

**Hemodialysis Remains a Feasible Option for Renal Replacement Therapy in Elderly Who Are over Eighty-Five Years** Pratima Ghimire, Eli A. Friedman, Moro O. Salifu, Mary C. Mallappallil. *Dept of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

**Background:** There are limited studies on effectiveness of initiating hemodialysis (HD) on elderly patients above 85 years. We studied the quality of life and outcome of hemodialysis among patients who are 85 years and above.

**Methods:** Among 290 adult prevalent hemodialysis patients, we conducted cross-sectional study on 11 patients who had age 85 years and above. Five of eleven were over the age of 90 years. We looked at factors that contributed to the longevity despite needing hemodialysis.

**Results:** The mean age of the group was 88.9 ± 3.5 years. Four of them were men and seven were women. Only two patients were from nursing homes, the rest were from home. Out of eleven patients, seven had arteriovenous fistula, two had arteriovenous grafts and two had permanent cuffed catheters. One of the patients who had a catheter had failed multiple other accesses including a lower extremity arteriovenous graft. Two patients had low ejection fraction, both of whom currently had cuffed dialysis catheters. The mean hemoglobin and serum albumin were 10.6 ± 1.0 gm/dL and 3.5 ± 0.4 gm/dL respectively. Seven were diabetic and only one had peripheral vascular disease. The vintage on dialysis ranged from less than one year to twenty five years, most of them have been on dialysis for more than 3 years and most had initiated dialysis after the age of 85.

**Conclusions:** Most of our patients, who are 85 years and above, come from home, are diabetic, have working accesses, have normal cardiac function and have laboratory parameters that indicate stable dialysis. Age alone should not prevent initiation of hemodialysis in the very elderly even over the age of eighty five.

**TH-PO677**

**Dialysis in Elderly Patients in a Multi-Ethnic Population: A Single Centre Unit's Experience** Alvin Kok Heong Ng. *Renal Medicine, Changi General Hospital, Singapore.*

**Background:** The incidence of elderly dialysis patients is rising worldwide as life expectancy improves with more advanced and better access to healthcare. We analyzed our multi-ethnic population of elderly dialysis patients, and describe their demographics, dialysis treatment and access at initiation, and mortality data.

**Methods:** This is a retrospective longitudinal analysis of incident dialysis patients from 1 January 2010 to 30 April 2013, aged 70 and above. Cases were identified from our unit's database. Outcomes were identified from electronic case notes and follow-up is from date of initiation of dialysis to death or 30 April 2013. The data was then analyzed via SPSS programme.

**Results:** 43 patients aged 70 years and above were started on long-term dialysis. There were 62.8% females and 37.2% males. Ethnicity was divided to Chinese, Malay, Indian and Others with a distribution of 79.4%, 14%, 2.3% and 4.7% respectively. Patients aged 70-74.99 years and 75-79.99 years contributed to 34.9% each, 18.6% were aged 80-84.99 years and finally 11.63% at 85 years and above.

The predominant cause of end stage kidney disease (ESKD) was diabetic nephropathy (62.8%). Initiation of dialysis in the elderly involved temporary vascular catheter in 41.9% and 30.2% of patients required continuous renal replacement therapy (CRRT). None of our patients had a functioning dialysis access during their initiation of dialysis. 20.9% of the patients were established on peritoneal dialysis and 79.1% hemodialysis. Median time to arteriovenous fistula or graft (AVF/AVG) creation was 81 days and to Tenckhoff insertion was 23 days.

There were only 3 deaths at 90 days (11%) from a total of 27 patients who had follow-ups of at least one year. Death rates were not calculated as the small number of patients may skew the results.

**Conclusions:** Diabetes remains a predominant aetiology of ESKD in elderly population with a large proportion requiring temporary vascular access and CRRT. Time to dialysis access creation was shorter with Tenckhoff catheter than AVF/AVG creation. Number of deaths appeared to be low but may not be accurate. We hope to improve the access to dialysis treatment, timely intiation, and care in our elderly population with the above findings.

**TH-PO678**

**Unplanned Dialysis Start Despite a Follow-Up by a Nephrologist in Elderly Patients Reaching End Stage Renal Disease. The PSPA Cohort Study** Olivier Moranne,[1,3] Cecile M. Vigneau,[2,3] Cécile Couchoud.[3,4] *[1]Nephrology, CHU Nice, France; [2]Nephrology, CHU Rennes, France; [3]For PSPA Investigators, France; [4]French Registry REIN, La Plaine St Denis, France.*

**Background:** Elderly (i.e > 75 yrs) represent about 40% of incident dialysis patients in the French REIN Registry. These patients have an higher risk of unplanned starting dialysis (30%) which is documented as a strong prognosis factor [REIN 2011]. Unplanned starting dialysis in elderly patients despite a follow-up by a nephrologist needs to be better understood. The objective of this study is to identify predialysis patient characteristics and therapeutic project associated with unplanned vs planned dialysis start from the French prospective multicenter cohort PSPA study [Moranne et al, J Gerontol A Biol Sci Med 2012].

**Methods:** From the 581 elderly patients followed by nephrologist included in the PSPA study (mean MDRD at initiation 14±4ml/min/1.73m²), 253 (43%) started dialysis during 3 years-follow-up. Patient's clinical characteristics, way of life, share therapeutic project about the option for dialysis at inclusion in the cohort and medical reasons to start dialysis between groups starting unplanned vs planned dialysis were analysed.

**Results:** Median age was 82 [79-85] yrs with 64% men and 57(23%) started dialysis in an unplanned manner (for life-threatening risk). Those patients were significantly older, had more often active cancer, lower functional mobility, lived more often in institution. They had more often a "non dialysis option" as therapeutic project, because of patient's request or stable GFR consideration according to nephrologist. Nondialysis option at nephrologist's request was not associated with unplanned dialysis. The starting dialysis reason for those patients were significantly more often anemia and hyperkalemia.

**Conclusions:** These results show that elderly reaching ESRF followed by nephrologists start unplanned dialysis in 23% of cases. Unplanned dialysis start was associated with frailty and institutionalized patients and if therapeutic project about the option for dialysis was postponed by patient or according to stable GFR by nephrologist. The results should alert nephrologist about the necessity of share therapeutic project and better enhanced the organization of initiation of dialysis.

*Funding:* Government Support - Non-U.S.

**TH-PO679**

**Efficacy of Intradialytic Rehabilitation in Renal Anemia among Elderly Patients** Yoshimi Ueda,[1,2] Junitiro Kato,[1,2] Keitaro Yokoyama,[2] Takashi Yokoo.[2] *[1]Tokyu Hospital, Tokyo, Japan; [2]The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Increasing numbers of elderly patients (Pts) with end-stage renal disease are starting dialysis. Previous reports have shown that the initiation of dialysis is associated with a substantial and sustained decline in functional status among them. The impact of a lower functional status may be reduced by offering rehabilitation to dialysis Pts. Correction of anemia and physical activity are effective for at least moderate improvements in physical function although these studies have been conducted primarily in younger Pts. The effect of exercise on renal anemia in elderly Pts is unclear. Therefore, we examined the efficacy of intradialytic rehabilitation (IDR) to prevent functional decline and renal anemia in elderly Pts.

**Methods:** We evaluated 16 hemodialysis outpatients over 65 yr of age who participated in our IDR program since September 1, 2009 to Feburary 28, 2013. All Pts underwent our program during each dialysis session for at least 12 weeks. Pts who were initiated hemodialysis within 3 months were excluded. We prescribed the stretch of the lower limbs and progressive resistance exercise using an elastic band and the gizzard. We assessed following parameters at the start and following 3 months of our program: physical tests (e.g. muscular strength, 10-meter walking test), anthropometrics, nutritional status, erythropoiesis stimulating agents (ESA) dose and hemoglobin (Hb), and data known as risk factors for ESA hyporesponsiveness.

**Results:** Pts were mean age 74.7 ± 6.0 years, 78.5% male, 18% diabetic; dialysis vintage was 125.4 ± 120.3 months. 3 Pts withdrew because of offering decline from the program, and of admission due to fall and severe hypotension. In more than half of Pts, physical strength was maintained after our program. Weekly ESA dose significantly declined from 6923IU to 5423IU ($p = 0.021$), while mean Hb concentrations increased from 10.8g/dl to 11.4g/dl at the start and 3 months, respectively.

**Conclusions:** IDR for elderly Pts has a potential not only to prevent their functional decline, but also to improve renal anemia.

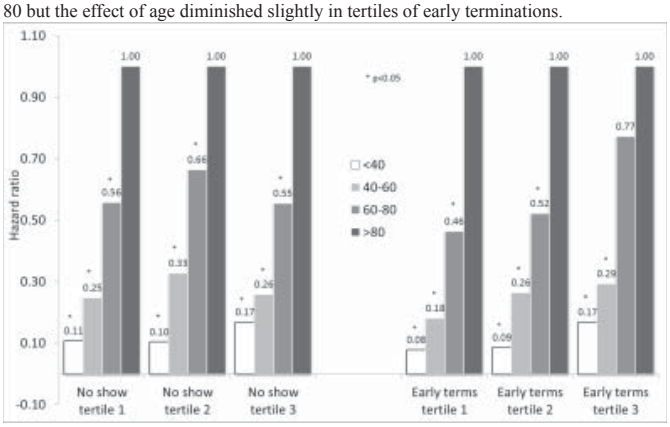
**TH-PO680**

**Relationship of Non-Compliance and Outcomes Stratified by Age Groups in Dialysis Population** Suzette Thompson,[1] Penny Faith Sheppard,[1] Len A. Usvyat,[2] Mary T. Sullivan,[1] Peter Kotanko,[1] Paul M. Zabetakis,[1] Nathan W. Levin.[1] *[1]RRI, NY, NY; [2]FMCNA, Waltham, MA.*

**Background:** Non-compliance is a significant issue in US hemodialysis (HD) patient population. We aim to understand whether non-compliance rates are different in different age groups as well as whether age affects relative risk of death in different groups of non-compliance.

**Methods:** All RRI in-center HD patients with >100 treatments and treated for >365 days between Jan 1, 2010 and Feb 28, 2013 were selected. Patients were stratified based on age (<40, 40-60, 60-80, >80) at start of the study period. For each patient, we computed % of treatments with unexcused no shows as well as % of treatments with early terminations (defined as terminated >15 minutes prior to their prescribed treatment time) during entire study period. Patients were divided into tertiles of no show and early termination rates. To determine if hazard risks of death are different at different levels of non-compliance, we constructed Cox proportional hazards models in each tertile of no shows and early terminations separately.

**Results:** We studied 7029 patients. Non-compliance was inversely related to age (not shown) as previously shown (Vega, EDTA 2011). In each tertile of no shows and early terminations, older patients had poor survival. Compared to patients >80 years, patients <40 years had similar although increasing hazard ratios in no show tertiles 1, 2, and 3 (0.11, 0.10, 0.17) for no shows respectively and for early termination tertiles 1, 2, and 3 (0.08, 0.09, 0.17). As expected, hazard risks of death were higher in age group 40 to 60 and 60 to 80 but the effect of age diminished slightly in tertiles of early terminations.




**Conclusions:** Older patients appear to have better treatment compliance than younger patients. Relative impact of age on survival appears similar across tertiles of no shows but diminishes in tertiles of early terminations.

**TH-PO681**

**Safety and Feasbility of Structured Exercise Training in Older Deconditioned Adults with CKD** Stephen L. Seliger,[1,2] Jamie Giffuni,[2] Leslie I. Katzel,[1,2] Andrew M. Well,[3] Christopher W. Washington,[2] Kieran Reid,[4] Daniel E. Weiner.[3] *[1]Medicine, U Maryland, Baltimore, MD; [2]Medicine, Baltimore VAMC, Baltimore, MD; [3]Medicine, Tufts NEMC, Boston, MA; [4]Human Nutrition Rsch Cntr Aging, Tufts Univ, Boston, MA.*

**Background:** CKD is associated with lower physical performance, especially in older adults. The feasibility and safety of structured aerobic and resistance exercise training in these patients is unclear.

**Methods:** The AWARD study is an ongoing randomized trial among community-dwelling sedentary adults aged 60+ years with stage 3b-4 CKD, comparing the effects of 12 months exercise training vs. health education (control). Thrice weekly in-center treadmill-based exercise sessions are prescribed, with intensity and duration titrated over the first 8 weeks to goal of 40 minutes/session at 70-80% of the heart rate reserve. Additional resistance training is performed twice weekly. Aerobic capacity ($VO_2$peak) is measured with graded treadmill testing, and submaximal gait with the 6-minute walk test.

**Results:** 28 patients have been enrolled to date. Mean age and BMI were 68.8 years and 30.3 kg/m$^2$, mean eGFR 37 cc/min/1.73m$^2$, and 64% had diabetes. $VO_2$peak was 18.8±5.7 mL/kg/min (32% lower than predicted for healthy older adults), and distance walked in 6 minutes was 1330±334 feet (24% lower than predicted). 15 participants were randomized to exercise, with 803 individual exercise sessions prescribed to date. Of these, 76% were attended, with median individual adherence 87%. Target heart rate was achieved or surpassed in 55% of individual sessions. A total of 5 non-serious adverse events (AE) occurred that were potentially related to exercise (minor muscle injuries and post-exercise hypoglycemia). No serious AE's related to exercise occurred.

**Conclusions:** Thrice weekly training with aerobic and resistance training is feasible and safe for sedentary older adults with non-dialysis CKD. Adherence rates are excellent/high when compared with previous long-term exercise trials in frail older adults. Final results of this trial will determine whether such training is effective at improving cardiorespiratory fitness and/or lower extremity function, parameters which are markedly impaired in these patients.

*Funding:* NIDDK Support

**TH-PO682**

**Association of Chronic Kidney Disease with Falls in Nursing Home Residents** Rasheeda K. Hall,[1,2] Ann M. O'Hare,[3,4] Ruth A. Anderson,[1] Cathleen Colon-Emeric.[1,2] *[1]Duke Univ; [2]Durham VA; [3]VA Puget Sound; [4]Univ of Washington.*

**Background:** Chronic kidney disease (CKD) has been independently associated with falls in community dwelling older adults, but it is not known if this is also the case for nursing home (NH) residents. CKD may be associated with falls in NH residents because it comes with known fall risk factors (i.e., anemia, Vitamin D deficiency). To better understand falls risk in the growing population of NH residents with CKD, this study examines the association of estimated glomerular filtration rate (eGFR) with fall rate.

**Methods:** Secondary analyses of NH resident data obtained in a multicenter randomized controlled trial of NH staff-based fall prevention strategies (2009-2012). In each participating NH, residents with at least 1 fall during either of 2 6-month periods underwent chart abstraction for fall risk factors, serum creatinine, and subsequent fall events. We calculated eGFR using the Modification of Diet in Renal Disease equation, and CKD was defined by an eGFR less than 60ml/min/1.73m$^2$. To test for independent association of eGFR with falls, our multivariable regression covariates included demographics, fall risk factors, resident's time at risk, and location.

**Results:** A total of 510 residents with falls were identified from 9 unique NHs. The mean (± SD) resident age was 77.2±11.5, 27% were female, and 63% resided in Veterans Affairs NHs. Overall median eGFR was 72 ml/min (IQR=50-95) while 35% of residents had CKD. Residents with CKD were older (79.3±11 vs. 76.1±11.6) and more likely to be female (34 vs. 23%). Mean fall rate was 5.6 falls per 100 patient days and did not differ by CKD status (p =0.40). In unadjusted analyses, lower eGFR was not associated with an increase in falls (p=0.58). In adjusted analyses, this relationship was unchanged.

**Conclusions:** In this study of NH residents who fall, one-third had low eGFR, but eGFR was not associated with the risk of recurrent falls. The absence of association suggests that CKD does not further increase falls risk in this high risk population. Given the increasing prevalence of CKD in NHs, this evidence suggests that current NH fall prevention strategies do not need to be altered for those with CKD.

*Funding:* Other NIH Support - NIA, NINR

**TH-PO683**

**Patients' and Caregivers' Perspectives on Palliative Care in Chronic Kidney Disease: Systematic Review and Thematic Synthesis of Qualitative Studies** Allison Tong,[1,2] Katharine Cheung,[3] Sumi Sukumaran Nair,[3] Manjula Kurella Tamura,[3] Jonathan C. Craig,[1,2] Wolfgang C. Winkelmayer.[3] *[1]Sydney School of Public Health, The Univ of Sydney, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Australia; [3]Div of Nephrology, Stanford Univ.*

**Background:** While dialysis offers life-prolonging treatment for end-stage kidney disease, up to 25% of deaths are attributed to dialysis withdrawal. Difficult decisions to withhold or discontinue dialysis may be necessary when the treatment burden outweighs

the benefits. This study aims to describe patients' and caregivers' perspectives on palliative care in chronic kidney disease (CKD).

**Methods:** We conducted a comprehensive literature search in Medline, Embase, PsycINFO, CINAHL and reference lists to May 2013. Thematic synthesis was used to analyze the findings.

**Results:** Twenty-four studies involving over 440 patients and 161 caregivers were included. We identified five themes: relief from suffering (bodily deterioration, loss of freedom and independence, pervasive fatigue and pain, resignation, treatment burden and harm); personal vulnerability (imminence of death, fear of misunderstanding and judgement, maintaining autonomy and dignity, medical abandonment, valuing safety and trust); relational responsibility (being a burden, demonstrating loyalty, protecting others from grief); negotiating existential tensions (accepting natural course of life, disrupted aging, worthlessness, living on borrowed time, respecting sanctity of life, life satisfaction, preserving self-identity); and preparedness (decisional clarity, information support, spirituality and hope).

**Conclusions:** Patients with CKD can experience physical and psychosocial frailty, and feel ambivalent about prolonging life. Caregivers believe in providing relief from suffering but are uncertain about making decisions regarding palliative care. CKD management should encompass palliative care strategies that promote emotional resilience, sense of well-being and self-value. Also, respectful and attentive communication is needed so patients are empowered to convey their values and preferences in treatment decision making.

## TH-PO684

**Understanding Patterns of Place of Death in Patients with CKD 4-5** Fliss E. Murtagh,[1] Natasha B. Lovell,[1] Chris Jones,[2] Baynes Ingrid Dawn,[2] Sarah J. Dinning,[2] Catherine Susanna Vinen.[2] *[1]Dept of Palliative Care, Policy and Rehabilitation, Kings College London, London, United Kingdom; [2]Kings Renal Unit, Kings College Hospital, London, United Kingdom.*

**Background:** Meeting preference regarding place of death is an important marker of quality of care; systematic review shows 42% end stage kidney disease patients prefer home death. These patients have high mortality, yet little research has been done to study place of death. The aim of this study was to understand patterns of place of death in patients with Chronic Kidney Disease (CKD) 4-5 known to a UK renal team.

**Methods:** A retrospective cohort study of CKD patients stage 4-5 known to a UK renal team, who died over 4 years (2009-2012). Demographic and clinical variables were collected, including management pathway and living circumstances. Multi-variable regression analysis was undertaken to determine relationship with place of death.

**Results:** 721 patients were included (mean age 73.9, SD 13.65). 59.6% male. 70.2% died in hospital (95% CI 66.8%-73.4%), 21.9% died in their usual residence (95% CI 19.0%-25.0%), and 7.9% died in an inpatient palliative care unit (95% CI 6.1%-10.1%). 1) Management pathway and 2) Residential setting were most strongly associated with place of death. 1) Patients on the conservative (non-dialytic) pathway had 4.6 times the odds of dying out of hospital (OR 4.6, 95% CI 2.8-7.3, P<0.01). 2) Patients living alone were less likely to die out of hospital (OR 0.6, 95% CI 0.4-1.0, P<0.05). Those where living status was not documented were less likely to die out of hospital (OR 0.5, 95% CI 0.3-0.8, P<0.01). Patients living in a care home had 9 times the odds of dying out of hospital (OR 9.3, 95% CI 3.4-25.7, P<0.01).

**Conclusions:** Hospital death predominated all groups, but place of death varied by management pathway, consistent with national data. Out-of-hospital deaths were higher in the conservative group. This may represent impact of palliative care service development for conservatively managed patients in this unit. This work highlights importance of considering management pathway and living circumstances, so we meet preference for place of death in other management pathway groups, including patients on haemodialysis.

## TH-PO685

**Symptom Scores in End-Stage Kidney Disease: Can They Help U.S. with Timing of Anticipatory Care Planning?** Fliss E. Murtagh,[1] Natasha B. Lovell,[1] Katherine Bristowe,[1] Kate A. Shepherd,[2] Heather Jane Brown,[1] Beverley Matthews,[1] Donal O'Donoghue,[1] Catherine Susanna Vinen.[2] *[1]Kings College London, Cicely Saunders Institute, London, United Kingdom; [2]Kings College Hospital, London, United Kingdom.*

**Background:** Patients with end stage kidney disease report high fatigue (weighted mean prevalence 71%) and up to 58% experience depression (Murtagh AJKD 2007). Good symptom management and being prepared are patient priorities but professionals find timing of discussions challenging. We aimed to identify if routine symptom assessment had potential to inform timing.

**Methods:** Mixed-methods study, including experience survey, and symptom assessment (validated renal Palliative care Outcome Scale). 3 questions concerned anticipatory care planning (ACP): Q1)Have you thought about where you would like to be cared for if you were not able to get better? Q2)Have you thought about who you would like to be involved in decisions about your medical care? Q3)When you raise these concerns do you feel they are taken seriously?

**Results:** 91 dialysis patients, 64.4% male, 30% aged 75 and above. 49.5% white. Fatigue and depression showed significant differences in question response: Q1 responses: 19.1%(95% CI 11.5-28.8) 'yes', 15.7%(95% CI 8.9-25.0) 'to some extent', 58.4%(95% CI 47.5-68.8) 'no', and 6.7%(95% CI 2.5-14.1) 'don't know'. Patients with high depression scores more often considered future care options ($\chi 2$=8.569;p=0.036;n=87). Q2 responses: 55.6%(95% CI 44.7-66.0) 'yes', 21.1%(95% CI 13.2-31.0) 'to some extent', 20.0%(95% CI 12.3-30.0) 'no', and 3.3%(95% CI 0.7-9.4) 'don't know'. Patients with high fatigue more often considered who they wanted involved in decisions ($\chi 2$=9.798;p=0.02;n=85). Q3 responses: 53.5%(95% CI 42.4-64.3) 'yes', 32.6%(95% CI 22.8-43.5) 'to some extent', 8.1%(95% CI 3.3-16.1) 'no', and 5.8%(95% CI 1.9-13.0) 'not applicable'. Patients with high fatigue felt less sure concerns were taken seriously ($\chi 2$=8.799;p=0.032;n=81).

**Conclusions:** These results highlight need to consider symptoms when timing ACP discussion. Routine regular symptom assessment may help inform readiness of patients, particularly if reporting fatigue/ depression. This work is a key component in a project led by NHS Kidney Care.

*Funding:* Government Support - Non-U.S.

## TH-PO686

**Depression, Social Support, Self-Efficacy, and Fluid Adherence in Older Adults on Hemodialysis** Tiffany R. Washington. *Social Work, Univ of North Carolina at Chapel Hill, Chapel Hill, NC.*

**Background:** Controlled fluid intake is an important aspect of kidney disease self-management, yet depression, the most common psychiatric issue affecting older end-stage renal disease (ESRD) patients, negatively impacts fluid adherence. Factors that mitigate depression's deleterious effects are underidentified, and if identified, can aid in the design and development of psychosocial interventions to reduce its negative impact and improve fluid adherence. This paper examines four logistic regression models to determine mediating factors between depression and fluid adherence.

**Methods:** A total of 107 ESRD patients from four hemodialysis facilities in the southeast aged 50 and older were interviewed about their kidney disease self-management behaviors. Four logistic regression models were analyzed: 1) the primary independent variable (depression); 2) the primary independent variable (depression) with a secondary independent variable (social support); 3) the primary independent variable (depression) with a secondary independent variable (self-efficacy); and 4) the primary independent variable (depression) with both secondary independent variables (self-efficacy and social support).

**Results:** Age was associated with an increase in fluid adherence (adjusted odds ratio [AOR] = 1.08, 95% confidence interval [CI] = 1.02-1.14), and depression was associated with a decrease in fluid adherence (AOR = 0.82, 95% CI = 0.67-0.99). When self-efficacy was entered into the model, the association between depression and fluid adherence disappeared. Race comparisons showed that depression was negatively associated with fluid adherence ($r = -0.26$, $p < .05$), and self-efficacy and age were positively associated with fluid adherence among black participants ($r = 0.24$, $p < 0.05$ and $r = 0.32$, $p < 0.01$, respectively).

**Conclusions:** The findings from this study suggest that self-efficacy and age are important factors in fluid adherence, and self-efficacy can potentially mediate the negative effects of depression in older ESRD patients. Advanced statistical methods and longitudinal studies are required to determine the indirect effect of depression to fluid adherence mediated by self-efficacy.

*Funding:* Private Foundation Support

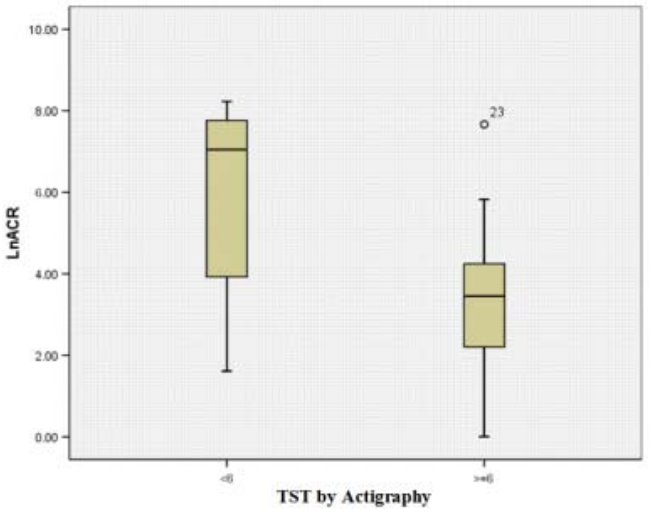
## TH-PO687

**Short Sleep Duration and Albuminuria in Advanced Chronic Kidney Disease** Andrew M. Well,[1] Jamie Giffuni,[2] Stephen L. Seliger,[2] Roger A. Fielding,[1] Leslie I. Katzel,[2] Daniel E. Weiner.[1] *[1]Tufts Univ, Boston, MA; [2]Univ of Maryland, Baltimore, MD.*

**Background:** Short sleep duration (SSD) is a predictor of cardiovascular disease and mortality, and, in one study of younger individuals with normal eGFR, self-reported SSD was associated with dipstick proteinuria. The association between sleep duration and albuminuria has not been evaluated in older individuals with advanced CKD.

**Methods:** AWARD is a randomized controlled trial evaluating the effects of exercise vs. health education in individuals 60 years and older with an eGFR of 15 to 45 ml/min/1.73m$^2$. Participants' total sleep time (TST) at baseline was quantified by actigraphy worn for 5 consecutive nights and by self report using the Pittsburgh Sleep Quality Index. Urine albumin creatinine ratio (ACR) was assessed at baseline.

**Results:** Of 28 participants who have completed baseline testing by May 15, 2013, 27 have complete sleep data and were included. Mean age is 69 ± 7 years, 15% are women, 67% diabetic and 56% on ACEi or ARB therapy; mean eGFR is 37 ± 11 ml/min/1.73m$^2$ and median [interquartile range] ACR is 49 [17 – 878] mg/g. Mean actigraphy TST was 6.6 ± 1.6 hours with 41% sleeping <6 hours. Participants with TST <6 hours on actigraphy had a median ACR of 1152 [66–2580] mg/g while those with a TST ≥6 hours had a median ACR of 31 [9–63] mg/g (p=0.02).




Greater TST on actigraphy was correlated with lower Ln-transformed ACR in univariate (β = -0.443, p=0.02) and multivariable analyses controlling for age, BMI, eGFR, diabetes and ACE/ARB use (β= -0.463, p=0.007). Self reported TST was not associated with ACR (β= -0.08, p=0.70).

**Conclusions:** Among older adults with CKD Stage 3b-4, objectively measured sleep duration is associated with higher ACR. The elevated ACR in SSD may suggest increased inflammation or vascular risk factor burden due to SSD.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO688

**Chronic Pain in Hemodialysis Patients** Sharmeela Saha, Robert E. Olivo, Robin L. Hensley, Julia Lewis. *Nephrology, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** We conducted a retrospective analysis of narcotic prescription practices in our outpatient hemodialysis (HD) units. The purpose was to examine management of chronic pain and to develop strategies to improve patient safety.

**Methods:** We studied HD outpatients in 2 units from January 1, 2012 until January 1, 2013. We collected information regarding patient demographics, narcotic prescriptions and prescribers, and documentation of analgesic indication by examining the Tennessee Monitoring Database and Vanderbilt medical records.

**Results:** 52 of 191 (27%) HD patients were supplied with narcotics for at least a continuous 90 day period. Almost 40% of patients in the 40-49 and 50-59 age groups were treated for chronic pain. There was no predominance by gender but 32% of African Americans and 19% of Caucasians received chronic narcotics. Pain medications were most common among patients on HD for over 5 years. Arthritis and diabetic neuropathy were the most prevalent comorbidities. Hydrocodone with acetaminophen was the most commonly prescribed medication. The most frequently recorded indication was peripheral polyneuropathy; however, 52% (27/52) of patients had no documented indication. 90% of patients on chronic pain meds did not have a narcotics contract. 21% of patients had their narcotics solely prescribed by their nephrologist.

**Conclusions:** There is suboptimal documentation of the indication for pain meds for our patients on chronic narcotics; specifically, for over 50% of identified patients we were unable to find a recorded reason for therapy. We propose that the narcotics prescriber indicate semiannually an assessment of the pain and maintain a narcotics contract with the patient. Moreover, the Tennessee Monitoring Database allows providers to determine what other narcotic prescriptions the patient holds and reviewing the database monthly may help control for polypharmacy as well as prevent patients from obtaining narcotics from multiple providers. We hope to improve patient safety by addressing pain management infrastructure including maintaining annual narcotic contracts, regular documentation of indication for prescription and selection of a primary provider who manages pain control.

## TH-PO689

**Renal Function Decline in Community-Dwelling Older Adults** Jennifer Yi-Chun Lai,[1] Mindy Katz,[3] Peter X.K. Song,[2] Richard B. Lipton,[3] Markus Bitzer.[1] *[1]Medicine, Univ of Michigan, Ann Arbor, MI; [2]Biostatistics, Univ of Michigan, Ann Arbor, MI; [3]Neurology, Albert Einstein College of Medicine, Bronx, NY.*

**Background:** Decreased kidney function is associated with increased morbidity and mortality in older adults. The rate of renal function decline varies, but risk factors for accelrated decline are not well-defined in older adults. Therefore, we examined rate of renal function decline and associated clinical factors in community dwelling older adults enrolled in Einstein Aging Study (EAS), a community-based longitudinal study of aging.

**Methods:** We identified 250 subjects (>68 years of age), who were followed up to 7 years since 2005 and had at least three serum creatinine measurements separated by at least 11 months. We calculated creatinine-based eGFR by different formulas (CG, MDRD, CKD-EPI, and Berlin initiative study1 (BIS1)). Rates of eGFR decline were determined by mixed effect modeling. Multivariate linear or logistic regression was used to identify factors associated with mixed effect eGFR decline rate.

**Results:** The mean age of the study subjects was 79 years (60% are female). Mean baseline eGFR was 55 to 74 min/ml/1.73m$^2$ based on different formulas. The mean mixed effect eGFR annual percentage change was between -3.0% to -1.3%. Cross-sectional analysis revealed that higher homocysteine were significantly associated with lower baseline eGFR (P<0.0001). Longitudinal analysis showed that in linear regression, baseline eGFR was the only factor associated with eGFR annual percentage change (β= 0.01-0.04, P<0.0001). In logistic regression, subjects with lower baseline eGFR were more likely to have progressive eGFR decline (OR~16-40, P ≤ 0.001 for CG, MDRD and BIS1; no significance was detected using CKD-EPI formula).

**Conclusions:** The average annual renal function decline in this older adult cohort is 1 to 3 % per year. Baseline eGFR is an independent risk factor for faster eGFR decline. Homocysteine levels are associated with cross-sectional eGFR, but not with eGFR decline. Neverthelss, additional markers are needed to identify subjects with similar eGFR at risk for future loss of renal function, in particular with CKD stage III.

*Funding:* NIDDK Support

## TH-PO690

**Assessment of Renal Function in Elderly with Chronic Kidney Disease Using CKD-EPI and MDRD Equations** Eleni Chelioti,[1] Dimitrios Athanasopoulos,[2] Evangelia Gkalitsiou,[1] Evdokia Efthimiou,[1] Maria Sotiraki,[1] Alexia Papalexandrou,[1] Maria Tsilivigou,[1] Gabriel Papadakis.[1] *[1]Dept of Nephrologr, General Hospital of Piraeus, Athens, Greece; [2]Health Centre of Dimitsana, General Hospital of Tripoli, Tripoli, Greece.*

**Background:** Kidney function loss in the elderly results from physiologic aging of the kidney and lifelong pathologic insults. To estimate glomerular filtration rate (eGFR), MDRD and CKD-EPI formulae are the most frequently use.

**Aim** of the study was to assess the renal function in elderly with chronic kidney disease using the MDRD and CKD-EPI equations.

**Methods:** A cross sectional study was carried out. The study included elderly patients that attended the outpatient nephrology clinic within one year. The patients divided in 2 groups: eGFR>60 and eGFR<60ml/min/1.73m$^2$. Estimated GFR was calculated using MDRD and CKD-EPI formulae on time 0 and 12months.

**Results:** 224 patients were studied (52%men, 42% diabetics, 83% hypertensive, mean age70±13years-old, mean SAP 133,6±15mmHg, mean DAP 73,4±12mmHg, mean serum creatinine 1,8±0,1mg/dl). There are not statistically differences between mean CKD-EPI and MDRD GFR on time 0(43±20 and 42±22 respectively) and 12 months (43±20 and 43±19 respectively) in patients with eGFR>60ml/min/1.73m2. Not statistically differences found between mean CKD-EPI and MDRD on time 0 (30±18 and 31±17 respectively) and 12 months (32±18 and 32±18 respectively)in patients with eGFR<60ml.min/1.73m2.

However, we found that there is a statistically significant correlation between eGFR >60 or eGFR<60 and sex (p=0,001) in both time 0 and 12. Also, the correlation between reduced eGFR and hypertension was statistically significant (p=0,01)in both groups and independent by the using of MDRD or CKD-EPI equations.

**Conclusions:** Our results suggest that at ages>70 years there is not difference between the equations for the assessment of renal function.

## TH-PO691

**Prevalence of Stage 3-5 Chronic Kidney Disease and Anaemia in Elderly and Very Elderly** Eleni Chelioti,[1] Dimitrios Athanasopoulos,[2] Evdokia Efthimiou,[1] Maria Sotiraki,[1] Alexia Papalexandrou,[1] Evangelia Gkalitsiou,[1] Maria Tsilivigou.[1] *[1]Dept of Nephrologr, General Hospital of Piraeus, Athens, Greece; [2]Health Centre of Dimitsana, General Hospital of Tripoli, Tripoli, Greece.*

**Background:** Chronic kidney disease (CKD) is a growing problem due to our aging population, many of who have increased comorbidities.Cross-sectional studies in the general population demonstrate that CKD is seen in up to 15,3% including 39% of those over the age of 65 years.

**Aim** of the study was to examine the prevalence of stage 3-5 CKD in elderly and very elderly at an outpatient nephrology clinic and its association with anemia.

**Methods:** The cohort was defined as all patients that attended the outpatient nephrology clinic within the 2012.The patients divided in 3 age groups (AG):65-75AG1,75-80AG2 and AG3>80 years old.Estimated glomerular filtration rate (eGFR) was calculated using MDRD and CKD-EPI formulae.

**Results:** A total of 231 patients were studied (52%men,mean age76±6years,mean eGFR 31±18ml/min/1.73m$^2$,meanHb 11,98±1,7g/dl).According to the 3 age groups the percentage of patients was:50%AG1,27%AG2 and 23%AG3.There are not statistically difference between mean CKD-EPI and MDRD GFR.The percentage of stage 3,4 and 5 CDK was higher at AG1,AG3 and AG1 respectively.

| Age groups (y) | CKD-EPI GFR ml/min/1.73m2 mean (SD) | MDRD GFR ml/min/1.73m2 mean (SD) | p | Hb(g/dl) mean (SD) | Stage 3 % | Stage 4 % | Stage 5 % |
|---|---|---|---|---|---|---|---|
| 65-75 | 33,5(20) | 33,7(20) | NS | 12,1(1,9) | 43,8 | 23,2 | 23,2 |
| 75-80 | 30,5(17,6) | 30,3(17,5) | NS | 11,9(1,8) | 33,9 | 39 | 18,6 |
| ≥80 | 28,3(12,3) | 30(12,3) | NS | 11,8(1,4) | 29,4 | 52,9 | 15,7 |

The percentage of all patients who had Hb<11,5g/dl or<10g/dl was 41,5% and 10,3% respectively.Correlation between decline of eGFR and Hb was statistically significant (p<0,0001) in all age groups and not statistically significant with the age(p=0,7).

**Conclusions:** Our results suggest that the prevalence of stage 3 CKD is higher in the elderly but the prevalence of CKD stage 4 is highest in very elderly and lower in stage 5.Also the anemia in the elderly and very elderly seems to be in association with the decline of GFR and not with the aging.

## TH-PO692

**Hospitalized Elderly with Acute Kidney Injury Are at Greater Risk of Death and Progressive Chronic Kidney Disease** Jia Liang Kwek, Hui Zhuan Tan, Cynthia Ciwei Lim, Manish Kaushik. *Dept of Renal Medicine, Singapore General Hospital, Singapore, Singapore.*

**Background:** To determine the patient and renal outcomes in elderly patients with AKI as defined by Acute Kidney Injury Network (AKIN) criteria.

**Methods:** A single-center retrospective cohort study on elderly ≥ 65 years old admitted during first week of January 2011. Demographic data, co-morbidities and laboratory findings were retrieved from the electronic medical records. Patients with <2 serum creatinine values were excluded. AKI was present if serum creatinine increased ≥ 26.4 µmol/L within a 48h during hospitalization. The primary outcome was kidney function measured by MDRD estimated glomerular filtration rate (eGFR) at latest visit. The secondary outcomes were in-hospital mortality and incident end-stage renal disease (ESRD) requiring dialysis. Patients were followed up until 31st December 2012.

**Results:** Of 709 elderly admitted, 324 (median age 77, male 48.6%, diabetes mellitus 46%, hypertension 78.7%, ischemic heart disease 38.3%) were studied. Baseline serum creatinine was available in 264 patients (81.5%). Median baseline eGFR was 71.3 ml/min/1.73m$^2$ (range 2.7-228.7) and median eGFR on admission was 58.6 ml/min/1.73m$^2$ (range 1.5-292.6). 46 patients (14.6%) had AKI at median 2 days (range 1-33) during hospitalization. 3 patients (0.9%) required dialysis. Comparing AKI group (n=46) with non-AKI group (n=278), the only significant risk factor for AKI found in this study was hypotension (34.8% vs 13.7%, p=0.001). Median follow-up period was 14.5 months (range 0-24). 52.2% of AKI group had eGFR < 30 ml/min/1.73m$^2$, compared to 20.5% of non-AKI group (p <0.001). Significantly, 34.8% of AKI group had decreased eGFR from baseline by ≥ 50%, as compared to 11.9% of non-AKI group (p<0.001). In-hospital mortality was increased for AKI group (21.7% vs 3.6%, p < 0.001). However, there was no difference in incident ESRD requiring dialysis (4.3% vs 1.1%, p=0.149).

**Conclusions:** This study confirmed that even mild AKI is associated with significant risk of deterioration in renal function and death. Future interventional study can target preventive measures and timely treatment of hospitalization AKI to lower the risk of adverse outcomes.

## TH-PO693

**Triamterene-Hydrochlorothiazide Is Associated with Chronic Kidney Disease in Hypertensive Elderly Patients** Manish K. Saha, Ann L. Rinehart. *Nephrology, HealthPartners, St. Paul, MN.*

**Background:** Triamterene-HCTZ (TR-HCTZ) is a commonly prescribed antihypertensive medication combination. TR can cause abnormal urinary sediment, nephrolithiasis, and acute renal failure but has not been implicated in causing chronic kidney disease (CKD).

**Methods:** A case series of 95 patients referred for CKD to a single nephrologist at a tertiary center in St. Paul, MN from Jan 1995 to June 2010. All received TR-HCTZ for hypertension (HTN). Data was collected for: serum creatinine (CR) prior to TR-HCTZ exposure, at drug cessation, and at 1-3 and 6-12 month follow up; age, BMI, gender, and race; TR-HCTZ dosage and duration; patient reported NSAID use; UA and urinary microalbumin; ace inhibitor (ACE) or angiotensin receptor blockade (ARB) use; HgbA1c; mean outpatient blood pressure (BP) readings in prior year; BP uncontrolled if mean > 140/90; history and physical exam; IV contrast or other nephrotoxin exposures; and renal ultrasound.

**Results:** 42 patients had no alternative CKD diagnoses identified except for TR-HCTZ exposure (24% TR-HCTZ 37.5/25 mg vs 76% 75 mg/50 mg) with or without NSAID use. Mean age was 71.8 yrs (range 51-92) with 81% female, 17% diabetic without microalbuminuria, and 31% taking ACE/ARB, which was discontinued with TR-HCTZ and resumed after 6 months in all patients. Median TR-HCTZ exposure was approximately 10 years (range 2.3-20). 19 used NSAIDs > 2 times weekly; 1 patient with unavailable data. Mean (SD) eGFR (ml/min/1.73m$^2$)

| | n=17 | n=25 | NSAIDs: none or < 1x/wk (n=22) | NSAID > 2x/wk (n=19) |
|---|---|---|---|---|
| eGFR pre TR-HCTZ | 65.9 (19.7) | NA | NA | NA |
| eGFR at TR-HCTZ discontinuation | 33.5 (10.2) | 32.3 (9.8) | 32.7 (10.1) | 33.2 (9.9) |
| eGFR at 1-3 months post TR-HCTZ | 43.2 (12.3) | 40.8 (12.6) | 43.4 (13.5) | 40.5 (11.4) |
| eGFR at 6-12 months post TR-HCTZ | 46.1 (10.6) | 43.6 (15.0) | 46.6 (15.5) | 42.6 (10.1) |

**Conclusions:** Excluding other causes of CKD, TR-HCTZ is associated with CKD in primarily female elderly hypertensive patients with or without NSAID use. Its cessation resulted in partial improvement in renal function, even when ACE/ARB and HCTZ were resumed. TR should be avoided in elderly patients to reduce the risk of chronic kidney disease. Its role in hypertension management should be further studied and reassessed.

## TH-PO694

**Use of Beers Criteria Medications and Opioids among Very Elderly Hemodialysis Patients** Eric D. Weinhandl,[1] Wendy L. St. Peter,[1,2] Anne M. Murray.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Univ of Minnesota, Minneapolis, MN.*

**Background:** The very elderly constitute the most rapidly growing segment of dialysis patients in the United States and cognitive impairment (CI) is highly prevalent among them. Beers Criteria (BC) include several medication classes that are recommended to be avoided in older adults with CI. Opioids are commonly prescribed to dialysis patients and may further worsen cognitive function. We used Medicare Part D data to assess use of both potentially inappropriate medications in CI and opioids in very elderly hemodialysis patients in 2011.

**Methods:** Included patients carried Medicare coverage during all of 2010; initiated dialysis no later than September 30, 2010; received hemodialysis from October 1 to December 31, 2010; were age ≥80 years on January 1, 2011; and carried Part D coverage from January 1, 2011, to the earliest of death or December 31, 2011. We categorized patients as unsubsidized (UN); subsidized and not institutionalized (SUB); or subsidized and institutionalized (SUB+IN).

**Results:** We identified 8,459 UN; 7,196 SUB; and 3,052 SUB+IN patients. Regarding BC, 44.7% of patients used ≥1 medication that is recommended to be avoided in older adults with CI, including agents with anticholinergic effects (27.8%), H-2 receptor antagonists (12.1%), zolpidem (11.1%), and antipsychotics (7.0%). Factors associated with use of ≥1 BC medication were age ≥90 vs. 80-84 years (adjusted odds ratio, 0.75), white vs. black race (1.33), female vs. male sex (1.33), diagnosed dementia (1.28), SUB vs. UN status (1.54), and SUB+IN vs. SUB status (1.48). Regarding opioids, 51.0% of all patients used ≥1 medication; estimates of opioid use in patients with and without diagnosed dementia were 50.9% and 51.1%, respectively. Opioid use was associated with diagnosed dementia (0.88), SUB vs. UN status (1.27), and SUB+IN vs. SUB status (1.30).

**Conclusions:** Use of potentially inappropriate medications in cognitive impairment was common among very elderly hemodialysis patients and almost 30% more likely among patients with diagnosed dementia. Use of opioids was also common. Studies are needed to determine whether these medications are prescribed appropriately.

*Funding:* NIDDK Support

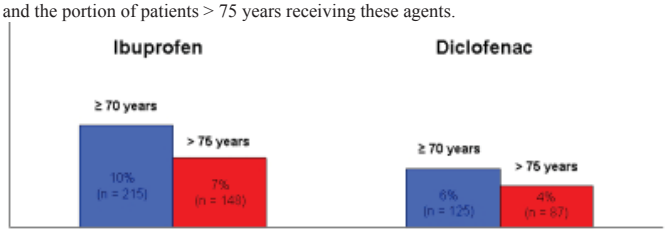
## TH-PO695

**Non Steroidal Anti-Inflammatory Drugs and Kidney Function in the Elderly** Antonios Douros,[1] Natalie Ebert,[2] Olga Jakob,[3] Reinhold Kreutz,[1] Elke Schaeffner.[2] *[1]Clinical Pharmacology, Charite, Berlin, Germany; [2]Nephrology, Charite, Berlin, Germany; [3]Clinical Epidemiology, Charite, Berlin, Germany.*

**Background:** There are several limitations regarding the use of non steroidal anti-inflammatory drugs (NSAIDs) in the elderly among which impaired kidney function (KF) represents an important clinical problem. The present study investigates the relationship between intake of NSAIDs and KF in people ≥ 70 years.

**Methods:** Individuals included in this analysis were participants of the Berlin Initiative Study (BIS). BIS is a population-based cohort study initiated in 2009 in Berlin, Germany, in order to evaluate KF in individuals ≥ 70 years. Medication was assessed through personal interviews and coded using the Anatomical Therapeutic Chemical Classification System. For estimation of glomerular filtration rate (eGFR) we used the CKD-EPI equation.

**Results:** From overall 2070 individuals 440 subjects (21%) received NSAIDs, either regularly or on demand. Mean age of the NSAIDs population was 79.8±6.4 years (females 62%). Figure 1 illustrates the frequency of use for the two most commonly taken NSAIDs, and the portion of patients > 75 years receiving these agents.




In comparison to the overall cohort analysis of the subpopulation with eGFR < 30 ml/min/1.73m$^2$ revealed no considerable difference concerning NSAIDs use, i.e. 20% of subjects.

**Conclusions:** As acute or chronic pain conditions are frequent in advanced age, use of NSAIDs in the elderly is a common topic of debate. Our data show that about 20% of this population take NSAIDs. Most of these patients were > 75 years, despite the recent classification of nearly all NSAIDs as potentially inappropriate medication for this age group by the American Geriatric Society. Presence of severe renal impairment did not seem to affect the prescription behavior of physicians regarding NSAIDs, although this represents a formal contraindication.

**TH-PO696**

**Antiviral Dose and Hospitalization with Urgent Computed Tomography Scan and Mortality in Older Patients with and without Chronic Kidney Disease – A Population-Based Study** Ngan Lam,[1,2] Jamie L. Fleet,[1] Eric McArthur,[2,3] Stephanie Dixon,[2,3] Amit X. Garg.[1-3] *[1]Nephrology, Western Univ, London, Canada; [2]Epidemiology and Biostatistics, Western Univ, London, Canada; [3]Institute for Clinical Evaluative Sciences, Toronto, Canada.*

**Background:** Higher versus lower doses of antiviral drugs used to treat herpes zoster infection may lead to more adverse events in older adults, particularly those with chronic kidney disease (CKD) where drug elimination is reduced.

**Methods:** We conducted a matched retrospective population-based cohort study of older adults (mean 77 years) in Ontario, Canada who were initiated on a higher (n = 23,256) versus lower (n = 3876) dose of one of three study oral antivirals (acyclovir, valacyclovir, or famciclovir) for the treatment of herpes zoster infection. Patients were enrolled between 2002 and 2011. The primary outcome was 30-day hospitalization with evidence of an urgent head computed tomography (CT) scan (a proposed proxy for acute delirium). The secondary outcome was 30-day all-cause mortality. We stratified our results based on the presence or absence of CKD.

**Results:** A higher dose of antiviral drug was not associated with a higher risk of hospitalization with CT head scan (247 [1.06%] events with higher dose versus 43 [1.11%] events with lower dose, relative risk 0.96, 95% confidence interval 0.69 to 1.33, p-value 0.79). There was also no significant difference in mortality between the two dose groups. Results were consistent in all subgroups, including those with and without CKD (p-value for interaction 0.25).

**Conclusions:** In this study, a higher vs. lower dose of antiviral drug for the treatment of herpes zoster was not associated with a higher risk of hospitalization with CT head or mortality in older patients with and without CKD.

**TH-PO697**

**Serum Anion Gap Is Predictive of Mortality in an Elderly Population** Shin-Young Ahn, Seon Ha Baek, Jiwon Ryu, Ho Jun Chin, Ki Young Na, Dong Wan Chae, Sejoong Kim. *Internal Medicine, Seoul National Univ Bundang Hospital, Seongnam-si, Gyeonggi-do, Republic of Korea.*

**Background:** An elevated serum anion gap is known to be associated with hypertension, low cardiorespiratory fitness, and decreased renal function. We evaluated whether serum AG might be predictive of elderly mortality in a community-based cohort in Korea.

**Methods:** We analyzed the available data from 862 elderly people in the Korean Longitudinal Study on Health and Aging. In the baseline study of the KLoSHA, the participants were required to visit Seoul National University Bundang Hospital on two occasions for comprehensive interviews and laboratory tests.

**Results:** Over a 5-year observational period, 151/862 (17.5%) participants died, and a high albumin-adjusted anion gap (SAAG) was associated with an increased risk of all-cause mortality in unadjusted analyses (hazard ratio [HR], 1.96; 95% confidence interval [CI], 1.41 - 2.71) and fully adjusted analyses (HR, 1.77; 95% CI, 1.24 - 2.52), compared with a low SAAG group. In particular, the participants with high SAAG had higher cardiovascular and infection-related mortality rates than those with low SAAG (HR, 2.11; 95% CI, 1.06 - 4.19, and HR, 9.69; 95% CI, 1.12 – 83.4, respectively).

**Conclusions:** High SAAG may be an independent predictor of mortality and can affect cardiovascular- and infection-related mortality in the elderly Korean population.

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**TH-PO698**

**Polypharmacy Is a Risk Factor for Acute Renal Failure in the Elderly Population** Osman Z. Sahin,[1] Fatih Sumer,[2] Teslime Ayaz,[2] Kadir Ilkk?l?c.[2] *[1]Nephrology, Recep Tayyip Erdogan Univ Faculty of Medicine, Turkey; [2]Internal Medicine, Recep Tayyip Erdogan Univ Faculty of Medicine, Turkey.*

**Background:** Because of the rapid growth in elderly population, polypharmacy has become a serious public health issue worldwide. Particularly, angiotensin coverting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) are increasingly used in the elderly for heart failure and hypertension. Acute renal failure (ARF) is one negative consequence of polypharmacy. We therefore aimed to assess the impact of polypharmacy, especially usage of ACE inhibitors and/or ARBs on the development of ARF in elderly poupulation.

**Methods:** 42 consecutive patients who were older than 65 years and admitted to our emergency unit or Nephrology outpatient clinic between January 2010 and June 2012 for ARF included in this study. Polypharmacy was defined as more than 5 prescriptions per day. All the patients were using an ACE inhibitor or ARB for at least 3 months. We recorded all the other medications especially diuretics and nonsteroidal anti-inflammatories (NSAIDs). We especially noted the change in medication for 10 days prior to admission. ARF was defined as a sudden increase in serum creatinine from normal to more to 2 mg/dl or an increase 50% above the baseline within 3 months.

**Results:** The study population consisted of 44 patients, aged 65-86 years (mean: 74.27 ± 6.6) and 54.5% of them were male. 61.4% of them had hypertension, 22.7% had diabetes, 11.4% had congestive heart failure and 22.7% had chronic renal failure. 83.3% of the patients were receiving ACE-I or ARB and 52.4% of them were usingNSAIDs. Mean number of prescriptions per day was 6.1. Basal urea and creatinine levels of the patients were 4.71±2.67 mg/dl and 49.2±57.76 mg/dl respectively. Potassium levels were ≥6 mEq/lt in 22.5% of the patients. ACE-I or ARB and NSAIDs were stopped. 15.9 % the patients were undergone on hemodialysis. % 40.9 patients had recovered and serum creatinine level came back to normal.

**Conclusions:** We observed an association between the polypharmacy, usage of ACEI or ARBs and the occurrence of ARF in elderly population.

**TH-PO699**

**Initiatives by the Geriatric Nephrology Advisory Group (GNAG) of the American Society of Nephrology (ASN)** Markus Bitzer,[1] Mark E. Williams.[2] *[1]Internal Medicine, Univ of Michigan, Ann Arbor, MI; [2]Joslin Diabetes Center, Harvard Medical School, Boston, MA.*

**Background:** There is growing interest in the care of older adults with kidney disease among Nephrologists and the increasing use of dialysis and kidney transplantation in older adults has attracted the attention of geriatrics and palliative care.

**Methods:** We describe resources and initiatives of the ASN's Geriatric Nephrology Advisory Group (GNAG) to improve care for older adults with CKD.

**Results:** To improve the quality of dialysis rounds of geriatric patients GNAG, in partnership with the Association of Specialty Professors (ASP) and under the direction of Mark Williams, created a video series that discusses critical aspects of care for elderly patients, including patient assessment, dialysis care, recognition of physical and mental decline, quality of life issues, and sharing decision-making information, which is freely available at www.asnrounds.org and has already over 5,000 views. To enhance education of Nephrology fellows in Geriatric Nephrology, GNAG developed and updates an on-line Geriatric Nephrology curriculum with teaching tools in the diagnosis and management of various geriatric entities (with ASP support). For an in-depth discussion of specific aspects of Geriatric Nephrology, the GNAG with support of the ASN Post-Graduate Education Committee has organized the Dimitrios G. Oreopoulos Memorial Program in Geriatric Nephrology, a two-day course on Geriatric Nephrology included in the annual meeting of the ASN since 2008. The 2013 course is entitled "Patient-Centered Care for Older Adults with Kidney Disease". To provide guidance in the care of older adults with kidney disease and to promote scientific collaborations to advance research in Geriatric Nephrology GNAG established the Dimitrios G. Oreopoulos Visiting Professor Program, which supports visits from experts in Geriatric Nephrology to Nephrology fellowship training programs, with ASP support. A Career Development Grant in Geriatric Nephrology is supported by the ASN Foundation for Kidney Research.

**Conclusions:** The GNAG has implemented a variety of initiatives by which health care providers can improve care of older adults with kidney disease.

*Funding:* NIDDK Support

**TH-PO700**

**Elevated Prorenin Induces Dose-Dependent Podocyte Injury in cyp1a1-Prorenin Transgenic Rats** Chunyan Gu, Alfred K. Cheung, Yufeng Huang. *Div of Nephrology & Hypertension, Dept of Internal Medicine, Univ of Utah School of Medicine, Salt Lake City, UT.*

**Background:** Plasma prorenin levels are commonly elevated in diabetic patients and appear to predict the development of albuminuria and progression of DN. Albuminuria is often the result of podocyte injury. However, the potential pathogenic role of prorenin in podocyte injury is unclear. In this study, we examined the associations of plasma prorenin levels in transgenic animals in which prorenin is produced in the liver with damages to podocytes.

**Methods:** Cyp1a1 prorenin transgenic rats at 12 wks of age were fed with normal diet containing incremental amounts of indole-3-carbinol (I3C) at 0.05% (n=6), 0.15% (n=6) or 0.3% (n=4) respectively. Wild-type rats with normal diet (n=6) served as healthy controls.

**Results:** Transgene induction by I3C for 4 wks increased plasma prorenin levels in the transgenic rats by 7-, 18-, and 24-fold respectively. However, plasma renin levels are not increased. The induction of prorenin expression by I3C resulted in a dose-dependent decrease in expression of nephrin and podocin, and WT-1-positive podocytes in the kidney as determined by immunofluorescent staining, associated with segmental podocyte foot process effacement and podocyte body hypertrophy assessed by transmission electron microscope. These data strongly suggest that prorenin induces podocyte injury. There were also significant increases in the mean arterial pressure and urine albumin/creatinine ratio in the prorenin transgenic rats treated with I3C, although the dose-dependency of these phenomena was less apparent.

**Conclusions:** These findings suggest a novel link between elevated plasma prorenin levels and structural changes in diabetic kidney. Of note, treatment of transgenic rats with either amlodipine (10mg/kg/d by gavage)or enalapril (200mg/L in drinking water) for 6wks prevented the development of hypertension and ameliorated, but did not prevent, the development of podocyte injury and albuminuria. Our findings further indicate that hypertension in this model is Ang II-dependent. However, the prorenin-induced podocyte injury is only partially dependent on hypertension and Ang II, suggesting additional Ang II-independent effects.

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## TH-PO701

### Critical Role of Proximal Promoter Elements and Their Interacting Factors in the Transcription of Angiotensin II Binding and Inhibitory Protein in Renal Tubule Cells

Kouichi Tamura. *Dept of Medical Science and Cardiorenal Medicine, Yokohama City Univ Graduate School of Medicine, Yokohama, Japan.*

**Background:** The Angiotensin II (Ang II) type 1 receptor (AT1R)-associated protein (ATRAP/Agtrap) promotes constitutive internalization of the AT1R so as to specifically inhibit the pathological activation of its downstream signaling but yet preserve the baseline physiological signaling activity of the AT1R. Thus, tissue-specific regulation of *Agtrap* gene expression is relevant to the pathophysiology of cardiovascular and renal disease. However, the regulatory mechanism of *Agtrap* gene expression has not been fully elucidated yet.

**Methods:** In the present study, we examined whether the proximal promoter of the mouse *Agtrap* gene, which contains the X-box, E-box and GC-box consensus motifs, is able to elicit substantial transcription of the *Agtrap* gene. We focused our analysis on a functional role of the E-box and characterization of its putative binding transcription factors *in vitro* and *in vivo*. We also examined a possible role of the E-box of the human *AGTRAP* proximal promoter in the transcriptional regulation.

**Results:** We showed that the E-box-Usf1/Usf2 binding regulates *Agtrap* gene expression, because: (1) mutation of the E-box so as to prevent Usf1/Usf2 binding reduces *Agtrap* promoter activity; (2) knockdown of Usf1 or Usf2 affects both endogenous Agtrap mRNA and Agtrap protein expression and (3) the decrease in Agtrap mRNA expression in the afflicted kidney by unilateral ureteral obstruction (UUO) is accompanied by changes in Usf1 and Usf2 mRNA. Furthermore, the results of siRNA transfection in mouse distal convoluted tubule cells and those of UUO in the afflicted mouse kidney suggest that Usf1 decreases but Usf2 increases the *Agtrap* gene expression by binding to the E-box. The results also showed a functional E-box-USF1/USF2 interaction in the human *AGTRAP* promoter.

**Conclusions:** Collectively, these results demonstrated that an interplay between E-box and Usf1/Usf2 is important for *Agtrap* gene regulation. A strategy of modulating the E-box-Usf1/Usf2 interaction may have novel therapeutic potential.

*Funding:* Government Support - Non-U.S.

## TH-PO702

### T Type Ca-Channel Blocker Exerts Anti-Albuminuic Effect through Amelioration of eNOS Uncoupling in Hypertensive Kidney Disease Model

Seiji Itano, Kengo Kidokoro, Minoru Satoh, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

**Background:** Tetrahydorbiopterin (BH4), an essential cofactor for endothelial Nitric oxide (NO) synthase (eNOS), is easily oxidized by oxidative stress. In such condition, eNOS generates superoxide rather than NO (eNOS-uncoupling), thus further aggravates oxidative stress. Certain class of calcium channel blocker (CCB) has shown to improve endothelial dysfunction by 'recoupling' eNOS. We investigated the molecular mechanisms underlying recoupling of eNOS and reno-protective effects by two different classes of CCBs, Benidipine(T/L type) and Amlodipine(L type).

**Methods:** We used 6 week-old male Dahl salt sensitive (Dahl) rats and treated them with either Benidipine (BN:3mg/kg/day) or Amlodipine (AM:3mg/mg/day) for 4 weeks. Urinary albumin excretion, glomerular BH4 level, expression of GTP cyclohydrolase 1 (GTPCH I), a rate-limiting enzyme of BH4 synthesis, were evaluated in the kidney tissues. Production of NO and reactive oxygen species (ROS) in the kidney tissues were imaged by confocal laser microscopy after renal perfusion with two types of fluorescent dyes, DCFH-DA and DAR-4M AM, ROS and NO indicators respectively.

**Results:** With elevation of blood pressure, increased UAE were observed in Dahl group. Furthermore, glomerular BH4 level and GTPCH I expression were decreased in the kidney tissues of Dahl group. Exacerbated ROS production and diminished bioavailable NO were noted in the glomeruli of Dahl group. Western analysis revealed eNOS uncoupling in Dahl kidney. No significant difference in blood pressure was observed between BN group and AM group. However, all the above mentioned changes were ameliorated to a greater degree in BN group. The expression of ICAM-1, VCAM-1 mRNA and the number of infiltrated ED-1 positive cells in glomerulus were also significantly suppressed in BN group. AMP-activated protein kinase activity which attenuates GTPCH I degradation was significantly higher in BN group compared with AM group.

**Conclusions:** T/L type CCB, Benidipine attenuates hypertensive kidney injuries via improvement of eNOS uncoupling by maintenance of BH4 and GTPCH I level.

## TH-PO703

### Valsartan Improves Myocardial Cellular Viability in Renovascular Hypertension

Xin Zhang,[1] Zi-Lun Li,[1] John A. Crane,[1] Stephen C. Textor,[1] Amir Lerman,[2] Lilach O. Lerman.[1,2] *[1]Nephrology and Hypertension, Mayo Clinic; [2]Cardiovascular Diseases, Mayo Clinic.*

**Background:** Renal artery stenosis (RAS) leads to renovascular hypertension and alterations in cardiac structure and function. The Angiotensin II receptor blocker Valsartan (VAL) lowers blood pressure and is cardioprotective, although the exact mechanisms remain unclear. We hypothesized that processes of cellular self-digestion (autophagy) and mitochondrial digestion (mitophagy) develop in the myocardium in response to renovascular hypertensive stress. We tested the hypothesis that Val would alleviate autophagy and improve left ventricular (LV) myocardial cellular viability in swine RAS.

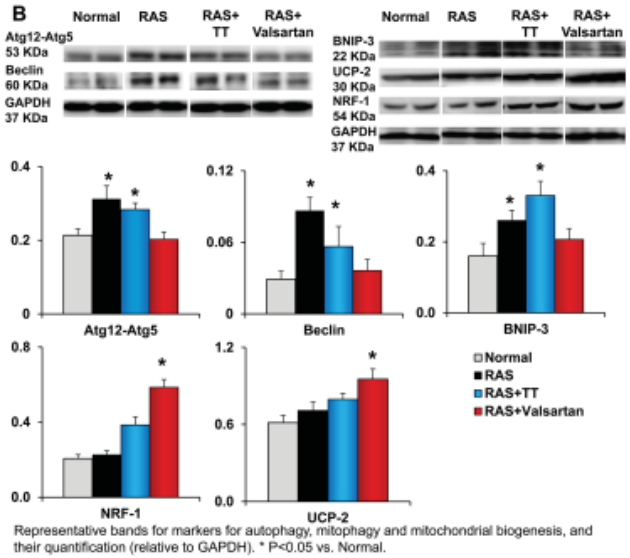
**Methods:** Domestic pigs were randomized to control, unilateral RAS, and RAS treated with Val (320 mg/day; RAS+Valsartan) or conventional triple therapy (Reserpine+hydralazine+hydrochlorothiazide, RAS+TT) for 4 wks post 6-wks of RAS (n=7 each). Kidney and LV function were assessed by CT, myocardial cellular autophagy by expression of Atg12-Atg5 and Beclin-1, signaling for mitophagy by BNIP-3, and mitochondrial biogenesis by NRF-1 and UCP-2.

**Results:** Valsartan and TT similarly decreased blood pressure, but did not alter renal function. RAS induced LV hypertrophy (LVH), accompanied by increased markers of myocardial autophagy and mitophagy. Val alleviated LVH, lowered myocardial autophagy, normalized mitophagy, and increased mitochondrial biogenesis. In contrast, TT did not improve LVH, or affected the cellular and mitochondrial viabilities.

A

| | Normal | RAS | RAS+TT | RAS+Valsartan |
|---|---|---|---|---|
| Mean arterial pressure (mmHg) | 107±5 | 163±12* | 127±9.0*$ | 130±5*$ |
| Stenotic kidney GFR (ml/min) | 82±7 | 66±9* | 57±6* | 62±5* |
| LV muscle mass (g/kg ) | 80±6 | 115±13* | 102±38* | 91±16*$ |
| LV-EDV ($mm^3$) | 70±5 | 62±7 | 74±3 | 73±7 |
| Ejection fraction (%) | 62±5 | 67±9 | 63±4 | 59±5 |

GFR: glomerular filtration rate. LV: left ventricle. EDV: end diastolic volume.
*P<0.05 vs. Normal; $: p<0.05 vs. RAS.




Representative bands for markers for autophagy, mitophagy and mitochondrial biogenesis, and their quantification (relative to GAPDH). * P<0.05 vs. Normal.

**Conclusions:** Val-induced improvement in cardiac structure in renovascular hypertension was associated with improvement in myocardial cellular viability. Valsartan may promote cardiac repair in RAS by directly improving cell survival by mechanisms independent of blood pressure control.

*Funding:* NIDDK Support, Other NIH Support - HL085307 and HL77131

## TH-PO704

### Renal Dopamine-Induced Diuresis and Natriuresis Is Mediated by CYP Epoxygenase 2c44

Ming-Zhi Zhang, Bing Yao, Yinqiu Wang, Kimberly Fisher, Jorge H. Capdevila, Raymond C. Harris. *Medicine, Vanderbilt Univ School of Medicine, Nashville, TN.*

**Background:** We have recently demonstrated that intrarenal dopamine plays an important role in preventing the development of systemic hypertension. Similarly, renal cytochrome P450 (CYP)-epoxygenase-derived arachidonic acid metabolites, epoxyeicosatrienoic acids (EETs) are also anti-hypertensive. The current studies investigated potential interactions between renal dopamine and epoxygenase systems.

**Methods:** COMT-/- mice with increased intrarenal dopamine levels and ptAADC-/- with renal dopamine deficiency were treated with low-salt diet or high-salt diet for 2 weeks. Wild type or Cyp2c44-/- mice were treated with gludopa, which selectively increased renal dopamine levels.

**Results:** In low-salt treated mice, renal EET levels correlated with renal dopamine levels, being highest in COMT-/- mice and lowest in ptAADC-/- mice. In high-salt treated mice, total EET and individual EET levels in both kidney and urine were also highest in COMT-/- mice and lowest in ptAADC-/- mice. Selective increases in renal dopamine in response to gludopa administration led to marked increases in both total and all individual EET levels in the kidney without any changes in blood levels. qPCR and Western analysis indicated that gludopa increased renal Cyp2c44 mRNA and protein levels. Dopamine also increased Cyp2c44 mRNA levels dramatically in cultured mouse proximal epithelial cells. Gludopa induced marked increases in urine volume (ml/16-h: control: 0.95 ± 0.11; gludopa: 3.77 ± 0.23, $P < 0.001$, $n = 4$) and urinary sodium excretion (μM/16-h: control: 99.65 ± 9.93; gludopa: 302.08 ± 23.43, $P < 0.001$, $n = 4$) in wild type mice. In contrast, gludopa did not induce significant increases in urine volume (ml/16-h: control: 0.67 ± 0.11; gludopa: 0.90 ± 0.30, $P > 0.05$, $n = 4$) or urinary sodium excretion (μM/16-h: control: 116.04 ± 7.83; gludopa: 157.71 ± 21.72, $P > 0.05$, $n = 4$) in Cyp2c44-/- mice.

**Conclusions:** These studies demonstrate that renal EET levels are maintained by intrarenal dopamine, and Cyp2c44-derived EETs play an important role in intrarenal dopamine-induced natriuresis and diuresis.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO705

**Sorting Nexin 1 Knockout Mice Manifest with Hypertension and Impaired Natriuretic Response** Van Anthony M. Villar, Laureano D. Asico, Jun B. Feranil, Ines Armando, John Edward Jones, Pedro A. Jose. *Univ of Maryland, School of Medicine, Baltimore, MD.*

**Background:** Dopamine is important for the regulation of blood pressure (BP), sodium balance, and renal function by engendering natriuresis via the inhibition of tubule NaCl reabsorption. The dopamine $D_5$ receptor ($D_5R$) interacts with sorting nexin 1 (SNX1), a protein involved in agonist-activated receptor trafficking.

**Methods:** We evaluated the effect of a congenital absence of Snx1 on the regulation of BP and sodium excretion in mice through an array of *in vitro* (confocal microscopy, RNAi, biochemical assays) and *in vivo* approaches.

**Results:** SNX1 depletion in human renal proximal tubule cells impaired receptor trafficking and $D_5R$-mediated Ga activation, cAMP response, and sodium transport, indicating that SNX1 loss results in impaired $D_5R$ function. To evaluate the *in vivo* correlates of these *in vitro* observations, we used an innovative approach to acutely silence the renal Snx1 via subcapsular infusion of Snx1-specific siRNA in mice, which resulted in 50% knockdown of expression. Renal depletion of Snx1 in salt-sensitive C57BL/6J and salt-resistant BALB/cJ mice resulted in high BP (ΔSBP of 13 and 25 mm Hg, and ΔDBP of 15 and 23 mm Hg from baseline, respectively; P<0.05, n=3-4/group), conceivably due to perturbed $D_5R$ function; $Drd5^{-/-}$ mice also develop hypertension. Moreover, salt-loaded Snx1-depleted BALB/cJ mice failed to excrete sodium (ΔUNaV of -13.8±13.4% and +40.5±24.1% from baseline in Snx1-depleted vs. control BALB/cJ mice, respectively; P<0.05, n=3-4/group) in response to $D_1R$ and $D_5R$ agonist treatment. We next extended our studies to $Snx1^{-/-}$ mice, which have a congenital absence of Snx1. We found that adult male $Snx1^{-/-}$ mice, compared to wild-type littermates, are hypertensive (131.3±6.3/107.3±5.4 mm Hg vs. 105.5±6.4/84.2±8.9 mm Hg, P<0.05, n=3/group) and have impaired natriuretic response (ΔUNaV of -17.011.5 ±13.4% and +201.5±40.1% from baseline, respectively; P<0.05, n=3/group), similar to the phenotypes observed in acutely (Snx1)-depleted mice.

**Conclusions:** These data demonstrate a novel and crucial role for SNX1 in direct $D_5R$ and indirect $D_1R$ regulation in the pathogenesis of essential hypertension.

*Funding:* NIDDK Support

## TH-PO706

**PACAP and Stress-Mediated Regulation of Renal Plasminogen Activator Inhibitor-1 Gene Expression *In Vivo*** Neill A. Gingles,[1,2] Marc A. Olivier,[1,2] Lindsey A. Miles,[3] Robert J. Parmer.[1,2] *[1]Univ of California; [2]VA San Diego Healthcare System; [3]The Scripps Research Institute, La Jolla, CA.*

**Background:** Sympathetic nervous system (SNS) hyperactivity contributes to renal disease progression; however, the specific factors mediating SNS-related renal injury have not been fully elucidated. Pituitary adenylate cyclase-activating polypeptide (PACAP), a member of the VIP/secretin/glucagon family, is a major effector of sympathoadrenal physiologic responses and is a potent transcriptional activator. Also, plasminogen activator inhibitor 1 (PAI-1), the major inhibitor of fibrinolysis, has been shown to be a key mediator of renal fibrosis in a variety of disorders, including hypertensive nephrosclerosis, diabetic nephropathy, experimental glomerulonephritis, and transplant rejection.

**Methods:** To explore potential links among SNS stress responses, the PACAP peptidergic system, and renal PAI-1 biosynthesis, we investigated PAI-1 expression in response to stress and to PACAP *in vivo*. PAI-1 expression was monitored by quantitative RT-PCR in kidneys from C57BL/6 mice that were either unstressed or subjected to sympathoadrenal activation by restraint stress for 2 hours.

**Results:** Renal PAI-1 mRNA expression was markedly increased in stressed mice (3.2±0.3 fold, n=5, compared to controls, P<0.001). PACAP1-38 agonist (0.006 to 0.6 mg/kg IP) resulted in a dose-dependent increase in renal PAI-1 mRNA (up to 6.5±0.8 fold induction versus 1.0±0.3, n=5, for vehicle treated mice, P<0.001). Renal PAI-1 protein expression also increased significantly (by 3.1±0.2 fold, P<0.001). Neither restraint stress nor PACAP1-38 affected renal expression of plasminogen activators, suggesting that local tPA/PAI-1 or uPA/PAI-1 biosynthetic balance is markedly altered by stress. The observed increases in PAI-1 mRNA during stress were substantially blunted (by 62±11%, P<0.05) by pretreatment with the specific PACAP receptor antagonist, PACAP6-38, compared to pretreatment with vehicle.

**Conclusions:** Renal PAI-1 expression is substantially increased by stress and by PACAP, suggesting key links among the SNS, the PACAP peptidergic signaling pathway, and renal PAI-1 biosynthesis, which may contribute to SNS-mediated renal injury.

*Funding:* Other NIH Support - NHLBI, Veterans Affairs Support

## TH-PO707

**Compensatory Activation of the Sympathetic Nervous System after Deletion of Angiotensin Type 1 Receptors from Vascular Smooth Muscle Cells** Matthew A. Sparks, Johannes Stegbauer, Susan B. Gurley, Thomas M. Coffman. *Div of Nephrology, Duke Univ Medical Center, Durham, NC.*

**Background:** Vasoconstriction is a key physiological action of angiotensin II (Ang II). Precisely how $AT_1$ receptors ($AT_1R$) on VSMCs contribute to vasoconstriction *in vivo* has not been completely defined.

**Methods:** In order to define the role of direct actions of $AT_1R$ in VSMCs, we generated mice with deletion of $AT_{1A}R$ from VSMCs using a floxed *Agtr1a* allele and a mouse expressing *Cre* only in VSMCs.

**Results:** We compared vascular responses to acute infusions of Ang II in mice lacking $AT_{1A}R$ in VSMCs (SMKOs) and controls. Surprisingly, brisk vasoconstrictor responses to 1μg/kg Ang II were preserved in SMKOs with peak increases in MAP only modestly reduced to ~75% of control levels (15±2 vs. 23±1 mmHg P<0.05). Robust systemic vasoconstriction to Ang II was observed whether elimination of $AT_{1A}Rs$ was carried out *in utero* with a constitutively expressed SM-Cre or in adult mice with a tamoxifen-inducible SM-Cre. By contrast, Ang II-dependent vasoconstriction in the renal circulation was largely abrogated in SMKOs. Moreover, despite preserved systemic vascular responses to acute Ang II in SMKOs, baseline BPs was reduced and susceptibility to Ang II-dependent hypertension was significantly attenuated. To determine if the minor $AT_{1B}R$ isoform might be responsible for their residual vascular responses, we generated SMKOs on an $AT_{1B}$-null background (SMKO $1B^{-/-}$); the absence of $AT_{1B}Rs$ did not affect this response (SMKO $1B^{-/-}$ 16±2 vs. SMKO-$1B^{+/+}$ 15±2 mmHg; ns). As an alternative explanation, we tested for an involvement of the sympathetic nervous system (SNS) by infusing 400μg/kg of the α-blocker phentolamine (PT) 5 min before Ang II. While PT had no effect on Ang II-dependent vasoconstriction in controls (pre-PT: 23±1 vs. post-PT: 23±3 mm Hg), it almost completely abrogated vasoconstriction in SMKOs (pre-PT: 15±2 vs. post-PT: 3±1 mm Hg; P<0.005).

**Conclusions:** Thus, we find a significant capacity for the activation of the SNS to maintain acute Ang II-dependent vasoconstriction when $AT_1R$ signaling in VSMCs is absent. This pathway induces brisk vasoconstriction in the systemic but not the renal circulation.

*Funding:* Private Foundation Support

## TH-PO708

**Renal Denervation Deriving Cardiorenal Protection beyond Blood Pressure Control via Sympathetic Regulation of Local Renin-Angiotensin System** Masahiro Eriguchi,[1] Kumiko Torisu,[1] Toshiaki Nakano,[1] Kosuke Masutani,[1] Kazuhiko Tsuruya,[2] Takanari Kitazono.[1] *[1]Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Dept of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

**Background:** Recently catheter-based renal sympathetic denervation (DNx) is beginning to be applied for clinical aim. The sympathetic nerve system (SNS) and renin-angiotensin system (RAS) would be suggested as possible cardio-renal mediators. The mechanisms behind the beneficial effects of DNx for cardio-renal vicious cycle are not fully elucidated but may include disruption of local RAS in addition to the vicious cycle of SNS-RAS.

**Methods:** We tested the effects of renal denervation on cardiorenal injury induced by chronic administration of L-NAME (nitric oxide synthase inhibitor) in Wistar rats. The rats were divided into four groups (control, L-NAME, L-NAME with bilateral DNx and L-NAME with hydralazine group). Cardiorenal injury, SNS, circulating RAS and local RAS (heart and kidney angiotensin II (AII) and urinary angiotensinogen (AGT)) were evaluated. We also observed rats treated with L-NAME + unilateral DNx to confirm the sympathetic direct regulation of intrarenal RAS. Serial measurements of kidney AII and urinary AGT of both kidneys were performed to examine the laterality of local RAS in the body of the same individuals.

**Results:** Bilateral DNx ameliorated over-activity of SNS and circulating RAS accompanied with downregulation of heart and kidney local RAS, and prevented cardiorenal injury. Cardiorenal protective effects of bilateral DNx were greater than the hydralazine treatment that blood pressure (BP) was kept to the same level. In unilateral DNx model, kidney AII and urinary AGT from the denervated kidneys were smaller than the innervated kidneys under the same circulating conditions and renal injury of the denervated kidneys were alleviated compared to the innevated kidneys.

**Conclusions:** DNx derives the beneficial BP-independent effects associated with local RAS amelioration in cardiorenal syndrome.

## TH-PO709

**Fructose Stimulates Sodium/Hydrogen Exchanger 3 Activity via Protein Kinase C Activation in the Proximal Tubule** Pablo D. Cabral, Jeffrey L. Garvin. *Physiology and Biophysics, Case Western Reserve Univ, Cleveland, OH.*

**Background:** Consumption of high-fructose corn syrup as a sweetener has increased dramatically. Fructose has been implicated in the epidemic of diabetes, obesity and salt-sensitive hypertension. However, the mechanisms are poorly understood. The proximal nephron reabsorbs 60-70% of the fluid and Na, and most of the filtered bicarbonate via Na/H exchanger 3 (NHE3). It has been shown that protein kinase C (PKC) stimulates fluid and bicarbonate absorption in this segment. Enhanced proximal nephron transport has been implicated in several forms of hypertension. We hypothesized that fructose stimulates NHE3 activity in the proximal tubule via a PKC-dependent mechanism.

**Methods:** To test our hypothesis we isolated and perfused proximal tubules from Sprague Dawley rats. NHE3 activity was measured as the recovery of intracellular pH after an NH4Cl acid pulse using the pH sensitive dye BCECF. The rate of pH recovery was measured in Fluorescent Units per second (FU/sec).

**Results:** In the presence of a 5.5 mM glucose-containing physiological saline the basal rate of pH recovery was 3.1 ± 0.8 FU/sec. When the luminal solution was exchanged to a 0.6 mM glucose + 5 mM fructose-containing physiological saline in a second period, the rate of pH recovery increased to 5 ± 1 FU/sec ($p$<0.03, $n$=8).To study whether this effect was due to the addition of fructose or the subtraction of glucose to the lumen, we performed a separate set of experiments where 5 mM glucose was substituted for 5 mM fructose. In the presence of 0.6 mM glucose the basal rate of pH recovery was 3.6 ± 1.5 FU/sec. When 5 mM fructose was added the rate of pH recovery increased to 5.9 ± 2 FU/sec ($p$<0.02, $n$=5). Control experiments showed no differences between periods when 5 mm glucose was added back to the luminal perfusate. Finally, we tested the effect of the general PKC inhibitor staurosporine on the effect of fructose. In the presence of the general PKC inhibitor staurosporine $10^{-10}$ M Fructose did not stimulate NHE3 activity (from 6.0 ± 1.2 to 6.5 ± 1.7; $n$=6).

**Conclusions:** We conclude that acute treatment with fructose stimulates NHE3 activity in the proximal tubule via a PKC-dependent mechanism.

*Funding:* Other NIH Support - HL28982, HL70985, and HL90550, Private Foundation Support

## TH-PO710

**CXCR6 Plays a Critical Role in Angiotensin II-Induced Renal Injury and Fibrosis** Yunfeng Xia, Yanlin Wang. *Dept of Medicine, Div of Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** Recent studies have shown that inflammation plays a critical role in the pathogenesis and progression of hypertensive kidney disease. However, the signaling mechanisms underlying the induction of inflammation are poorly understood. In this study, we examined the role of CXCR6 in Ang II-induced renal injury and fibrosis.

**Methods:** Wild-type (WT) and CXCR6-KO mice were treated with Ang II via subcutaneous osmotic minipumps at 1500 ng/kg/min for up to 4 weeks. To accelerate renal injury, all mice were subjected to unilateral nephrectomy and received 1% NaCl in drinking water.

**Results:** WT and CXCR6-KO mice had virtually identical blood pressure at baseline. Ang II treatment led to an increase in blood pressure that was similar between WT and CXCR6 knockout mice. CXCR6 knockout mice were protected from Ang II-induced renal dysfunction, proteinuria, and fibrosis. Deletion of CXCR6 suppressed bone marrow-derived fibroblast accumulation and myofibroblast formation in the kidneys of Ang II treated mice, which was associated with a reduction in extracellular matrix protein expression. Furthermore, deletion of CXCR6 inhibited infiltration of $F4/80^+$ macrophages and $CD3^+$ T cells in the kidney of Ang II treated mice compared with WT mice. Finally, deletion of CXCR6 reduced gene expression of proinflammatory cytokines in the kidney of Ang II treated mice.

**Conclusions:** Our results demonstrate that CXCR6 plays a pivotal role in the development of Ang II induced renal injury and fibrosis through regulation of macrophage and T cell infiltration and bone marrow-derived fibroblast accumulation. These findings suggest that inhibition of CXCR6 signaling could constitute a novel therapeutic target for hypertensive kidney disease.

*Funding:* NIDDK Support

## TH-PO711

**Suppression of Insulin Resistance by Renal Sympathetic Denervation in Obese Rats** Akira Nishiyama, Daisuke Nakano. *Dept of Pharmacology, Kagawa Univ Medical School, Kagawa, Japan.*

**Background:** Recent studies have shown that renal sympathetic denervation improves glucose metabolism and insulin sensitivity in patients with resistant hypertension. To elucidate its precise mechanism, studies were performed to examine the effect of renal sympathetic denervation on glucose metabolism and blood pressure in obese rats.

**Methods:** Uninephrectomized obese Otsuka Long Evans Tokushima Fatty (OLETF) underwent renal denervation (RDX) at 6 weeks of age (prediabetic stage).

**Results:** At 22 weeks of age, RDX resulted in undetectable kidney tissue norepinephrine (NE) levels and decreased plasma NE levels, but did not significantly change blood pressure (measure by telemetry system) in type 2 diabetic OLEFT rats. As compared to sham-operated OLETF rats, RDX OLETF rats showed lowered blood glucose and plasma insulin levels as well as decreased areas under the glucose response curvesafter oral glucose loading during the oral glucose tolerance test. Whole body insulin sensitivity was also assessed by the hyperinsulinemic-euglycemic clamp study at 20 week of age, and RDX OLETF rats showed an improved glucose infusion rate. Furthermore, RDX improved in vivo glucose uptake by adipose tissues, soleus muscle and liver. Interestingly, RDX also decreased proximal tubular SGLT2 expression and increased urinary glucose excretion rate in OLETF rats.

**Conclusions:** Renal sympathetic denervation at a prediabetic stage improves glucose metabolism and insulin sensitivity through suppression of glucose uptake and enhancement of urinary glucose excretion.

*Funding:* Government Support - Non-U.S.

## TH-PO712

**The Transcription Factor ETS-1 Mediates Renal Injury in Salt Sensitive Hypertension** Wenguang Feng,[1] Phillip H. Chumley,[1] Huma Fatima,[2] Gabriel Rezonzew,[1] Ping Hua,[1] Edgar A. Jaimes.[1,3] *[1]Medicine/Nephrology; [2]Pathology, Univ of Alabama at Birmingham; [3]VA Medical Center.*

**Background:** ETS-1 regulates the expression of several growth factors and cytokines and mediates pro-fibrotic effects of Angiotensin II. Herein, we tested the hypothesis that increased expression of ETS-1 in Dahl salt-sensitive (DS) rats mediates renal injury in this model of hypertension.

**Methods:** DS rats (n=6 per group) were fed normal salt diet (0.5%, NS) or a high salt diet (4%, HS) for 4 weeks. Four additional groups of HS rats received: ETS-1 dominant negative peptide (HS/DN, 10mg/kg/day), a control ETS-1 mutant peptide (HS/MU, 10mg /kg/day), the AT1 receptor blocker Candesartan (HS/ARB 10 mg/kg/day) or a combination of DN and ARB (HS/DN/ARB).

**Results:** HS rats had a 3 fold increase in the cortical expression of ETS-1. Treatment with DN, MU, ARB or DN/ARB resulted in non-significant reductions in BP. HS increased proteinuria, glomerular injury score (GIS), fibronectin expression (western blot), urinary TGF-β (ELISA) and macrophage (ED1) infiltration, that were improved by DN (table). ARB partially reduced proteinuria, GIS and macrophage infiltration. Treatment with DN/ARB resulted in further improvements as compared to DN or ARB.

**Conclusions:** Increased expression of ETS-1 mediates renal injury in salt sensitive hypertension.

| | NS | HS | HS/DN | HS/MU | MS/ARB | HS/DN/ARB |
|---|---|---|---|---|---|---|
| BP(mmHg) | 133.5±4.9 | 175.1±5.7* | 156.5±5.3* | 166.1±6.8* | 153.6±7.3* | 164.1±3.5* |
| Proteinuria(mg/mg Cr.) | 44.8±3.9 | 91.3±6.1* | 65.3±6.6*# | 82.8±16.3* | 51.9±3.2# | 42.5±3.0§ |
| GIS | 3.2±0.6 | 9.6±2.0* | 3.0±0.5# | 9.9±3.4* | 3.7±0.8# | 2.6±0.6# |
| TGF-β(pg/24 hrs) | 13.0±8.8 | 417.4±87.6* | 142.2±64.9*# | 498.3±138.3* | 604.1±94.9*# | 23.0±9.3§ |
| Fibronectin(AU) | 0.3±0.01 | 1.7±0.2* | 0.3±0.2# | 1.3±0.2* | 1.2±0.3* | 0.1±0.07§ |
| Macrophage(cell/mm$^2$) | 10.8±1.4 | 43.0±4.7* | 15.2±4.9# | 47.2±7.8* | 27.2±3.3# | 10.6±0.7§ |
| *p<0.05 vs LS; #p<0.05 vs HS; §p<0.05 vs DN and ARB | | | | | | |

*Funding:* Veterans Affairs Support

## TH-PO713

**Differential Regulation of Epithelial Sodium Channel (ENaC) and Sodium-Chloride Cotransporter (NCC) by Gilz** Priyanka Rashmi, Michael Ng, David Pearce. *Div of Nephrology, Dept of Medicine, Univ of California, San Francisco, CA.*

The renin-angiotensin-aldosterone (RAAS) system is the central regulator of $Na^+$ reabsorption and $K^+$ secretion primarily through the tubular effects of aldosterone. Among the multiple transporters implicated in electrolyte homeostasis and regulated by the RAAS are the $Na^+Cl^-$ cotransporter (NCC) and epithelial sodium channel (ENaC).Electrogenic $Na^+$ reabsorption via ENaC stimulates $K^+$ excretion, while electroneutral reabsorption of $Na^+$via NCC inhibits $K^+$secretion by competing with ENaC for $Na^+$ reabsorption. Serum- and glucocorticoid- induced kinase 1 (Sgk1) is an aldosterone regulated serine-threonine kinase that phosphorylates the ubiquitin ligase Nedd4-2, and Raf-1 kinase to stimulate ENaC. *In vitro* evidence from our laboratory shows that another aldosterone regulated protein; glucocorticoid-induced leucine zipper protein 1 (Gilz1) acts in concert with Sgk1 to stimulate ENaC cell surface expression. We now show that in contrast to its effect on ENaC, Gilz blocks Sgk1-mediated stimulation of NCC, with important consequences for $Na^+$ and $K^+$ handling *in vivo*. When placed on a low salt diet, Gilz knock out mice are able to come to sodium balance more rapidly than the wild type animals suggesting an upregulation of another sodium transporter. Surface expression of NCC and NKCC2 (as well as phosphorylated NKCC2) is increased in plasma membrane fractions of kidneys from Gilz knock out animals while the expression of ENaC is decreased. In cultured HEK293 cells, Sgk1 stimulates the surface expression of NCC while simultaneous co-expression of Gilz abrogates the effect of Sgk1 in a dose dependent manner. Overexpression of Gilz in mpkDCT tubular epithelial cells results in downregulation of NCC while simulataneously inducing ENaC surface expression. Furthermore, Gilz1 overexpression inhibits the interaction between WNK4 and Sgk1 allowing WNK4 mediated inhibition of NCC. Taken together, these results suggest that: Gilz1 is part of a "shift" mechanism, which allows the differential regulation of ENaC and NCC in order to balance $Na^+$ reabsorption and $K^+$ secretion.

## TH-PO714

**Early Hypertensive Renal Injury Is Associated with Capillary Hypertrophy, Preceding Pathological Angiogenesis and Capillary Loss** Timo Rademakers,[1] Floortje Steegh,[1] Petra Hautvast,[1] Marcella M. Baldewijns,[1] Sylvia Heeneman,[1] Victor L. Thijssen,[3] Mat Daemen,[4] Ben Janssen,[2] Carine Peutz-Kootstra.[1] *[1]Pathology, Maastricht Univ Medical Center, Maastricht, Netherlands; [2]Pharmacology, Maastricht Univ, Maastricht, Netherlands; [3]Medical Oncology, VU Medical Center, Amsterdam, Netherlands; [4]Pathology, Academic Medical Center, Amsterdam, Netherlands.*

**Background:** Chronic hypertension predisposes for end-organ damage in e.g. the kidney. Both patients and animal models have shown peritubular capillary (PTC) loss in chronic hypertensive renal disease, yet PTC rarefaction in early disease is poorly understood. We hypothesized that in early hypertensive renal disease PTC hypertrophy precedes PTC loss, in association with a distinct angiogenic profile.

**Methods:** Patient study: Healthy renal tissue from patients undergoing nephrectomy (n=50; 31 hypertensive, 19 non-hypertensive) was included. Tissue was scored histologically and PTC per tubule (PTC/tub) was assessed by CD31/CD34-staining. Animal study:

C57Bl/6 mice underwent one-sided nephrectomy, after which mice (n=6/group) were left untreated (control), treated with DOCA and/or angiotensin-II (ATII). Renal tissue was scored histologically. RNA for angiogenic profiling via qPCR was isolated from the cortex.

**Results:** Patients with early hypertension showed increased PTC/tub (1.08±0.18 vs. 0.97±0.15; p=0.04) compared to controls. DOCA-treated mice resembled these patients, showing 1.3-fold increased PTC/tub (p=0.05) and 2.2-fold increased VEGFR-2 expression (p=0.04) by angiogenic profiling. DOCA+ATII-treatment, resembling chronic damage, reduced PTC/tub 1.3-fold compared to DOCA-treatment (p=0.04), reduced angiopoietin (ang)-1/ang-2 ratio 5.5-fold (p=0.01), and 2-fold increased PDGFR-β/ang-1 ratio (p=0.02), indicative of pathological angiogenesis and capillary loss.

**Conclusions:** We showed that capillary hypertrophy occurred in human hypertensive renal disease, preceding capillary loss. Murine hypertensive renal disease revealed specific angiogenic profiles associated with capillary phenotype, pending validation in the human cohort. Further study will allow more insights into disease processes and potential targets for therapy.

*Funding:* Clinical Revenue Support

## TH-PO715

**The Role of Nitric Oxide in Blood Pressure Regulation by $D_3$ Dopamine Receptor** Zhenyu Diao, Laureano D. Asico, John Edward Jones, Jun B. Feranil, Ines Armando, Pedro A. Jose, Xiaoyan Wang. *Univ of Maryland, Baltimore.*

**Background:** $D_3$ dopamine receptor ($D_3R$)-deficient ($Drd3^{-/-}$) mice have increased blood pressure (BP); the increase BP in $Drd3^{-/-}$ mice is associated with activation of the renin-angiotensin system. However, the renal production of reactive oxygen species (ROS) is normal.

**Methods:** We measured BP, renal protein expression of nitric oxide synthases (NOS) and nitrotyrosine, and nitrite/nitrate (NOX) excretion in $Drd3^{-/-}$and wild-type ($Drd3^{+/+}$) mice on normal (0.8%) and high (1.6 or 4%) NaCl diets (1week).

**Results:** The systolic BPs were higher in $Drd3^{-/-}$(114±1 mm Hg, n=4, under anesthesia) than $Drd3^{+/+}$(92±2 mm Hg, n=6) on normal salt intake. The renal expression of inducible NOS (iNOS, 127±14 vs. 100±14, % of $Drd3^{+/+}$mice) tended to increase in $Drd3^{-/-}$compared to $Drd3^{+/+}$ (n=5/group). Extracellular NOS (eNOS, 158±12 vs. 100±17) was increased in $Drd3^{-/-}$ relative to $Drd3^{+/+}$(t-test, P<0.05). The renal expression of nitrotyrosine, a product of ROS and NO interaction, was not altered in $Drd3^{-/-}$ compared to $Drd3^{+/+}$(107±30 vs 100±10). But L-NAME (0.5 mg/kg, i.v. x 1) markedly increased systolic BP in $Drd3^{-/-}$ and slightly in $Drd3^{+/+}$(145±5 vs. 113±6 at 10 min; 151±1 vs. 107±10 at 30 min, n=3-4/group, P<0.05, ANOVA, Holm-Sidak test) under normal salt intake, suggesting an increase in eNOS activity. Increasing the salt diet to 4% NaCl increased systolic BP (>10% over 0.8% NaCl diet, via telemetry) in $Drd3^{-/-}$, but not in $Drd3^{+/+}$. 1.6% NaCl diet normalized renal expression of iNOS (96±6 vs. 100±4, n=4/group) and eNOS (91±12 vs. 100±4) and did not affect BP in either strain. However, nitrotyrosine was decreased in $Drd3^{-/-}$(49±8) relative to $Drd3^{+/+}$(100±14, t-test, P<0.05); NOX excretion was similar between the 2 mouse strains (2.5±1.88 vs. 3.9±1.9, mM/mg Cr) at baseline but it was increased in $Drd3^{+/+}$ (13.6±4.6, P<0.05), not in $D_3$-/- (8.2±1.8) (ANOVA, Holm-Sidak test, n=4-5/group).

**Conclusions:** Our studies suggest that the increased renal expression of eNOS in $Drd3^{-/-}$ mice may be a compensatory mechanism for the increased BP seen with germline deletion of the Drd3 gene and the normalization of eNOS with high salt diet may contribute to the salt sensitivity in $Drd3^{-/-}$ mice.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

## TH-PO716

**Salt Sensitivity of Blood Pressure in Nephrotic Syndrome Rats** Yan Qin, Lijun Mou, Xuemei Li, Xuewang Li. *Nephrology Dept, Peking Union Medical College Hospital, Beijing, China.*

**Background:** The prevalence of salt sensitivity of blood pressure is striking in CKD patients. The kidney is the key peripheral organ involved with salt sensitity. In this present study we found the Adriamycin Nephropathy Rats (ADRs) represent as an useful mouse model to study sodium sensitivity of BP in CKD.

**Methods:** In this study the circadian characteristics of MAP, SBP, DBP, HR, pulse pressure (PP) and locomotor activity were measured in conscious and unrestrained 12-week old ADRs and age-matched SD control rats by the radiotelemetry system. After baseline studies were obtained, the rats were were provided a high salt diet (8.0%) for a 1-wk period prior to the 7 day telemetry study.

**Results:** 1. The ADRs presented with the reversed circadian rhythms of MAP, SBP, DBP, and PP compared with SD control rats. 2.In the ADRs, the circadian rhythm of the urine sodium excretion was disturbed, the RUNa in Dark period was significantly lower than that in the Light period of the same group[(14.69 ±3.65) v.s (27.66± 5.84) μmol/h, *P*=0.001] and also significantly lower than that in the Dark period of the control group[(14.69 ±3.65)v.s (39.49±22.44) μmol/h,*P*=0.023].3.In the ADRs, the FENa in Dark period was significantly lower than that in the Light period of the same group(0.15±0.06 v.s 0.29±0.06,*P*=0.008) and also significantly lower than that in Dark period of the control group(0.15±0.06 v.s 0.31±0.19,*P*=0.050).4.Under high salt diet, SBP and MAP in Dark period increased significantly by 19.8mmHg and 18.5mmHg respectivelyin ADRs (*P*<0.001); In the control group, the SBP and MAP in Dark period increased significantly by 8.4mmHg and 6.2mmHg respectively. In the ADRs, the SBP and MAP in Light period increased significantly by 20.1mmHg and 17.7mmHg respectively(*P*<0.001); In the control group, the SBP and MAP in Light period increased by 7.2mmHg and 3.0mmHg respectively, but both differences were not significant.

**Conclusions:** We concluded that the ADR nephropathy rats showed a striking salt sensitive of blood pressure. The ADR rat was a suitable CKD animal model with disturbed circadian BP rhythm and sodium sensitivity.

## TH-PO717

**Simvastatin Induces a Central Hypotension Effect via Ras-Mediated Signaling to Integrate Endothelial Nitric Oxide Synthase Upregulation** Wen-Yu Ho,[1] Wen-Han Cheng,[2] Ching-Jiunn Tseng.[2] *[1]Dept of Internal Medicine, Kaohsiung Medical Univ Hospital, Kaohsiung Medical Univ, Kaohsiung City, Taiwan; [2]Dept of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung City, Taiwan.*

**Background:** Clinical studies indicate that statins have a blood pressure (BP)-lowering effect for hypertensive hypercholesterolemic patients. Statins would modulate BP through the upregulation of endothelial nitric oxide synthase (eNOS) activation in the brain. However, the signaling mechanisms through which statins enhance eNOS activation remain unclear. Therefore, we examined the possible signaling pathways involved in statin-mediated BP regulation in a BP-control center of brain stem, nucleus tractus solitarii (NTS).

**Methods:** BP responses of simvastatin administered intracerebroventricularly in spontaneously hypertensive rats (SHRs) were measured in the presence or absence of various inhibitors, inculinding farnesylthiosalicylic acid (FTS), geranylgeranyltransferase inhibitor (GGTI-2133), LY294002 or PD98059. Western blotting was used to confirm the activation of various signaling molecules in the NTS.

**Results:** Pretreatment with a Ras-specific inhibitor, FTS, significantly attenuated the depressor effect and nitric oxide (NO) production evoked by simvastatin. Additionally, immunoblotting and pharmacological studies further showed that inhibition of Ras activity by FTS significantly abolished simvastatin-induced extracellular signal-regulated kinases 1/2 (ERK1/2), ribosomal protein S6 kinase (RSK), Akt phosphorylation and decreased eNOS phosphorylation. Likewise, administration of Akt and ERK1/2 signaling inhibitors into the NTS attenuated the depressor effects evoked by simvastatin. Furthermore, the addition of simvastatin also decreased Rac1 activation and the number of ROS-positive cells in the NTS.

**Conclusions:** These results suggest that distinct Ras and Rac1 signaling-mediated simvastatin control of central BP regulation in SHRs. Both Akt and ERK1/2-RSK signaling at least partly contribute to the eNOS activation of simvastatin.

*Funding:* Government Support - Non-U.S.

## TH-PO718

**Fidelity of Podocyte Number in Preeclampsia due to Podocyte Regeneration** Maria Elisabeth Penning,[1] Kitty Bloemenkamp,[2] Tom T. Van der Zon,[1] Joke M. Schutte,[3] Jan A. Bruijn,[1] Ingeborg M. Bajema,[1] Hans J. Baelde.[1] *[1]Pathology, Leiden Univ Medical Center, Leiden, Netherlands; [2]Obstetrics, Leiden Univ Medical Center, Leiden, Netherlands; [3]Obstetrics, Isala Clinics, Zwolle, Netherlands.*

**Background:** Preeclampsia (PE) is characterized by increased numbers of podocytes in the urine. This observation led to our hypothesis that there may be a decreased number of podocytes in the glomerulus in PE. Furthermore, we evaluated possible parietal epithelial cell activation as this could play a role in the replacement of injured and lost podocytes.

**Methods:** We performed a search for renal autopsy-tissues using a nationwide computerized database (PALGA) to collect a unique large cohort ofwomen who died during pregnancy or postpartum due to PE (n=11). Three control groups were included consisting of young women who died during pregnancy without hypertension (n=25) and non-pregnant controls with (n=14) and without (n=13) chronic hypertension. WT-1 staining was used to quantify the numbers of podocytes. To evaluate cellular proliferation Ki-67 staining was performed. To investigate parietal epithelial cell migration, but to exclude CD44-positive leukocytes, a double staining with CD44 and CD45 was performed.

**Results:** No significant difference was found between the numbers of podocytes in the preeclamptic group and the controls. However, preeclampsia was significantly (p<0.05) more often associated with intraglomerular Ki-67 positivity. Furthermore, the numbers of Ki-67 positive parietal cells were significantly (p<0.05) higher in the women with PE than in the pregnant- and hypertensive controls. CD44 positive (and CD45 negative) cells were observed within the glomerular tuft.

**Conclusions:** This study shows for the first time that, although PE is characterized by significantly increased numbers of podocytes in urine, glomerular podocyte numbers remain stable. Significantly increased intraglomerular Ki-67 positivity and CD44 positive parietal cells suggest that regeneration of podocytes may play an important role in the maintenance of the glomerular filtration barrier during PE.

## TH-PO719

**Enhanced Phosphorylation of NKCC2 by SPAK/OSR1 in a Murine Model of Diet Induced Obesity** Matthew R. P. Davies,[1,2,3] Marina Katerelos,[1] Kurt Gleich,[1] Scott Andrew Fraser,[1] Peter F. Mount,[1] David A. Power.[1,2,3] *[1]Nephrology, Austin Health, Heidelberg, Victoria, Australia; [2]Institute of Breathing and Sleep, Victoria, Australia; [3]Medicine, Univ of Melbourne, Victoria, Australia.*

**Background:** Obesity promotes salt-sensitive hypertension. Tubular mechanisms of enhanced sodium retention in obesity are not well defined.

**Methods:** C57Bl/6 mice were fed 40% (high fat, HFD) or 12% (control, CD) fat diets for 14 weeks. Abundance and phosphorylation of proteins were determined by western blotting. Surface expression was analysed with immunofluorescence and confocal microscopy. *In vitro* studies used stimulation of AMPK with 100uM A769662, and stimulation of WNK/SPAK/OSR1 with 26mM $Cl^-$ solution, in murine embryonic fibroblasts (MEF's) from β1-AMPK-/- and β1-AMPK+/+ mice.

**Results:** HFD mice gained weight and developed hyperinsulinaemia & hyperleptinaemia. Cortical expression of NKCC2 was reduced but activating phosphorylation (T96/T101) was increased. No change in expression or phosphorylation of NCC, or expression of α- or γ-ENaC was found. Surface localisation of transporters was unchanged. SPAK/OSR1 is known to phosphorylate NKCC2 on T96/101. Phosphorylation of SPAK/OSR1 on S373/325 was increased, consistent with increased activity of the WNK/SPAK/OSR1 pathway. AMPK inhibition is involved in mediating obesity-related renal injury. Active AMPK (phosT172) was reduced in HFD mouse cortex. SPAK/OSR1 and AMPK were found to co-immunoprecipitate with NKCC2, indicating a possible kinase-kinase interaction. *In vitro*, activation of AMPK led to a reduction in S373/325-phos SPAK/OSR1 in β1-AMPK+/+ MEF's. No effect was seen in β1-AMPK-/- MEF's, indicating a specific AMPK-mediated effect. Low $Cl^-$ solution invoked a significantly greater increase in S373/325-phos SPAK/OSR-1 in β1-/- than in β1+/+ MEF's, supporting an inhibitory role of AMPK in modulating the WNK/SPAK/OSR1 pathway.

**Conclusions:** NKCC2 is the most important sodium co-transporter in this model of obesity-related hypertension. Enhanced phosphorylation of NKCC2 occurs due to activation of SPAK/OSR1, which itself may be secondary to AMPK inhibition . These data identify NKCC2, SPAK/OSR1 and AMPK as therapeutic targets in obesity-related hypertension.

*Funding:* Other NIH Support - Funding from NHMRC, Australia

## TH-PO720

**Effects of L/N-Type $Ca^{2+}$ Channel Blocker Cilnidipine on the Cardiac Histological Remodeling and Inducibility of Atrial Fibrillation in the High-Salt-Fed Rats**

Eri Harada,[1,2] Kazumi Sugino,[1,2] Akira Takahara,[2] Makoto Shiozaki.[1] *[1]Ajinomoto Pharmaceuticals Co., Ltd., Japan; [2]Dept of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Toho Univ, Japan.*

**Background:** High-salt intake has been shown to induce hypertrophy and fibrosis in the atria and ventricles, which would cause the development of atrial fibrillation (AF). Whereas development of AF is suggested to be prevented by RAS inhibitors, recent findings have indicated that the preservation is closely associated with their antihypertensive effects. In this study, we investigated whether the L/N-type $Ca^{2+}$ channel blocker cilnidipine counteracts salt-induced atrial and ventricular remodelings and inducibility of AF.

**Methods:** Seven-week-old Dahl salt-sensitive rats were fed an 8% NaCl diet. Seven weeks later, cilnidipine of 5 or 10 mg/kg (Cil-L or Cil-H group) was administered orally for 5 weeks, and then echocardiography, electrophysiological evaluation and histological analyses were performed. The effects were compared with those of the L-type $Ca^{2+}$ channel blocker amlodipine (1.5 or 3 mg/kg; Aml-L or Aml-H group).

**Results:** Following the 8% NaCl diet intake, the BP and urinary protein levels increased, and fibrosis was induced in the atria and ventricles. Cilnidipine decreased the BP in a dose-dependent manner, and the extents in the Cil-L and Cil-H groups were similar to those in the Aml-L and Aml-H groups, respectively. Cilnidipine and amlodipine significantly ameliorated increases in urinary protein excretion. Cilnidipine produced a greater decrease of the fibrosis-area than amlodipine in the atria and ventricles. The fractional shortening and ejection fraction were greater in the Cil-H group than those in the Aml-H group. The 8% NaCl diet intake tended to induce shorteratrial effective refractory period and longer AF cycle length. The Cil-H group had tendency to suppress AF duration increased by NaCl intake. Plasma noradrenaline levels in the Cil-H group were lower than Aml-H group.

**Conclusions:** Cilnidipine effectively suppressed salt-induced atrial and ventricular remodelings and dysfunction at the dose causing antihypertension and renoprotection, probably through its N-type $Ca^{2+}$ channel-blocking actions.

*Funding:* Pharmaceutical Company Support - Ajinomoto Pharmaceuticals Co., Ltd.

## TH-PO721

**Indoxyl Sulfate Stimulates Cardiac Fibrosis with Enhanced Oxidative Stress and Reduced Anti-Oxidative Defense in Hypertensive Rats**

Toshimitsu Niwa,[1] Maimaiti Yisireyili,[1] Hidehisa Shimizu,[1] Shinichi Saito,[1] Fuyuhiko Nishijima.[2] *[1]Nagoya Univ Graduate School of Medicine; [2]Biomedical Research Laboratories, Kureha Co.*

**Background:** Cardiovascular disease (CVD) is common in chronic kidney disease (CKD) patients. Indoxyl sulfate (IS) is a nephrovascular uremic toxin that induces oxidative stress in kidney and vascular system. The present study aimed to examine the effect of IS on fibrosis and oxidative stress in rat heart.

**Methods:** The effects of IS on heart were examined by Masson's trichrome (MT) staining and immunohistochemistry using; (1) Dahl salt-resistant normotensive rats (DN), (2) Dahl salt-resistant normotensive IS-administered rats (DN+IS), (3) Dahl salt-sensitive hypertensive rats (DH), and (4) Dahl salt-sensitive hypertensive IS-administered rats (DH+IS).

**Results:** DH+IS rats showed significantly increased heart weight and left ventricle weight compared with DN. DH and DH+IS rats showed significantly increased diameter of cardiomyocytes, increased MT-positive fibrotic area, increased staining for transforming growth factor (TGF)-β1, α-smooth muscle actin (SMA), type 1 collagen, NADPH oxidase Nox 4, malondialdehyde (MDA), and 8-hydroxydeoxyguanosine (8-OHdG) and decreased staining for nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase-1 (HO-1) in the heart compared with DN. More notably, DH+IS rats showed significantly increased diameter of cardiomyocytes, increased fibrotic area, increased staining for TGF-β1, SMA, type 1 collagen, Nox4, 8-OHdG and MDA, and decreased staining for Nrf2 and HO-1 in the heart compared with DH.

**Conclusions:** IS aggravates cardiac fibrosis and cardiomyocyte hypertrophy with enhanced oxidative stress and reduced anti-oxidative defense in hypertensive rats.

## TH-PO722

**Reduced Firing Activity of Afferent Renal Innervation in the 2Kidney/1Clip Model of Hypertension**

Wolfgang Freisinger,[1] Annalena Karl,[2] Tilmann Ditting,[2] Sonja Heinlein,[2] Roland E. Schmieder,[2] Karl F. Hilgers,[2] Johannes Schatz,[2] Jens Lutz,[1] Roland Veelken.[2] *[1]Nephrology, Med. Clinic, Universitätsmedizin Mainz, Mainz, Germany; [2]Nephrology and Hypertension, Med. Clinic 4, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany.*

**Background:** Renal denervation has been shown to be effective in hypertension. Recently, we found that afferent renal neurons show a distinctive feature, exhibiting predominantly a sustained firing upon current injection due to a specific expression of TTX resistant Na-channels. So far, the firing pattern of these specific sensory neurons in hypertension is unclear. Hence we wanted to test the hypothesis that the firing pattern of renal afferent neurons is altered in the 2K/1C model of hypertension.

**Methods:** Hypertension was induced by unilateral clipping of the renal artery in male Sprague Dawley rats 3 weeks prior to experiments. Labelling (DiI) allowed the identification of renal afferent Dorsal root ganglion (DRG) neurons. Current clamp was used to characterize neurons as "tonic", i.e. sustained action potential (AP) firing or "phasic", i.e. <5 APs. Electrophysiological parameters and AP properties were determined in neurons of hypertensive animals and compared to controls.

**Results:** Renal DRG neurons of hypertensive animals (n=88) showed a significant decrease in tonic firing compared to controls (44.3% [39/88] vs. 59.5% [50/84], $p< 0.05$). Current Clamp analysis revealed no significant change in action potential shape e.g. overshoot, firing threshold in hypertensive animals. Tonic cells had a higher capacity in hypertensive neurons (124pF vs 87.8pF, $p<0.01$), other cell parameters were equal. Renal morphology in hypertensive animals was equal in the clipped vs. non-clipped kidney.

**Conclusions:** For the first time we could show that excitability of afferent renal neurons in the 2Kidney/1Clip model of hypertension is significantly altered, as renal afferent DRG neurons exhibit less sustained firing upon stimulation. Sustained high blood pressure is possibly characterized by a generally decreased afferent renal activity with impaired sympathoinhibition. The underlying mechanisms causing a "switch" from tonic to phasic need further elucidation.

## TH-PO723

**Increased Neurokinin Release from Afferent Renal Nerves Is Accompagnied by Decreased Afferent Electric Activity**

Tilmann Ditting,[1] Kristina Rodionova,[1] Christian Ott,[1] Johannes Schatz,[1] Sonja Heinlein,[1] Roland E. Schmieder,[1] Wolfgang Freisinger,[2] Karl F. Hilgers,[1] Roland Veelken.[1] *[1]Med Clinic 4, Nephrology & Hypertension, Friedrich-Alexander Univ Erlangen, Erlangen, Germany; [2]Med Clinic 1, Dept of Nephrology, Johannes Gutenberg Univ Mainz, Mainz, Germany.*

**Background:** Afferent renal nerves exhibit a dual function. They influence intrarenal immunological processes by release of neurokinins like CGRP and control central sympathetic outflow via afferent electrical activity. The former seems to be important in renal inflammation whereas the sympathetic modulation by afferent electrical activity is not fully understood. Hence, we hypothesized that augmented effects of CGRP in renal inflammation occur with increased afferent renal nerve activity.

**Methods:** As inflammatory model, normotensive renal inflammation (RI) was induced by i.v. injection of 1.75 mg/kg BW OX-7 antibody to rats. These were investigated neurophysiologically and pathomorphologically using standard techniques 6 days after RI induction.

**Results:** Blood pressure (BP) was normal, hence confounding BP effects were unlikely. RI rats exhibited albuminuria (61±6 µg/24h), infiltration of macrophages in the interstitium (26 ± 4 cells/hp field) and glomeruli (3.7±0.6 cells/glomerular X-section). Pretreatment with the CGRP antagonist rCGRP 8-37 (15nmol/kg) significantly reduced proteinuria and macrophage infiltration suggesting increased activity of CGRP. In an *in-vitro* assay, renal RI tissue exhibited increased CGRP release (14 ± 3 pg/ml vs. controls 6.8 ± 2.8 pg/ml; $p<0.05$, n=8). RI tissue from renally denervated rats showed no CGRP. Afferent renal nerve activity (ARNA; spikes/sec) was unexpectedly lower in RI compared to controls (8.0 ± 1.8 vs. 27.4 ± 4.1 Hz*,n=6, $p<0.05$). On the other hand, the burst-frequency renal sympathetic nerve activity (RSNA) in RI was significantly higher than in controls (14.7 ± 0.9 vs. 11.5 ± 0.9 bursts/sec*; n=6, *$p<0.05$).

**Conclusions:** In contrast to our hypothesis increased release of neurogenic CGRP aggravating RI occurred with decreased ARNA. Increased RSNA pointed to a reduced tonic inhibition by ARNA. The latter mechanism might worsen sympathetic dysregulation and renal damage.

## TH-PO724

**The Prorenin Receptor (PRR) Mediates Compensatory Responses to Buffer the Actions of the Renin-Angiotensin System (RAS) in the Collecting Duct through Cyclooxygenase-2 Upregulation in the Renal Medulla**

Alexis A. Gonzalez,[1] Minolfa C. Prieto.[2] *[1]Instituto de Química, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile; [2]Physiology, Tulane Univ, New Orleans, LA.*

**Background:** Angiotensin (Ang) II-induced hypertensive rats exhibit augmented secretion of prorenin and prorenin receptor (PRR) mRNA, which contrast with the reduced levels of full-length PRR in the renal medulla. The urine of these rats contains high levels of the soluble form of PRR (sPRR). PRR stimulates cyclooxygenase-2 (COX-2) in the

intercalated cells (IC) via ERK1/2. However, whether the PRR-mediated stimulation of COX-2 in the renal medulla varies during the course of AngII-induced hypertension remains unknown.

**Methods:** We examined the temporal changes of renin, full-length PRR, sPRR, ERK1/2 and COX-2 during chronic AngII infusion for 3, 7, and 14 days in the rat renal medulla and in cultured inner medullary collecting duct (IMCD) cells.

**Results:** AngII infusion (80 ng/min) increased full-length PRR and COX-2 protein levels, phospho-ERK1/2 and $PGE_2$ during the early normotensive phase of the AngII infusion (day 3). In contrast, during the established hypertensive phase (day 14), no changes were observed. At day 14, the urines of AngII-infused rats showed increased renin, sPRR and AngII. In cultured IMCD cells, AngII treatment (100 nM, 6 hrs) increased prorenin, full-length PRR, phospho-ERK1/2 and COX-2. AT1R blockade abolished these effects. Interestingly, after 16 hours of AngII incubation, the sPRR became detectable in cell culture media.

**Conclusions:** These results suggests that during early phase of AngII-induced hypertension, the collecting duct harbors compensatory mechanisms directed to buffer the actions of the RAS, in which the secretion of prorenin by the principal cells exerts a paracrine activation of PRR-membrane bound in IC, stimulating COX-2 and PGE2. By contrast, during the late phase, sPRR secretion into the collecting duct exerts hypertensinogenic effects by binding local prorenin, increasing intratubular AngII.

## TH-PO725

**SIRT1 Activation Protects the Endothelial Dysfunction by Inhibiting PDGF and TGF-β Generation** Hideyuki Negoro. *Medicine, Harvard Medical School, The Graduate School of Project Design, Boston.*

**Background:** SIRT1 is a conserved NAD(+)-dependent deacetylase and possesses beneficial effects against aging-related diseases, but little information is available on a putative role of SIRT1 in endothelial and vascular homeostasis. Endothelial senescence causes endothelial dysfunction to promote atherosclerotic change and contribute to age-related vascular diseases. Growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β can be produced by additional cells involved in the pathogenesis of arteriosclerosis and play an important part in the progression of age-related vascular diseases.

**Methods:** We established an in vitro senescence model by prolonged culture of primary endothelial cells isolated from bovine aorta. The freshly isolated young endothelial cells gradually underwent senescence during 1 month of repetitive passages. We knocked down SIRT1 to evaluate the protein levels of LKB1, phosphorylated AMPK, PDGF and TGF-β in the knocked down cells.

**Results:** It was observed that protein expressions of SIRT1 were decreased time dependently in the senescent endothelial cells. In contrast, the protein levels of LKB1, a serine/threonine kinase, the phosphorylation of its downstream target AMP-activated protein kinase (AMPK), PDGF and TGF-β generation were dramatically increased in the senescent cells. On the other hand, resveratrol activated SIRT1 in the endothelial cells. SIRT1 activation with resveratrol inhibited the increase of LKB1, AMPK. At the same time, SIRT1 activation with resveratrol reduced PDGF and TGF-β generation in the endothelial cells significantly. We knocked down SIRT1 and the protein levels of LKB1, phosphorylated AMPK, PDGF and TGF-β elevated in the knocked down cells. The protein levels of LKB1, phosphorylated AMPK, PDGF and TGF-β generation did not changed in the SIRT1 knocked down cells even if they were stimulated with resveratrol.

**Conclusions:** These findings indicate that activation of SIRT1 provides beneficial effects against the endothelial dysfunction to promote atherogenesis by inhibiting PDGF and TGF-β generation.

*Funding:* Government Support - Non-U.S.

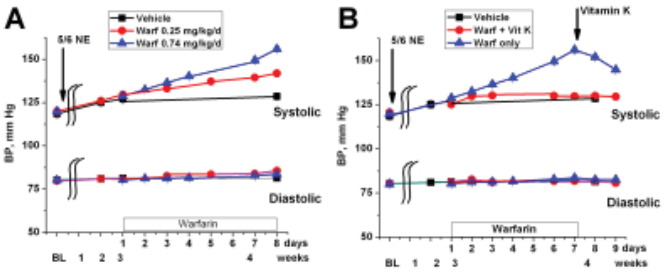
## TH-PO726

**Oral Warfarin Increases Blood Pressure in Control and 5/6 Nephrectomy Rats** Jay C. Vance, Kyle M. Ware, Zahida Qamri, Lee A. Hebert, Anjali A. Satoskar, Gyongyi Nadasdy, Iouri Ivanov, Tibor Nadasdy, Brad H. Rovin, Sergey V. Brodsky. *Depts of Pathology and Medicine, The Ohio State Univ, Columbus, OH.*

**Background:** Hypertension is a common comorbidity in patients with chronic kidney diseases (CKD). In our animal model of warfarin-associated nephropathy we found that the blood pressure (BP) of rats with CKD increased with warfarin treatment. Because an effect of warfarin on BP has not been previously reported, the aim of this study was to examine the relationship of warfarin therapy to hypertension.

**Methods:** Sham-operated (control) and 5/6 nephrectomy rats (5/6NE) were treated with different doses of warfarin. Blood pressure (BP) was measured by a tail cuff.

**Results:** Warfarin increased BP in control and 5/6 NE rats in a dose-depended manner. Warfarin affected predominantly systolic BP with little or no change in diastolic BP (Figure 1, A, effects in 5/6 nephrectomy rats, 3 weeks after the ablative surgery are shown). The hypertensive effects of warfarin were more prominent in animals with progressing CKD. Treatment with vitamin K attenuated the effects of warfarin on BP (Figure 1, B, 0.74 mg/kg/day of warfarin was used. Vitamin K 40 mg/kg was injected i.p. daily in the co-treatment with warfarin (red) or once after 1 weeks of 0.74 mg/kg/day warfarin (blue)).




**Conclusions:** Warfarin increases systolic, but not diastolic, BP in both control and CKD rats. Warfarin effects are Vitamin K dependent. The pathogenesis of this effect is not yet clear. Warfarin is the most commonly prescribed anticoagulant and is used extensively in patients with CKD.Our data indicate that BP should be carefully monitored in patients on warfarin therapy, especially those with CKD.

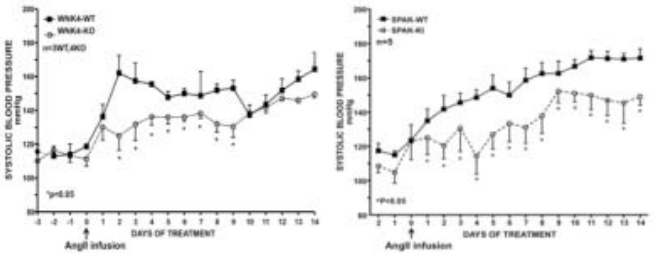
## TH-PO727

**Disruption of the WNK4/SPAK Pathway Reduces the Hypertension Induced by Angiotensin II** Luz Graciela Cervantes-Perez,[1] Maria Castañeda-Bueno,[1] Norma Hilda Vázquez,[1] Dario Alessi,[2] Norma Bobadilla,[1] Gerardo Gamba.[1] *[1]Molecular Physiology Unit, INNSZ-IIB, UNAM, Mexico City, Mexico; [2]MRC Phosphorylation and Ubiquytilation Unit, Dundee Univ, Dundee, United Kingdom.*

**Background:** Angiotensin II (AngII) induces systemic hypertension by its renal effects in reducing salt excretion. The effect of AngII is associated with increased phosphorylation of the renal NaCl cotransporter, NCC. The AngII activation of NCC requires the presence of an intact WNK4-SPAK pathway. We thus analyzed the effect of AngII infusion on blood pressure in genetically altered mice either lacking WNK4 ($WNK4^{-/-}$ mice) or expressing a version of the SPAK kinase that cannot be activated by WNKs ($SPAK^{T245A/T245A}$ knockin mice).

**Methods:** $WNK4^{-/-}$ and SPAK knockin mice and their respective controls were infused with AngII at 1440/μg/kg/day by osmotic minipumps during 14 days. $WNK4^{-/-}$ mice and their respective wild type controls were resistant to the AngII-inducing high blood pressure at the infused dose. To over come this, unilateral nephrectomy was performed in this colony. Blood pressure was assessed by radiotelemetry from three days before the beginning of AngII infusion and during the next 14 days after infusion. At the end of the infusion period mice were sacrificed and renal tissue was used for protein extraction to assess total NCC and phosphoT58-NCC with specific antibodies.

**Results:** Figure 1 shows the effect of AngII infusion in $WNK4^{-/-}$ and SPAK-KI and their controls. The response to AngII in $WNK4^{-/-}$ was reduced during the first week, but eventually was similar, owing in part to a reduction of AngII effect in wild type mice. In contrast, the response to AngII was clearly reduced in SPAK-knockin animals. AngII induced NCC phosphorylation in wild type mice but not in $WNK4^{-/-}$ or SPAK-KI mice.




**Conclusions:** Hypertension induced by AngII infusion is due in part to NCC activation through WNK4-SPAK pathway.

*Funding:* Government Support - Non-U.S.

**TH-PO728**

**Treatment with Azilsartan and Chlorthalidone Lowers Blood Pressure and Reduces Renal Inflammation in a Rodent Model of the Metabolic Syndrome**
Chunhua Jin, David M. Pollock, John Jason White. *Medicine, Georgia Regents Univ, Augusta, GA.*

**Background:** The metabolic syndrome (metS) and chronic kidney disease are global health issues. The metS induces hypertension (HTN) and commonly results in renal damage. The optimal therapy for HTN in the metS is unknown. JNC7 recommends thiazide diuretic as first line therapy for all patients with HTN. However, these drugs may have untoward metabolic effects. This study was conducted to investigate the effects of azilsartan (AZL), chlorthalidone (CLTD), and the combination (AZL + CLTD) on blood pressure and renal injury in a rodent model of metabolic syndrome.

**Methods:** In order to mimic the metS in a hypertensive model, male Dahl salt sensitive rats were fed a high fat (36% fat) high salt (4% NaCl) diet. Groups of rats were then treated with vehicle, AZL (3 mg/kg/day), CLTD(5 mg/kg/day) or AZL+CLTD. Mean arterial pressure (MAP) was recorded continuously by telemetry. After 4 weeks, rats were sacrificed and kidneys were harvested for histology.

**Results:** After 4 weeks, both AZL and CLTD attenuated the rise in blood pressure compared to vehicle, and the combination further reduced blood pressure compared to CLTD alone. All treatments reduced proteinuria and albuminuria. However, only groups treated with AZL prevented nephrinuria (a podocyte injury marker). The nephrinuria was 57% lower, and proteinuria was 47% lower with combination therapy compared to AZL alone. All treatments reduced the number of ED1+ (monoclonal antibody) cells in the kidney. Plasma monocyte chemoattractant protein-1(MCP-1) was significantly lower only in the AZL group.

**Conclusions:** In our rodent model of the metS, AZL and CLTD lower blood pressure and exhibit renal protective effects. Treatment with either reduces albuminuria and renal cortical inflammatory cell infiltration. AZL treatment offers additional protection as evidenced by lower nephrinuria and plasma MCP-1. These findings indicate that combination therapy afforded the greatest protective effects and thus may be the best choice for hypertensive therapy in the metS.

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**TH-PO729**

**Systemic PPARγ Deletion in Mice Leads to Sympathoactivation**
Tianxin Yang,[1] Mi Liu,[2] Zhanjun Jia.[1] *[1]Internal Medicine, Univ of Utah and Veterans Affairs Medical Center, Salt Lake City, UT; [2]Institute of Hypertension, Sun Yat-sen Univ School of Medicine, Guangzhou, Guangdong, China.*

**Background:** PPARg and sympathetic nerve activity (SNA) antagonistically regulate energy metabolism and cardiovascular function but their precise relationship is not known.

**Methods:** Systemic inactivation of PPARg was generated constitutively by using Mox2-Cre mice or inducibly by using the tamoxifen system (TM KO). Sympathetic activity was evaluated.

**Results:** Radiotelemetrydemonstrated consistent increases in resting heart rate (HR) in both strains of null mice; this was associated a more than 10-fold increase in urinary epinephrine and norepinephrine. Subsequently, more detailed analysis of SNA was performed in TM KO mice. In response to a bolus ip injection of the gangalion blocker pentolinium at 7.5 mg/kg, the decreases in HR and MAP were greater inthe null mice than in the flox controls (delta HR:-254.2 vs. 92.3 beat/min; delta MAP: -67.8 vs. 33.4 mmHg).The HR decrease in response to the beta-blocker metaprolol was greater in the null mice than in the flox controls so was the MAP decease in response to the alpha1-blocker prazosin. Baroreflex sensitivity (BRS) assessed by the HR responses to bolus injections of sodium nitroprusside (NP-BRS) or phenylephrine (PE-BRS) showed normal PE-BRS but blunted NP-BRS. Echocardiographydemonstrated that the ejection fraction (EF) was 11% lower after administration of metaprolol contrastingto a drop of only 1.5% for the flox controls, in parallel with a greater reduction of HR in the KO mice. In contrast, the increase in HR following administration of the beta1 agonist dobutamine was less in the KO mice than in the flox controls.

**Conclusions:** Together, PPARg deletion causes the SNA activation, accompanied with suppressed beta1 receptor activity.

*Funding:* NIDDK Support

**TH-PO730**

**Imatinib Mesylate Has a Protective Effect on Angiotensin II-Induced Podocyte Injury**
Qian Yang, Zhilong Ren, Xinghua Chen, Wei Liang, Guohua Ding. *Div of Nephrology, Renmin Hospital of Wuhan Univ, Wuhan, Hubei, China.*

**Background:** c-Abl is a non-receptor tyrosine kinase involved in cytoskeleton formation, cell proliferation and apoptosis. Ang II stimulates c-Abl expression in kidneys and podocytes, suggesting that c-Abl participates in Ang II-induced damage. Imatinib mesylate is a 2-phenylaminopyrimidine-based inhibitor of the Abl tyrosine kinase family. In this study, we evaluated the effect of imatinib mesylate on Ang II-induced podocyte injury.

**Methods:** 36 male Wistar rats were randomly assigned to receive Ang II (400 kg/kg/min), Ang II + imatinib mesylate (50 mg/kg/day) or saline by osmotic mini-pumps. SBP and 24 hr Uprot were measured weekly. Kidneys were collected at 14 and 28 d. Glomerular expression of c-Abl was evaluated by immunotechniques and Western blotting. immortalized mouse podocytes were treated with Ang II (10-8M) in the presence or absence of the imatinib mesylate (0.5-20ug/ml). Cytoskeleton formation was evaluated by FITC-phalloidin staining and apoptosis by flow cytometry and Hochest-33258 staining. Western blotting and immunofluorescence assays were used for the expression of c-Abl and p-c-Abl (phosphorylation at Y245 and Y412).

**Results:** Ang II-infused rats had hypertension and proteinuria, and podocyte damage, which was alleviated by imatinib mesylate. C-Abl was localized along capillary loops. Imatinib mesylate attenuated the expression of c-Abl in Ang II rats .($P<0.05$). A significantly positive correlation was found between the c-Abl expression and the amount of Uprot ($r=0.665$, $P<0.05$). Ang II significantly stimulated c-Abl expression in cultured podocytes, and increased its phosphorylation at Y245 and Y412. In controls, c-Abl staining in the nucleus was increased by Ang II, with no changes in cytoplasmic levels. Cytoskeleton reorganization and apoptosis were observed in Ang II-stimulated podocytes. Pretreatment with Imatinib mesylate prevented these effects.

**Conclusions:** c-Abl specific inhibitor Imatinib mesylate has a protective effect on Ang II-Induced podocyte injury, which conforms that c-Abl plays an important role in Ang II-induced podocyte injury.

*Funding:* Government Support - Non-U.S.

**TH-PO731**

**Immunoreactive (Pro)renin Receptor in the Human Plasma**
Kazuhito Totsune,[1] Takuo Hirose,[1] Shiro Oguma,[2] Itiro Ando,[1] Hiroshi Sekino,[3] Hiroshi Sato,[4] Kazuhiro Takahashi,[5] Yutaka Imai.[1] *[1]Planning for Drug Development and Clinical Evaluation, Tohoku Univ Grad Sch of Pharm Sci and Med; [2]Koujinkai Nagamachi Clinic; [3]Koujinkai Central Clinic; [4]Labotratory of Clinical Pharmacology and Therapeutics, Tohoku Univ Grad Sch of Pharm Sci and Med; [5]Endocrinology and Applied Med Sci, Tohoku Univ Grad Sch of Med, Sendai, Japan.*

**Background:** (Pro)renin receptor ((P)RR) is a new member of renin-angiotensin system. It is reported that soluble type of (P)RR (s(P)RR) with 28 kDa is present in human blood. However, the characteristics of immunoreactive (P)RR (IR-(P)RR) in plasma have not been clarified yet.

**Methods:** We therefore examined a profile of IR-(P)RR in human plasma by a gel chromatography and two types of assay systems: a competitive enzyme immunoassay (CEI-assay) which we reported previously (Kidney Week 2011) and a commercially available s(P)RR sandwich ELISA kit (s(P)RR-assay) (IBL Co., Ltd.). Plasma IR-(P)RR levels also were measured in 10 non-dialyzed subjects and 16 hemodialysis patients.

**Results:** Gel chromatography of human plasma showed a major peak of IR-(P)RR at the elution position of human γ-globulin (158 kDa) by CEI-assay, and in contrast a small peak at 158 kDa accompanied with a major peak at 29 kDa by s(P)RR-assay. These data indicate that IR-(P)RR in human plasma is composed of two components, one with a large molecular size and s(P)RR. By s(P)RR-assay, which turned out to be sensitive to s(P)RR, plasma IR-(P)RR levels in HD patients were 2.0-fold higher than non-HD subjects ($P<0.01$), and the levels significantly increased during HD sessions in diabetic HD patients from 1.04±0.04 to 1.14±0.07 pmol/ml (mean±SE, n=8, $P<0.05$), whereas, the levels decreased in non-diabetic HD patients from 1.19±0.06 to 1.04±0.05 pmol/ml (n=8, $P<0.05$). There was no significant correlation between plasma IR-(P)RR levels measured by CEI-assay and those by s(P)RR-assay.

**Conclusions:** These results suggest that measurement of IR-(P)RR in plasma, s(P)RR in particular, is important to clarify the role of (P)RR in the cardiovascular regulation.

*Funding:* Government Support - Non-U.S.

**TH-PO732**

**Co-Assembly of GABA(A) Receptor π, α1 and β3 Subunits and Its Expression in Rat and Human Kidney**
Kozue Takano,[1] Junichi Yatabe,[2] Midori Sasaki Yatabe,[1] Hironobu Sanada,[3] Pedro A. Jose,[4] Tsuyoshi Watanabe,[3] Junko Kimura.[2] *[1]Dept of Pharmacol, Fukushima Med Univ Sch Med, Japan; [2]Dept of CKD Initiatives, Fukushima Med Univ Sch Med; [3]Dept of Nephrol, Hypertens, Diabetol, Endocrinol and Metabol, Fukushima Med Univ Sch Med, Japan; [4]Univ of Maryland.*

**Background:** Gamma aminobutyric acid (GABA) lowers blood pressure and induces diuresis/natriuresis. We have reported characteristic expressions of GABA-related molecules in rat kidney. In contrast to brain, GABA(A) receptor π subunit may be dominant in the kidney, constituting $\alpha1/\alpha1/\beta3/\beta3/\pi$ pentamers. In this study, coexpressions, intra-renal localizations and relative expressions of the subunits in addition to GABA-related molecule expressions in human kidney were examined.

**Methods:** Renal cortices of Wistar-Kyoto rats (WKY) were used for immunohistology and immunoprecipitation. Renal cortical messenger RNA expressions of WKY on normal diet and on high-salt diet (8%, 7 days) were compared by quantitative RT-PCR. Quantitative RNA assays using human brain and kidney RNA were also performed for those GABA-related molecules found in rat kidney.

**Results:** GABA(A) π subunit mRNA in WKY kidney was more than 100 times that in the brain. Costaining of α1, β3 and π subunits was observed mainly on the apical side of cortical tubules. There was significant π subunit signal in collecting duct, suggesting coexpression with GABA(B) receptor. Kidney protein immunoprecipitated with π subunit antibody revealed α1 and β3 subunits, with molecular weights suggesting dimerization. Salt loading reduced π subunit mRNA in WKY kidney. In human kidney, mRNA of GABA(A) α1, β3 & π subunits, GABA(B) R1 & R2 subtypes, and GABA(C) ρ1 & ρ2 subunits in addition to GABA transporter, GAT2, GABA producing enzymes, GAD65 & GAD67, and GABA degrading enzyme, ABAT, were found similar to WKY kidney.

**Conclusions:** Characteristically high expression of GABA(A) π subunit in the kidney suggests its role in mediating kidney-specific actions of GABA. Components for GABA

synthesis, action, and dedgradation exist also in human kidney. Further analyses of GABA receptor composition, distribution, and function may elucidate novel blood pressure regulatory mechanism in the kidney.

## TH-PO733

**Effect of Gender and Diabetes in Circulating ACE2 and ACE Activity in Streptozotocin-Induced Mice** Sergi Clotet-Freixas, Maria Jose Soler, Julio Pascual, Marta Riera. *Hospital del Mar - IMIM, Barcelona, Spain.*

**Background:** Male gender predisposes to chronic kidney disease. We showed before increased circulating ACE2 and ACE in diabetic male mice. We study gender differences and effect of diabetes and gonadectomy(GDX) in circulating ACE2 and ACE in STZ-induced mice.

**Methods:** Study groups:Control and diabetic females(fCONT,fDB),males(mCONT,mDB) and GDX males(mCONT-GDX,mDB-GDX).GDX was performed 12 days after STZ injection.At the end of the study(19wks postSTZ) blood glucose(BG),body weight,kidney/body weight,blood pressure and urinary albumin excretion(UAE) were measured.Serum ACE2 and ACE activity were determined by fluorometric assays.

**Results:** Hyperglycemia was observed in STZ groups.BG was significantly higher in males as compared to females.GDX significantly decreased BG in mDB(298.3±17.0 vs 431.5±21.8mg/dL; p<0.05).ACE2 and ACE in fDB and mDB were increased as compared to controls.Male control mice showed significantly higher ACE2 and ACE than females. GDX resulted in significant reduction of ACE2(in both mCONT and mDB) and ACE(only in mDB).We found direct correlation between BG and ACE2 and ACE(r=0.6/r=0.5,p <0.01).

| | fCONT (n=11) | mCONT (n=18) | mCONT-GDX(n=7) | fDB(n=10) | mDB(n=9) | mDB-GDX (n=9) |
|---|---|---|---|---|---|---|
| ACE2(RFU/µl/h) | 60.0±4.8 | 75.4±4.4* | 50.9±1.6$ | 132.6±32.6& | 130.6±15.8& | 63.7±1.7$& |
| ACE(RFU/µl/min) | 1546.5±111.5 | 2143.9±133.4* | 1856.6±172.0 | 2279.7±181.9& | 2984.1±198.1*& | 2123.3±107.5$ |
| KW/BW(%) | 0.91±0.02 | 0.97±0.03* | 0.79±0.03$ | 1.44±0.08& | 1.32±0.06& | 0.89±0.02$& |
| SBP(mmHg) | 98.2±2.1 | 96.4±1.6 | 101.0±1.4$ | 106.0±3.8 | 97.6±1.9* | 97.8±1.7 |
| UAE(µgAlb/mgCrea) | 14.8±3.2 | 18.5±2.5 | 19.6±7.9 | 86.4±26.2& | 308.0±133.9*& | 22.2±5.6$ |
| p | *p<0.05vs.female;$p<0.05vs.male;&p<0.05vs.CONT | | | | | |

**Conclusions:** STZ injection increased BG and ACE2 and ACE activity in all experimental groups.GDX diminished BG and circulating ACE2 and ACE.Thus,increased levels of circulating ACE2 and ACE in diabetic male mice may be ascribed to a modulation of male sex hormones.

*Funding:* Government Support - Non-U.S.

## TH-PO734

**Physiological and Pharmacological Concentrations of Angiotensin II Yield Distinct Differences in AT1 Receptor Signaling** Lena Scott,[1] Kristoffer Bernhem,[2] Hjalmar Brismar,[2] Anita Aperia.[1] *[1]Karolinska Institutet; [2]Royal Institute of Technology.*

**Background:** Studies on the desensitization of AT1R and the interaction between AT1R and calcium channels are generally done using pharmacological concentrations of angiotensin II (AngII).

**Results:** Here we show that repeated physiological AngII doses will, in contrast to the desensitizing effect of pharmacological AngII concentrations, result in an enhanced calcium response and a redistribution of AT1R receptors. We have recorded the $Ca^{2+}$ response to repeated doses of physiological (1 nM) and pharmacological (100 nM) Ang II in $AT_1R$ expressing HEK293a cells at (three time points) 0 min, following a 5 min and following an additional 30 min recovery. At 100 nM Ang II a significant desensitization was seen in response to the 2nd dose with partial recovery observed at the 3rd dose. At 1 nM Ang II no desensitization was observed to the 2nd dose, and the response to the 3rd dose was 2-fold enhanced. This enhanced response could be explained by increased recruitment of AT1R to the plasma membrane. To study redistribution of AT1R we used HEK293a cells expressing AT1R with an extracellular hemagglutinin tag and a C-terminus Venus tag. The ratio between the intensities from these two tags was 2.5-fold higher at the time of the 3rd dose of 1 nM Ang II as compared to base line expression. Voltage Gated Calcium Channels (VGCC) are therapeutical targets in hypertension and are partially controlled by angiotensin. We have investigated the effect of inhibiting VGCC with Nifedipine on AT1R calcium signaling. Nifedipine did not change the basal level of $[Ca^{2+}]$, but had a strong boosting effect on the $Ca^{2+}$ response to the first 1 nM Ang II dose. This was followed by a gradual desensitization to the 2nd and 3rd pulse.

**Conclusions:** In summary this study shows new aspects of the $AT_1R$ response to physiological angiotensin concentrations and will have several important clinical implications.

*Funding:* Private Foundation Support

## TH-PO735

**The Imaging of Leakage of Red Blood Cells from Submucosal Capillary of the Bladder in Bladder Overdistension** Hideki Mizuno,[1,2] Tokunori Yamamoto,[2] Momokazu Gotoh.[2] *[1]Urology, National Hospital Organization Nagoya Medical Center, Nagoya, Japan; [2]Urology, Nagoya Univ Graduate School of Medicine, Japan.*

**Background:** We previously reported that the effect of tamsulosin on bladder microcirculation in a rat ischemia-reperfusion model, evaluated by pencil lens charge-coupled device microscopy system(Mizuno H et al Urology 2010). In the present study, to elucidate the causes of macrohematuria after bladder ischemia-reperfusion injury.

**Methods:** Changes in blood flow through a submucosal capillary of the rat bladder were measured during bladder filling using the PLCMS. One week after starting infusion of either physiological saline or tamsulosin, blood flow in the bladder was halted by bladder overdistension via an infusion of physiological saline. The bladder was then emptied to be reperfused with blood. Changes in blood flow through a submucosal capillary of the bladder during ischemia and reperfusion were measured using a PLCMS, and the data obtained for the control group and tamsulosin group were compared. Moreover, we investigated 56 cases who had a medical examination in our hospital because of acute urinary retention from September 2009 to August 2012.

**Results:** The leakage of red blood cells from capillary vessels in model rat of bladder ischemia-reperfusion injury could be clearly visualized using the PLCMS. The percentage of the leakage in the tamsulosin group decreased compared to the control group.

In acute urinary retention patients, average residual urinary volume were significantly increased in microhematuria(+) compared to microhematuria(-). The percentage of $\alpha_1$-Adrenoceptor antagonists treated patients was 13.3% in macrohematuria(+) and 42.9% in macrohematuria(-).

**Conclusions:** The PLCMS image showed that leakage of red blood cells from submucosal capillary of the bladder in bladder overdistension. The results of the present study suggest that tamsulosin hydrochloride protects the submucosal capillaries of the bladder from ischemic injury.

## TH-PO736

**Effect of Exercise Training on Angiotensin II, Sodium Excretion, and the Abundance of Sodium Transporters and Channels in Two-Kidney One-Clip Hypertensive Rats** Noreen F. Rossi, Bruce E. Linebaugh, Haiping Chen, Kevin B. Ginsburg, Robert A. Augustyniak. *Internal Medicine and Physiology, Wayne State Univ School of Medicine, Detroit, MI.*

**Background:** Two-kidney one-clip (2K1C) hypertension is characterized by elevated sympathetic tone. Efferent renal sympathetic nerves regulate renin-angiotensin (Ang) and Na reabsorption via norepinephrine actions at the macula densa and renal tubules. One mechanism whereby exercise training decreases mean arterial pressure (MAP) is by decreasing sympathetic output. We hypothesize that Na transporter/channel abundance will be increased in 2K1C rats and that exercise training will decrease mean arterial pressure (MAP), plasma Ang II, Na excretion and increase transporter abundance.

**Methods:** Six-week old sham-clipped (SC) and 2K1C rats were assigned to sedentary housing (standard cages. SED) or exercise training (cages with running wheels, EXT) and pair fed for 12 weeks. MAP was monitored by telemetry. GFR, UNaV and FENa were assessed by standard methods. Renal cortex was harvested, homogenized and Na transporters/channels analyzed by western blot.

**Results:** MAP was 133±3 mmHg in SC SED rats and did not change with EXT. In 2K1C rats, MAP was lower in 2K1C EXT 170±6 mmHg vs 2K-1C SED 191±10 mmHg, $p < 0.05$. Plasma Ang II in 2K1C EXT was 48.9 ± 7.5 fmol/ml which was similar to that in SC rats and lower than 2K1C SED 187.4 ± 43.6 fmol/ml, $p < 0.05$. UNaV and FENa were higher in the non-clipped kidney of 2K1C SED vs either SC SED or EXT groups. UNaV and FENa from the clipped kidney of 2K1C SED rats were similar to those of SC rats. Both clipped and non-clipped kidneys of 2K1C EXT rats had UNaV and FENa similar to SC SED and EXT rats, and were lower than 2K1C SED rats ($p < 0.05$). The abundance of cortical NaK-ATPase and NCC was higher in clipped vs non-sclipped kidneys. NaK-ATPase, 72 kD subunits of αENaC and γENaC and the 95 kD γENaC were increased in 2K1C EXT rats vs 2K1C SED.

**Conclusions:** Exercise training decreases arterial pressure and Ang II in 2K1C rats. 2K1C SED rats display pressure natriuresis. The expression of tubular Na transporters and channels is consistent with the urinary Na excretion pattern observed in sedentary and exercise trained 2K1C rats.

*Funding:* Other NIH Support - HL-079102, Veterans Affairs Support

## TH-PO737

**Additive Renoprotective Effect of Renal Liver Type Fatty Acid Binding Protein on Angiotensin II Type 1a Receptor Loss in Renal Injury due to RAS Activation** Daisuke Ichikawa,[1] Atsuko Ikemori,[2] Yugo Shibagaki,[1] Takashi Yasuda,[1] Kenjiro Kimura.[1] *[1]Hypertension, Dept of Internal Medicine, St. Marianna Univ School of Medicine, Kawasaki, Japan; [2]Dept of Anatomy, St. Marianna Univ School of Medicine, Kawasaki, Japan.*

**Background:** The aim of this study was to assess the additive renoprotective function of renal human L-FABP (hL-FABP) on angiotensin II (Ang II) type 1a receptor (AT1a) loss in renal injury due to renin-angiotensin system (RAS) activation.

**Methods:** We established hL-FABP chromosomal transgenic mice ($Tg\text{-}AT1a^{+/+}$), crossed the $Tg\text{-}AT1a^{+/+}$ with AT1a knock down homo mice ($AT1a^{-/-}$), generated Tg-AT1a hetero mice ($Tg\text{-}AT1a^{+/-}$). After the back-cross of these cubs, $Tg\text{-}AT1a^{-/-}$ were obtained. In order to activate the renal RAS, wild type mice ($non\text{-}Tg\text{-}AT1a^{+/+}$), $Tg\text{-}AT1a^{+/+}$, $non\text{-}Tg\text{-}AT1a^{+/-}$, $Tg\text{-}AT1a^{+/-}$, $non\text{-}Tg\text{-}AT1a^{-/-}$ and $Tg\text{-}AT1a^{-/-}$ were administered high dose systemic Ang II infusion and were given a high salt diet for 28 days.

**Results:** In the $non\text{-}Tg\text{-}AT1a^{+/+}$, Ras activation ($non\text{-}Tg\text{-}AT1a^{+/+}$-Ras) caused hypertension and tubulointerstitial damage. In the $Tg\text{-}AT1a^{+/+}$-Ras, tubulointerstitial damage was significantly attenuated compared with non $Tg\text{-}AT1a^{+/+}$-Ras. In the AT1a partial ($AT1a^{+/-}$) or completed ($AT1a^{-/-}$) knock out mice, Ras activation led to a significantly lower degree of renal injury compared with $non\text{-}Tg\text{-}AT1a^{+/+}$-Ras or $Tg\text{-}AT1a^{+/+}$-Ras mice. Renal injury in $Tg\text{-}AT1a^{+/-}$-Ras mice was significantly attenuated compared with $non\text{-}Tg\text{-}AT1a^{+/-}$-Ras mice. In both $non\text{-}Tg\text{-}AT1a^{-/-}$-Ras and $Tg\text{-}AT1a^{-/-}$-Ras mice, renal damage was rarely found. The degrees of renal hL-FABP expression and urinary hL-FABP levels increased by Ras activation and gradually decreased along with reduction of AT1a expression levels.

**Conclusions:** Renal hL-FABP expression attenuated tubulointerstitial damage in addition to the renoprotective action depending on the decreasing in AT1a expression in the renal injury due to Ras activation.

### TH-PO738

**The Effect of Nifedipine and Captopril on the Production of Pro-Inflammatory Cytokines by Peripheral Blood Mononuclear Cells**
Gyu Tae Shin, Ka Young Jung, Eunjung Kang, Inwhee Park, Heungsoo Kim. *Dept of Nephrology, Ajou Univ School of Medicine, Suwon, Kyunggi, Republic of Korea.*

**Background:** In the present study, we investigated the effect of nifedipine and captopril on the production of tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) by lymphocytes, monocytes and dendritic cells (MoDCs) derived from peripheral blood mononuclear cells (PBMCs).

**Methods:** PBMCs obtained from healthy volunteers (n = 20) were separated into lymphocytes and monocytes. Monocytes were cultured with GM-CSF and IL-4 to generate MoDCs. Lymphocytes were stimulated with ionomycin and phorbol myristic acetate and monocytes and MoDCs were stimulated with lipopolysaccharide to measure cytokines by ELISA and flow cytometry. The activation of mitogen activated protein kinases (p38, JNK, ERK) was measured by western blot. Throughout the experiment, 100 uM of nifedipine and 10 mM of captopril were used.

**Results:** In lymphocytes, TNF-alpha and IL-6 concentrations were significantly suppressed by nifedipine and captopril and notably the combined treatment of nifedipine plus captopril suppressed TNF-alpha significantly more than the monotherapy of each drug. In monocytes, similar results as seen in lymphocytes were observed for TNF-alpha, whereas only the combination treatment was effective in suppressing IL-6 production. In MoDCs, both TNF-alpha and IL-6 levels were significantly suppressed by captopril but not by nifedipine. Flow cytometry analysis showed that TNF-alpha expression in CD4 cells was significantly suppressed by nifedipine but not by captopril. In CD8 cells, TNF-alpha expression was significantly suppressed only by the combination treatment of both drugs but not by monotherapy of each drug. Western blot showed that p38 activity was significantly decreased by nifedipine but not by captorpil.

**Conclusions:** We first demonstrated that nifedipine and captopril exert differential effects in the suppression of TNF-alpha and IL-6 depending on the cell types of PBMCs and the combination treatment of both drugs has additive effects on the suppression of these cytokines. We also showed that the effect of nifedipine on these cytokines may be partly mediated by p38 MAPK pathways.

### TH-PO739

**Loss of *Gstm1* Alters Blood Pressure Homeostasis and Augments Angiotensin II-Induced Hypertension**
Thu H. Le, Rosa Chan, Sylvia Cechova. *Univ of Virginia.*

**Background:** *GSTM1* gene encodes an enzyme that belongs to a superfamily of glutathione-S-transferases that metabolize xenobiotic and a broad range of reactive oxygen species and electrophilic compounds formed as secondary metabolites during lipid peroxidation. *Gstm1* has been reported to be a positional and functional candidate gene for hypertension in spontaneously hypertensive stroke-prone (SHRSP) rats. We hypothesized that *Gstm1* may influence blood pressure (BP) regulation through its central role in modulating redox homeostasis. The objective of this study was to determine the response of *Gstm1* knockout (KO) mice to 2 models of hypertension that are mediated in part by oxidative stress: 1) high salt (HS) model, and 2) angiotensin II (Ang II) model.

**Methods:** *Gstm1-/-* (KO) mice were generated on the 129S6 background through conventional gene targeting strategy. Hypertension was induced using HS diet (6% NaCl) for 2 weeks or by infusion of Ang II via osmotic pump at dose 1000 ng/kg/min for 3 weeks. Systolic BP (SBP) at baseline and under HS condition was measured using radio-telemetry. Tail-cuff manometry was used to measure SBP in Ang II induced hypertension. Urinary isoprostane (index of oxidative stress) was measured using ELISA assay. For all studies, $n \geq 4$ per group.

**Results:** By radiotelemetry, *Gstm1* KO mice display a modest but significantly higher baseline SBP compared to their wild type (WT) littermates: KO: 138.8 ± 1.3; WT: 132.1 ± 1.1, $p < 0.01$. On HS diet, both WT and KO mice displayed salt sensitivity, and the difference in SBP was maintained between the two genotypes (KO: 148.7 ± 1.6; WT: 143.8 ± 1.3, $p < 0.05$). Urinary isoprostane (ng/100 mg of body weight) is significantly higher in KO mice than WT mice at baseline as well as during HSD (baseline: KO 15.1 ± 2.9; WT: 8.0 ± 0.8, $p < 0.04$; HSD: KO 27.2 ± 2.0; WT: 21.0 ± 1.3, $p = 0.04$). Ang II infusion augments the difference in SBP between WT and KO mice (185.0 ± 3.1; WT mice 175.4 ± 1.4, $p < 0.05$).

**Conclusions:** In an experimental setting, loss of GSTM1 enzymatic activity results in elevated baseline BP and augmented Ang II induced hypertension. The altered BP homeostasis from deficiency in GSTM1 may be mediated by a decreased capacity to handle oxidative stress.

*Funding:* NIDDK Support

### TH-PO740

**Kallikrein Kinin System and Blood Pressure Modulation by Aldosterone**
Pablo J. Azurmendi, Natalia Corbera, Ayelen R. Toro, Fernando Raul Ibarra, Rodolfo S. Martin, Elisabet Monica Oddo, Elvira Arrizurieta. *Lab. Riñón Experimental, IIM Alfredo Lanari, UBA-CONICET, Buenos Aires, Argentina.*

**Background:** Previous results have shown that after gonadectomy (Gx) kallikrein kinin system activity and mineralocorticoid levels increase in both, male and female SHR. A gender difference in urinary kallikrein activity (UKa) was also observed, being UKa higher in female rats.

**Methods:** To further explore the interaction between KKS activity and mineralocorticoid levels, the effect of an inhibitor of Aldosterone receptor (Spironolactone, S) upon kallikrein synthesis (KLK), renal content (RC) and UKa was studied in 12 weeks old SHR, that were Gx at weaning. UKa and RC were measured using a chromogenic tripeptide substrate and KLK expresión by semiquantitative reverse transcription polymerase chain reaction (RT-PCR).

**Results:** UKa (nkat/d/gK) diminished in Gx rats after S treatment from 36.29±2.4 and 41.3±6.5 to 23.4±3.9 and 22.8±2.4 in male and female rats ($p<0.001$), respectively. RC (nkat/gK) instead, did not change in male and female Gx animals by the treatment (18.3±0.56 and 17.1±3.27 to 18.9±1.24 and 17.6±2.02, respectively). S treatment increased RC by 50% in Gx and intact male and female as well while UKa diminished. KLK diminished after S treatment in Gx male and female rats from 0.70±0.02 and 0.73±0.01 to 0.54±0.02 ($p<0.001$) and 0.68±0.03 (NS), respectively.

**Conclusions:** Present results suggests that Aldosterone allows the release of renal tissue kallikrein into urine, since, S treatment increases RK while diminishes UKa. This finding is less evident in Gx animals, as it is, KLK synthesis. This may indicate that sexual hormones could also contribute to KKS modulation.

*Funding:* Government Support - Non-U.S.

### TH-PO741

**The Role of A1AR in Hypertension Induced by Hyperuricemia**
Lin Liu,[1] Jinling Hao,[1] Lanping Jiang,[1] Junping Fan,[2] Xuejun Zeng,[2] Xuewang Lee,[1] Limeng Chen.[1] *[1]Nephrology, Chinese Academy of Medical Science & Peking Union Medical College Hospital, Beijing, China; [2]Internal Medicine, Chinese Academy of Medical Science & Peking Union Medical College Hospital, Beijing, China.*

**Background:** Hyperuricemia is known as an independent risk factor of hypertension with renin activation. Adenosine and A1 adenosine receptor (A1AR) play an important role in tubuloglomerular feedback (TGF) and regulation of renin secretion. We aimed to investigate the possible role of A1AR in the hypertension induced by hyperuricemia.

**Methods:** Hyperuricemia in wild type (WT) and A1AR$^{-/-}$ mice was induced by gavage of 250 mg/kg/d oxonic acid. Arterial blood pressure and urine electrolyte excretion was determined.The expression of renin, α-SMA, COX2, and nNOS in the juxtaglomerular apparatus (JGA) was assessed by immunohistochemistry and confocal laser microscopy.

**Results:** Hyperuricemia was successfully induced in WT and A1AR$^{-/-}$ mice. SBP was increased in hyperuricemic WT mice (106.6 ± 7.4 vs. 114 ± 11 mmHg, $P$=0.017), accompanied by lower expression of nNOS in macula densa cells and higher expression of α-SMA in afferent arterioles. The percentage of renin-positive JGA was increased in hyperuricemic WT mice compared to control and allopurinol treated mice (31.0±6.3% vs. 16.8±6.3% and 19.1±4.7%, $P$=0.008 and 0.013, respectively). Urinary excretion of potassium and chloride was augmented in hyperuricemic mice. In contrast, A1AR$^{-/-}$ mice with similar elevation of serum uric acid did not show hypertension ($P$<0.05), thickening of afferent arteriole smooth muscle, or enhanced excretion of urine electrolytes.

**Conclusions:** A1AR may be involved in the hypertension induced by hyperuricemia. The mechanisms underlying this effect need to be studied further.

*Funding:* Government Support - Non-U.S.

### TH-PO742

**Exenatide and Human Glucagon-Like Peptide-1 (7-36 Amide) Are Diuretic, Natriuretic, and Positive Inotropes**
Melanie K. Shadoan,[1] Donald L. Anderson,[1] Mary J. Numerick,[2] Neetu Rajpal,[1] Jane G. Binz,[1] Sharon Y. Weng,[1] Shane G. Roller,[1] Mandy L. Bergquist,[3] Andrew A. Young.[1] *[1]Metabolic and Cardiovascular Therapeutic Area, GlaxoSmithKline, Research Triangle Park, NC; [2]Laboratory Animal Science, GlaxoSmithKline; [3]Quantitative Sciences, GlaxoSmithKline.*

**Background:** We have developed a comprehensive *in vivo* screen in the anesthetized rat to simultaneously measure multiple renal, cardiovascular and pharmacokinetic properties of test agents.

**Methods:** Inulin (for glomerular filtration rate; GFR), para-amino hippurate (for renal plasma flow; RPF), and test article were continuously infused intravenously (jugular) via 4-way adapter at 3.5 mL/hr. Arterial blood samples and pressure (MAP and total peripheral resistance; TPR, derived from τ) were obtained via Ohmeda pressure transducer. The left ureter was cannulated for timed urine collections, and snares are placed around renal vessels for each kidney for ligation (acute nephrectomy) at desired timepoint.

**Results:** Administration of exenatide (5 nmol/hr) or human glucagon-like peptide-1 (7-36) amide (GLP-1, 50 nmol/hr) increased excretion of $Na^+$ and clearance of free water. Hemodynamic changes included reduced peripheral resistance, increased inotropy, increased

mean arterial pressure (MAP) and increased heart rate (HR). Pharmacokinetic analysis showed that exenatide concentrations in plasma substantially increased following renal ligation, affirming predominantly renal clearance of this peptide.

| | Urine Flow | $Na^+$ Excretion | Free Water Clearance | GFR | RBF | Peripheral Resistance | Inotropy | MAP | HR |
|---|---|---|---|---|---|---|---|---|---|
| Exenatide | ↑23 X | ↑70 X | ↑82 X | ↑30% | ↑3 X | ↓24% | ↑67% | ↑28% | ↑32% |
| GLP-1 | ↑24 X | ↑144 X | ↑54% | ↔ | ↔ | ↓37% | ↑48% | ↑49% | ↑37% |

**Conclusions:** This protocol allows collection of over 120 physiological cardiorenal and pharmacokinetic parameters in association with intravenous administration of test agents.

*Funding:* Pharmaceutical Company Support - GlaxoSmithKline

## TH-PO743

### Impact of Proton-Pump Inhibitors and Diuretics on the Risk of Hypomagnesemia in Patients Admitted to the Emergency Department

Spyridon Arampatzis,[1,2] Markus Mohaupt,[1] Alexander Benedikt Leichtle,[3] Andreas Pasch,[1] Aristomenis K. Exadaktylos,[2] Gregor Lindner.[2] *[1]Dept of Nephrology, Hypertension and Clinical Pharmacology, Inselspital, Univ of Bern, Bern, Switzerland; [2]Dept of Emergency Medicine, Inselspital, Univ of Bern, Bern, Switzerland; [3]Center of Laboratory Medicine, Univ of Bern, Bern.*

**Background:** The aim of this study was to evaluate the risk of hypomagnesemia under concomitant use of proton pump inhibitors (PPIs) and diuretics and explore the role of hypomagnesemia as a risk factor for adverse outcome in a cohort of emergency department (ED) patients.

**Methods:** Cross-sectional study in 4,919 patients who presented to a large tertiary care ED between 01 January 2009 and 31 December 2010 with measurements of serum magnesium on admission. Hypomagnesemia was defined as serum magnesium concentration of <0.75 mmol/L. Demographic data, electrolyte disorders, data on medication, morbidities and outcome in terms of length of hospital stay and mortality were documented.

**Results:** The mean serum magnesium concentration was 0.81 mmol/L (SD 0.1); 1,195 patients (24%) showed hypomagnesemia on admission. Magnesium levels tended to be lower in patients under diuretic and PPI medication and were significantly lower in subjects taking both drugs ($p<0.001$). Use of loop diuretics ($p=0.002$) and thiazide diuretics ($p=0.02$) was a predictor for hypomagnesemia. In multivariable regression analyses, PPIs (OR 1.7, $p<0.0001$), diuretics (OR 1.3, $p<0.011$) and the presence of diabetes mellitus (OR 2.6, $p<0.0001$) were independently associated with hypomagnesemia. While mortality was not increased in patients with hypomagnesemia alone ($p=0.83$), patients with concomitant hypokalemia had a significantly higher mortality rate ($p=0.03$).

**Conclusions:** Hypomagnesemia is common in patients presenting to the ED and is associated with use of PPIs, diuretics, and the presence of diabetes. Hypomagnesemia with concomitant hypokalemia was an independent predictor of in-hospital mortality.

## TH-PO744

### Mutations in *PCBD1* Are Associated with Hypomagnesemia, Renal Magnesium Wasting and MODY

Jeroen H.F. De Baaij, Silvia Ferrè, Joost Hoenderop, René J. Bindels. *Dept of Physiology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands.*

**Background:** Mutations in *PCBD1* are causative for transient neonatal hyperphenylalaninemia and primapterinuria (HPABH4D). Until now HPABH4D has been regarded as a transient and benign neonatal syndrome without complications in adulthood.

**Methods:** We assessed 3 patients with PCBD1 for blood magnesium levels and diabetes. Expression levels and localization of PCBD1 in mouse kidney and pancreas was evaluated. Moreover, the ability of PCBD1 wild type and PCBD1 mutants to co-activate the FXYD2 promoter was investigated by luciferase-reporter assays. Additionally, we examined the subcellular localization of PCBD1 wild type and mutants in HEK293 cells.

**Results:** In our study, two adult patients with homozygous mutations in the *PCBD1* gene were diagnosed with hypomagnesemia and renal $Mg^{2+}$ loss. One patient also developed diabetes with characteristics of maturity onset diabetes of the young (MODY). Our results suggest that these clinical findings are related to the function of PCBD1 as dimerization cofactor for the transcription factor HNF1B. Mutations in the *HNF1B* gene have previously been shown to cause renal malformations, hypomagnesemia and MODY. Gene expression analysis in the kidney showed that Pcbd1 is co-expressed with Hnf1b in the distal convoluted tubule (DCT) where *Pcbd1* transcript levels are upregulated by a low $Mg^{2+}$-containing diet. Overexpression in a human kidney cell line demonstrated that wild-type PCBD1 binds HNF1B to co-stimulate the *FXYD2*-promoter, whose activity is instrumental in $Mg^{2+}$reabsorption in DCT. Five out of seven *PCBD1* mutations previously reported in HPABH4D patients caused proteolytic instability leading to a reduced *FXYD2*-promoter activity. Furthermore, HNF1B mutations may disturb PCBD1 localization in the nucleus, since PCBD1 showed an increased cytosolic localization when co-expressed with HNF1B mutants.

**Conclusions:** Overall, our findings establish PCBD1 as an important co-activator of the HNF1B-mediated transcription necessary for fine-tuning of *FXYD2* transcription in DCT. Thus, patients with HPABH4D should be monitored for previously unrecognised late complications, such as hypomagnesemia and MODY diabetes.

*Funding:* Government Support - Non-U.S.

## TH-PO745

### Characterization of Osteoclast-Specific NCX1 Knock-Out Mice

Daniel G. Fuster,[1] Olivier Bonny.[2] *[1]Div of Nephrology, Hypertension and Clinical Pharmacology, Univ of Bern, Bern, Switzerland; [2]Institute of Pharmacology and Toxicology, Univ of Lausanne, Lausanne, Switzerland.*

**Background:** Bone is dissolved by a polarized cell, the osteoclast. Previous studies indicated that sodium/calcium exchanger (NCX) inhibitors decrease bone resorption in a dose dependent manner in vitro. In addition, siRNA-mediated knock-down of NCX1 significantly suppressed osteoclastic bone resorption in vitro, indicating a critical role of NCX1 in osteoclast-mediated bone resorption. To test the role of NCX1 in osteoclasts in vivo, we generated mice with osteoclast-specific deletion of NCX1.

**Methods:** Mice with a floxed exon 11 of NCX1 were crossed with mice expressing Cre-recombinase under the influence of the cathepsin K promoter to generate osteoclast-specific NCX1 knock-out mice (herein named NCX1 $^{\Delta OC/\Delta OC}$ mice).

**Results:** Osteoclasts differentiated from NCX1 $^{\Delta OC/\Delta OC}$ mice displayed a 80 % reduction of NCX1 mRNA and protein compared to wild-type mice. NCX2 was not expressed in osteoclasts. NCX3 was expressed a low levels in osteoclasts but was not upregulated in NCX1 $^{\Delta OC/\Delta OC}$ osteoclasts. NCX1 expression was unaltered in extraosseus tissues in NCX1 $^{\Delta OC/\Delta OC}$ mice. Structural bone parameters, analyzed by high-resolution microcomputed tomography (μCT) were not different in 12 week old male and female wild-type and NCX1 $^{\Delta OC/\Delta OC}$ mice. Similarly, no differences were observed when we assessed osteoclast differentiation or bone resorption in vitro of cells isolated from wild-type and NCX1 $^{\Delta OC/\Delta OC}$ mice, respectively. Finally, to stimulate osteoclast-mediated bone resorption, we performed surgical ovarectomy in 12 week old female mice. Ovarectomy-induced bone loss, however, was identical in wild-type and NCX1 $^{\Delta OC/\Delta OC}$ mice at 3,6, 9 and 12 weeks after the operation.

**Conclusions:** Thus, our data indicate that genetically induced deficiency of NCX1 in osteoclast-precursors and mature osteoclasts does not affect osteoclast differentiation and bone resorption in vitro. Furthermore, osteoclast-specific deletion of NCX1 seems not seem to affect bone volume in 12 week old mice or ovarectomy-induced bone loss in female mice until 12 weeks after the operation.

## TH-PO746

### Angiotensin II and PDGF Decrease the Phosphorus-Induced Calcification in Vascular Smooth Muscle Cells *In Vitro*

Carmen Herencia,[1] Maria Encarnacion Rodriguez ortiz,[1] Juan R. Muñoz-Castañeda,[1] Julio Manuel Martínez Moreno,[1] Rocio Canalejo,[2] Addy Rosa Montes de Oca Gonzalez,[1] Carmen Marin,[1] Antonio Canalejo,[2] Mariano Rodriguez,[3] Yolanda Almaden Peña.[1] *[1]IMIBIC, Spain; [2]Univ of Huelva, Spain; [3]Reina Sofia Univ Hospital.*

**Background:** High Phosphorus(HP) plays a key role in the pathogenesis of vascular calcification(VC), which occurs through a Wnt/β-catenin(BCTN) induced osteoblastic differentiation of vascular smooth muscle cells(VSMCs). The involvement of AngiotensinII(AII) and platelet derived growth factor(PDGF) in the development of VC is controversial.We evaluated the effect of AII and PDGF on VC in human VSMCs in vitro.

**Methods:** VSMCs were cultured with HP alone or supplemented with $10^{-5}$M AII or 20ng/ml PDGF for 9 days. Calcium deposition (spectrofotometry),osteogenic genes expression(RT-PCR) and BCTN nuclear translocation(confocal microscopy) were measured.

**Results:** High P induced calcification was associated with BCTN nuclear translocation. An increase in mRNA levels of BMP2,Runx2,Osterix and ALP was produced. AII and PDGF supplementation reduced calcification levels,osteoblastic genes expression and BCTN translocation. *p<0.05 vs control.#p<0.05 vs HP.

| | Calcif (μgrCa /mgrProt) | Nuclearβ catenin (D coef. Mander vs Control) | BMP2 (U.Ratio vs Control) | Runx2(U.Ratio vs Control) | OTX (U.Ratio vs Control) | ALP(U.Ratio vs Control) |
|---|---|---|---|---|---|---|
| **Control** | 0.55±0.05 | 1.0±0.03 | 1.0±0.08 | 1.0±0.06 | 1.0±0.04 | 1.0±0.08 |
| **HP** | 3.20±0.38* | 3.50±0.32* | 2.14±0.07* | 1.35±0.05* | 1.74±0.17* | 1.38±0.07* |
| **HP+AII** | 1.86±0.38# | 2.22±0.49# | 1.40±0.07# | 0.90±0.10# | 0.93±0.10# | 0.62±0.16# |
| **HP+PDGF** | 1.08±0.21# | 2.31±0.20# | 1.17±0.11# | 0.33±0.05# | 0.29±0.06# | 0.26±0.06# |

**Conclusions:** AII and PDGF decrease the Phosphorus-induced calcification in human VSMCs in vitro. This appears to be mediated by a reduction in osteogenic genes expression and an inhibition in Wnt/BCTN pathway.

## TH-PO747

### Calcium Salts Affect Acid-Base Balance by Stimulating Differently Type A and B Intercalated Cells in Mouse Kidney Collecting Ducts

Yukiko Yasuoka,[1] Yuichi Sato,[2] Hiroshi Nonoguchi,[3] Katsumasa Kawahara.[1] *[1]Physiol., Kitasato U. Sch. of Med, Sagamihara, Japan; [2]Mol. Diag., Kitasato U. Grad. Sch. of Med. Sci., Sagamihara, Japan; [3]Internal Med., Kitasato U. Medical Center, Kitamoto, Japan.*

**Background:** Calcium salts (calcium carbonate (CaC) and calcium phosphate (CaP)) are widely used for patients with chronic kidney disease (CKD) and osteoporosis. These salts have the potential to affect acid-base balance via the Ca-sensing receptor (CaSR) expressed in type B intercalated cell (IC-B) of the collecting ducts (Yasuoka Y, ASN meeting, 2011).

**Methods:** C57Bl/6J mice (10 weeks, male) fed with different Ca supplements and acid-base conditions (+2.5% $NH_4Cl$ diet and +0.28 M $NaHCO_3$ drinking) for 6-7 days. By using a quantitative in situ hybridization technique, we evaluated the levels of expression

of anion exchanger type 1 (AE1), Pendrin (an apical $HCO_3^-/Cl^-$ exchanger), and CaSR along the collecting duct. Blood and urine samples were also analyzed.

**Results:** Plasma Ca concentration and urinary Ca excretion increased slightly, but not significantly in mice with 2.5% CaC (n=7) and 2.5% CaP diets (n=8). In contrast, these measurements significantly increased from 7.7 to 9.9 (mg/dl) and from 0.12 to 3.21 (mg/d) in mice with 2% $CaCl_2$ diet (n=12). In addition, urine pH significantly decreased from 6.73 to 5.78 ($CaCl_2$), increased from 6.58 to 7.72 (CaC), and was unchanged (CaP). In IC-A, as expected, the level of the AE1 mRNA expression (an index of acid excretion) increased, decreased, and remained unchanged, respectively, in mice with $CaCl_2$, CaC, and CaP supplements. On the other hand, in IC-B, the levels of the pendrin (an index of alkali excretion) and CaSR mRNAs expression decreased due to acidosis caused by the $CaCl_2$ containing diet, whereas they increased in mice showing normal plasma pH consuming CaC and CaP diets.

**Conclusions:** These data strongly suggest that CaC may work as an alkali as well as a Ca supplement, and may increase urinary alkali excretion by stimulation of pendrin mediated by basolateral CaSR in IC-B. Therefore CaC may be the best Ca supplement for patients with CKD and osteoporosis with regards to Ca metabolism and acid-base balance.

## TH-PO748

**β1-Adrenergic Receptor Signaling Activates the Epithelial Calcium Channel, TRPV5, via the Protein Kinase A Pathway** Kukiat Tudpor, Eline A.E. Van der Hagen, Sjoerd Verkaart, René J. Bindels, Joost Hoenderop. *Physiology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands.*

**Background:** Epinephrine (Epi) and norepinephrine (NE) are present in the pro-urine. β-adrenergic receptor (β-AR) blockers administered to counteract sympathetic overstimulation in patients with congestive heart failure (CHF). Even though Epi and NE are secreted in the pro-urine no effects of these hormones through signaling via β-ARs on renal active $Ca^{2+}$ transport have been reported. Active $Ca^{2+}$ reabsorption in the late distal convoluted and connecting tubules (DCT2/CNT) is initiated by $Ca^{2+}$ influx through the transient receptor potential vanilloid type 5 (TRPV5) $Ca^{2+}$ channel.

**Methods:** Expression levels and localization of β-adrenergic receptors was investigated in mouse kidney by immunohistochemistry and DCT2/CNT were isolated using the COPAS technology. TRPV5 was expressed in HEK293 cells and the intracellular $Ca^{2+}$ concentration was monitored by spectrofluorometry using Fura-2AM. Cell surface expression of TRPV5 was addressed using biotinylation assays.

**Results:** Although it was reported that β-ARs are present in the DCT2/CNT region, their role in active $Ca^{2+}$ reabsorption remains elusive. Here we revealed that β1-AR, but not β2-AR is colocalized with TRPV5 in DCT2/CNT. Treatment of TRPV5-expressing mouse DCT2/CNT primary cell cultures with the β1-AR agonist dobutamine showed enhanced apical-to-basolateral transepithelial $Ca^{2+}$ transport. In human embryonic kidney (HEK293) cells, dobutamine was shown to stimulate cAMP production, signifying functional β1-AR-expression. Fura-2 experiments demonstrated increased activity of TRPV5 in response to dobutamine, which could be prevented by the PKA inhibitor H89. Moreover, non-phosphorylable T709A-TRPV5 and phosphorylation-mimicking T709D-TRPV5 mutants were unresponsive to dobutamine. Surface biotinylation showed that dobutamine did not affect plasma membrane abundance of TRPV5.

**Conclusions:** In conclusion, activation of β1-AR stimulates active $Ca^{2+}$ reabsorption in DCT2/CNT by increased TRPV5 activity via PKA phosphorylation of residue T709 possibly. These data may suggest a calciotropic role in addition to the inotropic property of the β1-AR.

*Funding:* Government Support - Non-U.S.

## TH-PO749

**The Pathophysiology of Aortic Calcification in an In Vitro Model: The Role of Mineral Imbalance** Navid Shobeiri,[1] Julie Cruanes,[1] Rachel M. Holden,[2] Michael A. Adams.[1] *[1]Dept of Biomedical and Molecular Sciences, Queen's Univ, Canada; [2]Medicine, Queen's Univ, Canada.*

**Background:** Vascular calcification (VC) is accelerated in patients with chronic kidney disease (CKD), resulting in increased risk of cardiovascular disease and mortality. Mineral imbalance in CKD contributes to this process, and elevated phosphate is a major risk factor.

**Methods:** An in vitro model of VC was developed to determine the impact of bone minerals (phosphate, magnesium, calcium). Aortas were incubated in pro-calcification DMEM media with or without elevated magnesium. Calcification was assessed by tissue calcium (Ca) levels and visualized with von Kossa staining.

**Results:** The degree of calcification was dependent on time-course and phosphate concentration. Aortic rings calcified in media phosphate concentrations above 3.0mM (>25fold increase in Ca), however elevated calcium media did not cause calcification. Aortas in 3.8mM phosphate media demonstrated accumulation of calcium at day 2 (78.2±17.2nmol/mg), but an accelerated accumulation by day 4 (528.9±62.4nmol/mg). This calcification was associated with the tunica media (histology). Increasing the media magnesium levels (0.8mM to 2.5mM) blunted tissue calcification significantly (□93±4%, p<0.05). Delaying 2.5mM magnesium treatment continued to be effective if the increase occurred prior to day 4 of a 6 day incubation period (addition of magnesium at day 2 □66±14% and day 3 □35±49%tissue Ca). Two day pretreatment with 2.5mM magnesium also prevented calcification. That is, incubation of these vessels for only 2 days in high magnesium prevented *de novo* calcification when transferred to pro-calcification media without high magnesium (+4 days) (10.5±1.3nmol/mg vs 175.5±28.9nmol/mg tissue Ca, p<0.5). Consequently, tissue magnesium remained elevated at day 6 (12.6±1.2nmol/mg vs 2.4±.8nmol/mg, p<0.05).

**Conclusions:** Elevated phosphate appears to be a key signal in the development of pathological calcification. This process is blocked by magnesium, but only if calcification has not already begun.

## TH-PO750

**Estrogen Directly Downregulates NaPi-IIa through the Activation of Both Estrogen Receptor Isoforms (ERα and ERβ) in Rat Kidney Proximal Tubule** Hassane Amlal,[1] Rose P. Webster,[1] Rashma Faroqui,[1] Moshe Levi,[2] Sulaiman Sheriff.[3] *[1]Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Internal Medicine, Univ of Colorado, Denver, CO; [3]Dept of Surgery, Univ of Cincinnati, Cincinnati, OH.*

**Background:** Estrogen depletion in postmenopausal women is associated with hyperphosphatemia. We have demonstrated that 17β-estradiol (EST) downregulates NaPi-IIa and causes phosphaturia and hypophosphatemia in ovariectomized (OVX) rats. However, the signaling mechanism(s) mediating this effect remain unknown.

**Methods:** The expression of ERα and ERβ in the proximal tubule (PT) was examined using RT-PCR. The respective roles of these receptors in EST-induced downregulation of NaPi-IIa were studied using OVX rats placed in metabolic cages and treated with specific agonists of either ERα (PPT) or ERβ (DPN). A cell line (U20S) stably expressing ERα or ERβ or both under doxycycline control and transiently transfected with rat NaPi-IIa was also used. In these cells, ERα and ERβ are expressed in a functional ERα/β heterodimers.

**Results:** RT-PCR data indicate that both ERα and ERβ are expressed in the cortex and PT cells. PT suspensions harvested from OVX rats and incubated on a shaker in a cell culture incubator for 24hrs exhibited a significant downregulation of NaPi-IIa expression (RNA + protein) in the presence of EST vs. vehicle. Treatment of OVX rats with either PPT or DPN alone did not affect NaPi-IIa expression and did not cause phosphaturia. However, combined treatment with PPT+DPN mimicked the effect of estrogen and caused a sharp downregulation of NaPi-IIa along with significant phosphaturia. U20S cells expressing both ERα and ERβ showed a significant downregulation of NaPi-IIa protein in response to EST vs. vehicle only when pre-treated with doxycycline. Interestingly, NaPi-IIa protein abundance was not altered by EST in U20S cells bearing either ERαor ERβ alone.

**Conclusions:** These studies demonstrate that rat PT cells express both ERα and ERβ and that EST downregulates NaPi-IIa by directly acting on PT cells. This effect is mediated via a complex mechanism involving the activation, and likely heterodimerization of ERα and ERβ.

*Funding:* NIDDK Support

## TH-PO751

**Estrogen Downregulates NaPi-IIa in Mice Kidney through a Mechanism Involving the Activation of Estrogen Receptor Isoform α (ERα) and 3'UTR Region of NaPi-IIa mRNA Transcript** Hassane Amlal,[1] Sulaiman Sheriff,[2] Rashma Faroqui,[1] Rose P. Webster.[1] *[1]Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Internal Surgery, Univ of Cincinnati, Cincinnati, OH.*

**Background:** 17b-estradiol (EST) downregulates NaPi-IIa mRNA and protein and causes phosphaturia in rats likely by activating both EST receptor isoforms ERα and ERβ (published report and accompanying abstract). To confirm these findings, we sought to study the effect of EST on NaPi-IIa using mice with genetic deletions of ERα or ERβ.

**Methods:** Females ERα knockout (KO), ERβ KO and their wild-type (WT) mice were placed in metabolic cages with free access to food and water and treated with EST or its vehicle for 3 days. Food and water intake, urine volume and urinary Pi excretion were measured daily. A cell line (U20S) stably co-expressing both ERα and ERβ under doxycycline control and transiently transfected with different mouse NaPi-IIa constructs (mNaPi-IIa) was also used. Molecular studies examined the expression of NaPi-IIa in the kidney or U20S cells.

**Results:** Like rats, EST-treated WT mice exhibited a significant reduction in food intake along with increased renal phosphate wasting. However, unlike rats, the phosphaturic effect of EST resulted from a sharp downregulation of NaPi-IIa protein without affecting its mRNA expression levels. Interestingly, EST-induced phosphaturia and downregulation of NaPi-IIa protein were also observed in ERβ KO but not in ERα KO mice. U20S cells expressing ERα and ERβ treated with EST for 24hrs showed a significant downregulation of NaPi-IIa protein abundance when transfected with a plasmid containing ORF-3'UTR but not 5'UTR-ORF of mNaPi-IIa transcripts. This effect is observed only when cells were pre-treated with doxycycline.

**Conclusions:** EST causes phosphaturia in mice despite a reduction in food intake. This effect results from the downregulation of NaPi-IIa protein abundance with no change in its mRNA expression levels, and is mediated through the exclusive activation of ERα. A cis-acting element in the 3'UTR region of mNaPi-IIa mRNA likely plays an important role in the inhibition of its translation by EST.

*Funding:* NIDDK Support

## TH-PO752

**Conditionally Immortalized Human Proximal-Tubular Epithelial Cells Isolated from the Urine of a Healthy Subject Express Functional Calcium-Sensing Receptor (CaSR)** Annarita Di Mise,[1] Grazia Tamma,[1] Marianna Ranieri,[1] Maria Svelto,[1] Elena N. Levtchenko,[2] Giovanna Valenti.[1] *[1]Dept Biosciences, Biotechnologies and Biopharmaceutics, Univ of Bari, Bari, Italy; [2]Dept of Pediatric Nephrology, Univ Hospital Gasthuisberg, Leuven, Belgium.*

**Background:** The calcium-sensing receptor (CaSR) is a G protein coupled receptor, which plays an essential role in regulating $Ca^{2+}$ homeostasis.

Here we show that conditionally immortalized proximal tubular epithelial cell line (ciPTEC) obtained by immortalizing and subcloning cells exfoliated in the urine of a healthy subject expresses functional endogenous CaSR.

**Methods:** Primary cells isolated from human urine sediment were infected with SV40T and hTERT vectors. Subconfluent cell layers were transferred to 33°C and selected by antibiotics for 15 days. Cells were subcloned and expanded to 70% confluence at 33°C. After maturation at 37°C for 10 days, the cloned cells were used.

**Results:** The obtained ciPTEC cells expressed ZO-1 protein and aquaporin 1 thus confirming their epithelial and PT origin respectively. The expression of the endogenous CaSR in ciPTEC was confirmed by Western blotting revealing the immunodetection of both forms at 130 and ~200 kDa, corresponding to the monomeric and mature receptor. Of note, functional studies with Fura2-AM indicated that the physiological agonist, calcium ($Ca^{2+}$), and the calcimimetic NPS-R568, induced a significant increase in cytosolic calcium, proving a high sensitivity of the endogenous receptor to low concentrations of its agonists. Cytosolic calcium levels were 46.2±2.22% (vs ATP 100%) after stimulation with 2.5µM $Ca^{2+}$ and to the 37±1.76% (vs ATP 100%) after stimulation with 2.5µM NPS-R568. Calcium depletion from the ER using CPA (cyclopiazonic acid) abolished the increase in cytosolic calcium elicited by NPS-R568 confirming the origin of calcium exit from intracellular stores.

**Conclusions:** We conclude that human proximal tubular ciPTEC cells express functional CaSR and respond to its activation with a release of calcium from the ER. These cell lines represent a valuable tool for research into the disorder associated with gain or loss of function of the CaSR by producing cell lines from patients.

*Funding:* Government Support - Non-U.S.

### TH-PO753

**Deranged Phosphate Metabolism, Intestinal $Na^+$ Loss, and Volume Depletion in Janus Kinase 3-Deficient Mice** Michael Föller, Anja Umbach, Dong Luo, Bingbing Zhang, Hajar Fahkri, Zohreh Hosseinzadeh, Shefalee Bhavsar, Bernd Pichler, Florian C. Lang. *Univ of Tübingen.*

**Background:** Janus kinase JAK3 participates in the triggering of dendritic cell apoptosis. Lack of JAK3 increases the abundance of dendritic cells and triggers inflammatory bowel disease. Dendritic cells and macrophages express 1 hydroxylase and may thus generate $1,25(OH)_2D_3$, a major regulator of phosphate metabolism. Inflammatory bowel disease is in addition known to affect colonic ENaC activity leading to salt loss. The present study thus analyzed the impact of JAK3 deficiency on $1,25(OH)_2D_3$ formation, phosphate metabolism, extracellular volume and blood pressure.

**Methods:** Gene-targeted mice lacking functional JAK3 ($jak3^{-/-}$) were compared to wild type mice ($jak3^{+/+}$). Serum $1,25(OH)_2D_3$, PTH, FGF23 and aldosterone concentrations were determined by immunoassays, serum, fecal and urinary phosphate and $Na^+$ utilizing photometry, colonic ENaC activity by Ussing chamber experiments and blood pressure by the tail cuff method.

**Results:** $1,25(OH)_2 D_3$ and FGF23 plasma levels were significantly higher in $jak3^{-/-}$ mice than in $jak3^{+/+}$ mice. Intestinal phosphate absorption as well as absolute and fractional renal phosphate excretion were higher in $jak3^{-/-}$ mice than in $jak3^{+/+}$ mice. Bone density was not different between the genotypes. Colonic ENaC activity was reduced in $jak3^{-/-}$ mice resulting in fecal $Na^+$ loss and reduced blood pressure despite compensatory aldosterone release and renal $Na^+$ retention.

**Conclusions:** JAK3 deficiency causes profound derangement of phosphate metabolism and decreased colonic ENaC activity which results in compromised extracellular volume maintenance and in arterial hypotension.

### TH-PO754

**VS-501, a Novel Phosphate Binder, Is Highly Efficacious in 5/6 Nephrectomized Hyperphosphatemic Rats** J. Ruth Wu-Wong, Yung-Wu Chen, Jerry Wessale. *Vidasym.*

**Background:** Inadequate control of serum phosphate in chronic kidney disease (CKD) can lead to pathologies of clinical importance including cardiovascular complications, renal osteodystrophy, and increased mortality. On-market phosphate binders are mainly used in dialysis patients, but their effectiveness is limited by safety concerns and low compliance due to high pill burden and gastrointestinal (GI) discomfort. VS-501 is a proprietary, non-absorbed, calcium- and aluminum-free, chemically-modified, plant-derived compound that effectively adsorbs phosphate in the GI tract.

**Methods:** The 5/6 nephrectomized (NX) male Sprague Dawley rats with established uremia (as indicated by elevated serum creatinine and BUN) were fed a high-phosphate diet (normal diet plus $KH_2PO_4$ at 0.67% and $K_2HPO_4$ at 0.33% by dry weight) with or without VS-501 or sevelamer carbonate added to food for 4 weeks.

**Results:** Increasing dietary phosphate led to an increase in serum phosphate (3.26 ± 0.10 to 3.63 ± 0.13 mmol/L, $p<0.05$) in the group treated with vehicle (unmodified polymer), but no significant changes in serum phosphate were observed in rats treated with VS-501 or sevelamer carbonate at 0.2 – 2%. Hypophosphatemia was observed in rats treated with 5% VS-501 or sevelamer. Urinary phosphate increased from 73 ± 20 to 687 ± 45 µmol/24 hr ($p<0.001$) in the vehicle-treated group; VS-501 or sevelamer (0.02 - 5% in food) reduced urinary phosphate in a dose-dependent manner. VS-501's density is about two-fold higher than that of sevelamer, suggesting a significantly reduced pill size. VS-501 exhibits a low swelling volume when exposed to simulated gastric fluid, a feature likely to mitigate GI discomfort. For example, on adding 5 ml of simulated gastric fluid to 0.1 g (dry powder) of VS-501 or sevelamer, the volume of sevelamer increased to ~4 $cm^3$ (~21-fold) within 20 min at 37°C, but the volume of VS-501 increased to only ~0.2 $cm^3$ (~2-fold).

**Conclusions:** These results strongly support the conclusion that VS-501 effectively controls serum phosphate levels by adsorbing phosphate in the GI tract with minimal swelling volume. VS-501 exhibits a compelling product profile suitable for clinical development as a medical food or prescription drug.

*Funding:* NIDDK Support

### TH-PO755

**Lanthanum Carbonate: Safety Data after 9 Years** Marc E. De Broe,[1] Rosamund J. Wilson,[2] J. Brian Copley.[3] *[1]Dept of Nephrology, Antwerp Univ, Antwerp, Belgium; [2]Spica Consultants, Marlborough, United Kingdom; [3]Shire Pharmaceuticals, Wayne, PA.*

**Background:** Concerns over the safety of lanthanum carbonate (LaC) are based on fears that long-term La intake may result in accumulation and toxicity in the GI tract, bone, liver, and CNS. The aim of this abstract is to summarize key data from the ongoing clinical development program pertaining to the long-term safety profile of LaC.

**Methods:** Shire PV and Risk Management performs continuous signal detection and monitoring of safety data from worldwide sources for all Shire products, including LaC. SPD405-402 (NCT00557323) is a 5-year observational study with the purpose of collecting safety data on subjects with up to 6 years of treatment with LaC.

**Results:** As of January 2013, estimated worldwide patient exposure to LaC amounts to 497,762 person-years and over 6000 patients in phase 2–4 clinical studies. Pre-clinical and clinical studies of LaC have demonstrated strong evidence of efficacy and safety, confirmed by 9 years of post-marketing monitoring. **GI tract.** The majority of LaC ADRs are related to the GI tract, but are primarily mild/moderate in severity. SPD405-402 does not suggest any change in the GI safety profile of LaC. **Bone.** Bone biopsy studies out to 5 years showed no evidence of aluminium-like accumulation or toxicity, and improved bone formation rates and histology. SPD405-402 showed that long-term LaC use is not associated with bone fracture. **Liver.** Patients treated with LaC for up to 6 years showed no clinically relevant changes in liver enzymes or bilirubin levels; and no increase in the incidence of liver-associated AEs. SPD405-402 does not suggest a change in the hepatic safety profile of LaC. **CNS.** Animal toxicology studies demonstrated that La does not cross the intact blood-brain barrier. Clinical data show no evidence of an effect of LaC on cognitive function.

**Conclusions:** After ~500,000 person-years of exposure, there is no evidence at this time that LaC is associated with adverse safety concerns in the GI tract, bone, or liver; or that La crosses the intact blood-brain barrier. GI tract ADRs seen with LaC are mild/moderate and consistent with those observed with other phosphate binders.

*Funding:* Pharmaceutical Company Support - Shire Pharmaceuticals

### TH-PO756

**Double-Blind, Dose-Ranging, Study of Lanthanum Dioxycarbonate (SPI-014, RenaZorb) in Healthy Volunteers Shows High Phosphorus Binding Capacity** William F. Finn,[1] Cynthia J. Denu-Ciocca,[2] Melanie S. Joy,[3] Guru Reddy,[4] Shanta Chawla,[4] Armando Garsd,[4] Ronald Goldwater.[5] *[1]Consultant, Chapel Hill, NC; [2]Univ of North Carolina, Chapel Hill, NC; [3]Univ of Colorado, Denver, CO; [4]Spectrum Pharmaceuticals, Irvine, CA; [5]Parexel International, Baltimore, MD.*

**Background:** Hyperphosphatemia continues to be a significant issue among dialysis patients - partly related to the limited phosphorus binding capacity of available agents. Lanthanum dioxycarbonate (SPI-014, RenaZorb), a second generation lanthanum-based phosphate binder, is in development for the treatment of hyperphosphatemia in these patients. Preliminary studies have shown an excellent phosphorus binding profile.

**Methods:** To determine the pharmacodynamic (PD) and pharmacokinetic (PK) parameters of SPI-014, healthy subjects were placed on a phosphorus controlled diet and randomized 3:1 to receive SPI-014 or placebo in 4 sequential cohorts of 8 subjects each. SPI-014 doses were: 1500, 3000, 4500, and 6000 mg/day (p.o. in 3 divided doses within 15 minutes pc). 24-hour urine and feces samples were collected at baseline and on-treatment to determine phosphorus content. PD of SPI-014 was evaluated by comparing changes from baseline in urine and fecal phosphorous excretion. PK samples were collected at pre-specified time points.

**Results:** Enrolled subjects (N=32) were healthy, predominately African American (n=21) and male (n=26) with median age of 40 years (range 22-61). Compared to placebo, all 4 doses of SPI-014 significantly reduced daily urinary phosphorous excretion. The reductions in urine phosphorus (mean; 95% CI) were 202 (24, 380), 350 (172, 528), 420 (242, 598), 441 (263, 619) mg/24 hours for SPI-014 doses of 1500, 3000, 4500 and 6000 mg/day, respectively. Increased fecal phosphorous excretion compared to baseline was observed at each dose. Systemic absorption of lanthanum was negligible.

**Conclusions:** SPI-014 resulted in a statistically significant reduction in daily urinary phosphorous in a dose-related fashion concomitant with increased fecal phosphorus excretion. The pharmacodynamics profile suggests that the binding capacity of SPI-014 exceeds that of currently available agents.

*Funding:* Pharmaceutical Company Support - Spectrum Pharmaceuticals

### TH-PO757

**Barriers and Facilitators to Dialysis Patient Self-Management of Phosphate Binders** Teri Browne,[1] Joseph R. Merighi,[2] Tamara Savage,[1] Diana Clynes,[3] Kerri L. Cavanaugh.[4] *[1]College of Social Work, Univ of South Carolina, Columbia, SC; [2]School of Social Work, Univ of Minnesota, Minneapolis, MN; [3]American Association of Kidney Patients, Tampa, FL; [4]Div of Nephrology, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** Dialysis patients encounter difficulties self-managing their medication regimes, in particular phosphate binders that make up the majority of patient pill burden. It is critically important to identify ways that dialysis professionals can help patients self-

manage their phosphate binder medications to improve patient outcomes. To achieve this goal, a qualitative study was conducted to pinpoint dialysis patient-centered facilitators and barriers to phosphate binder self-management.

**Methods:** Primary data were gathered from five, 90-minute, small-group interviews with hemodialysis patients (N = 17) in Atlanta, GA. The majority of patients were female (70%), African American (76%), had a dialysis vintage = 87 months (SD=59), and a daily pill burden of 16 pills (SD=13). A constant comparative method was used to identify themes that emerged from a line-by-line review of the interview transcripts.

**Results:** Participants identified three facilitators to self-managing their phosphate binders: social support; placing binders in multiple locations; and seeing images that depict health consequences associated with high serum phosphorus. Patients also identified three main barriers to self-management of phosphate binders: financial burden of medications, privacy concerns about taking medications in a public setting, and medication regimen complexity.

**Conclusions:** The study findings provide the basis for the development of future research and targeted interventions that can improve patient self-management of phosphate binders and improve dialysis patient health outcomes.

## TH-PO758

### At What Stage of CKD Phosphate Binder Treatment Should Be Started?

Juergen Bommer,[1] Martina Fliser,[2] Heinz Juergen Roth,[2] Daniel Saure.[3] *[1]Medical Univ Hospital Heidelberg, Dialysis Center Heidelberg, Heidelberg, Germany; [2]Limbach Laboratory, Heidelberg, Germany; [3]Institute of Medical Biometry and Informatics Univ Heidelberg, Heidelberg, Germany.*

**Background:** Disturbed mineral metabolism plays a major role in vascular calcification followed by an increased mortality. The constant serum P concentration until eGFR decreases to about 30 ml/dl was explained by increasing phosphaturic effect of increasing PTH and FGF 23 in patients with progressing renal failure. Recently an increased vascular calcification {CAC} score was reported in early CKD (eGFR 90-60ml/min) compared with the group with normal renal function This raisesthe issue at which point in the evolution of CKD interventions in mineral metabolism should be started.

To adress this issue we studied in a large cohort of unselected subjects of the outpatient units the association between eGFR/creatinine clearance and serum concentration as well as 24 hr urinary excretion of Ca and P.

**Methods:** Serum concentrations of Ca, P and Crea were measured photometricaly in 26 097 unselected individuals, 60.4% males, a mean age of 68.8± 13.7 years (range 19-99 years). In addition 24 hr urine of 2758 subjects, 59.3% males, mean age of 56.1±14.2 years (range 15-96 years) was collected to quantify excretion of Ca, P and creatinine photometricaly. eGFR was estimated by the MDRD formula.

**Results:** Mean and median of serum Ca and P concentration remained constant at eGFR > 30 ml/min. When eGFR decreased to <30 ml/min serum phosphorous increased ($p < 0.0001$) and serum Ca decreased ($p<0.0001$). In parallel with the decrease of eGFR from 120 down to <20 ml/min a continuous decrease was found for Ca excretion {from 5.3±3.03 to 1.16±0.98 mmol /24 hr ($p<0.0001$)} as well as P excretion {from 27.2±9.5 to 15.4±6.8 mmol/day ($p<0.0001$)}.

**Conclusions:** Serum Ca and P remain constant until eGFR is decreased to levels below 30 ml /min. However, 24 hr. urinary excretion of Ca and P start to decrease at much earlier stages of CKD . These findings may be related to the early onset of soft tissue calcification in CKD patients. In view of these findings the optimal start of P-binder treatment requires further investigation.

## TH-PO759

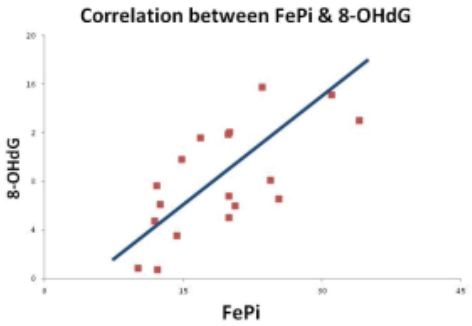
### Effect of High and Low Phosphate Intake on Oxidative Stress Levels

Essam F. Elsayed, Maram Museitif, Makoto Kuroo. *UT Southwestern.*

**Background:** High phosphate (Pi) intake has been shown to be associated with premature aging and increase in oxidative stress in animals and many of these abnormalities are rescued by a low phosphate diet. In humans, serum phosphorus levels positively correlate with mortality but the mechanism by which Pi can be toxic is not clear.

**Methods:** The participants were randomly assigned to a low Pi or a high Pi phase then after a washout period; they were crossed over to the other arm. We used a Pi supplement during the high Pi phase and a Pi binder during the low Pi phase. Pi intake, as measured by fraction excretion of Pi (FePi), was used as the independent predictor and the outcome was the level of 8-hydroxydeoxyguanosine (8-OHdG) as a marker of DNA oxidative stress.

**Results:** The mean age of the 19 participants was 53 years, 100% were men and 60% were African American. The participants had a mean serum phosphorus of 3.7mg/dL, mean FePi of 19.1% and mean eGFR of 73 mL/min.1.73m$^2$. Almost half (47%) of the participant (n=9) were stage 3 CKD with mean e-GFR of 49 mL/min.1.73m$^2$ At baseline, urinary Pi was significantly correlated with 8-OHdG levels (Pearson correlation coefficient of 0.59, P= 0.02).




]In the proportional hazard model, a 1% increase in FePi was associated with a 0.7 ng/mL increase in 8-OHdG (P= 0.008). Repeated observations model, models using 24 hours urinary Pi and models adjusting 8-OHdG to creatinine excretion had similar results. There was no significant effect for randomization group and there was a trend for the interaction between Pi intake and CKD (P-value = 0.07).

**Conclusions:** The novel concept of "phosphotoxicity" is supported by studies in animal models. Induction of high levels of oxidative stress and oxidative DNA damage are potential mechanisms by which Pi might affect cellular aging. This pilot study brings similar finding to the human level as it shows significant association between Pi intake and oxidative stress levels.

*Funding:* Veterans Affairs Support

## TH-PO760

### Effects of Dietary Phosphorus Load on the Postprandial Blood Glucose and Glucose-Regulating Hormone Levels

Michiyo Yamasaki, Misaki Katsumoto, Yutaka Taketani, Hisami Okumura, Eiji Takeda. *Dept of Clinical Nutrition, Univ of Tokushima, Japan.*

**Background:** Dietary phosphorus (P) loading implicates bone and mineral disturbance in CKD patients. However, disturbance of P metabolism also affects other nutrient metabolism including glucose and lipid metabolisms. In this study, we examined the effects of dietary P loading on the postprandial blood glucose and glucose-regulating hormone levels.

**Methods:** 24 healthy young men (21 to 27 years old) were alternately served with high glycemic index (GI) high P meal (HGHP; white rice, P 1200mg), high GI low P meal (HGLP; white rice, P400mg), low GI high P meal (LGHP; barley, P 1200mg) or low GI low P meal (LGLP; barley, P 400mg). Blood and urine samples were collected before and at 0, 15, 30, 60 and 120min after the meal ingestion and measured serum levels of P, intact-parathyroid hormone (iPTH), glucose, insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), and fractional urinary P excretion.

**Results:** In the low GI group (LGHP and LGLP), dietary high P loading significantly increased the postprandial blood glucose levels at 15-30min compared to low P loading ($P<0.05$). Serum insulin and GLP-1 levels tended to increase after high P loading. On the other hand, there was no significant change in serum GIP levels in response to P loading. The area under the curve (AUC) at 0-120min of serum P was positively associated with the change of serum GLP-1 concentration at 0-30min in low GI group ($P<0.05$). However, these associations were not clearly observed in high GI group (HGHP vs HGLP, P=0.90).

**Conclusions:** Our study demonstrates that dietary high P loading can affect the blood glucose concentration and the glucose-regulating hormone such as GLP-1 when glucose loading is not so much.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO761

### Minute-to-Minute Regulation of Plasma Calcium and Plasma Phosphate after Experimental Induction of Hypercalcemia and Hyperphosphatemia

Ewa Lewin,[1] Eva Gravesen,[2] Jacob Hofman-Bang,[2] Klaus Olgaard.[2] *[1]Nephrological Dept B, Herlev Hospital, Univ of Copenhagen, Copenhagen, Denmark; [2]Nephrological Dept P, Rigshospitalet, Univ of Copenhagen, Copenhagen, Denmark.*

**Background:** Bone is the main storage of calcium (Ca) and phosphate (P). A 'labile' Ca storage pool, which is in equilibrium with blood Ca, exists on the bone surface, which is supposed to serve as a 'buffer', temporarily storing excess calcium, hereby keeping plasma ionized Ca ($Ca^{2+}$) very stable on a minute-to-minute basis. Whether a similar rapid regulation of plasma P exists is not known. Our purpose was to evaluate the rapid minute-to-minute regulation of plasma $Ca^{2+}$ and P after induction of acute hypercalcemia and hyperphosphatemia.

**Methods:** Acute hypercalcemia or hyperphosphatemia was induced by intravenous (i.v.) injections, and then the rapid recovery of p-$Ca^{2+}$ and P were followed for 80 min. in normal rats and acute nephrectomized (NX) rats.The control group had NaCl.

**Results:** The i.v. bolus of Ca increased p-$Ca^{2+}$ significantly from 1.29±0.03 to 1.64±0.03 mM/L ($P < 0.01$). Within 40 min p-$Ca^{2+}$ was normalized. Another group of rats had a very large Ca bolus, increasing p-$Ca^{2+}$ to 2.35±0.08 and p-$Ca^{2+}$ became normalized within 80 min. Acute hyperphosphatemia was induced by an i.v. bolus of P, which increased p-P significantly from 2.42±0.19 to 5.54±0.17 mM/L ($P < 0.01$). Within the 60 min p-P

decreased to a level of 3.66±0.10 and the remained stable at this level, significantly higher that basal P level (P<0.01). In NX rats the p-P declined to 3.68±0.18 and then increased again significantly to 4.23±0.37 mM/L ($P < 0.05$).

**Conclusions:** The results of the present investigation clearly show that plasma $Ca^{2+}$ levels on a minute-to-minute basis are very tightly regulated. Even extremely severe acute hypercalcemia recovered to normal levels within 80 minutes. Phosphate levels, however, remained significantly elevated after induction of acute hyperphosphatemia in both normal and nephrectomized rats, indicating that the possible exchangeable pool for phosphate is differently regulated from that of calcium.

## TH-PO762

**Denosumab for Hypercalcemia of Malignancy in Renally Impaired Patients** Ilya Glezerman,[1] Karl Insogna,[2] Mimi Hu,[3] Cecile N. Chougnet,[4] Rasim Gucalp,[5] Waldemar Misiorowski,[6] Bennett W. Yu,[7] Paul Zorsky,[7] Diego Tosi,[8] Alberto Bessudo,[9] Arnaud Jaccard,[10] Giuseppe Tonini,[11] Wendy Ying,[12] Ada Braun,[12] Rajul K. Jain.[12] *[1]Memorial Sloan-Kettering Cancer Ctr, Weill Cornell Medical College, New York, NY; [2]Yale School of Medicine, New Haven, CT; [3]MD Anderson Cancer Ctr, Houston, TX; [4]Institut Gustave Roussy, Villejuif, France; [5]Montefiore Medical Ctr-Bronx, New York, NY; [6]Medical Ctr for Postgraduate Education, Warsaw, Poland; [7]Peninsula Regional Medical Ctr, Salisbury, MD; [8]Centre Val d'Aurelle Paul Lamarque, Montpellier, France; [9]California Cancer Associates for Research & Excellence, Encinitas, CA; [10]Centre Hospitalier Universitaire de Limoges-Hôpital Dupuytren, Limoges, France; [11]Policlinico Universitario Campus Biomedico, Rome, Italy; [12]Amgen Inc., Thousand Oaks, CA.*

**Background:** Hypercalcemia of malignancy (HCM), caused primarily by tumor-induced bone resorption, is often treated with IV bisphosphonates (BPs); BPs may not be appropriate for patients (pts) with renal impairment. Denosumab binds to RANKL to inhibit osteoclast-mediated bone resorption.

**Methods:** In this single-arm open-label study, pts with HCM (albumin-corrected serum calcium [CSC] >12.5 mg/dL) despite IV BP treatment ≥7 and ≤30 days (d) before screening received SC denosumab 120 mg on d 1, 8, 15, and 29, then every 4 weeks. The primary endpoint was rate of response (defined as CSC ≤11.5 mg/dL) within 10 d of denosumab initiation.

**Results:** The study enrolled 33 pts (64% men, mean age 60 [SD 15] years). At baseline, estimated creatinine clearance (CrCl; Cockcroft-Gault formula) was <60 mL/min for 11 pts (33%), ≥60 mL/min for 17 pts (52%), and missing for 5 pts (15%). By d 10, 10/17 pts (59%) with baseline CrCl ≥60mL/min and 6/11 pts (55%) with baseline CrCl <60 mL/min had a response. By d 10, 7/17 pts (41%) with baseline CrCl ≥60 mL/min and 3/11 pts (27%) with CrCl <60 mL/min had a complete response (defined as CSC≤10.8 mg/dL). Over the course of the study, 10/17 pts (59%) with baseline CrCl ≥60mL/min and 8/11 pts (73%) with baseline CrCl <60 mL/min had a complete response.

**Conclusions:** In this study of pts with persistent or relapsed HCM despite recent IV BP treatment, similar proportions of pts responded to denosumab treatment regardless of renal function.

*Funding:* Pharmaceutical Company Support - Amgen Inc.

## TH-PO763

**Relationship between Urinary Phosphate with Cardiovascular Disease and Mortality in the AUSDIAB Cohort** Nigel David Toussaint,[1] Eugenia Pedagogos,[1] Stephen G. Holt,[1] Steven J. Chadban,[2] Kevan Polkinghorne.[3] *[1]Nephrology, The Royal Melbourne Hospital, Parkville, Victoria, Australia; [2]Nephrology, Royal Prince Alfred Hospital, Sydney, Victoria, Australia; [3]Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.*

**Background:** Serum phosphate is associated with cardiovascular disease (CVD) and mortality in the general population. Greater dietary phosphorus intake may lead to higher serum phosphate and adverse outcomes. Urinary phosphate excretion is a marker of intestinal phosphorus absorption and may be a more reliable marker of phosphate homeostasis. Studies report good correlation between urine phosphate-creatinine ratio (uPiCr) and 24-hour urinary phosphate excretion, but whether uPiCr is associated with risk of CVD or mortality is uncertain. We aimed to assess the association between urinary phosphate excretion measured by uPiCr and outcomes in a large population-based cohort.

**Methods:** Using the nationally representative AusDiab cohort, uPiCr was determined from available urine samples (n=11,116). Participant baseline characteristics were compared across quartiles of uPiCr and regression analysis was used to determine associations. Relationships between uPiCr and all-cause mortality were determined using Cox proportion hazards regression with uPiCr modelled using fractional polynomials.

**Results:** Mean age 51+/-14y, 45% males and 9.6% had chronic kidney disease (CKD-Epi equation). During a median follow-up 12.1 years there were 1265 deaths. Mean uPiCr was 1.45+/-0.7mmol/mmol. Participants with higher uPiCr were older, more likely female, had higher albuminuria, lower GFR, greater prevalence of CVD and hypertension (all p<0.001) and higher BMI (p=0.002). With increasing quartiles of uPiCr the hazard ratios (HR) for all-cause mortality were 1.27 [95%CI 1.07,1.52], 1.54 [1.30,1.83], and 2.07 [1.76,2.44] (compared to the lowest quartile). When modelled using fractional polynomials, both low and high urinary phosphate were independently associated with increased risk.

**Conclusions:** Low and high uPiCr are associated with increased all-cause mortality in a general population cohort and uPiCr may serve as a useful marker for interventions aimed at improving phosphate balance.

## TH-PO764

**Effects of Gastric Bypass on Mineral Homeostasis and Skeletal Measures in Obese Rats** Benjamin Canales,[1] Anne Schafer,[2] Dolores Shoback,[2] Thomas Carpenter.[3] *[1]Urology, Univ of Florida, Gainesville, FL; [2]Medicine, Div of Endocrinology, San Francisco Veterans Affairs Medical Center, San Francisco, CA; [3]Medicine, Div of Endocrinology, Yale Univ, New Haven, CT.*

**Background:** Due to the invasiveness of bone biopsy and questionable reliability of dual-energy X-ray absorptiometry during surgical weight loss, the skeletal impact of Roux-en-Y gastric bypass (RYGB) surgery is unclear. To better understand bone loss mechanisms, we evaluated mineral homeostasis and skeletal phenotype in diet-induced obese (DIO) rats subjected to RYGB or sham surgery.

**Methods:** DIO male Sprague Dawley rats underwent sham (n=4) or RYGB (n=8) surgery. Animals were then placed on high-fat, normal calcium diets and euthanized after 20 weeks. Calciotropic hormones and bone turnover markers (BTM) were measured pre- and post-surgery. Femurs were analyzed using micro-computed tomography (uCT) and light microscopy (LM).

**Results:** Compared to post-operative controls, RYGB animals had significantly lower body weight, serum calcium, and 25-hydroxyvitamin D with increased parathyroid hormone levels, reflecting vitamin D deficiency and secondary hyperparathyroidism (SHPT). In controls, aging led to coupled decreases in BTM of formation [P1NP] and resorption [CTx]. In contrast, RYGB animals had evidence of relatively high CTx but lower P1NP levels. uCT confirmed reduced trabecular bone volume, number, and thickness as well as reduced cortical volume, thickness, and moment of inertia in RYGB rats relative to controls. Histomorphometric evidence by LM of increased RYGB bone resorption included increased osteoclasts and lower bone volume. RYGB rats also had increased osteoid volume with increased osteoblasts and osteoblastic surface yet serum formative markers were markedly lower than controls.

**Conclusions:** In our DIO model, RYGB-associated bone resorption appears to be driven by vitamin D malabsorption and SHPT. However, bone uncoupling with decreased osteoblastic activity suggests other mechanisms, such as gut hormone signaling, may play a role in bone mass differences. Further mechanistic research to explore these differences may identify targets for RYGB bone loss prevention.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Ethicon Endosurgery, Private Foundation Support

## TH-PO765

**Clinical and Biological Data of Homozygous and Heterozygous Patients with CYP24A1 Mutations** Marie-Lucile Figuères,[1] Maryvonne Hourmant,[1] Gwenaelle Roussey,[2] Bertrand Knebelmann,[3] Amélie Ryckewaert,[4] Marie-Laure Kottler,[5] Sandra Mercier,[6] Emma Allain-Launay.[2] *[1]Nephrology, Nantes Univ Hospital, Nantes, France; [2]Pediatric Nephrology, Nantes Univ Hospital, Nantes, France; [3]Nephrology, Necker Univ Hospital, Paris, France; [4]Pediatric Nephrology, Rennes Univ Hospital, Rennes, France; [5]Medical Genetic, Caen Univ Hospital, Caen, France; [6]Medical Genetic, Nantes Univ Hospital, Nantes, France.*

**Background:** Loss-of-function mutations of CYP24A1, a vitamin D metabolizing enzyme, have recently been involved in infantile hypercalcemia. We report here clinical, biological and genetic data of a french cohort of 17 patients with CYP24A1 homozygous or heterozygous mutations.

**Methods:** 6 homozygous patients (2 adults, 4 children), their 10 heterozygous relatives and one heterozygous patient discovered on clinical manifestations are included in the study.

**Results:** Inaugural manifestations, that began in the first year of life in 5/6 homozygous patients, were symptomatic hypercalcemia (3.99±1.00mM) and medullary nephrocalcinosis. One patient only had nephrolithiasis. Hypercalcemia was associated with hypercalciuria, indetectable PTH, normal 25OH vitamin D, normal or elevated $1,25OH_2$vitamin D. Hypercalcemia occurred with usual doses of oral vitamin D and normal calcium load. Outside these episodes, calcemia usually remained at the upper limit of the normal range with normal or low PTH level, especially in summer, but persistent hypercalciuria. The 2 adults presented extrarenal manifestations (calcic corneal deposits, osteoporosis) and had chronic kidney insufficiency. In the heterozygous patients with the exception of one who had similar abnormalities than the homozygous cases, the phospho-calcic product was normal in 8, one had hypercalciuria and one a slightly increased 1,25OH vitamin D.

**Conclusions:** Hypercalcemia, together with nephrocalcinosis, low PTH, and high $1,25OH_2$vitamin D were the main parameters leading to the suspicion of CYP24A1 mutation. Renal prognosis was associated with nephrocalcinosis. Response to vitamin D load in heterozygous patients still have to be determined.

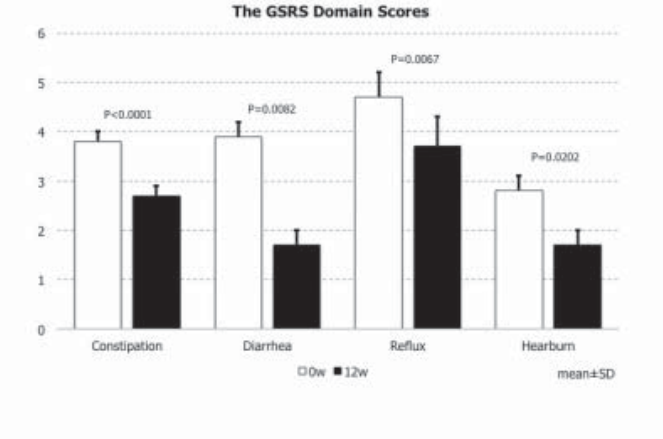
## TH-PO766

**Treatment of Hyperphosphatemia with Bixalomer in Hemodialysis Patients with Sevelamer-Associated Gastrointestinal Symptoms** Hiroaki Ogata,[2] Chiaki Kumata,[1] Kae Ito,[1] Kanji Shishido,[1] Akiko Takeshima,[2] Masahide Mizobuchi,[3] Eriko Kinugasa,[2] Tadao Akizawa.[3] *[1]Internal Medicine, Kawasaki Clinic, Kawasaki, Japan; [2]Dept of Internal Medicine, Showa Univ Northern Yokohama Hospital, Yokohama, Japan; [3]Div of Nephrology, Dept of Medicine, Showa Univ School of Medicine, Tokyo, Japan.*

**Background:** Bixalomer (Bix) is an amine-functional polymer, non-calcium (Ca) containing phosphate (P) binder and has been clinically available in Japan recently. Bix is expected to cause fewer gastrointestinal (GI) side effects as compared with sevelamer hydrochloride (SH), because of less expansion of Bix in the GI tract.

**Methods:** In this retrospective study, we evaluated changes in GI symptoms by the Gastrointestinal Symptom Rating Scale (GSRS) score in long-term hemodialysis outpatients with SH-associated GI symptoms, who switched to Bix from SH. Total 114 patients (age 63.7±10.8 year (mean±SD), female 65.5%, vintage 11.2±8.6 years, diabetes mellitus 27.4%) were enrolled. The GSRS score was checked at 0 and 12 weeks after the start of Bix. Bix was started at the initial dose of 750mg/day, and then was titrated.

**Results:** Serum albumin, P, Ca, and iPTH levels did not significantly change during Bix treatment period. However, serum LDL-cholesterol and bicarbonate levels significantly increased at 12 w as compared with those at 0w (73.1±20.0 vs 92.4mg/dL±23.9, P<0.0001 in LDL-c; 18.7±2.0 vs 19.5±2.3mEq/L, P<0.0001 in $HCO_3$). In GSRS score, total and each domain scores including constipation, diarrhea, and reflux, heartburn, significantly reduced in 12w after Bix treatment as compared with 0w.




**Conclusions:** Bix is an effective P binder in long-term hemodialysis patients. In addition, Bix may be less often associated with gastrointestinal symptoms as compared with SH.

## TH-PO767

**Are Allograft Microcalcifications a Marker of Mineral Bone Disease?** Sarah Margaret Moran,[1] Kate O'Connor,[2] Marek J. Mazur.[1] *[1]Nephrology, Cork Univ Hospital, Ireland; [2]Histopathology, Cork Univ Hospital, Ireland.*

**Background:** Renal allograft calcification has been previously described, however its significance remains unclear. We aim to assess whether renal transplant allograft calcification is associated with mineral bone disease.

**Methods:** A computerized search of histopathological records was conducted. Data on primary renal diagnosis, allograft biopsy diagnosis, transplant & patient outcome and comorbidities were obtained. Serum calcium, phosphate, bicarbonate and parathyroid hormone (PTH) within 3 months of biopsy and serial creatinine measurements were obtained. Pathological slides were assessed by a Consultant Nephropathologist for calcification using a validated scoring system.

**Results:** We identified a total of 32 allograft biopsies in 25 patients. Time to biopsy ranged from 1 to 180 months. Calcification was present in 46.9% (15/32). The number of foci ranged from 1 to 42, mean 7.5. Calcifications were located in tubules in 93.3% (14/15), with 0 to 42 foci, mean 6.3 foci. Interstitial calcification was present in 20% (3/15), 0 to 15 foci, mean 1.1 foci. There was a trend towards an association between calcification and PTH levels which did not reach statistical significance (p=0.3). Mean PTH in those with calcification was 261.71pg/ml (SD 309.5), mean PTH in those without calcification was 185.65pg/ml (SD 173.89). PTH levels in those with calcification were subanalysed into allograft outcome which revealed a trend towards elevated PTH levels but did not reach statistical significance. Mean PTH level in allograft recovery was 197 pg/ml (SD 266.9) and 378.2pg/ml (SD 277.5) in allograft failure (p=0.31). Serum phosphate levels three months prior to biopsy ranged from 0.66 to 1.94 mmol/L (mean 1.12 mmol/L). Serum corrected calcium levels three months prior to biopsy ranged from 2.13 to 3.3 mmol/L (mean 2.52 mmol/L). There was no association between calcium (p=0.8) or phosphate (p=0.72) levels and allograft calcification.

**Conclusions:** This is the first study to examine the significance of microcalcifications in non-protocol allograft biopsies up to 180 months. Non-significant trends were observed towards associations with allograft outcome and elevated PTH levels.

*Funding:* Clinical Revenue Support

## TH-PO768

**PTH Inhibits Type IIa Sodium Phosphate Cotransporter mRNA Expression through Both Protein Kinase A and Protein Kinase C-Dependent Mechanisms** Rebecca Murray,[1] Nina W. Lesousky,[2] Syed J. Khundmiri,[1,2] Barbara Clark,[3] Eleanor D. Lederer.[1,2,4] *[1]Physiology & Biophysics, Univ of Louisville, Louisville, KY; [2]Medicine, Univ of Louisville, Louisville, KY; [3]Biochemistry & Molecular Biology, Univ of Louisville, Louisville, KY; [4]Robley Rex VAMC, Louisville, KY.*

**Background:** We have previously shown that PTH decreases NpT2a mRNA stability through an effect dependent on both transcription and translation. However, little is known about the signaling pathways responsible for destabilization of NpT2a mRNA by PTH. The PTH receptor activates both cAMP/PKA and PLC/PKC pathways, and both pathways have been shown to decrease proximal tubule phosphate reabsorption. A genome-wide analysis by Zhang et al (PNAS, 2005) identified a half-CRE within the promoter region of the NpT2a gene.

**Methods:** We hypothesize that PTH produces chronic down-regulation of NpT2a mRNA through activation of the PKA pathway. To address this hypothesis, we treated opossum kidney cells, a proximal tubule cell line featuring PTH-sensitive sodium-phosphate cotransport, with 8-bromo-cAMP (8-br) or phorbol myristate acetate (PMA) to activate the PKA and PKC pathways, respectively, and measured Npt2a mRNA expression by qRT-PCR.

**Results:** 2h treatment of OK cells with 10 μM 8-br decreased NpT2a mRNA by 45.46 (±4.12)%, and 8h treatment decreased NpT2a mRNA expression by 55.25 (±4.64)%. Treatment over a period of 8h with 100 nM PMA resulted in no significant decrease versus control. Pretreatment with the PKA inhibitory peptide (PKAi) 2μM to block the activation of PKA followed by PTH 100 nM produced a 65% reduction in NpT2a mRNA expression relative to control. 1 μM PTH(3-34), an analogue that selectively activates the PKC pathway, significantly decreased NpT2a mRNA expression at 6h and 8h by 52.59 (±11.76)% and 49.66 (±9.33)%, respectively, but not at shorter treatment times.

**Conclusions:** We conclude that both PKA and PKC pathways may contribute to regulation of NpT2a mRNA by PTH. Funding provided by VA to EDL.

*Funding:* Veterans Affairs Support

## TH-PO769

**Urinary Phosphate and Serum FGF-23 Predict Endothelial Function: A Single Blind Cross-Over Trial** Kathryn K. Stevens, Rajan K. Patel, Patrick B. Mark, Christian Delles, Alan G. Jardine. *Insitute of Cardiovascular and Medical Sciences, Univ of Glasgow, United Kingdom.*

**Background:** Acute phosphate loading with a single meal impairs endothelial function but the effect of sustained phosphate loading has not been studied. We studied the effect of sustained phosphate loading and phosphate binding medication on endothelial function in healthy volunteers.

**Methods:** Healthy volunteers attended for 3 visits. Prior to each visit a 24 hour urine collection was performed for urinary electrolytes and cGMP. Bloods were drawn for routine biochemistry and FGF-23. Flow mediated dilatation (FMD) was recorded at each visit. Volunteers were randomised at visit 1 to receive phosphate sandoz (PS) or lanthanum carbonate (LC) for two weeks prior to visit 2. After a wash out period, volunteers took the other drug before attending for a final visit.

**Results:** There were 19 participants. At baseline, mean age was 42±14 years, serum phosphate 1.05±0.18mmol/L and fractional excretion of urinary phosphate (FeP) 14.3±3.4%. Median FMD was 8.4% (IQR 6.2-11.6%). After PS, there was a non-significant trend towards a higher serum phosphate within the normal range, FGF-23 and FeP rose significantly compared to baseline (p=0.013, p<0.001) and FMD reduced significantly (3.38% (IQR 2.57-5.26%), p<0.001). With LC, FMD also reduced significantly (p=0.033). Randomization order had no effect. Urinary cGMP correlated negatively with serum phosphate (p=0.003). In a regression model, higher FGF23 and FeP independently predicted reduced FMD.

**Conclusions:** This is the first study to show that sustained phosphate loading impairs endothelial function. Serum phosphate was unchanged but the deleterious effect seen with PS may be explained by elevated total body phosphate. Elevated FeP and FGF-23 are likely surrogate markers of higher total body phosphate. Urinary cGMP, as a marker of endothelial dysfunction negatively correlates with serum phosphate. This supports the hypothesis that phosphate increases cardiovascular risk by impairing endothelial function, possibly via the nitric oxide pathway and shows that sustained phosphate loading is directly detrimental to the vasculature even when serum phosphate remains within the normal range.

*Funding:* Other NIH Support - British Heart Foundation

## TH-PO770

**Spurious Hyperphosphatemia due to High Bilirubin Levels** Talal A. Khan, Azka Arif, Muhammad R. Syed, Ayesha Waqar, Karthik M. Ranganna, Gregory Malat, PharmD, Bette Seamonds, Parmish Lalit Kohli, Stephen Guy, Alden Michael Doyle. *Drexel Univ College of Medicine, Philadelphia, PA.*

**Background:** We report a case series of spurious hyperphosphatemia in patients with end-stage liver disease (ESLD) that was associated with highly elevated serum bilirubin levels. Hyperphosphatemia was noted with one type of laboratory analyzer, but the results could not be duplicated utilizing a different analyzer that is based on a different calibration method . As treatment of spurious hyperphosphatemia may lead to complications, awareness of this issue may have important ramifications for the care of ESLD patients.

**Methods:** Four patients with ESLD who were listed for liver transplantation presented with severe hyperbilirubinemia. In each patient, unexpectedly high Phosphorus ($PO_4$) levels triggered the use of PO4 binders. Suspicion for an incorrect diagnosis arose because the degree of hyperphosphatemia did not correlate with either the degree of kidney dysfunction or oral intake of $PO_4$. Intact PTH levels were unremarkable & hemolysis was ruled out. Samples were re-analyzed using our Beckman LX20 & DxC analyzers. They were sent out and analyzed on a Roche Integra analyzer as well. We found that the original measurements of PO4 were falsely elevated.

**Results:** The initial mean serum PO4 level was 7.9mg/dl (SD=±2.31) when assayed using the Beckman analyzers. Results on the Roche Integra analyzer revealed a mean PO4 level of 3.9 mg/dl (SD=± 0.43). Mean PTH and Calcium levels were 50pg/ml and 9mg/dl respectively. Mean Total Bilirubin level was 23.7mg/dl(SD=±3.19.

| Patient | Serum PO4 (Beckman) mg/dl | Serum PO4(Roche) mg/dl | Serum Calcium mg/dl | Total Bilirubin mg/dl | Serum PTH pg/dl | Serum Creatinine mg/dl |
|---|---|---|---|---|---|---|
| 1 | 5.9 | 4.1 | 9.2 | 26.4 | 69 | 1.1 |
| 2 | 6.8 | 3.9 | 9.0 | 19.4 | 65 | 4.1 |
| 3 | 7.8 | 3.3 | 8.4 | 23.1 | 23 | 0.66 |
| 4 | 11.2 | 4.3 | 9.9 | 25.9 | 44 | 0.76 |

**Conclusions:** Hyperphosphatemia should be carefully evaluated in ESLD patients with severe hyperbilirubinemia. Analyzers that use time-dependent photometric methods to measure serum PO4 can be affected by high bilirubin levels. In contrast, analyzers that use end-point photometric method remain accurate even in the face of high bilirubin because the blank sample allows for interference correction.

## TH-PO771

**The Etiology of Low Bone Mineral Density in Hypercalciuric Children** Maria Goretti M. G. Penido,[1] Marcelo S. Tavares,[1] Uri S. Alon.[2] *[1]Pediatric Nephrology Unit, Dept of Pediatrics, Federal Univ of Minas Gerais, Belo Horizonte, MG, Brazil; [2]Bone and Mineral Disorders Clinic, Pediatric Nephrology, The Children's Mercy Hospital, Kansas City, MO.*

**Background:** Some children with idiopathic hypercalciuria (IH) develop bone mass reduction but the reason for the decreased bone mineral density (BMD) remains undefined. The aim of this study was to look for the etiology of bone mass reduction in children with IH comparing them to a control group of normal children (C).

**Methods:** We evaluated the data of 88 children (50 males) with IH aged 2.2–17.8 years (median 9.4); and 29 controls (13 males) aged 4.0–16.3 years (median 9.8). 24h urine collections were analyzed for volume, creatinine, calcium, citrate, phosphate, oxalate, magnesium, hydroxyproline. Urine N-telopeptides (NTX) and pH were analyzed on a fasting random sample. Simultaneous blood samples were tested for creatinine, electrolytes, calcium, phosphorus, alkaline phosphatase, magnesium, PTH, osteocalcin, blood gases. Bone densitometry was performed by DXA using a Lunar DPX-IQ 2516 device. Clinical, laboratory, and bone densitometry data were expressed as median, minimum and maximum values. We use the Kruskal-Wallis test to compare the groups.

**Results:** There were no differences in age, BMI Z-scores and gender between the 2 groups. Lumbar spine (L1-L4) BMC, BMC corrected for height and for width of the vertebra, areal BMD, volumetric BMD and Z-score BMD were significantly lower in IH. Serum phosphorus and alkaline phosphatase, and urinary calcium and NTX were significantly higher in the IH group, whereas their serum PTH, urinary volume and citrate were significantly lower compared with C.

**Conclusions:** The calciuria associated with decreased BMD in IH children seems to be due to increased bone resorption, independent of PTH. Further studies should investigate the possible association of increased bone resorption with citraturia and other factors like $1,25(OH)_2$vitamin D and nutrition.

## TH-PO772

**Decreased Bone Mineral Density in Hypercalciuric Children Is Not Related to the Presence of Urolithiasis** Maria Goretti M.G. Penido,[1] Marcelo S. Tavares,[1] Uri S. Alon.[2] *[1]Pediatric Nephrology Unit, Dept of Pediatrics, Federal Univ of Minas Gerais, Belo Horizonte, MG, Brazil; [2]Bone and Mineral Disorders Clinic, Pediatric Nephrology, The Children's Mercy Hospital, Kansas City, MO.*

**Background:** The association between idiopathic hypercalciuria (IH) and decreased bone mineral density (BMD) has been described in adults and children. The risk for low bone density in hypercalciuric children with urolithiasis has not yet been well defined. The aim of this study was to explore if hypercalciuric children with urolithiasis are at higher risk for low BMD compared to those without stones.

**Methods:** We evaluated the data of 88 children with IH. They were divided to group 1 (G1) consisted of 49 children with urolithiasis (29M) aged 9.6±3.6 years, and group 2 (G2) consisted of 39 without urolithiasis (21M) aged 7.5±2.4 years. Group 3 (G3) was a control group composed of 29 children (13M) aged 10.3±3.8 years. 24h urine collections were analyzed for volume, creatinine, calcium, uric acid, citrate, phosphate, oxalate, magnesium, cystine, hydroxyproline. Ntelopeptide and pH were analyzed in a fasting urine sample. Venous blood samples were analyzed for creatinine, electrolytes, calcium, phosphorus, alkaline phosphatase, uric acid, magnesium, PTH, osteocalcin, blood gases and bicarbonate. Bone densitometry was performed by DXA using a Lunar DPX-IQ 2516 device. We use the Kruskal-Wallis and Dunn's multiple comparison tests to compare the groups.

**Results:** There were no differences in age, gender, BMI Z-scores, biochemical and mineral parameters between G1, G2 and G3. The 24h urine volume was higher in G2 when compared to G1 ($p<0.01$) while urine oxalate was lower ($p<0.01$). There were no differences in areal BMD and BMD Z-score of the lumbar spine (L1–L4) between G1 and G2, but values in G3 were higher then in both G1 ($p<0.01$) and G2 ($p<0.001$).

**Conclusions:** The analysis of hypercalciuric children with and without urolithiasis did not show any difference regarding their lower BMD Z-score of the lumbar spine. The only observed difference between patients' subgroups was a higher urinary level of oxalate and lower urine volume, both contributing to stone formation without an effect on bone metabolism.

## TH-PO773

**Increased Arterial Stiffness and Osteopenia in Calcium Nephrolithiasis: A Dangerous Cardiovascular Risk Liaison** Antonia Fabris,[1] Antonio Lupo,[1] Chiara Caletti,[1] Pietro Manuel Ferraro,[2] Gabriele Comellato,[3] Giovanni Gambaro.[2] *[1]Nephrology, Univ of Verona, Verona, Italy; [2]Nephrology, Catholic Univ, Rome, Italy; [3]Geriatrics, Univ of Verona, Verona, Italy.*

**Background:** Recent studies have described high incidence of cardiovascular (CV) disease in calcium nephrolithiasis (CNL). The mechanisms of such an association are not known since a role of obesity, hypertension, diabetes, gout has been ruled out. An inverse relationship between bone density and arterial stiffness (AS) has been reported in osteoporosis and CKD. CNL is often associated with metabolic bone disease (BMD). AS is a predictor of CV mortality. We hypothesized that CNL because of the coexistent BMD have abnormal AS and hence increased CV risk. We analyzed AS and BMD in CNL pts and in healthy controls.

**Methods:** 128 recurrent CNL pts and 39 controls. Exclusion criteria were: hypertension, chronic inflammatory diseases, intestinal and metabolic diseases, diabetes, primary hyperparathyroidism, smoking or menopause since >18 months. Biochemical tests, total body bone densitometry and pulse waive velocity (PWV) (Complior and Augmentation Index) were evaluated. Statistical analysis: Wilcoxon rank sum test.

**Results:** Both carotid-radial and carotid-femoral PWVs are higher in CNL than in controls. In multivariate analysis BMD is an independent predictor of both PWVs being responsible for 70% of the variability.

| | Stone formers (128) | Controls (39) | p |
|---|---|---|---|
| Age (yr) (SD) | 47 (14) | 39 (12) | |
| Gender M (n,%) | 61 (48) | 13 (33) | |
| BMI (Kg/m2)* (SD) | 25 (4) | 23 (3) | |
| Log-PWV carotid-radial (CI 95%) | 8.6 (8.3-8.8) | 7.8 (7.5-8.2) | 0.004 |
| Log-PWV carotid-femoral (CI 95%) | 7.5 (7.2-7.8) | 6.5 (6.1-7.0) | < 0.001 |
| Log-Peripheral Pulse Pressure (CI 95%) | 45 (42-48) | 42 (38-46) | 0.22 |
| Log-Augmentation index (CI 95%) | 9.4 (8.0-10.9) | 9.4 (6.9-12.8) | 0.96 |
| Prevalence osteopenia/osteoporosis | 70% | 23% | |

**Conclusions:** These results show the existence of an association between BMD and functional modification of the elastic arteries in CNL independent of the common CV risk factors. This observation may explain the increased CV risk observed in stone formers.

## TH-PO774

**Nephrolithiasis and Risk of Incident Bone Fracture** Eric N. Taylor,[1,2] Gary C. Curhan.[2,3] *[1]Div of Nephrology and Transplantation, Maine Medical Center, Portland, ME; [2]Channing Div of Network Medicine, Brigham and Women's Hospital, Boston, MA; [3]Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Higher urine calcium is a central feature of calcium nephrolithiasis and is associated with lower bone mineral density in individuals with kidney stones (KS). Previous reports, albeit small and unadjusted for dietary intakes, suggest that individuals with KS may have higher risk of bone fracture.

**Methods:** We prospectively examined independent associations between a history of KS and incident fracture in the Nurses' Health Study I (N= 77,055 women; 30 years of follow-up) and the Health Professionals Follow-up Study (N=50,982 men; 24 years of follow-up). We excluded pre-menopausal women, men < 45 years old, and individuals who reported osteoporosis at baseline. Medical record review confirmed > 95% of self-reported KS in each cohort, and the majority of stones (> 77%) were predominantly calcium oxalate. We included only cases of incident wrist (distal radius) or incident hip (proximal femur) fractures due to low or moderate trauma. Validated food frequency questionnaires were used to assess dietary intake every four years. Cox proportional hazards regression was used to adjust for age, BMI, thiazide use, supplemental calcium, dietary intake, and other factors.

**Results:** At baseline, participants with KS had higher BMI and were more likely to use a thiazide diuretic than participants without KS. During follow-up there were 3741 wrist and 1160 hip fractures in women and 859 wrist and 634 hip fractures in men. The multivariable relative risk of incident wrist fracture in participants with a history of KS compared to participants without was 1.36 (95 % CI 1.17 to 1.57) in women and 1.22 (95% CI 1.00 to 1.48) in men. The multivariable relative risk of incident hip fracture in participants with KS was 0.99 (95 % CI 0.75 to 1.29) in women and 0.98 (95% CI 0.77 to 1.24) in men.

**Conclusions:** Nephrolithiasis is associated with a significantly higher risk of incident wrist but not hip fracture. Our study is limited by a relatively low number of hip fractures in participants with KS.

*Funding:* NIDDK Support

## TH-PO775

### Decreased Renal Clearance of Uric Acid Is Present in First Time Female Kidney Stone Formers

Hemamalini Ketha, John C. Lieske, Eric J. Bergstralh, Xujian Li, Rajiv Kumar, Andrew D. Rule. *Dept of Medicine, Mayo Clinic, Rochester, MN.*

**Background:** Elevated serum uric acid (UA) occurs in Ca stone formers (SF), but the mechanism has not been elucidated. We investigated whether serum UA levels in SF differ by gender and are impacted by UA clearance.

**Methods:** Serum UA and urinary pH, UA, sulfate, and the fractional excretion of UA were measured in a sample of first time SF (females (F) = 142, males (M) = 121) and in age-matched controls (C) (F = 151, M = 166) from the general population.

**Results:** In F (mean age 43.5 years), serum UA and FEUA were higher and urinary pH was lower in SF compared to C (Table 1). Despite the lower urine pH, F SF had lower urinary sulfate compared to controls (13.2 vs 18.7 mmol/24 h, p=0.004) suggesting lower protein intake. Urine volume and sodium were similar in F SF vs C. In M (mean age 48.2 years), serum uric acid, %FEUA, pH, and urinary sulfate did not differ between SF and C.

**Conclusions:** Higher serum UA levels among first time SF were only evident in F. Unlike controls, F SF have altered UA metabolism compared to M SF. The higher serum UA in female SF cannot be attributed to increased protein intake as urinary sulfate excretion is lower in SF vs C. Instead, an increase in the reabsorption of UA from tubular fluid, or failure in the excretion of UA into tubular fluid, accounts for the increase in serum UA.

**Table 1**. Serum and urinary parameters in F and M, SF and C

| | Controls (317, F=151, M=166) | Stone Formers (263, F=142, M=121) | P Value |
|---|---|---|---|
| *Serum uric acid, mg/dl* | | | |
| F | 4.2 ± 1.2 | 5.6 ± 1.2 | <0.0001 |
| M | 5.9 ± 1.3 | 5.6 ± 1.3 | 0.14 |
| *FEUA (%)* | | | |
| F | 8.2 ± 3.7 | 6.3 ± 3.4 | <0.0001 |
| M | 6.4 ± 2.9 | 6.2 ± 3.1 | 0.14 |
| *pH* | | | |
| F | 6.3 ± 0.6 | 6.1 ± 0.6 | 0.004 |
| M | 6.2 ± 0.6 | 6.1 ± 0.5 | 0.21 |
| *Urine Volume, mL/24/h* | | | |
| F | 1710 ± 807 | 1560 ± 875 | 0.15 |
| M | 1894 ± 795 | 1717 ± 694 | 0.06 |
| *Urine Na, mmol/24h* | | | |
| F | 103 ± 50 | 101 ± 49 | 0.8 |
| M | 154 ± 74 | 147 ± 72 | 0.49 |

*Funding:* NIDDK Support, Other NIH Support - Arthritis and Musculoskeletal Diseases, Private Foundation Support

## TH-PO776

### The Anti-Phosphaturic Effect of 1,25(OH)2D Is Impaired in Nephrolithiasis

Hemamalini Ketha, Ravinder Singh, Stefan Grebe, Eric J. Bergstralh, Xujian Li, Andrew D. Rule, John C. Lieske, Rajiv Kumar. *Mayo Clinic, Rochester, MN.*

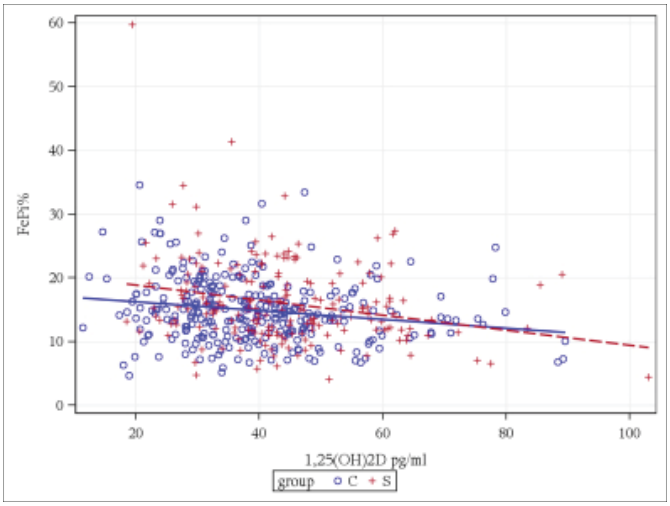
**Background:** Phosphate homeostasis is regulated by parathyroid hormone (PTH) and $1,25(OH)_2D$. Previous studies have shown an increased renal clearance of phosphate in kidney stone formers (SF) with normal serum PTH levels.

**Objectives:** 1. To investigate whether renal phosphate (Pi) clearance is increased in first time kidney SF. 2. To determine the relationship between the renal fractional excretion of Pi (FEPi), serum $1,25(OH)_2D$ and PTH.

**Methods:** Serum $1,25(OH)_2D$ (measured by mass spectrometry), intact PTH, Pi and urinary chemistries were measured in 263 first time SF and 317 controls (C) sampled from the general population. FEPi, $1,25(OH)_2D$, PTH and urine analytes were compared between SF and C groups.

**Results:** SF compared to C showed significantly higher FEPi (mean 16.0% vs 14.9% P = 0.04), higher $1,25(OH)_2D$ (43 vs 39 pg/ml, P = 0.003) and similar PTH (42.2 vs 39.5 pg/mL, P = 0.13). The higher FEPi in SF remained significant after adjusting for age, gender and $1,25(OH)_2D$ (P = 0.0176) (Fig. 1). The FEPi declined by 0.64% when $1,25(OH)_2D$ increased by 10 pg/ml (P = 0.0004) in both SF and C, with no evidence of an interaction (P = 0.21). No association between FEPi% and PTH was observed in SF or C.

**Conclusions:** The anti-phosphaturic response of the kidney to circulating concentrations of $1,25(OH)_2D$ is impaired in SF compared to C. The results suggest a systemic alteration in vitamin D and Pi metabolism common to many first time SF in the community.




*Funding:* NIDDK Support, Other NIH Support - Arthritis and Musculoskeletal, Private Foundation Support

## TH-PO777

### Evidence for Altered Responsiveness of the Vitamin D Endocrine System to Endogenous Circulating Parathyroid Hormone in Kidney Stone Formers

Hemamalini Ketha, Ravinder Singh, Stefan Grebe, Eric J. Bergstralh, Xujian Li, Andrew D. Rule, John C. Lieske, Rajiv Kumar. *Mayo Clinic, Rochester, MN.*

**Background:** Elevated $1,25(OH)_2D$ concentrations have been reported in some kidney stone formers (SF). Additionally, reduced $24,25(OH)_2D$ concentrations have been associated with elevated $1,25(OH)_2D$ and hypercalciuria in some patients with nephrolithiasis.

**Objective:** To investigate the associations between serum $1,25(OH)_2D$, $24,25(OH)_2D$ and PTH concentrations in a cohort of first time adult SF compared to controls (C).

**Methods:** Serum $1,25(OH)_2D$ and $24,25(OH)_2D$ were measured by mass spectrometry in SF (N = 263) and C (N = 317). Serum full-length parathyroid hormone (PTH), calcium (Ca) and inorganic phosphorus (P), and urinary chemistries and supersaturations were measured in the same subjects. Logistic regression was performed to determine if serum vitamin D metabolite, PTH, Ca and P, and urine analytes and supersaturations (SS) differed in SF versus C.

**Results:** In multivariate analysis adjusting for age and gender, mean serum $1,25(OH)_2D$ concentrations were significantly higher in the SF versus C (43.40 vs 39.22pg/mL, P<0.0001). Mean serum $24,25(OH)_2D$ was similar between the two groups (2.92 vs. 3.16 ng/ml, P = 0.175). Serum PTH concentrations were also similar in the two groups (42.16 vs. 39.63 pg/ml, P = 0.053). A higher concentration of $1,25(OH)_2D$ (P = 0.009), and a lower concentration of $24,25(OH)_2D$ (P = 0.055), was observed at any concentration of PTH in SF versus C. Changes in vitamin D metabolites and PTH did not correlate with serum or urinary minerals or SS.

**Conclusions:** Elevated $1,25(OH)_2D$ concentrations were present in a large group of first time SF. Serum $24,25(OH)_2D$ concentrations were similar in the two groups. A higher concentration of $1,25(OH)_2D$ and a lower concentration of $24,25(OH)_2D$ was noted for any level of PTH in SF versus C, suggesting altered responsiveness of the vitamin D endocrine system to circulating PTH.

*Funding:* NIDDK Support, Other NIH Support - Falk Foundation, Private Foundation Support

## TH-PO778

### Sclerostin Is Associated with Age, Weight, BMI and Ionized Calcium Levels in Recurrent Kidney Stone Formers

Nilufar Mohebbi,[1] Daniel Rodríguez Gutiérrez,[1] Rudolf P. Wuthrich,[1] Carsten A. Wagner.[2] *[1]Div of Nephrology, Univ Hospital of Zurich, Zurich, Switzerland; [2]Institute of Physiology, Univ of Zurich, Zurich, Switzerland.*

**Background:** Kidney stones are common in industrialized countries. Calcium containing stones are the most frequent stone type. Many factors predispose or contribute to the development of kidney stones, including genetic background, diet, and metabolic factors. Sclerostin is secreted by osteocytes and inhibits bone formation at the level of osteoblasts. In humans, Sclerostin mRNA is expressed in several tissues, with high levels in the kidney whereas Sclerostin protein is restricted to osteocytes. The effect of sclerostin on bone formation may not be unique. A potential role in other diseases where bone homeostasis may be disarranged such as nephrolithiasis may be very likely.

**Methods:** We performed a cross-sectional observational study in 96 male and female recurrent kidney stone formers (rKSF) to analyze the potential role of sclerostin.

**Results:** 66 of the patients were male and mean age of all patients averaged to 45±13 years. Mean serum creatinine, phosphate, total calcium levels and venous bicarbonate were 78±1.6 umol/l, 0.95±0.01 mmol/l, 2.41±0.01, and 27±0.2 mmol/l, respectively. Mean 25-OH-Vitamin D levels were 57.1±2.2 ng/ml and PTH levels averaged to 48±2 pg/ml. Consistent with data in healthy adults, sclerostin levels were significantly higher in male than in female rKSF (82.2 ±14.7 vs. 67.3±12.3 pmol/l, p<0.0001). Similar to healthy adults,

we also found a positive correlation between sclerostin and age, weight and BMI ($p<0.05$). Additionally, sclerostin was negatively correlated with ionized calcium. Hypercalciuria (>6.2 mmol/24h) or hyperphosphaturia (>22.6 mmol/24h) were not correlated with sclerostin levels in rKSF. Surprisingly, patients with uric acid stones presented with highest sclerostin levels (50.8±14.2 pmol/l, p=0.0072) when compared to patients with calcium oxalate stones.

**Conclusions:** In recurrent kidney stone formers, sclerostin serum levels are positively correlated with age, weight, BMI, and negatively correlated with ionized calcium. Future studies are required to clarify the role of sclerostin in this population.

## TH-PO779

**Greater Loss of Bone Mineral Density with Exogenous $1,25(OH)_2D_3$ in Genetic Hypercalciuric Stone-Forming Rats** Kevin K. Frick,[1] Adeline Ng,[2] Madison Cohen- McFarlane,[2] John R. Asplin,[3] Christopher D. Culbertson,[1] Kelly Kyker-Snowman,[1] Nancy S. Krieger,[1] Marc Grynpas,[2] David A. Bushinsky.[1] *[1]Medicine, Univ of Rochester, Rochester, NY; [2]Samuel Lunenfeld Research Institute, Toronto, Canada; [3]Litholink Corporation, Chicago, IL.*

**Background:** Genetic hypercalciuric stone-forming (GHS) rats, bred to maximize urine (U) calcium (Ca), have, like humans with idiopathic hypercalciuria, increased intestinal Ca absorption and bone Ca resorption and reduced renal Ca reabsorption, leading to hypercalciuria compared to parental SD rats. GHS rats have increased vitamin D receptors (VDR) at these sites, with normal levels of $1,25(OH)_2D_3$ (1,25D), indicating their VDR is undersaturated with 1,25D. Exogenous 1,25D induces a greater increase in UCa in GHS than SD.

**Methods:** To determine the effect of the increased VDR on the bone response to 1,25D, we fed GHS and SD rats ample Ca and injected them with 1,25D (12.5 or 25ng/100 g body wt/d) or vehicle (veh) daily for 16d. Femurs and vertebrae were examined by DEXA, μCT and histomorphometry.

**Results:** Vertebral areal bone mineral density (aBMD, by DEXA) was decreased from 0.092±0.006 g/cm$^2$ in SD to 0.082±0.005 g/cm$^2$ in GHS+veh ($p<0.05$) and further decreased to 0.075±0.004 in GHS+25ng 1,25D ($p<0.05$). Femoral aBMD was decreased from 0.133±0.003 g/cm$^2$ in GHS+veh to 0.123±0.003 in GHS+12.5ng 1,25D ($p<0.05$) and to 0.123±0.004 in GHS+25ng 1,25D ($p<0.05$) while there was no effect of 1,25D on SD. Both vertebral and femoral volumetric BMD (by μCT) were lower in GHS vs SD and further reduced by 25ng 1,25D. Osteoclast number was decreased from 4.7±0.9/mm bone surface in GHS+veh to 2.0±1.5 in GHS+25ng 1,25D ($p<0.05$). In GHS tibiae, 25ng 1,25D increased trabecular thickness and number; osteoid volume increased 12-fold while bone volume increased only 3-fold. Bone formation rate was decreased from 21.8±1.1mm$^2$/mm$^2$/yr in GHS+veh to 9.8±0.7 in GHS+25ng 1,25D ($p<0.02$).

**Conclusions:** In GHS rats, exogenous 1,25D leads to loss of BMD due to a mineralization defect, which contributes to increased hypercalciuria and should decrease bone strength. The enhanced effect of 1,25D in GHS indicates that the increased number of VDR is biologically active.

*Funding:* NIDDK Support

## TH-PO780

**Calcium Oxalate Crystals Induced Changes in Secretion of Proteins from Basolateral Compartment of Renal Tubular Cells That, in Turn, Enhanced Crystal Invasion** Visith Thongboonkerd, Wararat Chiangjong. *Siriraj Hospital, Mahidol Univ, Bangkok, Thailand.*

**Background:** Calcium oxalate monohydrate (COM) crystals cause kidney stone disease by unclear mechanisms. One of hypotheses was that COM crystals induce changes in various cellular mechanisms, leading to enhancement of crystal invasion into renal interstitium. The present study thus aimed to characterize changes in secretion of proteins from basolateral compartment of renal tubular epithelial cells after exposure to COM crystals and then examined functional significance of these changes in association with the stone pathogenesis.

**Methods:** Polarized MDCK cells were cultivated in Transwell containing serum-free medium with or without 100 μg/ml COM crystals for 20 h. Secreted proteins in culture medium from the lower chamber (basolateral compartment) were then collected, desalted by dialysis against deionized water, and finally concentrated by lyophilization. The recovered proteins from five individual cultures in each group with equal amount were resolved in individual 2-D gels and visualized by Deep Purple stain. Proteins with significantly differential levels were then identified by tandem mass spectrometry. The proteomic data were confirmed by Western blot analysis. Finally, functional analysis was performed to validate the significant role of the identified altered proteins in crystal invasion using a recently established crystal invasion assay.

**Results:** Trypan blue exclusion assay revealed comparable cell viability between the two groups of cells. Spot matching and intensity analysis revealed six protein spots with significantly altered levels in the basolateral compartment of COM-treated cells. The altered secreted proteins were then successfully identified by LC-Q-TOF-MS/MS, including enolase-1, phosphoglycerate mutase-1, actinin, 14-3-3 protein epsilon, alpha-tubulin 2 and ubiquitin-activating enzyme E1. The proteomic data were successfully confirmed by Western blot analysis. Finally, functional analysis revealed that some of the identified secreted proteins played an important role in crystal invasion.

**Conclusions:** These data provide an important basis for further elucidation of sophisticated mechanisms of kidney stone disease.

*Funding:* Government Support - Non-U.S.

## TH-PO781

**Adenosine Signaling Inhibits Oxalate Transport by Human Intestinal Caco2-BBE Cells** Hatim A. Hassan, Yong-Chul Jung, Ruhul Amin, Sireesha Ratakonda, Sohee Jeon, Sapna Sharma. *Univ of Chcago.*

**Background:** 80% of kidney stones are composed of calcium oxalate, and minor changes in urine oxalate affect the stone risk. Intestinal oxalate secretion mediated by anion exchanger SLC26A6 (A6) plays a crucial role in limiting net absorption of ingested oxalate; thereby preventing hyperoxaluria and calcium oxalate kidney stones. We recently reported (AJP-Cell Physiol, 2013) that the extracellular nucleotides ATP and UTP inhibit oxalate transport by human intestinal Caco2-BBE cells (C2) through signaling pathways that likely include the $P2Y_2$ purinergic receptor, PLC, and PKC-δ. Since extracellular nucleotides are rapidly degraded (ATP□ ADP□ AMP □ adenosine) by ectonucleotidases, we therefore examined whether intestinal oxalate transport is subject to regulation by one or more of these degradation products.

**Results:** To this end, we measured $^{14}$C-oxalate uptake in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity, ≥ 50% of which is mediated by A6 in C2 cells. We found that ADP, AMP, and adenosine significantly inhibited oxalate transport by C2 cells, by > 26%, 27%, and 31%, respectively. The inhibitory effects of adenosine were partially and significantly blocked by pretreatment with the PKC inhibitor Gö6983 (by > 50%) and the PLC inhibitor U73122 (by >80%). The nonselective (8-p-sulfophenyltheophylline: 8-SPT) adenosine receptor antagonist partially and significantly blocked (by > 40%) adenosine-induced inhibition of oxalate transport by C2 cells. Using selective pharmacological inhibitors, we found that ERK1/2, PKA, and Src kinases are not involved in the observed adenosine-mediated suppression of oxalate transport by C2 cells. These findings are of potential importance because intestinal cells are known to be exposed to adenosine, ADP, and AMP under physiological, as well as pathological (e.g. IBD and therefore might have potential relevance to the IBD-associated hyperoxaluria) conditions.

**Conclusions:** We conclude that adenosine signaling inhibits oxalate transport by C2 cells through signaling pathways that likely include PKC, PLC, and one or more of the known adenosine receptors. ADP and AMP also significantly inhibit oxalate transport by C2 cells.

*Funding:* NIDDK Support

## TH-PO782

**Proinflammatory Cytokines Regulate Oxalate Transport by Intestinal Epithelial Cells** Hatim A. Hassan, Ruhul Amin, Yong-Chul Jung, Sireesha Ratakonda, Sapna Sharma, Sohee Jeon, Peili Chen, F. Gary Toback. *Univ of Chicago.*

**Background:** 80% of kidney stones are composed of calcium oxalate, and minor changes in urine oxalate affect the stone risk. The mammalian intestine plays a crucial role in oxalate homeostasis. Intestinal oxalate absorption is largely passive and paracellular, while anion exchanger SLC26A6 (A6) plays a critical role in active intestinal oxalate secretion. Proinflammatory cytokines (PCs) disrupt intestinal barrier function and increase paracellular flux, as well as inhibit several intestinal transporters. We therefore initiated studies to test the hypothesis that PCs could enhance intestinal oxalate absorption and/or reduce intestinal oxalate secretion.

**Results:** The PCs IFN-γ [IFN] and TNF-α [TNF] caused > 3-fold increase in mucosal to serosal $^{14}$C-oxalate & $^{3}$H-mannitol (a paracellullar marker) absorptive fluxes in human intestinal Caco2-BBE (C2) cells grown on snapwell inserts and mounted in Ussing chambers (UCs). The PC-induced increased absorptive fluxes were completely blocked by pretreatment with AMP-18 and GLP-2, peptides known to improve intestinal barrier function. Importantly, pretreatment of BALB/c mice with TNF before isolating and mounting jejunal segments in UCs led to > 1.9-fold increase in $^{14}$C -oxalate & $^{3}$H-mannitol absorptive fluxes, effects completely blocked by pretreatment with AMP-18 and GLP-2. IFN and TNF also significantly inhibited (by 30-40%) apical $^{14}$C-oxalate uptake, measured as Cl-oxalate exchange, by C2 cells through mechanisms involving reduced A6 mRNA/total protein expression. Another PC, IL-6, also significantly inhibited apical oxalate uptake by C2 cells, while IL-1β, 2, & 8 had no effect. These findings are of potential relevance to pathophysiology of IBD- and obesity-associated hyperoxaluria, in which higher local and systemic PCs levels, respectively, are seen.

**Conclusions:** PCs significantly enhance intestinal oxalate absorption, and that AMP-18 and GLP-2 have therapeutic potential in this process. PCs also significantly reduce oxalate uptake by C2 cells by lowering A6 mRNA/protein expression. Since C2 cells closely resemble the native epithelium, such regulation would likely lead to reduced intestinal oxalate secretion in vivo.

*Funding:* Private Foundation Support

## TH-PO783

**Lithogenic Factors and Inflammatory Bowel Disease: Importance of Disease Severity** Cristina Beato Henriques Outerelo,[1] Fernando Teixeira e Costa,[1] Pedro Figueiredo,[2] Joao Freitas,[2] Aura Ramos.[1] *[1]Nephrology, Hospital Garcia Orta, Almada; [2]Gastrenterology, Hospital Garcia Orta, Almada.*

**Background:** Lithogenic factors are prevalent in patients with inflammatory bowel disease (IBD). Their correlation with the severity of the underlying bowel disease is not yet established. The aim of this study was to evaluate weather the prevalence of known lithogenic factors is related to IBD severity.

**Methods:** Single centre prospective study of eighty-seven consecutive outpatients with IBD. Clinical data was obtained through questionnaire and file consultation; nephrolithiasis

was defined as history of symptomatic renal colic. Routine indices of kidney function were assessed as well as serum concentrations of calcium, phosphorus, magnesium, uric acid and potassium. The 24h urine excretion of these electrolytes was measured, plus oxalate and citrate. Disease severity was defined as 1) need for hospitalization, 2) bowel surgery or 3) imunossuppression (azatioprine, infliximab or adalimumab).

**Results:** Mean age 46±17 years, 43.5% male, 59.8% with Crohn´s disease, median time of disease of 11 years (IQR 5-15). Symptomatic nephrolithiasis had occurred in the past in 19.5%. Patients with at least one severity criteria (62.1%) showed a significantly lower magnesium excretion (64.74±35.02 vs 81.80±40.47, p=0.041), lower calcium excretion (median 2.77 IQR 1.80-4.35 vs 4.25 IQR 2.65-6.32, p=0.015), a trend to higher oxaluria (0.37±0.16 vs 0.31±0.11, p=0.073) but no differences in history of renal colic episodes (16.7% vs 24.2%, p=0.387). Considering the calcium excretion among the different severity variables, only the use of adalimumab was significantly associated with low calciuria (median 2.05 IQR 0.94-2.87 vs 3.34 IQR 2.10-5.40, p=0.008).

**Conclusions:** In spite of no differences found in the prevalence of symptomatic nephrolithiasis, lower urinary magnesium excretion was significantly related with more severe IBD; there was also a trend to higher urinary oxalate excretion. Interestingly, lower urinary calcium excretion was observed, which seemed to be associated with adalimumab use. The impact of these factors on stone formation, renal colic episodes and chronic interstitial nephritis remains to be established.

## TH-PO784

### Total Stone Volume on Renal CT Predicts Future Symptomatic Stone Events

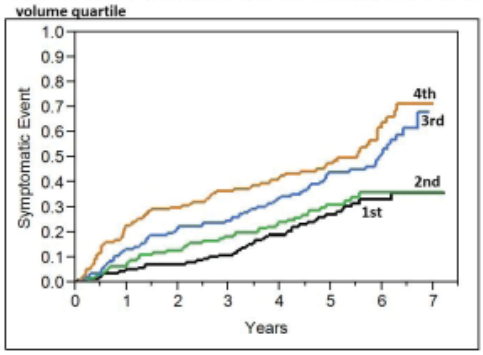
Michael G. Selby,[1] Terri J. Vrtiska,[2] John C. Lieske,[1] Amy E. Krambeck,[3] Eric J. Bergstralh,[4] Andrew D. Rule.[1] *[1]Nephrology, Mayo Clinic, Rochester, MN; [2]Radiology, Mayo Clinic, Rochester, MN; [3]Urology, Mayo Clinic, Rochester, MN; [4]Statistics, Mayo Clinic, Rochester, MN.*

**Background:** The optimal method to quantify radiographic stone burden by renal computed tomographic (CT) scans in order to predict future symptomatic stone events is unknown.

**Methods:** A mailed survey was sent in 2012 to stone formers who had undergone evaluation at the Mayo Clinic between 2005-2009 that included a renal CT scan to evaluate their stone disease while aysmptomatic. The renal CT scan was reviewed to determine the number of kidney stones, largest stone diameter, total stone volume (by automated algorithm), and presence of bilateral stones. Using the survey and electronic medical record, the first symptomatic kidney stone event after the CT scan was identified.

**Results:** The survey was completed by 550 stone formers (55% male, mean age 55 y) of which 43% had a subsequent symptomatic stone event a mean 2.9 years after their CT scan. Stone burden by quartiles was 0-1, 2-3, 4-6, ≥7 for number of stones, 0-2, 3-4, 5-7, ≥8 mm for largest stone diameter, 0-8, 9-70, 71-280, and ≥ 281 $mm^3$ for total stone volume. Forty-eight percent had bilateral stones. The hazard ratio (HR) for a symptomatic stone event by stone burden per quartile level was 1.30 (p<0.001) for the number of stones, 1.26 (p<0.001) for largest stone diameter, and 1.38 (p<0.001) for total stone volume. The HR for an event was 1.80 (p<0.001) for the presence of bilateral stones. On multivariable analysis with all 4 CT characteristics, only total stone volume was an independent predictor of symptomatic events (HR=1.31, p=0.03).

**Figure 1: Risk for future symptomatic stone event by total CT stone volume quartile**




Findings remained similar and statistically significant with exclusion of rare stone types (cystine).

**Conclusions:** An automated total stone volume on renal CT scan optimally predicts future symptomatic stone events.

*Funding:* NIDDK Support

## TH-PO785

### Consecutive CT Scan Monitoring of Total Stone Volume Is More Useful in Patients Who Are Having Interim Symptomatic Stone Events

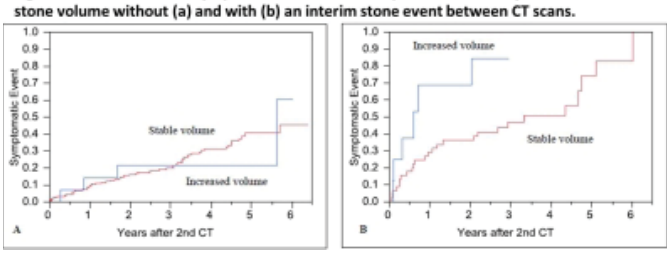
Michael G. Selby,[1] Terri J. Vrtiska,[2] John C. Lieske,[1] Amy E. Krambeck,[3] Eric J. Bergstralh,[4] Andrew D. Rule.[1] *[1]Nephrology, Mayo Clinic, Rochester, MN; [2]Radiology, Mayo Clinic, Rochester, MN; [3]Urology, Mayo Clinic, Rochester, MN; [4]Statistics, Mayo Clinic, Rochester, MN.*

**Background:** The goal of this study was to determine whether the change in kidney total stone volume between two renal computed tomographic (CT) scans was predictive of future symptomatic stone events.

**Methods:** A mailed survey was completed in 2012 by stone formers who had undergone evaluation at the Mayo Clinic that included two renal CT scans between 2005 and 2012 to evaluate their stone disease while asymptomatic. Rare stone types (cystine) were excluded. Both CT scans were reviewed to determine total stone volume using an automated algorithm. The survey and electronic medical record identified any symptomatic stone events between or after the two CT scan dates.

**Results:** The survey was completed by 239 stone formers (62% male, mean age 57 y) with two renal CT scans a median 1.1 years apart. An interim event occurred in 21% and 36% had a symptomatic stone event a mean 3.2 years after their second CT scan. Total stone volume increased a median 17 $mm^3$/year with 10% having a rapid increase in stone volume (>560 $mm^3$/year). The risk of symptomatic stone recurrence was increased in patients with a rapid increase in stone volume who also had an interim stone event (n=50, HR=3.1, p=0.03) but not in those without an interim stone event (n=189, HR=0.49, p=0.26).

**Figure 1: Risk for a future symptomatic stone event in patients with a rapid increase in total stone volume without (a) and with (b) an interim stone event between CT scans.**




Test for interaction was significant (p=0.01).

**Conclusions:** There appears to be a higher risk of symptomatic events in stone formers who have a rapid increase in total stone volume between annual renal CT scans despite passing stones between scans. Annual renal CT scans may not be as helpful in stone formers who are not passing stones.

*Funding:* NIDDK Support

## TH-PO786

### Dietary Acid Load and Risk of Incident Kidney Stones

Ernest I. Mandel,[1,2] Eric N. Taylor,[2,3] Gary C. Curhan.[1,2] *[1]Renal Div, Dept of Medicine, Brigham and Women's Hospital, Boston, MA; [2]Channing Div of Network Medicine, Dept of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [3]Div of Nephrology and Transplantation, Maine Medical Center, Portland, ME.*

**Background:** Higher dietary acid load may reduce urinary citrate excretion, which is associated with an increased risk of kidney stone formation. However, the association between dietary acid load and risk of incident kidney stones has not been explored in population-based studies of individuals.

**Methods:** We prospectively examined the association between dietary acid load, using the diet-estimated net endogenous acid production (NEAP) and potential renal acid load (PRAL), and the risk of incident kidney stones in the Health Professionals Follow-up Study (n=44,581 men; 24 years of follow-up), Nurses' Health Study (n=73,154 older women; 26 years of follow-up), and Nurses' Health Study II (n=91,509 younger women; 16 years of follow-up). We used Cox proportional hazards regression to generate hazard ratios (HRs) adjusted for age, race, BMI, dietary, lifestyle, and medical factors.

**Results:** We identified a combined total of 6361 incident cases of kidney stones during 4,411,939 person-years of follow-up. After adjusting for potential confounders, the multivariable HRs (95% confidence interval (CI)) for kidney stone formation for individuals in the highest quintile of diet-estimated NEAP compared to those in the lowest quintile were 1.59 (1.20 to 2.10; p for trend=0.002) for men, 1.48 (1.11 to 1.99; p for trend =0.05) for older women, and 1.53 (1.18 to 1.98; p for trend=0.007) for younger women. After adjusting for potential confounders, the multivariable HRs (95% CI) for kidney stone formation for individuals in the highest quintile of diet-estimated PRAL compared to those in the lowest quintile were 1.54 (1.19 to 1.98; p for trend=0.003) for men, 1.08 (0.84 to 1.40; p for trend=0.49) for older women, and 1.30 (1.04 to 1.62; p for trend=0.04) for younger women.

**Conclusions:** Higher dietary acid load, especially as measured by diet-estimated NEAP, is independently associated with a higher risk of kidney stone formation.

*Funding:* NIDDK Support, Private Foundation Support

**TH-PO787**

**Lime Powder Regimen Is a Novel Alternative for Medical Treatment of Nephrolithiasis** Piyaratana Tosukhowong,[1] Chanchai Boonla,[1] Thasinas Dissayabutra,[1] Kriang Tungsanga.[2] *[1]Biochemistry, Chulalongkorn Univ, Pathumwan, Bangkok, Thailand; [2]Medicine, Chulalongkorn Univ, Pathumwan, Bangkok, Thailand.*

**Background:** Lime powder regimen (LPR) is a limeade-based regimen containing active pharmaceutical components of 21 mEq potassium and 63 mEq citrate. Our preclinical and phase 1 studies showed LPR was a safe regimen for daily consumption and delivered the citraturic, urine alkalinizing and antioxidative effects.

**Methods:** 84 nephrolithiasis patients were recruited post-operative stone-free condition, and were randomly allocated into two treatment arms. Eight patients (10%) were dropout, then 33 were allocated into LPR and 32 in placebo group. Urinary citrate, oxalate and uric acid were measured by enzymatic method, total antioxidant status (TAS) by ABTS, Sodium, potassium, magnesium and calcium by ICP-OES.

**Results:** Age and gender distribution between LPR and placebo groups were not significantly different. In LPR group, urinary citrate and pH were significantly increased, while urinary calcium, oxalate and uric acid were reduced, after 6-month treatment compared to the pre-treatment baseline. In placebo group, urinary calcium, uric acid and volume were significantly reduced while urinary citrate trend to reduce after the 6-month treatment. Urinary total antioxidant status was significantly increased for both LPR and placebo treatments. Plasma protein carbonyl content was significantly reduced only in the LPR group. No adverse effect was found in both groups.

**Conclusions:** LPR was warranted to be a safe regimen in the nephrolithiasis patients. The current data indicated that 6-month LPR treatment delivered citraturic, urine alkalinizing and antioxidative effects in nephrolithiasis patients. These medicinal effects potentially reduce the propensity of kidney stone recurrence. Therefore, the phase 3 clinical trial needs to be further conducted to actually evaluate the efficacy of LPR treatment for reducing the recurrence rate.

*Funding:* Government Support - Non-U.S.

**TH-PO788**

**Trend of Incident and Prevalent Kidney Stone Disease in a Large Health Care Organization 1997-2007** Jie Tang,[1,3] Angela Keniston,[1] John R. Holmen,[2] Michel Chonchol.[3] *[1]Medicine, Denver Health Medical Center, Denver, CO; [2]Intermountain Health Care, Murray, UT; [3]Medicine, Univ of Colorado, Denver, CO.*

**Background:** Recent epidemiological trend of kidney stone (KS) disease is unclear. We aim to determine the KS incidence and prevalence trends through time in an integrated healthcare system serving a defined geographical region.

**Methods:** Incident and prevalent kidney stone (IKS & PKS) rates were obtained using Intermountain Healthcare (IHC) Data Warehouse. IHC is a healthcare organization serving 2.4 million Utah and Idaho residents. IKS formers were defined as patients having first ever diagnosis of KS disease and were identified by ICD9 codes 592 & 594.0 assigned from 1997 to 2007. PKS formers were defined as patients having prior ICD9 codes 592, 594.0 or V13.01. Negative binomial regression analyses were used to examine the incidence and prevalence trends.

**Results:** 53,045 IKS formers and 342,150 PKS formers were identified. Adjusted for age, gender, race and imaging use, there were no significant changes in KS incidence rates from 1997 (0.49%) to 2007 (0.52%), p=0.2. However, the adjusted KS prevalence rates increased significantly from 1997 (1.45%) to 2007 (4.9%), p<0.0001. For men, age, race and imaging use adjusted incidence rates did not change significantly from 1997 (0.67%) to 2007 (0.63%), p=0.9, but the adjusted prevalence rates increased significantly from 1997 (1.98%) to 2007 (6.15%), p<0.0001. For women, the adjusted incidence rates did not change significantly from 1997 (0.35%) to 2007 (0.44%), p=0.3, but the adjusted prevalence rates increased significantly from 1997 (1.04%) to 2007 (3.93%), p<0.0001. Age, gender and imaging use adjusted incident rates did not change significantly in any of the race categories from 1997 to 2007. However, the adjusted prevalence rates increased significantly in all race categories (p<0.0001) except in African Americans (p=0.06) from the same time period.

**Conclusions:** Even though IKS rates did not change from 1997 to 2007, the PKS rates increased significantly. The causes of these findings remain to be determined.

*Funding:* NIDDK Support

**TH-PO789**

**Adenine Phosphoribosyltransferase Deficiency: Two Novel Genetic Mutations and United Kingdom Experience** Gowrie Balasubramaniam,[1] Monica Arenas Hernandez,[2] Lynette D. Fairbanks,[2] Anthony Marinaki,[2] Emilia Escuredo Polo,[2] Sarah Mapplebeck,[1] Michael K. Almond.[1] *[1]Southend Univ Hospital, Southend, Essex, United Kingdom; [2]Purine Research Laboratory, Guy's and St. Thomas' NHS Trust, London, United Kingdom.*

**Background:** Adenine phosphoribosyltransferase (APRT) deficiency causes 2,8-dihydroxyadenine (2,8-DHA) crystals and renal failure. We present three cases with two novel mutations from a single centre. UK data of APRT is limited; we undertook a cross-sectional survey of patients identified at the Purine Research Laboratory.

**Methods:** An 18-year old man of presented with a history of recurrent nephrolithiasis. 2,8-DHA stones were identified by UV spectrophotometry. 11 years after his initial symptoms, he re-presented with chronic kidney disease stage (CKD) 4. His younger brother presented with loin pain and acute kidney injury. Renal biopsy showed an acute tubulointerstitial nephritis with crystalline deposits. A 48-year man with a history diabetes and hypertension presented with an acute on chronic kidney injury. Renal biopsy showed minimal diabetic features but chronic tubulointerstitial nephritis with crystalline deposits. We sent questionnaires to obtain clinical information of the identified cases between 1979-2013.

**Results:** All three cases had 2,8-dihydroxadenine in urine and complete APRT deficiency. Genetic studies identified a homozygous novel mutation in the APRT gene from Case 1 and 2; APRTc.543 A>T, p.181X>C, and case 3 APRTc.380a>G, p.127D>G. We identified only 17 patients (12 M) from 14 families. Mean age at diagnosis was 26 (range 2-70). The commonest reason for testing was nephrolithiasis (11/17). Outcome information was not available in 2 patients (1 had ESRD). Of the remaining 15 patients; 3 patients were deceased (1 had ESRD); 8/12 had normal renal function and 4/12 had CKD.

**Conclusions:** APRT deficiency is a rare disease with varied presentation that is currently under-diagnosed in the UK. Stone analysis and family screening are important ways to identify cases. Diagnosis should be sought if unexplained crystalline deposits are found on renal biopsy. It is easily treatable and a more concerted approach should be undertaken to diagnose patients in the UK.

**TH-PO790**

**Profound Life Threatening Hypokalemia as a Manifestation of Topiramate Induced Renal Tubular Acidosis** Osama W. Amro, Narittaya Varothai, Anita Abhyankar. *Nephrology, Tufts Medical Center, Boston.*

**Background:** Topiramate is used for migraine prophylaxis and seizure disorders. One potential side effect is the development of renal tubular acidosis (RTA). We are reporting a case of Topiramate induced RTA that presented with profound hypokalemia.

**Methods:** A 29 year old lady with history of Migraine presented with muscle aches and generalized weakness, patient had no fever, diarrhea, nausea, or vomiting. Physical examination revealed an obese lady in a euvolemic state with no focal neurological signs. Initial studies showed serum K of 1.7, PO4 2.2, HCO3 16, Cl 107, Na 137, CK 5000. ABG showed PH of 7.37, PCO2 28, PO2 105 and HCO3 19. Urinalysis showed a PH of 6.5 and was otherwise unremarkable. Urine anion gap was negative and the TTKG was 8. Renal ultrasound showed 8 mm calculus in the right renal pelvis. The patient received a total of 600 mEq KCL and serum K normalized. The patient's historical blood tests revealed chronic hypobicarbonatemia and hypokalemia for the last 2 years with levels between 17 to 21 and 2.7 to 3.3 respectively. The 2 year period correlates with the time in which Topiramate was initiated (100 mg twice daily for migraine prophylaxis). The patient was taken off Topiramate and a repeat blood level done in 48 hours showed normalization of serum bicarbonate and K levels. Repeat urinalysis showed a urine PH of 5.5. Muscle weakness and rhabdomyolysis resolved.

**Conclusions:** Topiramate can induce mixed RTA picture with features of RTA 1 and 2. This can be explained by the ability of Topiramate to inhibit Carbonic Anhydrase (CA) II. CA II plays an essential role in the reabsorption of HCO3 by the proximal tubule and the urinary acidification by the distal tubule. In our patient Proximal RTA is suggested by non-anion gap metabolic acidosis with negative urine anion gap. The presence of high urine PH in the steady state along with nephrolithiasis are features of distal RTA. The development of metabolic acidosis at the time Topiramate initiation and its resolution following discontinuation of the treatment confirms the diagnosis of Toperimate induced RTA. The case emphasizes the importance of electrolytes monitoring following initiation of Topiramate.

**TH-PO791**

**Extreme Fluctuation of Serum Potassium Level during Hypothermia Induction** Osama W. Amro, Geetha Narayan. *Nephrology, Tufts Medical Center, Boston, MA.*

**Background:** Induction of hypothermia following cardiac arrest is being used to decrease the risk of permanent neurological damage. This method is usually safe but it carries few potential complications including hypokalemia. Hypokalemia is related to intracellular potassium shift. We are reporting a case of extreme fluctuation of serum K level before and after induction of hypothermia.

**Methods:** A 27 year old previously healthy male presented with general weakness to the ER and had witnessed cardiac arrest. Fortunately he was revived with prompt CPR. Initial blood work showed K of 6.4, Cr 2.2, CK of 4000. Repeat electrolytes confirmed serum K level. Following the cardiac arrest patient became anuric mostly secondary shock. Hypothermia was induced and upon reaching target temperature of 32 degree repeat electrolytes showed k level of 2.3. Patient started to develop multiple premature ventricular beats and was given 40 meq KCL IV. Cautions repletion of K was chosen to avoid rebound hyperkalemia following rewarming. No further K supplements were given and repeat K level during rewarming phase showed k levels between 4-5.5. Next day patient urine output and kidney function improved and serum K level remained normal.

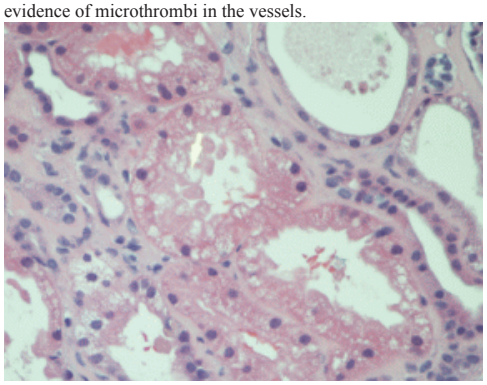
**Conclusions:** Severe hypokalemia may be seen in hypothermic patients, which represents intracellular potassium shift rather than a true depletion. Cautious repletion of hypokalemia is necessary to avoid fatal arrhythmia from extreme hypokalemia or hyperkalemia. Hyperkalemia can occur during rewarming phase. The case we are reporting illustrates the importance of carful electrolytes monitoring during and after induction of hypothermia. In our patient we encountered extreme fluctuation of serum potassium level during hypothermia induction. Potassium shift is the only mechanism explaining fluctuation of serum k level in the case we are reporting as the patient was anuric and had no diarrhea.

## TH-PO792

**Hypokalemic Nephropathy: Rare but Reversible** Rohini Prashar, Sandeep Vetteth, Deepak K. Malhotra, Imran Shafique. *Univ of Toledo Medical Center, Toledo, OH.*

**Background:** Chronic hypokalemia is well known to be associated with renal insufficiency and tubulointerstitial disease. We describe a case of progressive renal failure in a patient with thrombotic thrombocytopenic purpura which was initially thought to be thrombotic microangiopathy but eventually proven to be hypokalemic nephropathy from prolonged hypokalemia, successfully treated by correcting serum potassium.

**Methods:** A 50-year-old female was referred to us for evaluation of progressive increase in serum creatinine from 0.8 mg/dL to 3.7 mg/dL over the last two years. She had a history of thrombotic thrombocytopenic purpura diagnosed 14 years ago, which had been in remission for 4 years, and hypertension, well controlled with hydrochlorothiazide for 5 years. Her blood pressure was 105/65 mm Hg and physical examination was unremarkable. Review of old records revealed that she had been persistently hypokalemic (2.4 meq/l) and hypomagnesemic (1 meq/l) for the last 4 years. Other workup for chronic kidney disease was negative. She had high urinary potassium loss, which persisted even after hydrochlorothiazide was held. Kidney biopsy showed tubular vacuolation and chronic interstitial nephritis consistent with hypokalemic nephropathy. Interestingly, there was no evidence of microthrombi in the vessels.



Her renal function markedly improved with correction of potassium (3.8 meq/l), and creatinine was 2.1 mg/dl within 2 months of follow up.

**Conclusions:** We hypothesize that our patient had nephropathy from severe hypokalemia from the use of thiazides. Late-onset Gitelman's syndrome is also a strong possibility, which might have clinically manifested following the use of hydrochlorothiazide. Our case not only highlights the importance of obtaining renal biopsy in patients with unexplained renal failure, but also emphazises the need for monitoring electrolytes in patients on chronic diuretic therapy.

## TH-PO793

**Refractory Hypokalemia with Sustained Ventricular Tachycardia as the Presenting Sign of Metastatic Cancer** Sajan K. Eapen, Ruchir D. Trivedi. *Nephrology, Univ of Connecticut Health Center, Farmington, CT.*

**Background:** Hypokalemia usually has a readily apparent etiology. However, it can be the first sign of a complex process. We present a patient with refractory hypokalemia in the setting of metastatic disease.

**Methods:** An 84 year old female was repeatedly defibrillated by her AICD. Serum potassium (K+) level was 1.9meq/L and bicarbonate ($HCO3^-$)level 41 meq/L. Physical examination revealed blood pressure of 170/76 mmHg, obesity, and bilateral pitting edema. Urine specific gravity was 1.020, pH 7, otherwise negative for protein or active sediment. Relevant medication use included daily loop diuretics as well as laxatives and calcium citrate. Despite cessation of these medications, her metabolic alkalosis and hypokalemia persisted. 24 hour urine had shown K+ loss of 216meq/day. Mineralocorticoid excess was considered and workup was undertaken. Serum cortisol was 127 µg/dl (3-25). Serum aldosterone ranged from 7.9 - 11.6ng/dl (0 - 44 ng/dl). High-dose dexamethasone suppression test revealed cortisol levels of 138, 126, 119 µg/dl at 0, 30, 60 minutes, respectively. Urinary cortisol measured 15158 µg/d (n- <45µg/day). Serum adrenocorticotropic hormone (ACTH) level was measured at 192 pg/ml (6 -58pg/ml) suggestive of an ACTH mediated process. Imaging studies revealed the presence of multiple lung nodules consistent with metastatic disease as well as a pancreatic mass. Ectopic ACTH syndrome (EAS) was determined to be the underlying disease process.

**Conclusions:** Ectopic ACTH syndrome led to severe hypercortisolism. The mineralocorticoid-like activity of cortisol led to profound hypokalemia as well as metabolic alkalosis and volume overload, mediated both by distal hydrogen secretion as well as sodium reabsorption. EAS is one possible diagnosis of hypokalemia and metabolic alkalosis since similar inherited and acquired pathologies exist. A structured approach is necessary to reveal the underlying diagnosis.

## TH-PO794

**Metformin-Associated Lactic Acidosis in an Alcoholic with Fluorescent Urine** Ian Matthew Rivera, Jorge I. Martinez Osorio, Christina M. Yuan, Dustin J. Little. *Dept of Medicine, Nephrology Section, Walter Reed National Military Medical Center, Bethesda, MD.*

**Background:** Lactic acidosis is a rare but serious complication of metformin use. Patients with comorbidities such as renal insufficiency or liver disease may be at increased risk.

**Methods:** A 63 y.o. diabetic male with a history of alcoholism presented to the ED with abdominal pain. He was hypothermic (31°C) and lethargic but alert with otherwise stable vitals. Initial labs showed serum creatinine 2.64 (baseline 1.0mg/dL), glucose 29mg/dL, arterial pH 6.71, serum bicarbonate 2meq/L, PaCO2 16.5mmHg, lactate 28.5mmol/L, anion gap 45mmol/L, osmolar gap 20, AST/ALT/AlkPhos 396/140/133 U/L, PT 17.1 (11.6-14.2), Tbili 0.4mg/dL, and MCV 109fL. The patient's urine fluoresced brightly under ultraviolet light. Hemodialysis was initiated (only 1 treatment required, 6 hrs duration), and one dose of 4-methylpyrazole was administered. Serum levels of ethanol, toxic alcohols, salicylates, and acetaminophen were normal/negative. Carnitine deficient transferase and urine ethanol metabolites suggested recent heavy ethanol consumption. Serum metformin was 4.7 (therapeutic 1-2µg/mL). The patient's labs normalized and he required no further hemodialysis. He was discharged on hospital day 6. Urine fluoresced for the remainder of his hospital stay, but not on follow-up.

**Conclusions:** This patient survived following prompt initiation of supportive care and hemodialysis. His alcoholism may have predisposed him to metformin-associated lactic acidosis through associated B vitamin deficiencies and liver dysfunction and increased NADH generation by alcohol dehydrogenase. His osmolar gap was likely secondary to AKI and lactic acidosis, and the etiology of his urine fluorescence is unknown. This case suggests that providers should consider alternatives to metformin in patients at risk for alcohol abuse, and highlights the nonspecific nature of urine fluorescence.

"Disclosure: The views expressed are those of the authors and do not reflect the official policy of the Department of the Army, the Department of the Navy, the Department of Defense, or the United States government."

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## TH-PO795

**Pseudoacidosis after Cardiac Catheterization** Annamalai Arunachalam, Shreya Patel, Sreedhar A. Mandayam. *Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** Metabolic acidosis is a common problem in hospitalized patients. It is usually detected by a lowering of serum bicarbonate level or an accompanied increase in anion gap. The ADVIA chemistry CO2 concentrated Reagan method used to measure serum CO2 levels in venous blood is affected by hyperlipidemia. To minimize this interference, labs use "Lipoclear Centrifuge Method".

It is unclear other substances also interfere with this method. We report a case of spuriously low CO2 levels in a patient after exposure to contrast agent.

**Methods:** 50 year old gentleman with a history of hypertension, diabetes mellitus, hyperlipidemia was admitted with chest pain. BMP on admission was normal, triglyceride levels 507 mg/dL. Cardiac catheterization was performed. Next day bicarbonate level were less than 10. Arterial blood gas showed ph of 7.41, pC02 41 and Hco3 of 26. The BMP and the ABG were repeated twice with similar results. Nephrology & Clinical Chemistry were consulted. Next day BMP showed normal bicarbonate levels and patient was discharged.

| | admission | precath | d1 post cath | repeat | day 2 |
|---|---|---|---|---|---|
| Creatinine | 1.07 | 0.8 | 0.93 | 0.78 | 0.99 |
| HCO3 | 22 | 25 | <10 | <10 | 27 |
| pH | | | 7.41 | 7.43 | |
| pCO2 | | | 40 | 39 | |
| HCO3 (ABG) | | | 25 | 25 | |

**Conclusions:** ADVIA chemistry CO2 concentrated Reagan method is based on phosphoenolpyruvate carboxylase catalyzed reaction followed by indicator reaction which uses malate dehydrogenase as a catalyst for the oxidization of the reduced NADH analog. Lipemic samples alter bicarbonate levels. Thus the Lipoclear centrifuge method is used. In our patient, bicarbonate levels were normal on admission but turned falsely low for 24 hours despite using the lipoclear centrifuge method prior to running the sample via the routine chemistry analysis. This was repeated twice under supervision. Within 24 hrs, the BMP normalised. As his ABG did not show any evidence of acidosis during his entire stay & his CO2 returned to normal in 24 hrs, we hypothesize that Radiocontrast agent used for heart cath (Omnipaque 350: 90% cleared 24 hrs) interfered with the ability of Lipoclear to clear the serum & lead to an abnormally low CO2 level. Spuriously low venous CO2 levels can be seen in patients with hyperlipidemia after contrast study for 24 hours.

## TH-PO796

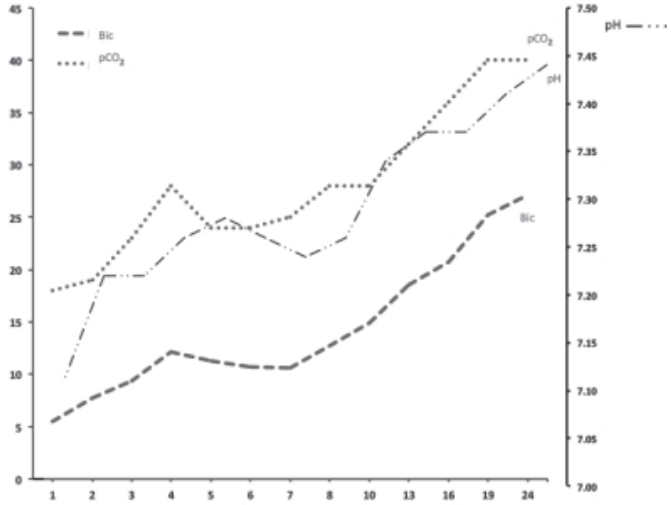
**Severe Ketoacidosis in a Patient with Spinal Muscular Atrophy Type II** Peter Bertke, Spyridon Arampatzis. *Dept of Nephrology, Hypertension and Clinical Pharmacology, Inselspital, Uni. of Bern, Bern, Switzerland.*

**Background:** We describe a patient with spinal muscular atrophy type II resulting in severe ketoacidosis due to starvation.

**Methods:** A 23-y/o Caucasian male with muscular atrophy type II underwent an arm-amputation due to rhabdomyosarkoma. Subsequently, a worsening, severe metabolic acidosis was noted. Laboratory findings included:blood-pH 7.15, Bic:4.6mEq/l, $pCO_2$:14mmHg, Anion-Gap:15mmol/l, creatinine:0.07mg/dl, urea:8.4mg/dl, ketonuria>15mmol/l. The

patient, was fasting overnight due to secondary surgery planned the following day. Other causes being excluded, a diagnosis of starvation-ketoacidosis (SK) was made. Correction by Na+[/sup]-bicarbonate and glucose infusions normalized the severe metabolic disorder within 24h. After starting enteral feeding and despite i.v. substitution of K+[/sup] and phosphate, a transient refeeding-syndrome dyselectrolytemia was corrected. Ketonuria instantly disappeared.

| | Initial | 12h | 15h | 18h | 21h | 24h | Units |
|---|---|---|---|---|---|---|---|
| Phosphate | 0.73 | 0.30 | 0.32 | 0.79 | 1.23 | 1.83 | mmol/l |
| Calcium | 0.81 | | 1.9 | 1.88 | 1.84 | 1.75 | mmol/l |
| Potassium | 3.3 | 3.4 | 3.2 | 3 | 3.5 | 4.9 | mmol/l |




**Results:** The diagnosis of SK was on the rapid therapeutic response without further preexisting conditions.The remarkable low serum creatinine was due to spinal muscular atrophy which could also have aided to the reduced capability of providing energy supplies other than by lipolysis further worsened by the release of excess stress hormones given the surgical intervention.

**Conclusions:** SK should be considered in the differential diagnosis of severe metabolic acidosis. Correction of SK exacerbated by stress need careful monitoring due to the potential development of refeeding-related disturbances.

## TH-PO797

**Acute Severe Lactic Acidosis Associated with Venlafaxine** Chaitanya Iragavarapu, Tanush Gupta, Treta Purohit, Savneek S. Chugh. *Internal Medicine, New York Medical College.*

**Background:** Lactic Acidosis that is not secondary to tissue hypoperfusion or hypoxemia (Type B lactic acidosis) is a rare but potentially fatal condition that has been associated with drugs like Metformin, Linezolid and Nucleoside Reverse Transcriptase Inhibitors (NRTIs). Here, we report a case of severe type B lactic acidosis caused by overdose of a Serotonin-Norepinephrine Reuptake Inhibitor (SNRI), Venlafaxine, which to our knowledge has not been published in current literature.

**Methods:** A 55 year old man with no significant past medical history was brought to our emergency room by the police after intentional ingestion of around eighty capsules of Venlafaxine (a total dose of over 6000 mg) in an attempt to commit suicide. He was awake and alert; his only complaint being mild nausea. On admission, he had a blood pressure of 168/101, pulse rate 115, respiratory rate 16/min and saturating 97% on room air. Physical examination was otherwise unremarkable. Pertinent laboratory findings at admission included bicarbonate of 13 mEq/L with an anion Gap of 22. Other results including complete blood count with differential, blood urea nitrogen, creatinine, creatine kinase and hepatic function panel were normal. Arterial blood gas revealed a pH of 7.39, a $pCO_2$ 19 mm Hg, p02 119 mmHg, calculated bicarbonate 11.5 mEq/L and lactate level of 8.6 mmol/L. He received aggressive intravenous hydration with normal saline along with oral Charcoal and Sorbitol. Repeat blood work after four hours showed an improvement in anion gap (15) and serum lactate (5.6 mmol/L). Patient remained asymptomatic and hemodynamically stable throughout his hospital stay and his lactic acidosis resolved in 24 hours.

**Conclusions:** Type B Lactic acidosis is due to impairment in lactic acid generation and/or utilization at the cellular level. In our case, venlafaxine was the most likely cause of lactic acidosis given the absence of hypotension, hypoxemia, kidney or liver dysfunction, myopathy, malignancy or other medication use. Rapid improvement of acidosis was probably related to clearance of the drug. Venlafaxine is a commonly prescribed medication and physicians should be aware of this potentially lethal consequence from overdose.

## TH-PO798

**Mind the Gap: A Subtle Clue as a Diagnostic Aid** Prashant Kolar. *Nephrology, Univ of Missouri, Columbia, MO.*

**Background:** We present a case of unknown alcohol intoxication with elevated anion and osmolal gap. Patient had a blood lactate level that was significantly higher on arterial blood gas (ABG) compared to serum, suggesting an apparent lactate gap (LG). This finding is an indirect marker of ethylene glycol poisoning which can be a helpful clue, especially in patient with mixed alcohol intoxications. Knowledge of artifactual elevation of lactate is helpful in avoiding unnecessary expensive work up for lactic acidosis including a potential laparotomy for possible bowel ischemia.

**Methods:** A 65 y/o male on gastroenterostomy tube feeding for previous complicated cholecystectomy, was brought to hospital with unsteady gait & shortness of breath. He had respiratory distress without hypoxemia, requiring mechanical ventilation. Lab data showed serum lactate level of 6.6 mmol/L, but the lactate on ABG using auto analyzer showed value of 12.8 mmol/L. This suggest apparent lactate gap, seen in ethylene glycol overdose. The measured ethylene glycol level was 44mg/dl.

**Conclusions:** The metabolites of ethylene glycol, glycolic acid and glyoxylic acid can cross react with the analytical reagent L-lactate oxidase, used in many blood gas analyzers(1). Other methods using lactate dehydrogenase may not be affected by these metabolites(2). Thus finding a "lactate gap" using 2 different methods can help to differentiate ethylene poisoning from true lactic acidosis. Interestingly in the setting of potential ethylene glycol poisoning, the use of the lactate oxidase may be a simple method to "monitor" level of metabolites(3). Treatment with dialysis can be stopped, once the gap disappears. Knowledge of potent artifactual lactate results was the clue to the diagnosis of ethylene glycol poisoning in our case but the mode and motive of intoxication remained an enigma.

1. Shirey T, Sivilotti M. Reaction of lactate electrodes to glycolate. Crit Care Med 1999;27:2305-7; 2. Graïne H, Toumi K, Roullier V, et al. Interference of ethylene glycol on lactate assays. Ann Biol Clin 2007;4:421-4; 3. Porter WH, Crellin M, Rutter PW, et al. Interference by glycolic acid in the Beckman synchron method for lactate: a useful clue for unsuspected ethylene glycol intoxication. Clin Chem 2000;46:874-5.

## TH-PO799

**Two Cases of Acute Renal Injury and Fatal Lactic Acidosis due to Germanium Ingestion as a Supplement** Ikuyo Narita, Michiko Shimada, Takeshi Fujita, Yuko Shimaya, Reiichi Murakami, Norio Nakamura, Hideaki Yamabe, Ken Okumura. *Nephrology, Hirosaki Univ, Hirosaki, Japan.*

**Background:** Germanium supplements have been used as elixir. However, serious adverse effects have been rarely reported. Here, we describe 2 cases of fatal lactic acidosis occurred in a married couple.

**Methods:** Case 1: A 59-year-old female who had been suffering with erythematous rashes for 1 month and gastrointestinal symptoms for 2 weeks visited a clinic and laboratory studies revealed renal dysfunction. She was consulted to nephrology and admitted to our hospital. Her blood pressure was 78/50 mmHg, heart rate was 110/min and her conscious was clear on admission. Laboratory data were as follows:WBC 5640/μl, Hb 14.9g/dl, Plt $6.0x10^4$/μl, BUN 27 mg/dl, serum creatinine 2.5 mg/dl, AST 100 IU/l, ALT 102 IU/l, CPK 6464 IU/l, CRP 16.2 mg/dl. Blood gas analysis: pH7.41, $pO_2$ 95 mmHg, $pCO_2$ 18 mmHg, $HCO_3$ 11.3 mmol/l, lactic acid 10.7 mmol/l. On the 2nd hospital day, she was admitted to the intensive care unit and continuous hemodiafiltration was iniciated. Her general conditions were exacerbated due to low blood pressure, liver damage, renal failure, respiratory distress and disseminated intravascular coagulation. Lactic acidosis was progressively exacerbated and the data finally became pH 6.8 and lactic acid 22 mmol/l. She died on the 6th hospital day. Case2: A 65-year-old male who is the husband of case 1 was hospitalized 2 days after the admission of his wife due to the similar symptoms. His general conditions and lactic acidosis were similarly exacerbated and he died on the 8th hospital day. In both cases, intensive search for infection revealed no positive results. There was no evidence of illicit drug use. However, they had been taking supplements containing germanium for 10 years. Analysis of the hair and nail in both cases revealed very high germanium concentrations. The autopsy of case 1 revealed severe fatty degeneration of the liver and renal tubular epithelial cells. Pellagrous changes were seen in the brain.

**Conclusions:** The symptoms in these cases were compatible with the previous reports of germanium intoxication. These symptoms suggested profound mitochondrial dysfunction.

*Funding:* NIDDK Support

## TH-PO800

**High Anion Gap Metabolic Acidosis and Chronic Acetaminophen Use: An Under Recognized Association** Olutayo T. Olabige, Mamoon Rasheed, Ewalola A. Ijaduola, Sudhanshu Jain, Leroy Herbert, Herman L. Anderson, Jeffrey D. Wallach. *Harlem Hospital Center, New York, NY.*

**Background:** High anion gap metabolic acidosis (HAGMA) is a severe metabolic disorder with diverse causes including lactic acidosis, ketoacidosis, metabolites from various toxic substances (methanol, glycols or paraldehyde) and renal failure. Accumulation of 5-oxoproline (pyroglutamic acid) is another rare and underdiagnosed cause of HAGMA. Chronic acetaminophen ingestion causes depletion of glutathione in malnourished patients (via glycine deficiency) leading to disruption of the gamma-glutamyl cycle with accumulation of 5-oxoproline.

**Methods:** A 55-year old African American male with history of metastatic lung cancer, hypertension, diabetes mellitus, congestive heart failure, atrial fibrillation,and chronic pain presented with worsening dyspnea. Home medications included acetaminophen/codeine and aspirin. Initial ABG showed a pH 7.32, pO2 78mmHg, pCO2 18mmHg HCO3 9mmol/L. He was intubated and placed on mechanical ventilation. Electrolytes showed Na 143 K 4.2 Cl 108 HCO3 11 anion gap(AG) 24. BUN 12mg/dL, creatinine 0.8mg/dL, glucose 108mg/dl, serum osmolar gap 2, AST/ALT normal, and albumin 2.2g/dL. Salicylate 18mg/dL, lactate 2.0mmol/L. Serum acetone was positive initially and rapidly cleared with no improvement in HAGMA. Urine AG +92. Patient developed septic shock and oliguric acute kidney injury

(AKI) requiring continuous renal replacement therapy. Acetaminophen was discontinued, N-acetylcysteine empirically started to replete glutathione stores and his HAGMA and AKI resolved within 72 hours.

**Results:** Urine organic acid assay was positive for 5-oxoproline at concentration of 256 mmol/mol creatinine (NL 15-59), homovanillic acid 275 mmol/mol creatinine (NL 1-5). Glycolic acid and other organic acids were normal.

**Conclusions:** 5-oxoproline accumulation is an increasingly recognized cause of HAGMA. It should be considered in patients with chronic acetaminophen use, especially in the context of malnutrition and multiple comorbidities. A high index of suspicion is necessary to make an accurate diagnosis and institute appropriate supportive therapy. A urinary organic acid screen can confirm the diagnosis rapidly.

## TH-PO801

**Renal Fanconi's Syndrome and Hypophosphatemic Osteomalacia Induced by Low-Dose Adefivir Dipivoxil Therapy for Hepatitis B** Rikako Hiramatsu, Yoshifumi Ubara, Keiichi Sumida, Junichi Hoshino, Tatsuya Suwabe. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

**Background:** Adefovir dipivoxil (ADV) at a daily dose of 10 mg is commonly used as an antiviral agent to treat chronic hepatitis B. The safety of a daily dose of 10 mg ADV is advocated by the registration trial. We present here two cases of renal Fanconi's syndrome together with hypophosphatemic osteomalacia induced by low-dose ADV in chronic hepatitis B (CHB) patients.

**Methods:** The first patient was a 67-year-old woman who complained of severe bone pain involving her knees and ankles. She had a history of CHB, and had been treated with ADV for the past 53 months. Laboratory tests showed severe hyphophosphatemia (serum P; 0.8 mg/dL) with urinary phosphate wasting, glucosuria, aminoaciduria, metabolic acidosis, and serum alkaline phosphatase (ALP) elevated to 819 IU/L. Serum creatinine and 1,25-hydroxyvitamn D3 level were normal. A whole bone scan showed intense uptake in both knees, ankles and bilateral ribs.

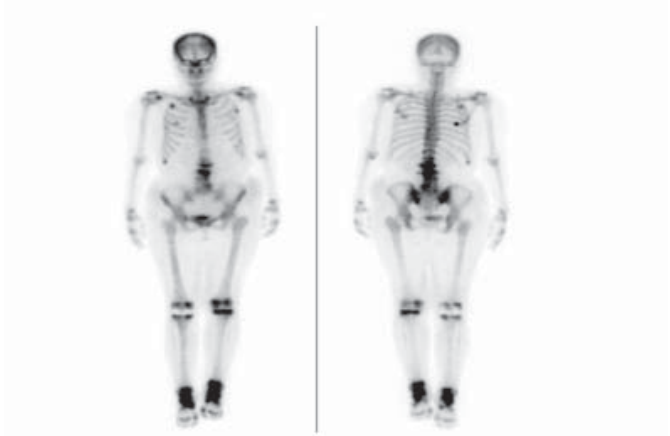


Fig. 1

The second patient was a 65-year-old woman with CHB who complained of pain in several bilateral ribs and ankles. She had been treated with ADV for 56 months before occurrence of bone pain. She was also found to have hyphophosphatemia (serum P; 1.2 mg/dL) with proximal renal tubule dysfunction and elevation of serum ALP. In both cases, bone biopsy revealed osteomalacia. After discontinuation of ADV and phosphate supplementation, their symptoms resolved, laboratory abnormalities normalized and imaging studies showed improvement in both patients.

**Conclusions:** Despite large clinical trials advocating the safety of ADV at 10 mg daily, long-term use of this agent can be nephrotoxic and in rare cases, can cause renal Fanconi's syndrome and severe hypophosphatemic osteomalacia. Clinicians should be aware of this infrequent but severe complication of low-dose ADV in CHB treatment.

## TH-PO802

**Hydrochloric Acid to Treat Extreme Metabolic Alkalosis** Jose Jesus Perez, Rajeev Raghavan. *Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** Anorexia-bulimia nervosa are eating disorders that are associated with electrolyte and acid-base disturbances. Extreme alkalosis with a pH greater than 7.65 has a high risk of mortality. We present a bulimic patient with such alkalemia and our subsequent medical management.

**Methods:** A 27-year old female presented to the ER with complaints of nausea, vomiting, weakness, palpitations, and abdominal pain. She denied having an eating disorder, and did not take medications. Within an hour of presentation, she developed ventricular tachycardia requiring cardioversion. Initial resuscitation included 2 liters of normal saline, 2 grams magnesium sulfate, 4 grams calcium gluconate and 20 meq of KCl. She continued to have ventricular arrhythmias including Torsades de Pointes. On exam, blood pressure 96/50 mm Hg, pulse rate 84 beats/min, respiratory rate 20 breaths/min, temperature 98.1°F, weight 90 lbs. The patient was thin, had poor skin turgor, dry mucous membranes, with enamel erosions.

| | Time 0 | 2 hours | 4 hours | 10 hours |
|---|---|---|---|---|
| Sodium (mmol/L | 109 | 110 | 118 | 120 |
| Potassium (mmol/L) | 2.1 | 1.8 | 2.3 | 3.5 |
| Chloride (mmol/L) | <50 | <50 | 65 | 78 |
| CO2 (mmol/L) | >45 | 43 | 35 | 27 |
| BUN (mg/dL) | 12 | 11 | 10 | 10 |
| Creatinine (mg/dL) | 0.6 | 0.6 | 0.5 | 0.6 |
| Calcium (mg/dL) | 9.1 | 8.5 | 16 | 12.8 |
| Phosphorous (mg/dL) | 1.8 | 1.4 | 1.9 | 6 |
| Magnesium (mg/dL) | 2.4 | 1.9 | 8.5 | 3.6 |
| pH | - | 7.89 | 7.71 | 7.60 |
| pCO2 (mmHg) | - | 32 | 25.5 | 40.4 |
| pO2 (mmHg) | - | 399 | 217 | 222 |

The patient had persistent metabolic alkalosis with cardiac instability. Since her serum sodium had corrected by 9 mmol within 4 hours and she had been adequately volume resuscitated, we were hesitant to give normal saline and proceeded to treat with intravenous hydrochloric acid. Preparation of infusion was made with 7.5ml of 10% HCl in D5W infused at 50cc/hr, providing a total of ~21mEq of HCl. Electrolytes were aggressively repleted. Sedation was increased to achieve a target pCO2 of 40. The patient's family later reported a long history of anorexia-bulemia.

**Conclusions:** Patients with eating disorders may develop severe metabolic derangements that could potentially be life threatening. This vignette represents a case of extreme metabolic alkalosis in which the patient was successfully treated with hydrochloric acid and able to survive and recover.

## TH-PO803

**Vancomycin-Induced Nephrotoxicity: A Pitfall of Overestimating GFR for Drug Dosing by Using Serum Creatinine** Ekamol Tantisattamo, Jason Cobb, Tahsin Masud. *Renal Div, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** Widely used as a surrogate of overall renal function, serum creatinine (SCr) is one of the most practical markers of eGFR. We report a case of an amyotrophic lateral sclerosis (ALS) man with very low SCr presenting with vancomycin toxicity from an overestimation of eGFR.

**Methods: Case Description:** A 39 year-old Caucasian man with a history of ALS complicated with chronic respiratory failure and ventilator-dependence, was diagnosed with staphylococcal bacteremia and treated with vancomycin 1,500 mg every 12 hours. His baseline BUN and SCr was 3 mg/dL and <0.3 mg/dL, respectively. Serum albumin was 2.4 g/dL. His weight was 81 kg and BMI was 24.95 kg/m$^2$. Three days prior to admission, he had decreased urine output and lethargy. SCr was elevated to 2.98 mg/dL. Vancomycin level was supratherapeutic (>120 ng/ml 10 days after vancomycin was started). SCr continued to rise up to 3.43 mg/dL and vancomycin level was persistently high. Four days later, he was oliguric and was started on hemodialysis for 3 days. Vancomycin level decreased to therapeutic level 6 days after initiation of hemodialysis and SCr returned to baseline.

**Conclusions:** eGFR is relatively accurate for most patients; however, it cannot be applied to our patient with muscular atrophy from underlying ALS. His lean body mass (LBM) decreases while total body fat increases, leading to artificially decreased SCr. As volume of distribution of relatively hydrophilic drugs such vancomycin correlates very well with LBM, estimated LBM in the patients losing muscle mass should be taken into account when initiating vancomycin. LBM which determines SCr level needs to be considered in estimating GFR when dosing medications. Creatinine clearance from 24-hour urine collection may be warranted. Close monitoring of renal function and vancomycin level is crucial for early detection of vancomycin toxicity. Moreover, as high level of remaining vancomycin in the setting of acute kidney injury can perpetuate the toxicity, low threshold of initiating hemodialysis should be considered to mitigate the toxicity.

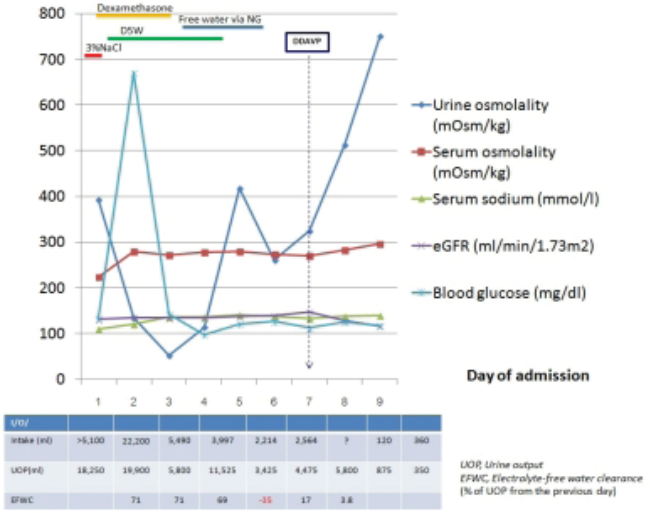
## TH-PO804

**Concomitant Polyuria and Hyponatremia in a Glucocorticoid Deficiency Patient with Transient Nephrogenic Diabetes Insipidus** Ekamol Tantisattamo, Frederic F. Rahbari-Oskoui, John Doran. *Renal Div, Grady Memorial Hospital, Emory Univ, Atlanta.*

**Background:** Hypernatremia and polyuria are classic manifestations of diabetes insipidus (DI). However, glucocorticoid can affect urinary dilution leading to hyponatremia. We report a man with corticosteroid deficiency presenting with symptomatic severe hyponatremia and transient partial nephrogenic DI.

**Methods: Case description:** A 27-year-old man with polysubstance addiction presented with seizures and was intubated. He took no medications. Serum sodium (SNa) was 109 mmol/l and serum osmolality (SO) was 224 mOsm/kg. Blood glucose (BG) was 133 mg/dl. Urine osmolality (UO) were 339 mOsm/kg and urine Na (UNa) was 76 mEq/L. 3% NaCl 30 ml/h was started. Ten hours later, SNa rose to 128 mmol/l and urine output (UOP) was 760 ml/hour. 3%NaCl was switched to D5W and the rate matched with UOP. SNa ranged 130-135 mmol/l and UOP was 18 L over 24 hours. Labs on the following days were shown in the figure. On day 5, D5W was discontinued and UOP decreased to 3 L/day. UO were 418 and SO was 279 mOsm/kg. He was intubated so primary polydipsia was excluded. Water deprivation test was not performed. Desmopressin was given. UO slightly rose to 472 and 481 mOsm/kg 1 and 2 hours after desmopressin administration, respectively. All pituitary hormones were normal except low cortisol of 2 mcg/dl. Dexamethasone was started. He was discharged on day 10 with normal SNa and no polyuria or neurological deficit.

**Conclusions:** Concomitant DI and hyponatremia can occur in glucocorticoid deficiency impairing urinary dilution via an ADH-independent mechanism. Apart from DI, replacement the UOP with D5W led to osmotic diuresis and subsequently persistent polyuria. Glucocorticoid deficiency should be considered as one of the causes of hyponatremia in DI. IV fluid need to be cautiously replaced to avoid prolonged polyuria from iatrogenic hyperglycemia.




## TH-PO805

**Anabolic Steroid-Induced Liver Injury and Cholemic Nephropathy**
Greg T. Means, Leybelis Padilla, Fernanda Payan Schober, Rachel Cianciolo, Marc Richards, Patrick H. Nachman. *Nephrology, Univ of North Carolina, Chapel Hill.*

**Background:** The use of anabolic steroids has increased in both athletes and non-athletes. Acute Liver injury is a well-recognized consequence of androgenic steroid use. Severe hyperbilirubinemia has been hypothesized to cause acute kidney injury through bilirubin tubular cast formation, a phenomenon referred to as cholemic nephropathy. We report a unique case of anabolic-steroid induced cholemic nephropathy.

**Methods:** A 35 year old male with remote history of alcohol abuse presented with a 2 month history of jaundice, fatigue, vomiting, unintentional 7 kg weight loss, and a serum creatinine of 4 mg/dL. There was no history of hypotension (BP 123/59), exposure to IV contrast or NSAIDs. He reported taking 'Epistane', a readily available pro-anabolic steroid 'food supplement', and N-actetyl cysteine for 4 weeks prior to presentation. His total bilirubin was 40 mg/dL with near normal transaminases and intact synthetic liver function (albumin 4.5 g/dL, INR 1.0). Hepatitis serologies, HIV screen, and urine toxicology tests were negative. Abdomen/pelvis CT scan and renal ultrasound were unremarkable. Urine sediment showed darkly pigmented, bile stained granular casts. A renal biopsy revealed diffuse moderate to severe tubular injury in association with bile casts consistent with a bile cast nephropathy. Despite cessation of Epistane, the hyperbilirubinemia persisted. His renal function deteriorated and hemodialysis was started 3 weeks later. Two weeks later, his bilirubin decreased to 15 mg/dL and GFR recovered to 39 ml/min/1.73m2. One month later, bilirubin was 2.2 mg/dL and GFR >60 ml/min/1.73m2.

**Conclusions:** Supplements containing anabolic steroids can be hepatotoxic leading to isolated hyperbilirubinemia, which is implicated in acute tubular injury and cast nephropathy, especially when bilirubin levels are >20 mg/dL [1,2]. Cholemic nephropathy should be considered in the absence of other causes of renal damage. This case suggests the possible benefit of decreasing serum bilirubin concentrations with dialysis to preserve and recover renal function.

[1] Bomzon A. Seminars in Nephrology 1997; 6: 549-62
[2] Sitprija V. Kidney International 1990; 38: 948-55.

## TH-PO806

**A Rare Complication of BCG Therapy**
Kristin M. Corapi,[1] Imran Sajjad,[1] David B. Mount,[2] Robert J. Hamburger.[2] *[1]Renal, MGH/BWH, Boston, MA; [2]Renal, VA Boston, West Roxbury, MA.*

**Background:** We present a rare case of hypercalcemia and acute kidney injury (AKI) following intra-vesical Bacillus Calmette-Guerin (BCG) therapy.

**Methods:** A 64-year-old man with history of bladder cancer was admitted with lethargy, nausea, and vomiting. His lab work revealed AKI and hypercalcemia. His cancer was treated with TURBT and intra-vesical chemotherapy; he remained on maintenance BCG treatments. His most recent dose was complicated by fevers, dysuria, and hematuria. He received Ciprofloxacin for a presumed UTI. On admission his physical exam was unremarkable. His lab results are below.

| Test | Result | Normal Range |
|---|---|---|
| BUN; mg/dL | 52 | 7-25 |
| Creatinne; mg/dL | 3.8 | 0.5-1.5 |
| Ionized Calcium; mg/dL | 6.6 | 4.5 - 5.3 |
| PTH; pg/mL | 12.8 | 10-65 |
| PTHrp; pg/mL | 33 | 14-27 |
| 25 OH Vitamin D; pg/mL | 39 | 10-65 |
| 1,25 OH Vitamin D; pg/mL | 100 | 18-72 |
| ACE; U/L | 163 | 3-67 |
| Alkaline Phosphatase; U/L | 433 | 40-150 |
| Gamma GTP; U/L | 721 | 10-65 |

His course was complicated by pulmonary edema, pancytopenia, and elevated alkaline phosphatase. Renal ultrasound showed no hydronephrosis. A chest CT revealed bilateral reticulo-nodular opacities. Both a bronchoscopy and bone marrow aspirate were normal with negative AFB cultures. Renal biopsy was notable for active interstitial nephritis and chronic vascular changes. Lastly, a liver biopsy was diagnostic of granulomatous hepatitis from disseminated BCG. He was started on prednisone, INH, Rifampin, and pyridoxine with improvement in his labs abnormalities. BCG exerts anti-tumor effects by creating local inflammation; granulomas may form as a hyersensitivity reaction. Hypercalcemia results from extra-renal calcitriol production in the granuloma macrophages.

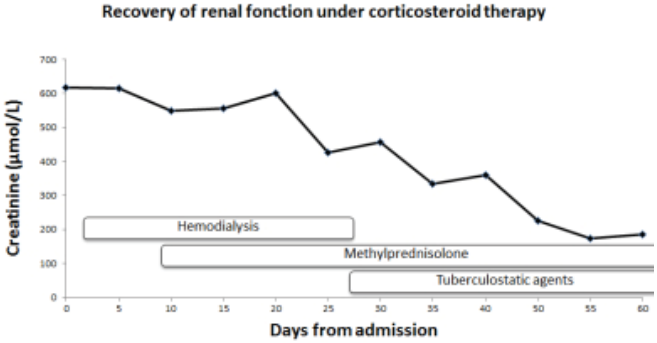
**Conclusions:** BCG is used to treat bladder cancer and is generally well tolerated. The most common side effects are urinary complaints or flu like symptoms. It is important to consider systemic granulomatosis if such a patient develops hypercalcemia, look for granuloma's, and treat with anti-Tb therapy.

## TH-PO807

**Prevention of Kidney-Lung Failure due to Intra-Bladder BCG Therapy**
Quentin Mat, Steffy Larroze, Rim Kada, Olivier Lmc MAT. *Nephrology - Dialysis, EpiCURA, Ath, Belgium.*

**Background:** We observed ARF 9 months after intravesical BCG adjuvant treatment for T1 bladder Transitional Cell Carcinoma (TCC). *Mycobacterium bovis* was identified by PCR on lung biopsy and tubulo-interstitial nephritis (TIN) was proved. He responded well to steroids and tuberculostatic tri-therapy. Prevention requires the eviction of risk factors and regular control of renal function (RF) during therapy.

**Methods:** A 76-year-old man presented ARF. TCC was treated by 10 adjuvant BCG intravesical instillations during one year. Laboratory revealed serum creatinine 699 µmol/L, ACE 140 IU/L. Hemodialysis was started. Chest CT confirmed a "ground glass" interstitial syndrome. A 10 IU PPD skin test was negative. Kidney biopsy showed TIN with eosinophilic infiltrate without granuloma. Lung biopsy demonstrated moderate lympho-neutophilic infiltration, few aspecific granulomatous lesions. Sarcoïdosis was suspected and oral methylprednisolone was started. Serum creatinine decreased to 220 µmol/L; hemodialysis was discontinued. On day 27, Mycobacterium bovis was identified on lung samples by PCR; tuberculostatic agents were initiated. Corticosteroids was gradually stopped.




**Conclusions:** Intravesical BCG is safely used as adjuvant treatment for bladder TCC despite flu-like syndrome in 20%. Dissemination occurs after traumatic bladder instillation and cumulative doses. Prophylactic Isoniazid doesn't prevent BCG complications. Contraindications include recent bladder surgery or radiotherapy, polypectomy, active cystitis, tuberculosis, immunosuppressive treatment. Medline system reported 12 associated ARF : 9/12 TIN, 5/12 granuloma; glomerulonephritis are exceptional. Our case combines the presence of causal Mycobacterium on lung samples. Prevention requires avoiding the risk factors and regular control of RF during prolonged therapy. Corticosteroids and tuberculostatic therapy should always be considered.

## TH-PO808

**Acute Kidney Injury (AKI) due to Multiple Myeloma (MM) Successfully Reversed by Bortezomib: A Medical Emergency Requiring a High Index of Suspicion and Prompt Diagnosis by Plasma Free Light Chains (PFLC)**
Usman Z. Bhutta,[1] Saurabh Dasgupta,[2] Kai Lau.[1] *[1]Nephrology Section, OUHSC, Oklahoma City, OK; [2]Medicine/Pediatrics, OUHSC, Oklahoma City, OK.*

**Background:** MM is caused by plasma cell dyscrasia & excess M protein. It can cause renal failure by cast nephropathy or light chain (LC) deposition. We report a case with AKI where MM was promptly diagnosed & renal failure rapidly reversed by bortezomib, a proteasome inhibitor selective against plasma cells.

**Methods:** We cared for a 56-year-old man with AKI noted in evaluating his back pain. Serum creatinine (Cr) rose from 0.8 to 4.6 mg/dL in 2 months. Serum protein electrophoresis (SPEP) had a small M spike. Urine PEP showed 2 large M spikes (3.1 & 78.9% of 5.1 g protein/d), but dipstick only 1+ positive. PFLC κ was up 2 fold (from renal failure), but λ pathologically up 70fold to 1890 mg/L (vs. 26 in normal),yielding a low κ: λ ratio of 0.02, diagnostic for MM. Other causes for AKI were excluded. 50% λ-restricted plasma cells in the marrow confirmed MM. He got plasma exchange, steroids & 4 doses of bortezomib in 2 weeks. Cr peaked at 9.9 but needed no dialysis. Bortezomib dropped his PFLC λ to 15 mg/L in 10 days & controlled it over next 4 months. Cr also fell in 10 days & hit 2.4, 1.5 , & 1.2-1.4 mg/dL after 0.5, 2, & 3.5-5 months respectively of the 1st dose.

**Conclusions:** 1. Our case illustrates the need for a high index of suspicion for MM in adult AKI.

2. PFLCs are sensitive & specific, useful in monitoring therapy & progression. Myeloma proteins are missed by urine dipstick but detected & quantifiable by UPEP.

3. Contrary to other causes of AKI where therapy is largely supportive, preventing complications & averting nephrotoxins, bortezomib is effective in halting synthesis of LC, the specific culprit, thus reversing AKI in MM. His gratifying response confirms & extends the published short list of similar patients diagnosed & effectively treated by bortezomib without dialysis.

4. Recent experience suggests a much improved outcome in MM despite severe renal failure vs. the era before bortezomib. Myeloma kidneys should be managed as a treatable medical emergency.

## TH-PO809

### Encephalopathy and Profound Hyponatremia due to SIADH Induced by Bortezomib (Velcade)

Usman Z. Bhutta,[1] Kent David Stuber,[2] Kai Lau.[1] *[1]Nephrology Section, OUHSC, Oklahoma City, OK; [2]Medicine/Pediatrics, OUHSC, Oklahoma City, OK.*

**Background:** Velcade-induced hyponatremia is not rare, at times symptomatic though under-recognized & underdiagnosed. We report a case to illustrate its insidious onset & slow spontaneous recovery needing brief tolvaptan therapy.

**Methods:** We consulted on hyponatremia in a 72-year-old woman getting outpatient velcade for symptomatic multiple myeloma. She was admitted for confusion, ataxia and a fall. A month ago, the 1st of 7 doses of velcade was given. Serum (S) Na (in mM) was 140 after the 4th but fell to 125 after the 6th & to 121 after the 7th dose. Though vital signs, volume status & neurologic exam were normal, urine (U) Na (<10) & BNP (13 pg/ml) were low, prompting normal saline for presumed salt depletion. Despite gaining 5.5 L of fluid & accruing edema, SNa peaked only at 127 & then quickly fell to 122. On day 3, U osmolality (osm) (in mOsm/kg) remained high at 567 vs. S osm of 258, despite high UNa of 102. On day 4, when S osm was low at 251-254, plasma ADH was inappropriately normal at 1.1 pg/ml. SIADH was diagnosed. Other known etiologies of SIADH were systematically excluded. To prevent worsening hyponatremia, she got 15 mg oral tolvaptan, which promptly dropped U osm to 50, led to 3 L diuresis in 48 h, & briefly raised SNa to 132-139. By restricting fluids to <1.2 L/day, SNa hovered ~ 130 from days 7-12, at which point, U osm spontaneously fell to 228 & 119. 12 days after the 7th & last dose of velcade, SNa was 130, slowly climbed to 135 & remained normal for the next month on ad lib fluids.

**Conclusions:** 1. Our case illustrates the potential of symptomatic severe hyponatremia due to velcade-induced SIADH.

2. Frequent & continued monitoring of SNa is key as hyponatremia may emerge insidiously, late after multiple doses, & even in outpatients.

3. Ample hydration during velcade may be unwise.

4. She took > 2 weeks after onset to recover spontaneously, suggesting a brief course of tolvaptan is needed if hyponatremia is severe or fluid restriction ineffective.

5. We need more studies to define the mechanism & address the feasibility of resumption after recovery, with or without concurrent tolvaptan prophylaxis.

## TH-PO810

### A Case of Acute Kidney Injury and Calcium Oxalate Deposition Associated with Synthetic Cannabinoids

Mahmoud Kamel, Bijin Thajudeen. *Nephrology, Univ of Arizona, Tucson, AZ.*

**Background:** We report a case of acute kidney injury(AKI) and renal calcium oxalate deposition after synthetic cannabinoids ingestion.

The use of these synthetic cannabinoid preparations has increased significantly in the United States over the past few years, and the incidence of acute kidney injury is underestimated. Also we are pointing to cannabinoids as a cause of hyperoxaluria based on results of renal biopsy and after we excluded all other causes of oxalosis.

**Methods:** 65 yr old male patient, paraplegic with neurogenic bladder, who was brought by EMS for altered mental status for which he was intubated in emergency department for airway protection. Upon presentatin, he was found to have AKI ( Cr 6.3 ) and hyponatremia 113.He was treated with Vanomycin/Zosyn for possible pneumonia.His PMH only includes neurogenic bladder. No previous abdominal surgeries. His home medications include methadone, oxycodone, nortriptyline and pregabalin. He reported recent Cannabis intake. His physical exam is significant for diminished breath sounds at both bases. Normal heart sounds, soft and lax abdomen with no palpable masses. His labs are significant for Na:113, K:5,CL:84,CO2: 14, BUN:88, Cr: 6.3, WBC 8.4 (normal differential), HB: 12.9 and platelet 178. urine sediment was bland. Renal us showed normal sized kidney and no hydronephrosis His pneumonia got better after receiving IV antibiotics and he was extubated but his cr continues to worsen and it peaked to 11.2, and at that time renal biopsy was performed which showed an evidence of active tubulointerstitial nephritis with marked tubular injury and calcium oxalate crystals present within tubular lumen.

**Conclusions:** Review of literature showed two reports demonstrated 14 and 30 patients respectively diagnosed with Acute kidney injury associated with SPICE use. A new article in Alabama showed also 4 cases of AKI assocaited with synthetic Cannabinoids. Here, we present a case of AKI and calcium oxalate deposition in a patient with synthetic cannabinoids use, pointing to cannabis as a cause of secondary oxalosis. Synthetic cannabinoids use is a potential cause for acute kidney njury/acute insterstitial nephritis and can lead to hyperoxaluria.

## TH-PO811

### Acute Kidney Injury Associated with Synthetic Cannabinoids Use

Ali Khalil, Jonathan W. Bazeley, Brian S. Decker. *Indiana Univ.*

**Background:** Synthetic cannabinoids (SC) include a variety of compounds publicized as harmless legal substitutes for Marijuana. SC are increasingly used and have serious toxicities including acute kidney injury (AKI) and myocardial infarction (MI). In this case we describe a patient with AKI and MI associated with use of SC.

**Methods:** A 20-year old previously healthy man, known to be a heavy SC user, had a witnessed seizure minutes after inhaling SC. On hospital presentation he was combative, suffered repeated seizures and was intubated. His blood pressure was 95/56 mmHg and pulse was136 beats/min. Physical exam was otherwise normal. Labs included serum creatinine (SCr) of 1.4 mg/dL, bicarbonate of 18mEq/L, creatine kinase of 149 ng/mL. Urinalysis showed specific gravity of 1.009, protein 100 mg/dL, glucose 30 mg/dL. Due to hypotension and oliguria, he was given normal saline intravenously. His SCr a day later was 5.32 mg/dL and renal replacement therapy was begun. Ultrasound showed normal kidney size and echotexture. Echocardiography revealed an ejection fraction (EF) of 20% with diffuse hypokinesis. Twelve days after initiation, dialysis was stopped. A coronary angiogram was normal with EF of 55%. SCr 4 weeks after presentation was 0.7 mg/dL.

**Conclusions:** The first SC were created by John Hoffman at Clemson. They are similar in structure to tetrahydrocannabinol but are up to 800 times more potent. Commercial names include K2, Spice, Legal Funk, and Gold. Formulations have included dried leaves or powder, typically a mixture of SC and herbal additives. The mechanism of kidney injury is unclear. Renal biopsy was not performed in our case but in a case series of four SC-exposed patients with AKI (Bhanushali et al., CJASN, 2013), three biopsies showed ATN, possibly due to a direct nephrotoxic effect of SC. Poor cardiac output may contribute to AKI in some cases, including ours. Cannabinoid receptor activation may lead to norepinephrine release and parasympathetic blockade, which could cause the cardiomyopathy. SC are also associated with coronary artery syndromes, severe hypotension, seizures and psychosis. Our goal with this report is to promote awareness among clinicians of the increasing use and toxicities of SC, including AKI.

## TH-PO812

### Acute Kidney Injury as a Result of Drug Elution from Antibiotic Impregnated Spacer Device in a Patient with Chronic Kidney Disease

Feroz Abubacker,[1] Prerna Ganjoo,[2] Pooja Singh.[2] *[1]Nephrology, Thomas Jefferson Univ Hospital, Philadelphia, PA; [2]Nephrology, Thomas Jefferson Univ Hospital, Philadelphia, PA; [3]Nephrology, Thomas Jefferson Univ Hospital, Philadelphia, PA.*

**Background:** Antibiotic impregnated cement spacers have been used commonly for the treatment of infected prosthetic hip and knee joints. Vancomycin and aminoglycosides are the most commonly used antibiotics in spacer devices. This modality of treatment provides high concentration of the antibiotic at the infected joint, while minimizing the systemic adverse effects. We present a case of acute kidney injury progressing to end stage renal disease from combined vancomycin and tobramycin toxicity in this setting.

**Methods:** A 78 year old woman, with history of left hip replacement (10 years ago), complicated by chronic periprosthetic infection, hypertension, chronic kidney disease (CKD) stage III , baseline serum creatinine 1.2mg/dl presented with 3 days of nausea and vomiting. Patient had undergone resection arthroplasty, with insertion of antibiotic spacer (3.6gm tobramycin + 3gm vancomycin per pack) to the left hip 3 weeks prior to admission. On examination, she was afebrile, hemodynamically stable, with negative orthostasis. Initial labs revealed elevated serum creatinine of 3.1 mg/dl, magnesium 1.1 meq/l, urinalysis with 1.7gm proteinuria with no active sediment, and normal renal imaging .Serum Tobramycin and Vancomycin levels were elevated at 8 mg/l, and 25 mg/l respectively. Patient developed oliguria and worsening renal function, and had to be commenced on hemodialysis(HD). 12 hour post HD Tobramycin and Vancomycin levels remained elevated, demonstrating continued drug elution. Serum antibiotic levels diminished only after the spacer was removed. The patient, however had progressed to end stage renal disease by this time.

**Conclusions:** Antibiotic spacer devices are currently classified as medical devices, and undergo a less stringent approval process by the FDA. Physicians should be cognizant of nephrotoxicity from drug elution in patients undergoing antibiotic impregnated prosthetic devices and monitoring protocols should be implemented to assess for renal function and drug elution.

**TH-PO813**

**A Case of Acute Tubular Necrosis in the Setting of IgG Kappa Multiple Myeloma: A "Sticky Situation" Solved with Plasmapheresis**
Emmett D. Ratigan, Nancy Miller, Reza Elahimehr, Dena E. Rifkin. *Nephrology, Univ of California San Diego, San Diego, CA.*

**Background:** Renal failure is a frequent complication amongst patients with multiple myeloma and is a well-known predictor of morbidity and mortality. Here we report a case of a patient with IgG kappa multiple myeloma (MM) and chemotherapy-induced acute kidney injury in whom high-flux hemodialysis proved impossible due to intractable clogging of hemodialysis membranes. Hemodialysis was only successful after plasmapheresis pretreatment.

**Methods:** A 72 year-old man with refractory IgG kappa MM was admitted with oliguric renal failure, due to acute tubular necrosis, two days after initiating a salvage regimen of CC (carfilzomib-cyclophosphamide-dexamethasone). He became progressively hyperkalemic and oliguric, prompting hemodialysis catheter placement and initiation of intermittent dialysis using a F160NR high-flux filter. Within minutes of treatment initiation his dialyzer clogged, which recurred on subsequent attempts despite heparin and TPA anticoagulation. A trial of continuous venovenous hemodiafiltration (CVVHD) using regional citrate anticoagulation also failed, as filters clogged within 120 minutes. Heparin-induced platelet antibodies and a hypercoagulability screen were negative. As serum light chain levels were elevated (163 mg/dl) we speculated they were aggregating and sludging through the filter. To acutely reduce their level, we then performed plasmapheresis (100% plasma volume replacement), after which he was able to complete a full hemodialysis treatment without use of anticoagulation. Following three days of paired treatments, he recovered renal function and became dialysis-independent. Following additional treatment with CCd, he redeveloped acute kidney injury necessitating hemodialysis, which we again primed with plasmaphersis. Four months later, he has no evidence of myeloma-related kidney disease, despite persistently elevated serum light chains.

**Conclusions:** Studies have demonstrated that light chains can aggregate and deposit in hemodialysis filters. In cases complicated by frequent filter clogging, plasmapheresis may be a useful tool to facilitate short-term hemodialysis.

**TH-PO814**

**Chronic Urine Leak Causing Acute Kidney Injury due to Abdominal Compartment Syndrome: An Unusual Case Masquerading as Hepatorenal Syndrome I**
Elhami N. Hannan,[1] Jeanie Park.[2] [1]*Nephrology, Emory Univ, Atlanta, GA;* [2]*Nephrology, Emory Univ, Atlanta, GA.*

**Background:** We present a case of a patient with acute onset Ascites, Spontaneous Bacterial Peritonitis ( SBP ), heavy alcohol intake and acute kidney injury ( AKI ), was initially presumed to be due to Type I Hepatorenal Syndrome ( HRS I).

**Methods: Case Description**A 76 year male presented to hospital with abdominal pain, increased abdominal girth and decreased urine output ( UOP ) for 2 weeks. His past medical history was significant for chronic kidney disease stage III, of unknown etiology. He also, has a history of Bladder cancer, was surgically removed, and a construction of neo bladder two decades ago. On exam, he was noted to have a tense ascites. Labwork revealed AKI with serum Creatinine 8.2 mg/dl. Paracentesis done with peritoneal fuid analysis consistent with SBP. Abdominal ultrasound revealed normal appearing kidneys, large ascites and atrophic liver. The patient started on treatment for presumed HRS I, was given Albumin, Octereotide and Midodrine. He did get hemodialysis as he became uremic. The patient remained oliguric and noted to have rapid reaccumulation of ascites, even after large volume paracentesis. Subsequently, MRI of abdomen and pelvis revealed a defect in the posterior wall of the neobladder. Ascitic fluid was then sent for Creatinine level , confirming that the ascitic fluid was urine. A foley catheter was inserted. The patient had significantly increased UOP, resolution of ascites, and a rapid drop in his serum Creatinine to normal. He was discharged home with urological follow up.

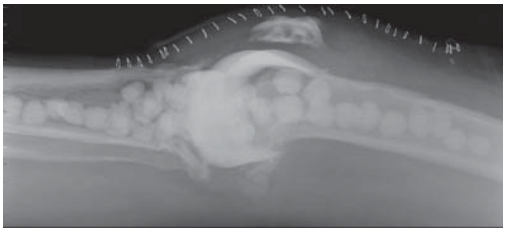
**Conclusions:** The patient presented with typical case of HRS I, in an alcoholic patient and ascites with SBP. However, his diagnosis was a much rarer case of AKI due to abdominal compartment syndrome from urine leak, that resolved with Foley insertion.

**TH-PO815**

**Permanent Renal Damage after Implantation of Tobramycin Beads during Orthopedic Surgery**
Cristy Gianna Martinez, Juan C. Lemos, Eddie M. Rodriguez, Carlos Antonio Cortes Sanchez, Carlos S. Rosado-Rodriguez, Hector R. Cordova. *Medical Service, VA Caribbean Healthcare System, San Juan, PR.*

**Background:** Antibiotic-impregnated cement or beads are used in surgeries of infected hip and knee arthroplasties. The incidence of systemic side effects of antibiotics is low because the antibiotic is delivered locally.

**Methods:** A 79 y/o man with history of total left knee arthroplasty in 2008 was admitted to Orthopedic service due to sudden pain and swelling of the knee. His baseline serum creatinine level was 1 mg/dl. Septic arthritis was documented with arthrocentesis. Antibiotic therapy consisted of vancomycin followed by tygecycline. The infected prosthesis was surgically removed. The wound was irrigated and spacer created with tobramycin–impregnated cement. Two strings of 16 tobramycin-eluting beads were also inserted in the metaphyseal cavities of the femur and tibia as shown in this film:



Only the joint fluid was positive for methicillin-sensitive Staph aureus. Thirteen days after surgery, the serum creatinine level increased to 4.7 mg/dl. Renal sonogram was negative. U/A showed 1+ protein, hyaline and granular casts, WBC's, RBC's and bacteria. Gallium scan showed intense tracer concentration involving the tibial and femoral components of the left knee without significant tracer concentration in the kidneys. Hemodialysis was initiated. Serum tobramycin levels were 1.1 and 0.7 mg/l, 17 and 28 days after surgery, respectively. The patient continues on chronic hemodialysis six months after arthroplasty.

**Conclusions:** Serum tobramycin levels above 0.1 mg/L and a dose of tobramycin >2 gm per 40gm of cement have been associated with AKI. Both of these factors were present in our case. Physicians should be cognizant of the potentially severe nephrotoxic effect of the use of aminoglycoside-eluting cement and beads in patients undergoing restorative orthopedic surgery.

*Funding:* Veterans Affairs Support

**TH-PO816**

**Warfarin Associated Nephropathy**
Amita Vasudeva, Muhammad R. Mustafa, Sana R. Akbar. *Nephrology, West Virginia Univ, Morgantown, WV.*

**Background:** Warfarin induced nephropathy is a relatively newly diagnosed disease entity. We hereby present a case of warfarin induced nephropathy.

**Methods:** 79 year old white male with history of Hypertension, atrial fibrillation, mechanical aortic valve replacement on coumadin for 14 years underwent cardiac catheterization due to anginal symptoms, it showed minimal CAD. Home medications were Losartan, Lasix, Bactrim for cystitis, Imdur, Hydralazine, lopressor. Baseline labs were BUN 28, Cr was 1.1. Ten days after cardiac catheterization, he developed gross hematuria. BUN and Cr increased to 39 and 2.4 mg/dl respectively, with INR of 6. Exam was normal without edema. Losartan and Lasix were stopped with no change in kidney function. Ultrasound was normal. Cr worsened to 6.13 mg/dl with BUN 90. Urine sediment showed many normal RBCs. Urine protein to Cr ratio was 2.5mg/ mg. ANCA, Anti GBM antibodies and ANA were negative. Serum C3, C4 were normal. He had to be started on hemodialysis due to uremia. Kidney biopsy showed red blood cells in Bowman's space (one glomerulus), red blood cells in tubular lumina withmild interstitial fibrosis. Immunofluorescence was negative and there were no electron dense deposits. Findings were consistent with the diagnosis of warfarin-related nephropathy. Pt remains dialysis dependent months later.

**Conclusions:** Brodsky et al. reviewed renal biopsies from 9 patients with elevated serum creatinine level (mean, 4.3±0.8 mg/dL) and abnormal INR (mean, 4.4±0.7 IU) after excluding acute/active glomerulonephritis. The biopsy specimens revealed RBCs in both Bowman spaces and tubules. All of these patients had previous underlying kidney disease. Recovery was incomplete in six of nine patients. It appears that in patients with pre-exiting CKD, warfarin may precipitate AKI via either glomerular hemorrhage and intra-tubular obstruction in addition to other processes.

References: 1. Brodsky, Sergey V., Anjali Satoskar, Jun Chen, Gyongyi Nadasdy, Jeremiah W. Eagen, Mirza Hamirani, Lee Hebert, Edward Calomeni, Tibor Nadasdy. (2009) Acute kidney injury during warfarin therapy associated with obstructive tubular red blood cell cases: A report of 9 cases. Am J Kidney Dis. 54:1121-1126.

**TH-PO817**

**A Case of Acute Kidney Injury in a Young Male with Recurrence of Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) Syndrome during Steroid Taper**
Nima Naimi. *Dept of Nephrology, Washington Univ, St. Louis, MO.*

**Background:** Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a rare disease occurring 2 to 9 weeks after exposure to drugs such as antiepileptics, antibiotics, and allopurinol. The most common organs affected include the liver, kidney, and lungs, although any organ may be involved. Ten to 30 percent of patients with DRESS syndrome present with kidney involvement, with age and underlying kidney disease being risk factors.

**Methods:** A 19 year old male with a history of acne was admitted with a fever, pruritic rash, and diffuse lymphadenopathy 3 weeks after starting minocycline for his acne. He was found to have a white blood cell count of 29.7 with 40% eosinophils, alanine aminotransferase of 103, and a creatinine of 1.1. Infectious and rheumatologic work up was negative. He was seen by dermatology who diagnosed him with DRESS syndrome and recommended discontinuation of the minocycline along with prednisone 80mg daily with a taper over 6 weeks. He was discharged home after 2 days once his fevers resolved and his rash improved. Two weeks later, he was readmitted after a routine follow up lab demonstrated a creatinine of 3.28. The patient also noticed a recurrence of his rash when his prednisone was tapered from 80 to 60mg daily. He was initially started on methylprednisolone and then switched to 80 mg of prednisone with a 10 mg per week

taper for a total of 8 weeks. His rash improved, and his creatinine decreased to 2.06 upon discharge. He was seen in the renal clinic a month later with complete resolution of his rash and normalization of his creatinine.

**Conclusions:** Acute kidney injury (AKI) usually occurs in the initial phase of DRESS syndrome. However, AKI can occur during recurrence of DRESS syndrome while patients are tapering their steroids, thus highlighting the importance of a slow taper. The current recommendation is to wean steroids over 8 to 12 weeks once clinical improvement and normalization of laboratory values have been achieved. Serial liver and kidney function monitoring is imperative during this time.

## TH-PO818

**Renal Failure Caused by Drug Reaction with Eosinophilia and Systemic Symptoms Syndrome (DRESS)** Jie Cui, David J. R. Steele. *Nephrology Dept, Massachusetts General Hospital, Boston, MA.*

**Background:** Drug reaction with eosinophilia and systemic symptoms syndrome (DRESS) is a severe adverse drug reaction with rash, fever and multi-organ damage. Kidney involvement of DRESS is usually mild with moderate elevated creatinine. However, severe renal damage required renal replacement therapy can also occur in severe cases.

**Methods:** A 42 year-old healthy male presented with daily fever, diffuse myalgia, sore throat and erythematous, pruritic rash. His lab was unremarkable on initial presentation. He was diagnosed with scarlet fever and his symptoms went away after 5-day course of penicillin VK. However, 7 days after he stopped penicillin, his rash, fever and myalgia returned. Physical exam was remarkable for T 102F, inguinal adenopathy and pinpoint erythematous papules all over the body. Labs are shown in table1.

| | initial presentation | on admission | 6 months after presentation |
|---|---|---|---|
| WBC (per mm$^3$) | 9600 | 8700 | 7600 |
| Eosinophila% | 4 | 5 | 2 |
| Hg (g/dl) | 14.7 | 10.7 | 14.2 |
| Platelet (per mm$^3$) | 199,000 | 144,000 | 211,000 |
| AST(U/liter) | 23 | 37 | 14 |
| ALT(U/liter) | 45 | 168 | 33 |
| Creatinine (mg/dl) | 1.0 | 7.6 | 1.0 |

He was started on ceftriaxone owing to suspicion of infection. However, all infectious work up were negative including throat culture, HIV, parvovirus, coxsackie, HSV, EBV, hepatitis A, B, C, Ehrlichia, lyme, blood culture and urine culture. ANA, RF, schistocytes and renal ultrasound were negative. Patient's renal function rapidly declined, and hemodialysis was initiated. Renal biopsy was performed, which showed severe active interstitial nephritis. Skin biopsy showed interface and spongiotic dermatitis, which was consistent with DRESS syndrome. All antibiotics were discontinued and patient was started on prednisone. His renal function slowly recovered and hemodialysis was discontinued 7 days later. Patient's renal function returned to his baseline 6 months after presentation.

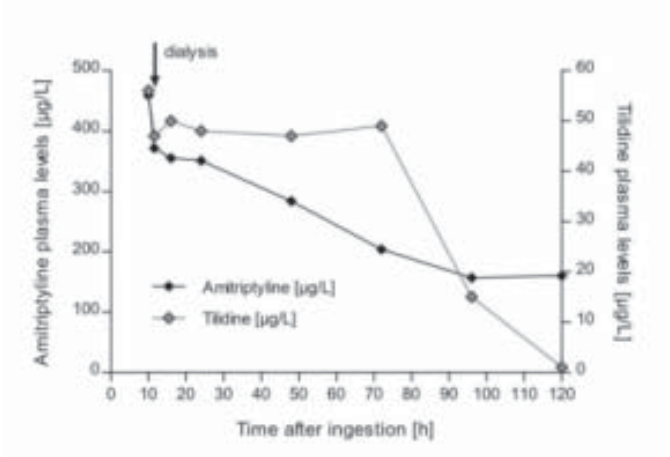
**Conclusions:** DRESS is a potential life threatening hypersensitive reaction that can cause multi-organ involvement. Severe renal failure required renal replacement therapy is a rare complication. Prompt recognition and removal of the offending medication is crucial to limit further end organ damage.

## TH-PO819

**Successful Treatment of Life-Threatening Intoxication with Amitriptyline and Tilidine Using High Cut-Off Dialysis** Anna Bertram,[1] W. Nikolaus Kühn-Velten,[2] Hendrik Suhling,[3] Jan T. Kielstein.[1] *[1]Nephrology, Hannover Medical School, Hannover, Germany; [2]Medical Laboratory of Bremen, Bremen, Germany; [3]Pneumology, Hannover Medical School, Hannover, Germany; [4]Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany.*

**Background:** The antidepressant amitriptyline (MW 313D; protein binding 96%) and the opioid tilidine (MW 273D; PB 10%) are both considered non-dialyzable. New high cut-off dialyzers have not been used for their removal so far. We report a case of mixed intoxication, in which we eliminated amitriptyline and tilidine using a high cut-off dialyzer.

**Methods:** A 54 y old woman was admitted to our hospital 6h after ingesting an unknown amount of amitriptyline and tilidine in suicidal attempt. The patient was deeply unconscious with a GCS of 5, blood pressure was 140/80mmHg and heart rate 94/min. ECG showed a broad QRS complex (134ms) and prolonged QTc (517ms), representing increased risk for ventricular arrhythmias. Based on the clinical condition we started a 5h dialysis using a high cut-off Ultraflux® EMiC2-dialyzer (polysulfone; 1.8m$^2$; dialysate and blood flow 300ml/min). Immediately afterwards slow extended dialysis was started. Plasma levels and samples pre and post dialyzer were drawn at different time points.




Three lines of evidence show that amitriptyline and tilidine are removed by high cut-off dialysis: Dialyzer clearances for amitriptyline and tilidine were 58ml/min and 67ml/min. Reduction rate of amitriptyline and tilidine by 1$^{st}$ dialysis were 27% and 41%. Into the 6$^{th}$ hour of treatment the patient regained consciousness and was transferred to psychiatric care on the 3$^{rd}$ day.

**Conclusions:** We conclude that use of a high cut-off membrane is a feasible method to accelerate elimination in cases of life-threatening poisonings with amitriptyline and tilidine, for which conventional hemodialysis is considered ineffective.

## TH-PO820

**Hyporeninemic Hypoaldosteronism and Renal Insufficiency after Unilateral Adrenalectomy for Primary Hyperaldosteronism** Shing Li, John A. Walker. *Medicine/Nephrology, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.*

**Background:** Treatment for primary hyperaldosteronism may be complicated by persistent hyporeninemic hypoaldosteronism and apparent acute kidney injury.

**Methods:** A 49 year old man with a history of longstanding hypertension and hypokalemia (2.7 mEq/L) was diagnosed with primary hyperaldosteronism secondary to an aldosterone producing adenoma (APA) on the bases of undetectable plasma renin activity (PRA), elevated plasma aldosterone concentration (PAC), and abdominal CT showing a 2.5cm right adrenal mass. Baseline renal function was normal (serum creatinine 0.9mg/dL; eGFR 122mL/min). A laparascopic right adrenalectomy was performed and hypertension improved thereafter off medications. However, serum creatinine doubled (creatinine clearance 76.5mL/min) and acidosis and hyperkalemia were noted (6.3 mEq/L); PRA and PAC were undetectable. This pattern persisted for several months. Treatment with fludrocortisone 0.05mg/day resolved the hyperkalemia and acidosis, but only partially attenuated the azotemia.

**Conclusions:** The incidence of persistent hypoaldosteronism with hyperkalemia after adrenalectomy for APA has been reported as 5.5%; concomitant hyporeninemia was even less frequent at 1.8%. Prolonged mineralocorticoid replacement therapy is often necessary. In one study, patients with APA treated with either adrenalectomy or spironolactone had short-term decreases in GFR of 13.6mL/min. Our patient's hyporeninemic hypoaldosteronism in combination with the magnitude of his GFR reduction make this case unique. Though the hyperkalemia is explained by the hypoaldosteronism, the reduction in renal function is more puzzling and has negative prognostic implications. We surmise our patient's postoperative decline in GFR may reflect a combination of 1)diminished renal blood flow due to an abrupt reduction in serum aldosterone, and 2)CKD due to underlying hypertensive nephrosclerosis, previously masked by hyperaldosterone-driven hyperfiltration. Clinicians should be alert to this potential outcome in patients undergoing adrenalectomy for APA, and monitor postoperative laboratory parameters accordingly.

## TH-PO821

**Liddle's Syndrome Caused by a Novel Mutation in the β-Subunit of the Epithelial Sodium Channel Gene SCNN1B** Satomi Nakashima, Keitaro Yokoyama, Masatsugu Nakao, Izumi Yamamoto, Yudo Tanno, Ichiro Ohkido, Takashi Yokoo. *Div of Kidney and Hypertension, Dept of Internal Medicine, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Liddle's syndrome has been known as a disorder associated with abnormal sodium reabsorption in the distal tubule, and is transmitted as a rare autosomal dominant trait. The epithelial sodium channel (ENaC) was established as a cause of this syndrome by hyper-sensitizing the channel. The ENaC is caused by mutations in the SCNN1B or SCNN1G gene, which truncates the cytoplasmic carboxyl terminus of the β and γ subunit of the ENaC.

**Methods:** The case, a 24-year-old woman, was diagnosed with hypertension, and was started on an antihypertensive agent 8 years ago. But, her blood pressure was not controlled, therefore she was referred to our hospital for further treatment . She showed hypertension , hypokalemia, metabolic alkalosis, low renin and aldosterone levels, hence pseudo-aldosteronism was suspected. Spironolactone was not effective, but triamterene was effective in controlling her blood pressure with an improvement in her serum potassium concentration.

There was no history of familial Liddle's syndrome, therefore sporadic Liddle's syndrome was suspected. Therefore a genetic test was performed . However the previously reported gene muation for Liddle's syndrome was not detected. Further DNA analyses showed the point mutation(Cytosine□Guanine) in the PY (Proline-Proline-x-Tyrosine) motif of the β subunit in the ENaC. Screening for specific mutations of the SCNN1B gene in relatives of patients with Liddle's syndrome can be used to identify previously unrecognized cases within the family. A new nonsense mutation(Y604X) of the SCNN1B gene is the likely cause of Liddle's syndrome in this case.

**Conclusions:** Based on direct DNA sequencing, a novel heterozygous nonsense mutation at codon 604 of the SCNN1B gene from TAC (Tyrosine) to stop codon (TAG) was detected as a cause of Liddle's syndrome in this case.

## TH-PO822

**Reverse of Congestive Heart Failure in Patient with Primary Aldosteronism** Narittaya Varothai, Erdal Sarac. *Internal Medicine, St. Elizabeth Health Center, Youngstown, OH.*

**Background:** Primary Aldosteronism (PA) is a disorder causing hypokalemia, sodium retention, hypertension and cardiovascular damage which can culminate into congestive heart failure. Early adrenectomy reccomended to improve cardiac function. We are reporting a PA case who has progressive CHF with underlying ischemic heart disease which cardiac function improve after delayed adrenectomy for 10 years.

**Methods:** A 70 year old Caucasian male with history of HTN, CAD s/p CABG, chronic Atrial Fibrillation s/p ablation and cardioversion, hypothyroidism. During hospitalization in 2000 for atrial fibrillation, patient had HTN and persistent hypokalemia. Laboratory studies showed PAC 37.1 ng/dL, PRA 0.51 ng/ml/hr with PAC/PRA ratio of 72.74(more than 20). CT abdomen showed hyperplasia of left adrenal gland. Initial treatment included potassium supplement and spironolactone. Patient's clinical status got worse in 2009 when progressive swelling and hypokalemia occurred. A repeat CT scan showed new presence of 2 cm adrenal gland tumor. No Surgery was done uncontrolled atrial fibrillation and marked decline in left ventricular ejection from 43 % in 2003 to 25 % with global hypokinesis in 2011. Subsequently, patient had recurrent admissions with decompensated CHF during 2011-2012 despite optimized medical treatment of CHF. Finally, patient underwent laparoscopic adrenelectomy on 2012 after which there was significant clinical improvement of CHF with NY functional status improving from IV to II. Within 2 months, his blood pressure control improved with number of antihypertensive agents decreasing from five to two. and diuretic dependence resolving. Repeat 2D echo after 1 year showed EF of 55%.

**Conclusions:** PA can cause cardiovascular damage from uncontrolled BP and also induce cardiac hypertrophy and fibrosis. Recommended treatment in PA with possible unilateral disease is unilateral laparoscopic adrenectomy. Although medical therapy could be option for patient who is not surgical candidate (unwilling or poor clinical condition), delayed surgery still help to improve clinical outcome and reverse cardiac function even in ischemic heart patient. So we should consider early adrenelectomy to prevent all the consequences.

## TH-PO823

**Chronic Hyponatremia Associated with Levamisole Adulterated Cocaine (LAC) Use** Anju A. Oommen,[1,2] Harold A. Franch.[1,2] *[1]Nephrology, Atlanta VAMC; [2]Emory Univ, Atlanta, GA.*

**Background:** There are no reports of hyponatremia associated with cocaine use in adults before 2011. In 2002, anti-helminthic Levamisole was identified in cocaine. With the increasing use of Levamisole as an adulterant, a number of complications including acute hyponatremia have been reported among cocaine users. In 2008, Levamisole was found in 69% of cocaine seized and studies report 78% of the cocaine positive urine in hospitalized patients had Levamisole.

**Methods:** A 65 y/o male with hypertension and polysubstance abuse (cocaine, alcohol and tobacco) referred to renal clinic for chronic asymptomatic hyponatremia. Since his presentation in 2005 to our health care system, his serum sodium (Na+) has ranged from 136mmol/L to 124mmol/L. Urine drug screens done along the same time frame always tested positive for cocaine. Review of systems was negative for any causes of hyponatremia. His physical examination was unremarkable. Labs were pertinent for serum Na+ of 130mmol/L, serum osmolality 280 and inappropriately high urine osmolality at 430 mosm/kg. Urine Na+ was 52mmol/L and serum uric acid level was low at 3.1mg/dl. His renal function, TSH, cortisol were normal. Chest X-ray was unremarkable. He was not on schizoaffective medications. A diagnosis of SIADH was made. Despite fluid restriction and addition of salt tablets, serum Na+ continued to remain below 134mmol/L. Urine tested positive for Levamisole through analysis by high performance liquid chromatography/tandem mass spectrometry. Hyponatremia was attributed to LAC.

**Conclusions:** Levamisole was withdrawn from the US market in 2000 because of adverse effects, notably severe agranulocytosis. Levamisole is linked to hyponatremia in cancer patients. But there are no case reports of LAC causing chronic hyponatremia. Although the exact mechanism of hyponatremia is unknown, it has been postulated to cause SIADH. This case is limited by continued cocaine use in our patient and hence the inability to check for resolution of hyponatremia. As LAC becomes more prevalent, it is possible that we will see more cases of unexplained hyponatremia. This case of chronic hyponatremia from LAC suggests a potential complication and testing for Levamisole is indicated early.

## TH-PO824

**Obstructive Jaundice Associated Hyponatremia** Supriya Ravella, Gertrude S. Lefavour, Amay Parikh, Mary O. Carayannopoulos. *Nephrology, UMDNJ-RWJMS, New Brunswick, NJ.*

**Background:** Pseudohyponatremia is associated with a normal serum osmolality. Elevated lipid levels are a known cause of pseudohyponatremia. While the plasma water is approximately 93% of the plasma volume normally, in patients with hyperlipidemia or hyperproteinemia, the plasma water can decrease to 80% or lower leading to a reduced measured sodium concentration.

**Methods:** A 40-year-old man with a past medical history of autoimmune pancreatitis/ lymphoplamacytic sclerosing cholangitis, biliary stricture with stent placement, hyperlipidemia and diabetes mellitus presented with fever for two days. On exam, he was jaundiced and euvolemic. His serum sodium was 119 mEq/L, potassium was 3.3 mEq/L and chloride was 87 mEq/L. Total cholesterol was 2109 mg/dL. His lipid panel however demonstrated a LDL of 68 mg/dL, HDL of 36 mg/dL and triglycerides of 299 mg/dL. The large difference between the total cholesterol and its components prompted a lipoprotein electrophoresis, which detected lipoprotein X.

**Conclusions:** Lipoprotein X (Lp-x) is associated with the low density lipoprotein fraction. It is an abnormal lipoprotein which appears in the sera of patients with obstructive jaundice. Abnormally high levels of Lp-x can cause pseudohyponatremia. Serum sodium is commonly measured with analyzers that use indirect potentiometry with ion-selective electrodes. This utilizes a dilution of the sample. Increased lipid or protein concentrations alters the total water concentration. Then the dilution step and the subsequent calculation of concentration by the analyzer results in a falsely low sodium value. When factitious results are suspected, serum sodium should be measured by direct potentiometry ion-selective electrodes. When serum sodium levels are low in patients with cholestatic jaundice either due to autoimmune pancreatitis or graft versus host disease after stem cell or bone marrow transplantation, the suspicion for pseudohyponatremia should be high.

## TH-PO825

**Hyponatremia Case due to Cerebral Salt Wasting and Underlying Syndrome of Inappropriate Antidiuretic Hormone Secretion: Diagnostic and Therapeutic Challenges** Oana C. Badescu, Khurram Saleem, Farhang Ebrahimi. *Richmond Univ Medical Center, Staten Island, NY.*

**Background:** The existence of cerebral salt wasting (CSW) is controversial, but hyponatremia in patients with central nervous system (CNS) injury, particularly subarachnoid hemorrhage, has been recognized in literature. We present a case of hyponatremia due to CSW complicated with underlying syndrome of inappropriate antidiuretic hormone secretion (SIADH). The coexistence of these conditions has been rarely reported.

**Methods:** We present a 45 year old male with chronic ventriculo-peritoneal shunt admitted for cervical spine injury secondary to seizure induced fall. Patient was found to have hyponatremia. Urine osmolality (UOsm) and urine sodium (UNa) were elevated, and there was hypouricemia. Diagnosis of SIADH was considered. Fluid restriction, Demeclocycline, and combination of normal saline (NS) and diuretics were associated with worsening of hyponatremia. We noticed large volume diuresis in the presence of hemodynamic instability and signs of hypovolemia. Possibility of CSW was considered. Serum sodium was 116 mEq/L, UOsm 833 mOsm/Kg, and UNa 200 mEq/L. Hyponatremia responded to 3% hypertonic saline (HS) and volume expansion with normal saline, with partial correction of serum sodium. Each time we held HS, while on NS alone, hyponatremia worsened, and UOsm would not decrease below 300 mOsm/Kg. This was consistent with underlying SIADH. Patient remained quadriplegic and required long term maintenance HS and NS and eventually serum Na stabilized after addition of large doses of mineralocorticoid therapy. Other unique manifestations of CSW are the absence of hypokalemia despite natriuresis and significant hypouricemia, which both were present in our patient.

**Conclusions:** Our case illustrates the coexistence of CSW and SIADH, which poses challenges to the physician. If the mainstay of therapy in CSW is replacement of sodium and water, in SIADH it is water restriction. Early recognition of the main pathology could reduce complications of inappropriate therapy. Also the unique positive response to mineralocorticoids could help physicians not familiar with this therapy in patients with this rare combination of disorders.

## TH-PO826

**Lamivudine Induced Nephrogenic DI** Mohamad Mudar Morad, Michelle W. Krause, Dumitru Rotaru, Gracen Elizabeth Hauk. *Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

**Background:** Acquired NDI in adults is most commonly caused by lithium, hypercalcemia or hypokalemia. Less frequent causes are medications like: cidofovir, foscarnet, vasopressin V2 receptor antagonists, amphotericin B, demeclocycline, ifosfamide. We are reporting a case of acute NDI induced by lamivudine.

**Methods:** A patient with AML admitted for autologous stem cell transplant (ASCT). His medications included prophylactic antibiotics, antifungals, Acyclovir and Lamivudine. The hospital course was complicated by acute polyuria with 12 L of UOP/day followed by pre-renal AKI with creatinine increasing from 0.9 to 2.2. Urine analysis was normal except low specific gravity of 1.001 and urine sediment was bland. During this time, the patient also had low urine osmolality and high serum osmolality. Patient received repeated doses of DDAVP with no significant change in urine osmolality or urine volume. A diagnosis of acute

nephrogenic diabetes insipidus (NDI) was made. In our patient we stopped Lamivudine and started Maxzide. Over the next 48hrs patient UOP decreased gradually to 2 l/day, his urine osmolarity increased gradually to 400 at 72 hrs and his renal function improved to normal.

**Conclusions:** In the absence of common etiologies we explored the possibility of antivirals causing acute NDI in our patient. We performed a literature research for NDI associated with Acyclovir and/or Lamivudine. We found previous reports of Didanosine causing NDI. Based on similarities between Lamivudine and Didanosine which are both nucleoside analogue drugs with comparable mitochondrial toxicity profile we presumed that Lamivudine was the likely cause of NDI in our patient. Tenofivir use was also reported to increase the concentration and toxicity of Didanosine through competition for OAT1 binding for urinary excretion. The same OAT1 is involved in both Acyclovir and Lamivudine excretion and we propose that Acyclovir use in our patient may have also contributed to increased Lamivudine levels. Further studies may be needed to assess the incidence of acute NDI with Lamivudine.

## TH-PO827

**Supra-Therapeutic Doses of 1-Deamino-8-D-Arginine Vasopressin to Treat a Severe Case of Lithium Induced Nephrogenic Diabetes Insipidus: Back to the Basics** Shaifali Sandal, Catherine A. Moore, Matthew D. Gross. *Nephrology, Univ of Rochester, Rochester, NY.*

**Background:** Nephrogenic diabetes inspidus (NDI) is an inadequate response of the kidneys to the antidiuretic hormone, resulting in polyuria, and water and electrolyte imbalances. We report the successful use of high doses of 1-deamino-8-D-arginine vasopressin (dDAVP) as a successful treatment strategy.

**Methods:** A 51-year-old male with history of schizoaffective disorder on long-term lithium therapy causing NDI (with baseline normal sodium levels) was hospitalized with a 3 day history of confusion and gait instability. Workup revealed serum sodium of 165mmol/L, lithium level of 1.3 mmol/L, acute kidney injury, and a new diagnosis of thrombotic thrombocytopenic purpura (TTP). Lithium was discontinued and patient required aggressive fluid administration due to continued polyuria at 9 to 10 L/day with urine osmolality of 60-80 mosmol/Kg H20. dDAVP at "physiologic dose" of 2mcg was given intravenously with no significant improvement. Plasma exchange was initiated with clinical improvement in TTP, however polyuria persisted at a range of 10-16 L/day despite initiation of a thiazide diuretic. High dose dDAVP was then administered at 4 mcg subcutaneously twice a day, with resultant increase in urine osmolality to 275 mosm/Kg and decrease in urine output to 3-5 L/day.

**Conclusions:** Up to 40% of individuals treated with lithium develop NDI as a side effect via an unknown molecular mechanism. Traditionally, treatment of NDI has involved the use of thiazide diuretics and stopping lithium. In the 90's several different studies were published demonstrating the successful use of indomethacin with or without desmopressin for treatment. However, this fell out of favor potentially due to the impaired renal function caused by indomethacin. Several new therapies are being proposed to treat NDI such as vaptans, statins and heat shock protein 90. We have demonstrated higher doses of dDAVP as a successful treatment modality in a case of severe NDI. Our patient likely had a partial form of NDI, hence the response to higher doses of dDAVP. Through this case we encourage physicians to go back to the basics when treating NDI.

## TH-PO828

**Kidney Transplant Exposing Central Diabetes Insipidus** Todd W. Robinson, Anthony J. Bleyer. *Wake Forest School of Medicine, Winston-Salem, NC.*

**Background:** Central diabetes insipidus (DI) leads to an inability to maximally concentrate the urine. Patients with DI and CKD have less water delivery to the collecting duct (CD), causing less urine output. We describe a patient with central DI whose low GFR was fixed with living related kidney transplantation, resulting in abrupt onset of polyuria.

**Methods:** A 45 year old peritoneal dialysis patient with ESRD due to Wegener's Granulomatosis presented for kidney transplantation. On post-operative day 2 the patient developed urine output between 15 and 16 liters daily without diuretics. The patient did not complain of polydipsia. The serum sodium was 149 mEq/l., bicarbonate 21 mEq/l, chloride 116 mEq/l, BUN 16 mg/dl, and creatinine 1.6 mg/dl. Urine osmolality was measured and noted to be very low at 79 mOs/kg with a serum osmolality of 297 mOs/kg. Immediately, the urine output of the donor was assessed and found to be < 3 liters/day. In the recipient, a water deprivation test was initiated and there was minimal elevation of the urine osmolality with respect to baseline values. With exogenous ADH administration, the patient's urine osmolality markedly increased with a significant decrease in urine volume. MRI of the brain revealed no evidence of neoplasm, mass lesion, or white matter disease. At discharge, he was continued on desmopressin nasal spray with good results.

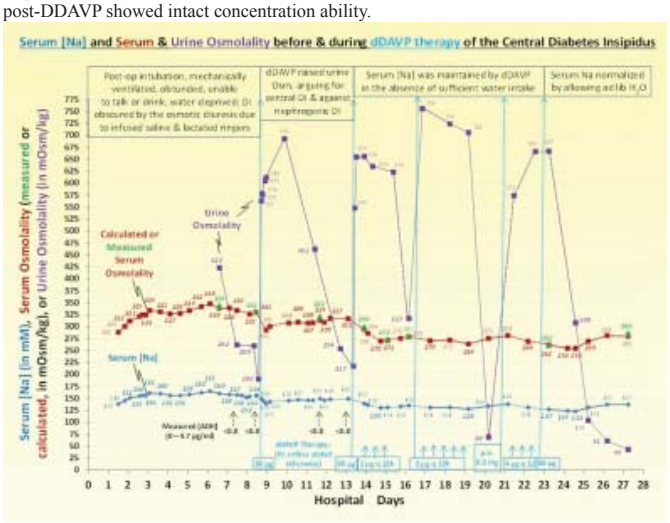
**Conclusions:** This case illustrates the important interaction between GFR and polyuria in DI. Prior to transplant, the GFR was low, with diminished water delivery to the CD and only moderate urine output. Post-transplantation, the increased GFR resulted in increase water delivery to the CD with resulting polyuria. Interestingly, the diagnosis of central DI was made quite rapidly, because determination of the urine output of the donor ruled out nephrogenic DI. The poor perception of thirst may have been due to the free availability of water at the bedside in the hospital. With the urinary catheter, the patient did not realize the extent of urine output. The etiology of central DI in this case was likely granulomatous involvement of the posterior pituitary, which has been described in a small number of Wegener's cases.

## TH-PO829

**Central Diabetes Insipidus (CDI): Undiagnosed for 3 Decades Till Unmasked by Dehydration due to Endotracheal Intubation and Stupor** S .Damera, Laxmi Gadde, Kai Lau. *Nephrology, OU Health Sciences Center, OK.*

**Background:** DI is marked by excessive dilute urine,usually controlled by polydipsia. We here report a man with chronic CDI undiagnosed till revealed by severe hypernatremia from inadvertent water deprivation due to prolonged intubation & stupor.

**Methods:** A 54yo man with strangulated inguinal hernia had emergent bowel resection. He stayed intubated for more surgery on day(d)2 & 3,all under sevoflurane.On d1, urine SG was 1.005.He had since been polyuric to 4-9 L/d.Serum (S)Na,139 mM 2y ago & 135 on d1, rose to 165 by d6.By deleting IV Na & giving more $H_2O$, SNa fell to 154 by d7.Polyuria (10-11 L/d) remained & Uosm fell from 423 Osm/kg on d6 to 190 by d8.Despite Sosm of 331,ADH was repeatedly <0.8 pg/ml,confirming DI.Sevoflurane via F can cause NDI. To differentiate CDI from NDI,he got 80µg IV DDAVP & raised Uosm to 563, 578, 691 respectively by 3rd5th& 31sth.But Uosm fell to 462 by d 3 & 217 by d5.Peak Uosm of 756 post-DDAVP showed intact concentration ability.




On MRI, posterior pituitary had no T1 hyperintensity (i.e. no ADH) confirming CDI. With delirium from SNa & dysphasia from intubation,he could not drink to prevent dehydration.Thus he got 11d of DDAVP to keep SNa 139-149 & allow extubation.When conscious & talking,he denied history of DI but admitted to 12L of $H_2O$/d for 30 years. For 7d before discharge, SNa was normal by ad lib fluids.

**Conclusions:** This man shows classical elusive nature of DI due to usually effective polydipsia.He became dehydrated when prolonged intubation precluded drinking and perpetuated his inability to voice & quench thirst & aggravated SNa,which in turn caused more encephalopathy.Once lucid, drinking kept SNa normal better than DDAVP.CDI was diagnosed by >500 mOsm/kg rise in Uosm with DDAVP, but peak Uosm of only 756 belied a partial NDI.Loss of MRI T1 hyperintensity in posterior pituitary confirms its utility to diagnose CDI.

## TH-PO830

**A Rare Cause of Diabetes Insipidus** Sujata Kale, Neera K. Dahl. *Section of Nephrology, Yale School of Medicine, New Haven, CT.*

**Background:** Central Diabetes Insipidus (CDI) is a disorder characterized by reduced or absent Anti Diuretic Hormone (ADH) presenting with polyuria/polydipsia. Idiopathic DI is the most common cause of CDI. It can also occur secondary to neurosurgery or trauma or from infiltrative diseases such as Langerhans cell histiocytosis (LCH). Patients with LCH with hypothalamic-pituitary disease may develop CDI.

**Methods:** A 35-year-old woman was originally diagnosed with idiopathic CDI in Puerto Rico in February 2010. She was successfully treated with DDAVP. In addition, she had a history of a spontaneous pneumothorax in 2003 requiring chest tube placement, as well as a history of amenorrhea. She had no history of headaches, visual problems or hypothyroidism. On her initial evaluation in Puerto Rico she presented with the following: normal TSH, elevated LH (20.9), borderline high prolactin (23.3), with an undetectable ADH and normal serum osmolality (282). An MRI scan revealed a small pituitary gland. In 2012, she presented for further evaluation and management of CDI. She was doing well on DDAVP. Her vital signs were within normal limits. Her visual fields were full to confrontation, with a normal lung and heart exam. Her repeat LH and FSH were in the low normal range (5-6), cortisol was normal at 11.6, prolactin was now normal at 8, and the TSH normal at 0.98, serum sodium was 141, and a urine specific gravity was 1.015, while on DDAVP. Given her history of amenorrhea and central DI a repeat MRI of the brain was obtained which showed a thickened pituitary stalk. The combination of pituitary stalk lesion with a history of spontaneous pneumothorax raised the possibility of Langerhans cell histiocytosis, which was further substantiated with a high resolution chest CT showing cystic changes. Taken together, these findings established a unifying diagnosis of Langerhans cell histiocytosis. This is a rare autoimmune disorder and a definitive diagnosis is made with tissue biopsy and histology. Her lung function was found to be normal and bone scans were negative for fractures/lytic lesions. The patient has remained stable on DDAVP.

## TH-PO831

**Oxcarbazepine Therapy for Central Diabetes Insipidus** Basmah A. Abdalla,[1] Tara C. Lagu,[1] Daniel L. Landry,[2] Gregory Lee Braden.[2] *[1]Dept of Medicine, Baystate Medical Center/Tufts Univ School of Medicine, Springfield, MA; [2]Dept of Medicine, Div of Nephrology, Baystate Medical Center/Tufts Univ School of Medicine, Springfield, MA.*

**Background:** Oxcarbazepine causes hyponatremia in up to 51% of patients. Mechanisms for this effect have not been completely elucidated.

**Methods:** A 39 year-old female suffered a severe traumatic brain injury in a motor vehicle accident which left her with seizures and complete central diabetes insipidus (CDI). At the time, her serum sodium (SNa) was 158 mEq/L & a urinary osmolality was 137 mOsm/kg. ADH levels by RIA were undetectable. Serum ACTH & cortisol levels were normal. She received intranasal DDAVP 10mcg twice daily & oxcarbazepine 600mg twice daily. 2 years later she developed refractory seizures. Sna was 133mEq/L and oxcarbazepine level was 21 ug/mL (therapeutic). The oxcarbazepine dose was up-titrated to 900 mg twice daily. Concomitantly, Sna gradually fell to 131 mEq/L. DDAVP was discontinued, yet the Sna level declined to 121 mEq/L on day 9. After treatment with 3% saline and fluid restriction, Sna rebounded to 138 mEq/L in 2 days. Serum cortisol and free T4 levels were normal. The patient was discharged on oxcarbazepine 900 mg twice daily, without DDAVP. ADH was undetectable. 9 months later, the patient remained off DDAVP with a normal Sna. Monotherapy with oxcarbazepine effectively controlled her epilepsy and CDI.

**Conclusions:** Proposed mechanisms of oxcarbazepine induced hyponatremia include: SIADH, prolonged ADH half-life, enhanced sensitivity of the renal tubules to ADH, & resetting the osmotic threshold for ADH release in the hypothalamus. Mechanisms of hyponatremia from oxcarbazepine appear to be either direct stimulation of the V2 receptor in renal collecting tubules in absence of ADH or co-stimulation of the V2 receptor in the presence of micromolar ADH concentrations undetectable by current assays. We conclude oxcarbazepine effectively managed both the seizure disorder & complete CDI in our patient and may be a useful alternative to DDAVP in patients with complete CDI.

## TH-PO832

**Use of Continuous Renal Replacement Therapy for Dabigatran Toxicity – Does It Work?** Asif A.K. Ansari, Diana L. Deitzer. *Cleveland Clinic Foundation.*

**Background:** Dabigatran is an oral direct thrombin inhibitor is approved for anticoagulation of non-valvular atrial fibrillation (AF). Drug toxicity in combination with lack of a reversal agent in cases of bleeding has been a major concern, especially in patients with impaired renal function. Extra corporeal therapy (ECT) is considered an effective modality for drug clearance in cases of toxicity, though data supporting its use is sparse. We report a case of critically ill patient with bleeding complications due to toxic levels of Dabigatran that was successfully treated with IHD sessions after inadequate clearance with several days of CRRT.

**Methods:** 70 year old man with AF on Dabigatran 150 mg twice daily that was appropriately dosed for chronic kidney disease stage 3 presented with intra cardiac defibrillator lead infection with enterococcus fecalis leading to septic shock and multi organ failure. He was aggressively treated with mechanical ventilation, pressor support and intravenous antibiotics. His illness was complicated gastro-intestinal bleeding with elevated Dabigatran level of 573 ng/dl and elevated thrombin time >120 seconds requiring multiple blood products. Intriguingly, Dabigatran was discontinued 21 days previously and the patient was receiving continuous renal replacement therapy (CRRT) for the past 15 days for worsening renal failure with fragile hemodynamics. He was then successfully treated with 3 sessions of intermittent hemodialysis (IHD) with resumption of CRRT afterwards that resulted in adequate clearance of the drug. Although bleeding and the indices improved, patient succumbed eventually to multi organ failure.

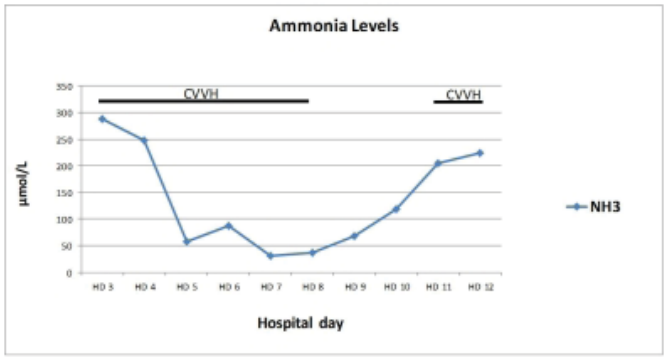
**Conclusions:** Dabigatran eliminates the need for therapeutic drug level monitoring but must be dosed according to the renal reserve to avoid toxicity. Limited data available supports the use of multiple sessions of IHD for drug removal in cases of toxicity; whereas success with CRRT has been variable. In our patient Dabigatran levels remained elevated despite several days of CRRT, suggesting that CRRT is not a reliable modality for drug removal and must not be used in such cases.

## TH-PO833

**Fatal Valproic Acid-Induced Hyperammonemia Despite Continuous Renal Replacement Therapy** Nwamaka Denise Eneanya, Sushrut S. Waikar. *Renal Medicine, Brigham and Women's Hospital, Boston, MA.*

**Background:** Valproic acid can be associated with severe hyperammonemia leading to neuronal injury and cerebral swelling. We report a case of fatal hyperammonemia in a young woman taking Valproic acid despite early initiation of continuous renal replacement therapy.

**Methods:** A 38 y/o female with history notable only for schizoaffective disorder presented with headache and confusion. She was taking Valproic acid with a recent dose increase due to behavioral outbursts. Upon presentation, the patient had frothing of the mouth, large pupils and lower extremity rigidity. Labs revealed normal toxicology screen, renal and liver function. Head CT showed gray-white matter loss and swelling of cerebral hemispheres. Ammonia level, checked on hospital day 3, was 288 µmol/L. Urgent continuous veno-venous hemofiltration (CVVH) was initiated. Additional therapy included scavenging agents (sodium phenylacetate/sodium benzoate), hypertonic saline, and a standard cooling protocol. On hospital day 8, ammonia levels decreased to 34 and CVVH was discontinued.




These levels, however, eventually increased to 205 and CVVH was re-initiated on hospital day 11. Repeat head CT scan on hospital day 12 showed increased intracranial pressure and uncal herniation. Comfort measures only were established for the patient. Although serum orotic acid was elevated to 2.5 mmol/mol-Cr, subsequent genetic testing showed no known ornithine transcarbamylase deficiency.

**Conclusions:** There are currently no published guidelines as to timing of initiation or length of renal replacement therapy in the setting of severe hyperammonemia. Despite reduction of ammonia levels to normal, there was no evidence of neurologic improvement in this patient. Early detection of ammonia levels in encephalopathic patients on Valproic acid should be coupled with urgent renal replacement therapy and frequent monitoring of neurological status.

## TH-PO834

**Dapsone Induced Methemoglobinemia Related Kidney Injury in a Kidney Transplant Recipient** Aditya Kadiyala,[1] James M. Pullman,[2] Madhu C. Bhaskaran,[1] Kellie R. Calderon.[1] *[1]Nephrology, Hofstra North Shore LIJ School of Medicine, Great Neck, NY; [2]Pathology, Montefiore Medical Center/Albert Einstein School of Medicine, Bronx, NY.*

**Background:** Methemoglobin is an oxidative product of hemoglobin which is actively reduced by enzymatic processes in normal state of health. Dapsone, due to its oxidative properties, has been shown to cause methemoglobinemia, a rare cause of kidney injury. It has been shown only in rats to be associated with pigment-induced tubular injury. Here we report a case of dapsone-induced methemoglobinemia causing kidney injury in a kidney transplant recipient. Renal biopsy findings show the similarity of this injury to that in the rat model.

**Methods:** A 65 year old female underwent deceased donor kidney transplantation. Maintenance immunosuppression was achieved with standard triple therapy of tacrolimus, mycophenolic acid and prednisone. A history of sulfa drug allergy precluded the use of trimethoprim/sulfamethoxazole; hence dapsone was used for Pneumocystis Jiroveci prophylaxis. Eleven months post transplant the patient discontinued prednisone against medical advice. Creatinine levels subsequently rose from 0.8mg/dL to 1.7mg/dL and a kidney biopsy was performed. The biopsy revealed acute tubular injury with depositions of brown pigment primarily in the medullary thick ascending limb. Renal parenchyma was otherwise intact with no evidence of rejection or other changes. Laboratory data revealed elevated lactate dehydrogenase, undetectable haptoglobin, macrocytic anemia and signs of hemolysis (appropriately elevated reticulocyte count and erythropoietin levels). Methemoglobin level was elevated at 3.5% (reference range 0-1.5%) and dapsone was immediately discontinued. Serum creatinine declined over the next 10 days to 1.2mg/dL.

**Conclusions:** Acute kidney injury due to methemoglobinemia is documented as a complication of therapy with dapsone. Chronic, low grade hemolysis from methemoglobinemia may lead to tubular toxicity in solid organ transplant recipients receiving dapsone.

## TH-PO835

**Ceftaroline-Induced Allergic Interstitial Nephritis: An Atypical Presentation** Bhupinder Sangha, Judy L. Locati, Ishwinder Sidhu, Karina Sulaiman. *Medicine, LSUHSC-Shreveport, Shreveport, LA.*

**Background:** Allergic interstitial nephritis has commonly been reported with cephalosporins. Ceftaroline is a fifth generation Cephalosporin approved by the FDA for use in community acquired pneumonia and acute bacterial skin structure and skin infections. It has activity against MRSA and has demonstrated non-inferiority to Vancomycin and therefore has been used in off label use for bacteremia. There are no reported cases of AIN with ceftaroline thus far. We describe a case of a patient with atypical presentation of AIN due to suspected use of ceftaroline.

**Methods:** A 52 year old white male with PMH of DM and HTN who presented to the hospital with fever, chills and fatigue was found to have MRSA bacteremia without an identifiable source. He was started on Vancomycin and blood cultures remained positive showing resistance to Vancomycin. Patient was then started on IV Ceftaroline. The serum creatinine was subsequently noted to be elevated from baseline. Urine Wright stain was positive for eosinophils. He was discharged on a six week regimen of IV Ceftaroline. Two weeks following discharge sputum cultures were positive for Mycobacterium kansasii and he was started on four drug regimen. He presented again to the hospital twenty days after completion of Ceftaroline therapy with anasarca and his renal function had improved but had not returned to his baseline. The repeat Wright stain continued to show eosinophils and patient was noted to be in persistent AKI. Renal biopsy showed acute tubular interstitial

nephritis with eosinophils consistent with hypersensitivity reaction. We attributed the nephritis to Ceftaroline and continued the therapy for mycobacterium kansasii. His renal function improved after discontinuation of Ceftaroline.

**Conclusions:** Cephalosporins have long been known to be associated with AIN. Experience with Ceftaroline is limited in clinical practice. We present a case of AIN associated with Ceftaroline use. In this case the offending agent was continued for a prolonged period causing interstitial fibrosis along with tubular damage that subsequently lead to ineffective natriuresis. This may explain his anasarca.

## TH-PO836

**Transient Pigment Induced Nephropathy following Percutaneous Rheolytic Thrombectomy** Rapeepat Lekkham, Eric J. Bloom. *Medicine, Einstein Medical Center, Philadelphia, PA.*

**Background:** The AngioJet® rheolytic thrombectomy system is a catheter-based system for the removal of intravascular thrombi. Hemolysis and hemoglobinuria following the use of AngioJet® has been reported. We present the rare case of severe acute kidney injury following the use of this device that was successfully treated conservatively.

**Methods:** A 49-year-old female with HTN presented with the sudden onset of a severe headache secondary to subarachnoid hemorrhage. Cerebral angiography revealed a ruptured anterior communicating artery aneurysm. She underwent emergency craniotomy with clipping of the aneurysm. Prior to a repeat cerebral angiogram, the AngioJet® device was used to remove a thrombus in the right external iliac artery adjacent to the common femoral sheath. She received IV hydration prior to and during the contrast procedure. The patient was given a total of 85 ml of Iohexol. Immediately following the thrombectomy, her urine became red and progressively decreased in volume from 80-200 cc/hr to anuria within 6 hours. Her serum Cr increased from 0.6 mg/dL to 1.5 mg/dL. A repeat Hb dropped from 11.7 g/dL to 6.9 g/dL. The patient's serum LDH level was elevated and the serum haptoglobin level was low consistent with acute intravascular hemolysis. The urinalysis was strongly positive for blood with only 9 RBC/HPF on microscopy. Centrifugation of a urine sample produced a red supernatant which was positive for heme. Her urine myoglobin was negative. Centrifugation of a blood sample yielded red plasma consistent with a diagnosis of hemoglobinemia. Aggressive hydration with isotonic saline solution was continued without alkalinization or diuretic. The patient did not require renal replacement therapy as her urine output improved significantly over the next 24 hours after thrombectomy. The serum Cr level returned to normal 10 days after thrombectomy.

**Conclusions:** Acute kidney injury caused by intravascular hemolysis and hemoglobinuria can occur following the use of the AngioJet® system. Aggressive hydration may be the best strategy to prevent and minimize the risk of this complication. The risk of AKI should be explained to patients before undergoing thrombectomy by use of AngioJet®.

## TH-PO837

**Propofol Induced Rhabdomyolysis** Pavan K. Annamaraju, James I. McMillan. *Nephrology, VA Loma Linda Healthcare System, Loma Linda, CA.*

**Background:** Propofol induced rhabdomyolysis (RM) is a rare cause of acute kidney injury (AKI), especially in adults. A case of AKI from propofol induced RM is reported.

**Methods:** A 63 year old man was admitted to the hospital for atypical chest pain and dyspnea. Past history included diabetes, hypertension and obesity. Myocardial infarction was ruled out, but he continued to have worsening dyspnea. He developed fever, leukocytosis and pulmonary infiltrates. On hospital day 6, he was transferred to the intensive care unit for respiratory failure and sepsis, likely from pneumonia. He was intubated, placed on mechanical ventilation and given empiric IV antibiotics and vasopressors. Because of agitation, propofol 2.4 mg/kg/hr was added to midazolam and fentanyl on day 14, and subsequent doses of 1.5-1.8 mg/kg/hr were used. From day 19, patient was noted to have progressive transaminitis and worsening renal function with electrolyte disturbances and developed anuria by day 24. RM was confirmed by rise in Creatine kinase (CK) levels and urine blood positivity without urine red blood cells. Emergent hemodialysis was begun on day 24. Propofol induced RM was suspected and drug discontinued. CK level trended down within 72 hrs after stopping propofol, returning to normal in five days. Anterior thigh muscle biopsy showed a monophasic insult pattern of injury excluding chronic inflammatory injury. Dialysis was discontinued on hospital day 40 with subsequent full renal recovery.

| Hospital Day | Serum Creatinine (0.64-1.27 mg/dL) | Serum K (3.6-5.1 mg/dL) | Serum Phosphorus (2.4-4.7 mg/dL) | AST (15-41 IU/L) | Urine RBC (0-2/HPF) | Urine Blood (Neg) | Creatinine Kinase (49-397 IU/L) |
|---|---|---|---|---|---|---|---|
| 1-2 | 1 | 4 | 5.5 | 29 | 1 | Neg | 222 |
| 19 | 2 | 6 | 7.5 | 44 | 1 | Large | |
| 24 | 4.2 | 7.6 | 16.4 | 841 | Anuric | Anuric | >13,200 |

**Conclusions:** Propofol induced RM is rare, and is usually seen at doses > 4 mg/kg/hr over a prolonged time. Our patient developed RM and AKI with doses of only 2.4 mg/kg/hr or less. Differential diagnosis included statins, neuroleptics, paralytics, critical illness myopathy, and pressure-induced ischemia and these were excluded. Discontinuation of propofol led to prompt recovery.

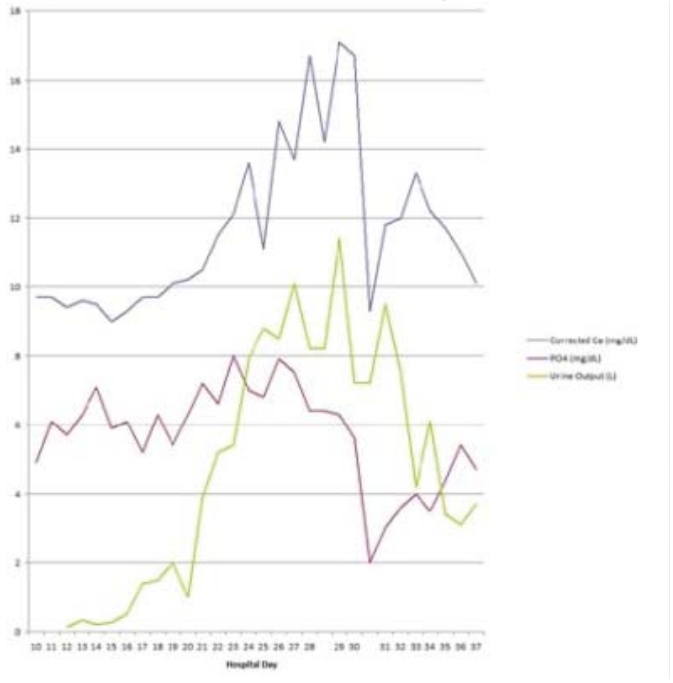
*Funding:* Veterans Affairs Support

## TH-PO838

**Profound Hypercalcemia in Rhabdomyolysis Associated Acute Kidney Injury** Lisa Aimee Hechanova, Seyed-Ali Sadjadi. *Jerry L. Pettis VA Medical Center, Loma Linda, CA.*

**Background:** Rhabdomyolysis is a common phenomenon which results from muscle breakdown due to trauma, extreme exertion, or myotoxins, and results in acute kidney injury (AKI) in 13-50% of cases. Early hypocalcemia is often seen due to precipitation of calcium with phosphate released from lysed muscle cells, and occasionally hypercalcemia can be seen during the recovery phase due to mobilization of calcium phosphate deposits. Herein we present a case of rhabdomyolysis complicated by AKI and profound hypercalcemia.

**Methods:** A 23-year-old male developed AKI after an episode of severe rhabdomyolysis from a car accident which caused extensive crush injuries to his legs, leading to bilateral lower extremity compartment syndrome, requiring several fasciotomies and eventual left above the knee amputation. The rhabdomyolysis was associated with severe hyperkalemia causing multiple episodes of ventricular tachycardia requiring cardioversion, acute respiratory failure requiring mechanical ventilation, and anuric AKI requiring hemodialysis. Renal function recovered 3 weeks after his initial injury, and he entered into the polyuric phase of AKI. During this phase, his serum calcium started rising, reaching a peak of 17.1mg/dL, causing nausea, weakness, drowsiness and polyuria with urine output up to 11L per day. He was treated with IV fluids, calcitonin, pamidronate, and dialysis with a calcium free dialysate. A technetium pyrophosphate scan showed extensive calcium deposition in his left thigh muscles, where he had severe compartment syndrome. His hypercalcemia lasted 16 days, after which his serum calcium level and urine output normalized.




**Conclusions:** It is important to remember that patients with rhabdomyolysis are not out of danger when serum creatinine and urine output start to improve; one must also watch for severe hypercalcemia that is rare but may occur during the diuretic phase of rhabdomyolysis induced AKI.

## TH-PO839

**Biopsy-Induced Tumor Lysis Syndrome** Sharad Virmani, Timothy Muchayi, David Roth. *Nephrology and Hypertension, Univ of Miami Miller School of Medicine, Miami, FL.*

**Background:** Tumor lysis Syndrome (TLS) results from rapid and massive lysis of malignant cells leading to life threatening electrolyte abnormalities. Our case involves the onset of TLS 12 hours following the biopsy of a suspicious tumor.

**Methods:** A 74 y/o Hispanic man with an orthotopic heart transplant in 2009 was admitted with abdominal distention and diarrhea for one month. The patient was hemodynamically stable with baseline creatinine 1.3mg/dl. On day two a computed tomography (CT) scan with contrast revealed a 10.6 x 11.0 x 10.3 cm heterogenous mass in the right lower quadrant causing a small bowel obstruction. Serum labs indicated little concern on day five.

| Day | Potassium | Uric Acid | Calcium | Phosphorous | Creatinine | BUN | LDH |
|---|---|---|---|---|---|---|---|
| Five | 4.4 | N/A | 8.4 | 3.3 | 1.27 | 10 | N/A |
| Six | 9.4 | 40.1 | 5.9 | 17 | 1.94 | 45 | 2,584 |

On day six, A CT guided needle biopsy of the mass yielded seven core samples. Twelve hours post procedure the patient experienced hypotension and tachycardia with peaked T waves. Serum labs showed a potassium of 9.4 mmol/L, bicarbonate of 17 mmol/L, phosphorous of 16.1 mg/dl, and uric acid of 40.9 mg/dl. Hemodialysis was initiated immediately to treat the hyperkalemia, and intravenous fluid resuscitation with one dose of

Rasburicase was given. On day seven CVVHD was started to correct the phosphorous and uric acid. Pathology of the mass showed Burkitt Lymphoma on day 7. Cyclophosphamide and vincristine was initiated to treat the lymphoma.

**Conclusions:** Eectrolyte abnormalities of TLS include hyperuricemia, hyerkalemia, hyperphosphatemia, and hypocalcemia. Clinical consequences include arrhythmias, acute kidney injury, seizures and death. Triggers of TLS can be cytotoxic and biological agents or ischemia. Spontaneous TLS has been documented in Burkitt's lymphoma however little evidence exists demonstrating TLS from biopsies of lymphoma masses. We propose CT-guided biopsies of our patient's abdominal mass caused an acute TLS requiring renal replacement therapy. TLS and AKI should be considered a risk factor when undergoing biopsy of bulky tumors. Patients should be prophylactically treated with allopurinol and hydration.

## TH-PO840

**Prophylaxis of Tumour Lysis Syndrome in Patients Receiving Bendamustine Chemotherapy: A Cautionary Tale** William Petchey,[1] Kristian M. Bowles,[2] Gillian E. Turner,[2] Mahzuz Karim.[1] *[1]Renal Dept, Norfolk & Norwich Univ Hospital, United Kingdom; [2]Haematology Dept, Norfolk & Norwich Univ Hospital, United Kingdom.*

**Background:** Bendamustine is an alkylating agent used to treat Chronic Lymphocytic Leukaemia (CLL). Food and Drug Administration (FDA) approval was granted in 2008, but the product literature was updated in 2009 following post-marketing reports of serious skin complications (Toxic Epidermal Necrolysis [TEN] and Stevens-Johnson Syndrome [SJS]) associated with co-administration with allopurinol. The amended literature no longer recommended prophylactic allopurinol (previously advocated to prevent tumour lysis syndrome [TLS]) and stated that TLS could be mitigated by adequate hydration and careful biochemical monitoring. In a phase III trial of bendamustine, hyperuricaemic prophylaxis was given to all 162 patients in the bendamustine arm. 9/162 (0.06%) developed adverse skin reactions; however only 3 were grade 3 hypersensitivity reactions, and notably no grade 4 reactions, TEN or SJS were reported. The post-marketing incidence of TEN/SJS has not been quantified by the FDA, but is likely extremely low, and the relationship with allopurinol remains only associative.

**Methods:** In view of the concerns regarding skin toxicity we ceased treating our bendamustine patients with allopurinol. However, within 2 months we admitted 3 patients with significant TLS and acute kidney injury: 2 had life-threatening hyperkalaemia, one requiring urgent haemodialysis. All 3 were treated with rasburicase (recombinant urate oxidase) and intravenous hydration. Although renal function improved in all patients, one was left with residual renal impairment. We have since routinely used low dose rasburicase (0.2mg/Kg) in all patients commenced on bendamustine and have observed no further cases of TLS.

**Conclusions:** Bendamustine is an effective treatment for patients with CLL, but we propose conservative measures to prevent TLS are insufficient. Allopurinol is associated with a small but significant risk of life-threatening skin complications; however a single dose of rasburicase pre-cycle 1 of chemotherapy appears an effective alternative in preventing TLS but may not be associated with skin toxicity.

## TH-PO841

**Case Series of Rituximab-Induced Tumor Lysis Syndrome** Venu Velagapudi, Mazen O. Al-Qadi. *Critical Care, Mayo Clinic, Rochester, MN.*

**Background:** Tumor lysis syndrome (TLS) is characterized by hyperuricemia, hyperkalemia, hyperphosphatemia, hypocalcemia, acute kidney injury and metabolic acidosis. Rituximab-induced TLS is extremely rare with only 7 cases reported in the literature. We report a case series of 2 cases of TLS following Rituximab therapy in lymphoproliferative diseases.

**Methods: CASE-1** An 84-year-old female with CLL presented for chemo-therapy with rituximab. After rituximab, she had severe rigors, tachycardia and tachypnea. TLS was diagnosed on the basis of fulfilling Cairo-Bishop laboratory criteria for potassium, phosphorus, uric acid and calcium. She also had pancytopenia, lactic acidosis and acute kidney injury. Clinical grade 2 Cairo-Bishop criteria were fulfilled for renal impairment and arrhythmia. Despite IV fluids, rasburicase, and antibiotics, she developed respiratory failure, *Klebsiella pneumoniae septicemia* and died after withdrawal of care. **CASE-2** An 82-year-old man with angioedema, Gamma heavy chain disease from lymphoplasmacytic lymphoma developed TLS 24 hours after Rituximab. The diagnosis was made by fulfilling 3 laboratory Cairo Bishop Criteria for Phosphorous of 7.2 mg/dl from 5.1 mg/dl, Uric acid 9.6 mg/dl from 5 mg/dl, calcium 6.4 mg/dl from 8.2 mg/dl despite allopurinol and IV fluids. He also fulfilled clinical criteria for grade 2 TLS with serum creatinine of 1.9 mg/dl (>2.3 X Upper limit) and atrial fibrillation requiring treatment. He also had lactic acidosis and respiratory failure. He recovered from TLS, but opted for hospice care later.

**Conclusions:** Rituximab is an anti-CD20 monoclonal antibody that mediates destruction of CD20+ B-lymphocytes. Serious reactions including TLS are seen in 2% with lymphoproliferative disorders (typically high grade lymphomas). Rituximab-induced TLS in patients with low-grade lymphoproliferative diseases is exceedingly rare. We report 2 rare cases of rituximab-induced TLS in low grade lymphoproliferative diseases. TLS may mask sepsis and associated with high mortality in older patients. We suggest diligent monitoring, suspicion and need for prophylaxis when using rituximab in these disorders.

## TH-PO842

**Immobilization-Induced Hyperphosphatemia and Functional Hypoparathyroidism Successfully Treated with Oral Bisphosphonates** Disha D. Trivedi,[1] Elvira Gosmanova,[1] Barry M. Wall.[1,2] *[1]UTHSC, Memphis, TN; [2]VAMC, Memphis, TN.*

**Background:** Immobilization can induce hypercalcemia with suppression of PTH. Functional hypoparathyroidism is an infrequent cause of hyperphosphatemia.

**Methods:** A-46 year-old previously healthy male with 5-month history of paraplegia after motor vehicle accident developed persistent hyperphosphatemia with serum phosphorus levels (Pi) of 6.5-6.7 mg/dL after the injury. Physical examination was remarkable for T5 –T6 level paraplegia and left elbow calcification. Oral intake consisted of tube feeding with 1 Liter per day of "Peptamen" containing 667mg of Pi per L. Laboratory values showed total serum calcium (Ca) of 10.6mg/dL, ionized Ca of 1.32mmol/L (normal range 1-1.35mmol/L), serum bicarbonate 33 mEq/dL, PTH level of 10.5pg/mL (normal range 16.5-70 pg/ml), 25-hydroxy vitamin D level of 16.7ng/ml. Serum albumin, magnesium, cortisol, and TSH levels were normal. Urine Ca to creatinine ratio was 0.149, fractional excretion of Pi (FEPi) 5.2%, and TmP/GFR 7.64md/dL. Serum and urine immunofixation electrophoresis were negative for monoclonal protein. FGF 23 level was 263Ru/mL (normal range 44–215Ru/mL), PTHrP was undetectable. The patient was started on sevelamer 2.4 g with meals; with no improvement in Pi levels. The diagnosis of persistent hyperphosphatemia due to functional hypoparathyroidism from immobilization-induced hypercalcemia was made. Administration of teriparatide 60mcg lead to appropriately increased FEPi, from 14% to 28%. Bone specific markers supported increased bone resorption (N-telopeptide to creatinine ratio of 248, normal range 3-51), and bone turnover (bone-specific alkaline phosphatase 24.7 mcg/L, normal 0 – 20.1 mcg/L), findings characteristic for immobilization. After 3 wk of oral alendronate (70mg weekly), PTH, Pi and Ca levels normalized to 77 pg/ml, 3.2 mg/dL and 8.4 mg/dL, respectively. This was accompanied by decrease in N-telopeptide to creatinine ratio to 112 and bone-specific alkaline phosphatase to 20 mcg/L.

**Conclusions:** Immobilization-induced hyperphosphatemia from increased bone resorption and functional hypoparathyroidism was successfully treated with oral bisphosphonate.

## TH-PO843

**Horsetail Tea Ingestion and End-Stage Renal Disease: An Unrecognized Form of Silica Associated Nephropathy?** Adam Safdi, Shubhada N. Ahya, Yashpal S. Kanwar, Sheldon C. Chen. *Northwestern Univ.*

**Background:** To date, horsetail tea has not been associated with renal disease. Silica is a main component of the plant horsetail, genus Equisetum. Silica is thought to have direct nephrotoxic and inflammatory effects in the kidney. This is a case of a 64 year old woman who presented with accelerated end stage renal disease in the setting of diabetic nephropathy, but renal progression might have been hastened by silica in horsetail tea.

**Methods:** Our 64 year old woman was recently diagnosed with type 2 diabetes and started on insulin therapy 1 month prior to presentation. At the time, her serum creatinine was 1.2 mg/dl, and she was told she had a 'kidney problem.' While vacationing in Mexico, her family encouraged her to drink horsetail tea to 'build up her kidneys.' The tea is made by boiling crushed horsetail which contains copious amounts of silica. For 3 weeks, she drank horsetail tea daily, until she became increasingly ill with tinnitus and head fullness. She was hospitalized and found to have bilateral hemorrhagic strokes and severe kidney failure for which hemodialysis was initiated. Renal biopsy demonstrated findings consistent with diabetic nephropathy, but atypical findings included ATN that was characterized by cytoplasmic vacuolar degeneration. Histologic findings of silica in kidney disease include glomerulonephritis with immune deposits, proximal tubular sloughing, cytoplasmic vacuolization, which might contain aggregates of dense osmiophilic particles. Increased silica in kidney tissue has also been reported. Thus, we suspect that silica contributed to her acute-on-chronic renal failure.

**Conclusions:** Silica can incite or hasten renal injury, acting as a direct nephrotoxin or an inflammatory adjuvant. Though silica nephropathy is usually thought of as an occupational hazard from inhalation (e.g., sandblasting), this is the first report of the disease associated with environmental exposure from purposeful ingestion. We are pursuing various methods to detect increased silica in this patient's kidney tissue. Diagnosing silica-induced renal disease requires a high index of clinical suspicion, because history is paramount and could be the only clue to a potential role of horsetail.

## TH-PO844

**A Fulminant Case of Hypermagnesemia** Mary Muoneke, Ginius Pradhan, Kankam Charity, Deetu Simh. *Internal Medicine, St. Vincent Charity /Case Western Reserve Univ, Cleveland, OH.*

**Background:** The efficiency of renal response to Magnesuim load is such that hypermagnesemia is primarily seen in impaired renal function & iatrogenic (when a large Mg load is taken IV,orally,enema). The symptoms are well known but under-recognized. We report a case of hypermagnesemia presenting with fall& paralytic ileus complicated by cardiopulmonary arrest.

**Methods:** 82 yr old lady presented with fall,nausea&constipation. PMH:HTN,schizoaffective disorder. Meds:cogentin,benazepril,seroquel,haldol. She had stable vital signs,distended abdomen,hypoactive bowel sounds,hard stools in the rectum & diminished reflexes in all extremities. Labs Na.135, K.4.4, Cl.97.Hco3 21, BUN.31 Cr.1.0 KUB:distended bowel suggestive of paralytic ileus. She got enema & surgical consult. Further history revealed intake of large doses of magnesium citrate to relieve constipation. Magnesium level was 15.3mg/dl. She got IV fliuds,lasix,was more lethargic,hypotensive

&bradycardic followed by a cardiac arrest. She was resuscitated successfully as per ACLS protocol. She got IV calcuim gluconate & urgent hemodialysis.Despite these interventions,she coded again& 2nd resuscitation attempt was futile.

**Conclusions:** Magnesuim is a cofactor in more than 300 enzymatic reactions. Homeostasis is maintained by intestines& kidneys. Massive oral ingestion may cause hyperMg if the absorbed Mg exceeds renal capacity. The index patient took large doses of magnesium to relieve constipation. It resulted in a vicious cycle of hypermagnesemia causing paralytic ileus which further enhanced Mg absorption. Hypermagnesemia manifested as fall due to muscle weakness,bradycardia ,hypotension &cardiopulmonary arrest. Deterioration of her renal function is explained by hypermagnesemia-induced hypotension &reduced cardiac output leading to reduced GFR &worsening Mg clearance. Treatment:Removal of exogenous Mg, IV fluids, IV Calcuim carbonate & if therapy fails,hemodialysis against a low Mg bath. She presented with non specific symptoms which retrospectively points to high Mg as the culprit. This case illustrates that hypermagnesemia can occur in patients with normal renal function especially in elderly patients who present with paralytic ileus & generalized weakness.

## TH-PO845

**FGF-23 Mediated Phosphaturia – A Rare Paraneoplastic Disease in Carcinoma** Rupal Mehta, Eudora Eng. *Nephrology, Northwestern Univ, Feinberg School of Medicine, Chicago, IL.*

**Background:** Fibroblast growth factor (FGF) 23, a crucial regulator of serum phosphate and 1,25(OH) vitamin D, is secreted by osteocytes and osteoblasts and is regulated by oral phosphate load and vitamin D levels.

In oncogenic osteomalacia, abnormal secretion of FGF-23 represents a paraneoplastic syndrome of abnormal phosphate and vitamin D metabolism. It is typically described in tumors of mesenchymal origin and presents as hypophosphatemia secondary to renal phosphate wasting.

**Methods:** A 51 year old Chinese male with metastatic nasopharyngeal carcinoma to the bone presented for additional therapy of a pericardial effusion. He was noted to have a phosphorus level of 1.1 mg/dl. Investigation of his hypophosphatemia revealed normal renal function, vitamin D level, parathyroid hormone, and calcium. A fractional excretion of phosphorus was 22.7% (reference < 5% in hypophosphatemia) and phosphorus excretion on 24 hour urine collection was 681.4 mg. A FGF-23 level of 540 RU/ml (reference <181 RU/ml) confirmed the suspected diagnosis of osteogenic osteomalacia. Aggressive oral and IV phosphorus supplementation was started for persistent symptomatic hypophosphatemia.

| Hospital Day | Phosphorus | Phosphorus Repletion in mmol |
|---|---|---|
| 1 | 1.1 | 16 |
| 2 | 1.7 | 21 |
| 5 | 1.9 | 126 |
| 8 | 1.8 | 8 |
| 11 | 1.8 | 60 |
| 13 | 2.7 | 48 |
| 20 - Discharge Day | 1.6 | |

**Conclusions:** Oncogenic osteomalacia is an entity seen mostly in malignancies of bone and soft tissue with definitive treatment being resection. The half life of FGF-23 is approximately 58 minutes, thus, phosphate levels can quickly normalize postoperatively. We present the first reported case of osteogenic osteomalacia in a patient with nasopharyngeal carcinoma. He had exhausted all surgical, chemotherapeutic and radiation options for treatment of his malignancy and no proven nonsurgical therapies exist for treatment of osteogenic osteomalacia. Cinacalcet, an agonist of the calcium sensing receptor, has been used as a potential treatment given that FGF-23 may be PTH dependent however, our patient had normal PTH levels. With limited therapeutic options, this disease entity was extremely difficult and cumbersome to manage.

## TH-PO846

**A Case of Severe Hypocalcemia Induced by Sprue-Like Enteropathy Associated with Olmesartan** Keita Hirano, Kumiko Shimasaki, Masahiko Nagahama, Yasuhiro Komatsu. *Div of Nephrology, Dept of Internal Medicine, St. Luke's International Hospital, Japan.*

**Background:** In this report, we describe a rare case of hypocalcemia with tetanic symptoms. The cause of hypocalcemia in hospitalized patients is most often caused by disorders of parathyroid hormone (PTH) or vitamin D. symptomatic hypocalcemia is as a first sign of celiac sprue.Celiac sprue is an autoimmune disorder of the small intestine. Chronic diarrhea and calcium and vitamin D malabsorption are characteristics of celiac sprue. They cause hypocalcemia.

**Methods:** A previously healthy 64-year-old woman was referred to our hospital because of severe diarrhea and tetanic symptoms. She had diabetes and hypertension treated with olmesartan. Her serum ionized calcium ($Ca^{2+}$) level was 1.63 mmol/L and she presented with high intact-PTH and very low 25-hydroxy-vitamin D levels. Physical examination indicated no abnormalities. Fecal calcium excretion indicated malabsorption of vitamin D, and biopsy of the small intestine demonstrated pathologic changes characteristic of celiac sprue. However, the initiation of a gluten-free diet did not result in correction of all biochemical abnormalities. The results of anti-transglutaminase antibody and anti-endomysium antibody were negative which revealed that the case was not celiac sprue. In August 2012, Rubio-Tapia A et al reported that severe sprue-like enteropathy may be associated with olmesartan (Rubio-Tapia A., Mayo Clin Proc. 2012 Aug;87(8):732-8.). After discontinuing her olmesartan use, her diarrhea improved and calcium level normalized. The final diagnosis was severe hypocalcemia induced by sprue-like enteropathy associated with olmesartan.

**Conclusions:** This patient presented with severe hypocalcemia and diarrhea and showed clinical response and histologic improvement after suspension of olmesartan which was suspected to be causing the sprue-like enteropathy.

## TH-PO847

**Tetany, an Unusual Cause!** Reem Daloul, Abhishek Swami. *William Beaumont Hospital, Royal Oak, MI.*

**Background:** Tetany is a sustained involuntary muscle contraction that often occurs as a result of hypocalcemia or severe alkalosis. Here we report a case of severe acute tetany with a very unusual etiology.

**Methods:** A 34 year old female nurse with history of anxiety disorder and depression presented to the hospital for diffuse tetany. Symptoms started suddenly while driving with numbness and tingling of extremities followed by diffuse spasm and contraction of arms, legs, jaw and chest. Her home medications included seroquel and sertraline. On presentation, the patient was normotensive. She had wide opened eyes, locked jaw, flexed wrists and digits, and flexed posture. Laboratory work revealed severe hypokalemia with potassium of 1.9 mmol/L, and metabolic alkalosis with a bicarbonate level of 37 mmol/L. Ionized and total calcium as well as magnesium levels were normal. Administration of intravenous potassium chloride resulted in prompt muscle relaxation. Further investigations revealed a high urinary potassium indicating renal potassium loss. Urine for diuretic screen was negative. The patient was discharged in stable condition on oral potassium supplement with plan for further evaluation to establish the etiology of hypokalemia as out patient.

**Conclusions:** Neuromuscular effects of hypokalemia typically include generalized weakness, fatigue and muscle paralysis. Tetany, which represents muscle irritability appears to be a paradoxical effect. It has been described in standard text books and there are rare reports in the literature. In our patient, the prompt muscle relaxation following potassium supplements administration indicates that hypokalemia was the principle causative factor. Hypokalemia needs to be considered in the differential diagnosis of acute tetany as early recognition and treatment is needed for this potentially fatal condition.

## TH-PO848

**Acute Development of Resistant Hypertension following Octreotide Therapy for Carcinoid Syndrome** Douglas W. Schwartz,[1] Barry M. Wall.[1,2] *[1]Univ of Tennessee Health Science Center, Memphis, TN; [2]Veterans Affairs Medical Center, Memphis, TN.*

**Background:** Octreotide has been effective in managing symptoms of carcinoid syndrome. Both hypotension and acute hypertensive crises associated with carcinoid syndrome have responded to octreotide therapy. Although carcinoid tumors can produce a number of vasodilatory products, the development of refractory hypertension during octreotide therapy for carcinoid syndrome has not been reported.

**Methods:** 61 yr old male with well controlled hypertension (Lisinopril/HCTZ) was confirmed to have carcinoid syndrome during evaluation for chronic diarrhea and facial flushing. Biopsy of liver lesions showed a carcinoid neuroendocrine tumor. 5-HIAA level was markedly elevated at 26 mg/day. Within 3 days of initiating long acting ocreotide (20mg IM) blood pressure became acutely elevated, 224/101mmHg with no change in heart rate. Despite progressive addition of metoprolol, hydralazine, and amlodipine, systolic BP remained > 150mmHg. Workup for secondary causes of hypertension included: normal hemoglobin, normal serum potassium, creatinine (1.3 mg/dl), urinalysis, and urinary metanephrine excretion. Imaging studies revealed normal adrenal glands and kidneys. Plasma renin activity was not elevated. Volume status was unchanged. During long term monthly octreotide therapy, diarrhea and flushing rapidly resolved and have not recurred. BP control improved (140/80 mmHg), but continues to require multi- drug therapy.

**Conclusions:** Octreotide has not been reported to have direct vasoconstrictor effects. Acute hypertensive crisis has been reported to occur following octreotide therapy in 2 prior patients (pheochromocytoma and diabetic associated diarrhea) that resolved following discontinuation of the drug. The development of refractory hypertension during octreotide therapy for carcinoid syndrome has not been reported. The most likely etiology for the accelerated hypertension in our patient is increased systemic vascular resistance, resulting from decreased production of vasodilatory substances from the carcinoid tumor.

## TH-PO849

**Midaortic Syndrome: An Unusual Cause of Resistant Hypertension in Adults** Prerna Ganjoo, Maria P. Martinez Cantarin. *Medicine-Nephrology, Thomas Jefferson Univ Hospital, Philadelphia.*

**Background:** Resistant hypertension(RH) is defined as BP>140/90 (general population),and >130/80 (chronic kidney disease) despite concurrent use of 3 antihypertensives. The prevalence of RH may be as high as 20% in the hypertensive population.

**Methods:** A 52year old Hispanic male with history of resistant hypertension (diagnosed at age 8), hemorrhagic stroke and chronic kidney disease (CKD) stage III with baseline creatinine 1.7 mg/dL, was admitted with chest pain and severe hypertension. Examination revealed blood pressure in left arm 208/89 mmHg, and right arm 225/118 mmHg, cardiac holosystolic murmur and an abdominal bruit. A work up to rule out aortic dissection was initiated. CTA thorax showed elongated funnel-shaped stenosis with severe luminal narrowing involving the distal descending thoracic aorta. Cardiac workup was negative for ischemia. Further testing showed negative renal artery stenosis, normal bilateral renal size and echotexture, negative ANA, RPR, and urine toxicology screen. Carotid artery ultrasound showed less than 50% diameter reduction. A diagnosis of supraceliac midaortic syndrome was made. Patient underwent Aorto-aorto (descending thoracic aorta to infrarenal

aorta) bypass. At discharge, patient's creatinine was 1 mg/dL, and he required only 2 antihypertensive medications. Nine months after surgery, creatinine and anti-hypertensive regimen remains unchanged from time of discharge.

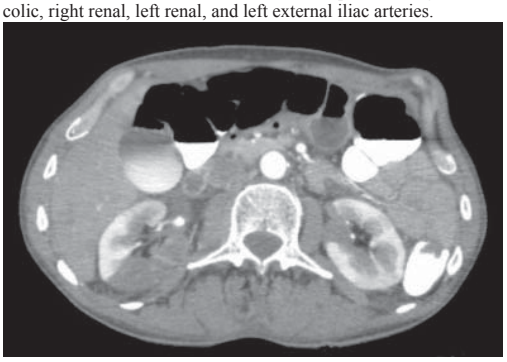
**Conclusions:** Midaortic syndrome (MAS) is a rare anomaly accounting for 0.5% to 2.0% of all aortic coarctations. It is most common in pediatric population, and young adults. MAS is usually congenital, but can also be associated with Takayasu's or temporal arteritis, neurofibromatosis and Williams syndrome. Serious complications of renovascular hypertension, including CKD, heart failure or ischemia, intracranial hemorrhage and aortic rupture may ensue.Treatment is predominantly surgical. We report an unusual case of idiopathic MAS in an adult patient with history of CKD, intracranial hemorrhage and resistant hypertension, with normalization of renal function, and improvement in blood pressure control post surgical repair.

## TH-PO850

**Bilateral Renal Infarction due to Fibromuscular Dysplasia** Taha Ayach, Amir Kazory. *Nephrology, Hypertension and Renal Transplantation, Univ of Florida, Gainesville, FL.*

**Background:** While Fibromuscular dysplasia (FMD) is an established cause of secondary hypertension, its association with renal infarction, which often has a subtle presentation, is less well recognized. Here, we report a case of FMD presenting with acute abdomen, bilateral renal infarcts, and severe hypertension.

**Methods:** A 53-year-old man presented with hematuria and acute severe abdominal and back pain radiating to the scrotum. Physical examination was unremarkable except for a blood pressure of 194/129 mmHg and right lower quadrant abdominal tenderness. Heart rate was regular and there were no skin lesions. Laboratory studies showed a serum creatinine level of 1.3 mg/dL, leukocyturia, and hematuria. Contrast-enhanced abdominal CT showed bilateral renal infarcts and multiple short segment dissections of the middle colic, right renal, left renal, and left external iliac arteries.



A comprehensive immunologic workup as well as hypercoagulability tests were all negative. Echocardiogram did not show any vegetation or thrombus. Conservative management was associated with improvement in symptoms and control of blood pressure.

**Conclusions:** The clinical presentation of FMD depends on the anatomic distribution as well as the extent of vascular involvement and is often asymptomatic or subtle. Although renal arteries are the most commonly involved vascular bed, renal infarction is seen in only 0.9% of the patients. Bilateral renal infarction is extremely rare and has so far been reported in a handful of cases. Revascularization is reserved for patients with resistant hypertension or progressive deterioration in renal function. This case, coupled with previous reports, illustrates the high variability in the presentation of FMD and this diagnosis should be considered in patients presenting with otherwise unexplained renal ischemia/infarction and involvement of multiple arteries.

## TH-PO851

**Weeping Kidney Syndrome** Ankit Rawal, Stuart M. Sprague. *Nephrology, Univ of Chicago - NorthShore, Evanston, IL.*

**Background:** Weeping kidney syndrome has a very low incidence rate with few reported cases and there is confusion in the definition. This report presents one rare case and discusses features of this disease.

**Methods:** A 41 year old african american female with past medical history of type I diabetes mellitus nine years post kidney and pancreatic transplant presents with recurrent abdominal pain, vomiting, and inability to tolerate oral intake. On initial examination, heart and lungs were normal. On abdominal examination, there was distention and soft ballotable swelling of the right lower quadrant. Routine blood examination was normal with the exception of an acutely elevated serum creatinine of 2.0 mg/dL. Radiological evaluation revealed new onset ascites with a cystic lesion in the transplanted kidney causing ureteral obstruction. A therapeutic paracentesis was perfomed which gave the patient symptomatic relief and revealed a high serum-ascites albumin gradient. The patient subsequently presented 3 days later with similar complaints. Imaging studies demonstrated a complex fluid collection throughout the pelvis with a loculated collection around the transplanted kidney. Serological evaluation of liver function and possible causes of hepatic dysfunction all returned negative. An abdominal drain was then placed which drained approximately 1.5 liters of yellow serous fluid per day. This provided the patient minimal relief. A liver biopsy was performed and returned negative. Further ascitic fluid analysis revealed a peritoneal fluid creatinine of 6.7 mg/dL and with a corresponding serum creatinine of 3.1 mg/dL. At this time it was thought the ascites may be nephrogenic in nature or due to urinary leak. She underwent a diagnostic laparoscopy which showed a slow, continuous leak of lymphatic fluid from the transplanted kidney, diagnostic for renal lymphangiectasia. The patient then underwent marsupilization and wrapping of the weeping kidney in omentum. This procedure proved to be successful and resolved her presenting complaints from six months prior.

**Conclusions:** Renal lymphangiectasia is a rare disease of the renal lymphatics with approximately 40 cases being described since 1890. This case depicts a rarely reported complication of renal transplantation.

## TH-PO852

**Chronic Kidney Disease from Functional Urinary Tract Obstruction** Anshul Kumar, Vibha Agrawal, Pradeep Dhakarwal, Joseph C. Guzzo, Henry L. Schairer. *Nephrology, LVHN, Allentown, PA.*

**Background:** Functional obstructive uropathy should be considered in patients with CKD and polyuria/polydipsia, especially who have evidence of central or nephrogenic DI, psychogenic polydipsia or use of anti-cholinergic medications.

**Methods:** 74 year old female was seen for worsening creatinine which rose from 1.7mg/dl in 2007 to 2.4mg/dl in 2011. Her main complaint was polyuria and polydipsia. In early childhood she was diagnosed with Hand Schuller Christian disease. On evaluation she had stable vitals, bilateral nerve deafness and a palpable urinary bladder. Her serum Cr was 2.5mg/dl, serum Na 145meq/l and UA showed a specific gravity of 1.005. Renal USG showed bilateral collecting system dilatation and post void residual of 578ml. Urine osmolality rose from 146mosm/kg to 365mosm/kg 2 hours following a 2mcg i.v. DDAVP. She was taught self catheterization and started on 0.5mg of DDAVP orally daily. Follow up labs showed Na between 133-140meq/l and Cr around 1.9mg/dl. Repeat USG showed mild bilateral collecting system fullness. Her urine output and fluid intake had markedly decreased improving her quality of life.

**Conclusions:** Large urine volumes lead to bladder distension and hypertrophy with subsequent intramural obstruction of the distal ureters. Bladder contractility is compromised, ureteric peristalsis diminishes and large residual volumes worsen this functional obstructive uropathy. Social embarrassment leading to voluntarily urine retention exacerbates it. Corrective therapy, even after prolonged period can lead to improvement in urinary tract dilatation. CKD can occur but ESRD is unknown. Our patient though had central DI did not reach the maximum concentrating ability following DDAVP test. A part maybe due to age related decrease in concentrating ability. Also long standing obstructive uropathy can result in acquired nephrogenic DI. The exact patho-physiology is not known but severe hyposthenuria indicates an intact diluting capability and locates the defect distal to the loop of Henle. Increased collecting duct pressure causes damage to the tubular epithelium resulting in insensitivity or partial sensitivity to the action of AVP.

## TH-PO853

**Perceptions of Nephrology among Internal Medicine Residents** Ivan E. Porter,[1] Hope Kincaid,[2] Stuart J. Hartman,[2] Nabeel Aslam,[1] Sharon E. Maynard.[2] *[1]Nephrology & Hypertension, Mayo Clinic, Jacksonville, FL; [2]Lehigh Valley Health Network, Emmaus, PA.*

**Background:** As interest in nephrology declines, we sought to identify factors influencing nephrology career interest among internal medicine (IM) residents.

**Methods:** This is a cross-sectional survey of IM residents to assess perceptions of nephrology and the impact of these perceptions on career decisions. All categorical IM residents at two institutions (Lehigh Valley Health Network and Mayo Clinic Florida) were invited to participate, prior to the initiation of a nephrology fellowship program. The survey was adapted from a tool previously validated in Australian medical trainees.

**Results:** 58 of 78 residents completed the survey (74% response rate). 53% of the residents were male and 84 % were US graduates. 14% of residents indicated an interest in a nephrology career. Cardiology (32%) and Hospitalist (41%) were the most sought after careers. While 43% of residents stated that their choice of residency was affected by the presence of a fellowship program at their institution, 41% first became interested in their specialty of choice during residency and 55% became certain of their specialty of choice during residency. Figure 1 shows whether subjects agreed or disagreed with several negative statements about Nephrology, and the impact of each statement on the residents' career decision. Factors with the most impact on career choice were that nephrologists have long work hours (42%) and frequent/difficult call (57.9%).

| | % Perceptions | | | % Impact | | |
|---|---|---|---|---|---|---|
| **Statements about Nephrology** | Disagree | Neutral | Agree | Little | Moderate | High |
| Not Interesting | 87 | 7 | 5 | 71 | 26 | 3 |
| Unappealing due to chronic nature of disease | 63 | 14 | 23 | 62 | 35 | 3 |
| Pathophys too complex | 48 | 32 | 20 | 63 | 37 | 0 |
| Poorly prepared in medical school | 52 | 24 | 24 | 86 | 14 | 0 |
| No positive role models | 87 | 7 | 6 | 77 | 14 | 9 |
| No previous Nephrology experience | 71 | 11 | 18 | 79 | 21 | 0 |
| Poorly Paid | 27 | 64 | 9 | 71 | 26 | 3 |
| Few opportunities for procedures | 32 | 34 | 34 | 79 | 13 | 8 |
| Long work hours | 23 | 45 | 32 | 58 | 26 | 16 |
| Not conducive to part time work | 29 | 59 | 12 | 79 | 16 | 5 |
| Frequent/Difficult Call | 24 | 36 | 40 | 42 | 42 | 16 |
| Fellowship long hours/difficult call | 18 | 48 | 34 | 53 | 37 | 10 |

**Conclusions:** Most residents become certain of their specialty choice during residency. We found several negative perceptions of Nephrology among IM residents, which may impact career decisions. Efforts to increase interest in Nephrology careers should focus on improving these negative experiences and perceptions among residents.

**TH-PO854**

**More Women Choose Nephrology, but Publications by Female Senior Authors Have Not Increased** Suzanne M. Norby, LaTonya J. Hickson, Amy W. Williams. *Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** Over the past decades, the number of female physicians has increased, yet women are promoted to associate professor and professor levels at lower rates than for men. Differences in number of peer-reviwed publications by female and male authors likely contribute to the discrepancy. To explore this further in nephrology, this study compared the number of female nephrology fellows in 1994 and 2011 as well as the number and relative percentage of publications by female authors in two nephrology journals.

**Methods:** The number of female nephrology fellows in the U.S. was obtained from published data in the the Journal of the American Medical Association. The tables of contents of regular issues of the Journal of the American Society of Nephrology and the American Journal of Kidney Diseases from 1994 and 2012 were reviewed. The study was limited to articles with first or last authors listing a U.S. institutional affiliation. The gender of first and last authors for original articles was determined, using internet search strategies when gender was not obvious by first name.

**Results:** In 1994, 121/637 (19%) of nephrology fellows were women compared with 336/918(40%) in 2011 (p=<0.0001). 520 original article entries were reviewed. 316 were from U.S. institutions: 168/244(69%) in 1994 and 148/276(54%) in 2012. In 1994, 34(20%) of articles had a female first author compared with 60 articles(40%) in 2012 (p=0.005). Proportion of articles with a female last author was not significantly different; 23(14%) in 1994 compared with 24(16%) in 2012. Female first authors did not increase significantly in JASN [21/91 (23%) to 30/79(38%) ] but did in AJKD [13/77(17%) to 30/69 43%), p=0.014]. Female last authors did not increase significantly for JASN (12% vs 19%) and decreased for AJKD (16% vs 13%).

**Conclusions:** Although the proportion of female nephrology fellows doubled between 1994 and 2011, the number of publications by women in two high-impact nephrology journals does not demonstrate a parallel increase. Since there is a lag between completion of training and achieving academic productivity, the increase in number of female first authors is encouraging. Further analysis is needed to examine trends.

**TH-PO855**

**Documentation of Counseling Performed at Clinic Visits, a CMS EHR Incentive Program Requirement** Anna Burgner, Julia Lewis. *Div of Nephrology, Vanderbilt Univ School of Medicine, Nashville, TN.*

**Background:** One of the requirements of Stage 2 for the CMS Medicare and Medicaid Electronic Health Records (EHR) Incentive Programs is to provide clinical summaries to the patient for each office visit within one business day (meaningful use core measure #8). Part of these clinical summaries includes written documentation of counseling/instructions given to the patients during the visit. Frequently the same counseling can be appropriate for multiple patients during a clinic session.

**Methods:** Using our electronic medical record system, we have created a set of templates in those areas that we frequently counsel our patients, and these can be easily auto-imported into the clinical summary. They include topics such as ACE-I and ARB use and teratogenicity, starting immunosuppression, sodium and fluid restricted diet, low potassium diet, and low phosphorus diet. Each template can be modified with patient specific information, such as the name of their ACE-I or the degree of fluid restriction, allowing for flexibility in the templates and more patient centered care.

**Results:** We find that utilizing preformed templates leads to a more efficient completion of the clinical summary. It saves documentation time. It also allows for the patient to get a consistent, well written, and educational set of counseling at each visit.

**Conclusions:** The use of modifiable templates to document counseling and instructions given during the clinical visit in the clinical summary allows for improved efficiency in clinic, while delivering a consistent, educational directive to the patient.

**TH-PO856**

**A Pilot Renal-Palliative Care Curriculum: Improving Knowledge and Skills among Nephrology Fellows** Katharine Cheung,[1] Sumi Sukumaran Nair,[1] Emilee R. Wilhelm-Leen,[1] Manjula Kurella Tamura.[2] *[1]Stanford Univ School of Medicine; [2]Palo Alto VA Health Care System.*

**Background:** Nephrologists frequently care for frail, older patients with high annual mortality. But the majority of trainees feel unprepared to have end-of-life discussions. This quality improvement project aims to describe the knowledge, attitudes and skills of nephrology fellows at a single center and track their progress through an innovative, renal-palliative care curriculum.

**Methods:** We conducted a focus group of nephrology fellows to assess their knowledge gaps in palliative care. Using that baseline, we collaborated with experts in Palliative Care and Geriatric Nephrology to devise a pilot curriculum. We assessed knowledge, attitudes and skills with surveys before and after the pilot and each learning module. Survey data was collected using Qualtrics survey tool.

**Results:** The curriculum consisted of four lectures (hospice, dialysis withdrawal, cross-cultural care, non-pain symptom management), two workshops (pain management and communication skills) and one journal club (prognostication in kidney disease) over six months. In the pre-test, four of nine trainees reported having no prior training in communication skills, end-of-life or palliative care for renal patients but 90% of trainees reported a sense of responsibility to be trained in these areas. When asked to rate knowledge and/or skills on 21 specific areas (e.g., knowledge of hospice services available to dialysis patients), the mean score ranged from 2.1-3.5 on a five-point scale, with 5 being the best score. Trainees reported particular need for teaching in planning for withholding dialysis. In general, trainees' scores on content-based pre-tests were low (mean correct across three tests 34%) and there was improvement in post-tests (mean correct across three tests 75%). Trainees reported a high score for usefulness of the content as well as their likelihood of recommending the sessions to others (means 4.7 and 4.8 respectively on a five-point scale).

**Conclusions:** Renal fellows have limited training in palliative care, but desire this training. A curriculum covering key topics in palliative care increases content knowledge and is rated by trainees as highly useful.

**TH-PO857**

**Nephrology Elective Experience during Medical Residency: A U.S. Nephrology Fellowship Training Program Directors' Survey** Hitesh H. Shah,[1] Nancy Day Adams,[2] Aditya Kadiyala,[1] Kenar D. Jhaveri.[1] *[1]Nephrology, Hofstra North Shore–LIJ School of Medicine, Great Neck, NY; [2]Univ of Connecticut Health Center, Farmington, CT.*

**Background:** Interest in nephrology careers is declining in the United States (US). The type of nephrology elective that internal medicine (IM) residents experience may play an important role in enhancing interest in nephrology careers. We do not know what types of nephrology electives are offered in IM programs or whether nephrology electives are representative of what practicing nephrologists do.

**Methods:** An anonymous online survey was created & subsequently distributed to all US nephrology fellowship training program directors (N-TPDs) in May 2013.

**Results:** So far, 50 US N-TPDs have responded to our survey (34% response rate). 86% of the programs offered a 4-week nephrology elective at their institution. 48% of the programs did not offer any outpatient (OP) nephrology experiences to their IM residents during a typical 4-week elective. Nearly 90% of the programs did not rotate their residents in either their outpatient HD unit or PD clinic. Only 30% rotated their residents in their OP kidney transplant program. When asked what was most effective in fostering interest in nephrology careers, N-TPDs identified nephrology elective experience (78%) & nephrology faculty mentoring (78%). 72% felt that a 4-week elective would provide adequate exposure to nephrology during residency. 92% of the N-TPDs thought that an "ideal" nephrology elective experience should include both inpatient (IP) & OP experiences. "Inpatient service needs" (48%) and "too much work to coordinate various OP experiences" (26%) were some of the reasons identified by N-TPDs for none or limited OP nephrology experiences for IM residents. Over half of the N-TPDs responded that their elective structure was not representative of what nephrologists do in practice.

**Conclusions:** Nearly half of the responding programs did not offer any OP nephrology experiences to IM residents. To enhance knowledge and interest in nephrology careers during IM residency, a combined exposure to both IP & OP nephrology (including outpatient dialysis and transplantation) should be strongly considered by the training community.

**TH-PO858**

**Creating Interest in Nephrology Careers: A Novel Nephrology Elective Experience for Medical Students** Hitesh H. Shah, Kenar D. Jhaveri. *Nephrology, Hofstra North Shore–LIJ School of Medicine, Great Neck, NY.*

**Background:** Interest in nephrology careers is declining in the United States (US). The type of nephrology elective that US medical students experience may play an important role in creating interest in nephrology careers.

**Methods:** We created a novel 4-week nephrology elective experience for visiting medical students at our institution. Our redesigned elective included both 2-week inpatient (IP) & 2-week outpatient (OP) nephrology experiences. The OP rotation included 10 half-days of various nephrology clinic experiences, 2 half-days of immediate post-transplant clinic, 1 half-day of kidney donor evaluation clinic, 2 half-days of PD clinic and 3 half-days of outpatient HD unit rounding. Our redesigned elective also included educational conferences. From 6/2012 to 12/2012, five 4th year medical students (all from different US medical schools) completed our redesigned nephrology elective. An anonymous online survey was created to evaluate the novel elective experience and was subsequently distributed to all students in June 2013.

**Results:** All students responded to our survey. All reported adequate OP nephrology exposure. 80% of the students had worked with one or two faculty members during the IP setting. In comparison, 80% were exposed to at least four different faculty members during the OP experiences. All students had interacted with at least 3 fellows. All reported that the elective experience enhanced their exposure and knowledge in nephrology and that they would recommend this elective to other medical students. They also thought that this elective provided them with a better insight into what nephrologists do in practice. 80% of the students reported that this elective experience created an interest in nephrology career. Majority (60%) of the students responded that they would consider nephrology as one of their 3 top career choices as a result of this elective experience.

**Conclusions:** We believe that the restructured nephrology elective will provide the medical student with a much needed and realistic exposure to nephrology. Based on our experience, we recommend all nephrology training programs to consider this elective structure for medical students.

**TH-PO859**

**Characteristics of U.S. Adult Nephrology Fellowship Training Program Directors** Hitesh H. Shah, Divya Monga, Aditya Kadiyala, Anna Mathew. *Nephrology, Hofstra North Shore–LIJ School of Medicine, Great Neck, NY.*

**Background:** Characteristics of US adult nephrology fellowship training program directors (N-TPDs) are not known. We do not know how long N-TPDs have served in their role as TPDs and if they had undergone fellowship training in the same program where they were serving as TPD.

**Methods:** Data were abstracted in June 2013 from public websites of Accreditation Council for Graduate Medical Education-Accreditation Data System, Electronic Residency Application Service, Healthgrades.com and webpages of individual nephrology fellowship programs. Data was analyzed for 146 N-TPDs**.**

**Results:** As of June 2013, analysis of this data showed that the average age of N-TPDs was 52.5 years with majority (76%) of them being males. Age stratified data available for 138 N-TPDs showed that only 5.1% were in the younger age group of 30-40 years of age with 19.6% being more than 60 years old. 43.5% were between 41-50 years of age while 31.8% were between 51 to 60 years old. Out of the 146 N-TPDs, 71.2% were United States (US) medical graduates with the remaining 28.8% being international medical graduates (IMG). Interestingly, 42.9% of this IMG group underwent medical school training in India. Half (50.7%) were found to be serving in the role of N-TPD for at least 5 years with 26% having served more than 10 years. 24.5% have served as N-TPD for less than 2 years at the time of this analysis. Out of the data available for 134 N-TPDs, 39.6% held the academic rank of professor of medicine, with 40.3% being associate professors and 20.1% being assistant professors. Also, 33.6% were found to have completed their fellowship training in the same program that they were serving as N-TPD.

**Conclusions:** Majority of the N-TPDs were males and had undergone medical school training in the US. A significant percentage of N-TPDs who were trained in international medical schools were from India. Majority of this group has served as TPD for over 5 years and nearly one fourth have served for more than 10 years. Interestingly, one-third of the N-TPDs had undergone fellowship training in the same program where they were serving as TPD.

**TH-PO860**

**ASN Dimitrios G. Oreopoulos Visiting Professor Program in End-of-Life Care: Results of an Educational Intervention** Jamie Alton Green,[1] Alvin H. Moss,[2] Michael F. Schultz,[1] James E. Hartle,[1] Ion D. Bucaloiu.[1] *[1]Dept of Nephrology, Geisinger Medical Center, Danville, PA; [2]Center for Health Ethics and Law, West Virginia Univ, Morgantown, WV.*

**Background:** Prior studies show that nephrology fellows receive inadequate training in end-of-life care.

**Methods:** We performed a half-day workshop led by a nationally recognized expert in end-of-life care to improve fellow preparedness in this area. The workshop included formal didactic lectures and case-based discussions pertaining to renal palliative medicine. Participants were 1st and 2nd year nephrology fellows at Geisinger Medical Center in Danville, Pennsylvania (n=5). Self-reported preparedness was measured pre- and post-workshop using a 5-point Likert scale (0=not at all prepared to 4=very prepared). Paired t-tests were used to assess changes in fellow preparedness based on the workshop.

**Results:** 60% of the fellows were male and 40% had received formal training in palliative care at baseline. Mean level of preparedness increased significantly for all skills (Table). 100% of fellows "strongly agreed" that the program significantly increased their knowledge and comfort regarding end-of-life care in nephrology. 100% of fellows also "strongly agreed" that based on the program they would be more likely to estimate and discuss prognosis with their kidney patients. Table. Changes in Fellow Preparedness.

| Skill | Pre | Post | p-value |
|---|---|---|---|
| Care for dying patients | 3.2 | 4.0 | 0.02 |
| When to refer to hospice | 2.8 | 4.0 | 0.004 |
| Tell a patient he/she is dying | 3.0 | 4.0 | 0.03 |
| Assess prognosis | 2.8 | 4.0 | 0.004 |
| When to refer to palliative care | 2.6 | 3.8 | 0.03 |
| Respond to requests to stop dialysis | 3.0 | 4.0 | 0.03 |
| Discuss dialysis options including no dialysis | 2.8 | 4.0 | 0.03 |
| When to use the "surprise" question | 2.8 | 4.0 | 0.03 |
| Role of shared decision-making | 2.6 | 4.0 | 0.005 |
| Assess and manage pain | 2.4 | 4.0 | 0.003 |
| Assess and manage depression | 2.0 | 3.8 | 0.001 |
| Assess and manage nausea | 2.6 | 3.8 | 0.03 |
| Assess and manage pruritus | 2.4 | 4.0 | 0.003 |
| **All skills combined** | **2.7** | **4.0** | **<0.001** |

**Conclusions:** A brief educational intervention performed by a nationally recognized expert improved fellow preparedness in end-of-life care in nephrology. These results will be used to implement a more formal renal palliative care training program.

**TH-PO861**

**Formal Clinical Research Training during Fellowship Is Valuable in Initiating a Career in Academic Nephrology** Shilpa Sharma, J. Kevin Tucker. *Brigham & Women's Hospital.*

**Background:** In a survey of ASN members which included an equal representation of academic/private practice nephrologists, most respondents indicated they had "little or no training" in clinical research (Berns et al. cJASN 2010). 11.1% to 35.2% of respondents reported feeling well trained and competent in funding through federal, industry, or foundation/society sources. Traditionally, nephrology fellowship has been 2 yrs in duration, the minimum training period required by ACGME. An increasing number of fellowship programs are requiring fellows to pursue three/four yrs of training with an emphasis on research. One factor that may hinder future funding is lack of formal research training in epidemiology, biostatistics, and related fields. We sought to study the impact of formal clinical research training during fellowship in forming successful academic nephrology careers, within a single large fellowship program based at two tertiary medical centers in Boston.

**Methods:** We conducted a retrospective cohort study including all clinical research fellows over a 10 yr period utilizing a database maintained by the program director. The fellows have an option of pursuing formal research training at the Harvard School of Public Health and affiliates after their first year.

**Results:** 41 clinical research fellows trained from 1998 to 2008, of which 51.2% underwent additional research training, obtaining degrees during fellowship. During fellowship, 57.1% of fellows with additional training were funded compared to 10% of fellows without additional training (p-value <0.05). Within 5 yrs of fellowship completion, 57.1% of the additionally trained fellows obtained independent investigator funding compared to 5% of the fellows without additional training (p-value <0.001, OR 25.3). 95.2% of the additional training group fellows joined academic institutions after completing fellowship compared to 45% of fellows without additional training (p-value<0.001,OR 24.4).

**Conclusions:** This study highlights the value of formal clinical research training during fellowship. For future clinical investigators, formal clinical research training may be a critical factor in improving the chances of acquiring funding and promoting careers in academia.

**TH-PO862**

**Prevalence and Demographic Associations of Health Literacy in Pre-Dialysis Chronic Kidney Disease** Julie A. Wright Nunes,[1] T. Alp Ikizler,[2] Kerri L. Cavanaugh.[2] *[1]Nephrology, Univ of Michigan, Ann Arbor, MI; [2]Nephrology, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** Limited health literacy is associated with poor clinical outcomes. Data is limited describing prevalence and demographic associations of health literacy in patients with non-dialysis dependent chronic kidney disease (CKD).

**Methods:** Adult patients with CKD Stages 1-5 not on maintenance dialysis were enrolled in a cross-sectional study from April 2009 to October 2010. The Rapid Estimate of Adult Literacy in Medicine (REALM) was used and an assessment of ≥9th grade reading level was considered adequate health literacy.

**Results:** 556 patients were enrolled. The mean (SD) age was 57 (16) years. 53% were male, 81% were Caucasian, and 78% had CKD Stage 3-5. Nineteen percent of patients had < 9th grade health literacy. Univariate analysis revealed men were less likely than women (OR 0.50[CI 0.32,0.79];p<0.01) to have adequate literacy, as were patients of non-white race compared to white (0.34[0.21,0.54];p<0.01). Patients of higher income had higher odds (1.76[1.33,2.34];p<0.01) of adequate health literacy, as did patients with eGFR ≥60 (2.87[1.48,5.54];p<0.01), and those with higher formal educational attainment (6.53[3.25,13.14];p<0.01). In analysis adjusted for age, sex, race, income, education, and kidney function, these relationships remained significant. In particular, men (0.45[0.27,0.75];p<0.01 vs. women) and patients of non-white race (0.33[0.19,0.57];p<0.01 vs. white) continued to have a lower odds of having adequate health literacy, despite adjustment for income, kidney function and formal education.

**Conclusions:** Low health literacy is associated with less desirable clinical outcomes. More research is needed to determine factors responsible for gender and racial differences in health literacy, and to develop literacy-sensitive interventions for populations at risk.

*Funding:* NIDDK Support, Other NIH Support - T32, Private Foundation Support

**TH-PO863**

**Nephrology Exposure during Medical Residency: A U.S. Internal Medicine Program Directors' Survey** Karen M. Warburton,[1] Kenar D. Jhaveri,[2] Ankit B. Patel,[3] Stephanie A. Call,[4] Vineet Arora,[5] Hitesh H. Shah.[2] *[1]Nephrology, Univ of Pennsylvania; [2]Nephrology, Hofstra NSLIJ School of Medicine; [3]Medicine, Weill Cornell Medical College; [4]Medicine, Virginia Commonwealth Univ; [5]Medicine, Univ of Chicago.*

**Background:** Amid concerns of a workforce shortage in the field of nephrology, little is known about the types of nephrology exposure offered during internal medicine (IM) residency in the US. The relationship of this exposure to success in recruiting future nephrologists from residency programs is also unknown. It is unclear if US IM program directors (IM-PDs) are aware of the annual ASN Kidney Week Training Program (KWTP) for residents.

**Methods:** An online survey was created and distributed to US IM-PDs as part of the 2012 APDIM spring survey.

**Results:** 214 US IM-PDs responded to our survey (54% response rate). 55% of programs had a nephrology fellowship program, and 48% had a nephrology transplant program at their institution. Inpatient nephrology consult service was a required rotation for 41% of programs. 46% of programs did not have a dedicated ambulatory nephrology rotation. Nearly two-thirds of the programs did not have a kidney transplant or outpatient hemodialysis unit experience for IM residents. When asked what was most valuable in fostering nephrology interest among IM housestaff, IM-PDs identified nephrology attending role modeling (69%), nephrology consult experience (67%) and nephrology faculty mentoring (59%). Only 61% of IM-PDs reported familiarity with the ASN KWTP.

**Conclusions:** While nearly two thirds of IM-PDs thought that nephrology consultative experience was important to foster interest in nephrology careers, this experience was not

required by the majority of programs. The majority of programs did not offer a kidney transplant or outpatient dialysis rotation for IM residents. To enhance exposure to and interest in nephrology careers among IM residents, a combined exposure to inpatient and outpatient nephrology, increased offerings in transplant or outpatient dialysis experiences, and increased awareness of opportunities such as the ASN KWTP for residents should be strongly considered by the training community.

## TH-PO864

**Development and Implementation of Nephrology Fellow OSCEs for End-Stage Renal Disease Counseling** Mark G. Parker, Christine Mallar, Jennifer Gammon, Patricia L. Cantlin. *Maine Medical Center, Portland, ME.*

**Background:** Observed Structured Clinical Encounters (OSCEs) are established assessment tools in medical education, but there is little published experience on their use in nephrology fellowships. Surveys of nephrology fellowship graduates indicate that many feel inadequately prepared to counsel patients about dialysis options and palliative care. We have developed two OSCEs to assess these skills.

**Methods:** Two scenarios with trained standardized patients (SP) were administered to fellows in months 7 and 19 of training. The first is a mid-age woman with advanced chronic kidney disease (CKD) and no immediate transplantation prospects. The second is an elderly man with advanced CKD, considerable co-morbidity, and limited life expectancy. Two trained faculty members observed the encounters. Fellows received feedback from SP and faculty, and completed a 7-question learner feedback survey using a 5-point Likert scale for responses (1=strongly disagree, 5= strongly agree) and a narrative self-reflection.

**Results:** 6 unique fellows were assessed over a two-year period. Mean learner feedback survey scores ranged from 4.17-5.0 for questions about the quality of the OSCE administration and 4.33-4.67 for questions about utility of the OSCE for analyzing skills and behaviors. Thematic analysis of narrative self-reflections suggests that fellows identified strengths in their communication skills and core knowledge of dialysis options. However, they identified challenges in their knowledge of nuances of dialysis care, such as financing and home therapy details, as well as description of resources for palliative care. Challenges were greatest for month 7 fellows.

**Conclusions:** We have demonstrated feasibility and potential utility of OSCEs for assessment of dialysis and palliative care counseling skills of nephrology fellows. Our fellows find the experience to replicate true clinical experiences satisfactorily and to provide useful information about their strengths and challenges for improvement. Limitations include the single center nature of this experience and small number of observations. We are initiating a multi-center prospective study to address these limitations.

## TH-PO865

**Testing Strategies to Education Primary Care Physicians on Chronic Kidney Disease** Laura J. Maursetter. *Div of Nephrology, Univ of Wisconsin School of Medicine and Public Health, Madison, WI.*

**Background:** It has been shown that there is a knowledge gap in identification and management of chronic kidney disease (CKD) by primary care providers. In an effort to improve this practice the Wisconsin Research and Education Network (WREN) developed a research project aimed to test ways that can help primary care practitioners improve identification of patients with CKD and implement guidelines for CKD management through a local learning collaborative.

**Methods:** All WREN sites were invited to the learning collaborative. A survey was distributed that asked the primary providers if a group would be useful and to identify topics that would be of interest to the group. Teleconferencing and blackboard collaboration were used as methods of communication. A group facilitator, a nephrologist and a lead family medicine physician were part of each meeting. After 3 months of meetings, a participation survey was distributed to determine the utility of the group.

**Results:** 29% of the 84 sites that had been a part of the CKD project responded to the invitation to be a part of the learning collaborative with 46% of the responders indicating that they would be interested in participating in a monthly virtual statewide learning collaborative. After 3 meetings with 1-4 sites participating during each meeting, a follow up survey was done. The results of the follow up survey showed that there was little interest in continuing these meetings with the most stated reason being scheduling conflicts.

**Conclusions:** Despite a group of primary care providers that was primed to be interested in the topic of CKD, there was a very low rate of participation in the learning collaborative. The overwhelming reason for low participation stated it was due to a rigorous schedule. Teleconferencing and blackboard collaborative were used as strategies to bring together those in various geographic areas across the state of Wisconsin but this did not seem to improve participation. It is likely that an asynchronous method of learning may increase participation and ultimately improve recognition and management of CKD.

*Funding:* Other U.S. Government Support

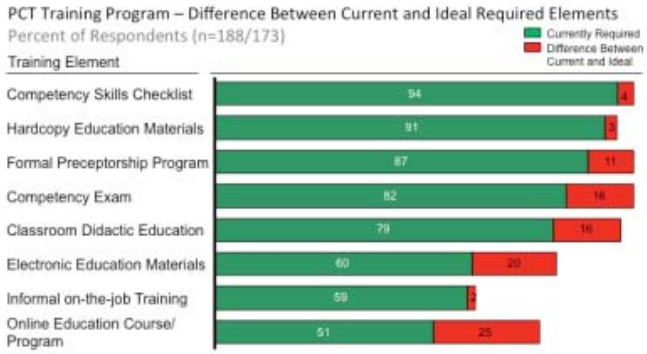
## TH-PO866

**Most U.S. Patient Care Technician Training Programs Leverage Several Required Elements but Fall Short of Ideal and Are Not Sourced from Independent Third Parties** Alex Yang, Jennifer Vavrinchik. *NCS Research.*

**Background:** CMS instituted certification requirements for patient care technicians (PCTs). In this study, we evaluate PCT training programs.

**Methods:** >2000 nephrology professionals were screened against inclusion criteria: US nephrology professional, dialysis practice setting, >1 yr of nephrology experience, and consent. 428 eligible study candidates were invited with eventual 188 study participants (48%). Elements of PCT training programs were evaluated. Analyses were conducted across all study participants with no excluded data for any reason.

**Results:** Percentages of current elements in PCT training programs and difference from ideal are shown (Figure).




>80% of study participants believe patient care would improve if their PCT training program included the ideal elements. Current and ideal percentages of independent 3rd-party sourced elements for PCT training programs are shown (Table 1).

**Table 1: Independent 3rd Party Source for PCT Training Elements**

| Elements of PCT Training Program | Current Actual (Percent) | Ideal (Percent) | Difference (Percent) |
|---|---|---|---|
| Competency Exam | 6 | 62 | 56 |
| Online Education Course/Program | 6 | 62 | 56 |
| Electronic Education Materials | 6 | 62 | 56 |
| Hardcopy Education Materials | 4 | 55 | 51 |
| Competency Skills Checklist | 1 | 48 | 47 |
| Classroom Didactic Education | 2 | 44 | 42 |
| Formal Preceptorship Program | 1 | 44 | 43 |
| Informal on-the-job Training | 0 | 35 | 35 |

**Conclusions:** Although most PCT training programs utilize several elements, study participants believe more PCT training elements would be ideal and would improve patient care. Furthermore, 3rd party independent sources of PCT training/education are currently rarely used but roughly 1/3 to 2/3 of study participants believe it is important for PCT training and education elements to be independent. Efforts to standardize PCT certification should be coupled with efforts to standardize the elements, the content, and the independence of PCT training/education.

## TH-PO867

**Medical Student Test Performance during a Second-Year Kidney Pathophysiology Course Improves with Participation in Small Group Active Learning** Elizabeth J. Brant, Elizabeth W. Dehmer, Gerald A. Hladik. *UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC.*

**Background:** We assessed the impact of participation in active learning on test scores during a second-year medical student kidney pathophysiology course. We hypothesized that participation in active learning would have a favorable impact on test scores.

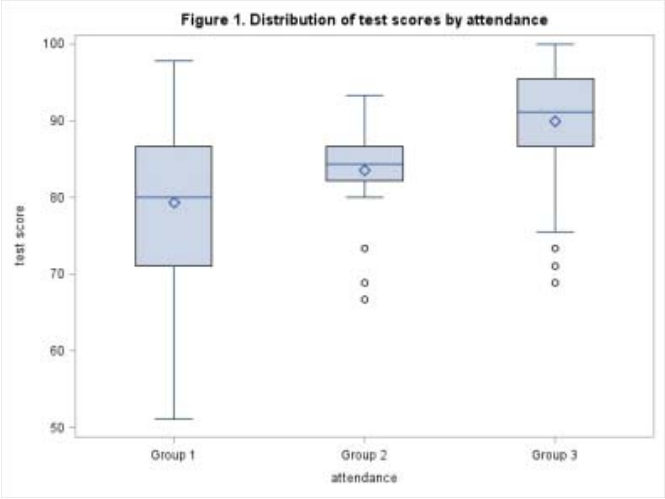
**Methods:** The second-year kidney pathophysiology course consisted of 20 hours of traditional lecture and 20 hours of optional active learning sessions. During active learning, students worked through clinical cases under the supervision of nephrology faculty, with a faculty:student ratio of 1:15. Of the 133 students enrolled in the course, 74 (56%) participated in active learning. Participation in small groups was classified as follows: Group 1: Participation in <30% of sessions; Group 2: Participation in 30-60% of sessions; Group 3: Participation in >60% of sessions. One-way analysis of variance was used to compare participation and mean test scores.

**Results:** The distribution of students according to level of participation in active learning sessions and corresponding test scores is shown in Table 1.

Table 1
Mean test scores according to level of participation in active learning sessions

| Group | Number of students N(%) | Mean test score (standard deviation) |
|---|---|---|
| 1 | 59 (44) | 79.3 (10.4) |
| 2 | 21 (16) | 83.6 (7) |
| 3 | 53 (40) | 89.9 (7.7) |

Participation at the small group active learning sessions was associated with higher mean test scores ($p<0.001$). Tukey's test showed significant differences in scores between Group 3 and each of the other groups (Fig. 1).




**Conclusions:** Student participation in active learning small groups during a second-year kidney pathophysiology course was associated with superior test performance. Limitations of the study include that this was a retrospective analysis at a single center.

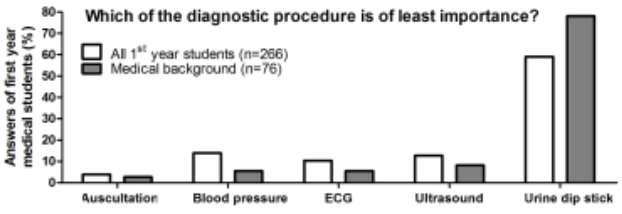
## TH-PO868

**Mind the Dip Ranking of Diagnostic Procedures – An Online Survey among 266 First Year Medical Students at Two German Universities**
Philip Bintaro,[1] Heike Kielstein,[2] Volkhard Fischer,[1] Jan T. Kielstein.[1] *[1]Hannover Medical School, Hannover, Germany; [2]Martin Luther Univ Halle-Wittenberg, Halle, Germany.*

**Background:** Urine dipstick analyses pH, specific gravity, proteinuria, bilirubinuria, glucosuria, ketonuria and nitrite. It is one of the most frequently used diagnostic tests and a screening tool for many diseases as well as part of a guideline based work up ranging from urinary tract infection to assessment of cardiovascular risk and CKD. The aim of our study was to investigate the attitude of first year medical students (in a 6 year curriculum) towards urine analysis and ranking it among other frequently used diagnostic tests and procedures.

**Methods:** 549 first year medical students were invited to fill out an online questionnaire consisting of several questions concerning medical diagnostic tests. The return rate was 47.4 %. Of the 266 participants 64.9 % were female. One question was "Which of the following diagnostic procedures is of least importance?" allowing to select ONE of the following: 1) Auscultation 2) Blood pressure 3) Electrocardiogram 4) Ultrasound 5) Urine dip stick.

**Results:** Urine dip stick and blood pressure were viewed as the least important tests.




Auscultation was selected as the most important test. Concordingly 98.1 % of the medical students selected the stethoscope as the most important medical equipment conveying the image of a doctor, followed by doctors coat (95.5 %) and syringe (25.5 %). Among the 76 students with prior medical training (nurse, paramedic) blood pressure measurement was considered to be more important than in the whole cohort, however, 78.1 % thought that urine dipstick is the least important diagnostic test.

**Conclusions:** Weighing the importance of diagnostic procedures should be an integral part of medical education, keeping in mind that auscultation is closely related to the self (and perceived) image of physicians.

*Funding:* Clinical Revenue Support

## TH-PO869

**Developing a New Pediatric CKD Educational Material: The Increasing Kidney Disease Network (IKAN) Transplant Project**
Bessie A. Young,[2] Bonnie K. Harp,[2] Janice Sabin.[1] *[1]Biomedical Informatics and Medical Education, Univ of Washington, Seattle, WA; [2]Nephrology/Dept Medicine, VA Puget Sound, Univ of Washington, Seattle, WA.*

**Background:** Much research has been done to evaluate disparities in transplantation rates for racial/ethnic populations; however, much less research has focused on determining the best methods by which to overcome educational barriers and increase transplantation awareness among pediatric patients and their families within these groups.

**Methods:** We convened a focus group to assess content and design of a new kidney disease education modality in comic book format. Focus group participants (N=6) included researchers, community members (one kidney transplant recipient), and a nephrologist. Participants were African American (50%) and white (50%). Participants' reviewed the culturally tailored comic book for the African American patient population, aimed primarily at adolescents and young adults with CKD and their families, the final version of which will be evaluated in a pilot study using focus groups at Seattle Children's Hospital.

**Results:** The comic book was reviewed for content, reading level, language, whether it is culturally appropriate, age appropriate, for quality of visual presentation, and organization of the information. The focus group concluded that fact checking of dialog in the book for authenticity with a group of African American adolescents is important, medical terms should include a phonetic pronunciation, the comic book should have a glossary, and the book should be divided into chapters. Comments from the group include; "comic book [will be used] as reference material"; "it hit on all four cylinders"; "as a kidney transplant patient…it is good to have it all in one place"; "the underlying message is that transplant is the best option."

**Conclusions:** Culturally tailored and health literacy-appropriate education materials that use innovative delivery of information are an important tool for both adults and pediatric patient populations. These innovative and unique materials will be field tested and finalized for use in future research among pediatric patients and their families with CKD and ESRD.

*Funding:* NIDDK Support, Veterans Affairs Support

## TH-PO870

**A Novel Journal Club Experience during Nephrology Fellowship Training**
Kenar D. Jhaveri, Shailaja Chidella, Hitesh H. Shah. *Nephrology, Hofstra North Shore–LIJ School of Medicine, Great Neck, NY.*

**Background:** Journal Club (JC) is a forum for fellows to present topics of current interest including clinical and cutting edge research. Despite critical review of the published article during JC, questions may remain unanswered. The lead author's perspective during JC might help better understand the article being reviewed. At our institution, we embarked on an innovative journal club format that included a discussion with the author of the JC article.

**Methods:** At our institution, nephrology JC is a biweekly conference prepared by fellows covering research papers in nephrology. Following the JC article presentation(introduction and presentation for 45 min), the last 15 minutes are traditionally devoted to critically discuss the manuscript. During the 2012-2013 academic year, we introduced a novel JC experience for our group. Many leading or corresponding authors of JC papers discussed were invited few weeks prior to the JC session by our fellows to participate in a 15 minutes teleconference with all fellows and faculty members. An anonymous online survey was created to evaluate this novel JC format. The survey was subsequently distributed to all fellows and participating faculty members in May 2013.

**Results:** So far, 7 out of 8 fellows and 5 faculty members responded to our survey. On a 5-point scale (where 1 being least and 5 being most), respondents rated this experience innovative (4.58), thought provoking (4.17) and relevant (4.0). 75% felt that this format enhanced their understanding and knowledge of the article being reviewed at JC. Two-thirds (66.7%) felt that the direct discussion with the author enhanced their critical thinking skills. All enjoyed participating in this novel JC format and nearly two-thirds responded that this format would increase their JC participation. When asked to rate this format as compared to the traditional JC, the average rating was 4.3 on a 5-point scale (1 being least valuable, 5 being most). All respondents wanted to see such "author participated" JC sessions in the future.

**Conclusions:** This novel "author participated" JC sessions enhanced our JC experience. Based on our experience, we encourage all medical educators to consider this format of JC.

## TH-PO871

**Do Medical Trainees Receive Adequate Training in Understanding, Managing and Treating Chronic Kidney Disease and Its Complications**
Mansoor N. Ali. *Renal Medicine, York Teaching Hospitals Foundation NHS Trust, York, Yorkshire, United Kingdom.*

**Background:** The objective of this survey was to gauge the understanding and the level of competence amongst junior trainees; in treating and managing Chronic Kidney disease (CKD) and its complications. This survey was generated as a result of our previous work in understanding the training needs amongst trainees in understanding Acute Kidney Injury (AKI).

**Methods:** This survey was conducted between December 2012 to March 2013 at York Teaching Hospitals. It was conducted as part of the teaching session on CKD for the trainees with the target group being the Core medical trainees on a medical rotation. The questionnaires were handed out before the lecture and the audience were asked to fill it in during or after the session. Total responses were based on the total number of attendances and were thirty eight in total. The Questionnaire revealed grade and place of work whether acute or rehab setting including subspecialties such as Renal/Gastro/Haematology.

**Results:** The first question asked about the definition of CKD and none were able to answer correctly (CKD classification was accepted). Only 20 had come up with their own definitions of CKD and others left it blank. 34 trainees responded by saying that they have managed more than 16 patients with CKD whereas 4 responses were for < 8-16. On a scale between 1 and 5 where 1 being not confident and 5 being confident/competent no one replied that they were competent (5) to manage CKD; 8 people replied saying they felt confident at (4) as they had managed to do renal rotations where as 30 people thought that they are confident to manage at level (3).

**Conclusions:** The survey highlighted the fact that most trainees felt that teaching on CKD were inadequate at both undergraduate and postgraduate levels. There was evidence of the lack of understanding of the complications of CKD and its management. There also seemed to be a consensus that most junior trainees would like to know how to treat and manage CKD rather than leaving it to the nephrologists. Many have responded by agreeing to do a renal placement and some who have already done one found it very useful.

## TH-PO872

**Development and Validation of a Shared Decision Making Tool for Mortality on Dialysis Compared to Kidney Transplantation: iChoose Kidney**
Rachel E. Patzer,[1] Mohua Basu,[1] William M. McClellan,[2] David H. Howard,[3] Yijian Huang,[3] Kimberly Arriola.[3] *[1]Emory Transplant Center, Atlanta, GA; [2]Dept of Medicine, Emory Univ, Atlanta, GA; [3]Rollins School of Public Health, Atlanta, GA.*

**Background:** End Stage Renal Disease (ESRD) patients have two primary treatment choices: dialysis or kidney transplant (KTx). Predictive models to compare individualized risks of mortality of dialysis or KTx have not yet been developed to help patients and providers make informed decisions about treatment choices.

**Methods:** We examined mortality among a cohort of 721,571 ESRD patients 2005-2001 in the United States Renal Data System (USRDS) surveillance registry who received 1) dialysis vs. 2) KTx. Multivariable logistic regression was used to derive separate risk models for patients on dialysis and for patients who received a living or deceased donor transplant. Data were divided into 50% derivation and 50% validation datasets. Models were evaluated using concordance statistics (c-statistics) and measures for model discrimination and calibration for three-year outcomes.

**Results:** Among dialysis patients (n=663,900), 47.4% of patients died vs. 5.9% of transplant recipients (n=57,671) over the 7-year study period (p<0.0001). Factors significantly associated with a higher odds of death for dialysis and KTx patients included older age; minority race/ethnicity; longer dialysis vintage; non-private insurance; delayed access to pre-ESRD nephrology care; no erythropoietin use prior to dialysis; smoking and drug use; and comorbidities, including cardiovascular disease and congestive heart failure. The discriminatory ability of the models for 1-year and 3-year mortality was moderately high (c-statistics ranging from 0.69-0.72). We used these validated, risk prediction models to develop an electronic, user-friendly, mobile application (iPad) tool called iChoose Kidney.

**Conclusions:** Risk prediction estimates for mortality on dialysis compared to KTx may help assist in identifying patients at high risk for poor outcomes, communicating knowledge about risks of mortality to patients and providers, and encouraging lifestyle or behavioral changes to promote health.

*Funding:* Other NIH Support - National Center for Advancing Translational Sciences (NCATS)

## TH-PO873

**Towards Harmonizing Renal Guidelines: An International Survey of Major Guideline Bodies**
Maria C. Haller,[1] Sabine N. Van der Veer,[1] Evi V. Nagler,[1] Raymond C. Vanholder,[1] Jonathan C. Craig,[2] Wim Van Biesen.[1] *[1]European Renal Best Practice, Ghent Univ Hospital, Ghent, Belgium; [2]KHA-CARI, School of Public Health, Univ of Sydney, Australia.*

**Background:** Several organizations produce renal guidelines, resulting in duplication of effort while many topics remain uncovered. A collaborative work plan based on common, Institute of Medicine-endorsed methods would improve efficiency and impact, and reduce inconsistency. ERBP and KHA-CARI aimed to identify organizational and methodological differences between major renal guideline bodies that might hamper their collaboration.

**Methods:** We invited the chairs of KHA-CARI, CSN, ERBP, KDIGO, KDOQI, SLANH and UK-RA to complete an electronic 62-item survey, based on Institute of Medicine standards.

**Results:** All 7 groups completed the survey. Funding arises from their governing bodies (N=5) or directly from industry (N=2). In no case do funders have any influence on topic selection or guideline content. The available budget to develop one guideline varies from $2,000 to $ 500,000. 3 groups seek patient perspectives and 4 consider health economics. All groups provide training for guideline development groups, 6 communicate their methods to the public. All groups try to avoid overlap by considering guidelines already planned or published by others. 5 conduct systematic searches for evidence, 2 include detailed critical appraisal and all use the GRADE framework. 5 groups organize public review of the guideline draft. All update their guidelines in case of new evidence, and when a pre-set time frame has passed (N=3). Commentaries or position statements on guidelines published by others are produced by 5, with the ADAPTE framework (N=1) and the AGREE II (N=2) used by some.

**Conclusions:** Overall there was considerable commonality in methods and approaches but important differences remain. Increased collaboration will require substantial consensus building activities. The financial and in-kind human resource costs of guideline production are high, and to maximize impact and develop a sustainable work plan, a collaborative approach is now required. This study is a promising first step towards harmonization of guideline development in renal medicine.

## TH-PO874

**Patient and Staff Perspectives of Intradialytic Cycling**
Amy L. Clarke,[1] Hannah M.l. Young,[1] Maurice Dungey,[1] Nicky Hudson,[2] James O. Burton,[1] Alice C. Smith.[1] *[1]Leicester Kidney Exercise Team, Univ Hospitals of Leicester, United Kingdom; [2]School of Applied Social Sciences, De Montfort Univ, Leicester, United Kingdom.*

**Background:** Evidence increasingly shows that exercise benefits haemodialysis (HD) patients, but lifestyle restrictions limit participation. Intradialytic cycling (IDC) is an attractive option but setting up and sustaining a programme can be challenging, partly due to patient and staff resistance. Addressing the user's point of view is key to successful implementation of new initiatives. This study explored patient and staff perspectives prior to the introduction of a new IDC programme, and again when the programme was established.

**Methods:** Ahead of IDC initiation, focus groups were conducted with a purposive sample of 24 HD patients and 9 staff. 6 months after IDC introduction, semi-structured interviews were held with 11 patients and 8 staff. Audiofiles were transcribed verbatim, translated where necessary, and subjected to qualitative framework analysis.

**Results:** Prior to IDC, patients anticipated that the positive experiences of peers would encourage them to participate, whilst post-IDC they were motivated by their own perceived improvements. Staff initially felt making IDC part of the existing routine of the unit would facilitate implementation and later commented that a collaborative approach helped to sustain the programme. Both staff and patients wanted education on the benefits and principles of exercise. Anticipated patient barriers were initially concerns regarding safety and the negative effects of fatigue and depression. None of these concerns proved influential for patients after implementation. Both patients and staff were concerned about IDC adding to the staff workload. This continued to be an issue for both groups post implementation and proved to be the most influential barrier.

**Conclusions:** This study provides a unique perspective into HD patient and staff views on initiating and sustaining an IDC programme. Education is the key element for initial patient and staff engagement and for patients this education is best facilitated through peer interaction. Long term sustainability requires IDC to become a routine part of the work plan of HD unit staff.

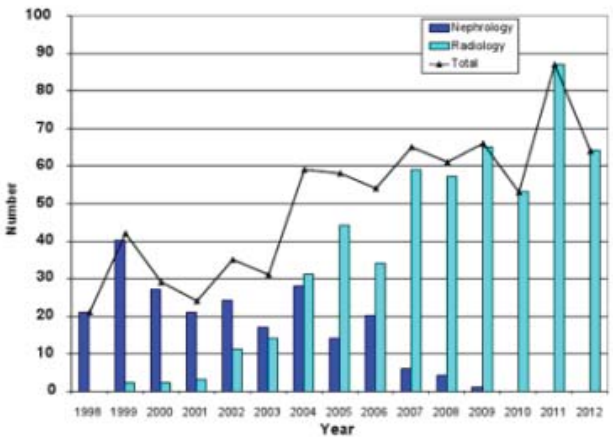
## TH-PO875

**Comparison of Glomerular Yield and Complication Rates between Nephrology Performed and Radiology Performed Percutaneous Renal Biopsy**
Paul D. Sanders,[1] Klaudia Barbara Jumaa,[3] David C. Holland,[2] Ben Mussari.[1] *[1]Dept of Radiology, Queens Univ, Kingston, Canada; [2]Dept of Nephrology, Queens Univ, Kingston, Canada; [3]Dept of Medicine, Queens Univ, Kingston, Canada.*

**Background:** Percutaneous renal biopsy (PRB) is being more commonly performed by Interventional Radiology (IVR) causing concern over lost skills for Nephrology Fellows, safety and post-procedural care. Our study compares glomerular yield and complication rates between IVR and Nephrology following PRB.

**Methods:** A retrospective chart review was performed. Patient charts were analyzed for total glomerular yield, number of passes taken per biopsy and post-biopsy complications that required blood transfusion, endovascular or surgical intervention.

**Results:**




18% of biopsies performed by Nephrology yielded less than 10 total glomeruli vs 9% for IVR (p < 0.001). Complications requiring blood transfusion occurred in 3.1% of Nephrology biopsies vs 1.9% for IVR (p=0.298). Endovascular intervention was required in 1.3% of the Nephrology group vs 0.8% for IVR (p=0.431). No patient required surgical intervention.

| | Total | Nephrology | Radiology | |
|---|---|---|---|---|
| | 749 | n = 223 | n = 526 | p |
| Total Yield (mean # of glomeruli ± SD) | 23.9 ± 13.3 | 20.1 ± 11.9 | 25.6 ± 13.5 | <0.001 |
| Yield per Pass ± SD | 8.6 ± 5.2 | 9.7 ± 6.8 | 8.2 ± 4.3 | <0.001 |
| # with total yield <10 glomeruli (%) | 84 (11.2%) | 40 (17.9%) | 44 (8.6%) | <0.001 |
| Blood Transfusion (%) | 17 (2.3%) | 7 (3.1%) | 10 (1.9%) | 0.298 |
| Endovascular Intervention (%) | 7 (0.9%) | 3 (1.3%) | 4 (0.8%) | 0.431 |

**Conclusions:** Our results show that there is no significant difference in severe complication rates between IVR vs Nephrology performed PRB; however, there were fewer biopsies containing less than 10 total glomeruli in the IVR group.

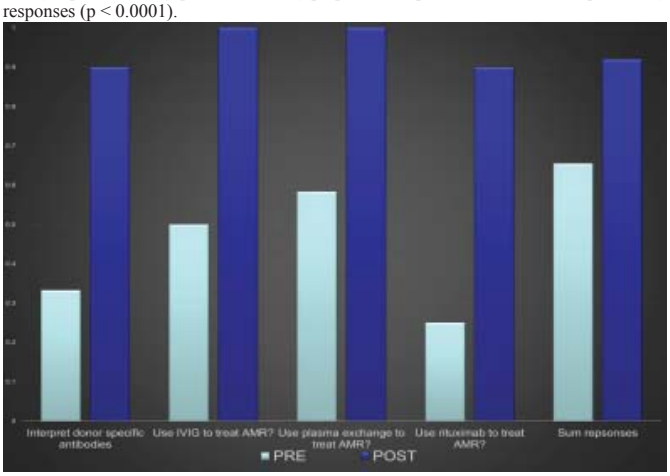
**TH-PO876**

**Improving Self-Reported Provider Preparation to Evaluate and Treat Antibody-Mediated Renal Transplant Rejection** Dustin J. Little,[1] Austin Parker,[3] Mark D. Poirier,[2] Amy J. Zwettler,[1] David J. Yoo,[1] Bruce Reinmuth,[1] Kevin C. Abbott,[1] Christina M. Yuan,[1] Lisa K. Prince.[1] *[1]Walter Reed National Military Medical Center; [2]San Antonio Military Medical Center; [3]Naval Medical Center Portsmouth.*

**Background:** Antibody-mediated kidney transplant rejection (AMR) is a major cause of allograft loss. We designed and implemented a performance improvement project aimed at optimizing the evaluation and treatment of AMR at our institution.

**Methods:** Medical literature on AMR was reviewed, as were AMR cases treated at our institution from 2006-2011. An electronic survey was administered to the nephrology staff and fellows, which included ten questions assessing provider preparation to evaluate and treat AMR. Lectures were given on the evaluation and treatment of AMR and findings of review of institutional AMR cases. An educational electronic "AMR Toolkit" was created, and an identical post-survey was administered 4 months following the pre-survey. Fisher exact test was used to compare pre and post-survey responses, with a p value of 0.05 used to determine significance.

**Results:** Twenty-one cases of AMR were identified in 16 patients. No patients died and two experienced graft loss. Pre and post-survey response rates were 12/13 and 10/12, respectively. Significantly more respondents reported feeling quite or extremely prepared on the post-survey for 5 of the 10 items. Ninety-one of 99 total responses on the post-survey reported feeling quite or extremely prepared, compared to 78/119 of total pre-survey responses ($p < 0.0001$).




**Conclusions:** AMR presents a dilemma, in part because of limited data regarding treatment options. We implemented a comprehensive education strategy, following which providers reported feeling better prepared to evaluate and treat AMR. Our results suggest that this model should be considered for other challenging clinical problems.

**TH-PO877**

**A Pilot Trial of a Computerized Renal Template Note to Improve Resident Knowledge and Documentation of Kidney Disease** Shayan Shirazian, Ray Wang, Dennis Moledina, Vladimir Liberman, Joseph H. Zeidan, Joseph Mattana. *Medicine, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Kidney disease is under-documented in physician notes suggesting under-recognition, especially at earlier stages of chronic kidney disease (CKD). The objective of this study was to determine whether a computerized renal template note (RTN) with clinical documentation support (CDS) improves resident knowledge and documentation of kidney disease.

**Methods:** In this prospective IRB-approved study, first year residents (PGY1s) were encouraged to use the RTN with CDS for documentation over a 1 month period. The RTN included an option for classification of patients into CKD and acute kidney injury (AKI) categories. The CDS consisted of a link to standard classifications of CKD and AKI. Knowledge of CKD and AKI was tested with a quiz given before and after implementation of the RTN. Study investigators determined whether CKD or AKI was appropriately documented in 100 RTNs and 100 historical internal medicine progress notes of patients with known AKI or CKD from the same time period in the previous year. RTN and historical notes were compared using Fischer's exact test. Quiz scores before and after RTN utilization were compared using paired t-testing.

**Results:** 18 PGY1s completed all study procedures. Their average age was 28, 12 were male, and 7 had taken a prior nephrology elective. At the end of the study, approximately 2,436 inpatient encounters were documented using the RTN. Accurate staging of CKD-3 was significantly improved following implementation of the RTN when compared to historical notes (9/46 vs. 0/29, p=0.01), whereas accurate staging did not improve for CKD-4, CKD-5 or AKI. End of study quiz scores were higher than pre-study scores, however this difference did not reach statistical significance (29.6% vs. 20.6%, p=0.13).

**Conclusions:** A computerized RTN with CDS significantly improved documentation of stage 3 CKD though without correspondingly increased knowledge. Given these findings and the opportunities provided by increasing electronic health record use, further studies of the use of computerized RTN with CDS to improve CKD recognition and staging may be warranted.

**TH-PO878**

**Stress and Burnout among Hemodialysis Nurses: A Single Center Survey Study** Ayman Karkar, Betty Mandin, Mohammed Abdelrahman. *Kanoo Kidney Center, Dammam Medical Complex, Dammam, Saudi Arabia.*

**Background:** Hemodialysis (HD) nurses are prone to specific types of stress and burnout, which are capable of having a detrimental impact on organizational productivity, and pose a serious health and safety hazards on the job. We aimed in this study to determine the type and level of stress and the amount of burnout among our HD nurses, evaluate the coping skills, and the impact of stress on their work performance.

**Methods:** The study was conducted using modified stress and burnout questionnaires. The questionnaires consisted of stress and burnout scale, outcomes in workplace, and coping skills. A descriptive correlational design was used to determine if there was a relationship between stress and burnout. There were 93 female dialysis nurses (19 national and 74 expatriate) who participated in this prospective study. Their years of employment and practice varied between 1 to 26 years in the same HD unit. Pearson's product-moment correlation was applied to test the correlation between stress and burnout.

**Results:** Our results show that most nurses (79% national and 68% expatriate) experienced a mild level of stress (42%) and moderate level of burnout (38%), respectively. The most common stressor among national nurses was technological breakdowns of machines (15.9%) and job insecurity (16.9%) among expatriates. The majority of national nurses (21%) coped with by increased sick leaves, whereas majority (25%) of expatriates responded by becoming easily frustrated. The most utilized coping skill among both groups was the relaxation methods (20.8% versus 24.9%) and the least utilized was denial (3.9% versus 0.5%), respectively. There was no correlation between stress/burnout and duration of employment.

**Conclusions:** A dialysis nurse who delivers HD treatment to end-stage renal failure patients with complex social, emotional, psychological and physical needs must have a positive emotional well-being in order to be positively reflected on the quality of care delivered. The support of hospital and nurse managers can provide a healthy atmosphere with an almost stress free environment that would be positively reflected on clinical performance, quality care, productivity and patient and staff safety.

*Funding:* Private Foundation Support

**TH-PO879**

**Raising Public Awareness of Chronic Kidney Disease through the Kidney Disease Screening and Awareness Program (KDSAP)** Laura C. Polding,[1] Andrew J. Pan,[1] Ryan Lindeborg,[1] Jake Cohen,[2] Paul Kim,[2] Michael Ho-Young Ahn,[1] Manjinder Singh Kandola,[1] Tzongshi Lu,[3] Kenneth Lim,[3] Jennie Kuo,[3] Li-Li Hsiao.[3] *[1]Harvard College; [2]Brookline High School; [3]Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** KDSAP has two main objectives: 1) increasing public awareness and early detection of chronic kidney disease (CKD) in the community and 2) student career development. Here, we describe an initiative called the Boston Kidney Health Series (BKHS) as part of KDSAP's community outreach efforts. BKHS is an entirely student-run, day-long program that aims to provide free public education on a range of topics surrounding CKD.

**Methods:** KDSAP students developed a program that contained CKD-related topics with speakers from multidisciplinary health care professions involved in the care of kidney patients, including nephrologists, a renal dietician, a social worker, and a patient. Students designed flyers, compiled a phone directory listing local community organizations, and solicited their support to promote this free event. A survey was then conducted to evaluate the program.

**Results:** BKHS's established directory contains over 100 greater Boston community centers and organizations, including libraries, churches, YMCA, health organizations, and social media. Attendees included CKD patients, kidney transplant recipients and donors, caretakers, family members, and friends from diverse communities around Boston. Survey feedback was highly positive, with 73% and 60% of surveyed participants rating the event usefulness and likelihood of future attendance, respectively, a 5 on a 5-point Likert scale. Furthermore, student volunteers reported that BKHS increased their exposure to and interest in nephrology.

**Conclusions:** KDSAP's BKHS is an effective model to raise CKD awareness. It is unique in including topics and speakers from multidisciplinary health care professions and being entirely student-run. Organizing BKHS provides KDSAP members valuable opportunities for career development, leadership and mentorship. Under the supervision of nephrologists, BKHS can serve as a model to both raise public awareness of CKD and cultivate interest in nephrology among undergraduate students.

*Funding:* Private Foundation Support

## TH-PO880

**Nephrology Blogosphere: An Analysis of Productivity, Longevity, and Consistency** Joel Topf. *Medicine, St. John Hospital and Medical Center, Detroit, MI.*

**Background:** Blogs are web sites with multiple discrete chronologic entries (typically displayed with the most recent at the top and older entries displayed below). There are numerous inexpensive and easy to use tools that allow blogs to be published with little technical friction. There are dozens of blogs that focus on nephrology and nephrology education; while most are still producing content some have ceased publishing. Little is known about the size, productivity, and lifespan of nephrology focused blogs.

**Methods:** Every nephrology blog written in English was assessed for productivity, longevity and inconsistency. Productivity was measured by the number of posts per month. Longevity was measured by duration of publication from first post to either the final post or through May 2013 for active blogs. Inconsistency was defined as the number of months without any posts.

**Results:** From March 2005 through May 2013 we found 30 nephrology blogs that published 10,198 posts. Twenty blogs were still publishing as of May 2013. Mean productivity was 339.9 (95% CI 175.2-504.7) posts per blog or 9.0 posts per month (95% CI 5.7-12.4). Longevity ranged from 1 to 73 months with a mean duration of 31.2 months (95% CI 24.1-38.3). Inconsistency was not common, with a mean of 4.6 months without posts per blog (95% CI 1.8-7.5). 11 blogs never missed a month, and 4 only missed 1 month.

Regression versus time show that most blogs lose productivity over time. Of the 25 blogs with at least 6 months of publication, 15 (60%) had a decreasing productivity over time ($p<0.05$) versus only 1 (4%) with a increasing productivity, 9 (36%) showed no association between time and productivity ($p>0.05$). Despite decreasing productivity in most individual blogs, the entire nephrology blogosphere showed a significant increase in the number of posts per month, R^2 0.38 $p<0.001$, slope 1.17.

There was no association between inconsistency and termination of the blog (R=0.18, p-0.35).

**Conclusions:** Blogging is a new means for distributing nephrology education. The number of blogs is increasing. Most blogs have decreased productivity over time however the nephrology blogosphere in total is growing both in number of voices and monthly posts.

## TH-PO881

**Conditional Inactivation of Exocyst Sec10 in Mouse Kidney Epithelium Causes Primary Cilia Defects and Kidney Cysts** Noemi Polgar,[1] Amanda J. Lee,[1] Vanessa H. Lui,[1] Xiaofeng Zuo,[2] Joshua H. Lipschutz,[2] Ben Fogelgren.[1] *[1]Anatomy, Biochemistry, and Physiology, Univ of Hawaii; [2]Medicine, Univ of Pennsylvania.*

**Background:** The pathogenesis of polycystic kidney disease (PKD) is dependent on disruptions in primary cilia function in renal epithelial cells. Despite intense study, it remains poorly understood how proteins are targeted and delivered to cilia.

**Methods:** In cultured renal epithelial cells, we have previously shown that the eight-protein exocyst complex regulates the length and signaling of the primary cilia. Using the Cre-Lox system, we have generated a novel transgenic mouse allowing tissue-specific deletion of the Sec10 gene, a central component of the exocyst complex.

**Results:** To investigate the role of Sec10 and the exocyst in renal development and primary cilia signaling, we crossed this floxed-Sec10 strain with the KspCadherin-Cre mouse strain to inactivate Sec10 in ureteric bud derived epithelium. Most mice died hours after birth due to upper urinary tract obstructions. However, mice that escaped urinary obstruction and hydronephrosis displayed numerous renal cysts with defects in primary cilia number and length in Cre-expressing tubules.

**Conclusions:** This is the first *in vivo* evidence of the exocyst regulating cilia assembly and trafficking in mammalian development, and the first conditional allele for any exocyst component. Given the known biochemical and genetic interactions between the exocyst and other ciliary proteins, it is likely that the exocyst is an important mechanism by which cells regulate ciliary trafficking.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO882

**Nuclear Distribution C Homolog Regulates Mitotic Spindle Orientation and Tubulogenesis** Maoqing Wu,[1] JingJing Zhang,[1] Wassim El-Jouni,[1] Rebecca Powell,[2] Tomoko Obara,[2] Faran E. Ghumman,[1] Maria Rasmussen,[1] Xuefeng Su,[1] Shixuan Wang,[1] Jagesh V. Shah,[1,4] Li-Yuan Yu-Lee,[3] Jing Zhou.[1] *[1]Harvard Center for Polycystic Kidney Disease Research and Renal Div, Dept of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Dept of Cell Biology, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK; [3]Dept of Medicine, Baylor College of Medicine, Houston, TX; [4]Dept of Systems Biology, Harvard Medical School, Boston, MA.*

**Background:** Polycystic kidney disease (PKD) is a common cause of renal failure. Misoriented cell division and dysregulated ciliogenesis have been observed in both autosomal dominant and recessive forms of PKD. To understand the cystogenic pathways, we searched for binding partners of PKD proteins.

**Methods:** Mass spectrometry analysis was used to identify proteins co-immunoprecipitated with a PKD protein in kidney tubular epithelial cells. Small hairpin-mediated mRNA depletion, three-dimensional tubulogenesis assay in inner medullary collecting duct (IMCD) cells, and anti-sense morpholino (MO) gene knockdown in zebrafish were used as functional assays.

**Results:** Mass spectrometry analyses identified nuclear distribution C homolog (NudC), a microtubule-associated protein necessary for dynein-mediated nuclear migration in Aspergillus nudulans. NudC is expressed in various phases of mitotic kidney epithelial cells. NudC depletion leads to multiple mitotic defects and misoriented mitotic spindle in kidney cells. In non-mitotic cultured kidney tubular cells and in postnatal kidneys, NudC is localized to the primary cilium. NudC depletion leads to elongated cilia after 48hr serum withdrawal in interphase cells, and exclusive cyst formation in a three-dimensional culture system. Knockdown of nudc in zebrafish resulted pronephric cyst, body curvature, hydrocephalus and pericardial edema, which are typically seen in other cilia mutants.

**Conclusions:** Our work identifies NudC as a novel regulator of mitotic spindle orientation, ciliogenesis, and tubulogenesis. We propose that NudC is a novel candidate for ciliopathy.

*Funding:* NIDDK Support, Private Foundation Support

## TH-PO883

**Nephronophthisis Centrosomal Protein *CEP164* Regulates Cell Cycle Progression, Apoptosis, and Epithelial-to-Mesenchymal Transition** Gisela G. Slaats,[1] Amiya K. Ghosh,[4] Lucas Falke,[2] Stephanie Le Corre,[5] Indra Shaltiel,[3] Marianne C. Verhaar,[1] Roel Goldschmeding,[2] Tri Q. Nguyen,[2] Iain A. Drummond,[5] Friedhelm Hildebrandt,[4] Rachel H. Giles.[1] *[1]Nephrology and Hypertension, Univ Medical Center, Utrecht, Netherlands; [2]Pathology, Univ Medical Center, Utrecht, Netherlands; [3]Medical Oncology, Univ Medical Center, Utrecht, Netherlands; [4]Internal Medicine, Univ of Michigan, Ann Arbor, MI; [5]Genetics, Harvard Medical School, Charlestown, MA.*

**Background:** We recently reported that mutations in centrosomal protein 164 (*CEP164)* in nephronophthisis (NPHP) patients affect cilia and the DNA damage response. Here, we address the role of *CEP164* in NPHP and concomitant fibrosis.

**Results:** Live cell imaging of RPE FUCCI (fluorescent, ubiquitination-based cell cycle indicator) cells after siRNA knockdown of *CEP164* reveals a quicker cell cycle than control cells despite a longer S phase. FACS experiments with renal IMCD3 cells confirm that *Cep164* siRNA knockdown cells accumulate in S-phase, but we demonstrate that this can be rescued by human WT *CEP164* but not disease-associated mutants. siRNA of *CEP164* causes a proliferation defect over time, as measured by xCELLigence, CyQuant as well as PrestoBlue cell assays. We hypothesized that the discrepancy between accelerated cell cycle and inhibited overall proliferation could be explained by induction of apoptosis or epithelial-to-mesenchymal transition (EMT). Indeed, reduction of *CEP164* levels induces apoptosis in immunofluorescence, RT-QPCR and FACS experiments, and this phenomenon can be partially rescued by WT *CEP164*. Zebrafish injected with *cep164* morpholinos show developmental abnormalities and p53-independent apoptosis *in vivo*. Furthermore, knockdown of *Cep164* or overexpression of dominant negative mutant allele *CEP164 Q525X* induced EMT as we demonstrated by RT-QPCR, migration assay and western blot. Accordingly fibrosis mRNA markers are upregulated after *Cep164* knockdown.

**Conclusions:** We reveal a novel role for *CEP164* in NPHP pathogenesis, in which mutations causing ciliary defects are coupled with apoptosis and EMT.

*Funding:* Government Support - Non-U.S.

## TH-PO884

**RUVBL1 Is Part of Disease-Associated Protein Complexes at the Ciliary Base and Essential for Tubular Architecture *In Vivo*** Max C. Liebau,[1,2] Claudia Dafinger,[1] Ingolf Schmedding,[1] Benjamin Schairer,[1] Sandra Habbig,[1,2] Thomas Wunderlich,[3] Oliver Rinner,[4] Thomas Benzing,[1] Bernhard Schermer.[1] *[1]Dept of Internal Medicine 2, Univ Hospital of Cologne, Cologne, Germany; [2]Dept of Pediatrics, Univ Hospital of Cologne, Cologne, Germany; [3]Institue for Genetics, Univ of Cologne, Cologne, Germany; [4]Biognosys AG, Schlieren, Switzerland.*

**Background:** Cystic kidney diseases including Nephronophthisis (NPH) and ARPKD are common genetic causes of end stage renal failure during childhood and adolescence. However, the cellular protein functions of the affected gene products remain poorly understood. Recent work has linked ciliopathies to DNA damage response signaling.

**Methods:** Using immunoprecipitation-based mass spectrometry approaches we recently identified candidates for components of the Nephrocystin-1 and the Fibrocystin protein complex. Various candidates were followed up by independent methods in vitro and *in vivo.*

**Results:** Our candidates include an AAA ATPase with known roles in the regulation of cilia-associated signalling pathways and DNA damage response signalling, called RUVBL1. The interaction of RUVBL1 with various ciliary disease-associated proteins was confirmed by independent measures. In addition to other subcellular compartments, RUVBL1 localizes to the ciliary base during interphase. A newly generated tubule-specific Ruvbl1-knockout mouse shows high neonatal mortality and cystic kidney disease. Work on the cellular mechanisms resulting in this phenotype is ongoing.

**Conclusions:** We identified a member of the Tip60 DNA damage signalling complex as a cysto-protein at the ciliary base and as a component of disease-associated protein complexes. The link between ciliary signaling and DNA damage response signalling requires further investigation.

*Funding:* Government Support - Non-U.S.

## TH-PO885

**Modified Molecular Inversion Probe Analysis in Patients with Nephronophthisis-Related Ciliopathies** Markus Schueler,[1] Jan Halbritter,[1] Dan Doherty,[2] Ian Phelps,[2] Daniela A. Braun,[1] Jonathan Porath,[1] Heon Yung Gee,[1] Neveen Soliman,[3] Marwa Mohamed Nabhan,[3] Jay Shendure,[4] Brian J. O'Roak,[4] Edgar Otto,[5] Friedhelm Hildebrandt.[1,6] *[1]Dept of Medicine, Boston Children's Hospital, Boston, MA; [2]Dept of Pediatrics, Univ of Washington, Seattle, WA; [3]Egyptian Group for Orphan Renal Diseases, EGORD, Cairo, Egypt; [4]Dept of Genome Sciences, Univ of Washington, Seattle, WA; [5]Dept of Pediatrics, Univ of Michigan, Ann Arbor, MI; [6]Howard Hughes Medical Institute, Chevy Chase, MD.*

**Background:** Nephronophthisis is a recessive cystic kidney disease that progresses to terminal renal failure during the first three decades of life. In a cohort of 384 children diagnosed with a nephronophthisis-related ciliopathy two different high-throughput mutation analysis methods were used to identify disease-causing mutations within coding regions of 12 established NPHP genes and compared to each other.

**Methods:** Exon targeted mutation analysis was carried out using a modified molecular inversion probe (MIPs) technique. Previously we had performed mutation analysis in the same cohort using PCR-based Access Array microfluidic technology (AAMT) from Fluidigm™ with consecutive next-generation sequencing (NGS). Significant mutations were confirmed by Sanger sequencing and shown to segregate with the affected status.

**Results:** When using the MIP technology 100 pathogenic mutations were found, revealing the molecular cause in 64 out of 384 families (16%) and discovering 20 novel mutations in the genes NPHP1 (3), INVS (2) >NPHP3 (3), IQCB1 (1), CEP290 (3), RPGRIP1L (3), TMEM67 (1) and TTC21B (4). Whereas, when using the Fluidigm™/NGS approach, bioinformatics analysis of the identical cohort yielded only 84 pathogenic mutations in 52 out of 384 families (13.5%) including 21 novel mutations. Interestingly, only in 37 families the disease causing mutations were detected with both methods.

**Conclusions:** We conclude that the combination of two different high-throughput methods (AAMT and MIP) increases the mutation detection sensitivity in cohorts with broadly heterogeneous Mendelian disorders.

## TH-PO886

**Renal Oxylipin Alterations in the pcy Mouse Model of Nephronophthisis and Modulation by Therapeutic Intervention with Dietary Flaxseed Oil Rich in α-Linolenic Acid** Shizuko Nagao,[1] Clara R. Lysecki,[2] Ashleigh Reid,[2] Tamio Yamaguchi,[2] Jessay Gopuran Devassy,[2] Melissa Gabbs,[2] Mai Sasaki,[1] Amir Ravandi,[2] Harold M. Aukema.[2] *[1]Education and Research Center of Animal Models for Human Diseases, Fujita Health Univ, Toyoake, Aichi, Japan; [2]Dept of Human Nutritional Sciences, Univ of Manitoba, Winnipeg, Canada.*

**Background:** Nephronophthisis (NPHP) is a juvenile form of renal cyst disease characterized by cyst enlargement and interstitial fibrosis. It is the most common cause of end stage renal disease in children. Renal cytosolic phospholipase $A_2$ and cyclooxygenase (COX) 1 are elevated in pcy mice, an orthologous model of NPHP, suggesting that oxylipin formation may be altered. COX is elevated in another PKD model, the Han:SPRD-Cy rat, and COX inhibition reduces COX-derived oxylipins and ameliorates disease progression. If oxylipins are similarly altered in pcy mice, there may be potential for treatment. Flax oil contains the n-3 fatty acid α-linolenic acid and thus may alter oxylipin formation. Therefore, in the current study renal oxylipins were determined early in the disease process, and the effect of dietary flax oil on disease progression and oxylipin alterations were examined.

**Methods:** Kidney homogenates from normal and pcy mice at 15, 30, 60 days were used for initial oxylipin analyses by HPLC-tandem mass spectrometry. In a second study, pcy mice were given dietary soy (control) or flax oil for 16 wk from weaning. Kidney sections were stained with Masson's trichrome for fibrosis analysis and kidneys were analyzed for oxylipins.

**Results:** The earliest significant oxylipin differences between normal and diseased kidneys were observed at 30 days, when several COX derived oxylipins were higher. Lipoxygenase oxylipins were not different until 60 days of age, when several were lower in diseased kidneys. Dietary flax oil treatment significantly reduced kidney weight and interstitial fibrosis and ameliorated many of the renal oxylipin alterations.

**Conclusions:** Renal oxylipins are altered in NPHP and dietary flax oil rich in ALA reduced the progression of renal interstitial fibrosis, possibly via amelioration of oxylipin abnormalities.

*Funding:* Government Support - Non-U.S.

## TH-PO887

**Sheep as a Large Animal Model of a Recessive Ciliopathy** Cherie Stayner,[1] May Pilanthananond,[1] Julia A. Horsfield,[1] David M. Markie,[1] John C. McEwan,[3] C. Anthony Poole,[2] Robert J. Walker,[2] Michael R. Eccles.[1] *[1]Pathology, Univ of Otago, Dunedin, New Zealand; [2]Medicine, Univ of Otago, Dunedin, New Zealand; [3]Invermay Research Centre, AgResearch, Mosgiel, New Zealand.*

**Background:** Autosomal recessive polycystic kidney disease (ARPKD) predominately affects infants and young children. Mutations can arise in genes that cause a number of different recessive syndromes, Meckel-Gruber Syndrome (MKS) showing the earliest lethality. We have identified a sheep model of ARPKD with features that resemble MKS. Affected lambs die *in utero*, or in the early neo-natal period, and show extensive cyst formation and fibrosis in the kidney.

**Methods:** Homozygosity mapping was chosen to map candidate disease loci. DNA from affected lambs was analyzed using a single nucleotide polymorphism (SNP) Beadchip. A knockdown model in zebrafish was employed to investigate the pathogenicity of the missense changes identified in the ovine *Mks3* gene. Zebrafish *mks3* was knocked down by morpholino injection of embryos and rescue experiments were performed by co-injection with mRNA for either the wildtype, or mutant, sheep *Mks3*.

**Results:** A candidate region identified on chromosome 9 contained the *Tmem67*/*Mks3* gene, and sequencing revealed two novel single nucleotide sequence variants in exon 20 of *Mks3* in affected sheep. These lead to missense amino acid substitutions (I680N and I686S) in the cilia-located transmembrane receptor protein, meckelin. Modelling of these sequence variants predict that either variant would have a deleterious effect on protein function. Wildtype sheep *Mks3* was able to rescue the embryonic defects caused by knockdown of zebrafish *mks3* expression. Co-injection of an *Mks3* mRNA that contained the two missense changes found in our affected sheep, was not able to rescue these defects.

**Conclusions:** Two amino acid changes in exon 20 of the *Mks3* gene, discovered in sheep with recessive polycystic kidney disease, have been demonstrated as being potentially pathogenic, and are likely to be the cause of the kidney phenotype in these sheep. A large animal model of renal cystic disease can offer important advantages for screening of novel therapeutics for polycystic kidney disease.

*Funding:* Government Support - Non-U.S.

## TH-PO888

**Loss of Function Mutation of FAT1 May Represent a Novel Disease Causing Gene in Nephronophthisis with Nephrotic Syndrome Overlap** Jonathan Porath,[1] Carolin E. Sadowski,[1] Jan Halbritter,[1] Rannar Airik,[1] Daniela A. Braun,[1] Markus Schueler,[1] Heon Yung Gee,[1] Edgar Otto,[2] Friedhelm Hildebrandt.[1,3] *[1]Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Pediatrics, Univ of Michigan, Ann Arbor, MI; [3]Howard Hughes Medical Institute, Chevy Chase, MD.*

**Background:** Nephronophthisis-related ciliopathies (NPHP-RC) comprise a group of autosomal recessive cystic kidney diseases. As the disease-causing gene has only been identified in 40-60% of individuals with NPHP-RC, we performed whole exome resequencing (WER) to identify novel disease causing genes.

**Methods:** We performed homozygosity mapping as well as WER (NimbleGen SeqCap EZ™ Exome ver2) followed by next generation resequencing (Illumina HiSeq 2000) in a cohort of 84 families with NPHP-RC. We then screened an additional 720 individuals with nephrotic syndrome using array based multiplex PCR (Fluidigm Access Array™) and next generation resequencing (Illumina MiSeq).

**Results:** A homozygous truncation (p.P1032Cfs*11) was identified in the gene FAT1 (FAT atypical cadherin 1) in a consanguineous Turkish family with one affected child. This individual has a complex phenotype including nephrotic range proteinuria, glomerular sclerosis, and histologic features of nephronophthisis leading to end-stage renal disease at the age of 15 years old. There were also extrarenal manifestations of bilateral ptosis, pulmonary artery stenosis, intellectual disability, pachygyria, and dilated Virchow-Robin spaces. We additionally identified a homozygous truncation of unknown significance in the gene PIDD in this individual. In addition, we identified two compound heterozygous FAT1 mutations (p.N2951S and p.E4374K) in a child with congenital nephrotic syndrome and renal histology of diffuse mesangial sclerosis. FAT1 has been shown to play a role in WNT signaling in which defects have previously been shown to be a mechanism for renal disease. The Fat1$^{-/-}$ mouse displays abnormal podocyte foot processes, brain developmental defects, and eye abnormalities.

**Conclusions:** We identified mutations in FAT1 in individuals with an overlapping phenotype of NPHP-RC and nephrotic syndrome.

## TH-PO889

***CCDC41* Is Mutated in Nephronophthisis-Related Ciliopathy** Heon Yung Gee,[1] Jan Halbritter,[1] Jonathan Porath,[1] Daniela A. Braun,[1] Markus Schueler,[1] Edgar Otto,[2] Friedhelm Hildebrandt.[1,3] *[1]Div of Nephology, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Dept of Pediatrics, Univ of Michigan, Ann Anbor, MI; [3]Howard Hughes Medical Institute.*

**Background:** Nephronophthisis-related ciliopathies (NPHP-RC) are degenerative recessive diseases that affect, retina, or brain (1). More than 15 genes are associated with NHPH-RC, accounting for about 40% of all cases (1). About 60% of cases are molecularly unsolved.

**Methods:** To find additional single-gene cause of NPHP-RC, we screened a worldwide cohort of over 700 individuals by multiplex PCR followed by barcoding and next-generation resequencing (1).

**Results:** We identified a homozygous frameshift mutation (p.E669Dfs*14) in CCDC41 (coiled-coil domain containing 41) in an affected individual with NPHP, macrocephaly and heart anomalies. CCDC41 is a component of the distal appendages of centrioles and was shown to be required for ciliary vesicle docking to the mother centriole (2). Loss of CCDC41 blocks centriole-to-membrane docking and inhibits ciliogenesis (3).

**Conclusions:** We identified a mutation in CCDC41 as causing NPHP-RC, adding another component of the distal appendages of centrioles as defective in cystic kidney diseases.

**References** 1. Halbritter et. Al., Hum Genet, in press; 2. Tanos et. Al., Genes Dev, 27:163, 2013; 3. Joo et. Al., Proc Natl Acad Sci U S A, 110:5987, 2013.

## TH-PO890

**A Novel *Cep290* Collecting Duct Tubule Cell Line of the Ciliopathy Joubert Syndrome** Ann Marie Hynes,[1] Rachel H. Giles,[2] Lorraine Eley,[1] Colin Miles,[1] John Andrew Sayer.[1] *[1]Institute of Genetic Medicine, Newcastle Univ, Newcastle upon Tyne, United Kingdom; [2]Univ Medical Center Utrecht, Univ Medical Center Utrecht, Utrecht, Netherlands.*

**Background:** Joubert Syndrome related disorders (JSRD)are autosomal recessive ciliopathies characterised by retinal degeneration, cystic kidney disease and cerebellar vermis aplasia. Mutations in CEP290 are the most common cause of JSRD. Here we described the isolation and characterisation of collecting duct cell lines, isolated from kidneys of $Cep290^{LacZ/LacZ}$ mice backcrossed with H-2Kb-tsA58$^{+/-}$ "immorto" mice. These cell lines have been used to investigate ciliary signallin and disease pathogenesis.

**Methods:** *$Cep290^{LacZ/LacZ}$::H-2Kb-tsA58$^{+/-}$* (mutant) and *$Cep290^{+/+}$::H-2Kb-tsA58$^{+/-}$* (wild-type) collecting duct cells were isolated from kidneys of one month old transgenic mice. Kidney samples were digested in 0.1% collagenase type II andenriched on 6 well plates coated with 10 mg/ml Dolichos Biflorus Agglutinin at 33°C. PCR for collecting duct cell markers, western blotting and staining for cilia were used to characterise these cells under a range of conditions.

**Results:** Mutant and wild type collecting duct cells displayed collecting duct morphology and expressed both the mineralocorticoid receptor and the epithelial sodium channel alpha subunit. There was a loss of primary cilia in Cep290 mutant cells compared to wild type cells (5% vs. 55%, $p < 0.0001$). The cilia that were evident in Cep290 mutant cells were also shortened when compared with the wild type cells (mean length 2.4μm vs. 3.5um). Western blotting showed disrupted signalling in mutant cells. 3D culturing of Cep290mutant cells confirmed a loss of primary cilia and disrupted epithelial polarity.

**Conclusions:** The conditionally immortalised wild type and Cep290 mutant collecting duct cells formed epithelial cell layers and expressed collecting duct cell markers. Cep290 mutation leads to either a loss of cilia or shortened cilia compared to wild type controls. Disrupted signalling in Cep290 mutant cells suggests that these cell lines provide a valuable tool for understanding cystic kidney disease and for drug testing.

*Funding:* Private Foundation Support

## TH-PO891

**Tolvaptan Reduces the Mortality and Ameliorates the Progression of PKD in DBA/2:FG-*pcy* Mice** Miki Aihara, Katsuji Hattori, Koji Ohmoto, Hiroshi Mizuguchi, Hiroyuki Fujiki, Makoto Ishikawa, Keisuke Nagano, Yoshitaka Yamamura. *First Institute of New Drug Discovery, Otsuka Pharmaceutical Co.,Ltd., Tokushima, Japan.*

**Background:** Tolvaptan slowed the increase in total kidney volume and the decline in renal function in patients with ADPKD in the TEMPO 3:4 clinical trial. There still remain the questions of whether tolvaptan has a dose-related effect and whether long-term treatment with tolvaptan is effective in delaying the end stage of the disease.

**Methods:** We examined the dose-related effect of tolvaptan by short-term treatment (0.01% to 0.3% via diet; 5 to 15 weeks of age) and the long-term effect of tolvaptan by whole-life treatment at the optimal dose (0.1% via diet; 5 to 29 weeks of age) in DBA/2:FG-*pcy* mice, an animal model of nephronophthisis.

**Results:** By short-term treatment, at 15 weeks of age tolvaptan dose-dependently prevented increases in kidney weight, kidney cyst volume, kidney fibrosis volume, and mitotic index, and also significantly reduced renal cyclic AMP level and ERK activity, suggesting that the mechanism for these effects was antagonism of vasopressin signals. Urinary NGAL level was also suppressed dose-dependently. Based on the overall efficacy profile, the optimal dose was considered to be 0.1% via diet. In the long-treatment study, left kidney volume was monitored by MRI over time, and tolvaptan significantly inhibited the enlargement of left kidney volume by as much as 50% of the control ($p < 0.01$, repeated measures ANOVA) and also significantly inhibited urinary albumin excretion during the experimental period. Additionally, tolvaptan significantly reduced mortality rate to 20% ($p = 0.0418$, log-rank test) versus 60% in control DBA/2:FG-*pcy* mice.

**Conclusions:** These studies showed for the first time that long-term treatment with tolvaptan at the optimal dose was highly effective in inhibiting cystogenesis, preventing deterioration of renal function, and improving mortality in DBA/2:FG-*pcy* mice. Furthermore, these data strongly indicate that vasopressin-mediated cAMP elevation plays a key role in the pathogenesis of PKD and that tolvaptan ameliorates the progression of PKD by V2 receptor antagonism, providing a promising treatment for PKD.

## TH-PO892

**Loss-of-Function Mutations in *SLCO4C1* as a Cause of Hypertension in Chronic Kidney Disease** Daniela A. Braun,[1] Heon Yung Gee,[1] Eikan Mishima,[2] Jan Halbritter,[1] Jonathan Porath,[1] Markus Schueler,[1] Edgar Otto,[3] Takaaki Abe,[2,4] Friedhelm Hildebrandt.[1,5] *[1]Div of Nephrology, Boston Children's Hospital, Boston, MA; [2]Dept of Clinical Biology, Tohoku Univ Graduate School of Medicine, Miyagi, Japan; [3]Dept of Pediatrics, Univ of Michigan, Ann Arbor, MI; [4]Div of Medical Science, Tohoku Univ Graduate School of Biomedical Engineering, Miyagi, Japan; [5]Howard Hughes Medical Institute, Chevy Chase, MD.*

**Background:** Nephronophthisis (NPHP) a recessive cystic kidney disease represents the most frequent genetic cause of kidney failure in the first decades of life. Although in more than 10% of the cases NPHP is associated with extra-renal involvement, hypertension is characteristically absent from individuals with NPHP.

**Methods:** We performed homozygosity mapping and whole exome resequencing (WER) in a consangineous family with two affected children with NPHP and hypertension.

**Results:** By homozygosity mapping and WER we identified a homozygous missense mutation in the gene SLCO4C1 (p.M1-start loss). SLCO4C1 (solute carrier organic anion transporter family member 4C1) is a kidney-specific organic anion transporter that plays a major role in the excretion of uremic toxins and has been implemented in the pathogenesis of hypertension[1]. By IHC we confirm localization of SLCO41 to the basolateral membrane of proximal tubular epithelium. We show in vitro that the observed mutation represents a functional null allele by ablating the translation initiation codon and causes loss of function.

**Conclusions:** In a consangineous family with two affected siblings we identified a homozygous missense mutation in SLCO4C1 as causing hypertension in children with NPHP.[1] Toyohara et al, JASN 20: 2546, 2009.

## TH-PO893

**Dynamics of E-Cadherin Localization and β-Catenin Activation following Unilateral Ureteral Obstruction in Wild Type and Pkhd1$^{del4/del4}$ Kidneys** Rachel Gallagher,[1] Seung H. Lee,[1] Stefan Somlo.[1,2] *[1]Internal Medicine, Yale School of Medicine, New Haven, CT; [2]Genetics, Yale School of Medicine, New Haven, CT.*

**Background:** Studies on *Pkhd1* have primarily focused on its role in autosomal recessive polycystic kidney disease (ARPKD), however its role in renal homeostasis has yet to be addressed. We have previously described that *Pkhd1$^{del4/del4}$* mice had impaired recovery with sustained tubule dilation when subjected to unilateral ureteral obstruction (UUO) (ASN2010, F-PO1765). In this study, we sought to investigate the molecular basis for the poor recovery in *Pkhd1$^{del4/del4}$*kidneys.

**Methods:** *Pkhd1$^{del4/del4}$* mice and littermate controls were subjected to 3 days of UUO followed by recovery at 14 days post injury. Kidneys were analyzed by immunohistochemistry, BATgal reporter activity, and quantitative PCR (qPCR).

**Results:** Immunohistochemical analysis showed that E-cadherin expression was reduced at the lateral membrane and was re-localized to the cilia following 3 days of UUO in both wild type and *Pkhd1$^{del4/del4}$*mice. β-catenin expression was detected in both the cytoplasm and at the lateral membrane after injury and there was no difference between wild type and *Pkhd1$^{del4/del4}$*injured kidneys. The lateral membrane was repopulated with E-cadherin after 14 days repair in the wild type kidneys but not in *Pkhd1$^{del4/del4}$*kidneys in which very little was detected at the lateral membrane. Since loss of E-cadherin has been shown to release β-catenin from the membrane where it can translocate to the nucleus and activate TCF/lef transcription, we examined whether there was β-catenin-dependent transcriptional activation using qPCR and the BATgal reporter mouse. qPCR analysis showed elevated levels of β-catenin transcriptional targets in the *Pkhd1$^{del4/del4}$*injured kidneys, however the reporter studies showed that the transcriptionally active cells were primarily in the fibroblasts and not in the collecting duct cells.

**Conclusions:** The data show abnormal localization of E-cadherin in cilia during UUO injury and the occurrence of sustained β-catenin activity in fibroblasts in *Pkhd1$^{del4/del4}$* kidneys after 14 days of recovery from UUO. Response to injury may be a feature of *Pkhd1* activity in adult kidney homeostasis.

*Funding:* Private Foundation Support

## TH-PO894

**Ectopic Expression of the Human *PKHD1* Gene Product Can Rescue the Cystic Phenotype of *Pkhd1* Knockout Mice** Ao Li,[1,2] Yuan Li,[1,2] Haichao Liu,[1,2] Wei Li,[1,2] Dan Liang,[2] Guanqing Wu.[2] *[1]State Key Laboratory of Molecular Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences, Beijing, China; [2]Depts of Medicine, Vanderbilt Univ, Nashville, TN.*

**Background:** We have recently produced a transgenic mouse in which a human *PKHD1*-full-length ORF cDNA is controlled by a pCAGGS expression vector.

**Methods:** The pCAGGS expression vector has been reported to express the reporter gene at an early stage of embryonic development and possesses ubiquitous expression in all tissues and organs of the mice. Sixty-six offspring from foster mothers were screened by PCR, and transgene founders were detected in five of them. Four founders (tg5, 20, 46 and 63) can go through germline transmission to produce offspring with the transgene. By qPCR testing, tg5 and 20 do not show human *PKHD1* mRNA overexpression in the disease-affected tissues and organs. Southern analyses indicate that there are approximately three copies of the transgene in tg63 (*PKHD1$^{tg63}$*) and two copies in tg46 (*PKHD1$^{tg46}$*) mice. We used *PKHD1$^{tg63}$* mice for our further studies.

**Results:** *PKHD1$^{tg63}$* mice do not show any abnormal phenotypes during mouse development. Using a mating strategy, *PKHD1$^{tg63}$* mice were crossmated with *Pkhd1$^{-/-}$* mice (Kim et al, *JASN* 2008:19;455) to produce *Pkhd1$^{-/-}$:PKHD1$^{tg63}$* mice. Kaplan-Meier analysis illustrated that approximately 25% of *Pkhd1$^{-/-}$:PKHD1$^{tg63}$* mice survive until 6 months, compared with only 18% of *Pkhd1$^{-/-}$* mice, suggesting that human *PKHD1* transgene can rescue partially lethal phenotype in *Pkhd1$^{-/-}$* mice. By pathological analysis, we also found that the *Pkhd1$^{-/-}$:PKHD1$^{tg63}$* mice (n=5) exhibited significantly decreased cystic number and volume in the pancreas, liver and kidneys, compared to *Pkhd1$^{-/-}$* littermates, suggesting that an increased expression level of human *PKHD1* gene product can significantly rescue cystic phenotypes in the mice model of ARPKD. *Pkhd1$^{-/-}$* mice with *PKHD1$^{tg63}$* also exhibitmuch better improved hepatorenal functions than *Pkhd1$^{-/-}$* mice alone.

**Conclusions:** Our results indicate that ectopic expression of the human *PKHD1* gene product can rescue the cystic phenotypes of *Pkhd1* knockout mice, and underlies a therapeutic potential for gene therapy of ARPKD.

*Funding:* NIDDK Support

**TH-PO895**

**Smad3 Phosphorylated at Both Linker and COOH-Terminal Regions in Cyst-Lining Epithelia in cpk Mouse, a Model of ARPKD**
Taketsugu Hama,[1] Koichi Nakanishi,[1] Hironobu Mukaiyama,[1] Hiroko Togawa,[1] Masashi Sato,[1] Yuko Shima,[1] Masayasu Miyajima,[2] Kandai Nozu,[3] Shizuko Nagao,[4] Hisahide Takahashi,[4] Kazumoto Iijima,[3] Norishige Yoshikawa.[1] *[1]Pediatrics, Wakayama Medical Univ, Wakayama, Japan; [2]Laboratory Animal Center, Wakayama Medical Univ, Wakayama, Japan; [3]Pediatrics, Kobe Univ, Kobe, Japan; [4]Education and Research Center of Animal Model for Human Disease, Fujita Health Univ, Aichi, Japan.*

**Background:** Cystic epithelia acquire mesenchymal features in PKD. In the context of this alteration, however, TGF-β/Smad3 pathway remains not to be fully investigated. TGF-β signaling involves Smad3 phosphorylated at linker regions (pSmad3L), COOH terminal regions (pSmad3C) and both (pSmad3L/C). pSmad3L/C has an important role in colorectal cancer. Mesenchymal-phenotype-specific cell response in TGF-β/Smad3 pathway may be involved also in PKD.

**Methods:** We confirmed mesenchymal features and examined Smad3 phosphorylation profile in *cpk*. Kidneys from 5 *cpk* and control mice (each on days 14 and 21) were harvested. Kidney sections were stained with antibodies to mesenchymal markers and domain-specific phospho-Smad3. TGF-β, pSmad3L, pSmad3C, JNK, CDK4 and c-Myc were evaluated by western blotting (WB). Cophosphorylation of pSmad3L/C was assessed by immunoprecipitation (IP) with WB.

**Results:** Vimentin and α-SMA were *de novo* expressed in *cpk*, showing mesenchymal features, while not expressed in controls. pSmad3L was increased in*cpk*, while no significant staining in controls. pSmad3L was predominantly expressed in nuclei of tubular epithelial cells in cysts. On the other hand, pSmad3C was similarly expressed in both *cpk* and controls. There was no difference in TGF-β level. WB showed that pSmad3L, JNK, CDK4 and c-Myc levels in cell nuclei were significantly higher in *cpk*. IP with WB showed that most Smad3 was cophosphorylated (pSmad3L/C) in *cpk*.

**Conclusions:** These findings suggest that upregulating c-Myc through JNK/CDK4-dependent pSmad3L/C pathway in cell nuclei may be a key pathophysiology in *cpk*. In conclusions, qualitative rather than quantitative abnormality of TGF-β/Smad3 pathway seems to involve PKD, and may be a target for a disease-specific intervention.

*Funding:* Government Support - Non-U.S.

**TH-PO896**

**Glycogen Synthase Kinase-3 Inactivation Ameliorates Polycystic Kidney Disease in Cys1$^{cpk}$ Mice**
Reena Rao,[1] Erin Suderman,[1] Shixin Tao.[1] *[1]Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS; [2]Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS; [3]Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Polycystic kidney diseases (PKD) are a family of inherited disorders characterized by the formation of multiple fluid filled cysts primarily arising from the vasopressin responsive renal collecting ducts. Vasopressin mediated increase in cAMP is mitogenic in cystic epithelium. The glycogen synthase kinase-3 (GSK3) family of protein kinases, consisting of GSK3α and GSK3β plays a crucial role in multiple cellular signaling pathways. In previous studies we found that GSK3β inactivation reduces vasopressin-mediated renal cAMP generation. Hence, we evaluated the therapeutic potential for GSK3β inactivation as a novel approach to treat PKD.

**Methods:** Renal GSK3β expression and activity were increased in the Cys1$^{cpk}$ murine model of autosomal recessive PKD. To examine the effect of GSK3 inactivation on cyst development and progression, Cys1$^{cpk}$ mice were administered a GSK3 inhibitor, TDZD-8 from postnatal days 3 to 14, or GSK3β was knocked out in renal collecting ducts of Cys1$^{cpk}$ mice.

**Results:** Pharmacological inhibition of GSK3 or gene knockout of GSK3β in renal collecting ducts of Cys1$^{cpk}$ mice significantly diminished development and progression of PKD, indicated by decreased kidney size, cyst area and fibrosis, and improved renal function. GSK3 inactivation also reduced renal cAMP concentrations, proliferation of cyst-lining epithelial cells and proliferation factors, c-myc and cyclin-D1, independent of canonical WNT signaling.

**Conclusions:** We conclude that GSK3β has a significant role in promoting renal cyst growth in response to cAMP and suggest that GSK3 inhibitors may be therapeutically useful to reduce cyst expansion and preserve renal function in PKD.

*Funding:* NIDDK Support

**TH-PO897**

**Role of the Epithelial $Na^+$ Channels in Development of Autosomal Recessive Polycystic Kidney Disease**
Alexander Staruschenko, Vladislav Levchenko, Daria Ilatovskaya, Oleg Palygin, Tengis S. Pavlov. *Physiology, Medical College of Wisconsin, Milwaukee, WI.*

**Background:** Autosomal recessive polycystic kidney disease (ARPKD) is characterized by the development of renal cysts of tubular epithelial cell origin. Dysfunction and aberrant regulation of the epithelial $Na^+$ channel (ENaC) leads to a spectrum of diseases ranging from hyper- and hypotension associated with improper renal $Na^+$ conservation and wasting, respectively, to cystic fibrosis. Members of the epidermal growth factor (EGF) family have direct actions on $Na^+$ reabsorption in the distal nephron causing long-term reduction of ENaC activity. It has also been reported high EGF concentration in cystic fluid and that EGF receptor (EGFR) is overexpressed and mislocalized to the apical membranes in the cystic epithelia.

**Methods:** Here we tested whether ENaC plays a role in cyst development in PCK rats, an animal model of ARPKD. 4 weeks old PCK rats were treated with selective ENaC inhibitor benzamil (or vehicle) in drinking water *ad libitum* (15 mg/L) for additional 4 or 12 weeks. In the end of experiments, the kidneys were collected to assess cystic area percentage and kidney to total body weight ratio. PCK and Sprague Dawley rats were also used for patch clamp analysis of ENaC activity in isolated cysts and tubules and immunohistochemical assay.

**Results:** 4 and 12 weeks treatment with benzamil given in drinking water exacerbates cyst development compared to vehicle-treated PCK rats. Patch clamp studies of the freshly isolated tissues revealed that ENaC activity in the cystic epithelia was significantly lower than in the non-cystic cortical collecting ducts of the PCK or Sprague-Dawley rats. Immunohistochemical analysis confirms that β-ENaC expression is decreased compared to the non-cystic cortical collecting ducts within PCK rat kidneys.

**Conclusions:** We conclude that abnormal EGF signaling leads to inappropriately low ENaC activity which contributes to cyst formation in PCK rats. Thus, the disruption of the EGF-EGFR axis reported in ARPKD is linked to suppression of normal $Na^+$ reabsorption, fluid accumulation in cysts and further progress of this kidney disease.

*Funding:* Other NIH Support - NHLBI R01-108880, Private Foundation Support

**TH-PO898**

**ADAM17 Regulates Mitochondrial Bioenergetics in Polycystic Kidney Disease (PKD)**
Monika Gooz,[1] Eduardo Maldonado,[1] Yujing Dang,[1] May Y. Amria,[1] Hanna E. Abboud,[2] John Lemasters,[1] P. Darwin Bell.[1] *[1]Medical Univ of South Carolina, Charleston, SC; [2]Univ of Texas Health Science Center, San Antonio, TX.*

**Background:** Previously, we identified ADAM17 as the main sheddase of growth factors in activating the epidermal growth factor receptor and switching on the proliferative extracellular signal-regulated kinase (ERK) signaling pathway in cilia(-) collecting duct cells originating from the Ift88 hypomorph mouse. In this study we investigated the contribution of increased ADAM17 activation to mitochondrial bioenergetics in PKD because recent data showed altered mitochondrial metabolism in several proliferative diseases including cancer.

**Methods:** We assessed ERK activation by Western blotting and ADAM17 activity by a fluorogenic substrate. We determined mitochondrial respiration by a Seahorse XF Analyzer, and measured nicotinamide adenine dinucleotide (NADH) autofluorescence by confocal multiphoton microscopy. Mitochondrial membrane potential ($\Delta\Psi$) was measured by tetramethyl rhodamine methyl ester fluorescence.

**Results:** Expression and enzyme activity of ADAM17, and level of phosphorylated ERK were higher in the highly proliferative cilia(-) cells compared to the less proliferative cilia(+) cells. Cilia(-) cells had also higher basal respiration than cilia(+) cells: 440±31 versus 278±39 pmol $O_2$/min/10,000 cells. Addition of the uncoupler CCCP (5 μM), induced higher maximal respiration in cilia(-) cells compared to cilia(+) cells. NADH level was 36±15% higher in cilia(-) cells compared to cilia(+) cells. Interestingly, $\Delta\Psi$ was 50% lower in cilia(-) cells compared to cilia(+) cells. After chemical inhibition of ADAM17 (10 μM TMI-005, Pfizer) basal respiration (365±9 pmoles/min) and NADH production in cilia(-) cells decreased to the level of cilia(+) cells. Additionally, the ERK inhibitor thiazolidinedione (50 μM) decreased basal mitochondrial respiration to 383±10 pmoles/min in cilia (-) cells.

**Conclusions:** Our data suggest that ADAM17 and ERK activation enhances mitochondrial metabolism in cilia (-) cells compared to cilia (+) cells. Thus, ADAM17-dependent cellular bioenergetics can be an important contributor to the pathobiology of PKD.

*Funding:* NIDDK Support, Veterans Affairs Support

**TH-PO899**

**Chronic and Excessive Netrin-1 Expression in Renal Tubular Epithelium Induces Polycystic Kidney Disease in Mice**
Riyaz Mohamed,[1] Calpurnia Jayakumar,[1] Andreas D. Kistler,[2] Ganesan Ramesh.[1] *[1]Vascular Biology Center, Georgia Regents Univ, Augusta, GA; [2]Div of Nephrology, Univ Hospital Zurich, Zurich, Switzerland.*

**Background:** The mechanism of cyst development and pathogenesis leading to end-stage renal disease is not entirely understood due to lack of animal models that closely mimic human disease. This study was done to characterize the new animal model for PKD and determine the role of netrin-1 in PKD.

**Methods:** Two transgenic mice lines (Tg3 and Tg6) that overexpresses netrin-1 in the kidney proximal tubules using L-fatty acid binding protein promoter and their wild-type littermate were used in this study. Netrin-1 overexpression, signaling pathways that are activated in the kidney and role of netrin-1 in PKD was determined by Western blot, RT-PCR, ELISA and siRNA infusion.

**Results:** The transgene chicken netrin-1 excretion in urine is 100 fold more in Tg6 as compared to Tg3 mice and over 1000 fold as compared to WT mice. Tg6 but not in Tg3 transgenic mouse developed multiple cysts at the age of 4 weeks and cyst growth accelerated rapidly reaching a kidney weight of 15-17gram by 32 weeks of age that was accompanied by kidney dysfunction (BUN: 35±5 mg/dl, $p<0.001$ vs. WT), polyuria, albuminuria, increased epithelial cell proliferation and increased excretion of cAMP. Surprisingly, cyst formation in the kidney was restricted to male animals with a penetrance of 85%. Transcription factors (Atf3, NFAT, Cebpb and Fos) and signaling pathways (ERK, focal adhesion kinase) that are associated with cAMP and polycyst formation in human were upregulated in Tg6 mice kidney. Interestingly, p53 expression was increased but present in an inactive unphosphorylated form. To determine whether the observed effects were due to netrin-1 overexpression, netrin-1 expression was suppressed with siRNA infusion. siRNA

mediated suppression of netrin-1 expression completely suppressed polycyst formation in Tg6 animals. Consistent with animal studies, human ADPKD patients excrete significantly more netrin-1 than healthy patients.

**Conclusions:** Our result suggests chronic and excessive overexpression of netrin-1 induce polycyst formation through dedifferentiation and suppression of p53 activation.

*Funding:* NIDDK Support

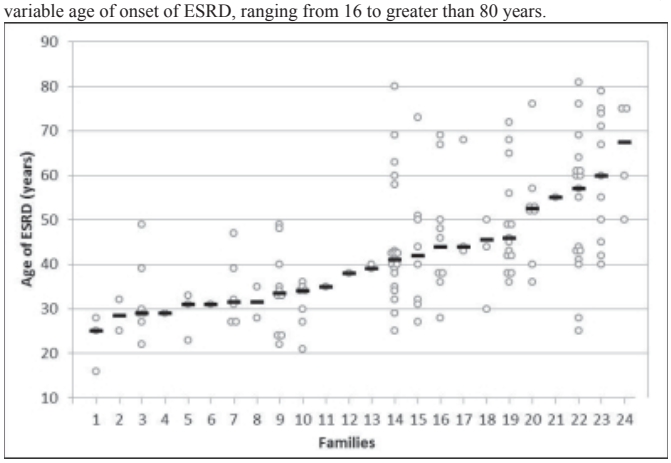
## TH-PO900

**Variable Clinical Presentation in Individuals with a MUC1 Mutation Causing Medullary Cystic Kidney Disease Type 1** Anthony J. Bleyer,[1] Stanislav Kmoch,[2] Kendrah O. Kidd,[1] Victoria C. Robins,[1] Katerina Hodanova.[2] *[1]Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; [2]Institute of Inherited Metabolic Disorders, Charles Univ in Prague, Prague, Czech Republic.*

**Background:** A cytosine insertion in the *MUC1* gene was recently identified as the most common cause of medullary cystic kidney disease type 1 (MCKD1). The clinical expression of this mutation has not been well characterized.

**Methods:** Genotyping and evaluation of clinical characteristics were performed on families with a history of *UMOD*- and *REN*-negative autosomal dominant interstitial kidney disease.

**Results:** 25 of 36 families tested were identified with a *MUC1* mutation (m*MUC1*) . Of 179 members of m*MUC1* families, m*MUC1* was identified in 95 individuals, and 84 individuals did not have a mutation. There were 110 individuals identified as historically affected. Individuals with m*MUC1* suffered from chronic kidney failure with a widely variable age of onset of ESRD, ranging from 16 to greater than 80 years.




There appeared to be 2 types of families. In 13 families, the age of start of dialysis was <50 in almost all individuals, with many young individuals with ESRD. In the other 12 families, there was much more variability in the age of ESRD, with some patients not reaching dialysis until after 70. 38/38 renal ultrasounds of m*MUC1* individuals showed no medullary cysts. While *MUC1* is expressed in many tissues, there were no clinical manifestations of the *mMUC1* in the breasts, skin, respiratory or gastrointestinal tract.

**Conclusions:** *MUC1* mutations are the predominant cause of *UMOD* negative autosomal dominant interstitial kidney disease. Individuals with m*MUC1* have highly variable expression, with some proceeding to dialysis as teens, while others remain dialysis-free into their 80's. While *MUC1* is expressed in many tissues, it only results in clinical abnormalities in the kidney.

## TH-PO901

**Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Medullary Cystic Kidney Disease (MCKD) in an Australian Chronic Kidney Disease (CKD) Cohort** Andrew John Mallett,[1,2,3] Anne Salisbury,[1,2,3] Zaimin Wang,[1,2] Helen G. Healy,[1,2,3] George T. John,[1,3] Wendy E. Hoy.[1,2] *[1]CKD.QLD; [2]Centre for Chronic Disease, School of Medicine, Univ of Queensland, Brisbane, Queensland, Australia; [3]Dept of Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.*

**Background:** 5.7% and 0.3% of Australian and New Zealand patients commencing renal replacement therapy (RRT) in 2011 had ADPDK or MCKD respectively as a Primary Renal Diagnosis. Those with CKD are not well described within Australasia.

**Methods:** To define the frequency and characteristics of CKD patients with APKCK and MCKD. CKD.QLD is a registry and research platform involving all consenting patients in public renal practices in Queensland (~10,800). Primary Renal Disease coding on the first 2359 patients at 5 hospital sites were searched for ADPKD and MCKD. These patients were compared to all patients in the CKD.QLD registry for age, gender and CKD stage.

**Results:** 93 ADPKD and 9 MCKD patients were identified representing 3.9% and 0.4% of the total. Distribution was similar between all 5 CKD.QLD sites. 48% of ADPKD patients and 6/9 MCKD patients were female.

The most common CKD Stages in ADPKD patients, MCKD patients and the broader CKD.QLD registry were 3b (23.7%, 44%, 28%) and 4 (21.5%, 44%, 22%). Men with ADPKD were most commonly CKD Stage 3b (29%). Women with ADPKD were most commonly CKD Stage 4 (28.8%) with suggestion of bidmodal age distribution.

The mean age of ADPKD and MCKD patients were 54.6 and 52.8 years respectively. The most common ADPKD age group was 45-54 years (23.6%) with that for MCKD being 65-74 years (44%). For the full CKD.QLD population mean age was 65.5 years with most common age group 65-74 years (29%).

**Conclusions:** The proportions of patients with CKD due to ADPKD and MCKD are similar to those suggested by RRT data. These patients are younger than the general CKD population. Further patient accrual and analysis is required to corroborate these findings. Future molecular genetic investigation may identify distinct ADPKD and MCKD subgroups for further analysis.

*Funding:* Government Support - Non-U.S.

## TH-PO902

**Identification of Novel Tubulogenesis Genes by Microarray Analysis in 3D-Cultured MDCK Cells** Maria F. Chacon-Heszele,[1] Xiaofeng Zuo,[1] Sarah McKenna,[1] Liwei Huang,[1] Soo Young Choi,[1] Joshua H. Lipschutz.[1,2] *[1]Depts of Medicine, Univ of Pennsylvania; [2]Philadelphia VAMC, Philadelphia, PA.*

**Background:** The kidney originates from two types of "building blocks", cysts and tubules. Cysts (e.g. the renal vesicle during development) are spherical monolayers of epithelial cells surrounding a lumen. Tubules, also lumen-containing monolayers, are cylindrical. Although differently shaped, cysts and tubules are topologically equivalent, as both have an apical surface facing the lumen, a lateral surface with cell-cell contacts, and a basal surface interacting with the basement membrane. Tubules, therefore, in many ways, are morphogen-induced cysts.

Determining the signaling pathways controlling cystogenesis and tubulogenesis is critical for understanding kidney formation and repair. Due to the transitory nature of these processes, it is difficult to study them in vivo.

Although widely used, we hypothesize that traditional two-dimensional (2D) in vitro cell culture models (e.g. cell monolayers on plastic) do not adequately replicate the process of tubulogenesis in vivo. Therefore, we used a 3D cell culture system, where cells are grown to the cyst stage in collagen, and are then induced with HGF to undergo tubulogenesis.

**Methods:** Here, we sought to identify differentially regulated genes involved in early tubulogenesis, which might be missed with 2D studies, and to determine which of these genes are dependent on MAPK signaling. We used microarray analysis of 2D and 3D grown Madin-Darby canine kidney (MDCK) cells treated with HGF and MAPK inhibitors to analyze changes in gene expression during tubulogenesis, and confirmed these results with RT-PCR.

**Results:** We identified 1,857 "tubulogenes". Of these, 840 genes were identified as being MAPK-dependent.

**Conclusions:** Our results show that expression of putative tubulogenes, identified in previous 2D studies, do not always change during tubulogenesis in 3D culture. In contrast, some of the genes we identified as tubulogenes in this study, such as MMP1, were not identified in 2D studies. Supporting our findings we determined that shRNAmediated knockdown of MMP1 inhibited cyst and tubule formation in MDCK cells cultured in a 3D matrix.

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support

## TH-PO903

**Tuberous Sclerosis in Children: Its Variable Renal Presentation and Oucome** Isabel Roberti, Shefali Vyas. *Children's Kidney Center, Saint Barnabas Medical Center, Livingston, NJ.*

**Background:** Tuberous sclerosis (TS) is a multisystem disorder encompassing a wide spectrum of renal lesions. Renal involvement is often asymptomatic but renal failure can affect 1% of adults with TS. A variable number of renal cysts and/or angiomyolipomas have been reported in children with TS but renal failure and renal CA are not expected.

**Methods:** This is a retrospective analysis of children with TS referred to our Pediatric Nephrology Center in the past 15 years. Demographic info, clinical data, therapy and outcome were reviewed.

**Results:** We identified **22 children**, aged 1 day to 14 yrs (median= 5 yrs), half were males, 13 C, 5 AA, 5H. **Reason for referral**: abnormal renal sonogram or MRI= 21 (3 pre-natal, 2 with enlarged abdomen with palpable kidneys, 16 routine), CKD = 1. **FH** + in 9 (41%). **PMH** + for cardiac rabdomyomas. **GFR @ presentation**: ESRD=1, Stage 2 CKD =1, Stage 3 CKD =2; **HTN** =4 (18%); **Proteinuria** =1, **MA** =1. All had **CNS involvement** by brain MRI; 17 (77%) had seizures and developmental delay (varied from mild to autism). **Skin lesions** were seen in 15 (68%). **Renal sonogram @ presentation**: few cysts=4, bilateral cysts (some complex)= 7, angiomyolipomas= 11, hamartomas =2, increased echogenicity= 3, enlarged kidneys (>15 cm)= 2. **Follow-up time** (N=18): 5 ms to 10yrs (median=3 yrs). **Renal outcome**: **3 ESRD** (**1 on PD since birth** died at 9 mos with aortic aneurysm unable to have a txp, **2 txp**: 1 s/p renal CA and nephrectomies died due to seizures and 1 with C1QN with no renal TS), **1 stage 3 CKD** and **14 (77%) stage 1 CKD. Renal sono f/u (16):** 5 stable sonogram; 10 with worse #/size lesions, 1 with less lesions on everolimus. **Meds**: 14 on anti-seizure, 3 on everolimus (2 due to CNS astrocytomas, 1 due to complex renal masses + CKD), 5 on enalapril. Five had partial brain lobectomies.

**Conclusions:** We report 22 children with TS with various degrees of renal and CNS involvement, including an infant with ESRD. Our incidence of CKD/ESRD was higher than expected at 18%. Renal lesions typically progressed with time. Everolimus significantly ameliorated the renal dysfunction in 1 child. Further studies detailing the long-term f/u of such children and the possible benefit of everolimus for ameliorating renal disease are needed.

**TH-PO904**

**Aberrant Glycosylation and Localization of Polycystin-1 Cause Polycystic Kidney in AQP11-Knockout Mice** Yuichi Inoue,[1] Eisei Sohara,[1] Katsuki Kobayashi,[2] Tatemitsu Rai,[1] Kenichi Ishibashi,[3] Shigeo Horie,[4] Xuefeng Su,[5] Jing Zhou,[5] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Tokyo Medical and Dental Univ; [2]Chiba East National Hospital; [3]Meiji Pharmaceutical Univ; [4]Juntendo Univ; [5]Brigham and Women's Hospital.*

**Background:** We previously reported that the disruption of aquaporin-11 (AQP11) gene in mice resulted in cyst formation in the kidney. However, the mechanism of cyst formation in the AQP11(-/-) mouse is still unknown.

**Methods:** To investigate the mechanism, we analyzed the AQP11(-/-) mouse and AQP11 BAC transgenic ($Tg^{AQP11}$) mouse that expresses 3xHA–tagged AQP11, by focusing on the polycystic kidney disease-related gene products.

**Results:** Immunofluorescence of the kidney from $Tg^{AQP11}$ mice revealed that 3xHA-AQP11 was localized in the cytoplasm of proximal tubule cells. Double immunofluorescence with organelle markers and immunoblots of isolated ER fraction of the kidney of $Tg^{AQP11}$ mice revealed that 3xHA-AQP11 localizes to ER in vivo. Since ER is essential for quality control and trafficking of newly synthesized proteins, we hypothesized that the absence of AQP11 in ER could result in impaired quality control and aberrant trafficking of polycystins, responsible proteins for autosomal dominant polycystic kidney disease. We found an increase in protein expression level of PC-1 and a decrease in protein expression level of PC-2 in AQP11(-/-) mice kidney, compared with wild-type mice. We confirmed higher molecular weight of PC-1 in AQP11(-/-) mice kidney, caused by impaired N-glycosylation processing of PC-1. In addition, density gradient centrifugation of kidney homogenate revealed impaired membrane trafficking of PC-1 in AQP11(-/-) mouse. Finally, we demonstrated that the $Pkd1^{+/-}$ background results in increased severity of polycystic kidney disease in AQP11(-/-) mice kidneys.

**Conclusions:** Our data demonstrated that impaired glycosylation processing and aberrant membrane trafficking of PC-1 in AQP11(-/-) mouse could be a key mechanism of cyst formation in AQP11(-/-) mice.

*Funding:* Government Support - Non-U.S.

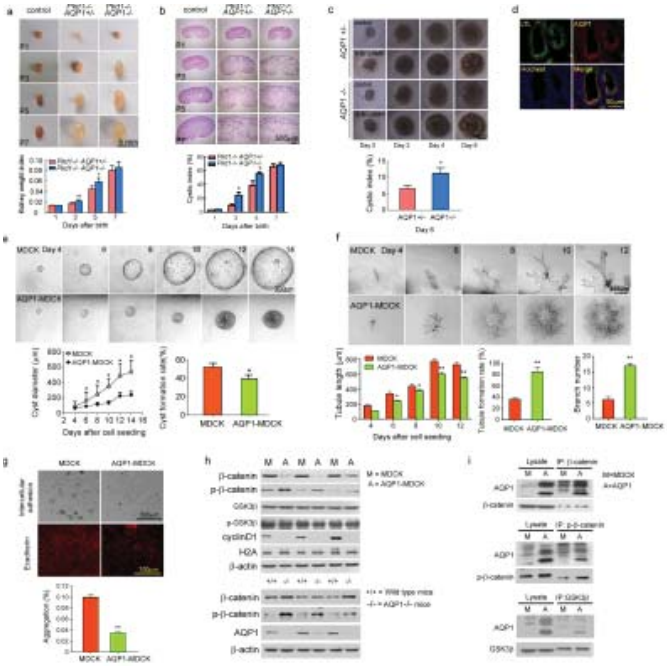
**TH-PO905**

**Aquaporin1 Inhibits Renal Cyst Formation and Enlargement by Down-Regulating Wnt Signaling** Weiling Wang, Baoxue Yang. *State Key Laboratory of Natural and Biomimetic Drugs, Dept of Pharmacology, Peking Univ, Beijing, China.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the progressive enlargement of cysts caused by mutations in *Pkd1* or *Pkd2*. Polycystin-1 encoded by *Pkd1* undergoes multiple cleavages that intervene in several signaling pathways involved in cellular proliferation and differentiation mechanisms.Aquaporin1 (AQP1) is a water channel protein, widely expressed in epithelial and endothelial cells. Recent research revealed AQP1 interacted with Lin7 to regulate cytoskeleton remodeling in melanoma cell. Our aim was to study the role of AQP1 in ADPKD development.

**Methods:** We analyzed kidney index and cyst index in both AQP1 null PKD mice and embryonic kidney cyst model.We evaluated β-catenin, p-β-catenin, CyclinD1, GSK3β and p-GSK3β expression by Western blot and immunofluorescence in AQP1 over-expression MDCK cell and MDCK cell as well as AQP1 null mice.

**Results:** With AQP1 null PKD mice, We found AQP1 deficiency significantly promoted renal cyst development. Similarly, 8-Br-cAMP induced cyst enlargement was promoted in AQP1 null embryonic kidney model.Over-expression of AQP1 inhibited cyst development and promoted tubulogenesis in MDCK tubule model. It was found that AQP1 impaired the extracellular adhesion and decreased E-cadherin. AQP1 down-regulated β-catenin expression and up-regulated β-catenin phosphorylation in AQP1-MDCK cells. Furthermore, CyclinD1 was hardly detected in AQP1-MDCK cells. The kidneys from AQP1 knockout mice showed high level of β-catenin.The level of p-GSK3β was lower in AQP1-MDCK cells. It was also found that AQP1 co-immunoprecipitated with β-catenin, p-β-catenin as well as GSK3β.



**Conclusions:** AQP1 may involve in maintaining the stability of the "degraded complex" to promote β-catenin phosphorylation and suppress the Wnt signaling pathway. We speculate that AQP1 might be a new target for ADPKD therapy.

*Funding:* Other NIH Support - National Natural Science Fouccation of China 81261160507 and 81170632

**TH-PO906**

**Implication of Inflammasome Activation via Mitochondrial Reactive Oxygen Species in the Development of Renal Interstitial Fibrosis Induced by Aldosterone** Hiroyuki Kadoya, Minoru Satoh, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

**Background:** The inflammasome plays an important role in a variety of diseases, including atherosclerosis and chronic kidney disease (CKD). The inflammasome is a cytoplasmic multiprotein complex that activates caspase-1, through interaction with ASC (Apoptosis-associated Speck-like Protein Containing a Caspase Recruitment Domain), and finally leads to the processing and secretion of the pro-inflammatory cytokines, such as IL-1β and IL-18. Aldosterone (Aldo) has been indicated to induce kidney damages through activation of pro-inflammatory signaling pathway. We hypothesized that Aldo induces renal damages via activation of inflammasome.

**Methods:** ASC-deficient mice (ASCKO) and control C57Bl/6 mice (WT) were used. All animals were received left uninephrectomy and given drinking water with 1% NaCl. The mice were divided into the following groups: WT-vehicle, WT-Aldo (Aldo, 0.25 mg/kg/day, osmotic pump), WT-Aldo treated with eplerenone (WT-Aldo+Eple; Eple, 100 mg/kg/day, gavage), and ASCKO-Aldo. Three weeks after drug administration, mice were sacrificed. We also examined the mitochondrial superoxide production by Aldo and its possible involvement in inflammasome activation in cultured mouse peritoneal macrophages.

**Results:** Tubulointerstitial damage and increased expressions of inflammasome components, NLRP-3 and ASC, were demonstrated in WT-Aldo. Increased Caspase-1 activity and concomitant overproduction of IL-1β and IL-18 were also demonstrated in the WT-Aldo. Eple treatment suppressed these changes. Tubulointerstitial injuries were significantly attenuated in the ASCKO-Aldo. Increased Caspase-1 activity and expressions of IL-1β and IL-18 were also attenuated in ASCKO-Aldo. Aldo induced mitochondrial superoxide production and resulted in inflammasome activation in cultured macrophages obtained from peritoneal cavity. This was completely blocked by eplerenone.

**Conclusions:** Our results indicate that Aldo induced interstitial fibrosis via activation of inflammasomes in infiltrated macrophages. Thus, inflammasome activation in macrophages could be a new therapeutic target for CKD.

**TH-PO907**

**Altered Excitability of Afferent Renal Innervation in an In-Vivo Model of Renal Inflammation** Wolfgang Freisinger,[1] Annalena Karl,[2] Nadja Tzinis,[2] Tilmann Ditting,[2] Sonja Heinlein,[2] Roland E. Schmieder,[2] Jens Lutz,[1] Roland Veelken.[2] *[1]Nephrology, Med. Clinic, Universitätsmedizin Mainz, Mainz, Germany; [2]Nephrology and Hypertension, Med. Clinic 4, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany.*

**Background:** Renal innervation is most likely important in renal inflammatory disease. Recently, we found that afferent renal neurons show a distinctive excitability, exhibiting predominantly a sustained firing upon current injection that depends on specific sodium

channel expression. So far, excitability of these neurons under inflammatory conditions in vivo is unclear. Hence we wanted to test the hypothesis that in a model of renal inflammation, Thy1.1 nephritis, the specific firing pattern of renal afferent neurons is altered.

**Methods:** Thy1.1 nephritis was induced (OX7, 1.2mg/kg) in Sprague Dawley rats 7 days before harvesting neurons, controls received vehicle. Nephritis was confirmed by proteinuria and histologically.Labelling (DiI) identified renal afferent neurons situated in dorsal root ganglia (DRG). Current clamp was used to characterize neurons as "tonic", i.e. sustained action potential (AP) firing or "phasic", i.e. <5 APs. AP properties were determined in renal and non-renal neurons of nephritic rats and controls.

**Results:** Nephritic rats had all proteinuria and displayed histologically inflammation. Untreated renal afferent DRG neurons (n=58) exhibited in 64% a tonic firing pattern. In Thy1.1 nephritic rats, renal DRG neurons (n=112) exhibited significantly less a tonic firing pattern (43% vs. 64%, *$p<0.05$). Whereas the characteristic "tonic" vs. "phasic" changed, neither electrophysiological parameters (resistance, cell capacity, membrane potential) nor action potential morphology was altered.

**Conclusions:** We could show that in an in vivo model of renal inflammation, renal afferent neurons exhibited a significantly decreased excitability upon electric stimulation. An altered expression of sodium channel subtypes might lead to a faster inactivation of these channels and decreased firing activity. In consequence, altered afferent renal activity in vivo could lead to sympathetic dysregulation aggravating renal inflammation.

## TH-PO908

**Nlrp3 Inflammasome Activation Promotes Renal Tubulointerstitial Inflammation in Albumin-Overload Nephropathy** Dan Liu,[1] Bi-Cheng Liu.[1] *[1]Institute of Nephrology, Southeast Univ, Nanjing, China; [2]Institute of Nephrology, Southeast Univ; [3]Institute of Nephrology, Southeast Univ.*

**Background:** Nlrp3 inflammasome are implicated in recognizing certain non-microbial origin 'danger-signals'. This study aimed to investigate whether overload albumin induce Nlrp3 inflammasome activation and contribute to tubular cell stress and tubulointerstitial inflammation.

**Methods:** Albumin-overload nephropathy was induced in adult male Wistar rats that were uninephrectomized or sham operated 5 days before starting bovine serum albumin injection. Rats were given daily intraperitoneal injections of 5g/kg/d BSA (n=10) or sham sterile normal saline (UXN: n=6, Sham: n=6) injections for 9 weeks. Blood samples were taken on weeks 0, 2, 5, 7, 9 after collection of a 24-h urine. At the end of the study, rats were anesthetized and tissue were kept for subsequent analysis. Nlrp3 inflammasome and inflammatory cytokines were examined by real time RT-PCR and Western blotting.

**Results:** Urinary protein excretion assay shows that proteinuria were increased in albumin-overload rats compared to saline-injected controls ($p<0.05$). Tubular injury was confirmed by ELISA examination of NGAL, which increased in albumin-overload rats than controls ($p<0.05$). Immunofluorescence showed Nlrp3 activation in proximal tubular cells. Albumin triggered the upregulation of Nlrp3 inflammasome, which subsequently lead to IL-1β and IL-18 secretion (Nlrp3: $p<0.05$, IL-1β: $p<0.05$, IL-18: $p<0.01$). Real time RT-PCR indicated Nlrp3 mRNA expression increased of renal cortex. Meanwhile, proximal tubular epithelial cells show cell atrophy observed by PAS staining and mitochondrial dysfuntion measured by mitochondrial membrane potential reduction by JC-1 dye after albumin overload ($p<0.01$). Immunohistochemical staining shows that inflammatory cell infiltration in tubulointerstitium was significantly greater in BSA injection group than saline group.

**Conclusions:** Our study demonstrated that albumin caused the tubular injury and tubulointerstitial inflammation through the mitochondrial dysfunction that activate Nlrp3 inflammasome with subsequent secretion of inflammatory cytokines in albumin-overload nephropathy.

## TH-PO909

**Human Leukocyte Antigen-G Attenuates Renal Inflammation in a Mouse Model of Lupus Nephritis** Onkar Kulkarni, Shrikant R. Mulay, Murthy Narayana Darisipudi, Santhosh Kumar VR, Hans J. Anders. *Medizinische Klinik und Poliklinik IV, Univ of Munich, Munich, Bavaria, Germany.*

**Background:** Human leukocyte antigen (HLA-G) interacts with PIR-B expressed on mouse leukocytes. Administration of microbeads coated with HLA-G5-B2m has been shown to induce tolerance of DCs and inhibit DC maturation in transplantation mouse models. PIR-B is the negative regulator of TLR-9 in B1 cells which are primarily responsible for rheumatoid-factor (RF) release. B1 cells depletion attenuate the progression of lupus nephritis in MRL-lpr mice. PIR-B knockout mice have exaggerated release of RF, anti-DNA antibodies and reduced survival. We hypothesized that administration of HLA-G5-B2M absorbed on microbeads will improve renal inflammation by inhibiting DCs maturation and B1 cells activation in MRL-lpr mice.

**Methods:** We injected microbeads coated with HLA-G-B2m (2microgram/week/mouse) for 10 wks starting from week 12 of age. Plasma, urine, tissue samples were collected at the end of the treatment period. Kidney tissues were processed for histological analysis. Various lymphocyte subsets were analyzed by FACS from spleen and kidney. Part of isolated tissue was analyzed for expression analysis by RT-PCR.

**Results:** HLA-G treatment attenuated lupus nephritis, determined by the activity and chronicity index of histomorphological damage. HLA-G treatment improved renal function as observed by reduced BUN levels in the plasma. HLA-G treatment reduced the intra-renal accumulation of CXCR3 positive T cells. IL17- and IFN-gamma-producing T cells were also reduced in the kidney significantly in the HLA-G treated group. Infiltration and activation of dendritic cells and macrophages as well as expression of pro-inflammatory mediators in the kidney was significantly reduced by HLA-G treatment. Plasma levels of RF, IL12p40, TNF-alpha and IL17 were significantly reduced by the treatment. HLA-G treatment did not have any significant effect on the leukocytes subsets in the spleen and autoantibody production.

**Conclusions:** HLA-G5 treatment significantly suppresses lupus nephritis of MRL-lpr mice, mainly by reducing systemic and intra-renal inflammation.

## TH-PO910

**Renal Cytokine Expression during Sepsis Is Repressed in Conditional NFAT5-Knockout Mice** Christoph Kueper,[1] Wolfgang Neuhofer.[1,2] *[1]Dept of Cellular Physiology, Univ of Munich; [2]Dept of Nephrology, Univ of Munich.*

**Background:** The osmosensitive transcription factor NFAT5 (also known as TonEBP) regulates the expression of osmoprotective genes (AR, HSP70) and urinary concentrating genes (AQP-2, UT-A, ClC-K1) in the kidney. Besides, NFAT5 is also involved in the expression of cytokines in immune cells during LPS stimulation. The aim of the present study was to evaluate, if NFAT5 activation is also involved in cytokine expression in renal cells in response to LPS. For this purpose, we generated conditional NFAT5 knockout mice, in which NFAT5 can be deleted in mature animals.

**Methods:** In the genome of the conditional knockout mice, exon 4 of the NFAT5 gene is flanked by two loxP sites; additionally, the animals express the Cre recombinase under control of a tamoxifen-inducible promoter. At the age of 6 weeks, male conditional knockout mice were treated with tamoxifen to induce Cre-mediated NFAT5 knockdown. Knockdown efficiency and expression of defined renal NFAT5 target genes (AR, AQP-2, UT-A, ClC-K1) was measured by qRT-PCR and immunoblotting. In additional experiments the NFAT5-knockout mice or wildtype-mice were treated with LPS (5 mg/kg bw) for 24 h. Expression of various cytokines (CCL-2, CCL-5, TNF-a, IL-6) was measured by qRT-PCR or ELISA.

**Results:** Expression of renal NFAT5 in tamoxifen-treated animals was diminished to approximately 10% compared to control animals. In accordance, expression of NFAT5 target genes AR, AQP-2 and ClC-K1 was also significantly decreased. In a LPS/sepsis model, expression of cytokines CCL-2, CCL-5, TNF-a and IL-6, both in the kidney and in the spleen of NFAT5 knockout animals was significantly reduced compared to control animals.

**Conclusions:** We have developed a mouse model, in which the expression of NFAT5 can be efficiently repressed in mature animals. This mouse model may turn out to be very helpful for future studies investigating NFAT5 function in vivo. First results with this model demonstrate that several cytokines are downregulated in the kidney of NFAT5-knockout animals during LPS treatment, indicating that NFAT5 is an important factor of the inflammatory process in the kidney.

## TH-PO911

**Characterization of Reparative Macrophages in the Kidney Using a Novel IL10 Reporter Mouse: F4/80$^{hi}$ CD11b$^{+}$ Macrophages as a Major Source of IL10 in the Repair Phase of Kidney Inflammation** Julia Lichtnekert, Takahisa Kawakami, Bryce Gordon Johnson, Jeremy Stuart Duffield. *Div of Nephrology, Univ of Washington, Seattle, WA.*

**Background:** IL-10 is a potent anti-inflammatory cytokine that plays an important role in the regulation of immune responses, thereby preventing damage to the host. In various acute and chronic models of kidney diseases, IL-10 administration protected against renal injury. To study the source and characteristics of IL10-producing cells in the kidney in steady state and injury/repair, we analyzed IL-10 production *in vivo* using a novel IL-10-beta lactamase reporter mouse (ITIB).

**Methods:** Single cells from ITIB mouse kidney after systemic LPS administration, bilateral renal ischemia-reperfusion (IRI) and unilateral ureteral obstruction (UUO) were analyzed by flow cytometry analysis.

**Results:** IIL-10 is expressed by 3 distinct mononuclear phagocyte (MPC) populations: CD11b$^{hi}$CD11b$^{low}$, CD11b$^{hi}$CD11c$^{lo}$ and CD11b$^{int}$CD11c$^{int}$. Analyzing IL-10 producing cells using ITIB mice, most MPCs do not synthesize IL-10 in steady state, but a proportion of resident CD11b$^{int}$CD11c$^{int}$F4/80$^{hi}$ MPCs generate IL-10 in healthy kidneys, suggesting they may have a unique role in preventing inflammatory responses. Next, we investigated IL-10 up-regulation in the kidney after systemic LPS administration. After 48h, CD11b$^{+}$Ly6G$^{+}$ neutrophils and 40% of MPCs showed IL-10 production. Analysis of subpopulations indicated that CD11b$^{int}$CD1c$^{int}$F4/80$^{hi}$ and CD11b$^{hi}$CD11c$^{low}$ MPCs are major sources of IL-10. Furthermore, we investigated IL-10 expression after UUO and bilateral IRI. At day 4 after UUO, significantly increased levels of IL-10 producing cells could be detected, which were CD11b$^{int}$CD11c$^{int}$ and CD11b$^{hi}$CD11c$^{hi}$ MPCs, whereas at day 7 CD11b$^{hi}$CD11c$^{low}$ and CD11b$^{hi}$CD11c$^{hi}$ expressed IL-10. Interestingly, these latter subpopulations were positive for F4/80. After bilateral IRI, the major sources of IL-10 were CD11b$^{hi}$CD11c$^{low}$ and CD11b$^{hi}$CD11c$^{hi}$ MCP subpopulations at day 4 and 7 of which the majority was F4/80 positive.

**Conclusions:** These studies demonstrate for the first time that IL-10 is produced *in vivo* in renal reparative macrophages in kidney inflammation and injury.

*Funding:* Government Support - Non-U.S.

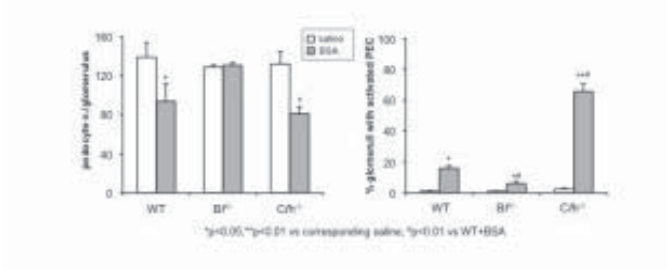
**TH-PO912**

**Alternative Pathway (AP) of Complement (C) Activation Triggers Glomerular Podocyte and Parietal Epithelial Cell (PEC) Dysregulation in Proteinuric Nephropathy** Marina Morigi,[1] Monica Locatelli,[1] Daniela Corna,[1] Simona Buelli,[1] Mauro Abbate,[1] Marina Noris,[1] Ariela Benigni,[1] Giuseppe Remuzzi,[1,2] Carlamaria Zoja.[1] *[1]IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; [2]Unit of Nephrology and Dialysis, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy.*

**Background:** We previously showed that protein overload led to glomerular injury, accumulation of C3 in podocytes and podocyte damage promoting C-dependent dysfunction of glomerular filtration barrier, which accelerated progressive renal injury. Here we sought to evaluate: 1) which of the C pathways were involved in glomerular C3 deposition and podocyte loss; 2) whether C-dependent glomerular injury was related to signs of PEC activation. To this aim,protein overload-proteinuria was studied in mice deficient for the cofactor of the AP-C3 convertase factor B or for the AP inhibitor factor H.

**Methods:** Wild type (WT), *Bf*$^{-/-}$ and *Cfh*$^{-/-}$ mice underwent uninephrectomy and 5d later received daily i.p. injections of saline or BSA up to 28d. Podocyte number was assessed as WT1+cells. Activated PEC were defined as NCAM+/claudin+ cells forming adhesion between capsule and tuft, and glomerulosclerosis (GS).

**Results:**




WT-BSA mice showed glomerular C3 deposits associated with podocyte loss (32%) and PEC activation (16% glomeruli), which were prevented by factor B deficiency (Fig.), indirectly showing that AP of C was involved in both phenomena. Additional evidence of AP involvement was provided by *Cfh*$^{-/-}$-BSA mice that instead had more proteinuria, greater C3 deposits (score, 4±0 vs 2±0.1, p<0.01), more podocyte loss (39%), more severe PEC alterations (66% glomeruli) and higher % of glomeruli with GS (20±3% vs 9±3%, p<0.05) than WT-BSA mice.

**Conclusions:** Inhibiting AP of C attenuates glomerular injury in proteinuric mice, while its uncontrolled activation leads to more PEC-related glomerular sclerotic lesions.

*Funding:* Private Foundation Support

**TH-PO913**

**Interleukin 17-Receptor A on Leukocytes and Tissue Cells Mediates Inflammation in a Murine Model of Crescentic Glomerulonephritis** Joanna R. Ghali,[1,2] Stephen R. Holdsworth,[1,2] A. Richard Kitching.[1,2] *[1]Centre for Inflammatory Diseases and Dept of Medicine, Monash Univ, Clayton, Victoria, Australia; [2]Dept of Nephrology, Monash Health, Clayton, Victoria, Australia.*

**Background:** Interleukin (IL)-17A and IL-17F are inflammatory cytokines which signal through IL-17 Receptor A (IL-17RA), expressed on many cell types, including renal tissue cells (TC). We aimed to explore the role of IL-17RA in experimental glomerulonephritis (GN).

**Methods:** Necrotising, crescentic GN was induced by intravenous administration of sheep anti-mouse glomerular basement membrane globulin, thereby planting sheep globulin (SG) in glomeruli. Mice were culled at day 21. Wild type C57BL/6 (WT), IL-17RA-/- and bone marrow (BM) chimeric mice were used.

**Results:** IL-17RA-/- mice had reduced crescent formation (WT 17±3 vs IL-17RA-/- 9±3%; P<0.05), fewer glomerular neutrophils (1.2±0.1 vs 0.76±0.0 cells/glomerular cross section [c/gcs]; P<0.05), and macrophages (2.0±0.2 vs 1.1±0.2c/gcs; P<0.05). IL-17RA-/- mice had lower circulating anti-SG antibodies than WT mice (IgG $OD_{450}$ 1:100; 0.34±0.06 vs 0.17±0.02; P<0.05). BM chimeric mice (chimerism 96%) were generated, permitting assessment of selective IL-17RA deficiency in BM or TC. Compared to BM+TC+ mice, BM-TC+ mice had reduced glomerular segmental necrosis (BM+TC+ 49±5 vs BM-TC+ 27±5%; P<0.05) and urinary protein:creatinine ratios (BM+TC+ 2.0±0.3 vs BM-TC+ 1.1±0.3mg/mmol; P<0.05), but BM+TC- mice did not. Mice with BM or TC IL-17RA deficiency had impaired cellular immunity (ELISPOT: BM+TC+ 91±19 vs BM-TC+ 41±6 IFNγ+ spots/2x10$^6$ SG-stimulated splenocytes; BM+TC+ 91±19 vs BM+TC- 43±8; both P<0.05). Glomerular neutrophil recruitment did not differ between BM+TC+ and BM-TC+ or BM+TC- mice, but differed between chimeric groups (BM-TC+ 0.6±0.1 vs BM+TC- 1.1±0.1c/gcs; P<0.05). No difference in glomerular macrophage or T cell recruitment was found. Compared to BM+TC+ mice, BM+TC- mice had impaired humoral immunity (IgG $OD_{450}$ 1:100: BM+TC+ 0.58±0.10 vs BM+TC- 0.18±0.03; P<0.05).

**Conclusions:** IL-17A/F signalling promotes glomerular injury. Leukocyte-derived IL-17RA promotes cellular immunity and injury, while IL-17RA on radio-resistant cells enhances antigen-specific cellular and humoral immunity.

*Funding:* Government Support - Non-U.S.

**TH-PO914**

**FMS-Like Tyrosine Kinase 3 Ligand Does Not Protect Mice from Experimental Glomerulonephritis, Despite Inducing Regulatory T Cells** Joanna R. Ghali,[1,2] Stephen R. Holdsworth,[1,2] A. Richard Kitching.[1,2] *[1]Centre for Inflammatory Diseases and Dept of Medicine, Monash Univ, Clayton, Victoria, Australia; [2]Dept of Nephrology, Monash Health, Clayton, Victoria, Australia.*

**Background:** FMS-like tyrosine kinase 3 ligand (FLT3-L) is a growth factor that can expand regulatory T cells (Tregs) and plasmacytoid dendritic cells (pDC). We hypothesised that FLT3-L-induced Tregs would protect mice from experimental glomerulonephritis (GN).

**Methods:** Naive C57BL/6 mice were injected with FLT3-L 10mg or PBS intraperitoneally daily for 10 days. To determine whether FLT3-L altered immune responses to foreign antigen, FLT3-L/PBS was injected for 10 days then mice were primed with sheep globulin (SG) and culled 4 days later. To induce GN, mice were primed with SG, given intravenous sheep anti-mouse glomerular basement membrane (GBM) globulin at day 4 (planting SG in glomeruli), and culled at day 14.

**Results:** Compared to PBS, FLT3-L-treated mice had increased splenic Treg proportions (CD4+Foxp3+/CD4+: FLT3-L 21.4±1.9 vs PBS 11.1±0.8%; P<0.05). Four days after SG priming, FLT3-L-treated mice had higher proportions of lymph node pDC (PDCA-1+/CD11c+: 10.0±1.6 vs 5.0±0.9%; P<0.05) and enhanced SG-specific dermal delayed type hypersensitivity (0.31±0.08 vs 0.09±0.02mm; P<0.05), without an increase in Tregs, suggesting that ceasing FLT3-L therapy enhanced cellular immunity. Mice were given FLT3-L/PBS for 10 days prior to inducing GN. FLT3-L-treated mice were not protected from renal injury (segmental necrosis: 48.3±15.3 vs 46.0±13.2%; P=0.91, crescents: 7.0±3.4 vs 4.0±2.0%; P=0.48). When FLT3-L/PBS was administered daily throughout the GN model, FLT3-L-treated mice developed substantial mortality compared to controls (87.5% vs 12.5%; P<0.05) at day 13. Five days after anti-GBM globulin, FLT3-L-treated mice had enhanced cellular immunity (IL-17A ELISPOT: 12.8±1.8 vs 6.4±0.8 spots/1x10$^6$ SG-stimulated splenocytes; P<0.05), more activated T cells (CD4+CD25+/CD4+: 15.4±0.8 vs 12.9±0.5%; P<0.05) and conventional DC in the spleen (PDCA-1-/CD11c+: 93.4±0.5 vs 83.5±2.3%; P<0.05).

**Conclusions:** FLT3-L therapy enhanced systemic T cells responses, demonstrating that attempts to enhance protective immunity in GN via DC manipulation may be harmful.

*Funding:* Government Support - Non-U.S.

**TH-PO915**

**PKC-Theta Modulates Activation of Murine Primary Macrophages via Regulation of Proinflammatory Cytokine Expression** Anna Bertram, Torsten Kirsch, Hermann G. Haller, Nelli Shushakova. *Dept of Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** Systemic inflammation is a hallmark of sepsis, which is afflicted with a high mortality. A better understanding of underlying mechanisms might lead to new therapeutic targets in inflammatory diseases. Macrophage activation and subsequent release of proinflammatory mediators are critical steps in inflammation. We investigated the role of PKC-theta in the activation of murine primary macrophages.

**Methods:** Peritoneal macrophages were obtained by peritoneal lavage of SV129 wild type and PKC-theta gene-deficient mice and stimulated with LPS. Expression of PKC-theta and cytokines was quantified using RT-PCR, and release of cytokines was measured with ELISA. Confocal microscopy was used to visualize nuclear translocation of NFkB. Peritonitis was induced by intraperitoneal injection of 3% thioglycollate in wild type and PKC-theta gene-deficient mice.

**Results:** PKC-theta is expressed in peritoneal macrophages of SV129 wild type mice. Stimulation of isolated PKC-theta gene-deficient peritoneal macrophages with 100 ng/ml LPS resulted in a significantly impaired secretion of TNF-alpha and MIP-2 after 2, 3 or 4 h in comparison to wild type peritoneal macrophages (44% for TNF-alpha, 34.2% for MIP-2 after 4 h). Consistently, mRNA levels of TNF-alpha and MIP-2, which show a peak 1 h after LPS stimulation, were markedly decreased in PKC-theta gene-deficient macrophages (55.2% for TNF-alpha, 49.8% for MIP-2). However, nuclear translocation of NFkB was not defective, but rather accelerated in PKC-theta gene-deficient macrophages, suggesting a downstream target of PKC-theta. These *in vitro* findings correlated with decreased neutrophil accumulation in a model of experimental peritonitis in PKC-theta gene-deficient vs. wild type mice (41.4%).

**Conclusions:** We conclude that PKC-theta modulates the activation of murine primary macrophages by regulating the expression of proinflammatory cytokines. Further experiments are required to pinpoint the molecular site of action of PKC-theta. Inhibition of PKC-theta may be a novel therapeutic target to modulate the inflammatory response in sepsis and other inflammatory disorders.

**TH-PO916**

**Effection of CagA Protein on the Production and Underglycosylation of IgA1 in DAKIKI Cells** Junming Fan,[1,2] Fugang Li,[2] Li Liu,[2] Man Yang,[2] Zi Li,[1] Wei Qin.[1] *[1]Dept of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China; [2]Div of Nephrology, Dept of Internal Chinese Medicine, Luzhou Medical College, Luzhou, Sichuan, China.*

**Background:** IgA nephropathy (IgAN) is the most common form of glomerulonephrits in the world. The infection rate of Helicobacter pylori (Hp) is very high in China and the Asia Pacific region.Hp infection is associated with IgAN, but the underlying molecular mechanism remains unclear. This study was to investigate the effects of cytotoxin associated gene A protein (CagA), a major virulence factor of Hp, on the production and underglycosylation of IgA1 in DAKIKI, a B cell line.

**Methods:** DAKIKI, was cultured and incubated by recombinant CagA protein. The cells were cultured for 48h with different CagA dose (0.4 ug/ml, 0.8 ug/ml, 1.6 ug/ml, 3.2 ug/ml) in dose-dependent test, and for 24, 48 and 72hs in time-dependent test. The cell proliferation was examined by CCK-8 assay and cell counting. The production and glycosylation of IgA1 in supernatants were determined by ELISA and helix aspersa (HAA) lectin binding assay. For the mechanism of O-glycosylation , cells were incubated with 1.6 ug/ml CagA, lipopolysaccharide (LPS) or Bovine serum albumin(BSA) for 48h, and the protein expression of C1GALT1 and Cosmc were measured by Western blot.

**Results:** For dose-dependent test, while the dose within the range of 0.8~1.6 ug/ml, CagA stimulated cell proliferation. However, 3.2 ug/ml of CagA inhibited cell proliferation. Moreover,CagA promoted the production and underglycosylation of IgA1 in a dose-dependent manner. For time-dependent test, 1.6 ug/ml of CagA stimulated the production and underglycosylation of IgA1 in a time-dependent manner. The protein expression of C1GALT1 and Cosmc is significantly lower in cells stilimulated by CagA and LPS than control or BSA.

**Conclusions:** CagA promoted the B cell proliferation and IgA1 secretion in vitro. CagA protein also induced underglycosylation of IgA1,and which might be resulted from a downregulation of C1GALT1 and Cosmc expression.These results suggested that CagA might play an important role in the pathogenesis of IgAN, and that anti-Hp treatment may be a new target for prevention of IgAN in clinic.

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## TH-PO917

**Transfer and Substrate Specificity of Neutrophil Serine Proteases towards Endothelial Cells** Uwe Jerke,[1] Daniel Perez Hernandez,[2] Brice Korkmaz,[3] Gunnar Dittmar,[2] Ralph Kettritz.[1,4] *[1]Experimental and Clinical Research Center, a Joint Cooperation between the Charité and the Max-Delbrück Center for Molecular Medicine (MDC); [2]MDC; [3]3INSERM U-1100/EA-6305 Univ Francois Rabelais; [4]Nephrology and Intensive Care Medicine, Campus Virchow.*

**Background:** Active neutrophil serine proteases (NSPs) are released from neutrophils during inflammation and participate in vascular diseases, including ANCA vasculitis. NSPs consist of proteinase 3 (PR3), human neutrophil elastase (HNE) and cathepsin G (CG). We hypothesized that enzymatically active NSPs are acquired by endothelial cells (EC) and exert common, but also distinct cleavage patterns towards EC substrates.

**Methods:** We used western blotting, flow cytometry, confocal microscopy and Boc-Ala-Pro-Val substrate assay to monitor NSP transfer from neutrophils to EC and terminal-amine isotopic labeling of substrates (TAILS) for substrate characterization.

**Results:** Activated neutrophils released enzymatically active NSPs into the supernatant and this activity was transferred to EC. EC acquired Alexa488-labeled purified NSPs in a time- and concentration-dependent mannor by flow cytometry and confocal microscopy. Importantly, PR3 showed diffuse cytoplasmic staining whereas HNE and CG localized to granular compartments. Approximately 3,000 potential substrates for NSPs were suggested using peptide model libraries. Employing TAILS, active NSPs, and endothelial cell lines we identified native substrates: 93 for PR3, 48 for HNE and 65 for CG. Clustering the substrate sequences showed characteristic extended recognition patterns. PR3 cleaved after valine and isoleucine with an unpolar amino acid (aa) cluster upstream and an acidic cluster downstream of the cleavage site. HNE cleaved after isoleucine or valine with acidic aa upstream and hydrophobic aa downstream, and CG showed a leucine or phenylalanine residue at the cleavage site with downstream aliphatic amino acids.

**Conclusions:** Thus, our data indicate distinct specificities and cellular localization of the three NSPs suggesting distinct biological effects towards EC. The findings could be of therapeutic relevance in inflammatory vascular diseases.

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## TH-PO918

**Different Functions of Soluble and Membrane-Bound TNF in Nephrotoxic Serum Nephritis** Martin B. Müller, John M. Hoppe, Andrei Bideak, Nuru Eltrich, Volker Vielhauer. *Nephrologisches Zentrum, Medizinische Klinik und Poliklinik IV, Ludwig-Maximilians-Univ, Munich, Germany.*

**Background:** TNF is a proinflammatory cytokine mediating inflammatory renal diseases such as immune complex glomerulonephritis. The two receptors TNFR1 and TNFR2 play distinct roles in this process, with TNFR2 strongly required for induction of disease. TNFR2 is predominantly activated by membrane-bound TNF (memTNF), but not soluble TNF (solTNF). Thus, we examined the functional role specifically of memTNF in heterologous nephrotoxic serum nephritis (NTN).

**Methods:** NTN was induced by i.v. injection of anti-GBM sheep serum into C57BL/6 wild-type and memTNF knock-in mice with two uncleavable Δ1-9, K11E TNF alleles, which express memTNF, but no solTNF (memTNF mice). At day 5 functional renal parameters, renal histology, leukocyte infiltrates and cytokine production were analysed in the two groups. In addition, we performed stimulation experiments in vitro with glomeruli isolated from wild-type and memTNF mice.

**Results:** NTN induced in memTNF mice was exacerbated compared to wild-type, indicated by increased albuminuria, higher serum levels of urea and more severe glomerulosclerosis and tubular cell injury. Surprisingly, this was accompanied by significantly lower renal leukocyte infiltrates in memTNF mice and correlated with reduced renal mRNA expression of inflammatory chemokines and cytokines. Decreased cytokine production was also present in isolated mTNF glomeruli stimulated with LPS or IL-1β, demonstrating that absent solTNF in the presence of intact mTNF decreases production of proinflammatory mediators in intrinsic glomerular cells.

Furthermore, wild-type mice treated with the TNF antagonist etanercept during NTN exhibited decreased renal leukocyte infiltrates comparable to untreated and treated memTNF mice. This identifies solTNF as a major mediator of renal leukocyte recruitment.

**Conclusions:** In summary, we demonstrate that soluble TNF predominantly mediates renal leukocyte recruitment, whereas uncleaved memTNF is an important inflammatory mediator of renal tissue damage in murine glomerulonephritis. These findings underline the importance of memTNF in inflammatory kidney disease as a possible therapeutic target.

*Funding:* Government Support - Non-U.S.

## TH-PO919

**Kinin B1 Receptor Deficiency Abrogates Induction of Anti-Myeloperoxidase Induced Crescentic Glomerulonephritis in Mice** Peiqi Hu, Hong Xiao, Masao Kakoki, Ronald J. Falk, J. Charles Jennette. *Pathology and Laboratory Medicine, Univ of North Carolina, Chapel Hill, NC.*

**Background:** Anti-myeloperoxidase (anti-MPO) IgG causes crescentic glomerulonephritis (CGN) in mice that is a model for human CGN caused by ANCA (anti-neutrophil cytoplasmic autoantibodies). Neutrophils play a critical role in CGN induction in this model. The B1 and B2 receptors, two G-protein-coupled kinin receptors, are expressed on neutrophils and involved in modulation of neutrophil functions including neutrophil migration, activation and apoptosis. In this study, we investigated the role of these kinin receptors in anti-MPO induced CGN.

**Methods:** Anti-MPO IgG was purified from the sera of MPO-/- mice that had been immunized with murine MPO. Mice with knock out B1 receptor (B1 KO) and knock out of both B1 and B2 receptors (B1/B2 KO), and normal wild-type C57BL/6j mice (WT B6) were injected i.v. with 50ug/g body weight anti-MPO IgG. Circulating anti-MPO IgG was monitored by ELISA. Proteinuria, hematuria and leukocyturia were monitored, and mice were sacrificed at day 6 and kidney tissue obtained for pathologic examination.

**Results:** At day 6, all 3 groups of mice that received anti-MPO IgG showed similar levels of circulating anti-MPO. All WT B6 mice developed hematuria and CGN (mean 12% glomeruli with crescents). In contrast, B1 KO mice and B1/B2 KO mice had normal urine and less CGN with mean 1% crescents in B1 KO mice (p=0.01 compared to WT) and 2% crescents in B1/B2 KO mice (p=0.01 compared to WT).

**Conclusions:** Absence of the B1 receptor ameliorates anti-MPO induced CGN. Slightly more severe CGN induction in B1/B2 KO mice than B1 KO mice suggests that B2 receptor may modulate the B1 effect, but additional studies are needed to clarify this. Kinin B1 receptors play an important role in the pathogenesis of CGN induced by anti-MPO antibodies, probably by recruitment and activation of neutrophils. Antagonists of kinin B1 receptors may have a therapeutic role in ANCA disease.

*Funding:* NIDDK Support

## TH-PO920

**19F-MRI for Noninvasive Visualization of Renal Inflammation in a Murine ANCA-Induced Glomerulonephritis Model** Adrian Schreiber,[1,2] Ralph Kettritz,[1,2] Min-Chi Ku,[3] Helmar Waiczies,[3] Sonia Waiczies,[3] Thoralf Niendorf,[3] Andreas Pohlmann.[3] *[1]Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine, Berlin, Germany; [2]Clinic for Nephrology and ICU, Medical Faculty of the Charité, Berlin, Germany; [3]Berlin Ultrahigh Field Facility (BUFF), Max Delbrück Center for Molecular Medicine, Berlin, Germany.*

**Background:** ANCA-activated neutrophils and monocyte cause necrotizing crescentic glomerulonephritis (NCGN). Renal inflammation is assessed in patients biopsies or in murine kidneys after animals were sacrificed. Non-invasive methods with potential for serial studies are needed. We tested the hypothesis that 19F-MRI visualizes renal inflammation in a murine ANCA-induced NCGN model.

**Methods:** 19F MRI.

**Results:** First we studied 19F labeling and tracking of neutrophils and monocytes in a murine thioglycolate peritonitis model. 19F-nanoparticles (100ul, 50mM, 153nm) were injected i.v., cells harvested from the peritoneal cavity (4h for neutrophils, 48h for monocytes), and the 19F-MR signal was assessed by MR spectroscopy. Monocytes were more efficiently labeled compared to neutrophils (neutrophils 2.7±0,2 AU vs. monocytes 25.8±3.2 AU). Next, a murine bone-marrow (BM) transplantation model of ANCA-induced NCGN was employed. 8 weeks after BMTx mice showed severe NCGN with 17.4±3.5% crescents and 9.9±1.8 necrosis (n=9). Immunohistochemistry demonstrated monocyte (0.35±0.23 cells/glomerulus) and neutrophil infiltration (0.10±0.05 cells/glomerulus). 9.4T MR revealed a renal 19F-signal in all animals of the experimental group, a much weaker signal in the control BMTx group and no signal in unchallenged mice. Ex vivo MR-analysis indicated a strong cortical signal pattern. Clinical application requires efficient 19F-nanoparticle uptake by human myeloid cells. Phagocytosis of 19F-DiL-fluorescence nanoparticle showed stronger ingestion by human monocytes compared to neutrophils (monocytes: 455±119 MFI in whole blood and 290±96 MFI in isolated cells; neutrophils: 331±107 MFI in whole blood and 94±44 MFI in isolated cells).

**Conclusions:** Our data suggest that 19F-MRI provides a non-invasive method to monitor renal inflammation in animal models and in patients with ANCA-induced NCGN.

*Funding:* Government Support - Non-U.S.

## TH-PO921

**Tissue-Type Plasminogen Activator Promotes Macrophage Mobility through a Novel Signaling Cascade** Ling Lin, Kebin Hu. *Dept of Medicine, Penn State Univ College of Medicine, Hershey, PA.*

**Background:** Macrophage accumulation in the renal interstitium is one of the hallmarks of progressive kidney disease. Our recent work discovered that tissue-type plasminogen activator (tPA) promotes macrophage infiltration and renal inflammation in a model of chronic kidney injury. However, the underlying mechanism remains largely unknown.

**Methods:** We investigated the role of tPA in macrophage mobility and elucidated the underlying signaling mechanism using both in vitro and in vivo approaches.

**Results:** It was found that tPA promoted macrophage migration through its protease-independent function. tPA activated focal adhesion kinase (FAK) and Rac-1 signaling in macrophages in a temporal order. Both FAK and Rac-1 were indispensable to tPA-induced macrophage migration as either infection of macrophages with FAK dominant-negative inhibitor adenovirus or pretreatment of macrophages with Rac-1-specific inhibitor abolished the effect of tPA. We also investigated the role of tPA in macrophage mobility in vivo. Macrophages derived from bone marrow were labeled by fluorescence and adoptively transferred into tPA knockout and wild-type mice, followed by unilateral ureteral obstruction for 7 days. It was found that the number of infiltrated fluorescence-labeled macrophages in the obstructed kidneys from tPA deficient mice was significantly less than that from the wild-type mice. In addition, tPA knockout mice displayed reduced Rac-1 activity in comparison with their wild-type counterparts.

**Conclusions:** Therefore, it is clear that tPA promotes macrophage migration through a novel signaling cascade involving FAK and Rac-1 pathway.

*Funding:* Private Foundation Support

## TH-PO922

**Uric Acid-Induced NLRP3 Inflammasome Contribute to Diabetic Kidney Injury** Su-Mi Kim, Kyung Hwan Jeong, Sang Ho Lee, Chun-Gyoo Ihm, Ju Young Moon. *Div of Nephrology, Dept of Internal Medicine, Kyung Hee Univ, College of Medicine, Seoul, Korea.*

**Background:** Despite of the fact that hyperuricemia is frequently found in a diabetic nephropathy, the definite cause and effect between hyperuricemia and kidney injury have not well investigated yet. Recently, reports showed IL-1β secreting NLRP3 inflammasome in cytoplasm plays a role as a sensor of the innate immune injury in metabolic disease. Therefore, we investigated the cause and effects of hyperuricemia and kidney injury in diabetic nephropathy to demonstrate the role of NLRP3 inflammasome in uric acid-induced kidney injury in diabetes.

**Methods:** We designed four animal groups as following; 1) LETO (Long Evans Tokushima Otsuka); 2) OLETF (Otsuka Long Evans Tokushima Fatty); 3) OLETF + HFD (high fructose diet) for 16 weeks; 4) OLETF + HFD + allopurinol (10mg/dL in drinking water). HK-2 (Human renal proximal tubule cells) and THP1 (Human acute monocytic leukemiacell line) were cultured and stimulated with uric acid.

**Results:** OLETF + HFD group showed a higher serum uric acid ($1.4 \pm 0.1$ vs $2.2 \pm 0.4$ mg/dL) and urinary albumin creatinine ratio ($350 \pm 72$ vs $594 \pm 102$ μg/mg) than OLETF group. In the OLETF + HFD group, the increase trend of NLRP3 and IL-1β expression in kidney was observed. Immunohistochemical staining of $CD68^+$ cells showed significant increase in HFD group compared to OLETF group. Allopurinol attenuated HFD induced hyperuricemia and NLRP3 activation-related renal inflammation. Uric acid-induced NLRP3 activation and IL-1β secretion in THP1 cells were also observed. During the THP1 and HK-2 co-culture it is confirmed that IL-1β secreted in THP1, plays a pivotal role in activating IL-1βR1, MyD88 and IRAK4 signaling in HK-2 cells. This up-regulated IL-1βR1 signaling was resulted in NF-κB activation in HK-2 cells.

**Conclusions:** According to these results we can conclude that hyperuricemia activates NLRP3 inflammasome of macrophage and contribute in renal injury by secretion of IL-1β.

## TH-PO923

**Phenotype Transition of Endothelial Cells with an Activation of NRLP3 Inflammasome by Globotriaosylceramide (Gb3) in Fabry Mice Kidney** Yea-Jin Choi, Hyun-Soo Shin, Kyu Bok Choi, Duk-Hee Kang. *Div of Nephrology, Ewha Womans Univ School of Medicine, Seoul, Republic of Korea.*

**Background:** The lysosomal storage disorder Fabry disease is characterized by Gb3 accumulation in kidney. Defective lysosomal alpha-galactosidase A is responsible for Gb3 accumulation, and vascular endothelium is one of the sensitive cells to the effects of Gb3 accumulation. Recently, NLRP3 inflammasome activated by cellular signals is known to play a role in endothelial dysfunction and renal fibrosis. We aimed to investigate whether Gb3 induced phenotype transition of endothelial cells and activated NLRP3 inflammasome in endothelial cells and Fabry mouse.

**Methods:** Endo-MT was evaluated by the changes in morphology and a comparison of the expression of the endothelial markers, VE-cadherin or CD31 and the mesenchymal marker, α-SMA by real time PCR, WB and ICC in HUVEC exposed to Gb3. Activation of inflammasome was evaluated by real time PCR of NLRP3 and ASC. Effects of recombinant Gla (rGla), anti-oxidant and NO donor on Gb3-induced Endo-MT were investigated. Endo-MT in the kidney of Fabry mouse was examined by double IHC of CD31 and α-SMA.

**Results:** HUVEC with Gb3 (0-40 μM) down-regulated the expression of CD31 and VE-cadherin with an up-regulation of α-SMA in a dose-dependent manner. Gb3 induced a differential phosphorylation of $^{S1177}$eNOS and $^{T495}$eNOS in HUVEC with a decrease in NO production. Gb3 also activated NLRP3 and ASC expressions. rGla or N-acetyl cysteine ameliorated Gb3-induced endo-MT and NLRP3 inflammasome. Although NO donor inhibited Gb3-induced endo-MT, the expression of NLRP3 and ASC was not altered by NO donor. In Fabry mouse, Gb3 accumulation was observed in glomular podocyte, tubular cell and peri-tubular capillaries (PTC) with vacuolar generation in renal tubules. Immunostaining with CD31 and α-SMA revealed capillary rarefaction both in glomerular and PTC with de-novo expression of α-SMA in PTC, suggesting endo-MT in the kidney of Fabry mouse.

**Conclusions:** These findings suggested that Gb3 induced a phenotypic transition of endothelial cells and the activation of NLRP3 inflammasome, which could be the mechanisms of Gb3-induced endothelial dysfunction and nephropathy in Fabry disease.

## TH-PO924

**Lactoferrin Is a Suppressor of Neutrophil Extracellular Traps in Inflammation** Koshu Okubo,[1] Masaomi Nangaku,[1] Toshiro Fujita,[2] Junichi Hirahashi.[1] *[1]Graduate School of Medicine, The Univ of Tokyo, Bunkyo, Tokyo, Japan; [2]Div of Clinical Epigenetics Research Center for Advanced Science and Technology, The Univ of Tokyo, Meguro, Tokyo, Japan.*

**Background:** Neutrophils are endowed with microbicidal functions including phagocytosis, degranulation and neutrophil extracellular traps (NETs), recently identified web-like structures composed of chromatin fibers and serine proteases that protect against invading pathogens. On the other hand, NETs are associated with the development of autoimmune and/or inflammatory diseases and thrombosis. We found that lactoferrin(Lf), one of the components of NETs, is a suppressor of NET formation.

**Methods:** We isolated neutrophils from healthy donors for assay of NET formation. To silence the expression of Lf, we treated Human Leukemia-60 cells with Lf small interference RNA. We observed NET formation under a confocal microscopy with time lapse analysis. We utilized two fluorescence probes, HPF and HySOx, to hydroxyl radical and hypochlorous acid, respectively, to examine the effects of Lf on reactive oxygen species(ROS) generation during NETosis. Spontaneous model of autoimmune small-vessel vasculitis(SCG/kj), localized Shwartzman reaction(LSR), and the model of immune complex(IC)-induced NET formation in the cremaster muscle were used.

**Results:** Lf translocated from the cytoplasm to the inner plasma membrane of neutrophils and suppressed NET formation without effects on ROS upon stimulation. Furthermore exogenous Lf shrunk the chromatin fibers in the released NETs and bound to NETs through charge-charge interaction. In SCG/kj mice plasma DNA release and ANCA titer were decreased and the survival rate improved in Lf-treated mice. Subcutaneous bleeding in LSR decreased, and thrombus and erythrocytes extravasation were less in Lf-treated mice. Furthermore, Lf suppressed the IC-induced NET formation. These observations suggest that Lf serves as an intrinsic inhibitor of NETs and prevents DNA release into the circulation.

**Conclusions:** We found that Lf suppresses NET formation and DNA release into the circulation in inflammation. Our data indicated that Lf may represent a therapeutic lead for controlling NET release in autoimmune and/or inflammatory diseases.

## TH-PO925

**Potential Immunological Role of Renal Intercalated Cells via the P2Y14 Receptor** Anie Azroyan, Virna F. Cortez-Retamozo, Ye Chun Ruan, Mikael Pittet, Dennis Brown, Sylvie Breton. *Program in Membrane Biology, Center for Systems Biology, Nephrology Div, Massachusetts General Hospital, Harvard Medical School, Boston, MA.*

**Background:** Epithelial cell injury leads to inflammation in the kidney. UDP-glucose is a damage-associated molecular pattern (DAMP) molecule that is released from injured cells and signals through the P2Y14 receptor to initiate the release of pro-inflammatory chemokines (PIC).

**Methods:** Immunofluorescence (IF) labeling was used to examine the expression of P2Y14 in mouse kidney sections. Downstream effectors of P2Y14 were analyzed by RT-qPCR and IF in the MDCK-C11 cell line and in intercalated cells (ICs) isolated by FACS from B1-EGFP mice. Infiltration of immune cells in the kidney was assessed by flow cytometry.

**Results:** We showed high expression of P2Y14 specifically in intercalated cells (ICs), identified by their positive labeling for the V-ATPase B1 subunit. RT-qPCR showed P2Y14 mRNA expression in FACS-isolated EGFP-positive ICs, but not in EGFP-negative cells. In vitro stimulation with UDP-glucose increased ERK-phosphorylation (p-ERK) and up-regulated PIC mRNA transcripts in isolated EGFP-positive ICs (CXCL1, CXCL2, CCL2 and CCL3) and in MDCK-C11 cells (IL8, CCL2). Inhibition of p-ERK with a MEK1 inhibitor prevented the UDP-glucose-dependent PIC mRNA increase in MDCK-C11 cells. Tail vein injection of mice with UDP-glucose increased ERK phosphorylation and PIC mRNAs in ICs isolated by FACS after 4 hours, and induced an increased infiltration of neutrophils into the renal medullary regions surrounding collecting ducts after 48 hours.

**Conclusions:** In summary, our data provide evidence for the participation of ICs as DAMP sensors via P2Y14, and indicate the role of ICs as novel mediators of inflammation in the kidney.

*Funding:* NIDDK Support

## TH-PO926

**Estradiol Reduces Ischemic Renal Atrophy in the 2K1C Model in Female ApoE KO Mice** Carolyn M. Ecelbarger, Chelsee Holloway, Nikhil Sharma, Hong Ji, Kathryn Sandberg, Lijun Li. *Dept of Medicine, Georgetown Univ, Washington.*

**Background:** The incidence of atherosclerosis increases in post-menopausal women likely as the result of the fall in circulating estradiol ($E_2$) levels, although the mechanisms remain obscure. The two-kidney, one clip model (2K1C) of renal ischemia has been demonstrated to lead to marked systemic inflammation in the Apoliprotein E (ApoE) knockout (KO) mouse, a mouse model of atherosclerosis. We aimed to elucidate the protective effects of $E_2$ on kidneys in the 2K1C Apo E KO mouse.

**Methods:** Left renal artery incomplete ligation (LRAL) was performed on 12-week-old, female ApoE KO mice. In the same surgery, mice were ovariectomized (OVX) and physiologically replaced with 17ß estradiol ($E_2$, 2µg/day, slow-release pellet) or placebo pellet (P, n = 6/group). Urine (24-hour) was collected in mouse metabolic cages. Kidneys, blood, liver, and vasculature were obtained at euthanasia after 60 days. Histology was done on fixed tissue sections and blinded sections scored for analysis.

**Results:** $E_2$ replacement did not lead to differences in final body weight (BW, g): 24.5 ± 0.4 (OVX + P) versus 23.7 ± 0.5 (OVX + $E_2$), p = 0.22. However, left kidney (LK) atrophy was markedly attenuated by $E_2$ (LKW, g): 0.058 ± 0.016 (OVX + P) versus 0.125 ± 0.017 (OVX + $E_2$), p = 0.018. In contrast, the contralateral right kidney was similarly sized in both groups (RKW, g): 0.185 ± 0.008 (OVX + P) versus 0.185 ± 0.018 (OVX + $E_2$), p = 0.97. Urine volume (24-hour) was also significantly higher (~30%) in the $E_2$-treated mice, p = 0.007. Semi-fasting (4-hour fast) glucose levels were: 150 ± 6 and 121 ± 13 in the OVX + P and OVX + $E_2$ respectively, not significantly different, p = 0.07. In addition, $E_2$ reduced qualitative indices of renal damage including glomerular injury, tubular atrophy, interstitial fibrosis and interstitial inflammation, with lymphocyte and macrophage infiltration in the LK, as compared to placebo-treated mice.

**Conclusions:** $E_2$ replacement to OVX female ApoE KO in 2K1C mice significantly attenuated atrophy and renal injury due to ischemia. We suggest these differences might result from alterations in inflammatory cascades and/or differential blood pressure control.

*Funding:* Private Foundation Support

## TH-PO927

**Extracellular Histones Drive the Progression of Inflammation and Crescent Formation in Necrotizing Glomerulonephritis** Santhosh Kumar VR, Onkar Kulkarni, Shrikant R. Mulay, Dana Thomasova, Murthy Narayana Darisipudi, Hans J. Anders. *Klinische Biochemie, Universität München, Munich, Bavaria, Germany.*

**Background:** Crescentic glomerulonephritis is characterized by extensive glomerular necrosis. Dying cells release intracellular proteins that act as DAMPs to activate innate immunity. Earlier we demonstrated that dying tubular cells release histones which drive tubulointerstitial inflammation in septic or post-ischemic AKI by activating TLR2/4. Here we speculated that extracellular histones also elicit similar pathogenic effects in necrotizing glomerulonephritis.

**Methods:** Necrotic glomerulonephritis was induced in mice by single i.v. injection of 100µl of anti-GBM serum. Effect of histone neutralisation was evaluated on day7.

**Results:** Anti-GBM treated mice show increased proteinuria, plasma creatinine and BUN levels. This was associated with reduced number of podocytes, increased crescentic glomeruli and infiltration of neutrophils/macrophages in kidney. Neutralization of extracellular histones significantly reduced proteinuria with significant less damage to podocytes. This was associated with improved renal function defined by lower plasma creatinine and BUN levels. Also improved renal inflammation marked with less infiltration and activated neutrophils/ macrophages in kidney. Histone blockade also significantly reduced renal mRNA expression of TNFα and reduced fibrinogen in glomerular capillaries, which was associated with less glomerulosclerosis, crescents and tubular atrophy. In contrast, the autologous variant of the Anti-GBM model that lack necrotizing glomerular lesions did not benefit from anti-histone antibody treatment. In-vitro studies demonstrated that, stimulation of BMDCs with histones significantly increased the TNFα and IL6 levels. Histones show dose dependent cytotoxic effect on GEnC and increased podocyte detachment. Neutralization of histones reverses these cytotoxic effects.

**Conclusions:** We conclude that the release of histones from dying glomerular cells contribute to renal immunopathology and dysfunction during crescentic glomerulonephritis. This may either relate to their direct toxic effects on endothelial and epithelial cells or potential to activate innate immunity via TLR2/4.

## TH-PO928

**LPS-Induced Delayed Preconditioning Is Mediated by Hsp90 and Involves the Heat Shock Response** Peter Hamar,[1] Mária Godó Godó,[1] Csaba Revesz,[1] Miklos M. Mozes,[1] Tamás Kaucsár,[1] Csaba S?ti.[2] *[1]Institute of Pathophysiology, Semmelweis Univ, Budapest, Hungary; [2]Dept of Medical Chemistry, Semmelweis Univ, Budapest, Hungary.*

**Background:** The kidney is an important shock organ, often beeing responsible for the death of septic patients. We and others demonstrated previously that endotoxin (LPS) pretreatment protected from subsequent lethal LPS or from ischemia-reperfusion injury (IRI) of the kidney. The heat shock response is an ancient and essential cellular defense mechanism, which plays a role in resistance to, and recovery from, diseases. The major heat shock protein Hsp90 is as a constituent of the LPS receptor. Here, by using the specific pharmacological inhibitor novobiocin, we investigated the role of Hsp90 and the heat shock response in this delayed-type cross-tolerance model.

**Methods:** Male C57/Bl6 mice were treated with preconditioning (p: 2 mg/kg) LPS alone (p) or in combination with subsequent lethal (p+L: 10 mg/kg) dose of LPS alone (LPS(L)) or in combination with novobiocin (NB: 100 mg/kg) (LPS(p)+NB, NB+LPS(L), LPS/NB+LPS(L)). Controls received saline (C) or NB withouth LPS (NB). Survival and renal HSP expresion were determined.

**Results:** Preconditioning LPS conferred protection from a subsequent lethal LPS treatment. LPS induced a marked heat shock response as demonstrated by Northern and Western blots of Hsp70 and Hsp90 mRNA and protein levels, respectively. Importantly, the protective effect of LPS-preconditioning was completely abolished by a concomittant NB treatment. NB also stimulated Hsp70 and Hsp90 mRNA expression, however, both Hsp proteins were significantly downregulated in NB-treated mice. Similarly, heat shock protein induction in LPS-treated mice was abolished by a concomittant NB treatment, demonstrating a NB-induced impairment of the heat shock response to LPS preconditioning.

**Conclusions:** LPS-induced heat shock protein induction and endotoxin tolerance to a subsequent lethal LPS treatment was prevented by novobiocin. Our findings demonstrate a critical role of Hsp90 and a potential involvement of the heat shock response in LPS-induced delayed preconditioning. Funding: OTKA, K 81972.

*Funding:* Government Support - Non-U.S.

## TH-PO929

**Genetic Susceptibility to Experimental Autoimmune Glomerulonephritis** Stephen Paul McAdoo, Zelpha D'souza, Jacques Behmoaras, Frederick W.K. Tam, H. Terence Cook, Charles D. Pusey. *Imperial College London.*

**Background:** The WKY rat strain is susceptible to experimental glomerulonephritides, including nephrotoxic nephritis (NTN) and experimental autoimmune glomerulonephritis (EAG), whereas the LEW strain is resistant. In previous studies by our group, genome-wide screening identified a QTL on Ch13 (crgn1) linked to disease severity in both models. A QTL on Ch16 (crgn2) was additionally linked to disease severity in NTN. We have previously shown that introgression of LEW.crgn1 onto a WKY background conferred partial protection from disease in EAG. We sought to (1) examine the additional effect of introgressing LEW.crgn2 onto a WKY background in this model, and (2) establish if susceptibility to EAG could be conferred to LEW rats by co-introgression of WKY.crgn1 and WKY.crgn2 to a LEW background.

**Methods:** Reciprocal double-congenic (DC) rats were generated - LEW.DC (LEW.WKYcrgn1,2) and WKY.DC (WKY.LEWcrgn1,2) - and immunized with recombinant rat α3(IV)NC1, and assessed for EAG development.

**Results:** Summarised in the table below, at day 28 after disease induction. Expressed as mean/group. Statistical analysis was by one-way ANOVA with Newman Keuls Comparison test.

| | WKY | WKY.DC | p value (WKY v WKY.DC) | LEW | LEW.DC | p value (LEW v LEW.DC) |
|---|---|---|---|---|---|---|
| Proteinuria (mg/day)* | 90 | 8 | <0.05 | 0 | 0 | ns |
| % Crescents* | 76 | 16 | <0.05 | 0 | 0 | ns |
| Macrophages/glomerulus* | 14 | 4 | <0.05 | 1 | 0 | ns |
| Anti-GBM antibody (au)** | 195 | 312 | ns | 144 | 143 | ns |

ns=not significant

**Conclusions:** Additional introgression of LEW.crgn2 onto a WKY background confers greater protection from EAG than seen with LEW.crgn1 alone (Reynolds *et al*, Am J Pathol 2012). This confirms our previous observations in NTN, and highlights the importance of macrophage activation (regulated by crgn2) in these models, and as a potential therapeutic target. Despite making similar levels of anti-GBM antibodies at WKY rats, LEW.DC did not develop disease, suggesting that additional genetic factors contribute to disease susceptibility in the parental WKY rat in this model.

## TH-PO930

**Potential Role of Lysophosphatidic Acid in HIV-Induced Tubular Cell Microcyst Formation** Kamesh R. Ayasolla, Partab Rai, Tejinder Singh, Ashwani Malhotra, Guohua Ding, Praveen N. Chander, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, New York, NY.*

**Background:** HIV-associated nephropathy is characterized by microcyst formation and collapsing glomerulopathy. Lysophosphatidic acid (LPA) and its receptors (LPA1-4) have been implicated in the tubular cell fibrosis (TIF) and cyst formation in autosomal dominant polycystic kidney disease (ADPKD). We hypothesized that LPA might be contributing to tubulointerstitial lesions in HIV milieu.

**Methods:** Primary human proximal tubular cells (HRPTCs) were transduced with either empty vector or HIV (NL4-3). C/HRPTCs, EV/HRPTCs, and HIV/HRPTCs were incubated in media containing either buffer, Ki 16425 (LPA inhibitor), AACOCF3 (LPA synthesis blocker) or DAG kinase inhibitor for 24 hours (n=3). Subsequently, protein blots were probed for TGF-β, cTGF, α-SMA, fibronectin, collagen Ia, III, and IV. The same blots were reprobed for actin. Since Integrin Linked Kinase (ILK-1), focal adhesion kinase (FAK), and G–protein coupled receptor (GPCR) serve as molecules involved in intermediate signaling leading to upregulation and enhanced binding of NFkB, we evaluated expression of these molecules on tubular cell injury in LPA/HIV milieu.

**Results:** Both LPA and HIV milieus altered tubuar cell expression of cTGF, a-SMA, collagen I, III, IV and fibronectin. HIV/HRPTCs also displayed enhanced expression of p-ILK-1, p-FAK, pPI3K,p-Akt, and pP38 MAPK. Moreover, HIV enhanced increased transcriptional binding activity of NF-kB in HRPTCs; however, LPA inhibitors attenuated these effects of HIV.

**Conclusions:** Lysophosphatidic acid may be contributing to HIV-induced tubular cell injury.

*Funding:* NIDDK Support

### TH-PO931

**Protective Effect of Valproic Acid on Adriamycin-Induced Nephropathy by Regulation of Histone Acetylation** Qin Dai, Jian Liu, Fang Zhong, Lili Xu, Weiming Wang, Nan Chen. *Dept of Nephrology, Ruijin Hospital, Shanghai Jiao Tong Univ, Shanghai, China.*

**Background:** Inflammation and podocyte injury are involved in glomerulosclerosis. Histone acetylation takes a part in the renal disease. In the study, we explored whether HDAC inhibitor valproic acid (VPA) has a protective role in the adriamycin-induced nephropathy (ADN) and podocyte injury.

**Methods:** VPA were administered for 6 weeks after ADN modelling. Serum creatinine, urine creatinine, urinary protein, the total acetylation level of histone H3,H4 and mRNA of CTGF, PAI-1, COL-I, COL-III in the renal tissue were measured .Histone acetylation levels were detected by ELISA. HDAC2 and EP300 mRNA expression were detected by realtime-PCR methods. Renal immflamtion were assesed by evaluating MCP-1, STAT3, suppressor of cytokine signaling-1 (SOCS-1) and SOCS-3 changes in the renal cortex by realtime-PCR. ChIP - qPCR was used to detect H3 and H4 acetylation levels in the promoter of MCP-1 and STAT3. Mouse podocytes were cultured to investigate the in vitro effect of VPA against ADR-induced injury, which were detected by MTS assay, scratch wound assay and F-actin changes. ChIP-qPCR and realtime-PCR were applied to explore the histone acetylation of the MCP-1 and integrin-linked kinase (ILK) and mRNA expression.

**Results:** Compared with ADR group, urinary albumin/creatinine was decreased significantly in VPA group ($P<0.05$); serum creatinine, urinary albumin/creatinine, the total level of histone H3, H4 acetylation, the expression of mRNA of CTGF, PAI-1, COL-III were decreased significantly ($P<0.05$) in renal tissue in VPA group. VPA intervention could reduce glomerulosclerosis, change histone acetylation levels and the expression of HDAC, HAT and inflammatory cytokines. VPA could change the MCP-1 expression by regulating the SOCS-1 and SOCS-3 expression.VPA intervention can reduce podocyte death induced by ADR, increased podocyte proliferation, reduce podocyte motility and improve the structural changes of podocytes.

**Conclusions:** Histone acetylation modification participates in the process of glomerular sclerosis, and regulation of histone acetylation may be a promising mean of intervention in progression of kidney disease.

### TH-PO932

**Interferon Regulatory Factor-4 (IRF-4) Prevents Chronic Kidney Disease after Acute Kidney Injury by Modulating Innate Immunity** Falk Bernhard Batz, Maciej Lech, Georg Lorenz, Heni Susanti, Nora Stigrot, Hans J. Anders. *Clinical Biochemistry, Medizinische Klinik und Poliklinik IV, LMU, München Univ of Munich, Munich, Bavaria, Germany.*

**Background:** Acute kidney injury (AKI) is defined as a rapid decrease in the glomerular filtration rate with an accumulation of serum urea and creatinine. If patients recover from AKI they are still at a greater risk of developing a chronic kidney disease, probably due to incomplete regeneration and persistent renal inflammation. Cells of the innate immune system such as macrophages and dendritic cells play a crucial role in initiating the inflammatory responses and the healing of the tubular compartment. IRF-4 is an immune cell specific regulatory transcription factor which suppresses the TLR-signaling by competitive blocking of the IRF-5 binding site. We hypothesize that genetic factors such as IRF-4, which regulate the activation state and subtype of renal macrophages, have influence on inflammatory processes and regeneration after AKI.

**Methods:** We induced AKI by transient unilateral renal artery clamping for 45min (ischemia/reperfusion kidney surgery) in wild type (WT) and IRF-4 knockout mice (IRF4 -/-). After 5 weeks of IR injury, we evaluated kidney size, histology, inflammatory markers and fibrosis in a chronic phase.

**Results:** After 5 weeks, the IRF4-/- kidneys shrank to 1/3 of their original size due to a loss of proximal tubules. Atubular glomeruli occurred at high density and there were significantly more F4/80+ macrophages compared to the WT kidneys. In IRF4-/- kidneys the mRNA expression levels of proinflammatory and damage markers, also the number of CD11b+ Ly6Chigh cells, were significantly increased. In contrast, WT kidneys showed complete recovery from AKI, with maintained kidney size and improved histology and functional parameters.

**Conclusions:** We conclude that IRF4 suppresses the persistent activation of renal proinflammatory M1-macrophages upon transient renal ischemia. This inhibits intrarenal overexpression of the proinflammatory and proapoptotic cytokines and promotes the recovery of tubular injury. Thus, IRF4 is an important transcription factor for maintaining homeostasis during AKI.

*Funding:* Government Support - Non-U.S.

### TH-PO933

**Role of Homeostatic Chemokine SDF1/CXCL12 in Glomerulosclerosis** Simone Romoli, Onkar Kulkarni, Hans J. Anders. *Nephrology Unit, Univ of Munich, Munich, Germany.*

**Background:** Stromal Derived Factor (SDF1) is a homeostatic chemokine, which promotes the homing of hematopoietic stem cells to the bone marrow and innate immune cells to the sites of tissue injury. Therefore, we investigated the role of SDF1 in acute glomerular disorders such as anti-GBM nephritis.

**Methods:** Anti-GBM nephritis was induced by single intravenous injection of anti-GBM serum in C57BL/6 male mice at 6-7 week of age. Anti-GBM injected mice were sacrificed for evaluation on day 2 (acute phase) and day 28 (late phase). Control mice received inactive inhibitor and treatment mice received anti-SDF1 (NOX-A, 50mg/kg s.c. Noxxon), thrice a week. Murine immortalized renal progenitor cells (mPECs) and human PECs (hPECs) were used for in-vitro cell culture studies. In-vivo activity was evaluated by histology,ELISA,FACS,qRT-PCR.

**Results:** Anti-GBM serum induced proteinuria in a dose dependent manner in C57BL/6 mice. Treatment with NOX-A significantly reduced the proteinuria levels at acute phase compared to inactive inhibitor treated group. SDF1 blockade reduced the homing, migration and number of innate immune cells into the kidney during acute phase. After SDF1 blockade FACS analysis of peripheral blood showed an increased number of neutrophils and monocytes, while significant reduction in number of neutrophils and macrophages were detected in the kidney in the acute phase. Additionally, we observed a significantly less podocyte loss and reduction of glomerular lesions during acute injury in the treatment group. However, the SDF1 blockade did not affect the extent of proteinuria and renal histology at later phase of the disease. In-vitro, recombinant SDF1 markedly decreased the proliferation capability of mPECs as well as hPECs in MTT assay but drives the healing in scratch assay. SDF1 inhibited the differentiation of mPECs and hPECs.

**Conclusions:** Blocking SDF1 reduced the infiltration of neutrophils and macrophages into the kidney and inflammation during the acute phase of glomerular disease. Also we observed amelioration of the number of glomerular lesions and more number of podocytes in treatment group.Thus, SDF1 blockade is beneficial in acute phase of the Anti-GBM nephritis for actual mechanisms further studies are required.

### TH-PO934

**SCAP Is a Dual Regulator for Both Cholesterol Homeostasis and Inflammatory Stress in Macrophages and Renal Proximal Tubule Cells** Lung-Chih Li,[1,2] Xiong Z. Ruan.[1] [1]*Centre for Nephrology, John Moorhead Renal Research Laboratory, Centre for Nephrology, Univ College London (UCL), London, United Kingdom;* [2]*Nephrology Dept, Chang-Gung Memorial Hospital-Kaohsiung Medical Centre, Kaohsiung, Taiwan.*

**Background:** Both macrophages and renal proximal tubular cells play important roles in the progression of chronic kidney disease (CKD). Sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) is a cholesterol sensor that regulates LDL receptor (LDLr) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoAR) transcription and maintains the intracellular cholesterol homeostasis. The aim of this study is to investigate if SCAP regulates inflammatory response.

**Methods:** SCAP in PMA-activated THP-1 and HK2 cells was knocked down using siRNA or over-expressed by gene transfection. Intracellular cholesterol content was assessed by Oil Red O (ORO) staining and quantitative assay. The mRNA and protein expression of SCAP, pro-inflammatory cytokines, LDLr and HMGCoAR were examined by real-time quantitative RT-PCR and Western blotting. Nuclear p65, phosphorylated IκB and JNK were investigated.

**Results:** Over-expression of SCAP increased, while knockdown of SCAP decreased LDLr and HMGCoAR expression in both cells. Intracellular cholesterol content was significantly increased after over-expression of SCAP and remarkably reduced after knocking down SCAP. Interestingly, over-expression of SCAP also increased the pro-inflammatory cytokines IL-6 and TNF-α. LPS increased IL-6 and TNF-α expression as expected. However, knocking-down SCAP abolished the up-regulatory effects by LPS on IL-6 and TNF-α, accompanying with reduction of phosphorylation of IκB (rather than JNK phosphorylation) and nuclear p65 levels, indicating that SCAP mediates inflammatory response in THP-1 and HK2 cells via IκB phosphorylation.

**Conclusions:** SCAP is not only a cholesterol sensor but also a key regulator for inflammatory response in THP-1 and HK2 cells. SCAP may serve as a novel target for both lipid-lowering and anti-inflammatory therapies in renal diseases.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

### TH-PO935

**Overexpression of Leukocyte Kv1.3-Channels Promotes Renal Fibrosis in Rats with Advanced Chronic Renal Failure** Itsuro Kazama. *Physiology I, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan.*

**Background:** Leukocytes, such as lymphocytes and macrophages, predominantly express delayed rectifier $K^+$-channels (Kv1.3), and the channels play crucial roles in the activation and proliferation of the cells. Since lymphocytes are activated in patients with end-stage renal disease (ESRD), the channels expressed in those cells would contribute to the progression of renal fibrosis in advanced stage chronic renal failure (CRF).

**Methods:** Male Sprague-Dawley rats that underwent 5/6 nephrectomy followed by a 14-week recovery period were used as the model of advanced CRF. Age-matched sham-operated rats were used as controls. Cellular proliferation of leukocytes and the expression

of Kv1.3-channels in the kidneys were examined. Histopathological features of the kidneys and the expression of cell cycle markers were also examined before and after treatment with margatoxin, a selective Kv1.3-channel inhibitor.

**Results:** In the cortical interstitium of advanced CRF rat kidneys, leukocytes proliferated *in situ* and overexpressed Kv1.3-channel protein in their cytoplasm. Treatment with margatoxin significantly suppressed the number of leukocytes and slowed the progression of renal fibrosis with a significant decrease in the cortical cell cycle marker expression.

**Conclusions:** This study demonstrated for the first time that the number of leukocytes was dramatically increased in rat kidneys with advanced CRF. The overexpression of Kv1.3-channels in the leukocytes was thought to contribute to the progression of renal fibrosis by stimulating cell cycling and promoting cellular proliferation.

*Funding:* Government Support - Non-U.S.

## TH-PO936

**Modulation of Endothelial Function Attenuates Kidney Fibrosis by Regulation of Soluble Epoxide Hydrolase** Jae-Yoon Park,[3] Jung Pyo Lee,[1] Seung Hee Yang,[2,3] Chun Soo Lim,[1] Yon Su Kim,[2,3] Yun Kyu Oh.[1] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea; [2]Seoul National Univ Kidney Research Institute, Seoul, Korea; [3]Dept of Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea.*

**Background:** Soluble epoxide hydrolase (sEH) in endothelial cells catalyses the degradation of epoxyeicosatrienoic acids (EETs), which may act as vasoactive agents to control vascular tone. Kidney fibrosis is the final common pathway for most progressive kidney diseases. Here we show that kidney fibrosis was reduced by regulation of sEH activity.

**Methods:** Unilateral ureteral obstruction (UUO) was used as a model of kidney fibrosis in C57BL/6 mice. sEH activity was controlled by continuous release of the sEH inhibitor 12-(3-adamantan-1-ylureido)-dodecanoic acid (AUDA) (8mg/kg/day) for 1 or 2 wks.

**Results:** AUDA treatment restored significantly improved tubulointerstital fibrosis. Fibroblast marker FSP-1 and TGFβ expressions were significantly decreased. sEH inhibition increased the expression of endothelial markers vWF, VE-cadherin, and CD31. On flow cytometric analysis, UUO increased FSP-1 positive cell population and AUDA treatment reduced CD 31 proportion in FSP-1 postive cell. Similarly, sEH inhibition reduced vWF/FSP-1 double positive cells. In an endothelial-to-mesenchymal transition (EndMT) in-vitro model using HUVECs, AUDA treatment restored the morphologic change and reduced the expression of FSP-1. Interestingly, AUDA-activated HUVEC significantly restored the epithelial-to-mesenchymal transition (EMT) of using tubular epithelial cell (TEC) in co-culture system using the Transwell™ system.

**Conclusions:** The results of this study suggest that direct inhibition of EndMT and indirect inhibition of EMT by treatment with sEH inhibitors may be an effective therapy for delaying the progression of fibrosis associated with CKD.

## TH-PO937

**Low Dose Lipopolysaccharide (LPS) Sensitizes Macrophages (MO) and Aggravates Inflammation: An Effect Blocked by Polymixin (PM) and Curcumin (CU)** Siddhartha S. Ghosh, Sam Righi, Todd W. Gehr. *Nephrology, VCU, Richmond, VA.*

**Background:** LPS has been seen in the circulation following high fat diet (HFD) in men and mice. Our studies have shown increased levels of plasma LPS in uremic mice and high fat diet fed mice. Although, the amount of LPS in circulation following HFD or uremia is too low to cause significant inflammation, studies demonstrate that both HFD and uremia are inflammatory disorders. To understand the mechanism we hypothesized that low levels of LPS sensitizes MO to toxins such as Palmitic acid (PA) from HFD and TNFα (from HFD & uremia) and elicits synergistic activation of NFkB.

**Methods:** MO cell line J774 was transduced with 5 MOI of adenovirus with NFkB promoter and luciferase reporter and adenoviral vector containing beta Gal as control. To see if PA or TNF have synergistic effect on the activation of NFkB by LPS, the cells were treated with 50 pg/ml of LPS (a dose which did not activate NFkB) and after 3 hours treated with 50 ng/ml of TNFα and/or 150 μM PA. In a separate experiment J 774 cells were treated with 50 pg/ml LPS to see if this dose could also increase TNFα in MO. TNFα mRNA was measured in MO by qPCR. PM which binds to LPS and CU (inhibits NFkB and reduces renal failure) were used as antagonists. The antagonists were added 2 hours before the addition of LPS.

**Results:** In the absence of LPS, TNFα and PA activated NFkB by 2 and 3 fold, respectively (P<0.05). However, in the presence of LPS the activation was 5 and 8 fold, respectively suggesting synergistic interaction. CU (10 μM) and PM (50μM) significantly inhibited NFkB activity (p<0.05). To understand the mechanism we incubated cells with low dose LPS and found that there was 4 fold increase (P<0.05) in TNFα mRNA in the MO after 18hrs.

**Conclusions:** Although high dose LPS can render MO resistant to LPS stimulation by downregulating TLR4, our study suggest that low dose LPS can potentiate the inflammatory effect of fatty acids and cytokines likely by sensitizing the MO and/or releasing TNFα. This can significantly potentiate inflammation. CU which also blocks TLR4 counteracts the sensitizing effect. Agents such as PM may be used to sequester and block the effect LPS mediated MO sensitization.

*Funding:* Private Foundation Support, Clinical Revenue Support

## TH-PO938

**Effect of Curcumin (CU) and Polymixin (PM) on Renal Failure and Glucose Intolerance in LDLR$^{-/-}$ ¾ Nephrectomy Mice** Siddhartha S. Ghosh, Shobha Ghosh, Todd W. Gehr. *Nephrology, VCU, Richmond, VA.*

**Background:** Uremia and high fat diet (HFD) alters gut microbiota and intestinal permeability resulting in the absorption of lipopolysaccharide (LPS). LPS aggravates inflammation which worsens renal function and promotes glucose intolerance. Our previous studies have shown CU ameliorates renal failure. Since CU is poorly absorbed we speculated that it can mediate its effect by altering the gut absorption of LPS. To examine this hypothesis we compared CU to a nonabsorbable antibiotic, PM, a known inhibitor of LPS.

**Methods:** LDLR-/- mice fed either normal chow or HFD underwent ¾ nephrectomy (Nx) (control,n=6; Control+Nx,n=6; HDF+Nx,n=6). All groups also received either CU (75 mg/kg,n=5) or PM (15 mg/L in drinking water,n=4).

**Results:** Creatinine (CR), BUN and urinary albumin excretion (ACR) was significantly elevated in Nx and Nx+HFD (p<0.01). The parameters shown in table were measured. CU and PM significantly reduced BUN and ACR in Nx and Nx+HFD (p<0.05). CR in all the groups was significantly reduced by CU (p<0.05) but not by PM. Cu and PM treatment significantly improved GTT, IL6 and TNFα in HFD,Nx and Nx+HFD groups (p<0.05). LPS was reduced by 45% by Cu (p<0.01) and 75% by PM (p<0.001).

| | Control | HFD | Nx | Nx+HFD |
|---|---|---|---|---|
| GTT (mg.min/dl) | 36321±2207 | 52133±1278* | 44453±1801* | 608351±2207*,T,♦ |
| LPS (EU/ml) | 0.44±.04 | 2.3±0.22* | 1.5±0.22*,T | 4.0±0.37*,T,♦ |
| IL6 (% control) | 100±10.4 | 183±12.9* | 322±29*,T | 798±233.4*,T,♦ |
| TNFα(% control) | 100±5.8 | 156±18.1* | 217±17.1*,T | 519±52.4* ,T,♦ |

*P<0.05 from Control; T P<0.05 from HFD; □p<0.05 from Nx.

**Conclusions:** In LDLR-/- mice CU and PM effectively blocked the inflammatory response to HFD and Nx and improved glucose tolerance test as measured by GTT. Renal function was also improved by both CU and PM, albeit CU was better than PM. Since PM is confined to the gut we contend that CU might have significant local effect on the gut and that its anti-inflammatory effect is partly mediated through alteration in gut permeability or inhibition of LPS absorption.

*Funding:* Private Foundation Support

## TH-PO939

**Chronic Interstitial Nephritis after Brief Mild Adenine Excess Despite Scarce Crystal Deposition** Ricardo P. Mazzonetto, Orestes Foresto-Neto, Camilla Fanelli, Simone C.A. Arias, Raquel L. Borges, Viviane D. Faustino, Claudia R. Sena, Vivian L. Viana, Denise M.A.C. Malheiros, Niels O.S. Camara, Roberto Zatz, Clarice K. Fujihara. *Univ of Sao Paulo, Brazil.*

**Background:** We showed recently that, in rats on excess adenine (ADE) for 3 weeks, intratubular crystals (Crys) migrate to the interstitium (INT), causing INT nephritis. Here we studied whether renal damage caused by 1 wk ADE would regress/stabilize upon ADE withdrawal or would instead progress to chronic kidney disease (CKD).

**Methods:** Adult male Munich-Wistar rats (n=42) received no treatment (C), or ADE, 0.5% in chow for 1 week, after which Crys/mm$^2$, serum creatinine ($S_{Cr}$, mg/dL), tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/day), % glomerulosclerosis (%GS), INT macrophages (MΦ, cells/mm$^2$), and % INT collagen-1 (%COLL) were assessed. Measurements were repeated 4 and 24 wks later.

**Results:**

| | Crys | $S_{Cr}$ | TCP | ALB | %GS | MΦ | %COLL |
|---|---|---|---|---|---|---|---|
| 1 week ADE | | | | | | | |
| C | - | 0.6±0.1 | 130±4 | 3±1 | 0±0 | 10±3 | 5±1 |
| ADE | 0.3±0.2 | 1.1±0.1$^a$ | 156±6$^a$ | 2±1 | 0±0 | 115±16$^a$ | 5±1 |
| 4 weeks after ADE withdrawal | | | | | | | |
| C | - | 0.6±0.1 | 134±4 | 2±1 | 0±0 | 8±1 | 4±1 |
| ADE | 0.3±0.1 | 0.6±0.1$^b$ | 133±3$^b$ | 2±1 | 0±0 | 61±11$^{ab}$ | 9±2 |
| 24 weeks after ADE withdrawal | | | | | | | |
| C | - | 0.6±0.1 | 137±3 | 16±4 | 0.4±0.2 | 21±3 | 4±1 |
| ADE | 0.2±0.1 | 0.6±0.1$^b$ | 153±4$^{ac}$ | 51±24$^{abc}$ | 2.1±1.0$^{abc}$ | 63±13$^{ab}$ | 12±2$^{ab}$ |

Mean±SE, $^{a,b,c}$, p<0.05 vs C, 1wk and 4 wk, respectively. At 1 wk, Group ADE exhibited, despite very few Crys, $S_{Cr}$ elevation, hypertension, and INT MΦ infiltration, without excess COLL or GS. Four wk after ADE withdrawal, MΦ regressed, while TCP and $S_{Cr}$ were normalized. However, ALB, TCP, %GS and %COLL had risen 24 wk after ADE withdrawal, while MΦ remained elevated.

**Conclusions:** Despite regression of acute renal inflammation/function loss shortly after ADE was ceased, INT fibrosis and GS occurred in the long run, indicating that even light ADE excess can cause changes that lead to self-perpetuating CKD.

FAPESP/CNPq.

*Funding:* Government Support - Non-U.S.

**TH-PO940**

**Galactose-Deficient IgA1 Production Enhanced by Abnormal Cytokine Signaling in IgA1-Secreting Cells of Patients with IgA Nephropathy Can Be Controlled by STAT Inhibitors** Koshi Yamada,[1,2] Zhi Qiang Huang,[1] Milan Raska,[1,3] Joshua Anderson,[1] Hitoshi Suzuki,[2,1] Colin Reily,[1] Hiroyuki Ueda,[1] Zina Moldoveanu,[1] Krzysztof Kiryluk,[5] Yusuke Suzuki,[2] Robert J. Wyatt,[4] Jiri F. Mestecky,[1] Yasuhiko Tomino,[2] Ali G. Gharavi,[5] Bruce A. Julian,[1] Christopher D. Willey,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Juntendo Univ, Tokyo, Japan; [3]Palacky Univ, Olomouc, Czech Republic; [4]Univ of Tennessee, Memphis, TN; [5]Columbia Univ, New York, NY.*

**Background:** IL-6 is a B-cell differentiation factor that regulates multiple functions through JAK/STAT pathway. Here, we assessed the effects of IL-6 on IgA1 glycosylation using IgA1-secreting cells derived from the circulation and tonsils of IgA nephropathy patients (IgAN-C and IgAN-T) and of healthy controls (HC-C and HC-T), and characterized signaling pathways associated with the enhanced production of galactose-deficient IgA1 (Gd-IgA1).

**Methods:** IgA1-secreting cells were stimulated with IL-6. Gd-IgA1 levels were determined by lectin ELISA. The role of STAT3 in enhanced production of Gd-IgA1 was assessed by STAT3 siRNA knock-down. The findings were confirmed by specific inhibitors of JAK/STAT pathway. Signaling was assessed in cell lysates from IgA1-secreting cells after IL-6 stimulation, with or without an inhibitor of STAT3 signaling, by kinome profiling using PamStation® 12 PTK (tyrosine kinome PamChip) and Western blotting.

**Results:** Gd-IgA1 production by IgAN-C stimulated with IL-6 was significantly higher than that by HC-C. IL-6 induced STAT3 phosphorylation at Y705 (P-STAT3), and P-STAT3 was greater in IgAN-C than in HC-C (2-fold; $p<0.019$). Increase in Gd-IgA1 production after IL-6 stimulation of IgAN-C decreased after siRNA STAT3 knock-down ($p<0.05$). A specific inhibitor of STAT3 signaling reduced IL-6-induced production of Gd-IgA1 in both IgAN-C and IgAN-T. This effect was associated with down-regulation of P-STAT3. Kinome profiling confirmed STAT-signaling pathways as the main target of the inhibitor.

**Conclusions:** IL-6 enhanced production of Gd-IgA1 in IgAN-C and IgAN-T. The IL-6-signaling pathway with abnormal activation of P-STAT3 may represent another pathogenetic process in IgAN.

*Funding:* NIDDK Support, Government Support - Non-U.S.

**TH-PO941**

**Elevated Soluble Galectin-3 and Monocyte Galectin-3 Levels in Experimental Uraemia Are Reversed by Anti Advanced Glycaemic End Products (AGE) Therapy** Andrew Duncan Stewart Findlay, Petros Andrikopoulos, Steven Michael Harwood, Julius Edward Kieswich, Magdi Yaqoob. *Translational Medicine, William Harvey Research, London, United Kingdom.*

**Background:** Galectin-3 expressing monocytes and macrophages are implicated in the pathogenesis of chronic inflammatory and fibrotic diseases. Moreover, In human studies elevated soluble plasma Galectin-3 levels are associated with Advanced Glycaemic End product (AGE) and determine poor cardiovascular outcomes.

**Methods:** A murine model of progressive tubulointerstitial nephritis - The Adenine Diet (AD)- was used to define Galectin-3 levels in circulating murine leucocytes and tissue resident macrophages (by FACS analysis and Real Time Quantitative PCR) subject to progressive uraemia. Plasma AGE was quantified by ELISA in AD compared to sham diet (SD) at 0 and 2 weeks. Mice were then given the anti-AGE compound Pyridoxamine in drinking water (400mg/kg) or standard drinking water for 2 weeks of 0.25% Adenine diet and leucocyte Galectin-3 expression was re-evaluated.

**Results:** Plasma Galectin-3 is significantly raised at 7 days to 28 days in AD fed mice(n=33) vs SD (n=30). Circulating monocytes and granulocytes in AD fed mice(n=5) demonstrate significantly higher Galectin-3 Median Fluorescence Intensity(MFI) on FACS analysis at 14-28 days vs SD(n=5) ($p=0.0317$ AD vs SD at 28 days). At 28 days resting peritoneal macrophages and cardiac homogenate failed to show increased expression in Galectin-3 MFI and Galectin-3mRNA levels respectively. Plasma AGE is significantly raised at 2 weeks 0.25% Adenine Diet and significantly reduced by supplementation with 400mg/kg of pyridoxamine in drinking water(n-5) vs standard drinking water(n=5)($p=0.0079$). Pyridoxamine supplementation also significantly reduced adenine diet induced Galectin-3 expression of circulating monocytes at 14 days.

**Conclusions:** In experimental uraemia only circulating Galectin-3 monocyte levels are increased with accumulation of AGE's. Targeting elevated Galectin-3 levels by anti-AGE strategies may be important given Galectin-3's pathogenic role in inflammatory cardiovascular disease.

*Funding:* Clinical Revenue Support

**TH-PO942**

**Oleanolic Acid Attenuates Renal Interstitial Fibrosis in Unilateral Ureteral Obstructive Nephropathy by Facilitating Nuclear Translocation of Nrf2** Sung Jun Kim, Hye Eun Yoon, Seok Joon Shin, Cheol Whee Park, Sungjin Chung. *Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** Renal interstitial fibrosis is a common final pathological process in the progression of renal disease. This is primarily due to oxidative stress, which contributes to renal inflammation and fibrosis. Nuclear factor-eythroid-2-related factor 2 (Nrf2) is known to coordinate induction of genes that encode antioxidant enzymes. We investigated the effects of oleanolic acid, a known Nrf2 activator, on oxidative stress-induced renal inflammation and fibrosis.

**Methods:** Oleanolic acid treatment was initiated one week before unilateral ureteral obstruction (UUO) in C57BL6/J mice, and was continued until 3, 7, and 14 days after UUO. Renal inflammation and fibrosis, markers of oxidative stress, and changes in Nrf2 expression were subsequently evaluated.

**Results:** Oleanolic acid significantly decreased tululointerstitial fibrosis score that was increased in the kidneys of UUO mice. Furthermore, oleanolic acid attenuated UUO-induced collagen deposition and macrophage infiltration on day 14. Additionally, significantly less apoptosis, a lower ratio of Bax to Bcl-2 expression, and fewer apoptotic cells on TUNEL staining were observed in the obstructed kidneys of oleanolic acid-treated mice. Oleanolic acid increased nuclear Nrf2 as well as the expression of HO-1, and decreased lipid peroxidation in the obstructed kidneys. By contrast, olenolic acid did not affect the expression of catalase and the level of tissue hydrogen peroxide. There were no changes in the expression of total Nrf2 and Kelch-like ECH-associated protein 1 (Keap1), indicating that oleanolic acid enhanced nuclear translocation of Nrf2.

**Conclusions:** These results suggest that oleanolic acid may exert beneficial effects in renal fibrosis by increasing nuclear translocation of Nrf2 and subsequently reducing renal oxidative stress.

**TH-PO943**

**Proteinase-Activated Receptor-2 Transactivation of EGF- and TGFβ-Receptor Signalling Pathways Contributes to Renal Fibrosis** Hyunjae Chung, Morley Hollenberg, Daniel A. Muruve. *Medicine, Univ of Calgary, Calgary, Canada.*

**Background:** During renal injury, kidney-localized proteinases can signal by cleaving and activating proteinase-activated receptor-2 (PAR2), a G-protein-coupled receptor involved in inflammation and fibrosisthat is abundantly expressed in renal tubular cells. We investigated the role for PAR2 in the pathogenesis of renal fibrosis.

**Methods:** (i) We evaluated tubular injury and progression of fibrosis in a murine unilateral ureteral obstruction (UUO) model using both wildtype and PAR2-null mice. Renal tissues were evaluated histopathologically for morphology (H&E), collagen deposition (Masson's Trichrome, collagen assay) and biochemically (western blot) for α-smooth muscle actin (αSMA). (ii) We examined the mechanism of PAR2 signaling that regulates fibrosis and the production of the profibrotic cytokine, connective tissue growth factor (CTGF) was measured using primary human proximal tubular epithelial cells (HPTC). Cells were activated with PAR2-activating peptides (PAR2-AP) in presence or absence of signaling pathway inhibitors for TGFβRI (SB431542), EGFR (AG1478) and MAPKinase (UO126).

**Results:** Following UUO, PAR2-null mice displayed reduced renal tubular injury, fibrosis, collagen synthesis and aSMA gene expression at 7 days, compared to wildtype controls. In HPTC *in-vitro,* PAR2 stimulation with PAR2-AP alone significantly up-regulated the expression of CTGF and did so synergistically to augment TGF-β-induced CTGF. Consistent with these findings, stimulation of HPTC with PAR2-AP induced Smad2/3 phosphorylation in the canonical TGF-β signaling pathway. The Smad2 phosphorylation and the CTGF induction by PAR2 activation were downregulated by the use of inhibitors for TGFβRI and EGFR suggesting that PAR2 utilizes transactivation mechanisms of both receptors to initiate fibrogenic signaling.

**Conclusions:** Our data demonstrate that (i) PAR2 plays a role in the early stages of renal fibrosis in a murine UUO model and that (ii) PAR2 utilizes transactivation mechanisms of EGFR and TGFβR to enhance Smad2 activation and the production of CTGF. PAR2 appears to be a contributor to renal injury and fibrosis in vivo and represents a potential therapeutic target for patients with CKD.

*Funding:* Government Support - Non-U.S.

**TH-PO944**

**Oral Activated Charcoal Adsorbent, AST-120 Improves Intestinal Environment and Microbiota in CKD Rats** Ayumi Yoshifuji, Shu Wakino, Junichiro Irie, Kozi Hosoya, Hitoshi Minakuchi, Kazuhiro Hasegawa, Hirobumi Tokuyama, Koichi Hayashi, Hiroshi Itoh. *School of Medicine, Keio Univ, Tokyo, Japan.*

**Background:** Although gut microbiota and colon barrier function were deteriorated in CKD state, the detailed mechanism or pathological relevance has not been elucidated. Oral activated charcoal adsorbent (AST-120) has been reported to delay the progression of CKD by adsorbing uremic toxin from the intestine. However, the effects of AST on the gut environments in CKD have not been fully elucidated.

**Methods:** Six-week-old spontaneously hypertensive rats (SHR) were rendered CKD by 5/6$^{th}$ nephrectomy. Rats were divided into four groups (n=10 each); sham-operated SHR (SHR); SHR given AST-120 (SHR+AST); 5/6$^{th}$ nephrectomized SHR (Nx); Nx given AST-120 (Nx+AST). AST-120 was orally given for 12 weeks.The distribution of the intestinal flora was examined by T-RFLP and real time PCR.

**Results:** Serum level of indoxylsulfate, serum creatinine levels and urinary protein excretion were increased in Nx. Glomerular sclerosis and tissue fibrosis were increased in Nx. These changes were attenuated in Nx+AST. The histological analysis of the colon tissue revealed that the number of goblet cells and the protein expression of mucin-2 were decreased in Nx. These decreases were restored by AST. The expression levels of tight junction protein ZO-1 and Claudin-1 were also decreased in Nx, which were mitigated in Nx+AST. The analysis of the gut flora showed the decrease in the number of *Lactobacillus* in Nx. This decrease was also reversed in Nx+AST.

**Conclusions:** It has been reported that tight junction proteins were downregulated and intestinal barrier function was impaired in mucin-2 deficient mice. Mucin-2 enhances the growth of *Lactobacillus* by inducing their adhesion to the colon. Our data demonstrated that AST-120 improved the gut environment favorable to *Lactobacillus* which affected the expressions of the tight junction proteins. Recent study demonstrated that probiotics that supplement a part of intestinal flora had favorable effects on renal function. These effects provide a novel mechanism whereby AST120 or probiotic therapy improves renal function through modulating the gut environment.

## TH-PO945

**Genetic and Pharmacological Manipulations of Sphingosine Kinase 2, Diminishes Renal Inflammation/Renal Fibrosis in Response to Unilateral Ureteral Obstruction** Shobha D. Thangada,[1] Mallika Ghosh,[1] Herald Yamase,[3] Cynthia J. D'Alessandri,[2] Ferrer Fernando.[1&2] *[1]Vascular Biology, UCHC, Farmington, CT; [2]Urology, CCMC, Hartford, CT; [3]Pathology, UCHC, Farmington, CT.*

**Background:** Sphingosine Kinase-2 (*Sphk-2*) is a metabolizing enzyme responsible for production of bioactive lipid, Sphingosine-1 Phosphate (S1P), which plays a major role in tissue injury. In the present study we address in vivo significance of *Sphk-2*, in renal inflammation/ fibrosis in response to unilateral ureteral obstruction.

**Methods:** For Genetic model studies, *Sphk-2* $^{-/-}$ mice and for pharmacological model studies (treated with novel SphK2 inhibitor, SKX002411, obtained from SphynKx Therapeutics, VA) , wild type (WT) C57BL/6 mice were subjected to complete UUO. Obstructed and unobstructed kidneys were examined for histological changes and expression of key renal injury markers, such as TGF ß and alpha SMA by immunohistochemistry and western blotting. Immune cell profile by flowcytometry and inflammatory cytokine expression by qRT PCR were assessed.

**Results:** Histological examination reveals that WT mice had extensive renal damage with elevated levels of TGF ß and alpha SMA in interstitial spaces when compared to *Sphk-2* $^{-/-}$ mice. Flowcytometric analysis reveals that *Sphk2* $^{-/-}$ mice demonstrated a reduction in pro-inflammatory macrophages (M1) and a corresponding increase in anti-inflammatory (M2) phenotype. Mice treated with SphK2 inhibitor exhibited diminished renal injury histologically, in addition to decreased levels of TGF ß , alpha SMA and reduced infiltration of inflammatory monocytes and macrophages. This observation was further supported by diminished expression of inflammatory cytokines, such as MCP-1, TNF a, CXCL2, IL b1in the drug treated group.

**Conclusions:** The results demonstrate that, genetic and pharmacological manipulation of Sphingosine kinase-2 significantly decreases inflammatory and fibrotic responses, resulting in diminished renal injury. Suggesting that therapeutic modulation of SphK2 signaling pathway may prove beneficial in renal injury representing a novel class of therapeutics.

*Funding:* Private Foundation Support

## TH-PO946

**Changes in Hemodynamic Forces on Glomerular Endothelial Cells following Partial Nephrectomy** Nicholas J. Ferrell,[1] Ruben M. Sandoval,[2] Bruce A. Molitoris,[2] William Fissell.[1] *[1]Nephrology, Vanderbilt Univ, Nashville, TN; [2]Nephrology, Indiana Univ, Indianapolis, IN.*

**Background:** Renal hyperfiltration following loss of functional nephron mass is thought to drive disease progression. Changes in blood flow dynamics in addition to compensatory structural adaptations in the nephron transiently alter the mechanical forces on cells that line the glomerular capillary wall. Therefore, we have characterized disease related changes in fluid shear stress on the surface of glomerular endothelial cells following significant loss of renal mass using the 5/6 nephrectomy (5/6 Nx) model.

**Methods:** Red blood cell (RBC) velocities in the glomerular capillaries of Simonsen Munich Wistar rats were measured by intravital microscopy under physiologic conditions, immediately after 5/6 Nx, and two weeks after 5/6 Nx. Shear forces on the surface of glomerular endothelial cells were calculated based on the measured RBC velocity by accounting for the change in hematocrit (Fahraeus effect) and effective viscosity of blood (Fahraeus-Lindqvist effect) in small diameter vessels.

**Results:** Under normal physiological conditions, RBC velocity was measured at 0.16±0.08 cm/sec and shear stress at the surface of glomerular endothelial cells was 23.6±11.9 dyn/cm$^2$. Blood flow rapidly increased in remnant nephrons prior to structural remodeling of the glomerulus and proportionally increased RBC velocity to 0.22±0.13 cm/sec and shear stress in the glomerular capillaries to 29.9±15.4 dyn/cm$^2$ ($p<0.05$). Shear stress on glomerular endothelial cells further increased at two weeks to 35.8±16.5 dyn/cm$^2$ ($p<0.05$) but not to the extent that would be predicted in the absence of glomerular structural remodeling.

**Conclusions:** These data indicate that mechanical forces on cells in the glomerular capillary rapidly increased following significant loss of functional renal mass. Structural remodeling of the nephron following injury partially normalized these changes but not to levels that are typical of normal physiological conditions. These mechanical and structural alterations may have an effect on chronic renal disease progression.

*Funding:* NIDDK Support

## TH-PO947

**Differential Effects of Oncostatin M on Proinflammatory and Profibrotic Gene Expression in Human Proximal Tubular Cells** Markus Pirklbauer, Rita Sarkozi, Gert J. Mayer, Herbert Schramek. *Dept of Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical Univ, Innsbruck, Austria.*

**Background:** Accumulating evidence indicates that inflammatory mechanisms play a role in initiation and progression of diabetic nephropathy. Recently we reported that oncostatin M (OSM) exerts potent antifibrotic effects in the human proximal tubular cell line HK-2. In this study we investigated OSMs effects on mRNA expression of CCL5/RANTES, thrombospondin-1 (TSP-1) and tenascin C (TNC) induced by TNF-α, IL-1β or TGF-β1.

**Methods:** Human kidney-2 (HK-2) cell culture, real-time PCR.

**Results:** 10 ng/ml of TNF-α or IL-1β led to a time-dependent upregulation of TSP-1 and TNC mRNA expression, which was highest after 48 h and 6 h, respectively. After 24 h of incubation, induction of TSP-1 mRNA was 5.9-fold and 3.2-fold in TNF-α- and IL-1β-treated HK-2 cells but 12.4-fold in TGF-ß1-stimulated ones. At the same time point, TNC mRNA expression was hardly affected by TNF-α or IL-1β, but showed strong induction after administration of TGF-β1. After 24 h of co-incubation, 10 ng/ml OSM inhibited mRNA expression of TSP-1 and TNC induced by either one of the three ligands. In contrast to these two profibrotic genes, induction of proinflammatory CCL5 mRNA expression by TNF-α or IL-1β started as early as 3 h after incubation and was highest after 48 h. TGF-β1 did not significantly affect CCL5 mRNA expression at any of the time points investigated. While CCL5 mRNA expression was unaffected by OSM alone, OSM led to a strong additive stimulatory effect after 6 h and 24 h when administered together with either TNF-α or IL-1β.

**Conclusions:** In human proximal tubular HK-2 cells, OSM exerts a strong additive effect on TNF-α- and IL-1β-stimulated mRNA expression of proinflammatory CCL5. In contrast, OSM inhibits TNF-α-, IL-1β- and TGF-β1-induced mRNA expression of the profibrotic genes TSP-1 and TNC after long-term incubation. Thus, OSM may have the ability to act both as pro-inflammatory ligand and anti-fibrotic mediator.

## TH-PO948

**A Serine Protease Inhibitor, Camostat Mesilate, Attenuates the Progression of CKD through Its Antioxidant Effects** Yoshikazu Miyasato,[1] Miki Ueda,[1] Kohei Uchimura,[1] Yuki Narita,[2] Teruhiko Mizumoto,[1] Tomoaki Onoue,[1] Rika Yamazoe,[1] Jun Morinaga,[1] Manabu Hayata,[1] Yutaka Kakizoe,[1] Sakai Yoshiki,[3] Kenichiro Kitamura.[1] *[1]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan; [2]Center for Clinical Pharmaceutical Sciences, Kumamoto Univ, Kumamoto, Japan; [3]Research Headquarters, Ono Pharmaceutical Co., Ltd., Osaka, Japan.*

**Background:** Previously we demonstrated a renoprotective effect of camostat mesilate (CM) in the remnant kidney model. However, the precise mechanisms by which CM delays the progression of CKD still remain poorly understood. Here, we investigated the effect of CM on the progression of CKD in the adenine-induced CKD model and explored the renoprotective mechanisms of CM.

**Methods:** Thirteen week-old SD rats were divided into four groups: 1) Control group, 2) dietary adenine-induced CKD (CKD) group, 3) CKD+CM (CM) group, and 4) CKD+hydralazine (HYD) group. Blood pressure measurements and 24 hr urine collections were made during the treatment period. Rats were sacrificed following 5 weeks treatment period. We also evaluated the radical scavenging activities of CM and hydralazine in vitro by electron spin resonance (ESR).

**Results:** At the end of the experiment, both proteinuria and serum creatinine levels were substantially increased in CKD group. Although CM group and HYD group showed similar reduction in the blood pressure levels, CM significantly reduced proteinuria and serum creatinine levels compared with HYD. CM remarkably decreased the mRNA levels of NADPH oxidase components and the reactive oxygen species generation in the kidney. Furthermore, the ESR-spin trapping method revealed substantial hydroxyl radical scavenging activity of CM in vitro.

**Conclusions:** We demonstrated that CM significantly ameliorated the progression of CKD through its antioxidant effects independently of blood pressure. Our current results suggest the possibility that CM could be a new therapeutic agent against the progression of CKD.

## TH-PO949

**Nrf2 Agonist Decreases Colonic Inflammation and Tight Junction Depletion in Rats with Chronic Kidney Disease** Wei Ling Lau, Jun Yuan, Shuman Liu, Mahyar Khazaeli, Zhenmin Ni, Nosratola D. Vaziri. *Nephrology, Univ of California, Irvine, Orange, CA.*

**Background:** Gut inflammation is prevalent in chronic kidney disease (CKD) and contributes to systemic inflammation via disruption of the epithelial tight junction barrier with subsequent endotoxin translocation. Nrf2 is a key anti-inflammatory transcription factor that is deficient in CKD. We hypothesize that treatment with a potent Nrf2 activator RTA 408 will decrease colonic inflammation and preserve expression of tight junction proteins.

**Methods:** Sprague-Dawley rats were divided into 3 groups: sham controls (n=5), CKD (n=8), and CKD+Nrf2 treatment group (n=8). CKD was induced via 5/6 nephrectomy. CKD+Nrf2 treatment was RTA 408 (2 mg/kg/day) via oral gavage x10 weeks.

**Results:** CKD was associated with elevated plasma malondialdehyde (MDA, a marker of systemic oxidative stress). CKD rats had histological evidence of colitis and depletion of all 3 tight junction proteins (Figure 1). Treatment with RTA 408 significantly raised

tissue Nrf2 levels, and was associated with decreased nuclear levels of NF-kB. Colon from CKD+Nrf2 treated rats had decreased inflammatory mediators (COX-2, MCP-1) and oxidative stress factors (iNOS, NOX4), and lower plasma MDA levels. Additionally, Nrf2 agonist treatment improved expression of tight junction proteins (Figure 1).

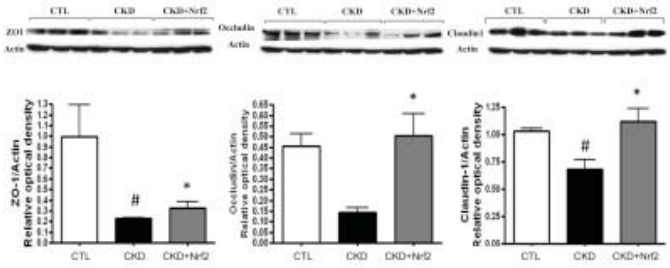



Figure 1. Representative Western blot data showing decreased expression of tight junction proteins (zona occludens-1, occludin and claudin-1) in the colon from CKD rats; this was ameliorated by treatment with a Nrf2 agonist. $P<0.05$ compared to controls (#) or non-treated CKD rats (*).

**Conclusions:** Treatment with a potent Nrf2 agonist decreased colonic inflammation and improved expression of tight junction proteins in CKD rats. There was concurrent decreased systemic oxidative stress. Thus, there are potential benefits of intestinal Nrf2 activation in CKD.

*Funding:* Pharmaceutical Company Support - Reata Pharmaceuticals; WL Lau funded by a Sanofi fellowship award, Private Foundation Support

## TH-PO950

**Tissue Kallikrein Mediates Pro-Inflammatory Pathways in Proximal Tubular Epithelial Cells** Wai Han Yiu,[1] Dickson WL Wong,[1] Joseph C. K. Leung,[1] Loretta Y.Y. Chan,[1] Hui Y. Lan,[2] Kar Neng Lai,[1] Sydney C.W. Tang.[1] *[1]Dept of Medicine, The Univ of Hong Kong, Queen Mary Hospital, Hong Kong, China; [2]Dept of Medicine and Therapeutics, Li Ka Shing Institute of Health Sciences, The Chinese Univ of Hong Kong, Hong Kong, China.*

**Background:** Tissue kallikrein (KLK1) expression is up-regulated in human diabetic kidney tissue. Since the kallikrein-kininsystem (KKS) has been linked to cellular inflammatory process in many diseases, we explore the role of KLK1 in tubular pro-inflammatory responses under the diabetic milieu.

**Methods:** Human proximal tubular epithelial cells (PTEC) were incubated with recombinant KLK1 protein to examine the expression of pro-inflammatory cytokines and the activation of signaling pathways. Cells were then transfected with KLK1-specific or control siRNA to investigate the effect of KLK1 on advanced glycation end products (AGE)-induced pro-inflammatory responses.

**Results:** Recombinant KLK1 stimulated the production of inflammatory cytokines including IL-8, ICAM-1 and CCL-2, and activated the phosphorylation of p42/44 and p38 MAPK in PTEC. Increased expression of KLK1 was detected in PTEC stimulated with AGE (0.5 mg/ml), and molecular knockdown of endogenous KLK1 expression attenuated AGE-induced tubular IL-8 and ICAM-1 productions.

**Conclusions:** Our data suggest for the first time that KLK1 mediates pro-inflammatory responses in renal tubule cells under a diabetic milieu, and pave the way for further investigation that targets KLK1 in ameliorating diabetic tubular injury.

Fund support: Research Grant Council of Hong Kong (GRF grant number 7796/11M).

## TH-PO951

**Regulatory T Cells in Nephrocalcinosis and Dystrophic Cardiac Calcinosis in DBA/2 Mice** Alexander H. Kirsch,[1] Alexander R. Rosenkranz,[1] Kathrin Eller,[1] Philipp Eller.[2] *[1]Clincal Div of Nephrology, Medical Univ of Graz, Graz, Austria; [2]Div of Angiology, Medical Univ of Graz, Graz, Austria.*

**Background:** Nephrocalcinosis is characterized by aberrant deposition of calcium in the kidneys and is seen in phosphate nephropathy, primary hyperparathyroidism, and distal renal tubular acidosis.

**Methods:** To further evaluate the specific pathophysiologic role of T cells in ectopic calcification, we used DBA/2 mice that are prone to develop nephrocalcinosis and dystrophic cardiac calcinosis. Female DBA/2 mice were depleted of T cells (n = 10) or regulatory T cells (Tregs) (n = 15) using either an anti-CD3ε or an anti-CD25 monoclonal antibody and compared with isotype-treated controls (n = 9; n = 15), respectively. After this immunomodulation, the DBA/2 mice were given a high-phosphate diet for 9 days and the degree of calcification was assessed by microcomputed tomography. Successful depletion was confirmed by flow cytometry of splenocytes.

**Results:** In DBA/2 mice, the high-phosphate diet induced a phenotype of nephrocalcinosis and dystrophic cardiac calcinosis. T-cell depletion significantly increased renal calcification in microcomputed tomography (P = 0.022). Concordantly, Treg depletion significantly deteriorated acute phosphate nephropathy (P = 0.039) and was associated with a significantly increased mortality rate (P = 0.004). Immunomodulation had no impact on the amount of cardiac calcification. Semiquantitative histopathologic evaluations with Alizarin Red staining independently confirmed the respective radiologic measurements.

**Conclusions:** In summary, our data suggest a pivotal role of T cells, particularly Tregs, in the progression of nephrocalcinosis and emphasize the fact that inflammation deteriorates the outcome in acute phosphate nephropathy.

*Funding:* Government Support - Non-U.S.

## TH-PO952

**Aberrant Activation of Neutrophils Allow ANCA to Aggravate Glomerulonephritis in Experimental Autoimmune Vasculitis** Go Kanzaki, Shinya Nagasaka, Yusuke Kajimoto, Seiichiro Higo, Kayori Tsuruoka, Akira Shimizu. *Dept of Analytic Human Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

**Background:** ANCA-neutrophil and neutrophil-endothelial cell interactions play an important role in the pathogenesis of ANCA-associated vasculitis (AAV). However, it is still unclear whether these interactions aggravate glomerulonephritis (GN) *in vivo*. In the present study, we used an experimental autoimmune vasculitis (EAV) rat model by generation of an immune response to exogenously administered human MPO (hMPO) in adjuvant. We tested this hypothesis in this rat model by using lipopolysaccharide (LPS), phorbol myristate acetate (PMA), or G-CSF as a stimulus for neutrophils. In addition, we analyzed gene expression of cytokines and adhesion molecules in this rat model.

**Methods:** Necrotizing and crescentic GN was induced in WKY rat by immunization with hMPO (1600 μg/kg). Blood and urine samples were obtained at every week. Quantitative RT-PCR was used to determine gene expression of cytokines and adhesion molecules were analysed in isolated glomeruli in 8 weeks after NCGN induction.

**Results:** Hematuria (2-3+) and proteinuria (300-1000 mg/dl) were noted at 4 weeks. hMPO-immunized rats had serum anti-hMPO antibody titers of 1:10000 by ELISA. We demonstrated that the induced anti-MPO antibodies cross-reacted with rat neutrophils inducing IL-1β in vitro. Several cytokines or adhesion molecules, which are mainly involved in neutrophil-endothelial interactions, were induced or up-regulated in glomeruli of hMPO immunized rat. The administration of LPS, PMA, or G-CSF exacerbated disease in EAV rat model, as assessed by both histological and functional parameters.

**Conclusions:** This study suggested that ANCA developed in EAV model rat aberrantly activated neutrophils in vivo. The excess stimuli for neutrophils or endothelial cells would develop glomerulonephritis in AAV.

## TH-PO953

**Innate Immunity Signal, S100 A8/A9, Are Danger Biomarkers of Lupus Nephritis** Yingyos Avihingsanon,[1] Pornpen Tantivitayakul,[2] Thitima Benjachat,[4] Vipawee Kittikovit,[3] Nattiya Hirankarn.[3] *[1]Medicine, Faculty of Medicine, Chulalongkorn Univ, Bangkok, Thailand; [2]Microbiology, Mahidol Univ, Bangkok, Thailand; [3]Faculty of Medicine, Chulalongkorn Univ, Bangkok, Thailand; [4]Biomedical Science, Interdisciplinary Program, Graduate School, Chulalongkorn Univ, Bangkok, Thailand.*

**Background:** S100A8 and S100A9 are members of S100 family proteins that involved innate immunity. The proteins are co-expressed on macrophages and infiltrating monocytes during an active involvement of skin and kidney in lupus patients. They can activate auto-reactive CD8+T-cell through the TLR4 signaling and can lead to the development of nephritis in lupus mice. In this study, we determined the non-invasive blood monitoring of S100A8/A9 mRNA levels and their usefulness for a prediction of therapeutic response.

**Methods:** We studied S100 messenger RNA levels from blood leucocytes of lupus nephritis patients (n=68) and compared with healthy control (n=15). A 6-month follow-up blood mRNA levels was then measured and correlated with therapeutic response to standard therapy. The 18s mRNA was used as house-keeping gene and the mRNA levels were expressed as log-transformation.

**Results:** All active lupus nephritis (n=38) were biopsy-proven proliferative class III/IV LN. Median (IQR) urine protein and serum albumin were 5.01 (3.03-5.69) g/day and 2.90 (2.30-3.25) g/dL. Serum creatinine was 0.85 (0.72-1.22) mg/dL. S100A8 and A9 mRNA levels from blood leucocytes were tightly correlated with renal disease activity (r = 0.33). Both S100 mRNA levels of active LN were higher than those of inactive LN and healthy control ($p<0.01$). In the study of pre/post treatment of induction therapy, there were 12 responders and 14 non-responders. In the responders, both S100 mRNA levels were significantly reduced after a 6-month course of immunosuppressive treatment.

**Conclusions:** This supports the importance of innate immune response in lupus nephritis. Expression of S100 A8 and A9 of blood leucocytes are potential non-invasive biomarkers of lupus nephritis.

*Funding:* Government Support - Non-U.S.

## TH-PO954

**Diagnostic Value of the Simultaneous Detection of Anti-C1q and Anti-dsDNA Antibodies in the Diagnosis of Active Lupus Nephritis** Katarzyna Smykal-Jankowiak, Zofia I. Niemir, Magdalena Polcyn-Adamczak. *Laboratory of Molecular Nephrology, Univ of Medical Sciences, Poznan, Poland.*

**Background:** Development of lupus nephritis (LN) is one of the most important factors that worsen the prognosis of systemic lupus erythematosus (SLE). Anti-C1q and anti-dsDNA antibodies (Abs) are candidates for non-invasive markers of renal disease, which may by alternative to kidney biopsy in the assessment of LN activity. We examined the prevalence and predictive values of anti-C1q and anti-dsDNA Abs in the judgment about the LN activity.

**Methods:** Sixty-three patients with LN and 86 healthy volunteers (C)were enrolled into the study. LN patients were divided into two sub-groups depending on the activity of SLE, scored according to the systemic lupus disease activity index-2K (SLEDAI-2K). Thirty-eight patients constituted the group with active LN (aLN), whereas the remaining 25 patients were in inactive phase of the disease (inLN). Standardized enzyme-linked immunosorbent assays were used to detect anti-C1q and anti-dsDNA Abs in sera. The C3 and C4 complement components in sera were measured by turbidimetry.

**Results:** Anti-C1q Abs were detected in 81.6% of patients with aLN and 48% of those with inLN ($p<0.01$). Positive anti-dsDNA Abs were found in 89.5% of patients with aLN, compared to 56% of those with inLN group ($p<0.01$). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of anti-C1q Abs in the diagnosis of aLN were 81.58%, 52.00%, 72.09% and 65.00%, respectively. For anti-dsDNA Abs, these values were 89.47%, 44.00%, 70.83% and 73.33%. In the whole LN group, both Abs were present in sera of 57.1% patients, compared to 11.1% of those with anti-C1q Abs only, 13.0% with anti-dsDNA only and 12.6% without both Abs ($p<0.001$). Simultaneous detection of both Abs increased the NPP to100%, but not PPV, which reached 75.00%. The addition of decreased levels C3 to the detection of both Abs resulted in 100% of sensitivity, 85% of PPV and 100% of NPV for aLN.

**Conclusions:** Absence of both anti-C1q and anti-dsDNA antibodies in sera of patients with LN seems to exclude the active phase of LN. The addition of C3 to this set of examinations strengthens these results.

*Funding:* Government Support - Non-U.S.

## TH-PO955

**Macrophage Migration Inhibitory Factor (MIF) in Active Lupus Nephritis (LN) – Response to Immunotherapy** Ratana Chawanasuntorapoj, Marie B. Condon, Tom Cairns, Megan Griffith, Frederick W.K. Tam, Liz Lightstone. *Kidney and Transplant Centre, Imperial College.*

**Background:** MIF plays a central role in inflammatory responses and elevated MIF levels have been detected in patients with inflammatory kidney diseases. Kerschbaumer et al ASN 2012 reported MIF in urine correlated with activity of LN, however this was in a relatively small pilot population. In this study, we investigate urinary MIF (uMIF) in a larger cohort looking at response to immunotherapy and it's predictive value at time of biopsy.

**Methods:** uMIF was measured by ELISA on 643 urine samples from 60 patients collected over 54 mths. All patients had biopsy proven ISN/RPS class III/IV/V LN, >75% received Rituximab +/- MMF +/- steroids; the other patients received MMF +/or cyclophosphamide +/- steroids. uMIF levels were normalised for urinary creatinine (creat). uMIF/creat +/- 5 wks of biopsy were compared with uMIF/creat in samples collected at 26-52 wks post biopsy; subdivided into complete remission (CR=uPCR<50mg/mmol+<15% rise in serum creat); partial remission (PR=uPCR 50-300mg/mmol) and non response (NR=uPCR>300mg/mmol).

**Results:** Samples analysed: 75 at biopsy; 82 at 26-52 wks post biopsy (53 CR; 27 PR; 17 NR). uMIF/creat is significantly reduced in all patients at 26-52 wks post biopsy compared with biopsy, however the more significant the clinical response the more significant the reduction (CR = 58% ($p<0.0001$); PR = 44% ($p=0.0002$); NR = 37% ($p=0.0175$)). There is a significant correlation between uMIF/creat and uPCR at time of active biopsy ($p=0.027$, Spearman's correlation R=0.26), but not with serum creat. uMIF/creat at biopsy did not show a significant correlation with time to CR or with class of LN.

**Conclusions:** uMIF is abundant in all patients with active LN on renal biopsy; as the disease goes into remission the levels fall significantly. uMIF is not reported in healthy subjects, any uMIF is pathological, maybe indicative of a "smouldering" disease process. As the treatment protocols at this centre are deemed to be "steroid sparing", the lack of demonstration of a predictive value of uMIF maybe due to this approach being effective at treating the MIF dependent aspect of the disease process as MIF may induce steroid resistance.

## TH-PO956

**Monitoring of Urinary Cytokine Levels as Biomarker for Disease Activity in Thai Lupus Nephritis** Peenida Skulratanasak,[1] Ratana Chawanasuntorapoj,[1] Boonyarit Cheunsuchon,[2] Pisal Parichatikanond,[2] Kriengsak Vareesangthip,[1] Chairat Shayakul.[1] *[1]Medicine, Siriraj Hosp.; [2]Pathology, Siriraj Hosp., Mahidol Univ.*

**Background:** Proliferative lupus nephritis (LN) is one of the most common and severe secondary glomerulonephritis (GN). Early detection and good monitoring are essential to provide optimal care, while delay of the appropiate treatment may worsen the outcome. Regarding the pathogenesis of LN, immune and inflammatory responses play an essential role. Several studies have demonstrated cytokines/chemokines in correlation with LN activities. Using a novel Luminex™ assay to detect such changes has maximized its efficiency and clinical advantages by simultaneous measurement of several cytokines within a short period. This study was to demonstrate the correlation of urinary biomarkers with LN activity and its application.

**Methods:** We measured urinary cytokines; monocyte chemoattractant protein-1(MCP-1), vascular endothelial growth factor(VEGF), interleukin-6 (IL-6), IFN-γ inducible protein 10(IP-10), platelet derived growth factor(PDGF) and interleukin(IL)-1β using Bio-plex assay, and related their levels to the clinical status among active LN and control groups. In active LN, we measured these cytokines along two years during Jan 2011 to Dec 2012.

**Results:** 40 biopsy-proven active proliferative LN were compared with 35 inactive LN, 35 other GN and 35 healthy controls. Urinary MCP-1, VEGF, IL-6, IP-10, PDGF and IL-1β were significantly higher in active LN than healthy and inactive LN. Our cross-sectional part found the sensitivity of MCP-1 to identify the active LN was better than routine serology. In 40 active LN, the clinical response at 6 months therapy were 12 complete, 15 partial, and 9 non-response. Urinary IL-1β, IP-10, PDGF, and VEGF significantly decreased and correlated with the clinical. We described the persistently high level of urinary PDGF at 3rd month could be predicting the non-responder to induction therapy at 6th month. During 2 years, renal flare occurred in 5 cases. Increase of IL-1β might be applied as predictor of renal flare.

**Conclusions:** According to our result, urinary biomarkers could be useful to monitor LN activity and might be minimize kidney re-biopsy in LN patients.

*Funding:* Government Support - Non-U.S.

## TH-PO957

**Soluble CR1 – Could It Be a New Marker of Lupus Nephritis Activity?** Aleksandra Rochowiak, Zofia I. Niemir, Magdalena Polcyn-Adamczak. *Laboratory of Molecular Nephrology, Univ of Medical Sciences, Poznan, Poland.*

**Background:** CR1, a membrane receptor for complement components (C3b/C4b), has a soluble form (sCR1) present in the serum and in the urine.The sources of sCR1 are probably erythrocytes and blood leukocytes.CR1 regulates the complement cascade activation and plays an important role in the removal of immune complexes coated with C3b/C4b. Increased serum concentrations of sCR1 were observed in patients with inflammatory diseases and hematologic malignancies. The aim of our study was the analysis of the sCR1 concentration in serum of healthy individuals and patients with primary glomerulonephritides (PGN) and lupus nephritis (LN).

**Methods:** The serum samples were obtained from 147 patients with PGN, 65 with LN and 67 healthy volunteers serving as a control group (C). The activity of the disease was determined by using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). Among patients with LN, 32 were in active phase of the disease (aLN) (SLEDAI score ≥10) and remaining 33 in inactive phase of the disease (inLN) (SLEDAI score <10). Serum sCR1 was evaluated by the standardized enzyme-linked immunosorbent assays.

**Results:** The highest level of serum sCR1 was noticed in patients with aLN compared to the results obtained in C ($p<0.01$), PGN ($p<0.01$) and inLN ($p<0.05$). A significant correlation was observed between sCR1 level and SLEDAI-2K score in LN ($r=0.2576$; $p<0.05$), while significant negative correlation appeared between sCR1 level and C3 ($r=-0.269$; $p<0.05$) and C4 concentration ($r=-0.319$; $p<0.001$) in LN.Serum sCR1 did not correlate with C3 and C4 serum level in C and PGN. We also have not found any correlations between sCR1 level and anti-dsDNA concentration in LN, as well as between creatinine serum level and sCR1 in LN and PGN.

**Conclusions:** Increased serum level of sCR1 in aLN and correlations observed between its concentration and C3 and C4, as well as sCR1 and SLEDAI-2K score in LN indicate that serum sCR1 may be a potential marker of lupus nephritis activity.

*Funding:* Government Support - Non-U.S.

## TH-PO958

**Elevated Levels of Lysophospholipid Mediators in Patients with Lupus Nephritis** Junya Yamahana, Kengo Furuichi, Yasunori Iwata, Norihiko Sakai, Miho Shimizu, Takashi Wada. *Dept of Nephrology, Kanazawa Univ Hospital, Kanazawa, Ishikawa, Japan.*

**Background:** Lysophospholipid mediators are key molecules to regulate cell proliferation, differentiation, and/or apoptosis in the living body. The mediators have also been reported to be involved in fibrosis of the liver, lung, and kidney. In this study, we evaluated the pathogenic involvement of the lysophospholipid mediators in chronic kidney diseases, including lupus nephritis.

**Methods:** Sixty-five cases of biopsy proven kidney diseases were evaluated in this study. Sixty-five cases contained lupus nephritis (LN, 40 cases), membranous nephropathy (MN: 18 cases), minimal change nephrotic syndrome/focal segmental glomerulosclerosis (MCNS/FSGS: 4 cases), and rapidly progressive glomerulonephritis (RPGN: 3 cases). Correlations between clinic-pathological findings, serum levels of autotaxin (ATX) and phosphatidylserine-specific phospholipase A1 (PS-PLA1) were evaluated. Furthermore, response of serum ATX and PS-PLA1 levels to steroid therapy was also analyzed.

**Results:** Serum levels of PS-PLA1 in female patients with LN were higher than those in the patients with MN, or MCNS/FSGS ($90.1 \pm 15.5$, $30.9 \pm 6.0$, $44.5 \pm 28.5$ mg/L, respectively, $p < 0.05$). Serum ATX levels were also higher in female LN patients ($1.06 \pm 0.12$ mg/L) than those in MN ($0.69 \pm 0.09$, $p<0.05$). In regard to the response of steroid therapy in LN, serum levels of ATX and PS-PLA1 were negatively correlated with the doses of oral prednisolone ($p < 0.01$). In cases receiving steroid pulse therapy in LN, serum levels of PS-PLA1 ($97.4 \pm 26.3$ vs. $53.4 \pm 7.4$, $p = 0.17$) and ATX ($1.1 \pm 0.1$ vs. $0.6 \pm 0.1$, $p < 0.01$) were decreased accompanied with prevention of disease activity (SLE Disease Activity Index: decrease from $14.5 \pm 2.2$ to $7.0 \pm 2.1$). Pathologic analysis of untreated LN patient revealed that serum levels of PS-PLA1 were correlated with chronicity index (correlation coefficient 0.629, $p<0.05$). In patients of LN with higher level of serum PS-PLA1, renal function tended to decline rapidly.

**Conclusions:** Serum levels of PS-PLA1 and ATX were elevated in patients with LN, and decreased in accordance with suppression of the disease activity by treatment.

**TH-PO959**

**Urinary Angiogenin – A Novel Biomarker in Lupus Nephritis**
Marie B. Condon, Ratana Chawanasuntorapoj, Megan Griffith, Tom Cairns, Liz Lightstone, Frederick W.K. Tam. *Kidney and Transplant Centre, Imperial College, London.*

**Background:** Angiogenin (Ang), also known as Ribonuclease 5, is a 123 amino acid protein, involved in stimulating RNA transcription after localisation into the nucleus, leading to angiogenesis and growth stimulation. The presence of Ang in glomerular disease is not known. This is the first report in relation to activity of lupus nephritis (LN). Treatment of lupus nephritis with the steroid sparing "Rituxilup" regimen has published outcomes and all patients were treated in a consistent manner.

**Methods:** Ang was measured by ELISA on 342 urine samples from 34 patients collected over a 4 year period. During that time all patients had at least 1 episode of biopsy proven ISN/RPS class III/IV/V LN, for which they received the steroid sparing "Rituxilup" regimen. Median age at time of biopsy 44 yrs (IQR 27 years). Urine Ang (uAng) levels were normalised for urinary creatinine (creat) level. The relationship between uAng/creat ratios and serum creat and urine protein/creat ratio (uPCR) in the samples taken within +/- 5 weeks of biopsy were investigated. Effect of immunotherapy on uANG/creat ratios +/- 5 weeks of achieving partial remission (PR=uPCR <300mg/mmol with a >50% reduction from baseline with <15% rise in serum creat) and complete remission (CR=uPCR<50mg/mmol with <15% rise in serum creat) were evaluated.

**Results:** 33, 28 and 30 samples analysed at biopsy, PR and CR respectively. There is a significant correlation between uAng/creat and serum creat at time of active biopsy (p=0.011, Spearman's correlation R=0.45), but not with uPCR. uAng/creat is significantly reduced in PR and CR by 51% (p<0.05) and 84% (p<0.001) respectively.

**Conclusions:** In a well described cohort, treated in a consistent manner, uAng is associated with the severity of renal impairment at time of biopsy of active lupus nephritis, and showed significant reduction in patients with remission following immunotherapy. The lack of correlation of uAng with uPCR at presentation suggested that uAng is not a simple consequence of proteinuria. The potential role of Ang in the development of LN will need further investigation.

**TH-PO960**

**Interobserver Agreement in Proliferative Lupus Nephritis**
Suzanne Wilhelmus,[1] H. Terence Cook,[2] Laure-Helene Noel,[3] Franco Ferrario,[4] Ron Wolterbeek,[5] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, LUMC, Leiden, Netherlands; [2]Pathology, Imperial College, London, United Kingdom; [3]Pathology, Necker Hospital, Paris, France; [4]Pathology, San Gerardo Hospital, Monza, Italy; [5]Medical Statistics, LUMC, Leiden, Netherlands.*

**Background:** In lupus nephritis the histological class plays a central role in guiding treatment. Although the classification is almost 40 years old and has undergone various revisions (latest ISN/RPS 2003), remarkably few studies have paid attention to the interobserver agreement among pathologists, in particular with respect to the clinically most relevant proliferative lesions. We aimed to determine the interobserver agreement for proliferative lupus nephritis among nephropathologists worldwide.

**Methods:** We distributed a survey with pictures of 30 glomeruli among members of the Renal Pathology Society. We received 34 responses from 12 countries. For each glomerulus it had to be decided if a lesion was present that put the biopsy in class III or IV. If so, additional parameters had to be scored. For each parameter, we calculated the kappa or intraclass correlation (ICC). A value < 0.4 was considered as poor, and between 0.4 – 0.6 as moderate agreement. Pathologists had to indicate their level of experience.

**Results:** The ICC for the presence of a lesion putting the biopsy in class III or IV was 0.39 (poor). The kappa/ICC for the additional parameters were: active, chronic or both 0,36; segmental vs global 0.39; endocapillary proliferation 0.46; inflammatory cell influx 0.32; endothelial cell swelling 0.46; extracapillary proliferation 0.57; crescent type 0.45; wire loops 0.35. Highly experienced pathologists consistently showed less variability for all parameters than those less experienced.

**Conclusions:** Even though the clinical consequences of classifying a biopsy into class III or IV are substantial, the agreement among pathologists as to what constitutes a proliferative lesion is poor. We think improvements can be made by training renal pathologists and by revising the definitions of proliferative lesions provided in the classification system. Our results also underline the importance of central review by at least two experienced pathologists in clinical trials.

**TH-PO961**

**Thrombotic Microangiopathy in IgA Nephropathy Is Strongly Associated with Chronic Lesions**
Jamie S. Chua,[1] Johan W. De Fijter,[2] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, Leiden Univ Medical Center, Leiden, Netherlands; [2]Nephrology, Leiden Univ Medical Center, Leiden, Netherlands.*

**Background:** Thrombotic microangiopathy (TMA) is an underappreciated phenomenon in IgA nephropathy (IgAN) (El Karoui, JASN, 2012). We present results of the occurrence of TMA in IgAN from a single center study incorporating 116 cases.

**Methods:** In this single center study, 116 consecutive renal biopsies from the period 2003-2013 were incorporated, of which 98 were previously diagnosed with IgAN and 18 with Henoch-Schonlein nephritis (HSN). Biopsies were re-evaluated according to the Oxford Classification for IgAN. TMA, arterial intimal sclerosis and arteriolar hyalinosis were scored according to the guidelines of El Karoui et al. Patient demographics and clinical data were collected retrospectively from medical records, including blood pressure, history of hypertension and renal function.

**Results:** TMA occurred in 21/116 biopsies (18%), in 19/98 of IgAN patients (19%) and in 2/18 HSN patients (10%). TMA lesions were acute in 10/21 cases (48%) and chronic in 11/21 cases (52%). TMA was primarily located in arterioles (n=16). Endocapillary hypercellularity, IFTA and arterial intimasclerosis were found more frequently in patients with TMA than in those without (X-square test, respective p-values: 0.014, 0.004 and <0.001). Mesangial hypercellularity, hypertension and serum creatinin at time of biopsy were not associated with TMA. All reported associations remained statistically significant when omitting patients with HSN and there were no significant differences between chronic and acute TMA cases.

**Conclusions:** In this single center study, we found fewer TMA lesions in IgAN (18%) than previously reported (53%). It was hypothesized by El Karoui et al. that the high frequency of TMA in IgAN in their study could be explained by the selection of patients from a hypertension-clinic. Our results contradict this hypothesis. In our study, TMA was associated with chronic lesions such as IFTA and arterial intimasclerosis, but not with hypertension.

**TH-PO962**

**Complement Factor C4d Deposition along Glomerular Capillary Walls: C4d beyond Antibody Induced Injury**
Jamie S. Chua,[1,2] A. Gasim,[1] Harsharan Kaur Singh,[1] Volker Nickeleit.[1] *[1]Pathology, Div of Nephropathology, The Univ of North Carolina, Chapel Hill, NC; [2]Pathology, Leiden Univ Medical Center, Leiden, South Holland, Netherlands.*

**Background:** C4d deposits along peritubular capillaries (ptc) mark humoral rejection in ABO compatible renal allografts. However, the diagnostic significance of C4d deposits along glomerular basement membranes (GBM-C4d), which can occur as isolated event in transplant glomerulopathy (TG), is poorly understood.

**Methods:** We analyzed 318 for-cause renal allograft biopsies from 217 patients (excluded: glomerulonephritis and ABO incompatibility). GBM remodeling was analyzed by light (Banff cg-score) and by electron microscopy. C4d staining by immunohistochemistry (IHC) and immunofluorescence (IF) was scored along the GBM and ptcs. Clinical data included donor specific antibody titers post transplantation. Controls: native kidneys with minimal change disease (n=9) and thrombotic microangiopathy (n=16).

**Results:** TG occurred in 52/318 biopsies (16%). By IF, 49/52 (94%) TG cases showed GBM-C4d; 36/52 (69%) had isolated GBM-C4d lacking concurrent ptc C4d deposits. By IHC, performed on 27/52 TG cases, fewer cases showed GBM-C4d (74%, 20/27 cases); 12/27 (44%) cases had isolated GBM-C4d. GBM-C4d staining was associated with GBM remodeling by light and electron microscopy (X-square tests, p <0.001). GBM-C4d staining intensity was associated with Banff cg-score (p=0.023). GBM remodeling by electron microscopy occured in 29 cases with normal GBM by light microscopy; 23/29 (79.3%) had GBM-c4d staining. Associations between GBM-C4d and GBM remodeling remained significant after omitting cases with donor specific antibodies, C4d staining along ptc, or transplant glomerulitis. Controls: Minimal change disease never had GBM-C4d. 13/16 (81%) thrombotic microangiopathy cases had GBM-C4d and GBM remodeling.

**Conclusions:** GBM-C4d staining is associated with GBM remodeling in native and transplant kidneys. It can precede light microscopic GBM changes and occur independent of donor specific antibodies. As complement seems to play a role in TG, blockage of the complement cascade may have future therapeutic implications.

**TH-PO963**

**Predicting Long-Term Outcomes of Henoch-Schönlein Purpura Nephritis in Adults Using the Oxford Classification of IgA Nephropathy**
Chan Ho Kim,[1] Ji Suk Han,[1] Tae ik Chang,[2] Hyung Jung Oh,[1] Seung Hyeok Han,[1] Tae-Hyun Yoo,[1,3] Dae-Suk Han,[1] Shin-Wook Kang.[1,3] *[1]Dept of Internal Medicine, College of Medicine, Yonsei Univ; [2]NHIC Ilsan Hospital, Gyeonggi-do; [3]Brain Korea 21, Yonsei Univ, Seoul, Korea.*

**Background:** Recently, there has been emerging concern that crescents, the main histologic feature of Henoch-Schönlein purpura nephritis (HSPN), merely reflect active inflammation, and may not be a useful predictor of long-term outcomes. We therefore conducted a single-center retrospective study to evaluate whether the new Oxford classification of IgA nephropathy (IgAN) can be used to predict the long-term outcome in adult patients with HSPN.

**Methods:** We included 61 adult biopsy-proven HSPN patients between January 1991 and August 2010. In addition to the International Study of Kidney Disease in Children classification, pathologic findings were also evaluated by the Oxford classification of IgAN. Primary outcome was defined as either the onset of estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m2 with a ≥30% decrease in baseline eGFR or ESRD.

**Results:** During a median follow-up duration of 49.3 months, 13 patients (21.3%) reached the primary endpoint. A Kaplan-Meier plot showed that renal survival rates were significantly higher in patients with <50% crescents compared to those with crescents in ≥50% of glomeruli (P=0.003). Among the Oxford-MEST lesions, patients with endocapillary hypercellularity (E1) (P=0.02) and tubular atrophy/interstitial fibrosis (T1/T2) (P=0.02) had significantly lower renal survival rates than those with E0 and T0. In a multivariate Cox model adjusted for clinical and pathologic factors, E1 (HR, 8.91; 95% CI, 1.47-53.9; P=0.02) and T1/T2 (HR, 8.74; 95% CI, 1.40-54.4; P=0.02) lesions were independently associated with the primary outcome, whereas the extent of crescentic lesions did not (HR, 3.27; 95% CI, 0.76-14.0; P=0.11).

**Conclusions:** The Oxford classification of IgAN can be used to predict the long-term outcomes of HSPN. The significance of crescentic lesions remains to be further explored, as its prognostic value was attenuated in a multivariate analysis.

## TH-PO964

**Clinicopathological Features and Renal Outcomes of IgA Nephropathy in the Elderly** Yusuke Okabayashi, Nobuo Tsuboi, Akira Fukui, Yoichi Miyazaki, Iwao Ohno, Tetsuya Kawamura, Makoto Ogura, Takashi Yokoo. *Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** The number of elderly patients with renal diseases is increasing in parallel with an increased longevity in the general population. However, information is limited regarding characteristics of each disease in the elderly. This study therefore investigated clinicopathological features of the elderly patients with IgA nephropathy (IgAN), the most common glomerulonephritis worldwide.

**Methods:** A total of 92 IgAN patients over 60 years old were retrospectively analyzed. Two hundred-fifty IgAN patients of 18 to 59 years of age, from the retrospective cohort of Ministry of Health, Labour and Welfare in Japan, were used as comparison (J Nephrol, 2012). Clinicopathological features at biopsy, therapies during the follow-up, renal outcomes and extra-renal complications were evaluated.

**Results:** The average values at diagnosis were age of 65 years, eGFR of 45 ml/min/1.73m$^2$, and urinary protein excretion (UPE) of 1.8 g/day. These features were more serious than those of the control group. Consistent with these clinical features, chronic pathological findings including glomerulosclerosis or interstitial fibrosis/tubular atrophy were more advanced than the control group, while degrees of acute lesions such as crescent formations were almost the same as the control group. As the result of appropriate treatments such as diet, RAS inhibitors, statins or steroids, more than 50% of the patients showed clinical remission (UPE<0.3g/day) at the last observation (median 4.1 years).On the other hand, 21 patients (23%) showed a 50% decrease in their eGFR or reached ESRD. The eGFR at biopsy and the proteinuria at one year were identified as independent factors that were associated with the slope of renal function.Extra-renal complications observed during the follow-up were hypertension (79%), diabetes (13%), dyslipidemia (35%), hyperuricemia (33%) and cardiovascular diseases (29%).

**Conclusions:** In spite of the advanced clinicopathological features and the presence of various extra-renal complications, therapeutic interventions may lead to better renal outcomes of the elderly patients with IgAN.

## TH-PO965

**Innate and Adaptive Immunity in Patients with IgA Nephropathy Who Underwent Tonsillectomy** Elisa Loiacono, Luca Vergano, Roberta Camilla, Rosanna Coppo, Roberto Albera, Daniel C. Cattran, Shubha Bellur, Ian Roberts, John Feehally, Stephan Troyanov, Licia Peruzzi, Alessandro Amore, Laura Morando, Maria Elena Donadio, Giovanni Cancarini, Roberta Tardanico, Loreto Gesualdo, Cristiana Rollino, Elisabetta Moggia, Gianna Mazzucco, Sara Ravera. *VALIGA Network, Città della Salute e della Scienza di Torino, Turin, Italy.*

**Background:** The benefits of tonsillectomy in IgA nephropathy (IgAN) is still debated. Tonsillectomy might remove pathogen sources and reduce the Mucosal Associated Lymphoid Tissue (MALT), limiting dimeric and undergalactosylated IgA1 production.

**Methods:** In the European network VALIGA 62/1147 IgAN underwent tonsillectomy (TxIgAN); 15 were tested and compared to 45 non-tonsillectomized IgAN (No-Tx) and 30 healthy controls (HC) for: a)degalactosylated IgA1; b)Toll-like receptors (TLRs) 2,3,4,9 mRNAs, c)proteasome (PS) and immunoproteasome (iPS) mRNAs; d) advanced oxidated protein products (AOPP).

**Results:** Levels of degalactosylated IgA1 were lower in Tx IgAN than in No-Tx IgAN (p=0.005), however still higher than in HC (p=0.03). TLR mRNAs were more expressed in Tx IgAN than in HC (TLR2,p=0.018;TLR4,p=0.007;TLR9,p=0.009), higher in Tx IgAN than in No-Tx IgAN (p<0.001 for TLR2,4,9). A switch from PS to iPS was detected in PBMC of Tx IgAN, higher than HC and no-Tx IgAN (LMP2/beta1 p=0.01 and LPM7/beta5 p<0.0001). The levels of AOPP in Tx IgAN were significantly higher in Tx IgAN than HC (P<0.0001)and no-Tx IgAN (p=0.0011).

**Conclusions:** Serum degalactosylated IgA1 were lower in tonsillectomized than non-tonsillectomized IgAN, however significantly higher than controls. Activation of innate immunity via TLRs and ubiquitin-proteasome and pro-oxidative milieu were not affected by tonsillectomy. Tonsillectomized patients had signs of mucosal immunity and oxidative stress higher than non tonsillectomized ones, possibily via extra-tonsillar MALT.

## TH-PO966

**Analysis of the Correlation between the Oxford Classification and Clinical Indicators of IgA Nephropathy** Qiuling Fan. *Dept of Nephrology, The First Affiliated Hospital of China Medical Univ, Shenyang, China.*

**Background:** Analyze the correlation and risk factors between clinical indicators and the four main pathological lesions of the Oxford classification in IgAN.

**Methods:** Clinical and pathological data were collected from 514 patients with biopsy-proven IgA nephropathy who were 18 years or older. Spearman's coefficient of rank correlation was performed to evaluate associations between the Oxford classification of IgAN and various clinical indicators. The independent risk factors affecting the pathological classification were analyzed by multivariate regression.

**Results:** In 514 IgAN patients, the average age was 35.70, and the average disease duration was 18.31±30.42 months. M0E0S0T0 was the major pathologic classification of isolated hematuria. Proteinuria and albuminuria were positively correlated with M lesion, serum albumin, C3 and PLT showed a negative correlation with M lesion. Proteinuria and blood platelet count were the independent risk factors for M lesion. The proportion of M1 in cases with proteinuria ≥3.5 g/d is much higher than that in cases with non-nephrotic range proteinuria. Age, SBP, uRBC, proteinuria, albuminuria were positively correlated with E lesion, Duration, serum albuminshowed a negative correlation with E lesion. Age and duration of nephritis were independent risk factors for E lesion. 73.3% of patients more than 60 years old showed endothelial proliferation. CKD stage, proteinuria were positively correlated with S lesion. Age, CKD stage, SBP, DBP, C4, TC, LDL-C, CRP, Fib, UA, Cys-C and proteinuria were positively associated with T lesion, Hb, serum albumin, IgGshowed a negative correlation with T lesion. High CRP levels, DBP more than 90 mmHg, hypoalbuminemia, high low density lipoproteinemia, and anemia were independent risk factors for T lesion.

**Conclusions:** 1. Proteinuria and blood platelet count were the independent risk factors for M lesion. 2. Age and duration of nephritis were independent risk factors for E lesion. 3. CKD stage, SBP and proteinuria were positively correlated with S lesion. 4. High CRP levels, DBP ≥90 mmHg, hypoalbuminemia, high low density lipoproteinemia, and anemia aggravate T lesion.

## TH-PO967

**Prognostic Significance of C1Q Deposition with Renal Outcomes among Patients with IgA Nephropathy** Abdulkareem Alsuwaida,[1] Sufia Husain,[1] Noura Aloudah,[1] Fayez F. Alhejaili,[2] Khaled Alsaad,[2] Hala M. KFoury,[1] Mohammed A. Al-Ghonaim.[3] *[1]King Saud Univ, Saudi Arabia; [2]King Abdulaziz Medical City, Saudi Arabia; [3]Prince Salman bin Abdulaziz Chair for Kidney Research, Saudi Arabia.*

**Background:** It has been reported in a study that mesangial C1q deposition in the glomerulus is associated with a poor renal outcome and severe pathologic features in patients with IgAN. The objective was to investigate the predictive power of co-deposition C1q in predicting long term outcome in patients with IgAN.

**Methods:** Participants with IgAN were divided into two groups based on the renal tissue C1Q status and their baseline and long-term outcome of worsening renal function (WRF) were compared. The rate of WRF was defined as increase in serum creatinine of ≥25% from baseline.

**Results:** Fifty five patients had IgAN and 54.5% had a positive C1Q. The baseline proteinuria was significantly higher among those with positive C1Q ($P$=0.03) while serum creatinine at baseline was not significantly different ($P$=0.3). The distribution of mesangial hypercellularity (M), segmental sclerosis (S), endocapillary proliferation (E), tubular atrophy and interstitial fibrosis (TF) were equally distributed among those with C1q positive and negative group.

Table 1. Clinical and histological feature.

| | C1Q Positive (n=30) | C1Q Negative (n=25) |
|---|---|---|
| Age (Years) Mean±SD | 31.4 (11.1) | 32.9 (13.0) |
| Clinical Presentation(%) | | |
| Elevated serum creatinine | 23 | 44 |
| Hypertension | 16.7 | 48 |
| Nephritic syndrome | 56.7 | 44 |
| Baseline creatinine μmol/L* | 107 (191±216) | 131 (164±110) |
| Last creatinine μmol/L* | 98 (178±196) | 113 (243±255) |
| Oxford classification(%) | | |
| M1 | 76.6 | 72 |
| E1 | 16.7 | 24 |
| S1 | 76.6 | 52 |
| TF1 | 23.3 | 36 |
| TF2 | 13.3 | 20 |
| *Median (Mean±SD); All $P$ values are not significant. | | |

Among 36 patients whom they were follow up greater than one year and a median of 5 years, the rate of WRF was seen in 29.4% for those with C1Q positive and 31.6% for C1Q negative (p=0.8).

**Conclusions:** The tissue C1Q status among patients with IgAN has no long term prognostic significance.

*Funding:* Government Support - Non-U.S.

## TH-PO968

**Renal PLA$_2$R Is a Valuable Biomarker for Idiopathic Membranous Nephropathy** Qionghong Xie, Yan Li, Yueheng Ren, Xiaoye Zhu, Weiyu Zhu, Jianyong Zhong, Jun Xue, Chuan-Ming Hao. *Huashan Hospital, Fudan Univ, Shanghai, China.*

**Background:** The present study characterized PLA$_2$R expression in the kidney and examined its role as a biomarker for idiopathic membranous nephropathy (iMN).

**Methods:** Patients with MN without identifiable secondary causes were included. Anti-PLA$_2$R antibody was detected by indirect immunofluorescence (IF) and renal PLA$_2$R examined using a specific antibody. PLA$_2$R expression has been optimized by testing different antigen retrievals. Microwaving was used in this study unless specified. We compared paraffin-embedded and frozen section of 40 iMNs, 35 were positive and 3 were negative on both sections, 2 were negative on paraffin but positive on frozen section, suggesting frozen sections are more sensitive.

**Results:** In normal kidney, PLA$_2$R was negative by IF or IHC in both paraffin-embedded and frozen section. After antigen retrieval by autoclave, PLA$_2$R was detected in glomeruli along the GBM. However, in renal biopsies with iMN, 47/50 (94%) showed granular staining of PLA$_2$R along the GBM with microwave retrieval. All these 47 tissue samples showed IgG4(+), while the three PLA$_2$R negative samples were IgG4(-) but IgG1(+). In contrast, non-MN primary GN (40) exhibited negative PLA$_2$R staining except one (FSGS) that showed weak PLA$_2$R. In 30 lupus nephritis, 14 exhibited weak PLA$_2$R staining, and in a more scattered pattern.

We then examined the correlation of serum autoantibody and kidney $PLA_2R$ expression. Of 41 MNs with both serum anti-$PLA_2R$ antibody data and renal biopsy $PLA_2R$ (paraffin) available, 24 were anti-$PLA_2R$ antibody positive (59%) and 17 were negative (41%). Among these 17 negative autoantibody cases, 9 exhibited kidney $PLA_2R$(+) staining. Of 24 serum $PLA_2R$ antibody positive cases, only one showed negative for kidney $PLA_2R$.

**Conclusions:** (1) Normally PLA2R is expressed in podocytes, but can only be detected after antigen retrieval (2) In iMN but not non-MN GN, PLA2R immunofluoresecnt becomes positive. (3) Some serum PLA2R autoantibody negative iMN patients show renal tissue PLA2R staining. (4) Kidney PLA2R can also be detected in lupus nephritis. Conclusion: Renal tissue PLA2R can provide additional information for MN diagnosis.

## TH-PO969

**Excellent Long-Term Prognosis and Its Contributing Factors in Korean Idiopathic Membranous Nephropathy Patients** Hyuk Huh, Hajeong Lee, Jung Pyo Lee, Dong Ki Kim, Yun Kyu Oh, Yon Su Kim, Chun Soo Lim. *Internal Medicine, Seoul National Univ Hospital, Seoul, Korea.*

**Background:** Recently, prognosis of idiopathic membranous nephropathy (iMN) has been improved by using antiproteinuric therapies including immunosuppressive agents and renin-angiotensin system (RAS) blockades. In this study, we aimed to explore the long-term prognosis and its contributing factors in Korean iMN patients.

**Methods:** We included consecutive biopsy-proven iMN patients at Seoul National University Hospital. Patients who were younger than 18 years with known secondary causes of MN were excluded. Primary outcome was defined as composite of end-stage renal disease, doubling creatinine. The secondary outcome was complete or partial remission of proteinuria.

**Results:** A total of 302 patients met these criteria between 1979 and 2012. Median length of follow up was 136 months (80 – 249). Mean age was 49 ± 12 years and 183 (60.6%) patients were male. In these patients, 70.9% of patients were treated with immunosuppressant, 74.5% of patients received RAS blockades. Among them, 9.3% of patients reached the primary outcome. Although relapse was observed in 17.3% of patients who was reached remission with primary therapy, patients who reached remission finally was 89.2%. Rate of immunosuppressive agent induced remission was 80.9% and spontaneous remission appeared in 19.1% of patients. RAS blockade significantly improved remission rate even adjusted by age, hypertension, anemia, serum albumin, proteinuria, estimated GFR (eGFR), immunosuppressants (adjusted odd ratio (OR) 4.000, 95% confidence interval (CI) 1.215 – 13.167, P = 0.023). However, increased age and decreased eGFR (eGFR < 60mL/min/1.73m$^2$) (adjusted OR 0.072, 95% CI 0.014 – 0.387, P = 0.002) at the time of biopsy were a significant risk factor for poor response. Also, hypertension (adjusted OR 0.305, 95% CI 0.094 – 0.987, P = 0.048), anemia (adjusted OR 0.200, 95% CI 0.058 – 0.691, P = 0.011) could predict poor response.

**Conclusions:** Patients of Korean iMN had excellent prognosis in comparison with Caucasian. This study suggests RAS blockades ameliorate renal outcome of Korean idiopathic membranous nephropathy.

## TH-PO970

**Subclass Analysis of Glomerular IgG Deposit in Patients with Various Types of Glomerulonephritis** Raima Nakazawa, Noriko Kaneyama, Hisae Tanaka, Qiong Wu, Masayuki Endoh, Masafumi Fukagawa. *Dept of Internal Medicine, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan.*

**Background:** IgG subclass restriction is known to be observed in some glomerular IgG deposit. However it is not established as a routine clinical procedure for the diagnosis of glomerular diseases to study IgG subclasses. In order to evaluate usefulness of routine staining of IgG subclasses for renal biopsy specimen, we performed IgG subclass staining for two hundred fifty eight glomerulonephritis (GN) patients who had IgG deposit in their glomeruli shown by ani-whole human IgG antibody.

**Methods:** One hundred thirty nine patients with idiopathic membranous nephropathy (IMN), 67 of lupus nephritis [in which 17 cases are membranous lupus nephritis (MLN)], 20 of IgA nephropathy, 13 of anti-GBM nephritis and 19 of other GN were included in this study. Immunofluorescent microscopy using FITC labeled mouse monoclonal anti-human IgG1, IgG2, IgG3 and IgG4 antibodies (Southern Biotechnology, USA) was used to detect IgG subclasses in the glomeruli of these patients.

**Results:** Fifty two percent of IMN shows IgG1 and IgG4 combination deposit, whereas only 5.9 % of MLN shows IgG1 and IgG4 combination. Specificity of IgG1+IgG4 combination deposit in IMN is 94 %. Patients with LN show various combination of IgG subclasses. Fifty nine percent of LN are shown to have IgG1+IgG2+IgG3 combination. Ninety two percent of anti-GBM nephritis are revealed to be only IgG1 subclass antibody mediated diease. Sixty five percent of IgG deposit in IgA nephritis and Henoch-Shoenlein nephritis was shown to be IgG1 restricted. Three cases of proliferative glomerulonephtitis with monoclonal IgG deposit (PGNMID) were found in this study. All three cases are shown to have IgG3-k deposit.

**Conclusions:** It is concluded that routine IgG subclass staining in renal biopsy is useful tool for definite diagnosis of various types of glomerulonephritis which have glomerular IgG deposit. Further study is mandatory to elucidate the pathogenesis and the mechanism of restricted IgG subclass deposition in each type of glomerulonephritis.

*Funding:* Private Foundation Support

## TH-PO971

**Dutch Transplantation in Vasculitis (DUTRAVAS)-Study: Outcome of Renal Transplantation in ANCA-Associated Vasculitis** Arda Goceroglu,[1] Chinar Rahmattulla,[1] Annelies Evaline Berden,[1] Marlies Reinders,[1] Marcory van Dijk,[2] Anoek A.E. de Joode,[2] Carine Peutz-Kootstra,[3] Maarten H.L. Christiaans,[3] Iris Noorlander,[4] Roel Goldschmeding,[5] Arjan D. Van Zuilen,[5] Eric Steenbergen,[6] Luuk Hilbrands,[6] Lorraine Harper,[7] Mark Alan Little,[8] Ernst C. Hagen,[9] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Leiden Univ MC; [2]Univ MC Groningen; [3]Maastricht Univ MC; [4]Erasmus MC Rotterdam; [5]Univ MC Utrecht; [6]Radboud Univ MC Nijmegen; [7]Univ of Birmingham; [8]Trinity College Dublin; [9]Meander MC Amersfoort.*

**Background:** We present a multi-center study on the outcome of renal transplantation in ANCA-associated vasculitis (AAV) patients focusing on renal disease recurrence and graft survival within 5 years after transplantation.

**Methods:** In total 113 AAV patients (first renal transplantation) were included from 6 university hospitals. Clinical data and transplant biopsies were collected through PALGA (Dutch national pathology database) and a center-specific search. Three patients had immediate graft loss and were excluded from analysis. Transplant biopsies were scored with Banff '09. Renal disease recurrence was scored with the histopathologic classification of ANCA-associated glomerulonephritis. We focused on the first 5 years after transplantation.

**Results:** Sixteen grafts were lost due to acute rejection (3), renal disease recurrence (4), IFTA (4), sepsis (2), infarction (1), cyclosporine toxicity (1), and post-transplant lymphoproliferative disorder (1). Five year graft survival was 82.8%. Thirteen patients had 16 disease relapses; 14 (11 patients) involved the graft. The risk for a first disease recurrence in the graft was 2.8% per patient year within 5 years follow-up. Ten recurrences were classified as: 5 focal, 1 crescentic and 4 mixed class. Four recurrences were clinically diagnosed (no histological confirmation). Of the 4 recurrences that led to graft loss, 3 could be classified: 1 focal and 2 mixed class.

**Conclusions:** This largest cohort study to date shows that a substantial proportion of patients with renal disease recurrence of AAV lost their graft due to the recurrent disease.

## TH-PO972

**The Histopathologic Classification of ANCA Associated Glomerulonephritis Is Associated with the Occurrence of Renal Relapse** Arda Goceroglu,[1] Marta Fiocco,[1] Annelies Evaline Berden,[1] Jan A. Bruijn,[1] David R.W. Jayne,[2] Ingeborg M. Bajema.[1] *[1]Leiden Univ MC; [2]Addenbrooke's Hospital Cambridge.*

**Background:** Patients with ANCA-associated glomerulonephritis (AAGN) at high risk of relapse should be identified early to improve clinical management. We investigated whether the histopathologic classification of AAGN is associated with the occurrence of renal relapse.

**Methods:** Diagnostic renal biopsies and clinical data of 113 patients with mild-severe AAGN from 2 European randomized clinical trials were included. Renal relapse was defined as new hematuria or proteinuria and/or a >30% rise in serum creatinine attributable to active AAGN. We used 2 competing risks methods (applied when >1 competing endpoints are possible). Competing endpoints were ESRF and death. For an etiological association we used the cause-specific proportional hazards method. For a predictive association we used the Fine & Grey method. We analysed 2 predefined multivariate models. Model 1 included class, age, creatinine and plasmapheresis. Model 2 included class, creatinine, diagnosis and ANCA-type.

**Results:** CAUSE-SPECIFIC PROPORTIONAL HAZARDS METHOD: In both models the histopathologic class was the only independent baseline parameter associated with renal relapse. Focal (model 1: HR=0.10, 95%CI 0.02-0.60, p=0.01; model 2: HR=0.09, 95%CI 0.02-0.55, p=0.01) and crescentic class (model 1: HR=0.21, 95%CI 0.07-0.62, p<0.01; model 2: HR=0.21, 95%CI 0.07-0.64, p=0.01) were associated with less patients experiencing renal relapse compared to sclerotic class. FINE & GREY METHOD: The histopathologic class was not associated with renal relapse in both models. Only higher age (model 1) was associated with lower risk for renal relapse (HR=0.97, 95%CI 0.94-0.99, p=0.02).

**Conclusions:** Results from the analyses indicate that the histopathologic class of AAGN has a role in the etiology of renal relapse, but does not identify patients at high risk for renal relapse. In sclerotic class patients renal relapses may be more readily detected because of the limited compensatory capacity of the kidney. Alternatively, different classes may represent different phenotypes of AAGN in which renal relapses are combined with more chronic lesions.

**TH-PO973**

**ENT Involvement Is Related to Better Renal Function in Patients with ANCA-Associated Vasculitis (AAV)** Chinar Rahmattulla,[1] Robert A. De Lind van wijngaarden,[2] Annelies Evaline Berden,[3] Herbert Hauer,[4] Oliver Flossmann,[5] David R.W. Jayne,[6] Niels Rasmussen,[7] Laure-Helene Noel,[8] Franco Ferrario,[9] Ruediger Waldherr,[10] Charles D. Pusey,[11] Ernst C. Hagen,[12] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Leiden UMC; [2]Erasmus MC Rotterdam; [3]Bronovo Hospital; [4]MC Leeuwarden; [5]Royal Berkshire Hosp.; [6]Addenbrooke's Hosp.; [7]Statens Seruminstitut; [8]Necker Hosp.; [9]San Gerardo Hosp.; [10]Univ of Heidelberg; [11]Imperial College London; [12]Meander MC.*

**Background:** It has been suggested that ear, nose, and throat involvement (ENT+) can prompt early diagnosis of AAV and that ensuing rapid instigation of therapy can prevent renal impairment. Therefore, this study investigates whether ENT+ AAV patients have better renal function and fewer chronic renal histopathologic lesions at time of diagnosis than ENT- patients.

**Methods:** 441 newly diagnosed AAV patients with renal involvement from 4 international, prospective, multicenter trials were included. Age, ENT+, ANCA type, interstitial fibrosis and tubular atrophy (IFTA), tubulitis, interstitial infiltrates and the histopathologic class for ANCA-associated glomerulonephritis (AAGN) (n=151) were correlated with eGFR at diagnosis (eGFR0).

**Results:** Multivariate analysis revealed that ENT+ (n=212) ($r=0.25, p<0.001$), age ($r=-0.34, p<0.001$), IFTA ($r=0.16, p<0.001$), tubulitis ($r=0.16, p=0.001$), interstitial infiltrates ($r=0.20, p<0.001$) and the classification ($r=4.1p<0.001$) were associated with eGFR0. A prespecified sensitivity analysis including only PR3-ANCA-positive patients showed similar results. ENT+ patients had a higher eGFR0 (60 vs. 44 mL/min, $p<0.001$), less IFTA ($p=0.001$) and more often class I/II than class III/IV in their renal biopsies ($p<0.001$). Increasing numbers of BVAS ENT parameters correlated with increasing renal function ($p<0.001$).

**Conclusions:** In AAV, ENT+ is associated with better eGFR0 and characterized by class I/II on renal histopathology, also in the PR3-ANCA subgroup. These findings indicate that ENT+, possibly because symptoms are clinically overt, leads to a prompt diagnosis of AAV. Reduced diagnostic delay means identifying renal impairment at an early stage, which – with prompt start of treatment – has a known favourable effect on renal outcome.

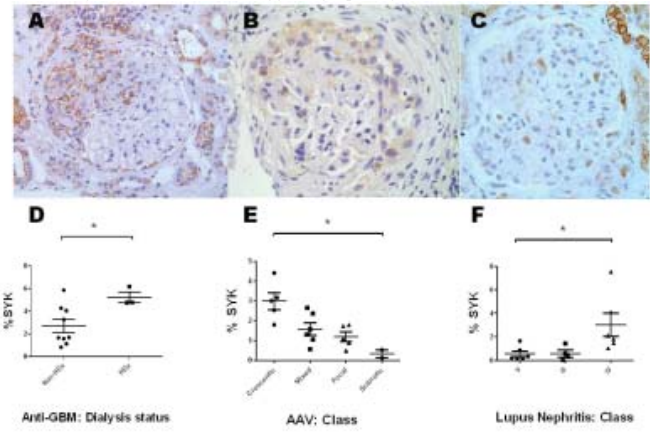
**TH-PO974**

**Spleen Tyrosine Kinase (SYK) Expression Correlates with Disease Activity and Outcome in Glomerulonephritis** Stephen Paul McAdoo, Gurjeet Bhangal, Anisha Tanna, Liz Lightstone, H. Terence Cook, Charles D. Pusey, Frederick W.K. Tam. *Imperial College London.*

**Background:** Spleen tyrosine kinase (SYK) has a critical role in immunoreceptor signaling. SYK inhibition has shown efficacy in animal models of immune-mediated renal injury, including murine lupus and, in work previously reported by our group, autoimmune glomerulonephritis and nephrotoxic nephritis. However, SYK expression in human renal tissue is not well characterised.

**Methods:** We conducted immunohistochemical analysis for total and phosphorylated SYK in clinical biopsy specimens (thin basement membrane, n=4; minimal change, 4; anti-GBM disease, 12; ANCA vasculitis, 18; lupus nephritis, 16). SYK staining was quantified using automated image analysis software and related to histological class and clinical outcome.

**Results:** Glomerular staining for total SYK was positive in anti-GBM disease, AAV and lupus nephritis (Fig A,B,C respectively), but not thin basement membrane disease or minimal change. SYK expression appeared to localise to inflammatory lesions (areas of crescent formation or endocapillary proliferation). SYK expression correlated with disease severity and/or histological class (Fig D,E,F). In anti-GBM and AAV, glomerular staining for phosphorylated SYK was positive and localized to areas of crescent formation, demonstrating SYK activation and suggesting a pathogenic role.




*$p<0.5$.

**Conclusions:** SYK is expressed in a variety of autoimmune renal diseases, and this is the first report that glomerular expression levels appear to correlate with disease activity and outcome. Clinical studies targeting SYK in glomerulonephritis are warranted.

**TH-PO975**

**Immuno-Histological Detection of Parietal Epithelial Cells Distinguishes Early FSGS from Minimal Change Disease** Bart Smeets,[1] Hermann-Josef Groene,[2] Rutger J. Maas,[3] Vivette D. D'Agati,[4] Agnes B. Fogo,[5] Toin Van Kuppevelt,[3] Jürgen Floege,[1] Jack F. Wetzels,[3] Marcus J. Moeller.[1] *[1]Univ Hospital of the RWTH Aachen; [2]German Cancer Research Center (DKFZ); [3]RUNMC, Nijmegen; [4]Columbia Univ; [5]Vanderbilt Univ Medical Center.*

**Background:** Recently, we have shown that parietal epithelial cells (PECs) are crucially involved in the development of FSGS lesions. In the presents study we investigated whether immuno-histological detection of (activated) PECs present on the glomerular tuft can be used to distinguish early FSGS from minimal change disease (MCD).

**Methods:** 95 renal biopsies containing 8 or more glomeruli from 38 adult patients diagnosed with "early" FSGS and 57 patients with newly diagnosed MCD were stained for claudin-1 (PEC marker), CD44 (PEC activation) and LKIV69 (PEC matrix). Unaware of the original diagnosis, the glomeruli were revaluated. Biopsies showing staining of one or more of the PEC markers in a visceral location were assigned as FSGS. Those negative for the PEC markers were assigned as MCD.

**Results:** Revaluation of the biopsies with the PEC markers confirmed the original diagnosis (FSGS or MCD) in 76 biopsies (80% of total) and could confirm in 33 of 38 (87%) biopsies diagnosed with FSGS the presence of glomerulosclerosis. PECs were involved in the development of FSGS lesions from the earliest stages of disease. In the remaining 5 biopsies diagnosed as FSGS, no PEC marker staining was detected – most likely due to the focal nature of the lesions and because only a single slide of each biopsy was evaluated. Interestingly, in 14 of 57 (25%) biopsies originally diagnosed as MCD we detected the presence of FSGS lesions. These lesions were typically small and difficult or non-detectable in PAS stained sections but could be detected by PEC matrix marker LKIV69.

**Conclusions:** Immunohistological detection of PECs on the glomerular tuft very likely improves the sensitivity of the FSGS diagnosis. In particular, the detection of parietal matrix using the LKIV69 antibody may be a more sensitive tool to detect early FSGS lesions, which might otherwise be missed on PAS stained sections.

*Funding:* Government Support - Non-U.S.

**TH-PO976**

**Kidney Disease in the Obese: The Full Biopsy Spectrum** Steven Salvatore, Surya V. Seshan. *Pathology, Weill Cornell Medical College, New York, NY.*

**Background:** Obesity related glomerulopathy (ORG) is a well-recognized pathologic process attributed to hyperfiltration podocyte injury in which glomerular hypertrophy, perihilar segmental sclerosis, and partial foot process effacement lead to subnephrotic proteinuria in patients with excess body mass. Renal biopsies are performed in obese patients with clinical renal disease which may be directly related to obesity or secondary to associated or unassociated medical conditions.

**Methods:** Native kidney biopsies from 2000-2012 were retrospectively reviewed from patients with obesity, body mass index >30. Glomerular diameter was measured using a standard micrometer in 1-7 glomeruli cut through the hilum (mean 4). The clinicopathologic characteristics are analyzed.

**Results:** Of 6702 total native kidney biopsies, 287 (4%) were obtained from obese patients (mean: weight 122kg, BMI 40.3kg/m2). Associated factors such as insulin resistance (31%), hypertension (60%), and obstructive sleep apnea (9%) were frequent. Indication for biopsy was proteinuria in 93%, nephrotic (40%) and subnephrotic (53%). 52% also had renal insufficiency, defined as Cr >1.5mg/dL. Typical lesions of ORG were seen in 41% of cases: focal segmental glomerulosclerosis (FSGS) 28% - perihilar variant (9%), and glomerulomegaly alone 13%. Diabetic nephropathy was seen in 22% of all obese patients and 70% of the obese diabetic patients. Immune complex (IC) disease 15%, tubulointerstitial (TI) disease 5%, vascular disease 5%, thin basement membranes 5%, minimal change disease 3%, idiopathic FSGS 2%, and collapsing glomerulopathy 2% were seen. Glomerulomegaly, glomerular diameter greater than 180 μm, was present in 84% of cases (mean 224 μm) vs normal size in 11% (mean 157 μm). Proteinuria was highest in patients with idiopathic FSGS (mean 8g/24hrs) and IC GN (mean 7.4g/24hrs) and was subnephrotic in obesity-related FSGS (61%), glomerulomegaly-alone (82%), and TI diseases (80%). Creatinine levels were highest in TI diseases (mean 8.4mg/dL) and progressive DN (mean 2.5mg/dL).

**Conclusions:** Diverse kidney pathology superimposed on ORG is present in obese patients with clinical renal disease. Kidney biopsy is important for accurate diagnosis, prognosis and treatment.

**TH-PO977**

**Glomerular Lesions in Chronic Smoking and Hypertension Are Not Always Nodular Sclerosis** Steven Salvatore,[1] Megan L. Troxell,[2] Douglas Hecox,[3] Kevin R. Sperling,[4] Surya V. Seshan.[1] *[1]Weill Cornell Medical College, New York, NY; [2]Oregon Health and Science Univ, Portland, OR; [3]Renal Care Consultants, Medford, OR; [4]Crozer-Chester Medical Center, Chester, PA.*

**Background:** Idiopathic nodular glomerulosclerosis (ING) is a well-recognized entity associated with chronic smoking and hypertension leading to glomerular mesangial nodule formation which clinically and pathologically mimics that seen in nodular diabetic nephropathy (DN). However, like DN, diffuse mesangial sclerosing changes may occur in this setting in the absence of nodules.

**Methods:** The clinicopathologic features of 8 non-diabetic patients with long smoking history found in 5139 native kidney biopsies from 2003-2012 showing diffuse mesangial glomerulosclerosis (5) or ING (3) were analyzed.

**Results:** The patients had similar clinical characteristics, 7/8 were men, age 58-84 years with smoking history of 15-72 pack-years. None of the patients manifested obesity or diabetes, but all had hypertension. Other cardiovascular comorbidities were also present including peripheral vascular disease, heart disease and stroke. At the time of biopsy, the mean creatinine was 1.8mg/dL (range 1.4-5.4) and the mean proteinuria was 3.7g/24hrs. The renal pathologic findings were similar in all patients except mesangial nodules in ING. Global glomerulosclerosis was seen in 6-65% of glomeruli (mean 29%), glomerulomegaly in 7/8, and the range of interstitial fibrosis was 10-80% (mean 40%). Moderate (25%) and severe (75%) arteriosclerosis and arteriolar hyalinosis (86%) were also observed. Glomerular hilar neovascularization was prominent. Capillary wall double contours, subendothelial widening, endothelial swelling and new basement membrane formation suggesting chronic healing thrombotic microangiopathy (TMA) was noted in 88%. However, no immune complexes were localized.

**Conclusions:** Kidney biopsies showing either diffuse or nodular mesangial sclerosis from patients with proteinuria and chronic renal insufficiency may be due to prolonged smoking and hypertension. Chronic glomerular endothelial injury may result from smoking and cause a healing TMA appearance in the appropriate setting, even in the absence of mesangial nodule formation.

## TH-PO978

**Endothelial Cell Injury May Involved in Pathogenesis of Secondary Focal Segmental Glomerulosclerosis Associated with Myeloproliferative Disease** Emiko Fujita,[1] Akira Shimizu,[2] Megumi Fukui,[1] Akiko Mii,[1] Shuichi Tsuruoka.[1] *[1]Dept of Nephrology, Nippon Medical School, Bunkyo-ku, Tokyo; [2]Dept of Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

**Background:** Recently, JAK2V617F mutation may cause myeloproliferative disease(MPD) including polycythemia vera (PV), essential thrombocythemia (ET). The frequency of JAK2 mutation may determine which cell lineages increase, possibly leading to the diversity of MPD. Several reports describe MPD cases have some degree of proteinuria due to biopsy proven focal segmental glomerulosclerosis (FSGS). The pathogenesis of FSGS associated with MPD remains unknown. Endothelial cell abnormalities with mutation of JAK2V617F were reported in PV patients. We herein report three cases of FSGS associated with MPD with endothelial cell injury.

**Methods:** We observed three cases with persistent proteinuria, two of them are nephritic range, after a 8 to 10-year history of MPD. The type of MPD was PV in two patients, unclassifiable MPD in one. Renal biopsy was performed and assessed the pathological characteristics of these cases. We examined serum thrombomodulin(TM) and VEGF to assess endothelial cell injury.

**Results:** Renal biopsy revealed some glomeruli have segmental sclerosis with foam cell infiltration and podocyte proliferation, double contours, and mesangial sclerosis and hypercellularity on light microscopy. Arteriosclerosis was seen in all cases and ranged from mild in unclassifiable MPD, to severe in PV case which was looks like onion skin thickening. Electron microscopy showed endothelial cell proliferation, loss of fenestra, and separation of endothelial cells from capillary basement membrane with subendothelial edema. Serum VEGF and TM level was elevated in PV cases accompanied by JAK2V617F mutation, whereas normal in JAK2 mutation –negative unclassifiable MPD. Endothelial cell injuries in glomeruli and small arteries were prominent in the cases with the mutation of JAK2V617F.

**Conclusions:** We described three cases of glomerulopathy with MPD patients revealed FSGS accompanying endothelial cell injuries in glomeruli and small arteries. Endothelial cell abnormalities with mutation of JAK2V617F may have involvement in pathogenesis in FSGS associated with MPD.

## TH-PO979

**Histopathologic Findings Associated with *APOL1* Risk Variants in Chronic Kidney Disease** Christopher Patrick Larsen, Josephine M. Ambruzs, Larry N. Cossey, Nidia Cordeiro Messias, Patrick D. Walker. *Nephropath, Little Rock, AR.*

**Background:** African Americans (AA) have a greatly increased risk of kidney disease which can be explained by the presence of risk alleles in the *APOL1* gene. The *APOL1* disease spectrum includes primary FSGS, non-diabetic ESKD, and collapsing glomerulopathy. We sought to perform an *APOL1* genotype-phenotype correlation in renal biopsies from AA with CKD.

**Methods:** AA patients with an eGFR<60 mL/min/1.73 m$^2$ for 3 months with a diagnosis of arterionephrosclerosis on biopsy were included. *APOL1* risk allele genotyping was performed using TaqMan assays. 114 biopsies were included including 58 biopsies which had 2 risk alleles and 56 biopsies with 0 risk alleles. A blinded morphologic evaluation of each compartment of the kidney was carried out.

**Results:** We found significantly less obsolescent glomerulosclerosis, more thyroidization-type tubular atrophy and more microcystic tubular dilatation in the patients with 2 risk alleles. There was more arteriosclerosis in the 0 risk allele group. The degree of foot process effacement was not significantly different in these two groups.

| Morphology | 0 risk alleles | 2 risk alleles |
|---|---|---|
| Glomeruli, mean (range) | 19.2 (8-48) | 15.8 (8-50) |
| Global sclerosis, mean (%) | 9.4 (49%) | 9.7 (61%) |
| -Obsoclescent, mean (%) | 5.3 (56%) | 3.0 (31%) |
| -Solidified, mean (%) | 2.3 (25%) | 3.4 (35%) |
| -Disappearing, mean (%) | 1.8 (19%) | 3.3 (34%) |
| FSGS, mean (%) | 1.5 (8%) | 1.6 (10%) |
| Degree GBM thickening | 1.1 | 0.9 |
| Degree foot process effacement | 1.3 | 0.9 |
| Degree tubular atrophy | 2.3 | 2.7 |
| -Degree thyroidization-type TA | 0.4 | 0.9 |
| -Degree endocrie-type TA | 0.2 | 0.2 |
| -Degree classic-type TA | 1.7 | 1.6 |
| % cases with microcystic tubular dilitation | 7% | 31% |
| Degree arterial intimal fibrosis | 2.4 | 2.1 |
| Degree arteriolar hyalinosis | 1.5 | 1.0 |

**Conclusions:** We present the first detailed description of the biopsy changes associated with APOL1 risk alleles in the setting of CKD. These findings suggest that APOL1-related CKD is not driven by arteriosclerosis or a primary podocytopathy. Additionally, they allow prediction of the presence of APOL1 risk alleles in this demographic based on renal biopsy findings.

## TH-PO980

**Prediction of Renal Outcome in Patients with Diabetic Nephropathy Using the Pathological Classification** Yu An, Feng Xu, Yongchun Ge, Cai-Hong Zeng, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing Univ School of Medicine.*

**Background:** In 2010, a pathologic classification of diabetic nephropathy (DN) was launched by Tervaert et al. But its value in the prediction of clinical outcome is largely undetermined. Our aim was to evaluate its predictive power in type 2 diabetes mellitus (T2DM).

**Methods:** We enrolled 414 T2DM patients who had biopsy-confirmed DN and were followed for at least a year after biopsy. All cases were categorized according to the pathologic classification of Tervaert et al.Some common pathological changes of DN were also examined. The relevancies between pathological findings and renal outcome were assessed. A renal event was defined as eGFR<15ml/min/1.73m$^2$.

**Results:** Cox regression showed that the glomerular classes, </a>interstitial fibrosis and tubular atrophy (IFTA) and interstitial inflammation can significantly influence renal survival in these patients. Scores of arteriolar hyalinosis and arteriosclerosis were not significant variables. More than one area of arteriolar hyalinosis was commonly found in 95.4% of these patients, indicating that this index may not be suitable for classification. Patients with nodule lesions, glomerular microaneurysms, atubular glomeruli, segmental sclerosis, hyaline caps, fibrinoid exudation, foam cells within loops, glomerular inflammation, crescents, segmental endothelial proliferation and acute tubular injury had a worse renal survival. Multivariate COX analysis showed that the glomerular classes, IFTA and acute tubular injury were independent risk factors for renal prognosis, even when adjusted for proteinuria, blood pressure and eGFR.

**Conclusions:** The glomerular classification and IFTA are significantly associated with renal outcome in patients with T2DM, independently of proteinuria, blood pressure and eGFR. However, the vascular indexes in the classification are incapable to reflect the difference in vascular lesion severity of the patients and can't be used for renal prognosis, suggesting a necessity to redefine them. Finally, acute tubular injury is also an independent risk factor of the progression of DN to ESRD.

*Funding:* Government Support - Non-U.S.

## TH-PO981

**Misdiagnosing Renal Amyloidosis as Adult Minimal Change Disease: Is It Time for a Change in Practice?** Rabya H. Sayed,[1,2] Janet A. Gilbertson,[2] Paul Steven Bass,[1] Philip N. Hawkins,[2] Helen J. Lachmann,[2] Julian D. Gillmore.[2] *[1]Centre for Nephrology, UCL. Royal Free Campus, United Kingdom; [2]National Amyloidosis Centre, UCL Div of Medicine, Royal Free Campus, London, United Kingdom.*

**Background:** Minimal change disease (MCD) accounts for 10-15% of all adult nephrotic syndrome (NS) cases and diagnosis relies on normal renal light microscopy with foot process effacement on electron microscopy (EM). Renal amyloid deposits (RAD) are identified by red-green birefringence under cross-polarized light after staining with Congo Red (CR) and fibrils seen on EM support the diagnosis further. Late diagnosis and delayed treatment of renal amyloidosis negatively impacts on renal and overall prognosis.

**Methods:** A retrospective analysis of 2116 patients referred with histologically proven renal amyloidosis to the UK National Amyloidosis Centre (NAC) between 2001 and 2013 was performed. Twenty-nine patients were identified in whom the initial renal histology was reported as MCD before subsequent identification of RAD. All available biopsies from these patients were reviewed by two independent specialists.

**Results:** Mean age at MCD diagnosis was 58.6 years and median time period between the diagnosis of MCD and amyloidosis was 1 year (range 2 weeks to 10 years). MCD was diagnosed in the absence of EM in 13 of 29 cases. EM pictures were subsequently obtained/reviewed in 9 such cases, all of which showed fibrils typical of amyloid. MCD was diagnosed in the absence of CR staining in 17 cases which was diagnostic of amyloid in each of 13 cases that were retrospectively stained. The mean number of renal biopsies taken prior to reaching a diagnosis was 1.6. The overriding reason for reviewing or repeating renal biopsies was steroid-resistance of proteinuria, which averaged 7.8 g/24hr at presentation.

**Conclusions:** This analysis clearly demonstrates the importance of undertaking EM and CR staining in adults with NS and the value of reviewing original biopsies with EM and CR prior to undertaking another renal biopsy. Early diagnosis of amyloid is likely to limit morbidity and mortality associated with a prolonged nephrotic state, progression of untreated amyloidosis and repeated renal biopsies.

*Funding:* Government Support - Non-U.S.

## TH-PO982

**Medullary Epithelial Mass Morphometry in Human Renal Biopsies: Progressive Injury and Relationship to Cortical Epithelial Mass**

Alton Brad Farris,[1] Diane H. Lawson,[1] Cynthia Na Cohen,[1] Seymour Rosen.[2]
*[1]Emory Univ; [2]Harvard Univ/Beth Israel Deaconess Medical Center.*

**Background:** The outer medulla has two major zones: outer stripe (OS) and inner stripe (IS). OS cellular mass is primarily formed by proximal tubules and is limited in extent. IS consists of an epithelial cell mass (EPCM) formed mostly by thick ascending limbs and collecting ducts. OS and IS zonal delineation depends on recognizing corticomedullary junction connective tissue/vascular components, adjacent OS, and vasa recta. To characterize injury in these regions, we conducted a morphometric study.

**Methods:** Consecutive native renal biopsies (n=37) were examined; and the sole inclusion criteria was the availability of medulla (primarily outer medulla). Trichrome, PAS, and cytokeratin (CK) immunostained slides were digitally scanned to obtain whole slide images. For the entire cortex and entire medulla, EPCM was quantitated using a positive pixel count algorithm tuned to detect the brown chromogen of the CK stain and the red staining of trichrome. In addition, pathologist visual assessment was recorded for % tubular atrophy (%TA) and % composed of EPCM.

**Results:** The EPCM (mean±standard deviation [SD]) of the cortex and medulla, respectively, were 61±12% and 45±12% on trichrome; 55±11% and 53±11% on CK, and 46±24%; and 24±15% on pathologist assessment. Cortical and medullary EPCM declined together, with linear regression showing direct relationships between cortical and medullary EPCM by trichrome ($r=0.64$ between cortex and medulla, $p<0.0001$) and CK ($r=0.87$, $p<0.0001$). The OS and IS width was 1.7±0.9 and 4.7±2.7 mm (mean±SD), respectively. Notably, IS width inversely correlated with trichrome EPCM ($r=-0.64$, $p<0.0001$ for all tissue and $r=-0.58$, $p=0.002$ for the medulla).

**Conclusions:** Medullary and cortical EPCM are related, diminishing together. Furthermore, IS expansion is associated with reduced EPCM. Thus, as epithelial cell elements are lost, reactive/fibrotic responses cause interstitial expansion and IS zonal widening. Analyzing and quantitating EPCM in medullary injury may provide a unique perspective to examine human chronic kidney disease.

## TH-PO983

**The NEPTUNE Digital Pathology Morphologic Profiling of Nephrotic Syndrome**

L. Barisoni,[1,2] C. Avila-Casado,[2] A. Gasim,[2] J. Troost,[2] S. Bagnasco,[2] J.B. Hodgin,[2] J. Charles Jennette,[2] D.B. Johnstone,[2] Jeffrey B. Kopp,[2] Catherine M. Conway,[2] Stephen M. Hewitt,[2] Cynthia C. Nast.[2] *[1]U Miami; [2]NEPTUNE.*

**Background:** Pathologic classifications of nephrotic syndrome historically have been performed by light microscopy using known diagnostic entities. The Nephrotic Syndrome Study Network (NEPTUNE) has taken a novel approach by a) applying digital pathology review on renal biopsy whole slide imaging (WSI) and immunofluorescence (IF) and electron microscopy (EM) digital images from FSGS, MCD and MN patients; b) morphologically profiling renal biopsies using "descriptors" of injury for all renal compartments.

**Methods:** We defined 49 histologic, 10 IF and 14 EM descriptors. Glomerular histologic descriptors were applied to individually identified, digitally-annotated multilevel-reconstructed glomeruli. As proof of concept and to test descriptor-based profiling reproducibility of WSI and digital EM images, podocyte descriptors (see table) were evaluated in 133 FSGS/MCD biopsies by 3 pathologists: junior, mid-career and senior (P1, P2 & P3). We used Kendall's coefficient of concordance to measure inter-reader reliability on an ordinal scale across all 3 pathologists as well as pairwise comparisons between each pathologist.

**Results:**

| Kendall's coefficient of concordance for each comparison | | | | |
|---|---|---|---|---|
| | Effacement (0-4+) | Cytoskeletal condensation (0-2+) | Microvillous transformation (0-2+) | Loss of primary processes (0-2+) |
| P1, P2, P3 | 0.923 | 0.631 | 0.714 | 0.445 |
| P1 vs P2 | 0.939 | 0.709 | 0.763 | 0.609 |
| P1 vs P3 | 0.925 | 0.741 | 0.788 | 0.514 |
| P2 vs P3 | 0.964 | 0.720 | 0.804 | 0.617 |

**Conclusions:** NEPTUNE descriptor-based morphologic profiling applied to a web-based information system provides comprehensive analysis of renal structures. Preliminary inter-observer reproducibility ranges from excellent (effacement) to good (loss of primary processes), independently from the reader experience. The granularity of morphologic profiling may improve correlation with clinical and molecular signatures, provide more robust information regarding the significance of specific lesions for prognosis and therapeutic response, and set the bases for new classification systems.

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## TH-PO984

**Granulomatous Interstitial Nephritis Secondary to Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma**

Samih H. Nasr, Tait Shanafelt, Curtis Hanson, Sanjeev Sethi, Lynn D. Cornell, Mary E. Fidler, Nelson Leung.
*Mayo Clinic.*

**Background:** Granulomatous interstitial nephritis (GIN) is an uncommon lesion with a native kidney biopsy incidence of 6%. Established causes of GIN include drug hypersensitivity, infection (particularly fungi), sarcoidosis, and TINU syndrome. Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) has not been previously recognized as a potential cause of GIN.

**Methods:** We report the clinicopathologic findings and outcome of 4 CLL/SLL patients who developed GIN.

**Results:** The cohort consisted of 2 males and 2 females, all Caucasians, with a mean age of 72.8 years at renal biopsy. CLL/SLL was diagnosed concurrently with GIN in 1 patient and 2-8 years prior to GIN in the remaining 3. CLL/SLL stage at the time of GIN diagnosis was 0 in two patients, 1 in one, and 3 in one. All patients presented with severe renal impairment with mean serum creatinine at renal biopsy of 4.7 mg/dl. Three patients had leukocyturia and 1 hematuria.

Histologically, all cases showed interstitial inflammation (diffuse in 3 and patchy in 1), lymphocytic tubulitis, non-necrotizing granulomas with multinucleated giant cells, and focal acute tubular injury. Interstitial eosinophils were not prominent in any case. In one case, the granulomatous reaction also involved arteries. Focal interstitial involvement by CLL/SLL was noted in 3 cases. One patient also had granulomatous reaction in skin lesions and lymph nodes.

GIN was treated with steroids alone in 2, steroids and cyclophosphamide in 1, and sequential treatments with steroids, cyclophosphamide, rituximab, vincristine, and alemtuzumab in 1. Duration of follow up ranged from 2 to 66 months. All 4 patients had a partial response to therapy (mean follow up serum creatinine 2.6 mg/dl, mean decrease in serum creatinine 5.1 mg/dl). One patient had multiple relapses of GIN occurring during CLL/SLL flares which responded to lymphoma chemotherapy.

**Conclusions:** We report a novel association between GIN and CLL/SLL. GIN secondary to CLL/SLL may occur with or without interstitial lymphomatous infiltration and extrarenal granulomatous reaction. Steroids (with or without lymphoma directed chemotherapy) leads to partial improvement of kidney function.

## TH-PO985

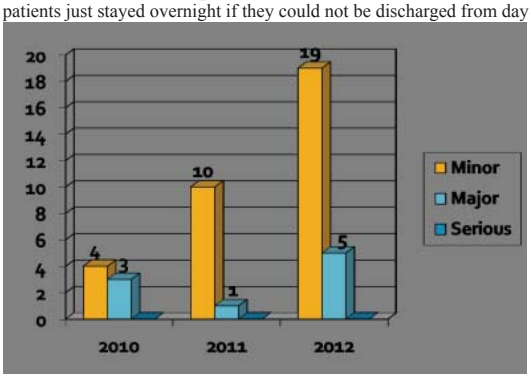
**Day Case Renal Biopsy: Single Centre Experience**

Roshni Rathore.
*Renal Medicine, Royal Preston Hospital, Preston, United Kingdom.*

**Background:** There has been an increase in number of renal biopsies being performed on day case basis in recent years. Day case renal biopsy offers advantage of patients being discharged home same day. We aim to look at our practice in the last 3 years at Royal Preston Hospital, specifically looking at minor and major complications, biopsies with adequate tissue yield, patients discharged home same day and mean length of stay if admission was required.

**Methods:** We retrospectively analysed 312 renal biopsies done between 2010-2012. Data was gathered using Quadramed system and histopathology database. Percutaneous renal biopsies were performed under ultrasound guidance using spring loaded biopsy gun. Minor complications included macroscopic haematuria or perinephric haematoma. Need for transfusion or intervention was regarded as major complication. Serious complications included death, loss of kidney or life-threatening haemorrhage. If complication occurred after discharge, it was regarded as a delayed complication.

**Results:** Number of biopsies has increased with each consecutive year. In more than 93% of biopsies, adequate tissue yield was obtained. While minor complications increased as biopsies increased, major complications remained few. Only 3 patients required intervention in the form of CT angiography in 3 years; only 1 out of these 3 needed embolisation. Most patients just stayed overnight if they could not be discharged from day case unit.




**Conclusions:** If patients suitable for day case renal biopsy are selected using set criteria, they are more likely to be safely discharged from day case unit on the same day. If haematuria warrants inpatient admission, overnight stay is sufficient for most of the patients. With ultrasound guided technique using automated biopsy gun, number of complications is small and the diagnostic yield is reasonably good.Therefore,day case renal biopsy remains a safe procedure in majority of patients.

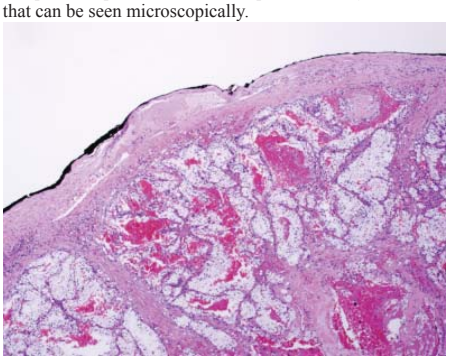
## TH-PO986

**Critical Analysis of the Pseudocapsule after Robotic Assisted Enucleation Partial Nephrectomy** Maria M. Picken,[1] Gopal N. Gupta.[2] *[1]Pathology, Loyola Univ Medical Center, Maywood, IL; [2]Urology, Loyola Univ Medical Center, Maywood, IL.*

**Background:** The optimal surgical margin for small renal masses (SRMs) is contested. Enucleation of SRMs is gaining acceptance as it maximally preserves nephrons and allows for zero warm ischemia time. However, there remains a concern about the oncologic adequacy of enucleation margins. Our objective was to critically examine the pseudocapsule of 7 consecutive SRMs treated with robotic assisted off clamp enucleation.

**Methods:** We retrospectively examined 7 consecutive SRMs that were extirpated utilizing a robotic assisted enucleation partial nephrectomy technique without hilar clamping. The tumor pseudocapsule was used to guide the extirpation. Specimens were reviewed by a genitourinary pathologist with particular attention to pseudocapsule characteristics.

**Results:** Mean pathologic tumor size was 3.1 cm (range 1.2–5.6 cm); clear cell RCC x4, papillary type II RCC x2, renal oncocytoma x1. Surgical margins were negative on all patients. The mean thickness of the pseudocapsule was 0.05 mm (range 0.02-0.15 mm). The pseudocapsule is made of compressed kidney tissue which creates a plane of dissection that can be seen microscopically.



The compressed tissue is mainly composed of sclerosed glomeruli and tubules. In 5 specimens arteries run parallel and in 2 cases perpendicular to the pseudocapsule; perpendicular veins were seen in 3 cases. There was no invasion of tumor into the pseudocapsule itself.

**Conclusions:** The ideal treatment of a SRM would be maximally nephron sparing with no warm ischemia time and be performed with minimally invasive techniques. Robotic assisted enucleation partial nephrectomy accomplishes these goals. It is important for surgeons and pathologists to understand the characteristics of the pseudocapsule which constitutes the oncological margin. Further study is warranted.

## TH-PO987

**Angiotensin II-Regulated Proteins in Human Kidney Cells as Markers of Renal Angiotensin II Activity** Ana Konvalinka,[1] Susan B. Gurley,[3] Thomas M. Coffman,[3] Shao-Ling Zhang,[4] Rohan John,[2] Eleftherios P. Diamandis,[2] James W. Scholey.[1] *[1]Div of Nephrology, Univ of Toronto, Toronto, Canada; [2]Pathology and Laboratory Medicine, Univ of Toronto, Toronto, Canada; [3]Div of Nephrology, Duke Univ Medical Center, Durham; [4]Faculty of Medicine, Univ of Montreal, Montreal, Canada.*

**Background:** Angiotensin II (AngII), the main effector of the renin angiotensin system, mediates kidney disease progression by signalling through AT-1 receptor (AT-1R), but there are no specific measures of renal AngII activity. Accordingly, we sought to define an AngII-regulated proteome in primary human proximal tubular cells (PTEC) in order to identify potential markers of AngII activity in the kidney.

**Methods:** We utilized stable isotope labelling with amino acids (SILAC) in PTECs to compare proteomes of AngII-treated and control cells. Our top hits were verified by Selected Reaction Monitoring (SRM), ELISA and qRT-PCR. We finally used bioinformatics to define key enriched functional protein groups, and validated our findings in AngII-treated mice.

**Results:** Of 4618 quantified proteins, 83 were differentially regulated by AngII. SILAC ratios for 18 candidates were confirmed by SRM. Both SILAC and SRM revealed heme oxygenase-1 (HO-1) as the most significantly upregulated protein in response to AngII stimulation. AngII-dependent regulation of HO-1 gene and protein was further verified in PTECs. In order to extend these in vitro observations, we utilized a bioinformatics approach, which demonstrated that five enriched functional groups containing HO-1 were common to our AngII-regulated proteins and differentially expressed kidney genes from AngII-treated wild type and AT-1R knock-out mice. Furthermore, HO-1 kidney expression and urinary excretion were reduced in AngII-treated mice with PTEC-specific AT-1R deletion compared to AngII-treated wild-type mice, thus confirming AT-1R-mediated regulation of HO-1.

**Conclusions:** Our in vitro approach identified novel markers of AngII activity and the animal studies demonstrated that these markers are relevant in vivo. These interesting proteins hold promise as specific markers of renal AngII activity in patients and in experimental models.

*Funding:* Pharmaceutical Company Support - This work was funded in part by the AMGEN Canada Investigator Sponsored Initiative Research Program.

## TH-PO988

**Urinary Exosomal Ceruloplasmin Is a Marker of Kidney Disease and a Potential Biomarker of Early Diabetic Nephropathy** Krishnamurthy P. Gudehithlu,[1] Jane Vernik,[1,2] Mark A. Kraus,[1,2] Peter D. Hart,[1,2] Jose A.L. Arruda,[3,4] George Dunea,[1,4] Ashok K. Singh.[1,3,4] *[1]Div of Nephrology, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL; [2]Dept of Internal Medicine, Rush Univ Medical College, Chicago, IL; [3]Section of Nephrology, Univ of Illinois at Chicago, Chicago, IL; [4]The Hektoen Institute of Medicine, Chicago, IL.*

**Background:** Urine exosomal proteins are fingerprints of the metabolic state of the kidney and offer new biomarkers for diagnosing early diabetic nephropathy. Previously we found that urinary exosomal ceruloplasmin, a redox enzyme sensitive to oxidative stress, was up-regulated several fold in nephrotic patients. We also observed that high exosomal cerulopasmin in these patients did not correlate with the ceruloplasmin levels in the urine suggesting that urinary exosomal ceruloplasmin was derived from the kidney and not from filtered ceruloplasmin. In this study we investigated whether urinary exosomal ceruloplasmin could also serve as a biomarker for diagnosing diabetic nephropathy prior to onset of microalbuminuria.

**Methods:** Urine samples from control subjects (n=15) and diabetic patients (n=37) were collected. Exosomal extracts were prepared by ultracentrifugation. Ceruloplasmin levels were measured in the exosomal extracts by ELISA. For analysis of results the diabetic patients were subdivided as: non-albuminuric (<0.03 g albumin/g of creatinine), microalbuminuria (0.03-0.3 g albumin /g of creatinine), subnephrotic (0.31-2.0 g albumin /g of creatinine), and nephrotic (>2.1 g albumin /g of creatinine).

**Results:** Compared to controls (1.3 ± 0.4 (mean ± SEM) ng ceruloplasmin/mg exosomal protein), ceruloplasmin levels increased 5 fold in diabetics with microalbuminuria ($p<0.05$) and remained at that level in subnephrotics and nephrotic groups ($p<0.05$). Importantly, ceruloplasmin levels in the non-albuminuric diabetic patients were 3 fold higher ($p<0.05$) than controls, suggesting that urinary exosomal ceruloplasmin levels increased before the onset of microalbuminuria.

**Conclusions:** We conclude that early increase of urinary exosomal ceruloplasmin levels could be potentially predictive of onset of diabetic nephropathy.

*Funding:* Private Foundation Support

## TH-PO989

**Urine Exosomal Gelatinase – A Potential Biomarker of Early Diabetic Kidney Disease** Krishnamurthy P. Gudehithlu,[1] Carolyn S. Brecklin,[1,3] Jane Vernik,[1,2] Mark A. Kraus,[1,2] Peter D. Hart,[1,2] Jose A.L. Arruda,[3,4] George Dunea,[1,4] Ashok K. Singh.[1,3,4] *[1]Div of Nephrology, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL; [2]Dept of Internal Medicine, Rush Univ Medical College, Chicago, IL; [3]Section of Nephrology, Univ of Illinois at Chicago, Chicago, IL; [4]Hektoen Institute of Medicine, Chicago, IL.*

**Background:** Urinary exosomal proteins are fingerprints of the metabolic condition of kidney and as such offer early biomarkers for onset of diabetic nephropathy. One of the earliest pathological markers of diabetic nephropathy is the accumulation of mesangial matrix in the kidney. Decrease of tissue gelatinase, an enzyme which degrades matrix, is responsible for the matrix accumulation. We investigated whether the previously reported decreased tissue gelatinase would be reflected in decreased gelatinase in the urinary exosomes of diabetic patients.

**Methods:** Urine from diabetic (n=37) and non-diabetic controls (n=19) were collected. For analysis of results the diabetic patients were subdivided according to the level of albuminuria into three groups, microalbuminuria (0.03-0.3 g albumin/g of creatinine), subnephrotic (0.31-2.0 g albumin/g of creatinine), and nephrotic (>2.1 g albumin/g of creatinine). Exosomal extracts were prepared from urine by ultracentrifugation. Gelatinase was quantified by a fluorometeric assay using FITC-gelatin. Few random samples were analyzed by SDS-PAGE-zymography to determine the molecular sizes of the gelatinases in the exosomal extracts. Gelatinase activity was expressed in units/mg exosomal protein (1 unit =1 µg gelatin degraded/hour at 37ºC).

**Results:** Gelatinase activity as compared to controls (311 ± 24 units/mg exosomal protein, n=19) was reduced by 86% in nephrotic patients (; n=6; $P<0.05$), by 32% in subnephrotic patients (n=11; $P<0.05$) and by 20% in microalbuminuric patients (n=22; $P<0.05$). Zymography revealed that the exosomal gelatinase activity was largely confined to the 72 kD and 92 kD proteins in the extracts.

**Conclusions:** We conclude that gelatinase activity in urinary exosomes may serve as a potential biomarker for diagnosing early kidney disease.

*Funding:* Private Foundation Support

**TH-PO990**

**Plasma Pro-Enkephalin Is Associated with Acute Kidney Injury in Critically Ill Patients and Is Not Influenced by Sepsis** Joachim Struck,[1] Rossella Marino,[2] Olle Melander,[3] Andreas Bergmann,[1] Salvatore Di Somma.[2] *[1]Sphingotec GmbH, Hennigsdorf, Germany; [2]Sant'Andrea Hospital, Rome, Italy; [3]Skåne Univ Hospital, Malmö, Sweden.*

**Background:** Novel biomarkers for the detection of Acute Kidney Injury (AKI) in critically ill patients have not been established in clinical routine, as they are not sufficiently accurate, influenced by inflammation and/or detectable only in urine. Opioid receptors are highly expressed in the kidney, but it is not known, whether Enkephalin is associated with kidney function.

**Methods:** 101 consecutive ED patients with the final diagnosis sepsis, severe sepsis or septic shock were enrolled. Plasma samples were obtained on ED admission and on the following day. The 7 day mortality rate was monitored. Patients were categorized for severity of AKI according to the RIFLE criteria. Plasma Pro-Enkephalin (pro-ENK), a stable surrogate marker of the unstable Enkephalin stemming from the same precursor peptide, was measured by a novel immunoassay. For comparison, pro-ENK was measured in a large general population cohort (n=4453).

**Results:** In the general population the mean pro-ENK concentration was 44 pmol/L and the 99$^{th}$ percentile was 80 pmol/L. Pro-ENK was negatively correlated with eGFR (r=-0.33, p<0.0001). Key characteristics of the ED cohort were: median age: 78 y; final diagnosis severe sepsis or septic shock: 29%; 7 day mortality rate: 28%. Pro-ENK was negatively correlated with creatinine clearance (r=-0.74, p<0.0001) and was associated with 7 day mortality rate (survivors: 75 [47-124] pmol/L; non-survivors: 209 [77-499] pmol/L; p<0.001), whereas creatinine clearance was only borderline significant (p=0.071). Multivariate linear regression revealed creatinine clearance to be by far the strongest determinant of pro-ENK (partial $R^2$=0.17). Pro-ENK increased with severity of AKI (RIFLE staging; ANOVA p<0.0001). Importantly, pro-ENK levels in patients without kidney dysfunction or only risk were essentially in the normal range. In contrast, NGAL was markedly elevated over normal already in patients without kidney dysfunction.

**Conclusions:** Pro-ENK is a new plasma biomarker for AKI severity and independent from SIRS and infection.

**TH-PO991**

**The Proteome-Transcriptome-Combined Database of Kidney Distal Tubular Proteins for Novel Urinary Biomarker Discovery** Hidehiko Fujinaka,[1] Tadashi Yamamoto.[2] *[1]Institute for Clinical Research, Niigata National Hospital, Kashiwazaki, Niigata, Japan; [2]Structural Pathology, Institute of Nephrology, Niigata Univ, Niigata, Japan.*

**Background:** Several proteins have been proposed as new urinary biomarkers of kidney injuries, but they do not always identify the injured sites of kidney nephron segments. The aim of the present study is to discover new urinary biomarkers which identify the injured sites as **distal** tubule cells.

**Methods:** The proteome-transcriptome-combined database was completed with 3 databases; the human protein atlas (immunohisotochemistry, http://www.proteinatlas.org/), the microarray database of human kidney (Fujinaka, Nephrology 2010), and the urinary proteome database (http://141.61.102.16/urine/). By the atlas database, kidney proteins of tubule-predominant expression were searched and 468 proteins were selected. Two-hundred sixty eight genes of the 468 genes were verified as kidney tubule-predominant genes by the microarray database. Among them, 64 proteins were confirmed in the urinary proteome database. The localizations of these 64 proteins in kidney tubules were specified visually in the atlas database, and 14 proteins were defined as distal tubule originated urinary proteins. The expressions of these proteins were examined in rat kidney injury models and human kidney disease, by real-time PCR and/or immunohistochemistry of the kidney cortices, and Western Blotting of urine samples.

**Results:** In rat kidney cortices of UUO, mRNA expressions of 8 genes such as CAPG and ANXA3 were increased, while 3 genes such as CALB1 were decreased. Among them, the % change of the CALB1 mRNA was the most prominent. CALB1 protein expression in the kidney distal nephron segment was shown decreased extremely in UUO and gradually in rat anti-GBM GN. The urinary CALB1 protein levels of the anti-GBM GN rats were decreased concomitantly with the reduction in the kidney cortices. The human IgAN patients displayed decreased CALB1 protein expression in their dilated distal tubules, and some patients displayed decreased urinary calbindin 1 levels.

**Conclusions:** Our proteome-transcriptome-combined database may be a good tool to discover new urinary biomarkers which identify distal tubule injuries.

**TH-PO992**

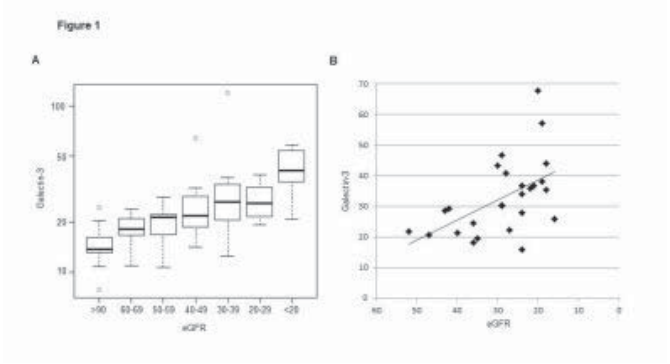
**Galectin-3, a Biomarker of Cardiovascular Risk, Inversely Correlates with eGFR** George Tidmarsh,[1] Pablo E. Pergola,[2] Bhupinder Singh,[3] James Rolke,[1] Stacey Ruiz,[1] Gerard John Smits,[5] Geoffrey A. Block.[6] *[1]La Jolla Pharmaceutical Company, Inc, San Diego, CA; [2]Clinical Advancement Center, San Antonio, TX; [3]Southwest Kidney Institute, Tempe, AZ; [4]Balboa Nephrology Medical Group, La Mesa, CA; [5]CSC, Inc, Santa Barbara, CA; [6]Denver Nephrology, Denver, CO.*

**Background:** Galectin-3,an important fibrosis mediator, is clinically regarded as a marker of CV risk. It has been shown to correlate with kidney function in patients with CV disease, however, alterations in patients without CV disease have not been studied.

**Methods:** We evaluated the relationship between galectin-3 and kidney function in a retrospective study of 99 subjects with an eGFR <20 to >90 mL/min/1.73m$^2$ and in a prospective study of 26 patients with an eGFR of 15 to 50 mL/min/1.73m$^2$. Galectin-3 was measured using an ELISA that exhibits no cross-reactivity with collagens or other galectin family members.

**Results:** In the retrospective study, galectin-3 and eGFR showed a significant inverse correlation (Figure 1A; r=-0.74, p<0.001). In the prospective study, a significant inverse correlation was also observed (Figure 1B; r=-0.56, p=0.003). No statistically significant difference between galectin-3 and eGFR was observed when comparing both retrospective and prospective data. Mean galectin-3 levels in subjects with eGFR >90 were 15±3.8 ng/mL. Using a threshold of 24 ng/mL (2 SD above the mean), 43% of subjects with eGFR 40-49 had elevated galectin-3 while 93% with eGFR <20 had elevated galectin-3. For reference, congestive heart failure patients are considered to be at highest risk when serum levels are >25.9 ng/mL.

**Conclusions:** We find that serum galectin-3 is substantially elevated in the setting of even mildly reduced eGFR. Mean values of galectin-3 were markedly elevated in individuals with eGFR <50. Prognostic utility of measuring galectin-3 remains to be validated in CKD and interventions aimed at reducing galectin-3 are of clinical interest.




*Funding:* Pharmaceutical Company Support - La Jolla Pharmaceutical Company

**TH-PO993**

**Plasma α-Klotho Levels and Its Clinical Implications in Patients with IgA Nephropathy** Hidenori Yamazaki, Fumihiro Tomoda, Kota Kakeshita, Taizo Nakagawa, Tsutomu Koike, Satoshi Kagitani, Hiroshi Inoue. *The Second Dept of Internal Medicine, Univ of Toyama, Toyama, Japan.*

**Background:** α-Klotho has protective actions against renal injury and its renal expression has been demonstrated to decrease in many experimental models of kidney disease. However, circulating α-Klotho levels and its clinical implications remained to be elucidated in human renal disease. In the present study, plasma α-Klotho levels in patients with IgA nephropathy and its relationship to disease severity were evaluated.

**Methods:** Twenty-seven patients with IgA nephropathywere enrolled into the study. Plasma circulating α-Klotho was measured using that commercial assay kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan). The disease severity was estimated by effective GFR (eGFR), urinary protein excretion and histological injury score using Oxford classification.

**Results:** Plasma α-Klotho correlated negatively with eGFR (r= 0.438, p=0.022) and positively with urinary protein excretion (r=0.549, p=0.003). Thus, the decrease in plasma α-Klotho was associated with reduced renal function and worsening of proteinuria in IgA nephropathy. Stepwise multiple regression analysis identified plasma α-Klotho as independent determinant factor for urinary protein excretion ($R^2$= 0.706), but not eGFR. Additionally, in histological appearances, the scores for mesangeal hypercellularity and tubular atrophy/interstitial fibrosis were greater in the patients with plasma α-Klotho< 450 pg/mL (i.e., the median α-Klotho value) than in those with plasma α-Klotho> 450 pg/mL.

**Conclusions:** Our cross-sectional study showed that decreased circulating α-Klotho was associated with the progression of IgA nephropathy, suggesting that α-Klotho may be a novel surrogate marker for severity of IgA nephropathy.

**TH-PO994**

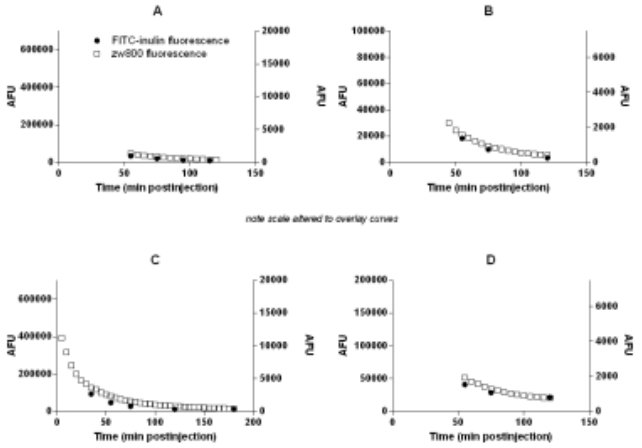
**Near-Infrared Fluorophore ZW800-1: A Potential Marker of Glomerular Filtration Rate (GFR)** Michael Hutchens,[1] Mizuko Ikeda,[1] Hak Soo Choi,[3] Sharon Anderson.[2] *[1]Anesthesiology & Perioperative Medicine, OHSU, Portland, OR; [2]Nephrology & Hypertension, OHSU, Portland, OR; [3]Hematology/Oncology, BIDMC, Boston, MA.*

**Background:** Noninvasive measurements of GFR using clearance of fluorescein-isothiocyanate–inulin (FITCI) and -sinistrin have been reported, but FITC use in injured animals may be limited by temperature- and pH-dependent fluorescence and hemoglobin absorbance.[1] ZW800-1 is a stable, zwitterionic fluorophore, MW 943Da, which is eliminated almost entirely in urine.[2] We hypothesized that elimination of ZW800-1 would be similar to elimination of FITCI.

**Methods:** C57BL/6 mice were used. Group 1: procedurally naïve mice received a jugular catheter under isoflurane anesthesia. On the imaging stage of a whole-animal imager, mice were coinjected with 11.5 μg of ZW800-1 and 10 mg of FITCI diluted in 300 μL of 0.9% saline. Images were acquired via the 800nm filter every 5m for 120m. Blood was collected via tail artery at 55,75,95, and 120m and analyzed for FITC fluorescence

after physiologic buffering. Groups 2&3: the above was performed 24h after cardiac arrest and cardiopulmonary resuscitation (CA/CPR) or in naïve controls. FITCI and ZW800-1 disappearance curves were fit.

**Results:** ZW800-1/FITCI coinjection was well tolerated. FITCI and ZW800-1 disappearance curves were similar.




Clearance halftime ($t_{1/2}$) was 19±2m for FITCI and 22±1m for ZW800-1 (mean±SEM,n=4,p=0.1). CA/CPR delayed ZW800-1 clearance (24±1 vs 17±1 m n=4,3 p=0.01). FITCI and ZW800-1 $t_{1/2}$ correlated in controls and CA/CPR-treated animals ($r^2$ =0.991,n=6 ,p<0.0001).

**Conclusions:** We demonstrate for the first time that near-infrared fluorophore ZW800-1 clearance is correlated with inulin clearance in mice with and without renal injury. ZW800-1 is a candidate marker of GFR. Further study is ongoing.

References: 1. Schock-Kusch D,et al. *NDT*.2009.
2. Choi HS,et al.*Angew Chem Int Ed Engl*.2011.

*Funding:* NIDDK Support

## TH-PO995

### Is Coagulation the Missing Pathway of Glomerulonephritis Pathophysiology?

Dimitrios Petras,[1] Glykeria Tsouka,[1] Ploumis Stavros Passadakis.[2] *[1]Nephrology Dept, Hippokration Hospital, Athens, Greece; [2]Dept of Nephrology, Democritus Univ of Thrace, Greece.*

**Background:** In experimental models of GN, plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor (vWf) are implicated to renal scaring and glomerulosclerosis, but in humans their role and prognostic value have not been elucidated yet. Therefore, we studied the expression of these proteins in human glomerulonephritis.

**Methods:** Renal biopsies, obtained from 87 patients with different types of primary GN and diabetic nephropathy, were scored for severity of glomerulosclerosis and interstitial fibrosis. Serum creatinine level, 24-H urinary protein excretion and other laboratory data were also collected. PAI-1 and vWf intraglomerular deposition was assessed immunohistochemically and expressed semiquantitatively on a 0-3 scale (normal, mild, moderate and severe respectively). 11 normal kidney samples were used as controls.

**Results:** Glomerular PAI-1 and vWf staining scores in glomerulonephritic samples were markedly higher compared to controls. Samples with moderate deposition of PAI-1 had higher levels of glomerulosclerosis (40.1±18.1%), comparing to samples with no (11.8±16.9%) or mild (21.3±14.7%) staining (p<0.001). Moderate/intense level of interstitial fibrosis was found in all specimens with moderate staining for PAI-1, but in only 18.8% of samples with normal staining (p<0.001). Serum creatinine level was significantly higher (3.4±2.1 mg/dl) in patients with moderate deposition of PAI-1, comparing to patients with no (1.4±1.3 mg/dl) or mild (1.5±0.6 mg/dl) deposition (p<0.001).

**Conclusions:** PAI-1 and vWf are deposited intraglomerularly and probably cooperate at human GN. Increased expression of PAI-1 is an ominous finding for renal function, as it is implicated to matrix expansion, fibrosis and glomerulosclerosis.

## TH-PO996

### Urine Screening for Kidney Injury Biomarkers in Children and Adults with Sickle Cell Disease with and without Albuminuria

Ofelia A. Alvarez,[1] Dima Hamideh,[1] Vimal Master Sankar Raj,[1] Thomas Harrington,[2] Monica T. Garcia-Buitrago,[3] Phillip Ruiz,[3] Gaston E. Zilleruelo.[1] *[1]Dept of Pediatrics, Univ of Miami, Miami, FL; [2]Dept of Internal Medicine, Univ of Miami, Miami, FL; [3]Dept of Pathology, Univ of Miami, Miami, FL.*

**Background:** Renal involvement in sickle cell disease (SCD) manifest in several forms. Presence of albuminuria is considered a hallmark of sickle cell nephropathy. The objective of this study was to evaluate kidney injury (KI) biomarkers in urine which could predict onset of sickle cell nephropathy.

**Methods:** We measured KI biomarkers in random urine samples of 38 children and adults with SCD with or without albuminuria in a cross-sectional analysis. Kidney injury molecule 1 (KIM-1), N-acetyl-ß-D-glucosaminidase (NAG), endothelin-1 (ET-1), and transforming growth factor-ß1 (TGF ß1) were measured by colorimetric assays. Prussian blue was used to identify intracellular hemosiderin in urine. Albuminuria was associated to the urine biomarkers by Pearson and Spearman correlation.

**Results:** Participants were classified into two groups based on the presence or absence of albuminuria. Significant differences between two groups by t-test is noted in the table. Patients with albuminuria were more likely positive for urine hemosiderin, and had significantly higher KIM-1 and NAG to urine creatinine ratios.

| Parameter, units | Albuminuria (N=19) Mean±SD | No albuminuria (N=19) Mean±SD | *P* value |
|---|---|---|---|
| KIM-1/urine creat, ng/mg | 0.93±0.73 | 0.35±0.31 | **0.004** |
| NAG/urine creat, unit/g | 10.33±8.25 | 4.26±2.97 | **0.006** |
| ET-1/urine creat, pg/mmol | 3.32±2.96 | 2.76±1.94 | 0.49 |
| Urine hemosiderin | 12/19 | 3/17 | **0.008** |

**Conclusions:** In patients with SCD and normal GFR, kidney injury biomarkers NAG and KIM-1 strongly correlated with albuminuria. Urine hemosiderin, indicating hemolysis, also was associated with albuminuria. Prospective trials will help establish the role of these biomarkers in early detection of renal disease in SCD.

*Funding:* Clinical Revenue Support

## TH-PO997

### Glomerular Injury in Patients with Acutely Decompensated Heart Failure and Chronic Kidney Disease

Michael G. Selby, Vesna D. Garovic. *Nephrology, Mayo Clinic, Rochester, MN.*

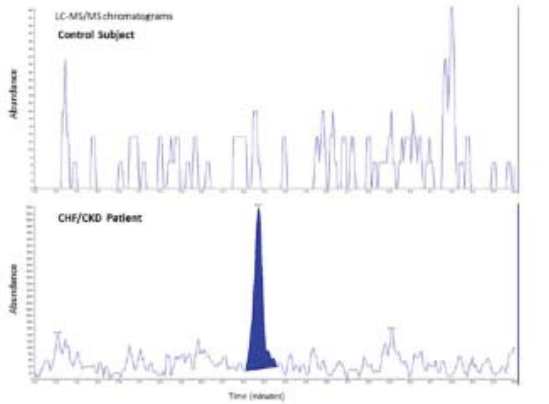
**Background:** Intrinsic renal alterations that underlie chronic kidney disease (CKD) in patients with chronic heart failure (CHF) have been partially investigated. In these patients, tubular injury noted by specific urinary/serum markers has been studied; however, glomerular injury has not. Urinary podocin is a marker of podocyte loss and injury. Accordingly, urinary podocin was assessed by quantification of a tryptic podocin peptide with high performance liquid chromatography (LC) coupled to tandem mass spectrometry (LC-MS/MS) in patients with CHF and CKD, and in controls.

**Methods:** Twenty-seven patients, who developed CKD while receiving treatment for CHF, were studied during an acute CHF decompensation. Patients were recruited at Tulane University (2010-2011). Urine samples were collected for podocin, creatinine, and protein. Eight healthy subjects served as controls. The LC-MS/MS technology assumes a 1:1 stoichiometric ratio between a tryptic fragment and its related protein. Urinary podocin was expressed in femtomole (fmol) of podocin/mg of creatinine.

**Results:** The mean patient's age was 57 y with 55% male. The majority were New York Heart Association Functional Class 3. Overall, the urinary podocin/creatinine ratio was significantly greater in CHF patients than controls: 0.37 ± 0.77 v. 0.06 ± 0.05 fmol podocin/mg creatinine, p=0.04. 40% of patients had a urinary podocin/creatinine ratio greater than the upper limit of normal (>0.2 fmol podocin/mg creatinine). 55% of patients had protein/creatinine ratio ≥ 0.15 (which correlates to estimated 24-hour proteinuria of > 150 mg). Urinary podocin/creatinine ratio was elevated in 54% of patients with proteinuria, and in 46% of patients without proteinuria.

**Conclusions:** During acute CHF decompensation, 40% of patients with CHF and CKD have evidence of glomerular injury and podocyturia as evidenced by an elevated urinary podocin/creatinine ratio.

Figure 1: LC-MS/MS chromatograms from fixed cells: age and sex matched control (top) and example CHF patient (bottom), with a calculated tryptic peptide concentration of 0.002 versus 1.459 fmol podocin/mg creatinine, respectively.




*Funding:* Private Foundation Support

**TH-PO998**

**Circulating suPAR in Japanese Patients with Glomerular Diseases: A Multicenter Cross-Sectional Study** Takehiko Wada,[1,2] Masaomi Nangaku,[1,2] Shoichi Maruyama,[2] Enyu Imai,[2] Kumi Shoji,[1] Sawako Kato,[2] Tomomi Endo,[2] Eri Muso,[2] Kouju Kamata,[2] Hitoshi Yokoyama,[2] Keiji Fujimoto,[2] Yoko Obata,[2] Tomoya Nishino,[2] Hideki Kato,[2] Shunya Uchida,[2] Yoshie Sasatomi,[2] Takao Saito,[2] Seiichi Matsuo.[2] *[1]Div of Nephrology and Endocrinology, Univ of Tokyo, Tokyo, Japan; [2]Japanese Refractory Nephrotic Syndrome Study Investigators, Japan.*

**Background:** Elevated serum soluble urokinase receptor (suPAR) levels have been described in focal segmental glomerulosclerosis (FSGS) patients in several different cohorts. However, it still remains to be determined if this is the case for Japanese patients and if circulating suPAR can be clinically useful as a diagnostic marker.

**Methods:** We measured serum suPAR levels in 70 Japanese patients with biopsy-proven glomerular diseases from 8 hospitals in a cross-sectional manner, using a commercially-available ELISA kit. We also analyzed the association between the suPAR levels and clinical/histological parameters obtained from medical record.

**Results:** The suPAR levels show a significant positive correlation with age ($R^2$=0.15, p=0.001) and negative correlation with eGFR ($R^2$=0.32, p<0.0001). After excluding patients with low eGFR (< 60 mL/min/1.73m$^2$), the difference in suPAR levels between the diseases was not significant. Analyses based on receiver operating characteristics (ROC) curve revealed that the serum suPAR level was not a potent clinical measure as a diagnostic marker. In a different cohort, patients with ANCA-associated glomerulonephritis had remarkably higher levels of serum suPAR (6791.3 ± 1513 pg/mL) compared with all of the primary glomerular diseases, which may be associated with inflammation and renal impairment.

**Conclusions:** Data in the current study suggest that suPAR may be accumulated in the patients with renal dysfunction. For patients with normal renal function, the difference of suPAR values was not significant among primary glomerular diseases. Assessment of statistical measures based on ROC curve analysis showed that the suPAR level has little value as a diagnostic biomarker.

*Funding:* Government Support - Non-U.S.

**TH-PO999**

**The Role of Inflammatory Marker MRP8/14 in CKD Patients** Tatsuki Matsumoto,[1] Yoshinori Taniguchi,[1] Yoshiko Shimamura,[1] Kosuke Inoue,[1] Taro Horino,[1] Kazu Hamada,[1] Yoshio Terada,[1] Kenji Yuasa,[2] Shinpei Fujimoto,[1] Koji Ogata.[1] *[1]Kochi Univ, Nankoku, Japan; [2]Kochi-Takasu Hospital, Kochi, Japan.*

**Background:** Myeloid-Related Protein 8/14 complex (MRP8/14) is an endogenic ligand of toll-like receptor (TLR)-4, and is considered to be an inflammatory marker. Although it has been reported that MRP8/14 related to arteriosclerosis and coronary lesion in type 2 diabetes, there are no reports about the relationship between MRP8/14 and chronic kidney disease (CKD). We studied the association between MRP8/14 levels and renal function or the other parameter in CKD.

**Methods:** A total of 432 patients (mean age 60±17) with CKD were enrolled. Serum samples were collected, and MRP8/14 levels were measured by using ELISA kit. Serum creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA), urine protein/Cr ratio, and the other parameter of renal function were also measured. This study was approved by Kochi Medical School review board. All patients provided written informed consent.

**Results:** MRP8/14 levels were positively associated with serum Cr (p=0.007, r=0.135), BUN (p<0.001, r=0.175), UA (p=0.011, r=0.127) levels, and urinary protein/Cr ratio (<p<0.001, r=0.212), and Body Mass Index (BMI) (p<0.001, r=0.189). MRP8/14 levels were inversely associated with eGFR (p=0.006, r=-0.137). MRP8/14 levels significantly increased in CKD stage 5 (p<0.05; vs stage1-4). Moreover, MRP8/14 levels in CKD patients with diabetes and hypertension were significantly increased (p<0.05), compared to patients without diabetes and hypertension. Stepwise multiple regression analysis showed that MRP8/14 levels correlated well with BMI, Hb and urinary protein levels.

**Conclusions:** Serum MRP8/14 significantly correlated with renal function and BMI in CKD patients, and might show that MRP8/14 is critical for disease progression and metabolic pathogenesis in CKD.

**TH-PO1000**

**Albumin and Advanced Glycation Endproducts Activated KIM-1 Release by Proximal Tubular Epithelial Cells through Distinct Kinetics and Mechanism** Ai Ing Lim,[1] Loretta Y.Y. Chan,[1] Sydney C.W. Tang,[1] Kar Neng Lai,[2] Joseph C. K. Leung.[1] *[1]Dept of Medicine, The Univ of Hong Kong, Hong Kong; [2]Dept of Health Assessment, Hong Kong Sanatorium and Hospital, Hong Kong.*

**Background:** Tubulointerstitial injury in diabetic nephropathy (DN) is an important factor in disease progression. Kidney injury molecule-1 (KIM-1) is expressed by proximal tubular epithelial cell (PTEC) and released upon kidney injury. In this study, we examine the kinetics and mechanism of KIM-1 release in PTEC under the activation by different mediators of DN, including human serum albumin (HSA), advanced glycation endproducts (AGE) and glucose.

**Methods:** The kinetics of KIM-1 and MMP-3 release by PTEC under the activation with HSA, AGE and glucose; was determined by RT-PCR and ELISA. The activation profiles of major signaling pathways in PTEC cultured with HSA, AGE and glucose, were identified by PCR array. Based on these array data, blockade experiments with various signaling inhibitors were designed to examine their regulatory roles on KIM-1 release.

**Results:** We have shown that HSA induce KIM-1 shedding in PTEC by MMP-3. We further addressed the potency and kinetics of AGE- and glucose-mediated KIM-1 shedding in PTEC as compared to that of HSA. Prompt shedding of KIM-1 was observed in PTEC cultured 4 h with HSA and AGE, but not with glucose. Long term culturing PTEC for 3 days with AGE exhibited sustained release of KIM-1 and this is not observed for HSA. In all experiments, glucose did not induce KIM-1 release by PTEC. HSA and AGE activated the ERK and NF-kB pathways in PTEC. The Jak-Stat pathway was activated by AGE but not by HSA. Incubation of PTEC with diphenylene iodonium, but not pyrrolidine dithiocarbamate, P98059 or fludarabine, blocked the short term release of KIM-1 mediated by HSA or AGE. Interestingly, fludarabine but not diphenylene iodonium, pyrrolidine dithiocarbamate or PD98059, diminished the long term KIM-1 release by PTEC cultured with AGE.

**Conclusions:** Our data suggest that KIM-1 release in PTEC was differentially up-regulated by HSA and AGE. The short term KIM-1 release was mediated by the reactive oxygen species while Jak-Stat pathway controls the long-term KIM-1 release mediated by AGE.

*Funding:* Pharmaceutical Company Support - HKSN Research Grant 2012

**TH-PO1001**

**Urinary Retinol Binding Protein Is a Marker of the Extend of Kidney Fibrosis** Nicolas Pallet,[1,2] Sophie Chauvet,[1] Charlene Levi,[1] Vannary Meas-Yedid,[1] Eric Thervet,[1] Alexandre Karras.[1] *[1]Nephrology, HEGP, Paris, France; [2]Biochemistry, HEGP, Paris, France; [3]Pathology, HEGP, Paris, France; [4]Institut Pasteur.*

**Background:** No specific urinary marker already implemented in routine clinical chemistry has been evaluated as a predictor of the extent of interstitial fibrosis. The aim of our study was to evaluate the diagnostic performance of the measurement of urinary concentration of low and high molecular weight proteins in estimating the extent of interstitial fibrosis.

**Methods:** The urine of 189 consecutive patients who underwent renal biopsy in the Nephrology Department of the Georges Pompidou European Hospital were analyzed. Kidney transplant recipients or patients with acute kidney injury were excluded. The percentage of IF was assessed using a numerical quantification software based on the colorimetric analysis of fibrous areas. Urinary biochemical analyzes were performed to determine total proteinuria, albuminuria, microalbuminuria, as well as urinary levels of retinol binding protein (RBP), alpha1-microglobulin (α1MG), beta 2 microglobulin (β2MG), transferrin, and IgG mmunoglobulins.

**Results:** We found a significant correlation between the degree of IF and RBP/creat ratio ($R^2$: 0.11, p <0.0001). In a lesser extent IF was associated with urinary b2MG and a1MG, but not with total proteinuria or with high molecular weight proteinuria. The correlation of interstitial fibrosis with RBP/creat remained significant after adjustment with estimated glomerular filtration rate, age, body mass index, a1MG and b2MG. The RBP/creat ratio was significantly higher in patients with IF> 30% when compared with patients with FI <30% (16.6 ± 3.6 mg/g vs 4.8 mg/g±1.2, p=0.0007). The specificity of the test for the diagnosis of fibrosis score> 30% of parenchyma was 95% when using a threshold of 30 mg/g creat.

**Conclusions:** We have demonstrated that increased expression of LMW, and especially RBP, is a reliable quantitative and non-invasive marker which may independently predict the extent of kidney IF. The fact that urinary RBP dosage is available in nearly all clinical chemistry departments is appealing for its implementation in the routine care of CKD patients.

**TH-PO1002**

**MicroRNA-27b, an Exosomal Biomarker, Is Required for Angiotensin II-Stimulated TGF-β1 mRNA Expression in NRK-52E Cells** Alejandro Alvarez-Prats, Jonathan Street, Taro Horino, Ana C. Souza, Robert A. Star, Peter S.T. Yuen. *NIDDK, NIH, Bethesda, MD.*

**Background:** Exosomes are secreted by many cell types and contain proteins, miRNAs, and transcription factors that can reflect cell physiology. We previously showed that urinary exosomal miR-27b correlates with renal fibrosis severity in a mouse folic acid model. As AngII can induce fibrosis via TGF-β signaling, we tested whether AngII or TGF-β1 can regulate exosomal miR-27b in vitro.

**Methods:** NRK52E cells were treated with 13ng/ml TGF-β or 100nM AngII. Media was replaced every 12h (exosomal miR-27b time course) until 48h when cells were harvested. Exosomes were isolated from media by 2-step centrifugation (17,000 xg for 15min, and 200,000 xg for 1h). RNeasy-purified miR-27b was measured by RT-qPCR (Taqman) using a pure, synthetic, mature miR-27b standard. TSG101 was quantified by Western blot. In another study cells were treated with vehicle or 5 μg/ml antagomiR-27b for 24h and media was replaced with vehicle or 100nM AngII until cells were harvested at 48h. TGF-β1 expression was measured by Taqman.

**Results:** AngII, but not TGF-β1, increased cellular miR-27b 2-fold. Net media exosomal miR-27b production was constant over time and unchanged by TGF-β1. Ang II increased media exosomal miR-27b, peaking by 12h with sustained elevation until a return to control levels at 48h. To determine if AngII increased the number of exosomes or the concentration of miR-27b per exosome, we measured the pan-exosomal protein TSG101 as a surrogate exosome biomarker. TSG101 levels were constant through 36h for control and both treatments. At 48h control TSG101 was unchanged, but AngII and TGF-β1 increased exosomal TSG101 by 2- and 3-fold, respectively. **If Ang II, but not**

**TGF-β1, stimulated miR-27b, yet both stimulated TSG101, could miR-27b mediate AngII-stimulated TGF-β1 signaling?** Ang II increased TGF-β1 mRNA expression 2-fold, which was completely inhibited by antagomiR-27b.

**Conclusions:** AngII regulates at least two exosomal miR-27b functions: early AngII-specific stimulation of exosomal miR-27b, and a late AngII and TGF-β stimulated exosomal release. miR-27b is essential for AngII-stimulated TGF-β1 mRNA expression.

*Funding:* NIDDK Support

## TH-PO1003

**Combined Cyclosporine and Prednisolone Therapy in Adult Patients with New-Onset Minimal-Change Nephrotic Syndrome** Sayuri Shirai, Daisuke Ichikawa, Kayori Tsuruoka, Naohiko Imai, Yugo Shibagaki, Tsutomu Sakurada, Takashi Yasuda, Kenjiro Kimura. *Nephrology and Hypertension, St. Marianna Univ School of Medecine, Kawasaki, Japan.*

**Background:** Cyclosporine (CyA) is beneficial for the management of steroid-resistant and frequently relapsing nephrotic syndrome. Previously we have reported that absoarption of CyA is improved when given CyA preprandially rather than postprandially and the preprandial administration of CyA would be useful in the managements for the refractory nephrotic syndrome in adults. In this study, we evaluated the therapeutic efficacy of the combined cyclosporine and prednisolone therapy in adult patients with the new-onset minimal-change nephrotic syndrome (MCNS).

**Methods:** Patients with new-onset of MCNS were randomly assigned to two groups, namely, the CyA (about 2mg/kg, C2:600-1200 ng/ml) + PSL (0.8 mg/kg/day) group (n = 10) and the PSL alone (PSL) (0.8 mg/kg/day) group (n = 10), and the clinical characteristics were compared between two groups. All patients used C2 for CyA monitoring.

**Results:** There were no significant difference of relapse rates among both group. The duration of complete remission (CR) preservation in the CyA + PSL group (593 days) was longer than that in the PSL group (565 days). C2 in non-relapsing group (902ng/ml) was higher than that in relapsing group (802ng/ml) in the CyA+PSL group. The total dose of PSL in PSL group was more than that in CyA+PSL group. Incidence rate of side effect in the CyA+PSL group was higher than that in the PSL group.

**Conclusions:** The combined cyclosporine and prednisolone therapy obtained longer CR preservation and reduced the PSL dose and side effects in adult patients with new-onset MCNS.

## TH-PO1004

**Study of Single-Dose Rituximab at an Interval of 6 Months during 24-Month Period on Steroid-Dependent Minimal-Change Nephrotic Syndrome in Adults** Yuko Iwabuchi, Takashi Takei, Takahito Moriyama, Kosaku Nitta. *Medicine, Kidney Center, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** Steroid dependent minimal-change nephrotic syndrome (MCNS) necessitates administration of prolonged courses of prednisolone, therefore, a paradigm shift from such toxic 'non-specific' therapies to selective immunomodulating regimens is necessary for these cases.

**Methods:** To assess the therapeutic effects of rituximab (an anti-CD20 antibody) in adult patients with steroid-dependent MCNS, we performed a prospective trial of the effects of a single dose of rituximab administered fourth at an interval of 6 months in 27 MCNS patients. We evaluated the biochemical parameters and compared the clinical findings between the 24-month period before and 24-month period after the first rituximab infusion.

**Results:** Significant reduction in the total number of relapses was observed during the 24-month period after the first rituximab infusion as compared with the findings during the 24-month period before the first rituximab infusion (110(100%) vs 8(7%), $p<0.001$). Twenty-one of 25 patients with prednisolone at the first rituximab were able to discontinue prednisolone after 24-month period ($p<0.001$). Complete remission was induced/maintained in all patients under ongoing B-cell depletion.

**Conclusions:** Our results revealed that rituximab therapy was associated with reduction in the number of relapses and in the total dose of prednisolone needed. Therefore, rituximab appears to be a useful therapeutic agent for adult patients with steroid-dependent MCNS. These results suggest that this treatment is rational and should be considered and important patients with steroid-dependent MCNS.

## TH-PO1005

**Nephrotic Syndrome Study Network Baseline Cohort** Debbie S. Gipson, Lawrence B. Holzman, Crystal A. Gadegbeku, John R. Sedor, J. Troost, Peter X.K. Song, Daniel C. Cattran, Fernando C. Fervenza, Patrick H. Nachman, Frederick J. Kaskel, Marva M. Moxey-Mims, Matthias Kretzler. *Nephrotic Syndrome Study Network.*

**Background:** The Nephrotic Syndrome (NS) Study Network (NEPTUNE) is an ongoing longitudinal observational study. Here we introduce the NEPTUNE cohort.

**Methods:** Eligibility: proteinuria >500 mg/d and initial renal biopsy. Comparison of the initial 361 subjects with baseline data and histology are compared between children and adults using two-sample t or chi-square tests. Continuous variables are presented as median (25,75th %tile) and frequencies as n(%). HTN is defined as BP>140/90 for adults and >95th %tile for children. eGFR was calculated using CKD-Epi or CKiD equations.

**Results:**

| | <18 yrs n=121 | >=18 yrs n=240 |
|---|---|---|
| **Age** | 11(9) | 44(28) |
| **Black**** | 43(36) | 34(14) |
| **Hispanic** | 24(20) | 42(18) |
| **Disease duration** (m) | 5.1(1.9,14.3) | 5.1(2.0,25.8) |
| **Edema** | 42(39) | 96(44) |
| **eGFR**** | 105.5(74.3,141.9) | 56.5(37.2,97.5) |
| **UPC*** <1 | 25(32) | 27(16) |
| 1-3 | 22(29) | 66(40) |
| >3 | 32(41) | 74(44) |
| **S. Albumin** (g/dL) | 3.2(2.2,4.0) | 3.2(2.6,3.9) |
| **Hypertension**** | 51(48) | 67(31) |
| **Biopsy Indication**** | | |
| FRNS/SDNS | 33(27) | 3(2) |
| SRNS | 16(13) | 1(<1) |
| Proteinuria | 65(54) | 213(89) |
| Other | 7(6) | 23(10) |
| **Histology**** | | |
| FSGS/MCD | 72(77) | 89(43) |
| MN | 1(1) | 46(22) |
| Other | 21(22) | 74(35) |
| **Pre-biopsy IST**** | 69(71) | 51(23) |
| **Post-biopsy IST**** | | |
| Steroids only | 22(23) | 42(23) |
| Second line agents | 44(47) | 28(14) |
| None (by m 4) | 14(15) | 93(50) |
| ***p<0.01, **p<0.001, IST=immunosuppression** | | |

**Conclusions:** NEPUTNE exemplifies the potential of enrolling glomerular disease patients into a complex observational protocol with tissue and biospecimen banking. Children and adults were significantly different by race, eGFR, biopsy indication, histology and IST. The low prevalence of MN may in part reflect the proteinuria eligibility criterion as well as a lower frequency of newly diagnosed MN than expected. Baseline data illustrate current practice patterns wherein 71% of children and 23% of adults were treated with IST prior to biopsy.

*Funding:* NIDDK Support, Other NIH Support - ORDR, Private Foundation Support

## TH-PO1006

**Clinicopathological Characteristics of Minimal Change Disease with Glomerular Foam Cell Infiltration** Emiko Fujita,[1] Akira Shimizu,[2] Akiko Mii,[1] Megumi Fukui,[1] Shuichi Tsuruoka.[1] *[1]Dept of Nephrology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan; [2]Dept of Pathology, Nippon Medical School, Bunkyo-ku, Tokyo.*

**Background:** Minimal change disease (MCD) is a major cause of idiopathic nephrotic syndrome. It is defined pathologically as glomerular disease with minimal or no glomerular alterations, no glomerular immune deposits, and extensive effacement of podocyte foot processes . We sometimes experience the cases of MCD with foam cell (FC) infiltration in glomeruli, but can not include the diagnostic criteria for FSGS.

**Methods:** In the present study, we examined renal biopsies of MCD in our department between 1990 and 2013, selected the cases of MCD with or without glomerular FC infiltration, and assessed the clinicopathologic characteristics of these cases.

**Results:** In 312 biopsies with a diagnosis of MCD, glomerular FC infiltration was detected in 10 cases (3.2%) [FC(+) group]. In histopathology, glomerular endothelial cell injuries were present in all FC(+) cases that were characterized by narrowing capillaries, increase number of endothelial cells, swelling of endothelial cells with loss of fenestra, separation of endothelial cells from capillary basement membrane with subendothelial edema, and macrophage infiltration in subendothelial and mesangial areas. In clinical findings, to compared with FC(-) group, FC(+) group showed that age of biopsy was older (46.2±22.0 vs 21.7±17.6, $p<0.01$), the selectivity index of urinary protein was lower (0.15±0.12 vs 0.07±0.10, $p<0.05$), systolic blood pressure was higher (134.4±26.0 vs 118.0±7.2, $p<0.05$), high frequency of complications including diabetes mellitus (2 case), hypertension (40% vs 17%), and limited response to steroid therapy (partial remission in 2 cases), but there was no significance in serum LDL-cholesterol level between two groups.

**Conclusions:** FC infiltration in MCD may be associated with the presence of glomerular endothelial cell injuries and macrophage infiltration into subendothelial and mesangial areas in milieu of nephrotic syndrome. MCD cases with glomrular FC infiltration include the cases of incomplete remission after steroid therapy.

## TH-PO1007

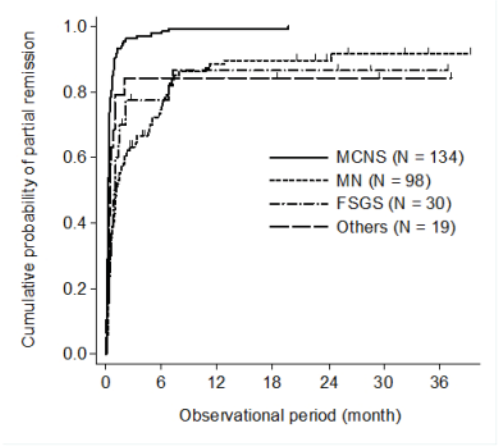
**Resistance to Immunosuppressive Therapy in Patients with Primary Nephrotic Syndrome: Japan Nephrotic Syndrome Cohort Study (JNSCS)** Ryohei Yamamoto,[1] Shoichi Maruyama,[2] Hitoshi Yokoyama,[3] Seiichi Matsuo,[2] Enyu Imai.[2] *[1]Geriatric Medicine and Nephrology, Osaka Univ, Suita, Osaka, Japan; [2]Nephrology, Nagoya Univ, Nagoya, Aichi, Japan; [3]Nephrology, Kanazawa Medical Univ, Uchinada-Cho, Ishikawa, Japan.*

**Background:** In primary nephrotic syndrome (NS), little information is available about the prevalence of resistance to immunosuppressive therapy (IS) and the optimal time to diagnose the resistance to IS.

**Methods:** Study design and setting: A nationwide prospective cohort study including 57 nephrology centers in Japan. Patients: Between 2008 and 2010, 396 primary NS patients were diagnosed by kidney biopsy as primary NS and were registered in JNSCS. The present study included 281 JNSCS participants with ≥3.5 g/day of the baseline urinary protein

(UP) at initiating IS. Outcome: Partial remission (PR) defined as UP <3.5 g/day. Statistics: 90% and 95% distribution of time to PR in minimal change disease (MCD), membranous nephropathy (MN), focal segmental glomerulonephritis (FSGS) and others were assessed.

**Results:** Median baseline ages of MCD (n=134), MN, (n=98), FSGS (n=30) and others (n=16) were 39 (range 12-81), 65 (14-84), 59 (20-83) and 49 (14-83) year, respectively. During median 13 days (range 2-1122) of the observational period, 100%, 88%, 83% and 84% achieved PR, and their cumulative probabilities of the resistance to IS at the final observation were 0.00, 0.08, 0.13 and 0.14.




Among 261 patients with PR, 90% (95%) time to PR were 29 (59), 207 (242), 66 (207) and 30 (60) days. Initial immunosuppressive drugs administered within 1 month were corticosteroid and, in some patients, cyclosporin (11%, 46%, 40% and 32%). Only 5 MN patients received cyclophosphamide.

**Conclusions:** Approximately 10% of primary NS were resistant to IS except MCD. Optimal time to diagnose the resistance may be 1-2, 7-8, 2-7, and 1-2 months in MCD, MN, FSGS and others.

*Funding:* Government Support - Non-U.S.

### TH-PO1008

**Oxidative Stress Markers in the Urine from Pediatric Patients with Nephrotic Syndrome** Yaeko Motoyoshi, Tomohiro Udagawa, Eriko Tanaka. *Pediatrics, Tokyo Medical and Dental Univ, Bunkyo-ku, Tokyo, Japan.*

**Background:** Oxidative stress has been shown to be involved in the renal damage especially in diabetes mellitus (DM). Beside DM, we previously found that massive urinary protein is reabsorbed by proximal tubule cells via megalin, and induces Heme oxygenase-1 (HO-1) in a mouse model of nephrotic syndrome (Kidney Int 74: 1262). However, oxidative stress in human patients with nephrotic syndrome has not yet been well studied. The aim of this study is to show that when large amount of protein is filtrated through glomeruli, proximal tubule cells require a great deal of energy to decompose the protein, and oxidative stress is produced according to the dose of urinary protein.

**Methods:** Urine was collected from 9 nephrotic syndrome patients (one to ten years old), every day from development or relapse of the disease until remission. 2 steroid sensitive, 4 steroid dependent, and 3 steroid resistant patients were included to this study. Their peak urinary protein /creatinine ratio (g/g) was 20.0-136.5.

**Results:** We plotted the progress of 3 oxidative stress markers, setting the day when urinary protein/cre ratio was highest as day 0. Urinary L-FABP/Cre ratio reached to peak on day-1 to 2. Urinary 8-OHdG/Cre ratio reached to peak on day 5 to 9. Urinary SOD activity/Cre ratio reached to peak on day -1 to 7. There was no correlation between 3 oxidative stress markers and urinary protein/cre ratio. In contrast, urinary L-FABP/Cre ratio was 1.8-fold higher and urinary SOD activity/Cre ratio was 2.0-fold higher in steroid resistant group compared with steroid dependent group. 3 markers were elevated in nephrotic syndrome patients with massive proteinuria. L-FABP excretion increased, then decreased at almost the same time as total protein change. Value of 8-OHdG and SOD activity ascended after urinary total protein started to decrease in most patients.

**Conclusions:** Although there was no correlation between 3 markers and the dose of urinary protein, we speculate that L-FABP excretion increased, reflecting tubular damage induced by massive urinary protein. SOD activity elevated to neutralize oxidative stress caused by urinary protein, and 8-OHdG excretion increased at last to repair DNA damage.

*Funding:* Private Foundation Support

### TH-PO1009

**Nephrotic Range Proteinuria Has No Significant Effect on Probability of CD19 Depletion following Rituximab Treatment** Emily Glover,[2] Alan D. Salama,[2] Aine Burns.[1] *[1]Centre for Nephrology Royal Free NHS Trust; [2]Univ College London.*

**Background:** Rituximab is used to treat an increasing number of autoimmune conditions. It is an expensive treatment and different units use different frequency and dosing regimens.

In our unit, circulating CD19 counts are routinely checked following first infusion to assess effective depletion (CD19 ≤5 x $10^9$) and to decide if a further dose is indicated. We have previously demonstrated that response to and duration of depletion using this protocol is similar to other published series in vasculitis patients using much higher doses, such as 1g two weeks apart or 375mg/$m^2$ weekly for 4 weeks (Little 2013).

We investigated whether there were any factors that could predict who would deplete following our 1st and 2nd doses of Rituximab treatment (375 mg/$m^2$) and importantly whether nephrotic range proteinuria reduced the chance of depletion.

**Methods:** 101 patients with different diagnoses and ethnicities were treated according to our standard protocol. Age, sex, ethnicity, diagnosis, proteinuria, serum albumin and serum IgG were analysed for predictors of first dose depletion. Of the 79 patients with data on proteinuria, 12 had nephrotic range (mean 5.88 g/L; range 4.21 to 13.38 g/L).

**Results:** Of the 101 patients analysed, 54 depleted (CD19 ≤5 x $10^9$) on the 1st infusion, 14 on the 2nd infusion, 18 did not deplete with either the 1st or 2nd infusion and 15 did not deplete on the 1st infusion and did not receive a 2nd infusion. There were 3 adverse drug reactions of which two involved respiratory distress. There was no statistically significant effect of age, sex, ethnicity or diagnosis on depletion. Critically, nephrotic range proteinuria, albumin and IgG had no predictive value for depletion following rituximab treatment.

**Conclusions:** The finding that nephrotic range proteinuria has no significant effect on probability of CD19 depletion following rituximab treatment is valuable information for reducing the cost of administration as higher doses or longer courses of rituximab would not appear to be indicated in these patients. Little, M et al. 2013. Induction treatment of ANCA associated vasculitis with a single dose of rituximab. *Rheumatology (Submitted)*.

### TH-PO1010

**Tacrolimus Monotherapy after Short Term Steroid Treatment in Treating Adults with Idiopathic Minimal Change Nephropathy: A Pilot Study** Heng Li, Xiayu Li, Jianghua Chen. *Kidney Disease Center, First Affiliated Hospital, College of Medicine, Zhejiang Univ, Hangzhou, Zhejiang, China.*

**Background:** Although steroids therapy leading to complete remission in most of patients with minimal change nephropathy (MCN), the wide range side effects of steroids make steroids therapy difficult to be accepted by the patients. This study aimed to observe the efficacy and tolerance of tacrolimus monotherapy after ten days' steroids treatment in patients with MCN caused nephrotic syndrome.

**Methods:** In this observational study, 25 patients with MCN were treated with tacrolimus monothery (Group A, n = 6) from the beginning or after 10 days' steroids therapy (Group B, n =19). Data on serum albumin, proteinuria, serum creatinine, eGFR and other biochemical results were collected during the at least 12 months' follow-up period.

**Results:** Three of six patients in Group A and all 19 patients in Group B achieved complete remission. Acute renal failure was occurred in two patients in Group A and one patient in Group B. One patient in Group A has no response to tacrolimus monotherapy for more than two months and achieved complete remission after treated with steroid. The adverse effects including gastrointestinal symptoms and pulmonary infections were mild and tolerated by the patients. One patient in Group A and 6 patients in Group B underwent 9 relapses during follow-up period.

**Conclusions:** Tacrolimus monotherapy after short-term steroid therapy is effective and safe in treating patients with MCN. It may become a novel method to treat MCN patients.

### TH-PO1011

**Efficacy and Safety of Low-Dose Rituximab on Steroid-Dependent Minimal Change Nephrotic Syndrome in Adults** Susumu Uda,[1] Keiko Kanemoto,[1] Eijin Ashikaga.[2] *[1]Div of Nephrology, Kanto Rosai Hospital, Kawasaki, Kanagawa, Japan; [2]Div of Nephrology, Yokohama Municipal Citizen's Hospital, Yokohama, Kanagawa, Japan.*

**Background:** Recently, B cell depleting therapy by rituximab (RTX) has been used successfully to treat therapy for steroid-dependent minimal change nephrotic syndrome (MCNS) in adults. However, given the cost, unexpected side-effects and the lack of data regarding the optimal dosage of RTX to MCNS patients, it would be useful to limit treatment to these patients. We investigated the effect of low-dose RTX and clinical response in adults with steroid-dependent MCNS.

**Methods:** Data on 7 adults patients receiving single dose of RTX (200mg (range 104-133 mg/$m^2$) IV) for biopsy-proven steroid-dependent MCNS after a steroid-induced complete remission and followed over 6-months is presented. Therapy with prednisone and other immune-suppressive drugs was tapered.

**Results:** To date a total of 8 cases included 1 relapsed patient had been studied (mean age 34±12 years, 6 male and 1 female, mean follow- up time 13±8 months). B cell depletion was found in all cases. At <6 months post-RTX, 7 (88%) cases maintained complete remission, allowing rapid prednisone & immunosuppressive agents taper or discontinuation (100%). Only one B cell depleted case (<5/mL) before RTX infusion relapsed under B cell depletion at 3 week. Two cases (25%) relapsed at 7-12 months after complete recovery of B cell. B cell recovery tendency from depleted state was detectable after 3 to 8 months. Two cases (25%) reached long term remission over 18 months without relapse. An increase of B cells was not always associated with relapse. No serious side effects were noted.

**Conclusions:** A single low-dose RTX was effective and safe for sustained complete remission in adult patients with steroid-dependent MCNS and maintained 6-months remission in about 90% cases.

*Funding:* Private Foundation Support

**TH-PO1012**

**Initial Steroid-Sensitivity Is a Highly Sensitive Predictor for Post-Transplant Recurrence of Steroid-Resistant Nephrotic Syndrome** Wen Y. Ding,[1] Ania Koziell,[2] Agnieszka Bierzynska,[1] Hugh J. McCarthy,[1] Corinne Antignac,[3] Olivia Boyer,[3] Moin Saleem.[1] *[1]Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom; [2]Nephrology, Evelina Children's Hospital, London, United Kingdom; [3]Hôpital Necker - Enfants Malades, INSERM, Paris, France.*

**Background:** The post-transplant recurrence risk of paediatric patients with a primary diagnosis of steroid-resistant nephrotic syndrome (SRNS) is between 30 to 50%. Accumulating evidence indicates that SRNS resulting from single gene mutations has no more than an 8% chance of responding to treatment but a significantly reduced risk of post-transplant recurrence. We therefore hypothesised that SRNS that progresses to end-stage renal failure (ESRF) could be classified into 2 categories: 'likely-genetic' - cases treatment-resistant from the outset and resulting from single gene mutations, and 'likely-circulating factor', - cases presenting at times with initial steroid sensitivity before treatment resistance developed. We posed the question whether these two categories might be linked to a differential risk of post-transplant recurrence.

**Methods:** We studied 153 consecutively transplanted patients with SRNS at 3 large paediatric centres in Europe. We compared a number of phenotypic factors including initial steroid-sensitivity, genetic mutations, extra-renal abnormalities and family history.

**Results:** 55 of 153 children (36%) with SRNS who were transplanted suffered from recurrence. Recurrence occurred in 26/28 (93%) patients who showed initial steroid-sensitivity ($p<0.0001$; OR = 130, 95% CI=6.7 to 2533.3) and 29/125 (23%) patients with primary steroid resistance. No patients with identified genetic mutations, extra-renal abnormalities or a positive family history (n=30) manifested initial steroid sensitivity or recurrence.

**Conclusions:** Our data indicates that initial steroid-sensitivity is the strongest predictor for post-transplant recurrence described to date, whereas SRNS associated with initial steroid resistance and documented or clinical features of a genetic mutation is highly protective. Clear clinical differentiation of these patient groups from the outset would enable accurate patient stratification for immunosuppression and transplantation strategies.

**TH-PO1013**

**The Efficacy of Adrenocorticotropic Hormone in Steroid-Dependent Nephrotic Syndrome: Old and New Therapy** Masao Kikuchi,[1] Yuji Sato,[1] Kazuo Kitamura,[1] Shouichi Fujimoto.[2] *[1]First Dept of Internal Medicine, Univ of Miyazaki, Miyazaki, Japan; [2]Dept of Hemovascular Medicine and Artificial Organs, Univ of Miyazaki, Miyazaki, Japan.*

**Background:** Recently, it has been reported that tacrolimus, mycophenolate mofetil and rituximab are effective therapies for patients with steroid-dependent nephrotic syndrome (SDNS). But, these drugs are off-label use in Japan as well as many countries. Adrenocorticotropic hormone (ACTH), widely used for nephrotic patients in the 1950s, has come under the spotlight for its antiproteinuric, lipid-lowering and renoprotective properties, which are not fully explained by its steroidogenic effects. In addition, ACTH might have the potential to recover adrenocortical suppression with long-term exogenous glucocorticoid therapy. We have reported the efficacy of ACTH in SDNS.

**Methods:** We treated with ACTH (tetracosactide acetate) for three patients who were diagnosed with minor glomerular abnormality on the basis of renal biopsy. Although they were treated with steroid pulse therapy, cyclosporine, cyclophosphamide, mizoribine and LDL-apheresis, they relapse easily after the steroid tapering. 0.5-1 mg ACTH was weekly given for them intramuscularly. We assessed their adrenocortical function before and after ACTH therapy by the rapid ACTH stimulation test.

**Results:** During the follow up (average 10 months), all three cases achieved complete remission after starting ACTH therapy. Their prednisolone dose could taper from 60.7 mg/day (before ACTH therapy) to 14.3 mg/day (after ACTH therapy). Their remission maintenance percentage of follow-up period increased from 12.3% (before ACTH therapy) to 85.3% (after ACTH therapy). There was no obvious adverse effect. Their responses to the rapid ACTH stimulation test after ACTH therapy recovered 2.8 times compared with those before ACTH therapy.

**Conclusions:** For ACTH helps steroid tapering and produces a recovery of adrenocortical function, ACTH is an effective therapy for steroid-dependent nephrotic syndrome.

**TH-PO1014**

**The Efficacies of Cyclosporine Combination Therapy on the First Attack of Minimal-Change Nephrotic Syndrome in Adults** Akira Fujiwara,[1] Nobuhito Hirawa,[2] Mari Katsumata,[2] Sanae Saka,[2] Keisuke Yatsu,[1] Yoshiyuki Toya,[1] Gen Yasuda,[2] Satoshi Umemura.[1] *[1]Medical Science and Cardiorenal Medicine, Yokohama City Univ School of Medicine, Yokohama, Kanagawa Prefecture, Japan; [2]Div of Nephrology and Hypertension, Yokohama City Univ Medical Center, Yokohama, Kanagawa Prefecture, Japan.*

**Background:** A combination of cyclosporine with prednisolone therapy (CPT) has been used as the treatment of frequently relapsing, steroid-dependent, or steroid-resistant minimal-change nephrotic syndrome (MCNS). However, few reports have examined the effects of CPT as first-line treatment of new-onset MCNS. Therefore, we conducted a clinical research to evaluate the efficacies and their safety by using CPT in new-onset MCNS in adults.

**Methods:** Forty-six adult patients with biopsy-proven MCNS, diagnosed for the first time, were analyzed retrospectively. We divided the patients into three groups. In the CPT group (n=17), they were treated with cyclosporine orally (2~3 mg/kg/day) with prednisolone (30 mg/day) after methylprednisolone pulse therapy (0.5 or 1.0 g/day for three days). In the MPP group (n=15), prednisolone (30 mg/day) after intravenous methylprednisolone pulse therapy was employed. The PSL group (n=14) was treated with prednisolone (40~60 mg/day) alone.

**Results:** Complete remission was obtained in all patients except one in the CPT group. Length of hospitalization was shorter in the CPT group ($P<0.05$). The average maintenance dosage of prednisolone at 6 months from the start of therapy was significantly low in the CPT group. During the nine months of following up, the CPT group experienced no relapse, whereas the MPP group and the PSL group experienced some relapses. Moreover, the adverse effects were mild and infrequent in the CPT group.

**Conclusions:** Cyclosporine combination therapy led to shorten the length of hospital stay and reduction of daily prednisolone dosage without severe adverse effects for the first attack adult-onset MCNS patients. Moreover, this combination therapy may be useful in inducing the complete remission in terms of the speed, and in reducing the relapse rates. Cyclosporine combination therapy may be a new treatment option for new-onset MCNS in adults.

**TH-PO1015**

**Clinicopathological Correlates in FSGS: A Single Tertiary Center Study** Maria T. Story,[1] Danniele Gomes Holanda,[2] Fadi Tohme,[1] Ramesh Nair,[2] Manish Suneja.[1] *[1]Internal Medicine, Univ of Iowa Hospital and Clinics, Iowa City, IA; [2]Pathology, Univ of Iowa Hospital and Clinics, Iowa City, IA.*

**Background:** Focal Segmental Glomerulosclerosis (FSGS) is a common pathological finding in kidney biopsies done to evaluate proteinuria. Distinguishing different FSGS pathological variants is helpful in guiding treatments. Guidelines for treating primary and secondary FSGS exist; however, heterogeneity abounds in practice. We investigated the clinical-pathological correlation in adult onset FSGS.

**Methods:** A retrospective analysis of biopsy proven FSGS between 10/2001 and 12/2009 was done. Patients with recurrent FSGS after transplant, age <16, immune complex glomerulonephritis, and on known nephrotoxic medications were excluded. All the remaining biopsies were classified according to the Columbia classification. Patients were than classified as either primary or secondary FSGS based on the presence of nephrotic syndrome (serum albumin<3.5, urine P/C ratio>3.5, edema, and hypercholesterolemia) and the pathology was correlated.

**Results:** 80 patients were initially identified with FSGS based on biopsy reports. After exclusion criteria were applied, we were left with 29 cases. 21 were classified as FSGS NOS, 3 perihilar, 3 collapsing, 1 cellular and 1 tip lesion variant. There were 5 cases of primary FSGS based on presence of nephrotic syndrome (3 collapsing, 1 cellular and 1 tip lesion). All secondary cases were either perihilar (n=3) or FSGS NOS (n=21). Of the cases of secondary FSGS 54% had BMI > 30 and 17% had a single kidney. Serum albumin of < 3.0 was significantly associated with primary FSGS ($p < 0.001$). 4 of the 5 primary cases and 3 of the 24 secondary cases were treated with steroids. One of the primaries and eight of the secondaries progressed to ESRD.

**Conclusions:** At our institution, primary/idiopathic FSGS is far less common than secondary FSGS (17% of FSGS biopsies). FSGS NOS, similar to perihilar lesions, is strongly associated with secondary FSGS. This observation is not reported previously. Low albumin is the strongest predictor of primary FSGS ($p < 0.001$). It is important to distinguish between primary and secondary FSGS to avoid unnecessary treatment with immunosuppressive therapy.

**TH-PO1016**

**Collapsing and Not Otherwise Specified FSGS Have Similar Response to Immunosuppressive Therapy** Louis-Philippe Laurin,[1] A. Gasim,[2] Vimal K. Derebail,[1] JulieAnne G. McGregor,[1] Susan L. Hogan,[1] Caroline J. Poulton,[1] J. Charles Jennette,[2] Ronald J. Falk,[1] Jason M. Kidd,[1] Patrick H. Nachman.[1] *[1]Div of Nephrology and Hypertension, Univ of North Carolina, Chapel Hill, NC; [2]Dept of Pathology and Laboratory Medicine, Univ of North Carolina, Chapel Hill, NC.*

**Background:** Idiopathic collapsing FSGS has been associated with poor renal prognosis. Minimal clinical data exist on the efficacy of immunosuppressive therapy.

**Methods:** Inception cohort study of biopsy-proven collapsing and not otherwise specified (NOS) FSGS patients diagnosed between 1984 and 2012. Statistical analysis performed with Mann-Whitney or Fisher's exact tests. Time to end-stage kidney disease (ESKD) and remission was reported with Cox regression hazard ratio (HR) with 95% confidence interval (CI).

**Results:** 174 patients were studied (Collapsing 46; NOS 128): age 38±20 years; 53% Black; 51% female; and follow-up time 40±39 mo.

| | | Collapsing | NOS | p |
|---|---|---|---|---|
| Onset eGFR (mL/min/1.73m$^2$) | | 51±29 | 66±36 | 0.02 |
| Proteinuria (g/d) | | 11.9±6.7 | 5.8±4.8 | <0.001 |
| Calcineurin inhibitor (CNI) (%) | | 44 | 47 | 0.7 |
| ESKD (%) | | 44 | 26 | 0.04 |
| Response to therapy[1] | | | | |
| | Complete Remission (CR) (%) | 40 | 42 | 1.0 |
| | Partial Remission (PR) (%) | 33 | 22 | 0.3 |
| | ESKD (%) | 31 | 27 | 0.6 |
| **Multivariate determinants of CR or PR[2]** | | HR (95%CI) | | |
| Collapsing variant | | 0.88 (0.38-2.02) | | 0.8 |
| CNI | | 2.31 (0.99-5.41) | | 0.05 |
| **Multivariate determinants of ESKD[3]** | | | | |
| Collapsing variant | | 1.84 (0.68-4.96) | | 0.2 |
| CNI | | 0.43 (0.20-0.95) | | 0.04 |

[1]steroids and/or CNI; [2]correcting for age, sex, race, onset proteinuria, onset albumin, renin-angiotensin inhibition and onset eGFR <30; [3]correcting for sex, race, onset proteinuria and onset eGFR <30.

**Conclusions:** Compared to NOS, collapsing FSGS presents with more severe nephrotic syndrome and lower GFR, but has a similar response to immunosuppression with CNI.

## TH-PO1017

**Clinical Features and Outcomes of Focal Segmental Glomerulosclerosis Pathologic Variants in Korean Adult Patients** Young Eun Kwon,[1] Ji Suk Han,[1] Hyung Jung Oh,[1] Seung Hyeok Han,[1] Shin-Wook Kang,[1,2] Tae-Hyun Yoo.[1,2] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul, Korea.*

**Background:** Focal segmental glomerulosclerosis (FSGS) is a common cause of nephrotic syndrome (NS) and ESRD, particularly in African-Americans. Many studies have shown that clinical characteristics and outcomes differ depending on pathologic variants of FSGS. However, these are not well defined in Asian populations.

**Methods:** We conducted a retrospective cohort study to evaluate clinical features and outcomes according to pathologic variants of FSGS in 111 adult patients. Primary outcome was the composite of doubling of baseline serum creatinine concentrations (D-SCr) or the onset of ESRD. Secondary outcome included the rates of complete remission (CR) or partial remission (PR).

**Results:** Not-otherwise specified (NOS), tip, perihilar, cellular, and collapsing variants were present in 70 (63.1%), 20 (18.0%), 17 (15.3%), 3 (2.7%), and 1 (0.9%) patients, respectively. Fifty patients (45.0%) presented NS, and among them 47 patients (94.0%) were treated with immunosuppressants. NS were more prevalent in patients with tip lesion than in those with other variants. The overall 5-year and 8-year renal survival rates were 76.8% and 56.3%, respectively. During a median follow-up duration of 34.5 months, only 1 patient (5.0%) with tip lesion reached the composite outcome compared to 2 (11.8%) and 12 patients (17.1%) with perihilar and NOS variants, respectively, but this difference did not reach statistical significance. CR and PR were achieved in 29 (26.1%) and 32 patients (28.8%), respectively. Tip lesion was associated with a significantly increased probability of achieving CR (P=0.044).

**Conclusions:** Similar to other populations, Korean adult patients with FSGS had distinct clinical features with the exception of a rare frequency of cellular and collapsing variants. Although pathologic variants were not associated with the overall outcome, tip variant exhibited favorable outcome in terms of achieving CR. Further studies with a larger sample size are required to delineate the long-term outcome and response to treatment according to the pathologic variants.

## TH-PO1018

**Tacrolimus Therapy in Adults with Steroid Resistant Focal Segmental Glomerulosclerosis** Raja Ramachandran, Harbir Singh Kohli, Manish Rathi, Vivekanand Jha, Krishan L. Gupta, Vinay Sakhuja. *Nephrology, PGIMER, Chandigarh, India.*

**Background:** Management of adults with steroid resistant (SR) focal segmental glomerulosclerosis (FSGS) steroids is a challenging task. Is tacrolimus (Tac) effective in this situation without serious adverse effects? This prospective study was done to answer this question.

**Methods:** This prospective observational study was done from January 2011 to April 2013. Patients with adult onset ( ≥18 yrs) FSGS with SR (prednisolone 1 mg/kg/day for at least 16 weeks) were enrolled. In patients with SR-FSGS, oral Tac was started targeting a trough level of 5-10 ng/ml along with oral prednisolone (0.15mg/kg/day). In patients with complete remission at 24 weeks, Tac dose was reduced to target 3-6ng/ml for next 24 weeks and hiked again if there was a relapse, while in partial responders Tac trough levels were kept at 5-10ng/ml for next 24 weeks. However, in patients with no remission at 24 weeks Tac was discontinued. Outcome viz complete remission (CR) (reduction of proteinuria to <0.3 g/d & sr albumin ≥ 3.5gm/dl), partial remission (PR) (reduction of proteinuria to 0.3–3.5 g/d & sr albumin ≥ 3.5gm/dl) were assessed at the end of 24 and 48 weeks. Relapses defined as increased proteinuria after complete or partial remissions were recorded. Adverse effects viz. nephrotoxicity (>25% rise in creatinine), cosmetic effects, infections and impaired fasting glucose were recorded every month and analysed at the end of 48 weeks.

**Results:** A total of 34 SR-FSGS completed the study. Of 34 patients CR and PR was achieved in 12 (35%) and 05 (15%) patients respectively. Tac resistance was seen in 17 (50%) patients. Time taken to achieve remission was 21.2±12.4 weeks. Two patients had relapse on tapering the dose after 24 weeks. Of 34 patients reversible nephrotoxicity was seen in 6 (17.6%), irreversible nephrotoxicity in 2 (5.8%), Tac related diarrhea in 6 (17.6%), infections in 12 (35.2%), impaired glucose tolerance/ DM in 10 (29.4%), tremors in 4 (11.7%) and gum hypertrophy in 2 (5.8%).

**Conclusions:** Tac is a very effective agent in the management of SR-FSGS. However, strict renal function and blood sugar monitoring is required due to its nephrotoxicity and diabetogenic potential.

## TH-PO1019

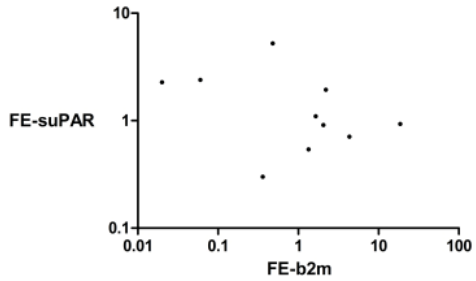
**Kidney Handling of Soluble Urokinase Receptor in Patients with Nephrotic Syndrome** Rutger J. Maas, Jeroen Deegens, Jack F. Wetzels. *Nephrology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands.*

**Background:** Serum suPAR was recently proposed as a specific biomarker of primary FSGS. However, subsequent studies have shown that eGFR is a major determinant of serum suPAR level in patients with FSGS and non-FSGS CKD. suPAR has a MW of 20-60kDa, which implies free glomerular filtration. Urinary suPAR excretion has been demonstrated. We studied kidney handling of suPAR in patients.

**Methods:** serum and urine samples of patients with FSGS (n=6) and MN (n=4) were obtained using a standardized protocol for measurement of urinary proteins. Urinary beta-2-microglobulin (b2m) was only measured if pH was >6.0. suPAR was measured with the Quantikine Human suPAR Immunoassay (R & D Systems, Minneapolis, MN). We compared fractional excretions (FE) of suPAR with b2m and albumin. eGFR was calculated with the 4 variable MDRD Study Prediction Equation.

**Results:** Median eGFR was 50 (IQR 23-71) ml/min/1.73m$^2$, and median proteinuria was 9.3 (IQR 6.6 -15.8) g /10mmol creatinine. Median serum suPAR was 6185 (IQR 5525-8828) pg/ml, median urinary suPAR was 695 (IQR 357-1355) ng/mmol. FE-suPAR did not correlate with FE-b2m, a marker of tubular reabsorption (figure). There was also no correlation of FE-suPAR with either FE-albumin or FE-IgG.

**Conclusions:** suPAR can be detected in urine. There was no correlation of FE-suPAR with FE-b2m. Thus, we found no evidence for tubular reabsorption of suPAR. FE-suPAR did not correlate with FE-albumin, which suggests that urine suPAR is also not simply a marker of glomerular permeability defect.




## TH-PO1020

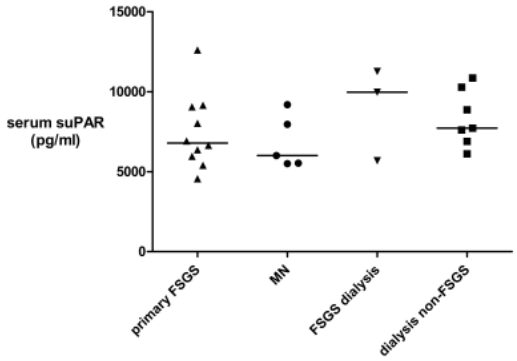
**Soluble Urokinase Receptor Levels in Serum and Urine of Patients with Primary Focal Segmental Glomerulosclerosis and Controls** Rutger J. Maas, Jeroen Deegens, Jack F. Wetzels. *Nephrology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands.*

**Background:** Serum suPAR was proposed as a diagnostic marker for primary FSGS and post-transplant FSGS recurrence. However, previous studies have shown a correlation between serum suPAR and eGFR, which is a potential source of bias. We investigated if serum or urinary suPAR levels can distinguish between primary FSGS and controls with comparable eGFR.

**Methods:** suPAR was measured with the Quantikine Human suPAR Immunoassay (R&D Systems, Minneapolis, MN) in serum and urine of patients with primary FSGS (n=10) and membranous nephropathy (MN; n=5) with nephrotic syndrome, and in dialysis patients with a history of recurrent (n=3) FSGS, and controls (n=7). eGFR was calculated with the 4 variable MDRD Study Prediction Equation.

**Results:** Median eGFR was 36 (IQR 15-69) ml/min/1.73m$^2$ in patients with primary FSGS and 37 (IQR 22-69) ml/min/1.73m$^2$ in patients with MN. Serum suPAR did not differentiate between patients with primary FSGS and MN (fig. 1). Median urinary suPAR was 822 (IQR 343-2150) ng/mmol creatinine in patients with FSGS and 636 (IQR 423-833) ng/mmol creatinine in patients with MN, a non-significant difference. In patients on dialysis, serum suPAR did not discriminate between patients with previous posttransplant FSGS recurrence and controls (fig. 1).

**Conclusions:** Serum and urinary suPAR levels were similar in patients with primary FSGS compared to eGFR-matched controls.




## TH-PO1021

**Circulating Soluble Urokinase Receptor Levels in Primary Nephrotic Syndrome Predict the Response to Therapy** Keiji Fujimoto, Hiroki Adachi, Hideki Yamaya, Hitoshi Yokoyama. *Nephrology, Kanazawa Medical Univ, Uchinada, Japan.*

**Background:** Circulating soluble urokinase receptor (suPAR) speculated to produce proteinuria and focal segmental glomerulosclerosis (FSGS). However, no studies have investigated the involvement of suPAR in the development of primary nephrotic syndrome (NS) in Japanese.

**Methods:** Circulating suPAR levels were measured by ELISA (R & D system) in 36 primary NS cases including 8 FSGS (4 with tip lesion, 2 with NOS type, and 2 with collapsing type), 14 minimal change nephrotic syndrome (MCNS), 12 membranous nephropathy (MN) and 2 membranoproliferative glomerulonephritis (MPGN), and 15 normal controls. Circulating suPAR levels and clinical parameters during the 6 months after immunosuppressive therapy were analyzed. In this study, refractory NS was defined as daily proteinuria did not decrease to <1 g after 6 months therapy.

**Results:** Circulating suPAR levels before therapy were significantly higher in NS patients than in normal controls (P <0.05), but not significantly different among those with FSGS, MCNS, MN, or MPGN. There was a significant negative correlation between the circulating suPAR levels and eGFR in the total NS, FSGS, and MCNS groups (total, r = -0.54, p <0.001, n=107; FSGS, r = -0.61, p=0.002, n=31; MCNS, r = -0.54, p=0.003, n=41) and a significant positive correlation between the circulating suPAR levels and CRP (r = 0.79, p <0.001, n=24) or age (r = 0.68, p=0.002, n=21) in MN. In addition, circulating suPAR levels decreased significantly in MCNS from 3,288 to 2,723 pg/mL after 2 months therapy (p=0.03), but not in FSGS or MN. Patients with refractory NS showed higher circulating suPAR levels with a tendency to increase during therapy. ROC analysis showed that refractory NS was defined by more than 3,324 pg/mL and the increase of circulating suPAR levels more than 226 pg/mL at 2 months after therapy (AUC 0.89, p=0.012, AUC 0.85, p=0.027, respectively).

**Conclusions:** These results suggested that alteration of circulating suPAR level and the levels at 2 months after the therapy reflected the response to therapy in patients with primary NS, even in MCNS.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO1022

**Renal Diagnoses and Outcomes in Women with Disease Identified during Pregnancy Compared with Non-Pregnant Controls – A Multicentre Cohort Study of 1486 Women** Philip Webster,[1] Kate Bramham,[2] Louise Webster,[2] H. Terence Cook,[1] Catherine Horsfield,[3] Isabelle Lydon,[2] Michele Homsy,[2] Raquel Vaz,[1] Clara Santos,[1] Liz Lightstone.[1] *[1]Imperial College Healthcare NHS Trust Renal and Transplant Centre, United Kingdom; [2]King's College London, United Kingdom; [3]Guy's and St. Thomas' NHS Foundation Trust, United Kingdom.*

**Background:** Chronic kidney disease affects approximately 2% of women aged 16-54yrs in England. Pregnancy is frequently the first time women are seen by health services, providing an opportunity to identify disease. Our aims were i) To compare the aetiology of renal disease diagnosed during/after pregnancy with disease identified in non-pregnant women of child-bearing age; ii) To compare long term renal outcomes in these groups. We present the largest and most comprehensive controlled cohort of patients to date.

**Methods:** Native renal biopsies from 2 London centres were studied (1997-2012). Renal disease identified in pregnancy-related (PR) biopsies included antenatal and postpartum biopsies. Controls included biopsies from all women of childbearing age (16 to 49yrs). First biopsies were included. Repeat and inadequate biopsies were excluded.

**Results:** 164 PR biopsies (19 antenatal, 145 postpartum) were identified; mean age at biopsy 32.7yrs. 1322 biopsies in control women; mean age at biopsy 35.3yrs. Focal segmental glomerulosclerosis (FSGS) was most frequently found in PR biopsies, and was more prevalent than in controls (p<0.0001). Lupus nephritis was more common in the control group (p<0.01). There were no significant differences in other diagnoses. Preliminary analysis of renal outcomes from one site (n=864) showed no difference in renal replacement free survival after biopsy in controls and those with disease identified in pregnancy, including those subsets with FSGS.

**Conclusions:** FSGS is the most common diagnosis in PR biopsies, but does not appear to be associated with worse long term renal outcomes compared with controls. It is unclear whether pregnancy reveals or causes FSGS, or whether lesions are primary or secondary. Further work is needed to explore this, and to define risk factors for progression in order to optimise antenatal and postpartum management.

## TH-PO1023

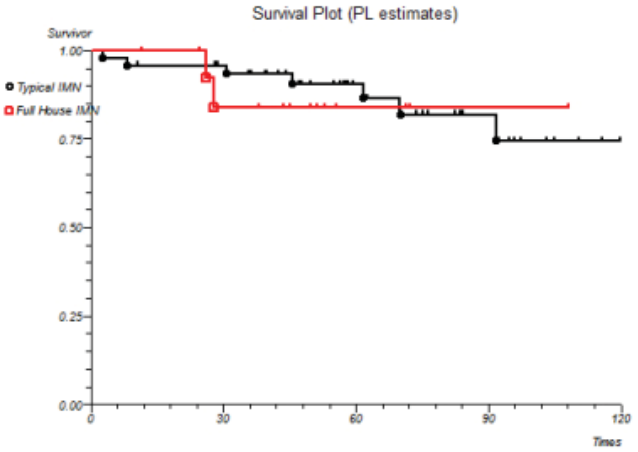
**Clinical Relevance of "Full House Idiopathic Membranous Nephropathy"** Shiv Bhutani, Durga A.K. Kanigicherla, Patrick Hamilton, Roshni Rathore, Mumtaz Patel, Paul E. Brenchley, Michael Venning. *Manchester Institute of Nephrology and Transplantation, Central Manchester Univ Hospitals NHS Foundation Trust, Manchester, United Kingdom.*

**Background:** Idiopathic Membranous Nephropathy is typically characterised by IgG and C3 deposits (Typical IMN, TIMN). Variants that include C1q, IgM, IgA (Full House IMN, FHIMN) without secondary causes at presentation are seen. Outcome in this subset is unclear.

**Methods:** Retrospective analysis of 62 consecutive adult patients with first diagnosis of IMN made by biopsy between 2003 & 2009 at our single center. Remission, CKD-5, and development of systemic diseases was recorded. Where archived serum was available, A-PLA2Rab was tested.

**Results:** 16 patients with FHIMN presented with preserved renal function. Renal survival at median follow up of 50 months was 87%. In comparison to 46 patients with TIMN, there was no significant difference in presentation or renal outcome.

| | Full House IMN | Typical IMN | p |
|---|---|---|---|
| n | 16 | 46 | |
| Initial Proteinuria (Median) | 7.1 | 8.7 | 0.31 |
| Initial eGFR | 82 | 74 | 0.20 |
| Immunosuppression (%) | 56 | 39 | 0.17 |
| Part Rem % | 75 | 83 | 0.32 |
| CKD5 % | 13 | 15 | 0.75 |
| A-PLA2Rab (%) | 50 | 68 | 0.29 |




3 patients with FHIMN (19%) developed secondary causes (SLE in 2 patients within 2 years / disseminated Esophageal malignancy in 1 - within 6 months of presentation). One patient with TIMN (2%), developed Esophageal malignancy, 25 months after presentation, whilst in partial remission (p=0.02). A-PLA2Rab was measured in 41 prevalent patients – positive in 5/10 patients with FHIMN (50%) and 21/31 with TIMN (68%) (p=0.29).

**Conclusions:** Patients with 'Full House IMN' without secondary causes at diagnosis had similar renal outcome as those with 'Typical IMN'. Point prevalence of A-PLA2Rab was similar in both groups. Development of significant co-morbidities suggests that in patients presenting with FHIMN targeted screening for secondary causes would be beneficial.

## TH-PO1024

**Low Serum Albumin and Elevated Serum Creatinine Levels Are Predictors for Thromboembolic Complications in Patients with Membranous Nephropathy** Elion Hoxha, Ina Ellen Thiele, Gunther Zahner, Ulf Panzer, Sigrid Harendza, Rolf A. Stahl. *III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

**Background:** Patients with Membranous Nephropathy (MN) have a high risk to experience thromboembolic complications (TEC). It is currently unclear whether there is a level of albuminuria or serum albumin which predict an increased risk of TEC. Secondly it is also unclear whether there exist differences in the risk for TEC between primary and secondary MN.

**Methods:** We analyzed in a prospective multicenter study 263 adult patients with MN up to 24 months (64.6% primary MN with $PLA_2R$-Ab positivity; 35.4% secondary MN). Patients were seen every three months for analysis of proteinuria, serum albumin and serum creatinine. TEC were assessed at every visit and categorized in emboly of lung arteries, thrombosis of leg veins, central veins, the dialysis fistula or renal venous thrombosis.

**Results:** 29 (11%) out of 263 patients developed a TEC. 17 patients had emboly of the lung arteries. 4 patients had thrombosis of leg veins, 2 thrombosis of central veins, 3 thrombosis of the dialysis fistulae, 3 patients had renal venous thrombosis. 23 patients developed the TEC before or at the time of the diagnosis of MN. There was no relative difference in the TEC between patients with primary or secondary MN (11.2% and 10.8% respectively).There was no statistically significant difference in the $PLA_2R$-Ab levels in patients with primary MN between patients with (221.4±451.0 U/ml) or without a TEC (198.9±341.6 U/ml). In patients with serum albumin <20g/L or proteinuria >8g/24h the incidence of a TEC was higher compared to the remaining patients ($p<0.05$). Surprisingly, patients with serum creatinine >1,2mg/dl also had a significantly higher risk of TEC ($p<0.05$).

**Conclusions:** Serum albumin levels < 20g/L, proteinuria >8g/24h and serum creatinine levels >1.2 mg/L are risk factors for the development of TEC in patients with MN. There was no difference between patients with primary or secondary MN. $PLA_2R$-Ab levels did not influence the incidence of TEC in patients with primary MN.

## TH-PO1025

**Spontaneous Remission of Proteinuria Is a Frequent Event in $PLA_2R$ Antibody Negative Patients with Membranous Nephropathy** Elion Hoxha, Ina Ellen Thiele, Gunther Zahner, Ulf Panzer, Sigrid Harendza, Rolf A. Stahl. *III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

**Background:** Membranous Nephropathy (MN) is the most common cause of nephrotic syndrome in adults. While 70-80% of patients with MN are positive for $PLA_2R$-Ab in the serum, little is known about the pathogenesis of MN in $PLA_2R$-Ab negative patients and whether the clinical course is different from patients with primary MN.

**Methods:** We performed a prospective multicenter study in order to study the clinical outcome in patients with $PLA_2R$-Ab negative MN. 60 adult patients with biopsy-proven MN were studied up to 24 months. Proteinuria, serum albumin, blood pressure and serum creatinine were studied in 3 monthly intervals. Immunosuppressive therapy or supportive medical care was assessed over the time of observation.

**Results:** 18 (30%) patients had non-nephrotic proteinuria (proteinuria <3.5g/24h) at the time of inclusion in the study. These patients had an excellent outcome without immunosuppressive treatment, 5 of them had a secondary cause of disease (3 Lupus Erythemathodes (LE), 1 Sjoergen's syndrome, 1 NSAID-Exposure). Of the 42 patients with proteinuria >3.5g/24h at inclusion in the study, 24 (57%) were treated with immunosuppressive agents (19 calcineurin inhibitors, 9 alkylating agents, 3 rituximab, 2 glucocorticoids). 7 of these patients had a secondary cause of disease (3 LE, 3 Tumor, 1 NSAID-Exposure). 9 patients received 2 or more different immunosuppressants during follow-up. The remaining 18 patients were treated with supportive medication only. 8 of them had a secondary cause of disease (2 LE, 5 Tumor, 1 neurologic chronic inflammatory disease). Patients treated with immunosuppressants had a similar age, gender distribution, proteinuria, serum albumin, and serum creatinine at the start of the study compared with patients on supportive treatment. After 24 months there was no significant difference in the levels of proteinuria and serum creatinine between patients receiving immunosuppressive therapy or supportive treatment only.

**Conclusions:** We conclude that a high number of $PLA_2R$-Ab negative patients with MN have a good prognosis and might not need immunosuppressive therapy.

## TH-PO1026

**Molecular Characteristics of Anti-PLA2R IgG in Japanese Patients with Idiopathic Membranous Nephropathy** Shin'ichi Akiyama,[1] Enyu Imai,[2] Seiichi Matsuo,[1] Shoichi Maruyama.[1] *[1]Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Aichi, Japan; [2]Nakayamadera Imai Clinic, Takaraduka, Hyougo, Japan.*

**Background:** We previously reported that a prevalence of auto-IgG against phospholipase A2 receptor (PLA2R) in Japanese patients with idiopathic membranous nephropathy (iMN) is remarkably lower (49%) than cases in Caucasian and Chinese patients (65%~) However, the molecular characteristics of anti-PLA2R IgG in Japanese patient with iMN have not been revealed. In this study, we studied antigenic reactivity and major subclass of anti-PLA2R auto-IgG in Japanese patients with iMN.

**Methods:** We performed Western blotting, under nonreducing or reducing condition, of recombinant PLA2R or the native PLA2R in human glomerular extract (HGE) with anti-PLA2R IgG positive serum specimens from 25 Japanese patients with iMN. To evaluate a influence of *N*-glycosylation of PLA2R to antigenic reactivity against anti-PLA2R auto-IgG, an enzymatic deglycosylated PLA2R was also used in Western blotting as antigen. The major subclasses of anti-PLA2R IgG were determined by ELISA with recombinant PLA2R and subclass specific secondary antibodies.

**Results:** None of the positive serum specimens retained reactivity to PLA2R when the HGE and recombinant PLA2R were electrophoresed under reducing conditions. All serum samples reacted with both native form and deglycosylated form of the PLA2R under nonreducing conditions. These results indicate that *N*-glygosilation of PLA2R is not necessary to bind with anti-PLA2R IgG, a native conformation of PLA2R is essential. The reactive serum samples are highly enriched in IgG4 that react with recombinant PLA2R or the native protein in HGE. Although IgG4 is predominant subclass of anti-PLA2R IgG, other subclasses are also present in smaller amounts.

**Conclusions:** The molecular properties, antigenic reactivity and major subclass, of anti-PLA2R IgG in Japanese patients with iMN are similar to those in Caucasian patients with iMN. The anti-PLA2R IgG in Japanese patients with iMN uniquely recognize a conformation-dependent epitope in PLA2R and their major subclass is IgG4.

## TH-PO1027

**Membranous Nephropathy after Allogeneic Hematopoietic Stem Cell Transplantation** Rikako Hiramatsu, Yoshifumi Ubara, Keiichi Sumida, Tatsuya Suwabe, Junichi Hoshino. *Nephrology Center, Toranomon Hospital, Tokyo, Minato-ku, Japan.*

**Background:** Renal involvement in patients with chronic graft-versus-host disease (cGVHD), presenting as nephritic syndrome, is rare following hematopoietic stem cell transplantation (HSCT). The most frequent form of post-HSCT glomerulonephritis is membranous nephropathy (MN); but data on its clinico-pathological features and long-term outcome are scarce.

**Methods:** From 2000 to 2012, the clinical characteristics, pathological features, and long-term outcome were evaluated in 5 patients with MN after HSCT. Kidney biopsies were analyzed by light microscopy (LM), immunofluorescence (IF), electron microscopy (EM), and IgG subclasses in glomerular deposits were identified.

**Results:** All patients had a history of cGVHD, with active cGVHD prior to MN diagnosis. Mean time between HSCT and diagnosis of MN was 21.8 months (range; 9–33). Serum creatinine was 1.0 mg/dL (0.8 to 1.3). Discontinuation of cGVHD prophylactic immunosuppressants prior to MN onset was a consistent feature, occurring at a median of 12.6 months (range; 2–25) before onset. Significant proteinuria (mean; 8.40 g/day, range; 5.93–13.0) was common. LM did not detect definite spike formation in capillary lumens. EM revealed subepithelial and intramembranous deposits, as well as mesangial deposits with extensive podocyte foot process effacement. Subepithelial deposits were segmental, small and electron-lucent. IF showed that IgG1 and IgG4 were the predominant IgG subclasses in glomerular deposits, which were negative for C3. All MN cases were initially treated with corticosteroids, resulting in complete remission (CR) at a median of 12.2 months (range; 5–24). Long-term follow up at a median of 92.4 months (range; 38–144) showed that relapse of MN paralleling the evolution of cGVHD occurred in 2 patients at 43 and 27 months after initial CR. One patient developed end-stage renal failure requiring hemodialysis.

**Conclusions:** This series of 5 patients with post-HSCT MN is the largest study so far. Initial response to treatment was favorable, but careful observation for MN relapse parallel to cGVHD evolution, and long-term renal monitoring, are necessary.

*Funding:* Government Support - Non-U.S.

## TH-PO1028

**Outcome of Renal Biopsy Proven Membranous Nephropathy Patients – Single Centre Experience** Muhammad A. Imtiaz,[1] Katie Ht Wong,[2] Rosa M. Montero,[1] Mona Wahba.[1] *[1]Dept of Renal Medicine, St .Helier Hospital, Surrey, United Kingdom; [2]Dept of Medicine, St. Peter Hospital, Surrey, United Kingdom.*

**Background:** Membranous Nephropathy(MN) is the commonest cause of nephrotic syndrome in adults. Number of studies that looked at the progression of MN showed variable rate of progression with 8-20% of patients become dialysis dependent.

**Methods:** Patients from 1995-2010 with MN were included.Urinary protein creatinine ratio(UPCR), eGFR, serum Creatinine(Cr), Cholesterol(Ch) and Albumin(Alb) levels were collected at 2, 5 and 10 years. Outcomes were measured as changes in baseline UPCR, baseline Cr, onset of renal replacement therapy(RRT) and patient survival. Significant change in creatinine was defined as 20% increase from baseline. Treatment groups were divided into Non Immunosuppression(Non Immuno) receiving angiotensin converting enzyme inhibitors(ACEi) or Angiotensin 2 receptor blockers(ARB) and a statin or Immunosuppressed(Immuno) group where they received additional immunosuppressive therapy.

**Results:** Non Immuno group included 77/125 patients(61%). 54 male, 23 female; Median age 60.5 years. Immuno group had 48 patients; 26 males. Median age was 59 years. In both groups UPCR reduced significantly by 2 and 5 years from baseline readings(both $p<0.001$). There was a significant increase in Cr at 2 and 5 years in the Non Immuno group from baseline ($p<0.05$, $p<0.01$, respectively) in contrast to the Immuno group where there was no significant change. There were 7 deaths in Non Immuno group compared with Immuno group that had 3 deaths over 10 years. 11 patients in the Non Immuno were on RRT by 10 years compared with 8 patients in the Immuno group that was not significant. There was a significant improvement in Ch and Alb levels from baseline in both groups that was maintained throughout the follow up period($p<0.001$, $p<0.001$, respectively).

**Conclusions:** Irrespective of immuno or non immuno group, patients with MN had similar clinical outcomes with regards to UPCR, Ch and Alb levels over 10 years. There was significant increase in Cr of Non Immuno group at 2 and 5 years. Overall 10/125(8%) died and 19/125(15.2%) became dialysis dependent over 10 years suggesting a survival advantage in the Immuno group.

## TH-PO1029

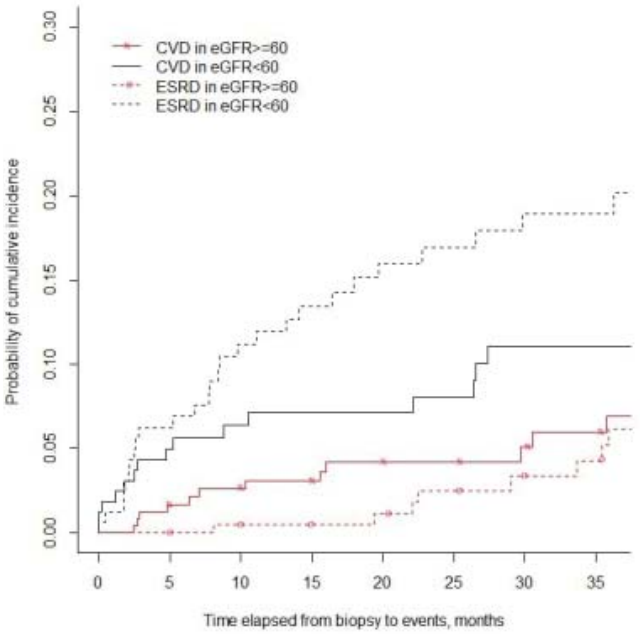
**The Risk of Cardiovascular Events (CVE) Is Similar or Exceeds That of End Stage Kidney Disease (ESKD) in the Early Years of Membranous Nephropathy (MN)** Taewoo Lee, Vimal K. Derebail, Shannon L. Mahoney, Abhijit V. Kshirsagar, Caroline J. Poulton, Susan L. Hogan, Ronald J. Falk, Patrick H. Nachman. *UNC Kidney Center, The Univ of North Carolina at Chapel Hill, Chapel Hill, NC.*

**Background:** We hypothesized that patients with primary MN have an increased risk for CVE. We explored the incidence, risk factors and time to CVE as compared to ESKD.

**Methods:** In an inception cohort of 404 patients with median follow-up of 23 m (IQR 9-50), we investigated the incidence of ESKD, all-cause mortality and CVE defined as acute coronary syndrome, stroke or peripheral artery thrombosis. Cumulative incidence rates of CVE before reaching ESKD were estimated using competing risk analysis. To

determine risk factors, competing risk regression analysis was performed with relevant covariates at baseline. A severity score of nephrotic syndrome (NS) determined by proteinuria ( ≥3.5 g/day) and/or hypoalbuminemia (<2.5 g/dL) at each visit was used as a time-dependent variable.

**Results:** 32 CVE occurred in a median of 8 m (IQR 3-29), 69% within 2 years. Cumulative incidence rates of CVE, ESKD, and death in the entire cohort were 5.7%, 8.9%, and 2.4% at 2 years and 9.1%, 19.4%, and 4% at 5 years, respectively. Among patients with baseline eGFR ≥ 60 ml/min, the risk of CVE [4.2% (95% CI 1.5-6.8)] exceeded that of ESKD [2.5% (0-5)] at 2 years; but not when eGFR <60 ml/min, [8% (3.6-12.4) vs 18.6% (12-25), respectively]. The severity of NS (HR 2.19), previous CVE, diabetes mellitus and age were significant risk factors of CVE after adjusting for smoking and CKD stages.




**Conclusions:** Early after diagnosis, the risk for CVE is similar to that of ESKD, and is greater than ESKD in patients with preserved renal function. CVE are an important complication of MN and may be as relevant an outcome measure as ESKD. CVE are associated with the severity of NS and traditional risk factors.

## TH-PO1030

**Development and Evaluation of a Patient-Reported Outcome (PRO) Measure for Idiopathic Membranous Nephropathy (IMN): The Membranous Nephropathy Quality of Life Tool (MNQoL)** Christine Barrett,[1] Jane E. De Vries,[1] Rachel B. Jones,[2] Lynda C. Doward,[3] Patrick Hamilton,[4] Michael Venning.[4] *[1]GlaxoSmithKline plc; [2]Vasculitis Unit, Addenbrooke's Hospital, Cambridge; [3]RTI-Health Solutions, Manchester; [4]Manchester Institute of Nephrology and Transplantation, Manchester Royal Infirmary, United Kingdom.*

**Background:** Regulators such as the FDA recognise that the patient perspective is important in evaluating new treatments for IMN. No PRO measures currently exist specific for nephrotic states including IMN. Our objective was to develop a new PRO tool, for use in IMN studies as an exploratory endpoint alongside the Kidney Disease Quality of Life 36 item survey (KDQoL-36), and to evaluate content validity of both tools.

**Methods:** Patient interviews (n=4) and a literature review yielded 12 key symptoms of IMN. A 14-item MNQoL was developed with 5 response levels asking the degree of "bother" associated with each symptom, treatment side effects and the quantity of medicines required to treat IMN. Cognitive debriefing was conducted with a new IMN patient sample (n=9) to determine content validity and ease of completion of the MNQoL and KDQoL-36 (study HO-12-955).

**Results:** Patients debriefed were 6M/3F, with median age=51/range 39-68, 4 active disease/5 remitted (partial or complete), mean years since diagnosis=6.3/SD 8.6. Cognitive debriefing demonstrated that the KDQoL-36 and MNQoL, used together, comprehensively represent symptoms and side effects experienced by IMN patients. Patients found the questionnaires relevant and easy to complete. Some minor comprehension issues were identified e.g. how to interpret "bother". Future versions of the MNQoL should also enquire if the patient actually experiences each symptom or side-effect. For the MNQoL to be used alone, additional symptoms would need to be added.

**Conclusions:** This research has shown the MNQoL and KDQoL-36 to be suitable instruments to assess the impact of IMN and its treatment from the patient's perspective. Further validation of the MNQoL is required within IMN clinical trials, as well as in other nephrotic conditions. This research will inform ongoing development of PROs as potential regulatory endpoints in IMN studies.

*Funding:* Pharmaceutical Company Support - GlaxoSmithKline plc

## TH-PO1031

**Serum Anti-Phospholipase A2 Receptor Antibody Testing for Idiopathic Membranous Nephropathy** Vinod Raman, Craig E. Gordon, Laurence H. Beck, Jean M. Francis. *Renal Section, Dept of Medicine, Boston Univ School of Medicine, Boston, MA.*

**Background:** Idiopathic membranous nephropathy (IMN) is diagnosed in 16-33% of kidney biopsies for nephrotic range proteinuria in adults. Anti-phospholipase A2 receptor antibody (anti-PLA2R) has been associated with idiopathic membranous nephropathy. The performance characteristics of the available diagnostic tests for anti-PLA2R have not been well defined. Using systematic review and meta-analysis, we aimed to identify the role of anti-PLA2R testing as a diagnostic tool for IMN in patients with nephrotic range proteinuria.

**Methods:** We performed a systematic review of the literature using Medline (last search: 5/7/2013) to identify studies reporting the use of serum anti-PLA2R as a diagnostic test for IMN in patients with nephrotic range proteinuria and biopsy confirmation of IMN. From reported data, we calculated the sensitivity and specificity for serum anti-PLA2R testing against reference standard of biopsy. IMN incidence data was used to estimate the positive and negative predictive values (PPV, NPV).

**Results:** Of 1125 retrieved studies, 10 studies provided data that allowed the calculation of sensitivity and specificity for serum anti-PLA2R testing. The 10 studies reported on total 620 unique patients with nephrotic range proteinuria. Patients had a mean age of 49.5y, 50% were male, mean proteinuria was 7.9g/g, and mean serum creatinine was 1.07mg/dl. Six studies used western blot and four used immunofluorescence. Sensitivity of anti-PLA2R testing ranged from 66-98% and specificity from 84-100%. Using the median value of IMN incidence in patients with nephrotic range proteinuria obtained from published literature (23%), PPV and NPV ranged from 92%-100% and 78-96%, respectively.

**Conclusions:** Our findings indicate that serum testing for anti-PLA2R is an important diagnostic tool in the evaluation of patients with nephrotic range proteinuria with a high sensitivity, specificity, and at expected incidence levels, a high positive and negative predictive value for IMN.

## TH-PO1032

**Circulating TNF Receptors Is a Significant Prognostic Biomarker for Idiopathic Membranous Nephropathy with Nephrotic Syndrome** Su Mi Lee,[1] Hyun Seop Cho,[2] Yun Jung Oh,[3] Dong Ki Kim,[1] Yun Kyu Oh,[4] Chun Soo Lim,[4] Shin-Wook Kang,[5] Jung Pyo Lee,[4] Yon Su Kim.[1] *[1]Seoul National Univ Hospital; [2]Gyeongsang National Univ Hospital; [3]Cheju Halla General Hospital; [4]Seoul National Univ Boramae Medical Center; [5]College of Medicine, BK21, Yonsei Univ.*

**Background:** Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults. There is no secure biomarker that can indicate the severity and predict the long-term prognosis of the MN. The aim of this study is to evaluate the clinical significance of TNF receptors (TNFRs) as a prognostic marker.

**Methods:** 94 patients had MN with nephrotic range proteinuria at the time of biopsy were enrolled. Circulating TNFR levels were measured using serum samples stored at the time of biopsy. The primary clinical endpoint was a remission. Secondary outcome was the decline of estimated glomerular filtration rate (eGFR; ≥30% decline compared to baseline).

**Results:** At the time of biopsy, eGFR and proteinuria worsened proportionally as circulating TNFR1 and TNFR2 increased (p<0.001). 73 of 94 patients reached primary outcome. Compared to non-responder group, responder group had significantly lower circulating TNFR 1 (1972.38 ± 1017.67 vs. 3743.32±2125.58, P=0.035). However, there was no significant difference of TNFR2 between responder and non-responder (4785.10±2215.38 vs. 7685.10±4210.6, P=0.074). The risk of reaching the secondary endpoint was significantly higher in the in the highest quartile of TNFR1 or TNFR2 compared with other quartiles by the Kaplan Meier method (P=0.001 and P<0.001, respectively). Highest TNFR1 was a significant risk factor for the secondary endpoint after adjusting for age, sex, amount of proteinuria, eGFR, treatment, and presence of remission (Hazard Ratio [HR] 1.51, 95% confidence interval [C.I] 1.14-2.02, P=0.005). Highest TNFR2 was also statistically significant (HR 1.50, 95% C.I 1.12-2.00, P=0.007).

**Conclusions:** This study showed circulating TNFR 1 could predict remission. In addition, circulating TNFR 1 and TNFR 2 could predict progression of CKD in patients with MN. Further studies would be needed to establish the role of circulating TNFRs to reflect the responsiveness of specific treatment.

## TH-PO1033

**Tacrolimus in Membranous Nephropathy** Paula Jara Caro Espada, Elena Gutierrez-Solis, Jorge Enrique Rojas-Rivera, Manuel Praga. *GLOSEN (Spanish Group for Study of Glomerular Diseases).*

**Background:** Tacrolimus (TAC) monotherapy has shown to be effective in the treatment of idiopathic membranous nephropathy (IMN), but observational studies involving a larger number of patients have not been published.

**Methods:** We performed a retrospective analysis of all IMN patients treated with TAC in 12 Spanish Hospitals. Primary outcomes were complete (CR) or partial (PR) remission. Secondary outcomes were relapses after TAC withdrawal and safety and tolerance to treatment.

**Results:** 122 patients were included. Median interval between renal biopsy and TAC treatment was 9 months (7-30). 43 patients (35%) had received other immunosuppressive treatments. Initial TAC dose was 0.049±0.12 mg/kg/day and only 10 patients were treated simultaneously with corticosteroids (CS). Mean duration of TAC treatment was 17±7 months, including a full-dose TAC period (12±5 months) and a tapering period (5±4). CR

or PR were achieved in 103 patients (84%). Non-responder patients (n=19) showed a non-significant trend for higher proteinuria and worse renal function at the onset of TAC than responders. Among responders, 60 patients (58%) achieved PR and 43 (42%) CR. Patients who achieved CR had a significantly higher percentage of females (44 vs 21%; p<0.015), a shorter time to remission (3 vs 6 months; p<0.018), and a lower proteinuria (6.7±3.2 vs 8.8±3.9 g/day; p=0.005) and serum creatinine (0.9±0.2 vs 1.1±0.3 mg/dL; p<0.0001) at baseline than PR patients. No influence of CS on the rate of remission was observed. TAC was well tolerated. Nephrotoxicity, leading to TAC withdrawal, was observed in 4 cases (3%), and minor/transient side effects in 21 pacientes (17.2%). After 30 (14-66 month) months of follow-up, 44% of patients who had achieved CR/PR, relapsed. Median time to relapse after TAC withdrawal was 9.5 months (3-62). Relapses were significantly more common among patients who had received CS (8% vs 0%). Patients who relapsed showed a higher although non-significant proteinuria (1.96±1.76 vs. 1.19±1.16 g/day; p=0.051) at the onset of TAC tapering.

**Conclusions:** TAC monotherapy induced a high number of remissions in IMN and was well tolerated. New strategies for avoiding frequent relapses after TAC withdrawal should be investigated.

## TH-PO1034

### Tacrolimus in Treating Hepatitis B Virus Associated Membranous Nephropathy with Nephritic Syndrome Who Failed Antiviral Monoyherapy

Jianghua Chen. *The First Affiliated Hospital, College of Medicine, Zhejiang Univ, Kidney Disease Center, Hangzhou, Zhejiang Province, China.*

**Background:** Hepatitis B virus associated membranous nephropathy (HBV-MN) may lead to renal failure in a significant proportion of patients and spontaneous remission is rare. Although antiviral treatment such as lamivudine is considered to improve renal outcome, its potential therapeutic impact on HBV-MN has not been characterized. When a proportion of patient with HBV-MN are resistant to antiviral monotherapy, it is a challenge to find other therapies that are effective.

**Methods:** This prospective observational study enrolled 14 adults with HBV-MN and nephritic syndrome who were no response to therapy with at least 3 months of lamivudine monotherapy. These patients received a regimen of tacrolimus combined with low dose prednisone (0.3 $mg.kg^{-1}.d^{-1}$ of prednisolone). Oral tacrolimus was administered (target trough levels of 5-10 ng/ml) for 24 weeks, and then reduced doses were given (target trough level of 3-6 ng/ml) for another 24 weeks. All patients continuously received lamivudine therapy.

**Results:** Following 24 weeks of therapy, Eight of 14 patients (57.1%) had complete remission (CR) and 4 of 14 patients (28.6%) had complete remission (PR). The mean time to partial remission and complete remission was 6.8 ± 4.7 weeks and 11.5 ± 6.6 weeks, respectively. Two patients developed resistance to TAC therapy due to no response with persistent severeproteinuria after 16 weeks of TAC therapy 3 (25%) of 12 patients who achieved remission experienced relapses during follow-up. Renal function remained stablein all patients with complete remission or partial remission. 1 of 2 non-responder progressed to doubling their baseline Scr. Elevation of AST occurred in 2 (14.3%), and 2 (14.3%) of 14 patients. The marker for HBeAg of all patients did not change during follow-up. HBV replicationwas seen in 1 (7.1%) of 14 patients due to HBV-DNA levelsincreased to $\geq 10^7$copies/mL.

**Conclusions:** The regimen of tacrolimus combined with low dose prednisone seems to beeffective and safety in treating patients with HBV-MN and nephritic syndrome who failed lamivudine monotherapy.

## TH-PO1035

### Efficacy and Safety of Rituximab Plus Cyclosporine in Idiopathic Membranous Nephropathy: Results of an Ongoing Prospective Trial

Meryl A. Waldman, Michelle Braun, Howard A. Austin. *NIDDK/Kidney Disease Section, National Institute of Health, Bethesda, MD.*

**Background:** Cyclosporine (Csa) has efficacy in reducing proteinuria in idiopathic membranous nephropathy (IMN) but partial remissions (PR), rather than complete (CR) are more common and relapse upon drug withdrawal is problematic. Extending treatment may increase remissions and reduce relapses but potential for nephrotoxicity exists. Rituximab (RTX) monotherapy has shown promise in IMN but PRs are more common and effect on proteinuria tends to be delayed. We are conducting a prospective phase 2 trial in 30 pts with IMN to investigate whether "induction" with RTX + Csa for 6 mos followed by "maintenance" RTX may achieve greater reduction in proteinuria than either agent alone, increase number of remissions (especially CR) & reduce relapse rates. Here we report interim data.

**Methods:** Patients with IMN, persistent high grade proteinuria despite conservative rx for min 6 mos & eGFR ≥40 ml/min/1.73 $m^2$ receive RTX (1 gm d 1, 15) + Csa x 6 mos, then tapered. A 2nd course of RTX is given after min of 6 mos & evidence of B cell recovery.

**Results:** To date, 11 pts are enrolled. Mean CrCl 61 ml/min(38 -90); Mean proteinuria 11g/d. Of 8 pts with minimum of 12 mos follow up, all showed response; 4 (50%) PR (defn:protein reduction ≥50% & ≤3.5g/d); 4 (50%) CR (≤0.3 g/d). 1 pt relapsed after achieving PR.

| Pt # | Baseline proteinuria g/24hr | 3 mos | 9 mos | 12 mos | 18-20 mos | 24 mos |
|---|---|---|---|---|---|---|
| 1 | 11.2 | 10.9 | 14 | 5.7 | 1.8 | 0.8 |
| 2 | 10.5 | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 |
| 3 | 14.1 | 11.3 | 2.1 | 1.4 | 0.8 | |
| 4 | 13.9 | 1.1 | 0.3 | 0.2 | 0.2 | |
| 5 | 8 | 1.9 | 0.7 | 0.5 | 0.6 | |
| 6 | 9.8 | 7.2 | 2.1 | 7.4 (relapse) | - | |
| 7 | 9.6 | 1.3 | 0.2 | 0.2 | 0.1 | |
| 8 | 12.6 | 3.5 | 0.3 | 0.2 | | |
| 9 | 15.9 | 2.9 | | | | |
| 10 | 8.3 | | | | | |
| 11 | 5.9 | | | | | |

Regimen was well tolerated.

**Conclusions:** "Induction" with RTX + Csa followed by "maintenance" RTX may be a treatment approach for IMN to achieve a greater number of remissions and may obviate the need for long term immunosuppression. It appears to be well tolerated. Enrollment continues and longer term follow up is needed.

*Funding:* NIDDK Support

## TH-PO1036

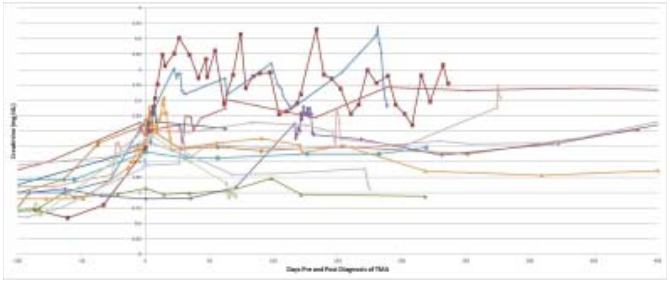
### Thrombotic Microangiopathy in Metastatic Melanoma Patients Treated with Adoptive Cell Therapy and Total Body Irradiation

Meryl A. Waldman,[1] Jennifer Tseng,[2] Michael S. Ring,[1] Howard A. Austin,[1] James Yang,[2] James E. Balow.[1] *[1]NIDDK/Kidney Disease Section, National Institute of Health; [2]NCI, National Institute of Health.*

**Background:** Adoptive cell therapy (ACT)using ex-vivo expanded autologous tumor infiltrating lymphocytes can mediate the regression of bulky metastatic melanoma when administered with high-dose IL-2 following a lymphodepleting preparative regimen of cyclophosphamide & fludarabine. A series of trials showed that addition of total body irradiation (TBI) 12 Gy to regimen led to improved response rates & survival. However,a complication encountered after TBI was thrombotic microangiopathy (TMA). Here we report incidence & course of TMA after TBI.

**Methods:** In the pilot trial, all pts received ACT with TIL + IL2 following non-myeloablative chemo & 12 Gy TBI. The RCT randomizes patients to ACT/ chemo regimen with or without TBI. Dx of TMA made by kidney bx in 3 pts; subsequently dx based on clinical presentation (new onset hemolytic anemia, rise in Scr &LDH, dec plts & haptoglobin).

**Results:** 25 pts enrolled in the pilot trial. To date, 89 pts enrolled in the RCT, 44 in TBI arm. Of 69 pts exposed to TBI in both trials, 16 (23%) were dx'd with TMA. No pts without exposure to TBI dev TMA. Median time to dx was 8 mos after TBI (range 4 -12). All had rise in Scr.



Mean LDH 255 U/L (nl value 113- 226); Proteinuria present in all, most subnephrotic; Urine Protein/cr : range 0.4 -3.6 (mean 1.8). All dev new onset htn or worsening of htn requiring multiple anti-htn agents. Cr values decreased in many over time but did not return to baseline. Low grade proteinuria persisted in almost all at last follow up.

**Conclusions:** Thrombotic microangiopathy is a complication of total body irradiation and occurred in almost 25% of pts exposed to 12 Gy dose. Although hematologic abnormalties are self limiting, most are left with residual renal dysfunction, proteinuria and persistent hypertension.

*Funding:* NIDDK Support, Other NIH Support - National Cancer Institute

## TH-PO1037

### Rituximab-Induced Continuous B Cell Depletion for Durable Remission in 28 Consecutive Patients with Idiopathic Membranous Nephropathy

William Franklin Pendergraft,[1-3] Charles T. Owens,[1-3] Andrew P. Murphy,[3] Colin M. Berry,[3] Karen A. Laliberte,[3] John Niles.[2,3] *[1]Joint Nephrology Fellowship Program, Massachusetts General Hospital (MGH) and Brigham and Women's Hospital, Boston, MA; [2]Div of Nephrology, MGH, Boston, MA; [3]Vasculitis and Glomerulonephritis Clinic, MGH, Boston, MA.*

**Background:** Great strides have been made with respect to the pathogenesis of idiopathic membranous nephropathy, and numerous groups are now actively working to develop an optimal strategy for induction and maintenance of remission. Induction therapy

with chlorambucil and corticosteroids appears to be more effective than with calcineurin inhibitors; however, rituximab may be more efficacious. We report here our experience to date with management of the disease.

**Methods:** We performed a retrospective analysis of clinical data from patients with biopsy-proven idiopathic membranous treated by our group with continuous B cell depletion.

**Results:** 28 patients (50% women) received induction of remission therapy for idiopathic membranous nephropathy. All patients received corticosteroids. 15 patients received cyclophosphamide and rituximab. Mean duration of therapy was 172 days (S.D. 139). Mean spot urine protein to urine creatinine ratios went from 9.0 (S.D. 4.8) at the start of therapy to 2.5 (S.D. 2.8). 13 patients received rituximab without concomitant cyclophosphamide. Mean duration of therapy was 301 days (S.D. 117). Mean spot urine protein to urine creatinine ratios went from 5.1 (S.D. 3.3) at the start of therapy to 2.3 (S.D. 3.1). One patient in the rituximab and corticosteroids group died five months into therapy after being institutionalized for advanced Alzheimer's. One patient developed GCSF-responsive neutropenia.

**Conclusions:** Over the past year, our group has seen a dramatic rise in the number of new cases. Induction therapy with cyclophosphamide, corticosteroids and rituximab was more effective than corticosteroids and rituximab alone in reducing proteinuria despite shorter overall duration of continuous B cell depletion. These preliminary results suggest that induction therapy with cyclophosphamide in the setting of B cell depletion may provide more rapid and complete induction of remission.

*Funding:* Clinical Revenue Support

## TH-PO1038

### Rituximab Therapy in Idiopathic Membranous Nephropathy with Poor Response to Tacrolimus: Long Term Follow Up

Montserrat M. Diaz Encarnacion. *Nephrology, Fundación Puigvert, Barcelona, Spain.*

**Background:** It is still unknown in which patients with idiopathic membranous (MN) Rituximab (RTX) could be a useful therapy.

**Methods:** In this study 17 patients with MN were included and met the following criteria: persistence of nephrotic syndrome (NS) after 6-8 months treatment with angiotensine II blockade and poor response to tacrolimus (Tc) (target level: 5-7ng/ml). RTX is introduced in case of persistence of NS after 4 months period of treatment with Tc (6 patients), partial response (PR) (at 46±26 meses; 9 pacients) and relapse or renal failure during Tc treatment (2 patients). 1 g of RTX was administered on day 1, 15 and every 6 months according to the response. Antibodies anti_PLAR2, B lymphocytes, renal function, proteinuria and serum albumin were evaluated at entry and at months 3,6,12, and 24.

**Results:** aPLAR2 antibodies were present in all patients (Western Blot immunoassay) and in 9 (IFI)

In all patient B cells were depleted (in 11/17 persistently)

Follow up after the first Rx dose was 36,3 months and 16±6 months after the end of treatment.

5/6 patients resistant to Tc achieved P or complete R

5/9 patients with PR achieved CR

Proteinuria decreased in 3 patients with PR

6/7 patients with a GFR<60ml/mn/1,73m2 improved their GFR (16±11ml/mn/ 1,73m2)

The 2 patients who relapsed or with Tc toxicity before RTX were free of relapse during the follow up and with CR.

At the end of follow up 8 patients presented a CR, 8 a PR (proteinuria = 0.7+/-0.4 g/d), 1 a NS.

RTX achieved a P or CR in 90% of patients with poor response to Tc. This response is still persisting after 16 months of follow up.

| | Pre-RTX | Post-RTX | End of followup | p value |
|---|---|---|---|---|
| proteinuria (g/d) mean+/-SD | 4.03+/-3.5 | 0.6+/-0.9* | 1.0+/-1.6 | 0.0009* 0.003 |
| Serum albumin (g/L) mean+/-SD | 33+/-8 | 46+/-5* | 45+/-5 | 0.00001* 0.00004 |
| GFR (CKD-EPI ml/min/1.73 m2) mean+/-SD | 62+/-20 | 72+/-20 | 73+/-20 | NS |

**Conclusions:** RTX could be a useful therapy for patient with poor response or resistant to calcineurin inhibitors therapy.

## TH-PO1039

### Long-Term Treatment of Idiopathic Membranous Nephropathy Based on Cyclosporine and Steroid-Free Regime: A Single Center Experience

Camila Barbosa L. Oliveira, Alline S. A. Oliveira, Carla Queiroz Neves, Clarissa Jacob Barros Carvalho, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

**Background:** Cyclosporine A (CyA) is an effective treatment for idiopathic membranous nephropathy (IMN) but the optimal dose and duration remains controversial. We aim to evaluate the effect of CyA as induction and long-term treatment for nephrotic patients with IMN.

**Methods:** Adult patients with IMN and nephrotic syndrome (NS) between 1997 and 2012 were evaluated. CyA was used for patients: NS > 6 months or proteinuria > 8 g/day or deteriorating renal function, at a dose of 3 mg/Kg/day without steroids.

**Results:** Demographic, clinical and prognostic characteristics are shown in Table 1.

| **Baseline characteristics** | **Treated** N = 33 | **Not Treated** N = 12 | **p-value** |
|---|---|---|---|
| Mean age, year | 39.7 ± 17.4 | 48.0 ± 17.4 | 0.168 |
| Male, n (%) | 21 (63.6) | 7 (58.3) | 0.503 |
| SCr, mg/dL | 1.1 (0.5) | 0.9 (0.4) | 0.279 |
| CrCl, ml/min/1.73m$^2$ | 75.5 ± 19.3 | 76.9 ± 18.8 | 0.500 |
| Serum albumine, mg/dL | 1.9 ± 0.7 | 2.0 ± 0.9 | 0.696 |
| Proteinuria, g/day | 8.32 ± 5.3 | 5.8 ± 1.5 | 0.001 |
| Hypertension, n (%) | 23 (69.7) | 7 (58.8) | 0.722 |
| ACEI/ARB, n (%) | 31 (94) | 10 (88.2) | 0.560 |
| **Outcome** | | | |
| Partial remission, n (%) | 16 (48.4) | 4 (33.3) | 0.502 |
| Complete remission, n (%) | 10 (30.3) | 5 (41.6) | 0.722 |
| Relapse, n (%) | 12 (36.3) | 1 (8.3) | 0.133 |
| Doubling Cr, n (%) | 10 (30.3) | 1 (8.3) | 0.239 |
| CrCl < 30, n (%) | 12 (36.3) | 0 | 0.019 |
| RRT, n (%) | 2 (6.1) | 0 | 1.000 |
| Follow-up, months | 60 ± 38.5 | 54.4 ± 53.6 | 0.746 |

Mean duration and dose of CyA was 23.8 months and 2.8 ± 0.67 mg/kg/day, respectively. In CyA group, developing any remission was associated with a reduced risk of doubling creatinine (p=0.016), CrCl < 30 ml/min/1,73m$^2$ (p<0.001) and RRT (p=0.039).

**Conclusions:** Long-term treatment with CyA induced remission in most of the patients. Achieving any remission in CyA group was associated with reduced risk of renal disease progression and RRT.

## TH-PO1040

### A Retrospective Analysis of Patients with Idiopathic Membranous Nephropathy Treated with Steroids and Intravenous Cyclophosphamide

Robin Ramphul, Raja Mohammed Kaja Kamal, David Makanjuola, Rebecca Suckling, Fiona E. Harris, Bhrigu Raj Sood. *Renal Unit, St. Helier Hospital, Carshalton, Surrey, United Kingdom.*

**Background:** The use of steroids and oral Cyclophosphamide in the modified Ponticelli regimen is a recognised treatment for patients with idiopathic membranous nephropathy however the role of intravenous cyclophosphamide for this indication is poorly described. We present our experience of intravenous cyclophosphamide as an alternative to oral cyclophosphamide in 12 patients with idiopathic membranous nephropathy treated between January 2003 and April 2012. The protocol is as follows: **Prednisolone - 40 mg/day for 30 days - months 1, 3 and 5**; **Cyclophosphamide - day 1 - months 2, 4 and 6** (dose as shown in table).

| **Age (years)** | **Creatinine (µmol/L)** | |
|---|---|---|
| | **<300** | **>300** |
| **<60** | 15 mg/kg | 12.5 mg/kg |
| **>60 and <70** | 12.5 mg/kg | 10 mg/kg |
| **>70** | 10 mg/kg | 7.5 mg/kg |

**Methods:** Data on creatinine, albumin and urine protein to creatinine ratio (UPCR) were collected. All patients included were followed-up for at least 6 months. Treatment failure was defined as ESRD, doubling of serum creatinine, switch to, or introduction of alternative immunosuppression. Complete remission (CR) was defined as a reduction in UPCR to < 50mg/mmol. Partial remission (PR) was defined as a reduction in UPCR to < 350mg/mmol and a 50% or greater reduction in UPCR.

**Results:** There were 12 men, age 68yrs (range 37 – 79); 1 died within 12 months. 10 patients had stable renal function, 2 doubled their serum creatinine and progressed to ESRD after 24 months. The mean UPCR reduced over the course of follow-up (mean UPCR at baseline = 1128.2 mg/mmol, 6 months = 849.2, 12 months = 615.3, 24 months = 93.5). 2 patients achieved CR of proteinuria and 3 had PR. 3 patients were switched to calcineurin inhibitor (CNI), 2 after 12 months and 1 after 18 months for worsening of UPCR - all 3 maintained stable renal function.

**Conclusions:** In our cohort of patients, the immunosuppressive regimen reduced the proteinuria and stabilised the decline in renal function. This suggests that pulsed intravenous Cyclophosphamide may be a viable alternative to oral Cyclophosphamide in the treatment of idiopathic membranous nephropathy.

## TH-PO1041

### Safety and Tolerability of Intravenous Cyclophosphamide as an Alternative to Oral Cyclophosphamide in Patients Treated for Idiopathic Membranous Nephropathy – A Single Centre Experience

Robin Ramphul, Raja Mohammed Kaja Kamal, David Makanjuola, Rebecca Suckling, Fiona E. Harris, Bhrigu Raj Sood. *Renal Unit, St. Helier Hospital, Carshalton, Surrey, United Kingdom.*

**Background:** The role of the modified Ponticelli regimen in managing idiopathic membranous nephropathy (IMN) is well recognised. With pulsed intravenous (iv) Cyclophosphamide, the total dose is reduced, but it is not clear whether this is safer, or better tolerated than oral Cyclophosphamide.

**Methods:** We retrospectively analysed the medical records for adverse events in 13 patients treated between January 2003 and April 2012 for IMN with immunosuppressive treatment according to the following treatment protocol: **Prednisolone - 40 mg/day for 30 days - months 1, 3 and 5**; Cyclophosphamide - day 1 - months 2, 4 and 6 (dose shown below).

| Age (years) | Creatinine (µmol/L) | |
|---|---|---|
| | <300 | >300 |
| <60 | 15 mg/kg | 12.5 mg/kg |
| >60 and <70 | 12.5 mg/kg | 10 mg/kg |
| >70 | 10 mg/kg | 7.5 mg/kg |

**Results:** The adverse events are tabulated below. 8/13 patients had no complications. In the 5 remaining patients, the majority of complications were infective. In 1 patient Chronic Lymphocytic Leukaemia (CLL) was diagnosed 5 years after treatment of the IMN. Subsequent treatment of the CLL with Chlorambucil resulted in remission of proteinuria. One patient died of a myocardial infarction.

| | |
|---|---|
| **Sepsis** | **5** |
| LRTI 3 | |
| UTI 0 | |
| GI – D&V 2 | |
| **Bladder toxicity** | **0** |
| **New onset diabetes** | **1** |
| **Malignancy** | **1** |
| Chronic Lymphocytic Leukaemia 1 | |
| **Death** | **1** |
| Myocardial infarction 1 | |
| **Complications necessitating alteration/cessation of treatment** - sepsis | **1** |
| **Hospitalisation** | **5** |
| LRTI 2 | |
| New onset diabetes mellitus 1 | |
| Dehydration caused by D&V 2 | |

**Conclusions:** Our data suggest that in the majority of patients, the use of iv Cyclophosphamide is well tolerated. However, in some patients the iv regimen was associated with significant morbidity. This highlights the importance, even with lower cumulative doses of Cyclophosphamide, of appropriate counselling for potential complications when planning treatment.

## TH-PO1042

### Immunosuppression for Idiopathic Membranous Nephropathy in North East England

Benjamin R. Beattie,[1,2] Shalabh Srivastava,[1,2] Alison Brown.[1,2]
*[1]Dept of Nephrology, Freeman Hospital, Newcastle-Upon-Tyne, United Kingdom; [2]Newcastle Univ, Newcastle-Upon-Tyne, United Kingdom.*

**Background:** Membranous nephropathy is the most common cause of adult-onset nephrotic syndrome in Europe, with an incidence of 5-10 per million population per year. The majority of cases are idiopathic (IMN) and it is now understood that IMN is an autoimmune disease. The current immunosuppressive (IS) protocol used for IMN patients in the North East of England is a variant of that described by Ponticelli using 6 months of steroid and chlorambucil treatment, but the effectiveness and safety of this regimen in populations with a high prevalence of diabetes and obesity, as in this region of the UK, are not well documented.

**Methods:** All patients with biopsy-proven IMN who had started IS before January 2013 were included in this retrospective cohort study. The following disease markers were recorded: serum creatinine and albumin, eGFR and urine protein:creatinine ratio (uPCR). Measurements of these markers were then repeated at completion of IS (or 6 months after commencing if they did not complete) and again at 6 months after completion of IS. Any reasons for non-completion and adverse events were recorded.

**Results:** 25 patients were included in the study. 17 (68%) were male and 8 (32%) were female. The mean age was 65.5 years. Of the 25 patients, 13 (52%) completed IS, 10 (40%) did not complete and 2 (8%) were currently undergoing treatment. Of those that completed, 3 (23%) completed as per protocol and 9 (69%) completed on a reduced dose. Of those that did not complete, 8 (80%) abandoned due to adverse effects and 2 (20%) died whilst on the regime. No patients achieved total remission (uPCR<20mg/mmol). 11 patients achieved partial remission (uPCR<200mg/mmol) by the end of IS and 13 patients were in partial remission 6 months after finishing treatment. There were 4 cases of sepsis and 3 cases of neutropaenia during IS.

**Conclusions:** The rates of remission in this study do not match the success of immunosuppression reported by Ponticelli and the high incidence of adverse events, non-completion and two deaths suggest that the safety of chlorambucil use in this population may warrant more concern than previously expected.

## TH-PO1043

### Maintenance Immunosuppressive Therapy in Membranous Nephropathy

Rosa M. Montero,[1] Katie Ht Wong,[2] Muhammad A. Imtiaz,[1] Mona Wahba.[1]
*[1]Dept of Renal Medicine, St. Helier Hospital, Surrey, United Kingdom; [2]Dept of Medicine, St. Peter Hospital, Surrey, United Kingdom.*

**Background:** Immunosuppressive therapy has been shown to be beneficial in the treatment of Membranous Nephropathy(MN). The benefits of maintenance immunosuppression therapy are unknown.

Aim is to assess outcome and progression of MN with different maintenance immunosuppression regimens.

**Methods:** Patients with biopsy proven MN in single centre from 1995-2010 had demographic data, urine protein creatinine ratio(UPCR in mg/mmol), baseline eGFR at 2, 5 and 10 years, onset of renal replacement therapy(RRT) and patient survival collected. Initial immunosuppressive regimens were; Prednisolone alone(P) and P+Cyclophosphamide(P+CYP). Maintenance therapy was; P+Azathioprine(AZA) or P+Mycophenolate mofetil(MMF).

**Results:** 48/125 were immunosuppressed; 26 male;22 female. 7/48 (14.6%) were on maintenance therapy; 5/7 P+AZA, 2/7 P+MMF. 2 male;5 female with median age 54 years. No significant difference seen in UPCR, Cr, eGFR, Cholesterol or Albumin between the maintenance groups over 10 years. 41/48 were immunosuppressed with no maintenance therapy. Comparing this group with those on maintenance, a significant reduction in UPCR was seen at 5 years compared with baseline($p<0.001$). There was no significant change in Creatinine or eGFR between the groups. Those on maintenance immunosuppression had a significant sustained improvement in albumin at 2, 5 and 10 years compared with baseline($p<0.05$, $p<0.001$, $p<0.01$, respectively). Improvement in albumin was seen at 2 years compared to baseline in those with no maintenance($p<0.01$). Maintenance immunosuppression had 100% survival and remained dialysis independent at 10 years. In contrast 11 of those on immunosuppression with no maintenance became dialysis dependent, 4 died within 2 years.

**Conclusions:** Those on maintenance therapy had 100% survival rates and remained dialysis independent. The cohort size on maintenance therapy is small and larger studies would be required to determine possible benefit of long term maintenance immunosuppression.

## TH-PO1044

### Clinicopathological Features of Membranoproliferative Glomerulonephritis under a New Classification

Preeti R. Nargund,[1] Neeraja Kambham,[2] Kshama Mehta,[1] Richard A. Lafayette.[1] *[1]Div of Nephrology, Stanford Univ School of Medicine, Palo Alto, CA; [2]Div of Pathology, Stanford Univ School of Medicine, Palo Alto, CA.*

**Background:** Membranoproliferative glomerulonephritis (MPGN) is a histologic pattern of glomerular injury, often associated with chronic infection or autoimmune disease. A recent classification relies on the presence of immunoglobulin and complement to help simplify diagnosis and point towards etiologies of the disease. To further substantiate this approach, we reviewed our own experience.

**Methods:** 281 patients diagnosed with MPGN at Stanford from 2000 to 2012 were identified. Patients with known hepatitis, systemic lupus erythematosus, myeloproliferative disorders were excluded. The clinicopathological findings of the remaining 71 patients were further analyzed using Student's t-test and Chi square tests.

**Results:** 51(72%) of the biopsy proven MPGN patients had a C3+/Ig- pattern by IF while 20 (28%) of them had a C3+/Ig+ pattern. Ig negative defined as zero to 1+ staining on IF. Both groups presented with nephrosis, hypertension, hematuria and depressed serum complement levels. There were no significant age or gender differences. While there was a trend for less proteinuria and higher creatinine in the C3+ /Ig- group, this did not reach significance (p=0.10, 0.16 respectively). By light microscopy, the vast majority of patients in both groups had ≤ 50% glomerulosclerosis and ≤50% cortical scarring and predominantly subendothelial deposits, suggesting opportunity for treatment. When the new classification was applied, of the patients in the C3+/Ig- group: 70.5% had C3GN, 11.8% had dense deposit disease, and the remaining had MPGN with exudative features. In the C3+/Ig+ group, 60% of the patients had an MPGN pattern with predominance of C3 on IF while the remaining had MPGN with exudative features (10%) or light chain (Kappa/Lambda) restriction with no known myeloproliferative disorder (30%).

**Conclusions:** The study highlights the clinicopathological features of patients with biopsy proven MPGN with no known historical etiological factors and sheds some light on the incidence of various etiological categories of MPGN, rapidly becoming a descriptive diagnosis.

## TH-PO1045

### Long Term Follow Up According to Reclassification of Membranoproliferative Glomerulonephritis in Multicenter Study

Hyo-Wook Gil,[1] Sungae Woo,[2] Soon Hyo Kwon,[2] Soo Jeong Choi,[3] Dong-Cheol Han.[2] *[1]Dept of Internal Medicine, Soonchunhyang Univ Cheonan Hospital, Cheonan, Chungnam, Korea; [2]Dept of Internal Medicine, Soonchunhyang Univ Seoul Hospital, Seoul, Korea; [3]Dept of Internal Medicine, Soonchunhyang Univ Bucheon Hospital, Bucheon, Gung-gi Do, Korea.*

**Background:** Membranoproliferative glomerulonephritis(MPGN) are progressive diseases and usually display poor prognoses. Recently, new reclassification into immunoglobulin mediated and complement mediated MPGN that respect pathogenesis was proposed. So we investigated clinical course of MPGN according to reclassification.

**Methods:** We conducted a retrospective study of patients diagnosed with MPGN at the three tertiary institution between 2001 and 2010. We investigated the incidence and prognosis of complement mediated MPGN. We analyzed factors contributing to poor renal function. Progressive renal dysfunction was defined as 50% reduction of GFR or need for renal replacement therapy.

**Results:** Among 3294 cases, 77 cases(2.3%) were diagnosed as MPGN. Thirty one cases were excluded because 7 patients were diagnosed as SLE, and the others were not followed until 12 months after diagnosis. Twenty cases(45.6%) had nephrotic range proteinuria. Mean GFR was 61.2±41.9ml/min/1.73m$^2$. Twenty eight cases were classified MPGN type I, eighteen cases were MPGN type III based on electron microscopic findings. Immune mediated MPGN was diagnosed in thirty nine cases and complement mediated GN in two cases(4.3%). Mean follow up duration was 82.8(12-310)months. Seventeen cases(39.6%) ended up with progressive renal dysfunction. All cases of complement mediated GN developed to progressive renal dysfunction. The progressive renal dysfunction was not influenced by Hepatitis B and C. Poor prognosis factors were male, hypertension, decreased GFR and hypoalbuminemia.

**Conclusions:** Complement mediated glomerulonephritis was 4.3% among previously diagnosed as MPGN cases. All cases of complement mediated GN developed to progressive renal dysfunction although the numbers were small. Decreased GFR, hypertension and hypoalbuminemia at the time of diagnosis may be factors predicting progressive renal dysfunction in MPGN patients.

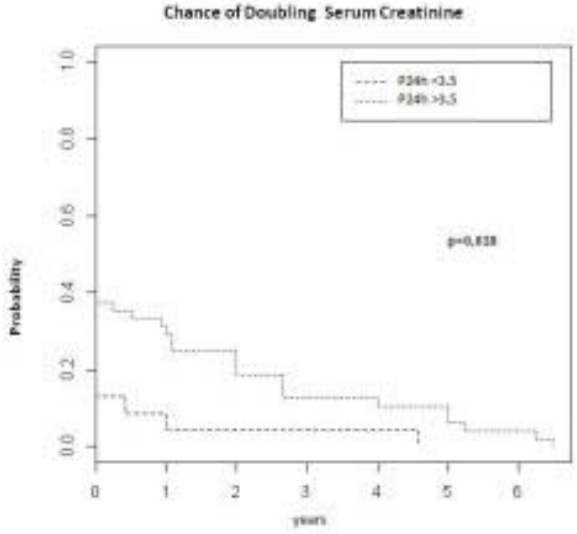
### TH-PO1046

**Factors Involved in the Progression of Membranoproliferative Glomerulonephritis: Retrospective Cohort of 74 Patients** Alline S. A. Oliveira, Camila Barbosa L. Oliveira, Carla Queiroz Neves, Clarissa Jacob Barros Carvalho, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

**Background:** Prognosis of membranoproliferative glomerulonephritis (MPGN) is generally considered unfavorable and it is not clear which factors are involved in long-term progression. Reports suggest that the prognosis is worse in cases of nephrotic syndrome compared with non-nephrotic proteinuria but some authors observed that although the trend is less favorable for the patients with nephrotic syndrome in the first years, this difference disappears later during the follow-up.

**Methods:** We evaluated the medical records of patients treated at the ambulatory of Nephrology diagnosed with MPGN between 1995 and 2013. Patients were considered with controlled blood pressure when at least 80% of measurements were below 140 and 90 mmHg.

**Results:** Seventy-four were patients were evaluated, 55% male with a median age of 44 ± 15 and mean follow-up time of 42.5 months. Initial assessment showed hematuria (70%), hypertension (58%) and 24-hour proteinuria (P24h) ≥ 3.5 g/day (61%). Blood pressure control was associated with a lower chance of developing ClCr < 30 (OR 0.34 p-value 0.04) and ESRD (OR 0.18 p-value 0.03). Moreover, for each additional gram on P24h, there was a 20% increase chance of duplicating serum Cr (p-value 0.03). Patients with P24h > 3.5, interstitial fibrosis and tubular atrophy were also correlated to a higher chance of doubling Cr (p-value of 0.018, 0.03 and 0.05, respectively). Male sex was associated with progression to ESRD (OR 8.79, p-value 0.03).




**Conclusions:** Failure to control blood pressure, male sex and high P24H as well as findings of tubular atrophy and interstitial fibrosis were related to poor renal outcomes in patients with MPGN.

### TH-PO1047

**Phenotypic and Genotypic Characteristics of C3 Predominant Glomerulonephritis** Carla M. Nester, Danniele Gomes Holanda, Kristin A. Tiedt, David A. Myers, Jennifer G. Jetton, Patrick D. Brophy, Richard J. Smith. *Univ of Iowa.*

**Background:** *C3 predominant glomerulonephritis* is the pathological designation used to distinguish the C3 Glomerulopathies (C3G) – a group of diseases defined by their immunofluorescent renal biopsy pattern and by an association with dysregulation of the alternative pathway (AP) of complement. The two major subcategories are dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). A portion of postinfectious glomerulonephritis patients will also present with a predominant C3 pattern on IF, however their clinical course will distingquish them from the C3Gs (hypocomplementemia less than 12 weeks and general renal recovery). We present the phenotypic and genotypic characteristics of the C3 dominant glomerulonephritic cases seen in our practice.

**Methods:** We performed a review of all patients seen and biopsied in our practice from 2007-2013 that met the criteria of C3 predominant staining on IF (C3 staining with a magnitude of 2 greater than other stains - except IgM) and at least 6 months of data for review.

**Results:** 26 patients met the criteria for review: 4 DDD, 17 C3GN and 4 PIGN. 100% of DDD pts and approximately 50% of C3GN and PIGN pts had hypertension at biopsy. The level of urine protein was not statistically different at presentation between the groups however was different at followup: DDD>3GN>PIGN. 40% of DDD, 18% of C3GN and 0% of PIGN pts required dialysis during their course with a trend toward worse eGFR for DDD pts at followup. 77% of pts had an abnormal C3 at presentation with 45% at 60 week followup (N = 18). All pts suspected of PIGN resolved their hypocomplementemia by 12 weeks. An AP genetic assessment was available on 15 of the 26 pts and DNA variants were found in 53%. No genetic abnormalities were found in the PIGN pts.

**Conclusions:** C3GN was the most common C3 dominant glomerulonephritis. Four of four PIGN patients who were biopsied for disease severity had a C3 dominant glomerulonephritis on biopsy. C3 dominant glomerular lesions of the non-PIGN type often follow a chronic glomerular disease course with nearly half of patients continuing to have hypocomplementemia. To our knowledge, this is the largest reported American cohort of C3Gs.

### TH-PO1048

**Bortezomib Is Highly Efficient in Monoclonal Immunoglobulin Deposition Disease** Camille Cohen,[1] Jean Paul Fermand,[3] Bertrand Arnulf,[3] Bertrand Knebelmann,[1] Frank Bridoux.[2] *[1]Nephrologie, Hopital Necker, Paris, France; [2]Nephrologie, CHU, Poitiers, France; [3]Immuno-Hematologie, Hopital Saint Louis, Paris, France.*

**Background:** Monoclonal immunoglobulin deposition disease (MIDD) is a rare complication of plasma cell disorders. Recent data from small case series suggest that novel anti-myeloma agents may improve patient and renal survival.

**Methods:** Thirty-patients with LCDD and 3 with HCDD, all treated with bortezomib plus dexamethasone (BD)–based chemotherapy, were retrospectively analyzed. Data were recorded at baseline and after completion of chemotherapy. Hematologic response was defined according to International Myeloma Working Group. Renal response was defined as a ≥50% decrease in 24H proteinuria (at least 0.5 g), in the absence of a reduction in eGFR ≥ 25% or an increase in serum creatinine ≥ 0.5 mg/dL.

**Results:** Median age at diagnosis was 57 years. Serum and/or urine monoclonal gammopathy was found in 32 patients (97%), 26 of whom (67%) had multiple myeloma and 1 had Waldenstrom macroglobulinemia. Serum free light chain (FLC) ratio was abnormal in all 32 patients tested, with raised kappa (28, 84%) or lambda (4, 12%) FLCs. At diagnosis, median serum creatinine was 213 mmol/l, with 24H proteinuria of 1,27g. Seven patients (21%) required hemodialysis at diagnosis. Patients received a median of 4 courses of BD, alone or reinforced with cyclophosphamide (n =11), thalidomide (n=2), or lenalidomide (n=1). In 14 patients (42%), treatment was followed by HDT/SCT. Hematological response (partial response or above) was achieved in 24 patients (73 %), including very good partial response in 19 cases, and complete response in 2 patients. After a median follow up of 27 months, 2 patients had died. In 23 patients with renal involvement, 11 (48%) had renal response, all of whom showed improved renal function. Two patients progressed to end stage renal disease. Out of 7 patients requiring hemodialysis dialysis at diagnosis, 1 died, and dialysis was withdrawn after BD in 1 case. Four patients had severe adverse effect including 1 encephalopathy.

**Conclusions:** BD-based regimens appear to be associated with high hematologic and renal response rates in MIDD. Further prospective studies are required to confirm these data.

### TH-PO1049

**Primary AL-Amyloidosis – Retrospective Study of 54 Patients, One Center Experience** Elena Zakharova. *Nephrology, City Clinical Hospital n.a. S.P. Botkin, Moscow, Russian Federation.*

**Background:** "Primary" AL-amyloidosis is a plasma-cell dyscrasia characterized by overproduction of monoclonal Ig light chains, most commonly affecting kidneys with nephrotic syndrome (NS) and renal failure (RF). Satisfactory treatment does not exist, current options include high-dose melphalan with autologous stem cell transplantation (TASC), melphalan-prednisone (MP), melphalan-dexamethasone (MD), bortesomib (B) containing regimens, and immunomodulators. We aimed to evaluate treatment results in our cohort.

**Methods:** Using electronic database for we searched patients with biopsy-proven "primary" AL-amyloidosis, treated in 1998-2013. Work-up included serum and urine immunochemistry, kidney biopsy with light microscopy and immunofluorescece ± electron microscopy, and bone marrow aspiration and/or biopsy with immunohistochemistry. Treatment regimens were TASC, MP, MD, B, and supportive treatment (ST) only.

**Results:** Study group included 54 patients, 29 (53.7%) male and 25 (46.3%) female, median age 57 [34;78] y.o. Clinical presentation and treatment are shown in table.

| | | |
|---|---|---|
| Duration before biopsy (months) | 12 | [1;92] |
| NS n | 46 | 85.1% |
| RF n | 29 | 53.7% |
| Creatinine µmol/l | 140 | [60;1200] |
| Kidneys only n | 15 | 27.7% |
| Kidneys+heart n | 14 | 25.9% |
| >2 organs n | 25 | 46.2% |
| TASC n | 9 | 16.6% |
| MP n | 13 | 24% |
| MD n | 6 | 11.1% |
| B n | 9 | 16.6% |
| 2 regimens n | 8 | 14.8% |
| ST n | 25 | 46.2% |
| Follow-up (months) | 5 | [1;94] |
| Alive not on dialysis n | 20 | 37% |
| Started dialysis n | 16 | 29.6% |
| Died n | 18 | 33.3% |

Among 29 patients, treated with chemotherapy, 14 (48.2%) achieved hematological remission, 11 (37.9%) – also organ remission, in 15 (51.7%) cases treatment failed, no significant differences between treatment groups was found.

**Conclusions:** Majority of patients in our cohort were diagnosed late and presented with nephrotic syndrome, notably about a half of them - with renal failure and multiorgan involvement, which limited chemotherapy choice and was associated with 33% of mortality. Treatment results in chemotherapy subgroup showed 48% of hematological and 38% of organ remissions, and did not depended on chemotherapy regimen.

## TH-PO1050

**Alchemy – A Large Prospective Study of Chemotherapy in Systemic AL Amyloidosis** Jennifer H. Pinney, Helen J. Lachmann, Ashutosh Wechalekar, Philip N. Hawkins, Julian D. Gillmore. *UK National Amyloidosis Centre, Div of Medicine, Royal Free Hospital, London, United Kingdom.*

**Background:** There are no large prospective clinical trials in AL amyloidosis.

**Methods:** ALchemy is a prospective observational study of chemotherapy in patients with AL amyloidosis. All patients with newly diagnosed AL amyloidosis requiring chemotherapy in the UK were eligible for study entry from September 2009. Study participants underwent assessment of their disease at baseline, after 3 cycles of chemotherapy and 6, 12, 18 and 24 months from baseline. Clonal disease was assessed after each cycle of chemotherapy and monthly thereafter. Details about tolerability and toxicity of chemotherapy were collected via a case record form.

**Results:** This the largest prospective study in AL amyloidosis worldwide; recruitment is ongoing. Here we present data from the first 616 patients. A primary renal presentation was seen in 49% and 24% had isolated renal disease. Median survival was best in patients with primary renal disease (median undefined) and worst in those with cardiac disease (median 9.3 months). There was no difference in patient survival when stratified for stage of CKD or amount of proteinuria at baseline. Intention to treat analysis of clonal response at 6 months showed that 20% achieved a CR, 23% a VGPR, 17% a PR, and 14% had no clonal response. 24% had died within 6 months of follow-up. In sub group analyses of patients with renal involvement, 59% of patients who presented with CKD stage 1-3 died or were on RRT by 24 months vs 91% of those with CKD stage 4-5 ($P < 0.01$). Renal progression or response occurred within the first 6 months in the majority of cases. By 24 months 46% of patients had renal progression, 26% were unchanged and 27% had a renal response. More patients were in CR/VGPR in the renal progression group compared to the renal response group (80% vs 50%, $P < 0.05$).

**Conclusions:** ALchemy is the largest prospective study in AL amyloidosis worldwide, and has provided a wealth of data to facilitate validation of clinical endpoints, to determine efficacy and toxicity of chemotherapy. Inclusion of all patients with all stages of disease indicates a persistently poor prognosis among a substantial proportion.

## TH-PO1051

**Glomerular Toxicity of Two Therapies Targeting the Vascular Endothelial Growth Factor Result from Distinct Mechanisms** Mario Ollero,[1] Hassane Izzedine,[2] Melanie Mangier,[1] André Pawlak,[1] Djillali Sahali.[1] *[1]INSERM, U 955, Equipe 21, Univ Paris-Est Creteil Val-de-Marne, Créteil, France; [2]Pitie-Salpetriere Hospital, Paris, France.*

**Background:** Renal toxicity constitutes a dose-limiting side effect of anticancer therapies targeting the vascular endothelial growth factor (VEGF), including anti-VEGF ligands and receptor tyrosine kinase inhibitors (RTKI).

**Methods:** We studied 29 patients having followed this kind of treatment. Eight of them developed minimal change nephropathy (MCN)/focal segmental glomerulopathy (FSG)-like lesions and 13 thrombotic microangiopathy (TMA). MCN/FSG-like lesions developed mainly under RTKI, whereas TMA complicated anti-VEGF therapy. We performed immunohistochemical and immunoultrastructural studies on kidney biopsies displaying TMA or MCN/FSG-like lesions and compared them with idiopathic forms. In parallel, we investigated the molecular mechanisms underlying these glomerular syndromes. The RTKI Sorafenib was tested in vitro on wild-type and RelA-deficient mouse embryonic fibroblasts (MEF), on a podocyte cell line, and on lymphocytes from healthy donors.

**Results:** TMA glomeruli exhibited high RelA expression in endothelial cells and podocyte nuclei, while c-mip was undetectable. Conversely, c-mip was highly abundant in MCN/FSG-like lesions, whereas RelA was scarcely detected. Electron microscopy showed major alterations in TMA glomeruli, including duplication of glomerular basement membrane, loss of fenestrations, detachment of endothelial cells and marked effacement of visceral epithelial cell foot processes in some areas. RelA gold particles where increased in podocyte nuclei, as well as in endothelial cells as compared with controls. Sorafenib upregulated c-mip at both transcript and protein levels in vivo and in vitro, at least partly due to inhibition of NF-kB activity. Notably, Sorafenib induced a profound cytoskeleton disorganization with depletion of stress fibers and impressive production of F-actin-rich membranes.

**Conclusions:** These results suggest that renal toxicity of anti-VEGF and RTKI therapies results from distinct mechanisms operating in podocytes and endothelial cells, in which RelA and c-mip play an antagonistic and mutually exclusive role.

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## TH-PO1052

**Care Gaps and Barriers to Guideline-Based Management of Glomerulonephritis: A Survey of Nephrologists** Sean Barbour,[1,2] Monica C. Beaulieu,[1,2] Jagbir Gill,[1,2] Heather N. Reich,[3] Adeera Levin.[1,2] *[1]Div of Nephrology, Univ of BC; [2]BC Provincial Renal Agency; [3]Div of Nephrology, Univ of Toronto.*

**Background:** There is substantial variability in the treatment of glomerulonephritis (GN) that contributes to poor patient outcomes and historically may be due to a lack of accepted guidelines. As such, we sought to better understand the need for and barriers to knowledge translation (KT) of the recent KDIGO GN guidelines into clinical practice.

**Methods:** We surveyed nephrologists in British Columbia, Canada, using a 40 question survey addressing physician characteristics, GN exposure, care gaps, barriers to guideline use and support for a regional GN registry.

**Results:** The response rate was 64% (47 of 73 nephrologists). Biannually a median of 6 (IQR 5,10) new cases of idiopathic GN are seen per physician, which is similar in urban vs rural and academic vs private practices. Self-reported treatment of GN is shown in the table. Most treat ANCA and membranous GN as per KDIGO guidelines; however 19% treat FSGS and 2g/d proteinuria with immunosuppression and only 56% treat FSGS and 5g/d proteinuria with prednisone (less often in those with >15 vs fewer years in practices, 21% vs 64-73% p=0.03). Over 90% feel that standardized care tools would improve patient care yet they are available to only 19-27%. Patient education tools and decision support are unavailable to 93% and 57%. Insurance for immune therapies is poorly accessible to 86% yet 98% feel this would improve care. Almost all physicians support a regional GN registry that would provide achievable benchmarks in GN clinical care.

| Immune Therapies | | | | | |
|---|---|---|---|---|---|
| | **No Immune Therapy** | **Prednisone Alone** | **Calcineurin inhibitor** | **Cyclophosphamide** | **Azathioprine or MMF** |
| MN with 8g/day | 2% | 0% | 33% | 63% | 2% |
| FSGS with 5g/day | 7% | 56% | 27% | 11% | 0% |
| FSGS with 2g/day | 81% | 14% | 5% | 0% | 0% |

| Conservative Management | | | | | |
|---|---|---|---|---|---|
| | **Never** | **Rarely** | **Some of the time** | **Most of the time** | **All of the time** |
| ACEI to reduce proteinuria | 0% | 0% | 0% | 35% | 65% |
| Combination RAS blockade to reduce proteinuria | 23% | 42% | 30% | 5% | 0% |
| Calcium / Vit D to prevent osteoporosis in those on steroids | 0% | 2% | 5% | 23% | 70% |
| Bisphosphonates to prevent osteoporosis in those on steroids | 5% | 16% | 44% | 28% | 7% |
| PCP prophylaxis in those on high dose immunosuppression | 0% | 2% | 0% | 49% | 49% |

**Conclusions:** We describe significant care gaps in the management of GN, emphasizing the need to promote KT of the GN guidelines. We identify barriers to guideline implementation and physician support for initiatives that address these barriers potentially improving patient outcomes.

## TH-PO1053

**Proteinuria Does Not Need to Be Standardized to Body Surface Area in Adults with Glomerulonephritis** Sean Barbour,[1,2] Daniel C. Cattran,[3] Gabriela Espino-Hernandez,[2] Michelle A. Hladunewich,[3] Adeera Levin,[1,2] Heather N. Reich.[3] *[1]Div of Nephrology, Univ of BC; [2]BC Provincial Renal Agency; [3]Div of Nephrology, Univ of Toronto.*

**Background:** Baseline proteinuria (Prot) is an important but poorly defined determinant of renal outcome in glomerulonephritis (GN). While it is conventional in children to adjust proteinuria to body surface area (ProtBSA) this is not common in adults. We sought to determine if ProtBSA is more closely associated with the risk of kidney failure than traditional unadjusted proteinuria.

**Methods:** We analyzed an adult cohort with IgA nephropathy (IgAN N=445), focal segmental glomerulosclerosis (FSGS N=472) and membranous nephropathy (MN N=434) from the Toronto GN Registry followed for a median of 54 months. The primary outcome (ESRD or 50% drop in eGFR N=385) was analyzed using Cox regression to compare model fit between Prot and ProtBSA at biopsy, which were log-transformed due to non-linearity.

**Results:** The median baseline Prot was 3.7g/d and ProtBSA was 3.5g/d/1.73m$^2$. As shown in the table, in MN and IgAN both Prot and ProtBSA were associated with a similar and increased risk of renal progression ($p<0.001$ for all HR). The $R^2$, AIC, and C-statistic were similar between Prot and ProtBSA and the continuous (c)NRI and IDI were very

small or not different from zero. As in previous studies neither baseline Prot nor ProtBSA were associated with renal progression in FSGS (HR=1.11 95%CI 0.94-1.32, HR 1.12 95%CI 0.95-1.33 both p=0.2).

| | HR | $R^2$ | AIC | C statistic | Δ C statistic | cNRI | IDI |
|---|---|---|---|---|---|---|---|
| **IgAN** | | | | | | | |
| Prot | 1.66 (1.36, 2.02) | 0.062 | 1343 | 0.65 (0.60, 0.71) | | | |
| ProtBSA | 1.71 (1.41, 2.08) | 0.071 | 1339 | 0.66 (0.61, 0.72) | 0.01 (0.001, 0.02) | 0.37 (0.046, 0.65) | 0.006 (-0.0005, 0.02) |
| **MN** | | | | | | | |
| Prot | 1.89 (1.39, 2.56) | 0.048 | 920 | 0.63 (0.56, 0.70) | | | |
| ProtBSA | 1.92 (1.40, 2.63) | 0.048 | 920 | 0.63 (0.55, 0.70) | -0.0004 (-0.01, 0.01) | 0.20 (-0.34, 0.52) | -0.0006 (-0.005, 0.005) |

**Conclusions:** In a large cohort of adults with GN, Prot and ProtBSA similarly predict the risk of renal outcome. BSA adjustment does not improve the prognostic value of baseline proteinuria measurements.

## TH-PO1054

**Immunohistochemical Staining of IgG4 in Glomeruli for a Range of Renal Biopsies** Stacy C. Wolforth,[1] Xu Zeng,[2] Michele T. Rooney,[1] Wei Li,[1] Ping L. Zhang.[1] *[1]Anatomic Pathology, William Beaumont Hospital, Royal Oak, MI; [2]Bostwick Laboratories, Orlando, FL.*

**Background:** Immunohistochemical (IHC) staining for IgG4 became available in our lab due to the discovery of a systemic autoimmune inflammatory disease. Other labs have used immunofluorescent (IF) methods to stain IgG4 to differentiate idiopathic membranous glomerulopathy (MGN) from lupus membranous nephritis (LMN). In this study, we used IHC methods to stain IgG4 for a range of renal biopsies with known IgG staining by IF method to determine the presence of IgG4 in various glomerulopathies.

**Methods:** Monoclonal IgG4 antibody was purchased from Cell Marque (dilution 1:100). Four control groups including normal renal tissue (C1 normal renal tissue n= 5), LMN (C2, n =9), monoclonal light chain nephropathy without heavy chain deposition (C3, n = 24), and ANCA associated crescentic glomerulonephritis (CGN) (C4, n = 8) . Four study groups were composed of MGN (S1, n = 14), heavy chain nephropathy with or without monoclonal light chain (S2, n = 3), mixed group (S3, post-infectious glomerulonephritis, n = 2, thrombotic microangiopathy, n =5 , membranoproliferative glomerulonephritis n =2), and anti-glomerular basement membrane (anti-GBM) CGN (S4 n =13). All cases were stained for IgG4 and their expression in each was graded from 0 to 3+ depending on staining intensity.

**Results:** All 4 control groups (C1 – C4, total n = 46) are entirely negative for IgG4 in glomeruli. In group S1, 64 % (9/14) of MGN showed granular membranous staining for IgG4. One of three from group S2 (33%) revealed linear IgG4 (3+) along glomerular loops and along tubular basement membranes, similar to its IgG pattern by IF method. Group S3 stained negatively for IgG4. In one of 13 cases in group S4 (7.7%), linear IgG4 (2+) was identified in all 7 glomeruli, similar to the pattern of IgG linear staining by IF method.

**Conclusions:** Our data using IHC staining for IgG4 has confirmed specific IgG4 staining in the glomeruli of a majority of MGN cases as reported by others using IF methods. In addition, heavy chain deposition disease and anti-GBM CGN can be IgG4 positive in the glomeruli but with differing staining patterns as compared to MGN.

*Funding:* Clinical Revenue Support

## TH-PO1055

**Histopathological Analysis of IgG4-Related Kidney Disease: Hints from an Autopsy Series of 5 Cases** Ichiro Mizushima,[1] Mitsuhiro Kawano,[1] Takako Saeki,[2] Yoshifumi Ubara,[2] Nobuya Ohara,[2] Yasuharu Sato,[2] Kazunori Yamada,[1] Hitoshi Nakashima,[2] Shinichi Nishi,[2] Yutaka Yamaguchi,[2] Satoshi Hisano,[2] Michio Nagata,[2] Takao Saito.[2] *[1]Rheumatology, Kanazawa Univ Hospital, Japan; [2]'IgG4-Related Kidney Disease' Working Group, Japan.*

**Background:** IgG4-related disease (IgG4-RD) is a systemic disease characterized by marked lymphoplasmacytic infiltrates with abundant IgG4-positive plasma cells (PCs), fibrosis, and obstructive phlebitis. Plasma cell-rich tubulointerstitial nephritis is a typical renal manifestation of this disease. However, histopathological evaluation of the whole kidneys in autopsy cases has not been previously reported.

**Methods:** We analyzed 5 autopsy cases using Hematoxylin and eosin, Periodic acid-Schiff, Periodic acid methenamine silver, and elastic Van Gieson stains. Immunohistochemical staining was performed using anti-IgG4 antibodies (Abs) and anti-CD138 Abs.

**Results:** Two patients died of associated cancer and they had undergone chemotherapy without corticosteroid (CS). Two patients were receiving maintenance CS treatment (Tx), and the remaining one was not. All patients had typical extrarenal involvement by IgG4-RD. Four patients fulfilled kidney infiltrating IgG4 counts (mean 78; range 38-116) and IgG4:CD138 ratios (mean 81; range 48-110) proposed by the consensus statement on the pathology of IgG4-RD [IgG4+ > 30/high power field (HPF); IgG4/CD138 > 40%], while one patient, who underwent long-term CS Tx, showed few lymphoplasmacytic infiltrates but marked periarterial fibrosis of interlobular arteries. Three patients without CS Tx or with only short-term CS Tx had bird's eye pattern fibrosis, dense lymphoplasmacytic infiltrates around renal or interlobular artery segments (width 200-500μm) and vein. In contrast, another patient with long-term CS Tx had mild patchy infiltration of lymphocytes and PCs, but had a large fibrotic area with disappearance of almost all tubules with scattered glomeruli and arteries remaining.

**Conclusions:** Periarterial and perivenous lesions with marked lymphoplasmacytic infiltrates are additional characteristic features of IgG4-related kidney disease.

## TH-PO1056

**Diffuse Effacement of Foot Processes of Podocytes in Stage I Membranous Nephropathy** Kayori Tsuruoka, Yusuke Kajimoto, Seiichiro Higo, Go Kanzaki, Shinya Nagasaka, Akira Shimizu. *Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

**Background:** Idiopathic membranous nephropathy (MN) is widely characterized pathologically by the spike formation in glomerular basement membrane in light microscopy (LM), glomerular capillary pattern of IgG and C3 deposition in immunofluorescence study (IF), and glomerular subepithelial electron dense deposits (EDD) in electron microscopy (EM). However, in stage I (Ehrenreich T and Churg J) of MN, we sometimes experience the cases that did not have typical features of these MN findings.

**Methods:** In the present study, in order to clarify the clinicopathological characteristics of MN stage I, we selected 33 cases (22.3%) of stage I of MN from 148 cases of idiopathic MN that we diagnosed in our department from 2001 to 2013.We assessed clinical and pathological characteristics including LM, IF, EM, and immuneno-EM.

**Results:** The 33 cases of stage I of MN were consisted of 22 males and 11 females. Ages at the time of biopsy ranged from 3 to 80 years (56.0 ± 20.1 years). Renal dysfunction was not detected in all cases. In addition, stage I MN was characterized by the presence of nephrotic rage proteinuria (4.84 ± 2.57g/day), nephrotic syndrome in 24 cases (72.7%), and short interval from disease onset (3.6 ± 4.1 months). In the pathology, the formation of spike on GBM was not detected in all cases. In EM, segmental subepithelial EDD was detected in all MN cases, but only a segmental and small subepithelial EDD was noted on GBM in 13 cases (39.4%). In these 13 cases, weak IgG deposition was evident with the degree of deposition of IgG > C3 in IF. The IgG subclass showed the predominant deposition of IgG4 > IgG1. In addition, IgG was only noted on subepithelial EDD on GBM in immuno-EM. Interestingly, diffuse effacement of foot process of podocytes was evident in stage I MN, even in the segmental and small subepithelial EDD on GBM.

**Conclusions:** In the stage I MN, diffuse effacement of foot process of podocytes was noted, even in the segmental and small subepithelial EDD on GBM. Subepithelial EDD and podocyte injury may be mediated heavy proteinuria in stage I MN.

## TH-PO1057

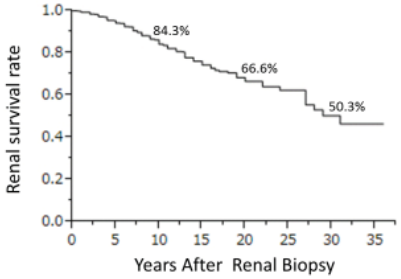
**Prognosis in IgA Nephropathy: 30-Year Analysis of 1,012 Patients at a Single Center in Japan** Takahito Moriyama, Kayu Tanaka, Chihiro Iwasaki, Yasuko Oshima, Takashi Takei, Kosaku Nitta. *Medicine, Tokyo Women's Medical Univ, Shinjyuku-ku, Tokyo, Japan.*

**Background:** Little is known about the long-term prognosis of patients with IgA nephropathy (IgAN). We therefore assessed the prognosis over 30 years of 1,012 patients with IgAN treated at our center since 1974.

**Methods:** This retrospective cohort analysis evaluated clinical and histological findings at the time of renal biopsy, initial treatment, patient outcomes over 30 years, and risk factors associated with progression.

**Results:** Of the 1,012 patients, 40.5% were male. Mean patient age was 32.9±12 years and mean blood pressure was 122±17/75±13 mmHg. Mean serum albumin was 3.9±0.5 g/dL, mean uric acid was 5.7±1.6 mg/dl, mean serum creatinine was 0.89±0.42 mg/dL, and mean estimated glomerular filtration rate (eGFR) was 71.1±23.5 ml/min/1.73m$^2$. Mean proteinuria was 1.19±1.61 g/day, and number of mean urinary red blood cells was 36.6±35.3 per high powered field. Histologically, mesangial hypercellularity was present in 47.6% of patients, endothelial hypercellularity in 44.3%, segmental sclerosis in 74.6%, and tubular atrophy/interstitial fibrosis in 28.8% by Oxford classification. Initial treatment consisted of corticosteroids in 26.9% of patients, immunosuppressive agents in 1.5%, RAS inhibitors in 28.9%, and tonsillectomy plus steroids in 11.7%. The 10-, 20-, and 30-year renal survival rates were 84.3, 66.6, and 50.3%, respectively.

*Cumulative renal survival rate from renal biopsy until ESRD in all 1,012 patients with IgAN.*




Cox multivariate regression analysis showed that higher proteinuria(HR 1.28, 95%CI 1.02-1.62, P=0.0372), lower eGFR (HR 1.86, 95%CI 1.43-2.43, P<0.0001), and higher uric acid concentration (HR 1.28, 95%CI 1.05-1.62, P=0.0372) at the time of renal biopsy were independent risk factors for the development of end stage renal disease (ESRD).

**Conclusions:** IgAN is not a benign disease, with over 50% of patients progressing to ESRD within 30 years despite treatment.

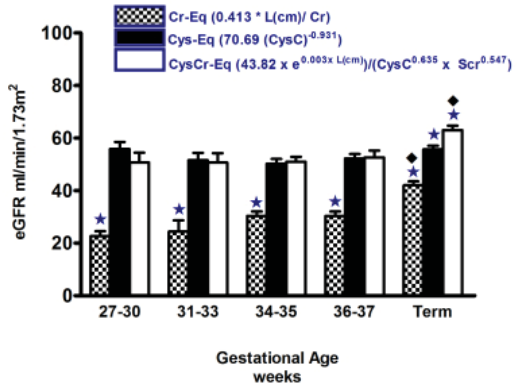
**TH-PO1058**

**Neonatal Kidney Size and Function in Preterm Infants: What Is a True Estimate of Glomerular Filtration Rate?** Carolyn L. Abitbol,[1] Wacharee Seeherunvong,[1] Marta G. Galarza,[2] Marissa J. Defreitas,[1] Teresa C. Cano,[1] Chryso P. Katsoufis,[1] Alcia D. Edwards- Richards,[1] Vimal Master Sankar Raj,[1] Jayanthi Chandar,[1] Shahnaz Duara,[2] Salih Y. Yasin,[3] Gaston E. Zilleruelo.[1] *[1]Pediatric Nephrology, Univ of Miami/ Holtz Children's Hospital, Miami, FL; [2]Neonatology, Univ of Miami/ Holtz Children's Hospital, Miami, FL; [3]Obstetrics & Perinatology, Univ of Miami/ Holtz Children's Hospital, Miami, FL.*

**Background:** Preterm birth is associated with early and late renal insufficiency that may be related to low nephron mass and altered maturation of renal function.

**Methods:** Sixty preterm and 40 term infants were enrolled at birth. Serum creatinine(Cr) and cystatin-C(CysC) were used to calculate estimated glomerular filtration rate (eGFR). Renal ultrasounds assessed kidney dimensions with calculation of total kidney volume (TKV) as a surrogate of nephron mass. Multiple regression analysis was applied to assess the relative impact of neonatal parameters on eGFR including TKV, gestational age (GA) and mean arterial pressure (MAP).

**Results:** Renal lengths correlated with GA and were within reference values for fetal ultrasounds at each GA. Estimation equations for GFR based on Cr, CysC and combined CysC+Cr demonstrated that Cr-based equations underestimated GFR; whereas, CysC and combined equations were consistent with the reference inulin clearance studies. TKV, GA and MAP correlated positively with eGFR; though, only MAP and GA remained significant when other measures were held constant.




**Conclusions:** Primary determinants of GFR in preterm infants are gestational age and mean arterial blood pressure. Serum creatinine is unreliable as a marker of renal function in preterm infants. Until more reliable markers are available, CysC should be used as the primary assessment of GFR in preterm infants.

*Funding:* Pharmaceutical Company Support - Gerber Foundation

**TH-PO1059**

**Birthweight Predicts Postnatal Kidney Function** Suad Ma Hannawi,[2] Issa A.L. Salmi.[1] *[1]The Renal Medicine Dept, The Royal Hospital; [2]The Medical Dept, AlBaraha Hospital, Dubai, United Arab Emirates.*

**Background:** Under nutrition in middle &late gestation programmes for later chronic diseases(CKD) or most importantly its risk factors.

**Methods:** A questionnaire about birthweight(BW) data was given to 11,247 participants,aged ≥25 years that were enrolled during a previous study. Children of VLBW due to prematurity were assessed for kidney function. This cohort &Control group were enrolled.Also, 412 CKD patients filled the questionnaire. Control, matched for gender &age, were selected for CKD patient(Salmi et al).

**Results:** O7,157 responded to our questionnaire &4,502(63%), reported BW data. BW ranged from 0.4 to 7.0kg & mean(SD) of 3.37(0.7). BW was lower for females, 3.28(0.6), compared to males, 3.5(0.7)kg. The eGFR strongly and positively associated with BW, & lowest sex-specific BW quintiles having the lowest mean eGFR. OR for eGFR<10$^{th}$ percentile,(<61.4 ml/min for females &<73.4 for males) for people in the lowest vs. the higher BW was 2.19(95% CI 1.14-4.2) in females &2.37(1.1-5.3) in males, after adjustment for other factors.

37 premature(PM) 23 fullterm children(FT). Gestational age for PM cohort was 26.7(2.6) &ranged from 23-35 weeks. Their BW was 867(248) g vs3433(285) for FT. The age of PM children was 12.1(3.8) years vs.11.5(3.5) years for FT. PM had lower kidney volumes (99.7 ml (89.3,110) vs131(111,151), P=0.01 and lower eGFR(87.0 (78.5,113) vs. 113(99.1,128), p<0.001.

Among CKD; mean BW was 3.27(0.62) vs3.46(0.6) kg for controls, p<0.001 and the proportions with BW<2.5 kg were 12.17% and 4.44%, p<0.001. Among CKD patients,22.8%, 21.7%, 8% &37.6% were in CKD stages 2,3,4 and 5 respectively. BW by CKD stage &their controls were as follows: 3.38(0.52) vs3.49(0.52), p=0.251 for CKD2; 3.28(0.54) vs3.44(0.54), p=0.121 for CKD3; 3.19(0.72) vs3.43(0.56), p= 0.112 for CKD4 and 3.09(0.65) vs3.47(0.67), p=<0.001 for CKD5.

**Conclusions:** LBW people were predisposed to higher rates of renal dysfunction in later stages of life. The awareness of the effect of LBW on development of renal dysfunction is of vital importance. A proactive public health strategy towards a better management of this group of population that are increasingly surviving into adulthood is of great importance.

**TH-PO1060**

**Tubular Dysfunction in Extremely Low Birth Weight Survivors** Kazuya Matsumura, Mariko Hida, Midori Awazu. *Dept of Pediatrics, School of Medicine, Keio Univ, Tokyo, Japan.*

**Background:** Extremely low birth weight (ELBW) survivors are known to develop glomerulosclerosis due to low nephron number. Microalbuminuria is regarded as an early marker of glomerular injury. Tubular damage may also occur, but little is known other than increased urine calcium excretion. We therefore studied tubular function of ELBW survivors.

**Methods:** Fifty-three subjects (30 boys and 23 girls, aged 7 months-19 years, median 42 months) were studied. The median gestational age and birth weight were 26 (22-32) weeks and 745 (316-999) g, respectively. Normal values were defined as urine calcium:creatinine ratio (Ca/Cr) <reference values by age, ß2 microglobulin:creatinine ratio (ß2M/Cr) <0.3 mg/g, N-acetyl-ß-d-glucosaminidase:creatinine ratio (NAG/Cr) <5 U/g), glucose:creatinine ratio (glu/Cr) <250 mg/g, uric acid:creatinine ratio (UA/Cr) <reference values by age, and microalbumin:creatinine ratio (malb/Cr) <30 mg/g. We assessed the association between urine markers and factors thought to predict renal injury such as asphyxia, nephrotoxic drugs, chronic lung disease etc. Univariate and logistic regression analyses were used to compare variables.

**Results:** Three subjects who had overt proteinuria were excluded. Ninety percent of subjects had at least one tubular dysfunction. Frequencies of elevated values were; NAG/Cr 75.5%, UA/Cr 53.1%, ß2m/Cr 38.2%, malb/Cr 28.2%, Ca/Cr 21.1%, and glu/Cr 20.5%. Urine ß2M/Cr and glu/Cr were negatively correlated with the gestational age. No association was observed between urine markers and the birth weight. There was a significant negative correlation between the current age and Ca/Cr, NAG/Cr, glu/Cr, and UA/Cr, suggesting a maturation of tubular function. On the other hand, ß2M/Cr and malb/Cr did not correlate with the current age, which may indicate persistent kidney injury. Significant correlations were observed between malb/Cr and ß2M/Cr, and malb/Cr and NAG/Cr. There was no association between urine markers and putative risk factors for renal injury.

**Conclusions:** Tubular dysfunction is quite common in ELBW survivors. Microalbuminuria is paralleled by increases in urine ß2M and NAG, which may suggest that it is, at least in part, tubular in origin.

*Funding:* Government Support - Non-U.S.

**TH-PO1061**

**Epidemiology of Acute Kidney Injury in Hospitalized Children: A Comparison of ICU and Non-ICU Patients** Elizabeth A.K. Hunt,[1,2] Michael A. Ferguson,[1,2] Sushrut S. Waikar.[2,3] *[1]Div of Nephrology, Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Acute kidney injury (AKI) in children has been primarily studied in intensive care units and following cardiac surgery. Less is known about the epidemiology of AKI in hospitalized, non-critically ill children.

**Methods:** We obtained clinical, demographic, and laboratory data from all children hospitalized at a large pediatric tertiary care center in 2011 who had at least one serum creatinine (Scr) measurement. We excluded critically ill neonates, patients aged ≥24, and those with ESRD. Estimated creatinine clearance (eCCl)was calculated using the modified Schwartz formula. AKI was defined and stratified using the pRIFLE criteria (risk (R)= eCCl decrease by 25%, injury (I) = decrease by 50%, failure (F) = decrease by 75%). We used pre-hospital Scr measurements or the nadir inpatient Scr to determine baseline eCCl.

**Results:** We studied 12,090 admissions of 8,706 patients. 5,699 patients had no Scr measurement and 5,485 had one Scr measurement. The incidence of AKI in ICU patients was 53% (23% R, 7% I and 23% F); 3.3% of patients with pRIFLE F required renal replacement therapy (RRT). Overall mortality in ICU patients was 2.8%, and ranged from 0.9% with no AKI to 8.6% in pRIFLE F. Length of stay (LOS) increased with higher stages of AKI. In non-ICU patients, the incidence of AKI was 25% (13% R, 3% I and 9% F); 2.7% of patients with pRIFLE F required RRT. Overall mortality was 0.15%, and ranged from 0.07% with no AKI to 0.97% in pRIFLE F**.** LOS increased with higher stages of AKI.

| | No AKI | pRIFLE R | pRIFLE I | pRIFLE F | p Value |
|---|---|---|---|---|---|
| ICU | (n =1101) | (n =535) | (n =166) | (n =545) | |
| Inicidence % | 47 | 23 | 7 | 23 | |
| LOS days (IQR) | 3 (1-5) | 5 (2.5-7.5) | 6 (2-10)) | 14 (1-27) | <.001 |
| RRT % | 0 | 0 | 0 | 3.3 | |
| Mortality % | 0.91 | 0.93 | 2.4 | 8.6 | <.001 |
| non-ICU | (n=7036) | (n=1237) | (n=313) | (n=826) | |
| Incidence % | 75 | 13 | 3 | 9 | |
| LOS days (IQR) | 2 (0.5-3.5) | 5 (1.5-8.5) | 7 (2-12) | 16 (2.5-29.5) | <.001 |
| RRT% | 0 | 0 | 0 | 2.7 | |
| Mortality % | 0.07 | 0.08 | 0 | 0.97 | <.001 |

**Conclusions:** AKI is common in both ICU and non-ICU patients. The incidence and outcomes of children with AKI outside of the ICU deserve further study.

*Funding:* Other NIH Support - T32 training grant

**TH-PO1062**

**Applying the KDIGO AKI Definition to a Pediatric Critical Care Population** David T. Selewski, J. Troost, Susan M. Hieber, Brett Ehrmann, Michael Heung, Timothy Cornell, Neal B. Blatt, Kera E. Luckritz, David B. Kershaw, Robert J. Gajarski, Thomas P. Shanley, Debbie S. Gipson. *Univ of Michigan.*

**Background:** The Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury working group has proposed a standardized definition of acute kidney injury (AKI). We evaluated the KDIGO AKI criteria and the relationship of AKI with relevant

outcomes in a single center tertiary pediatric intensive care (PICU) and cardiothoracic unit (PCTU) population.

**Methods:** Electronic health records for all discharges (N=3213) from 07/2011-02/2013 were extracted, validated and analyzed. The KDIGO creatinine (Cr) based criteria (Cr must be greater than 0.5 mg/dL) were used to stage AKI. Outcomes were ICU length of stay (LOS) and ICU mortality. Exclusion: ESRD, renal transplant or missing PRISM score (N=199). Univariate analysis utilized Chi Squared tests (N,%) for count and Kruskal-Wallis (Median, IQR) tests for continuous variables. Multivariate analysis were performed controlling for mechanical ventilation (yes/no), vasopressors (yes/no), age, and PRISM III score (severity of illness).

**Results:** AKI occurred in 560 (19%) of 3014 discharges (PICU N=1931, PCTU N=1083) among 2393 patients, staged as I=153, II=143, and III=264. Overall ICU mortality was 2.7%. Univariate results are presented in Table 1. On multivariate analysis AKI stage was associated with an increase in ICU LOS (Stage I β=49hrs, p=0.018, II β=80hrs, p<0.001, and III β=280hrs, p<0.001). AKI Stage III was associated with increased odds of ICU mortality (OR 4.2, 95% CI 2.4-7.6.)

**Conclusions:** Using the KDIGO criteria to define AKI we show there is a high prevalence of AKI, AKI is associated with increased ICU LOS and stage III AKI is associated with increased mortality. The KDIGO criteria adequately describes clinically meaningful AKI in a broad pediatric critical care population.

| | No AKI(N=2454) | AKI(N=560) | *p* |
|---|---|---|---|
| Age(yr) | 4(0,11) | 2(0,11) | <0.001 |
| PRISM Score | 2(0,6) | 9(5,13) | <0.001 |
| Vasopressor | 521(21%) | 390(70%) | <0.001 |
| Mechanical ventilation | 1089(44%) | 455(81%) | <0.001 |
| ICU LOS(hr) | 45(24,93) | 214(77,387) | <0.001 |
| ICU mortality | 27(1%) | 54(10%) | <0.001 |

*Funding:* Private Foundation Support

## TH-PO1063

**Acute Kidney Injury (AKI) in Non-Cardiac Neonates in the Pediatric Intensive Care Unit (PICU)** Disha S. Kriplani, James Schneider, Daniel E. Leisman, Christine Sethna. *Pediatrics, Div of Nephrology, Cohen Children's Medical Center of New York, New Hyde Park, NY.*

**Background:** AKI is common in neonates undergoing cardiac surgery. The incidence of AKI in non-cardiac neonates is unknown. The objectives were to define the incidence, assess independent risk factors and outcome of AKI in non-cardiac neonates in the PICU.

**Methods:** A retrospective study of neonates (age ≤28 days) admitted to a single, tertiary PICU from 2008-2012 was conducted. The incidence of AKI was determined based on the pRIFLE criteria. Logistic and linear regression were used to evaluate risk factors and outcomes related to AKI.

**Results:** 80 neonates, gestational age(GA) 38 (32-42) weeks (62.5% males) were reviewed. AKI was reported in 37 (46.2%) neonates. 5.4% reached pRIFLE category F. Neonates with AKI were born to diabetic mothers (13% vs 0%), were younger (6.1±7.8 vs 14.4±9.2), had higher max sodium values (146.9±6.2 vs 143±6), required umbilical catheter placement (64% vs 32%) and had longer duration of mechanical ventilation (15.6±21 vs 6.9±9.9), compared to those without AKI, (p < 0.05). On univariate analysis, variables of ECMO (p <0.001, OR 6.1, 95% CI 2.3 -16.1), max sodium value (p = 0.01, OR 1.12, 95% CI 1.02-1.23) and umbilical lines (p = 0.02, OR 3.6, 95% CI 1.2-10.7) were significantly associated with AKI; however, the associations did not hold after multivariate adjustment. Younger age was found to be an independent risk factor for AKI in a multivariate model (p <0.001, OR 0.89, 95% CI 0.85 – 0.95). Gender, ethnicity, GA, birth weight, sepsis and asphyxia were not associated with AKI. There was a significantly higher mortality rate in neonates with AKI compared to those without AKI (26% vs 7%, p = 0.02). In univariate analysis, AKI was associated with increased risk of mortality (p = 0.03, OR 6.2 95% CI 1.2-18.4) and longer duration of mechanical ventilation (ß 6.9, CI 1.9-11.9, p = 0.007); however, AKI was not an independent predictor of these variables after adjustment for age. AKI was not associated with hospital length of stay.

**Conclusions:** AKI is common in non-cardiac neonates in the PICU and is associated with increased morbidity and mortality. Age is an independent predictor of AKI.

## TH-PO1064

**Survival to Discharge after ECMO in Neonates with Preexisting CKD** Aaron G. Wightman,[2] Miranda Bradford,[3] Jordan M. Symons,[2] Thomas V. Brogan.[1] *[1]Pediatric Critical Care, Univ of Washington School of Medicine, Seattle, WA; [2]Pediatric Nephrology, Univ of Washington School of Medicine, Seattle, WA; [3]Children's Core for Biomedical Statistics, Seattle Children's Research Institute, Seattle, WA.*

**Background:** Little is known about the outcomes of neonates with preexisting chronic kidney disease (CKD) who receive ECMO (Extracorporeal Membrane Oxygenation) support. Limited data indicates some neonates with CKD have acceptable outcomes. We sought to investigate the rates and survival of children with preexisting kidney disease after placed on ECMO support.

**Methods:** We analyzed the ELSO (Extracorporeal Life Support Organization) registry of neonates (<30 days of age) with CKD noted at time of initiation of ECMO for survival to discharge from hospitalization. We compared survival to published norms and performed logistic regression to assess covariate associations with survival.

**Results:** 423 neonates (ECMO indication: 266 pulmonary, 130 cardiac, 27 ECPR) with preexisting CKD were identified in the ELSO registry from 1991-2012. Dialysis was required in 47% of neonates with pulmonary indication and 55% of neonates with a cardiac indication. Survival to discharge was lower in neonates with CKD compared to previously published norms of neonates for both pulmonary (42% vs. 85%, p<.001) and cardiac (20% vs. 40%, p<.001) indications for ECMO. Among infants with a pulmonary indication for ECMO, increased survival to discharge was associated with increased gestational age, increased weight at ECMO onset, treatment after 2000, VV vs. VA ECMO, and not having elevated creatinine or requiring dialysis. Survival was not associated with kidney diagnosis, ECMO duration, or age at ECMO onset. For neonates with a cardiac indication for ECMO, survival to discharge was not significantly associated with any covariates in logistic regression.

**Conclusions:** Neonates with preexisting CKD who require ECMO support have poorer chance of surviving to discharge than other neonates who require ECMO. This suggests that preexisting CKD should be considered when making ECMO initiation decisions.

## TH-PO1065

**Quality of Life after Renal Transplantation in Children** Aaron G. Wightman,[1] Kathryn B. Whitlock,[2] Marrie T. Ketchum,[1] Kristin L. Stockard,[1] Jodi M. Smith,[1] Ruth McDonald.[1] *[1]Pediatric Nephrology, Seattle Children's Hospital, Seattle, WA; [2]Core for Biomedical Statistics, Seattle Children's Research Institute, Seattle, WA.*

**Background:** Renal transplantation is the goal for children with end stage kidney disease providing them with the best opportunity for growth and development. Previous studies of quality of life (QOL) after renal transplant have yielded conflicting results. We sought to assess the impact of renal transplant on QOL.

**Methods:** We administered the PedsQL 3.0 ESRD module to patients and their parents following renal transplantation. The overall scores of transplant recipients were described and MANOVA analysis, accounting for correlation between parent and patient responses and among subscales, was used to analyze the association of demographic covariates with QOL scores. Where available, patient and parent pre-transplant QOL scores were collected. For those with pre and post transplant values within a year of transplant, the linear changes related to transplant were assessed.

**Results:** 32 patients and their parents were included in the analysis. Using MANOVA analysis, recipients who were white had higher scores on the "Treatment Problems" subscale of the module than non-white recipients (p<.015). Parents of children with living donor transplants had higher scores on the "Worry" and "Family and Peer Interaction" subscales than parents of children with deceased donor transplants (p<.005, p<.012). Parents of children who received a preemptive transplant had higher scores on the "Family and Peer Interaction" subscale than parents of children who did not receive a preemptive transplant (p<.019). Pre and post transplant QOL scores were available for 11 patients and their parents. In this subset, no significant changes were found over time in either the patient or parental total or subscale QOL scores.

**Conclusions:** Our study found that pediatric kidney transplant recipients score QOL differently depending on race. Parents of recipients score their child's QOL differently based on type of transplant. These differences in perceived QOL may provide opportunities for further study and implementation of targeted strategies to improve QOL in children after renal transplant.

## TH-PO1066

**Hyperuricemia Is Associated with Hypertension and Progression of Chronic Kidney Disease in Children** George J. Schwartz,[1] Michael F. Schneider,[8] Kyle Rodenbach,[1] Vikas R. Dharnidharka,[2] Donald J. Weaver,[3] Sahar A. Fathallah-Shaykh,[4] Marva M. Moxey-Mims,[5] Bradley A. Warady,[6] Susan L. Furth,[7] Mark Mitsnefes.[9] *[1]U. Rochester, Rochester, NY; [2]St. Lou. Child. Hosp., St. Lou., MO; [3]Levine Child. Hosp., Charleston, NC; [4]UAB, Birmingham, AL; [5]NIDDK, Bethesda, MD; [6]Child. Mercy Hosp., KC, MO; [7]Child. Hosp. Phila., Phila., PA; [8]J. Hopkins Sch. P. Hlth., Baltimore, MD; [9]Cin. Child. Hosp., Cincinnati, OH.*

**Background:** There is little data on the relationship of uric acid [Ur] to pediatric CKD.

**Methods:** We quantified the relationship between [Ur] and GFR, hypertension (Htn), and obesity among 617 subjects of the CKiD study. Htn was defined as systolic or diastolic BP>95$^{th}$ %ile and was separated from those whose BP was <95$^{th}$%ile on antiHtn meds. GFR was measured by iohexol plasma disappearance or estimated from CKID-published formulas. From [Ur] distributions subjects were divided into 2 groups: 1) girls and boys<13 years old, and 2) boys ≥13 years. 432 kids with >2 GFR measurements were studied by multivariate analysis of the effect of initial [Ur] on risk of a ≥30% GFR decline.

**Results:** Cross-sectional: Group 1: Hypertensives (n=73) or those on antiHtn meds (n=234) had mean [Ur] that was 0.35 [95% CI: -0.04, 0.73] and 0.50 [95% CI: 0.21, 0.78] mg/dL higher, respectively, than those with BP <95th %ile and on no antiHtn meds. A 5 mL decrease in GFR was associated with an increase of 0.15 mg/dL in [Ur]; this relationship was not modified by Htn. Obesity was associated with a 0.64 mg/dL (95% CI: 0.29, 0.98) greater [Ur]. Group 2: Hypertensives had comparable [Ur] to those with normal BP without antiHtn meds; those on antiHtn meds had marginally higher [Ur]. Obesity and low GFR were associated with higher [Ur]. Longitudinal: Group 1: [Ur] >7 mg/dL, SBP or DBP >95$^{th}$ %ile, and 5 mL/min lower initial GFR were associated with 2.16 [95% CI: 0.95, 4.90], 4.37 (95% CI: 2.04, 9.38), and 1.18 (95% CI: 0.99, 1.41) times greater risk for ≥30% GFR decline, respectively. Group 2: only initial GFR predicted ≥30% decline in GFR.

**Conclusions:** [Ur] is associated with Htn, obesity, and lower GFR in pediatric CKD. High [Ur] results in increased risk of 30% decline in GFR in girls and young boys with CKD, independent of other factors.

*Funding:* NIDDK Support

**TH-PO1067**

**Prediction of Chronic Kidney Disease (CKD) Progression in Children by Urinary Neutrophil-Associated Lipocalin (NGAL)** Anke Doyon,[1,2] Aysun Karabay Bayazit,[2] Daniela Kracht,[2] Rene Zeller,[2] Ali Anarat,[2] Anja Christine Sander,[2] Anette Melk,[2] Uwe Querfeld,[2] Franz S. Schaefer.[1,2] *[1]Pediatric Nephrology, Center for Pediatrics and Adolescent Medicine, Heidelberg, Germany; [2]4C Study Group.*

**Background:** NGAL, a novel biomarker of acute kidney injury, has been suggested in animal studies and work in adult patients to be predictive also of CKD progression. The aim of this work was to evaluate whether NGAL povides useful information about progression risk in children with CKD.

**Methods:** Urinary NGAL was measured in 385 children with CKD (mean age 12.5±3.3 yrs, eGFR 31±10 ml/min/1.73m²) followed in the 4C Study for a mean of 20±7 months. NGAL/creatinine ratio was calculated and log-transformed (NGAL-log). Short-term CKD progression was defined as either attainment of end-stage renal disease (ESRD) or >10% eGFR loss within the first year of follow-up. Long-term CKD progression was defined as ESRD or >50% eGFR loss at any time during the observation period.

**Results:** NGAL-log was correlated inversely with eGFR (r= -0.4, p<0.0001) and positively with albuminuria (r=0.3, p<0.0001) and CRP (r=0.2, p<0.0001). There was no association to age, BMI or blood pressure (BP).

Short-term CKD progression was noted in 187 patients (49%). These patients were not different from stable patients in age or baseline eGFR, but had higher BP and CRP levels. NGAL-log was a strong predictor of short-term CKD progression by both univariate and multivariate Cox regression (HR 1.11, p<0.02, adjusted for baseline GFR, BP, age, albuminuria and CRP).

Long-term progression was evident in 81 patients (21%) during the whole follow-up period. While NGAL-log significantly predicted long-term progression by univariate analysis, this association was not sustained when correcting for baseline GFR, BP, albuminuria, age and CRP in the multivariate analysis.

**Conclusions:** Urinary NGAL is an independent predictor of short-term progression of CKD in children, independent of baseline eGFR and other classic risk factors. There was no additional impact of NGAL on prediction of long-term CKD progression.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

**TH-PO1068**

**Serum Hepcidin Is a Superior Indicator of Functional Iron Status in Children with Chronic Kidney Disease (CKD)** Anke Doyon,[1,2] Franz S. Schaefer.[1,2] *[1]Pediatric Nephrology, Univ of Heidelberg, Germany; [2]4C Study Group.*

**Background:** Hepcidin inhibits intestinal iron uptake as well as release from internal iron stores. It is upregulated in CKD and may thereby contribute to functional iron deficiency and anemia. Little is known about the distribution of serum hepcidin in pediatric CKD. The aim of this work was to evaluate the range and determinants of serum hepcidin concentrations observed in children and adolescents with CKD and to assess its usefulness in the evaluation of anemia in pediatric CKD.

**Methods:** Serum hepcidin, hemoglobin (Hb), and measures and determinants of iron status were measured in 130 children and adolescents with CKD (age 6 - 17 years, eGFR 10 - 60 ml/min/1.73 m²) followed in the 4C Study. Current use and dosage of erythropoetin stimulating agents (ESA, administered in 47% of patients) and iron supplements (prescribed to 10 %) was recorded. Hepcidin, ferritin and CRP levels were log-transformed for multivariate regression analysis.

**Results:** Median (IQR) serum hepcidin was 26.2 (37.6) ng/ml in boys and 28.0 (20.9) ng/ml in girls (p=n.s.). Hepcidin was correlated inversely with eGFR (r=-0.45, p<0.0001) and age (r=-0.18, p<0.05), and positively with serum ferritin (r=0.70, p<0.0001) and CRP (r=0.2, p<0.05) by univariate and multivariate analysis. No significant association with transferrin saturation (TSAT) was found. Also, serum hepcidin levels were not affected by iron supplementation or ESA therapy according to multivariate analysis.

A multivariate model adjusting for age, eGFR, gender, ESA and iron therapy identified hepcidin but not TSAT, ferritin or CRP as a significant predictor of Hb levels (p<0.05). High hepcidin levels were the only significant associate of Hb levels in non-ESA treated patients (p<0.01), whereas none of the parameters of iron status was predictive of Hb in patients receiving ESA.

**Conclusions:** Serum hepcidin increases in proportion to renal failure in children with CKD. It is linked to CRP and ferritin but not TSAT in children with CKD, suggesting its usefulness as a marker of functional iron deficiency in CKD associated anemia. Hepcidin appears superior to traditional indicators of iron status and inflammation in predicting Hb levels.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

**TH-PO1069**

**Renal Hyperfiltration and Urinary Cytokines/Chemokines in Adolescents with Type 1 Diabetes Mellitus** Ronnie Lok-Hang Har,[1] James W. Scholey,[1] Denis Daneman,[2] Farid H. Mahmud,[2] Rahim Moineddin,[3] Masha Ostrovsky,[2] Livia Deda,[2] Laura Motran,[2] Yesmino Elia,[2] Etienne Bertrand Sochett,[2] Heather N. Reich,[1] David Cherney.[1] *[1]Univ Health Network, Univ of Toronto, Canada; [2]Hospital for Sick Children, Univ of Toronto, Canada; [3]Univ of Toronto, Canada.*

**Background:** In adults with type 1 diabetes (T1D), renal hyperfiltration is associated with high intraglomerular pressure, and with high levels of urinary cytokines/chemokines, which may contribute to the initiation of diabetic nephropathy. In children with T1D, the interaction between hyperfiltration and inflammation remains unknown. Our objective was to determine whether renal hyperfiltration is associated with increased levels of urinary cytokines/chemokines in adolescents with T1D.

**Methods:** Blood pressure, estimated glomerular filtration rate using cystatin C and a panel of 41 urine cytokines/chemokines using a Luminex platform were measured in individuals recruited to the Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT). T1D patients with hyperfiltration (DM-H, n=81, GFR ≥135 ml/min/1.73m²) or normofiltration (DM-N, n=88, GFR<135 ml/min/1.73m²) were compared to a group of healthy controls (n=59).

**Results:** Compared to controls and after correcting for age, gender and weight, DM-H and DM-N exhibited elevated levels of urinary FGF-2, Flt-3, IL-12, -13 and -15, TNFα and TNFb (p≤0.042). These factors were not different in the two T1D groups. In contrast, some markers were higher in control vs. DM-H and DM-N, including eotaxin, GM-CSF, IL-7 and -17, PDGF-AA and PDGF-AB/BB, RANTES, VEGF and MCP-3 (p≤0.031). Other interleukins, IFN, IP-10, MCP-1, TGF, MDC and MIP1α were similar in the three groups.

**Conclusions:** Normotensive, normoalbuminuric T1D children exhibit differences in urinary cytokines/chemokines compared to healthy controls. In contrast with previous work in adults, hyperfiltration in T1D children is not associated with increased urinary cytokine/chemokine excretion. Further work is warranted to determine 1) if this early renal biomarker profile changes over time; 2) whether these factors respond to drug therapy; 3) the correlation between these factors and longer term outcomes.

*Funding:* Private Foundation Support

**TH-PO1070**

**Response to Standard Vitamin D Treatment among Children with Chronic Kidney Disease and Primary Hypertension** Bandana Paudyal, Gail Prado, Morris J. Schoeneman, Manoj Kumar Nepal, Valeriya M. Feygina, Vaishali Bansilal, Hanan K. Tawadrous, Anil K. Mongia. *Pediatrics Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

**Background:** Children with vitamin D deficiency are treated with either ergocalciferol or cholicalciferol. Vitamin D treatment is standard fixed dose and duration as per serum 25 (OH) D levels, irrespective of age and weight. That means, the same dose might be sub therapeutic for some patients and toxic for other patients. However, limited studies are available looking at response of 25(OH) D level post treatment.

**Methods:** We enrolled 144 patients aged 2 to 19 yrs, 34 Primary Hypertension(PH), 95 Chronic kidney disease (CKD) 23 Control(C). Among CKD, 58 were (CKD I-IV), 16(Transplant), 21(Dialysis). We collected retrospective data on age, sex,race, cause of kidney disease, eGFR, Ht, Wt, BMI, BP z-scores, lipid panel, 25(OH) D level, PTH, Calcium, Phosphorus, Magnesium, type and dosing of Vitamin D Supplements and post treatment 25(OH) D level.

**Results:** Mean age (yrs) was (13.02±4.73). 76% African american. eGFR (ml/min/1.73m2 was significantly lower among CKD (61.23±44.10) than PH and C. SBP z-score (0.7±1.8) was higher among PH (2.1 ±1.9) than CKD (0.26±1.75), p=0.001 and controls (0.39±1.27), p=0.01. BMI z-score) was significantly higher among PH (1.61±1.22) and Control (1.20±1.21) than CKD (0.37±1.58) p= 0.04.Prevalence of Vitamin D deficiency was (91%); 60% had level <20 ng/ml. Mean pretreatment 25(OH) D (ng/ml) was (19.07±10.44), no significant difference among PH (18.5±9.7), CKD (18.8± 11.33) and Control (20.6±7.5). Pretreatment 25(OH) D didn't correlate with PTH, eGFR, BMI, SBP/DBP Z-score, serum cholesterol. Mean post treatment 25(OH) D (ng/ml) was (24.5±911.16). After completion of standard treatment almost 76 % patients had 25 (OH) D level <30 ng/ml, mean 25 (OH) D (24.5±11.16); PH (24.57±11.7) and CKD (24.9±9.9) and control (21.8±5.67). None were in toxic range.

**Conclusions:** The standard treatment dose of Vitamin D doesn't normalize 25(OH) D level in majority of patients. Newer treatment guidelines may be needed to optimize the treatment.

**TH-PO1071**

**Prevalence and Determinants of 25-Hydroxyvitamin D Deficiency in the Chronic Kidney Disease in Children Cohort** Juhi Kumar,[1] Lisa Aronson Friedman,[3] Alison G. Abraham,[3] Valerie L. Johnson,[1] Frederick J. Kaskel,[3] Bradley A. Warady,[3] Susan L. Furth,[3] Michal L. Melamed,[2] Anthony A. Portale.[3] *[1]Pediatrics, Weill Cornell Medical College, New York, NY; [2]Medicine, Albert Einstein College of Medicine, Bronx, NY; [3]Chronic Kidney Disease in Children(CKiD) Investigator.*

**Background:** Deficiency of 25-hydroxyvitamin D (25OHD) is highly prevalent in healthy children. Children with chronic kidney disease (CKD) are at higher risk for 25OHD deficiency, although the risk factors are poorly defined.

**Methods:** We measured 25OHD levels at baseline and after 1.5 years and 3.5 years of follow-up in 504 children with CKD stages 2-4 enrolled in the Chronic Kidney Disease in Children (CKiD) study. We used generalized estimating equations to determine the associations of demographic, nutritional, anthropometric and biochemical factors with 25OHD deficiency, defined as level <20 ng/ml; 25OHD sufficiency was defined as level ≥30 ng/ml.

**Results:** Median participant age was 12.2 years, and 22 % were African-American. Overall, 64 % of participants were 25OHD deficient at one or more visits. The prevalence of deficiency decreased with subsequent visits; 28% were deficient at baseline, 24% at 1.5 and 23% at 3.5 years of follow-up. Nutritional vitamin D supplement use was low, 1.5% at baseline, 3% at 1.5, and 15% at 3.5 years of follow-up. By multivariable analysis, 25OHD deficiency was significantly associated with older age (OR=1.1, p <0.05), African-American race (OR=4.4, p <0.001), higher BMI (OR=1.1, p <0.001), less than daily milk intake (OR=2.6, p <0.001), insufficient sunlight exposure (winter season/higher latitude of blood draw, OR=2.8, p<0.001), lower serum albumin (OR=2.5, p <0.01), baseline vs. 1.5 year follow up visit (OR=2.0, p<0.05) and absent use of nutritional vitamin D supplements (OR=6.6, p<0.01). Gender, maternal education, income, history of low birth weight, GFR, proteinuria, albumin-corrected calcium, PTH, and FGF 23 levels were not associated with 25OHD deficiency.

**Conclusions:** Risk factors for 25OHD deficiency in children with CKD are similar to those in normal children with age, race, BMI, sunlight exposure, milk intake, and use of nutritional vitamin D supplements being significant predictors.

*Funding:* NIDDK Support

## TH-PO1072

### Trends in Control of Hypertension (HTN) in Childhood Chronic Kidney Disease (CKD), 2005-13

Joseph T. Flynn,[1,2] Christopher B. Pierce,[2] Mark Mitsnefes,[2] Gina-Marie Barletta,[2] Joshua A. Samuels,[2] Susan L. Furth,[2] Bradley A. Warady.[2] *[1]Seattle Children's Hospital; [2]CKiD Investigators.*

**Background:** Early data from the chronic kidney disease in children (CKiD) cohort study demonstrated poor control of HTN. Enrollment of a second CKiD cohort allows examination of whether HTN control has improved over time.

**Methods:** Baseline casual and ambulatory blood pressure (BP) data were compared between participants enrolled in 2005-09 (cohort1) and those enrolled in 2011-13 (cohort2). Using auscultatory casual BP (CBP), we examined the prevalence of pre-HTN and HTN, as well as the prevalence of controlled (<90th %tile) and uncontrolled (90th %tile) CBP among those on antihypertensive medications. Ambulatory BP data were used to classify subjects as having confirmed normotension, WCH, ambulatory HTN or masked HTN. Cumulative logistic regression with adjustment for glomerular filtration rate (GFR), race, sex, age and body mass index was performed to examine the effect of cohort on BP status. Analyses were stratified by CKD diagnosis (glomerular and non-glomerular).

**Results:** Cohort characteristics were similar except for significantly higher GFR in cohort2. Unadjusted BP results are displayed in the table.

| | | Glomerular | | | Non-glomerular | |
|---|---|---|---|---|---|---|
| BP status, % | cohort1 | cohort2 | P | cohort1 | cohort2 | P |
| Systolic CBP | | | 0.05 | | | NS |
| Normotensive | 64 | 77 | | 78 | 73 | |
| Pre-HTN | 18 | 16 | | 10 | 11 | |
| HTN | 17 | 17 | | 12 | 17 | |
| Diastolic CBP | | | 0.049 | | | NS |
| Normotensive | 77 | 90 | | 78 | 80 | |
| Pre-HTN | 10 | 6 | | 9 | 9 | |
| HTN | 13 | 5 | | 13 | 11 | |
| Ambulatory BP | | | NS | | | NS |
| Normotensive | 47 | 63 | | 45 | 37 | |
| WCH | 2 | 5 | | 5 | 0 | |
| Masked HTN | 33 | 21 | | 35 | 36 | |
| Ambulatory HTN | 19 | 11 | | 14 | 15 | |

Rates of uncontrolled CBP in those on antihypertensive medications were similar in the two cohorts. After adjustment, no effect of cohort on BP status could be demonstrated.

**Conclusions:** HTN remains unrecognized and under-treated in children with CKD. Interventions to improve the recognition and treatment of HTN in pediatric CKD are urgently needed.

*Funding:* NIDDK Support, Other NIH Support - NHLBI, NICHD

## TH-PO1073

### The Contributions of Glomerular versus Non-Glomerular Diagnoses and Marked Proteinuria to Neurocognitive Outcomes in Children and Adolescents with CKD

Stephen R. Hooper,[1] Arlene C. Gerson,[2] Marc Lande,[3] Matthew Matheson,[2] Christopher Cox,[2] Bradley A. Warady,[4] Susan L. Furth.[5] *[1]U. North Carolina; [2]Johns Hopkins; [3]U. Rochester; [4]Children's Mercy; [5]CHOP.*

**Background:** The presence of pediatric chronic kidney disease may be associated with abnormal neurocognitive function. The type of kidney disease (glomerular [G] vs. non-glomerular [NG]) and marked proteinuria are two potential risk factors we have considered in assessing functional outcomes. Marked proteinuria has previously been shown to be related to lower neurocognitive functioning. However, to date, there are no available data to show how these two factors (diagnosis and proteinuria) interact to affect neurocognitive outcomes. This study investigated (1) the group differences between those with G versus NG, with and without marked proteinuria; and (2) how this interaction contributes to neurocognitive functioning.

**Methods:** Cross-sectional data from the NIDDK/NHLBI/NICHD-funded CKiD Study were employed for both questions. The G group included 43 subjects with marked proteinuria (uP/C>2) and 123 without, while the NG group included 39 with marked proteinuria and 460 without. Median Iohexol-based GFR for the sample was 50.44 ml/min/1.73m$^2$. Measures at study entry included IQ, inhibitory control, attention regulation, problem solving, working memory, and ratings of overall executive functioning at study entry.

**Results:** In comparing G vs NG groups with and without proteinuria at study entry, univariate results showed the 4 groups to be significantly different only for IQ ($p < .009$), with the groups having marked proteinuria having the lowest IQ scores. After adjusting for demographic and disease-related variables, neither diagnosis nor proteinuria was predictive of neurocognitive outcomes, although there was a trend for proteinuria to be associated with IQ ($p < .088$). The interaction between diagnosis and marked proteinuria was nonsignificant.

**Conclusions:** While a diagnosis of G vs. NG does not seem to affect neurocognitive outcomes cross-sectionally, proteinuria does show a modest effect on IQ regardless of diagnosis. A diagnosis X marked proteinuria interaction on neurocognitive functions was not detected.

*Funding:* NIDDK Support

## TH-PO1074

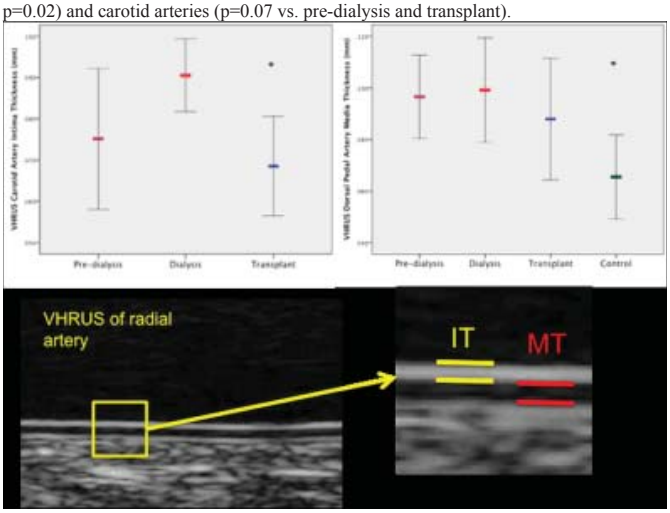
### Very High Resolution Ultrasound Reveals Peripheral Arterial Changes in Both Intima and Media in Children with Chronic Kidney Disease

Frida Dangardt,[1] Rukshana Shroff,[2] Marietta Charakida,[1] John E. Deanfield.[1] *[1]Vascular Physiology, Univ College London, London, United Kingdom; [2]Dept of Nephrology, Great Ormond Street Hospital for Children, London, United Kingdom.*

**Background:** Children with chronic kidney disease (CKD) have multiple risk factors for vascular damage that will contribute to the very high cardiovascular morbidity and mortality seen in this group. We hypothesize that children with CKD develop changes in their arterial intima and media, associated with different risk factors and that varies between vascular beds.

**Methods:** Intimal (IT) and medial (MT) thickness of the carotid, radial and dorsal pedal arteries were measured using very high resolution ultrasound (VHRUS) (40-55MHz) in 22 children with CKD (14.6 ± 3.0 years) (n=9 pre-dialysis CKD stages 4-5, n=7 dialysis, n=6 post transplant) and compared to radial and dorsal pedal arteries from 17 matched healthy controls. Results were compared with conventional carotid intima-media thickness (cIMT) and biochemical data in CKD patients.

**Results:** In CKD children, there was widespread medial thickening, most marked in dorsal pedal arteries (0.095±0.03 vs. 0.065±0.03 in controls; p=0.004). In children on dialysis, increased IT was also seen in radial arteries (0.060±0.01 vs. 0.056±0.01 in controls; p=0.02) and carotid arteries (p=0.07 vs. pre-dialysis and transplant).




Radial IT and carotid MT were both associated with serum calcium in these patients (r=0.42, p=0.05 and r=0.89, p=0.02). Intimal and medial changes could not be distinguished by conventional cIMT.

**Conclusions:** VHRUS reveals changes of both intima and media, not only in the central, but also in peripheral arteries. These changes are more pronounced in children on dialysis, and associated with calcium levels. This suggests that, in CKD children, strategies to target both metabolic and atherosclerosis risk factors should be initiated from an early age.

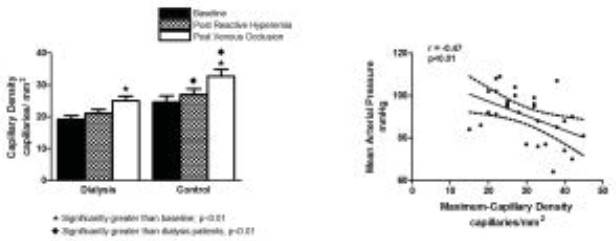
**TH-PO1075**

**Carotid Intima Media Thickness and Nailfold Capillary Density in Pediatric Hemodialysis Patients** Alcia D. Edwards-Richards,[1] Nao Sasaki,[2] Chryso P. Katsoufis,[1] Wacharee Seeherunvong,[1] Vimal Master Sankar Raj,[1] Marissa J. Defreitas,[1] Jayanthi Chandar,[1] Michael Freundlich,[1] Gaston E. Zilleruelo,[1] Carolyn L. Abitbol.[1] *[1]Pediatric Nephrology, Univ of Miami/ Holtz Children's Hospital, Miami, FL; [2]Pediatric Cardiology, Univ of Miami/ Holtz Children's Hospital, Miami, FL.*

**Background:** Pediatric patients with chronic kidney disease (CKD) on hemodialysis (HD) are at increased risk of cardiovascular disease (CVD) and cardiac death. Early detection of microvascular abnormalities may prompt timely therapeutic interventions to reduce these risks.

**Methods:** Nailfold capillary density (NCD), an early marker of microvascular disease, was assessed by capillaroscopy in 19 pediatric HD patients (aged:16.6±3.5 years) and 16 age-matched controls. Carotid intima media thickness (cIMT) and left ventricular mass index (LVMI) were measured by echocardiography and compared to healthy adolescent controls. Laboratory assessments included demographics of ethnicity and gender, dialysis vintage, mean pre-dialysis and post-dialysis blood pressures and cardiac biochemical markers such as pro-Brain natriuretic peptide (pro-BNP) and high sensitivity C-reactive protein (hsCRP).

**Results:** NCD was significantly lower in HD patients and correlated significantly with mean arterial pressures (Figure A & B). LVMI was greater than that of controls (36.5±19 versus 29.2±27 g/m$^{2.7}$; p<0.01) and cIMT was significantly less (0.37±.05 versus 0.41±.07 mm; p=0.03). All other biochemical markers of CVD injury were elevated above normal limits but did not correlate with NCD or echocardiographic measures.




**Conclusions:** In the present cross-sectional pilot study, pediatric HD patients had significantly lower NCD measurements than age-matched controls. NCD appears to be a valuable non-invasive tool to detect early subclinical CVD risk in young hemodialysis patients. Further studies are very much warranted.

**TH-PO1076**

**Peritoneal Membrane Transformation: Results from the International Pediatric Peritoneal Biopsy Study** Bradley A. Warady,[1] Betti Schaefer,[2] Stephan Macher-Goeppinger,[2] Maria Bartosova,[2] Benjamin L. Laskin,[3] Stefan Holland-Cunz,[2] Franz S. Schaefer,[2] Claus P. Schmitt.[2] *[1]The Children's Mercy Hospital, Kansas City; [2]Univ of Heidelberg, Heidelberg, Germany; [3]The Children's Hospital of Philadelphia, Narberth.*

**Background:** The peritoneal membrane has not yet been studied systematically in healthy children and adults, and age dependent differences are unknown. In adults, acidic PD fluids with abundant glucose degradation products (GDP) induce major alterations of the peritoneum. The impact of exposure to these same fluids or to "biocompatible PD fluids" in children is unknown.

**Methods:** Standardized peritoneal specimens were obtained from 56 healthy children (0.1-16.6 yrs), 9 healthy adults (19-62 yrs), 56 children at time of PD catheter insertion (0.1-19.6 yrs) and 51 children on PD (0.3-20.1 yrs) from 26 centres in 14 countries. 46 patients had low GDP fluids.

**Results:** In controls, the mesothelial cell layer was mostly intact, but in young children calretinin negative, indicating immaturity. Submesothelial compact zone was absent in 95% of samples, and submesothelial fat was missing in children under 2 yrs of age. Total submesothelial zone thickness was 269(207-370) µm. Capillaries were located in 3 layers 56(19-88), 162(69-301) and 323(157-774) µm below the mesothelial surface, depending on age and submesothelial thickness. Capillary surface area decreased with age. In patients, the mesothelial cell layer was intact in 68/58/21% at onset, after 0-2 and after > 2 yrs of PD. Submesothelial thickness increased to 250(90-700), 430(350-700) and 625(600-650) µm after 0-2, 2-4 and > 4 yrs of PD, despite low GDP fluid. Whereas capillary density increased in a subgroup of patients, vessel morphology remained unaltered. ASMA+ fibroblasts and CD45/CD68+ leucocytes were present in 23/11% of controls, in 31/26% at PD onset and in 55/69% of children on chronic PD. VEGF/TGF-ß abundance increased with PD. No biopsy related complications were reported.

**Conclusions:** Our findings reflect major, age dependent differences in peritoneal structure in non-uremic controls, as well as substantial time dependent peritoneal membrane transformation, despite low GDP fluid usage.

*Funding:* Pharmaceutical Company Support - Fresenius Medical Care, Bad Homburg, Germany; University Medical Faculty Funding, Government Support - Non-U.S.

**TH-PO1077**

**The Bioelectric Impedance Analysis Using a Body Composition Monitor Allows Adequate Determination of Body Fluid Volume in Children and Adolescents following Peritoneal Dialysis** Rainer Büscher. *Univ Hospital Essen, Dept of Pediatrics II, Essen, Germany.*

**Background:** The adequacy of body fluid volume in children requiring dialysis is difficult to assess and a gold standard for the best method of measurement is missing. Chronic overhydration or hypovolemia are serious side effects of an inadequate dialysis treatment and a major risk for cardiovascular sequelae. This study was performed to determine whether body composition monitoring (BCM) or sonographic measurement of the inferior vena cava diameter (IVCD) is a valid means to study overhydration in children following peritoneal dialysis (PD).

**Methods:** Fourteen children (7 male) undergoing PD (CCPD n=11, CAPD n=3), mean age 14.5±3.1 years (male) and 12.2±1.2 years (female), age at onset of dialysis 11.7± 5.8 (male) and 11.1 ± 3.4 years (female) were included. Using a BCM device (Fresenius, Bad Homburg, Germany) we determined resistance (Ω) as well as sonographic IVCD (mm) in supine position before and following a CCPD interval. In addition, SDS-blood pressure was monitored using a 24-h ambulatory blood pressure monitor (ABPM). Data are presented as mean±SD.

**Results:** Resistance as a marker of fluid reduction increased significantly following a CCPD interval (male: mean 545±79 Ω before dialysis, 605±76 Ω after dialysis; female: mean 523±76 Ω before dialysis, 593±62 Ω after dialysis). Accordingly, IVCD significantly decreased following a CCPD-interval (male: mean 12.1±3.4 mm before dialysis, 10.2±3.0 mm after dialysis; female: mean 11.1±1.8 mm before dialysis, 8.8±2.2mm after dialysis). ABPM revealed a significant reduction of SDS-blood pressure following a CCPD-interval (male: 2.2±1.7 before dialysis, 0.4±1.1 after dialysis, p<0.0001; female: 2.1±1.2 before dialysis, 0.9±1.2 after dialysis, p<0.0001).

**Conclusions:** Bioelectric impedance analysis is an easy and reproducible method in children undergoing PD to determine body fluid volume on a daily basis. In contrast, determination of the IVCD is more laborious and combined with a greater observer variance. Although both methods simplify the assessment of fluid balance in children, the uncritical use of normograms leads to false high dry weights with subsequent overhydration.

*Funding:* Clinical Revenue Support

**TH-PO1078**

**Current Achievement of the PD Adequacy Target in the Pediatric ESRD Population** Sylvia Paz B. Ramirez,[1] Alissa Kapke,[1] Natalie Scholz,[2] Bradley A. Warady.[3] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]Univ of Michigan, Ann Arbor, MI; [3]The Children's Mercy Hospital.*

**Background:** Clinical practice guidelines in the pediatric (age<18) peritoneal dialysis (PD) population recommend a target weekly Kt/V of 1.8. While this target is based on prior studies in the pediatric and adult PD populations, there is consensus that greater solute clearance may be associated with improved outcomes in the pediatric PD population. We sought to examine the current achievement of this Kt/V target in the population.

**Methods:** Medicare claims data submitted for pediatric PD patients from 2011-2012 were assessed to determine how often patients achieved a weekly Kt/V ≥1.8 at the patient and facility-level.

**Results:** Claims were submitted for a total of 675 pediatric PD patients over two years resulting in 4,009 monthly observations (patient-months). A total of 140 patients (21%; 2,630 patient-months) did not have a single Kt/V reported. Among patient-months with Kt/V, 76% had a Kt/V ≥1.8 but this was 48% when patient-months without reported Kt/V were included. On facility-level analysis, the median %patient-months with weekly Kt/V ≥1.8 was 52% (interquartile range [IQR]:16%-78%). Facilities with <10 pediatric PD patients had a higher percentage with Kt/V ≥1.8 (median=56%;IQR:17%-79%), compared to facilities with >10 patients (median=49%;IQR:7%-71%). Better achievement was observed in urban facilities, hospital satellite facilities, and independent facilities [Table 1].

**Conclusions:** Many pediatric PD patients do not report Kt/V. When including patient-months without reported Kt/V, over 50% of patient-months did not meet the target. Facilities with more PD pediatric patients, rural facilities, hospital or freestanding facilities, and chain-associated facilities were less likely to meet the Kt/V target.

**Table 1. Percent patient-months with Kt/V>=1.8 by facility characteristics**

| Category | # Facilities | Median % with Kt/V>=1.8 (Q1-Q3) |
|---|---|---|
| **# PD pediatric pts** | | |
| 1-10 | 125 | 56 (17, 79) |
| 11+ | 19 | 49 (7,71) |
| **Urban/Rural** | | |
| Urban | 124 | 56 (15, 79) |
| Rural | 12 | 44 (8, 73) |
| **Facility Type** | | |
| Hospital-Based | 50 | 57 (15, 79) |
| Free-Standing | 80 | 50 (16, 78) |
| Hospital-Satellite | 10 | 63 (29, 85) |
| **Ownership Type** | | |
| Chain | 71 | 50 (17, 78) |
| Independent | 69 | 56 (15, 81) |

*Funding:* Other U.S. Government Support

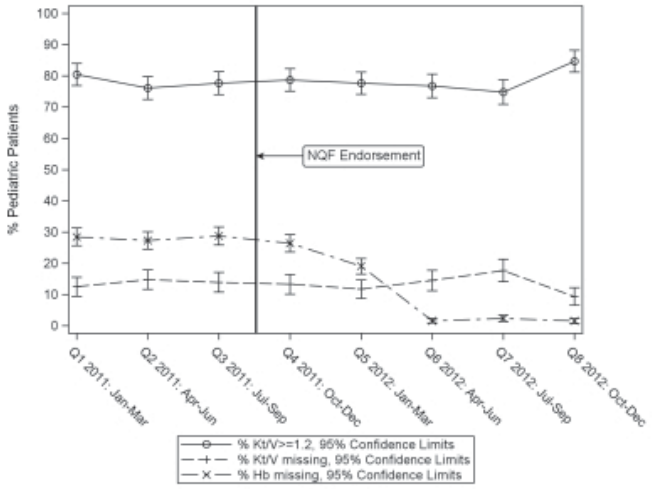
**TH-PO1079**

**Performance of Quality Measures for Pediatric Hemodialysis Patients**
Sylvia Paz B. Ramirez,[1] Alissa Kapke,[1] Jeffrey Pearson,[1] Jordan Jahnke,[1] Bradley A. Warady.[2] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]The Children's Mercy Hospital, Kansas City, MO.*

**Background:** Published studies suggest improvement in mortality among pediatric patients receiving maintenance dialysis over time but mortality remains high; improved quality of dialysis care in this fragile patient group may further improve survival. Several pediatric ESRD quality measures received endorsement from the National Quality Forum (NQF) in August 2011 and in June 2012 were proposed for inclusion the CMS ESRD 2015 QIP. This study aims to examine the change in performance of these measures between 2011 and 2012.

**Methods:** Monthly hemoglobin (Hb) and dialysis adequacy measurements, and the achievement of spKt/V ≥1.2 were evaluated using Medicare claims data from 2011-2012. Monthly claims were aggregated by quarter for reporting purposes.

**Results:** Performance for the adequacy measures remained stable from Q1 through Q7. In Q8, the %spKt/V ≥1.2 increased nearly 5% over all other quarters to 85%, while the %monthly Kt/V not measured decreased by 5% to 11.5%. The %patients whose monthly Hgb was not reported remained over 25% through 2011 and decreased to 19% after August 2011. In April 2012, Medicare claims reporting instructions changed to require facilities to enter a Hgb value or 99.99 if Hgb was not reported, which appeared to be associated with a marked increase in reporting of Hgb levels (approximately 98% reporting). Figure 1. Quarterly performance of pediatric ESRD quality measures.




**Conclusions:** The pediatric ESRD quality measures analyzed in this study show some improvement in clinical performance over a two year period when several important steps were undertaken to implement the quality measures. Future analyses are needed to evaluate whether improvement in clinical performance measures is associated with improved health outcomes.

**TH-PO1080**

**Inpatient Citrate Based Hemodialysis in Pediatric Patients**
Rita D. Sheth, Cheryl P. Sanchez, Drew C. Cutler, Shobha Sahney. *Pediatric Nephrology, Loma Linda Univ, Loma Linda, CA.*

**Background:** Heparin use in inpatient hemodialysis is limited in critically ill or perioperative patients. Citrate based dialysate allows heparin avoidance.

**Methods:** Pediatric inpatient hemodialysis treatments (HDRx) were performed at a single institution over 1 year with a citrate based dialysis solution. Ica were monitored hourly for initial 3 HDRx. Rates of catheter malfunction, dialyzer and chamber clotting were monitored. HDRX characteristics and iCa levels were compared between AKI & CKD patients.

**Results:** 18 inpatients (10 male, ages: 0.01-18.13 yrs, 4.5 -57 kgs) were dialyzed with Citrasate®: 6 AKI, 2 new & 10 established CKD 5 pts. 119 HDRx over 21 admissions were performed;1-16 HDRx per patient. 10 pts (64 HD Rx) had a cuffed semi-permanent HD catheter, 8 pts (54 HDRx) had a temporary HD catheter and 1 pt (1 HDRx) an AVF. 10 HD Rx done with a blood prime. All patients were dialyzed heparin-free.

| | Overall | AKI | CKD | p value |
|---|---|---|---|---|
| Lost Circuit /Clotted | 3/119 (2.5%) | 3/54 HD Rx (5.6%) | 0/65 HD Rx (0%)* | <0.01* |
| Catheter malfunction | 2/119 (1.7%) | 2/54 D Rx (3.7%) | 1/65 HD Rx (1.5%) | NS |
| Decreased HD time | 7/119 (5.9%) | 5/54 HD Rx (9.3%) | 2/65 HD Rx (3.1%)* | <0.01* |
| Chamber clots | 62/119 (52.1%) | 32/54 (59.2%) | 32/65 (49.2%) | NS |
| Dialyzer clots | 50/119 (42.0%) | 28/54 (51.9%) | 23/65 (35.4%)* | <0.01 |
| Mean iCa hour 1 (mmol/L) | 0.97±0.12 | 0.92±0.11 | 1.0±0.11 | <0.05 |
| Mean iCa hour 2 (mmol/L) | 0.98±0.13 | 0.95±0.15 | 0.98±0.10 | NS |
| Mean Δ iCa hour 2 (mmol/L) | -0.01±0.17 | -0.03±0.22 | -0.02±0.12 | NS |
| Single iCa <1.0 (mmol/L) | 40/49 (81.6%) | 13/16 (81.1 %) | 25/33 (75.8%)* | <0.01 |
| ICa <1.0 entire HD Rx | 22/49 (44.9%) | 11/16 (68.8%) | 12/33 (36.4%)* | <0.01 |

* p<0.05

No patients had symptomatic hypocalcemia, lowest iCa was 0.76, 0.72 mmol/L at hour 1 and 2 respectively.

47% vs. 40% HDRX with a chamber clot had a iCa > 1 mmol/L, p=0.04; 56% vs. 55% HDRx with dialyzer clot had a iCa > 1 mmol, p=NS.

**Conclusions:** Citrate based acute HD is a safe and useful alternative and allows the avoidance of heparin in sick hospitalized patients. Risk for significant circuit loss is low. Most patients had mild asymptomatic hypocalcemia with no adverse reactions. Higher iCa values >1 mmol/L increased the risk for clotting.

**TH-PO1081**

**The Use of Nafamostat Mesilate as an Anticoagulant in Pediatric Continuous Renal Replacement Therapy**
Heeyeon Cho, Sang Taek Lee. *Pediatrics, Samsung Medical Center, Seoul, Korea.*

**Background:** Nafamostat mesilate, a synthetic serine protease inhibitor, has been used as an anticoagulant during continuous renal replacement therapy (CRRT) for the patients with high risk of bleeding. However, there is little data about its clinical utility in pediatric patients. The aim of this study was to evaluate the ideal dosage, efficacy and safety of nafamostat mesilate in CRRT among critically ill pediatric patients.

**Methods:** We conducted a retrospective study in pediatric patients who received CRRT from January 2011 to January 2013. Thirty seven patients were enrolled in this study (22 males with the median age at the initiation of CRRT of 8.3 years). The median duration for CRRT was 12.9 days and the survival rate 37.8 %. In the 6 pediatric patients without the risk of bleeding, heparin was used during CRRT. In the 31 patients with the risk of bleeding, we started CRRT without anticoagulation, and nafamostat mesilate was used if hemofilter lifespan was less than 12 h or the activated clotting time (ACT) was less than 200. The starting dosage of nafamostat mesilate was 0.25 mg/kg/h and titrated according to ACT.

**Results:** Of the 31 patients with the risk of bleeding, 14 (45.2 %) patients received nafamostat mesilate and the average hemofilter lifespan was improved from 7.8 h to 28.6 h after the use of nafamostat mesilate ($p < 0.001$). The average hemofilter life span with nafamostat mesilate was significantly greater than heparin (28.6 h vs. 11.3 h, $p<0.001$). The average hemofilter lifespan was 42.1 h in patients without anticoagulation. No patients experienced major bleeding, agranulocytosis, hyperkalemia or anaphylaxis while treated with nafamostat mesilate.

**Conclusions:** These data suggest that nafamostat mesilate is a good alternative as an anticoagulant in critically ill pediatric patients with high risk of bleeding who require CRRT.

**TH-PO1082**

**Long-Term Hemodialysis Therapy in Neonates and Infants with End Stage Renal Disease**
Shirley Pollack,[1,2] Israel Eisenstein,[1,2] Daniella Magen,[1,2] Mahdi Tarabeih,[1] Israel Zelikovic.[1,2] *[1]Pediatric Nephrology, Rambam Medical Center; [2]Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel.*

**Background:** Peritoneal dialysis (PD) is the preferred mode of renal replacement therapy (RRT) in infants with end stage renal disease (ESRD). Hemodialysis (HD) is seldom used in neonates and infants because of the major complications of this procedure in the very young.

**Methods:** We analyzed demographic, clinical, laboratory and imaging data in all infants younger than 12 months with ESRD who received HD therapy in our Pediatric Dialysis Unit between 1997-2013. RRT- primarily HD- is performed in all infants with ESRD admitted to our service without exclusion.

**Results:** Eighteen infants (M:F 6/12; Arabs/Jews 14/4) with ESRD (median age, 5 months ; median wt, 3.8 kg) received HD through a tunneled central venous catheter (CVC) for a total of 16,292 days. Seven (39%) were neonates (< 1 month of age ; group 1) and 11 (61%) were infants (1-12 months; group 2) who received HD for a cumulative 7141 days (44% of total) and 9151 days (56%), respectively. In 6 of the patients, the initial mode of RRT was PD for 1-3 months. Five (28%) of the patients had serious comorbidities. Thirty eight CVC were inserted (34 angiographically). There were 5 episodes of CVC infection- a rate of 0.3/1000 CVC days. Median catheter survival time was 428 days. Five (28%) of the infants (2 in group 1) underwent renal transplantation and 6-10 year graft survival was 80%. Seven (38%) of the patients died. Most infants had good oral intake and only 4 (22%) required gastric tube. Thirteen (72%) of the infants displayed normal growth. Eight (44%) of the patients (5 in group 1) had delayed psychomotor development. Of these infants, 5 (3 with comorbidities) had abnormal brain imaging, 4 had seizures, and 3 had sensorineural deafness.

**Conclusions:** Long term HD in neonates and infants with ESRD, performed in the appropriate setting, is technically feasible, can be implemented without major complications, carries a very low rate of CVC infection and malfunction, and results in good nutrition, growth and survival. Future efforts should aim to improve neurodevelopmental outcome and lower mortality rate, especially in treated neonates.

*Funding:* Clinical Revenue Support, Government Support - Non-U.S.

**TH-PO1083**

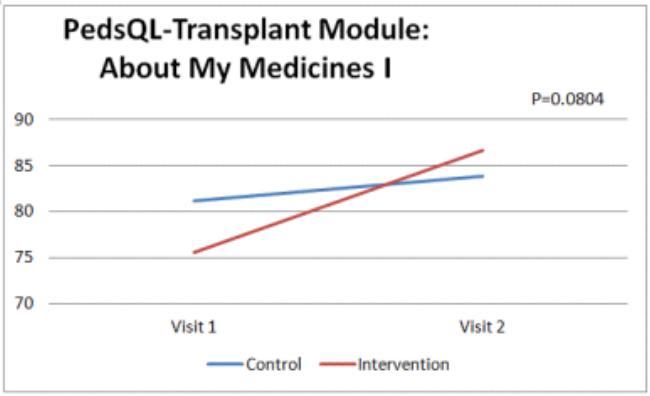
**Peers4PATH: Testing Peer Support as a Mechanism to Improve Transplant Outcomes among Adolescents with Solid Organ Transplants**
Sandra Amaral,[1,2] Nina Foster,[1] Amy Yang,[2] Justine Shults,[1,2] Susan L. Furth.[1,2] *[1]Pediatrics, Children's Hosp of Phila, Phila, PA; [2]Biostatistics and Epidemiology, Univ of Pa, Phila, PA.*

**Background:** Medication nonadherence contributes to inferior allograft outcomes among adolescents with solid organ transplants (SOT). Factors intrinsic to adolescence such as wanting to feel "normal" like peers may impact medication taking behavior. Peers4PATH

is an NIDDK-sponsored randomized clinical trial to examine peer mentoring as a means to improve medication taking behavior and health-related quality of life (HRQOL) among adolescents with SOT.

**Methods:** SOT recipients 14-23 years old at a single center are randomized to receive a gender- and race-matched peer mentor vs. usual care. Mentors meet in-person with mentees at 1, 6 and 12 months and communicate with mentees by phone, text, email or FaceBook at least once weekly. Mentors provide social support and positive role modeling. Primary outcomes are 1) change in percent medication adherence (by pharmacy refill data) and 2) change in HRQOL (PedsQL3.0 Transplant Module) over one year. Target enrollment is 60 subjects. This interim analysis examines initial feasibility and acceptability.

**Results:** Since April 2012, 30 subjects have enrolled: 17 kidney, 7 liver, and 6 heart transplant recipients. 14 subjects have completed six months of the study (5 intervention, 9 control). Thus far, mentees report that: "my mentor understands me", "is there for me" and "makes me feel important." Mentees (vs. controls) also report greater interim improvements in HRQOL related to problems with medication taking.




**Conclusions:** Peers4PATH is one of the first clinical trials to examine peer support as a means to improve medication adherence and HRQOL among adolescents with SOT. In its early phase, this study has shown feasibility and good acceptability by subjects. Further data are needed to assess the efficacy of the intervention.

*Funding:* NIDDK Support

## TH-PO1084

**Health Literacy in Caregivers of Children and Adolescents with Renal Transplant – Association with Adherence** Smitha R. Vidi, Tamar Y. Springel, Nataliya Zelikovsky, Susan L. Furth. *Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA.*

**Background:** Health literacy in caregivers and patients is associated with poor outcomes in multiple medical conditions. Little is known about health literacy in pediatric kidney transplant recipients and their parents. As poor adherence is linked to poor graft function and graft loss in solid organ transplant recipients, we sought to determine the association between lower health literacy and markers of poor adherence.

**Methods:** In this cross sectional study, we assessed the health literacy of 24 participants using validated measures; S-TOFHLA (Short Test of Functional Health Literacy in Adults), and REALM (Rapid Estimate of Adult Literacy in Medicine)-Teen & Transplantation. We compared the health literacy levels of adolescent patients and their parents based on S-TOFHLA and REALM scores. Adherence was measured both by self-report using MAM (Modified Adherence Module) and by calculating variability of immunosuppressant levels over a period of 6 months, by retrospective chart review. We used >2.5 standard deviation (SD) and >30% co-efficient of variation (CV) of immunosuppressant levels as markers of poor adherence, as shown in previous studies.

**Results:** Among 17 caregivers and 7 transplant recipients with median age 16.9 (IQR 15.7-19.7) yrs, all participants had adequate functional health literacy with S-TOFHLA scores >23. Five out of 24 participants (20%) had 7th/8th grade equivalent level of health literacy (REALM score:45-60) although they had higher levels of education. All participants had a MAM score of >20%, which indicated good adherence by self-report. Lower health literacy levels (REALM scores ≤ 61) was associated with >2.5 SD of immunosuppressant level in 4/7 and >30% CV in 5/7 participants. Comparing 5 adolescent patients and their parents, lower health literacy levels of either of them, is associated with significant variability in immunosuppressant levels (SD>2.5 and CV%>30).

**Conclusions:** Lower health literacy of both caregivers and adolescent kidney transplant recipients are associated with poorer adherence. Improving health literacy and using targeting interventions in this population may improve transplant outcomes.

*Funding:* Pharmaceutical Company Support - Fellowship support from an unrestricted educational grant from Amgen.

## TH-PO1085

**Transfer from Pediatric Nephrology to Adult Nephrology Goes along with Deterioration of Transplant Function** Christina Taylan, Michaela Gessner, Gesa Schalk, Rasmus J.C. Ehren, Lutz Thorsten Weber. *Pediatric Nephrology, Univ Hospital of Cologne, Cologne, Germany.*

**Background:** The transfer to adult nephrology implies a decisive turning point in the medical life of a patient in most cases.

**Methods:** We present data of 24 renal transplant recipients (21 ±4 years of age) who had been transferred to adult nephrologists between 2010 and 2012 (follow-up 24 – 3 months). Mean follow-up after renal transplantation (RTx) was 31 (6-113) months. The following parameters directly before transfer and the most recent follow-up were analyzed: retention values, number of rejections, blood pressure, change of medication, personal contentment with the actual situation, obtained through telephone survey.

**Results:** In 20/24 patients plasma creatinine levels had increased 0,65 (0,24 - 4,32) mg/dl. Five patients had to be hemodialyzed intermittently following the transfer, four of which were admitted back to the dialysis program. One patient died 1,5 years after the transfer from a PTLD. 7/24 patients had to be treated for acute rejection after transfer versus 4/24 before. Medication changes were numerous due to newly occurred acidosis, followed by changes in blood pressure medication. 19/24 patients were content, but reported a temporary sense of insecurity after the transfer.

**Conclusions:** Deterioration of renal function and increased risk of acute rejection after transfer underline the need for optimization of the transition process. The potential reasons for the described problems after transfer are manifold and comprise lower consultation frequency, higher expected self-reliance, limited team support, larger patient numbers etc. A refinanced standardized program for young adult chronic kidney patients might help the patients to reveal deficits in coping with their condition and prepare better for the transition to adult patient care.

## TH-PO1086

**Adherence to Cysteamine Therapy and Renal Outcomes in Cystinosis** Larry A. Greenbaum,[1] Ben Cadieux.[2] *[1]Pediatric Nephrology, Emory Univ, Atlanta, GA; [2]Raptor Pharmaceuticals, Novato, CA.*

**Background:** Early initiation and adherence to cysteamine therapy are essential in delaying progression to ESRD in cystinosis. Immediate-release cysteamine (IR-C) requires chronic, lifelong Q6H dosing. Objective measures of long-term adherence are challenging to quantify. Adherent patients are believed to significantly delay the onset of ESRD (beyond 10 yrs observed prior to cysteamine).

**Methods:** We examined 12 years (7/1/97 to 6/30/09) of Florida Medicaid claims to characterize IR-C adherence among cystinosis patients (ICD-9 270 and ≥1 IR-C claim).

**Results:** Among more than 7 million Florida Medicaid enrollees, we identified 12 pts (8 male) ages 1 to 22 years with cystinosis. Of these, 9 pts were born after FDA approval of IR-C: median age of diagnosis was 18 mos (range 11-42 mos). The majority (8/9) initiated IR-C within 3 mos of dx (delayed 14 mos in 1 pt). Duration of IR-C follow-up ranged from 11 mos to more than 11 yrs in 3 pts (total of 729 mos). Monthly refills for IR-C were skipped 296 times (41% of eligible months; ranged from 0 to 93%). Number of refill gaps averaged 1.3/yr (no gaps in 1 pt; <4 gaps in 4 patients; 12-25 gaps in 4 pts). Two pts appeared to have discontinued IR-C (gaps of 33 and 117 mos from last refill to last non-IR-C claim). Two pts reached ESRD at 9 and 11 years (skipped refill rates of 41 and 93%, respectively). Two pts reached CKD stage IV, each at 13 years (skipped refill rates of 22% and 53%, and each with an extended gap of 12 and 17 months, respectively).

**Conclusions:** Retrospective claims analysis provides a means of assessing adherence to cysteamine in patients with cystinosis. Adherence with IR-C in this small group of Florida Medicaid recipients with cystinosis is poor, and is associated with adverse renal outcomes. Future analysis will correlate adherence with health outcomes, and analyze changes in adherence following the recent introduction of delayed release cysteamine (q12h dosing formulation). Ongoing studies of adherence in this population are warranted.

*Funding:* Pharmaceutical Company Support - Raptor Pharmaceuticals

## TH-PO1087

**Trends in Hospitalizations, Emergency Room Visits and Economic Burden of Pediatric Nephrolithiasis** Kirsten A. Kusumi, Brian Becknell, Andrew L. Schwaderer. *Pediatric Nephrology, Nationwide Children's Hospital, Columbus, OH.*

**Background:** Nephrolithiasis poses a significant health burden with an approximately 10% lifetime risk and over 2 billion dollars in annual hospital charges for adults. The goal of this study was to examine national trends in hospitalization, emergency room utilization, secondary diagnoses, and charges associated with pediatric nephrolithiasis.

**Methods:** Nationally weighted data from the Kids' Inpatient Database of the Healthcare Costs and Utilization Project (HCUP) were evaluated from 1997-2009 for the total number of discharges, age, gender, length of stay, and hospital charges associated with nephrolithiasis. Secondary diagnoses were available on the basis of clinical classification software codes from 2003-2009. Data were also collected from the HCUP National Emergency Room Sample from 2006-2010 to yield the total number of pediatric Emergency Department (ED) visits associated with nephrolithiasis.

**Results:** The average number of pediatric discharges over the past 12 years with a primary diagnosis of nephrolithiasis increased by 30%. Mean hospital charges increased by 155% over this time frame, with aggregate hospital charges of $24,911,605 in 2009. 60% of patients were female, 47.5% were 15-17 years old, and length of stay was constant over this time period (2.3-2.7 days). Likewise, the number of pediatric ED visits increased by 13% from 359,771 in 2006 to 407,430 in 2010. The most common associated secondary diagnoses associated with nephrolithiasis between 2003-2009 were: urinary tract infections (628/4473, 14%); asthma (314/4473, 7%); epilepsy (180/4473, 4%); paralysis (178/4473, 4%). In 2009, secondary diagnoses of mood disorders and attention-deficit, conduct, and disruptive behavior disorders each occurred in 4% of children with nephrolithiasis.

**Conclusions:** Hospitalizations and inpatient health care charges associated with pediatric kidney stone disease continue to rise. Pediatric nephrolithiasis is associated with comorbid diagnoses of UTI, asthma, epilepsy, ADHD, disruptive behaviors and mood disorders. This raises the possibility that some of these conditions and/or medications used in their management may contribute to increased kidney stone frequency.

## TH-PO1088

**Urinary Stone Risk Factors in Pediatric Patients in Eastern North Carolina** Axita C. Patel,[2] Basema I. Dibas,[1] Guillermo Hidalgo,[1] Leonard Curtis Hymes,[1] Hsiao L. Lai.[1] *[1]Pediatrics, Div of Pediatric Nephrology, East Carolina Univ, Greenville, NC; [2]Undergraduate Honors College, East Carolina Univ, Greenville, NC.*

**Background:** The incidence of pediatric stone disease is rising. We hypothesized that the current trend of BMI elevation may be linked to increased urinary stone risk. Using Litholink 24-hr urinary analysis we identified stone risk distribution within pediatric patients in eastern North Carolina.

**Methods:** 143 consecutive Litholink collections from 100 patients obtained over a 2-year period from a single pediatric nephrology practice were analyzed with respect to BMI, gender and race. Indications for Litholink were history of kidney stone or findings of hematuria, hypercalciuria or crystalluria on spot urinary testing.

**Results:** The average age was 11 ± 4.2 years, 50% female, 56% White, 13% Black, and 27% Hispanic. 108 collections met volume adequacy criteria and were included in analysis. Prevalence of 3, 4, or 5 plus urinary risk factors were 32.4%, 15.7%, and 9.3%. BMI did not correlate with number of stone risk factors although male patients with BMI >85$^{th}$ percentile did have higher prevalence of uric acid supersaturation (17.6 vs 5.5%) compared to lean counterparts. Predominant risks were high urinary sodium excretion (69.4%), low urine volume (45.4%) and supersaturation of calcium phosphate (31.5%). Low urinary citrate was found in 38% of females and 22.2% of males, with especially high rate in Hispanic females (62.1%). Hispanic patients in general had lower supersaturation rates of calcium oxalate and calcium phosphate.

**Conclusions:** A high incidence of multiple urinary risks was observed, however we did not find the expected correlation of BMI with high urinary stone risk. The results of this study emphasize raising urine volume and lowering dietary sodium intake for all patients with stone risk. Hispanic patients appeared to have some protection against stone formation with lower urine saturation levels, but this was balanced by low urinary citrate levels. In female patients, especially Hispanic females, addressing low urinary citrate is a good strategy.

## TH-PO1089

**The Agony and the Ecstasy of a Career in Pediatric Nephrology** William A. Primack,[1] Larry A. Greenbaum,[2] Holly S. Ruch-Ross,[5] Suzanne Kirkwood,[3] Carrie Radabaugh,[3] Kevin E.C. Meyers.[4] *[1]UNC Kidney Center, Univ of North Carolina School of Medicine, Chapel Hill, NC; [2]Emory Children's Center, Emory School of Medicine, Atlanta, GA; [3]American Academy of Pediatrics, Evanston, IL; [4]Nephrology, Children's Hospital of Philadelphia and Univ of Pennsylvania, Philadelphia, PA; [5]Self Employed, Chicago, IL.*

**Background:** There is a shortage of pediatric nephrologists in the U.S., and close to 20% of trained pediatric nephrologists no longer practice pediatric nephrology (PN). Defining the perceived strengths and weakness of a career in PN may help to address this critical manpower shortage.

**Methods:** The AAP in collaboration with the ASPN sent an 88 item electronic survey in the Spring of 2013 to all physicians who are either working as pediatric nephrologists or were ever American board eligible in PN. One area of interest explored was: "How would you rate the following aspects of pediatric nephrology?" with completely positive rating=7 and completely negative=1.

**Results:** 500 responses were received (response rate 65.8%). 445 respondents provided data for this question.

| | mean | SD |
|---|---|---|
| intellectual stimulation | 6.53 | 0.76 |
| teaching opportunity | 6.03 | 0.98 |
| transplantation | 5.94 | 1.10 |
| critical care nephrology | 5.94 | 1.04 |
| academic setting | 5.59 | 1.27 |
| chronic dialysis | 5.59 | 1.24 |
| leadership opportunity | 5.07 | 1.38 |
| research opportunity | 5.04 | 1.51 |
| job opportunities | 4.81 | 1.54 |
| prestige | 4.71 | 1.41 |
| administrative | 4.64 | 1.29 |
| geographic distribution | 4.27 | 1.50 |
| hours worked | 4.06 | 1.74 |
| work-life balance | 3.85 | 1.65 |
| compensation | 3.61 | 1.56 |

**Conclusions:** Intellectual stimulation, the opportunity to teach, and the clinical areas of transplantation and critical care nephrology are the perceived strengths of a career in PN. However, cirtical lifestye issues such as work-life balance, compensation and hours worked are potent detractors. Efforts to address these issues may help to alleviate the shortage of pediatric nephrologists.

## TH-PO1090

**First-in-Man Study with BAY 85-3934 – A New Oral Selective HIF-PH Inhibitor for the Treatment of Renal Anemia** Michael-Friedrich Boettcher,[1] Silvia Lentini,[1] Andreas Kaiser,[2] Ingo Flamme,[3] Dagmar Kubitza,[1] Georg Wensing.[1] *[1]Clinical Sciences, Bayer Pharma AG, Wuppertal, Germany; [2]Global Biostatistics, Bayer Pharma AG, Berlin, Germany; [3]GTR-CH Acute Care Research, Bayer Pharma AG, Wuppertal, Germany.*

**Background:** Renal anemia is mostly caused by insufficient erythropoietin (EPO) synthesis in CKD patients. BAY 85-3934, a selective hypoxia-inducible factor prolylhydroxylase (HIF-PH) inhibitor, has shown to increase EPO dose-dependently in animal models thereby stimulating erythropoiesis.

**Methods:** This first-in-man study was a randomized, single-blind, placebo-controlled, group-comparison dose escalation study in healthy male subjects (18-45 years). Single oral doses of 5, 12.5, 25, 37.5 and 50 mg BAY 85-3934 PEG solution were administered to 12 subjects each (9 active, 3 placebo) to investigate safety, tolerability, pharmacokinetics and pharmacodynamics (EPO, reticulocytes, hemoglobin, and packed cell volume (PCV)).

**Results:** 59 (45 active, 14 placebo) subjects participated in the study. All 5 dosages were well tolerated. 14 subjects experienced 23 treatment-emergent adverse events (AE). 14 AEs were considered drug-related and all of mild intensity. The most common AEs were throat irritation (n=5) and headache (n=3). Frequency and quality of AEs were not related to dose. BAY 85-3934 was rapidly absorbed. Mean $C_{max}$ and AUC of BAY 85-3934 increased dose-proportional. Mean terminal half-lifes ($t_{1/2}$) ranged from 4.6 to 10.4 hours. The dose-dependent EPO increase resulted in a reticulocyte rise after 37.5 and 50 mg BAY 85-3934, whereas changes in hemoglobin and PCV were not measurable. Peak EPO values of 14.8 U/L (placebo; geo. mean) and of 39.8 U/L (50 mg) were observed about 12 hours post dose and reached baseline after approx. 24 hours. Vital signs, ECG and clinical laboratory parameters were not influenced.

**Conclusions:** The HIF-PH inhibitor BAY 85-3934 was well tolerated in healthy male subjects and EPO increased EPO dose-dependently, with a time course resembling the physiological diurnal variation of EPO. Therefore, it promises to be a valuable new treatment option for patients with renal anemia.

## TH-PO1091

**Effect of Different Doses of GQ-16, a Novel Partial Peroxisome Proliferator Activated Receptor Gamma Ligand, on Metabolic Parameters in Mice with Obesity and Insulin Resistance Induced by High Fat Diet** Alexandre Martini, Michela Soares Coelho, Francisco R. Neves. *Pharmaceutical Sciences Laboratory of Molecular Pharmacology, Univ of Brasilia, Brasilia, DF, Brazil.*

**Background:** Thiazolidinediones (TZDs) were used for the treatment of type 2 diabetes (T2D), and they act by activation of peroxisome proliferator-activated receptor γ (PPARγ). Despite their clinical efficiency, their use is limited by side effects such as weight gain and edema. This has prompted the search for novel PPARγ agonists with reduced side effects. We have previously described a novel partial PPARγ ligand (QG-16) with similar anti-diabetic efficacy as ROSI, yet in the absence of weight gain, in obese and insulin-resistant mice treated with GQ-16.

**Methods:** Mice were fed a normal-fat diet (NFD, 10% kcal fat) or HFD (60% kcal fat) since weaning. At the age of 16 wk, mice were randomly assigned into six groups and received GQ-16 (5, 10 or 20mg/kg/d), ROSI (4mg/kg/d) or vehicle by gavage daily for 2 weeks (groups: NFD, HFD, HFD+ROSI, HFD+QG+5, HFD+GQ+10 or HFD+GQ+20). Body weight (BW), BW gain, food, water and energy intake, metabolic efficiency, and fasting blood glucose were measured daily or weekly. Serum aspartate (AST) and alanine (ALT) transaminase levels were evaluated in adulthood. White adipose tissue (WAT) fat pad was excised and weighed for adiposity determination.

**Results:** BW, BW gain, WAT fat mass content, blood glucose and food intake were greater in the HFD group (BW: 67.4±2.2) compared to the NFD group (BW: 51.8±2.6g). AST and ALT transaminase levels did not differ among the six groups. GQ-16 treatment reduced BW and WAT fat pads in the HFD+GQ+20 group (BW: 45.9±3.2g), whereas ROSI treatment increased it (HFD+ROSI group, 74.1±3.6g). In HFD group, weight gain was reduced in all groups that treated with GQ-16, but increased by ROSI treatment. GQ-16 decreased blood glucose similarly to ROSI in the HFD+GQ+10 (91.3±1.4) and HFD+GQ+20 groups (85±5.5), when compared to the HFD group (123±6.4mg/dL).

**Conclusions:** Our results indicate that GQ-16 decrease blood glucose and visceral adiposity, modify body weight in mice with obesity and insulin resistance, with more favorable effects on weight, as compared to ROSI.

## TH-PO1092

**Therapeutic Monitoring of Serum Mizoribine (MZ) Concentration Is Effective for Preventing Adverse Events and Successive Engraftment in Renal Transplant Recipients** Kazufumi Iwata,[1] Kentaro Ueda,[1] Keiji Aizawa,[1] Kazuki Shimoishi,[1] Sachiko Jingami,[1] Eiko Fukunaga,[1] Hideyuki Saito.[2] *[1]Depts of Pharmacy, Japanese Red Cross Kumamoto Hospital, Kumamoto, Japan; [2]Dept of Pharmacy, Kumamoto Univ Hospital, Kumamoto, Japan.*

**Background:** MZ is an oral immunosuppressive agent for the prevention of rejection in renal transplantation. MZ exhibits a low incidence of cytomegalovirus (CMV) infection and severe adverse events, such as myelosuppression, hyperuricemia and hepatotoxicity, making it useful in long-term immunosuppression therapy. MZ is excreted predominantly in the urine (>80%), and its therapeutic window is considered to be set at 1.0 - 3.0 μg/mL

of trough serum concentration. However, the effectiveness of therapeutic monitoring of serum MZ has not been evaluated in practice. This study was to investigate retrospectively the relationship between serum MZ levels and adverse events in patients with renal transplantation.

**Methods:** Serum MZ concentration was determined in 12 male and 6 female recipients with renal transplantation, in those MZ was administrated once a day at an average dosage of 145 mg. The toxicity grade was evaluated by CTCAE version 4.0 criteria.

**Results:** The mean trough serum concentration of MZ in the whole recipients was2.5±1.7μg/mL. The significant relationship was found between the trough MZ level and serum creatinine (r=0.83), eGFR (r=0.65) and hemoglobin (r=0.63), whereas there was no correlation between the MZ levels and the data of white blood cell, platelet, uric acid, AST and ALT. The average trough level (3.7 μg/mL) of MZ in the recipients with >Grade 2 anemia was significantly higher than the level (2.3 μg/mL) in those with Grade 1/0 anemia. There was no recipient that experienced rejection or severe adverse events except for anemia. Two recipients with Grade 3 anemia was recovered by reducing the dosage or discontinue of MZ considering serum MZ levels.

**Conclusions:** In conclusion, therapeutic monitoring of the serum MZ level is suggested to be effective for maintaining the adequate serum levels to prevent both adverse events and rejection, as a clinical tool for personalized therapy in renal transplantation.

## TH-PO1093

### Analysis of 424 NEPharm Database Consultations on Drug Dose Adjustment in Kidney Patients

Belal Awad, Frieder Keller. *Internal Medicine 1, Nephrology, Univ Hospital, Ulm, Germany.*

**Background:** Half of all drugs and their metabolites are excreted renally and approximately 30 % of all adverse effects have a renal cause or a renal consequence [Arch Intern Med. 2005;165:790]. However, 70 % of all drugs lack any recommendation for dose adjustment in the case of renal dysfunction [Clin Pharmacol Ther. 2000;67:196].

**Methods:** We have been building up the NEPharm database since 1978 and have extracted 12,000 PubMed citations [Clin J Am Soc Nephrol. 2010;5:314]. Based on NEPharm, we have developed tables for dose recommendations (http://www.uni-ulm.de/nephrologie/fkd.html) and regularly receive consultant queries on drug doses which were evaluated here. The dose we recommend is generally targeting the peak level, for pharmacodynamic reasons. This was compared with the dose resulting from the proportionality rule according to Dettli [Ann Oncol. 2010;21:1395] where the individual dose (D) is obtained from the normal dose, the renally eliminated fraction (fren) and the individual glomerular filtration rate (GFR).

**Results:** In the period between 2002 and 2013, there was a total of 424 queries, 286 concerned cytostatic and anti-cancer drugs (68 %), 26 concerned anti-infective agents (6 %), 47 concerned other drugs (11 %) and 65 concerned the dosage table (15 %). Overall, queries came from 76 hospitals and from 130 physicians, of which 20 hospitals (26 %) and 30 physicians (19 %) also posed repeat queries. The number of queries increased by 8.6 times, from 8 in 2002 to 77 in 2012. Of the 359 queries regarding dose recommendations, 280 queries were for patients requiring hemodialysis (78 %), 9 queries for patients with peritoneal dialysis (2.3 %), 60 queries for patients with chronic renal failure (17 %) and a mean GFR of 45 +/- 19 ml/min. Compared with 100 % of the standard dose, our dose recommendation is 92 ± 16 %; by contrast, according to Dettli, it is only 69 % ± 19 % ($p < 0.0001$).

**Conclusions:** Based on pharmacodynamic considerations, we made individual dose recommendations which were higher than the porportional dose adjustment according to Dettli. Because of the increasing and repeated queries, it can be assumed, however, that the recommendations given were helpful.

## TH-PO1094

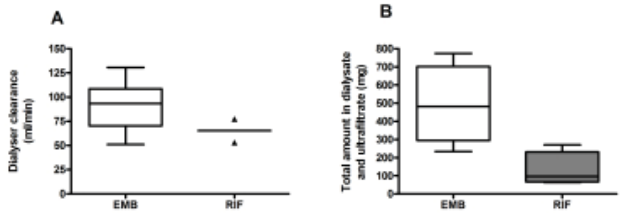
### Single and Multiple Dose Pharmacokinetics of Ethambutol and Rifampin in a Patient Undergoing Extended Daily Dialysis and ECMO

Ann-Kathrin Strunk, Julius Schmidt, Olaf Boenisch, Eva Schönenberger, Jan T. Kielstein. *Dept of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

**Background:** ARDS in tuberculosis is associated with an almost 100 % mortality, if acute kidney injury ensues. Dosing of tuberculostatic drugs is limited to outpatient dialysis. PK data for ICU patients receiving extended dialysis ED and ECMO are missing. We report single and multiple dose pharmacokinetics of ethambutol - EMB (renally cleared 80 %) and rifampin RIF under combined ED and ECMO.

**Methods:** A 42 year-old male (62 kg) i.v. drug user was admitted with progressive dyspnea and fever. Respiratory failure lead to intubation and ventilation. As adequate paO2 could not be achieved the patient was transferred to our center for ECMO support. Due to acute kidney injury extended daily dialysis (1.3 m² polysulfone dialyser, blood and dialysate flow of 230 ml/min, 10 hrs per day) was initiated. A bronchalveolar lavage yielded mycobacterium tuberculosis. Treatment with RIF (600 mg/d), EMB (1000 mg/d), pyrazinamid and moxifloxacin was started. Single and multiple dose PK were obtained. Diialyzer clearances were calculated. Amount of both drugs in the collected spent dialysate as well as pre and post the ECMO oxygenator were measured.

**Results:** EMB trough levels at 1000 mg/d were 1.5 mg/l, subtherapeutic (2-5 mg/l), promting the doubeling of the dose to 2000 mg/d. This lead to therapeutic levels of EMB (2.8-3.1 mg/l). Dialyser clearance of EMB was 91 ml/min, i.e. higher than previously reported A.




About 25% of EMB could be recovered in the spent collected Dialysate. There were no detectable effects of the ECMO membrane on the removal of both drugs.

**Conclusions:** Therapeutic drug monitoring is recommended for patients with mycobacterium tuberculosis infection undergoing renal replacement therapy as adherence to outdated dosing recommendations yields subtherapeutic plasma levels.

*Funding:* Clinical Revenue Support

## TH-PO1095

### Optimized Dosing of Cefazolin in Patients on Nocturnal Home Hemodialysis

Marisa Battistella,[1,2] Robert M. Richardson,[1,2] Christopher T. Chan.[1,2] *[1]Univ Health Network, Toronto, Canada; [2]Univ of Toronto, Toronto, Canada.*

**Background:** The uptake of Nocturnal Home Hemodialysis (NHD) use is increasing; however the dosing of antimicrobials with this modality has not been described. We hypothesize that intensive hemodialysis will augment dialytic clearance of cefazolin, consequently, the dosing of cefazoin may need to be titrated appropriately. Subtherapeutic dosing may lead to treatment failure and microbiological resistance, while high concentrations of cefazolin may cause seizures. Our objective was to determine the pharmacokinetics of cefazolin during NHD and propose an appropriate dosing regimen.

**Methods:** We conducted a prospective, single arm pharmacokinetic study of cefazolin in 15 patients on NHD. Both blood and dialysate flow rates were set at 300ml/min. Patients received an intravenous dose of 2 grams of cefazolin over 30 minutes after their NHD. The following day patients were dialyzed at the NHD unit for 6 hrs and blood samples were drawn pre HD, 60, 180, and 360 (end of HD). Patients then received a second dose of 2 grams of cefazolin after HD and blood samples were drawn at the end of the 30 minute infusion, 30 and 60 minutes post infusion; dialysate samples were also drawn pre- HD, at 3 hours and then at the end of HD. Cefazolin levels were measured by HPLC.

**Results:** The mean age of the study population was 46+7.8 years; 9 males and 6 females were enrolled in the study. The mean percentage cefazolin removal by 6hrs of HD was 70±10% and clearance of cefazolin was 1.7±0.57 L/h while on dialysis; off HD, the clearance was 0.26±0.09 L/hr. Mean t½ of cefazolin on HD was 3.8±1.33h, while off HD, mean t1/2 was 23.2±4.5h. The volume of distribution was 9.0±2.9L.

**Conclusions:** Although 70% of the drug was removed during a 6 hour NHD session, serum drug concentrations remained above the target interval for microbiological eradication. Our study validates that 1 g of cefazolin dosed at the end of each dialysis is an appropriate approach to antimicrobial dosing in NHD. Monte Carlo simulation can now be utilized to design empirical dosing regimens that ensure the greatest probability of achieving the pharmacodynamic targets associated with maximal antimicrobial response.

*Funding:* Private Foundation Support

## TH-PO1096

### Gentamicin Dosing Protocol for Empirical Therapy in Hemodialysis Patients

Lavern M. Vercaigne,[1,2] Robert E. Ariano,[3,1] Sheryl Zelenitsky.[1,3] *[1]Faculty of Pharmacy, Univ of Manitoba, Winnipeg, Canada; [2]Manitoba Renal Program, Winnipeg Regional Health Authority, Winnipeg, Canada; [3]St. Boniface General Hospital, Winnipeg, Canada.*

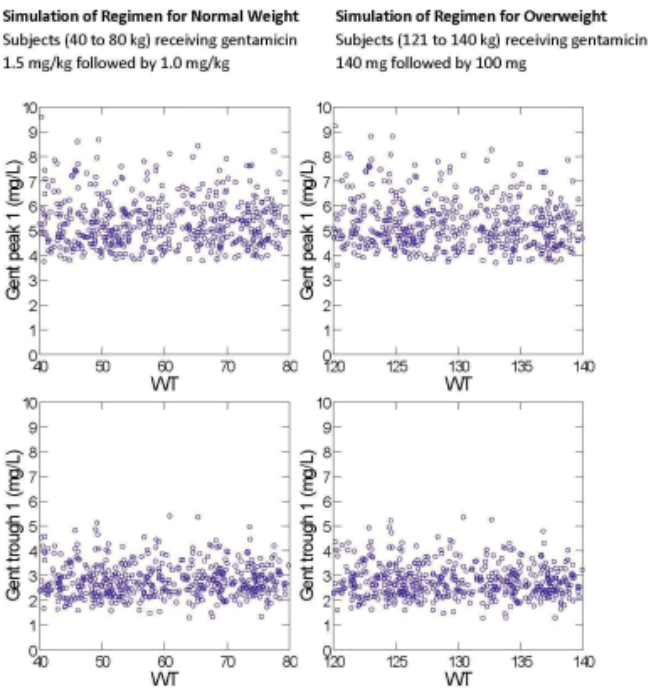
**Background:** Gentamicin is an important antibiotic for empirical treatment of Gram-negative infections in hemodialysis (HD) patients.

The goal of this study was to determine an effective and convenient empirical dosing strategy which avoids inadequate or excessive gentamicin exposure in overweight patients.

**Methods:** Monte Carlo simulation methods were used similar to those described previously *(Clin Infect Dis 2012;55:527) us*ing pharmacokinetics estimated from a population-pharmacokinetic model developed in the HD population *(Clin Pharmacokinet 2004;43:205).* The dosing regimen used by the Manitoba Renal Program (1.5 mg/kg load followed by 1.0 mg/kg post-dialysis) was tested as the baseline in subjects with normal body weights (40-80 kg). A dosing strategy to achieve similar gentamicin concentration profiles and exposures in patients with heavier body weights was determined (Table 1). Table 1.

| BODY WEIGHT (kg) | LOADING DOSE | SECOND DOSE (after next dialysis session) |
|---|---|---|
| 40-80 | 1.5 mg/kg | 1 mg/kg |
| 81-100 | 120 mg | 80 mg |
| 101-120 | 130 mg | 90 mg |
| 121-140 | 140 mg | 100 mg |

**Results:** The proposed dosing protocol provided very similar predicted peaks and troughs in patients ranging from 40-140 kg. Figure 1 shows first dose results for 500 simulated patients in the lower and upper weight categories. Figure 1.




As an index of gentamicin exposure, the $AUC_{0\text{-}48}$ (mg*h/L) was similar in the 40-80 kg group (180 ± 37 mg*h/L) compared with the 121-140 kg group (182 ± 38 mg*h/L).

**Conclusions:** The proposed dosing protocol provides similar predicted peak and trough gentamicin values in patients with body weights ranging from 40-140 kg. The protocol is suitable for empiric dosing of gentamicin over the first two HD sessions.

## TH-PO1097

**Clearance of Drugs for Multiple Myeloma Therapy during *In Vitro* High Cut-Off Hemodialysis** Detlef H. Krieter,[1] Eric Devine,[2] Christoph Wanner,[1] Markus Storr,[3] Bernd Krause,[3] Horst-Dieter Lemke.[2] *[1]Nephrology, Univ Hospital, Würzburg; [2]eXcorLab GmbH, Obernburg; [3]R&D, Gambro, Hechingen, Germany.*

**Background:** Current chemotherapy in multiple myeloma is based on bortezomib (BOR), dexamethasone (DEX) and thalidomide (THA). Purpose of the present study was to study their clearance during high cut-off (HCO) hemodialysis and to accordingly apply the results on the dialytic removal of protein-bound substances in general.

**Methods:** During *in vitro* hemodialysis with human blood (blood/dialysate/ultrafiltration flow rate 250, 500 and 5 mL/min, resp.) comparing a highly permeable HCO (Theralite™, 2.1 m²) to a high-flux dialyzer (HFHD; 2.1 m²), ultrafiltered volume was replaced by infusion of saline containing 30 g/L urea. After recirculation for equilibration, BOR was injected and arterial and venous samples were drawn after 10, 11 and 12 min to measure the plasma clearance (K) of both urea and BOR. The same procedure was performed with THA and DEX. By mathematical simulation, the influence of varying plasma albumin concentrations ($C_{HSA}$) on the protein bound drug fraction (PBF) and K was assessed.

**Results:** Plasma K of HCO and HFHD for THA, BOR, and DEX were about 40% (80±7 vs. 65±6 mL/min; P<0.05), 70% (40±8 vs. 33±4 mL/min; P<0.05), and 65% (47±11 vs. 38±7 mL/min; P<0.05), resp., lower (P<0.0001) compared to urea (125±7 vs. 122±5 mL/min). K was highest (P<0.0001) for THA. K negatively correlated with $C_{HSA}$ ($C_{HSA}$, THA, $r^2$=0.58, P<0.001; BOR, $r^2$=0.24, P<0.05; DEX, $r^2$=0.22, P<0.05). $C_{HSA}$ continually decreased (P<0.05) over time only with HCO resulting in lower calculated PBF. Compared to BOR and DEX (minimum 72 and 56%, resp.), the PBF of THA (37%) was much lower (P<0.001). Mathematical simulation based on K of urea and drug reliably estimated PBF ($r^2$=0.886, P<0.001).

**Conclusions:** Drugs for multiple myeloma therapy are significantly removed with both HCO and HFHD. This has important implications on the dosing and timing of application particularly in patients with cast nephropathy receiving extended dialysis. When knowing $K_{urea}$ of a dialyzer and the PBF of any given drug $K_{drug}$ can be reliably estimated by mathematical simulation.

*Funding:* Pharmaceutical Company Support - Gambro Dialysatoren

## TH-PO1098

**A Meta-Analysis of Extracorporeal Ultrafiltration versus Intravenous Loop Diuretics in Patients with Acute Decompensated Heart Failure (ADHF)** Fahad S. Alqahtani,[1] Khagendra Dahal,[2] Ioannis Koulouridis,[2] Maher Abulfaraj,[2] Paweena Susantitaphong,[3] Bertrand L. Jaber.[2] *[1]Robert C. Byrd Health Sciences Center, West Virginia Univ, Morgantown, WV; [2]Kidney and Dialysis Research Laboratory, Div of Nephrology, Dept of Medicine, St. Elizabeth's Medical Center, Boston, MA; [3]Extracorporeal Multiorgan Support Dialysis Center, Div of Nephrology, Dept of Medicine, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn Univ, Bangkok, Thailand.*

**Background:** The efficacy of ultrafiltration in patients with ADHF has been evaluated in several randomized controlled trials (RCTS) with conflicting results. We conducted a meta-analysis to assess the efficacy and safety of ultrafiltration compared to intravenous diuretics in hospitalized patients with ADHF.

**Methods:** We searched MEDLINE and EMBASE (through March 2013) and prior meta-analyses for RCTs comparing ultrafiltration/hemofiltration versus intravenous loop diuretic regimens. Random-effect model meta-analyses were used to examine several endpoints, including mean difference in fluid removal and weight loss, hospital length of stay and readmission, worsening of kidney function, and mortality.

**Results:** We identified 7 RCTs (578 patients). Fluid loss was significantly greater among patients receiving ultrafiltration compared to diuretics (-1998 mL; 95% confidence interval [CI] -3099, -897; P=0.0004). Weight loss was also significantly greater in among patients receiving ultrafiltration (-2.45 kg; 95% CI -4.01, -0.90; P=0.002). There was a non-significant trend toward a decrease in hospital length of stay (-2.4 days; 95% CI -5.12, 0.31; P=0.08), but there was no effect on more meaningful endpoints such as all-cause mortality (pooled risk ratio 1.05; 95% CI 0.68, 1.61; P=0.83), hospital readmission (P=0.86), and worsening of kidney function (P=0.39).

**Conclusions:** While extracorporeal ultrafiltration resulted in greater fluid and weight loss among hospitalized patients with ADHF compared to loop diuretics, other potential short- and long-term benefits of this invasive therapy could not be demonstrated.

## TH-PO1099

**Membrane Therapeutic Plasma Exchange (mTPE) Performed by Nephrologists: Early Technical and Clinical Experience** Casey N. Gashti. *Rush Univ Medical Center, Chicago, IL.*

**Background:** Separation of plasma using a highly permeable filter, referred to as mTPE, has undergone considerable investigation in Europe and Japan. Since nephrologists are well-trained in the management of extracorporeal blood purification and the advancement in technology has allowed performing mTPE using dialysis equipment, mTPE is gaining popularity in the US. We report our early single-center technical and clinical experience using hollow fiber plasma separator with dialysis equipment.

**Methods:** Data was prospectively collected on all pts receiving mTPE since program inception in January of 2013. Procedures used the Asahi Plasmaflo™ OP filter with the NxStage® System One™ machine. The mTPE was performed by dialysis technicians in cooperation with nursing staff. Albumin, Fresh Frozen Plasma (FFP) or a combination were used as replacement fluid (RF). Central venous access with targeted blood flow (QB) rate of 200 mL/min was obtained. The Filtration Fraction (FF) was monitored (RF/QB). Anticoagulation was not used for the significant majority of the procedures.

**Results:** A total of 200 mTPE procedures were performed in 41 pts over a five month period. The patients were 50±14 years old with a F:M ratio of 1.6:1. The pts had neurologic (44%), nephrologic including renal transplant (32%) and hematologic (17%) indications for therapy. RF was albumin in 57%, FFP in 38% and a combination of the two in the remaining treatments. Mean acheived QB was 179 mL/min. An average of 3746±909 mL of plasma was exchanged over 117±46 minutes (exchange rate of 35±10 mL/min). The average FF was 25%. Filter clotting was observed in 6% of treatments without the use of anticoagulation. This rate was similar (7%) in the small number of treatments that used anticoagulation (2 in 28 treatments). Paresthesia and cramping associated with FFP infusion was observed in 2% of all treatments. Hypotension was rare (1%). There were no central line-associated blood stream infections.

**Conclusions:** Nephrologists can safely and efficiently perform mTPE as an alternative to centrifuge-based therapeuic plasma exchange (cTPE). Advantages over cTPE include lack of anticoagulation and faster exchange rates, which could allow for shorter treatment time.

## TH-PO1101

**The Effect of N-Acetylcysteine on Methotrexate-Induced Nephrotoxicity: In Vitro Study in Renal Tubular Cells** Rikin Kartikbhai Shah, Ruchi Singh, Sudha P. Chennasamudram, Grant C. Weinheimer, Curtis W. Turner, Osvaldo Regueira, Tetyana L. Vasylyeva. *Dept of Pediatrics, Texas Tech Univ Health Science Center, Amarillo, TX.*

**Background:** High Dose Methotrexate (HDMTX) is effective treatment of childhood and adult cancers including acute lymphocytic leukemia, lymphoma and osteosarcoma (OS). Despite aggressive use of alkaline fluids and Leucovorin, renal dysfunction is a common side effect of the therapy. Mechanism of methotrexate(MTX) induced renal damage is unclear. MTX has been shown to increase the oxidative stress in rat kidney tissues. N-acetylcystiene(NAC) is a known antioxidant used in acetaminophen toxicity.Our objective is to evaluate the effects of N-acetylcysteine (NAC) on MTX induced toxicity in renal tubular cells and on MTX antitumor activity on the B- cell lymphoma cells.

**Methods:** Renal tubular cells (MDCK) and B-Lymphoma cells were exposed to MTX (10μM) alone and with NAC (2.5 mM). Reactive oxygen species (ROS) generation was measured by 2',7' –dichlorofluorescein diacetate (DCFDA) and flow cytometer. Percentage apoptosis was also measured using flow cytometer. Calorimetric measurement of GSH production was assayed by glutathione assay kit (Cayman Chemicals) as per manufacturer's instructions.

**Results:** Results are summarized in table below.

| Treatment | ROS generation in MDCK cells at 4 hrs | GSH generation in MDCK cells at 4 hrs | Apoptosis in MDCK cells at 24 hrs | Apoptosis in B-cell lymphoma cells at 24 hrs |
|---|---|---|---|---|
| MTX 10 μM | Increases | Increases | Increases | Increases |
| MTX 10 μM + NAC 2.5 mM | Significantly decreases then MTX alone | Significantly increases then MTX alone | Significantly decreases then MTX alone | No significant change |

**Conclusions:** NAC may prevent MTX induced renal tubular cytotoxicity by reducing ROS and increasing GSH production. Using NAC for kidney protection might not interfere with the anti-tumor activity of MTX. Further preclinical and clinical studies should be conducted to check this effect.

## TH-PO1102

### Safety, Tolerability and Efficacy of Febuxostat in Patients with Hyperuricemia and Severe Renal Impairment: A 24-Week Prospective, Open-Label, Non-Controlled Study

Yugo Shibagaki,[1] Iwao Ohno,[2] Tatsuo Hosoya,[2] Kenjiro Kimura.[1] *[1]Div of Nephrology and Hypertension, St. Marianna Univ Hospital, Kawasaki, Japan; [2]Div of Nephrology and Hypertension, Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Hyperuricemia (HU) is a very common comorbid condition in patients with chronic kidney disease (CKD). The novel xanthine oxidase inhibitor, febuxostsat, has been shown to be efficacious and well tolerated in patients with HU. However, safety, tolerability and efficacy of febuxostat are not well determined in patients with HU and severe renal impairment.

**Methods:** We conducted a 24-week prospective, non-controlled study to investigate the safety, tolerability and efficacy of febuxostat in patients with HU and moderate to severe CKD (stage 3b to 5). 71 prevalent adult outpatients (55 males with average age of 66.3 years, N=18, 33, and 19 in stage 3b, 4, and 5, respectively) at two tertially care hospitals who met the inclusion criteria [serum urate > 8 mg/dl, estimated GFR (eGFR) < 45 ml/min/1.73m$^2$, non-use of urate lowering drug in the previous month] were recruited from November 2011 to April 2012. All the participant received febuxostat. Starting dose of febuxostat was 10 mg qd, increased to 20 mg, 40mg qd in week 4, and week 8, respectively. We observed during the study period the adverse events and adherence as a primary outcome and levels of serum urate as a secondary outcome.

**Results:** Baseline serum urate level (mg/dl) were 9.9, 10.6, and 9.8, in stage 3b, 4, and 5, respectively. 14 out of 71 participants (19.7%) were dropped out of the study. 8 adverse events (constipation, chest pain, edema, gout attack, palpitation, numbness, neuropathic pain, skin rash) occurred in 6 patients (8.5%). Out of 8 adverse events, 1 occurred in CKD stage 3b (5.6%), 5 in stage 4 (9.1%), and 2 in stage 5 (10.5%). Most adverse events were mild without permanent sequelae. Average serum urate in each stage of CKD reached below the target of 6 mg/dl by 8 weeks in stage 5, and by 12 weeks in stage 3b and 4. More than 70% of participants reached this target by 24 weeks.

**Conclusions:** Treatment of hyperuricemia with febuxostat was safe, tolerable and efficacious in patients with CKD stage 3b to 5.

## TH-PO1103

### Carcoxylesterase1 (CES1) Single Nucleotide Polymorphism (SNP) Significantly Reduces Mycophenolic Acid (MPA) Associated Leucopenia in Renal Transplant Recipients (RTR)

Natalie L. Borman,[1] Anthony Marinaki,[2] Gopalakrishnan Venkat-Raman.[1] *[1]Wessex Renal and Tranplantation Unit, Portsmouth; [2]Guy's and St. Thomas, London.*

**Background:** Mycophenolic acid precursors(MPAP) are widely used in transplantation. Leucopenia is a common side effect predisposing to severe infection. CES1/2 are thought to play a role in hydrolysis of MPAP to MPA and hence SNP in the genes may play an important role in activation/ tolerability of MPA. **Aim:**Examine associations of SNPs in CES gene and clinical response to MPAP following renal transplant(RTx).

**Methods:** 287 RTR were studied in first year post RTx for primary outcome measure of leucopenia defined as reduction in white cell count to<3.0 x10$^9$/L on 2 or more consecutive blood tests or a single test if resulted in change of MPA dose. Secondary outcome measure was time to leucopenia. Exome sequencing was carried out using Illumina Human exome Beadchip. Associations were sought between SNP's in CES gene and leucopenia.

**Results:** All participants received CNI(64.1% CyA, 35.9% Tac) and MPAP(91.3% MMF 8.7% MPS), 60.9% male, 93.4% Caucasian, mean age at RTx 47 years (range 17-79). Primary outcome frequency- 25.8%. Results were analysed using Fisher's exact test for allele frequency, dominant and recessive models. Logistical regression used to correct for potential confounders. Kaplan-Meier survival plot was produced for time to event analysis and Cox regression analysis to correct for confounders.

| Gene | Dom/rec | p value | P value post logreg | Exp(B) | 95%CI | %↓odd of event |
|---|---|---|---|---|---|---|
| CES1 | Dom | 0.02 | 0.008 | 0.46 | 0.26-0.82 | 54.2 |
| CES1 | Rec | 0.015 | 0.038 | 0.34 | 0.12-0.94 | 66.5 |
| **Time to Leucopenia KM Log rank** | **Coxreg Homozy vs wild** | **% risk reduction** | **Coxreg hetrozy vs wild** | **% risk reduction** | **Coxreg wild vs non-wild** | **% risk reduction** |
| p=0.008 | p=0.008 Exp(B)0.27 (95% CI 0.1-0.7) | 73 | P=0.02 Exp(B)0.55 (95% CI 0.33-0.91) | 44.9 | p=0.02 Exp(B)0.48 (95% CI 0.3-0.76) | 52.5 |

**Conclusions:** This SNP in CES1 is significantly associated with leucopenia in MPA treated RTR. Non wild phenotype significantly reduced risk of leucopenia and time to leucopenia. This SNP would appear to have important clinical implications in this cohort.

## TH-PO1104

### Influences of Single Nucleotide Polymorphisms (SNP) on Mycophenolic Acid Tolerance and Side Effects in Renal Transplant Recipients (RTR)

Natalie L. Borman,[1] Anthony Marinaki,[2] Gopalakrishnan Venkat-Raman.[1] *[1]Wessex Renal and Transplantation Unit, Portsmouth; [2]Guy's and St Thomas, London.*

**Background:** Mycophenolic Acid precursors(MPAP) are widely used in transplantation. Adverse drug reactions are dose dependent and improve with reduction/cessation, but with increased rejection risk and poorer long-term graft survival. Genetic variability accounts for 20-90% of the individual variation in response to immunosuppression. Given these challenges, interest is growing in the use of pharmacogenetics to individualise drug regimens **Aim:**To identify and investigate associations of SNP's with clinical response to MPAP in RTR.

**Methods:** 287 RTR were studied for primary outcome measures of biopsy proven acute rejection(BPAR) leucopenia(wcc<3) anaemia(Hb<10) gastro-intestinal side effects(GISE) and infection in the first year post transplant. Exome sequencing was carried out using Illumina Human exome Beadchip. Associations were sought between SNP's and primary outcome measures. Extensive clinical data was collected.

**Results:** All participants received CNI(64.1% Cyc A, 35.9% Tac) and MPAP(91.3% MMF 8.7% MPS) 60.9% male, 93.4% Caucasian, mean age at transplant 47 years(range 17-79). Primary outcome event rates were between 10.8 % and 34.5%. Results were analysed for associations between SNP's in candidate genes and primary outcomes. Fisher's exact test was used for allele frequency, dominant and recessive model analysis. Logistical regression was used to correct for confounders. 20 statistically significant results(P<0.05) were found in 13 SNP's across 3 genes(UGT, SLCO1B1, CES1). Associations were with SNP's and GISE, BPAR, anaemia, leucopenia and MPAP reduction. Example results are shown below, full results to be presented at conference.

| Gene | SNP | Event | P value | P value(log reg) | Exp(B) | 95% CI |
|---|---|---|---|---|---|---|
| UGT1A9 | 1 | GISE | 0.027 | 0.025 | 0.54 | 0.31-0.92 |
| SLCO1B1 | 2 | BPAR | 0.005 | <0.0001 | 4.37 | 1.93-9.89 |
| CES1 | 3 | WCC<3 | 0.02 | 0.036 | 0.46 | 0.26-0.82 |

**Conclusions:** This study has shown several SNP's in genes known to be associated with MPA metabolism/excretion have a significant and important impact on clinical outcome after renal transplantation.

## TH-PO1105

### Effect of Erythropoietin on Hepatic Expression and Function of Cytochrome P450 Drug Metabolizing Enzymes in an Adenine-Fed Model of Chronic Kidney Disease

David A. Feere,[1] Thomas Velenosi,[1] Anzel Hennop,[1] Brad Urquhart.[1,2,3] *[1]Schulich School of Medicine and Dentistry, Dept of Physiology and Pharmacology, Western Univ, London, Canada; [2]Lawson Health Research Institute, London, Canada; [3]Medicine (Clinical Pharmacology/Nephrology), Western Univ, London, Canada.*

**Background:** Chronic kidney disease (CKD) causes decreased hepatic drug metabolism secondary to a decrease in expression of drug metabolizing enzymes (DMEs). As kidney function declines, it loses the ability to produce erythropoietin (EPO) leading to anemia. Consequently, CKD patients are treated with recombinant EPO and the effect of this on hepatic drug metabolism is unknown.

**Methods:** Male Wistar rats were placed on an adenine (n=16) or chow diet (n=16) for 4 weeks prior to receiving 150U/kg IP injections of EPO or vehicle saline every other day for 2 weeks. Hepatic DME expression was determined by real-time PCR. Rat liver microsomes were isolated to assess enzymatic activity of cytochrome P450 DMEs. Testosterone was used as a probe substrate for CYP activity and resulting metabolites were quantified by ultra-performance liquid chromatography with PDA detection.

**Results:** CYP3A2 expression was significantly decreased in EPO treated rats relative to controls (P<0.01). Significant reduction in CYP3A2 expression was also seen in CKD and CKD EPO treated rats relative to both control and control EPO injected animals (P<0.05). CYP2C11 expression was significantly decreased, relative to control, in CKD and CKD EPO but not in EPO control animals (P<0.001). Maximal enzyme velocity of CYP3A was significantly decreased (P<0.05) in control EPO, CKD and CKD EPO groups relative to control (67%, 49% and 39% of control, respectively). CYP2C11 mediated metabolism of testosterone was significantly decreased (P<0.001) in the CKD and CKD EPO groups relative to control and control EPO (13% and 8% vs. 100% and 89% of control, respectively).

**Conclusions:** EPO administration causes a significant decrease in CYP3A2 mRNA expression and enzyme activity. Our data suggest that EPO and CKD both decrease hepatic drug metabolism and that EPO administration to manage anemia in CKD could affect drug metabolism and clearance of CYP3A substrates.

### TH-PO1106

**Expression and Function of Hepatic Drug Metabolizing Enzymes and Transport Proteins in End-Stage Renal Disease (ESRD)** David A. Feere,[1] Thomas Velenosi,[1] Melisa Gaspar,[1] Brad Urquhart.[1,2,3] *[1]Schulich School of Medicine and Dentistry, Dept of Physiology and Pharmacology, Western Univ, London, Canada; [2]Lawson Health Research Institute, London, Canada; [3]Medicine (Clinical Pharmacology/Nephrology), Western Univ, London, Canada.*

**Background:** Pharmacokinetics of several drugs are altered in end-stage renal disease (ESRD). Non-renal drug clearance pathways such as altered expression/activity of drug metabolizing enzymes and transporters have been implicated in rodent models of kidney disease however human data is controversial. In this study we assessed expression and function of hepatic drug metabolizing enzymes and drug transport proteins in liver samples from human patients with ESRD.

**Methods:** Liver samples were collected from ten recently deceased patients with ESRD and eleven recently deceased controls. Relative gene expression of drug metabolizing enzymes, transport proteins and nuclear receptors were determined by real-time PCR.

**Results:** P-glycoprotein, OATP1B1, BCRP and CYP2E1 expression were significantly decreased ($P<0.05$) in ESRD livers compared to control. Hepatic OATP1B3 expression was significantly increased in ESRD livers relative to controls ($P<0.05$). Significant decreases in nuclear receptor expression were observed for farnesoid X receptor, hepatocyte nuclear factor 4$\alpha$ and retinoid X receptor $\alpha$ ($P<0.05$).

**Conclusions:** Our results demonstrate that select drug metabolizing enzymes and transport proteins are down-regulated in ESRD. Current studies are evaluating protein expression and enzymatic activity of hepatic microsomes from ESRD patients using specific P450 probe substrates. Our results suggest that drug transporter activity may be especially impacted in ESRD and likely explains the decrease in non-renal drug clearance observed in patients with ESRD.

### TH-PO1107

**Midazolam and Fexofenadine Pharmacokinetics Are Altered in Patients with Chronic Kidney Disease** Brad Urquhart,[1,2] Benjamin Ka Thomson,[2] Thomas D. Nolin,[3] Thomas Velenosi,[1] David A. Feere,[1] Michael J. Knauer,[1] Linda J. Asher,[2] Andrew A. House.[2] *[1]Physiology & Pharmacology, Western Univ; [2]Medicine (Clinical Pharmacology/Nephrology), Western Univ; [3]Univ of Pittsburgh.*

**Background:** To clarify the impact of chronic kidney disease (CKD) on drug disposition, we investigated the pharmacokinetics (PK) of the CYP3A4 probe midazolam (MDZ) and the transporter substrate fexofenadine (FEX).

**Methods:** Healthy controls (HC, n=8), CKD patients with an eGFR of <40 ml/min/1.73m$^2$ (CKD, n=8), hemodialysis patients (HD, n=10) and nocturnal automated peritoneal dialysis patients (PD, n=8) were recruited. Patients were given 1 mg of MDZ IV and 120 mg of FEX PO. Serial blood samples were collected over 8 hours for determination of PK parameters.

**Results:** MDZ clearance (Cl) and volume of distribution (Vd) were significantly decreased in CKD (190.9+/-90.3 mL/min, 12.5+/-7.7 L) and HD (95.7+/-68.5 mL/min, 5.0+/-6.3 L), compared to control (283.3+/-77 mL/min, 31.5+/-22.2 L), respectively (Figure 1a, $P<0.05$). In PD patients the Cl (434.3+/-169.9 mL/min) and Vd (53.8+/-18.3 L) were significantly greater than HC, CKD and HD. There was no change in MDZ half-life or terminal elimination rate. FEX Cl and and half-life were significantly different in HD, PD and CKD patients compared to the HC group (Figure 1b, $P<0.05$).

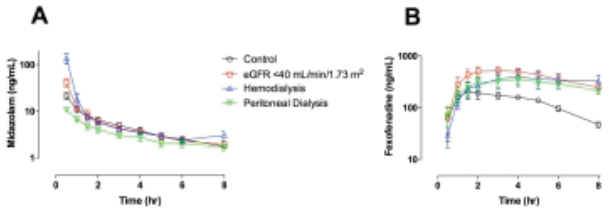



**Figure 1**

**Conclusions:** We show a significant reduction in MDZ Cl and Vd without changes in half-life suggesting decreased Vd and not hepatic intrinsic Cl alter MDZ PK in CKD. In addition, we show that the PK of MDZ is significantly different between HD and PD patients indicating that dialysis modality plays an underappreciated role in the PK of some drugs. In contrast, FEX pharmacokinetics are similarly altered in CKD, HD and PD patients. Collectively, our data indicate that dose adjustment for drugs that are not predominantly renally cleared warrants consideration in CKD.

*Funding:* Private Foundation Support

### TH-PO1108

**Hepatic Drug Metabolizing Enzymes Are Downregulated by the Uremic Toxin Indoxyl Sulfate** Thomas Velenosi,[1] David A. Feere,[1] Andrew Kai Cheong Wong,[1] Brad Urquhart.[1,2] *[1]Physiology and Pharmacology, Western Univ, London, Canada; [2]Medicine (Clinical Pharmacology/Nephrology), Western Univ, London, Canada.*

**Background:** Patients with chronic kidney disease (CKD) require many medications to treat their CKD and associated co-morbidities. The majority of prescribed medications are metabolized by the drug metabolizing enzymes CYP3A and CYP2C. CYP3A and CYP2C expression is decreased in CKD; however the factors causing this downregulation are unclear. As kidney function declines, organic waste products accumulate in the blood resulting in uremia. It has been suggested that these waste products affect drug metabolizing enzyme function and expression. The objective of this study was to screen various uremic toxins to determine which toxin(s) affect hepatic drug metabolizing enzyme downregulation in CKD.

**Methods:** Huh7 human hepatoma cells were treated with selected uremic toxins as well as a cocktail of all selected toxins and CYP3A4 mRNA expression was determined. Male Wistar rats were given 0.1% indoxyl sulfate in their drinking water for 4 weeks. Hepatic drug metabolizing enzyme function and expression was determined in these rats.

**Results:** CYP3A4 mRNA expression was decreased when cells were treated with a cocktail of selected uremic toxins ($P<0.05$). Indoxyl sulfate (300 $\mu$M) was the only uremic toxin in the cocktail to independently decrease CYP3A4 expression resulting in a 51% decrease ($P<0.05$). Cell viability was unaffected at indoxyl sulfate concentrations routinely observed in CKD patients. Indoxyl sulfate treated rats had approximately 3-fold greater plasma indoxyl sulfate level than controls. Hepatic CYP3A2 and CYP2C11 mRNA levels were decreased 49% and 34%, respectively compared to controls. Results for the rat study are represented as n = 3 and are currently ongoing.

**Conclusions:** We demonstrate for the first time that the uremic toxin, indoxyl sulfate, causes downregulation of CYP3A and CYP2C hepatic drug metabolizing enzymes. Our results show that indoxyl sulfate causes a dose dependent decrease in CYP3A4 mRNA expression in human liver cells and may also affect hepatic drug metabolizing enzymes *in vivo*.

### TH-PO1109

**Tacrolimus Dose Requirement Based on the CYP3A5 Genotype in Renal Transplant Patients** Jianghua Chen. *Zhejiang Univ.*

**Background:** To determine CYP3A5*1 and CYP3A5*3 genotype could be the predictive index of tacrolimus dose requirement.

**Methods:** We tested archival peripheral blood of 69 kidney recipients for CYP3A5 genotyping with PCR-SSP. We measured the dose-normalized blood concentrations of tacrolimus at 1 and 2 months after the renal transplantation.

**Results:** The frequency of CYP3A5*1/*1 was 6/69, CYP3A5*1/*3 was 22/69, and CYP3A5*3/*3 was 41/69. CYP3A5*1 was associated with significant lower tacrolimus dose adjusted concentration at 1 and 2 months after transplantation. Hepatic and renal function showed no significant effect on tacrolimus dose adjusted concentration at 1 and 2 months after transplantation. Gender did not show a significant impact on tacrolimus dose. Carriers of CYP3A5*1 allele had lower predicted measures for tacrolimus dose adjusted concentration and higher predicted measures for volume of distribution.

**Conclusions:** These results indicate that CYP3A5*1 carriers need higher tacrolimus dose than CYP3A5*3 homozygote to achieve the target blood concentration. CYP3A5 genotyping is a new approach for detecting tacrolimus dose requirement in kidney recipients.

*Funding:* Government Support - Non-U.S.

### TH-PO1110

**Influence of Kidney Function on Overanticoagulation Related Hemorrhage Risk in Warfarin Users** Nita A. Limdi,[1] Sarah Booth,[2] Thomas D. Nolin,[3] Marisa B. Marques,[1] Michael R. Crowley,[1] Michael Allon,[1] Timothy Mark Beasley.[1] *[1]Univ of Alabama at Birmingham; [2]Tufts Univ; [3]Univ of Pittsburgh.*

**Background:** Patients with chronic kidney disease (CKD) require lower warfarin dose, have poor anticoagulation control and a higher hemorrhage risk than those without CKD. Herein we evaluate if CKD patients have a differentially higher risk of hemorrhage associated with the episodes of over-anticoagulation (INR>4); and slower over-anticoagulation reversal rate.

**Methods:** We evaluated the influence of kidney function on over-anticoagulation and hemorrhage in 1270 warfarin users. Reversal of over-anticoagulation was evaluated in a subset of 79 patients. Warfarin and metabolite concentrations, clotting factors (II, VII, IX and X), and prothrombin induced in vitamin K antagonism (PIVKA-II) and INR levels were assessed at presentation with high INR and after reversal. The influence of the CKD-INR interaction on hemorrhage risk was assessed by Cox proportional hazards regression. The influence of CKD on anticoagulation reversal (rate of INR decline) was assessed using linear regression and path analysis. Multivariable analysis accounted for clinical (age, BMI, vitamin K dose) and genetic factors (CYP2C9, VKORC1, CYP4F2, GGCX) known to affect warfarin response.

**Results:** CKD patients experienced more frequent episodes of over-anticoagulation ($p<0.0001$) and a higher risk of hemorrhage ($p<0.0001$). The risk of hemorrhage was similar among patients with eGFR>60 and those with eGFR=45-59 ml/min/1.73 m$^2$, and was not modified by the INR at the time of the event ($p=0.79$). In contrast, the risk of hemorrhage was higher if the INR was >4 (vs. <4) for patients with eGFR 30-44 (HR 2.7; 95%CI 1.1-

6.9; p=0.03) and eGFR <30 ml/min/1.73m² (HR 5.2; 95%CI 2.6-10.7; p<0.0001) after adjustment for clinical and genetic factors. Finally, the adjusted rate of anticoagulation reversal was slower in CKD patients (p=0.018).

**Conclusions:** Impaired kidney function is associated with more frequent episodes of over-anticoagulation (INR>4) and a higher risk of hemorrhage in warfarin users. Moreover, reversal of over-anticoagulation is slower in patients with CKD, thereby prolonging their hemorrhagic risk.

*Funding:* Other NIH Support - RO1HL092173-S2; RO1HL092173, Pharmaceutical Company Support - Stago Diagnostica provided test reagents/ kits and consumables for analysis of clotting factors and Protein Induced by Vitamin K Antagonism-II (PIVKA-II)

### TH-PO1111

**Anti-Depressant Dose and Adverse Outcomes in Chronic Kidney Disease**
Varun Dev,[1] Stephanie Dixon,[1,2,3,4] Jamie L. Fleet,[2] Sonja Gandhi,[1,2,3] Amit X. Garg,[1,2,3,4] Ziv Harel,[7] Arsh Jain,[1,2,3] Salimah Z. Shariff.[1,4] *[1]Schulich School of Medicine, Western Univ, London, Canada; [2]Div of Nephrology, Western Univ, London, Canada; [3]Dept of Epidemiology and Biostatistics, Western Univ, London, Canada; [4]Institute for Evaluative Sciences, Canada; [5]Leslie Dan Faculty of Pharmacy, Univ of Toronto, Toronto, Canada; [6]Keenan Research Centre, Li Ka Shing Knowledge Institute, Toronto, Canada; [7]Div of Nephrology, Univ of Toronto, Toronto, Canada.*

**Background:** A lower dose of certain anti-depressants is recommended in patients with chronic kidney disease (CKD) to prevent drug accumulation from reduced elimination. In routine care this recommendation is often ignored.

**Methods:** We conducted a retrospective population-based cohort study to describe the 30-day risk of delirium and mortality in older adults from Ontario, Canada who initiated a higher vs. lower dose of three anti-depressants (paroxetine, mirtazapine or venlafaxine). We defined delirium using the proxy of hospitalization with an urgent head computed tomography (CT) scan. We also determined if CKD status modified the risk of delirium and examined 30-day all-cause mortality.

**Results:** We identified older adults (mean age 75 years) who started a new study anti-depressant at a higher dose (n=36,651; 31%) or a lower dose (n = 81,160; 69%) between 2002 and 2011. Initiating a higher vs. lower anti-depressant dose was not associated with a higher risk of hospitalization with head CT (incidence 1.09% vs. 1.27%; adjusted relative risk 0.90; 95% CI 0.80 to 1.02). This relative risk was not modified by the presence of CKD (interaction P value = 0.16). Initiating a higher vs. lower anti-depressant dose was associated with a lower risk of all-cause mortality (incidence 0.76% vs. 0.97%; adjusted relative risk 0.82; 95% CI 0.71 to 0.95). This relative risk was not modified by the presence of CKD (interaction P value = 0.68).

**Conclusions:** We did not observe an increase in adverse outcomes when study anti-depressants were initiated at a higher dose in patients with CKD. Contrary to our hypothesis, the 30-day risk of mortality was lower in patients initiating a higher dose.

### TH-PO1112

**Exposure-Response Relationship for Atrasentan in Patients with Diabetic Nephropathy**
Nael M. Mostafa, Chih-Wei Lin, Aksana Kaefer, Blai Coll, Dennis L. Andress, John J. Brennan, Cheri E. Klein, Walid Awni. *AbbVie, North Chicago, IL.*

**Background:** The pharmacokinetics (PK) and efficacy of atrasentan (ATR) in patients (pts) with diabetic nephropathy (DN) was studied in Phase 2 clinical trials (2 in Western and 1 in Japanese pts). The objective of this analysis was to characterize the exposure-response (ER) relationship between ATR concentration ($C_{ATR}$) and the decrease in urinary albumin to creatinine ratio (UACR).

**Methods:** A population pharmacokinetic (PPK) model for ATR was developed in NONMEM and utilized in the ER analysis using data (947 $C_{ATR}$ and 4709 UACR values) from pts with DN (N=257) who received ATR or placebo. The $C_{ATR}$ vs UACR ER model had additive terms for placebo (exponential) and drug effects (direct $E_{max}$). Likelihood ratio test was used for nested models comparison and visual predictive check (VPC) was employed for model qualification. Covariates were tested for their potential effect on ATR PK or ER.

**Results:** A two-compartment PPK model with first order absorption and elimination, fixed effects for weight on clearance (CL/F) and volume of distribution of the central compartment (V/F) and exponential inter-individual variability terms best described ATR PK. Mean estimates for CL/F and V/F were 17.5 L/hr and 1210 L, respectively. Structural parameters were well estimated with 5-48% relative standard errors (RSE) except for Ka. For the ER model, both $E_{max}$ and $EC_{50}$ for the $C_{ATR}$ vs UACR ER were well estimated (RSE 10-39%). The estimated $E_{max}$ corresponded to 46% drop in UACR and mean predicted $EC_{50}$ was 0.58 ng/mL. VPC for both PPK and ER models showed model adequacy as the majority of observations were within the 90% prediction interval of the simulated datasets. Study effect was not a significant covariate for PPK or ER models indicating that PK and ER are consistent between Western and Japanese pts.

**Conclusions:** A PPK model was developed for ATR PK and a statistically significant ER relationship between $C_{ATR}$ and UACR was characterized. ER analyses confirmed the efficacy of ATR in reducing UACR in DN pts with an estimated $E_{max}$ of 46% drop in UACR and mean predicted $EC_{50}$ of 0.58 ng/mL. ATR PK and ER relationship were similar between Western and Japanese pts.

*Funding:* Pharmaceutical Company Support - AbbVie

### TH-PO1113

**Identification of SULF-2 by Transcriptome-Wide Sequencing as a Candidate Nephrosis Factor in Childhood Nephrotic Syndrome**
Richard F. Ransom,[1,3,4,8] Milan Popovic,[1,3] Audrey Carol Papp,[6,7,8] Amy Webb,[5,8] Saraswathi Sundararajan,[2,3] Shipra Agrawal,[1,3] Juan Luis Fernandez Martinez,[9] Rainer Benndorf,[1,3,4,8] Andrzej Kloczkowski,[2,3] Wolfgang Sadee,[6,7,8] William E. Smoyer.[1,3,4,8,10] *[1]Center for Clinical and Translational Research; [2]Battelle Center for Mathematical Medicine; [3]The Research Institute at Nationwide Children's Hospital; [4]Pediatrics; [5]Biomedical Informatics; [6]Pharmacology; [7]Program in Pharmacogenomics, Spain; [8]The Ohio State Univ; [9]Dept of Mathematics, Univ of Oviedo, Spain; [10]The Midwest Pediatric Nephrology Consortium.*

**Background:** Glucocorticoids (GC) induce remission of nephrotic syndrome (NS) in most children, though ~20% present with or develop GC resistance. This study was designed to identify candidate genes (nephrosis factors) that are associated with NS by comparing gene expression in circulating leukocytes between children with steroid-sensitive (SSNS) and steroid-resistant nephrotic syndrome (SRNS).

**Methods:** cDNA libraries were produced from mRNA from leukocytes collected at presentation (S1) and after the ~8 weeks of GC therapy (S2) from children with SSNS and SRNS. Deep sequencing yielded whole transcriptome sequencing data over a wide range of transcript expression. The collected complex data sets were then analyzed by statistical, 'Extreme Learning Machine' (ELM), and 'Particle Swarm Optimization' (PSO) approaches to obtain candidate nephrosis factors.

**Results:** Transcriptome-wide deep sequencing identified numerous known GC-regulated genes. Expression analyses by the ELM and PSO algorithms identified the gene "extracellular endoglucosamine-6-sulfatase 2" (SULF2) to be associated with clinical responsiveness to GC in these children. The biological relevance of these findings to NS was then confirmed by demonstration that the plasma SULF2 enzyme activity ratio (S2/S1) was greater in children with SSNS vs. SRNS.

**Conclusions:** SULF2 is a secreted enzyme necessary for full activity of a variety of growth factors, and known to be crucial for podocyte homeostasis. Our findings suggest that SULF2 may act as a "positive" nephrosis factor, where a deficiency in circulating SULF2 activity may result in the development of NS.

*Funding:* Other NIH Support - Supported in part by NIGMS U01 GM092655

### TH-PO1114

**Transcriptome Profile from Leukocytes of Children with Steroid Sensitive and Resistant Nephrotic Syndrome**
Shipra Agrawal,[1] Amy Webb,[2] Richard F. Ransom,[1] Audrey Carol Papp,[3] Milan Popovic,[1] Rainer Benndorf,[1,4] Wolfgang Sadee,[5,6] William E. Smoyer.[1,4,7] *[1]Center for Clinical & Translational Research, The Research Institute at Nationwide Childrens Hospital; [2]Biomedical Informatics; [3]Pharmacology; [4]Pediatrics; [5]Pharmacy; [6]Program in Pharmacogenomics, The Ohio State Univ; [7]The Midwest Pediatric Nephrology Consortium.*

**Background:** RNA-sequencing is emerging as a powerful tool for transcriptome analysis and identification of disease biomarkers. The present study aims to generate the transcriptome landscape from children with steroid sensitive and resistant nephrotic syndrome (SSNS and SRNS) and to explore its potential in identifying biomarkers for SRNS. This is important because ~20% of NS cases present with or develop resistance to steroid therapy, due to largely unknown reasons.

**Methods:** RNA-sequencing was performed with SOLiD™ technology from SRNS and SSNS leukocytes collected at disease presentation and after ~8 weeks of steroid therapy. After alignment with Lifescope, transcript expression was measured with Cufflinks. The Mann-Whitney U test was used to obtain candidate genes for expression differences associated with SRNS. Alternative transcripts were identified by splice junction alignment, SNPs detected by Samtools and AEI calculated. Databases and genes for steroid sensitivity/resistance in leukocytes and of relevance to NS are being integrated into the transcriptome data.

**Results:** Of 23,080 gene transcripts (including non-coding) studied, 4018 showed >1.4 fold ratio change between SRNS and SSNS. These included *PTGS2* and *CNTNAP3* (type of Neurexin), potentially relevant to NS, and *FKBP5* and *BMPRII,* which are associated with steroid sensitivity. 1109 genes were identified with alternative transcript expression, and 1908 SNPs were detected from 1234 genes with AEI >1.5 in >2 samples. The Mann-Whitney U test identified candidates associated with nephropathy or actin polymerization.

**Conclusions:** Generation of the transcriptome landscape for SSNS and SRNS has identified numerous differences among these groups along the varied parameters (i.e. expression, AEI, splice variants) and provides the potential to identify novel biomarkers for SRNS.

*Funding:* Other NIH Support - Supported in part by NIGMS U01 GM092655

### TH-PO1115

**Intravital Multiphoton Microscopic Imaging of the Effect of Chronic Kidney Disease on Hepatocellular Transport**
Brian S. Decker, Jennifer Ryan, Ken Dunn. *Indiana Univ School of Medicine.*

**Background:** Animal studies have shown that the uremic solutes of chronic kidney disease (CKD) attenuate CYP450 enzyme and hepatocyte transporter activity. These animal studies incubated hepatocytes with uremic serum and measured the extent of mRNA transcription of the specific CYP450 enzyme or drug transport protein. Though the cultured cells provide a reproducible system, they cannot duplicate the cellular interactions

that occur in living organisms. A more powerful research tool is intravital multiphoton microscopy (IVM) which provides direct visualization of drug transport and anatomic detail in live animals in a real-time fashion. The purpose of this study was to investigate the effect of uremia on hepatic drug transport in a validated animal model of CKD using intravital multiphoton microscopy.

**Methods:** Chronic kidney disease was induced in six Sprague-Dawley rats using a two-staged, five-sixths nephrectomy. Three Sprague-Dawley rats received sham surgeries and served as controls. On day 42 post surgery, the CKD and control rats were prepared for IVM. The IVM procedure consisted of anesthetizing the animals and exteriorizing the liver. Subsequently, each animal was placed on the microscope stage and injected intravenously with sodium fluorescein (Na-F) which is a probe for the OATP1 and MRP2 transporters. Intravital multiphoton fluorescence microscopy was then conducted using a Bio-Rad MRC-1024MP laser scanning multiphoton scanner. Hepatocyte uptake of Na-F was assayed using 3D imaging of two liver lobules in time series immediately after intravenous injection of Na-F. Uptake was quantified as the increase in fluorescence in the cytosol and secretion as the increase in fluorescence of the canaliculi over time.

**Results:** Six CKD rats and 3 control rats completed the study. We found no significant difference between the CKD and control rats in the mean fluorescence of sodium fluorescein over time in the hepatocyte cytosol and canaliculi.

**Conclusions:** We successfully utilized IVM to study the effect of uremia on hepatic drug transport in an animal model of CKD. CKD does not decrease the function of OATP1 and MRP2 transporters.

*Funding:* NIDDK Support

## TH-PO1116

**Renal Tissue Engineering Based on Decellularized Matrix Scaffolds** Barbara Bonandrini,[1] Marina Figliuzzi,[1] Evangelia Papadimou,[1] Marina Morigi,[1] Fabio Sangalli,[1] Ariela Benigni,[1] Giuseppe Remuzzi,[1,3] Andrea Remuzzi.[1,2] *[1]IRCCS - Mario Negri Institute, Bergamo, Italy; [2]Univ of Bergamo, Dalmine, Italy; [3]Papa Giovanni XXIII Hospital, Bergamo, Italy.*

**Background:** Chronic kidney disease is a leading cause of mortality and morbidity. New strategies to replace function of severely damaged kidneys are needed due to limited regenerative capacity and organ donor shortages. The concept of using decellularized animal kidneys for engineering a functional organ is a potential future treatment option. The aim of the present study was to produce three-dimensional whole-kidney scaffolds by a decellularization process and to recellularize the scaffolds with mouse embryonic stem (mES) cells under physiological perfusion condition.

**Methods:** We obtained whole organ scaffolds with intact 3D geometry and vasculature by decellularization of rat and porcine kidneys, as shown by histological examination. TEM and SEM analysis confirmed the preservation of kidney architecture. Micro-CT scan established integrity, patency and connection of the vascular network. Immunohistochemistry demonstrated preservation of native expression patterns of extracellular matrix proteins, including collagen IV, laminin and fibronectin.

**Results:** We repopulated acellular rat kidneys with mES cells seeding the cells through the renal artery and perfusion of cell medium for up to 3 days. Seeded cells were completely retained into the organ and uniformly distributed in arterial tree and in glomerular capillaries, without major signs of apoptosis. mES cells lined the luminal surface of arterial vessels and express endothelial markers. Occasionally mES cells reached peritubular capillaries and tubular compartment.

**Conclusions:** Our findings indicate that rat and porcine kidneys can be successfully decellularized to produce intact renal extracellular matrix scaffolds. These scaffolds maintain their basic components and show intact vasculature. We also demonstrated the ability of the decellularized matrix to support the engrafment of mES cells in a continuos perfusion system. This is an important step toward development of a tissue engineered kidney.

## TH-PO1117

**Aqp2 Is the Missing Progenitor Cell Marker of Renal Principal and Intercalated Cells** Lihe Chen,[1] Zhou Xiao,[1,2] Qiaoling Zhou,[2] Wenzheng Zhang.[1] *[1]Univ of TX Medical School at Houston; [2]Central South Univ.*

**Background:** While the renal principal and intercalated cells regulate the electrolyte/fluid and acid-base balance, respectively, the progenitors of these cells remain mysterious, because a specific lineage-tracing marker is still not available.

**Methods:** We developed an Aqp2-lineage tracing mouse model. These mice harbor a targeted mutation of the Gt(ROSA)26Sor locus with a loxP-flanked STOP cassette preventing transcription of a CAG promoter-driven red fluorescent protein (Rosa-RFP). They also carry an Aqp2Cre transgene that permits Cre expression under the control of Aqp2 regulatory elements. A series of double immunofluorescence staining experiments were performed.

**Results:** In adult mice, RFP labeled almost all of principal cells (Aqp2$^+$and Calbindin-D28k$^+$), and vast majority of intercalated cells (CAII$^+$, V-ATPase B1B2$^+$, AE1$^+$,and Pendrin$^+$) throughout the whole kidney. However, RFP was detected in none of cells positive for proximal tubule markers (Aqp1 and Megalin) and thick ascending limb marker (uromodulin); 2) At E14.5, Aqp2$^+$ cells were occasionally observed, with no or weak RFP expression; 3) At E16.5, RFP$^+$ cells formed tubular structures. In these tubules, most of RFP$^+$ cells expressed Aqp2, others lost Aqp2 and gained B1B2 and CAII expression. No RFP$^+$ Pendrin$^+$ or RFP$^+$ AE1$^+$ were found; and 4) At day 7 after birth, most of Pendrin$^+$ and AE1$^+$ cells in the cortex were RFP$^+$.

**Conclusions:** 1) RFP faithfully recapitulates the temporal and spatial expression pattern of the endogenous Aqp2; 2) Expression of Aqp2 is detectable at E14.5; 3) Aqp2-lineage cells give rises to Aqp2$^+$ principal cells and intercalated cells (V-ATPase B1B2+ and CAII$^+$) at E16.5; 4) Most of α-IC (AE1$^+$) and β-IC (Pendrin$^+$) are derived from Aqp2-lineage cells around day 7 after birth; 5) Aqp2 is the missing progenitor cell marker of renal principal and intercalated cells; and 6) The double transgenic mice (Rosa-RFP Aqp2Cre) is a valuable model to study cell differentiation under normal and pathological conditions.

*Funding:* NIDDK Support

## TH-PO1118

**Activated Omentum Slows Progression of Chronic Kidney Disease** Ignacio Garcia-Gomez,[2,3] Nishit Pancholi,[2] Jilpa Patel,[2] Krishnamurthy P. Gudehithlu,[1] Perianna Sethupathi,[2,3] Peter D. Hart,[1,2,4] George Dunea,[1,2,3] Jose A.L. Arruda,[1,2,3] Ashok K. Singh.[1,2,3] *[1]Div of Nephrology, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL; [2]Hektoen Institute of Medicine, Chicago, IL; [3]Section of Nephrology, Univ of Illinois Medical Center at Chicago, Chicago, IL; [4]Internal Medicine, Rush Univ Medical College, Chicago, IL.*

**Background:** We examined whether attaching the omentum (a source of mesenchymal stem cells) to a subtotally nephrectomized kidney could slow the progression of chronic kidney disease.

**Methods:** Two groups of rats were studied, an experimental group which underwent 5/6 nephrectomy (removing left kidney and 2/3 of the remaining kidney), and a control group that underwent 5/6 nephrectomy as well as complete omentectomy. Polydextran gel particles were added intraperitoneally only in the experimental group in order to activate the omentum and facilitate its attachment to the injured kidney. Control omentectomized rats did not receive polydextran particles.

**Results:** After 12 weeks the experimental rats having omentum attached to the remnant kidney had 30% lower plasma creatinine and 50% lower urea nitrogen levels, 30% less glomerulosclerosis, 30% less tubulointerstitial injury, and reduced extracellular matrix and thickening of basement membranes. A fusion zone formed between the injured kidney and the omentum abounded in mesenchymal stem cells positive for sca-1, Wt-1 and CD 34, suggestive of an active and healing tissue. Kidney extracts showed a four-fold increase in VEGF levels at 1-4 weeks as well as significant increases of HGF and IGF-1 levels, increased number of proliferating cells especially at the injured edge, 2-fold increase of Wt-1 cells in the glomeruli and 5-fold increase in the gene expression for Wt-1.

**Conclusions:** These results suggest that the activated omentum attached to the injured kidney slowed the progression of chronic kidney disease. The effect appears to be brought about by the presence of stem cells and their secretory products in the vicinity of the injured kidney.

*Funding:* Private Foundation Support

## TH-PO1119

**Stem Cells in the Repair of the Damaged Mesangium: An Ultrastructural Study** Jiamin Teng, Elba Turbat-Herrera, Guillermo A. Herrera. *Pathology, LSU Health, Shreveport, LA.*

**Background:** In previous studies our group has shown the role of stem cells in repairing the damaged mesangium in a model of light chain-mediated mesangial injury. Mechanistically, the stem cells selectively home to the damaged mesangial areas and proceed to repair them.

**Methods:** Rat kidneys (n=10) were procured and maintained physiologically intact using a perfusion model previously described by us. Using this ex-vivo renal perfusion system, the renal artery was perfused with purified glomerulopathic light chains (GLC) obtained from the urine of patients with renal biopsy-proven AL-amyloidosis and light chain deposition disease. 10 um/ml of purified light chains were used to perfuse the renal artery. The perfused GLC reached the mesangium and were allowed to interact with mesangial cells and surrounding matrix for 24 hours. Subsequently, mesenchymal stem cells were perfused through the renal artery. Tissue for light microscopic, immunofluorescence, and ultrastructural evaluation was obtained at different time frames up to 96 hours post-stem cell perfusion.

**Results:** Using transmission electron microscopy, stem cells were observed in glomerular capillaries and in the neighborhood of damaged mesangial areas where there was either amyloid or increased extracellular matrix. Furthermore, the light chains engaged in phagocytosis of amyloid and altered mesangial constituents, as they cleaned the damaged areas. With time, stem cells which normally have a very small amount of cytoplasm essentially devoid of organelles, were transformed into primitive mesangial cells as they acquired cytoplasm with contractile elements.

**Conclusions:** This study provides conclusive evidence of the crucial action of stem cells in the repair of the damaged mesangium. Stem cells are attracted to the damaged areas, clear the damaged mesangium, and differentiate into mesangial cells eventually laying down new mesangial matrix.

## TH-PO1120

**Repair and Regeneration of the Diabetic Kidney** Tariq Javed, Kuntal Mohare, Himanshu Vashistha, Leonard G. Meggs. *Nephrology, Ochsner Clinic Foundation, New Orleans, LA.*

**Background:** The adult kidney retains intrinsic regenerative potential, but the identity of adult stem cells remains in question. Src homology transforming protein 1 (p66) controls mitochondrial metabolism and cellular responses to oxidative stress, aging and apoptosis. Our working hypothesis is kidney mesenchymal stem cells (MSC) genetically deficient in p66 (p66$^{-/-}$MSC) will be resistant to senescent and apoptosis phenotype(s) associated with diabetes, participating in organ maintenance and repair through self renewal, autocrine/paracrine mechanisms and regenerative properties.

**Methods:** Akita ($Ins2^{+/C96Y}$) diabetic mice were crossed with $p66^{-/-}$ (KO) mouse to generate p66 KO-Akita mice. Kidney MSC were isolated and expanded in culture. By immunocytochemistry 90% of MSC expressed stem cell antigen-1 (Sca-1 ) but did not express hematopoietic markers c-kit, CD31,CD34, CD45, CD106.

**Results:** $p66^{-/-}$ MSC show no increase in ROS metabolism, when maintained at high ambient glucose (HG), whereas wild type (WT) MSC show robust ROS signal. Growth curves for WT MSC at HG, were markedly attenuated by day 6. By contrast, $p66^{-/-}$ MSC remained in active growth phase up to 12 days. Consistent with this analysis, WT MSC show upregulation of senescent associated proteins (p21, p53 and $p^{16INK4a}$), DNA damage and apoptosis; all of which were suppressed $p66^{-/-}$MSC. We identified $Sca\text{-}1^{+}CD45^{-}lin^{-}$ MSC and $Ki67^{+}$ cells in kidneys of p66 KO Akita, but these cells were rarely encountered in WT and Akita. Senescent phenotypes associated with diabetes (glomerulosclerosis; interstitial fibrosis; tubular atrophy), were barely detectable in p66 KO Akita, with near normalization of urine albumin excretion (UAE), whereas in Akita, kidney lesions and UAE were substantially increased.

**Conclusions:** Kidney MSC genetically enhanced by p66 null mutation offer a potential strategy to repair and regenerate the diabetic kidney.

*Funding:* Private Foundation Support

## TH-PO1121

### Bone Marrow-Derived Mesenchymal Stem Cells Transplantation Repairs Glomerular Podocytes in Mouse with Puromycin Aminonucleoside-Induced Nephrosis

Jian-Xin Wan. *Nephrology, The First Affiliated Hospital of Fujian Medical Univ, Fuzhou, Fujian, China.*

**Background:** To investigate the effects of bone mesenehymal stem cells (BMSCs) on nephropathy of glomeruli in puromycin amino nuclear glucoside (PAN) nephritic mice models.

**Methods:** Isolating bonemesenehymal stem cells from the bone of mice. The model of mice was established by PAN. Then the mice were randomly divided into three groups: Control group, PAN group and BMSCs group. The 24-hour urinary protein was obtained from each group after modeling, and urinary protein excretion was determined. The mice were killed for the blood samples and kidney specimens after the tenth day of modeling. The blood sample were collected for measuring Scr and Bun. The one part of kidney specimens was taken for observing the pathological changes by HE staining and electronmicroscope and the other part is for detecting the expression of the protein and mRNA of Nephrin, CD2AP, synaptopodin, TRPC6 by the methods of real-time quantitative PCR, western-blot and immunohistochemistry.

**Results:** After tail vein injection of PAN (0.5 mg/g) into the BALB/C mice, foot process fusion phenomenon was detected by electron microscopy, and the 24hr urinary protein excretion increased significantly than control mice on day3, day7 and day10 ($P<0.05$). The fusion of foot processes in glomerulus was ameliorated after the transplantation of BMSCs, and the 24hr urinary protein decreased ($P<0.05$). The expression of nephrin, CD2AP, synaptopodin in the glomerular slit diaphragm (SD) were up-regulated than PAN nephropathy model mice ($P<0.05$) while TRPC6 was down-regulated ($P<0.05$).

**Conclusions:** Administration of PAN induced foot process fusion and proteinuria, thus successfully established minimal change nephropathy models. BMSCs transplantation could reduce foot process fusion and urine protein, and protect the podocyte damage caused by PAN.

## TH-PO1122

### Methodology for Decellularization and Cell Seeding of Pig Kidneys for Tissue Regeneration

Edward A. Ross,[1] Bradley J. Willenberg,[1] Jose Antonio Oca Cossio,[1] William L. Clapp,[1] Naohiro Terada,[1] Dale R. Abrahamson,[2] Gary W. Ellison,[1] Chris Batich.[1] *[1]Univ of Florida, Gainesville, FL; [2]Univ of Kansas, Kansas City, KS.*

**Background:** Models in our and most investigations of seeding cells into acellular whole organ scaffolds for tissue regeneration have used rodent kidneys. For human applications, however, there is a paucity of research decellularizing swine kidneys, which due to their larger size present logistic difficulties. The vasculature and collecting systems need to be kept intact so that they can be populated separately without leakage and mixing of cells of different lineages.

**Methods:** We tested modifications of rat decellularization techniques in pigs using ranges of: ages and weights; ionic and nonionic detergent concentrations; positive and negative pressures; and flow rates. Antegrade arterial and retrograde ureteral seeding was studied to optimize pressure flow parameters using red and green fluorescent microspheres (10 μm polystyrene) or cells (HeLa and mouse lung fibroblasts). Architecture preservation and seeding efficacy were studied by fluorescence and light imaging using fresh and fixed tissue samples.

**Results:** Histology showed optimal decellularization was obtained using a 3 day protocol with serial 1% Triton X100 and 0.75% SDS, water rinses and DNase. Larger organs needed further time to remove residual detergents. Fluid limited to approx 40 mm Hg and 800 ml/hr was safe and effective for pig ages 2 wk (5kg), 8 wk (15 kg) and adults (45 kg). Particulate seeding was accomplished with positive pressure of 60 mm Hg, but was improved by applying 80 mm Hg vacuum: imaging proved that they could reach (antegrade) the glomerular tufts and (retrograde) Bowman's space. Histology confirmed preservation of architecture and did not show leakage from the vascular and collecting system compartments nor mixing of those constituents with these pressure and flow parameters.

**Conclusions:** With the ultimate goal of xenotransplantation using swine scaffolds and human cell seeding, we have developed techniques for decellularization that preserve delicate architecture and allow the vascular and collecting systems to be repopulated separately, and then incubated for cell growth.

*Funding:* Private Foundation Support

## TH-PO1123

### Role of AT1 and AT2 Receptors in AFSC Mediated Podocyte Preservation

Sargis Sedrakyan, Astgik Petrosyan, Stefano Da Sacco, Roger E. De Filippo, Laura Perin. *Urology, Children's Hospital Los Angeles, Los Angeles, CA.*

**Background:** Emerging evidence strongly support the notion of podocyte depletion as a major mechanism driving glomerulosclerosis. We have previously demonstrated that stem cells derived from amniotic fluid (AFSC) significantly slow down chronic kidney disease progression in x-linked alport syndrome (XLAS) mice via preservation of podocyte numbers among other mechanisms. We hypothesize that AFSC protect podocyte injury through blockade of angiotensin II (Ang II) actions in podocytes acting through Ang II receptors types I (AT1) and II (AT2) with involvement of integrin linked kinase (ILK) and downstream elements of Wnt/b-catenin signaling pathway.

**Methods:** Primary cultures of glomerular cells, mainly podocytes were subjected to Ang II exposure for 4 hours then treated with AFSC. Cell characterization and gene expression were evaluated by Immunohistochemistry and qPCR. In vivo, $Col4a5^{-/-}$ XLAS mice were injected with AFSC. Whole kidneys, glomeruli and glomerular cells derived from injected mice were harvested and analyzed by qPCR, immunohistochemistry and western blotting.

**Results:** Ang II upregulated Glycogen synthase kinase-3 (GSk3b) and b-catenin in glomerular cells and downregulated tight junction proteins heavily present in podocytes such as nephrin and p-cadherin which was mitigated in the presence of AFSC. **Activation of this pathway involved both AT1 and AT2 receptors and blockade of one or the other resulted in a dampening of the response.** In vivo, injection of AFSC in Alport mice resulted in a significant downregulation of ILK protein levels at 10 weeks post treatment. More specifically, in glomeruli injected with AFSC lower expression of ILK was detected when compared to a non-treated sibling, indicating that AFSC regulates ILK expression in glomerular cells including the podocyte. Treated kidneys also had less presence of b-catenin at protein level.

**Conclusions:** AT1 and AT2 receptors play a significant role in mediating Ang II induced podocyte injury. Taken together our data suggest that AFSC contribute to podocyte preservation by preventing the activation of Ang II receptors, which trigger injurious mechanisms that result in podocyte depletion.

*Funding:* Other U.S. Government Support, Private Foundation Support

## TH-PO1124

### Development of Renal Regeneration with Human Renal-iPS Cells Using Epigenetic Memory

Osamu Takase, Keiichi Hishikawa. *Dept of Advanced Nephrology and Regenerative Medicine, Graduate School of Medicine, Univ of Tokyo, Tokyo, Japan.*

**Background:** We have investigated about the renal regeneration with human Renal-iPS (R- iPS) cells using epigenetic memory. Recently as epigenetic memory, it has been suggested that iPS cells derived from specific tissue is easy to differentiate into the original cells. We have already established original three R-iPS cells derived from tubular cells, mesangial cells, and proximal tubular cells. On DNA-methylation analysis in epigenetics, these R-iPS cells were different DNA-methylation from Fibroblastic-iPS (F-iPS) cells.

**Methods:** Here we investigated whether R-iPS cells were tendency to differentiate toward kidney of the source. As the differentiated-iPS cells, cells were cultured on conditional DMEM medium without basic-FGF and feeder cells. We studied the undifferentiated and differentiated makers in each iPS cells. Further to modify epigenetics, iPS cells were pre-treated with HDAC inhibitor (TSA). As application to in vivo, each iPS cells were injected into renal surface of SCID mouse.

**Results:** All R-iPS cells were not change the colony formation and increased in undifferentiation markers such as ALP staining, Oct3/4, and NANOG expression as compared with F-iPS cells. Differentiated-iPS cells lost undifferentiation character and expressed differentiation markers, especially Tubular-iPS cells expressed strongly the kidney-associated markers such as Pax-2, Sall-1, and AQP1. Further to pre-treat with TSA, Pax-2 and Sall-1 expression were further induced in Tubular-iPS cells. Interestingly in teratoma formation into the kidney, the injected Tubular-iPS cells differentiated into the renal component like glomerulus, and were increased in the expressions of AQP1, nephrin, and Epo, which are the specific kidney-associated markers.

**Conclusions:** Our results have suggested that the R-iPS cells differentiated advantageously toward kidney. We expect that R-iPS cells can treat to various CKDs and end-stage renal failure. Also the research with iPS cells using epigenetic memory may bring new strategy for various regenerative medicines.

**TH-PO1125**

**Developmental Expression of Stem Cell Factor Receptor c-kit Is Lost in Adult Renal Cortex, but Detected following Renal Injury in Glomerular Cells** Jan Sradnick,[1] Anika Luedemann,[1] Vladimir T. Todorov,[1] Michael Kotlikoff,[2] Christian Hugo,[1] Bernd Hohenstein.[1] *[1]Div of Nephrology, Internal Medicine III, Univ Hospital CGC, TU Dresden, Germany; [2]Biomedical Sciences Dept, College of Veterinary Medicine, Cornell Univ, Ithaca.*

**Background:** Stem cell factor receptor c-kit is a central marker of hematopoietic stem cells (HSC) and we previously showed that HSC are recruited to the kidney upon injury. c-kit was described as a marker of resident stem cells in the heart. c-kit expression has been studied in prenatal mice and can be detected in the renal medulla (collecting duct). Less is known about the localization and fate of c-kit expressing cells after birth and during renal injury. We now used c-kit eGFP transgenic mice to investigate expression patterns of c-kit positive cells in the kidney and after endothelial injury.

**Methods:** We studied c-kit eGFP transgenic mice during late renal development in healthy adult mice and after selective endothelial injury. Mice were sacrificed 4-6 days post-partum, and control and injured 10 week old mice were examined 1, 3 and 5 days after selective endothelial injury using the ConcanavallinA (conA)/anti-conA model. Kidneys were perfused with PFA and harvested for further assessment.

**Results:** Around day 5, a large set of cortical renal cells demonstrated c-kit expression. Many c-kit+ cells could be found in the medulla, which was described before in renal development. Tubules as well as cells of Bowman's capsule and many glomerular cells expressed c-kit, while adult mice lack c-kit+ cells in the cortex and glomeruli (except collecting duct cells). Following renal injury, c-kit positivity could be detected in tubules and glomeruli, localized in endothelial and podocytic positions. In addition, c-kit+ cells were also detected outside tubules along peritubular capillaries.

**Conclusions:** Many renal cells express c-kit during development, while adult mice virtually lack c-kit+ cortical cells. After injury, c-kit+ cells increased, which is consistent with our previous data on c-kit+ HSC using FACS analysis. Whether these c-kit+ cells immigrate from outside or consist of c-kit expressing resident renal cells needs to be clarified.

**TH-PO1126**

**Endothelial Progenitor Cells Recruited to the Kidney Do Not Originate from Bone Marrow** Jan Sradnick, Anika Luedemann, Lisa Maria Magyar, Charlotte Starke, Vladimir T. Todorov, Christian Hugo, Bernd Hohenstein. *Div of Nephrology, Internal Medicine III, Univ Hospital CGC, TU Dresden, Germany.*

**Background:** Recent literature implies that bone marrow (BM) derived endothelial progenitor cells (EPC) might participate in repair upon renal injury. Most of this data are based on *in vitro* experiments. We have previously shown that mainly endothelial outgrowth cells (EOC, ECFC) can be detected in the kidney after selective endothelial injury. To investigate the origin of such cells *in vivo*, we induced chimeric mice via BM transplantation to trace these cells.

**Methods:** BM cells were isolated from 8-10 week old ubiquitous tdTomato expressing reporter mice. Recipients were C57Bl6 mice of the same age. Endothelial injury was induced 6-8 weeks later in 20 of the 25 chimera. 5 mice were used as controls. Kidney, spleen, blood and BM were harvested on days 1, 3, 5 and 7 (n=5 per group) and analyzed using multicolor FACS-analysis and histology. EPC/ECFC (CD34, Flk-1, CD31, CD105, CD146, CD45, CD133, CD115, CD14), hematopoietic stem cells (c-kit+ Sca-1+ lin-), macrophages (F4_80+ CD11b+ CD11c- GR1-), dendritic cells (CD11c+ CD11b+ GR1-), B-cells and T-cells were measured using FACS-analysis.

**Results:** In injured kidneys the percentage of macrophages (d3: 0.5% vs ctrl: 0.1%), dendritic cells (4.4% vs 1.5%) and T-cells (CD4 0.7% vs 0.2%; CD8 0.3% vs 0.05%) increased significantly on d3 ($p<0.05$). B-cells were unchanged. ECFC were increased on d3 (1.7% vs 0.4% $p<0.001$) and HSC on d1 (0.6% vs 0.2% $p<0.05$). Compared to controls, significantly more tdTomato positive cells were recruited to injured kidneys (d1-d7: 4.3-8.3% vs 2.0%. Almost all macrophages (94.4%±6.4), dendritic cells (96.6%±1.5), neutrophils (99.7%±0.4) and B-cells (99.2%±0.7) were tdTomato positive, while only the minority of T-cells (CD4 34.5%±12.3; CD8 42.0%±11.0) and HSC (14.7%±11.0) were tdTomato positive. Basically none of the ECFC (0.3%±0.7) recruited to the kidney were tdTomato positive.

**Conclusions:** The present study demonstrates that EPC recruited to the kidney are not of BM origin, while HSC might partially emanate from BM. Further studies will have to define the niche for these stem cells in or outside the kidney.

**TH-PO1127**

**Sequential Activation of mTOR Network Components Determines Progression of Vascular Calcification** Theres Schaub,[1] Bjoern Hegner,[1] Claudia Lange,[2] Tobias B. Huber,[3] Duska Dragun.[1] *[1]Charité Universitätsmedizin Berlin; [2]Univ Medical Center Hamburg; [3]Univ Medical Center Freiburg.*

**Background:** Conversion of mesenchymal stromal cells (MSC) towards osteoblast like phenotype is instrumental for the development of uremic calcifying vasculopathy. mTOR kinase contained in mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) translates microenvironmental signals into cell differentiation responses and controls cell fate including authophagy and apoptosis. We hypothesized distinct roles of mTORC1 and mTORC2 during osteoblastic transformation of MSCs and examined temporal sequence of mTOR signaling operative in cell fate programs. In addition, we studied when and how pharmacologic mTOR targeting with Rapamycin (rapa) may interfere with these processes.

**Methods:** Human MSC were cultured in osteoblast induction medium with or without rapa. Osteoblastic differentiation, calcium deposition, induction and modulation of mTORC1 and mTORC2 signaling, cellular senescence, autophagy, and apoptosis were assessed every 3rd day.

**Results:** Induction of cellular senescence as indicated by elevated levels of p16INK4a in parallel to dormant autophagy was the earliest events accompanied by increased mTORC1 (Thr389pp70S6) and low mTORC2 (Ser473pAkt) activity. Alkaline phosphatase, a key enzyme of osteoblast differentiation, rose to a peak after 15 days paralleled by deposition of calcified extracellular matrix starting at day 6. Rapa treatment blocked mTORC1 activity while increasing mTORC2 activity resulting in inhibition of senescence and apoptosis and induction of autophagy. Rapa attenuated and delayed osteoblast differentiation with calcium deposition. Abrogation of mTORC2 by lentiviral shRNA transfer reversed protective effect of rapa by induction of apoptosis and increased extracellular calcium content.

**Conclusions:** Our findings reveal induction of mTORC1 and deregulation of mTORC2 signaling as early events closely linked to activation of different cell fate programs operative during osteoblastic MSC transformation. Modulation of mTOR signaling with rapa may evoke induction of alternative cell fate sequences and confer protection from accelerated osteoblastic transformation occurring during uremic vasculopathy.

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**TH-PO1128**

**Cellular Characterization of Nephron Regeneration after Acute Kidney Injury in the Zebrafish** Kristen K. McCampbell, Rebecca A. Wingert. *Dept. of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

**Background:** Acute kidney injury (AKI) is a devastating and often lethal condition in which kidney nephron cells are destroyed by damage from ischemia or toxin exposure. While nephron epithelial cells can regenerate after some forms of damage, there is currently a poor understanding of the cellular and molecular events that mediate nephron regeneration.

**Methods:** The zebrafish is an attractive and viable system to study the molecular pathways responsible for nephron regeneration, as its nephrons are simple, yet they maintain the biological complexity inherent to that of higher organisms including mammals. Previous studies have demonstrated that gentamicin-based chemical injury in zebrafish mimics human AKI, but detailed analysis of the cellular events associated with damage was not reported. We generated a novel toolkit of cellular and molecular protocols to perform this analysis in the zebrafish.

**Results:** Next, we extensively characterized the cellular changes resulting from gentamicin injury in the adult zebrafish using our platform of histology and immunohistochemistry techniques. This work has established the timing of renal cell death after injury, identified proliferative compartments within the kidney, and led to the assessment of gene expression changes associated with the regenerative response of proliferating cells.

**Conclusions:** Taken together, these data have provided a greater understanding of the full cycle of regenerative events. Insights from this work can be applied in future studies toward the design of chemical genetics screens in the adult and/or embryonic zebrafish to identify renal regeneration pathways and provide novel insights into the signals that orchestrate kidney epithelial regeneration.

*Funding:* Other NIH Support - NIH Director's New Innovator

**TH-PO1129**

**Amniotic Fluid Kidney Progenitors as a Tool to Study the Glomerular Filtration Barrier In Vitro** Stefano Da Sacco, Astgik Petrosyan, Sargis Sedrakyan, Kevin V. Lemley, Roger E. De Filippo, Laura Perin. *Children's Hospital Los Angeles, Los Angeles, CA.*

**Background:** The glomerular filtration barrier (GFR), essential for blood ultrafiltration, is formed by three major components: the podocyte, the glomerular basement membrane (GBM) and the fenestrated endothelial cell. While podocytes are the only cell in the glomerulus to produce collagen IV, endothelial cells play an important role in the formation of the GBM. However, the possibility to recapitulate the formation of a human GFR in vitro to study diseases and possible treatments, is still lacking. We have previously shown that human amniotic fluid progenitor cells (hAKPC) can differentiate into mature podocytes producing collagen IV alpha3-4-5 chains in vitro. In this study, we have evaluated the ability of hAKPC and glomerular endothelial cells to form in vitro a functional filtration barrier in vitro.

**Methods:** hAKPC were differentiated in VRADD media for 20 days. Immunofluorescence, qPCR, SEM and TEM were performed to confirm formation of slit diaphragm (SD) structures. Selective filtration of hAKPC seeded on transwells was tested by dextran permeability assay. To study their ability to form a functional GBM in vitro, hAKPC were co-cultured with human glomerular endothelial cells in 2D and 3D systems and analyzed by various means including PCR, Western Blotting, immunofluorescence, TEM and SEM.

**Results:** Upon differentiation, hAKPC express a full range of SD proteins and are capable of selective filtration. When co-cultured with human glomerular endothelial cells in 2D systems they depose collagen IV, laminin and other essential GBM components. When co-cultured with endothelial cells in 3D hanging drops, hAKPC are forming spheroids, express kidney and podocyte specific markers and form peculiar morphological structures.

**Conclusions:** In conclusion, hAKPC can be induced to differentiate into mature podocytes in vitro and exhibit the ability to form a functional SD capable of filtration. Moreover, they form, together with endothelial cells, a GBM-like structure. Based on these results, hAKPC might prove useful for a future cellular therapy aimed at understanding the mechanisms underlying recover of GBM in chronic kidney disease.

**TH-PO1130**

**C-kit+ Cells Restore Podocyte Function in a Model of Acute Glomerulonephritis** Erika B. Rangel,[1] Samirah Abreu Gomes,[1] Rosemeire Kanashiro-Takeuchi,[1] Phillip Ruiz,[1] Jochen Reiser,[2] Joshua M. Hare.[1] *[1]Univ of Miami; [2]Rush Univ.*

**Background:** C-kit-kidney derived cells exhibit the properties of stem cells and regenerate epithelial tubular cells following ischemia-reperfusion injury. We hypothesize that c-kit$^+$ cells have the capacity to improve podocyte function in a model of acute glomerulonephritis induced by puromycin aminonucleoside (PAN).

**Methods:** A single dose of PAN (15 mg/100g) was administered intraperitoneally into rats. After 5 days, animals were injected with saline or c-kit cells. Metabolic parameters (serum creatinine, blood urea nitrogen [BUN] and urinary ratio of albumin/creatinine), and histologic and gene expression analyses were performed at 10 and 21 days. A single dose of PAN (15 mg/100g) was administered intraperitoneally into rats. After 5 days, animals were injected with saline or c-kit$^+$ cells. Metabolic parameters (serum creatinine, blood urea nitrogen [BUN], urinary ratio of albumin/creatinine), histologic and gene expression analyses were performed at 10 and 21 days.

**Results:** C-kit treated animals exhibited lower values of BUN after 10 days compared to the saline group (92 mg/dL vs 58.2 mg/dL, P<0.001). At 21 days, kidney weight was lower in c-kit-treated animals (0.5 g/100 g body weight [BW]) compared to saline treated animals (0.54 g/100 g BW, P=0.024). Urinary ratio of albumin/creatinine was lower in c-kit treated animals (0.77±0.46 g/mg) compared to the saline group (1.15±0.77 g/mg). A few c-kit$^+$ cells were engrafted into the tubule-interstitial compartment. Expression of CD2AP and α-actin 4 (involved in podocyte cytoskeleton) and mTOR (mammalian target of rapamycin) were up-regulated after 21 days in the c-kit treated group. Of importance, Raptor/Rictor ratio was lower in c-kit treated animals compared to the saline group (1.17 vs 1.78, P = 0.029). Rictor expression was comparable between c-kit treated animals and normal kidneys, but was higher than the expression observed in the saline treated group (P<0.05). Autophagy was less efficient in the saline group compared to the c-kit treated group when the number of autophagosomes/autophagolysosomes was analyzed (4.3±0.6 vs 7.2±0.8, respectively, P=0.0088).

**Conclusions:** C-kit$^+$ stem cells preserve podocyte cytoskeleton in a model of PAN-induced glomerulonephritis apparently by up-regulating the mTOR-Rictor pathway and modulating the autophagic pathway. Taken together, these results suggest that c-kit$^+$ stem cells may have important biological and therapeutic properties. C-kit$^+$ cells preserve podocyte cytoskeleton in a model of PAN-induced glomerulonephritis apparently by up-regulating the mTOR-Rictor pathway and modulating the autophagic pathway. Taken together, these results suggest that c-kit$^+$ cells may have important therapeutic implications.

**TH-PO1131**

**Evaluation of Adipose Tissue-Derived Mesenchymal Stem Cells for Kidney Regeneration in Long-Term Dialysis Patients** Shuichiro Yamanaka,[1] Shinya Yokote,[1] Luna Izuhara,[2] Yuichi Jimmy Katsuoka,[2] Makoto Ogura,[1] Tetsuya Kawamura,[1] Takashi Yokoo.[1] *[1]Div of Kidney and Hypertension, Dept of Internal Medicine, Jikei Univ School of Medicine, Shinbashi, Minato-ku, Japan; [2]Div of Regenerative Medicine, Jikei Univ School of Medicine, Shinbashi, Minato-ku, Japan.*

**Background:** We previously demonstrated that mesenchymal stem cells (MSCs) differentiate into kidney cells with glomerular filtration function and erythropoietin production capacity. Therefore, MSCs may potentially be used for kidney regeneration. However, in patients on long-term dialysis, MSCs may have been exposed to uremic toxins, which can decrease their viability and differentiation capacity. In this study, MSCs from patients with end-stage kidney disease (ESKD) and individuals with normal renal function were compared in terms of gene expression profiles, differentiation capabilities, proliferation potential, and senescence.

**Methods:** MSCs were isolated from adipose tissues of ESKD patients (MSC-KD) and controls for phenotypic and functional comparisons. All ESKD patients were on long-term dialysis (mean duration, 58.1 ± 41.0 months). After confirming MSC surface marker expression, data regarding comprehensive gene expression profiles, proliferation potential, and senescence were obtained using real-time polymerase chain reaction array, Western blotting, population doubling time, and senescence assays. MSCs were differentiated into adipocytes, osteoblasts, and chondrocytes, following which histological analyses and/or functional assays were performed.

**Results:** A significant difference in gene and protein expression of transforming growth factor beta-3 (Tgfb3; upregulated) and p300/CBP-associated factor (PCAF; down-regulated) was observed between MSC-KD patients and controls. Meanwhile, no significant differences were observed in MSC differentiation, proliferation potential, or senescence.

**Conclusions:** Some differences in gene expression of stemnessmarkers were noted in MSC-KD patients. Therefore, long-term exposure of MSCs derived from adipose tissues to uremic toxins may render them inappropriate asa source of cells for regenerative therapy.

**TH-PO1132**

**Differentiation of Human Embryonic Stem Cells into Kidney Specific Protein Positive Cells** Shintaro Yamaguchi, Koichiro Homma, Toshiaki Monkawa, Ryuji Morizane, Sayuri Suzuki, Shizuka Fujii, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Dept of Internal Medicine, Keio Univ School of Medicine, Shinjyuku, Tokyo, Japan.*

**Background:** Kidney specific protein (KSP) is expressed in the ureteric buds, developing nephrons, mesonephric tubules, Bowman's capsules, proximal tubules, loops of Henle, distal tubules and collecting tubules. Recently, we succeeded in isolating mouse embryonic stem (ES) cells derived KSP-positive cells that could be differentiated into renal tubular lineage cells through tubular formation. Human ES cells possess many characteristics distinct from those of mouse ES cells, and the investigation of human ES cells is indispensable to clarify the kidney differentiation process in humans. In the present study, we utilized KSP as a marker to investigate the ability of human ES cells to differentiate into renal tubular lineage cells.

**Methods:** We performed monolayer multistep culturing of the human ES cell line KhES-1 on collagen substrate. As a first step, human ES cells were induced to differentiate into mesodermal lineage cells using a GSK-3β inhibitor. As a second step, further differentiation was induced under the low serum and several growth factors. Then the KSP-positive cells were sorted by flow cytometry at day 10.

**Results:** Quantitative RT-PCR analysis showed that significant amounts of intermediate mesoderm markers were expressed in human ES cells cultured with a GSK-3β inhibitor. Western blotting, immunocytochemistry, and flow cytometry showed that human ES cells cultured with a GSK-3β inhibitor were positive for KSP at day 10. On the other hand, human ES cells cultured without a GSK-3β inhibitor showed less protein expression of KSP, indicating that GSK-3β inhibition is essential to produce KSP-positive cells in this differentiation technique. Furthermore, the KSP-positive cells were able to form tubular-like structures when grown in a 3D culture in Matrigel.

**Conclusions:** We showed that human ES cells have the potential to differentiate into KSP-positive cells. Although the characteristics of KSP-positive cells should be investigated, the present technique is a promising step to obtaining renal tubular lineage cells from human ES cells.

**TH-PO1133**

**Early Endothelial Outgrowth Cells (eEOCs) in Murine Diabetic Nephropathy** Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology & Rheumatology, Univ Hospital of Göttingen, Göttingen, Niedersachsen, Germany.*

**Background:** Early Endothelial Outgrowth Cells (eEOCs), a major subpopulation of EPCs (Endothelial Progenitor Cells) have reliably been shown to protect mice from acute ischemic renal failure. These effects can significantly be improved by BMP-5. Chronic diabetic nephropathy is characterized by renal fibrosis during later stages of the disease, a process which partly results from mesenchymal transition of mature endothelial cells (EnMT). Aim of the current study was to analyze consequences of eEOC treatment of diabetic nephropathy in the context of BMP-5 actions on the cells.

**Methods:** Male C57/Bl6N mice were repeatedly injected with STZ. Animals received either untreated or BMP-5 pretreated syngeneic murine eEOCs at 2 and 7 days after the last STZ administration. Renal function, proteinuria, renal fibrosis and EnMT were analyzed 8 weeks later.

**Results:** STZ treatment induced significant and persistent increases of blood glucose levels, the animals additionally displayed renal dsyfunction and proteinuria at 8 weeks after the last STZ administration. Renal function and proteinuria significantly improved after injection of untreated and BMP-5 pretreated eEOCs. Reduction of proteinuria was more pronounced with BMP-5 treated eEOCs. Renal fibrosis was diminished by eEOCs as well. All experimental groups were characterized by increased expression of aSMA by CD31+ endothelial cells, indicating mesenchymal transition of the endothelium. BMP-5 treated eEOCs diminished aSMA expression in a statistically significant manner.

**Conclusions:** Early Endothelial Outgrowth Cells protect mice from diabetic nephropathy. In this situation BMP-5 augments antiproteinuric and antimesenchymal cell effects.

**TH-PO1134**

**Pretransplant Dialysis Duration Is a Risk Factor for Death after Kidney Transplantation also in the Current Era** Ilkka Helanterä,[1] Kaija Salmela,[2] Lauri Kyllönen,[2] Petri Koskinen,[1] Carola Gronhagen-Riska,[1,3] Patrik Finne.[1,3] *[1]Dept of Medicine, Div of Nephrology, Helsinki Univ Central Hospital, Helsinki, Finland; [2]Dept of Transplant Surgery, Helsinki Univ Central Hospital, Helsinki, Finland; [3]Finnish Registry for Kidney Diseases, Helsinki, Finland.*

**Background:** Although longer pretransplant dialysis has been associated with poor kidney transplant outcome, no data about this association exist from the current era or Europe. We studied the association of pretransplant dialysis duration with outcomes after kidney transplantation across different time periods.

**Methods:** All recipients of first kidney transplantation between 1964 and 2010 in Finland were included (N=5205) in this observational follow-up study of an inception cohort. Pre- and posttransplant data, and outcome data were collected from the Finnish Registry for Kidney Diseases. The association of the duration of pretransplant dialysis with patient and graft survival after transplantation was analyzed with multivariable Cox

regression and competing risk analyses. The association of pretransplant dialysis duration with the risk of specific causes of death (cardiovascular, infectious, or other causes) was analyzed using competing risk analysis.

**Results:** Longer duration of pretransplant dialysis was an independent risk factor for patient death after transplantation (Risk ratio 1.14 per one year increase) in the whole study population, but not for graft loss. Risk of death was increased in patients with more than 12 months of pretransplant dialysis. Longer duration of dialysis was an independent predictor of death due to cardiovascular diseases (RR 1.13 per one year increase), but not for other causes. After further adjustment in patients transplanted in 2000-2010, longer duration of dialysis remained an independent risk factor (RR 1.23 per one year increase). After stratification according to the era of transplantation, the risk of death associated with dialysis duration did not decrease over time.

**Conclusions:** Dialysis duration remains an independent predictor of patient death after kidney transplantation also in the current era, because of increased risk of death due to cardiovascular diseases.

*Funding:* Government Support - Non-U.S.

## TH-PO1135

**Associations of Pre-Transplant Narcotics Use with Death and Graft Loss after Kidney Transplantation** Krista L. Lentine,[1] David A. Axelrod,[2] Daniel C. Brennan,[3] Vikas R. Dharnidharka,[3] Mark Schnitzler.[1] *[1]Saint Louis Univ; [2]Dartmouth Univ; [3]Washington Univ.*

**Background:** Limited data are available on the implications of narcotics use for outcomes after kidney transplantation (KT).

**Methods:** We examined a novel database wherein OPTN identifiers for KT recipients were linked to pharmacy fill records from a large U.S. pharmaceutical claims clearinghouse (2005 to 2010). For this study, we selected adult KT recipients with 1yr of captured pre-transplant pharmaceutical fill records (N=29,882). Pharmacy fills for narcotics in the year prior to KT were normalized to Morphine Equivalents (ME), and expressed as average mg/kg/d exposures. Associations of ME with post-transplant graft and patient survival (adjusted hazards ratio, aHR) were quantified by multivariate Cox regression including recipient, donor and transplant clinical factors in the OPTN registry.

**Results:** 28.9% of the sample filled narcotics in the year before KT. Among narcotic users, the 25th, 50th and 75th percentiles of ME were: 0.01, 0.02, and 0.06 mg/kg/d, respectively. 3-yr survival of deceased donor (DD) recipients declined with higher quartiles of ME exposure, from 88.9% among non-users to 87.6% (Q2), 85.3% (Q3), 83.2% (Q4) (P<0.0001). After multivariate adjustment, the risks of post-transplant death and graft loss in DD recipients with the highest quartile ME exposure were 1.6-times and 1.4-times that of narcotic non-users, respectively (Table). Patterns were similar among live donor KT recipients, with the upper quartile of pre-transplant narcotic exposure predicting 2.4 times the risk of death (P<0.0001) and 1.8 times the risk of all-cause graft loss (P<0.0001) (Table).

**ADJUSTED ASSOCIATIONS of PRE-TRANSPLANT NARCOTIC USE with 3yr PATIENT & GRAFT SURVIVAL after DECEASED DONOR KT**

| | Death | Death-Censored Graft Failure | All-Cause Graft Loss |
|---|---|---|---|
| | aHR (95% CI)* | aHR (95% CI)* | aHR (95% CI)* |
| Non-User | Reference | Reference | Reference |
| Quartile 1 ME | 1.02 (0.81-1.28) | 0.72(0.44-1.17) | 0.94 (0.79-1.12) |
| Quartile 2 ME | 1.23 (1.01-1.48)* | 1.10 (0.76-1.59) | 1.21 (1.06-1.39)* |
| Quartile 3 ME | 1.34 (1.13-1.58)† | 1.47 (1.09-1.97)* | 1.26 (1.11-1.43)† |
| Quartile 4 ME | 1.56 (1.32-1.84)‡ | 1.12 (0.79-1.60) | 1.38 (1.21-1.57)‡ |

**ADJUSTED ASSOCIATIONS of PRE-TRANSPLANT NARCOTIC USE with 3yr PATIENT & GRAFT SURVIVAL after LIVE DONOR KT**

| | Death | Death-Censored Graft Failure | All-Cause Graft Loss |
|---|---|---|---|
| | aHR (95% CI)* | aHR (95% CI)* | aHR (95% CI)* |
| Non-User | Reference | Reference | Reference |
| Quartile 1 ME | 1.34 (0.88-2.02) | 0.46 (0.11-1.89) | 1.34 (1.00-1.79) |
| Quartile 2 ME | 1.07 (0.71-1.60) | 1.12(0.48-2.62) | 0.95 (0.71-1.27) |
| Quartile 3 ME | 1.54 (1.10-2.14)* | 1.10 (0.50-2.44) | 1.37 (1.07-1.75)* |
| Quartile 4 ME | 2.43 (1.79-3.32)‡ | 2.10 (1.06-4.18)* | 1.82 (1.42-2.33)‡ |

*Based on Cox's regression, adjusted for recipient, donor and transplant factors in the SRTR transplant survival equations. †P<0.05 . KT, kidney transplant; ME, morphine equivalents

**Conclusions:** While these associations may in part reflect underlying conditions that require pain management, KT recipients who use chronic narcotics should be identified as a high risk group.

## TH-PO1136

**Pre-Transplant Geriatric Nutritional Risk Index (GNRI) and Outcomes after Kidney Transplantation** Mikiko Yoshikawa, Kentaro Nakai, Yuriko Yonekura, Hideki Fujii, Shinichi Nishi. *Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Hyogo, Japan.*

**Background:** Malnutrition is a prevalent condition in chronic dialysis patients and seems to be a risk factor for mortality. The association between pre-transplant nutritional assessments andpost-transplant outcome is unclear. Geriatric nutritional risk index (GNRI: 1.489×albumin (g/dL)+41.7×body weight/ ideal body weight)is a simple nutritional assessment tool for chronic dialysis patients. We hypothesized that pre-transplantlower GNRI might be associated with the worse post-transplant renal outcomes.

**Methods:** We studied a retrospective cohort of 79patients who received first kidney transplantation at our center from April 2003 to March 2011 and surveyed their clinical courses for 12 months after transplantation. Chi-squared test,t-test (Mann-Whitney test), Pearson correlation coefficient, and multivariate logistic regression model were used to analyze the data.

**Results:** The GNRI was significantly correlated with eGFR changes at 12 months. (r= 0.281, p=0.012) Patients were divided into two groups; "High-GNRI group">99 and "Low-GNRI group" = or <99. Low-GNRI group did not predict delayed graft function and acute rejection, but were independently associated with eGFR deterioration (HR=3.00, [95% confidence interval: 1.168-7.706]), and every graft failure in Low-GNRI group developed within 5 years. There was no significant relationship betweenthe episodes of post-transplant complications such as infection and cardiovascular events.

**Conclusions:** Lower pre-transplant GNRI is an independent risk factor for the deteriorating post-transplant graft function.

## TH-PO1137

**Left Ventricular Systolic and Diastolic Dysfunction and Mitral Regurgitation Are Key Determinants of Pulmonary Hypertension in Chronic Kidney Disease Patients** Aditi Puri, Randal K. Detwiler, Abhijit V. Kshirsagar, Hubert James Ford, Alan L. Hinderliter. *Univ of North Carolina Chapel Hill.*

**Background:** Pulmonary hypertension (PH) is common in patients with chronic kidney disease (CKD), and is associated with reduced survival in patients who undergo renal transplant. The exact mechanism of PH in CKD patients is unclear. Potential contributing factors include high cardiac output due to anemia and the presence of a graft or fistula; chronic inflammation or other adverse effects of hemodialysis (HD); and a high left atrial (LA) pressure due to mitral regurgitation (MR) or left ventricular (LV) systolic or diastolic dysfunction.

**Methods:** To gain insight into mechanisms underlying PH in CKD patients, we examined clinical, laboratory, and echocardiographic characteristics of 349 patients who were referred for cardiac evaluation prior to kidney transplant at UNC. A diagnosis of PH was based on measurement of tricuspid regurgitant jet velocity (TRV) by Doppler echocardiography, and defined as TRV ≥ 2.8 m/s.

**Results:** The average age of our patients was 56±10 years. Most (75%) had end-stage renal disease (ESRD); 60% were on HD. The prevalence of PH was 17%. LV systolic dysfunction (ejection fraction ≤ 40%) (odds ratio 4.12, 95% confidence interval 1.73-9.81), moderate or severe LA dilation (OR 3.53, 95% CI 1.89-6.56), ≥ grade 2 LV diastolic dysfunction (OR 3.09, 95% CI 1.68-5.67), and moderate or severe MR (OR 9.85, 95% CI 2.78-34.9) were significant predictors of PH. Age, race, gender, body mass index, ESRD, HD, hematocrit, C-reactive protein, and LV hypertrophy were not associated with PH. Diastolic dysfunction and MR remained significant independent predictors in a multivariable model.

**Conclusions:** There was a high prevalence of PH in our cohort of patients with CKD undergoing kidney transplant evaluation. LV systolic and diastolic dysfunction, LA dilation, and MR were predictors of PH. These findings suggest that LV systolic and diastolic dysfunction and MR may play important roles in development of PH in CKD patients. PH may be associated with poor outcomes in part because it is a marker of advanced left heart disease.

*Funding:* Other NIH Support - Clinical and translational research center sponsored by NIH

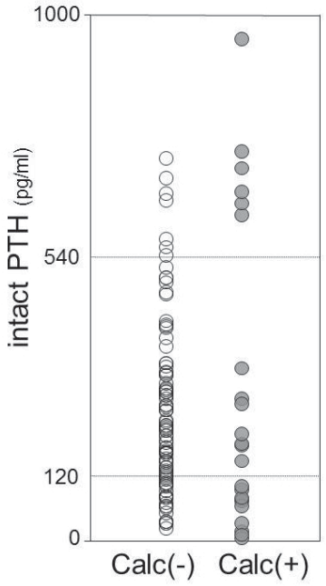
## TH-PO1138

**Preoperative Recipient Parathyroid Function Affects Intratubular Calcification in Transplanted Kidney Grafts** Junichiro J. Kazama,[1] Emiko Kono,[1] Michihiro Hosojima,[1] Suguru Yamamoto,[1] Kazuhide Saito,[2] Ichiei Narita.[1] *[1]Clinical Nephrology and Rheumatology, Niigata Univ, Niigata, Japan; [2]Urology, Niigata Univ, Niigata, Japan.*

**Background:** Intratubular calcification is occasionally found in transplanted kidney grafts. Its clinical significance remains unknown.

**Methods:** Among adult CKD patients who received kidney transplantation in Niigata University Medical and Dental Hospital between 2006 and 2012, those in whom the absence of intratubular calcification had been confirmed in the biopsied kidney graft tissue obtained immediately after the operation were retrospectively studied. The second biopsy of kidney graft was performed 4 weeks after the first biopsy.

**Results:** Of the 116 patients (M70F46, 43.4 ± 12.7yo, dialysis vintage 82.0 ± 97.3M) analyzed, intratubular calcification was found in 23 (19.8%) in the second biopsy specimen.




Those with calcification showed a significantly lower inulin clearance level than those without calcification at the time of second biopsy (50.6 ± 15.2 vs 40.4 ± 8.8 ml/min, p<0.05). Compared to the incidence of intratubular calcification among patients whose preoperative intact PTH level was within the KDIGO guideline's standard range of 120-540 pg/ml, both of those with intact PTH more than 540 pg/ml and with less than 120 pg/ml showed a significantly higher incidence of postoperative intratubular calcification (9.7 *vs* 46.2%, p<0.01 and 9.7 *vs* 32.3%, p<0.01, respectively). Hypercalcemia developed in patients with intratubular calcification with high PTH, but not in those with low PTH.

**Conclusions:** Intratubular calcification is a potential harm for a transplanted kidney graft. Both hyperactivated and suppressed preoperative parathyroid functions are associated with increased risk of the incidence of intaratubular calcification.

**TH-PO1139**

**Utility of Thrombophilia Screening in Children Awaiting Kidney Transplantation** Margret E. Bock,[1] Amy Bobrowski,[1] Rukhmi Bhat.[2] *[1]Kidney Diseases, Northwestern Univ; [2]Hematology, Northwestern Univ, Chicago, IL.*

**Background:** Vascular thrombosis is one of the more common causes of early allograft loss in children after kidney transplantation (KTx). The utility of screening for acquired & inherited thrombophilia is controversial. We posited that universal pre-transplant screening for thrombophilia was of low predictive value for post-operative outcomes.

**Methods:** We reviewed 84 children, aged 1-18 years, who underwent comprehensive thrombophilia evaluations prior to KTx & were then followed longitudinally.

**Results:** Pre-operative screening demonstrated thrombophilia in over 90% of patients (76/84). Low protein C, S & anti-thrombin 3 (AT) were found in 22.5%, 10.7% & 9.5% patients, respectively. Homocysteine was elevated in 25.8%. Plasminogen activity was abnormal in 16.5%. Antiphospholipid antibodies & lupus anticoagulant were positive in 20.8% & 62.1%, respectively. Heterozygosity for Factor V Leiden (FVL) & prothrombin gene mutations were found in 7.6% & 1.3% children, respectively. 14.5% patients had homo- & 36.8% patients had heterozygous MTHFR mutations. Patients were followed on average 4.2 yrs (SD: 1.8) after KTx. In 10/84 (11.9%) children a bleeding (5/84, 6%) or clotting (7/84, 8.3%) event occurred after KTx. Of the 5 bleeding events, 3 occurred during peri-operative anti-coagulation. A pre-KTx thromboembolic (TE) event occurred in 8/84 patients (9.5%). Post-operative events were associated with history of pre-KTx TE events (p = 0.019), FVL mutation (p = 0.002) & low AT level (p = 0.016). History of a pre-KTx TE event was associated with poorer graft survival at 3 yrs (p < 0.0001); FVL mutation was associated with poorer graft survival at 3 mos (p < 0.0001); low AT levels were associated with poorer graft survival at both 1 & 3 yrs (p < 0.0001 for both). Two of 84 grafts were lost to TE events.

**Conclusions:** Analysis of long-term follow-up data demonstrates that the value of universal comprehensive thrombophilia evaluation before KTx is low. Focused universal investigation, however, in addition to more detailed testing in those with a previous TE event, may be advisable, as these children may benefit from tailored peri-operative anti-coagluation.

*Funding:* Other NIH Support - Margret Bock is supported in part by Grant Number K12 HD055884 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development

**TH-PO1140**

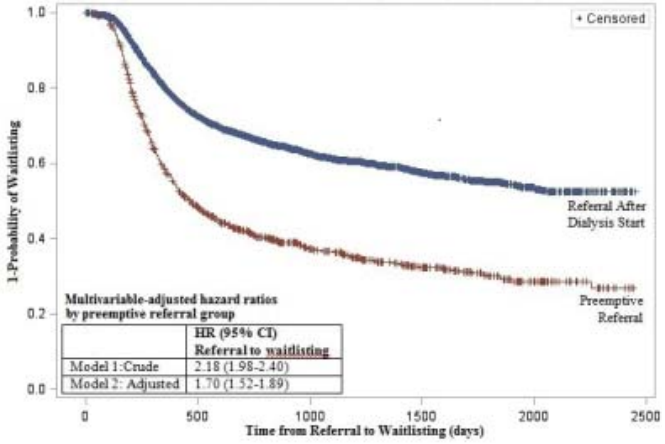
**Impact of Preemptive Referral for Kidney Transplant Evaluation on Waitlisting and Transplant** Mohua Basu, Brendan P. Lovasik, Justin D. Schrager, Stephen O. Pastan, Rachel E. Patzer. *Emory Univ School of Medicine.*

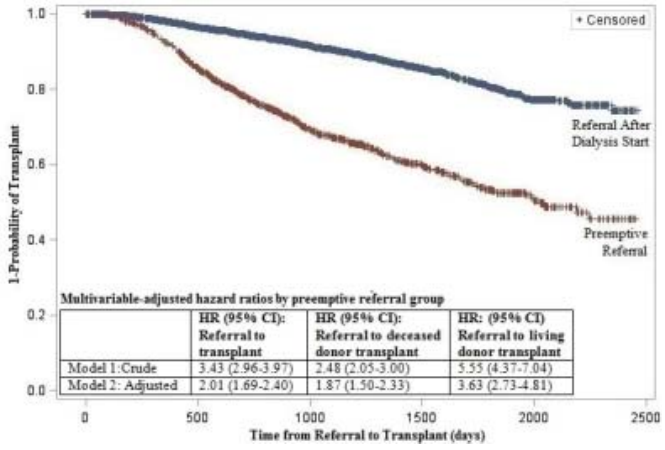
**Background:** Little is known about how preemptive referral, or referral for kidney transplant (KTx) evaluation prior to initiating dialysis, is associated with access to waitlisting and KTx in ESRD (end-stage renal disease) patients.

**Methods:** KTx center-level data for 4,914 patients with no previous transplant referred to a Southeastern transplant center for KTx evaluation from 2005-2010 were linked with United States Renal Data System baseline and follow-up data through September 2011. Patient addresses were geocoded and linked with 2009 Census poverty data. Cox models were used to examine the association between preemptive referral and access to 1) waitlisting and 2) KTx.

**Results:** Of 4,914 referred patients, 934 (19.0%) were preemptively referred. Among referred patients, 1,977 (40.2%) were waitlisted (62.8% of preemptively referred patients and 34.9% of patients referred after dialysis start), and 725 (14.8%) patients received KTx (35.0% preemptively referred and 10.0% referred after dialysis start). In analyses adjusted for demographic factors (age, sex, race, distance to KTx center), clinical factors (ESRD etiology, body mass index, tobacco use, cardiovascular disease, cancer, pre-ESRD nephrology care, erythropoietin use, hemoglobin and albumin levels), and SES factors (insurance and poverty), preemptively referred patients had a significantly higher rate of waitlisting (HR=1.70; 95% CI: 1.52-1.89) and KTx (HR=2.01; CI=1.69-2.40) compared to patients referred after initiating dialysis (Figure). Preemptive referral was associated with improved access to both deceased (HR=1.87; 95% CI: 1.50-2.33) and living donor KTx (HR=3.63; 95% CI: 2.73-4.81).

**Figure: Kaplan-Meier Curves for Access to Waitlisting and Transplant by Preemptive Referral Group**




**Conclusions:** Preemptive referral for KTx evaluation is associated with improved access to waitlisting and both deceased and living donor KTx. Interventions to increase early referral may improve access to KTx.

## TH-PO1141

**Knowledge and Attitudes toward Kidney Transplantation among Non-Physician Dialysis Providers: An Opportunity to Improve Kidney Transplantation Education and Access** Fidel Barrantes,[1] Elizabeth Evans,[1] Akeela Oaris,[2] Milagros D. Samaniego-Picota,[3] Fu L. Luan.[3] *[1]Renal Medicine Associates, Albuquerque, NM; [2]Internal Medicine, Presbyterian Health System, Albuquerque, NM; [3]Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

**Background:** Non-physician dialysis staff (NPDS) are the primary sources of education for patients with end stage renal disease (ESRD). However, many feel uncomfortable answering questions related to kidney transplantation (KT) and thus hinder early referral.

**Methods:** This survey was designed to evaluate NPDS knowledge and attitudes toward KT referrals. Survey was validated before implementation.

**Results:** We surveyed 259 providers nationwide. Amongst, 75% worked for for-profit dialysis organizations, 19.3% locally owned , and 5% were university affiliated ; 51% dialysis nurses, 18.5% nurse practitioners, and 11.4% technicians. The average dialysis working years was 12.5 . Only 58.1% stated that KT was the best renal replacement therapy option for ESRD patients , whereas 15.4% opted daily home hemodialysis, 11.8% peritoneal dialysis, 8.1% in-center nocturnal (3/week), and 8.1% intermittent hemodialysis. One third of participants thought kidneys from young living donors last 5-10 years. About 50% thought that kidneys from young deceased donors last 5-10 years and those from older donors <5 years.

NPDS identified two main reasons for non-referral: “patient too sick for transplant” or “non adherent to dialysis”. They identified that older patients, multiple co-morbidities and non-adherence to hemodialysis affect transplant outcomes; and reported that infection , non-compliance, failed cardiac clearance, morbid obesity, >3 co-morbidities and cancer as reasons transplant centers decline candidates. Only 40% of NPDS encouraged their patients to pursue evaluation once in the last 6 months.

Survey showed one third of NPDS never received KT education or had it >10 years ago.

**Conclusions:** NPDS displayed inadequate knowledge about KT, and identified factors that are associated with lack of referrals. Education in KT for NPDS may have the potential to avoid misinformation to patients with ESRD and to improve referral access to transplantation.

## TH-PO1142

**Achieving Preemptive Living Donor Kidney Transplantation in the United States for End Stage Renal Disease** Kevin C. Mange,[1] Francis L. Weng.[2] *[1]Barnabas Health, West Orange, NJ; [2]Saint Barnabas Medical Center, West Orange, NJ.*

**Background:** Preemptive living donor kidney transplantation (PLDKT) has been associated with survival advantages. However, the likelihood of preemptively wait-listed patients with ESRD in the US to undergo successfully PLDKT instead of initiating dialysis prior to LDKT is unknown.

**Methods:** To determine the likelihood of preemptively listed patients to undergo PLDKT, we examined UNOS data from 23,061 patients ≥18 years old who received a LDKT between 2000-12 where the transplant occurred either 1) preemptively or 2) within 90 days of starting dialysis yet the patient was preemptively listed. Estimates of income were obtained from 2011 American Community Survey by US Census Bureau. Univariate descriptive analyses were conducted to examine potential associations for logistic regression modeling.

**Results:** Potential PLDKTs represented 8.4% of all living donor transplants in the US. During 2000-12, PLDKT has become more frequent, and there was a 3-fold increased frequency of being listed before PLDKT (P<0.0001). Several factors have unadjusted associations. However, even though listed preemptively, Black or Hispanic race was more likely to get a non-PLDKT vs Caucasians (OR 1.40;OR 1.53, P<0.0001 for both).

| | Listed Preemp & PLDKT (N=21,123) | Listed Preemp & Non-PLDKT by <90 Days (N=1,938) | |
|---|---|---|---|
| Mean (SD) Days on Dialysis Pre-Transplant | ---- | 44.3 (25) | |
| Mean Recipient Age (SD) | 48.3 (13.6) | 47.1 (13.1) | <0.0001 |
| Female (%) | 41.0 | 38.9 | .08 |
| Recipient Ethnicity (%)<br>Caucasian<br>Black<br>Hispanic<br>Asian | <br>76.6<br>9.6<br>9.1<br>3.7 | <br>72.2<br>11.6<br>11.5<br>3.7 | <0.0001 |
| Cause of ESRD (%)<br>Diabetes<br>Hypertension<br>Glomerulonephritis<br>Chronic Interstitial Nephritis | <br>19.0<br>14.1<br>28.8<br>4.5 | <br>20.6<br>15.0<br>32.3<br>3.3 | <0.0001 |
| Primary Payer at Transplant<br>Private<br>Public<br>Other | <br>76.6<br>22.1<br>1.3 | <br>75.5<br>24.2<br>0.35 | <0.0001 |
| Median Household Income (2011 $) | 57,426 | 57,479 | 0.748 |
| Donor Relationship (%)<br>Parent<br>Child<br>Full Sibling<br>Spouse<br>Other | <br>6.9<br>18.1<br>26.9<br>16.0<br>32.1 | <br>7.1<br>15.1<br>22.6<br>15.8<br>39.4 | <0.0001 |

**Conclusions:** Based on US national data, PLDKT has become a more frequent treatment for ESRD. Even though listed preemptively, there are barriers to ultimately undergo PLDKT and realize a potential increase in allograft survival, especially among Blacks and Hispanics.

## TH-PO1143

**The Effect of Donor Service Area (DSA)-Specific Median Waiting Time on Waitlist Outcomes** Alireza Mehrnia, Mandana Kamgar, Marcelo Santos Sampaio, Edmund Huang, Suphamai Bunnapradist. *Medicine - Nephrology, UCLA - David Geffen School of Medicine, Los Angeles, CA.*

**Background:** Waiting time for deceased donor kidney transplant is increasing, but waiting time varies considerably in each DSA. We studied the impact of DSA waiting time on waitlist outcomes.

**Methods:** Primary adult kidney transplant candidates waitlisted from 2003 to 2012 were included. The cumulative incidence (CI) of a) receiving a transplant, b) being delisted, and c) dying was calculated using competing risks methods and is presented below. 201,937 candidates were included and followed for 5. DSA’s were stratified into quartiles by median waiting time calculated by the Kaplan-Meier method (Q1-Q4). Data was obtained from the OPTN/UNOS as of 31 December 2012.

**Results:** By 5 years, the CI of receiving a transplant was higher in Q1 (68.4%) compared to Q4 (35.1%). The CI of death was 10.8% in Q1 and 18.7% in Q4. The CI of delisting was mostly similar across quartiles. The CI of death was higher than delisting in the highest quartile (Q4).

**Conclusions:** Among 58 DSA’s, median waiting time varied considerably from 12 to 102 months. Death rate was higher among patients who were listed in centers with longer median waiting time, and was almost double in the quartile with the highest median waiting time (Q4) compared to the lowest (Q1).

| Quartiles by median DSA waiting time (min-max) | Transplanted (95% Confidence Interval) | Death (95% Confidence Interval) | Delisted (95% Confidence Interval) |
|---|---|---|---|
| **Q1** (12-31) | 68.4 (67.9-68.9) | 10.8 (10.5-11.1) | 13.5 (13.1-13.9) |
| **Q2** (32-43) | 59.2 (58.7-59.8) | 12.7 (12.3-13.0) | 13.8 (13.5-14.2) |
| **Q3** (44-50) | 50.7 (50.1-51.2) | 15.0 (14.6-15.4) | 14.8 (14.4-15.2) |
| **Q4** (51-102) | 35.1 (34.6-35.5) | 18.7 (18.3-19.1) | 13.8 (13.7-14.2) |

## TH-PO1144

**Access to Transplantation in the Elderly: Defining the Unmet Need** Elizabeth Hendren,[1] Elke Schaeffner,[2] Jagbir Gill,[1] John S. Gill.[1] *[1]Univ of British Columbia; [2]Charité Univ Medicine.*

**Background:** To what extent the low use of transplantation in the elderly is justified because of a high burden of comorbid disease and limited life expectancy is unclear.

The objectives of this USRDS analysis were:

1)To determine the proportion of incident ESRD patients ≥ 65 years that are transplant eligible (TE) and examine their access to transplantation (defined as wait-listing or transplantation).

2) To determine if the Medicare and Medicaid Services (CMS) requirement to report discussion of transplant treatment options at dialysis initiation (implemented in May 2005) was associated with an increase in access to transplantation.

**Methods:** We defined transplant eligibility using the following conservative criteria: 1) ESRD Survival ≥ 5 years, 2) no relative contraindications to transplantation, 3) no comorbid conditions and 4) no inability to ambulate or transfer.

**Results:** Overall 8.5% of elderly incident ESRD patients met our TE criteria, and 15.9% of these patients were ever wait-listed or transplanted. Among 65-9 year olds, 14% were transplant eligible and 30% of these patients gained access to transplantation. Among patients ≥ 70 years, 7% were TE but only 9% gained access to transplantation.

| Age (years) | All incident patients in USRDS 2000-2 | Survived 5 years | No contraindications or comorbid conditions | Access to transplantation (wait-listed or transplanted) |
|---|---|---|---|---|
| All ≥ 65 | 131,926 | 25,531 | 11,108 (8.5%) | 1772 (15.9%) |
| 65-69 | 25,107 | 8004 | 3627 (14%) | 1069 (30%) |
| 70-75 | 41,047 | 9561 | 4211 (10%) | 597 (14%) |
| 76-80 | 32,550 | 5129 | 2134 (7%) | 96 (4.1%) |
| ≥80 | 33,222 | 2837 | 1136 (3%) | 10 (1%) |

In a separate analysis, the proportion of TE elderly patients who gained access to transplantation within one year of first ESRD treatment increased by 100% in the two years after the implementation of the CMS requirement.

**Conclusions:** We conclude that 85% of TE elderly ESRD patients never gain access to transplantation. However, simple interventions such as required reporting of transplant discussions at dialysis initiation may increase access to transplantation among elderly patients.

*Funding:* Clinical Revenue Support

## TH-PO1145

**Incorporating Uncertainties and Contingencies in a Paired Donation Program** Mathieu Bray,[1] Wen Wang,[1] Peter X.K. Song,[1] Alan B. Leichtman,[1] Michael A. Rees,[2] John Kalbfleisch.[1] *[1]Univ of Michigan, Ann Arbor, MI; [2]Univ of Toledo, Toledo, OH.*

**Background:** A kidney paired donation (KPD) pool consists of transplant candidates and their incompatible donors along with altruistic donors (ADs). In a match run, exchanges are arranged among pairs in the pool and chains created from ADs. A problem of importance is how best to arrange exchanges and chains to optimize the number of transplants performed. This study examines allocation schemes in a realistic model of a KPD system, incorporating into the scheme probabilities (PR) that chosen transplants may not be completed, as well as allowing for contingency plans (CON) when the optimal solution fails.

**Methods:** The simulations use data from the Alliance for Paired Donation and the University of Michigan KPD program. Simulations compared optimization schemes in 200

replications over 8 months, with 30 pairs and 1 AD added each month. A virtual crossmatch was used to identify potential donations; PRs were specified from the literature. At each match run, the optimal set of cycles and chains was determined for each scheme, with and without PR and with and without CON, and the number of realized transplants was then obtained. Cycles of size 2 and 3 were considered and simulations were done for chains of length 2 to 6. Two schemes for contingencies (CON1 and CON2) were used. Calculations for longer chains were too complex with present algorithms for the CON options.

**Results:** Table 1 compares the allocation schemes in terms of ratios of average number of transplants to a standard where chains are of length 2, without PRs or CONs. Approaches that aim to maximize the number of transplants without allowing for PRs are suboptimal and deliver diminishing returns for longer chain lengths. On average, 17-18% more transplants are realized based on chains of size 3 or 4 that account for PRs and CONs.

| Chain Length | No PR No CON | PR No CON | PR CON1 | PR CON2 |
|---|---|---|---|---|
| 3 | 1.06 | 1.11 | 1.17 | 1.18 |
| 4 | 1.08 | 1.13 | 1.17 | - |
| 5 | 1.07 | 1.11 | - | - |
| 6 | 1.03 | 1.11 | - | - |

**Conclusions:** It is advantageous to consider clinically relevant uncertainties and contingency plans in allocation schemes in KPD.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## TH-PO1146

**Feasibility and Benefit of Kidney Paired Donation between Pools of Ethnic Difference** Kyung Sun Park, Dae Joong Kim. *Div of Nephrology, Internal Medicine, Samsung Medical Center, Sungkyunkwan Univ School of Medicine.*

**Background:** Kidney paired donation (KPD) is an established method to overcome ABO incompatibility and positive cross-match in living kidney transplantation. We hypothesized that the opportunities and quality of matching in KPD may be enhanced by using KPD pools of ethnic difference.

**Methods:** To identify the feasibility and effectiveness of international KPD, we analyzed ABO blood type and HLA-A,B, DR data of 2 donor-recipient pools (DRP) of USA (1 from Allied Paired Donation (APD), another from Methodist San Antonio (SA) and 1 DRP of Korea (KR). The calculated panel reactive antigen (cPRA) was defined as the proportion of donors who have same HLA as the recipient's anti-HLA antibodies.

**Results:** In APD, SA and KR DRPs, 627, 298 and 369 donors, and 515, 237, 369 recipients were registered respectively. Blood type of donors (P<0.001) and of recipients (P<0.001) were significantly different between USA and KR DRPs. The distribution of HLA typing of donors (P<0.001 for A, P<0.001 for B and P<0.001 for DR) and of recipients (P<0.001 for A, P<0.001 for B and P<0.001 for DR) were also different significantly. Mean cPRA of APD recipients (75.3% with APD donors) decreased significantly when calculated with KR donors (69.4%, P<0.001) in comparison with SA donors (75.8%). In SA recipients (80.7% with SA donors), cPRA decrease was more evident with KR donors (75.0%, P<0.001) than with APD donors (80.2%).

**Conclusions:** Our data showed that the distributions of blood type and HLA type were different between USA and KR DRPs and cPRA of US patients decreased significantly in pools of different country. Considering ethnic differences, the feasibility and efficacy of KPD could be improved by using international kidney exchange network.

## TH-PO1147

**Impact of Human Leukocyte Antigen-DR Mismatches on Poor Outcome of Living Unrelated Kidney Transplantation in Comparison with Living Related Kidney Transplantation** Kyung Sun Park, Hye Ryoun Jang, Jung Eun Lee, Wooseong Huh, Yoon-Goo Kim, Ha Young Oh, Dae Joong Kim. *Div of Nephrology, Dept of Internal Medicine, Samsung Medical Center Sungkyunkwan Univ School of Medicine, Seoul, Republic of Korea.*

**Background:** Living-unrelated donors (LURD) have been widely used for kidney transplantation (KT). We compared clinical outcomes of KT from LURD and from concurrent living-related donors (LRD), and identified risk factors associated with acute rejection, graft and patient survival in living KT.

**Methods:** We retrospectively reviewed 779 patients who underwent living donor KT (264 from LURD, 515 from LRD) at Samsung Medical Center from January 2000 to December 2012.

**Results:** Median follow-up was 67 months. Mean age (43.2 vs. 38.4 years, P<0.001), mean number of total HLA mismatches (4.1 vs. 2.5, P<0.001) and HLA-DR mismatches (1.2 vs. 0.8, P<0.001) were higher, and mean estimated glomerular filtration rate (eGFR) was lower (87.6 vs. 90.9 ml/min, P=0.007) in LURD. Acute rejection-free survival (64.9% vs. 72.7% at 5 years, P=0.018) and graft survival (92.9% vs. 96.5% at 5 years, P=0.025) were lower for LURD than LRD whereas patient survival rate was comparable (97.9% vs. 98.7% at 5 years, P=0.957). Cox-regression analysis showed that HLA-DR mismatches (OR 1.77, 95% CI 1.20-2.62, P=0.004 for 1 mismatches; OR 2.63, 95% CI 1.64-4.20, P<0.001 for 2 mismatches), donor eGFR (OR 1.33, 95% CI 1.02-1.73, P=0.033) and delayed graft function (OR 2.16, P=0.033) were significant risk factors for acute rejection. For graft survival, delayed graft function (OR 4.87, P=0.009), acute rejection (OR 2.84, P<0.001) and donor eGFR (OR 1.87, 95% CI 1.04-3.35, P=0.035) were significant. We also identified significant impact of diabetes (OR 3.61, P=0.012) and delayed graft function (OR 5.60, P=0.023) for patient survival. However, genetic relation was not significant for acute rejection (P=0.889), graft survival (P=0.205) and patient survival (P=0.745).

**Conclusions:** Our data suggest that HLA-DR mismatches and donor eGFR are independent risk factors for clinical outcomes of living KT. In living KT, these factors should be considered to prevent acute rejection and improve graft survival.

## TH-PO1148

**Addressing U.S. National Geographic Disparity in Kidney Transplantation by Creating Sharing Partnerships** Sanjay Mehrotra,[1] Ashley E. Davis,[1] John J. Friedewald,[2] Michael Abecassis,[2] Daniela Ladner.[2] *[1]Industrial Engineering, Northwestern Univ; [2]Surgery, Div of Organ Transplantation, Northwestern Univ.*

**Background:** In 1998, the Department of Health and Human Services' Final Rule requested the elimination of geographic disparities in organ allocation. Despite this action, geographic disparity persists for kidney transplantation. We aim to develop an alternative kidney sharing strategy to reduce geographic disparity over time without greatly altering current kidney allocation.

**Methods:** Kidney transplant patient and standard criteria donor kidney allocation dynamics were obtained from 2000 to 2009 at Donor Service Area (DSA) precision from Organ Procurement and Transplant Network data. Our kidney sharing strategy establishes regional and national sharing partnerships between DSAs to prioritize the offering of non-locally used kidney organs. Using simulation optimization techniques, we determine the optimal sharing partners for each DSA to best reduce geographic disparity over time. Local allocation levels are not affected. Geographic disparity is represented by the range in blood type specific DSA median waiting times to transplantation, transplant rates, and waitlist mortality rates.

**Results:** After ten years of use, our alternative kidney sharing strategy reduces geographic disparity significantly for all blood types. This reduction occurred when only 40% of shared kidney organs were redirected following our alternative strategy. Compared to actual 2009 kidney allocation, the range in DSA waiting times to transplantation fell by 31%, 38%, 27%, and 27% for blood types A, AB, B, and O kidney allocation, respectively. The range in DSA transplant rates also fell by 32%, 43%, 19%, and 26% for blood types A, AB, B, and O kidney allocation, respectively. Approximately 400 (9%) less waitlist mortalities took place each year over all blood types.

**Conclusions:** Alternative kidney allocation strategies are necessary to reduce geographic disparities in kidney transplantation. We provide a simple change to current kidney allocation policy that reduces geographic disparity over time without affecting current local kidney allocation levels.

## TH-PO1149

**An Evaluation of the ECD Classification in Canadian Kidney Transplant Recipients** Ann Young,[1,2] Charmaine E. Lok,[1,2] Stephanie Dixon,[2] Greg A. Knoll,[2] Amit X. Garg,[2] Joseph Kim.[1,2] *[1]Univ Health Network, Toronto, Canada; [2]Institute for Clinical Evaluative Sciences, Canada.*

**Background:** Although outcomes of kidney transplant recipients in Canada have been shown to be superior to their U.S. counterparts, a critical evaluation of the Expanded Criteria Donor (ECD) classification system has not been undertaken in a Canadian kidney transplant population.

**Methods:** This retrospective, population-based cohort study followed deceased kidney allograft recipients from Ontario, Canada from Jan/2005 to March/2011. Subjects were identified through a transplant database and linked to large administrative healthcare databases in Ontario (www.ICES.on.ca). ECD status was ascertained based on age, serum creatinine, history of hypertension, and death due to stroke. Outcomes were defined using reliable ICD codes compared to chart review. Multivariable survival analysis assessed total graft loss (i.e., graft loss or death), death-censored graft loss, graft loss with death as a competing event, and all-cause mortality.

**Results:** We studied 1,470 deceased donor kidney transplants, of which 286 were from ECD and 1136 from non-ECD. The median age was 64 vs. 46 years for ECD vs. non-ECD, respectively. There were significantly more donors in the ECD group with hypertension (62% vs. 24%) and death due to stroke (22% vs. 10%). Pre-terminal serum creatinine was not significantly different (71 vs. 74 µmol/L). Recipients of ECD kidneys were older (median: 62 vs. 54 years). The five-year cumulative incidence of total graft loss was 28.8% vs. 21.1% in ECD vs. non-ECD kidney transplants, respectively. There was an increased relative hazard for total graft loss (HR 1.42 [95% CI: 1.04, 1.94]) and death-censored graft loss (1.63 [95% CI: 1.05, 2.52]) in the ECD vs. non-ECD kidney transplants. Similar trends were observed for graft loss with death as a competing risk and total mortality.

**Conclusions:** The ECD classification identifies deceased donor kidneys at increased risk of graft failure among Canadian kidney transplant recipients. The performance of more granular measures of donor risk (e.g., KDRI) and its impact on allocation or organ utilization in Canadian patients requires further study.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## TH-PO1150

**Benefits of Pre-Emptive Kidney Transplantation in the Elderly Using Deceased Donor** Enrique Morales, Eduardo Gutierrez-Martinez, Manuel Praga, Amado Andres. *Nephrology, Hospital 12 Octubre, Spain.*

**Background:** During the last two decades we have seen a substantial increase in the incidence of elderly patients who require replacement therapy. In these patients, renal transplantation (RT) should be considered a treatment option. There are a large number of kidneys from elderly patients that are not used. The cadaveric pre-emptive RT has a better survival (SV) -graft and patient- in comparison to the patient on dialysis. The aim of our study was to analyze the evolution of elderly patients on end stage renal disease non-dialysis (ESRDND) after receiving a first RT.

**Methods:** Prospective observational study (years 2007-2012) analyzing pre-emptive RT from a cadaveric donor in our hospital unit. (ESRDND) aged 65 years with a glomerular

filtration rate (GFR) <15 ml / min at the time of inclusion. We established a control group of patients of the same age in hemodialysis (HD) of which 50% of the cases shared the same donor.

**Results:** We included 26 patients (ESRDND) patients (57.7% women) of mean age 74.3 ± 2.9 years, nephrosclerosis (38.5%) as the main cause of nephropathy. RT received a first donor mean age was 73 ± 7.2 years. Cerebral hemorrhage was the most common cause of death (76.9%) and had a GFR of 90.4 ± 19 ml / min at the time of extraction. The cold ischemia time was 21.4 ± 5.3 hours. Immunosuppression was basiliximab induction, tacrolimus, mycophenolate mofetil and steroids (96.2%) in both groups. All baseline characteristics were identical for both groups. At the end of follow-up time 35.5 (5-67) months, the GFR was similar in both groups (42.2 ± 11.7 vs 41.7 ± 11.2 ml / min, p0.72), delayed graft function was higher in the group HD (4 vs. 39.1%, p 0.03) and acute rejection was higher in the PRD group (23.1 vs. 3.8%, p 0.04). The patient SV was similar in both groups (78 vs 83%, p 0.79), however the SV graft was higher in the group ESRD non-dialysis or censoring death (100 vs 77, p 003 and 92 vs. 64%, p 0.03).

**Conclusions:** In conclusion, our study suggests that pre-emptive kidney transplant is an interesting alternative for elderly patients with ESRD non-dialysis. However, it is necessary to perform studies with more patients and longer follow-up to establish a stronger conclusion.

## TH-PO1151

**Long Term Outcomes of Kidney Transplants and Risk of Infection Transmission from High-Infectious Risk Donors** Mili Jay Shah,[1] Gaurav Gupta,[1] Martha Behnke,[2] Marc P. Posner,[2] Amit Sharma,[2] Adrian Cotterell,[2] Robert Fisher,[2] Anne L. King.[1] *[1]Nephrology, Virginia Commonwealth Univ, Richmond, VA; [2]Transplant Surgery, Virginia Commonwealth Univ, Richmond, VA.*

**Background:** The use of kidneys from deceased donors considered at high infectious risk based upon CDC criteria (CDCHR) represents a strategy to increase the limited donor pool. Concern over transmission of viral infections is reported to result in a higher discard rate of these kidneys. Prospective studies on virawl transmission rates are lacking. We report our experience with CDCHR kidneys.

**Methods:** All patients signed an informed consent to receive CDCHR kidneys. Nucleic acid testing (NAT) for HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) was performed on all CDCHR donors before kidney transplant (KTx). Per protocol, viral transmission testing using HBV surface antigen, HIV ELISA and HCV PCR was performed at least once during the first year and then annually post-KTx.

**Results:** There were 89 CDCHR KTx and 533 Non-high risk (NHR) KTx during the study period from June 2004 to Dec 2011. Although median waiting time was shorter for the CDCHR group (14.8 mths) as compared to the NHR group (20 mths), these results were not statistically significant. Nearly all patients underwent post-KTx screening for HIV (79/89; 89%), HCV (83/89; 93%) and HBV (82/89; 92%). Among patients tested, there was a 0% rate of viral transmission. When compared to the NHR group, CDCHR kidneys were more likely (26% vs. 40%; p<0.01) to be imports. Fewer CDCHR kidneys were from expanded-criteria donors (3/89; 3% vs. 129/533; 24%; p<0.01). CDCHR KTx were more likely to be HCV positive (34% vs 11%; p<0.01) and receive a HCV positive organ (28% vs. 7%; p<0.01). Graft and patient survival at 1, 3 and 5 years were similar between the two groups.

**Conclusions:** In this till date largest single-center report with prospective screening we demonstrate that CDCHR donor kidneys can be used safely with achievement of excellent renal function and graft survival. This data can guide counseling of potential kidney transplant recipients about the benefits of CDCHR kidneys which might also include shorter waiting times.

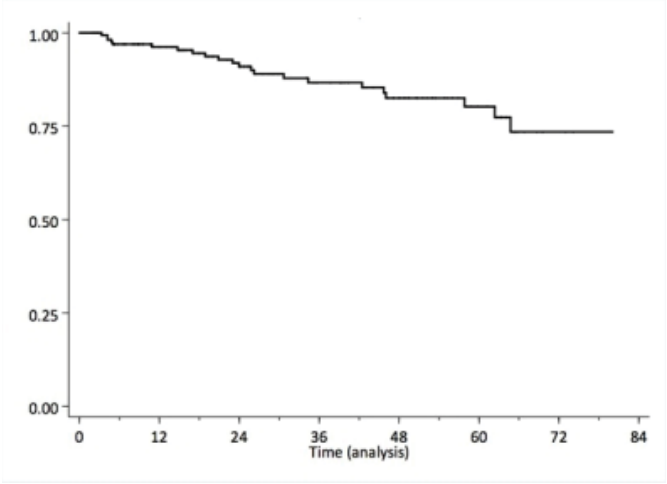
## TH-PO1152

**CDC High Risk Designation for Deceased Kidney Donors Is a Misnomer** Moya B. Gallagher, Demetra Tsapepas, Travis L. Samuels, Shefali Patel, Yael J. Coppleson, David J. Cohen, Lloyd Ratner, Sumit Mohan. *Columbia Univ Medical Center.*

**Background:** Approximately 10% of deceased donor kidneys meet the CDC criteria as "high-risk" for infection (HIV, HCV, HBV) and disease transmission. While this designation may lead to lower organ utilization by some centers, the risk of not detecting infection with modern nucleic acid testing appears low.

**Methods:** Since 2004, 170 patients received kidneys that meet CDC high-risk criteria at Columbia University Medical Center. In addition to standard pre-transplant serologic screening for HIV, HCV, and HBV these patients are also screened by antibody and PCR testing at 6, 12 and 24 weeks post transplant. All patients received our standard of care immunosuppressive protocol.

**Results:** Recipients were predominantly male (69.4%), Hispanic or White (68.8%), receiving a first transplant (80%) after waiting 1.7±1.2yrs and 27.7% experienced delayed graft function. Donors were 37±11.5yrs (10% extended criteria donors) and predominantly male (72.7%). Donor organs had 29.2±10.6hrs of cold ischemia, with a terminal creatinine of 2±1.7mg/dL. Among these donors, 57.1% had a history IVDU, 25.9% had high risk sexual behavior, 11.8% were incarcerated, 7.1% were men who had sex with men, and 4.7% had received multiple blood transfusions resulting in hemodilution. The majority (77.8%) were imported from other OPOs suggesting that other centers declined to use these organs. After a median of 2.4yrs of follow up, 86.5% of allografts were functioning (figure 2) with the most recent creatinine of 1.62±0.87mg/dL and no evidence of infectious seroconversion.




**Conclusions:** Our single center experience of 170 high-risk criteria organs demonstrates the relative safety of this important pool of deceased donor organs when screened by current methods. These organs should probably be labeled as "identified risk"; "high-risk" appears to be a misnomer.

## TH-PO1153

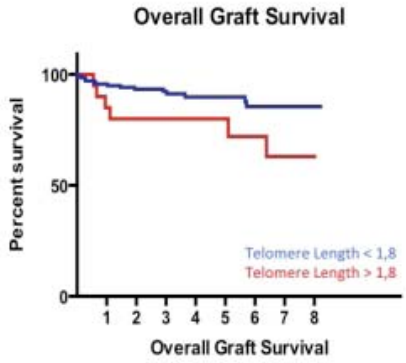
**The Discrepancy between Biological Age and Calendar Age: A Large Histology Study in Pre-Implantation Biopsies** Katrien De Vusser,[1] Nicky Pieters,[3] Evelyne Lerut,[2] Bjorn Meijers,[1] Dirk R. Kuypers,[1] Maarten Naesens.[1] *[1]Nephrology and Renal Kidney Transplantation, UZ Leuven, Leuven, Belgium; [2]Pathology, UZ Leuven, Leuven, Belgium; [3]Center for Environmental Science, U Hasselt, Hasselt, Belgium.*

**Background:** Replicative senescence (biological aging), associated with telomere shortening, plays an important roll in tissue aging. The relationship between calendar age, telomere length, markers of replicative senescence and renal histology is currently unknown.

**Methods:** DNA was extracted from a peripheral blood sample in 300 deceased kidney donors. Telomere length was measured by real-time PCR. All 300 kidneys were biopsied prior to implantation, and the histology was evaluated using the Banff classification. Kidney donor demographics and transplant outcome data were recorded prospectively. IHC was performed for p16, p53, KI-67 and Beta-galactosidase.

**Results:** Telomere length correlated with donor calendar age (p=0.0002). Older donor calendar age associated with IFTA grade and gs (both p<0.006), independent of the other donor characteristics. Telomere length predicted overall graft survival (p=0.02), but not death censored graft survival. Telomere length associated independently with intragraft arteriosclerosis (p<0.0001), independent of the other donor characteristics. Positive staining for p16 correlated (p=0,03) with longer telomere length, while intrarenal KI-67 expression with shorter telomere length. Thus, both molecules consistitute the renal senescent phenotype.

Figure 1: Overall graft survival Telomere Lenght.




**Conclusions:** This study indicates that biological aging and calendar age associate with different histological lesions, and likely represent different senescence pathways. We are currently expanding these studies in native kidney biopsies prior to transplantation, to investigate the molecular mechanisms underlying these observed discrepancies between calendar age and biological aging.

## TH-PO1154

**Peritubular Capillary (PTC) Number in Pre-Implantation Biopsies of Living Donors Is Associated with Cardiovascular Risk Factors and Later Graft Function** Floortje Steegh,[1] Marielle Gelens,[2] Marnix Van Agtmaal,[1] Elly Van Duijnhoven,[2] Mat Daemen,[3] Ernest Van Heurn,[4] Maarten H.L. Christiaans,[2] Carine Peutz-Kootstra.[1] *[1]Pathology, MUMC, Netherlands; [2]Nephrology, MUMC, Netherlands; [3]Pathology, AMC, Netherlands; [4]Surgery, MUMC, Netherlands.*

**Background:** Chronic transplant dysfunction (CTD) is a major cause of renal allograft loss and is associated with PTC loss and fibrosis in the renal biopsy (Bx). Recently we showed that PTC no. in the implantation Bx (M0 Bx) of postmortal and living donors (LDs) predicts PTC loss and graft function after transplantation (Tx). In this study we investigate the prognostic role of PTC no. in relation to CVD risk factors in an extended cohort of patients receiving kidneys from well characterized LDs.

**Methods:** 70 patients with representative protocollar Bx taken at implantation (M0) and 3 mo (M3) after kidney Tx with a LD kidney were included. All patients were treated with TAC-based immunosuppression. PTC no. per tubule at M0 and M3 was counted by 2 blinded observers (ICCs resp. 0.826 and 0.647). Statistical analysis was performed by T-test, Pearson correlations and multivariate analysis.

**Results:** In multivariate analysis we found that donor sex (women>men), positive family history for CVD and smoking of donor, are associated with higher PTC no. at M0. In contrast, high fasting glucose level of donor was associated with low PTC no. (r<sup style="font-family: Calibri; line-height: 17px;">2[/sup]=.327; p<.001, multiregression analysis). In this extended cohort we confirm our earlier finding that PTC no. is not significantly decreased after Tx for LDs. However, low PTC no. at M0 is associated with increased PTC loss the first 3 mo after Tx. Furthermore, PTC loss the first 3 mo after Tx is correlated with MDRD score at resp. 3 mo, and 1, 2 and 3 years after Tx (resp. r=.346,p<.001; r=.288,p=.028; r=.285,p=.030; r=.314,p=.025).

**Conclusions:** We confirm that low PTC no. in the M0 Bx is associated with more PTC loss the first months after Tx which is associated with renal function decline. Furthermore, we show that donor sex, but also CVD risk factors are associated with altered PTC density in the M0 Bx. Our findings indicate that CVD risk factors may affect PTC no. which can contribute to the progression of early CTD.

## TH-PO1155

**Glomerular Size on Time Zero Kidney Allograft Biopsies and Change in Kidney Function after Live Kidney Donation** Howard Hao Yan,[1] Elizabeth Hendren,[1] James Dong,[1] Michael Mengel,[2] John S. Gill.[1] *[1]Div of Nephrology, Univ of British Columbia, Vancouver, Canada; [2]Alberta Transplant Applied Genomics Centre, Univ of Alberta, Edmonton, Canada.*

**Background:** Identification of predictors of kidney function after live kidney donation may help expand the living donor pool or identify donors for long-term clinical follow-up.

**Methods:** In this study, we determined the association of demographic factors (donor age at transplantation, gender, race, body mass index), pre-donation kidney function (eGFR), and kidney allograft biopsy findings (glomerular diameter and volume as determined by Weibel-Gomez method) at time of transplantation (time zero) with change in kidney function (% decrease in eGFR 1 year after live kidney donation) among n=60 live kidney donors in our centre from 2000-2009.

**Results:** Mean±std pre- and post-donation eGFR were 93±14 and 61±15 ml/min/1.73m$^2$, respectively. Mean±std percent change in eGFR after donation was 34±12%, with 91% having ≥ 30% decrement in eGFR. Mean glomerular diameter was 163±17 µm, while mean glomerular volume was 2.81±0.98•10$^6$ µm$^3$. No donors had glomerulomegaly (defined as volume ≥6.81•10$^6$ µm$^3$). In regression analyses, contrary to previous reports, glomerular volume was not associated with donor age, gender, race or BMI. Donor age and overweight BMI were associated with percent decrease in kidney function after donation (3% decrement in pre-donation eGFR for each decade older, p=0.018; 6% decrease in overweight compared to normal BMI, p=0.04), but there was no significant association with pre-donation eGFR, glomerular diameter or volume, donor race or gender.

**Conclusions:** We conclude that eGFR percent decrement post-kidney donation is highly variable, and associated with donor age and overweight BMI at transplantation. eGFR decline in donors with increased BMI is independent of glomerular size. The absence of an association between glomerular diameter and volume with change in kidney function after donation in this study may be due to conservative donor selection. These parameters may still prove useful in the screening of higher-risk donors with other co-morbidities or more marginal levels of pre-donation kidney function.

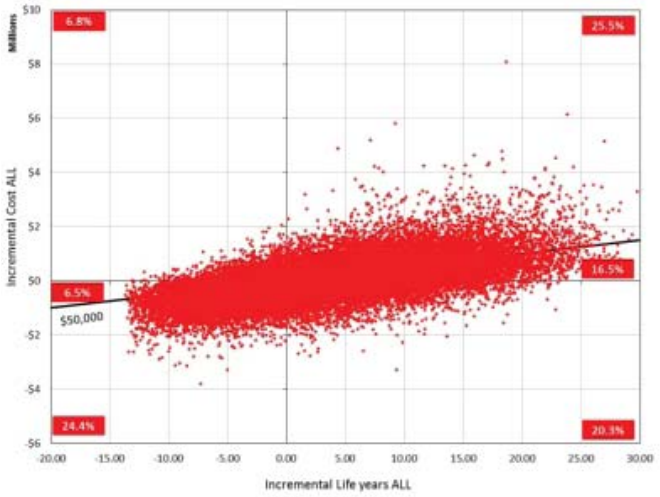
## TH-PO1156

**Is Deceased Donor Kidney Transplantation Cost-Effective across All End Stage Kidney Disease Patients?** Bekir Tanriover, Sumit Mohan, David J. Cohen. *Div of Nephrology, Columbia Univ College of Physicians & Surgeons, New York, NY.*

**Background:** Allocation of deceased donor kidneys is primarily based on waiting time in the US. The current system causes unnecessarily high re-transplantation and death with functioning graft resulting from survival mismatch between donor and recipient.

**Methods:** The United States Renal Data System (USRDS) linked Medicare database was analyzed to obtain real life outcomes, health care utilization related cost of wait-listed end stage kidney disease (ESRD) patients between 2000 and 2008. A probabilistic model was developed to simulate the natural history of a group of potential kidney transplant candidates (n = 100,000), stratified based on their underlying co-morbidities (low risk recipient –RL, defined as age <60 and no co-morbidities and high risk recipient – RH described as old age >60, diabetes, prior history of cardiovascular events including CABG, PTCA/stent, stroke, and peripheral arterial stent and bypass) and deceased donor categorized based on the Kidney Donor Risk Index-KDRI (low risk donor –DL, KDRI<1.6 and high risk donor –DH, KDRI ≥1.6) score.

**Results:** Under current waiting time for deceased donor transplantation, a potential recipient-donor pair achieves an average incremental cost-effectiveness ratio (ICER) of less than $50,000/LYG, except RH-DH group. In overall model, of all renal transplant recipients, 37.7% had worse survival and 38.8% cost more compared to matched wait-listed dialysis patients.




**Conclusions:** Our analysis suggests that renal transplantation is not cost-effective in all pairs compared with dialysis from third party-payers perspective and utilitarian grounds.

*Funding:* Other NIH Support - NIH-1KM1CA156709-01

## TH-PO1157

**Outcomes of Kidney Transplantation from Older versus Younger Living Donors: Expanding the Donor Pool** Himanshu V. Patel,[1] Pankaj R. Shah,[1] Aruna V. Vanikar,[2] Vivek Balkrishna Kute,[1] Manoj R. Gumber,[1] Hargovind L. Trivedi.[1] *[1]Nephrology and Transplantation, IKDRC-ITS, Ahmedabad, Gujarat, India; [2]Pathology, India, Ahmedabad, Gujarat, India.*

**Background:** The disparity between donor kidney availability and demand has increased utilization of kidneys from older living donors (OLD). We compared outcome of OLD kidney transplants (group-1) with younger live donor (YLD) (<60 years) kidney transplants (group- 2).

**Methods:** Outcome of renal transplants performed between 2007 to 2012 was compared in terms of demographics, survival and graft function.

**Results:** Out of 1457 transplants, 147(10.1% ) belonged to group-1 and 1310 (89.9% ) belonged to group-2. Mean donor/recipient age was 62.7/36.6 years in group-1 and 43.5/33.8 years in group-2 and HLA-match was 3.12 and 2.46 respectively. Post-transplant immunosuppression consisted of calcineurin inhibitor based regimen in both groups. Patient survival at 1, 3 and 5-years was 95.7% , 89.4% and 82.6% in group-1 and 93.8% , 89.1% and 83.1% in group-2 (P=0.785). Death-censored graft survival at 1, 3 and 5-years was 98.5% , 94.8% and 94.8% in group-1 and 96.1% , 92.9% and 89% in group-2(P=0.166). Biopsy proven acute rejections were and 21% and 16.8% (P=0.206) and chronic rejections 5.03% and 3.4% respectively (p= 0.542).

**Conclusions:** Patient, graft survival and immune injuries were comparable for recipients of OLD kidneys and YLD kidneys. This study suggests that in organ availability crisis, OLD kidneys can be utilized without compromising the recipient 5-year patient or graft survival. This facilitates in decreasing the transplantation waiting lists.

## TH-PO1158

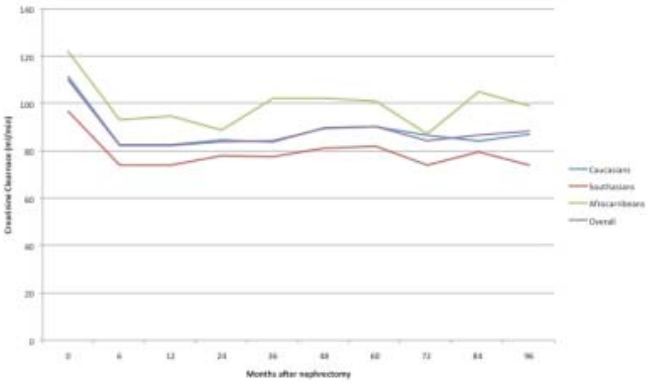
**Ethnicity Does Not Determine Long-Outcome after Donor Nephrectomy** Dimitrios Anestis Moutzouris, Rawya Charif, Jack W. Galliford, Jen McDermott, Harvinder Dulku, Honeylet Orr, David Taube, Marina Loucaidou. *Imperial College Kidney and Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

**Background:** Living kidney donor transplantation is the treatment of choice for ESRD. Although long term data suggests that survival and chronic kidney disease risk in living donors (LD) is similar to general populations, data is limited regarding ethnic groups, particularly South Asian(SA) and Afro-Caribbean(AC) populations. We previously reported 5-year data on LD from different ethnic groups in our centre.

**Methods:** We prospectively collected data on consecutive LD(2000-2012). We analyzed follow-up data for 24-hour Creatinine Clearance(CrCl) and protein excretion, and blood pressure control. We included LD with at least one year of follow-up. We used one-way and repeated measures ANOVA for analysis.

**Results:** We studied 555 LD. 424 completed 12-month,170 had 60-month and 52 had 96-month follow-up. 338(56.8%) were caucasian (CA),113(19%) were SA, 53(8.9%) were AC. There were 51 LD with history of hypertension (HTN). 48 donors developed HTN during the follow up period. AC patients were at increased risk for developing HTN(CA=30(10.2%),AC=9(21.4%) and SA=6(6.9%)(p<0.05).

The mean CrCl (mls/min) at baseline, 60 and 96 months was 110, 95 and 89 (CA=111±32, 90±26 and 87±27, SA=97±25, 82±17 and 74±9, AC=122±36, 101±40 and 99±23 (p<0.01, but for the rate of CrCl change p=NS).




There was significant ethnic group age difference (CA=48±12, SA=43±13 and AC=38±9 years,p<0.01). There was no difference in the degree of proteinuria at baseline and at five years (011±0.1 vs 012±0.1g/24h,p=NS),even in patients with pre-existing HTN (0.21±0.2 vs 0.18±0.1 g/24h,p=NS).

**Conclusions:** Donor nephrectomy in high risk ethnic groups is safe and not associated with poorer outcomes. Kidney function was preserved and proteinuria was minimal over time. AC population is at increased risk for developing HTN.

## TH-PO1159

**Impact of Donor Glomerular Filtration Rate on Post Transplant Donor and Recipient Renal Function** Aneesha A. Shetty, Eric Soukup, Emilio D. Poggio. *Dept of Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

**Background:** A glomerular filtration rate (GFR) greater than 80 ml/min/1.73m$^2$ independent of age and gender is considered suitable by certain living kidney donor selection guidelines. We studied the association of pre-transplant donor GFR with donor and recipient renal function post transplant to the impact of this screening criteria.

**Methods:** Pre-transplant $^{125}$I-iothalamate GFR was measured and pertinent demographic data was captured in 570 living kidney donors as part of donor evaluation. Follow up donor and recipient estimated GFR (eGFR) was calculated using the MDRD equation at 1, 2 and 3 years post-transplant.

**Results:** Mean pre-transplant donor BSA adjusted GFR (iGFR) was 106+/-23 ml/min/1.73m$^2$ and unadjusted GFR (uGFR) was 116+/-21 ml/min. Mean age was 43.5+/-11.6 years with 41.4% males and 14.4% African Americans. Mean donor eGFR was 63.8+/-12.3 ml/min, 66.4+/-15.8 ml/min and 68.7+/-10.6 ml/min, and mean recipient eGFR was 58.4+/-21.9 ml/min, 56.6+/- 24.5 ml/min and 55.1+/-23.5 ml/min at 1, 2 and 3 years of follow up.

Donor iGFR was a significant univariate predictor of donor eGFR at 1 and 2 years (p <0.05), but not at 3 years. Donor iGFR less than 90 ml/min/1.73m$^2$ was associated with lower donor eGFR at 1 year (p=0.01) but not at 2 or 3 years. In multivariate analyses donor iGFR was a significant predictor of donor eGFR at 1 year only (p = 0.003), while donor age remained a significant predictor of donor eGFR at 1 year (p=0.006) and 2 years (p=0.03). Gender and race were not significant predictors.

On recipient outcomes analyses donor uGFR was not associated with recipient eGFR over 3 years. In the multivariate model, however, donor age was negatively associated with recipient eGFR at 1 and 2 years (p<0.0001) but not at 3 years.

**Conclusions:** Recipient renal function up to 3 years post-transplant is independent of pre-transplant donor GFR. On the other hand, while donor GFR at 1 year post donation is lower for older donors with pre-transplant GFR less than 90 ml/min/1.73m$^2$, long term donor GFR appears to be independent of pre-donation GFR. Importantly, the compensatory effects of the remaining donor kidney GFR appears to be sustained or even increased over time.

## TH-PO1160

**Kidney Biopsy in Potential Donors with Non Visible Haematuria Is a Valuable Component of Donor Work-Up** Emma O'Lone, Abigail Lee, Ravindra Rajakariar, Raj C. Thuraisingham, Mark Blunden. *Nephrology, Barts and The London Hospitals, United Kingdom.*

**Background:** BTS guidelines recommend renal biopsy if non-visible haematuria (NVH) is >1+ on dipstick. Glomerular pathology precludes donation with the possible exception of thin basement membrane (TBM) disease. The level of evidence for these guidelines is graded as"moderate"; little data exists to support them. All biopsies performed on potential living kidney donors with NVH were reviewed.

**Methods:** A single centre review of prospectively collected data of patients between Jan 2008 and June 2013. Patients with undiagnosed NVH underwent biopsy. Percutaneous biopsy was performed under direct ultrasound (US) guidance using an 18 or 16 gauge needle. Samples were examined by a consultant histopathlogist by light microscopy (LM), immuno-histochemistry and electron microscopy (EM).

**Results:** 148 living donor transplants were performed in the 5 year period. 15 renal biopsies were done for undiagnosed NVH in 14 potential donors. Work-up included urine cytology, flexible cystoscopy, US and CT examination of urinary tract. Of those patients biopsied: Mean age 41.5 years and 33% male. Mean eGFR (by MDRD) of 94.5mls/min and mean corrected EDTA clearance 93.7mls/min. 11 (68.8%) biopsies had normal LM and immuno-histochemistry. Of these EM was normal in 6; TBM was diagnosed in 2 and minor paramesangial deposits seen in 2. 1 patient was found to have Zebra bodies on EM, Fabry's disease was excluded and the patient had previous exposure to chloroquine so donation went ahead. 4 biopsies precluded donation; 2 showed significant scarring, 1 a granulomatous TIN consistent with TB and repeat biopsy after treatment showed IgA nephopathy. Overall 11 patients were deemed suitable for donation and 4 biopsies (25%) precluded donation in 3 patients. All biopsies were performed as a day case and no complications were seen.

**Conclusions:** Biopsy for investigating NVH in potential donors is a safe and helpful investigation. It led to rejection of 25% of potential donors, despite acceptable EDTA GFR results but re-assured the transplanting team in 75% of cases. The long term significance of TBM disease and minor paramesangial deposits is uncertain in this scenario.

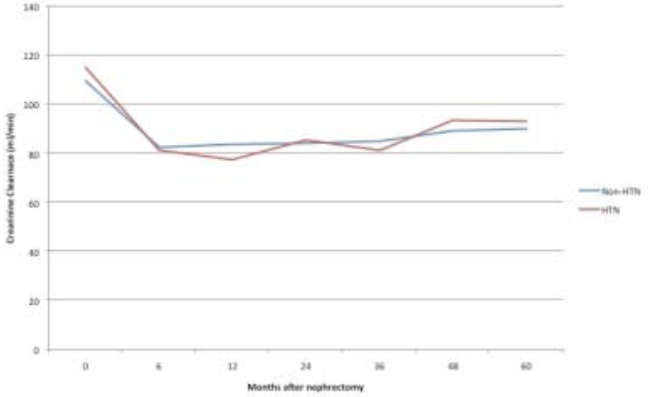
## TH-PO1161

**Hypertension Is Not a Contraindication to Living Kidney Donation** Rawya Charif, Dimitrios Anestis Moutzouris, Jack W. Galliford, Jen McDermott, Honeylet Orr, Harvinder Dulku, David Taube, Marina Loucaidou. *Imperial College Kidney and Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

**Background:** The increasing numbers of patients with ESRF on waiting list for a kidney transplant has been the main driving force for expanding the donor pool. We undertook living kidney donor transplantation from hypertensive donors after initial reports that short-term outcomes are safe. We report 5-yr data in this group and compare these with the outcomes of non-hypertensive donors from our centre.

**Methods:** Data were prospectively collected on 555 consecutive live donors(LD) from 2000-2012. The hypertensive group consists of LD presenting with a pre-existing diagnosis of hypertension(HTN), well-controlled on at least one antihypertensive agent and without evidence of end-organ damage. We analysed 5-yr follow-up data for 24 hr Creatinine Clearance(CrCl) and protein excretion as well as blood pressure (BP) control.

**Results:** 51 LD were hypertensive(H) and 504 non-hypertensive(NH). In the NH group 302(59.9%) were Caucasian (C), 104(20.6%) South Asian(SA), 49(9.7%) Afrocaribbean (AC) and 49(9.7%) of other ethnicity. There was a higher percentage of C in the H group (70.6%) but similar of SA and AC. Mean age was 45.5 years(yrs) (H=54.6, NH=44.6, p<0.001) with a mean weight of 75.6 Kg(H=78.6, NH=75.0, p=0.01). The mean CrCl (ml/min) was 110.1 (H=115.2, NH=109.6, p=0.26) at time 0 and 90.4 (H=93.0, NH=90.0, p=0.17) at 5 yrs.




We did not find evidence of developing proteinuria in with 24-hour protein excretion of 0.12 (H=0.17, NH=0.10, p=0.03) g/24hrs at 5 yrs. BP remained well-controlled in the H group at 5 yrs at 139/85 vs 135/81 in the NH group (p=0.25). 48 donors in the NH group developed HTN post donation.

**Conclusions:** Donor nephrectomy in well-controlled hypertensive donors with no evidence of end-organ damage is safe and does not result in poorer outcomes compared to non-hypertensive donors.

## TH-PO1162

**Screening for Adequate GFR in Obese Potential Kidney Donors** Sanjeev Akkina, Mahvesh K. Mahmud, Ignatius Yun-Sang Tang. *Medicine, Univ of Illinois at Chicago, Chicago, IL.*

**Background:** As organs become scarce, more obese donors are being considered for kidney donation. Assessment of glomerular filtration rate (GFR) is critical in the evaluation of these potential donors. Accurate measurement of GFR using exogenous markers is expensive and time-consuming which complicate the medical evaluation of potential kidney donors. The objective of this study is to identify potential obese donors that need formal measurements of GFR.

**Methods:** This is a single-center, retrospective analysis of potential kidney donors between 2009-2012. Estimation of GFR was by 24 hour urine collection (CrCl) or by using the Modification of Diet in Renal Disease (MDRD) and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. Measured GFR (mGFR) was either by camera-based technetium clearance or iohexol clearance. Sensitivity and specificity was calculated for mGFR > 80 and 90mL/min/1.73m2 using estimated cutoffs 10mL/min/1.73m2 above the corresponding measured cutoff to increase specificity. Groups were divided by BMI (<35, >35).

**Results:** There were 250 potential kidney donors with a mGFR. The average age was 38.0±11.9 years with 60% female and a racial composition of 44% Black, 26% White, and 20% Hispanic. The sensitivity/specificity analysis is shown in the table. Pre-donation proteinuria was similar between the groups as was post-donation microalbuminuria (n=135) and prevalence of those with eGFR < 60mL/min/1.73m2 (n=151) with median follow up time of 6 months (3-12 months).

| | BMI < 35 (n=176) | BMI > 35 (n=74) |
|---|---|---|
| | Sensitivity/Specificity | Sensitivity/Specificity |
| mGFR > 80, eGFR > 90 | | |
| 24hr CrCl* | 67/33 | 96/25 |
| MDRD | 47/94 | 59/62 |
| CKD-EPI | 71/61 | 85/50 |
| | | |
| mGFR > 90, eGFR > 100 | | |
| 24hr CrCl* | 56/46 | 91/33 |
| MDRD | 33/92 | 46/86 |
| CKD-EPI | 49/92 | 67/64 |

*72 individuals with a BMI < 35, 31 with a BMI > 35.

**Conclusions:** The MDRD equation with a cutoff of 10mL/min/1.73m2 higher than the required mGFR may identify both obese and non-obese potential kidney donors needing a mGFR. Results should be validated in an independent cohort before screening potential kidney donors.

*Funding:* NIDDK Support

## TH-PO1163

### The Change of Single Kidney Function in Kidney Donor after Uninephrectomy

Ji Hyun Yu,[2,3] Keun Suk Yang,[2,3] Seun Deuk Hwang,[2,3] Jong Hoon Lee,[3] Eun Nim Kim,[1,2,3] Cheol Whee Park,[1,2,3] Yong-Soo Kim,[1,2,3] Chul Woo Yang,[1,2,3] Bum-Soon Choi.[1,2,3] *[1]Transplant Research Center; [2]Div of Nephrology; [3]Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

**Background:** It was not fully investigated that how reserved capacity of single kidney for healthy kidney donor changes after unilateral nephrectomy. The aim of this study was to assess the change of remaining single kidney function after kidney donation and to evaluate predictive pre-donation factor for reserved single kidney capacity in donors.

**Methods:** Total 74 kidney donors who underwent $^{99m}$Tc-DTPA Scintillation-Camera renography before and after kidney donation were included in this study. By the renography, we measured single-kidney glomerular filtration rate (sk-GFR) of both kidney before donation and post-donation GFR of remaining kidney during 12 months. We investigated the factors that are associated with reserved capacity of remaining single kidney after donation.

**Results:** After uninephrectomy the mean of serum creatinine increased significantly (P=0.000, from 0.77 to 1.07 mg/dL) and the mean GFR measured by the renography declined (P=0.000, from 112.9 to 74.9 ml/min/1.73m$^2$). Nevertheless the mean of serum creatinine and mGFR was stabilized during 12 months follow-up period (mGFR at Post-donation, P=0.165 [6 month 74.9 ± 18.2 vs 12 month 81.4 ± 14.8 ml/min/1.73m$^2$]). The sk-GFR of remaining kidney significantly increased by 33.6 % after uninephrectomy (sk-GFR, P < 0.01 [Pre-nephrectomy 57.9 vs Post-nephrectomy 77.5 ml/min/1.73m$^2$]). By univariate linear regression, BMI, total mGFR, sk-GFR of remaining kidney and total kidney volume at pre-donation were included as independent predictors of change of sk-GFR. Among these, BMI (P=0.013) and sk-GFR of remaining kidney at pre-donation (P=0.019) were statistically related to reserved single kidney capacity in multivariate regression analysis.

**Conclusions:** After kidney donation, reserved single kidney capacity shows significant increase due to adaptive hyperfiltration. Especially in donor with lower BMI and lower sk-GFR of remaining kidney at pre-donation shows more compensatory response.

## TH-PO1164

### Kidney Transplant Outcomes among prior Live Organ Donors

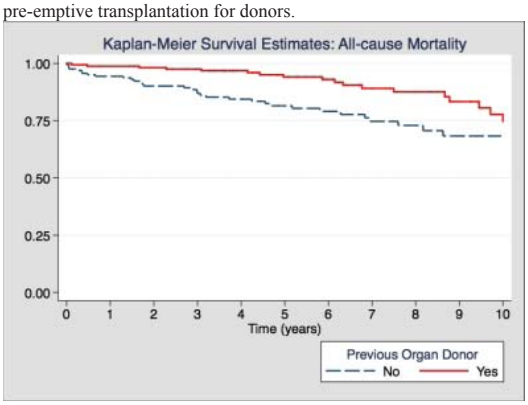
Vishnu S. Potluri, Meera Nair, Francis P. Wilson, Roy D. Bloom, Peter P. Reese. *Renal Div, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** OPTN gives high priority in organ allocation to wait-listed candidates who are previous organ donors. However, information about waiting time for kidney transplant, organ quality and post-transplant outcomes among former live donors has not been reported.

**Methods:** We assembled a cohort of candidates listed for a kidney transplant from 1996 to 2010 using Scientific Registry of Transplant Recipients data. We used multivariable Cox regression to examine the association of prior kidney donor status to time to kidney transplant. Among candidates who received a kidney transplant, we used a 1:1 propensity score approach to compare allograft quality as measured by the kidney donor risk index (KDRI) and post-transplant outcomes among prior organ donors to recipients with similar demographic and clinical characteristics.

**Results:** During the study period, there were 407,212 listings for a kidney transplant, of which 268 were prior organ donors. Among listed candidates, 82% (n=214) of donors received a deceased donor kidney transplant as compared to 34% (n=140,046) of non-donors. The median time to transplant for organ donors was 174 days (HR for transplant associated with prior organ donor status 4.8, p<0.001). Most prior donors received dialysis by the time of listing (68% vs 65%, p=.56). Compared to matched recipients, prior organ donors received higher quality allografts (Median KDRI 0.72 for donors vs 0.82 for non-donors), p<.001. Donors did not have better all-cause graft survival than non-donors (HR 1.3, p=.3), but had lower mortality (HR .32, p=.003).

**Conclusions:** OPTN policy has enabled transplant candidates who are previous organ donors to have a brief waiting time for kidney transplantation. However, >60% of donors had dialysis by wait-listing. Early nephrology referral and transplant evaluation may enable pre-emptive transplantation for donors.




## TH-PO1165

### Hypothermic Machine Perfusion versus Cold Storage for Deceased-Donor Organ Preservation – Systematic Review and Meta-Analysis

Manvir Kaur Hayer, Adnan Sharif. *Institue of Renal Medicine, Queen Elizabeth Hospital, Birmingham, West Midlands, United Kingdom.*

**Background:** Organ preservation helps optimise outcomes from deceased kidney donors, with hypothermic machine perfusion (HMP) thought to reduce the noxious effects of ischaemic reperfusion injury. However there is conflicting evidence on the benefit of HMP versus cold storage (CS). We performed a systematic review and meta-analysis of all prospective randomised controlled trials (RCTs) comparing HMP versus CS for preservation of deceased kidney donors.

**Methods:** Medline, Embase and the Cochrane Register of Controlled Trials were searched from 1966 to 2013 for relevant studies in English. We included all prospective RCTs. 2 reviewers independently extracted study data. RevMan 5 program was used to perform the meta-analysis with the odds ratio (OR) calculated for dichotomous data. Fixed-effect models were chosen for non-heterogeneous analyses ($I^2$<30%), assuming a similar treatment effect across studies. Where heterogeneity persisted ($I^2$ ≥30%), results from random-effect models are reported, assuming that treatment effect varies across studies.

**Results:** We identified 22 studies with data for 2236 kidney allograft recipients. 21 studies were original data; 1 was an extraction of extended criteria donor kidney allografts from a previously published study. Significant heterogeneity existed across studies on donor type and machine perfusion logistics. Delayed graft function (DGF) was lower with HMP than CS in all studies (OR 0.63 [95% CI 0.48-0.81] p=0.004) but there was no difference in graft loss (OR 0.81 [95% CI 0.52-1.25] p=0.35). Analysing the 5 studies using DCD kidneys only showed lower DGF rates (OR 0.54 [95% CI 0.35-0.82], p=0.004) but no difference in graft survival (OR 1.56 [95% CI 0.69-3.55], p=0.29). Analysing the 7 documented prospective RCTs showed similar reduction in DGF (OR 0.70 [95% CI 0.56-0.88] p=0.002) and a reduction in graft loss (OR 0.62 [95% CI 0.42-0.93] p=0.02).

**Conclusions:** HMP significantly reduces DGF rates but it is unclear if this results in lower kidney allograft attrition rates in the long term post transplantation. Further well designed, adequately powered trials are needed to ultimately investigate the benefits of HMP.

*Funding:* Private Foundation Support

**FR-PO001**

**Spit It Out to Check Your Kidneys: Saliva Urea Nitrogen Dipstick as a New Bedside Diagnostic Tool of Acute Kidney Injury**

Viviane Calice da Silva,[1,2] Marcos Alexandre Vieira,[1] Jochen G. Raimann,[3] Mary Carter,[3] John Callegari,[3] Nathan W. Levin,[3] Peter Kotanko,[3] Roberto Pecoits-Filho.[2] *[1]Pró-rim Foundation, Brazil; [2]School of Medicine, Pontificia Universidade Católica do Paraná, Brazil; [3]Renal Research Institute.*

**Background:** Kidney dysfunction can be identified non-invasively using a dipstick that semi-quantitatively determines saliva urea nitrogen (SUN) levels The SUN dipstick may be useful in areas with limited access to clinical chemistry facilities. Here, we evaluated the performance of SUN dipstick in patients with acute kidney injury (AKI; as per AKIN criteria).

**Methods:** Unstimulated saliva from hospitalized patients with pre-renal (PrR), renal (R) or post-renal (PoR) suspected AKI; blood urea nitrogen (BUN) was measured concomitantly. After collected, saliva was transferred to the SUN dipstick (Integrated Biomedical Technology, IN) and SUN's levels were read within one minute. The color of the test pad was compared to 6 standardized test pads indicating SUN concentrations: 5–14 (#1), 15–24 (#2), 25–34 (#3), 35–54 (#4), 55–74 (#5), and 75 (#6) mg/dL. Spearman's correlation test (Rs), areas under receiver operating characteristic curves (AUC ROC) and 95% confidence intervals evaluated the performances of SUN and BUN to diagnose AKIN 3. Youden's Index was used to compute thresholds to discriminate AKIN 3 from earlier AKI stages.

**Results:** 44 AKI patients (59.5±18 years, 58% female) (PrR: 67%; R: 24%; PoR: 9 %) with AKIN stage 1(33 %), 2 (27%), and 3 (40%) were enrolled. SUN and BUN levels were correlated (Rs= 0.77; p<0.001) irrespective of AKIN stage and AKI etiology. The diagnostic performance of SUN (AUC ROC: 0.76 (95% CI 0.61-0.91) and BUN (AUC ROC: 0.69 (95% CI 0.51-0.87) was comparable. Optimal diagnostic threshold levels were 72 mg/dL for BUN and 25-34 mg/dL (test pad #3) for SUN.

**Conclusions:** Our study indicates that SUN dipsticks discriminated AKIN 3 from earlier AKI stages which carry a lower probability to require dialysis. This low-resource technology may help to improve the diagnosis of advanced AKI and aid triaging patients in areas with limited access to healthcare facilities and may improve outcomes.

**FR-PO002**

**Biomarker Panels Can Predict and Classify Acute Kidney Injury**

Rajit K. Basu,[1] Catherine D. Krawczeski,[1] Lakhmir S. Chawla,[2] Derek Wheeler,[1] Stuart Goldstein.[1] *[1]Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Anesthesia and Critical Care, George Washington Univ, Washington, DC.*

**Background:** The ability to classify AKI severity or category of AKI (functional change or kidney damage) using biomarkers, a key directive from the 10th Acute Dialysis Quality Initiative (ADQI) conference, has yet to be demonstrated.

**Methods:** We retrospectively studied 359 children following cardiopulmonary bypass (CPB) surgery (pts) to determine if a biomarker panel taken 12 hours after CPB could differentiate the following AKI 'signatures': 1) severity of AKI or 2) AKI type: functional change or kidney damage. Severe AKI was defined as pRIFLE I-F (by creatinine) within the first seven days after CPB. AKI lasting only 1-2 days was deemed functional change. The functional change biomarker used was plasma Cystatin C (pCysC) while the kidney damage biomarker was urinary neutrophil gelatinase associated lipocalin normalized to creatinine (uNGAL/uCr). Sensitivity analyses identified cut-off values for both pCysC and uNGAL/uCr ("+").

**Results:** AKI occurred in 62 pts (17.3%). Pts with dual marker positivity were highly likely to develop severe AKI (likelihood ratio (+LR):24.6) while pts with dual marker negativity were highly unlikely (-LR:6.12). Pts with only (+)functional marker demonstrated a negligible increase in likelihood of severe AKI (+LR:1.04). In pts with AKI, a (+) functional/(-)damage panel carried a higher likelihood of functional injury (reversible), than any other biomarker combination and were unlikely to have persistent severe AKI (+LR:0.76). Addition of a positive damage marker (both positive) predicted a persistent AKI.

| **Outcome:** AKI ≤ 2 days (Reversible AKI) | | Damage Marker (uNGAL/uCr) | |
|---|---|---|---|
| | | Negative | Positive |
| Functional Marker (pCysC) | Negative | (+)LR: 0.00<br>(-)LR: 0.94<br>*No functional changes or damage* | (+)LR: 0.88<br>(-)LR: 1.04<br>*Damage without loss of function* |
| | Positive | (+)LR: 1.32<br>(-)LR: 0.97<br>*Functional AKI* | (+)LR: 0.92<br>(-)LR: 1.17<br>*Damage and loss of function* |

*Biomarker panel with ADQI-X phenotyped italicized.*
*Likelihood Ratios (LR) are for the outcome of AKI ≤ 2 days.*

**Conclusions:** Our data operationalizes the primary ADQI-X goal, combining functional and damage biomarkers into a panel which affords categorization of AKI severity and duration, predicting an AKI phenotype.

**FR-PO003**

**B-Type Natriuretic Peptide Reduces Acute Kidney Injury: A Meta-Analysis**

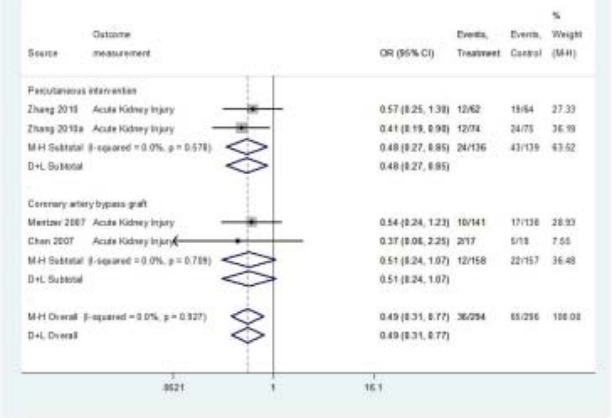
Sayyad F. Kyazimzade,[1] Daniel M. Pearlman,[1] Jeremiah R. Brown,[1] Alex L. Yerukhimov.[2] *[1]The Dartmouth Institute for Health Policy and Clinical Practice at the Geisel School of Medicine, Lebanon, NH; [2]Dartmouth College, Hanover, NH.*

**Background:** B-type natriuretic peptide (BNP) has been shown to improve renal function in individual clinical trials. However no systematic review or meta-analysis has been performed to determine whether BNP prophylaxis prevents acute kidney injury (AKI) in multiple randomized clinical trials involving coronary artery bypass graft (CABG) or primary percutaneous intervention (PCI). We hypothesized prophylactic treatment with BNP reduces the risk of AKI in cardiac procedures.

**Methods:** We conducted a meta-analysis for published randomized clinical trials involving treatment of BNP in percutaneous interventions or cardiac surgery procedures and reporting renal function. Our search identified 71 records, of which 5 RCTs, including 839 patients, met our pre-specified eligibility criteria. One article was excluded because BNP was administered after AKI was established. We calculated a random effects summary estimate for all trials and stratified by procedure type.

**Results:** Overall, only 12.2% of patients receiving BNP developed AKI compared 22.0% in controls with an odds ratio of 0.49 (95% confidence interval: 0.31 to 0.77, $P$ = 0.002 see figure). After stratifying this analysis by procedure type, risk of AKI remained significantly decreased for PCI (17.6% vs. 30.9%; OR, 0.48; 95% CI, 0.27 to 0.85; $P$ = 0.011) but not CABG (7.6% vs. 14.0%; OR, 0.51; 95% CI, 0.24 to 1.07; $P$ = 0.074). There was no observed heterogeneity (I-squared 0.0%; $P$ > 0.50).

**Conclusions:** Perioperative administration of BNP reduces the risk of AKI for patients undergoing PCI but not CABG surgery.




**FR-PO004**

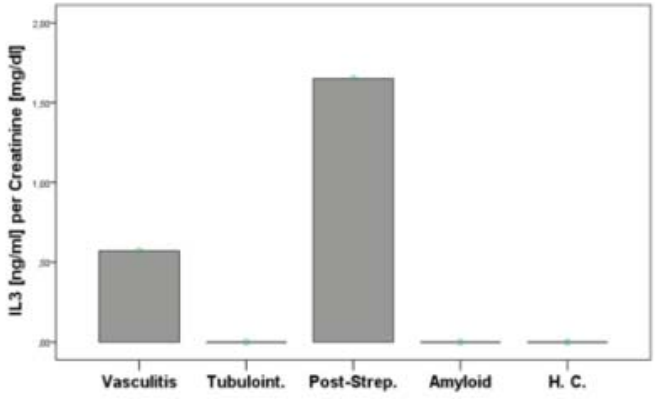
**Biomarker Profile in Biopsy-Proven Renal Diseases**

Natalie M. Otto,[1] Volker Schmitz,[1] Stephan Wolfgang Hanschke,[1] Joe F. Keenan,[2] Ralf Schindler.[1] *[1]Nephrology and Intensive Care, Charite, Berlin, Germany; [2]EKF Diagnostics Limited, Trinity Technology Campus, Dublin, Ireland.*

**Background:** The major tool to assess renal disease is the kidney biopsy. Efforts have been made to identify non-invasive markers to predict the type of renal damage. We investigated a variety of serum biomarkers to predict the category of renal histology obtained by biopsy.

**Methods:** 140 consecutive patients underwent renal biopsy as clinically indicated. Serum and urinary samples were prospectively collected and analyzed for 46 biomarkers. Serum levels of biomarkers were adjusted for serum creatinine. Biopsy results were categorized in 8 disease entities and biomarker levels compared in each category.

**Results:** Biopsy results were: minimal change/FSGS (n=38), vasculitis (n=19), IgA-nephropathy (n=20), membraneous nephropathy (n=19), diabetic nephropathy (n=11), hypertensive nephropathy (n=19), post-streptococcal nephritis (n=2), amyloidosis (n=4) and tubulointerstitial lesions (n=8). 10 healthy subjects without biopsy served as controls. There was a characteristic pattern of biomarker elevation for certain histological entities. For instance, IL-3 was elevated only in vasculitis and post-streptococcal nephritis (figure).




Several biomarker such as IL-6, IL-7, IL-8 were exclusively elevated in post-streptococcal nephritis. In contrast, tubulointerstitial disease was characterized by elevated levels of KIM-1 but marked decreased levels of biomarkers such as IL-23 or MMP-9. Hypertensive- and IgA-nephropathy did not show characteristic profiles of the investigated markers. Urinary biomarker results will also be presented.

**Conclusions:** In some types of renal disease serum levels of certain biomarkers are significantly different from controls. There appears to be a characteristic biomarker pattern for several disease entities that could help predicting the renal disease by straightforward and non-invasive analysis.

*Funding:* Government Support - Non-U.S.

## FR-PO005

**Urine Microscopy – A Golden Tool in Diagnosis of Acute Kidney Injury**

Pinaki Mukhopadhyay,[1] Piyali Banerjee,[2] Gautam Mukherjee.[3] *[1]Nephrology, NRS Medical College & Hospital, Kolkata, West Bengal, India; [2]Medicine, Bagnan Rural Hospital, Howrah, West Bengal, India; [3]Gynaecology & Obstretics, North Bengal Medical College, Siliguri, West Bengal, India.*

**Background:** Urine microscopy is very important diagnostic modality.The aim of the study was to review the role of urine microscopy and urine sediment examination in the differential diagnosis and outcome prediction of acute kidney injury (AKI ).

**Methods:** All patients admitted with AKI (RIFLE criteria) were included and urine was tested for different cast, including granular cast(GC),renal tubular epithelial cell(RTEC). AKI cause (pre renal/acute tubular necrosis ,ATN) was evaluated by nephrologist two times before urine testing and at discharge. A scoring system {Scoring 1(RTEC & GC=0),2(RTEC=0&GC=1to5 or RTEC=1to5 &GC=0),3(RTEC=1to5&GC=1to5 orRTEC=0 &GC 6to 10 orRTEC=6to20 & GC=0 )} was created based on casts and RTEC and evaluated its accuracy for differentiating ATN from prerenal AKI . Likelihood ratios (LR) were calculated for a diagnosis of both ATN and prerenal AKI.

**Results:** The urinary sediment scoring system was highly predictive of the final diagnosis of ATN. The odds ratio (OR) for ATN incrementally increased with an increase in severity of the scoring system (all compared with score 0; score 1: OR 9.7, 95% CI 5.3 to 18.6; score ≥2: OR 74.0, 95% CI 16.6 to 329.1; In patients with a high pretest probability of ATN (initial diagnosis of ATN), any granular casts or RTEC (score ≥2) resulted in very high Positive Predictive Value (100%) and low NegetivePredictiveValue (44%) for a final diagnosis of ATN. In patients with a low pretest probability of ATN (initial diagnosis of prerenal AKI), the lack of granular casts or RTEC on urinary sediment examination had a sensitivity of 0.73 and a specificity of 0.75 for a final diagnosis of ATN. The NPV of lack of granular casts or RTEC in patients with low pretest probability of disease was 91%.

**Conclusions:** urine microscopy on the day of nephrology consultation is a valuable diagnostic tool for strengthening the probability of a diagnosis of ATN. Furthermore, an ATN scoring system is useful for improving the differential diagnosis of ATN *versus* prerenal AKI.

## FR-PO006

**Urinalysis (UA) Dipstick Proteinuria (DP) at ICU Admission Predicts Mortality in Sepsis**

John Manllo, Javier A. Neyra, Fabrizio Canepa, Ghassan Bandak, Jerry Yee, Lenar T. Yessayan. *Dept of Medicine, Henry Ford Hospital, Detroit, MI.*

**Background:** Sepsis is one of the most common causes of admission to the ICU, with a high degree of mortality, early predictors for mortality are helpful in clinical practice, to ensure prompt intervention. The purpose of our study is to assess the utility of UA DP as a predictor of clinical outcome in patients with sepsis.

**Methods:** 5,558 consecutive patients admitted to the ICU between October 2007 and April 2012 with severe sepsis (N=4,032) or septic shock (N=1,826) were screened for DP within 24 h of admission. Exclusion criteria:>100 RBC/hpf on microscopic UA; bacteriuria; urinary tract infection diagnosed by 24 h of admission; and within 3 months pre-admission, absence of UA testing, UA DP, or documented proteinuria by other means.

**Results:** 229 patients fulfilled study criteria. Bivariate analysis revealed that UA DP was associated with ICU mortality OR=3.91 (1.72-8.86), p=0.001 and hospital mortality OR=3.65, (1.77-7.53), p=0.001. Associations persisted in a Multivariate model that adjusted for ICU severity score (APACHE II), vasopressor use, age, and level of creatinine in the first 24 h post-admission.

| | Odds Ratio of ICU Mortality | | Odds Ratio of Hospital Mortality | |
|---|---|---|---|---|
| Parameter | Odds Ratio (95 CI%) | P-value | Odds Ratio (95 CI%) | P-value |
| Proteinuria on admission | 2.62 (1.08- 6.37) | 0.034 | 2.27 (1.04-4.97) | 0.040 |
| Vasopressor use | 3.17 (1.48 6.79) | 0.003 | 2.94 (1.46- 5.91) | 0.002 |
| APACHE II | 1.04 (0.97-1.10) | 0.282 | 1.03 (0.97-1.09) | 0.366 |
| Creatinine (*mg/dl*) | 1.16 (0.85- 1.58) | 0.349 | 1.16 (0.87-1.55) | 0.508 |
| Age (years) | 0.98 (0.95- 1.00) | 0.084 | 0.992 (0.97-1.02) | 0.319 |

**Conclusions:** In severe sepsis or septic shock ICU patients UA DP bear significant mortality risk for ICU and/or hospital mortality.

## FR-PO007

**Urinary Biomarkers and Differential Diagnosis of Acute Kidney Injury in Patients with Cirrhosis**

Justin Miles Belcher,[1] Guadalupe Garcia-Tsao,[1] Arun Sanyal,[2] Joseph K. Lim,[1] Mark A. Perazella,[1] Aldo J. Peixoto,[1] Chirag R. Parikh.[1] *[1]Yale Univ; [2]Virginia Commonwealth Univ.*

**Background:** Acute kidney injury (AKI) is common in patients with cirrhosis and carries a poor prognosis. Effective treatment relies upon accurate differential diagnosis of the cause of AKI but distinguishing acute tubular necrosis (ATN) from hepatorenal syndrome (HRS) is challenging. HRS is a hemodynamically mediated disease not associated with significant tubular injury. Utilizing rigorous clinical adjudication as a gold standard, biomarkers of structural AKI may therefore identify ATN in these patients.

**Methods:** 76 prospectively enrolled patients with cirrhosis and progressive AKI were blindly adjudicated by three physicians. A diagnosis of HRS, ATN or prerenal azotemia (PRA) was retrospectively made upon review of medical records, including AKI outcome. Urine samples collected upon meeting AKIN criteria were measured for neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), liver-type fatty acid binding protein (L-FABP), fractional excretion of sodium (FENa) and albumin.

**Results:** 39 (53%) patients were diagnosed with ATN, 19 (26%) with PRA and 16 (22%) with HRS. Median values for NGAL, L-FABP and albumin were significantly higher in patients with ATN while IL-18 demonstrating a strong trend. While FENa was significantly higher in patients with ATN compared to HRS, it did not reach overall statistical significance.

| | PRA N=19 | HRS N=16 | ATN N=39 | P Overall | P ATN vs HRS |
|---|---|---|---|---|---|
| *Tubular injury markers* | | | | | |
| **NGAL (ng/ml)** | 78 (16-206) | 115 (51-373) | 565 (76-1000) | 0.001 | 0.01 |
| **IL-18 (pg/ml)** | 46 (15-106) | 37 (15-90) | 124 (15-325) | 0.06 | 0.05 |
| **KIM-1 (ng/ml)** | 4.6 (2.6-12.7) | 7.6 (4.5-10.1) | 8.4 (4.1-18.3) | 0.13 | 0.40 |
| **L-FABP (ng/ml)** | 10 (3-20) | 14 (6-20) | 27 (8-103) | 0.05 | 0.17 |
| *Tubular function marker* | | | | | |
| **FENA (%)** | 0.28 (0.04-0.43) | 0.10 (0.02-0.23) | 0.31 (0.12-0.065) | 0.10 | 0.03 |
| *Glomerular injury marker* | | | | | |
| **Albumin (mg/dL)** | 21 (5-65) | 24 (13-129) | 92 (44-253) | 0.004 | 0.06 |

**Conclusions:** Multiple urinary biomarkers including NGAL, IL-18, L-FABP, albumin and FENa may distinguish ATN from HRS in patients with cirrhosis. Further studies are needed to determine if such biomarkers can guide treatment decisions.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO008

**Insulin-Like Growth Factor-Binding Protein 7 and Tissue Inhibitor of Metalloproteinases-2 Provide Superior Risk Assessment for Acute Kidney Injury in Critically Ill Patients**

Azra Bihorac, Lakhmir S. Chawla, Jay L. Koyner, Michael Heung, Luis M. Ortega, George E. DeMuth, John A. Kellum. *On behalf of All Astute Medical Topaz Investigators.*

**Background:** In the multi-center Sapphire study we demonstrated excellent performance of the combination of two urinary biomarkers, insulin-like growth factor-binding protein 7 and tissue inhibitor of metalloproteinases-2 (TIMP2•IGFBP7), for risk assessment for acute kidney injury (AKI) in critically ill patients (area under the receiver operating characteristic curve (AUC) 0.80, 95% confidence interval (CI) 0.76-0.83).

**Methods:** We report on a new clinical trial, Topaz, in which we validated an optimal cutoff for urinary TIMP2•IGFBP7 for AKI risk assessment among 408 prospectively enrolled critically ill patients in 23 hospitals in the United States. The high-sensitivity cut-off of 0.3 ((ng/ml)$^2$/1000) was pre-selected from the Sapphire cohort. Urinary TIMP2•IGFBP7 was measured at admission to the ICU using a clinical assay platform (NephroCheck® Test, Astute Medical Inc, San Diego, CA, not available in the US). The diagnosis of AKI was established by a Clinical Adjudication Committee (CAC) of three expert nephrologists. We also compared the performance of TIMP2•IGFBP7 with that of paired serum creatinine (sCr) measurements.

**Results:** The prevalence of AKI diagnosed by the CAC was 17.4% in the Topaz cohort. Urinary TIMP2•IGFBP7 was superior (AUC 0.82, 95% CI 0.76-0.88) to paired sCr measurements (AUC 0.63, 95% CI 0.56-0.70) in detecting risk for AKI (p<0.0001). The cutoff of 0.3 was validated with high sensitivity, negative predictive value, and relative risk. The majority (~90%) of patients that manifested AKI was identified as being high risk using the cutoff of 0.3.

| Cut-Off | Study | Sensitivity (95% CI) | Negative predictive value (95% CI) | Relative Risk (95% CI) |
|---|---|---|---|---|
| 0.3 (ng/ml)$^2$/1000 | Sapphire | 0.89 (0.82-0.94) | 0.97 (0.96-0.99) | 7 (4-14) |
| 0.3 (ng/ml)$^2$/1000 | Topaz | 0.92 (0.85-0.98) | 0.96 (0.93-0.99) | 7 (4-22) |

**Conclusions:** The Topaz study confirms excellent performance of urinary TIMP2•IGFBP7 and externally validates a clinical cutoff for AKI risk assessment among critically ill patients.

*Funding:* Pharmaceutical Company Support - Astute Medical, INC

**FR-PO009**

**Evaluation of New Urine Biomarker TIMP-2 in Intensive Care Unit**

Tetsushi Yamashita, Kent Doi, Maki Tsukamoto, Yoshifumi Hamasaki, Masaomi Nangaku, Naoki Yahagi, Eisei Noiri. *The Univ of Tokyo, Tokyo, Japan.*

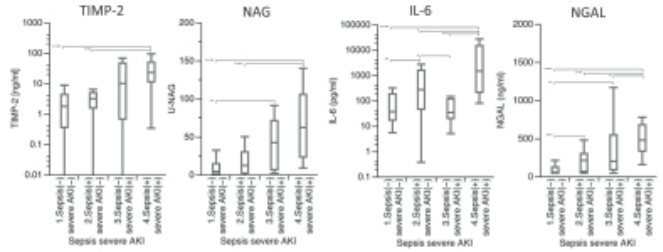
**Background:** Tissue inhibitor of metalloproteinases-2 (TIMP-2) was recently reported as a novel for predicting severe AKI in critical ill patients. We evaluated the performance of urine TIMP-2 in our adult mixed intensive care unit (ICU).

**Methods:** This study enrolled 98 patients who were admitted to the adult mixed ICU of The University of Tokyo Hospital from July 2011 to October 2011 by consecutive sampling. Urine TIMP-2 and NAG, and plasma NGAL and IL-6 were measured on ICU admission. This study was aimed to evaluate whether these biomarkers could predict AKI and its severity, and mortality by ROC analysis.

**Results:** AKI occurred in 42 (42.9%) patients including 27 (27.6%) severe AKI (KDIGO stage 2 or 3). The area under the ROC curve for each marker was shown in Table 1.

| AUROC | TIMP-2 | NAG | NGAL | IL-6 |
|---|---|---|---|---|
| AKI | 0.74 (0.63-0.83) | 0.84 (0.74-0.90) | 0.84 (0.74-0.91) | 0.72 (0.60-0.81) |
| Severe AKI | 0.80 (0.66-0.90) | 0.89 (0.78-0.94) | 0.87 (0.76-0.93) | 0.69 (0.59-0.80) |

Forty one (41.8%) patients was complicated with sepsis, including 19 (19.4%) severe AKI. In accordance with previous reports, plasma NGAL and IL-6 were increased by sepsis, however urine TIMP-2 and NAG was increased not by sepsis but by the presence of severe AKI.




In-hospital mortality was 15.3% in this cohort and urine TIMP-2 and NAG, and plasma NGAL were significantly higher in the non-survivors than the survivors, whereas plasma IL-6 was not significantly associated with mortality.

**Conclusions:** A new urine biomarker of TIMP-2 is increased especially in severe AKI and associated with mortality. Sepsis appeared to have a smaller impact on urine TIMP-2 and NAG compared with plasma NGAL and IL-6. This distinct feature of biomarkers will enable to evaluate the contribution of sepsis to the development of AKI.

*Funding:* Government Support - Non-U.S.

**FR-PO010**

**Biomarkers for Acute Kidney Injury in the Emergency Department**

Martin Kimmel,[1] Jing Shi,[2] Joerg Latus,[1] Niko Braun,[1] Mark Dominik Alscher.[1] *[1]Dept of Internal Medicine, Div of Nephrology, Robert-Bosch Hospital, Stuttgart, Germany; [2]Walker Bioscience, Carlsbad, CA.*

**Background:** There is a growing role of emergency departments (ED) for hospital admissions but there are only few studies addressing acute kidney injury in ED. Two novel urinary biomarkers of acute kidney injury (AKI) were recently reported for risk stratification for AKI and the combination of these biomarkers (TIMP2•IGFBP7) was found to be superior to other known biomarkers of AKI in critically ill hospitalized patients. We have performed an analysis of TIMP2•IGFBP7 in patients presenting to the emergency department.

**Methods:** We enrolled 397 patients in the Emergency Department at Robert-Bosch-Krankenhaus, Stuttgart. Daily serum creatinine values and urine output were recorded for AKI staging by KDIGO criteria (302 patients had urine output data). Biomarkers were analyzed in samples collected during the first day (at 0, 6 and 24h) after enrollment. Cystatin C, KIM-1, L-FABP and NGAL were measured in blood and urine and IGFBP7, TIMP2, and IL-18 were measured in urine samples. We examined the association between these biomarkers and KDIGO stage 2 or 3 within 12 hours of sample collection using univariate and multivariate generalized estimating equation (GEE) logistic regression.

**Results:** All urinary biomarkers tested had a statistically significant ($p<0.05$) univariate odds ratio for KDIGO stage 2 or 3 within 12 hours. Of the markers measured in blood, only serum creatinine and plasma cystatin C had a significant univariate odds ratio. In a multivariate GEE logistic regression model including all the urine markers and serum creatinine, only TIMP2•IGFBP7 and serum creatinine had statistically significant adjusted odds ratios (standardized odds ratio=2.4 (95% CI,1.4-4.4) and 1.9 (95% CI,1.3-2.9), respectively) for patients with urine output data.

**Conclusions:** In conclusion, our study is the first to report TIMP2•IGFBP7 in urine from a cohort of emergency department patients. Our results show that TIMP2•IGFBP7, associated with mechanisms recently implicated in the pathogenesis of AKI, provides valuable information for risk assessment in the emergency department.

*Funding:* Pharmaceutical Company Support - Astute Medical, Private Foundation Support

**FR-PO011**

**Diagnostic Utility of Biomarkers of Acute Kidney Injury in Critically Ill Patients**

Claire Hannon,[1] Patrick T. Murray.[2] *[1]Dept of Nephrology, Mater Univ Hospital, Ireland; [2]Univ College Dublin, Ireland.*

**Background:** Acute kidney injury (AKI) occurs in 5-10% of hospitalized patients, with a mortality of approximately 50% in a critical care setting. Diagnosis is based on changes in serum creatinine, a late marker of injury. Development of AKI biomarkers may permit earlier detection of AKI and allow improvements in its prevention and therapy.

**Methods:** All patients admitted to the intensive care unit (ICU) were screened for inclusion in this prospective cohort study. Clinical information and urine was collected daily for 7 days. Urine biomarkers analysed were neutrophil-gelatinase associated lipocalin (NGAL), $\alpha$ and $\pi$ glutathione-S-transferase (GST), albumin and creatinine. AKI was defined by KDIGO criteria using ICU admission creatinine as reference. ROC curves were generated for admission biomarkers and AUC's calculated for prediction of AKI and a composite endpoint of death and need for renal replacement therapy (RRT). Admission biomarkers were collected at enrolment, within 24 hours of ICU admission.

**Results:** 201 patients were enrolled, 90 patients (45.3%) developed AKI. AKI stages: stage 1 (19.9%, n=40), stage 2 (6%, n=12) and stage 3 (18.9%, n=38). Patients with AKI were significantly older (67 v 59 years, p=0.003), had a higher incidence of chronic kidney disease (24.4 v 7.3%, p=0.001) and a higher mortality (44.4 v 28.8%, p=0.011). 40% of patients with AKI needed RRT. Moderate to good AUC's for AKI prediction were seen with urine NGAL (0.723, $p<0.0001$) and albumin (0.689, $p<0.0001$). The AUC's of urine creatinine, $\alpha$ and $\pi$ GST were non-significant. Combination of admission urine NGAL and urine albumin and APACHE II score improved prediction of AKI with an AUC of 0.770 ($p=0.04$). Prediction of a composite end-point of death or dialysis using admission urine albumin and NGAL was moderate, with AUCs of 0.639 ($p=0.0008$) and 0.596 ($p=0.0186$) respectively.

**Conclusions:** Admission urine NGAL and albumin showed moderate to good ability at AKI prediction**,** combination with a clinical severity score further improved this. Biomarkers and clinical model combinations may improve AKI diagnosis and prediction.

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**FR-PO012**

**Positive Fluid Balance (PFB) and Hospital Mortality in Severe Septic Patients with or without Acute Kidney Injury (AKI)**

Javier A. Neyra,[1] Fabrizio Canepa,[2] John Manllo,[2] Beverley Adams-huet,[1] Robert D. Toto,[1] Jerry Yee.[2] *[1]Univ of Texas Southwestern Medical Center, Dallas, TX; [2]Henry Ford Hospital, Detroit, MI.*

**Background:** Severe sepsis causes increased capillary permeability, impaired renal excretion of exogenous sodium and slow vascular refilling, thereby promoting interstitial edema and adverse outcomes. AKI occurs in up to 70% of those with severe sepsis and intravenous fluid therapy is directed at restoring organ hypoperfusion. The aim of this study is to evaluate the relationship between PFB and hospital mortality by AKI status in non-CKD patients admitted to the ICU with severe sepsis.

**Methods:** A population-based linked administrative database of 2,786 septic patients was analyzed for the association of PFB (> 5 L) within the first 72 h of ICU admission and death. Our study group comprised 1,854 non-CKD patients (SCr < 1.5 mg/dL within 3 mo prior to admission) stratified by AKI status. Severe sepsis was defined by *Angus et al* criteria and AKI by Acute Kidney Injury Network. Multivariable logistic regression models were adjusted for age, gender, race, baseline SCr, comorbidities, vasoactive drugs, diuretic exposure, blood transfusion, and SOFA and APACHE II scores.

**Results:** Mean age (SD) was 65 (16) years: 39% were African American and 39% Caucasian. At time of admission, 47% (867/1,854) had AKI (Group A), 12% (232/1,854) developed AKI (Group B), and 41% (755/1,854) had no AKI within 72 h of admission (Group C). The mean cumulative fluid balance (SD) was 5.3 L (7.3) in Group A, 5.2 L (8.1) in Group B, and 2.8 L (5.4) in Group C. During hospitalization, 398 (21%) died: Group A, 219/867 (25%); Group B, 74/232 (32%); and Group C, 105/755 (14%). In stratified, adjusted models, PFB was significantly associated with hospital mortality in all groups: Group A, OR (95% CI) 2.4 (1.7, 3.4); Group B, 2.3 (1.2, 4.6); and Group C, 1.8 (1.1, 2.9).

**Conclusions:** In this study population of non-CKD patients with severe sepsis, PFB (> 5 L) was an independent predictor of hospital mortality in patients with or without AKI. These findings highlight the importance of individualized response-based fluid therapy, even in patients without significant increases in SCr within the first 72 h of ICU admission.

*Funding:* Clinical Revenue Support

**FR-PO013**

**Impact of Fluid Balance after Initiation of Renal Replacement Therapy (RRT) on Mortality in Critically Ill Patients with Acute Kidney Injury (AKI)**

Yee Lu,[1] Puja Goswami,[1] Robin L. Sands,[2] Michael Heung.[1] *[1]Dept of Medicine, Univ of MI, Ann Arbor, MI; [2]Univ of MI, Kidney Epidemiology and Cost Center, Ann Arbor, MI.*

**Background:** In critically ill patients with AKI, fluid overload (FO) at RRT initiation has been associated with increased mortality. However, the impact of fluid management during RRT is less clear. We examined fluid balance following RRT initiation for an association with hospital mortality.

**Methods:** Retrospective chart review of adult patients admitted to an intensive care unit (ICU) requiring RRT for AKI from January 2011 to January 2012 at a tertiary academic center. Patients initiated on RRT prior to ICU admission or for toxic ingestions were excluded. FO was defined as the cumulative fluid balance from ICU admit to RRT initiation expressed as a percentage of admit weight. Time-dependent Cox regression was used to assess the association between FO in the first eight days of RRT and hospital mortality, adjusted for sequential organ failure assessment score and history of malignancy.

**Results:** 149 ICU patients were included: mean age 54 ± 15y, 60% male, 74% white, mean baseline eGFR 81 ± 35 ml/min/1.73m$^2$ and mean SOFA score prior to RRT 13 ± 4. Hospital mortality was 56%. Mean FO at RRT start was 9.5 ± 11.3% and was correlated with FO on last day of RRT (r=0.83, p<0.0001). In unadjusted analysis, FO at RRT start was associated with an 80% higher risk among patients with FO>10.4% (3$^{rd}$ quartile) compared to patients with FO ≤ 3.4% (1$^{st}$ quartile; p=0.03); this association was not significant after adjustment. FO over the first eight days of RRT, adjusted for FO at RRT start, was significantly associated with risk of mortality in both unadjusted and adjusted analysis ($HR_{per\ 10\%}$=1.81; 95% CI 1.77-1.85, p<0.0001).

**Conclusions:** We found that FO at RRT initiation was associated with increased mortality, but this relationship was not significant after adjusting for severity of illness. Conversely, the ability to achieve a negative fluid balance following the initiation of RRT was associated with lower hospital mortality. Further studies are required to determine whether this finding represents an opportunity to positively intervene in this population.

## FR-PO014

### A New Urinary Scoring System for Differential Diagnosis of Acute Renal Injuries

Rong Chu,[1] Hua Xiao Liu,[1,2] Zi Jing Li,[1] Li Yang.[1] *[1]Renal Div, Peking Univ First Hospital, Beijing, China; [2]Renal Div, NingDe Hospital, NingDe, Fujian, China.*

**Background:** AKI is a clinical syndrome encompassing various etiologies that involve any of the renal tubules, interstitium, glomerulus or vasculatures. We developed a prospective cohort study to explore a new urinary scoring system that may help locate the pathogenic lesions of parenchymal acute renal injuries.

**Methods:** 165 AKI patients (2012-KDIGO) who were histologically proven as parenchymal acute renal injuries from Jun 2011 to June 2013 were enrolled. Urinary sediment examination was performed in the morning of renal biopsy by two independent nephrologists. 24hr-urinary protein was tested. A urinary scoring system was made from 50 AKI patients and validated in the following 115 AKI cases using histology as gold standard.

**Results:** The urinary scoring system originated from 15 cases with acute tubular necrosis (ATN, 13 with glomerular nephropathy, GN), 18 cases with acute tubulointerstitial nephritis (ATIN, 8 with GN), 15 cases with diffuse acute glomerular nephritic lesions (AGN, cellular crescentic GN etc) and 2 cases with vascular injuries.

| Pathological lesions | urinary indices | semiquantitative scores | scoring system |
|---|---|---|---|
| Glomerular (G) | RBC/HP | 0: <3<br>1: 3-10<br>2: 11-30<br>3: >30 or RBC casts | $G_{RBC}$+$G_{PRO}$<br>>=3: with GN<br><3: w/o GN |
| | Protein (g/24hr) | 0: <0.3<br>1: 0.3-2.0<br>2: 2.1-3.5<br>3: >3.5 | |
| Tubular injury (T) | Tubular epithelial cells /HPF | 0: <1<br>1: 1-5<br>2: >5<br>3: tubular epithelial cell casts | T<br>>=3: with ATN<br><1: w/o ATN |
| Inflammation (I) | WBC/HPF | 0: <5<br>1: 5-10<br>2:10-20<br>3: >20 or WBC casts | I<br>>=2: with inflammation<br><1: w/o inflammation |

Validation was performed in 35 ATN cases, 33 ATIN cases, 35 AGN cases and 12 vascular injuries cases. The urinary scoring system recognized 76.9% of ATN, 66.7% of ATIN and 91.2% of diffuse AGN. Patients with vascular injuries including TMA and malignant hypertension had "clean" urinary sediment examination and did not fit the scoring system.

**Conclusions:** Urinary scoring system may help differentiate acute renal parenchymal injuries. More cases are collecting to enlarge the sample size of the study. Urinary biomarkers, including NAG, a1-MG, KIM-1, NGAL, MCP-1 and IL-18, were tested. Analysis of combining the scoring system and biomarkers is processing.

## FR-PO015

### Glycosylated Protein CD147 Reflects Renal Dysfunction in Patients with Acute Tubular Necrosis

Hiroshi Kojima, Tomoki Kosugi, Mayuko Maeda, Kayaho Maeda, Yuka Sato, Hiroki Hayashi, Waichi Sato, Seiichi Matsuo, Shoichi Maruyama. *Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Aichi, Japan.*

**Background:** Acute tubular injury (ATN) describes a form of intrinsic acute kidney injury (AKI) that results from persistent hypoperfusion and subsequent inflammation in the kidney. A glycoprotein CD147 contributes to cell survival and cancer invasion. Recently, we demonstrated that CD147 is responsible for chronic inflammation in the kidney, using CD147 knockout mice. In addition, hypoxia induced CD147 expression in TECs. We therefore investigated whether plasma and urinary CD147 could reflect disease activity of ATN.

**Methods:** Experiment (Exp.) 1: Plasma and spot urine samples were collected from the 24 patients, who underwent renal biopsy between 2008 and 2012. They included pathological control (n=12) and ATN (n=12). Exp. 2: 40 patients are registered undergoing open surgery to treat abdominal aortic aneurysms (AAA) in 2004 at our hospital. We collected 160 urine samples from 7 and 33 patients with and without AKI, respectively. In both experiments, plasma and urinary CD147 levels were measured, and its expression in kidneys was examined by immunostaining. We further examined urinary L-fatty acid binding protein (L-FABP), ATP and 8-OHdG levels.

**Results:** Exp. 1: CD147 expression, mainly detected in TECs of healthy kidneys, was extremely lower in injured tubules of ATN patients. CD147 induction was found in macrophages and fibroblasts around damaged tubules and vessels. Both plasma and urinary CD147 values strikingly increased in ATN patients compared to control. Both levels were correlated with serum creatinine (Cre) and ischemia-related factors, including L-FABP, ATP and 8-OHdG. Surprisingly, plasma CD147 showed greater correlations with pathological injuries and renal dysfunction compared with L-FABP. Experiment 2: While there was no differences in CD147 values and Cre before AAA operation between patients with or without AKI, mean CD147 level in patients with AKI was significantly higher than those with non-AKI towards post-operative day 1.

**Conclusions:** CD147 may be a prime candidate for developing a new procedure for the evaluation of AKI.

## FR-PO016

### Urinary Excretion of Kidney Injury Molecule 1 Predicts the Need for Extra-Renal Replacement Therapy in Cirrhotic Patients Admitted to the Intensive Care Unit

Justine Devos,[1] Francois Jouret,[2] Yvan Fleury,[1] Pierre-françois Laterre,[1] Diego Castanares-zapatero.[1] *[1]Cliniques Universitaires Saint-Luc (UCL, Brussels); [2]Univ of Liege Hospital (ULg CHU, Liege).*

**Background:** Detecting acute kidney injury (AKI) requiring extra-renal replacement therapy (ERRT) remains challenging in cirrhotic patients admitted to the Intensive Care Unit (ICU). Still, AKI is associated with an increased mortality. Here, we prospectively investigated the usefulness of the urinary biomarker of kidney damage, kidney injury molecule 1 (uKIM-1), in predicting the need for ERRT in ICU patients with decompensated cirrhosis.

**Methods:** Consecutive patients admitted to the ICU of Cliniques Universitaires Saint-Luc, Brussels, for cirrhosis-related complications were prospectively enrolled. Patients with chronic kidney disease were excluded. Serum creatinine levels (sCr) and urinary excretion of KIM-1 were measured at 24h and 48h post admission. A multiple logistic regression for ERRT prediction was performed after adjustment for age, gender, sepsis, MELD score.

**Results:** The cohort included 109 patients. Main causes of admission were gastro-intestinal bleeding (38.5%) and sepsis (33.2%). Twenty-seven patients (24.7%) developed AKI requiring ERRT. No ERRT was initiated before day 2. Mortality rate (74.1% vs 35.3%, p<0.05) and MELD score (27.8 vs 20.1, p<0.05) were significantly higher in ERRT-treated patients. No correlation was found in these patients regarding the evolution of sCr and uKIM-1 within the first 48h. Indeed, sCr decreased over time, whereas uKIM-1 further increased. At day 2, uKIM-1 was significantly higher in patients who required ERRT (17.1 vs 9.7 ng/ml, p=0.027). In logistic regression analysis, uKIM-1 > 10 ng/mL at day 2 predicted the need for ERRT.

**Conclusions:** At day 2 post ICU admission, uKIM-1 helps better identify patients with decompensated cirrhosis at risk for ERRT.

*Funding:* Clinical Revenue Support

## FR-PO017

### The Monomer Is the Major Form of NGAL in Urine and Plasma in Severe Sepsis

Kristian Bangert,[1] Alexandra Baer,[1] Peter Buhl Hjortrup,[2] Anders Perner,[2] Lars O. Uttenthal.[1] *[1]BioPorto Diagnostics A/S, Denmark; [2]Rigshospitalet, Denmark.*

**Background:** Neutrophil gelatinase-associated lipocalin (NGAL) is a marker of acute kidney injury (AKI), but is also released from neutrophils on phagocytosis of bacteria and as part of the inflammatory response. It has been reported that the injured kidney releases NGAL in its monomer form. In contrast, the neutrophils are claimed to release NGAL primarily as homodimer. Patients with severe sepsis have both a high release of NGAL from the neutrophils and a high risk of AKI. We have measured the levels of NGAL monomer and homodimer in urine and plasma samples from patients suffering from severe sepsis.

**Methods:** NGAL monomer and homodimer were measured with specific ELISAs in sets of urine and EDTA plasma samples from 151 patients suffering from severe sepsis and compared with NGAL measured with a fully automated particle-enhanced turbidimetric immunoassay (The NGAL Test™, BioPorto Diagnostics A/S). AKI was defined as a 50% increase in plasma creatinine levels.

**Results:** AKI occurred in 83 of the 151 patients. The NGAL levels and the area under the receiver operating characteristics curve (AUROC) for the diagnosis of AKI are shown in the table:

| | NGAL immunoassay | Median value ng/mL | 25$^{th}$ and 75$^{th}$ percentiles ng/mL | AUROC | 95% confidence interval |
|---|---|---|---|---|---|
| **Urine** | Homodimer (% of total* NGAL) | 8.5 (1.2%) | 1.2 - 31.4 (1.0% - 2.6%) | 0.70 | 0.62 – 0.79 |
| | Monomer | 320 | 79.6 - 1914 | 0.75 | 0.67 – 0.82 |
| | The NGAL Test™ | 341 | 79.9 - 2012 | 0.74 | 0.66 – 0.82 |
| **EDTA plasma** | Homodimer (% of total* NGAL) | 31.5 (6.0%) | 17.2 - 51.4 (3.3% - 11.3%) | 0.59 | 0.50 – 0.68 |
| | Monomer | 455 | 230 - 850 | 0.78 | 0.70 – 0.85 |
| | The NGAL Test™ | 484 | 247 - 839 | 0.78 | 0.71 – 0.85 |

*Total NGAL = Monomer + Homodimer values.

**Conclusions:** NGAL monomer is the major form of NGAL found in urine and plasma from patients with severe sepsis. The occasional presence of low levels of NGAL homodimer has little impact on the interpretation of results obtained with monomer-reactive, homodimer cross-reactive NGAL assays in samples from patients with severe sepsis.

### FR-PO018

**Urine and Plasma Neutrophil Gelatinase Associated Lipocalin (NGAL), the Novel Biomarkers for Leptospirosis Associated Acute Kidney Injury (AKI): Result from the Multicenter Study** Nattachai Srisawat,[1,3] Kearkiat Praditpornsilpa,[1] Khajohn Tiranathanagul,[1] Sarinya Kumpunya,[1] Somchai Eiam-ong,[1] Kriang Tungsanga,[1] Visith Sitprija,[1,2] John A. Kellum.[3] *[1]Dept of Medicine, King Chulalongkorn Memorial Hospital, Thailand; [2]Queen Saovabha Memorial Institute, Thai Red Cross, Thailand; [3]The CRISMA Center, Dept of Critical Care Medicine, Univ of Pittsburgh School of Medicine.*

**Background:** AKI is one of the most serious complications of leptospirosis, an important zoonosis in the tropics. NGAL which represents as an early biomarker AKI has never been explored in this specific setting. In this multicenter study, we aimed to study the role of NGAL as an early marker and an outcome predictor of leptospirosis associated AKI.

**Methods:** Patients who presented with clinical suspiciousness of leptospirosis were prospectively enrolled in 11 centers. Blood and urine samples were serially collected on the first three days. We used three standard techniques (microscopic agglutination test, direct culture, and PCR technique) to confirm the diagnosis of leptospirosis. KDIGO criteria was used as the standard criteria for AKI diagnosis. Recovery was defined as alive and not requiring dialysis during hospitalization or having a persistent KDIGO staging at hospital discharge.

**Results:** Of the 89 recruited patients, 54 subjects were diagnosed as leptospirosis. Forty five percent had AKI. Median uNGAL and pNGAL levels in AKI group were significantly higher than non-AKI group [187.8 (51.7,724.6) vs 10.7 (2.2,35.9) ng/ml, $p < 0.001$] and [1300 (348,1300) vs 173 (86,238) ng/ml, $p < 0.001$], respectively. uNGAL and pNGAL levels predicted AKI with AUC-ROC of 0.89, and 0.90, respectively). Median uNGAL level in recovery group was significantly lower than non-recovery group [156.7 (38.0,551.2) vs 843.7 (256.8,922.2) ng/ml, $p = 0.039$], while pNGAL level was comparable. uNGAL and pNGAL predict renal recovery with AUC-ROC of 0.893 and 0.59, respectively).

**Conclusions:** From this multicenter study, uNGAL and pNGAL provided the promising result to be a marker for leptospirosis associated AKI. However, only uNGAL showed the potential role to be the predictor of renal recovery in this specific setting.

### FR-PO019

**The Renal Klotho Expression Is Associated with the Severity of Human AKI** Myung-gyu Kim, Sang-Kyung Jo, Won-Yong Cho. *Nephrology, Korea Univ Anam Hospital, Seoul, Republic of Korea.*

**Background:** Klotho is highly expressed in the kidney and known to have multiple functions including renoprotection from a variety of insult. Especially, its diagnostic and therapeutic roles in acute kidney injury (AKI) have been demonstrated recently in animal models. However, to date, there is no human study to determine renal Klotho in AKI. Therefore, here we examined renal Klotho expression in AKI patients and its clinical value.

**Methods:** We retrospectively collected kidney specimen and clinical data of AKI patients who underwent renal biopsy between January 2001 and December 2012 at Korea university hospital.The protein expression of Klotho was determined by immunohistochemical staining and clinical-pathological correlation was examined.

**Results:** Among 34 patients who were diagnosed with acute tubular necrosis or acute tubulointerstitial nephritis, a total of 21 patients without chronic histological lesion were included in the study. The mean age was 37.3±18.5years, and the causes of AKI were ischemic in 1 case(4.8%), infection in 7(33.3%), drug in 2(9.5%), rhabdomyolysis in 4(19.0%) and the others in 7(33.3%). Their peak creatinine level was 8.16±5.54mg/dL and 10 patients(47.6%) have received renal replacement therapy(RRT) temporarily. However, most of patients(81%) had functional recovery with creatinine level below 1.3mg/dL 3months after their discharge. When the Klotho expression was scored according to the percentage of area of positive cells, its low level was correlated to severe AKI. In addition, when the patients were divided into three groups according to the Klotho score (low, mid, high), the low group had the highest peak creatinine level, the longest hospital stay and the more RRT treatment significantly. In multivariate analysis, low Klotho score was also an independent factor associated with the development of severe AKI. However, Klotho score was not significant as a predictor for renal recovery or progression to chronic kidney disease.

**Conclusions:** This is a first human study which demonstrated that human renal Klotho expression was decreased significantly according to the severity of AKI regardless of etiology, and their low expression was associated with poor short-term outcome.

### FR-PO020

**Urinary NGAL (Neutrophil Gelatinase Associated Lipocalin) Excretion at Birth Is Predictive of Susceptibility to Acute Kidney Injury (AKI) in Very Low Birth Weight Infants** Licia Peruzzi,[1] Federica Chiale,[1] Roberta Camilla,[1] Giuliana Guido,[1] Claudio Martano,[2] Enrico Bertino,[2] Rosanna Coppo.[1] *[1]Nephrology Dialysis Transplanation, Regina Margherita Hospital, Italy; [2]Neonatology, Univ of Turin.*

**Background:** Preterm infants are susceptible to acute kidney injury (AKI); closure of patent ductus arteriosus (PDA) with prostaglandin inhibitors is a risk factor. Novel AKI biomarkers have not been assessed in their predictive value in very preterm infants.

We evaluated in very low birth weight (VLBW) infants the urinary excretion of NGAL as early AKI biomarker during treatment with ibuprofen for PDA.

**Methods:** We evaluated 50 VLBW (≤1500 g and/or gestational age ≤32 w) without congenital nephropaties, 19 treated with ibuprofen. Renal function was assessed by creatinine (sCr) (IDMS method) within 72 hours from birth, at 7 and 14 days and post ibuprofen (standard 3 doses). eGFR was calculated (Schwartz formula, k=0.413). uNGAL was measured with immunoassay (ARCHITECT®, Abbott) at the same time of sCr and 12-24h after each ibuprofen dose.

**Results:** Newborns with PDA had lower gestational age (28.37±2.79w. vs 30.61±2.17w, p 0.0045), lower APGAR score (6.63±1.83. vs 8.16±0.97, p 0.0009), required more ventilatory support (74% vs 16%, p<0.001) than untreated. At birth sCr and uNGAL were similar (sCr:1.02±0.34 mg/dl vs 0.91±0.19; uNGAL:81.65 ng/ml (4-1109) vs 41.40 ng/ml (13.30-215.1). p ns). In treated infants uNGAL rose significantly after first dose of ibuprofen (from 81.65 ng/ml to 188 ng/ml p 0.0067) and decreased after 7 days to 52.96 ng/ml (2.5-1046) (p 0.03), significantly higher than in untreated (52.96 vs 18.50 ng/ml,p 0.027).

9 children (7 treated, 2 untreated developed AKI according to AKIN criteria modified by Jetton2012: 4 stage 0, 3 stage 1, 2 stage 3 within the first week). uNGAL at birth was predictive of AKI, being higher in those who developed AKI (401.1±405ng/ml vs 41.60±52.3; p 0.03), while sCr was not predictive. ROC analysis showed a cut-off of 122.4 ng/ml discriminating infants at risk for AKI with specificity 76.92%, sensibility 75% and AUC values 0.75 (p 0.03).

**Conclusions:** uNGAL is a sensible and specific biomarker to monitor AKI in preterm infants treated with ibuprofen.

### FR-PO021

**Perioperative Plasma NGAL Measurement in LVAD Implantation Surgery** Maki Tsukamoto, Kent Doi, Minoru Ono, Naoki Yahagi, Masaomi Nangaku, Eisei Noiri. *The Univ of Tokyo.*

**Background:** Implantation of left ventricular assist device (LVAD) has recently been conducted for the treatment of severe heart failure. Perioperative complication of end-organ injury including acute kidney injury (AKI) is a frequent and serious problem in LAVD implantation. We evaluated an emerging biomarker plasma neutrophil gelatinase-associated lipocalin (NGAL), which can detect AKI before serum creatinine elevation, in a LVAD implantation cohort.

**Methods:** We prospectively studied 31 severe heart failure patients underwent LVAD implantation during the period of July 2011 to March 2013 at the University of Tokyo Hospital. Diagnosis of AKI was determined by the KDIGO creatinine criteria. Plasma NGAL was measured at pre-operation, just after operation (0 hr), and post-operative day (POD) 1, 7, 14, and 28. For severe AKI patients, plasma NGAL was continuously measured until discontinuation of dialysis or death.

**Results:** Causes of severe heart failure in this cohort were as follows; idiopathic dilated cardiomyopathy (n=20), ischemic cardiac disease (n=5), hypertrophic cardiomyopathy (n=3) and severe myocarditis (n=3). Seventeen (55%) patients were diagnosed as AKI and dialysis was required in 6 patients. Plasma NGAL values in the dialysis-requiring AKI group were significantly higher than the other patients at all the measurement time points, whereas the non-dialysis-requiring AKI group showed similar levels of plasma NGAL to the non-AKI group. AKI measured at pre-operation and ICU arrival after surgery (0 hr) could discriminate the dialysis-requiring AKI group from the non-dialysis-requiring AKI and the non-AKI groups (AUC-ROC 0.83 [95%CI 0.54-0.95] and 0.86 [0.66-0.95]). Dialysis could be discontinued in 4 of 6 severe AKI patients and their plasma NGAL levels decreased to 300 ng/ml or lower at termination of dialysis.

**Conclusions:** Measurement of perioperative plasma NGAL in LVAD implantation surgery will be useful for predicting severe AKI that requires dialysis and may potentially be used for predicting discontinuation of dialysis.

### FR-PO022

**Value of Plasmatic NGAL Levels in Assessing Severity and Outcomes in Acute Kidney Injury** Eva Rodriguez, Marta Riera, Maria Jose Soler, Clara Barrios, Julio Pascual. *Nephrology, Parc de Salut Mar-IMIM, Barcelona, Spain.*

**Background:** Plasmatic Neutrophil Gelatinase-Associated Lipocalin (NGAL) could be a good tubular biomarker for the diagnosis of Acute Kidney Injury (AKI), before creatinine elevation. Our aim was to assess if plasmatic NGAL at the time of AKI diagnosis is a good marker for identification of those patients who will develop a more severe form of AKI.

**Methods:** In a prospective cohort study in a tertiary hospital, 134 patients with different conditions known to be at high risk of AKI were included. One plasma-EDTA sample was collected at diagnosis, Four groups were pre-specified: septic patients (septic model of AKI, n=27,,52% with AKI vs 48% non-AKI), patients under colistin treatment (nephrotoxic model, n=50, 46% AKI vs 54% non-AKI), renal allograft recipients (ischemia-reperfusion model, n=40, 72.5% AKI vs 27.5% non-AKI), elderly patients with dehydration and ACE-I treatment (multifactorial-AKI, n=17). Plasmatic NGAL was measured by means of inmunofluorescence assay. Results were expressed in mean ± standard error.

**Results:** In our patient population, 52% had AKI and 48% normal kidney function. Plasmatic NGAL levels were significant higher in AKI patients (582 ± 44ng/mL vs 316± 41 ng/mL; $p<0.001$). NGAL levels were significantly higher in AKI-patients who needed renal replacement therapy during hospitalization compared with those who did not need such treatment (715±73 ng/mL vs 499±48 ng/mL; $p=0.014$) and significantly higher in AKI-patients who died (793±111 vs 442 ng/mL; $p<0.001$). Finally, NGAL levels were significantly lower in those AKI-patients who recovered renal function at time of discharge (258 ±48 ng/mL vs 336 ± 73 ng/mL, p=0.01). The receiver operating characteristic (ROC)

curve assessing the NGAL plasmatic value accuracy for the need of renal replacement treatment showed an AUC of 0.80 (CI 95% 0,57-0,87, p=0,03) and for the accuracy of death during AKI an AUC of 0,89 (CI 95% 0,54-0,90, p=0,01).

**Conclusions:** : Plasmatic NGAL could be useful to distinguish AKI patients who are at high risk for renal replacement therapy or will die during the event, and also to identify those patients who will recover renal function.

## FR-PO023

**Urine Stability Studies for Novel Biomarkers of Acute Kidney Injury** Chirag R. Parikh,[1] Isabel Butrymowicz,[1] Angela Yu,[1] Vernon M. Chinchilli,[5] Meyeon Park,[3] Chi-yuan Hsu,[3] William Brian Reeves,[5] Prasad Devarajan,[7] Paul L. Kimmel,[6] Edward D. Siew,[4] Kathleen D. Liu.[3] *[1]Yale School of Medicine; [2]Penn State College of Medicine; [3]Univ of California: San Francisco; [4]Vanderbilt Univ Medical Center; [5]NIDDK/NIH; [6]Cincinnati's Children's Hospital Medical Center, For the ASSESS-AKI Study.*

**Background:** The effect of variation in storage conditions on biomarker assays may affect accuracy and reliability of measurements. We examined how variations in short-term storage/processing affect measurement of urine Neutrophil Gelatinase-Associated Lipocalin (NGAL), Interleukin-18 (IL-18), Kidney Injury Marker-1 (KIM-1), Liver Fatty Acid Binding Protein (L-FABP) and Cystatin C in ASSESS-AKI, a multi-center study of hospitalized patients with and without AKI.

**Methods:** The impact of 3 processing conditions were tested: a) centrifugation and storage at +4°C for 48h before freezing at -80°C, b) centrifugation and storage at +25°C for 48h before freezing, and c) uncentrifuged samples immediately frozen. Biomarker values were log-transformed, and agreement with a reference standard of immediate centrifugation, aliquoting, and storage at -80°C was compared using concordance correlation coefficients (CCC).

**Results:** There was excellent CCC (>0.9) for all biomarkers for all processes, except IL-18 at +25°C.Good agreement was observed for IL-18 between samples stored at +25°C for 48h and the reference standard (0.81 (95% CI 0.66,0.96)).

| Processes | IL-18 | NGAL | KIM-1 | L-FABP | Cystatin C |
|---|---|---|---|---|---|
| A (Initial 48 hours: + 4ºC vs -80ºC) | 0.92 (0.85, 0.98) | 0.99 (0.98, 1.00) | 0.99 (0.99, 1.00) | 0.99 (0.98, 1.00) | 0.94 (0.89, 0.99) |
| B (Initial 48 hours: + 25ºC vs -80ºC) | 0.81 (0.66, 0.96) | 0.99 (0.98, 1.00) | 0.99 (0.99, 1.00) | 0.95 (0.92, 0.99) | 0.93 (0.87, 0.99) |
| C (Centrifuge vs No Centrifuge) | 0.98 (0.96, 1.00) | 0.99 (0.98, 1.00) | 0.99 (0.99, 1.00) | 0.99 (0.98, 1.00) | 0.99 (0.99, 1.00) |

**Conclusions:** All candidate markers tested showed high stability with short-term storage at +4°C or without centrifugation prior to freezing. For optimal fidelity, urine for IL-18 measurement should not be stored at +25°C before long-term storage.

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## FR-PO024

**No Increase in Kidney Injury Molecule-1 and Neutrophil-Gelatinase Associated Lipocalin Excretion following Intravenous Contrast Enhanced-CT** Judith Kooiman,[1] Wilke R. Van de Peppel,[1] Yvo W.J. Sijpkens,[2] Harald Brulez,[3] Jean-paul P.M. De Vries,[4] Mioara Alina Nicolaie,[1] Hein Putter,[1] Menno V. Huisman,[1] Sandra W. Van der Kooij,[1] Cees van Kooten,[1] Ton J. Rabelink.[1] *[1]Leiden Univ Medical Center; [2]Bronovo Hospital; [3]St. Lucas Andreas Hospital; [4]St. Antonius Hospital.*

**Background:** Although intra-arterial contrast injections have been associated with increased Kidney Injury Molecule-1 (KIM-1) and Neutrophil-Gelatinase Associated Lipocalin (N-GAL) excretion, it is unclear whether intravenous contrast enhanced-CT (CE-CT) is also accompanied by damage of tubular epithelial cells. We analyzed KIM-1 and N-GAL excretion post CE-CT in patients with chronic kidney disease (CKD).

**Methods:** Patients were enrolled in a trial on contrast induced-acute kidney injury (CI-AKI) preventing hydration. Blood and urine samples were taken at baseline, 4-6 and 48-96 hours post CE-CT. Urinary KIM-1 and N-GAL values were measured by ELISA, normalized for urinary creatinine levels, and presented as medians with 2.5-97.5 percentiles.

**Results:** 511 patients were included of whom 10 (2%) where lost to follow-up. CI-AKI, (serum creatinine increase >25% or >44 umol/L 48-96 hours post CE-CT) occurred in 3.9% of patients (20/501, 95%CI2.6-6.1%). Median KIM-1 values were 1.2 (0.1-7.7) at baseline, 1.3 (0.1-8.6) at 4-6 hours, and 1.3 ng/mg (0.1-8.1) at 48-96 hours post CE-CT (P=0.39). Median N-GAL values were 41.0 (4.4-3174.4), 48.9 (5.7-3406.1), and 37.8 ug/mg (3.5-3200.4), respectively (P=0.07).The amount of KIM-1 and N-GAL excretion in follow-up was similar for patients with and without CI-AKI (P-value KIM-1 = 0.08 and for N-GAL 0.73). In a linear regression analysis, baseline estimated glomerular filtration rate (eGFR) was associated with KIM-1 and N-GAL excretion in follow-up (estimates for log translated values -0.006, $p < 0.001$ and -0.012, $p < 0.001$, respectively).

**Conclusions:** KIM-1 and N-GAL excretion were unaffected by CE-CT in both patients with and without CI-AKI suggesting that CI-AKI was not accompanied by tubular injury.

*Funding:* Private Foundation Support

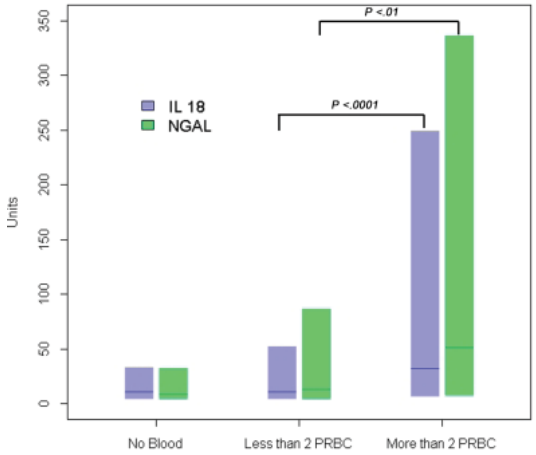
## FR-PO025

**Blood Transfusions Associate with New Biomarkers of Kidney Injury in Cardiac Surgery** Usman Ahmed Khan, Steven G. Coca, Kwangik Adam Hong, Jay L. Koyner, Amit X. Garg, Cary Steven Passik, Madhav Swaminathan, Susan Garwood, Uptal D. Patel, Mackenzie A. Quantz, Chirag R. Parikh. *Translational Research Investigating Biomarker Endpoints in Acute Kidney Injury (TRIBE-AKI) Consortium.*

**Background:** Blood transfusion is common in cardiac surgery and is associated with clinical acute kidney injury (AKI). We examined the effect of blood transfusion on biomarkers of kidney injury and serum creatinine in a clinical AKI in a large multicenter (TRIBE-AKI) cohort undergoing cardiac surgery.

**Methods:** 1210 adults underwent cardiac surgery and were divided into three groups based on the receipt of intraoperative packed red blood cell units: no blood (n=894), ≤ 2 PRBC (n=206) and > 2 PRBC (n=110). AKI was defined as: i) Doubling of serum creatinine from the baseline; ii) first post-operative urinary interleukin (IL)-18 and urinary neutrophil gelatinase-associated lipocalin (NGAL) in the 5th quintile. We determined the relative risk for AKI outcome according to PRBC group after adjusting for pre-operative and surgical variables. Using the Sobel test for mediation analysis, we also evaluated the role of biomarkers in causing AKI through indirect pathways.

**Results:** Urinary IL-18 and NGAL were higher in those who received >2 PRBC.




In patients receiving > 2 PRBC, the adjusted RRs were 2.3 (95% CI 1.2-4.4), 1.4 (95% CI 1.0-1.9), and 1.3 (95% CI 1.0-1.8) for doubling of serum creatinine, urinary IL-18 in the 5th quintile (>60 pg/ml), and urinary NGAL in the 5th quintile (>102 ng/ml), respectively. Furthermore, 17% of the effect of PRBC transfusion on AKI was mediated by IL-18 (P value for mediation 0.02).

**Conclusions:** Receipt of two or more PRBC during cardiac surgery is associated with a greater risk of AKI defined by serum creatinine and kidney injury biomarkers. Moreover, in part this effect is mediated by IL-18.

*Funding:* Other NIH Support - RO1HL085757 (CRP)

## FR-PO026

**Incidence, Risk Predictive Factors, and Clinical Outcomes of Acute Kidney Injury after Gastric Surgery for Gastric Cancer** Chang Seong Kim, Ha Yeon Kim, Yong Un Kang, Joon Seok Choi, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Dept of Internal Medicine, Chonnam National Univ Hospital, Gwangju, Republic of Korea.*

**Background:** Postoperative acute kidney injury (AKI), is a serious surgical complication in known to be common after cardiac surgery, however, reports on AKI after noncardiac surgery are limited. We sought to determine the incidence and predictive factors of AKI after gastric surgery for gastric cancer and its effects on the clinical outcomes.

**Methods:** We conducted a retrospective study of 4718 patients with normal renal function who underwent partial or total gastrectomy for gastric cancer between June 2002 and December 2011. Postoperative AKI was defined by serum creatinine change, as per the Kidney Disease: Improving Global Outcomes guideline.

**Results:** Of the 4718 patients, 679 (14.4%) developed AKI. Length of hospital stay and intensive care unit admission rates, and in-hospital mortality rate (3.5% versus 0.2%) were higher in patients with AKI than in those without. AKI was also associated with requirement of renal replacement therapy. Multivariate analysis revealed that male gender [odds ratio (OR) 1.75, 95% confidence interval (CI) 1.37-2.23], hypertension (OR 1.27, 95% CI 1.04-1.54), chronic obstructive pulmonary disease (OR 1.64 95% CI 1.15-2.35), hypoalbuminemia (< 4 g/dl) (OR 1.40 95% CI 1.11-1.77), use of diuretics (OR 2.39 95% CI 1.98-2.88), vasopressor (OR 1.87 95% CI 1.28-2.72) and contrast agent (OR 1.60 95% CI 1.29-2.00), and packed red blood cell transfusion (OR 1.72 95% CI 1.38-2.15) were independent predictors for AKI after gastric surgery. Postoperative AKI and vasopressor use entailed a high risk of 3-month mortality after multiple adjustments.

**Conclusions:** AKI was common after gastric surgery for gastric cancer and associated with adverse outcomes. We identified several factors associated with postoperative AKI; recognition of these predictors may help reduce the incidence of AKI after gastric surgery. Furthermore, postoperative AKI in patients with gastric cancer is an important risk factor for short-term mortality.

## FR-PO027

**Urine Angiotensinogen to Creatinine Ratio, a Marker of Intravascular Volume Depletion in Patients with Acute Kidney Injury** Line Malha, Joseph Alge, Nishant M. Bhensdadia, Morganne Phillips, Julie Teuber, Nithin Karakala, Christine M. Carr, John M. Arthur. *Medical Univ of South Carolina.*

**Background:** Intravascular volume depletion is a major risk factor for the development of acute kidney injury (AKI); its prompt identification may prevent the development of AKI. Urinary angiotensinogen/creatinine ratio (uAnCR) has been associated with worsening AKI. We hypothesized that uAnCR correlates with intravascular volume status.

**Methods:** 39 adult subjects were recruited in the emergency department. Volume status was determined by the examining physician's clinical impression. Patients were divided into two groups: intravascularly depleted (n=13) and non-depleted (n=26). The study is ongoing with a goal recruitment of 150 patients and a 1-year follow up chart review. Urinary angiotensinogen was measured by ELISA and corrected for urine creatinine in ng/mg.

**Results:** There was no difference in frequency between the intravascularly depleted and non-depleted groups by age, gender, use of Renin-Angiotensin System (RAS) blockade or AKI at the time of urine collection. 8 patients had AKI, 18 patients did not have AKI and 13 patients could not be assessed because they did not have a serum creatinine measurement. In the entire group, patients with volume depletion had a significantly higher uAnCR of 4.31±11.65ng/mg (median±interquatrile range) compared to 1.57±2.55ng/mg for the non-depleted patients (p=0.003). uAnCR accurately identified volume depleted patients, with an area under the receiver operating characteristic curve (AUC) of 0.793 (p= 0.003) for volume depletion. In the subgroup analysis, by AKI status, AUC was 1.0 (p=0.046) for the patients with AKI at presentation compared to an AUC 0.583 for the patients without AKI (p=0.57).

**Conclusions:** Intravascular volume depletion is associated with a higher UAnCR despite similar incidence of AKI at presentation and use of RAS blockade. The subgroup analysis suggests that the differences are driven entirely by patients with AKI. Larger numbers of patients will be required to determine if uAnCR can be used clinically to assess volume status among patients with AKI.

*Funding:* NIDDK Support

## FR-PO028

**Serum Cystatin C for Defining Acute Kidney Injury in Children Undergoing Cardiac Surgery** Michael Zappitelli,[1] Jason Henry Greenberg,[2] Steven G. Coca,[2] Catherine D. Krawczeski,[3] Simon Li,[4] Heather Thiessen Philbrook,[5] Gang Han,[2] Michael R. Bennett,[3] Prasad Devarajan,[3] Chirag R. Parikh.[5] *[1]McGill U.; [2]Yale U.; [3]U. Cincinnati; [4]Maria Fareri Child. Hosp.; [5]U. Western Ontario; [6]Yale U., Translational Research Investigating Biomarkers and Endpoints for AKI Consortium.*

**Background:** In theory, acute serum CysC change can be used to define acute kidney injury(AKI). If CysC, with its fewer limitations, defines AKI more accurately than serum creatinine(SCr) then AKI-biomarker associations will be stronger with CysC *vs.* SCr-AKI definition.

**Methods:** Three-center prospective study of children(1 mth-18 yrs) post-cardiac surgery. We measured pre and postop SCr, CysC and urine biomarkers(Fig.). We defined AKI based on KDIGO, using SCr- and CysC-AKI. We compared biomarker prediction of SCr and CysC-AKI (regression, diagnostic characteristics) and SCr- *vs.* CysC-AKI outcome associations.

**Results:** 287 children were included. There was poor agreement for SCr- and CysC-AKI Stage(St) 1(κ=0.38); good agreement for St 2 AKI(κ= 0.78). SCr- and CysC-AKI predicted longer hospital stay (SCr-AKI:7 *vs.* 4 d;CysC-AKI:7 *vs.* 5 d, adjusted[adj] p<0.001) and longer ventilation (SCr- and CysC-AKI:2 *vs.* 1 d, adj p<0.01). In general, 5th quintile (relative to 1st) 0-6hr postop biomarker concentrations predicted St 1 or worse CysC-AKI similarly or better than SCr-AKI (Fig, left, regression OR's); however, prediction was better for St 2 or worse SCr-AKI (Fig, right). Except for NGAL, 0-6hr postop biomarker AUCs were higher to predict St 1 or worse CysC-AKI (Fig, left); conversely, AUCs were higher to predict St 2 or worse SCr-AKI (Fig, right).

| AKI Definition | Urine Biomarker | Stage 1 AKI or worse | | Stage 2 AKI or worse | |
|---|---|---|---|---|---|
| | | Adj OR [95% CI] 5th vs 1st quintile | AUC ( SE) | Adj OR [95% CI] 5th vs 1st quintile | AUC ( SE) |
| SCr-AKI | IL-18 | 3.6 [1.3-9.7] | 0.66 (0.03) | 15.5 [1.8-135.8] | 0.75 (0.04) |
| | NGAL | 3.3 [1.1-9.6] | 0.69 (0.03) | 15.7 [1.7-145.7] | 0.76 (0.04) |
| | KIM-1 | 1.5 [0.6-3.5] | 0.58 (0.03) | 4.9 [1.2-20.6] | 0.69 (0.04) |
| | L-FABP | 3.4[1.2-9.6] | 0.66 (0.03) | 3.2 [0.8-13.5] | 0.73 (0.04) |
| | | | | | |
| CysC-AKI | IL-18 | 11.9 [2.3-62.2] | 0.74 (0.03) | 8.5 [0.9-81.8] | 0.72 (0.04) |
| | NGAL | 3.3 [0.9-13.5] | 0.66 (0.04) | 9.0 [0.9-90.8] | 0.72 (0.04) |
| | KIM-1 | 6.1 [1.5-25.2] | 0.65 (0.04) | 3.8 [0.8-17.7] | 0.67 (0.05) |
| | L-FABP | 5.3[1.4-19.9] | 0.68 (0.04) | 5.6 [0.8-36.8] | 0.70 (0.05) |

Biomarkers: Urine interleukin 18 (IL-18), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), fatty acid binding protein (FABP), measured 0-6 hours postoperatively. Odds Ratios are adjusted for age, gender, white race, cardiopulmonary bypass>120 min, surgical severity score, baseline eGFR percentile, site. AUC (area under the curve); SE (standard error).

**Conclusions:** AKI biomarkers predicted mild CysC-AKI better than mild SCr-AKI. For more severe AKI prediction, associations were stronger using SCr-AKI. Implications are relevant for future studies using CysC for AKI definition.

*Funding:* NIDDK Support

## FR-PO029

**Diagnosis of Acute Pyelonephritis in Renal Allograft: A Possible Role for MicroRNAs** Anjali A. Satoskar,[1] Steve Oghumu,[1] Uday S. Nori,[2] Sergey V. Brodsky,[1] Ronald Pelletier,[3] Tibor Nadasdy.[1] *[1]Pathology, Ohio State Univ Wexner Medical Center, Columbus, OH; [2]Internal Medicine, Ohio State Univ Wexner Medical Center, Columbus, OH; [3]Surgery, Ohio State Univ Wexner Medical Center, Columbus, OH.*

**Background:** Acute pyelonephritis (APN) versus acute rejection (AR) is a frequently encountered diagnostic and therapeutic dilemma in clinical transplantation. Biopsy and urine culture are important diagnostic tools, but they may not always correlate. Variable culture results, overlapping histologic features with AR, persistent graft dysfunction despite antibiotics are frequently encountered. We explored the utility of intragraft microRNA profiles to distinguish between allograft APN and AR.

**Methods:** Between 2003 and 2011, we identified 49 patients with features of APN on biopsy, performed within the first two years post-transplant. MicroRNA profiling was performed on 20 biopsies (normal kidney, n=4; unequivocal AR, n=5; features of APN, n=11).

**Results:** Only 32% (16/49) of the patients had concomitant positive urine cultures at biopsy and in 8/16 patients colony count was less than $10^5$ CFU/ml. In 14/49 patients, positive urine culture did not coincide with the biopsy and in 19/49 patients, cultures were negative. On microRNA profiling, good clustering was seen among the normal kidneys and among AR biopsies. Among the eleven biopsies with features of APN, four biopsies showed good clustering and a pattern distinct from AR; (these four patients recovered graft function with antibiotics); 7/11 biopsies with features of APN showed heterogeneity in microRNA profiles. Three of these seven patients recovered only after steroid treatment, three lost their grafts within one month of the biopsy despite antibiotics and one patient expired. We identified a panel of 21 microRNAs showing significant difference in expression between AR and APN. MiR-99b, miR-23b and miRs from let-7, miR-30 family, show promise as markers to distinguish APN from AR.

**Conclusions:** Allograft pyelonephritis can be a diagnostic and therapeutic challenge. In addition to histology and cultures, differential intragraft microRNA expression may prove helpful to distinguish APN from AR in renal allografts.

*Funding:* Clinical Revenue Support

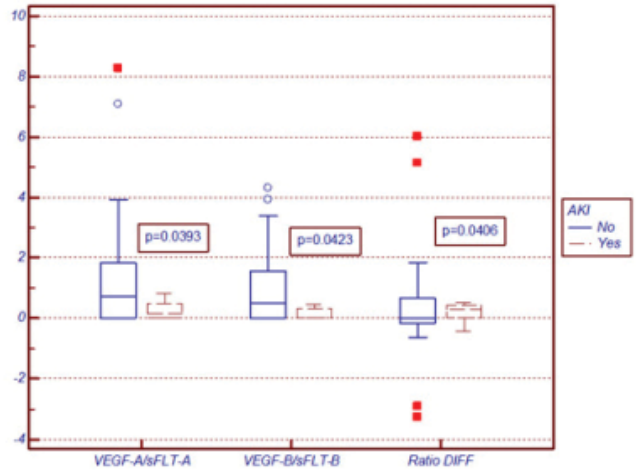
## FR-PO030

**Low VEGF/sFlt Ratio on Admission May Portend the Development of AKI in Critically Ill Patients with Sepsis** Arghya Majumdar, Aditi Jain. *Nephrology, AMRI Hospitals, Kolkata, India.*

**Background:** Microvascular alterations in the form of endothelial dysfunction and capillary leak play a key role in sepsis induced AKI.

**Methods:** To see whether VEGF/sFlt ratio on admission has a correlation with the development of AKI, in critically ill patients with sepsis. Methodology: Prospective observational study,conducted in a multi-specialty ICU, in a tertiary care hospital (AMRI), in Kolkata, India, for 1 year. Included: Adult patients with features of SIRS/sepsis admitted to ICU. Excluded: Patients‹18yrs old, brought in from other health facilities or transferred from wards after more than 24 hours of in-hospital stay, post-surgical patients, those anuric (for the first 6 hours after admission), with macroscopic hematuria, hemoglobinuria, pregnant or menstruating women, patients with neoplasm, known cases of CKD and macroalbuminuria. Within 6 hours of admission (A) and at 24 hours (B), VEGF and sFlt levels were measured from blood and microalbuminuria from urine samples. Clinical data was collated.

**Results:** An increase of microalbuminuria was noted in the first 24 hours,in the patients who went on to develop AKI, shock (requiring vasopressors) and organ dysfunction, more so in patients with sepsis, despite the fact that both the sepsis and SIRS group had similar APACHE IV and APS scores. The ratio of VEGF/sFlt had a significant correlation with AKI. In patients who developed AKI,VEGF/sFlt at (A) and 24 hours (B),after admission were lower than those who did not.



**Conclusions:** Endothelial dysfunction and capillary leak plays a pivotal role in pathogenesis of septic AKI. The ratio VEGF/sFlt on admission was inversely proportional to development of AKI. VEGF plays a key angio- protective role. Low VEGF levels may lead to endothelial cell apoptosis, capillary leak and AKI. Conclusion: Low VEGF/sFlt ratio may identify the septic patients at risk of developing AKI, on admission to ICU.

## FR-PO031

**Performance of Urinary Kidney Injury Molecule-1 (KIM-1) in Acute Kidney Injury (AKI): A Systematic Review** Paweena Susantitaphong,[1,2] Fouad Chebib,[1] Bertrand L. Jaber.[1] *[1]Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Medicine, Chulalongkorn Univ, Bangkok, Thailand.*

**Background:** Urinary KIM-1 is a proximal tubular cell injury marker that has been proposed as a biomarker for early detection of AKI. We conducted a systematic review of diagnostic studies that examined the performance of urinary KIM-1 in AKI.

**Methods:** We searched MEDLINE (through December 2012), Scopus, Google Scholar, Cochrane Central Register of Controlled Trials and ClinicalTrials.gov for human studies investigating the diagnostic and prognostic performance characteristics of urinary KIM-1 for predicting development of AKI, dialysis requirement, and mortality. We performed bivariate-model and random-effects model meta-analyses.

**Results:** Of the 41 cohort studies and 8 case-control studies, only 9 prospective cohort studies could be meta-analyzed (2,647 patients). For the diagnosis of AKI, the estimated sensitivity of urinary KIM-1 was 64.7% (95% CI (50.9, 76.5%) and the specificity 81.6% (95% CI 76.0, 86.1%), with a diagnostic odds ratio DOR of 1.83 (95%CI, 1.04, 3.25; $P$=0.04). For prediction of dialysis requirement (2 studies, 545 patients), the estimated sensitivity of urinary KIM-1 was 42.6% (95% CI 25.7, 61.5%), and the specificity 91.0% (95% CI 49.2, 99.1%). For prediction of mortality (2 studies, 577 patients), the estimated sensitivity of urinary KIM-1 was 71.5% (95% CI 6.4, 98.9%), and the specificity 77.9% (95% CI 62.0, 88.4%).

**Conclusions:** Although KIM-1 is a promising urinary biomarker in AKI, its potential role needs to be examined in large cohort studies and across a broad range of clinical settings.

## FR-PO032

**Kidney Injury Molecule-1 and Monocyte Chemotactic Protein-1 as Sensitive Biomarkers for Detecting Cisplatin-Induced Nephrotoxicity in Patients with Lung Cancer** Haruka Shinke,[1] Yasuaki Ikemi,[2] Masami Tadehara,[2] Kazuo Matsubara,[1,2] Yosuke Togashi,[3] Young Hak Kim,[3] Michiaki Mishima,[3] Takaharu Ichimura,[4] Joseph V. Bonventre,[4] Satohiro Masuda.[1,2] *[1]Dept of Clinical Pharmacology and Therapeutics, Kyoto Univ Hospital, Kyoto, Japan; [2]Dept of Pharmacy, Kyoto Univ Hospital, Kyoto, Japan; [3]Dept of Respiratory Medicine, Kyoto Univ Hospital, Kyoto, Japan; [4]Renal Div, Brigham and Women's Hospital, Boston.*

**Background:** Acute kidney injury (AKI) is a common and serious adverse event after cisplatin-based chemotherapy. To identify urinary biomarkers for the accurate detection of cisplatin-induced AKI, we examined whether the recently discovered urinary biomarkers for AKI can detect cisplatin-induced nephrotoxicity in patients with lung cancer.

**Methods:** A total of 50 patients were enrolled in this study after providing written informed consent. Patients diagnosed with AKI lacking urine samples at day 3 after treatment were excluded. The urine samples were collected the day before cisplatin (80 mg/sqr) administration and at days 3, 7, and 14. The diagnoses of AKI were based on our hospital's criteria. This study was approved by the institutional ethics committee.

**Results:** A comparison of the urinary biomarker concentrations between the AKI (+) group (n = 30 samples) and AKI (-) group (n = 12 samples), revealed that kidney injury molecule-1 (KIM-1) and monocyte chemotactic protein-1 (MCP-1), but not neutrophil gelatinase-associated lipocalin (NGAL), were significantly higher in AKI (+) than that in AKI (-). Composite area under the receiver operating characteristics curve (AUC-ROC) analysis showed that KIM-1 (AUC = 0.858, $p < 0.01$) and MCP-1 (AUC = 0.828, $p < 0.01$), but not NGAL (AUC = 0.552, $p > 0.05$), had high sensitivity for detecting cisplatin-induced AKI. Moreover, AUC-ROC of the combined estimation of KIM-1 and MCP-1 was higher than that of either one alone (AUC = 0.871, $p < 0.001$). The cutoff values of KIM-1 and MCP-1 were calculated as 2.45 and 0.25 ng/mg creatinine, respectively.

**Conclusions:** Bothurinary KIM-1 and MCP-1 provide sensitive and specific detection of cisplatin-induced nephrotoxicity in patients with lung cancer.

*Funding:* Government Support - Non-U.S.

## FR-PO033

**Urinary Kidney Injury Molecule-1 (KIM-1) Levels Immediately after Surgery Predict the Development of Tacrolimus-Related Chronic Kidney Disease in Liver Transplantation Recipients** Haruka Shinke,[1] Ayami Tsuchimoto,[1] Venkata Sabbisetti,[2] Miwa Uesugi,[1] Emina Hashimoto,[1] Kazuo Matsubara,[1] Yasuhiro Fujimoto,[3] Toshimi Kaido,[3] Shinji Uemoto,[3] Motoko Yanagita,[4] Takaharu Ichimura,[2] Joseph V. Bonventre,[2] Satohiro Masuda.[1] *[1]Dept of Clinical Pharmacology and Therapeutics, Kyoto Univ Hospital, Kyoto, Japan; [2]Renal Div, Brigham and Women's Hospital, Boston; [3]Dept of Surgery, Kyoto Univ Hospital, Kyoto, Japan; [4]Dept of Nephrology, Kyoto Univ Hospital, Kyoto, Japan.*

**Background:** The aim of the present study was to examine whether the urinary KIM-1 levels immediately after surgery associates with the occurrence of tacrolimus-related post-liver transplant chronic kidney disease (CKD).

**Methods:** Twenty-three patients who received de novo living-donor liver transplantation (LDLT) with tacrolimus as a primary immunosuppressant were enrolled after providing written informed consent. Urine samples were collected at postoperative day (POD) 1, immediately before the first administration of tacrolimus. Development of CKD was defined by a Scr level that was 1.5-fold higher than the preoperative level for 3 consecutive months between PODs 1 and 180 (KDOQI CKD guidelines).

**Results:** Urinary KIM-1 concentrations at POD 1 after LDLT were significantly higher than those of healthy subjects. The levels of urinary KIM-1 at POD 1 in patients who ultimately developed CKD were significantly higher than those who did not develop CKD ($P < 0.001$ by Student-t test). The median value of urinary KIM-1 at POD 1 of all patients was found to be 2.85 ng/mg creatinine. The risk of post-transplant CKD was greater in patients whose urinary KIM-1 values at POD 1 were higher than that median value in comparison with to patients with lower urinary KIM-1 level at POD 1 ($P = 0.0325$ by Fisher's exact test). However, the frequency of post-transplant CKD was similar when the patients were categorized by the median value of the urinary neutrophil gelatinase-associated lipocalin levels at POD 1 (29.4 ng/mg creatinine, $P = 0.582$).

**Conclusions:** Urinary KIM-1 on POD 1 is a prognostic biomarker for the development of tacrolimus-related CKD after liver transplantation.

*Funding:* Government Support - Non-U.S.

## FR-PO034

**Is Kim-1 Is a Valuable Diagnostic Tool for Early Diagnosis of Contrast Induced Nephropathy?** Derya Akdeniz,[1] Huseyin Tugrul Celik,[2] Hakki Yilmaz,[3] Fatmanur Kazanci,[2] Ayse Mukadder Bilgic,[3] Nuket Bavbek,[3] Ali Akcay.[3] *[1]Internal Medicine, Turgut Ozal Univ Faculty of Medicine, Ankara, Turkey; [2]Biochemistry, Turgut Ozal Univ Faculty of Medicine, Ankara, Turkey; [3]Nephrology, Turgut Ozal Univ Faculty of Medicine, Ankara, Turkey.*

**Background:** Contrast-inducednephropathy (CIN) is a common complication of diagnostic/therapeutic procedures. Serum creatinin levels are sensitive but often lead to diagnostic delays for acute kidney injury (AKI) and potential misclassification of actual injury status. Kidney injury molecule (KIM-1) is a novel early marker of AKI. The aim of our study was to evaluate KIM-1 levels in patients with CIN. We performed a single center, nested case-control study.

**Methods:** Four hundred eighty six patients who undergone coronary angiography were included into the study.Thirty patients were diagnosed CIN. The diagnosis of CIN was done according to KDIGO 2012 Acute Kidney Injury Guideline criteria.Urinary KIM-1 is measured by enzyme-linked immunosorbent assay before, 6$^{th}$ and 48$^{th}$ hours post contrast. Serumcreatinine was measured before, 24$^{th}$ and 48$^{th}$ hours postcontrast.

**Results:** We showed that KIM-1 levels were significantly increased in the patients with CIN significantly in 6$^{th}$ hour when compared to baseline ($p<0.01$; median levels 48 and 101.8 mg/dL).

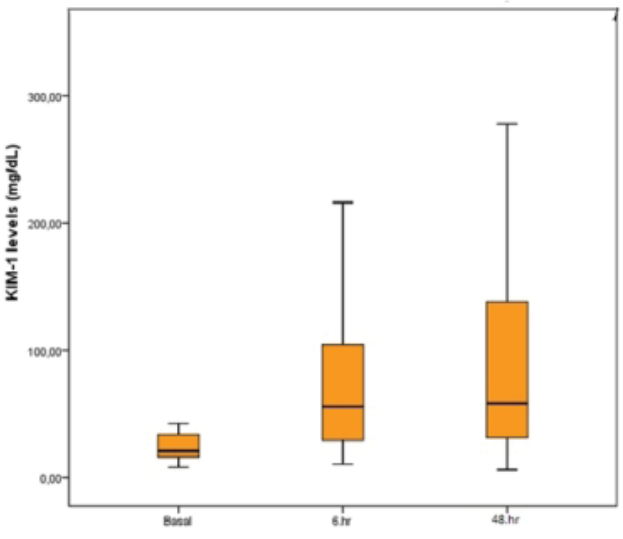


Figure 1 Comparison urinary corrected KIM-1 levels at baseline, 6th and 48th hour.

Pre-contrast and 48th hour KIM-1 levels were median levels were also significantly different(p= 0.001, median levels were 25, 101.8 respectively).

**Conclusions:** Because creatinine is a sensitive but a late marker of CIN, KIM-1 may be used for early diagnosis, early initiation of treatment and reduced risk of morbidity.

## FR-PO035

**Correlation of Increase in Urinary β2-Microglobulin and CD133 Staining in Renal Biopsy** Xu Zeng,[1] Guillermo A. Herrera,[1] Ping L. Zhang,[2] David G. Bostwick,[1] Deloar Hossain.[1] *[1]Nephrocor, Bostwick Laboratories, Orlando, FL; [2]Pathology, William Beaumont Hospital, Royal Oak, MI.*

**Background:** After filtration through the glomeruli, **β2-microglobulin is** reabsorbed by renal proximal tubules. Increase in urine **β2-microglobulin** indicates tubular injury, and measurement in urine sediment **is** useful to determine the source of kidney injury. CD133 has recently been characterized as a progenitor cell marker in the kidney, and also a marker for injured epithelial cells in proximal and distal nephron tubules. This study was designed to evaluate the correlation of urinary **β2-microglobulin and** CD133 with tubular injury.

**Methods:** Between 2009 and 2012, 47 patients with renal biopsies had prior RenalVysion™ analysis. Among these, 30 patients had increase in urinary **β2-microglobulin. The remainder 17 had normal urinary β2-microglobulin.** Immunohistochemical staining for CD133 (1:50 Miltenyi Biotec Inc.) was performed in corresponding renal biopsy specimens. CD133 staining in both proximal and distal tubules was considered positive. We evaluated the correlation of increase in urinary **β2-microglobulin** with positive CD133 staining in renal biopsy.

**Results:** 30 patients with increase in **β2-microglobulin** in the urine, and 25 of these also had CD133 staining positive proximal and distal tubules. In patients with normal **β2-microglobulin** in the urine, 11 of 17 patients were negative for CD133 staining. Using positive staining for CD133 as the end point for this analysis, sensitivity was 80.6%, specificity was 68.8%, positive predictive value was 83.3%, and negative predictive value was 64.7%.

| | CD133 (+) | CD133 (-) |
|---|---|---|
| β2-Microglobulin Increase | 25 | 5 |
| β2-Micorglobulin Normal | 6 | 11 |

**Conclusions:** Detection of **β2-microglobuline** in urine is a sensitive test of tubular injury, largely paralleled with CD133 upregulation in injured renal tubules. Due to low specificity, this finding should be utilized in conjunction with the patient's clinical history, urine cytology findings (including casts, tubular cells, and inflammatory cells), urine and serum chemistry.

## FR-PO036

**Plasma Cystatin C Should Not Be Used to Diagnose AKI after Cisplatin Therapy** Timothy J. Pianta,[1,2] Philip Peake,[1,2] Nicholas Buckley,[1] John W. Pickering,[3] Melvin Chin,[1] Zoltan H. Endre.[1,2,33] *[1]Prince of Wales Clinical School, Univ of New South Wales, Sydney, NSW, Australia; [2]Dept Nephrology, Prince of Wales Hospital, Sydney, NSW, Australia; [3]Dept Medicine, Univ of Otago, Christchurch, New Zealand.*

**Background:** Increased plasma cystatin C (pCysC) has been proposed as an alternative to serum creatinine (sCr) for diagnose AKI. We assessed theutility of plasma cystatin C (pCysC) to diagnose AKI after cisplatin-based chemotherapy.

**Methods:** Plasma and urinary biomarkers were measured at 8 time-points before and for 2 weeks after chemotherapy in 26 patients without CKD. All patients also received dexamethasone, aprepitant and palonosetron antiemetics.

**Results:** pCysC increased in all patients between 3 and 7 days after cisplatin. pCysC increased by more than 50% (AKI) in 9 patients (35%) whereas only 2 (8%) showed a 50% increase in sCr (p=0.04). pCys increased more than 25% in 16 patients (61%) whereas only 3 (12%) had a greater than 25% increase in sCr ($p < 0.001$). The mean difference between the percentage increase in pCysC and sCr was 25.0% (95% CI: 15.8% to 34.3%). Kidney injury molecule (KIM)-1, clusterin, interleukin-18 and neutrophil gelatinase-associated lipocalin (NGAL) increased above baseline 3 to 7 days after cisplatin ($p<0.05$). Peak KIM-1 and clusterin correlated with peak rise in sCr (r=0.43, p= 0.03; and r=0.40, p=0.04 respectively) suggesting the observed modest increases in sCr represented renal injury. Increases in pCysC did not correlate with increases in any other urinary biomarker ($0.80<p<0.88$).

**Conclusions:** After cisplatin chemotherapy, pCysC concentrations increase independently of sCr and kidney injury biomarkers in patients treated with contemporary antiemetics. This suggests significant interference, possibly by corticosteroids, neurokinin-1 receptor antagonists or serotonin antagonists warranting further study.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## FR-PO037

**Mean Daily Fluid Balance and Outcome in Acute Kidney Injury** Anup Chaudhari, Hemant J. Mehta. *Nephrology, Lilavati Hospital, Mumbai, Maharashtra, India.*

**Background:** Acute Kidney Injury (AKI) in critically ill patients carry high mortality. Fluid overload with oliguria leads to increased need for dialysis support and ventilation. The aim of this study is to evaluate the impact of mean daily fluid balance (MDFB) on outcomes in adult ICU patients with AKI.

**Methods:** This was a prospective study, from January 2009 to December 2010; included 130 patients. Inclusion criteria: Age > 18 years with AKI, admitted to ICU. APACHE II and SAPS II scores were calculated. Diagnosis of AKI was based on RIFLE criteria. MDFB was calculated for each day during ICU stay. Outcome was analyzed as survived or dead. Statistical analysis was done.

**Results:** Males/females = 89/49 (62.3%/37.7%). Mean age group 57.9 years (range 20-95 years), 46/130 (35.4%) had sepsis as the cause of AKI, 19 (14.6%) malaria, 17 gastrointestinal cause, 16 cardiac cause, 8 had dengue, 6 (4.6%) contrast induced nephropathy. Staging by RIFLE showed 9 cases (6.9%) in Risk group, 54 (41.5%) in Injury group, 60 (46.2%) in Failure group and 7 (5.4%) in Loss group. Comparison of various variables between recovered and expired cases at each stages of R, I, F and L for ventilation days, ICU days, inotropic support days, number of inotropes, APACHE II score and SAPS II score showed significant difference. Comparison between positive and negative MDFB showed significant difference for mechanical ventilator therapy, inotrope support, and survival v/s death such that patients in negative MDFB had a significant survival benefit. Overall survival was seen in 57.95% and mortality was 42.1%.

**Conclusions:** There appeared to be a step wise increase in relative risk of death, going from risk to failure in AKI. A negative MDFB was independently associated with a significant reduction in mortality. Even though we have invasive monitoring for fluid status, a simple calculation of MDFB by calculating intake and output can be useful, easy to calculate and very helpful for developing countries where such facilities of invasive monitoring may not be available. A recent multicentre study in Critical care by NEFROINT group has also shown that higher fluid balance and lower urine volume were both important factors associated with 28 days mortality of AKI patients.

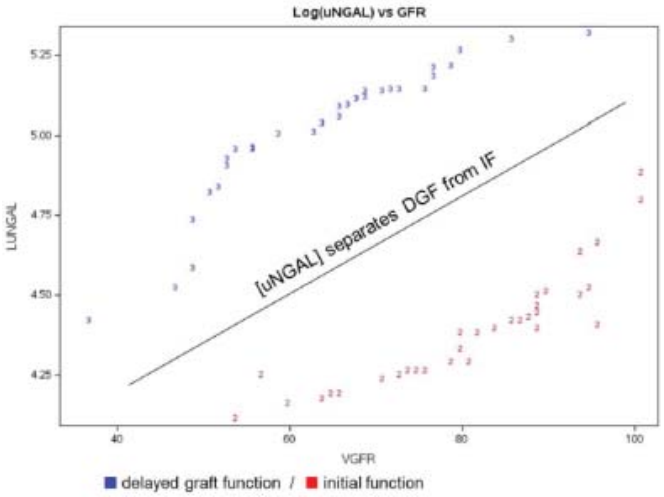
## FR-PO038

**Donor Neutrophil Gelatinase-Associated Lipocalin (NGAL) Concentration Predicts Post-Transplant Allograft Function after Kidney Tx** G. Junge, Wolfgang Arns. *The NGAL Study Group.*

**Background:** The donor pool for KTx has plateaued worldwide. Expansion of the pool by including LR-donations and donors who previously were not accepted is the only way to increase numbers. Data on specific parameters to predict success/failure of KTx are limited. It has been suggested that NGAL may serve as an early marker for renal injury, but have not as yet been investigated in kidney donors(KD). Therefore, it was the objective to evaluate NGAL in KD as a predictor of early allograft function after KTx.

**Methods:** This study prospectively evaluated NGAL (urine, serum) in healthy volunteers (n=30) to compare results with (1) brain dead organ donors (BDOD) before organ procurement (n=58), (2) living related (LR) KD (n=15), (3) KTx recipients who received an allograft from a BDOD (n=58) and (4) KTx recipients who received an allograft from LR donor (n=15). In addition, the study analyzed allograft function in associated kidneys in correspondent recipients that were classified into 2 groups depending on allograft function after KTx: initial function (IF) vs delayed graft function (DGF). The primary objective was to evaluate the predictive value of NGAL for post Tx allograft function. Secondary objectives were: (1) To compare NGAL levels in BDOD and CKD stage V patients on HD to healthy volunteers, (2) to evaluate NGAL evolution post (LR)KTx by visit, etc.

**Results:** Study results demonstrate that urine (and serum) NGAL levels in corresponding KD correlate with post transplant allograft function where higher NGAL levels where predictive for DGF.




**Conclusions:** In summary, our data indicates that NGAL represents a novel, sensitive and non-invasive urinary (and serum) biomarker predictive for primary graft function after KTx. Even in cases where our classical diagnostic parameters do not allow further differentiation of potential KD (marginal donors), NGAL seems to remain a stable and significant indicator.

## FR-PO039

### Effect of GFR Impairment on the Accuracy of BNP and NGAL as Markers of Cardiac and Renal Failure in Chronic Kidney Disease Patients

Carlo Donadio. *Clinical and Experimental Medicine, Univ of Pisa, Pisa, Italy.*

**Background:** Cardio-renal syndromes are characterized by the impairment of cardiac and renal functions. Plasma and urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL), and plasma B-type natriuretic peptide (BNP) are markers of acute kidney injury (AKI) and heart failure (HF), respectively.

The aim of this study was to assess, in stable chronic kidney disease (CKD) patients in the different functional stages of CKD, the effect of the reduction of GFR on plasma BNP and on plasma and urinary NGAL concentrations.

**Methods:** GFR ($^{99m}$Tc-DTPA), plasma BNP, and plasma and urinary concentrations of NGAL and LMWP were measured in 310 CKD patients, clinically stable, at functional stages from 1 to 5. Serum and urinary low-molecular-weight proteins (LMWP), and urinary tubular enzymes (Enz) were measured for comparison. Plasma BNP, NGAL and LMWP were measured in 31 maintenance hemodialysis (MHD) patients.

**Results:** Plasma NGAL increased with the reduction of GFR in CKD patientsfrom stage 2. In the different CKD stages modest differences were found for BNP values. Urinary NGAL slightly but significantly increased in patients at CKD stages 4&5, similarly to urinary LMWP. In MHD patients, plasma NGAL and BNP were markedly increased, and high-flux hemodialysis decreased significantly their plasma concentrations.

**Conclusions:** Plasma NGAL increases markedly the with reduction in GFR, generating a very high number of false positive diagnosis of AKI in stable CKD patients. GFR impairment and etiology of kidney disease have a lower effect on urinary NGAL and on plasma BNP. In any case, specific reference values of NGAL and BNP should be used in CKD patients, according to their functional stage, when assessing AKI, HF, and cardio-renal syndromes in patients with impaired GFR.

*Funding:* Government Support - Non-U.S.

## FR-PO040

### Is Mean Arterial Pressure or Use of Norepinephrine, during Cardiopulmonary Bypass, Associated with Acute Kidney Injury?

Kristian Kandler,[1] Mathias Ebbesen Jensen,[1] Christian Holdflod Møller,[1] Jens C. Nilsson,[2] Daniel Steinbrüchel.[1] *[1]Dept of Cardiothoracic Surgery, Rigshospitalet, Copenhagen, Denmark; [2]Dept of Cardiothoracic Anesthesia, Rigshospitalet, Copenhagen, Denmark.*

**Background:** Acute kidney injury (AKI) after cardiac surgery is common and associated with increased mortality. We wanted to investigate if the mean arterial pressure (MAP) or use of norepinephrine during cardiopulmonary bypass (CPB) were associated with AKI.

**Methods:** A retrospective analysis of patients who underwent elective or subacute CABG with or without concomitant procedures during the year 2012 was conducted. Exclusion criteria were baseline creatinine > 2.3 mg/dl (200 µmol/l), previous nephrectomy, selective cerebral perfusion and acute procedures within 24 hours of coronary angiography. AKI was defined, using the AKIN criteria, as a total increase in serum creatinine of ≥ 0.3 mg/dl (27 µmol/l) or > 50% compared to the baseline value, within the first 48 hours postoperative. Average MAP during CPB was gathered and calculated from electronic perfusion charts and entered in a binary logistic regression model together with pre- and intraoperative data.

**Results:** A total of 623 patients were included in the study of which 80% underwent isolated CABG. Mean age was 68.3 ± 9.7 years and 81% were males. AKI was observed in 177 patients (28.4%). Average MAP was 47 ± 6 mm Hg and 45 ± 6 mm Hg (p=0.003) in the AKI- and no-AKI group respectively. Norepinephrine was used, during ECC, in 19% of the AKI patients and 12% of no-AKI patients (p=0.024). Independent factors increasing the risk of AKI were higher age, body surface area, duration of CPB, Euroscore and hemodilution. Preoperative use of renin-angiotensin-aldosterone system inhibitors, hypertension and use of norepinephrine when leaving the operating room were also independently increasing the risk of AKI.

**Conclusions:** Although norepinephrine was used more frequently and the MAP was higher in the AKI group during ECC, the association was not found to be independent, using binary logistic regression analysis.

*Funding:* Private Foundation Support

## FR-PO041

### Diagnostic Value of Cystatin C in Contrast-Induced Nephropathy after Percutaneous Cardiovascular Intervention

Peng Li,[1] Wong Ka tong,[2] Chan Wai Hun,[1] Wong Ka Nam.[3] *[1]Internal Medicine, Kiang Wu Hospital, Macau, Macau; [2]Nephrology Association of Macau, Macau, Macau; [3]Emergancy Dept, Centro Hospitalar Conde de Sao Januario, Macau, Macau.*

**Background:** Contrast-induced Acute Kidney Injury (CIAKI) is one of most common complications in the patients undergoing percutaneous cardiovascular intervention(PCI) using contrast media (CM)[1]. Many researchers have illustrated that cystatin C was a more sensitive marker of kidney injury since it was less influenced by sex, race, age, protein intake and muscle mass compared with serum creatinine(SCr)[2], and also found that cystatin C might have a better diagnotic accuracy in CIAKI than SCr[3]. We investigated the variation of cystatin C in the patients undergoing PCI in order to further determine the diagnostic value of cystatin C in CIAKI.

**Methods:** SCr and cystatin C were measured at baseline before contrast exposure, 24 hours and 48 hours after contrast exposure,respectively.Cystatin C levels were detected by particle-enhanced turbidimetric immunoassay (PETIA).Serum creatinine increased >= 25% within 48 hours is defined as the criteria for the diagnosis of CIAKI[1]. ROC curve analysis was performed for the 24hr and 48hr serum cystatin C.

**Results:** Among 196 study patients (61 female,135 Male, mean age: 70.7±11.3yr), 29 patients developed CIAKIaccording to the creatinine criteria.The prevalence of CIAKI was 14.8% .The area under the ROC curve (AUC) of the vari ation of cystatin C at 48hr showed a better performance than the variation of cystatin C at 24hr [AUC: 0.783(95% confidence interval (CI): 0.701–0.865, P=0.000)vs 0.661(95% confidence interval (CI): 0.548–0.774, P=0.006). In the ROC curve of cystatin C at 48hr, the 10% elevation of cystatin C yielded 48.3% diagnostic sensitivity, 83.2% specificity and 90.3% negative predictive value. 25% elevation of cystatin C yielded 27.6% diagnostic sensitivity, 96.4% specificity and 88.5% negative predictive value respectively.

**Conclusions:** The variation of cystatin C at 48hr presents a better diagnostic value in CIAKI than that of cystatin C at 24hr. Meanwhile, 25% elevation of serum cystatin C at 48hr seems to acquire an excellent specificity for the diagnosis of CIAKI.

*Funding:* Government Support - Non-U.S.

## FR-PO042

### Clinical Adjudication Confirms TIMP2*IGFBP7 Results for Acute Kidney Injury

Mitchell H. Rosner,[1] Kathleen D. Liu,[2] Anitha Vijayan,[3] John A. Kellum.[4] *[1]Nephrology, Univ of Virginia, Charlottesville, VA; [2]Nephrology and Critical Care, Univ of California San Francisco, San Francisco, CA; [3]Renal Div, Washington Univ, St. Louis, MO; [4]Critical Care Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** We showed that the combination of two biomarkers (TIMP2•IGFBP7) was predictive of AKI as defined by KDIGO stages 2 and 3 (Kashani et al. Critical Care 2013, 17:R25). However, AKI is a clinical diagnosis that does not solely rely on consensus definitions. To further assess the performance of TIMP2•IGFBP7, we assembled a committee of 3 expert nephrologists to adjudicate moderate and severe AKI events.

**Methods:** Adjudiication was done in a cohort of 408 subjects using clinical judgement and consensus definitions. In cases of discordance, the final adjudication was determined by the majority (2 out of 3).

**Results:** The committee adjudicated 337 (83%) subjects as No AKI and 71 (17%) subjects as AKI. Concordance among adjudicators was 94% (97% for No AKI subjects and 79% for AKI subjects). The KDIGO 2-3 rate (18%) was similar to adjudicated AKI rate, and the agreement between KDIGO staging and adjudication was 97% (98% for No AKI and 92% for AKI subjects). Of the 13 subjects for whom the adjudication and KDIGO staging differed, concordance among the adjudicators was 38% compared to 96% for those subjects for whom the adjudication and KDIGO staging agreed. Adjudication (blinded to biomarker values) agreed more with TIMP2•IGFBP7 levels than did KDIGO staging. The median TIMP2•IGFBP7 value was significantly ($p<0.01$) higher for the 6 patients who were adjudicated as AKI but did not reach KDIGO 2-3 ($2.78x10^3$ (ng/mL)$^2$</a>) compared to the 7 patients who were adjudicated as No AKI but reached KDIGO 2-3 ($0.52x10^3$ (ng/mL)$^2$). The TIMP2•IGFBP7 area under the receiver operating characteristic curve was 0.82 for detection of AKI as adjudicated by the committee.

**Conclusions:** Clinical adjudication showed differences compared to non-adjudicated KDIGO staging. This result has important implications for the evaluation of biomarkers and stresses the importance of independent adjudication in biomarker performance trials.

*Funding:* Pharmaceutical Company Support - Astute Medical

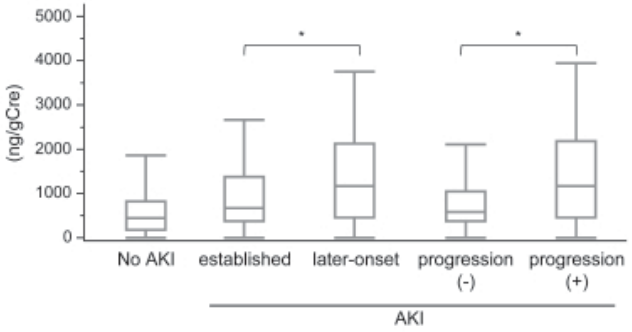
**FR-PO043**

**Urinary Semaphorin 3A Predicts the Progression of Acute Kidney Injury in an Adult Mixed Intensive Care Unit** Kent Doi,[1] Eisei Noiri,[1] Naoki Yahagi,[1] Calpurnia Jayakumar,[2] Ganesan Ramesh.[2] *[1]The Univ of Tokyo; [2]Georgia Health Sciences Univ.*

**Background:** Predicting the development of acute kidney injury (AKI) in critical care setting is challenging. Although several biomarkers showed moderately good performance for detecting established AKI even in heterogeneous disease oriented population, identification of new biomarkers that predict accurately the development of AKI is urgently required.

**Methods:** A single center prospective observational cohort study that was undertaken to evaluate for the first time the reliability of newly identified biomarker semaphorin 3A for AKI diagnosis in heterogeneous intensive care unit populations. Three hundred thirty-nine adult critically ill patients were recruited and urine samples were obtained at ICU admission.

**Results:** One hundred thirty one patients (39%) were diagnosed as AKI by the RIFLE criteria; 66 patients were diagnosed as AKI not at ICU admission but eventually within one week after (later-onset AKI). Eighty four AKI patients showed worsening the severity during one week observation (AKI progression). In addition to five urinary biomarkers of L-type fatty acid-binding protein (L-FABP), neutrophil gelatinase-associated lipocalin (NGAL), IL-18, albumin, and N-acetyl-β-D-glucosaminidase (NAG), urinary semaphorin 3A was measured at ICU admission. Although other five biomakers showed higher AUC-ROC values in detecting established AKI compared with detecting later-onset AKI or progression of AKI, semaphorin 3A was able to detect later-onset AKI and AKI progression better than established AKI (AUC-ROC for established AKI 0.64, later-onset AKI 0.71, AKI progression 0.71). Finally, sepsis did not have any impact on semaphorin 3A, while other urinary biomarkers were increased with sepsis.




**Conclusions:** A new AKI biomarker semaphorin 3A have a distinct predictive use for AKI progression from other AKI biomarkers.

*Funding:* Government Support - Non-U.S.

**FR-PO044**

**Identification of Superoxide Dismutase [Cu-Zn] as a Prognostic AKI Biomarker** Joseph Alge,[1] Nithin Karakala,[1] Benjamin Neely,[1] Michael G. Janech,[1] James A. Tumlin,[2] Lakhmir S. Chawla,[3] Andrew Shaw,[4,5] John M. Arthur.[1,6] *[1]Medical Univ of South Carolina; [2]Univ of Tennessee College of Medicine in Chattanooga; [3]George Washington Univ; [4]Duke Univ; [5]Durham VA Medical Center; [6]Ralph H Johnson VA Medical Center.*

**Background:** Prognostic biomarkers could be valuable tools for risk assessment during the early phases of AKI. We identified candidate prognostic biomarkers using the combined results of 5 discovery phase proteomics studies (3 human, 1 mouse, 1 rat).

**Methods:** Thirteen urinary proteins that appeared predictive of AKI across 5 proteomic analyses were measured by parallel reaction monitoring mass spectrometry in urine samples from 157 post cardiac surgery patients who were classified as AKIN stage 1 at the time of sample collection, 21 of whom met the primary outcome of AKIN stage 3 or death. Prediction of the primary outcome was evaluated using the area under the ROC curve.

**Results:**

| Table 1. Results of Parallel Reaction Monitoring Assay for Prediction of AKIN stage 3 or Death | | | |
|---|---|---|---|
| **Protein** | **AKIN 1 or 2/Survivedc** | **AKIN 3/Deathc** | **AUC** |
| Superoxide dismutase [Cu-Zn]a | 9.9 (4.9-20.3) | 22.0 (13.9-104.8)* | 0.74* |
| Angiotensinogenb | 75.4 (40.4-193.4) | 312.3 (122.0-493.8)* | 0.73* |
| L-FABPa | 12.1 (5.7-21.4) | 19.7 (15.0-58.4)* | 0.72* |
| Nonsecretory ribonucleasea | 8.2 (5.4-2.7) | 11.2 (7.2-20.7) | 0.62 |
| Uromodulina | 14.6 (8.5-24.1) | 19.9 (12.9-31.2) | 0.60 |
| Pigment epithelium derived proteinb | 14.3 (4.4-145.3) | 67.2 (8.9-354.9) | 0.60 |
| Antithrombin IIIb | 96.0 (27.1-270.5) | 92.6 (56.3-430.4) | 0.58 |
| Complement C4Bb | 6.4 (3.3-28.3) | 8.0 (4.7-17.7) | 0.57 |
| ProEGFb | 77.2 (8.8-240.5) | 40.7 (10.0-160.4) | 0.51 |
| Apo A-IV# | ND | ND | ND |
| CD59# | ND | ND | ND |
| Myoglobin# | ND | ND | ND |
| KIM-1# | ND | ND | ND |

afmol/μg creatinine; battomol/μg creatinine; cmedian (IQR); AUC, area under the receiver operator characteristic curve; *P<0.05; #>90% of samples were below limit of detection; ND, Not determined

**Conclusions:** We identified superoxide dismutase [Cu-Zn] as a new biomarker of worsening AKI and confirmed angiotensinogen and L-FABP as prognostic AKI biomarkers. Several other candidates had marginal prognostic ability. No conclusions could be drawn regarding Apo A-IV, CD59, and myoglobin due to the large number of patients with undetectable protein concentrations.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO045**

**Fibroblast Growth Factor 23 Is a Potential Biomarker of Development of Acute Kidney Injury in Rats and Patients: Preliminary Results** Luis F. Michea,[1] Luis Toro.[1,2] *[1]Centro Estudios Moleculares de la Celula, Facultad de Medicina Universidad de Chile, Santiago, Chile; [2]Critical Care Unit, Hospital Clinico Universidad de Chile, Santiago, Chile.*

**Background:** Acute kidney injury (AKI) is a condition of high incidence in Critical Care Units (CCU). Retrospective studies have shown association between serum Fibroblast Growth Factor 23 (sFGF-23) and AKI in hospitalized patients. The objective of the study is to evaluate in prospective clinical studies and murine models whether sFGF-23 is a predictor of AKI.

**Methods:** 1) Prospective cohort of patients admitted in the CCU of our hospital with severe sepsis or septic shock. sFGF-23 was measured at admission, 24 and 48 hours after hospitalization, plus clinical and biochemical parameters. Primary outcomes evaluated were the development and severity of AKI, defined by AKIN criteria. 2) Rat model of AKI through unilateral nephrectomy (U-NPX), with measurement of sFGF-23 and biochemical parameters at baseline, 1, 2, 3 and 4 hours after intervention, with a sham surgery control group.

**Results:** 1) Preliminary results with 11 patients. 7 (64%) developed AKI during hospitalization. 4 of them developed AKIN 1, 2 had AKIN 2 and 1 had AKIN 3. sFGF-23 was significantly increased in patients with AKI, compared to non-AKI patients, where the difference was most relevant at admission (AKI: 70.8±31.7 pg/mL; non-AKI: 14.9±6.1 pg/mL; $p<0.01$). The variation of sFGF-23 in AKI patients compared to non-AKI patients was more pronounced that changes in serum creatinine (sCr). Also, sFGF-23 at admission was significantly higher in patients with AKIN 2 and 3, compared to AKIN 1 patients (AKIN 2+3: 80.4±24.6 pg/mL; AKIN 1: 36.3±8.0 pg/mL; $p<0.01$). 2) sFGF-23 increased significantly after U-NPX, compared to control rats and baseline values. The increase was detected as early as 3 hours after U-NPX (10.6x higher than Sham, $p<0.01$), with a more pronounced variation in sFGF-23 compared to sCr.

**Conclusions:** Serum FGF-23 is correlated with the presence and severity of AKI in rats and CCU patients. Due to its early increase and magnitude, it may potentially be useful as a more sensitive biomarker to evaluate AKI.

*Funding:* Pharmaceutical Company Support - FONDECYT 1130550,1090223, IMII P09/016F, Government Support - Non-U.S.

**FR-PO046**

**Utility of Fractional Excretion of Sodium (FeNa) Compared with the Old-Fashion Clinical History and Urinalysis in the Evaluation of Acute Kidney Injury** A.C. Ortiz-Guerrero,[1] R.R. Malchira,[1] C.S. Huang,[1] E. McCann,[1] A. Jittirat,[1] P.T.T. Pham,[2] P.C. Pham.[1] *[1]Nephrology, Olive View MC; [2]Kidney & Pancreas Transplant, UCLA, Los Angeles, CA.*

**Background:** Traditionally, it is taught that FeNa can be used in the evaluation of non-oliguric acute kidney injury (AKI) to differentiate the prerenal state from acute tubular necrosis. Accordingly, primary care physicians (PCPs) oftentimes solely focus on obtaining FeNa prior to consulting nephrology for AKI, but not necessarily a complete clinical history or urinalysis (UA) that could be of great diagnostic value. We compared the diagnostic value of FeNa versus those obtained from routine clinical history and UA in the AKI setting.

**Methods:** This is a prospective study. All consults involving AKI evaluated by the lead author from July 2012-June 2013 were included. Data collected: Age, gender, ethnic background, presenting laboratory findings including Na, K, Cl, HCO3, blood urea nitrogen, creatinine, FeNa, UA, clinical history, and final diagnosis of AKI, recorded as prerenal or "other." FeNa <1%, presence of hyaline casts, urine specific gravity >1.015, or BUN to creatinine ratio (BUN:Cr) = of > 20, were considered to be consistent with prerenal state. Sensitivity and specificity of using FeNa, clinical history, UA, either positive clinical history or UA, and BUN:Cr for identifying a prerenal cause of AKI were calculated.

**Results:** There were 35 cases: 17 females, 17 Hispanics, 11 Caucasians, 7 others, mean age: 51+/-14, estimated glomerular filtration rate (MDRD-4): 24+/-15 mL/min/1.73m$^2$.

| | FeNa | Clinical history | UA | Either Clinical history or UA | BUN:Cr ≥ 20 |
|---|---|---|---|---|---|
| Sensitivity (%) | 62 | 62 | 77 | 85 | 38 |
| Specificity (%) | 68 | 82 | 54 | 59 | 86 |

**Conclusions:** While many PCPs heavily rely on FeNa to evaluate AKI, we suggest that FeNa does not provide greater sensitivity or specificity in identifying prerenal state compared to the basic clinical history and urinalysis. Emphasis on obtaining a thorough clinical history and routine UA rather than the sole reliance on FeNa for AKI evaluation among PCPs is warranted. Of interest, BUN:Cr > 20 traditionally taught to indicate a prerenal state has low sensitivity but high specificity.

## FR-PO047

**Assessing Fractional Excretion of Urea for Early Diagnosis of Cardiac Surgery Associated Acute Kidney Injury** Federico Varela, Angel Medina Ayala, Gustavo Cristian Greloni, Matilde Josefina Navarro, Guillermo Javier Rosa diez. *Nephrology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.*

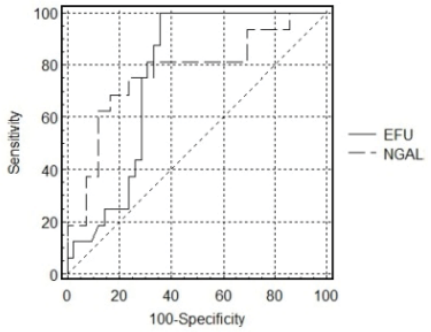
**Background:** AKI is a common complication after Cardiac Surgery (CS). Fraction Excretion of Urea (EFU) has been cited as a precise method to discriminate between early prerrenal and established AKI. The aim of our study is evaluate sensitivity and specificity of the EFU, in the early diagnosis of AKI in patients undergoing CS.

**Methods:** Prospective study of adult patients undergoing CS. AKI was defined by AKIN criteria. CKD patients were excluded. EFU sensitivity and specificity on predictive AKI diagnosis were tested on 1, 6 and 24 hours after CS. Same evaluation was conducted for urinary NGal. Comparation of AUC (ROC curve) for both methods was made.

**Results:** Sixty patients, 68 ± 11 years, 25% female. AKI prevalence 26.23%, mortality 3.28%. Staging AKI: 56% was AKIN I, 25% was AKIN II and was 19% AKIN III. Thirty-five of patients received diuretics during the first 48 hours. Patients with AKI had significantly lower EFU compared with normal kidney function group (13.3 ± 2.7 vs. 31.6 ± 3.4%, p<0.05) only at 6 hours after CS . N-Gal values also showed statistical difference between both groups at same period.

| | N Gal µg/l Hour 1 | EFU% Hour 1 | NGal µg/l Hour 6 | EFU% Hour 6 | NGal µg/l Hour 24 | EFU% Hour 24 |
|---|---|---|---|---|---|---|
| No AKI | 97.9 ± 43.1 | 31.5 ± 23.5 | 33.8 ± 38.9* | 31.6 ± 22** | 118.1 ± 279.4 | 40.9 ± 57 |
| AKI | 108.9 ± 59.7 | 25.9 ± 22.5 | 103.5 ± 104* | 13.3 ± 10** | 104.36 ± 127.88 | 16 ± 15 |

EFU showed sensitivity of 100%, specificity 65%, AUC of 0.768 and Yunden point of 28.04%. Comparision of AUC of both methods not showed statistical difference.




**Conclusions:** The results of this study support that EFU measured early after CS, predicts AKI in these patients.

## FR-PO048

**Renal Outcomes after Liver Transplantation in Fulminant Hepatitis A with Acute Kidney Injury: Comparison with Hepatorenal Syndrome** Jae-yoon Park,[1] Jung Pyo Lee,[2] Hyung Jung Oh,[3] Dong Ki Kim,[1] Chun Soo Lim,[2] Yon Su Kim.[1] *[1]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [2]Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea; [3]Internal Medicine, Yonsei Univ College of Medicine, Seoul, Korea.*

**Background:** Liver transplantation (LT) is the treatment of choice for hepatorenal syndrome (HRS). However, the outcome of LT is not well established in acute hepatitis A accompanied by acute kidney injury (AKI). We investigated the outcomes of LT in patients with AKI associated with acute hepatitis A compared with patients with HRS due to other causes.

**Methods:** We reviewed 1940 consecutive adult recipients undergoing LT at three liver transplantation centers in Korea between 2005 and 2012. 20 patients with acute hepatitis A and AKI (HAV group) were compared with 76 patients with HRS from other causes (HRS group).

**Results:** There was no difference in the model for end-stage liver disease (MELD) score between groups. Pretransplant estimated glomerular filtration rate (eGFR) was lower in the HAV group compared with the HRS group (20.6 ± 12.7 *vs.* 28.2 ± 12.8 mL/min/1.73m$^2$, p=0.021). Posttransplant patient and graft survival rates were similar between groups. More patients in the HAV group required posttransplant hemodialysis compared with the HRS group (65.0% *vs.* 38.2%, p=0.043). However, eGFR had been significantly higher in HAV group since posttransplant month 2 (p<0.05). In addition, the HAV group had superior recovery rate of kidney function (eGFR ≥60ml/min/1.73m$^2$) after adjustment for pretransplant renal function or liver function. No patients required long-term renal replacement therapy in either group.

**Conclusions:** Pre- and immediate posttransplant kidney function is worse in patients with AKI due to acute fulminant hepatitis A, compared to those with HRS due to any cause. However, posttransplant long-term renal outcomes may be better in the HAV group.

## FR-PO049

**GFR Distributions among Scheduled Contrast-Enhanced Computed Tomography before and after eGFR Self-Report** Yoshinari Yasuda, Kanako Shibata, Shoichi Maruyama, Seiichi Matsuo. *Nephrology/CKD Initiatives, Nagoya Univ, Nagoya, Japan.*

**Background:** Renal insufficiency is the most important risk factor for contrast induced nephropathy (CIN), however it is difficult to assess renal function only by serum creatinine (sCr). Estimated glomerular filtration rate (eGFR) is globally recommended for evaluation of renal function, and eGFR self-report may affect clinical decision making to prevent CIN. Since Nagoya University Hospital (NUH) started eGFR self-report in December 2009, we analyzed GFR distributions in out-patients prior to scheduled contrast-enhanced computed tomography (CE) in NUH before and after eGFR self-report.

**Methods:** Study subjects were 6,108 and 6,308 out-patients who were examined by scheduled CE in NUH in 2008 and 2011. Patients under 18 years of age and emergency CE cases were excluded. Age, gender and sCr before CE (until 4 months before) were collected from medical record and eGFR was calculated by Japanese eGFR equation. Implementation rates of sCr measurements and distributions of age, gender and eGFR categories (G1: 90 and above, G2: 60-89, G3a: 45-59,G3b: 30-44 ,G4: 15-29 and G5: less than 15 mL/min/1.73m2) were compared before and after eGFR self-report. And implementation rates preventive hydration were analyzed among CKD patients with eGFR below 45 mL/min/1.73m$^2$.

**Results:** Age and gender distributions were not different in 2008 and in 2011. Implementation rates of sCr measurements were 78.5% in 2008 and 78.4% in 2011. Proportion of G3b to G5 patients in 2011 was significantly smaller than that in 2008. Implementation rate of preventive hydration was dramatically increased in 2011 (86.7%) compared to 2008 (31.8%) , however hydration regimen was not appropriate in some cases.

**Conclusions:** Distributions of eGFR categories and implementation rate of preventive hydration among patients examined by scheduled CE were different before and after eGFR self-report in NUH, suggesting its preferable effect to prevent CIN.

*Funding:* Government Support - Non-U.S.

## FR-PO050

**SIRS, ALI, and AKI Are Established by Four Hours in Experimental Sepsis and Are Not Improved by Post-Sepsis Administration of TNF-α Antibodies** Sarah Faubel, Chris Altmann, Ana Andres-hernando, Rhea Bhargava, Kayo Okamura. *U of CO.*

**Background:** Acute kidney injury (AKI) and acute lung injury (ALI) are complications of sepsis, and the presence of either increases mortality. AKI is often viewed as a late complication of sepsis and some studies have suggested that AKI occurs as a result of ALI. Notably, the onset of AKI relative to ALI is unclear as routine measures of kidney function (BUN, creatinine) are insensitive and increase late. In this study, we hypothesized that AKI and ALI would occur simultaneously due to a shared pathophysiology (i.e., TNF-α mediated systemic inflammatory response syndrome [SIRS]), but that sensitive markers of kidney function would be required to identify AKI.

**Methods:** Sepsis was induced in male C57B/6 mice with 4 different one time doses of intraperitoneal (IP) endotoxin (LPS) (0.0001, 0.001, 0.01, or 0.25 mg). SIRS was assessed by serum inflammatory cytokines (TNF-α, IL-1β, CXCL1, IL-6), ALI was assessed by lung inflammation (lung MPO activity), and AKI was assessed by serum creatinine, BUN, and glomerular filtration rate (GFR) (FITC-labeled inulin clearance) at 4 hours. 20 µgs of TNF-α antibody (Ab) or vehicle was injected IP 2 hours before or 2 hours after LPS.

**Results:** Serum cytokines and lung MPO activity increased with all 4 doses of LPS; creatinine did not increase with any dose; BUN increased with 0.01 and 0.25 mg. Remarkably, GFR was reduced with all 4 doses of LPS and was 50% reduced in the 0.001 mg dose, demonstrating that dramatic loss of kidney function can occur in sepsis without a change in BUN or creatinine. Prophylactic TNF-α Ab reduced serum cytokines, lung MPO activity, and BUN; however, post-sepsis administration had no effect.

**Conclusions:** ALI and AKI occur together after sepsis and TNF-α plays a role in the pathogenesis of both. Although failure of anti-TNF-α therapy in clinical trials has generated doubt regarding the role of TNF-α in the pathophysiology of sepsis and called into the question the value of animal models, our data demonstrate that TNF-α mediated ALI and AKI are established early and not amenable to anti-TNF-α treatment; thus, even after just 2 hours, anti-TNF-α therapy cannot be expected to be successful in patients with sepsis.

*Funding:* Other NIH Support - NHLBI

**FR-PO051**

**Activation of Type 1 Angiotensin II Receptors on T Lymphocytes Limits TNF-α-Mediated Acute Kidney Injury** Jiandong Zhang, Mehul B. Patel, Jose Gomez, Matthew A. Sparks, Steven D. Crowley. *Duke Univ.*

**Background:** Although blockade of type 1 angiotensin ($AT_1$) receptors is a cornerstone of therapy for patients with chronic kidney disease, the role of the renin-angiotensin system (RAS) in the pathogenesis of acute kidney injury remains unclear. In the present experiments, we examined the actions of $AT_1$ receptors specifically on T lymphocytes during acute kidney injury in the cisplatin (CIS) nephropathy model.

**Methods:** To this end, we intercrossed mice carrying a floxed gene for the $AT_{1A}$ receptor (*Agtr1a*$^{flox/flox}$) with mice harboring *Cre recombinase* under the control of the CD4 promoter to remove $AT_{1A}$ receptor-mediated responses from T lymphocytes alone (*Cre*$^{+}$ *Agtr1a*$^{flox/flox}$ = TKO). Compared to *Cre*$^{-}$ *Agtr1a*$^{flox/flox}$ littermates (WT), the TKOs had a ~90% reduction in $AT_{1A}$ receptor mRNA expression solely in T lymphocytes ($p<0.0001$). We also generated CD4-Cre mT/mG (T cell GFP) mice in which T cells fluoresce green to trace renal infiltration of T lymphocytes.

**Results:** When we subjected T cell GFP mice to CIS injection (30mg/kg), we detected green signals in the kidney out to 72 hrs**,**confirming the renal infiltration of T lymphocytes during the window of nephrotoxicity. At 72hrs, BUN in CIS-treated WTs increased dramatically to 185±20mg/dL, compared to vehicle-treated WTs (25±0;,$p<0.001$), but CIS-treated TKOs had 30% higher BUNs than WTs (244±20; $p=0.008$). Similarly, CIS-treated TKOs had 50% higher serum creatinines (Scr) (1.85±0.21 vs. 1.24±0.23 mg/dL; $p=0.02$). Moreover, compared to CIS-treated WTs, TKOs had augmented renal mRNA expression for the injury marker NGAL (683±203 vs. 1796±645 au; $p=0.006$). TNF- α mediates CIS nephropathy and is produced by several cell lineages including T cells. Renal mRNA expression of TNF-α was increased in CIS- vs. vehicle-treated WTs (13.3±3.4 vs. 1.0±.21au), but even more profoundly in CIS-treated TKOs (30.8±5.2, $p=0.01$). By ELISA, protein levels of TNF- α were also higher in CIS-treated TKOs in the kidney (342±36 vs. 233±20 pg/mg protein, $p=0.004$) and the serum (169±52 vs. 59±20 pg/mL, $p<0.04$).

**Conclusions:** We concludethat $AT_1$ receptor stimulation on T lymphocytes ameliorates acute kidney injury by limiting the production of TNF-α.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO052**

**Selective Deletion of Endothelial Sphingosine-1-Phosphate Receptor 1 Exacerbates Kidney Ischemia-Reperfusion Injury** H. Thomas Lee,[1] Vivette D. D'Agati,[2] Mihwa Kim.[1] *[1]Dept of Anesthesiology, Columbia Univ, New York, NY; [2]Dept of Pathology, Columbia Univ, New York, NY.*

**Background:** Acute kidney injury (AKI) is a devastating clinical problem without effective therapy and renal ischemia reperfusion (IR) injury is a major cause of AKI. Although the renal protective effects of proximal tubule sphingosine-1-phosphate receptor 1 ($S1P_1R$) are well known, the role for endothelial $S1P_1R$ in AKI remains unclear as germline endothelial $S1P_1R$ deletion is embryonically lethal.

**Methods:** To test whether endothelial $S1P_1Rs$ play a protective role against AKI due to renal IR injury, we generated conditional endothelial $S1P_1R$ deficient mice by crossing mice with floxed $S1P_1R$ ($S1P_1R^{f/f}$) with mice that express a tamoxifen-inducible form of Cre recombinase (iCreER) under the transcriptional control of the Platelet-derived growth factor-b (Pdgfb) gene. We subjected tamoxifen-treated adult $S1P_1R^{f/f}$ $Pdgfb^{iCreER}$ mice as well as wild type ($S1P_1R^{f/f}$) mice to mild (20 min) renal IR injury.

**Results:** Mice with tamoxifen-induced deletion of endothelial $S1P_1R$ developed significantly worse kidney injury with large increases in plasma Cr (2.81+0.21 mg/dL, N=5) 24 hr after 20 min renal ischemia when compared to wild type $S1P_1R^{f/f}$ mice (Cr=0.95+0.22 mg/dL, N=5, $P<0.001$). Mice with conditional deletion of endothelial $S1P_1R$ ($S1P_1R^{f/f}$ $Pdgfb^{iCreER}$ mice) also had ~3 fold increase in kidney vascular permeability as well as increased renal tubular necrosis, neutrophil infiltration, pro-inflammatory cytokine expression and renal tubular apoptosis after ischemic AKI when compared to wild type mice. Moreover, conditional endothelial $S1P_1R$ deficient mice subjected to 20 min renal IR had increased hepatic injury (plasma ALT=160+20 U/L, N=5) compared to wild type mice (ALT=95+18 U/L, N=5, $P<0.05$). As a potential mechanism for exacerbated renal injury, conditional endothelial $S1P_1R$ null mice had markedly reduced (~90%) endothelial HSP27 expression compared to wild type mice.

**Conclusions:** Taken together, our studies demonstrate a protective role for endothelial $S1P_1R$ against ischemic AKI most likely by regulating endothelial barrier integrity and endothelial HSP27 expression.

*Funding:* NIDDK Support, Other NIH Support - NIGMS

**FR-PO053**

**Prior Splenectomy Increases the Susceptibility of Mice to Renal Ischemia-Reperfusion Injury** Joseph C. Gigliotti,[1] Liping Huang,[1] Eric Mace,[1] Amandeep Bajwa,[1] Hong Ye,[1] Alexander L. Klibanov,[2] Mark D. Okusa.[1] *[1]Div of Nephrology and CIIR; [2]Div of Cardiology, Univ of Virginia, Charlottesville, VA.*

**Background:** Several preventative therapies for acute kidney injury (AKI) in mice require an intact spleen. However, the interaction of the spleen with AKI is unknown. Therefore the objective of the current study was to determine how prior splenectomy (SPLX) modulates the susceptibility of mice to AKI.

**Methods:** Male 8-12 wk old C57BL/6 or $Rag1^{-/-}$ mice underwent SPLX or sham operation 7d prior to subthreshold, bilateral renal ischemia-reperfusion injury (subIRI). After 24h of reperfusion, AKI was assessed by plasma creatinine and histology. Renal inflammation was determined by FACS, immunohistochemistry, and renal CXCL1 and IL-6 mRNA expression. In separate experiments, phagocytic splenocytes were labeled by i.v. injection of fluorescently labeled liposomes 24h prior to unilateral IRI. Tissue samples were collected 1, 3, and 7d following reperfusion.

**Results:** 5d after SPLX, no difference ($P\geq0.25$) was observed in circulating leukocytes or total hematocrit compared to sham-operated mice. Plasma creatinine (mg/dL) in mice receiving subIRI alone was similar (0.4) to controls (0.3). However, SPLX+subIRI resulted in higher plasma creatinine (1.2, $P=0.001$) and acute tubular necrosis (15% of kidney section area) compared to subIRI alone (5%, $P<0.001$). SPLX+subIRI resulted in increased circulating ($P=0.009$) and infiltrating neutrophils ($P<0.001$) and renal mRNA expression of CXCL1 and IL-6 ($P\leq0.003$). SPLX $Rag1^{-/-}$ mice were also more susceptible to IRI ($P<0.001$), suggesting the effect of SPLX is not dependent upon T or B-cells. Fluorescently labeled cells were observed 3d after reperfusion and persisted only in the ischemic kidney through 7d, suggesting phagocytic cells originating from the spleen localize to ischemic renal tissue following AKI.

**Conclusions:** The spleen mediates the susceptibility of mice to IRI. Prior SPLX results in increased renal inflammation and damage following IRI. Trafficking of splenic phagocytes does not appear to influence AKI, since fluorescently labeled cells were not observed in the ischemic kidney until 3d after reperfusion.

*Funding:* NIDDK Support

**FR-PO054**

**Prior Ultrasound Exposure Improves Survival and Modulates the Early Inflammatory Response to Ischemia-Reperfusion Injury in Mice** Joseph C. Gigliotti,[1] Liping Huang,[1] Alexander L. Klibanov,[2] Diane L. Rosin,[1,3] Kambiz Kalantari,[1] Mark D. Okusa.[1] *[1]Div of Nephrology and CIIR; [2]Div of Cardiology; [3]Dept of Pharmacology, Univ of Virginia, Charlottesville, VA.*

**Background:** Inflammation mediates the tissue injury that occurs during acute kidney injury (AKI). We have shown prior ultrasound (US) exposure reduces AKI and inflammation in mice. How US modulates inflammation is unknown. Therefore, the objective of the current study was to determine if prior US exposure modulates the initial inflammatory response to AKI.

**Methods:** Male C57BL/6 mice were anesthetized and exposed to US (7MHz, MI = 1.2) using Siemens Acuson Sequoia 512 system with a 15L8w transducer. 24h after US exposure, mice were subjected to bilateral renal ischemia-reperfusion injury (IRI). For survival studies, mice were maintained for 7d after IRI. For acute studies, mice were euthanized and tissue samples were collected after 0h (no reperfusion) and 0.5h after IRI. AKI was assessed by measuring plasma creatinine. Neutrophils were quantified by FACS and renal IL1β mRNA expression was determined by RT-PCR.

**Results:** Exposure to US 24h prior to IRI significantly improved 7d survival as compared to IRI alone (mortality of 25% vs 87%, $P=0.004$). The protective effect of US was observed 0.5h after reperfusion, where mice pretreated with US had a 67% reduction ($P=0.02$) in plasma creatinine compared to mice receiving IRI alone. IRI resulted in a 3-fold increase in renal neutrophils, a phenomenon prevented with prior US exposure ($P=0.05$). However, prior US did not influence ($P=0.99$) circulating neutrophils 0.5h after reperfusion, suggesting that US modulates trafficking of neutrophils into the kidney. US reduced ($P=0.05$) kidney IL1β mRNA expression by 50% after ischemia alone (no reperfusion), suggesting prior US exposure modulates the response of the kidney to ischemic damage.

**Conclusions:** The results demonstrate that prior US exposure decreases the mortality associated with IRI in mice. This could be due to an US-induced reduction in ischemic tissue damage, which in turn reduced neutrophil accumulation following reperfusion. Identifying the mechanism responsible will shed light on new therapeutic targets for AKI.

*Funding:* NIDDK Support

**FR-PO055**

**Macrophage-Specific Deletion of Transforming Growth Factor-β1 Does Not Prevent Renal Fibrosis after Severe Ischemia-Reperfusion Injury** Sarah C. Huen, Gilbert W. Moeckel, Lloyd G. Cantley. *Yale Univ School of Medicine, New Haven, CT.*

**Background:** The persistence of macrophages is associated with tubulointerstitial fibrosis and progression of chronic kidney disease. Macrophages are known to be major producers of transforming growth factor-β1 (Tgf-β1). Tgf-β1 has long been implicated as a central mediator of kidney fibrosis. To determine the fibrogenic role of macrophage-derived Tgf-β1 we deleted *Tgfb1* from myeloid cells by establishing a *LysM-Cre;Tgfb1*$^{f/n}$ transgenic mouse and induced severe unilateral ischemic renal injury.

**Methods:** *LysM-Cre* mice were bred with *Tgfb1*$^{f/n}$ (*loxP* sites flanking exon 1 of the *Tgfb1* gene on one allele with the second allele being *Tgfb1* null) mice. Wild-type (*Tgfb1*$^{f/+}$) and whole body *Tgfb1* heterozygous with macrophage specific *Tgfb1* knockout (*LysM-Cre;Tgfb1*$^{f/n}$) male mice 8-10 weeks old were subjected to 35 minutes of unilateral renal ischemia. Kidneys were harvested 28 days after ischemia/reperfusion (I/R) for mRNA and protein analysis as well as histology for blinded fibrosis and tubular injury scoring.

**Results:** Both bone marrow derived macrophages and macrophages isolated from the kidney 28 days after I/R injury from *LysM-Cre;Tgfb1*$^{f/n}$ mice showed significantly decreased *Tgfb1* expression compared to macrophages from wild-type mice. Whole kidney *Tgfb1* mRNA expression increased by 6-fold after I/R injury in wild-type mice, while injured kidneys from *LysM-Cre;Tgfb1*$^{f/n}$ mice exhibit an overall reduction of *Tgfb1* by 75.5%. Consistent with the sustained increase in *Tgfb1* mRNA expression in wild-type kidneys, Smad2/3 was highly activated. In contrast, Smad2/3 activation was eliminated in injured kidneys from *LysM-Cre;Tgfb1*$^{f/n}$ mice. *LysM-Cre;Tgfb1*$^{f/n}$ injured kidneys showed a trend toward less fibrosis that did not reach statistical significance compared to wild-type injured kidneys. Markers of extracellular matrix and fibrogenic genes remain elevated in *LysM-Cre;Tgfb1*$^{f/n}$ kidneys and were only modestly decreased compared to wild-type kidneys.

**Conclusions:** Targeting macrophage-derived Tgf-β1 does not appear to be an effective therapy for attenuating progressive renal fibrosis after ischemic kidney injury.

*Funding:* NIDDK Support

## FR-PO056

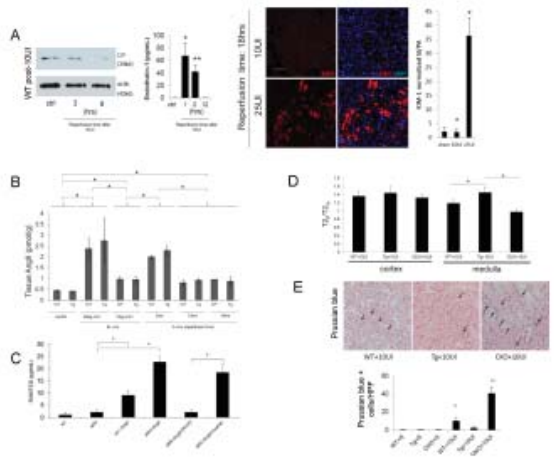
### RGS4 Inhibits Angiotensin II Signaling and Macrophage Localization during Renal Reperfusion Injury Independent of Vasomotor Tone

Paul Pang, Padsawan Khamlue, Joseph V. Bonventre, Andrew M. Siedlecki. *Medicine, Brigham and Women's Hospital, Boston, MA.*

**Background:** Vascular inflammation is a major contributor to the severity of acute kidney injury. The anti-inflammatory effect of RGS4 in angiotensin II-induced atherosclerosis is known (PNAS, 105:4277, 2008) but has not been studied in the setting of reperfusion injury in the kidney. We hypothesized that RGS4 expression in vascular smooth muscle cells is protective to the kidney after vascular injury.

**Methods:** We used RGS4 overexpressing (R4OX) and smooth cell-specific RGS4 deleted mice in a model of acute renovascular kidney injury. RGS4-dependent cell signaling was studied in vascuar smooth muscle cell (VSMC) culture.

**Results:** 18 hours after 10-minute unilateral renal artery clamping we observed: no tubular injury, normalized ET-1 levels, and reduced calponin-1 expression.




At the same timepoint blood flow by dynamic MRI was decreased in post-clamp wild type kidneys vs R4OX. WT and R4OX tissue- AngII levels were elevated in a sub-vasoconstrictive range in kidneys after 12 and 18hrs; confirmed by isolated perfused-kidney technique. By cytokine screen, AngII stimulated VSMC to secrete macrophage chemoattractant, RANTES. RANTES expression increased further when RGS4 expression was suppressed. AngIIR2 (AT2) inhibition decreased RANTES expression in RGS4-depleted cells implicating Gα protein activation in an AT2-RGS4-dependent pathway. Specificity of RGS4 function in VSMC was confirmed with VSMC-specific RGS4-/- that showed high macrophage density by T2 MR imaging compared to Tg and non-Tg. MΦ localized to the above region of blood flow disturbance. Tissue AngII in knockouts was also elevated at 12 and 18 hours but in a non-vasoactive range.

**Conclusions:** We conclude that RGS4 expression, specific to renal VSMC, inhibits AngII-mediated oxidative stress and macrophage recruitment during reperfusion injury.

*Funding:* NIDDK Support

## FR-PO057

### Regulatory T Cells Promote Renal Protection from Ischemia-Reperfusion Injury through Programmed Cell Death 1 (PD-1) Interactions with PD-L1 and PD-L2

Katarzyna Jaworska, Joanna Ratajczak, Brian K. Stevens, Liping Huang, Mark D. Okusa, Gilbert R. Kinsey. *School of Medicine, Div of Nephrology, UVA, Charlottesville, VA.*

**Background:** Regulatory T cells (Tregs) exert anti-inflammatory effects through diverse contact-dependent and -independent mechanisms. We have recently shown that Tregs protect against ischemia reperfusion (IR) injury and PD-1 expression on Tregs is required for their protective effect. We hypothesized that Treg PD-1 engagement by its ligands PD-L1 and PD-L2 promotes suppression of post-ischemic renal inflammation and dysfunction.

**Methods:** Naïve C57Bl/6 (WT) mice were injected with monoclonal blocking antibodies to PD-L1 or PD-L2 or isotype control antibodies 24 hr prior to bilateral renal ischemia followed by 24 hr reperfusion. In further experiments, WT Tregs were injected 6 hr after antibodies and 18 hr prior to IR.

**Results:** In a model of mild kidney IR (24 min ischemia) that does not result in significant renal dysfunction in isotype control antibody-treated mice (plasma creatinine (PCr) level: 0.5±0.1 vs. sham: 0.4±0.1) blockade of PD-L1 or PD-L2 prior to IR caused a significant reduction in renal function (PCr: 1.4±0.2 and 1.5±0.2, respectively) with enhanced acute tubular necrosis (ATN) and distinct patterns of inflammation in the kidney measured by flow cytometry, immunofluorescence microscopy and ELISA. Blocking both ligands simultaneously resulted in additive potentiation of renal inflammation and injury (PCr 2.4±0.1). As previously published, adoptively transferred WT Tregs offered near-complete protection from moderate renal IR (26 min ischemia) in the absence of PD-1 ligand blocking antibodies. In mice undergoing IR (24 min ischemia) in the presence of PD-L1 or PD-L2 blockade we observed moderate renal dysfunction (PCr: 1.5±0.2; 1.8±0.2, respectively) and adoptive transfer of WT Tregs in these mice did not protect from injury (PCr: 1.5±0.3 and 1.9±0.1, respectively), ATN or inflammation in the kidney.

**Conclusions:** These results suggest PD-L1 and PD-L2 have distinct and additive roles in protection from ischemic kidney injury and adoptively transferred Tregs must interact with both PD-L1 and PD-L2 to offer protection from kidney IRI.

*Funding:* NIDDK Support

## FR-PO058

### Effect of Endotoxemia on the Expression of the $PGE_2$ Synthetic System, and of Prostaglandin EP Receptors in the Kidney of the Mouse

Klaus Höcherl, Katharina Mederle, Hayo Castrop, Frank Schweda. *Univ of Regensburg, Institute of Physiology, Regensburg, Bavaria, Germany.*

**Background:** Sepsis is one of the leading causes of acute kidney injury (AKI). The present study was undertaken to characterize the biosynthetic pathway of prostaglandin (PG) $E_2$ and to examine the role of $PGE_2$ in the pathogenesis of endotoxemia-related AKI.

**Methods:** Male C57BL/6 mice, 8-10 wk old, were used. Endotoxemia was induced by the injection of lipopolysaccharide (LPS; 3 mg/kg; i.p.). The mRNA and protein levels were measured by q-RT-PCR and by immunoblotting, respectively. $PGE_2$ levels were determined by assay kits. Glomerular filtration rate (GFR) was determined by FITC-sinistrin clearance in conscious mice.

**Results:** LPS caused a time-dependent increase in renal COX-2 expression, whereas COX-1 expression was decreased. Endotoxemia increased the expression of mPGES-1, but not of mPGES-2 or cPGES. This was paralleled by an increase in tissue $PGE_2$ concentration. The COX-1 inhibitor SC-560 (20 mg/kg; i.p.) and the COX-2 inhibitor SC-236 (10 mg/kg; i.p.) decreased basal tissue $PGE_2$ levels. However, only pretreatment with SC-236 attenuated the LPS-induced increase in tissue $PGE_2$ concentration. Inhibition of COX-2 did not alter basal GFR, but augmented the LPS-induced decline in GFR. LPS caused an induction of the mRNA for the prostaglandin EP4 receptor, whereas the abundances of the prostaglandin EP1 and EP3 receptors were decreased. The mRNA expression of the EP2 receptor was not altered in response to LPS. In addition, $PGE_2$ exerted a dual effect on renal vascular tone, inducing vasodilatation at lower concentrations and vasoconstriction at higher concentrations in kidneys of control mice. In kidneys from endotoxemic mice, the vasodilatory component was more pronounced, whereas the vasoconstriction at higher $PGE_2$ concentrations was absent.

**Conclusions:** Our data provide evidence that an activation of the COX-2 / mPGES-1 synthetic pathway is responsible for the increased renal formation of $PGE_2$ in response to LPS. Our data further suggest that the vasodilatory effect of $PGE_2$ is enhanced in response to endotoxemia. Thus, agonism of EP2 and/or EP4 receptors may provide a basis for the treatment of sepsis-induced AKI.

*Funding:* Government Support - Non-U.S.

## FR-PO059

### Regulatory T Cells Recruited to the Kidney by *N,N*-Dimethylsphingosine Ameliorate Lipopolysaccharide-Induced Acute Kidney Injury

Koeun Lee,[1] Sang Heon Suh,[1] Chang Seong Kim,[1] Joon Seok Choi,[1] Eun Hui Bae,[1] Seong Kwon Ma,[1] Jongun Lee,[2] Soo Wan Kim.[1] *[1]Internal Medicine, Chonnam National Univ Medical School, Kwangju, Korea; [2]Physiology, Chonnam National Univ Medical School, Kwangju, Korea.*

**Background:** Regulatory T cells (Tregs) induce immunologic tolerance and prevent inflammatory diseases. The present study was aimed to examine whether Tregs recruited to the kidney by N,N-dimethylsphingosine (DMS), a sphingosine kinase inhibitor, prevents lipopolysaccharide (LPS)-induced kidney injury.

**Methods:** Mice were treated with LPS (20 mg/kg, i.p.) with or without DMS (0.4 mg/kg, i.p.). The degree of apoptosis was assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. The expression of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), endothelin-1 (ED-1), and Foxp3 was determined by immunoblotting and/or immunohistochemistry, while mRNA level of proinflammatory cytokines, chemokines, adhesion molecules, and Foxp3 was measured by real-time PCR. Using flow cytometry analysis, we investigated the expression of $CD4^+$ $CD25^+$ Tregs in the mice spleen and kidney.

**Results:** Plasma creatinine level was increased after LPS injection for 12 h compared with controls, which was attenuated in pretreatment with DMS. LPS treatment resulted in the elevation of both bax/bcl-2 ratio and number of TUNEL-positive cells in the kidney, which was counteracted by DMS pretreatment. The expression of COX-2, iNOS, and ED-1 was increased in LPS group, which was counteracted by DMS. DMS pretreatment also ameliorated LPS-induced increased mRNA expression of proinflammatory cytokines, chemokines, adhesion molecules,Administration of LPS upregulated $CD4^+$ $CD25^+$ Tregs in the spleen, while downregulated the cells in the kidney DMS cotreatment induced $CD4^+$ $CD25^+$ Tregs both in the spleen and kidney. The mRNA and protein levels of Foxp3 in the kidney were decreased in LPS group, which was prevented by DMS treatment.

**Conclusions:** In conclusion, it is supposed that DMS exerts its protective effects against LPS-induced kidney injury by suppression of apoptosis and inflammation in the kidneys, where modulation of Tregs by DMS might be involved in the underlying mechanisms.

**FR-PO060**

**Efficacy of C-C Chemokine Receptor 2 (CCR2) Antagonists CCX140 and CCX872 in a Mouse Model of Ischemia-Reperfusion Injury** Juan C. Jaen, Matthew J. Walters, Zhenhua Miao, Robert D. Berahovich, Jay P. Powers, Karen Ebsworth, Linda Ertl, Pirow Bekker, Thomas J. Schall. *ChemoCentryx Inc., Mountain View, CA.*

**Background:** Ischemia-reperfusion injury (IRI) is one of the most common causes of Acute Kidney Injury (AKI). In humans, IRI reflects poor renal perfusion in connection with major cardiothoracic surgery, such as coronary artery venous bypass grafting, and myocardial infarction. The CCR2 chemokine receptor is believed to regulate recruitment of circulating monocytes to the tubulointerstitial space in response to IRI-induced upregulation of the CCL2 chemokine by injured proximal tubular epithelial cells. We describe here the effects of 2 clinical-stage CCR2 inhibitors (CCR2-I) in a mouse model of renal IRI.

**Methods:** CCX872 was evaluated in male C57Bl/6 wt mice. Due to the high selectivity of CCX140 for human CCR2, CCX140 studies were performed in mice in which the mouse CCR2 coding region was replaced with the human CCR2 coding region (hCCR2KI). Mice underwent concurrent surgical clamping of both renal arteries for 45 min. Drug administration was initiated the day prior to surgery and continued until sacrifice 24 hrs later. Sham-operated animals were used as negative controls. Assessments included body weight, serum creatinine (sCRE) and BUN levels, histological assessment of tubular damage, and number of renal leukocytes.

**Results:** CCR2-I treatment significantly reduced sCRE, tubular damage and inflammatory cell infiltration in wild-type and hCCR2KI mice. sCRE: 1.47 and 2.02 mg/dL, CCX872 and vehicle control, respectively; $p<0.05$; Tubular Damage (% tubules): 41 and 62, CCX872 and vehicle control, respectively; $p<0.0001$; Total Leukocytes: 1,538 and 12,820 cells/kidney, CCX872 and vehicle control, respectively; $p<0.01$). Similar results were obtained with CCX140 using transgenic hCCR2KI mice.

**Conclusions:** Robust protection against AKI in a mouse model of IRI was obtained by prophylactic administration of either of two clinical-stage CCR2 inhibitors, one of which, CCX140, is currently being studied in two Phase-2 trials in subjects with T2D diabetic kidney disease. Thus, CCR2 inhibition may represent a novel therapeutic modality in the treatment of human AKI.

**FR-PO061**

**A Novel $S1P_1$ Agonist Protects Endothelial and Epithelial Cells and Preserves Renal Function in Acute Kidney Injury Models without Sustained Lymphopenia or Cardiovascular Effects** Bruno Poirier,[1] Véronique Briand,[1] Sylvain Ribeyro,[1] Marie-claire Philippo,[1] Alice Raffenne-devillers,[1] Catherine Cadrouvele,[1] Emilie Cornaire,[1] Isabelle Boitel-barbosa,[1] Florence Derdinger,[1] Michel Pelat,[1] Philippe Prigent,[1] Christine Girardot,[1] Jean-pierre Bidouard,[1] Valérie Fleury,[1] Philippe Beauverger,[1] Yi Shi,[4] Sylvie Lemarinel,[4] Meghan Clements,[2] Anna Zuk,[2] Souad Naimi,[4] Dieter Kadereit,[3] Matthias Schaefer,[3] Hartmut Ruetten,[3] Christina Soubrane,[1] Bérangère Thiers,[1] Alexandre Jagerschmidt,[1] Pierre Rosenzweig,[1] Ashfaq Parkar,[1] Steven R. Ledbetter,[2] Philip Janiak.[1] *[1]Tissue Protection and Repair Unit, Sanofi R&D, Chilly-Mazarin, France; [2]Tissue Protection and Repair Unit, Sanofi Genzyme, Framingham, MA; [3]Diabetes BU, Sanofi R&D, Frankfurt 65926, Germany; [4]SCP Biologics, Sanofi R&D, Vitry-sur-Seine, France.*

**Background:** We describe a novel $S1P_1$ agonist, $S1P_1$AGT, which is distinguished from the class of lymphopenic $S1P_1$ functional antagonists for multiple sclerosis.

**Methods:** $S1P_1$AGT was evaluated in cellular and in vivo models of AKI.

**Results:** $S1P_1$AGT does not desensitize $S1P_1$, which translates to an absence of sustained lymphopenia, and does not show any significant effect on heart rate and AV conduction. $S1P_1$AGT protected renal tubular epithelial cells from tunicamycin-induced apoptosis, inhibited the increase in VCAM, ICAM and P/E-selectins in TNFa-stimulated endothelial cells, and induced survival pathways (pAkt/pErk). In the setting of renal ischemia-reperfusion in rats, $S1P_1$AGT showed almost complete preservation of renal function and consistently reduced tubular necrosis, diminished renal inflammation, maintained endothelial barrier integrity and limited capillary rarefaction. The preservation of renal function was further confirmed in two separate mouse models of AKI induced by glycerol or tunicamycin.

**Conclusions:** $S1P_1$AGT displays endothelial protective effects which is in contrast to $S1P_1$ functional antagonists which are endothelial-damaging. The observed endothelial protective effects of $S1P_1$AGT, coupled with its epithelial protective and anti-inflammatory effects, represent a new and exciting approach for AKI treatment.

**FR-PO062**

**Natural IgM Protects against Renal Ischemia Reperfusion Injury by Altering Dendritic Cell Function** Peter I. Lobo, Kailo H. Schlegel, Liping Huang, Amandeep Bajwa, Mark D. Okusa. *Dept of Med/CIIR, Univ of Virginia, Charlottesville, VA.*

**Background:** We have shown in prior studies that natural IgM, especially IgM that binds to autologous leukocytes (IgM-ALA), protects against Renal IRI. These studies explore the mechanisms by which protection is conferred. Damage-associated molecular patterns (DAMPS), released following renal ischemia, activate Dendritic cells (DC), which present these autoantigens (especially glycolipids) to NK and NKT cells, initiating the inflammatory process resulting in magnified injury. As we observed high binding of natural IgM on DCs compared to other leucocyte population, we examined if the protective effect of IgM is mediated at the DC stage.

**Methods:** To test the effect of IgM on DC activation, cultured C57BL6 (B6) Bone Marrow Dendritic Cells (BMDC) were activated with LPS after pretreatment with either purified natural polyclonal murine IgM from serum, murine IgG, isotype murine IgM (lacking binding to mouse cells), or albumin. After 24 to 48 hours incubation, supernatant was taken for cytokine, and the BMDCs examined via flow cytometry. The effect of IgM on DC function was tested by I.V transfer of $5x10^5$ pre-treated BMDC into B6 mice given Renal IRI 24 hours later.

**Results:** IgM inhibited LPS induced TNFa secretion (from 7,150 pg/mL to 3,750 pg/mL) as well as decreasing the up-regulation of CD40 by approximately 40%. We observed no effect of IgM on BMDC expression of CD80, CD1d, MHC class II or on LPS induced TLR4 downmodulation. IgM+LPS pretreated DCs conferred significant protection against IRI compared to both control IRI or transfer of LPS treated DCs. Kid damage was evaluated by both plasma creatinine and histology.

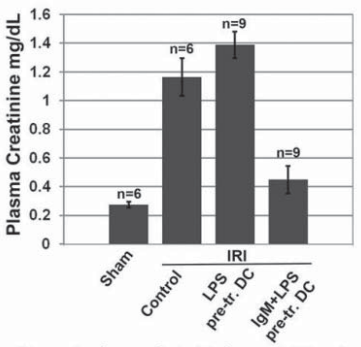



Figure 1 shows that 24 hr. post-IRI, mice receiving BMDC treated with IgM were protected from Renal IRI (p= 0.0006 for Control IRI vs IgM+LPS treated BMDC). This protection appeared to be IL-10 mediated, as an IL-10 neutralizing antibody given at time of IRI abolished the protective effect of IgM. Other experiments showed that pretreatment of BMDC with albumin, isotype IgM, or IgG conferred no protection. The effect appeared specific to DCs, as IgM pre-treatment and transfer of T or B cells did not protect.

**Conclusions:** These studies indicate that Natural IgM, especially IgM-ALA, can provide an innate regulatory mechanism by its effect on DCs.

*Funding:* NIDDK Support

**FR-PO063**

**Interleukin 17A Drives Acute Kidney Injury through the Recruitment of Innate Effector Cells** Shaun A. Summers,[1] Amy J. Chan,[1] Oliver M. Steinmetz,[2] A. Richard Kitching,[1] Stephen R. Holdsworth.[1] *[1]Nephrology and Medicine, Monash Health and Monash Univ, Melbourne, Victoria, Australia; [2]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

**Background:** Acute kidney injury (AKI) is a major cause of morbidity and mortality. Clinical and experimental data demonstrate that cytokines and leukocytes promote renal inflammation and injury. We sought to define the role of Interleukin (IL)-17A in cisplatin induced AKI.

**Methods:** We administered cisplatin (15mg/kg) to C57BL/6 wild type (WT) mice and measured kidney inflammation and injury. For reconstitution studies, CD4+ T cells were isolated using magnetic beads and injected into RAG1-/- mice, which lack adaptive immune cells.

**Results:** Kidney mRNA expression of IL-17A increased significantly after cisplatin treatment. Compared to WT mice treated with cisplatin, functional (serum urea: WT 72.2±10.7 vs. IL-17A-/- 28.2±8.2mmol/L, $P<0.01$) and histological injury (Injury score [0-4]: WT 3.3±0.2 vs. IL-17A-/- 1.0±0.2, $P<0.001$) were decreased in IL-17A-/- mice. While interstitial neutrophil recruitment (1.8±0.1 vs.1.0±0.1, cells/high power field, $P<0.001$) decreased in the absence of IL-17A, CD4+ T cell recruitment remained intact. Pre-emptive administration of anti-IL-17A antibodies to WT mice attenuated renal injury, serum urea (control antibodies: 54.6±10 vs. anti-IL-17A 25.9±11.2mmol/L, $P<0.05$). Neutrophil depletion prior to cisplatin treatment did not significantly reduce renal injury, which only decreased after additional IL-17A neutralization in neutrophil deficient mice. Depletion of both neutrophils and natural killer T cells significantly protected from AKI, but injury was not further reduced after additional anti-IL-17A treatment, linking these cells with IL-17A induced AKI. While reconstitution of RAG1-/- mice with CD4+ T cells prior to cisplatin treatment increased renal injury, there was no difference after reconstituting with WT or IL-17A-/- CD4+T cells, demonstrating that IL-17A- producing CD4+ T cells did not enhance AKI. Mice deficient in gammadelta T cells were not protected from AKI.

**Conclusions:** IL-17A drives AKI and represents a new therapeutic target.

*Funding:* Government Support - Non-U.S.

## FR-PO064

**Alpha-Lipoic Acid Attenuates Lipopolysaccharide-Induced Kidney Injury**
Sang Heon Suh,[1] Koeun Lee,[1] Eun Hui Bae,[1] Seong Kwon Ma,[1] Soo Wan Kim,[1] Chang Seong Kim,[1] Joon Seok Choi,[1] Jongun Lee.[2] *[1]Depts of Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea; [2]Depts of Physiology, Chonnam National Univ Medical School, Gwangju, Korea.*

**Background:** Kidney is one of the major target organs in sepsis, while effective prevention of septic acute kidney injury has not yet been established. We investigated the protective effect of α-lipoic acid (LA) on lipopolysaccharide (LPS)-induced kidney injury.

**Methods:** Two groups of rats were treated with LPS (20 mg/kg, i.p.), one of which being co-treated with LA (50 mg/kg), while the control group was treated with vehicle alone. Human renal proximal tubular epithelial cells (HK-2 cells) were cultured with or without LPS (10 μg/ml) in the presence or absence of LA (100 μg/ml) for 3 hours prior to LPS treatment.

**Results:** Serum creatinine level was increased in LPS-treated rats, which was attenuated by LA co-treatment.LPS treatment increased both cleaved caspase-3 expression and the number of terminal deoxynucleotidyl transferase dUTP nick end labelling-positive cells in the kidney, which was counteracted by LA. Protein expression of inducible nitric oxide synthase and cyclooxygenase-2 detected by immunoblotting and/or immunohistochemical staining, along with mRNA levels of pro-inflammatory cytokines detected by real time polymerase chain reaction, was increased in the kidney with LPS administration, which was ameliorated by with LA treatment. LA also protected LPS-induced tubular dysfunction, preserving type 3 $Na^+ / H^+$ exchanger and aquaporin 2 expressions in the kidney. Suppression of LPS-induced expression of cleaved caspase-3 by LA was also observed in HK-2 cells. 4', 6-diamidino-2-phenylindole staining also revealed heavy staining in HK-2 cells treated with LPS, which was prevented by LA. Increased protein expression of phospho-extracellular signal-regulated kinases 1/2 and c-Jun N-terminal kinases by LPS treatment was attenuated by LA pretreatment in HK-2 cells, while p38 was not affected by either LPS or LA treatment.

**Conclusions:** LA treatment attenuates LPS-induced kidney injury by suppression of apoptosis, inflammation, and renal tubular dysfunction.

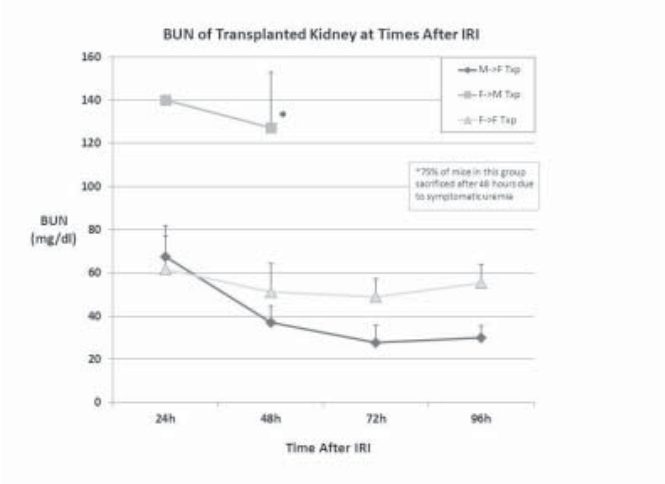
## FR-PO065

**Gender Differences in Murine Ischemia Reperfusion Tolerance Is Extrinsic to the Kidney Itself: Lessons from Transplantation**
Matthew H. Levine,[1] Zhonglin Wang,[1] Tricia Bhatti,[2] Wayne W. Hancock.[2] *[1]Surgery, Univ of Pennsylvania, Philadelphia, PA; [2]Pathology, Childrens Hospital of Philadelphia, Philadelphia, PA.*

**Background:** Gender differences are well described in murine basic renal ischemia reperfusion injury (IRI) models, with females being more tolerant of injury. Whether these differences are driven by differences in the kidney's resistance to IRI or whether they are due to differences in the host response to IRI are not known.

**Methods:** We utilized a syngeneic murine renal transplant model with male and female kidneys being transplanted into male and female recipients and then, after recovery, undergoing subsequent renal IRI by standardized 25 minute renal IRI protocol. Confirmatory cold ischemia renal experiments were performed with the same four groups, after storage of kidneys at 4 degrees C in UW solution for duration noted below and then subsequently transplanted with standardized transplantation times.

**Results:** Syngeneic kidneys transplanted into across genders with subsequent renal IRI showed that kidneys of both genders had similar and enhanced tolerance to IRI in a female host. Conversely, kidneys of both genders tolerated renal IRI equally poorly in a male host, with most mice euthanized in this group by 48 hours due to renal insufficiency.




Subsequent experiments demonstrated that male recipients of either gender kidneys after 18 hours of cold ischemia could not survive more than 48 hours after native nephrectomy due to renal insufficiency. Conversely, female recipients of either gender kidney tolerated 24 hours of cold ischemia and native nephrectomy with near total survival.

**Conclusions:** We conclude that female mice have significantly enhanced tolerance of renal IRI relative to males and that this tolerance is largely extrinsic to the kidney itself and is rather driven by gender differences in the inflammatory repsonse to IRI.

*Funding:* NIDDK Support

## FR-PO066

**Recombinant Alkaline Phosphatase Modulates Inflammation and Injury in Two Rat Models of AKI**
Can Ince,[1] Bulent Ergin,[1] Andrea Van van Elsas.[2] *[1]Translational Physiology, Academic Medical Center, Amsterdam, Zuid Holland, Netherlands; [2]AM Pharma, Bunnik, Netherlands.*

**Background:** Purified bovine alkaline phosphatase (AP) has shown intriguing clinical activity treating acute kidney injury in sepsis patients (Heemskerk et al. 2009, Crit. Care Med. 37: 417-23; Pickkers et al., 2012, Crit. Care 23: R14). A recombinant alkaline phosphatase was designed by swapping the crown domain from intestinal AP with that derived from placental AP enzyme generating a stable human chimeric called recAP.

**Methods:** To study its activity and mode-of-action in models of acute kidney injury (AKI), a single i.v. dose of 1000 U/kg recAP was administered to instrumented Wistar rats (7 per group) within 30 min. after ischemia-reperfusion (I-R) or LPS injection to induce AKI, and compared to saline (n=7). Systemic and local hemodynamics as well as kidney oxygenation were assessed for the duration of the experiment (3 h post-treatment). A control group underwent surgery and insertion of various probes without AKI induction.

**Results:** Saline treated animals developed mild (I-R model) or severe AKI (LPS model). Systemic hemodynamics was unaffected by recAP, however renal vascular resistance improved in the I-R model (control 2424 ± 370 dyn/s/cm2, 4314 ± 1182 in the saline group vs 2905 ± 1246 after recAP treatment) and renal blood flow significantly improved in the LPS model (control 5.02 ± 1.30 mL/min, saline 8.35 ± 2.98, recAP 6.28 ± 1.89, $p<0.01$). By immunohistochemistry, RecAP modulated inflammatory markers iNOS, IL-6 ($p<0.001$), as well as peroxidation marker MDA ($p<0.001$) in the LPS model. Compared to saline, recAP treatment reduced the number of infiltrating MPO+ leukocytes in the cortical peritubular areas ($p<0.05$). Moreover, recAP reduced expression of renal injury markers L-FABP, NGAL ($p<0.001$) and pro-apoptic Bax ($p<0.01$).

**Conclusions:** In conclusion, in rat AKI recAP demonstrated immediate pharmacological effect which included suppression of acute inflammation in the afflicted kidney and inhibition of tissue injury. This study provides first evidence that recAP is an active protein therapeutic in two rat models of AKI.

*Funding:* Pharmaceutical Company Support - AM Pharma, Bunnink, The Netherlands

## FR-PO067

**Protective Effect of Cilostazol and Probucol on Inflammatory Kidney Injury**
Peng He,[1] Harukiyo Kawamura,[1] Kunimasa Yan,[2] Minoru Takemoto,[1] Koutarou Yokote.[1] *[1]Clinical Cell Biology and Medicine, Chiba Univ Graduate School of Medicine, Chiba, Japan; [2]Dept of Pediatrics, Kyorin Univ School of Medicine, Tokyo, Japan.*

**Background:** The aim of this study is to clarify the protective effect of a combination of cilostazol (CSZ) and probucol (PBC), both of which are prescribed for cardiovascular diseases and exhibit anti-inflammatory properties on inflammatory kidney injury.

**Methods:** In vivo, we employed lipopolysaccharide (LPS) induced acute renal inflammation model mice. In brief, after pretreatment with CSZ, PBC aloneor in combination with each other (CSZ/PBC), mice were intraperitoneally injected with LPS. After 24 h, urinary albumin levels were measured by ELISA and expression of MCP1 in glomeruli was analyzed by immunostaining and real-time PCR. In vitro, we treated cultured podocytes with LPS (100 ng/mL) in the presence or absence of CSZ, PBC or CSZ/PBC and then we estimated activation of MAP kinase by immunoblotting, activation of NFκB by immunostaining and reactive oxygen species (ROS) by dihydroethidium (DHE) staining.

**Results:** In mouse glomeruli and cultured podocytes, LPS-induced albuminuria and MCP1 upregulation were considerably suppressed by CSZ/PBC. Among the native cells of glomeruli, podocytes expressed most of MCP1 compared with endothelial and mesangial cells. In cultured podocytes, CSZ and PBC suppressed LPS-induced NFκB activation and MCP1 expression, while the combination of them showed an additive suppressive effect. CSZ increased intracellular cAMP levels and PKA activation which in turn suppressed ERK activation and MCP1 expression downstream. On the other hand, PBC lowered LPS-induced NFκB activation and MCP1 expression by reducing LPS-induced activation of NADPH oxidase and intracellular ROS production.

**Conclusions:** In a LPS-induced acute renal inflammatory mouse model, we confirmed that both CSZ and PBC showed anti-inflammatory effects through different mechanisms. Moreover, the combination of these two drugs had a stronger protective effect on LPS-induced renal dysfunction and glomerular inflammation. This study indicated that combination of CSZ and PBC could be a prospective treatment for inflammatory kidney diseases.

## FR-PO068

### CXCR2 Knockout Mice Are Protected against Dextran Sulfate Sodium-Colitis Induced Acute Kidney Injury and Inflammation

Punithavathi Vilapakkam Ranganathan, Calpurnia Jayakumar, Ganesan Ramesh. *Vascular Biology Center, Georgia Regents Univ, Augusta, GA.*

**Background:** Organ cross-talk exists in many human diseases and in animal models. Recent study had demonstrated that inflammatory mediators can cause acute kidney injury (AKI) and neutrophil infiltration in the mouse model of acute colitis. However, the chemokines and their receptors that mediate distant organ effects in colitis are unknown.

**Methods:** We examined the hypotheses that KC-CXCR2 pathway mediates DSS-colitis induced AKI. Colitis was induced in 8-week old wild-type (WT) and CXCR2 knockout mice by feeding them with 3.5% DSS in drinking water for a period of 8 days. Animals were sacrificed 2 days after removal of DSS. Kidney function was monitored by measuring serum creatinine. Some mice received recombinant KC (100ng/animal) to determine whether exogenous administration of KC exacerbates acute kidney injury. Inflammation and immune cell infiltration were quantified by immunostaining, RT-PCR and ELISA.

**Results:** WT mice developed severe colitis with DSS treatment which was associated with inflammatory cytokine and chemokine expression and neutrophil infiltration in the colon. DSS-colitis in WT was accompanied by acute kidney injury (serum creatinine: 0.4±0.03mg/dl) and enhanced expression of cytokine (TNFa) and chemokines (MCP-1, IP-10 and KC) in the kidney. However, CXCR2 knockout mice were protected against DSS-colitis as well as acute kidney injury (serum creatinine: 0.2±0.02mg/dl). Moreover, the expression of cytokines and chemokines and neutrophil infiltration was significantly and completely blunted ($p<0.001$ vs. WT) in CXCR2 knockout mice in colon and kidney. Administration of recombinant KC exacerbated DSS-colitis induced acute kidney injury (0.6±0.03 mg/dl, $p<0.05$ vs. vehicle treated).

**Conclusions:** Our results suggest that KC and its receptor CXCR2 are critical and major mediators of organ cross talk between colon and kidney in DSS induced colitis and neutralization of CXCR2 will help to reduce the incidence of acute kidney injury due to ulcerative colitis and crohn's disease in humans.

*Funding:* NIDDK Support

## FR-PO069

### Genetic Deletion of Semaphorin 3A Render Resistance to Ischemia Reperfusion Induced Inflammation and Acute Kidney Injury in Mice

Punithavathi Vilapakkam Ranganathan, Calpurnia Jayakumar, Ganesan Ramesh. *Vascular Biology Center, Georgia Regents Univ, Augusta, GA.*

**Background:** Recent studies show that guidance molecules that are known to regulate cell migration during development may also play an important role in adult during normal and pathological state. One such molecule called semaphorin 3A (sema3A) is known to be expressed in the kidney and its patho-physiological role in the kidney is unknown.

**Methods:** To test the hypothesis that sema3A is a mediator of ischemia reperfusion of the kidney by increasing epithelial cell apoptosis. Ischemia reperfusion injury in the kidney was induced by clamping renal pedicle for a period of 35 minutes followed by 24hr of reperfusion in sema3A knockout (KO) and C3H control mice. Kidney function, inflammation, immune cell infiltration and apoptosis were quantified by measuring serum creatinine, immunostaining, RT-PCR, ELISA and TUNEL assay. Some wild-type received sema3A pathway inhibitor or sema3A blocking peptide 2hr before renal pedicle clamping to determine the effect of pharmacological inhibition of sema3A pathway on ischemic acute kidney injury (AKI).

**Results:** C3H mice subjected to ischemia reperfusion developed severe renal dysfunction as compared to sema3A KO mice (serum creatinine: 1.7±0.1 vs. 1.0±0.1 mg/dl, $p<0.01$), which was associated with histological alteration, neutrophil infiltration, increased cytokine and chemokine expression and excretion in urine. These changes were minimal in sema3A KO mice with ischemia reperfusion. Moreover, ischemia reperfusion induced extensive apoptosis in renal epithelial cells which was significantly suppressed in sema3A KO mice. Similarly, administration of sema3A inhibiting peptide or sema3A signaling inhibitor LOXblock-1 protected kidney against ischemia reperfusion induced AKI. Consistent with *in vivo* pathogenic role of semaphorin 3A, addition of semaphorin 3A to kidney epithelial cells *in vitro* increased cisplatin induced apoptosis.

**Conclusions:** Our data suggests that sema3A play a pathogenic role in acute kidney injury through increasing epithelial cell apoptosis and sema3A inhibition may provide protection against human AKI.

*Funding:* NIDDK Support

## FR-PO070

### Depletion of $CD25^+$ T Regulatory Cells Reverses Protection against Ischemic Renal Injury in Heat Shock Factor-1 Knock-Out Mice

Rajasree Sreedharan, Shaoying Chen, Dipica Haribhai, Calvin B. Williams, Scott K. Van Why. *Pediatrics, Medical College of Wisconsin, Milwaukee, WI.*

**Background:** We previously reported that Heat Shock Factor-1 knock-out (HSF-1 KO) mice, are protected against ischemic renal (IR) injury compared with Heat Shock Factor-1 wild type (WT). Further, HSF-1 KO mice have altered T cell infiltration immediately following IR, with significantly fewer $CD4^+$ and $CD8^+$ cells infiltrating HSF-1 KO kidneys than WT kidneys following IR. Our goal was to determine whether T regulatory cells play a role in the protection against IR injury seen in HSF-1 KO mice.

**Methods:** We compared T regulatory cells ($Foxp3^+/CD25^+$) in HSF-1 KO and WT kidneys in the sham condition and after reperfusion following 45 minutes ischemia by flow cytometry. The effect of depletion of T regulatory cells on IR injury was studied by subjecting HSF-1 KO mice to IR (45 minutes with 24 hours reflow) after treatment with PC61 anti-CD25 antibody or vehicle control group.

**Results:** There are significantly more $Foxp3^+$ T regulatory cells in sham operated kidneys of HSF-1 KO mice compared with WT ($p=0.02$). Early in reperfusion after renal ischemia, $Foxp3^+$ did not change in HSF-1 KO kidneys, but increased by 50% in WT kidneys. Treatment of HSF-1 KO mice with PC61 antibody significantly depleted the renal $Foxp3^+CD25^+$ cells when compared with vehicle control group in sham ($p=0.008$) and following IR insult ($p=0.01$). PC61 treatment did not affect serum creatinine compared to vehicle control group in sham operated HSF-1 KO mice. However PC61 treatment (causing depletion of intra-renal $CD25^+$ T regulatory cells) resulted in higher serum creatinine in HSF-1 KO than in vehicle treated HSF-1 KO following IR injury (0.89 mg/dL vs 0.48 mg/dL respectively).

**Conclusions:** Higher endogenous levels of renal T regulatory cells play a role in the protection afforded to HSF-1 KO mice against IR injury.

*Funding:* NIDDK Support, Other NIH Support - AIAID, Private Foundation Support

## FR-PO071

### Detailed Analysis of Double Negative (CD4-/CD8-) T Cells during AKI

Maria Noel Martina Lingua,[1] Samatha Bandapalle,[2] Sanjeev Noel,[2] Abdel Hamad,[1] Hamid Rabb.[2] *[1]Pathology, Johns Hopkins Univ, Baltimore, MD; [2]Nephrology, Johns Hopkins Univ, Baltimore, MD.*

**Background:** Double negative (DN) T cells lacking CD4 and CD8 have recently been described to be a resident kidney immune cell (*J Leuk Biol* 2008). The role of these cells in kidney and how they mediate immune responses are unknown. We hypothesize that DN T cells are innate T cells involved in regulating early immune response in kidney and have a suppressive function on cytokine production, potentially protecting kidney from injury.

**Methods:** Purified T lymphocytes from mice were analyzed for expressing surface markers and cytokines secretion. B6 wild type mice and acute kidney injury mice model were used. Surface and intracellular staining were done and analyzed with flow cytometry technology.

**Results:** Double negative T cells comprise a major population in kidney in the steady state (30%). This percentage shows a rapid and transient expansion early after IRI, increasing after 3h of ischemia up to 60% and decreasing after 24h to 10%. DN T cells showed an activated phenotype in the kidney, expressing markers like CD44 and CD69, and also higher level of co-stimulatory molecules like CD28 and CD40-L compared to CD4+ and CD8+ in kidney. DN T cells exhibit a unique cytokine secretion profile: in steady state express IL-17, IL-10, IFN-y, IL-4 (60%, 10%, 5% respectively) after in vitro stimulation with PMA/ion in, while in ischemic kidneys they increased IL-10 – IL-17 (18%, 68% respectively) secretion and decreased IL-4 (1%). They also express the transcription factor ROR-y up to 20% in steady state.

**Conclusions:** Our data shows that resident kidney double negative T cells produce a distinct cytokine profile during steady state. After AKI, DN T cells have a significant change in cytokine profileincluding a change in transcription factor ROR-y. DN T cells are a newly identified resident kidney cell that can improve our understanding of immune mediated kidney diseases including AKI, glomerulonephritis and transplant rejection.

*Funding:* Other NIH Support - R21 AI095484

## FR-PO072

### The Role of Toll-Like Receptors and Reactive Oxygen Species in the Development of Lupus Nephritis

Altaf-M Khan,[1] Jerome L. Maderdrut,[2] David H. Coy,[2] Eric E. Simon,[1,3] Vecihi Batuman.[1,3] *[1]Medicine, Section of Nephrology & Hypertension; [2]Peptide Research Laboratory, Tulane Univ School of Medicine, New Orleans, LA; [3]Veterans Affairs, SLVHCS, New Orleans, LA.*

**Background:** Lupus nephritis is a common and serious manifestation of systemic lupus erythematosus (SLE). We have explored the role of Toll-like receptors (TLRs) and reactive oxygen species (ROS) in the development of kidney disease in a mouse model of SLE.

**Methods:** We used the female New Zealand Black/New Zealand White (NZB/NZW) F1 hybrid (lupus-prone) mouse, which spontaneously develops SLE-like pathology, as a model for lupus nephritis. NZW mice served as the controls. Mice (n = 4) were euthanized at 22, 29 or 41 weeks (wk) of age, and blood and kidneys were harvested to assess renal function and pathology.

**Results:** Serum creatinine and cystatin C levels and mRNA levels of the genes for the kidney injury biomarkers KIM-1 and Nogo-B1 increased significantly in lupus-prone mice at 22 wk compared to controls and continued to rise through 41 wk. The mRNA levels of the genes for inflammatory cytokines (IFN-γ, MCP-1, TNF-α, and TGF-β1) and TLR2, TLR4, TLR6, TLR7, and TLR9 were significantly up-regulated in lupus-prone mice at 41 wk compared to controls at 41 wk. Activation of TLRs was propagated through MyD88-dependent but not TRIF-dependent signaling pathways. The messages for ROS-generating enzymes (iNOS, Nox2 and Nox4), for pro-apoptotic genes (FADD-1, Fas-1 and Aifm-1), and for the marker genes for monocyte/macrophage/neutrophil infiltration (CD11b, CD68 and MPO) were significantly increased, while the messages for the anti-apoptotic proteins Bcl-2 and Ube2v1 (a TLR effector protein) were significantly decreased at 41 wk in lupus-prone mice compared to controls. The protein levels for proinflammatory cytokines in the kidneys of lupus-prone mice increased significantly between 22 wk and 41 wk.

**Conclusions:** Lupus-prone mice develop renal pathology as early as 22 wk and could be a good model to study the role of innate immunity in lupus nephritis. These data suggest that modulation of TLR pathways and ROS suppression could be useful strategies for the treatment of lupus nephritis.

*Funding:* Private Foundation Support

**FR-PO073**

**Depletion of Macrophage Ameliorates Glycerol-Induced Acute Kidney Injury in Mice** Seong Eun Yun, Eunjin Bae, Yeojin Kang, Hyun Seop Cho, Se-Ho Chang, Dong Jun Park. *Internal Medicine, Gyeongsang National Univ Hospital, Republic of Korea.*

**Background:** The roles of macrophage in rhabdomyolysis mice model have not been well organized. This study was conducted to elucidate the relative contribution of renal macrophages to the development of acute kidney injury (AKI) by glycerol injection into mice.

**Methods:** The macrophages were depleted by liposomal encapsulated clodronate (LEC). Mice were distributed into four groups: control group (liposomal vehicle 100 *u*l, intravenously 24 hour prior to and just before glycerol injection), LEC group (LEC 100 *u*l, intravenously 24 hour prior to and just before glycerol injection), Gly (50% glycerol (10 ml/kg)), and Gly plus LEC. On day 24 hour, renal function, histology, flow cytometry for macrophages, Western blot and renal tissue immunohistochemistry were assessed.

**Results:** Glycerol injection increased serum creatinine and blood urea nitrogen (BUN) 24 hours after injection. LEC injections (100 *u*l/mouse via tail vein) administered 24 h prior to and just before glycerol injection prevented the deterioration of renal function and also tubular damages 24 hours after glycerol injection. Renal macrophages represented by $CD45^+CD11b^+Ly6c^+F4/80^+$ or $CD45^+CD11b^+Ly6c^+$ cells were significantly decreased by pretreatment of LEC in both normal and myoglobin injured kidney. LEC injection decreased apoptotic death of the tubular epithelial cells by decreasing pro-apoptotic caspase-9 protein and increasing anti-apoptotic Bcl-2 protein expression. LEC administration also attenuates activation of ERK and p53 expression 24 hr after glycerol administration. NF-kB, MCP-1, and ICAM-1 were also decreased in the damaged tubular epithelial cells by LEC injection. iNOS and COX-2 expression were decreased in LEC plus glycerol group, compared to glycerol only group.

**Conclusions:** These results support the hypothesis that depletion of macrophages is effective in prevention of renal dysfunction by abrogating apoptosis and attenuating inflammation in glycerol-induced AKI mice model.

**FR-PO074**

**PKC-α Offers Protection against Programmed Necrosis in Renal Proximal Tubules through Maintaining Functional MPT Pore** Grazyna Nowak. *Pharmaceutical Sciences, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

**Background:** We have shown that necrosis, but not apoptosis, is the mechanism of cell death in renal proximal tubular cells (RPTC) injured by oxidant. RPTC necrosis is preceded by mitochondrial membrane hyperpolarization and bioenergetics failure. PKC-α activation prevents mitochondrial hyperpolarization, reduces mitochondrial dysfunction and ATP deficits, and decreases necrosis in oxidant-injured RPTC. Overexpression of the inactive mutant of PKC-α induces mitochondrial membrane hyperpolarization in non-injured RPTC.

**Methods:** To determine specific mechanisms of oxidant-induced RPTC necrosis, monolayers were treated with inhibitors of three different pathways of regulated necrosis and cell viability was assessed following exposure to a model oxidant, tert-butylhydroperoxide. In some experiments, PKC-α was overexpressed and RPTC lysis assessed following TBHP exposure.

**Results:** TBHP induced 43% RPTC death at 24h after the exposure. Pretreatment of RPTC with cyclosporine A (inhibitor of cyclophilin D) offered no protection at concentration of 1 μM (41% lysis) and exacerbated cell lysis at higher concentrations (58% lysis). Iron chelation by deferoxamine and inhibition of ferroptosis by ferrostatin prevented TBHP-induced RPTC death (4% lysis). Necrostatin-1, an inhibitor of necroptosis, decreased RPTC lysis to 12% of controls. Overexpressing PKC-α in RPTC decreased oxidant-induced necrosis to 22% and this protective effect was reversed by cyclosporine A and PKC inhibitor, Go6976. Ferrostatin and deferoxamine augmented PKC-α-mediated protection against oxidant-induced injury (13% and 5% cell lysis, respectively).

**Conclusions:** These results demonstrate that oxidant-induced RPTC necrosis is iron-dependent and its major mechanism is through ferroptosis. Necroptosis is an additional mechanism involved in RPTC death. However, the opening of the mitochondrial permeability transition pore (MPTP) and loss of $\Delta\Psi_m$ are not mechanisms that mediate oxidant-induced programmed necrosis in RPTC. The protective effects of PKC-α are mediated through maintaining functional MPTP and preventing mitochondrial membrane hyperpolarization.

*Funding:* NIDDK Support

**FR-PO075**

**FGF/FGFR2 Signaling Protects against Tubular Cell Death and Acute Kidney Injury** Zhuo Xu, Weichun He, Junwei Yang, Chunsun Dai. *The Center for Kidney Disease, the Second Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

**Background:** Fibroblast growth factors (FGFs) are heparin-binding proteins involved in a variety of biological processes, including cell proliferation, differentiation, survival and angiogenesis. However, the role and mechanisms of FGFs/FGFR signaling in tubular cell survival and AKI need further investigation.

**Methods:** In this study, Ischemia/reperfusion or cisplatin injection was used to induce AKI in mice. A mouse model with tubule specific deletion of FGFR2 (Tubule-FGFR2-/-) was generated.

**Results:** In the kidneys with ischemia/reperfusion injury (IRI), the expression of FGFs including FGF2, FGF7, FGF10, FGF12, FGF13, FGF18, and FGF22 were up-regulated. In addition, phosphorylated ERK1/2, a downstream signaling of FGFR was also elevated in the IRI kidneys. To further explore the role of FGF signaling in tubular cell survival and AKI in mice, a mouse model with tubular cell specific FGFR2 gene disruption was generated by using Cre-LoxP system. The knockouts were born normal and no obvious kidney dysfunction was found within four months after birth. Specific deletion of FGFR2 in tubular cells in mice aggravated the tubular cell death as well as AKI induced by IRI or cisplatin. In cultured NRK-52E cells, recombinant FGF2 protein could induce Erk1/2 phosphorylation and inhibit cisplatin-induced cell death. PD98059, a specific MEK 1/2 inhibitor, abolished Erk1/2 phosphorylation and partly reversed the protective effect of FGF2 in ciaplatin-induced cell death.

**Conclusions:** Together, this study indicates that FGF/FGFR2 signaling is important for protecting against tubular cell death and AKI, which is partly through ERK1/2 signaling activation.

*Funding:* Government Support - Non-U.S.

**FR-PO076**

**Propidium Iodide Is Cheating on Necrosis Detection: A Multiphoton Microscopy Study** Matthias Hackl,[1] Andreas Linkermann,[2] Bernhard Schermer,[1] Thomas Benzing.[1] *[1]Dept II of Internal Medicine and Center for Molecular Medicine Cologne, Univ of Cologne, Cologne, Germany; [2]Clinic for Nephrology and Hypertension, Christian-Albrechts Univ, Kiel, Germany.*

**Background:** There is an ongoing debate, if apoptosis or necrosis is the more prevalent form of cell death after ischemia-reperfusion injury. Propidium iodide (PI) staining has been used as a marker of necrotic cells in many studies. Intact cell membranes prevent the entry of PI into viable cells. PI has been successfully used to detect necrotic nuclei *in vivo* in the heart, the eye and the brain. Here we tested if PI is a reliable marker of cell necrosis in the kidney *in vivo*.

**Methods:** 6 wk old C57BL6 mice were anaesthetized, an arterial catheter was placed and the left kidney was exteriorized for *in vivo* multiphoton microscopy. 30μg PI was injected during the acquisition of a time lapse movie. Blood vessels and nuclei were labeled by the injection of 70kDa Texas red dextrane and 150μg Hoechst 33342 respectively. Lucifer Yellow was injected to visualize tubular fluid handling. To stop tubular flow the left ureter was ligated three times with 6-0 silk and the animals imaged 2-7 days later.

**Results:** In healthy and uninjured kidneys a considerable amount of PI was taken up by tubular cells. Time-lapse imaging demonstrated that the nuclei turned red 30-60 minutes after the injection of PI into the circulation. Colabelling with Hoechst 33342 and studies of tubular handling of filtrate showed that only proximal tubular cells (PTC) take up PI. The uptake of PI was markedly reduced 3-7 days after an unilateral ureteral ligation, while Hoechst 33342 staining was unaltered. Imaging of liver and gut showed no uptake in these organs. After the induction of damage with the laser beam some cells immediately took up PI.

**Conclusions:** Our data clearly demonstrates that PI is taken up by viable PTC from the tubular fluid. Distal tubular cells, glomerular cells, the liver and the gut do not show uptake of PI, demonstrating that this uptake is restricted to PTC. Although cells with membrane damage may show a faster PI uptake than viable PTC, the use of PI as a marker for necrotic cells *in vivo* is limited by the false positive nuclei of viable PTC.

*Funding:* Government Support - Non-U.S.

**FR-PO077**

**SUMOylation Occurs in Acute Kidney Injury and Plays a Cytoprotective Role** Chunyuan Guo, Zheng Dong. *Dept of Cellular Biology and Anatomy, Georgia Regents Univ, Augusta, GA.*

**Background:** SUMOylation is a mechanism of post-translational modification where Small Ubiquitin-like Modifiers (SUMO) are covalently attached to target proteins to regulate their localization, stability and functions. SUMOylation has been demonstrated during hypoxia, oxidative, genotoxic and metabolic stresses, suggesting an important role of this modification in cellular stress response. However, it is largely unclear if SUMOylation contributes to the pathogenesis of kidney diseases, such as acute kidney injury (AKI).

**Methods:** We examined protein SUMOylation in two experimental models of AKI: cisplatin nephrotoxicity and renal ischemia-reperfusion in vitro and in vivo. In addition, we examined the regulation of SUMOylation during cisplatin treatment of cultured rat kidney proximal tubular cells (RPTC) by two ROS scavengers and a pharmacological p53 inhibitor. We further examined the role of SUMOylation during cisplatin treatment of RPTC cells by using ginkgolic acid (GA), a pharmacological inhibitor of the E1-activating enzyme in SUMOylation.

**Results:** We have demonstrated dramatic increases of protein SUMOylation in both experimental models of AKI. Cisplatin-induced SUMOylation in RPTC cells was partially suppressed by N-acetylcysteine and dimethyurea (two ROS scavengers), supporting a role of oxidative stress in the activation of SUMOylation. In addition, SUMOylation by SUMO-2/3, but not SUMO-1, was partially suppressed by pifithrin-alpha, a pharmacological inhibitor of p53, supporting a role of p53 in SUMOylation by SUMO-2/3. Pretreatment with GA suppressed SUMOylation during cisplatin incubation and importantly, GA enhanced apoptosis.

**Conclusions:** Taken together, the results demonstrate the first evidence of SUMOylation in AKI which may result from oxidative stress and p53 signaling and suggest that SUMOylation may play a cytoprotective role in kidney tubular cells.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO078**

**Two Independent Pathways of Regulated Necrosis Mediate Ischemia-Reperfusion Injury** Andreas Linkermann,[1] Jan Hinrich Braesen,[2] Maurice Darding,[3] Ana Belen Sanz,[4] Jan O. Heller,[1] Ricardo Weinlich,[5] Alberto Ortiz,[4] Henning Walczak,[3] Joel M. Weinberg,[6] Ulrich Kunzendorf,[1] Stefan Krautwald.[1] *[1]Clinic for Nephrology and Hypertension, Christian-Albrechts-Univ, Kiel, Schleswig-Holstein, Germany; [2]Institute for Diagnostic Histopathology and Cytopathology, Pathology Hamburg-West, Hamburg, Germany; [3]Centre for Cell Death, Cancer and Inflammation (CCCI), Cell Death and Inflammation Laboratory, Univ College London, Cancer Institute, London, United Kingdom; [4]IIS-Fundacion Jimenez Diaz, U Autonoma de Madrid, Redinren, FRIAT, Madrid, Spain; [5]Dept of Immunology, St. Jude Children's Research Hospital, Memphis, TN; [6]Div for Nephrology, Univ of Michigan Medical Center, Ann Arbor, MN.*

**Background:** Regulated necrosis (RN) may result from cyclophilin D (CypD)-mediated mitochondrial permeability transition (MPT) and receptor-interacting protein kinase 1 (RIPK1)-mediated necroptosis, but it is unclear whether there is one common pathway in which CypD and RIPK1 act in or if separate RN pathways exist.

**Methods:** Here, we demonstrate that necroptosis in ischemia-reperfusion injury (IRI) in mice occurs as primary organ damage, independent of the immune system, and that mice deficient for RIPK3, the essential downstream partner of RIPK1 in necroptosis, are protected from IRI.

**Results:** Protection of RIPK3-ko mice was significantly stronger than of CypD-deficient mice. Mechanistically, analysis of cisplatin-induced AKI and hyperacute TNF-shock models in mice suggested the distinctness of CypD-mediated MPT from RIPK1/RIPK3-mediated necroptosis. We therefore generated novel CypD-RIPK3 double-deficient mice that are viable and fertile without an overt phenotype and that survived prolonged IRI which was lethal to each single knockout. Combined application of the RIPK1 inhibitor necrostatin-1 (Nec-1) and the MPT inhibitor sanglifehrin A (SfA) confirmed the results with mutant mice.

**Conclusions:** The data demonstrate the pathophysiological co-existence and co-relevance of two separate pathways of RN in IRI and suggest that combination therapy targeting distinct RN pathways can be beneficial in the treatment of ischemic injury.

*Funding:* Other NIH Support - NIH-DK34275, Pharmaceutical Company Support - Novartis, Fresenius medical care, Interreg4A (MoMoTx),, Private Foundation Support, Government Support - Non-U.S.

**FR-PO079**

**Cdk2-Dependent Phosphorylation of Bcl-xL Promotes Cisplatin-Induced Renal Cell Death *In Vitro* and *In Vivo*** Peter M. Price,[1,2] Adel Tarcsafalvi,[1] Nang San Hti Lar Seng,[1] Judit Megyesi.[1,2] *[1]Univ AR Med Sci; [2]VA Med Ctr.*

**Background:** Expression of $p21^{WAF1/Cip1}$ *in vitro* and *in vivo* protects kidney cells from cisplatin cytotoxicity by inhibiting on Cdk2 activity. Similarly, inhibition of Cdk2 activity also protects from cisplatin-induced cytotoxicity.

**Methods:** *C*ultured mouse proximal tubule cells were exposed to cisplatin at 25 μM. Mice were administered cisplatin at 20 mg/kg body weight and were either wild-type (129/Sv), p21-transgenic, or DN-Cdk2 transgenic. Transgenes were induced specifically in kidney proximal tubules by implantation of testosterone pellets. Bax activation was assessed by a conformation-specific antibody. Bcl-xL and phosphomimic Bcl-xL were purified by Ni-affinity chromatography to His-tagged Bcl-xL. Specific phosphorylation of Bcl-xL by Cdk2 was by analogue-sensitive Cdk2/cyclin A. Localization of the Bcl-xL phosphorylation site was by MS/MS analysis.

**Results:** We show that after cisplatin exposure, Cdk2 activity is upregulated, that Cdk2 phosphorylates Bcl-xL, a pro-survival Bcl-2 family member, and that phosphorylated Bcl-xL has a lower affinity to bind and inhibit Bax, a pro-apoptotic Bcl-2 family member. There are several possible mechanisms proposed to explain Bax activation, which results in Bax-mediated MOMP and eventual cell death. A common factor in these hypotheses is the prevention of Bax activation by virtue of its binding to pro-survival Bcl-2 family member proteins, such as Bcl-xL. We provide evidence that Bcl-xL is phosphorylated by Cdk2 at a previously unreported site in the protein, and also that Bcl-xL phosphorylation after cisplatin exposure is dependent on Cdk2 *in vitro* and *in vivo*. Previous studies showed that Bcl-xL phosphorylation can cause dissociation of Bax/Bcl-xL heterodimers. The interaction of Bcl-xL and Bax prevents the structural change necessary for Bax activation, which is required for Bax oligomerization in the mitochondrial membrane and subsequent MOMP, caspase activation, and cell death.

**Conclusions:** We propose that Cdk2 phosphorylation of Bcl-xL interferes with Bax retrotranslocation, resulting in Bax accumulation on mitochondrial membranes, Bax activation, MOMP, and subsequent cell death.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO080**

**Nephron-Specific Kidney Injury Molecure-1 Overexpression Induces Tubular Damage and Kidney Failure in Transgenic Zebrafish** Wenqing Yin, Said Movahedi Naini, Dirk M. Hentschel, Joseph V. Bonventre. *Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Mammalian Kidney injury molecule-1 (KIM-1) is upregulated after kidney injury in proximal tubular cells, and serves as a highly sensitive and specific biomarker for acute kidney injury. KIM-1 is also upregulated in many subjects with chronic kidney disease. KIM-1 functions as a phagocytic receptor which mediates the uptake of apoptotic bodies as well as oxidized lipids. Data from our laboratory suggests that prolonged expression of KIM-1 in mice is maladaptive. Here, we characterized zebrafish KIM-1 (zKIM-1) family members, and studied the effect of zKIM-1 overexpression on the zebrafish pronephros.

**Methods:** We cloned zKIM-1 and related family members and compared biochemical and functional aspects with that of human KIM-1 (hKIM-1) using PCR, western blotting, in situ hybridization (ISH) and immunostaining. We created two zebrafish models of KIM-1 overexpression in the pronephros employing the cdh17 promoter. In one case the expression was constitutive. In the other case a cdh17:$Cre^{ERT2}$ transgenic fish line was created that allowed for tamoxifen-induced overexpression of KIM-1 in nephrons initiated at 72 hr post fertilization (hpf).

**Results:** As with hKIM-1, zKIM-1 was not expressed in healthy nephrons, and was markedly upregulated after gentamicin-induced injury. zKIM-1 also showed a conserved phagocytic activity comparable to hKIM-1. Tissue-specific constitutive overexpression of zKIM-1 in the pronephros under cdh17 promoter control caused pericardial edema, reduced GFR and a higher mortality with defects apparent by 48 hpf. Histologic examination revealed kidney tubular damage in nephrons overexpressing zKIM-1. Cre-mediated overexpression of zKIM-1 initiated at 72hpf caused a similar kidney maladaptive phenotype marked by tubular damage, edema and higher mortality.

**Conclusions:** zKIM-1 has high structural and functional similarities to hKIM-1. KIM-1 overexpression in the pronephros of the zebrafish results in tubular damage and kidney failure. Enhanced prolonged expression of KIM-1 in human proximal tubules may have maladaptive consequences due to KIM-1-mediated in phagocytosis of tubular components.

*Funding:* NIDDK Support

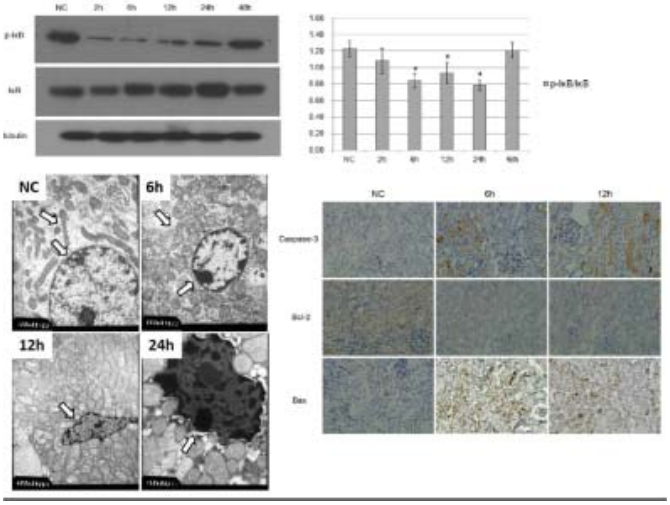
**FR-PO081**

**Melittin Induced Acute Kidney Injury through TNF-α/NF-κB/Mitochondria Dependent Apoptotic Pathway** Ling Zhang, Ping Fu. *West China Hospital of Sichuan Univ, China.*

**Background:** Mechanism of acute kidney injury (AKI) following multiple bee stings is not clear. Melittin, a main component of bee venom, can induce apoptosis in many cell types. We aimed to investigate the mechanism of melittin induced apoptosis in renal tubular epithelial cells through TNF-α/NF-κB signaling pathway.

**Methods:** AKI model was established by injecting melittin (4.0 μg/g) through the caudal veins of BALB/c mice (n=60). AKI was defined as creatinine (Cr) increased over 2 fold of that in control group. Blood and kidney samples were collected at 0h, 2h, 6h, 12h, 24h and 48h. Cr, urea nitrogen and hemoglobin were measured by Abbott i-STAT blood-gas analyzer. Creatine kinase was measured by immunochemiluminometric assays. Serum TNF-α was measured by ELISA method. Apoptosis of renal tubule cells was detected by TUNEL staining and transmission electron microscope. Expression of caspase-3, caspase-8, caspase-9, Bcl-2, Bax, and cytochrome c was detected by RT-PCR and/or immunohistochemical method. Expression of IκB and p-IκB was detected by Western Blot.

**Results:** AKI was diagnosed at 6h after injection of melittin (Cr: 1.1±0.3 mg/dl at 6h vs. 0.4±0.1 mg/dl at 0h, $p<0.05$). Serum TNF-α level increased significantly since 2h after injection. Apoptosis was detected since 2h, and was most notable at 12h. Western Blot showed that p-IκB/IκB ratio was significantly decreased in melittin group during 6h to 24h, indicating that NF-κB signal pathway was inhibited. RT-PCR showed that the Bcl-2/Bax ratio was significantly lower in melittin group than control group, indicating that the mitochondrial dependent apoptosis pathway was activated.




**Conclusions:** We successfully established melittin induced AKI in mice. Melittin might induce mitochondrial dependent apoptosis in renal tubular epithelial cells through activating TNF-α and inhibiting NF-κB signaling pathway.

**FR-PO082**

**DNA Damage Response in Renal Ischemia-Reperfusion and ATP-Depletion of Renal Tubular Cells** Zhengwei Ma, Zheng Dong. *Dept of Cellular Biology & Anatomy, Georgia Regents Univ, Medical College of Georgia, Augusta, GA.*

**Background:** Renal ischemia-reperfusion leads to acute kidney injury (AKI) that is characterized pathologically by tubular damage and cell death, followed by tubular repair, atrophy and interstitial fibrosis. Recent work suggested the possible presence of DNA damage response (DDR) in AKI. However, the evidence is sketchy and the role and regulation of DDR in ischemic AKI remain elusive.

**Methods:** In this study, DDR was studied in kidney tissues during ischemia-reperfusion in mice and renal proximal tubular cells (RPTC).

**Results:** We detected DDR in kidney tissues during ischemia-reperfusion in mice, as shown by increased expression of γH2AX and phosphorylation of ATM, Chk2 and p53. DDR was also induced In vitro during "reperfusion" of RPTC after ATP-depletion. DDR in this model was abrogated by supplying glucose (generate ATP via glycolysis), indicating that the DDR depends on ATP depletion. The DDR was suppressed to various extents by the ATM inhibitor Ku55933, the antioxidant N-acetylcysteine, the general caspase inhibitor z-VAD and overexpression of Bcl-2.

**Conclusions:** In conclusion, DDR occurs during renal ischemic AKI in vivo and ATP-depletion injury in vitro. ATM is a sensor in this DDR, which involves oxidative stress and apoptosis.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO083**

**Rictor/mTORC2 Protects against Cisplatin-Induced Tubular Cell Death and Acute Kidney Injury** Jianzhong Li, Weichun He, Junwei Yang, Chunsun Dai. *The Center for Kidney Disease, the Second Affiliated Hospital, Nanjing Medical Univ, Nanjing, Jiansu, China.*

**Background:** The mammalian target of rapamycin (mTOR) plays a critical role for cell growth and survival in many cell types. While substantial progress has been made in understanding the abnormal activation of mTORC1 in the pathogenesis of kidney disease, little is known about mTORC2 in kidney disease such as acute kidney injury (AKI).

**Methods:** Here a mouse model with tubule specific deletion of Rictor (Tubule-Rictor-/-) was generated and NRK-52E cell, a rat proximal tubular cell line, was used.

**Results:** The knockouts were born normal and no obvious kidney dysfunction or kidney morphologic abnormality was found within 2 months after birth. Cisplatin-induced AKI was generated in the mice and ablation of the Rictor in the tubular cells exacerbated cisplatin-induced AKI compared to those in the control littermates. Tubular cell apoptosis, Akt phosphorylation (Ser473) as well as autophagy were induced in the kidneys from the control littermates with cisplatin-induced AKI. Less cell autophagy or Akt phosphorylation and more cell apoptosis in the knockout kidneys were identified compared with those in the control littermates. In cultured NRK-52E cells, Rictor siRNA transfection sensitized cell apoptosis to cisplatin while reduced cisplatin-induced autophagy. Finally, metformin could abolish cell death induced by Rictor siRNA and cisplatin admininstration.

**Conclusions:** This study suggests that endogenous Rictor/mTORC2 protects against cisplatin-induced AKI, which is probably mediated by promoting cell survival through Akt signaling activation and autophagy induction.

*Funding:* Government Support - Non-U.S.

**FR-PO084**

**Rapamycin Prevents LPS-Induced Endothelial Cell Dysfunction** Giuseppe Castellano,[1] Alessandra Stasi,[1] Margherita Gigante,[1] Angelica Intini,[1] Paola Pontrelli,[1] C. Divella,[1] Claudia Curci,[2] Massimo Papale,[3] Enrico Fiaccadori,[4] G. Grandaliano,[2] Loreto Gesualdo.[1] *[1]DETO, Univ Bari, Bari, Italy; [2]Consorzio Carso, Univ Bari, Bari, Italy; [3]Med and Surg Sciences Dept, Univ Foggia, Foggia, Italy; [4]Clin and Exp Med Dept, Univ Parma, Parma, Italy.*

**Background:** During sepsis-induced acute kidney injury, LPS activates renal endothelial cells (ECs) resulting in cellular dysfunction. Aim of our study was to investigate the effects of LPS on ECs and the role of Rapamycin (Rp) in the modulation this pathogenic process.

**Methods:** Renal ECs were stimulated with LPS (4μg/ml) and Rp was used at 5nM. ECs were analyzed by MTT cell viability assay, FACS and western blot.

**Results:** We found that LPS did not induced apoptosis but a significant increase in ECs proliferation (.597 ± .03 vs basal .469 ± .004, p= .04; MTT assay). Moreover, LPS significantly decreased specific ECs markers such as CD31 (46.53% ± 9.65 vs basal p=.03) and VE-cadherin (15.2% ± 4.35 vs basal p=.001). On the contrary, LPS up-regulated markers of ECs dysfunction such as N-cadherin (81.51% ± 4.51 vs basal p=.002), Vimentin (71.34% ± 4.82 vs basal p=.01) and FSP-1 (64.66% ± 6.08 vs basal p=.01). Treatment with Rp normalized EC proliferation rate, without induction of apoptosis even in presence of LPS. Interestingly, the addition of Rp abrogated the LPS-induced ECs dysfunction by restoring the high expression of CD31 (R+LPS: 82.06% ± 3.4 vs LPS p=.01 ) and VE-cadherin (R+LPS: 55.33% ± 4.7 vs LPS p=.0003) and the low expression of N-cadherin (R+LPS:4%±2.1 vs LPS p=.0002), Vimentin (R+LPS:33,26%±4.1 vs LPS p=.03) and FSP-1 (R+LPS:9.83%±4.87 vs LPS p=.01). By western blot analysis, we observed that LPS induced a progressive and time dependent phosphorylation of ERK that was hampered by Rp treatment (R+LPS 60': .40±0.01 vs LPS 60' :1.15±0.32 p=.01).

**Conclusions:** Our study demonstrates that LPS caused EC dysfunction by activating EC proliferation and promoting phenotypic changes by ERK pathway. The inhibition of EC dysfunction by Rp may be a possible therapeutic strategy to preserve ECs during sepsis-induced acute kidney injury.

**FR-PO085**

**Protective Effects of Citrate in a Rat Model of Ischemic Acute Kidney Injury** Anja H. Bienholz,[1] Jonas Reis,[1] Frank Petrat,[1] Herbert De Groot,[1] Joel M. Weinberg,[2] Andreas Kribben,[1] Thorsten Feldkamp.[1,3] *[1]Univ Duisburg-Essen, Germany; [2]Univ of Michigan; [3]Christian-Albrechts-Univ Kiel, Germany.*

**Background:** Like other citric acid cycle metabolites, citrate can reverse the fatty acid-mediated energetic deficit impairing recovery of isolated proximal tubules subjected to hypoxia/reoxygenation at concentrations reached during clinical use as an anticoagulant for renal replacement therapy. In the present studies we used a rat model of ischemia/reperfusion (I/R)-induced AKI accompanied by intensive biomonitoring to assess renal protection by citrate in vivo.

**Methods:** I was induced in rats by bilateral clamping (40min) followed by R (3h). Citrate was infused at three different concentrations (in mmol/kg/h: 0.3; 0.6 and 1) continuously for 60min before and 45min after I. Normoxic and I/R control group received 0.9% NaCl. Ionized plasma calcium concentrations were kept stable by calcium gluconate infusion. Effects were assessed by biomonitoring, blood/plasma parameters and renal ATP.

**Results:** Renal I led to a drop in mean arterial blood pressure (end of I: 62±3 vs 85±2mmHg; p<0.01), a progressive rise in creatinine (end of R: 1.29±0.08 vs 0.49±0.04mg/dl; p<0.001) and a decrease in ATP (4.5±0.1 vs 9.0±0.4nmol/mg protein; p<0.001) in comparison to the normoxic control group. Citrate infusion stabilized blood pressure during I in concentration dependent fashion reaching statistical significance in the 1mmol/kg/h citrate group. Serum osmolarity was not changed during the experiment. Infusion of 1mmol/kg/h citrate lowered creatinine (1.04±0.04 vs 1.29±0.08mg/dl; p<0.05) and increased ATP (5.8±0.2 vs 4.5±0.1nmol/mg protein; p<0.05) at 3h of R compared to the I/R control group.

**Conclusions:** Citrate has protective effects in I-induced AKI, possibly by both beneficial cardiovascular effects and by directly limiting the energetic deficits. These data provide further rationale favoring the use of citrate in renal replacement therapy and encourages us to pursue citrate infusion with maintenance of stable calcium levels as a therapeutic treatment for AKI. They also indicate the importance of assessing hemodynamics in studies of protective maneuvers in renal I/R models.

*Funding:* Private Foundation Support

**FR-PO086**

**Arginase2 Is Up-Regulated in Renal Tubular Cells of AKI and Regulated NO In Vitro and In Vivo** Koji Ogata, Tatsuki Matsumoto, Kazu Hamada, Yoshinori Taniguchi, Yoshiko Shimamura, Kosuke Inoue, Taro Horino, Yoshio Terada. *Kochi Univ, Japan.*

**Background:** Arginase (AGase) is an enzyme that breaks down L-arginine and ornithine to urea, and was identified as nitric oxide (NO) metabolism-related factor. AGase2 is expressed constitutively in normal kidneys. However, because the biological role for AGase2 in acute kidney injury (AKI) is poorly understood, we studied the expression/modulation mechanism and role of AGase2 axis, and the aging effects.

**Methods:** To clarify the role of AGase2 in AKI, we used a rat ischemia/reperfusion (I/R) AKI model and cultured renal tubular cells (NRK-52E cells). To examine the effects of AGase2 in NO system, we used AGases2 expression vector and siRNA. Moreover, to assess aging effects, we also studied AGase2 expression in aged mice (42- and 87-week-old).

**Results:** Western blot analysis showed that AGase2 expression was increased at 12-48 h after I/R. The renal expression of AGase1 is very weak and not changed by I/R. Immunohistological examination revealed that AGase2 expression was increased in proximal tubules. In vitro AGase2 expression was also increased in NRK-52E cells under hypoxic stimulation. Overexpression of HIF-1α and Arnt resulted in increased AGase2 expression. When increased AGase2 under hypoxia was suppressed by AGase2 siRNA, phosphorylation of eNOS (Ser-177) was increased. Overexpression of AGase2 in NRK-52E cells resulted in suppressed phosphorylation of eNOS. Finally, AGase2 expression was significantly increased in 42- and 87-week-old aged mice, and showed further increase after AKI, in addition to the reduction of phosphorylation of eNOS.

**Conclusions:** In summary, AGase2 is up-regulated in renal tubular cells under AKI and hypoxic conditions. AGase2 might play a pivotal role on AKI pathogenesis via NO modulation by break down arginine and suppress eNOS activity. Furthermore AGase2 might be critical for aging-related AKI pathogenesis.

**FR-PO087**

**Inhibition of PKCδ Protects against Cisplatin-Induced Renal Tubular Cell Apoptosis by Activation of Autophagy through Directly Inhibition of AKT/mTOR Signaling** Zheng Dong.[1,2,3] *[1]The Second Xiangya Hospital, Central South Univ; [2]Dept of Cellular Biology and Anatomy, Medical College of Georgia, Georgia Regents Univ; [3]Charlie Norwood VA Medical Center, Augusta, GA.*

**Background:** Inhibition of PKCδ protects against cisplatin-induced acute kidney injury (AKI); however, the underlying mechanism remains[ensp]unclear. Autophagy has recently been recognized as an important mechanism of kidney protection. We hypothesized that inhibition of PKCδ may provide renoprotection by increasing autophagy.

**Methods:** In this study, we examined the role and signaling pathway of PKCδ in autophagy regulation during cisplatin treatment of renal proximal tubular cells (RPTC).

We further determined if the protective effect of PKCδ inhibitors depends on autophagy using renal proximal tubule–specific Atg7 knockout (PT-Atg7-ko) mice.

**Results:** PKCδ was activated rapidly by cisplatin in RPTC. After activation, PKCδ was shown to directly interact with Akt, resulting in Akt phosphorylation at serine-473. Consequently, Akt phosphorylated and activate mTOR, a major negative regulator of autophagy. Consistently, dominant negative and genetic inhibition of PKCδ during cisplatin treatment blocked mTOR activation, enhancing autophagy for cell survival. Rottlerin, a pharmacologic inhibitor of PKCδ, showed significant protective effect during cisplatin-induced AKI in mice, which was markedly abrogated in PT-Atg7-ko mice.

**Conclusions:** This study revealed a new signaling pathway mediated by PKCδ and Akt in the regulation of mTOR and autophagy. Moreover, it demonstrates the involvement of autophagy in the renoprotective effect of PKCδ inhibitors in AKI.

## FR-PO088

**Targeted Deletion of p53 from Proximal Tubules Protects against Ischemia-Reperfusion and Cisplatin-Induced Acute Kidney Injury** Zheng Dong.[1,2,3] *[1]The Second Xiangya Hospital, Central South Univ, Changsha; [2]Dept of Cellular Biology and Anatomy, Medical College of Georgia, Georgia Regents Univ; [3]Charlie Norwood VA Medical Center, Augusta, GA.*

**Background:** A role of p53 in acute kidney injury (AKI) has been suggested for over a decade but it remains controversial. Inhibition of p53 protects against AKI in rats, but it exacerbates ischemic AKI in mice as shown by recent work. One intriguing possibility is that p53 in different cell types in kidneys may have distinct or opposing roles: while tubular p53 may mediate injury in AKI, leukocyte p53 is renoprotective by limiting inflammation and inflammatory damage.

**Methods:** Test of this possibility requires the use of tissue or cell type specific p53-knockout models.

**Results:** Here we generated proximal tubule–specific p53 knockout mice, which were shown to be significantly resistant to both ischemic and cisplatin nephrotoxic AKI as indicated by renal function and histological analyses.Tubular apoptosis and associated gene expression were also attenuated.

**Conclusions:** Thus p53 in kidney tubules plays a general injury role in AKI.

## FR-PO089

**Valproic Acid, a Class I Histone Deacetylase Inhibitor, Protects Early Renal Function in the Renal Ischemia Reperfusion Injury Model** Elerson Costalonga, Andressa Daron, Filipe M. Silva, Irene L. Noronha. *Nephrology Div, Univ of Sao Paulo.*

**Background:** Ischemia reperfusion injury (IRI) is a major contributor to acute kidney injury (AKI). At present, there are no effective therapies to ameliorate injury or accelerate recovery after AKI. Histone deacetylases (HDACs) are a large family of epigenetic regulators, which catalyze the removal of acetyl groups from histones. Histone modifications have been shown to play a role in renal repair after IRI. The aim of the study was to analyze whether the valproic acid (VPA), a classe I HDAC inhibitor, could prevent renal IRI.

**Methods:** Male Wistar rats(250–300 g; n=24) were divided into three groups: **SHAM**: rats subjected to sham surgery; **IRI**: rats undergoing bilateral ischemia for 45 min; **IRI+VPA**: rats treated with VPA at 300 mg/kg twice daily 2 days before bilateral IRI. Blood urea nitrogen (BUN), serum creatinine(SCr), fractional excretion of sodium(FENa) and potassium(FEK), renal histology, number of interstitial macrophages (MØ)(immunohistochemistry), and TNF-α expression in the kidney tissue(real time PCR) were assessed 48 hours after IRI. The histopathological evaluation of the cortical acute tubular necrosis(ATN) used a grading scale of 0 to 5.

**Results:** VPA significantly attenuated renal dysfunction after ischemia, characterized by a decrease in BUN, SCr, FENa, and FEK in the IRI+VPA group compared with IRI. Kidney sections of the IRI+VPA group showed significantly lower ATN score with a marked reduction in the number of MØ, compared with the IRI group. Reduction of TNF-α mRNA levels was also observed.

| | SHAM | IRI | IRI+VPA |
|---|---|---|---|
| BUN(mg/dL) | 13±2 | 145±66* | 39±25# |
| SCr(mg/dL) | 0.3±0.05 | 2.7±1.6* | 0.5±0.2# |
| FENa(%) | 0.2±0.1 | 4.9±2.8* | 0.5±0.2# |
| FEK(%) | 22±8 | 129±56* | 17±4# |
| ATN Score | 0 | 2.3±0.9* | 1.1±0.4# |
| MØ(cells/mm$^2$) | 17±8 | 172±43* | 40±15# |
| TNF-α(mRNA level) | 1±0.1 | 2±0.2* | 1.4±0.1# |

*p<0.001 vs SHAM; #p < 0.001 vs IRI

**Conclusions:** These preliminary data suggest that HDAC inhibitors could prevent renal dysfunction and inflammation associated with IRI.

## FR-PO090

**ERK-Mediated Suppression of Cilia in Cisplatin-Induced Tubular Cell Apoptosis and Acute Kidney Injury** Shixuan Wang,[1] Zheng Dong.[1,2] *[1]Cellular Biology and Anatomy, Georgia Regents Univ, Augusta, GA; [2]Medicine/Renal, Second Xiangya Hospital, Changsha, Hunan, China.*

**Background:** In kidneys, each tubular epithelial cell contains a primary cilium that protrudes from the apical surface. Ciliary dysfunction was recently linked to acute kidney injury (AKI) following renal ischemia-reperfusion. Whether ciliary regulation is a general pathogenic mechanism in AKI remains unclear. Moreover, the ciliary change during AKI and its underlying mechanism are largely unknown.

**Methods:** We examined the change of primary cilium by immunofluorescence and its role in tubular cell apoptosis and AKI induced by cisplatin, a chemotherapy agent with notable nephrotoxicity.

**Results:** In cultured human proximal tubular HK-2 epithelial cells, cilia became shorter during cisplatin treatment, followed by apoptosis. Knockdown of Kif3a or Polaris (cilia maintenance proteins) reduced cilia and increased apoptosis during cisplatin treatment. We further subcloned HK-2 cells and found that the clones with shorter cilia were more sensitive to cisplatin-induced apoptosis. Mechanistically, cilia-suppressed cells showed hyperphosphorylation or activation of ERK. Inhibition of ERK by U0126 preserved cilia during cisplatin treatment and protected against apoptosis in HK-2 cells. In C57BL/6 mice, U0126 prevented the loss of cilia from proximal tubules during cisplatin treatment and protected against AKI. U0126 up-regulated Polaris, but not Kif3a, in kidney tissues.

**Conclusions:** It is suggested that ciliary regulation by ERK plays a role in cisplatin-induced tubular apoptosis and AKI.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO091

**Epidermal Growth Factor Receptor (EGFR) Inhibition with Erlotinib Attenuates Cisplatin-Induced Nephrotoxicity in Rats** Yukihiro Wada, Masayuki Iyoda, Kei Matsumoto, Yuki Shindo-Hirai, Yoshihiro Kuno, Taihei Suzuki, Ysutaka Yamamoto, Saitou Tomohiro, Ken Iseri, Takanori Shibata. *Div of Nephrology, Dept of Medicine, Showa Univ School of Medicine, Japan.*

**Background:** The effects of blocking the EGFR in acute kidney injury (AKI) are controversial. We investigated the renoprotective effect of erlotinib, a tyrosine kinase inhibitor that can block EGFR activity, on cisplatin (CP)-induced AKI.

**Methods:** CP nephrotoxicity (CP-N) was induced in 6-week-old male Sprague-Dawley (SD) rats (n=28) by intraperitoneal injection of CP (7 mg/kg) on day 0. Groups of animals were given either erlotinib (CP+E, 20 mg/kg, n=14) or vehicle (CP+V, n=14) daily by oral gavage from day -1 to day 3. Five SD rats were used as normal control (NC). All rats were sacrificed on day 4. In addition, we analized the effects of erlotinib in signaling pathways that are involved in CP-N by using human renal proximal tubular cells (HK-2).

**Results:** Compared to the NC rats, the CP+V rats exhibited marked AKI characterized by deterioration of renal function, severe tubulointerstitial (TI) damage, and increase in renal cortical mRNA expressions for proinflammatory cytokines, fibrogenic genes, and pro-heparin-binding EGF-like growth factor. Compared to vehicle, erlotinib treatment significantly prevented BW loss and increased urine volume. Erlotinib significantly improved renal function (sCr: 1.6 ± 0.3 vs. 0.8 ± 0.2 mg/dL, p<0.01) and ameliorated TI injury (the number of casts/HPF: 2.0 ± 0.7 vs. 0.7 ± 0.1, p < 0.01). PCNA-positive and TUNEL-positive cells were significantly reduced by erlotinib. Furthermore, renal cortical mRNA for TGF-β and Bax/Bcl-2 ratio were significantly reduced in CP+E rats compared to CP+V rats. Additionally, we observed that erlotinib significantly reduced the phosphrylation of ERK1/2, which was induced by CP in HK-2.

**Conclusions:** Our study shows that erlotinib attenuates the CP-induced AKI by degradation of tubular cell apoptosis and proliferation. The inactivation of ERK1/2 is likely one of the fundamental factors involved in apoptosis reduction. These results strongly suggest that erlotinib is useful for preventing AKI in patients receiving chemotherapy including CP.

## FR-PO092

**Truncated DNase I Acts as Dominant-Negative Inhibiting Kidney Cell Death Induced by Cisplatin** Alexei G. Basnakian,[1,2] Dmitry D. Zhdanov,[1] Tariq Fahmi,[1] Alena Savenka.[1] *[1]Pharmacology and Toxicology, Univ of Arkansas for Medical Sciences, Little Rock, AR; [2]Central Arkansas Veterans Healthcare System, John L. McClellan Memorial Veterans Hospital, Little Rock, AR.*

**Background:** DNA fragmentation, generally regarded as the hallmark biochemical event of apoptosis, is the attribute and important mechanistic step in many cell death modes that occurs in in vivo and in vitro models of kidney injury. DNA fragmentation induced by cisplatin toxicity to the kidney was shown to be mediated by deoxyribonuclease I (DNase I) in association with endonuclease G (EndoG). The latter, however, was later shown to induce alternative splicing of DNase I. The current study was aimed to determine the role of the alternatively-spliced truncated DNase I, delta4DNase I, generated by EndoG.

**Methods:** Real time RT-PCR, western blot, TUNEL assay, Annexin and flow cytometry.

**Results:** Our study demonstrated that cisplatin intoxication induced delta4DNase I in mouse kidneys. The DNase I isoform was shown to be expressed in many other mouse organs, and it was associated with low nuclear endonuclease activity. Recombinant delta4DNase I completely lacked endonuclease activity, and its actin-binding activity was also strongly diminished. The overexpression of delta4DNase I downregulated mRNA and protein expression of native DNase I and other apoptotic endonucleases in rat kidney tubular epithelial NRK-52E cells. It also suppressed total DNase activity in the cells, and provided protection against cisplatin toxicity measured by TUNEL assay or Annexin V/PI assay followed by flow cytometry.

**Conclusions:** This study identified a new native mechanism of kidney tubular epithelial cell protection by DNase I inactivation, which potentially can be applied in future therapies of cisplatin/toxic acute kidney injury.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO093**

**Epoxyeicosatrienoic Acids Increase Intrarenal Reoxygenation and Prosurvival Signaling and Protect against Renal Ischemia/Reperfusion Injury** Uwe Hoff,[1] Gordana Bubalo,[1] Maximilian Blum,[2] Mandy Fechner,[1] Ye Zhu,[1] Andreas Pohlmann,[2] Jan Hentschel,[2] Karen Arakelyan,[3] Erdmann Seeliger,[3] Bert Flemming,[3] Wolfgang Schneider,[2] Michael Rothe,[4] Thoralf Niendorf,[2] John Falck,[5] Wolf-hagen Schunck,[2] Duska Dragun.[1] *[1]Nephrology and Intensive Care Medicine, Campus Virchow-Klinikum, Center for Cardiovascular Research, Charité, Berlin, Germany; [2]Max-Delbrueck Center for Molecular Medicine and Berlin Ultrahigh Field Facility (B.U.F.F.), Berlin, Germany; [3]Institute of Physiology, Center for Cardiovascular Research, Charité, Berlin, Germany; [4]Lipidomix GmbH, Berlin, Germany; [5]Southwestern Medical Center, Univ of Texas, Dallas, TX.*

**Background:** Imbalances in cytochrome P450 (CYP)-dependent eicosanoids play a central role in ischemic acute kidney injury (AKI). We previously reported that inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) ameliorated ischemia/reperfusion (I/R)-induced AKI Now, we hypothesized that enhancement of epoxyeicosatrienoic acid (EET) actions may offset 20-HETE actions and contribute to cellular protection during immediate reperfusion.

**Methods:** Studies were performed in male Lewis rats that underwent right nephrectomy before 45-min clamping of left renal pedicle.

**Results:** CYP-eicosanoid analysis with liquid chromatography tandem mass spectrometry (LC-MS/MS) revealed pronounced postischemic 20-HETE release, yet only minor release of protective EETs. Therefore, we applied a synthetic EET agonist by intrarenal injection 5 min before ischemia. Continuous in vivo MRI monitoring in a 9.4 T MR-scanner showed markedly improved medullary reoxygenation after EET agonist application as assessed by T2* . EET-agonist treatment initiated fast rephosphorylation of key survival signaling PI3K dependent Akt (Thr308) and mTORC2 dependent Akt (Ser473) together with inactivation of pro-apoptotic GSK-3β, paralleled by reduced tubular apoptosis. Renal function was almost completely preserved upon EET intervention.

**Conclusions:** We demonstrate that EETs are responsible for induction of key survival mechanisms and improved intrarenal reoxygenation early after I/R. Renoprotective EET agonists offer a promising new option for prevention and treatment of ischemic AKI.

*Funding:* Government Support - Non-U.S.

**FR-PO094**

**Protein Kinase C-α Phosphorylates $F_0F_1$-ATPase and Contributes to Recovery of $F_0F_1$-ATPase Activity in Injured Renal Proximal Tubular Cells** Grazyna Nowak, Diana Bakajsova. *Pharmaceutical Sciences, Univ of Arkansas for Medical Sciences.*

**Background:** We have shown that overexpression of active PKC-α in mitochondria alleviates decreases in $F_0F_1$-ATPase activity and ATP content and promotes recovery of mitochondrial function in oxidant-injured renal proximal tubular cells (RPTC). This study tested if mitochondrial PKC-α interacts with $F_0F_1$-ATPase, changes its phosphorylation, and improves $F_0F_1$-ATPase recovery from injury.

**Methods:** Wild-type PKC-α (wtPKC-α) and inactive PKC-α mutant (dnPKC-α) were overexpressed in primary cultures of RPTC to increase their levels and cells were exposed to the model oxidant, *tert*-butylhydroperoxide (TBHP) or hypoxia. PKC-α was immunoprecipitated from mitochondria following RPTC injury and associated proteins and their phosphorylation were identified.

**Results:** Proteomic analysis demonstrated association of PKC-α with subunits α, β, and γ of $F_0F_1$-ATPase. The association of these subunits was predominantly with the active PKC-α in injured and non-injured RPTC. Overexpressing wtPKC-α maintained, whereas overexpressing dnPKC-α decreased, association of α, β, and γ subunits with PKC-α in mitochondria of injured RPTC. RPTC overexpressing wtPKC-α had increased levels of mitochondrial phospho-proteins. Proteomic analysis revealed increased levels of phosphorylated subunits α, β, and γ, which suggested that PKC-α is involved in their phosphorylation. Immunocapture of $F_0F_1$-ATPase and immunoblotting using phospho-(Ser) PKC substrate antibody showed phosphorylation of serine(s) present in PKC consensus site(s) on the γ subunit of $F_0F_1$-ATPase. Further, this phosphorylation increased in response to overexpression of wtPKC-α, but not dnPKC-α. Phospho-proteomic analysis of the immunocaptured γ subunit demonstrated phosphorylation of $Ser^{146}$ (TH**S**DQFLVTFK) of the γ subunit. $F_0F_1$-ATPase activity and ATP content decreased to 59% and 60% of controls, respectively, in injured RPTC. Overexpressing wtPKC-α, but not dnPKC-α, restored $F_0F_1$-ATPase activity and ATP content.

**Conclusions:** We conclude that active PKC-α associates with $F_0F_1$-ATPase in injured mitochondria, phosphorylates γ subunit, improves $F_0F_1$-ATPase activity, and promotes recovery of ATP content in injured RPTC.

*Funding:* NIDDK Support

**FR-PO095**

**The Injured Kidney Releases Factors into the Circulation That Cause Increased Mitochondrial Membrane Potential and Increased Anti-Oxidant Gene Expression *In Vitro* and *In Vivo*, Contributing to the Pathophysiology of Uremia** Jon D. Ahlstrom,[1] Florian Toegel,[3] Zhuma Hu,[1] Ping Zhang,[1] Christof Westenfelder.[1,2] *[1]Depts of Medicine, Univ of Utah and VA Medical Centers, Salt Lake City, UT; [2]Depts of Physiology, Univ of Utah and VA Medical Centers, Salt Lake City, UT; [3]Dept of Medicine, Brigham and Women's Hospital, Boston, MA.*

**Background:** Our laboratory has previously shown in animal models and in a Phase I Clinical Trial that Mesenchymal Stem Cells (MSCs) are a safe and effective therapy for acute kidney injury (AKI). It is well recognized that the changes in the "milieu interne" that result as a consequence of tissue injury adversely affect the function of distant organs, together leading to the multisystem manifestations of the "uremic syndrome". Largely unstudied and poorly understood is the impact that the kidney injury environment has on therapeutically administered MSCs, renal cells, or on other tissues and organs *in vivo*.

**Methods:** To characterize the response of cells exposed to a kidney injury environment, we treated cultured normal rat kidney cells (NRK, proximal tubular) or rat MSCs in 10% serum obtained from F344 rats 24 hrs post either 50 min IRI-AKI, bilateral nephrectomy (NPHX, uremic control without kidney injury) or SHAM controls. To verify these results *in vivo*, we also examined the mitochondrial function and gene expression of various organs from F344 rats following IRI-AKI, NPHX, or SHAM surgeries.

**Results:** The exposure of cell types to 10% AKI serum for 48 hours caused, compared to incubation with NPHX or SHAM animal sera, increased mitochondrial membrane potential (TMRE), up-regulation of anti-oxidant genes (catalase, HO-1), and increased reactive oxygen species activity (CM-H2DCFDA activity). Similar results were also observed in organs studied *in vivo* such as the kidney, heart, liver, and lung.

**Conclusions:** The mitochondrial and oxidative stress produced by yet to be identified factors, potentially electrophiles, released into the circulation by injured kidney tissue has the capacity to adversely affect kidney cells, other organs, and MSCs administered to treat AKI.

*Funding:* Veterans Affairs Support, Private Foundation Support

**FR-PO096**

**Oxygen-Sensing Prolylhydroxylase 1 (PHD1) in Acute Kidney Injury** Almut Grenz,[1] Raechel Peralta,[2] Uladzimir Shabeka,[1] Eunyoung Tak,[1] Sue Murray,[2] Shuling Guo,[2] Gene Hung,[2] Holger Eltzschig.[1] *[1]Anesthesiology, UC Denver, Denver, Aurora, CO; [2]ISIS Pharmaceuticals, Carlsbad, CA.*

**Background:** Acute kidney injury (AKI) due to ischemia is one of the most common complications with no treatment. Limited oxygen availability results in inhibition of three oxygen-sensing prolyl hydroxylases (PHD1-3) with subsequent stabilization of hypoxia-inducible factors (HIFs). Activation of HIF results in a transcriptionally regulated response that re-programs cellular metabolism towards hypoxia adaptation. *Thus, we hypothesize that genetic deletion or pharmacologic inhibition of PHDs mediates kidney protection from ischemia.*

**Methods:** Mice were studied in an ischemic model of AKI by utilizing a hanging weight system. Renal function was determined by inulin clearance, serum creatinine, urinary NGAL and KIM-1, renal cytokine levels, myeloperoxidase and histology.

**Results:** To pursue our hypothesis, we first treated wild type mice with a non-selective PHD inhibitor (DMOG, dimethyloxallyl glycine) before renal ischemia (30 min). Mice with DMOG treatment showed attenuated kidney injury. We then further characterized that the protection is specifically associated with PHD1but not PHD2 or PHD3 by utilizing gene-targeted mice (PHD1-3 KO mice). To confirm our findings in PHD1-/- mice, we explored a drugable approach using PHD1 specific antisense inhibitors (ASO1 and ASO2) which can reduce 90% and 60% kidney PHD1 RNA respectively in WT mice following systemic treatment. Interestingly, in the ischemic reperfusion model, both compounds showed comparable kidney protection as in PHD1-/- mice. To further elucidate cell type specificity of protection, we utilized conditional mice with depletion of PHD1 in tubular epithelia or endothelial cells. Indeed, mice with genetic deletion of PHD1 in tubular cells showed kidney protection from ischemia.

**Conclusions:** These studies identify tubular PHD1 inhibition or genetic depletion as key in protection from AKI due to ischemia. Furthermore, newly designed PHD1 inhibitors showed protection from AKI. If translatable from mice to human, these data have important therapeutic implications.

**FR-PO097**

**Stanniocalcin-1 Inhibits Renal Ischemia/Reperfusion Injury via an AMPK-Dependent Pathway** Jenny S. Pan,[1] Luping Huang,[1] Tatiana Belousova,[1] Qiang Tong,[2] David Sheikh-Hamad.[1] *[1]Nephrology Div, Dept of Medicine; [2]Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX.*

**Background:** Acute kidney injury (AKI) is common and is associated with increased morbidity, mortality, and development/progression of chronic kidney disease. Inflammatory, hypoxic and oxidative stresses play central roles in the pathogenesis of ischemic AKI; but while the pathogenesis is better defined, therapeutic options remain limited. We previously showed stanniocalcin-1 (STC1) suppresses superoxide generation through induction of mitochondrial uncoupling proteins (UCPs), and overexpression of STC1 in mice inhibits ROS and confers resistance to ischemia/reperfusion (I/R) kidney injury. Our observations reveal increased AMPK activity in STC1 transgenic (Tg) kidneys, and we hypothesize that STC1 protects from I/R kidney injury via AMPK activation.

**Methods:** We subjected wild-type (WT) and STC1 Tg mice to bilateral I/R kidney injury (clamping of renal pedicles for 30 min), concomitant with AMPK inhibition using compound C (CC; single IP injection at 20 mg/kg).

**Results:** Kidney baseline activity of AMPK correlated with the expression of stanniocalcins; with the highest activity in STC1 Tg, followed in decreasing order by WT, STC1 knockout (KO) and STC1/2 double KO. I/R in WT kidneys increased AMPK activity and expression of STC1, UCP2 and sirtuin-3 (SIRT3) proteins. AMPK inhibition with CC before I/R abolished AMPK activation and diminished SIRT3 and UCP2 expression without affecting STC1 protein expression. Moreover, the kidneys displayed increased production of superoxide and $H_2O_2$, and worse morphological changes. Pretreatment of STC1 Tg mice with CC restored susceptibility to I/R kidney injury characterized by: decreased creatinine clearance and urine output; morphological changes similar to those observed in WT kidneys after I/R; and increased ROS production. As observed in WT mice, AMPK inhibition in STC1 Tg mice attenuated UCP2 and SIRT3 expression at baseline and after I/R.

**Conclusions:** The data suggest that: 1) STC1 is critical for AMPK activation in the kidney; and 2) STC1-induced protection from I/R kidney injury and expression of UCP2 and SIRT3 are AMPK-dependent.

*Funding:* NIDDK Support, Other NIH Support - T32 Training Grant (DK062706), Private Foundation Support

## FR-PO098

**Development of Novel Nrf2 Activator to Treat Acute and Chronic Kidney Disease** Sanjeev Noel,[1] Samatha Bandapalle,[1] Rajesh Thimmulappa,[2] Shyam Biswal,[2] Hamid Rabb.[1] *[1]Dept of Medicine, Johns Hopkins Univ, Baltimore, MD; [2]School of Public Health, Johns Hopkins Univ, Baltimore, MD.*

**Background:** Oxidative stress induced by ischemia reperfusion (IR) participates in the development of AKI and its progression to CKD. Transcription factor Nrf2, is critical for the induction of antioxidant response during ischemic injury and therefore an attractive therapeutic target for pharmacological augmentation of antioxidant response in kidney diseases. Novel Nrf2 activators such as Trifluoromethyl 2'methoxychalcone (TMC) are needed which could be more potent and safer than bardoxolone.

**Methods:** Primary (RPTEC) and immortalized (HK-2) renal epithelial cells were treated with 5μM TMC for 6, 12 and 24h or 2.5, 5, and 10μM, TMC for 24h. Nrf2 activity was assessed by measuring HO-1 and NQO1 expression by qPCR and western blotting along with NQO1 enzyme activity. C57BL/6 male mice were treated with 40, 200 and 400mg/kg TMC for 6 or 24h to examine Nrf2 activity in mouse kidney.

**Results:** In comparison to vehicle, TMC treatment (5μM) resulted in significant ($p<0.01$) increase in *HO-1* gene expression at 6h (relative fold change (RFC) =1.2±0.3 vs 10.6±1.0) and 12h (RFC=1.0±0.02 vs 3.7±0.04) and showed a significant increase after 24h following 5 ($p=0.03$) and 10μM ($p<0.01$) TMC treatment in RPTEC cells (RFC=1.1±0.2 vs 2±0.2 and 7.3±0.2 respectively). Western blot analysis revealed 2.6, 3.4 and 3.6 fold increase in HO-1 protein expression in RPTEC cells following 2.5, 5 and 10μM TMC treatment respectively in comparison to vehicle. Similar observations were made for HO-1 expression in HK2 cells. *Ho-1* gene expression in mouse kidney increased significantly ($p<0.01$) after 200 and 400mg/kg oral TMC administration in comparison to vehicle at 6h (RFC=2.0±0.3 and 1.9±0.2 respectively) but returned to baseline at 24h. We observed significant increase in NQO1 expression and activity under these experimental conditions in HK2, RPTEC cells and mouse kidney.

**Conclusions:** These data demonstrates that TMC increases Nrf2 activity in renal epithelial cells and mouse kidney and appears to be a promising novel Nrf2 activator that merits evaluation for AKI and CKD treatment.

*Funding:* Other NIH Support - R01DK08445

## FR-PO099

**Crosstalk between p53-Sestrin2 and Hypoxia-BNIP3 (Bcl-2/Adenovirus E1B 19kDa-Interacting Protein3) Pathways in Regulating Autophagy, Mitophagy and Apoptosis in Renal Tubular Cells in Acute Kidney Injury** Masayuki Ishihara,[1] Madoka Urushido,[2] Kazu Hamada,[1] Yoshiko Shimamura,[1] Koji Ogata,[1] Kosuke Inoue,[1] Yoshinori Taniguchi,[1] Taro Horino,[1] Mikiya Fujieda,[3] Yoshio Terada.[1] *[1]Dept of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi Univ, Nankoku, Kochi, Japan; [2]Center for Innovative and Translational Medicine, Kochi Medical School, Kochi Univ, Nankoku, Kochi, Japan; [3]Dept of Pediatrics, Kochi Medical School, Kochi Univ, Nankoku, Kochi, Japan.*

**Background:** Little is known of the signaling pathways that regulate cell viability during acute kidney injury (AKI). We reported that hypoxia-Bcl-2/adenovirus E1B 19kDa-interacting protein 3 (BNIP3) and p53-sestrin2 pathways induced autophagy during AKI. The aim of this study is to investigate the crosstalk of two pathways during AKI.

**Methods:** We used rat ischemia/reperfusion (I/R) injury and cultured renal tubular cells (NRK-52E) as in vivo and in vitro models of AKI. We used TUNEL staining to evaluate apoptosis under oxidative stress in vitro AKI model. We transfected control siRNA or siRNA specific for BNIP3 and sestrin2, and measured LDH release following exposure of cells to hypoxic or oxidative stress.

**Results:** The expressions of BNIP3 and sestrin2 were upregulated after I/R. NRK-52E cells exposed to hypoxia showed increased expression of BNIP3 mRNA and protein. Sestrin2 mRNA and protein expression was upregulated following exposure to oxidative stress. Apoptosis was reduced by overexpression of sestrin2, and slightly reduced by BNIP3. These data are in accordance with the results of both the caspase 3 assay and Western blot analysis of cleaved caspase 3. Under hypoxic conditions LDH release was increased by BNIP3 siRNA and slightly increased by sestrin2. On the other hand, under oxidative stress, LDH release was increased by transfection with sestrin2 siRNA.

**Conclusions:** In summary of these data, both BNIP3 and sestrin2 induce autophagy. However, two molecules play some different role in apoptosis and cell death according to stress condition. These data the 2 autophagy-promoting signaling pathways regulate cell viability in different ways may be due to cell types or experimental condition.

## FR-PO100

**Structure and Function of the Polycystin-1 PLAT Domain** Yaoxian Xu,[1] Andrew J. Streets,[1] Peter J. Artymiuk,[2] Mike P. Williamson,[2] Albert C. Ong.[1] *[1]Academic Nephrology Unit, Univ of Sheffield, Sheffield, United Kingdom; [2]MBB, Univ of Sheffield, Sheffield, United Kingdom.*

**Background:** The PLAT (named after Polycystin-1, Lipoxygenase and Alpha Toxin) or LH2 (Lipoxygenase Homology 2) domain is located in the 1st intracellular loop of polycystin-1, the protein product of *PKD1*, the gene mutated in 90% of Autosomal Dominant Polycystic Kidney Disease (ADPKD) patients. This signature domain is found in all PC1 paralogues and shows evolutionary conservation in orthologues down to nematodes. PLAT has been predicted to be a lipid-binding domain based on homology to the lipoxygenases although little has been reported about its structure or function.

**Methods:** Nuclear Magnetic Resonance (NMR) studies of the polycystin-1 PLAT domain have resulted in assignment of 90% of its amino acid residues, which has allowed us to calculate a model of its structure. PLAT adopts a beta-sandwich fold composed of two four-stranded beta sheets, and is thus similar in structure to homologous domains in lipoxygenases.

**Results:** Lipid blot assays show that PLAT binds selectively to certain phospholipids, in particular phosphatidyl serine (PS) and phosphatidyl inositol 4-phosphate (PI4P). Using NMR, we have shown that these lipids have adjacent but different binding sites. We have also identified the binding site for calcium, and demonstrated that binding to PS is calcium dependent. In addition, we have identified a protein/protein interaction site, which is on the opposite face to the lipid binding interface. We also demonstrate that phosphorylation at a predicted protein kinase A (PKA) phosphorylation site dramatically internalizes the basolateral membrane-localized isolated PLAT domain and full length PC1 in kidney epithelial cell lines. Phosphorylation here abolishes the ability of PLAT to bind to PI4P, as predicted from the structure.

**Conclusions:** Based on these results, we propose a model for the function of PLAT, in which PLAT binds to specific membrane lipids, in a calcium- and PKA-dependent manner; binding controls the orientation of PLAT and thus the way in which it interacts with partner proteins. These interactions appear to be critical for the location and function of PKD1 and downstream effectors.

*Funding:* Private Foundation Support

## FR-PO101

**Cleavage of PC1 Is Dependent on the Unfolded Protein Response (UPR) Effector Xbp1** Sorin V. Fedeles,[1] Seung H. Lee,[1] Rachel Gallagher,[1] Ann-hwee Lee,[2] Stefan Somlo.[1] *[1]Internal Medicine, Yale School of Medicine, New Haven, CT; [2]Lab. and Exp. Medicine, Weill Cornell, New York, NY.*

**Background:** The unfolded protein response is a cellular mechanism that is activated when unfolded proteins accumulate in the ER, with Xbp1 being its most conserved effector. Active Xbp1s translocates to the nucleus and activates transcription of chaperones and proteins involved in ER-associated degradation. (ASN, 2011 TH-OR125) we have shown that Sec63, one of the ADPLD genes, and Xbp1 interact genetically in a polycystin-1 (PC1) dependent manner. In the current work we investigated the molecular underpinnings of the Sec63-Xbp1-Pkd1 interaction.

**Methods:** We examined processing of PC1 in kidney tubule cell lines isolated from mice carrying the ImmortoMouse and $Pkd1^{F/H}$-BAC transgenes in addition to inactivating mutations in Sec63 alone (SKO) or in Sec63 and Xbp1 together (DKO). We also analyzed kidney tissues from $Pkd1^{F/H}$-BAC SKO ($Sec63^{fl/fl}$;Ksp-Cre) and DKO ($Sec63^{fl/fl}$;$Xbp1^{fl/fl}$;Ksp-Cre) mice.

**Results:** Full length PC1 (PC1-FL) is cleaved at the GPS to yield the extracellular N-terminal fragment (PC1-NTF) and the intramembranous C-terminal fragment (PC1-CTF). The levels of PC1-CTF were reduced in SKO-$Pkd1^{F/H}$-BAC cells while they were almost completely absent in DKO cells where a concomitant increase in PC1-FL was observed, suggestive of a defect in GPS cleavage. Lysates from DKO-$Pkd1^{F/H}$-BAC mice exhibited further reduction in PC1-CTF beyond that seen in SKO-$Pkd1^{F/H}$-BAC kidneys. Re-expression of Sec63 in the DKO cells completely restored PC1-CTF while overexpression of XBP1 partially restored PC1-GPS cleavage in a dose dependent manner. Thus, activated XBP1s can compensate for Sec63 deficiency to promote PC1-GPS cleavage. XBP1s overexpression can also restore PC1 processing in $Prkcsh^{-/-}$;$Pkd1^{F/H}$-BAC cells suggesting that the effect of XBP1s on PC1-GPS cleavage is not limited to Sec63 null cells. Finally, the ER chaperone ERdj4, a XBP1s transcriptional target, modestly increased PC1-CTF levels in DKO cells.

**Conclusions:** The most conserved effector of the UPR response, XBP1, can modulate the cleavage of PC1 and this may represent a potential therapeutic avenue for PC1-misense mutant backgrounds.

*Funding:* NIDDK Support

## FR-PO102

**Functional Role of GPS Cleavage for Polycystin1 Biogenesis and Trafficking**
Feng Qian,[1] Almira Kurbegovic,[2] Hangxue Xu,[1] Hyunho Kim,[1] Julie Cruanes,[2] Marie Trudel.[2] *[1]Medicine, Univ of Maryland School of Medicine, Baltimore, MD; [2]Institut De Recherches Cliniques De Montreal, Montreal, Canada.*

**Background:** Autosomal dominant polycystic kidney disease is characterized by formation of renal cysts and caused mainly by mutations in PKD1, which encode polycystin-1 (PC1). PC1 is regulated in part by post-translational modifications via cleavage at the extracellular juxtamembrane GPS motif. We previously showed that this cleavage is frequently disrupted in ADPKD patients. $Pkd1^{V/V}$ mice homozygous for a "knock-in" missense change at cleavage site express a non-cleavable Pc1 ($Pc1^{V}$) and develop distal tubular cystic kidneys postnatally, leading to death by 1-month of age.

**Results:** Herein, we characterize the molecular composition of endogenous Pc1 resulting from GPS cleavage by various biochemical strategies. We show that GPS cleavage generates a previously unrecognized complexity of endogenous Pc1 products. Two distinct forms of cleaved Pc1 molecules are produced along with a small amount of uncleaved Pc1 ($Pc1^{U}$) in postnatal developing kidneys and other tissues: (1) heterodimeric $Pc1^{cFL}$ in which NTF is non-covalently associated with CTF; (2) $Pc1^{deN}$ in which NTF becomes detached from CTF. Both cleaved products traffic past the Golgi to substantial extents whereas $Pc1^{U}$ is restricted to the ER. Importantly, GPS cleavage is not a prerequisite for Pc1 trafficking. Only $Pc1^{deN}$ is found at the cell surface of renal epithelial cells. We have also analyzed the function of $Pc1^{deN}$ for kidney development by BAC-transgenic expression of a NTF fragment in $Pkd1^{V/V}$ mice. We then examined the molecular mechanism of $Pc1^{deN}$ biogenesis and trafficking using Pkd1 mutant mice with mutations in CTF. We show that proper $Pc1^{deN}$ trafficking requires intact CTF and propose a model in which $Pc1^{deN}$ is derived from $Pc1^{cFL}$.

**Conclusions:** Our data demonstrates that GPS cleavage is a critical regulator of Pc1 biogenesis and CTF plays a crucial role in Pc1 trafficking for all Pc1 products. This study thus provides novel insights into the complexity of Pc1 biogenesis and trafficking with functional roles in a cell type- and stage-dependent manner.

*Funding:* NIDDK Support

## FR-PO103

**PKC-Zeta Phosphorylates Polycystin-1 and Modulates Its Regulation of STAT3 Activity**
Nicholas Doerr,[1] Yidi Wang,[1] Markus M. Rinschen,[2] Jeffrey Talbot,[1] Alexis V. Gibson,[1] Bernhard Schermer,[2] Thomas Weimbs.[1] *[1]Univ of California Santa Barbara; [2]Univ of Cologne, Germany.*

**Background:** Mutations in the gene coding for polycystin-1 (PC1) cause autosomal-dominant polycystic kidney disease. We have previously shown that STAT3 is aberrantly activated in ADPKD and that PC1 regulates STAT3 activity by a dual mechanism. Here, we tested the ability of several kinases to bind PC1 and regulate PC1-mediated STAT3 activity.

**Methods:** Protein interactions were determined by co-IP. Phosphorylation of PC1 by PKC-zeta was examined in HEK293T cells and specific phosphorylation sites were determined by mass spectometry. Phosphorylation results were confirmed by *in vitro* kinase assays. Regulation of STAT3 activity was measured by luciferase reporter assay. Expression of PKC isoforms in ADPKD and mouse models was determined by western blot analysis.

**Results:** We found that atypical PKC (PKC-zeta) interacts with the cytoplasmic tail of PC1. We identified three sites within the PC1 tail that are phosphorylated by PKC zeta. The interaction between PC1 and PKC-zeta is enhanced by expression of the scaffold protein p62/SQSTM1, which was also found to bind the PC1 tail. PKC-zeta decreases the activation of STAT3 by membrane-anchored PC1 but promotes the activation of STAT3 by the cleaved, soluble PC1 tail, suggesting that it acts as a regulatory switch. Regulation of PC1-mediated STAT3 signaling is specific as PKC zeta does not alter IL6-induced STAT3 signaling nor PC1-mediated AP1 signaling. This signaling switch is likely dysregulated in the disease as PKC-zeta expression is decreased and STAT activation is increased in ADPKD kidneys and a PKD1 mouse model.

**Conclusions:** These data demonstrate a novel interaction between PKC-zeta, p62 and PC1. PKC-zeta binds and phosphorylates the PC1 tail and regulates its activation of STAT3. Whether PKC-zeta activates or represses STAT3 signaling depends on the state of proteolytic processing of PC1. Therefore together PKC-zeta and PC1 may serve as critical regulators of STAT3 signaling in the kidney and the pathogenesis of ADPKD.

## FR-PO104

**Polycystin-1 Accelerates Degradation of Polycystin-2 via the Aggresome Pathway**
Valeriu Cebotaru,[1] Liudmila Cebotaru,[2] Feng Qian,[3] William B. Guggino.[4] *[1]Johns Hopkins Univ; [2]Johns Hopkins Univ; [3]Univ of Maryland; [4]Johns Hopkins Univ.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder that presents with renal cysts. Mutations in PKD1 or PKD2 genes lead to ADPKD. Polycystin-1 (PC1) and polycystin-2 (PC2) are gene expression products of PKD1 and PKD2 respectively. Up-regulation or down-regulation PC1 or PC2 leads to polycystic kidney disease in mice suggesting that polycystins must be regulated within a functional window to avoid cyst formation. We have shown previously that PC1 regulates PC2 expression (un-published data). Here we further investigated the regulation of the PC2 degradation pathway by PC1. Misfolded and unfolded proteins are refolded by chaperones. Proteins that cannot be refolded usually end up degraded by proteasomes. Proteins that are not degraded by proteasomes precipitate as aggresomal particles and are transported by Histone deacetylase 6 (HDAC6) towards the aggresome for degradation.

**Methods:** We used MDCK cells that were stably transfected with an expression system where expression of full length PC1 or PC1-R4227X mutant can be induced by tetracycline.

**Results:** We found that inhibition of the aggresome degradation pathway with tubacin, a specific HDAC6 inhibitor, led to an up-regulation of PC2 expression when PC1 was overexpressed. Importantly, tubacin had no effect on PC1 expression. In addition we found that HDAC6 does interact with PC2 in control MDCK cells where PC1 expression has not been induced. Over-expression of PC1 not only decreased expression of PC2, but also greatly reduced the interaction of HDAC6 and PC2. On the other hand, PC1 does not interact with HDAC6, suggesting that PC1 accelerates the aggresomal degradation of PC2 via a second messenger that requires the presence of the C-terminal tail of PC1. Consistent with this concept is the observation that over-expression of the PC1-R4227X mutant, that lacks the last 76 amino acids, did not decrease PC2 expression, nor did it decrease the interaction between PC2 and HDAC6.

**Conclusions:** We have now uncovered a new pathway that explains how PC1 regulates PC2 expression via the aggresome degradation pathway.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO105

**Different Patterns of Pkd1 Deficiency Lead to Heart Dysfunction and Fibrosis in Mice**
Bruno E. Balbo, Andressa Godoy Amaral, Jonathan Mackowiak Fonseca, Vera Mc Salemi, Luiz F. Onuchic. *Nephrology, Univ of São Paulo, Brazil.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is a systemic disorder associated with cardiovascular manifestations, including hypertension (SAH) and ventricular hypertrophy. Although fibrosis impacts on kidney disease progression, its role in ADPKD cardiac phenotype remains uncertain.

**Methods:** Cardiac and renal phenotypes were analyzed in two *Pkd1*-deficiency mouse models: 10-13-wk-old noncystic $Pkd1^{+/-}$(HT) and its wild-type controls (WT); and 20-23-wk-old cystic $Pkd1^{cond/cond}$:$Nestin^{Cre}$(CY) and its noncystic controls ($Pkd1^{cond/cond}$, NC). Heart function and kidney cystic index (CI) were evaluated by high-resolution ultrasound.

**Results:** The HT and CY mice displayed lower fractional shortening (FS) and ejection fraction (EF) than their controls, findings consistent with systolic dysfunction (FS: 20.5±1.2 *vs* 28.8±1.9; 23.6±2.4 *vs* 31.8±1.8, $p<0.05$; EF: 42.3±2.2 *vs* 55.9±3.0; 47.2±4.3 *vs* 60.3±2.7, $p<0.05$). The myocardial performance index was also elevated in the HT and CY groups, expressing worsening of global ventricular function. In another study, we have shown that HT mice were not hypertensive at this evaluated age, while CY had SAH. No difference in BUN, serum creatinine ($S_{Cr}$), Na, K, fractional excretion of Na ($FE_{Na}$) and $FE_K$ were detected between HT and controls. CY mice presented lower $S_{Cr}$ than NC animals and a CI of 6.9% (IQR:4.1-9.5). A higher fibrosis index (FI) was observed in HT and CY cardiac tissue compared with their controls (0.84 *vs* 0.30%; 0.22 *vs* 0.01%, $p<0.01$), as well as higher apoptosis. Higher FI and increased cell proliferation rates were detected in HT and CY kidneys compared with their controls.

**Conclusions:** Our results demonstrate that two distinct profiles of *Pkd1* deficiency lead to heart dysfunction and fibrosis in mice. The absence of SAH in HT mice suggests the development of a primary cardiomyopathy associated with decreased polycystin-1 activity. Our findings suggest, on the other hand, that CY mice develop a cardiomyopathy based on two components: primary *Pkd1* deficiency and cardiac damage secondary to SAH. Our data provide significant insights into human ADPKD heart disease and prove useful for future investigations.

*Funding:* Government Support - Non-U.S.

## FR-PO106

**Over-Expression of the Polycystin 1 C-Terminal Tail Leads to Disappearance of Acetylated α-Tubulin**
Nikolay P. Gresko, Michael J. Caplan. *Cellular and Molecular Physiology, Yale Univ School of Medicine, New Haven, CT.*

**Background:** Autosomal dominant polycystic kidney disease is caused by mutations in the genes encoding polycystin-1 and 2 (PC1 and 2). PC1 is a massive multi-spanning membrane protein that is localized in part to the primary cilium in polarized epithelial cells. The primary cilium is composed of an axoneme containing 9 pairs of parallel microtubules. The tubulin monomers in these microtubules are modified through acetylation on lysine 40. This modification is controlled by the activities of acetylating and deacetylating enzymes and may contribute to the stability of cilia-associated microtubules. PC1 is subject to several cleavages that release various C terminal fragments. One of these fragments appears to include the 200 amino acids of the PC1 C terminal tail (CTT) and contains a functional nuclear localization sequence (NLS). We find that over-expression of a construct corresponding to the C terminal 200 amino acid residues of PC1 (P200) results in a dramatic reduction in tubulin acetylation.

**Methods:** Ciliated LLC-$PK_1$ cells were transfected to express P200 or a version of P200 lacking the NLS (p200ΔNLS). LLC-$PK_1$ cells transfected with an empty vector served as controls. Tubulin acetylation was assessed using anti-acetylated tubulin antibodies in western blot (WB) and immunofluorescence (IF) analyses.

**Results:** IF reveals that the cilia of control LLC-PK1 cells label brightly with anti-acetylated tubulin. Acetlyated tubulin is also detected by WB in lysates prepared from control cells. In contrast, P200 expression reduces the abundance of acetylated tubulin below the limits of detection of both the IF and WB assays. Interestingly, expression of p200DNLS significantly increases acetylated tubulin levels. Perhaps surprisingly, cilia appear to be structurally normal in the acetylated tubulin-deficient P200-expressing $LLCPK_1$ cells, as revealed through both light and electron microscopic analysis.

**Conclusions:** Over-expression of P200, but not of P200DNLS, results in dramatically decreased tubulin acetylation. Tubulin acetylation is not essential for the development or structural integrity of the primary cilium.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## FR-PO107

**Polycystin-1 Regulates Actin Cytoskeleton Organization and Directional Cell Migration through a Novel PC1-Pacsin 2-N-Wasp Complex**

Gang Yao,[1] Vy Nguyen,[1] Kristina A. Roberts,[1] Ayumi Takakura,[1] Markus Plomann,[2] Jing Zhou.[1] *[1]Harvard Center for Polycystic Kidney Disease Research, Brigham and Women's Hospital, Boston, MA; [2]Center for Biochemistry, Univ of Cologne, Cologne, Germany.*

**Background:** Formation of a tubule with defined length and lumen diameter is a fundamental step for epithelial and endothelial cells to make many vital organs. Autosomal dominant polycystic kidney disease (ADPKD), the most common life-threatening monogenic disease that manifests itself in cyst formation in multiple epithelial organs, is the result of the loss of control of tubule lumen size. One of the prerequisites for proper tubulogenesis in vitro and in vivo is directional cell migration.

**Methods:** In this study, we utilized yeast two-hybrid screens, biochemistry, cell biology, and live cell imaging to discover proteins that interacts with ADPKD protein polycystin-1 (PC1) and to study the consequence of the identified interaction.

**Results:** Yeast two-hybrid screens using the C-terminus of PC1 as bait, identified Pacsin 2, a cytoplasmic phosphoprotein that has been implicated in cytoskeletal organization, vesicle trafficking, cell intercalation during gastrulation, and more recently in kidney tubule development, and repair of ischemia-reperfusion injured kidneys. PC1 C-tail binds to a 107-residue fragment containing the a3 helix of the F-BAR domain of Pacsin 2. Pacsin 2 expression and localization are altered in *Pkd1* targeted kidneys. Kidney epithelial cells deficient in either PC1 or Pacsin 2 migrate at a slower speed with reduced directional persistency, and exhibit defects in lamellipodium formation and tubulogenesis in 3-dimentional (3D) collagen gels. We further demonstrate that PC1, Pacsin 2, and N-Wasp are in the same protein complex, and both PC1 and Pacsin 2 are required for N-Wasp/Arp2/3-dependent actin remodeling.

**Conclusions:** Our study suggests that PC1 modulates actin cytoskeleton rearrangements and directional cell migration during tubule development and regeneration through the Pacsin 2/N-Wasp/Arp2/3 complex, which consequently contributes to the establishment and maintenance of the sophisticated tubular architecture.

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## FR-PO108

**Polycystin Signaling Is Required for Lymphatic Development**

Patricia Outeda Garcia,[1] Gregory G. Germino,[2] Terry J. Watnick.[1] *[1]Dept of Medicine, Div of Nephrology, Univ of Maryland, School of Medicine, Baltimore, MD; [2]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.*

**Background:** Edema is a universal feature of all *Pkd1* and *Pkd2* targeted alleles reported in the literature to date and has been variably attributed to vascular fragility and/or cardiac defects. Since the lymphatic system plays a critical role in tissue fluid homeostasis, we speculated that a defect in the lymphatic vasculature might contribute to edema in *Pkd* mutant embryos.

**Results:** We harvested E14.5 $Pkd^{-/-}$ embryos and controls (WT) and performed whole mount staining of dorsal skin with the lymphatic endothelial cell (LEC) marker, Lyve1. We found that both $Pkd1^{-/-}$ and $Pkd2^{-/-}$ mice exhibited a more dilated and disorganized lymphatic network with decreased branching. Next, we focused on an earlier stage of lymphatic development and stained sections from the jugular area of E11.5 embryos with the LEC marker, Prox1. At this stage Prox1 positive LECs were budding from the dorsolateral aspect of the cardinal vein in both mutant and wild type embryos. In wild type embryos LECs were found in a narrow, restricted dorsolateral area. In contrast, in $Pkd^{-/-}$embryos the distribution of Prox1 positive cells appeared to be more random. We found that the area of distribution of Prox1 positive cells in the dorsolateral aspect of the cardinal vein was significantly increased in $Pkd^{-/-}$ embryos, suggesting an abnormality in polarized cell migration. We used a cell culture system to show that both *Pkd1* and *Pkd2* depleted endothelial cells have an intrinsic defect in directed migration that may contribute to the *in vivo* phenotype.

**Conclusions:** Our work establishes a role for polycystins in lymphatic development and identifies a new signaling pathway that is involved in lymphangiogenesis.

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## FR-PO109

**The Role of Phosphorylation in Polycysytin-2 Trafficking and Channel Activity**

Yiqiang Cai,[1] Ivana Y. Kuo,[2] Hongzhi Quan,[1] Kathryn Stone,[3] Ke Dong,[1] Xin Tian,[1] Ming Ma,[1] Seung H. Lee,[1] Jian Chen,[1] Barbara E. Ehrlich,[2] Stefan Somlo.[1] *[1]Internal Medicine, Yale Univ School of Medicine, New Haven, CT; [2]Pharmacology, Yale Univ, New Haven, CT; [3]WM Keck Biotechnology Resource Laboratory, Yale Univ, New Haven, CT.*

**Background:** Loss of polycystin-2 (PC2), a cation channel, results in autosomal dominant polycystic kidney disease (ADPKD). PC2 has been localized to cilia and is abundantly expressed in the endoplasmic reticulum (ER). Our previous studies have shown that PC2 is constitutively phosphorylated at residue $Ser^{812}$, and that loss of phosphorylation at $Ser^{812}$ results in a 10-fold decrease in sensitivity to $Ca^{2+}$ activation of PC2 but has no effect on PC2 localization at the ciliary membrane (Cai et al, 2004).

**Methods:** We identified additional phosphorylation sites in PC2 by phosphoproteomic analysis of TiO2 enriched immunoprecipitated PC2 prepared from either cultured epithelial cells stably overexpressing PC2 or from native mouse kidney tissues. Cultured renal epithelial cells stably overexpressing the PKD2 cDNAs with combinations of the mutations in the identified phosphorylation site(s) were examined for PC2 function. The functional assays examined the effects of PC2 phosphorylation on: 1) the association of PC2 with PC1 or with itself; 2) PC2 ciliary trafficking; 3) stabilization of PC1-CTF by PC2; and 4) cellular Ca2+ transient activity of PC2.

**Results:** A total of 3 Ser phosphorylation sites were identified in PC2. Loss of phosphorylation or pseudophosphorylation mutations at these sites had no effect on the association of PC2 with PC1 or PC2 itself, nor on the stabilizing effect of PC2 on the PC1-C terminal fragment.Loss of phosphorylation at one site resulted in increased ciliary expression of PC2. Loss of phosphorylation at another site resulted in higher baseline cytosolic Ca2+ in unstimulated cells likely effecting the ER channel activity of PC2.

**Conclusions:** Phosphorylation of PC2 plays a role in regulation of PC2 channel activity and trafficking to cilia. Understanding the molecular basis for the regulation of PC2 phosphorylation may enhance understanding the mechanism of cyst growth in ADPKD.

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## FR-PO110

**Sorting Nexin 3 Regulates the Trafficking and Surface Localization of Polycystin-2**

Shuang Feng, Andrew J. Streets, Albert C. Ong. *Academic Nephrology Unit, Univ of Sheffield, Sheffield, United Kingdom.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD), is caused by mutations in *PKD1* (85%) or *PKD2* (15%). Although the ADPKD proteins, polycystin-1 (PC1) and polycystin-2 (PC2) have been shown to form a receptor-ion channel complex, the surface expression of PC2 appears to be regulated by protein phosphorylation and interaction with key adaptor proteins.

**Methods:** To identify additional regulators of PC2 sorting and trafficking, we conducted a yeast-2-hybrid (Y2H) screen using the PC2 C-terminal domain as bait. Here, we report a functional interaction between PC2 and two isoforms of sorting nexin 3 (SNX3). The SNXs are a family of proteins first identified in yeast which share a Phox (PX) domain with affinity for PtdIns(3)P and which regulate endosomal sorting. In mammals, 30 SNXs have been identified. The retromer complex comprises two subcomplexes: a trimer (Vps26-29-35) which functions as a cargo-selective adaptor and a membrane deforming subcomplex consisting of a sorting nexin (SNX) heterodimer. In mammals, there are two distinct mammalian retromer complexes distinguished by either a SNX-BAR heterodimer or by monomeric SNX3.

**Results:** We identified a new SNX3 isoform (102aa, isoform 5) as a new binding partner to the C-terminus of PC2. This isoform lacks the PX domain present in the classical SNX3 isoform (162aa, isoform 1). In-vitro binding studies confirmed that this interaction was direct. Unexpectedly, we found that SNX3-162 could also bind to PC2 indirectly via the retromer complex; PC2 binds to VPS35 directly via its N-terminal domain. The localization of both isoforms was distinct with SNX3-102 expressed in clathrin-coated vesicles where it binds to AP2 whereas SNX3-162 is expressed in early endosomes where the retromer complex is expressed. Knockdown of both SNX3 isoforms in polarized kidney cells resulted in a two fold increase in surface PC2 expression by biotinylation.

**Conclusions:** Our results reveal for the first time that PC2 internalisation and recycling is regulated by a new SNX3 isoform as well as a SNX3-retromer complex. Since SNX3 expression can be modulated by different drugs, this could represent a potential new target for patients with ADPKD.

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## FR-PO111

**Deletion Mutant of *Pkd1* and *Pkd2* Gene Cause Cyst Formation in Transgenic Medaka**

Yasunobu Ishikawa,[1] Saori Nishio,[1] Tomotsune Miyamoto,[1] Sekiya Shibazaki,[1] Hisashi Hashimoto,[2] Yuko Wakamatsu,[2] Tatsuya Atsumi.[1] *[1]Medicine II, Hokkaido Univ, Sapporo, Hokkaido, Japan; [2]Bioscience and Biotechnology Center, Nagoya Univ, Nagoya, Aichi, Japan.*

**Background:** Autosomal dominant polycystic kidney disease is the most common monogenic disorder resulting in cystic kidneys and liver. Several genetic mechanisms probably contribute to the phenotypic expression of the disease. A two-hit mechanism has been proposed to explain how cysts develop. However, the dominant pattern of inheritance would argue for a gain of function, haploinsufficiency, or a dominant negative mechanism. We established four different transgenic medaka models to investigate whether a dominant negative mechanism can cause cyst formation.

**Methods:** We cloned the medaka *Pkd1* and *Pkd2* genes by homology search. Next we generated 4 transgenic medaka models named as *Pkd1* EF1α, *Pkd2* EF1α, *Pkd1* Tet-On and *Pkd2* Tet-On that expresses translation elongation factor 1 subunit alpha (EF1α) promoter linked to deleted exons 6-27 in *Pkd1* or exons 12-15 in *Pkd2* respectively. In lines *Pkd1* Tet-On and *Pkd2* Tet-On, we used the Tet-On system that induces gene expression upon doxycycline (DOX) administration. We carried out a series of analyses including gene expression by real time PCR, histology, cell proliferation and apoptosis in Medaka at specific time points of 1, 2, 3, 4 and 5 months of age .

**Results:** Pkd1 gene expression in the Pkd1 transgenic medaka ranged from 2- to 5-fold increase relative to Pkd1 endogenous levels from control medaka arbitrarily set at 1. Pkd2 expression levels in Pkd2 transgenic medaka ranged from 3- to 60-fold relative to Pkd2 endogenous levels from control medaka arbitrarily set at 1. All four models developed kidney cysts, however both Pkd1 EF1? and Pkd1 Tet-On showed very small and fewer cysts compared with Pkd2 EF1? and Pkd2 Tet-On. Proliferating Cell Nuclear Antigen positive cells increased in cyst lining cells of all models. But the overall number of apoptotic nuclei seen in low power fields in cystic kidneys was very low and did not differ significantly among all models.

**Conclusions:** Overexpression of of *Pkd1* and *Pkd2* deletion mutants in medaka results in cyst formation via a dominant negative mechanism.

## FR-PO112

### Biochemical Analysis of the ADPKD *PKD2-D511V* Patient Mutation

Alexis Hofherr, Michael Kottgen. *Div of Nephrology and General Medicine, Univ Medical Centre Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common lethal monogenetic disorder in humans. Mutations in the *PKD2* gene account for approximately one out of four cases. *PKD2* encodes the transient receptor channel polycystin-2 (TRPP2), a non-selective $Ca^{2+}$-permeable cation channel. There are multiple types of mutations causing ADPKD. The Mayo ADPKD Mutation Database lists a total of 268 unique *PKD2* mutations, including 77 frame-shift, 42 non-sense and 37 splice mutations as well as 26 substitutions. However, rather little experimental data investigating the disease mechanism of these mutations is available.

**Methods:** Here we analyze the biochemical properties of the pathogenic missense variant *PKD2-D511V*, which has been previously characterized as a channel-dead mutant. Cellular expression systems and a *Drosophila melanogaster PKD2* knock-out model are used for *in vitro* and *in vivo* studies, respectively.

**Results:** The *D511V* mutation causes significantly reduced (-85.2%) TRPP2 expression levels compared to wild-type protein in heterologous expression systems. The mutant protein is degraded in lysosomes. Treatment with the lysosomotropic agent chloroquine increases TRPP2 protein levels 14.6-fold, whereas incubation with the proteasome inhibitor MG132 has no effect. Notably, incubation of cells expressing TRPP2-D511V with an FDA-approved small molecule results in a significant rescue of reduced expression levels. To investigate the pathogenic mechanism of the *D511V* mutation *in vivo*, we introduced the corresponding mutation into the *D. melanogaster PKD2* homolog (*Amo-D627V*). This transgene does not rescue the *PKD2* knock-out phenotype and protein expression is likewise reduced compared to wild-type TRPP2 *in vivo*.

**Conclusions:** In conclusion, the *D511V* mutation seems to cause TRPP2 protein instability, which can be rescued by small molecules *in vitro*. Future studies will have to show whether this chemical rescue of protein instability results in increased TRPP2 function *in vivo*. The *D. melanogaster* model mimicking *PKD2-D511V* will be a valuable tool to test this hypothesis.

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## FR-PO113

### Roles of PKD2 mRNA 3'UTR in Its Translational Regulation

Wang Zheng, Jungwoo Yang, Xing-Zhen Chen. *Physiology, Univ of Alberta, Edmonton, Canada.*

**Background:** Mutations in PKD2 account for 10% autosomal dominant polycystic kidney disease (ADPKD) which is associated with elevated cell proliferation, apoptosis and de-differentiation, and affects over 10 million people worldwide. Either lose- or gain-function of PKD2 is pathogenic, indicating that the PKD2 protein level needs to be tightly regulated into a narrow range. However, how PKD2 steady-state level is regulated remains largely unclear. Here we reported two fragments in PKD2 mRNA 3' untranslated region (3UTR) that respectively mediate up- and down-regulation of PKD2 translation.

**Methods:** Dual luciferase assay was used to identify functional domains in PKD2 mRNA 3UTR which can regulate PKD2 translation in HEK and HeLa cells. Biotin-RNA pull down and mass spectrometry were used to identify protein partners that bind with these domains.

**Results:** By dual luciferase assays, we firstly found that PKD2 mRNA 3UTR significantly inhibits luciferase translation. We then divided the full-length 3UTR into two overlapping fragments and found that the first fragment strongly inhibits, while the second fragment stimulates, luciferase translation. We then narrowed down the inhibitory fragment to nt 691-1044 which was named 3FI and the stimulatory fragment to nt 1056 – 1129 which was named 3FS. Consistent with this, deletion of 3FI or 3FS from full-length 3UTR resulted in an increased or decreased luciferase activity, respectively. We then confirmed inhibitory/stimulatory effect of 3FI/3FS on luciferase and PKD2 expression by Western blotting. Using biotin-RNA pull down in combination with mass spectrometry, we isolated interacting partners of 3FI or 3FS. We are confirming the interaction between some identified potential partners and 3FI/3FS.

**Conclusions:** Two fragments (3FI and 3FS) in PKD2 mRNA 3UTR respectively inhibit and stimulate PKD2 translation and these regulations are presumably through RNA-binding proteins that interact with 3FI or 3FS. These translational regulations may provide novel insights into the mechanism of ADPKD pathogenesis. Supported by CIHR and AIHS (to XZC) and AITF (to WZ).

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## FR-PO114

### Regulation of Endoplasmic Reticulum Polycystin-2-Dependent Ca Signaling

Qian Wang,[1] Jungwoo Yang,[1] Wang Zheng,[1] Richard Zimmermann,[2] Xing-Zhen Chen.[1] *[1]Dept of Physiology, Univ of Alberta, Edmonton, Canada; [2]Dept of Medical Biochemistry and Molecular Biology, Saarland Univ, Homburg, Saarland, Germany.*

**Background:** Polycystin-2 (PC2), is a Ca permeable cation channel mutated in ~15% of the autosomal dominant polycystic kidney disease (ADPKD). PC2 mainly localizes in the endoplasmic reticulum (ER) membrane, but also presents in the plasma membrane and the primary cilium. The ER-resident PC2 was suggested to be an intracellular Ca release channel, but it is still controversial as to how PC2 regulates intracellular Ca concentration. In this study, we report the function of PC2 in the ER membrane and the regulation by its binding partners including filamin-A and PERK.

**Methods:** Ca imaging was used to measure the cytoplasmic Ca concentration. siRNA and shRNA were used for gene knockdown (KD).

**Results:** Here, we used cultured HEK, HeLa and mouse collecting duct (MCD) cell lines. We first showed that over-expression of PC2 in HeLa cells reduces Ca release from the ER in response to thapsigargin (Tg) treatment. Conversely, KD of PC2 by siRNA increased ER Ca release and the increased Ca response correlated with PC2 KD efficiency, indicating the specificity of PC2 siRNA. Our previous study showed that filamin-A, a large cytosolic actin-binding protein, physically interacts with PC2 and reduces its channel activity in a lipid bilayer system. Interestingly, HeLa cells with stable filamin-A KD showed a decreased response to Tg treatment, which can be rescued by KD of PC2. This is probably due to the fact that loss of inhibition on PC2 channel function in filamin-A KD cells increases the ER Ca permeability and leads to a lower ER Ca concentration, which results in reduced Ca release from the ER in response to Tg treatment. We are also obtaining data on the effect of PERK (pancreatic ER eIF2α kinase) on PC2-dependent Ca signaling.

**Conclusions:** ER-resident PC2 functions as a Ca channel, which reduces luminal Ca content thereby decreasing Ca release from ER lumen to cytoplasm upon Tg treatment. Filamin-A reverses these processes through inhibiting PC2 channel function. Our finding provides in vivo evidence on the regulation of ER PC2 channel function.

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## FR-PO115

### Polycystins Regulate Collagen Secretion in Polycystic Kidney Disease

Stephanie Le Corre, Iain A. Drummond. *Nephrology Div, Massachusetts General Hospital.*

**Background:** Mutations in Polycystins account for Autosomal Dominant Polycystic Kidney Disease (ADPKD), the most frequent heritable human genetic disease. ADPKD is characterized by the presence of multiple cysts in both kidneys but is also associated with vascular aneurysm, cardiac valve defects and abdominal wall hernia; these pathologies are linked to extracellular matrix defects.

**Results:** We have modeled ADPKD in zebrafish by polycystins mutation or knockdown and shown that the strong dorsal axis curvature mutant/morphant phenotype was caused by overproduction collagen II alpha1 protein in the notochord sheath. Indeed, *col2a1* knockdown rescued dorsal axis curvature in polycystin-deficient embryos. Surprisingly, overproduction of notochord collagens was not due to over-expression of *col2a1*, *col9a2*, or *col27a1* mRNA (qRTPCR). Instead, over-accumulation of collagen protein is more likely linked to enhanced collagen secretion or reduced degradation. One of the major post-translational modification of collagen is the cross-linking mediated by lysyl oxydase enzymes. We found that collagen type II cross-linking and expression of lysyl oxidase enzymes were not affected in polycystin2-deficient embryos. *MMP1*, *MMP9* and *MMP14* expression (qRTPCR) is increased in polycystin 2-deficient embryos. Therefore, collagen type II accumulation is not due to a defective cross-linking or a lack of degradation. Collagen secretion from the endoplasmic reticulum to the Golgi apparatus is mediated by the COPII complex (Coat Protein complex) that is made of Sec proteins. We found that *Sec23* and *Sec24* are upregulated in polycystin1 and polycystin2-deficient embryos (qRTPCR). Interestingly, when we knockdown *Sec24d* we are able to partially rescue the dorsal axis curvature observed in polycystin2-deficient embryos.

**Conclusions:** Our results support a model where Polycystins 1 and 2 are involved in the regulation of extracellular matrix secretion. A similar mechanism may contribute to disease pathogenesis in human ADPKD patients. Our project represents a re-examination of poorly understood ECM defects in polycystic kidney disease and may lead to the discovery of new therapeutic targets.

*Funding:* NIDDK Support

**FR-PO116**

**Ciliary Defects in Induced Pluripotent Stem Cells from Patients with Polycystic Kidney Disease and Ciliopathies** Benjamin S. Freedman,[1] Albert Q. Lam,[1,2] Jamie L. Sundsbak,[3] Rossella Iatrino,[1,4] Xuefeng Su,[1] Sarah J. Koon,[3] Maoqing Wu,[1] Peter C. Harris,[3] Jing Zhou,[1,2] Joseph V. Bonventre.[1,2] *[1]Harvard Center for PKD Research, Renal Div, Dept of Medicine, Brigham and Women's Hospital, Boston, MA; [2]Harvard Stem Cell Institute, Cambridge, MA; [3]Mayo Translational Polycystic Kidney Disease (PKD) Center, Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [4]Nephrology Div, Policlinico Universitario di Modena, Italy.*

**Background:** Induced pluripotent stem (iPSCs) are a powerful new technology for investigating human disease in diverse cell types. We tested the potential of iPSCs to reveal ciliary defects specific to polycystic kidney disease (PKD) or related ciliopathies.

**Methods:** Fibroblasts from patients with autosomal dominant (ADPKD) and autosomal recessive PKD (ARPKD), or the ciliopathies Bardet-Biedl Syndrome (BBS) and Meckel Syndrome (MKS), were retrovirally reprogrammed into iPSCs, sequenced for mutations, and compared to cells from healthy patients for PKD-related defects.

**Results:** PKD iPSCs elaborated primary cilia and expressed endogenous polycystin-1 (PC1), polycystin-2 (PC2), and fibrocystin/polyductin at levels similar to healthy controls. However, in ADPKD iPSCs or derived somatic epithelial cells, PC2 was detectable in cilia at only 25-50% of the frequency seen in ARPKD and healthy control lines. Unique *PKD1* heterozygous mutations were identified in fibroblasts from all three ADPKD patients, and iPSCs from two of these. A possible "second hit" was found at the mutation site in iPSC lines from one patient. Overexpression of wild-type PC1, but not a carboxy-terminal truncation mutant, increased endogenous ciliary PC2 ~3-fold in ADPKD iPSC-derived hepatoblasts or cultured mouse kidney cells. Neither BBS nor MKS iPSCs exhibited reduced ciliary PC2. However, MKS iPSC cilia were elongated two-fold and displayed curled ends.

**Conclusions:** PC1 enhances PC2 trafficking to cilia. Reduced ciliary PC2 may represent an early and highly penetrant metric of ADPKD, while other ciliopathies may have distinct ciliary phenotypes. iPSCs may provide novel mechanistic insights into the cellular pathophysiology of kidney disease.

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**FR-PO117**

**Fluid Shear Stress (FSS)-Mediated mTORC1 Regulation Is Aberrant in Cystic Epithelial Cells** Robin L. Maser,[1] Darren P. Wallace,[1] Xiangyi Lu.[2] *[1]Kidney Inst, Univ of Kansas Med Ctr, Kansas City, KS; [2]Inst of Environmental Health Sciences, Wayne State Univ, Detroit, MI.*

**Background:** Mammalian target of rapamycin complex 1 (mTORC1) is a major regulator of cell growth, division and metabolism. In autosomal dominant polycystic kidney disease (ADPKD), mTORC1 activity is increased and is thought to play a role in disease pathogenesis, but the underlying mechanism(s) is incompletely understood. For example, FSS has been shown to modulate mTORC1 by a primary cilium-dependent, but polycystin-2/calcium-independent mechanism, while polycystin-1 can inhibit mTORC1 via pathways independent of FSS. Since amino acid (AA) sufficiency is required for mTORC1 activation by all stimuli, we examined the relationship between FSS and AA-stimulated mTORC1 in both normal and cystic renal epithelial cells to uncover additional mechanisms of mTORC1 regulation.

**Methods:** Mouse inner medullary (IMCD3) and cortical (M1) collecting duct cell lines, and primary epithelial cells from a normal human (NHK) and an ADPKD kidney were grown under primary cilium-generating conditions. Cultures were subjected to either static or FSS (0.75 dyn/cm$^2$) conditions under a treatment regime that involved preconditioning in medium containing 1% serum, followed by a period of glucose- and AA-starvation. Starved cells were either lysed (-AA), or stimulated by addition of AA (+AA), and then lysed. Levels of phospho (p)- and total (t) p70 S6 kinase (S6K), an mTORC1 kinase substrate, were quantified by Western blot to determine relative mTORC1 activity.

**Results:** Under static conditions, +AA increased pS6K/tS6K levels in both mouse and human renal epithelial cells as compared to -AA. With FSS, +AA increases in pS6K/tS6K levels were significantly suppressed in M1 (61%; n=7; p<0.001), IMCD (77%; n=5; p<0.005), and NHK (54%; n=4; p<0.005) cells relative to static+AA levels. In contrast, FSS was unable to effectively suppress AA-activated mTORC1 in the human ADPKD cells (102%; n=3; p=0.470).

**Conclusions:** These studies reveal a FSS-dependent pathway of mTORC1 regulation that is aberrant in cyst-lining epithelial cells from an ADPKD kidney, and suggest that multiple mechanisms exist to modulate mTOR activity in renal epithelial cells.

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**FR-PO118**

**mTORC1 and 2 Signaling in Polycystic Kidney Disease** Kameswaran Ravichandran,[1] Iram Zafar,[1] Zhibin He,[1] Adam E. Mullick,[2] Charles L. Edelstein.[1] *[1]UC Denver; [2]ISIS Pharmaceuticals.*

**Background:** We have previously reported that Pkd2WS25/- mice, an orthologous model of human PKD, treated with a mTOR kinase antisense oligonucleotide (ASO) that inhibits both mTORC1 and 2 have a normalization of kidney function and a dramatic decrease in cyst volume density.The aim of the study was to determine the effect of combined mTORC1 and 2 inhibition on mTOR signaling in in vivo and in vitro models of cyst growth.

**Methods:** Pkd mice and wild types (WT) were treated with an mTOR (mTOR) or scrambled (SCR) ASO 50-100 mg/kg/wk IP from 4 to 16 weeks of age. In vitro, to inhibit both mTORC1 and 2, type 1 MDCK cells in which Rictor the functional component of mTORC2, was silenced using plasmid based shRNA vector, were treated with sirolimus a mTORC1 inhibitor.

**Results:** On immunoblot, the mTOR ASO resulted in a significant decrease in pS6, pAktser473 and pSGK1. On densitometry, the phospho/total protein ratio in PKD mice treated with SCR vs. mTOR ASO was 2.4±0.6 Vs 0.78 ± 0.3 for pS6 (p<0.05), 1.9 ± 0.3 Vs 0.9 ± 0.2 for pAktSer473 (p<0.05), 2 ± 0.6 Vs 0.5± 0.19 for pSGK1 (p<0.05). On PCNA staining the number of PCNA positive cells in normal tubules was 9 ± 0.5 in SCR and 2.7 ± 0.5 in mTOR ASO (p<0.01) and per cyst was 1.9 ± 0.2 in SCR and 0.8 ± 0.1 in mTOR ASO (p<0.01). On TUNEL staining the number of TUNEL positive cells in normal tubules was 3.6 ± 0.6 in SCR and 1.2 ± 0.1 in mTOR ASO (p<0.01) and per cyst was 4.4±1.3 in SCR and 1.4 ± 0.2in mTOR ASO (p<0.01). In MDCK cells, combined mTORC1 and 2 inhibition resulted in a complete inhibition of pAktSer473 on immunoblot. To determine the role of mTORC2-mediated production on pAkt Ser473, Akt1 was overexpressed in mTORC2 inhibited cells. Over-expression of Akt1 reversed the decrease in cyst size. On day 10 the cyst size (μm) was 251.4±6.5 in WT cells, 135.6 ± 7.2 in Rictor -/- cells and 216.5 ± 7.3 in Rictor -/- cells+Akt1.

**Conclusions:** Combined inhibition of mTORC1 and 2 results in a significant decrease in pAktSer473 both in in vivo and in vitro models of cyst growth. Ultimately mTOR inhibition decreases cyst growth in vivo by decreasing proliferation and apoptosis of both normal and cystic tubular cells.The role of Akt1 in cyst growth merits further study.

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**FR-PO119**

**Angiiotensinogen Inhibition Slows Cyst Growth in an Orthologous Model of Human Polycystic Kidney Disease** Kameswaran Ravichandran,[1] Iram Zafar,[1] Adam E. Mullick,[2] Zhibin He,[1] Charles L. Edelstein.[1] *[1]UC Denver; [2]ISIS Pharmaceuticals.*

**Background:** Renal cyst enlargement is associated with the activation of both circulating and intra-renal renin-angiotensin-aldosterone (RAAS) system. Angiotensin converting enzyme (ACE) inhibitors together with Angiotensin receptor blockers (ARB) are being tested in humans with PKD in HALT-PKD studies. Angiotensin is derived from its precursor molecule Angiotensinogen (AGT) which is primarily expressed in the liver, however renal AGT expression may also contribute to renal cystic disease. AGT is the only precursor for all angiotensin metabolites, which are the key effectors of RAAS. AGT inhibition is regarded as a novel therapy for hypertension in kidney disease. The aim of the study was to determine the effect of angiotensinogen inhibition in polycystic kidney disease.

**Methods:** An AGTantisense oligonucleotide (ASO) that inhibits both liver and renal AGT mRNA expression was injected at 50mg/kg/wk dose i.p. once weekly in PKD2WS25mice (an orthologous model of human ADPKD involving mutation of the Pkd2 gene) from 4 to 16 weeks of age. A scrambled (SCR) ASO that was similar to the AGT ASO but lacked antisense activity was used as a control.

**Results:** The 2K/TBW ratio in PKD mice treated with SCR Vs. AGT was 2.45 ± 0.31 vs. 1.54 ± 0.11 (p<0.01). The BUN in PKD mice treated with SCR vs. AGT was 46.71± 4.5 vs. 33.85 ± 3.3(p<0.01).The cyst volume density in PKD mice treated with SCR vs. AGT was 37.64 ± 5.7 vs. 21.97 ± 2.9 (p<0.01). The kidney weight in PKD mice treated with SCR vs. AGT was 0.66±0.08 vs. 0.47±.06 (p<0.01). The AGT treatment did not alter the body weight in PKD mice. The body weight in PKD mice treated with SCR vs. AGT was 27.28±1.6 vs. 29.42 ± 2.2 (NS).

**Conclusions:** In Summary, 1) Angiotensinogen inhibition resulted in significant decrease in kidney weight, 2K/TBW ratio and the cyst volume density in PKD mice,2) Angiotensinogen inhibition improved the kidney function. In conclusion the role of angiotensinogen inhibition at the precursor level in cyst growth merits further study.

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**FR-PO120**

**A Novel LBK-1 Activator Inhibits mTOR Signaling and Proliferation of Human ADPKD Cells** Gail Reif, Archana Raman, Bailee Lynn Slack, Cibele S. Pinto, Bhaskar Chandra Das, Darren P. Wallace. *Kidney Institute, Kansas Univ Medical Center, Kansas City, KS.*

**Background:** In autosomal dominant polycystic kidney disease (ADPKD), elevated mTOR activity contributes to the aberrant proliferation of cyst-lining epithelial cells. Stimulation of AMP-activated protein kinase (AMPK), an energy sensor that regulates cell growth, inhibits mTOR signaling and cell proliferation. AMPK also phosphorylates CFTR $Cl^-$ channels and inhibits $Cl^-$-dependent fluid secretion. Recently, AMPK activation was shown to inhibit mTOR and cyst growth in PKD animals. Liver kinase B1 (LKB1) is a tumor suppressor that directly phosphorylates and activates AMPK; and mutations that cause a loss-of-function of LKB1 are associated with elevated mTOR activity and cancer. Our goal was to determine if AMPK stimulation using a novel LKB1 activator inhibits $Cl^-$ secretion and mTOR-mediated proliferation of human ADPKD cells.

**Methods:** We designed and synthesized a small molecule LKB1 activator BIT-11 by incorporating an oxadiazole moiety into a unique 3-substituted 2H-chromene derivative. The effects of BIT-11 on phosphorylated AMPK (P-AMPK) and S6 kinase (P-S6K), a downstream target of mTOR, were determined by western blot analysis. To test the drug's effect on $Cl^-$ transport, ADPKD monolayers were incubated in the absence and presence of BIT-11 and then treated with forskolin, a cAMP agonist. Changes in $Cl^-$ transport were measured by short circuit current. For *in vitro* cyst growth assays, ADPKD cells were seeded within a collagen matrix and treated with EGF and forskolin ± BIT-11, and total surface area of cysts per well was calculated from individual cyst diameters.

**Results:** BIT-11 caused a concentration-dependent increase in P-AMPK/AMPK levels in ADPKD cells. BIT-11 (1 μM) decreased P-S6K/S6K and the rate of ADPKD cell proliferation, consistent with inhibition of mTOR signaling. The drug also inhibited cAMP-induced anion secretion across ADPKD cell monolayers and significantly reduced *in vitro* cyst growth.

**Conclusions:** AMPK activation with small molecule LKB1 activators, such as BIT-11, may be a therapeutic approach to reduce mTOR activity, cell proliferation and $Cl^-$-dependent fluid secretion in ADPKD.

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## FR-PO121

**Evaluation of Selective Inhibitors of Nuclear Export for the Treatment of ADPKD** Robert H. Weiss,[1] Hiromi Inoue Wettersten,[1] Matthew Tan,[1] Michael Kauffman.[2] *[1]UC Davis; [2]Karyopharm.*

**Background:** Capitalizing on the recent excitement in the ADPKD field with the connection between cancer and PKD, we have extended our RCC therapeutic innovations to evaluate the role of pharmacologic inhibitors of nuclear export to cystic disease characterized by uncontrolled RTE cell proliferation. We previously showed study that blockade of Exportin 1 (XPO1), also known as chromosome region maintenance protein 1 (CRM1), by a Selective Inhibitor of Nuclear Export (SINE) attenuates renal cell carcinoma (RCC) growth by confining p53 and p21 proteins to the nucleus and thus decreasing their degradation (J Urol. 2013, 189:2317). Given that low p21 levels are characteristic, and perhaps pathogenic, for PKD and may lead to increased proliferation and apoptosis of RTE cells (BMC Nephrol. 2007, 8:12), we asked whether SINE XPO1 antagonists attenuate key cell cycle regulatory proteins and exert salutary effects in ADPKD.

**Methods:** Immortalized human ADPKD cells (WT9-12 and WT9-7), human primary ADPKD cells, primary normal human kidney cells (NHK), mouse ADPKD cells ($pkd1^{v/v}$), and control mouse kidney cells were incubated with the SINE XPO1 inhibitors, KPT-251 and -330, and cell viability assay, cell cycle analysis, apoptosis assay, immunoblotting, immunofluorescence, and PCR were performed.

**Results:** Both KPT-251 and KPT-330 caused cell cycle arrest at G1/S and led to apoptosis in all ADPKD cell lines tested. While XPO1 inhibitors attenuated XPO1/CRM1 protein levels in ADPKD cells as was seen in RCC cells, the inhibitors did not affect p53 and p21 protein levels in ADPKD cells in vitro but decreased levels of the cell cycle-relevant proteins CDK4 and 6.

**Conclusions:** We introduce a novel therapeutic approach to the treatment of ADPKD, based on our earlier findings in RCC, using SINEs. These compounds attenuate key cell cycle regulatory proteins, and decrease proliferation, in human ADPKD cells. In vivo studies examining the efficacy of these XPO1 inhibitors in cyst growth and renal function preservation are currently underway; these studies have the potential to revolutionize ADPKD treatment.

*Funding:* NIDDK Support, Other NIH Support - NCI, Veterans Affairs Support

## FR-PO122

**Downregulating Hedgehog Signaling Attenuates Renal Cystogenesis in Mouse Models of Cystic Kidney Disease** Pamela Vivian Tran,[1] George Talbott,[2] Annick Doan,[2] Damon T. Jacobs,[1] Luciane M. Silva,[1] Michael P. Schonfeld,[1] Anindita Chatterjee,[1] Mary Prysak-gehrke,[2] David Beier.[2,3] *[1]Anatomy and Cell Biology and the Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS; [2]Genetics Div, Brigham and Women's Hospital/Harvard Medical School, Boston, MA; [3]Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA.*

**Background:** Mutations associated with renal cystic diseases reside in genes encoding proteins that localize to primary cilia. These cystoproteins can disrupt ciliary structure or cilia-mediated signaling, although molecular mechanisms connecting cilia function to renal cystogenesis remain unclear. The ciliary gene, *Thm1*, negatively regulates Hh signaling and is most commonly mutated in ciliopathies.

**Methods:** We examined renal cystogenesis in a *Thm1* conditional knock-out (cko) mouse, generated using a ubiquitous, tamoxifen-inducible Cre recombinase.

**Results:** *Thm1* deletion at E17.5 caused cystic kidney disease in adult mice, with elevated cAMP and increased proliferation. To determine if proliferation initiates *Thm1* cko renal cystogenesis, we examined kidneys at earlier time points. At P15, *Thm1* cko renal tubular dilations were evident and proliferation levels were high but similar to levels in wild-type kidneys. At P20, proliferation was markedly reduced in wild-type medullas, but not in *Thm1* cko kidneys. These data suggest that proliferation alone is insufficient to initiate renal cystogenesis and that the ciliary defect may keep the kidney in a developmental state. In *Thm1* cko cystic kidneys, expression of Hh signaling genes was upregulated. Importantly, transcripts of Hh target genes were also increased in *jck* and *Pkd1* cko cystic kidneys. To determine a functional role for enhanced Hh activity in renal cystogenesis, we are genetically downregulating Hh signaling in these mouse mutants. Preliminary data suggest that ablation of *Gli2*, the main transcriptional activator of the Hh pathway, attenuates *Thm1*, *jck* and *Pkd1* cystic kidney disease.

**Conclusions:** Thus enhanced Hh activity may play a general role in renal cystogenesis and may present a novel potential therapeutic target.

*Funding:* NIDDK Support, Other NIH Support - NIGMS

## FR-PO123

**Inhibition of Macrophage Migration Inhibitory Factor Delays Renal Cyst Growth in ADPKD** Li Chen,[1,2] Xia Zhou,[1,2] Katherine Swenson-Fields,[2,3] Xiaogang Li.[1,2] *[1]Dept of Internal Medicine; [2]Kidney Institute; [3]Dept of Anatomy and Cell Biology, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Macrophage migration inhibitory factor (MIF) can stimulate tumor cell proliferation, suppress apoptosis, facilitate invasion of the extracellular matrix and promote angiogenesis. Elevated levels of MIF have been found in the urine of ADPKD patients. However, the cellular origin of MIF in ADPKD and the potential contribution of this factor to disease progression have not been determined.

**Methods:** MIF expression was assessed in the kidneys and cells from Pkd1-deficient mice by RT-PCR and immunoblot analysis. The contribution of MIF to proliferation and proliferative signaling pathways was determined following treatment of cystic renal epithelial cells with either MIF siRNA or its inhibitor, ISO-1, in vitro. Then the functional effect of MIF on renal cyst growth was tested by treating Pkd1 conditional knockout mice with ISO-1.

**Results:** MIF was upregulated in Pkd1 mutant renal epithelial cells and tissues of Pkd1 conditional knockout mice vs Pkd1wild type mice. Furthermore, MIF was secreted and could be detected in the cyst fluid of $kd1^{flox/flox}$:Ksp-Cre mice and $Pkd1^{flox/flox}$:Pkhd1-Cre mice. Knockdown of MIF with siRNA or inhibition of MIF with ISO-1 in Pkd1 mutant renal epithelial cells: 1) decreased cystic renal epithelial cell proliferation as analyzed by the MTT assay; and 2) decreased the expression of Cyclin D1, phospho-Stat3, and phospho-S6, which are aberrantly upregulated and are known to contribute to cystic epithelial cell proliferation in ADPKD. These results suggest that MIF promotes cystic renal epithelial cell proliferation potentially through activation of these pathways. We also found that treatment with ISO-1 decreased monocyte chemoattractant activity in ADPKD cyst cell conditioned medium (CM). Most importantly, the administration of ISO-1 to $Pkd1^{flox/flox}$:Ksp-Cre mice delayed cyst growth and improved renal function.

**Conclusions:** Inhibition of MIF produces a potent anti-proliferative and anti-monocyte chemotactic effect in cystic renal epithelia and CM respectively, and delays cyst formation. These results support MIF as a potential therapeutic target in ADPKD.

*Funding:* NIDDK Support

## FR-PO124

**Effects of 2-OH Estradiol and Bicarbonate Loading on the Development of Polycystic Kidney Disease (PKD) in the PCK Rat** Xiaofang Wang, Hong Ye, Christopher James Ward, Vicente E. Torres. *Mayo Clinic.*

**Background:** 2-OH estradiol (2-OHE) alleviates PKD in Han:SPRD cy/+ rats by an undefined mechanism associated with up-regulation of P21 and mTOR inhibition (AJP Renal Physiol 302:F636, 2012). Since 2-OHE inhibits soluble adenylyl cyclase (SAC) *in vitro* and bicarbonate loading markedly aggravates PKD in PCK rats, likely by activating SAC (unpublished observation), we sought to determine whether 2-OHE administration would be protective in this *Pkhd1* model and prevent the detrimental effect of bicarbonate.

**Methods:** Four groups of PCK rats (10M and 10F per group) were treated with vehicle or 2-OHE (10 μg/kg/hr) via osmotic pumps and given water or 200mM sodium bicarbonate to drink between 3 weeks and sacrifice at 10 weeks of age. Linear regression analysis was used to assess the effect of treatment comparisons adjusted for gender.

**Results:** In PCK rats 1) 2-OHE modestly inhibited SAC and PKD development without affecting renal cAMP levels; 2) Bicarbonate loading markedly increased renal SAC activity, cAMP levels and PKD severity; 3) 2-OHE did not reduce cAMP levels or prevent the bicarbonate induced aggravation of PKD, possibly because of insuficient SAC inhibition; 4) 2-OHE stunted growth and had no effect on liver cystic disease.

| | Body Wt (g) | Kidney Wt (% BWt) | Cyst Volume (mls) | Fibrosis Volume (mls) | Renal SAC (pmol/min/ mg protein) | cAMP, (pmol/mg protein) | Pl Creat. (mg/dl) |
|---|---|---|---|---|---|---|---|
| M control | 394±20 | 1.38±0.21 | 1.07±0.59 | 0.28±0.13 | 44.8±14.0 | 16.1±6.2 | 0.34±0.06 |
| M 2-OHE | 325±33 | 1.12±0.19 | 0.42±0.32 | 0.13±0.07 | 30.9±10.3 | 17.0±6.2 | 0.38±0.03 |
| M Bicarb | 320±68 | 3.86±2.13 | 4.97±3.57 | 1.35±0.83 | 118.0±25.4 | 28.2±9.3 | 0.52±0.16 |
| M Both | 257±55 | 3.78±2.65 | 3.17±2.36 | 0.87±0.63 | 99.3±21.6 | 26.4±10.4 | 0.43±0.10 |
| F control | 249±20 | 1.35±0.11 | 0.57±0.17 | 0.27±0.13 | 51.4±15.2 | 15.3±2.2 | 0.37±0.03 |
| F 2-OHE | 223±19 | 1.21±0.08 | 0.36±0.09 | 0.20±0.10 | 41.9±10.9 | 14.9±2.6 | 0.36±0.04 |
| F Bicarb | 245±14 | 2.41±0.99 | 1.90±1.41 | 0.54±0.42 | 111.8±61.4 | 20.0±5.8 | 0.43±0.05 |
| F Both | 216±20 | 2.29±0.60 | 1.39±0.76 | 0.37±0.18 | 82.5±16.9 | 18.9±4.3 | 0.38±0.06 |
| P-values | | | | | | | |
| 2-OHE vs Cn | <0.001 | <0.001 | <0.001 | 0.003 | 0.006 | 0.886 | 0.264 |
| Bic vs Cn | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Both vs Bic | 0.003 | 0.860 | 0.124 | 0.083 | 0.047 | 0.571 | 0.050 |

**Conclusions:** Modulation of SAC activity by 2-OHE and bicarbonate loading may contribute to their effects on PKD development in PCK rats.

*Funding:* NIDDK Support

## FR-PO125

**COX-2 Inhibition in the PCK Rat** Xiaofang Wang, Hong Ye, Katharina Hopp, Peter C. Harris, Vicente E. Torres. *Mayo Clinic.*

**Background:** Cyclooxygenase (COX)-2 is up-regulated in the kidneys of Han:SPRD cy/+ rats and COX-2 inhibitors have a protective effect in this model (AJP Renal Physiol 293:F821, 2007; Mol Biol Rep 39:7743, 2012). Furthermore, antagonists of two PGE2 GsCPRs, EP2 and EP4, inhibit cAMP signaling in human and/or murine tubular epithelial cells and *in vitro* cystogenesis (AJP Renal Physiol 293:F1622-F1632, 2007; Prost & Lipid

Med 98:11, 2012). We sought to quantify renal mRNA expression of COX isozymes, PGE2 synthases (PGEs), and EP receptors and renal PGE2 levels in PCK compared to wild-type rats; and the effect of the COX-2 inhibitor celecoxib (CXB) on the development of PKD in this *Pkhd1* model.

**Methods:** mRNA expression was quantified using real-time PCR. Four groups of PCK rats (1 control, C, and 3 treatment groups fed 250, 500 and 1000ppm CXB in powdered chow, 10M and 10F per group) were studied between 3 weeks and sacrifice at 10 weeks of age. Linear regression analysis was used to assess the effect of treatment comparisons adjusted for gender.

**Results:** COX-1, -2, mPGEs-1, -2, cPGEs, and EP-1-4 mRNAs and PGE2 levels were significantly increased in PCK compared to wild-type kidneys. CXB administration was associated with relatively higher kidney volumes in both genders and blood pressures in male rats only (Table). Despite significant reductions in tissue PGE2, cAMP levels remained unchanged. CXB had no detectable effect on the liver cystic disease.

| | Body Wt (g) | Kidney Wt (% BWt) | Cyst Vol (ml) | Fibrosis Vol (ml) | Renal PGE2 (pg/mg prot) | Syst BP (mmHg) | Pl Creat (mg/dl) |
|---|---|---|---|---|---|---|---|
| M control | 390±11 | 1.39±0.28 | 1.02±0.48 | 0.14±0.05 | 1967±1274 | 123±5 | 0.49±0.05 |
| M 250 | 378±17 | 1.62±0.26 | 1.19±0.48 | 0.19±0.11 | 2100±1566 | 140±5 | 0.48±0.06 |
| M 500 | 382±18 | 1.66±0.26 | 1.11±0.32 | 0.11±0.05 | 962±328 | 140±6 | 0.47±0.05 |
| M 1000 | 381±16 | 1.59±0.21 | 0.86±0.42 | 0.15±0.05 | 675±352 | 141±4 | 0.45±0.06 |
| F control | 245±9 | 1.38±0.17 | 0.59±0.15 | 0.07±0.03 | 2356±1594 | 118±5 | 0.47±0.14 |
| F 250 | 245±9 | 1.52±0.18 | 0.73±0.20 | 0.07±0.04 | 1969±1623 | 121±4 | 0.44±0.08 |
| F 500 | 240±8 | 1.56±0.32 | 0.68±0.38 | 0.11±0.10 | 1386±680 | 118±3 | 0.41±0.12 |
| F 1000 | 231±16 | 1.58± 0.24 | 0.73±0.29 | 0.12±0.07 | 783±537 | 117±3 | 0.46±0.05 |
| P-Values | | | | | | | |
| 250 | NS | 0.015 | NS | NS | NS | < 0.001 | NS |
| 500 | NS | 0.011 | NS | NS | 0.043 | < 0.001 | NS |
| 1000 | 0.011 | 0.012 | NS | NS | 0.013 | < 0.001 | NS |

**Conclusions:** COX-2 mRNA expression and renal PGE2 levels are increased in PCK rats. However, inhibition of COX-2 increased BP in male rats and had no protective effect on PKD development, possibly because PGE2 acting on different receptors exerts antagonistic effects.

*Funding:* NIDDK Support

## FR-PO126

**Multi-Target Treatment in ADPKD** Yang Liu,[1] Johannes Loffing,[2] Alexandre Arcaro,[3] Andreas L. Serra.[1,4] *[1]Institute of Physiology, Univ of Zürich, Switzerland; [2]Institute of Anatomy, Univ of Zurich, Switzerland; [3]Dept of Clinical Research, Univ of Bern, Switzerland; [4]Div of Nephrology, Univ Hospital Zürich, Switzerland.*

**Background:** Targeting the mammalian target of rapamycin (mTOR) by mTOR inhibitors showed only a 30 to 50% reduction of kidney growth in polycystic kidney disease (PKD) animal model. The mTOR inhibitor treatment was proven futile in randomized clinical trials of ADPKD patients. A dual-negative feedback loop has been recently described in human cancer: treatment with mTOR inhibitor results in a hyperactive IRS-1/PI3K pathway lead to the pro-proliferative ERK and Akt pathway. We hypothesized that blockage mTOR in PKD lead to an upregulation of PI3K-dependent pathway rendering the polycystic kidney partial resistant to the anti-proliferative action of mTOR inhibitors.

**Methods:** We assessed the effect of mTOR inhibitor and placebo treatment in human and animal PKD on the activation status of the mTOR, PI3K/Akt and PI3K/ERK pathways in vitro and in vivo. Next we compare the effect of single-target mTOR and NVP-BEZ235, a multi-target mTOR/PI3K inhibitor, on PKD progression in Han:SPRD rats low and high dosage (NVP-BEZ235 15 and 50 mg/kg/day), and early and late treatment initiation in Pkd1 conditional knock-out mice (from D12 to 35 as early treatment, from D21 to 35 as late treatment).

**Results:** Continuous mTOR inhibitor treatment triggered a dual-negative feedback loop *in vitro*, in ADPKD patients and Han:SPRD rats. Multi-target mTOR/PI3K treatment prohibited these feedback loops and blocked completely PKD progression while single-target mTOR had only modest effects on PKD progression. Biomolecular studies revealed that the multi-target mTOR approach depressed the PI3K-dependent pathway dual feedback loop causing blockage of the mTOR and PI3K/Akt pathway, and ultimately blocking the pro-proliferative PI3K/ERK pathway.

**Conclusions:** mTOR inhibitors cause an upregulation of pro-proliferative PI3K-dependent singling pathways whereas mTOR/PI3K inhibitor treatment prevented these circumventions resulting in a complete normal renal function of morphology of PKD animal models suggesting an innovative effective therapeutic strategy for ADPKD.

## FR-PO127

**Combination Therapy with Tolvaptan and Pasireotide Has Enhanced Efficacy in the *Pkd1* R3277C Model** Katharina Hopp, Xiaofang Wang, Hong Ye, Cynthia J. Hommerding, Peter C. Harris, Vicente E. Torres. *Mayo Clinic.*

**Background:** Animal and human studies have identified elevated levels of cAMP as a basic defect in Autosomal Dominant Polycystic Kidney Disease (ADPKD). Further, indirect targeting of AC6 has been shown as an effective means to lower cAMP levels and slow the rate of cyst growth by using two therapeutics, tolvaptan and pasireotide, that act through antagonizing the stimulatory V2R or stimulating the inhibitory SSTRs, respectively.

**Methods:** Here, we utilize the *Pkd1* R3277C mouse model, which genetically and phenotypically mimics human ADPKD, to test the value of a combined treatment. Both drugs (tolvaptan, 0.1% via food; pasireotide, 10μg/hr/kg via osmotic pump) were administered over a 5-month period, starting at 1 month. Statistical analyses were performed by 2-Way ANOVA and a Tukey HSD post-hoc test.

**Results:** At 6 months the %KW/BW was 1.9±0.2 in the control (C, n=22), 1.7±0.2 in the tolvaptan (T, n=21), 1.6±0.1 in the pasireotide (P, n=18), and 1.4±0.2 in the T+P groups (B, n=20), highlighting a significant reduction of %KW/BW upon treatment (p=1.4E-9). Pairwise comparisons showed the greatest significance for the combined treatment (C vs: T, p=1.0E-2; P, p=2.3E-4; B, p=5.5E-10) with a marked additive effect over single treatments (B vs: T, p=1.9E-4; P, p=1.8E-2). Both treatments reduced cyst volume (μl, C: 91.8±38.1, T: 71.9±34.2, P: 50.4±20.6, B: 32.5±16.2), with significance of C vs P (p=1.8E-4), T vs B (p=3.2E-4) and C vs B (p=5.4E-8). Further, cAMP levels (pmol/mg protein) were significantly reduced to WT levels by the combined treatment (C: 4.5±1.5, T: 4.1±1.3, P: 3.9±1.4, B: 3.3±0.6, WT: 3.3±0.9; C vs B, p=1.7E-2). No significant differences were found between treatment with tolvaptan/pasireotide or males/females.

**Conclusions:** The strengths of this study are the use of an orthologous model with slowly progressive renal cystic disease and long-term treatment. Further, this study shows for the first time an additive effect between tolvaptan and pasireotide, reinforcing the central role of AC6 and cAMP in ADPKD pathogenesis and the likely benefit of combination therapy for ADPKD patients.

*Funding:* NIDDK Support

## FR-PO128

**Aliskiren Ameliorates Cyst Progression by Suppressing the Intrarenal Renin-Angiotensin System Activity in Autosomal Dominant Polycystic Kidney** Tasuku Nakagaki,[1] Saori Nishio,[1] Yasunobu Ishikawa,[1] Sekiya Shibazaki,[1] Akira Nishiyama,[2] Stefan Somlo,[3] Hiroyuki Kobori,[2] Tatsuya Atsumi.[1] *[1]Internal Medicine II, Hokkaido Univ Graduate School of Medicine, Sapporo, Hokkaido, Japan; [2]Dept of Pharmacology, Kagawa Univ School of Medicine, Kagawa, Japan; [3]Dept of Internal Medicine, Yale Univ School of Medicine, New Haven, CT.*

**Background:** Hypertension is a well-recognized complication of autosomal dominant polycystic kidney disease (ADPKD) and is significant independent risk factor for progression to end stage of renal disease. Involvement of the renin-angiotensin system (RAS) has been postulated, but no consistent relationship has been found between blood pressure and plasma renin activity.

The purpose of this study is to examine the effects of antihypertensive drugs for *Pkd1* conditional knockout mice and to evaluate the participation of intrarenal RAS in this model.

**Methods:** We generated *Pkd1* conditional knockout mice carrying the Cre transgene under the control of the *Mx1* promoter (*Pkd1* $^{flox/flox}$*;Mx1-Cre* mice). All mice were injected with polyinosinic-polycytidylic acid (pI:pC) to induce the expression of Cre recombinase and to inactivate *Pkd1* at 2 weeks of age. To evaluate the effects of antihypertensive drugs, *Pkd1* $^{flox/flox}$*;Mx1-Cre* mice were treated by osmotic mini-pumps with amlodipine or olmesartan or aliskiren, or vehicle for 12 weeks. Blood pressure was measured by the tail-cuff method. Kidney weight to body weight ratio (KW/BW),and renal cystic index were used to assess efficacy of treatment. Intrarenal RAS activity was examined by measurement of urinary angiotensinogen (AGT) excretion and immunohistochemistry for renin, angiotensin II, and AGT in the kidneys.

**Results:** Blood pressure was almost similar among the treatment groups. There was no difference in KW/BW and renal cystic index, between amlodipine, olmesartan and vehicle. Aliskiren treatment significantly reduced the KW/BW, renal cystic index, BUN, urinary albumin excretion, and intrarenal RAS activity.

**Conclusions:** These studies suggest RAS inhibition with aliskiren may be effective in slowing cyst growth by suppressing the intrarenal RAS activity in ADPKD.

## FR-PO129

**H2-Relaxin Modulates Expression of Fibrosis-Related Genes in Normal and Cystic Human Renal Epithelial Cells** Heather Hilary Ward,[1] Paul C. Grimm,[2] Angela Wandinger-ness.[3] *[1]Internal Medicine, Nephrology Div, Univ of New Mexico, Albuquerque, NM; [2]Pediatric Nephrology, Stanford Univ, Stanford, CA; [3]Pathology, Univ of New Mexico, Albuquerque, NM.*

**Background:** In autosomal dominant polycystic kidney disease (ADPKD), renal inflammation, abnormal extracellular matrix (ECM) deposition, and fibrotic progression are concurrent with cyst growth and collectively destroy renal architecture, resulting in irreversible loss of kidney function. To date, the opportunity to circumvent these additional factors that promote the loss of renal function has been insufficiently studied.

**Methods:** We used computer-assisted quantification of picrosirus red staining and real-time PCR to evaluate the effect of H2-relaxin on matrix metabolism in cystic kidneys and specific renal cell types.

**Results:** We demonstrate that administration of H2-relaxin in the Cy/+ rat model of ADPKD decreased expression of genes that regulate ECM synthesis and degradation, and these data correlated with trends of decreased renal interstitial matrix content. Relaxin acts directly on fibroblasts and endothelial cells, but little is known about relaxin-mediated signaling in epithelia. In vitro, real-time PCR data showed that human ADPKD renal epithelia expressed abnormal levels of ECM components in the absence of other fibrotic drivers. When normal and ADPKD renal epithelia were incubated with H2-relaxin in the presence of a fibrotic stimulus (TGFβ1), H2-relaxin differentially inhibited up regulation of matrix-synthesis genes, tissue inhibitors and mesenchymal markers in normal and ADPKD epithelia. In contrast, expression levels of genes related to ECM synthesis and degradation were strongly up regulated upon TGFβ1 stimulation in normal and ADPKD renal epithelia, when compared to respective vehicle-treated control cell types.

**Conclusions:** These studies provide the first detailed mechanistic analyses of the utility of relaxin for dissecting fibrotic mechanisms in renal epithelia. Pinpointing the mechanisms underlying fibrosis in ADPKD has important implications for identifying effective therapies, such as H2-relaxin, to prevent or reverse the process of fibrosis in ADPKD patients.

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## FR-PO130

**Pyrrolidine Dithiocarbamate Reduces Kidney Enlargement and Proteinuria in Experimental Polycystic Kidney Disease** Michelle Ta,[1] Padmashree Rao,[1] Mayuresh Korgaonkar,[2] Sheryl L. Foster,[2] Anthony Peduto,[2] David C. Harris,[1] Gopala K. Rangan.[1] *[1]Centre for Transplant & Renal Research, Westmead Millennium Institute, Sydney, Australia; [2]Brain Dynamics Centre, Westmead Millennium Institute, Australia.*

**Background:** The proinflammatory transcription factor family, nuclear factor (NF)-kB, is upregulated in experimental PKD, and NF-kB inhibition reduces cyst growth in *Pkd1*$^{-/-}$ murine kidney explants. To determine the *in vivo* effects of NF-kB, we hypothesised that pyrrolidine dithiocarbamate (PDTC, a NF-kB inhibitor) reduces disease progression in rodent PKD.

**Methods:** Male LPK rats (*Nek8/NPHP9* ortholog with diffuse collecting duct dilatation) received i.p.i of either vehicle (V) or PDTC [40mg/kg once (x1), or twice daily (x2)] from postnatal wks 4 until 11. Total kidney volume (TKV) was assessed by 3D MRI at wk 5 and 10.

**Results:** PDTC reduced proteinuria by 66%, but did not alter renal dysfunction (Table). By serial MRI, the relative within-rat increase in TKV was 1.3-fold greater in V vs PDTC (p=0.010). PDTC also attenuated 2KW:BW ratio by 25% and interstitial CD68+ cells (x1 only), but not %cyst area, Ki67+ cells or interstitial fibrosis.

| | Lewis+V N=3 | Lewis+PDTC(x2) N=4 | LPK+V N=9 | LPK+PDTC(x1) N=8 | LPK+PDTC(x2) N=9 |
|---|---|---|---|---|---|
| %2KW:BW | 0.7±0.1 | 0.8±0 | 6.4±0.7* | 5±0.9# | 4.8±1.1# |
| CrCl/BW (fold-Δ vs Lewis+V) | 1±0.1 | 0.8±0.2 | 0.5±0.1* | 0.5±0.1 | 0.5±0.2 |
| Urine Pr:Cr wk10 (g/mol) | 2±4 | 20±24 | 254±166* | 108±52 | 87±52# |
| %Cyst area | - | - | 52.2±7.3 | 51.1±8.2 | 56.0±5.9 |
| %CD68 | 5.3±5.3 | 16.3±15.9 | 39.8±33.2 | 11.7±3.8# | 40.1±34.8 |
| %Interstitial fibrosis (Sirius Red) | 0.6±0 | 0.8±0.4 | 4.8±3.3* | 3.4±1.4 | 2.7±1.5 |

Data as Mean±SD *p<0.05 vs Lewis+V; #p<0.05 vs LPK+V.

**Conclusions:** Chronic PDTC administration has selected renoprotective effects in LPK rats, suggesting that NF-kB may be a potential therapeutic target in PKD.

*Funding:* Government Support - Non-U.S.

## FR-PO131

**Phosphodiesterase 1A Rescue of Renal Cystogenesis and Examination of Additional PDEs in Zebrafish** Caroline R. Sussman, Christopher James Ward, Amanda Christine Leightner, Peter C. Harris, Vicente E. Torres. *Mayo Clinic.*

**Background:** Substantial evidence indicates the importance of elevated cAMP in polycystic kidney disease (PKD). Preclinical and clinical trials have shown the feasibility of therapies (vasopressin V2 receptor antagonists and somatostatin analogs) acting on G protein coupled receptors to inhibit Adenylyl Cyclase activity and production of cAMP.

**Methods:** To test whether modulating cAMP hydrolysis also affects renal cystogenesis, we have altered activity of the Phosphodiesterase, PDE1A, in zebrafish embryos by injecting morpholinos and RNA. We have additionally examined the involvement of cAMP/PKA signaling in zebrafish cystogenesis using the PKA inhibitor, H89. Finally, we have analyzed the expression of additional PDEs in zebrafish by RT-PCR.

**Results:** We have previously shown that *pde1a* morpholinos induce renal cysts, hydrocephalus, and body curvature in zebrafish embryos, and that PDE1A depletion aggravates body curvature phenotypes from morpholino-induced Polycystin-2 (PC2) depletion. We show here that human *PDE1A* RNA partially rescued PDE1A and PC2-depletion phenotypes (renal cysts, hydrocephalus, and body curvature). In addition, the PKA inhibitor, H89, partially rescued the formation of renal cysts, hydrocephalus, and body curvature in *pde1a* morphants. Besides *pde1a*, 2 day post-fertilization zebrafish embryos also expressed clear orthologs to human *pde1c, pde3a, pde3b,* and *pde4c*.

**Conclusions:** These data are consistent with previous data from PDE1A loss of function studies, providing further evidence of an integral role of PDE1A in renal cystogenesis in zebrafish. Data are consistent with PDE1A effects downstream of PC2 and upstream of PKA, and indicate the utility of zebrafish for analysis of additional PDEs. In addition, these data suggest a role for zebrafish in evaluating PDE-targeted therapy for PKD.

*Funding:* NIDDK Support

## FR-PO132

**Intravenous Renal Cell Therapy for Autosomal Recessive Polycystic Kidney Disease in Rats** Katherine J. Kelly,[1] Jesus H. Dominguez.[1,2] *[1]Medicine, IUMS, Indianapolis, IN; [2]Medicine, VAMC, Indianapolis, IN.*

**Background:** Polycystic kidney disease (PKD, including ADPKD1, ADPKD2, ARPKD) is a group of diseases with overlapping phenotypes and are not curable. They are enhanced by renal injury which accelerates progression to ESRD, and then the only hope to get off of dialysis is kidney transplantation, but the need for organs to transplant exceeds supply.

**Methods:** We have introduced intravenous renal cell transplantation (IRCT) in rats as a future alternative to kidney transplantation, and tested the hypothesis that IRCT with Serum Amyloid A protein (SAA) expressing normal adult renal cells improves structure and function in PKD by coordinating the re-orientation of adjacent host PKD cells.

**Results:** We tested this idea in the PCK rat, an orthologus model of ARPKD. We included four rat groups starting with surgery at 6 weeks of age and IRCT at 6, 8, and 10 weeks of age, termination was at 26 weeks of age: AS, were sham operated and given SAA negative cells, n=7. BS, were sham operated and given SAA positive cells, n=7. AI, had unilateral renal ischemia for 50 min and given SAA negative cells, n=8. BI, had unilateral renal ischemia for 50 min and given SAA positive cells, n=8. We followed renal function and structure with Dynamic Contrast Enhanced CT. Donor cells were found in abundant numbers on the cyst walls and damaged tubules, but not outside the kidneys. SAA positive "B" cells improved function and structure.

| | AS | BS | AI | BI |
|---|---|---|---|---|
| Kidney Weight (g) | 2.60±0.07 | 2.18±0.14* | 3.11±0.05 | 2.8±0.10§ |
| Albuminuria (g/g creat) | 1.93±0.14 | 1.26±0.11* | 2.40±0.16 | 1.42±0.13§ |
| BUN (mg/dl) | 24±0.6 | 19±0.6* | 29±1.5 | 23±0.3§ |
| Cyst Volume (ml) | 0.43±0.02 | 0.26±0.07* | 0.57±0.11 | 0.36±0.02§ |
| GFR (ml/min) | 1.08±0.05 | 1.28±0.08* | 0.97±0.09 | 1.32±0.09§ |
| *p<0.05 vs AS, §p<0.05 vs AI | | | | |

**Conclusions:** IRCT is an effective means to retard cyst development and progression (all "B" groups significantly different than corresponding "A" groups) in the PCK and has a great potential for PKD therapy.

*Funding:* NIDDK Support, Other U.S. Government Support, Veterans Affairs Support, Private Foundation Support

## FR-PO133

**Abnormal Upregulation of Notch3 Pathway in Polycystic Kidney Disease** Madhulika Sharma,[1] Trisha Home,[1] Lynn Magenheimer,[1] Gail Reif,[1] Brenda S. Magenheimer,[2] Robin L. Maser,[2] Darren P. Wallace,[1] James P. Calvet.[2] *[1]Internal Medicine (Nephrology and Hypertension), Univ of Kansas Medical Center, Kansas City, KS; [2]Biochemistry and Molecular Biology, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Aberrant proliferation of tubular epithelial cells is a hallmark of polycystic kidney diseases (PKD). Despite recent advances, a better understanding of the molecular mechanisms involved in cyst development is needed. Notch signaling plays an important role in cell proliferation and differentiation and has a key role in kidney development and in kidney diseases. However its role in PKD is not yet defined. We hypothesized that Notch signaling may be an important pathologic feature of autosomal dominant (ADPKD) and autosomal recessive (ARPKD) PKD.

**Methods:** Immunohistochemistry (IHC) was performed in kidney paraffin sections obtained from CPK mice (a model ARPKD) and Pkd1 null/HoxB7 conditional mice (a model of ADPKD) at one and two weeks of age. These mice develop rapidly progressive cystic disease and by 20 days of age the mice die due to end stage renal disease. We also obtained kidney sections from ADPKD patients undergoing a nephrectomy. We used commercially available antibodies for Notch1, Notch2, Notch3 and Notch4 (Notch receptors); Jagged1, Jagged2, Delta like1, Delta like3 and Delta like4 (Dll4) (Notch ligands); and Hes1 and HeyL (Notch targets). Signals from IHC were quantified using Image J Analysis. Comparisons were made with littermate controls or with normal human kidney samples.

**Results:** Notch3 was found to be distinctly upregulated in the cystic epithelia of all the models of PKD tested. Upregulation of Notch3 expression correlated with disease progression in CPK mice. Among the ligands, Dll4 expression was most significantly upregulated in the cystic epithelium. In addition both Hes1 and HeyL targets of Notch pathway were found to be significantly upregulated in the cystic epithelium of CPK, Pkd1null/HoxB7 mice and ADPKD patients.

**Conclusions:** Upregulation of the Notch signaling pathway in ADPKD and ARPKD suggests that small molecule inhibitors of this pathway may have therapeutic potential in the PKD setting.

*Funding:* Private Foundation Support

## FR-PO134

**Paricalcitol Attenuated In-Vitro Cyst Formation, Phenotype Transition and Apoptosis of ADPKD Cyst-Lining Epithelial Cells** Hyun-soo Shin, Yea-Jin Choi, Dong-Ryeol Ryu, Seung-Jung Kim, Kyu Bok Choi, Duk-Hee Kang. *Div of Nephrology, Ewha Womans Univ School of Medicine, Seoul, Republic of Korea.*

**Background:** Recent data demonstrated the reno-protective effect of vitamin D analogs via anti-inflammatory, immunomodulatory and anti-fibrotic effects. Polycystic kidney disease (PCK) is the most common inherited disease characterized by multiple cysts formation accompanied by renal fibrosis. However, there have been no studies investigating whether vitamin D imposes any effect on cyst formation, growth & renal fibrosis in PCK.

We aimed to examine the effect of active vitamin D analog, paricalcitol on in-vitro cyst formation as well as phenotype transition and apoptosis of cyst-lining epithelial cells.

**Methods:** 3D culture system of forskolin-treated Madin-Darby canine kidney (MDCK) cells was used to examine the effect of paricalcitol (20 nM) on cyst development. Effect of paricalcitol on TGF-β (10 ng/ml)-induced phenotype transition and apoptosis of ADPKD cyst-lining epithelial cells (WT9-12 cell) was also evaluated with an assessment of phosphorylation of p38 and ERK1/2 MAPK, GSK-3β and nuclear translocation of β-catenin.

**Results:** Paricalcitol significantly inhibited forskolin-induced cyst formation of MDCK cells with an attenuation of both ERK1/2 MAPK activation and GSK-3β phosphorylation. Paricalcitol also inhibited TGF-β-induced expression of vimentin and fibronectin in WT9-12 cells with an amelioration of p38- and ERK1/2 MAPK activation, GSK-3β phosphorylation and nuclear translocation of β-catenin. Paricalcitol protected ADPKD cyst-lining cells from TGF-β-induced apoptosis with down-regulation of Bax, Cytochrome C, cleaved caspase-3 and -9.

**Conclusions:** Paricalcitol ameliorated cyst formation, pro-fibrotic phenotype transition and apoptosis of renal tubular cells, which can be one of the therapeutic options targeting early and late mechanisms of renal disease progression in PCK.

## FR-PO135

**Macrophage-Cyst Cell Interaction in ADPKD Promotes Production of Fibrogenic Factors** Katherine Swenson-Fields, Jacqueline D. Peda, Sally M. Salah, Brad M. Davis, Darren P. Wallace, Timothy A. Fields. *The Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** In polycystic kidney disease (PKD), the destructive compression of parenchyma responsible for loss of kidney function is driven by both expanding cysts and fibrosis. We have shown that interaction between cyst epithelial cells and macrophages (MΦs) promotes cyst cell proliferation and expansion. MΦs are known to promote fibrosis in chronic kidney disease and thus are likely to do so in PKD. However, the specific contribution of MΦ/epithelial cell interactions to the creation of a pro-fibrotic environment in PKD has not been examined. In this study we co-cultured MΦs with primary human epithelial cells isolated from either PKD cysts or non-cystic kidneys to generate conditioned media (CM), which was assessed for fibrogenic effects on renal fibroblasts.

**Methods:** RAW 264.7 cells (mouse MΦ-like line) or MΦ-differentiated THP-1 cells (human monocytic line) were co-cultured with PKD cyst cells or collecting duct cells from non-cystic kidneys (NHK) either directly or in transwells (0.4 um pores). CM was collected and incubated with NRK-49F cells (rat renal fibroblast line), and the effects on cellular morphology and the production of fibrogenic markers [type-I collagen (COL1A1) and smooth muscle actin (SMA)] were assessed.

**Results:** CM from co-culture of MΦs and either PKD cells or NHK cells but not CM from any cell-type alone induced dramatic morphological transformation in fibroblasts. Fibroblasts treated with co-culture CM displayed a bipolar or stellate morphology with markedly elongated cellular extensions, which was similar to but distinct from that produced by TGFβ treatment. Also, treated cells accumulated in nodular foci. These effects were observed whether the MΦ-epithelial cell co-culture was direct or via transwell. Immunoblots showed COL1A1 was induced specifically by treatment with co-culture CM or with TGFβ. However, myofibroblast marker SMA was induced only by TGFβ.

**Conclusions:** Interaction between MΦs and renal tubule epithelial cells results in production of fibrogenic factors. This interaction may be a viable therapeutic target in PKD and other chronic kidney diseases.

*Funding:* Private Foundation Support

## FR-PO136

**Amphiregulin, an Epidermal Growth Factor Receptor Ligand, and BRP39 in the Pathogenesis in Cystic Kidney Fibrosis** Seung H. Lee,[1] Sung Hyun Son,[2] Sorin V. Fedeles,[1] Rachel Gallagher,[1] Stefan Somlo.[1] *[1]Dept of Internal Medicine, Section of Nephrology, Yale Univ, School of Medicine, New Haven, CT; [2]Dept of Internal Medicine, Section of Nephrology, BHS Han Seo Hospital, Busan, Republic of Korea.*

**Background:** Fibrosis plays an important role in the progression of polycystic kidney disease (PKD). Dysregulated EGFR signaling has also been implicated in the pathogenesis of PKD.We sought to determine whether there may be an inter-relationship between fibrosis signaling pathway, EGFR activation and mediators of the fibrotic/inflammatory response in PKD.

**Methods:** Fibroblast cells (NIH3T3) and kidneys from an ADPKD orthologous gene model ($Pkd1^{fl/fl}$;Pkhd1-Cre) were used to examine mRNA and protein levels of TGF-β1 targets (PAI-1, SMAD2), EGFR ligands amphiregulin (AR) and HB-EGF, EGFR downstream effectors Akt and ERK and the marker of tissue fibrosis/inflammation BRP39 by quantitative PCR (qPCR) and semi-quantitative Western blot.

**Results:** TGF-β1 activation of NIH3T3 cells resulted in increased AR expression and activation of EGFR targets p-Akt and p-ERk and associated fibroblast cell proliferation. The proliferative response to TGF-β1 was inhibited by EGFR inhibitor AG1478. We next examined P24 kidney tissues from cystic $Pkd1^{fl/fl}$;Pkhd1-Cre and non-cystic controls for evidence of up-regulation of these fibrosis related pathways. Cystic kidney tissue at P24 showed up-regulation of the TGF-β1 targets PAI-1 and AR and the fibrosis and inflammatory disease marker BRP39 by qPCR.

**Conclusions:** The data support further testing of the hypothesis that TGF-β1 mediated fibrosis in PKD may in part be mediated by amphiregulin and EGFR signaling and by the inflammatory mediator BRP39. These pathways may prove to be reasonable therapeutic targets for treating renal fibrosis in ADPKD.

*Funding:* Private Foundation Support

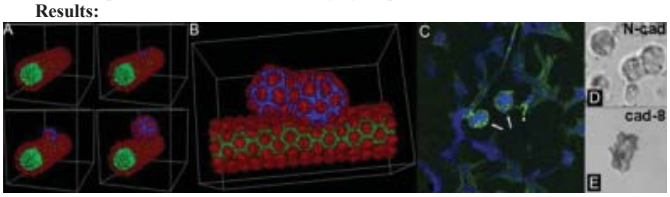
## FR-PO137

**Computer Model of Renal Cyst Formation** Julio M. Belmonte,[1] Sherry G. Clendenon,[1] James A. Glazier,[1] Robert L. Bacallao.[2] *[1]Physics/Biocomplexity Institute, Indiana Univ, Bllomington, IN; [2]Medicine, Indiana Univ, Indianapolis, IN.*

**Background:** Recently we have shown that ectopic cadherin-8 expression can initiate cyst formation. We utilized a 3D multi-cell simulation of renal tubules to investigate mechanisms behind this observation. Our model predicts that loss of cell-cell adhesion in a single cell suffices to trigger cysts and the pattern differs from contact-inhibition changes.

**Methods:** CompuCell 3D is an open source simulation environment for running multicell simulations. The program numerically solves matricies that model cell-cell, cell-ECM adhesion and cell/volume constraints. Cell adhesion was analyzed over a continuum of values and predictions confirmed by hanging drop assay.

**Results:**




**PKD simulation**. (**A**) Starting from a stable tubular structure we model cadherin-8 phenotype by changing macroscopic properties of the microinjected cell (blue cell in panel). Increased surface tension between wild type and cadherin-8 expressing cell leads to deactivation of contact-inhibition, proliferation and formation of a single ectopic cyst. (**B**) 3D view of a similar simulation showing two formed cysts. (**C**) Images of cysts (arrows) stained for cadherin- 8(green) (**D-E**) Hanging drop asays show (**D**) compacted spheres of N-cad expressing cells and (**E**) loosely associated sheets of cadherin-8 expressing cells. The prediction that cadherin 8 decreased cell-cell adhesion in renal epithelial cells was confirmed in hanging drop assays. Cadherin 8 transfected HK-2 cells had fewer cell aggregates and aggregate size was decreased compared to N-cadherin transfected cells.

**Conclusions:** in silico modeling of renal cyst formation makes discrete predictions about cell cell adhesion properties that are confirmed by cell-cell adhesion assays. This approach allows for rapid identification of biological pathways that alter normal epithelial morphogenesis pathways.

*Funding:* Government Support - Non-U.S.

## FR-PO138

**Microbiology and Outcomes of Central Line Associated Blood Stream Infections (CLABSI) in Chronic Hemodialysis Patients** LaTonya J. Hickson, Kirandeep K. Khangura, Muhammad Rizwan Sohail, Patricia J.M. Best, Robert C. Albright, Larry M. Baddour, Rodney L. Thompson. *Nephrology, Infectious Disease, Cardiology, Mayo Clinic.*

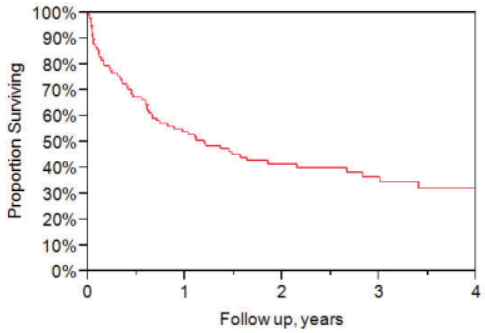
**Background:** Infections are the second most common cause for hospitalization in chronic HD patients. CLABSIs remain a major health concern and focus of quality improvement programs. However, CLABSIs continue to occur in select groups.

**Methods:** Review of baseline characteristics and outcomes of CLABSIs at a single tertiary care center.

**Results:** From 01/2007-06/2012, 99 chronic HD patients were treated for CLABSIs. CLABSIs were equally distributed over the 4.5 years; range 16-20/year. Mean age was 62±17 years, 52% male, and 77% Caucasian. Diabetes (55%), coronary artery disease (57%), and heart failure (64%) were frequent comorbidities. Others included: immunosuppression (24%), intracardiac device (22%), joint arthroplasty (15%), and prosthetic heart valve (10%). Diabetes was the main (28%) renal failure cause. The majority (94%) had tunneled central venous catheters (CVC) and few had temporary CVCs (6%). The most common pathogens are shown.

| | Prevalence |
|---|---|
| coagulase-negative staphylococci | 22% |
| *Enterococcus* species | 14% |
| methicillin-sensitive *Staphylococcus aureus (SA)* | 11% |
| methicillin-resistant *SA* | 9% |

CVCs were removed in 78% with salvage attempted in others. At index hospitalization, infective endocarditis (IE) was confirmed (5 patients) or suspected (6). Over a 6-month period, 2 were later diagnosed with IE. A second CLABSI occurred in 23 patients within 12 months. Overall survival rate was reduced at 6 (67%) and 12 months (47%).




**Conclusions:** Chronic HD patients hospitalized with CLABSI have a high prevalence of comorbidities that complicate decision-making. Even with being high risk, few patients developed IE from CLABSI. Better prediction tools to identify those at highest risk for CLABSI despite modern infection prevention protocols merit further investigation.

## FR-PO139

**Risk of Catheter-Related Bloodstream Infections in Elderly Patients on Hemodialysis** Mariana Murea, Kimone M. James, Gregory B. Russell, Anthony J. Bleyer, John M. Burkart, Barry I. Freedman. *Wake Forest School of Medicine, Winston- Salem, NC.*

**Background:** Elderly patients are more likely to require tunneled central vascular catheters (TCVC) for dialysis than younger patients. Unfortunately, there is little data on the risk of catheter-related bloodstream infections (CRBSI) in this population.

**Methods:** We collected data regarding CRBSI on prevalent and incident adult patients undergoing HD solely via TCVC at 9 Wake Forest Outpatient Dialysis centers between 2005 and 2007. Subjects who had a TCVC for <21 days were excluded. Cox proportional hazards regression analysis was performed with an event defined as CRBSI and censoring for death, end of study, or CRBSI no longer needed.

**Results:** 374 adult (age < 75 years) and 90 elderly (age ≥75 years) subjects of mean±SD age 54.8±12.3 and 81.3±4.9 years, dialysis vintage 1.8±3.3 and 1.5±2.9 years (p=0.47), and race distribution (European-American, African-American, other) 43.8%, 51.9%, 4.3%, and 73.3%, 26.7%, 0%, respectively (p<0.0001) were evaluated. CAD and CHF were more prevalent in elderly (51.1% and 48.9%) vs. adult patients (27.0% and 26.5%), p<0.001; while diabetes, HTN, and PVD had similar prevalence between groups. Mean total at-risk catheter-days were 272±243 in adults and 318±240 in the elderly. There were no significant differences in catheter site, use of anti-microbial catheter lock solutions, and bacterial culture results between groups. 208 total CRBSI events occurred (190 in adults, 18 in elderly), with CRBSI incidence per 100 catheter-days 0.20±0.46 in adults and 0.06±0.16 in elderly, p<0.0001. Using Cox regression analysis adjusted for sex, ethnicity, diabetes, catheter site and antimicrobial catheter lock solution, the hazard ratio (HR) for CRBSI in the elderly vs. adults was 0.33, (95% CI 0.20-0.54; p <0.0001). Risk of CRBSI declined by 11% per each 5 year age increment (HR, 0.89, 95% CI, 0.85-0.93, p<0.0001).

**Conclusions:** Elderly patients on HD using TCVC are at a significantly lower risk of CRBSI than younger patients. These results suggest that TCVC may be a more suitable option in elderly patients if permanent vascular access is problematic.

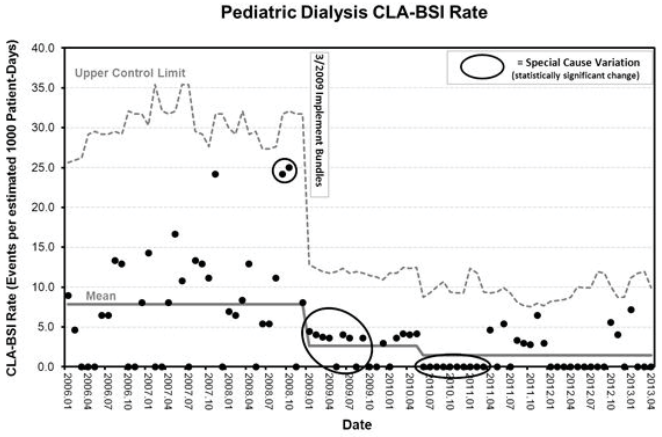
## FR-PO140

**Reduction in Catheter Associated Blood Stream Infections in a Pediatric Dialysis Unit** Kera E. Luckritz,[1] Maureen M. Shanley,[1] Theresa A. Mottes,[2] Matthew Niedner.[3] *[1]Pediatric Dailysis, Univ of Michigan, Ann Arbor, MI; [2]Center for Acute Care Nephrlogy, Cincinnati Children's Hospital, Cincinnati, OH; [3]Pediatric Critical Care, Univ of Michigan, Ann Arbor, MI.*

**Background:** Hemodialysis (HD) is the most common dialysis modality in children with a tunneled catheter as the vascular access in >75% of patients. The USRDS 2012 report cites a central line-associated bloodstream infection (CLABSI) incidence of 14.5% among pediatric patients. CLABSI can lead to morbidity including hospitalization and access failure, making prevention a priority in this population.

**Methods:** A CLABSI eradication team of pediatric nephrologists, dialysis/apheresis nurses, an infection control practitioner, a physician assistant who specializes in dialysis catheter insertions and an administrator was established. A bundle of evidence-based best practices was developed for the care of dialysis catheters and exit sites. A checklist of 10 steps was used as a tool to prompt desired practices and capture self-auditing process measures. Audits were performed with an "all or none" compliance methodology. Only trained dialysis/apheresis nurses who received 1:1 education regarding the accessing procedure from a member of the CLABSI team were allowed to access dialysis catheters. Audits were completed over 18 months and results were reviewed monthly by the team with feedback from the staff on challenges to compliance. After each infection, a root cause analysis was performed. Follow up communication with the dialysis staff emphasized lessons learned, preventability and patient impact.

**Results:** In the first 3 months, self-reported and observed compliance were 32% and 41% respectively, which rose to 82% and 67%. CLABSI rates are shown in a control chart, with statistically significant special cause variation patterns circled.




CLABSI rates fell from 7.9 to 2.6 per 1000-patient-days (66% reduction), and after a year of ongoing quality improvement work with rising compliance, rates dropped further to 1.5 per 1000-patient-days (81% reduction).

**Conclusions:** Minimizing the number of personnel accessing HD catheters and standardizing the technique with constant monitoring and feedback can reduce CLABSI rates.

## FR-PO141

**Fibrin Sheath Disruption during Catheter Exchange: Association with Bacteremia and Catheter Patency** Amanda M. Valliant, Muhammad K. Chaudhry, Alexander S. Yevzlin, Brad C. Astor, Micah R. Chan. *Univ of Wisconsin Hospitals and Clinics, Madison, WI.*

**Background:** Tunneled dialysis catheters are the most common form of vascular access among incident dialysis patients in the United States. Fibrin sheath formation is a frequent cause of late catheter dysfunction requiring an exchange procedure with balloon disruption of the fibrin sheath. It is unknown whether fibrin sheath disruption is associated with increased incidence of bacteremia or catheter failure.

**Methods:** We reviewed all tunneled dialysis catheter exchange procedures at the University of Wisconsin between January 2008 and December 2011. The primary outcome was incidence of bacteremia, defined as positive blood cultures within 2 weeks of the procedure. Catheter failure, requiring intervention or replacement, was examined as a secondary outcome. Baseline characteristics examined included diabetic status, gender, race and age.

**Results:** A total of 163 procedures were reviewed; 67 (41.1%) had fibrin sheath disruption and 96 did not. There was no difference in the prevalence of bacteremia among those with (3/67; 4.5%) and those without fibrin sheath disruption (3/97; 3.1%; p=0.65). Fibrin sheath disruption was not significantly associated with the risk of catheter failure (adjusted hazard ratio [AHR] = 1.34; 95% confidence interval [CI]: 0.87-2.10; p=0.18). Diabetes was associated with greater risk of catheter failure (AHR = 1.88; 95% CI: 1.19-2.95; p=0.006), whereas higher age was associated with a lower risk of catheter failure (AHR per 10 years = 0.83; 95% CI: 0.72-0.96; p = 0.01).

**Conclusions:** This study demonstrates for the first time, to our knowledge, that there is no significant association between fibrin sheath disruption and bacteremia or catheter patency. These results are encouraging given the large numbers of patients utilizing tunneled catheters for initial hemodialysis access and the known rates of fibrin sheath formation leading to catheter failure.

## FR-PO142

**MRSA and MSSA Carriage in a Cohort of Haemodialysis Patients: Prevalence, Eradication and Short Term Outcomes** Nadia Sarween, Anna Price, Jyoti B. Baharani. *Renal Medicine, Birmingham Heartlands Hospital, Birmingham, United Kingdom.*

**Background:** Haemodialysis (HD) patients with Staphylococcus aureus *(S.aurues)* bacteraemia have an increased risk of adverse outcome including death, compared to bacteraemia caused by other pathogens. Nasal colonisation of MRSA has been shown to increase the risk of subsequent endogenous infection with the organism in dialysis patients.

We performed a retrospective study in chronic haemodialysis patients to estimate the prevalence of *S.aureus* nasal carriage (for both MSSA and MRSA), to define patient groups at risk of colonisation and to look at the short-term outcomes following a decolonisation programme.

**Methods:** All established HD patients (666) were screened 4 monthly for 24 months (between June 2009 and May 2011)**.** At least two swabs; from the nose and access site were taken. Those with a positive result completed a course of decolonisation treatment. Data was gathered retrospectively using hospital electronic records and the renal unit data base for up to a period of 18 months following the date of the initial swab. Statistical analysis was performed using SPSS.

**Results:** The prevalence of colonisation with *S.aureus* was 47.7% (MRSA 9% MSSA 91%). 59% were male and median age was 65 years. 18.9% were in the high-risk co-morbidity group using Charlson Index. After decolonisation treatment 34% remained

successfully eradicated (eradicators). There was a significantly higher incidence of positive blood cultures in the non-eradicators (23%) compared with the eradicators (2%) but no difference was found in age, incidence of diabetes, co-morbidity score or number of hospital admissions between the two groups. 40% of patients died within 18 months without a difference in death rates between the two groups.

**Conclusions:** Decolonisation therapy was successful in eradicating carriage with *S.aureus* in 34% of our patients. Those that recolonised or remained colonised had a higher rate of bacteraemia with MSSA/MRSA compared to those that sucessfully eradicated. These patients are not easily identified from their demographics or co-morbidities. We would based on our findings advocate routine surveillance and aggressive attempts at eradication of *S.aureus* carriage in HD patients.

## FR-PO143

**Reduction of Catheter-Related Bloodstream Infections (CRBSI) after Implementation of an Endoluminal Catheter Colonization Surveillance Culture Protocol in Hemodialysis** Patricia Brañas,[1] Enrique Morales,[2] Francisco Rios,[1] Fernando Chaves.[1] *[1]Microbiology, Hospital 12 de Octubre, Madrid, Spain; [2]Nephrology, Hospital 12 Octubre, Madrid, Spain.*

**Background:** The colonization of the inner catheter lumen is the first step in the pathogenesis of most CRBSI. The aim of the study was to analyze the results of a protocol established in our hemodialysis (HD) unit, based on surveillance cultures (SCs), to prevent CRBSI.

**Methods:** Prospective study (April 2011-April 2012) in HD patients (pts) with tunnel cuffed central venous catheters (TCCs). Endoluminal colonization was assessed every 15 days by SCs: mixture of heparin (~ 2 mL) and blood (~ 3 mL) extracted from the TCC just before connecting the pts. SCs were inoculated into aerobic culture bottles and incubated 5 days (BacT/Alert, Biomerieux). Based on the microorganism and the time to positivity (TTP), an algorithm was designed: Group 1 (negative): continue surveillance; Group 2 (Coagulase-negative Staphylococcus [CoNS], TTP>14 hours): draw a new SC in a week. If the result was repeated, antibiotic lock therapy (ALT) was indicated (vancomycin or daptomycin 5 mg/mL, 2 weeks); Group 3 (CoNS, TTP<14 hours): ALT; Group 4 (other microorganism, any TTP): diagnosis of CRBSI and management according to guidelines.

**Results:** We included 104 pts with 129 TCCs. 56 pts were male and the median age was 73.0 (IR:54.5-80.0). The Charlson comorbidity index adjusted for HD was 7.0 (IR: 6.0-9.0), and the TCC vintage was118 days (IR:18-364). The most common causes of renal impairment were diabetic nephropathy (26.9%) and renal vascular disease (22.1%). The median of follow up was 262.5 days (IR: 135-365). A total of 1734 SCs were collected (median/pt: 18 [IR:10-24]) and 100 (5.8%) were culture positive. According to the protocol TCC colonization was detected in 28 occasions: 19 from groups 2 and 3, and 9 from group 4. Six pts carrying 7 TCCs had 7 CRBSI. The rate of CRBSI was 0.27/1000 catheter-days (1.65/1000 catheter-days in 2008).A total of 25 TCCs were removed, 11 for infection and 14 from other causes.

**Conclusions:** SCs, based on easily accessible samples, may contribute to reduce CRBSI in HD pts. Thereby, ALTs would be restricted to pts at high risk of CRBSI.

## FR-PO144

**Sustained Infection Reduction in Outpatient Hemodialysis Centers Participating in a Prevention Collaborative** Sarah H. Yi,[1] Stephanie L. Booth,[2] Virginia R. Bren,[2] Gemma Downham,[2] Sally Hess,[2] Karen Kelley,[2] Mary E. Lincoln,[2] Curt Lindberg,[2] Heather Weirich,[2] Alexander Kallen,[1] Priti Patel.[1] *[1]Div of Healthcare Quality Promotion, Centers for Disease Control and Prevention (CDC); [2]Steering Committee for the CDC Dialysis Bloodstream Infection Prevention Collaborative.*

**Background:** We reported previously reductions in bloodstream infections (BSIs) and access-related BSIs in a group of outpatient hemodialysis centers after implementation of a package of BSI prevention interventions. We sought to assess sustainability of these reductions, and to assess trends in intravenous (IV) antimicrobial use.

**Methods:** In 2009, 17 outpatient hemodialysis centers joined a CDC-sponsored collaborative project to prevent BSIs. Centers implemented CDC's Core Interventions for BSI Prevention, and reported BSIs, access-related BSIs, IV antimicrobial starts, and monthly patient census by vascular access type per CDC's National Healthcare Safety Network protocol. Up to 12 months of baseline and 36 months of intervention data were analyzed. Segmented regression analysis was used to assess initial change from baseline at intervention start and monthly rate changes during the intervention.

**Results:** The table contains actual and modeled rates.

| Measure | Pooled rates | | Modeled changes during intervention | | | |
|---|---|---|---|---|---|---|
| | | | Initial | | Monthly | |
| | Baseline | Intervention | Change | *P*-value | Change | *P*-value |
| BSI[a] | 1.09 | 0.83 | -24% | .16 | -0.71% | .36 |
| Access-related BSI[a] | 0.73 | 0.43 | -52% | <.0001 | -0.12% | .85 |
| IV antimicrobial start[a] | 4.38 | 3.36 | -17% | .22 | -0.67% | .25 |
| CVC use[b] (%) | 31.8 | 24.7 | -14.5%[c] | .02[d] | | |

[a]Count per 100 patient-months.
[b]Percent of patients with central venous catheter (CVC).
[c]Actual.
[d]Wilcoxon signed-rank test.

Using the modeled baseline trend, an estimated 147 of 298 expected access-related BSIs were prevented during the intervention.

**Conclusions:** Centers demonstrated an early drop in access-related BSIs that persisted through intervention month 36. Access-related BSIs may be preventable through implementation of and adherence to recommended prevention practices; improvements may be maintained at least three years after adoption.

*Funding:* Other U.S. Government Support

## FR-PO145

**Revision versus Exchange of Malfunctioned Tunneled Dialysis Catheters for Hemodialysis Lowers Infection Rate** Jackson Wang,[1] Tuan A. Nguyen,[1] Andrew I. Chin,[1,2] Jamie L. Ross.[1] *[1]Div of Nephrology, Univ of California Davis, Sacramento, CA; [2]Div of Nephrology, VA Healthcare Northern California, Mather Field, CA.*

**Background:** Malfunctioned catheter is defined as blood flow rate of <300 ml/min achieved in dialysis and not responding to tissue plasminogen activator. There are 2 methods of fixing a malfunctioned tunneled dialysis catheter (TDC): exchange vs revision. Exchange procedures involve TDC removal and exchange over a wire, using the same exit site and venotomy site, where diagnostic imaging or intervention are generally not performed. Revision procedures involve placement of a new TDC using the previous venotomy site and creating a new tunnel and exit site. The majority of revisions usually include diagnostic imaging and intervention in the central circulation if needed.

**Methods:** The choice of procedure was physician preference. A retrospective single review of 70 patients who underwent 97 TDC replacements from 2010 to early 2012 because of catheter malfunction. Endpoints of the study were either infection or malfunction within 30 days of the procedure.

**Results:** There were a total of 41 exchanges and 56 revisions out of the 97 procedures performed. There were 8 infections (documented by positive blood culture) in the exchanges (19.5%) and 1 in the revision group (1.8%). The need for an additional procedure due to malfunction was 10 in the exchange (24.4%) and 10 (17.8%) in the revision group.

| | TDC Exchanges (n = 31) | TDC Revisions (n = 56) | p-value |
|---|---|---|---|
| Infections (positive blood culture) within 30 days | 8 (19.5%) | 1 (1.8%) | 0.003 |
| Additional Procedures within 30 days | 10 (24.4%) | 10 (17.8%) | 0.432 |

**Conclusions:** Compared to an exchange, revision of a malfunctioned TDC resulted in lower infection rate and trended towards a lower malfunction rate within 30 days after the initial procedure. While the revision procedure may require more time and effort, the lower rate of subsequent infections may justify this approach to malfunctioning TDCs in hemodialysis patients.

*Funding:* Clinical Revenue Support

## FR-PO146

**Recirculation in Reverse Flow with a Split-Tip versus Symmetrical Tip Hemodialysis Catheter** Hariprasad S. Trivedi,[1] Tushar J. Vachharajani,[2] Nand K. Wadhwa,[3] Mark Vannorsdall,[4] Jean Lee,[5] Klemens B. Meyer.[6] *[1]Medical College of Wisconsin; [2]W. G.(Bill) Hefner VAMC; [3]Stony Brook Univ Med Ctr; [4]Maine Health; [5]Temple U; [6]Tufts Med Ctr.*

**Background:** When blood flow is low during hemodialysis (HD) line reversal is often undertaken, which can lead to high recirculation (RC). Catheter (C) tip design is considered a key factor that affects RC. We compared RC with a split-tip versus symmetrical tip C.

**Methods:** A prospective trial of split-tip and symmetrical tip tunneled C performance included a pre-planned ultrasound blood flow sub-study. Consenting adult chronic HD subjects were randomized to either: 1. A symmetrical tip catheter, Covidien's Palindrome™ catheter with side slots; 2. A split-tip catheter, Bard® Hemosplit® catheter. In 206 subjects ultrasound dilution velocity measurement was used to capture blood flow rates (BFR) and RC during week 1, 5 and 11 after C insertion. BFR and RC, and arterial (AP) and venous pressures (VP) were recorded in both forward flow (FF) and after line reversal at flow rates of 350, 400, and 450 mL/min, between 30 and 90 minutes following initiation of HD. The primary objective of the sub-study was RC in reverse flow (RF).

**Results:** Median RC in RF was 3.8% in the split-tip C and 3.2% in the symmetrical tip C (P=0.096). The following additional variables were analyzed. 1) RC in FF flow was higher in the symmetrical tip C (median 3.7 versus 0, P<0.0001); 2) The symmetrical tip C had a higher mean BFR during week 5 at all three pump settings (all $P \leq 0.038$) but no different at other measurements; 3) The difference between the prescribed and actual BFR was -20.4±25.9 vs. -9.8±21.1, split-tip vs. symmetrical tip C (P=0.018) during week 5 at setting of 350 mL/min and no different at all other measurements; 4) All measurements showed higher AP in the split-tip C (all $P \leq 0.03$) except during the week 11 setting of 450 mL/min (P = 0.9); 5) VP was higher in the split-tip C at pump settings of 350 and 400 mL/min during all weeks and at pump setting of 450 mL/min during week 1 (all $P \leq 0.032$).

**Conclusions:** There was no statistically significant difference in recirculation after line reversal between a split-tip and symmetrical tip HD catheter.

*Funding:* Pharmaceutical Company Support - Covidien

## FR-PO147

**Comparison of a Split-Tip versus Symmetrical Tip Hemodialysis Catheter**
Hariprasad S. Trivedi,[1] Tushar J. Vachharajani,[2] Nand K. Wadhwa,[3] Mark Vannorsdall,[5] Jean Lee,[4] Klemens B. Meyer.[6] *[1]Medical College of Wisconsin; [2]W. G.(Bill) Hefner VAMC; [3]Stony Brook Univ Medical Ctr; [4]Temple U; [5]Maine Health; [6]Tufts Med Ctr.*

**Background:** In a multi-center, randomized, open-labeled trial we compared the performance and longevity of a split-tip versus symmetrical tip hemodialysis (HD) catheter (C).

**Methods:** Adult subjects requiring chronic HD scheduled to receive a tunneled C for vascular access were considered for the trial. Key study criteria included a patent internal jugular vein and absence of active infection. Eligible and consented subjects were randomized in a 1:1 ratio to either: 1. A symmetrical tip catheter, Covidien's Palindrome™ catheter with side slots; 2. A split-tip catheter, Bard® Hemosplit® catheter. Subjects were followed until study completion which was defined as 35 weeks after catheter placement, study catheter removal, or death. The primary end-point was "time to catheter-induced complication or TCIC" defined as time to first incidence of intervention for malfunction due to thrombosis, poor flow requiring administration of tissue plasminogen activator (tPA), mechanical problems requiring surgical/radiological intervention, catheter displacement, or failure due to catheter related blood stream infection.

**Results:** 15 US centers enrolled 601 subjects of whom 568 had at least one post-baseline visit measurement for the primary end-point. Mean age was 59.4 ±14.8 years, 51.6% were female, 52.8% were African American, 8.3% were of Hispanic/Latino ethnicity. The most common cause of end-stage renal disease was type 2 diabetes mellitus (38.6%). 89 subjects completed the full 35 week follow-up. The most common cause of early termination was C removal. 42% (n= 252) subjects met the primary endpoint. The median TCIC for the symmetrical C group was 148 days and for the split-tip C group was 213 days. The 2 groups did not differ statistically in the composite primary end-point (log-rank test P=0.2).

**Conclusions:** There was no difference between a split-tip versus symmetrical tip hemodialysis catheter in time to catheter-induced complication comprised of a composite of important mechanical, thrombotic and infective complications.

*Funding:* Pharmaceutical Company Support - Covidien

## FR-PO148

**Exchange from Non-Tunneled to Tunneled Hemodialysis Catheter with over the Guidewire Can Be Performed without Increasing Complication Risk**
Hoon Suk Park, Kyungyoon Chang, Hyung Wook Kim, Bum-Soon Choi, Cheol Whee Park, Chul Woo Yang, Dong Chan Jin. *Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** Exchange from non-tunneled hemodialysis (HD) catheter to tunneled HD catheter over the guidewire using previous venotomy has advantage that it does not require new venipuncture so that it can prevent additional injury to vessel wall. Several previous studies demonstrated its safeties, but concerns that it can be associated with infection and bleeding prevent it from being chosen rather than de novo placement. Therefore, we investigated its safeties in our center.

**Methods:** Twenty five patients who started initially HD with non-tunneled catheter and then received catheter exchange procedure into tunneled catheter and 110 patients who initially received de novo placement of tunneled HD catheter within the same period were respectively assigned to exchange and de novo placement groups. Catheter survival, immediate and long term complication rates were compared.

**Results:** Catheter exchange into tunneled catheter was performed at 10±3 days after initial insertion of non-tunneled catheter. Immediate complication rates including exit site bleeding, bruise and hematoma were comparable (26.3 % in exchange group vs. 23.7 % in de novo placement group, p = 0.8). Long term complication rates including catheter dysfunction and catheter related infection (5.3 % in exchange group vs. 8.8 % in de novo placement group, p = 0.61) and catheter survivals (p = 0.7) were also comparable between 2 groups.

**Conclusions:** Exchanging procedure over the guidewire from non-tunneled to tunneled catheter was comparable with de novo placement of tunneled catheter in complication rates and catheter survival, in spite of merit in avoiding unnecessary additional vessel wall injury. Therefore it should be firstly considered in the cases where replacement non-tunneled catheter with tunneled one is required.

## FR-PO149

**Multi-Disciplinary Approach to Peripherally Inserted Central Catheter Avoidance in Hospitalized CKD Patients: A Single Center Experience**
Mireille El Ters,[1] Gauri Bhutani,[1] Amy Mahon,[2] Amy W. Williams,[1] Marie C. Hogan.[1] *[1]Nephrology, Mayo Clinic, Rochester, MN; [2]Nursing, Mayo Clinic, Rochester, MN.*

**Background:** The use of PICCs in chronic kidney disease is highly discouraged by several renal societies. Recent data show strong association of prior PICC with lack of functional arterio-venous fistula(AVF);however efforts at PICCs avoidance are so far ineffective.

**Methods:** 1.Creating an electronic alert of patients with ↓eGFR (<30 ml/min, CKD-EPI) at time of PICC order, stating the reason for its avoidance and providing alternate access (small bore internal jugular) with link to educational material.2.Education to nephrologists,dialysis nurses,hospitalists and intensivists in form of a lecture detailing the impact of PICCs on AVF success. We compared proportion of patients with ↓eGFR among 550 consecutive patients receiving PICC before (March 2010) and after the intervention (April 2013) using Fisher's exact test. We queried the electronic alert system to determine its effectiveness in reducing PICC prevalence in the target population.

**Results:** The proportion of patients with ↓eGFR receiving PICC before and after intervention was not statistically significantly different,except for a trend to a lower proportion of end stage renal disease patients after intervention. Of the 567 PICC requests during April 2013, 83 (14.6%) caused the alert to appear, leading 11 requests (13.3%) to be discontinued. The commonest reported indication for PICC despite the alert was need for central access,with ICU providers being commonest (23/30,76%).

| Intervention (Dec 2012) | Before (n=275) | After (n=275) | p value |
|---|---|---|---|
| All patients with eGFR < 30 ml/min | 35 (12.7%) | 30 (10.9%) | 0.51 |
| CKD | 17 (6.2%) | 16 (5.8%) | 0.99 |
| AKI | 18 (6.5%) | 14 (5.1%) | 0.58 |
| ESRD | 11 (4%) | 3 (1.1%) | 0.054 |

**Conclusions:** Despite educational measures on the impact of PICCs on future AVF there remains a continued reliance on PICC lines in our center in the CKD population especially in the critical care setting. Future efforts will concentrate on continued education of patients, critical care physicians and hospitalists and promotion of alternative small bore internal jugular lines.

## FR-PO150

**Decreasing the Incidence of Dialysis Catheter Infections Using Evidence Based Strategies**
Catherine C. Wells, Steven Wagner, Iasmina Craici, Mary Alice Luckett, Luis A. Juncos. *Dept of Medicine, Div of Nephrology, Univ of Mississippi, Jackson, MS.*

**Background:** Hemodialysis (HD) Catheter associated infections have recently become a focus for attention in outpatient HD quality control due to their role in morbidity and mortality. However, there are still no specific guidelines for inpatient HD catheter infection prevention. Additionally, since 2008 CMS payments are reduced for every incidence of preventable vascular catheter associated infection. Therefore, we implemented a Quality Improvement Project (QIP) to develop a protocol intended to reduce the burden of HD catheter associated infections.

**Methods:** The first intervention, performed in October 2009, was comprehensive education of all inpatient HD nurses based on the NKF KDOQI guidelines as well as limiting handling of HD catheters to HD nurses when possible. Nurses were taught using a slide presentation with pre- and post-testing, delivered via Healthstream online education system to ensure compliance. After education, audits of practice were conducted to ensure compliance. In Quarter 1 of 2010, a similar Healthstream slide presentation with post-testing was delivered to all hospital nurses. Education was repeated as indicated throughout the project. Chi Squared Trending test was used to evaluate significance; $p < 0.05$ was considered a significant reduction in hospital acquired infections.

**Results:** Quarterly evaluation of hospital acquired HD catheter infections from June 2009 to September 2012 fell from 25% to 3% ($p < 0.0001$). Annual incidence of HD catheter infections present on admission fell from 17% to 6%. At least 7 targeted infection prevention interventions were conducted during this time period, using the principles of the NKF KDOQI guidelines and restricting catheter use to HD nurses when possible.

**Conclusions:** Hospital-wide nurse education based on existing guidelines intended for outpatient HD catheter infection prevention, in addition to minimizing use of HD catheters by non-dialysis staff can successfully reduce the incidence of hospital acquired HD catheter infections. This was a QIP and required ongoing interventions to maintain the decline in hospital acquired infections.

*Funding:* Clinical Revenue Support

## FR-PO151

**Multidetector CT Angiography in the Evaluation of Catheter-Related Central Venous Stenoses in Hemodialysis Patients**
Yuliang Zhao, Tianlei Cui, Ling Zhang, Ping Fu. *Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan Province, China.*

**Background:** Multidetector CT angiography (MDCTA) is widely available in evaluating vascular tree, but its correlation with digital substraction angiography (DSA) in demonstrating catheter-related central venous stenoses among hemodialysis patients remains to be clarified. This study aims to compare MDCTA with DSA in the evaluation of catheter-related central venous stenoses in hemodialysis.

**Methods:** During 24 months, a total of 25 chronic hemodialysis patients with suspected catheter-related central venous stenoses were examined by MDCTA and 3-D image reconstruction, while subsequent DSA served as gold standard. The presence, site and number of stenoses or occlusions were accessed. According to the reduction in vessel lumen, the stenoses were classified as insignificant (<50%), significant (>50%) and occlusion. We calculated the sensitivity, specificity, accuracy, odds ratio (OR), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) for MDCTA as an alternative to DSA.

**Results:** 115 vascular segments in 25 patients were analyzed. MDCTA detected 22 significantly stenotic or occluded central veins. DSA verified 19 of them and demonstrated 25 additional ones.

| | | DSA | | |
|---|---|---|---|---|
| | | Significant stenoses/Occlusions | Insignificant stenoses | Total |
| MDCTA | Significant stenoses/Occlusions | 19 | 3 | 22 |
| | Insignificant stenoses | 25 | 68 | 93 |
| | Total | 44 | 71 | 115 |

MDCTA has a high specificity (95.8%) in diagnosing central venous stenoses as compared with DSA, but its sensitivity is low (43.2%). The PPV, NPV, PLR, NLR were 86.4%, 73.1%, 10.2, 0.59 respectively. The diagnostic OR is 17.2, with 75.7% accuracy.

**Conclusions:** MDCTA has a high specificity in detecting catheter-related central venous stenoses. Positive findings by MDCTA are helpful to clinical diagnosis. However, MDCTA might not be reliable in ruling out stenoses for its low sensitivity.

## FR-PO152

**Ethanol versus Heparin Catheter Locks for Cuffed Central Venous Catheters in Hemodialysis Patients: A Single-Center Randomized Controlled Trial** Xiaomeng Sun, Hong Liu, Bi-cheng Liu. *Institute of Nephrology, Southeast Univ, Nanjing, China.*

**Background:** Tunnelled central venous catheter use is significantly limited by the frequent occurrence of catheter-related bacteraemias (CRBs), which results in significant attributable morbidity and mortality. In this trial, we studied the efficacy and safety of 70% ethanol lock for the prevention of CRBs in hemodialysis patients with a tunnelled central venous catheter.

**Methods:** A randomized, prospective, controlled study was undertaken. Hemodialysis patients with tunnelled catheter were randomised assigned to receive 70% ethanol lock once a week and standard heparin locks for other dialysis days *vs* thrice per week heparin locks. CRBs and catheter dysfunction were recorded.

**Results:** From January 1, 2013 to June 1, 2013, 22 patients (aged 50 - 85 years) were enrolled. A total of 1484 catheter days were observed in the ethanol lock arm and 1568 catheter days in the heparin lock arm from trial entry. There was no significant difference in patient's age, gender, age of dialysis, the primary disease between the two groups. For ethanol locks, the incidence of CRBs per 1000 catheter days was 0.67 (1/1484), compared to 1.28 (2/1568) for heparin locks. Catheter dysfunction occurred at a rate of 0.67 (1/1484) vs 0.64 (1/1568) per 1000 catheter days in the heparin and ethanol groups respectively. No adverse symptoms attributable to ethanol occurred.

**Conclusions:** Our preliminary results suggested there was a trend towards a reduced rate of CRBs in a 70% ethanol lock without resulting in catheter dysfunction. However, these results should be confirmed with a larger trial.

## FR-PO153

**The Stuck Catheter—A Hazardous Twist to the Meaning of Permanent Catheters** Venkat Sainaresh Vellanki, Cynthia B. Bhola, Charmaine E. Lok. *Toronto General Hospital and the Univ of Toronto, Toronto, Canada.*

**Background:** Permanent central venous catheters (CVC) use is associated with significant complications that often require their rapid removal. Tunneled CVC's can usually be easily removed after dissecting out the retention cuff. However, a less often described complication is resistant removal of the CVC due to adherence of the CVC to the vessel wall which mandates use of invasive interventions for removal. The aim of this study is to describe the occurrence of the "stuck catheter" phenomenon and its consequences.

**Methods:** We performed a retrospective review from July 2005 to June 2013 at a single academic based HD center of all removed tunneled CVC's to determine the incidence of stuck catheters, the type of intervention needed to remove them and subsequent follow up. Data was retrieved from a prospective vascular access database and verified manually against patient charts.

**Results:** During the study period there were 1908 CVC removed in 923 patients. There were 16 cases (1.73%) of stuck CVC's (0.031/1000 days) needing interventional radiology (IR) or surgical intervention for removal. Clinical characteristics: 7 men (47%), 4 diabetic (27%), etiology of ESKD - 3 SLE, 1 ANCA vasculitis, 3 had secondary hyperparathyroidism needing parathyroidectomy. Catheter characteristics: 75% were in the right internal jugular vein, the mean CVC duration was 51.1 months (range 11-144). Three CVC could not be removed from the vessel, thus were embedded into the facial planes / mediastinum. The rest of the CVCs required dissection and traction by IR except 1 required surgical sternotomy. The two embedded catheters had 34 and 53 months of uneventful follow up. The other became infected with a fungal organism and the patient consequently died from infectious complications.

**Conclusions:** Our series demonstrated that 1.7% of hemodialysis patients with permanent catheter insertion were complicated by a vessel embedded catheter that required removal by IR or surgery. Prolonged catheter vintage could be a predisposing factor. Permanent catheter use should be avoided if possible to avoid complications, including this rare but important complication.

*Funding:* Clinical Revenue Support

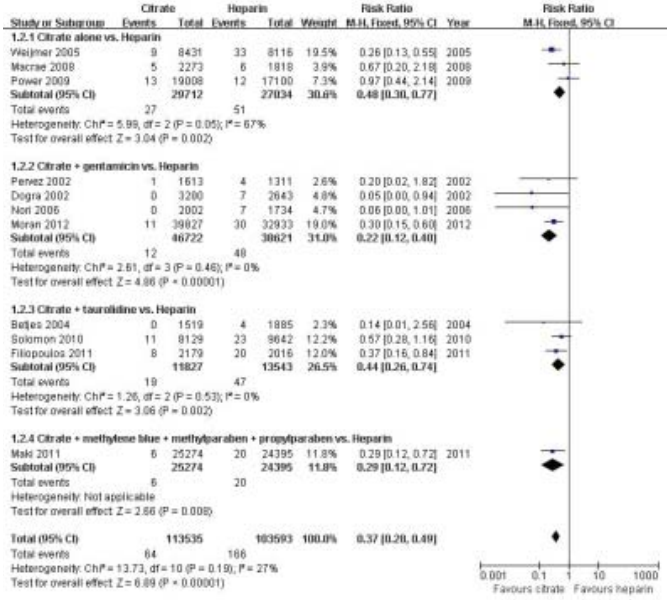
## FR-PO154

**Citrate versus Heparin Lock for Hemodialysis Catheter: A Systemic Review and Meta-Analysis of Randomized, Controlled Trials** Yuliang Zhao,[1] Ling Zhang,[2] Ping Fu.[3] *[1]Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan Province, China; [2]Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan Province, China; [3]Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan Province, China.*

**Background:** Citrate solution has been suggested as an effective and safe catheter lock in hemodialysis. However results from studies comparing citrate with heparin locks remain inconclusive.

**Methods:** We collected from PubMed, Ovid, the Cochrane Library, Web of Science databases and major nephrology journals. A systemic review and meta-analysis was performed of all eligible randomized controlled trials.

**Results:** 13 randomized controlled trials (1770 patients, 221064 catheter-days) met the inclusion criteria. Pooled analyses found that Citrate locks could significantly reduce the incidence of catheter related bloodstream infection (RR=0.37, 95%CI=0.28-0.49, *P*<0.001). Subgroup analysis showed citrate alone, citrate + gentamicin, citrate + taurolidine were all superior to heparin lock in the prevention of catheter related bloodstream infection (*P*=0.002, <0.001, =0.002 respectively).




Incidence of exit site infection(RR=0.67, 95%CI=0.47-0.95, *P*=0.03) and bleeding episodes (RR=0.47, 95%CI=0.30-0.74, *P*=0.001) were significantly lower in patients receiving citrate lock, while both groups were similar in terms of catheter removal for poor flow (*P*=0.91), thrombolytic treatment (*P*=0.77), all-cause death (*P*=0.21), catheter thrombosis (*P*=0.93), mean catheter duration (*P*=0.20), catheter related bloodstream infection-free catheter survival (*P*=0.22) and catheter-related readmission (*P*=0.51).

**Conclusions:** Citrate lock is better than heparin lock in the prevention of catheter-related infections and bleeding episodes, while both lock solutions show similar efficacy in preserving catheter patency. Our meta-analysis suggests wider applications of citrate lock in hemodialysis.

## FR-PO155

**Geographic Disparities in AV Fistula Placement in Patients Approaching Hemodialysis** Wajih A. Syed,[3] Bhanu K. Patibandla,[2] Akshita Narra,[1] Varun Chawla,[1] Yael Vin,[1] Tammy Hod,[1] Alexander S. Goldfarb-Rumyantzev.[1] *[1]BIDMC; [2]St. Vincent Hospital; [3]Metrowest Medical Center.*

**Background:** Arterio-venous fistula (AVF) is the preferred access for hemodialysis. However in the US, the number of patients starting HD with AVF is still relatively low. Several factors associated with AVF placement have been identified (e.g., age, sex, race, co-morbidities). In this project we hypothesized that geographic location of patient residence might be associated with the probability of AVF placement as the first access for initiation of hemodialysis.

**Methods:** We used the data from the USRDS database (2005-2008) linked to Medicare claims (2003-2008). Logistic regression was used to estimate specific characteristics of population associated with the AVF as first access placed or attempted for hemodialysis initiation. Our primary variable of interest was the geographic location and the multivariate model was adjusted for age, sex, race, BMI, primary cause of ESRD, duration of pre-ESRD nephrology care, comorbidities, employment status, substance abuse, and income. Geographic location of the patient residence was determined using the data collected by the RUCA project and divided population into metropolitan, micropolitan, and rural categories.

**Results:** Patients (n=111,953) identified from the USRDS data base with linked Medicare claims were examined. Rates of fistula placement in the metropolitan, micropolitan and rural population were 18.5%, 22.4% and 21.6% respectively. In comparison, patients who received catheter as the first access were 81.5%, 77.6% and 78.4% respectively. The odds ratio of AVF placement as a first hemodialysis access in the rural and metropolitan population compared to the micropolitan population were 0.96 (0.90-1.03) and 0.75 (0.71-0.79) respectively.

| Odds of having AVF as first access placed or attempted pre-dialysis compared to a catheter | | |
|---|---|---|
| | **OR (95% CI)** | **P value** |
| **Geography** | | |
| Rural | reference | |
| Urban | 0.76 (0.73-0.80) | 0.03 |
| Unknown | 0.49 (0.42-0.57) | <0.001 |

**Conclusions:** Our results indicate geographic disparities in AVF placement with decreased rates of AVF as the first access created in the metropolitan and rural populations compared to the micropolitan populations.

## FR-PO156

### Factors Predicting Failure of AV "Fistula First" Policy in the Elderly

Tammy Hod,[1] Bhanu K. Patibandla,[2] Yael Vin,[1] Robert S. Brown,[1] Alexander S. Goldfarb-Rumyantzev.[1] *[1]BIDMC; [2]St. Vincent Hospital.*

**Background:** An arteriovenous fistula (AVF) is the preferred access for HD. The goal of this study was to identify factors associated with AVF failure in the pre-dialysis period in an elderly HD population.

**Methods:** We used USRDS + Medicare claims data to identify incident HD patients ≥67 year old from 01/01/05 to 12/31/08 with first pre-dialysis access placed being an AVF (n=20,360; 76.2±6.02 year old, 58.5% males). AVF failure, defined as failure of using the AVF for the first outpatient dialysis, was used as the outcome. Logistic regression model was used to identify factors associated with AVF failure.

**Results:** Of patients who had an AVF placed pre-dialysis, 48% initiated dialysis using the AVF and 52% using a catheter or an AVG. The following variables were found to be associated with AVF failure when an AVF was created at least 4 months pre-HD initiation: age ≥67 yr, female gender, black race, prior history of diabetes mellitus, cardiac failure and shorter duration of pre-ESRD nephrology care. Odds ratio for AVF failure for the entire cohort showed similar findings.

| | Entire study population (n=20,360) | | 'Early' AVF placement group (n=11,258) | |
|---|---|---|---|---|
| | Odds Ratio (95% CI) | P Value | Odds Ratio (95% CI) | P Value |
| Age at ESRD onset | 1.01 (1.00-1.01) | 0.006 | 1.01 (1.00-1.02) | 0.003 |
| Female (vs male) | 1.55 (1.46-1.65) | <0.001 | 1.69 (1.55-1.83) | <0.001 |
| Black (vs white) | 1.33 (1.22-1.45) | <0.001 | 1.41 (1.26-1.58) | <0.001 |
| Diabetes | 1.20 (1.09-1.33) | 0.0003 | 1.22 (1.06-1.39) | 0.005 |
| Congestive Heart Failure | 1.30 (1.22-1.39) | <0.001 | 1.26 (1.15-1.37) | <0.001 |
| Pre-ESRD care | | | | |
| >12 months | Reference | | Reference | |
| 6-12 months | 1.24 (1.15-1.33) | <0.001 | 1.31 (1.18-1.43) | <0.001 |
| <6 months | 1.27 (1.16-1.39) | <0.001 | 1.22 (1.07-1.38) | <0.001 |
| Zero | 4.17 (3.58-4.86) | <0.001 | 4.09 (3.35-5.00) | <0.001 |

**Conclusions:** In an elderly HD population, there is an association of older age, female gender, black race, diabetes, cardiac failure and shorter duration of pre-ESRD care with predialysis AVF failure. These results might help to identify populations at risk in order to assure adequate AVF maturation or AVG creation as primary access.

## FR-PO157

### Optimal Time for AVF Placement in the Elderly. "The Earlier the Better"–A Myth or a Truth?

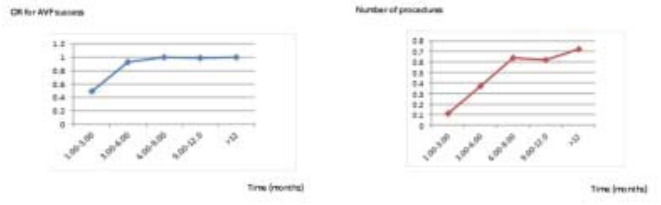
Tammy Hod,[1] Bhanu K. Patibandla,[2] Yael Vin,[1] Robert S. Brown,[1] Alexander S. Goldfarb-Rumyantzev.[1] *[1]BIDMC; [2]St. Vincent Hospital.*

**Background:** AVF is the preferred access for hemodialysis (HD). However, many AVFs fail to mature or develop stenosis/thrombosis prior to starting dialysis, especially in the elderly. That results an undesirable increased incidence of catheter use.

**Methods:** We used USRDS + Medicare claims data to identify incident HD patients ≥67 year old from 01/01/05 to 12/31/08 with first pre-dialysis access placed being an AVF (n=17,511; 76.1±6.0 year old, 58.3% males). AVF success is defined as using the AVF for the first outpatient dialysis. Our second outcome was the number of pre-dialysis vascular access procedures. Time between AVF placement and dialysis start was our primary variable of interest. Using a logistic regression model we further analyzed subgroups divided by gender, race, presence/absence of diabetes and CHF.

**Results:** Of patients who had an AVF placed pre-dialysis, 54.9% initiated dialysis using the AVF and 45.1% using a catheter or an AVG. The OR for success increased with longer time from AVF creation to HD initiation between 1-3, 3-6 and 6-9 months (OR of 0.49, 0.93 and 1) but stabilized after that so creation of AVF more than 6-9 months from the first HD session was not associated with a higher AVF success rate. The number of procedures increased with longer times between AVF placement and dialysis start. This trend was also present in the subgroups studied.

| Time (months) | OR for success (95% CI) | P Value | Procedures Mean (SD) | P Value |
|---|---|---|---|---|
| 1-3 | 0.49 (0.44-0.53) | <0.001 | 0.11 (0.64) | <0.001 |
| 3-6 | 0.93 (0.85-1.02) | 0.005 | 0.37 (1.38) | |
| 6-9 | 1 (0.90-1.11) | <0.01 | 0.64 (2.08) | |
| 9-12 | 0.99 (0.88-1.11) | 0.006 | 0.62 (1.82) | |
| >12 | 1 | | 0.72 (2.11) | |



**Conclusions:** We suggest that placing an AVF more than 6-9 months in advance of HD initiation in the elderly might be counterproductive as it is not associated with better AVF maturation, but might lead to greater number of access procedures.

## FR-PO158

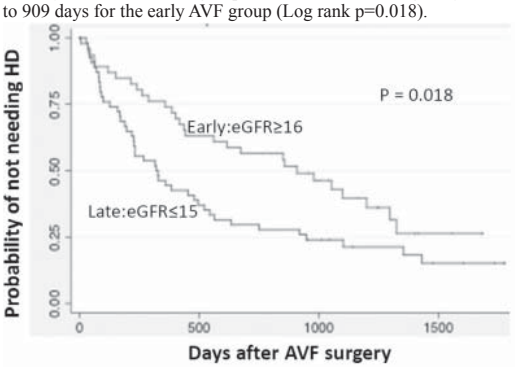
### Early versus Late Arteriovenous Fistula Creation in Pre-Dialysis Patients

Mardiana Lee, Matthew A. Roberts, Maree S. Ross-smith, Jason Chuen, Peter F. Mount. *Austin Health, Australia.*

**Background:** Optimal timing of arteriovenous fistula (AVF) surgery in pre-dialysis CKD patients is uncertain. Current guidelines recommend creation of an AVF in preparation for hemodialysis (HD) when the estimated glomerular filtration rate (eGFR) is 15-30 ml/min/1.73m$^2$.

**Methods:** We reviewed records of all CKD patients who had a first AVF creation for future HD at Austin Health from 01/01/2007-31/12/2009 and obtained follow-up data until 31/12/2011. Survival analysis was performed for the primary outcome of time from AVF creation to first need for HD.

**Results:** 100 patients had a first AVF created with a median eGFR at the time of AVF surgery of 15 ml/min/1.73m$^2$. Patients were classified as having had an early AVF if eGFR was ≥16 (n=46) or a late AVF if eGFR was ≤15 (n=54). The mean age was 63.7+13.7 years, 49 had diabetes and 39 were female. Over 2-5 years follow up 81% (44/54) of late AVF and 63% (29/46) of early AVF patients required HD (P=0.04). Median time to starting HD was 479 days. In the late AVF group median time to starting dialysis was 321 days compared to 909 days for the early AVF group (Log rank p=0.018).




At 3, 6 and 12 months respectively, 20%, 44% and 56% of patients with eGFR≤15 had needed HD compared to 11%, 20% and 26% with eGFR≥16. A dialysis catheter was required at HD commencement in 11% (5/44) of patients from the late AVF group and 24% (7/29) of patients from the early AVF group (P=0.20). Additional vascular access procedures prior to the need for HD were required for 35% of late AVF and 39% of early AVF patients.

**Conclusions:** A higher risk of AVF non-use was observed in patients having AVF surgery with an eGFR ≥16, whilst the rate of catheter use at HD start remained low in patients having AVF surgery with an eGFR ≤15. These data suggest that strict adherence to current guidelines might lead to AVF creation earlier than is clinically optimal for many patients.

## FR-PO159

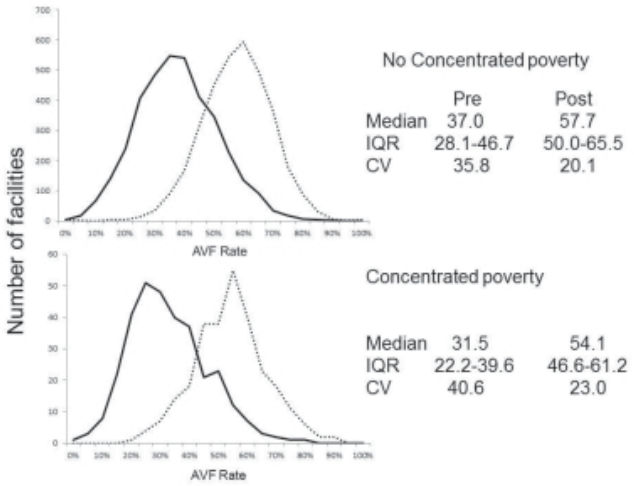
### Comparable Rates of Improved Arteriovenous Fistula Use Were Observed in Poor Communities during the Fistual First Breakthrough Initiative

William M. McClellan, Sumit Mohan, Janet R. Lynch, Edwin D. Huff. *For Fistual First Data Committee.*

**Background:** The Fistula First Breakthrough Initiative (FFBI) is a population-based intervention by the Centers for Medicare & Medicaid Services (CMS) to increase arteriovenous fistula (AVF) use. Variations in AVF have been noted among individual hemodialysis centers. This study examines the relationship between county-level poverty and baseline use and rates of improvement in facility AVF use.

**Methods:** This was a retrospective analysis of AVF use in 3,485 dialysis centers treating dialysis patients. Monthly data collected by the ESRD Networks was used to estimate rates of AVF use among prevalent adult hemodialysis patients. We calculated facility-specific slopes of prevalent AVF use over the study period. Counties with centers were categorized by the presence or absence of concentrated poverty and AVF slopes were compared between the two poverty groups after controlling for baseline case-mix factors.

**Results:** The Figure shows the proportion of a hemodialysis center's patients with a prevalent AVF was lower in counties that were part of a cluster of concentrated poverty than in other counties [difference (95% CI) = -8.4% (-10.3,-6.0 )].The slope of increased AVF use among centers was independent of county poverty and higher in those centers with lower baseline AVF use.The inverse association between the slope and lower baseline AVF use, which occurred more frequently in counties with concentrated poverty, resulted in substantial attenuation of poverty-associated disparities in AVF use at the end of the study illustrated by the narrowing of the differences between the two county categories interquartile ranges and coefficients of variation of the mean slopes (Figure).




**Conclusions:** Improvement rates of AVF use among hemodialysis centers were not significantly associated with poverty within the county where it is located.

*Funding:* Other U.S. Government Support

## FR-PO160

**Choice of Permanent Vascular Access for Dialysis in Elderly Patients (> 75 yo): A Multicenter Observational Study** Maurizio Gallieni,[1] Marcello Napoli,[2] Andrea Bandera,[2] Decenzio Bonucchi,[2] Antonio Granata,[2] Monica Spina.[2] *[1]Ospedale San Carlo Borromeo, Milano; [2]Scientific Committee, Vascular Access Study Group, Italian Society of Nephrology, Roma, Italy.*

**Background:** Currently, patients over 75 yo represent about 30% of the dialysis population in Italy. Median age at start of dialysis in Italian patients rose from 62.8 years in 1999 to 67.2 years in 2009. A concomitant increase in the prevalence of central venous catheters (CVC) was observed. This observational study aimed at better defining the status of vascular access and the clinical policies of Italian nephrologists in elderly dialysis patients.

**Methods:** In 31 dialysis units which spontaneously adhered to this observational study, data were collected regarding all permanent vascular access interventions (AV fistula, CVC, grafts) performed between Jan 2009 and Dec 2011 in a large cohort of elderly dialysis patients (over 75 years old). Both incident and prevalent hemodialysis patients were included.

**Results:** In 1346 patients (811 males, 60.3%), 1584 access interventions were recorded. Mean (±SD) age was 81.0±4.6 years. Comorbidities included diabetes mellitus (25%), heart disease (34%), cancer (13%), central or peripheral vascular disease (35%). In the total population, the chosen procedure was an AV fistula for 51,3% of patient, for 45,2% the insertion of a tunnelled CVC and for 3,5% a graft. Gender was a relevant factor in determining outcome: females had higher chances of having a CVC as dialysis access (54.4 vs. 39%, $p < 0.005$) and conversely lower chance of having an AV fistula (42.1 vs. 57.5%, $p < 0.002$), while grafts were similar (3.6 vs. 3.5%, p: ns). Presence of one or more comorbidities did not affect the patient distribution for dialysis access type. Among the 836 AV fistula interventions, 163 cases (19.5%) of early failure were recorded. None of the specific comorbidities affected AV fistula early failure, but the concomitant presence of 3 comorbid factors was associated with higher chances of early failure ($p < 0.01$).

**Conclusions:** In almost half of patients older than 75 years a tunnelled CVC is the dialysis access of choice. Elderly females, but not diabetics, are more likely to receive a tunnelled CVC.

*Funding:* Government Support - Non-U.S.

## FR-PO161

**A Non-Invasive Approach to Evaluation of Biofilm Burden in Hemodialysis Patients** Amy B. Pai,[1] Adinoyi O. Garba,[1] Jonathan Waldman,[2] Shaker Mousa.[3] *[1]Pharmacy Practice, Albany College of Pharmacy and Health Sciences, Albany, NY; [2]Medicine, Nathan Littauer Hospital, Gloversville, NY; [3]Pharmaceutical Research Institute, Rensselaer, NY.*

**Background:** Central venous catheters (CVCs) are rapidly colonized with Gram-positive bacteria that form intraluminal biofilm. Currently, there are no clinical assessments to detect biofilm in catheters. Lipoteichoic acid (LTA) is Gram-positive bacterial cell wall component that activates the toll-like receptor-2 resulting in transcription of pro-inflammatory cytokines. This pilot study evaluates relative concentrations of LTA in catheter aspirate, plasma and urine of hemodialysis patients.

**Methods:** Eligible patients had tunneled CVCs being used for dialysis access. Catheter aspirate from both ports was collected prior to dialysis initiation. Venous blood (plasma) from the dialysis circuit was collected 30 min after dialysis initiation. Urine was collected from non-anuric patients. Demographic, laboratory, infection and catheter data were collected. LTA concentration in aspirate, plasma and urine was measured by ELISA. For comparison, endotoxin concentration (ET, i.e. Gram-negative cell wall component) was measured by limulus amebocyte lysate (LAL) assay.

**Results:** Twenty-six patients were enrolled in the study. Baseline demographics included; median dialysis vintage 1.2 years (range 0.3 to 10) and mean (SD) duration of catheter use was 85(54.7) days. The median (range) concentration of LTA (ng/mL) and ET (EU/mL) in catheter aspirate was 2.7 (1.5-8.1) and 0.9 (0.23 to 35), respectively. In 10 (38%) of patients, plasma concentration of LTA after 30 minutes of dialysis was on average 7 fold higher ($p<0.001$) than the aspirate concentration. Plasma ET concentrations [median (range)] 0.7 (0.3 to 5.7) were on average lower than catheter aspirate ET. LTA and ET were also detected in all urine samples.

**Conclusions:** These data show that CVCs are likely colonized with Gram-positive and Gram-negative organisms. Systemic concentrations of LTA markedly increased in nearly half of the patients, suggestive of catheter biofilm instability after dialysis initiation. Biofilm as a source of inflammation should be further investigated.

*Funding:* Private Foundation Support

## FR-PO162

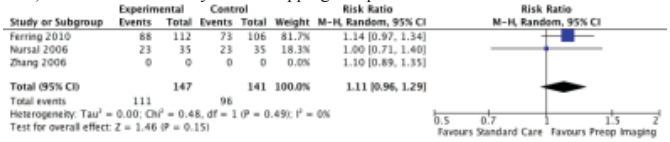
**Preoperative Hemodialysis Vascular Access Evaluation: A Cochrane Systematic Review** Sarah Daisy Kosa,[1] Ahmed A. Al-jaishi,[2] Louise M. Moist,[2] Charmaine E. Lok.[1] *[1]Toronto General Hospital and the Univ of Toronto, Toronto; [2]London Health Sciences Centre, London, Canada.*

**Background:** The high risk of arteriovenous fistula (AVF) failure to mature (FTM) and non-suitability for hemodialysis (HD) (20-60%) limits their greater use. Preoperative vascular access assessment by radiologic imaging (vessel mapping) may help identify vessels most suitable for AVF creation and may improve AVF maturation and use.

**Aim:** To compare the outcomes of preoperative vessel mapping vs. none (standard care) among adult HD patients requiring AVF creation.

**Methods:** We systematically searched for RCTs in the Cochrane Renal Group's specialised register, Cochrane Central Register of Controlled Trials (RCTs), MEDLINE, EMBASE and article refereencess without language restriction. Two reviewers assessed abstract and study inclusion, study quality and extracted data. Dichotomous outcomes were expressed as risk ratios (RR) and continuous outcomes as mean differences with 95% confidence intervals (CI). Outcomes were pooled using a random effects model.

**Results:** We screened 235 abstracts; 8 went to full text abstraction, and 3 were included in the meta analysis. A greater proportion of AVFs were mature at 6 months with vessel imaging (3 studies, 356 patients: RR 1.17, 95% CI 1.00 to 1.36); however, the proportion of AVF s successfully created (3 studies, 356 patients: RR 1.02, 95% CI 0.94 to 1.12) and the proportion of AVF s used for dialysis (2 studies, 288 patients: RR 1.11, 95% CI 0.96 to 1.29) were not increased by vessel mapping compared with standard care.




The proportion of patients requiring a catheter access and the rate of interventions needed to maintain patency did not differ between 2 groups.

**Conclusions:** We cannot recommend routine vessel mapping over standard care without imaging based on the current evidence. Uncertainty surrounds the benefits and there is a paucity of high quality data.

*Funding:* Clinical Revenue Support

## FR-PO163

**The Association of Pre-Dialysis Nephrology Care Intensity and Vascular Access at Hemodialysis Initiation** Vanessa Grubbs,[1] Bernard G. Jaar,[2] Jessica M. Ameling,[2] Raquel Greer,[2] Patti Ephraim,[2] Kerri L. Cavanaugh,[3] L. Ebony Boulware.[2] *[1]UCSF; [2]Johns Hopkins Univ; [3]Vanderbilt.*

**Background:** Despite the 2005 Fistula First Initiative, most patients initiating hemodialysis (HD) in the US continue to do so with catheters. While catheters are often thought the result of emergency initiation among patients with little or no pre-dialysis nephrology care, the role of the intensity of this care in achieving permanent vascular access (ateriovenous fistula or graft, AVF/AVG) has not been explored.

**Methods:** We conducted a retrospective chart review of 76 patients who received nephrology care in academically affiliated public hospital or private practice settings prior to initiating HD between 10/1/2011 and 9/30/2012. Using multivariable logistic regression adjusted for age and insurance, we examined the association of nephrology clinic visits within 6 months of initiating HD with provider action (pre-dialysis referral for AVF/AVG creation) or patient action (completion of at least one step—i.e. evaluation by a vascular surgeon, vein mapping or vascular surgery—toward AVF/AVG use at initiation).

**Results:** Patients' mean age at HD initiation was 60.7 (SD 14.3) years. 30.3% (n=23) had only Medicaid insurance and 18.4% (n=14) were uninsured. 29.0% (n=22) initiated with AVF/AVG. Of the remaining 71.0% (n=54) who initiated with catheter, 48.1% (n=26) had documented pre-dialysis referral for AVF/AVG creation. Among those referred, 69.2% (n=18) completed at least one action step toward AVF/AVG. Only 16.7% (n=9) of catheter patients missed any scheduled nephrology visit. Compared to catheter patients with 0-1 nephrology visits within 6 months of initiation (33.3%), those with 2-3 visits (37.0%) were similarly likely to have provider or patient action [odds ratio 4.0 (95% CI 0.8-20.2), p=0.09 or 3.9 (0.9-17.0), p=0.07], respectively, but those with 4+ visits (29.6%) were more likely to have provider and patient action [OR 6.9 (1.4-35.2), p=0.02 and 6.4 (95% CI 1.1-36.1), p=0.04], respectively.

**Conclusions:** More intense pre-dialysis nephrology care is associated with more provider and patient action toward permanent vascular access at hemodialysis initiation.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO164

**Barriers to Timely Dialysis Access Creation: South Texas University Health System Experience** Christina Mehanni, Faraz M. Khan, Padam Hirachan, Jonathan A. Gelfond, Shweta Bansal. *Div of Nephrology, Univ of Texas Health Sciences Center at San Antonio, San Antonio, TX.*

**Background:** An arteriovenous fistula (AVF), graft (AVG) and peritoneal dialysis catheter are the recommended accesses to start dialysis therapy, however, about 80% patients in the US start dialysis using a catheter. We assessed the rate of suboptimal dialysis start (defined as initiation of dialysis with a catheter) with the aim to identify barriers to timely access creation at the University Health System in San Antonio.

**Methods:** Charts of consecutive incident dialysis patients were reviewed from January 2010 to December 2012. A total of 154 patients were identified.

**Results:** Mean age ± SD of study population was 52.4 ± 13 years, 47% were males and 82% were Hispanics. End stage renal disease was attributed to diabetic nephropathy in 75% patients. Suboptimal starts occurred in 80% of the patients (19% had an immature AVF or AVG at the time of initiation). Patient's demographics, medical history and lab parameters were not different between the suboptimal and optimal start groups. Ninety-two percent patients in the optimal group were seen by a nephrologist for >6 months compared to 66% patients in the suboptimal group (p=0.003). Physician and nurse pre-dialysis education was noted for 86% and 84% patients, respectively in optimal starts versus 33% and 28%, respectively in the suboptimal starts (p<0.001). Among the suboptimal starts, lack of regular health care prior to initiation (40%) and patient related delays (36%) were major barriers to timely access creation. Wait times (median [IQR] in days) to nephrology consult (90[48, 169]), vascular mapping (23[12, 49]), surgery consult (41[27, 62]) and access creation (44[23, 106]) were same between the two groups. On multivariate regression analysis, care by a nephrologist for > 6 months was the only predictor for an optimal start (OR=13, 95% CI 2.3-72).

**Conclusions:** Initiation of dialysis with suboptimal access is common in our system. Lack of regular health care prior to initiation and patient related delays were the major barriers to timely access creation, which are partly modifiable. This study provides the opportunity to test methods to prevent suboptimal starts.

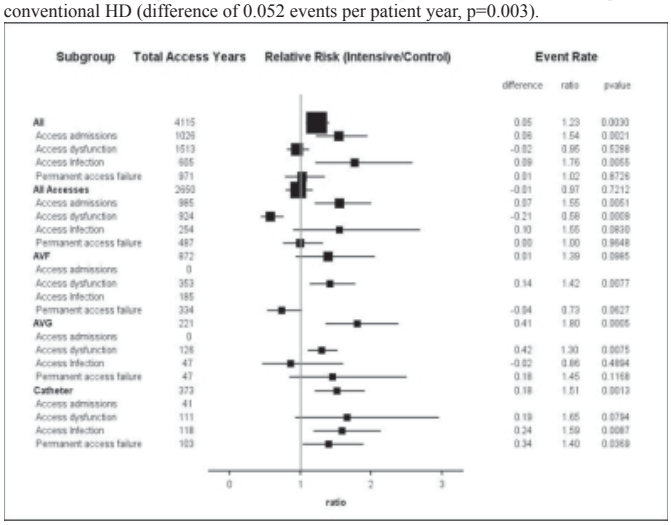
## FR-PO165

**Vascular Access Vulnerability in Intensive Hemodialysis: A Significant Achilles' Heel?** Tom Cornelis,[1] Len A. Usvyat,[2] Peter Kotanko,[2] Yuedong Wang,[3] Karel M.L. Leunissen,[1] Frank Van der Sande,[1] Jan Tordoir,[1] Jeroen Kooman.[1] *[1]Maastricht Univ Medical Center, Maastricht, Netherlands; [2]Renal Research Institute, New York; [3]Univ of California - Santa Barbara, Santa Barbara.*

**Background:** Frequent hemodialysis (HD) may be associated with an increased risk of vascular access complications as compared to conventional HD, especially for arteriovenous fistulas (AVF) and grafts (AVG) due to the increased cannulation frequency. Studies addressing vascular access outcomes in frequent HD so far have shown conflicting results.

**Methods:** We searched Medline and Embase for all trials looking at vascular access outcomes in frequent HD as compared to conventional HD.

**Results:** Nineteen studies met the inclusion criteria; 3 studies used a randomized design, 11 studies were prospective cohort studies, and 5 studies were retrospective cohort studies. The overall vascular access event rate (including access admissions, access dysfunction, access infection and permanent access failure) was higher in intensive HD as compared to conventional HD (difference of 0.052 events per patient year, p=0.003).




Event rates in the AVF group did not differ by HD intensity (conventional 0.155; intensive 0.161; difference 0.006, p=0.099). The overall event rates in the AVG (0.61 in conventional; 1.015 in intensive HD) and catheter (0.36 in conventional; 0.539 in intensive HD) group were higher than in the AVF group. Also, vascular access event rates were higher in intensive as compared to conventional HD patients for both the AVG (difference 0.405, p=0.001) and the catheter (difference 0.179, p=0.001) group.

**Conclusions:** Vascular access is the Achilles' heel also in intensive HD predominantly with AVG and catheters. Therefore, a Fistula First strategy is recommended also for intensive hemodialysis.

## FR-PO166

**Be Careful of Central Venous Stenosis in Chronic Dialysis Patients with a Well-Functioning Arteriovenous Fistula** Mingli Zhu,[1] Yaxue Shi,[2] Yucheng Yan,[1] Zhaohui Ni.[1] *[1]Renal, Renji Hospital, Shanghai Jiaotong Univ School of Medicine, Shanghai, China; [2]Vascular Surgery, Renji Hospital, Shanghai Jiaotong Univ School of Medicine, Shanghai, China.*

**Background:** It is not clear whether patient who is dialyzing with a well-functioning vascular access may be presence of venous stenosis. The aim of this study is assessment of venous stenosis/occlusion by digital subtraction angiography (DSA) in chronic hemodialysis patients with an ipsilateral well-functioning native AVF.

**Methods:** The data were comprised of prevalent hemodialysis patients with a well-functioning mature autogenous radio-cephalic forearm AVF in our single center by 7/31/2012. Total 54 patients were met the criteria of well-functioning AVF defined as adequate delivery of dialysis, no difficulty in cannulation, and no abnormal physical findings. All patients signed informed consents. We performed angiography examinations for these patients. Positive case was defined as any abnormality of relevant vein which was presence of stenosis, occlusion, or collateral branch.

**Results:** Of all 54 eligible patients, 30 (55.5%) were male, the median dialysis vintage was 7.74 (4.2, 12.38) years. The average of blood flow and venous pressure during dialysis session were 247.7±25.98 ml/min and 125.15±27.82 mmHg, and the mean URR and spKt/V were 77.33±4.61% and 1.83±0.27, respectively. Among 54 patients, 21 (39%) were detected positive cases by the angiography. 13 of 54 (24%) had mild central venous stenosis (stenosis <50% diameter with or without collateral branch), one of 54 (2%) had severe central venous stenosis (stenosis >50% diameter), 6/54 (11%) had upper arm vein system occlusion or stenosis, another one had anastomoty stenosis. No significant difference was observed between positive cases and negative cases (including BMI, BSA, hemoglobin, albumin, gender, primary disease, blood flow, venous pressure, URR and spKt/V, p>0.05).

**Conclusions:** The frequency of venous lesion is not low in hemodialysis patients with a well-functioning AVF. Although we don't recommend treating these cases by intervention approach immediately, we suggest to draw attention to these positive results and perform surveillance of access constantly.

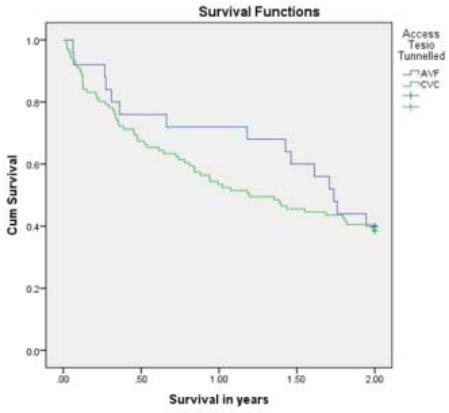
## FR-PO167

**Vascular Access Critical for Prolonged Survival in the over 80 Starting Haemodialysis** Adam Daniel Jakes,[1] Poonam Jani,[3] Archie Lamplugh,[2] Victoria L. Allgar,[3] Sunil Bhandari.[2] *[1]Leeds Teaching Hospitals NHS Trust, United Kingdom; [2]Renal Unit, Hull and East Yorkshire Hospital NHS Trust, United Kingdom; [3]Hull York Medical School, United Kingdom.*

**Background:** Initiating dialysis in patients over 80 years old carries a poor prognosis, but little is known about the most efficient vascular access method in this age group. The creation of an arteriovenous fistula (AVF) is both time consuming and expensive, requiring surgical insertion. A tunnelled central venous catheter (CVC) is a cheaper alternative and requires no surgical intervention but carries a higher risk of infection. We examined whether the creation of an AVF or CVC affected mortality for these patients.

**Methods:** Vascular access and survival data from patients within East Yorkshire was collated using regional databases. Patients must have started elective dialysis over the age of 80 years and received haemodialysis in hospital.

**Results:** 126 patients were included, with 101 initially being dialysed using a CVC and 25 by AVF. 78(62%) were male. The median age of starting dialysis was 82.7(IQR: 81.6-84.5) years and was similar in both CVC and AVF groups; 82.9(81.8-84.7) and 82.0(81.0-82.8) respectively. The median survival was 1.22(1.08-1.35) years, with patients using a CVC surviving 1.17(1.02-1.33) years compared with those using an AVF; 1.40(1.11-1.68) years (p=0.584). The mortality of patients using a CVC at 1 year was 47(47%) in contrast to an AVF; 7(28%), but this was not significant (p=0.094) and after 2 years the survival curves converged. Mortality at 2 years was; CVC 62(61%) and AVF 15 (60%)(p=0.899).




**Conclusions:** There was no statistical difference in survival associated with choice of vascular access for dialysis in elderly patients. Therefore a joint decision between patient and clinician should be considered, encompassing patient preference, fitness for surgery and realistic prognosis related to therapy.

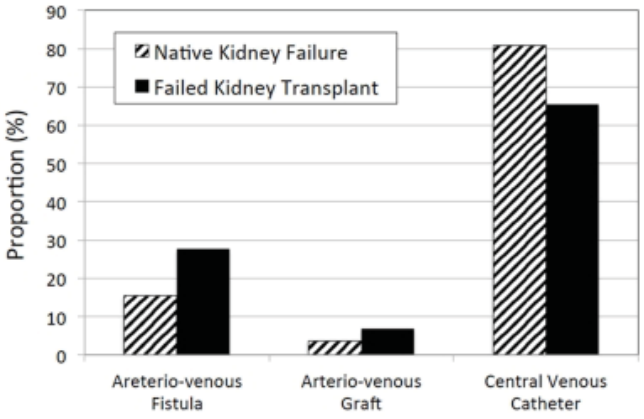
## FR-PO168

**Initial Vascular Access Type in Failed Renal Transplant Recipients** Micah R. Chan,[1] Ahmed I. Al-Absi,[1] Janet Bellingham,[2] Maureen J. Wakeen,[1] Alexander S. Yevzlin,[1] Brad C. Astor.[1] *[1]Nephrology, Univ of Wisconsin, Madison, WI; [2]Transplantation, Univ of Wisconsin, Madison, WI.*

**Background:** Permanent hemodialysis vascular access is crucial for ESRD patients and in those with failed renal transplants,as central venous catheters (CVC) are associated with greater risk of infection and mortality than arterio-venous fistulae (AVF) or arterio-venous grafts (AVG). As renal transplant patients are closely followed by transplant physicians,it would be expected that they have a higher prevalence of AVF/AVG at the time of hemodialysis initiation than those with native kidney failure.

**Methods:** We examined data from USRDS on 16,728 patients with failed renal transplant and 509,643 patients with native kidney failure who initiated dialysis between 1/1/2006 and 9/30/2011.

**Results:** At the initiation of dialysis, 27.7%(n=4,636) of patients with a failed transplant started with an AVF, 6.9%(n=1,146) started with an AVG, and 65.4% (n=10,946) started with a CVC. Conversely,80.8%(n=411,997) patients with native kidney failure initiated dialysis with a CVC (p<0.001).




Among those with failed transplant,predictors of CVC included female(adjusted [OR]=1.74; 95%[CI]: 1.63, 1.87),white race(OR=1.31; 1.22, 1.41), lack of referral to a nephrologist(OR=2.03; 1.74, 2.37), diabetes(OR=1.13; 1.05, 1.22),peripheral vascular disease(OR=1.30; 1.15, 1.47). Factors associated with lower odds of CVC use included public insurance(OR=0.76; 0.71, 0.81),current employment(OR=0.87; 0.80, 0.95),and having a prior failed transplant(OR=0.91; 0.85, 0.99).

**Conclusions:** Central venous catheters are used in more than half of failed renal transplant patients. This group of patients usually are being followed closely by transplant physicians before developing ESRD after a failed transplant,but the relatively low prevalence of AVF/AVG in this group at the initiation of dialysis needs to be investigated more thoroughly.

*Funding:* Clinical Revenue Support

## FR-PO169

**MicroRNA-30 Family InhibitsCalcineurin Signaling in Podocytes** Junnan Wu, Wanfen Zhang, Shaolin Shi, Zhi-hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing Univ School of Medicine, Nanjing, China.*

**Background:** Calcineurin signaling has recently been implicated in the injury of podocytes. Several reagents, including TGF-beta, Lipopolysaccharides (LPS) and puromycinaminonucleoside (PAN), can induce calcineurin signaling in podocytes, but the underlying mechanisms are unknown.We have recently found that miR-30 members are abundantly expressed in podocytes, but all downregulated by TGF-beta, LPS or PAN, leading to podocyte injury. Thus, miR-30s may protect podocytes by inhibiting calcium-calcineurin signaling, and downregulation of miR-30s by TGF-beta, LPS or PAN would enhance calcineurin signaling, leading to podocyte injury.

**Methods:** Conditionally-immortalized human podocyte cell line treated with TGF-beta, LPS or PAN,PAN-treated rats, and the biopsies of FSGS patients were used for the study. miR-30 target validations were performed by luciferase reporter assay and western blotting.

**Results:** We treated podocytes with TGF-beta, LPS or PAN, and found an increase of calcineurin activity, accompanied by downregulation of miR-30s and upregulations of calcineurin signaling components (PPP3ca and NFATc3, which are the predicted miR-30 targets) in the cells. However, exogenous miR-30 expression that sustained the overall level of miR-30s in the podocytes prevented the increase of calcineurin activity and upregulation of PPP3ca and NFATc3 in the treatment of TGF-beta, LPS or PAN. In PAN-treated rats, upregulation of PPP3ca and NFATc3 and downregulation of miR-30s were also observed in the podocytes. However, transferring exogenous miR-30a to the podocytes of the rats prevented the upregulation of PPP3ca and NFATc3 and ameliorated podocyte injury. Calcineurin (PPP3ca)upregulation was also found in the podocytes of FSGS patients, in which miR-30s were downregulated. Finally, luciferase reporter assays confirmed that PPP3ca and NFATc3 are the direct targets of miR-30s inpodocytes.

**Conclusions:** miR-30s inhibit calcineurin signaling in podocytes by directly targeting calcineurin signaling components. Downregulation of miR-30s and the consequent upregulation of calcineurin signaling may be an alternative mechanism by which TGF-beta, LPS or PAN damages podocytes.

*Funding:* Government Support - Non-U.S.

## FR-PO170

**The Paracrine Function of Myofibroblast Was Regulated by miRNA-21 in 5/6 Nephrectomy Mice** Yiwen Li. *ZheJiang Province Hospital.*

**Background:** Myofibroblast is very importment to maintain the normal cardiac function and the cardiac remodeling in pathologic condition. Our provious study found that the paracrine dysfunction of myofibroblast may be responsible for the left ventricular remodeling in uremic cardiopathoy. However, the mechanism of paracrine disregulation of myofibroblast is unknown.

**Methods:** CRF was induced in adult male mice by 5/6 nephrectomy. The mice were killed at 12 weeks after operation. Myofibroblast was derived from sham mice and 5/6 nephrectomy mouse. miRNA-21 was analysized by PCR. IL-1ß, IL-6, TNF-α,TGF-ß.were analysized by ELISA. p-AKT, p-ERK1/2, and p-STAT5 were analysized by west bloting. In the in vitro study, normal medium, and medium with uremic patients serum were used for the myofibroblast culture.

**Results:** Myofibroblas driverd from 5/6 nephrectomy mice showed a significant increased expression of miRNA-21 accompany increasing the concentration of IL-1ß, IL-6, TNF-α,TGF-ß in medium. The uremia patient serum increased the expression of miRNA-21 in normal cultured myofibroblas accompany increasing the expression of p-AKT, p-ERK1/2, and p-STAT5. Inhibited the miRNA-21 expression by siRNA significant reduced the overexpression of p-AKT, p-ERK1/2 and p-STAT5 caused by uremic serum. In same time the concentration of IL-1ß, IL-6, TNF-α,TGF-ß were also decreased in medium.

**Conclusions:** The current study elucidated that myofibroblast paracrine function was regulation by miRNA-21 through p-AKT, p-ERK1/2 and p-STAT5 signals pathways in 5/6 nephrectomy mice.

## FR-PO171

**Number and Function Impairment of Resident c-kit+ Cardiac Stem Cells in Mice with Renal Dysfunction Caused by 5/6 Nephrectomy** Yiwen Li. *ZheJiang Province Hospital.*

**Background:** Cardiac stem cell (CSC) dysfunction exists in various kinds of cardiovascular diseases, and may be responsible for the insufficient regeneration of cardiac myocytes and coronary vessels. However, whether chronic renal failure (CRF) affected CSC is unknown.

**Methods:** CRF was induced in adult male mice by 5/6 nephrectomy. The mice were killed at 12 weeks after operation. C-kit+ CSC numbers was evaluated by flow cytometer. Apoptosis and DNA damage of C-kit+ CSC in the control and CRF mice was analyzed by immunohistochemistry. In the in vitro study, normal medium, and medium with uremic rat serum were used for the CSC culture.

**Results:** CSC counts attenuated significantly in the chronic renal failure model, whereas apoptosis cells and 8-OHdG-positive cells significantly increased. CSC derived form 5/6 nephrectomy mice heart showed an impaired anti-oxidant potential. In the cultured cells, CSCs subjected to uremic rat serum showed a higher frequency of TUNEL stain-positive and 8-OHdG-positive cells. The uremia rat serum reduced the expression of hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) in CSC.

**Conclusions:** The current study elucidated that CSC number and function disorders existed in mice with chronic renal insufficiency. Apoptosis, oxidative stress and reduced angiogenic factors secretion caused by uremic toxins in serum are contributors to CSC dysfunction.

**FR-PO172**

**MicroRNA-22 Is a Master Regulator of Bone Morphogenetic Protein-7/6 Homeostasis in the Kidney** Jianyin Long,[1,2] Shawn S. Badal,[1,2] Yin Wang,[1,2] Farhad R. Danesh.[1,2] *[1]Dept of Medicine/Nephrology, Baylor College of Medicine, Houston, TX; [2]Section of Nephrology, The Univ of Texas MD Anderson Cancer Center, Houston, TX.*

**Background:** Accumulating evidence suggests that microRNAs (miRNAs) contribute to a myriad of kidney diseases. However, the regulatory role of miRNAs on the key molecules implicated in kidney fibrosis remains poorly understood. Bone morphogenetic protein-7 (BMP-7) and its related BMP-6 have recently emerged as key regulators of kidney fibrosis. Using the established unilateral ureteral obstruction (UUO) model of kidney fibrosis, we examined the regulatory role of miRNAs on BMP-7/6 signaling.

**Methods:** We employed several *in silica* analyses to identify conserved, putative, BMP-7/6 targeting miRNAs. Isolation of renal fibroblasts, real-time qPCR of mRNA and miRNA, miRNA *in situ* hybridization, oligonucleotide-directed mutagenesis, and luciferase reporter assays were carried out as previously described.

**Results:** miR-22 was predicted to target BMP-7/6 *in silica* and validated to downregulate BMP-7/6 expression *in vitro*. To further assess the effect of miR-22 *in vivo*, we generated a miR-22 null mouse using a targeted strategy to delete the whole exon containing *pri-miR-22*. We found that knockout of miR-22 significantly attenuated renal fibrosis in the UUO kidney fibrosis model. Interestingly, targeted deletion of miR-22 led to robust elevation of BMP-7/6 protein expression and downstream signaling *in vivo*. We further demonstrated that miR-22 directly binds to 3'-untranslated region (UTR) of BMP-7, BMP-6 and BMPR1B mRNAs *in vitro*. Importantly, transfection of miR-22 mimic into primary renal fibroblasts isolated from miR-22-deficient mice partially restored the pro-fibrogenic genes induced by TGF-β1. Finally, we found that miR-22 by itself is induced by BMP-7/6, and identified two BMP-responsive elements (BREs) in the proximal promoter region of miR-22 gene.

**Conclusions:** Our data suggest that miR-22 serves as a master regulator of BMP signaling cascade and renal fibrosis. Our findings also indicate that miR-22 and BMP-7/6 form a regulatory feedback circuit, where not only miR-22 inhibits BMP-7/6, but miR-22 by itself is induced by BMP-7/6 signaling.

*Funding:* NIDDK Support

**FR-PO173**

**The Mechanism of Pleiotropic Effect in Endothelial Cells with Pitavastatin Treatment** Tsuyoshi Inoue,[1,2] Yasuharu Kanki,[2] Tatsuhiko Kodama,[2] Youichiro Wada,[2] Masaomi Nangaku,[1] Takashi Maejima.[2] *[1]Div of Nephrology and Endocrinology, The Univ of Tokyo; [2]Laboratory for Systems Biology and Medicine, Research Center for Advanced Science and Technology, The Univ of Tokyo.*

**Background:** CKD is an important risk factor for progression of atherosclerosis related cardiovascular disorders. Statins are widely-used cholesterol lowering drugs. Multiple lines of evidence, including randomized clinical trials, suggested that statins also directly protect the kidney and vascular cells. These favorable, so-called "pleiotropic effects" are supposed to occur, in part, through alterations in vascular cell gene expression.

**Methods:** We used various epigenetic methods such as Chromatin Immunoprecipitation assay with deep sequencing (ChIP-seq), Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET), Chromosome Conformation Capture (3C)-qPCR assay and 3D-Fluorescence In Situ Hybridization (FISH).

**Results:** Histological analysis using ApoE knockout mouse showed that pitavastatin treatment reduced plaque and macrophage area in spite that the total cholesterol and triglyceride levels were not significantly affected. Repeated microarray analyses of HUVECs treated with pitavastatin identified KLF4 and KLF2 as the most highly induced genes. The up-regulation of these genes was confirmed with mRNA from aortic tissue of ApoE deficient mice. By series of knockdown experiments, MEF2C activated through MEK5/ERK5 pathway was involved in pitavastatin-dependent gene induction. Among MEF2 family, we focused on MEF2C, and identified a novel functional MEF2C binding site at 148 kb upstream of the KLF4 gene by ChIP-seq followed by luciferase assay. By applying whole genome and quantitative chromatin conformation analysis (ChIA-PET and 3C), we observed that the MEF2C-bound enhancer and transcription start site (TSS) of KLF4 took closer spatial proximity by pitavastatin. 3D-FISH imaging confirmed the conformational change in single cell manner.

**Conclusions:** These results showed that drug-responsive chromatin conformation change could be involved in one of the various gene induction mechanisms induced as some part of the pleiotropic effects of the statins in endothelial cells.

*Funding:* Government Support - Non-U.S.

**FR-PO174**

**The Mechanism of ANGPTL4 Induction, a Mediator of Nephrotic Syndrome, through Its Chromatin Conformational Change** Tsuyoshi Inoue,[1,2] Imari Mimura,[1] Takahide Kohro,[2] Toshiya Tanaka,[2] Hiroyuki Aburatani,[2] Tatsuhiko Kodama,[2] Youichiro Wada,[2] Masaomi Nangaku.[1] *[1]Div of Nephrology and Endocrinology, The Univ of Tokyo; [2]Laboratory for Systems Biology and Medicine, Research Center for Advanced Science and Technology, The Univ of Tokyo.*

**Background:** Angiopoietin-[zwj]like 4 (*ANGPTL4*) has drawn attention as a cause of minimal change nephrotic syndrome recently, but the mechanism of this gene induction has not been elucidated yet.

**Methods:** In addition to Chromatin Immunoprecipitation (ChIP) assay with deep sequencing (ChIP-seq) analysis of hypoxia inducible factor (HIF) 1α, peroxisome proliferator-activated receptor (PPAR) β/δ and RNA polymerase (Pol) II, we used epigenetic methods including ChIP-seq of histone marks, Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) and Chromosome Conformation Capture (3C)-PCR assay to dissect the molecular mechanism of the gene expression.

**Results:** Detailed investigation of HIF1 dependent gene expression led us to realize the importance of crosstalk between PPAR β/δ and HIF1 signaling axes. Migration assay showed that synergistic enhancement with these two stimuli, and *ANGPTL4* was identified as the common target gene by the combination of microarray and ChIP-seq analysis. The reporter assay combined with ChIP-seq identified the functional hypoxia responsive element (HRE) and PPAR responsive element (PPRE) in the promoter and intron regions of *ANGPTL4*. The profile changes of the enhancer histone mark (H3K27ac) under hypoxia, PPARβ/δ ligand, and dual stimulations suggested that the spatial proximity of two response elements was the principal cause of the synergistic transcription induction. ChIA-PET supported the interaction between the two regions, and further, newly developed quantitative 3C-PCR assay showed the quantitative change of the frequency of proximity.

**Conclusions:** These results imply that hypoxic and PPARβ/δ stimulations are important for activation of *ANGPTL4* transcription, and the synergistic activation is achieved by spatial proximity formation in *ANGPTL4* loci.

*Funding:* Government Support - Non-U.S.

**FR-PO175**

**Vascular Endothelial Growth Factor-C Production Stimulated by TNF-α through p38/HSP27 Pathway and Its Therapeutic Reduction in Human Proximal Renal Tubular Cells** Hideki Kimura,[1] Daisuke Mikami,[2] Kazuko Kamiyama,[2] Naoki Takahashi,[2] Kenji Kasuno,[2] Haruyoshi Yoshida,[3] Masayuki Iwano.[1] *[1]Dept of Clin Lab, Univ of Fukui Hosp, Fukui, Japan; [2]Div of Nephrol, Sch of Med, Univ of Fukui, Fukui, Japan; [3]Div of Nephrol, Obama Municipal Hosp, Fukui, Japan.*

**Background:** Vascular endothelial growth factor-C (VEGF-C) promotes lymphatic angiogenesis associated with renal inflammation and fibrosis in chronic and active kidney disease (CKD) where glucocorticoids and angiotensin II receptor type 1 blockers (ARB) are widely used as a main remedy. VEGF-C production status and its modification by the two main remedies have remained unclarified in human proximal renal tubular cells (HPTECs) under inflammation.

**Methods:** Confluent HPTECs were treated for 24h with putative VEGF-C inducers in abscense or presence of specific kinase inhibitors (SB203580, H89, PD98059), dexamethsone (DXA;100 nM) or Telmisartan (Telm; 1-10μM), an ARB with peroxisome proliferator-activated receptor (PPAR) activity. VEGF-C expression was assessed by RT-PCR and ELISA. Phosphorylation of signalling molecules and PPAR activity were examined by immunoblotting, and PPRE-luc assay and specific inhibitors, respectively.

**Results:** In HPTECs, TNF-α (1-10 ng/ml), advanced glycation endproducts, and high glucose induced VEGF-C by 2.8-fold, 1.3-fold, and 1.2-fold, respectively, while TGF-β (1-5 ng/ml) and hypoxia (1%O2) had no influence on the production. VEGF-C production by TNF-α was mediated via phosphorylation of p38 MAPK/HSP27 pathway but not ERK or NFkB pathway. HSP27 silencing by siRNA reduced VEGF-C expression. DXA reduced TNF-α-induced VEGF-C production at mRNA and protein levels by about 40% via decreasing p38/HSP27 phosphorylation. Like DXA, Telm also decreased basal and TNF-α-induced VEGF-C production by about 30%. Moreover, Telm served as a specific PPAR-δ activator, and reduced VEGF-C production which was partially explained by PPAR-δ-dependent suppression of p38 phosphorylation.

**Conclusions:** Renal parenchymal inflammation may induce VEGF-C production in proximal renal tubular cells, possibly facilitating the surrounding lymphatic vessel formation. Major remedies for CKD may modulate lymphatic angiogenesis as well as fibrotic status.

**FR-PO176**

**The Effects of Endothelial Microparticles Induced by Indoxyl Sulfate on TGF-β Signaling in Vascular Smooth Muscle Cells** Jung-hwa Ryu, Shina Lee, Dong-Ryeol Ryu, Duk-Hee Kang, Kyu Bok Choi, Seung-Jung Kim. *Dept of Internal Medicine, Ewha Womans Univ School of Medicine, Seoul, Republic of Korea.*

**Background:** Vascular access failure is one of the critical risk factor for the mortality or morbidity in hemodialysis (HD) patients. The venous neointimal hyperplasia (VNH) is the main pathogenesis of access stenosis, and recently it was reported that the proliferation of vascular smooth muscle cells (VSMCs) promoted by activation of TGF-β signaling

took a main portion for VNH. Endothelial microparticles (EMPs) are produced by various inflammatory stimulants associated with endothelial injury and increased in various vascular disease and uremic condition. However, it has not been established about the definite effects of EMPs on VSMCs. This study is aimed to isolate the EMPs induced by indoxyl sulfate(IS), well-known uremic toxin and to investigate whether the EMP directly stimulates the downstream of TGF-β signaling in VSMCs.

**Methods:** Human umbilical vein endothelial cells (HUVECs) were cultured and stimulated by IS of different concentrations. The culture media of HUVECs were collected and spun once and then ultracentrifugated at 100,000 Xg. The pellet was resuspended, incubated with fluorescent endothelial antibodies and analyzed by flow cytometry with comparing calibration beads. The near-confluent human aortic smooth muscle cells (HASMCs) were treated by this collected EMPs. After incubation, HASMCs were solubilized with lysis buffer and the activation of TGF-β signaling analyzed by western blotting for phophated-p38, -ERK1/2, -Akt, and -Smad3.

**Results:** The EMPs were well produced by IS, the quantity of them was similar to the amount generated by positive control, TNF-α(10ng/mL). In western blot analysis, EMPs produced by IS stimulated whole subsignals of p38, ERK1/2, Akt, and Smad3 in HASMCs with similar densities of blots stimulated by TGF-β.

**Conclusions:** The isolation of EMPs is confirmed by flow cytometry and these EMPs stimulated the subsignals of TGF-β in VSMCs. Further investigation is needed to demonstrate how EMPs stimulate the subsignals of TGF-β and affect on proliferation of VSMCs and to evaluate the importance of TGF-β signaling in vascular pathology.

## FR-PO177

### Microvesicles Released by Vascular Endothelial Cells Increase Hypoxia Inducible Factor Expression in Human Proximal Tubular HK-2 Cells

Javier Lucio,[1] Ana Valdehita,[1] Julia Carracedo,[2] Rafael Ramirez,[1] Ana Belen Fernandez.[1] *[1]Universidad Alcala, Spain; [2]Hospital Reina Sofia Cordoba, Spain.*

**Background:** Co-culture experiments suggest that vascular endothelial cells may influence transcriptional regulation of HK-2 cells and that several hypoxia-inducible factors (HIF)-dependent genes were upregulated (Am J Physiol 2008, 294:C543-C554). Here we aimed to investigate whether endothelial microvesicles (EMVs) increase the expression of HIF and the production of the putative renal protection factor HIF-regulated VEGF-A in HK-2 cells.

**Methods:** HK-2 cells were incubated for 0-48 h with 200.000 EMVs/ml (isolated from TNF-α—treated human umbilical vascular endothelial cells) and expression of HIF-1α and HIF-2α and production of VEGF-A were measured by WB and ELISA, respectively.

**Results:** Expression of HIF-1α and HIF-2α and production of VEGF-A increased over the controls. HIF inhibitor YC-1 partially prevented the increase in VEGF-A. We have recently found that the sequential activation of cyclooxygenases (COX), epidermal growth factor receptor (EGFR), mitogen- and stress.-activated kinase-1 (MSK-1) and retinoic acid receptor-β (RARβ) up-regulates HIF-1α This pathway was also involved in EMVs-induced increase in HIF since it was sensitive to diclofenac, AG1478, H89 and LE135 (which inhibit respectively COX, EGFR, MSK-1 and RARβ). Interestingly, we also found an increased content in EMVs in human urine samples taken from patients with diseases known to induce chronic vascular inflammation, which indicates that tubular cells are exposed to EMVs in vivo.

**Conclusions:** These results suggest that EMVs might influence the in vivo expression of HIF, and thereby that of its target genes, in proximal tubular cells.

*Funding:* Government Support - Non-U.S.

## FR-PO178

### Hyaluronic Acid Induces Lymphangiogenesis in Mouse Unilateral Ureteral Obstruction Model via Stimulation of Macrophage VEGF-C Production

Won Kim, Aesin Lee, Yujin Jung, Dal Kim, Kyung Pyo Kang, Sik Lee. *Internal Medicine, Research Institute of Clinical Medicine, Chonbuk National Univ Medical School, Jeonju, Republic of Korea.*

**Background:** Hyaluronic acid (HA) is one of important component of extracellular matrix proteoglycan. HA level is increased in chronic inflammation and has many biologic and pathologic effects such as inflammation, angiogenensis, wound healing and tissue remodeling. Our group reported that VEGF-C and VEGF-D from macrophages and tubular cells had induced lymphangiogenesis in mouse unilateral ureteral obstruction (UUO) model. However, there is few data about the effect of HA on lymphangiogenesis in renal fibrosis. To elucidate the role of HA in UUO-induced lymphangiogenesis, we investigated the expression of HA in UUO-induced lymphangiogenesis and the effect of HA on VEGF-C production in macrophages.

**Methods:** *In vitro* lymphangiogenic effects of HA in human lymphatic endothelial cells (hLECs) and *>in vivo* effect of HA on VEGF-C expression in UUO-induced lymphangiogenesis were evaluated.

**Results:** In UUO kidney, HA expression was increased in tubulointerstitial area 7 d after UUO. The density of LYVE-1-positive lymphatic endothelial cells was significantly increased in cortical interstitial fibrotic areas. HA expression correlates with lymphangiogenesis in UUO. VEGF-C expression was increased and F4/80-positive cells are costained with VEGF-C in kidney section after 1 w of UUO. About 35% of F4/80-positive macrophages was co-stained by HA and macrophage depletion with clodronate significantly decreased UUO-induced lymphangiogenesis. Our results also showed that low molecular weight HA induced capillary-like tube formation in the hLECs and treatment of HA in bone marrow derive marcrophages increased the expression of VEGF-C in a dose- and time-dependent manner.

**Conclusions:** These results suggest that HA has a lymphangiogenesis effects in UUO-induced renal fibrosis via stimulation of VEGF-C production in macrophages.

*Funding:* Government Support - Non-U.S.

## FR-PO179

### CD148 Tyrosine Phosphatase Promotes Cadherin Cell Adhesion

Keiko Takahashi, Anton Matafonov, Hideyuki Ito, Dana Zemel, Katherine Sumarriva, Rebecca S. Weller, Takamune Takahashi. *Vanderbilt Univ, Nashville, TN.*

**Background:** CD148 is a transmembrane tyrosine phosphatase that is expressed in renal endothelium and tubular cells. CD148 is accumulated at cell-cell contacts. Recent biochemical studies demonstrated that CD148 associates with VE- or E-cadherin/catenin complex, and p120 catenin serves as a substrate. However, the function of CD148 in cadherin adhesion is still unknown. We therefore addressed this issue using a series of stable cells and cell-based assays.

**Methods:** Wild type (wt) and catalytically inactive (c-s) CD148 was stably introduced to A431D (lacking cadherins), A431D/E-cad (expressing E-cadherin), and A431D/E-cadAAA764 (expressing p120-uncoupled E-cadherin) cells. The effects of CD148 on cadherin cell adhesion were assessed by $Ca^{2+}$ switch, cell aggregation, and cell density assays. Phosphorylation of E-cadherin/catenin complex and Rho family GTPase activities were examined using IP/Western blot or Rhotekin/PAK assay.

**Results:** CD148wt, but not CD148c-s, extended cadherin contact zone as early as 30 min after cadherin engagement ($Ca^{2+}$ switch assay) and remarkably strengthened cell-cell adhesion (aggregation assay) in A431D/Ecad cells, although CD148 introduction did not change E-cadherin/catenin levels and complex formation. These effects accompanied p120 and β catenin dephosphorylation and an increase in Rac1, but not RhoA and cdc42, activity. Interestingly, CD148wt increased phosphorylation of p120 Y228 (a Src kinase site) on cell contacts together with an increase in Src activity (well known CD148 activity). The effects of CD148wt to strengthen cell adhesion were abolished by Rac1 inhibition. CD148wt showed no effects in A431D and A431D/EcadAAA764 cells. Notes: β catenin was not dephosphorylated by CD148 in vitro, suggesting β catenin dephosphorylation in intact cells may be a secondary effect; CD148 showed similar effects in endothelial cells.

**Conclusions:** CD148 regulates p120 and β catenin phosphorylation upon cell adhesion via the direct and indirect mechanisms, increases Rac1 activity and extends cadherin contacts, strengthening E- (and VE-) cadherin adhesion. This CD148 effect requires Ecad-p120 interaction.

*Funding:* NIDDK Support

## FR-PO180

### Kidney Microvascular Endothelial Cells Exhibit Organ Specific Angiogenic Characteristics in 3D Culture

Takahide Aburatani,[1] Giovanni Ligresti,[1] Ying Zheng,[2] Jeremy Stuart Duffield.[1] *[1]Dept of Medicine, Univ of Washington, Seattle, WA; [2]Dept of Bioengineering, Univ of Washington, Seattle, WA.*

**Background:** The kidney microvasculature is frequently disrupted in Acute Kidney Injury and CKD and shows poor angiogenic responses, leaving a rarefied vasculature and a kidney prone to ischemia, tubular dysfunction and the development of CKD. The reason why the kidney microvascular is so susceptible are poorly understood, and one leading hypothesis has been this response is due to the nature of kidney pericytes. Kidney microvascular endothelial cells (KMVECs), however, have scarcely been studied because of difficulties in culturing them ex vivo.

**Methods:** We isolated, established primary cultures, and characterized KMVECs from 4-6 weeks old mice. The gene expression profile of mKMVEC was analyzed by RT-PCR and their characteristics evaluated by examining their responsiveness to cytokines including VEGF, and pericytes in 2D and 3D in vitro assays.

**Results:** We established methods for culturing mKMVEC isolated by FACS sorting. All cultures were used prior to passage four. In 2D culture they maintain expression of EC proteins. RT-PCR analysis showed that mKMVECs expressed S1P1 other than typical endothelial markers (CD31, VEGFR2, VE-cad, and Ang2). 2D assays such as wound scratch assay and Alamar blue proliferation assay showed that they migrate and proliferate in response to VEGF164 stimulation, but responses were blunted by VEGF120 and VEGF188. mKMVECs were evaluated in 3D angiogenesis assays. Sprouting with tip cell and lumen formation was induced by VEGF and further stimulated by adding macrophages in 3D collagen gel but was extremely poor compared to other EC cell types. By contrast mKMVEC avidly made tubes with tight functions in 3D collagen gel when stimulated by PMA or pericytes.

**Conclusions:** Isolated and cultured mKMVECs express unique genes, as well as typical EC genes. In addition they showed unique endothelial properties in vitro including markedly reduced angiogenic sprouting but a propensity to reassemble capillaries. These properties unique to kidney MVECs may contribute to poor angiogenic responses after kidney injury which predispose to CKD.

**FR-PO181**

**The Effect of Angiotensin (1-7) on Glomerular Angiopoietins-Tie-2 and Its Potential Mechanisms** Chengyan Xu,[1] Wei Ding,[1] Minmin Zhang,[1] Yong Gu.[1,2] *[1]Nephrology, Huahsan Hospital, Fudan Univ, Shanghai, China; [2]Nephrology, The Fifth People's Hospital of Shanghai, Fudan Univ, Shanghai, China.*

**Background:** Angiopoietins-Tie-2 is one of the important angiogenesis system in glomeruli. Though Ang (1-7) has been reported inhibiting endothelial cell tube formation in vitro in human umbilical vein endothelial cells, the research regarding its effect in glomerular angiogensis has not been fully understood. This study aimed to observe the effects of Ang (1-7) on glomerular angiopoietins-Tie-2 system and its potential mechanisms.

**Methods:** The mouse glomerular endothelial cells (MGECs) and podocytes were cultured. Under the condition with/without exogenous Ang II, the cells were treated with Ang (1-7), Losartan, A-779 and PD98059 respectively. Real-time PCR was applied to test the gene expression of Ang-1 in podocytes and Ang-2 in MGECs treated with different doses of Ang (1-7) and AngII. The protein expression of Ang-1, Ang-2, Tie-2, phosphorylate eNOS and ERK1/2 was detected by Western blot. The ability of endothelial cells tube formation was observed by Matrigel method.

**Results:** Losartan and Ang(1-7) co-inhibited the expression of aniopoietins-Tie-2 without exogenous Ang II. While under the condition of exogenous AngII, the downregulating effect of Ang(1-7) on angiopoietin-Tie-2 expression was even more significant ($P < 0.05$). However, the suppressing effect of Ang(1-7) on aniopoietins-Tie-2 could be reversed by Losartan, A-779 and PD98059 ($P < 0.05$). Furthermore, the eNOS pathway could be activated by Ang (1-7) ($P < 0.05$). However, under the condition of exogenous Ang II, the phosphorylate eNOS was downregulated whereas the phosphorylated ERK1/2 was upregulated, which could be reversed by L-NAME ($P < 0.05$).

**Conclusions:** Ang(1-7) inhibited angiogenesis and resisted the effect of AngII of promoting the expression of aniopoietins-Tie-2. With the pretreatment of exogenous AngII, Ang(1-7) inhibited the expression of angiopoietins-Tie-2 more remarkably via downregulating eNOS and activating ERK1/2 pathway, which could be reversed by Losartan.

*Funding:* Government Support - Non-U.S.

**FR-PO182**

**Inhibition of $Ca^{2+}$ Influx through Reverse-Mode $Na^+/Ca^{2+}$-Exchange (NCX) Preserves Endothelial Barrier Function in Response to Thrombin: Implications for CKD** Petros Andrikopoulos, Julius Edward Kieswich, Steven Michael Harwood, Magdi Yaqoob. *Translational Medicine and Therapeutics, William Harvey Research Institute, London, United Kingdom.*

**Background:** CKD patients exhibit high levels of plasma thrombin activity which could have a direct effect on the endothelium through its main receptor protease activated receptor-1 (PAR-1). This may result in loss of barrier function causing pulmonary and peripheral oedema that are hallmarks of CKD and exacerbate the severity of this multifaceted disease. We recently reported that in human endothelial cells (HUVECs), $Ca^{2+}$ influx through reverse-mode NCX is critical for ERK1/2 activation and angiogenesis by VEGF. Here, we investigated whether NCX activity also plays a role in ERK1/2 activation and endothelial barrier dysfunction by thrombin.

**Methods:** ERK1/2 activation was investigated by western blot, $Ca^{2+}$ transients were monitored with Fluo-4NW and permeability was assessed by FITC-dextran flux through an EC monolayer. *In vivo* permeability was determined by the extravasation of Evans blue-albumin (20mg/Kg) in mice stimulated with a PAR-1 agonist peptide (1mg/kg).

**Results:** ERK1/2 activation by thrombin required influx of $Ca^{2+}$ and was suppressed by the specific reverse-mode NCX inhibitors SN-6 and SEA0400 dose- and time-dependently. SN-6 or SEA0400 also attenuated thrombin-induced $Ca^{2+}$-transients. Knocking down NCX1 with siRNA suppressed thrombin-induced ERK1/2 activation. Conversely, loading ECs with $Na^+$ by inhibiting the $Na^+$-$K^+$-ATPase with ouabain, accelerated ERK1/2 activation. Reverse-mode NCX inhibitors also suppressed ERK1/2 activation in response to a peptide agonist of PAR-1. Inhibiting reverse-mode NCX with SN-6 and SEA0400 or knocking down NCX1 significantly preserved endothelial barrier function in response to thrombin. Finally, SEA0400 suppressed Evans Blue-Albumin extravasation to the lung and kidneys of C57bl/6 mice and attenuated oedema formation, in response to PAR-1 activation.

**Conclusions:** $Ca^{2+}$ influx through reverse-mode NCX could be a novel determinant of vascular permeability in response to thrombin and drugs targeting reverse-mode NCX could be beneficial in CKD patients with diminished endothelial barrier function.

**FR-PO183**

**1a, 25-Dihydroxyvitamin $D_3$ Inhibits TNF-α-Induced LOX-1 Expression via Suppression of Rac1 Activation in Human Aortic Smooth Muscle Cells** Wonseok Yang, Su-Kil Park. *Internal Medicine, Asan Medical Center, Seoul, Republic of Korea.*

**Background:** 1α, 25-Dihydroxyvitamin $D_3$($1,25D_3$)is used to treat secondary hyperparathyroidism associated with end-stage renal disease (ESRD). Atherosclerosis is highly prevalent in ESRD patients. Lectin-like oxidized low-density lipoprotein receptor-1(LOX-1) expressed on vascular smooth muscle cells is implicated in atherosclerosis.

**Methods:** We explored the effect of$1,25D_3$ontumor necrosis factor α (TNF-α)-induced LOX-1 expression in cultured human aortic smooth muscle cells.

**Results:** TNF-α increased LOX-1expression, while it was inhibited by preincubation with $1,25D_3$ in a dose-dependent manner. TNF-α-induced LOX-1expression was mediated by Rac1, reactive oxygen species (ROS) and spleen tyrosine kinase (Syk). TNF-α-induced Syk activation was inhibited by both NSC 23766 (Rac1 inhibitor) and N-acetylcysteine (NAC). $1,25D_3$ suppressed TNF-α-induced Rac1 activation, ROS expression and Syk activation. In summary, TNF-α-induced LOX-1 expression occurred through Rac1/ROS/Syk. $1,25D_3$ attenuated TNF-α-induced LOX-1 expression by inhibition of Rac1 activation and thereby subsequent downstream signals.

**Conclusions:** Through inhibition of LOX-1 expression on aortic smooth muscle cells, $1,25D_3$ may have an anti-atherosclerotic effect.

*Funding:* Private Foundation Support

**FR-PO184**

**Activation of mTOR Modulates SREBP-2 to Induce Foam Cell Formation in Vascular Smooth Muscle Cells through Increased Retinoblastoma Protein Phosphorylation** Kun Ling Ma, Jing Liu, Yang Zhang, Wu Yu, Jie Ni, Bi-Cheng Liu. *Institute of Nephrology, Southeast Univ School of Medicine, Nanjing, China.*

**Background:** Our previous studies demonstrated that inflammation contributes to foam cell formation through the disruption of low density lipoprotein receptor (LDLr) pathway. However, this effect is overridden by rapamycin, which is an inhibitor of mammalian target of rapamycin (mTOR). This study investigated the role of mTOR pathway in foam cell formation in vascular smooth muscle cells (VSMCs).

**Methods:** Rat VSMCs were treated with or without lipopolysaccharide(LPS) in the absence or presence of rapamycin or mTOR siRNA. Lipid accumulation in VSMCs was measured by Oil red O staining. The expression and protein phosphorylation of mTOR pathway, LDLr pathway, and retinoblastoma tumour suppressor protein (Rb) were checked by immunofluorescent staining, real-time PCR, and Western blotting.

**Results:** Inflammation increased lipid accumulation in VSMCs, which were correlated with increased expressions of LDLr, sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP), and SREBP-2, as well as with enhanced translocation of SCAP/SREBP-2 complex from endoplasmic reticulum (ER) to Golgi. Furthermore, inflammation increased both the percentage of cells in the S phase of cell cycle and protein expressions of the phosphorylated forms of Rb, mTOR, eukaryotic initiation factor 4E-binding protein 1 (4EBP1), and p70 S6 kinase. After treatment with rapamycin or mTOR siRNA, the activity of mTOR pathway was blocked, and the phosphorylation of Rb was also inhibited. Interestingly, the expression levels of LDLr, SCAP, and SREBP-2 and the translocation of SCAP/SREBP-2 complex from the ER to the Golgi in VSMCs were accordingly decreased even in the presence of inflammatory stress.

**Conclusions:** Inflammation disrupts LDLr feedback regulation through the activation of mTOR pathway. Increased mTOR complex-1activity was found to upregulate SREBP-2-mediated cholesterol uptake through Rb phosphorylation.

**FR-PO185**

**Activation of mTOR Modulates LDL Receptor at Transcriptional and Post-Transcriptional Level to Accelerate Lipid Accumulation in Fatty Livers of Apolipoprotein E Knockout Mice** Kun Ling Ma, Jing Liu, Yang Zhang, Wu Yu, Bi-Cheng Liu. *Institute of Nephrology, Southeast Univ School of Medicine, Nanjing, China.*

**Background:** Our previous studies demonstrated that inflammation exacerbates lipid accumulation in hepatic cells. However, these effects are overridden by rapamycin, which is an inhibitor of mammalian target of rapamycin (mTOR). This study aimed to investigate the role of mTOR pathway in lipid accumulation of fatty livers.

**Methods:** Apolipoprotein E knockout mice were subcutaneously injected with 10% casein to induce chronic inflammation. Serum levels of serum amyloid A, tumour necrosis factor-a, and lipid profiles were measured by enzyme-linked immunosorbent assay and clinical biochemistry assay. Lipid accumulation was evaluated by haematoxylin-eosin and filipin staining.The associated protein expression of mTOR and low-density lipoprotein receptor (LDLr) pathways were checked by immunohistochemical staining, immunofluorescent staining, and Western blotting.

**Results:** Inflammation significantly reduced serum levels of lipid profile compared to the controls. However, inflammation increased lipid accumulation in livers, correlated with increased expressions of LDLr, sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP), and SREBP-2, as well as with enhanced translocation of SCAP/SREBP-2 complex from endoplasmic reticulum (ER) to Golgi. Meanwhile, upregulated LDLr gene transcription recruited proprotein convertase subtilisin kexin 9 (PCSK9) with accordingly increased PCSK9 protein expression. Furthermore, inflammation increased the mTOR phosphorylation. After treatment with rapamycin, mTOR activity was inhibited, accompanied with accordingly decreased expression of LDLr, SCAP, and SREBP-2, decreased translocation of SCAP/SREBP-2 complex from the ER to the Golgi, and increased PCSK9 expression even in the presence of inflammatory stress.

**Conclusions:** The activation of mTOR pathway caused by inflammation disrupted the regulation of LDLr at transcriptional and post-transcriptional level, resulting in lipid accumulation and exacerbated the progression of fatty liver.

**FR-PO186**

**Effect of Cylcosporine Treatment on Akt-FOXO Signaling in $C_2C_{12}$ Muscle Cells** Jill Rahnert,[1] Bin Zheng,[1] Russ Price.[1,2] *[1]Dept of Medicine, Emory Univ, Atlanta, GA; [2]VA Medical Center, Atlanta, GA.*

**Background:** Muscle wasting is a consequence of chronic diseases such as chronic kidney disease (CKD) and type I diabetes (DM). Insulin/IGF-1 deficiency, combined with chronic elevation of glucocorticoids, promotes protein degradation by activating the FOXO

transcription factors. This results in increased expression of key components of multiple proteolytic systems including the ubiquitin-proteasome pathway. Insulin and IGF activate Akt which phosphorylates FOXO, thereby inhibiting its activity; FOXO activity is also repressed by the transcription coactivator, PCG-1α. Calcineurin (Cn), a $Ca^{2+}$-activated phosphatase, induces PGC-1α expression and promotes a slow-twitch fiber phenotype that is inherently less susceptible to atrophy. Although Cn itself does not directly regulate muscle mass, it is down-regulated in multiple models of muscle atrophy including CKD and DM, suggesting that Cn may indirectly affect protein degradation. This is an important point because renal transplant patients routinely take the Cn-inhibitor, cyclosporine A (CsA). Our experiments tested the hypothesis that acute inhibition of Cn with CsA sensitizes muscle to atrophic signals by altering upstream Akt/FOXO signaling.

**Methods:** $C_2C_{12}$ muscle cells were treated with phorbol 12-myristate 13-acetate (PMA) and thapsigargin (Tg) for 1 hour to increase intracellular $Ca^{2+}$ levels and activate Cn, with or without CsA.

**Results:** PMA/Tg increases the nuclear localization of the transcription factor NFATc3, the canonical target of Cn, 2.7-fold and this is prevented by CsA (PMA/Tg x CsA, p=0.013). PMA/Tg or CsA produce minimal changes in the phosphorylation status of Akt and FOXO. ERK mediated FOXO phosphorylation increases 1.6-fold with PMA/Tg and this is sensitive to CsA (PMA/Tg x CsA, p=0.046). However, neither Akt nor ERK signaling is sufficient to see significant changes in FOXO localization.

**Conclusions:** Together, these data do not support a role for Cn-mediated regulation of the Akt-FOXO axis. If down-regulation of Cn increases muscle fiber susceptibility to atrophy, it does so by a different mechanism.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO187

**Vitamin D Receptor (VDR) Expression Determines p53-Induced Tubular Cell Phenotype** Gautam Kishore Valecha, Nirupama Chandel, Partab Rai, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, New York, NY.*

**Background:** P53 displays a bimodal effect on cellular growth-p53 over expressing cells are prone to apoptosis, whereas, p53 mutant cells display proliferation mode. We hypothesize that vitamin D recepetor (VDR) plays a key role in p53-mediated bimodal tubular cell growth.

**Methods:** Human renal proximal tubular cells (HRPTC) were either transfected with siRNA-p53 (siRNA-p53/HRPTC), scrambled siRNA (SCR/HRPTC), or p53 plasmid (p53/HRPTC). Protein blots of C/HRPTCs, siRNA-p53/HRPTC, SCR/HRPTC, and p53/HRPTC were probed for VDR, angiotensinogen (Agt) renin, phospho-p66ShcA, and reprobed for total p66ShcA and actin. To confirm the activation of the renin angiotensin system (RAS), Ang II content of the cellular lysates was measured by ELISA.To determine the modulation of reactive oxygen species (ROS) generation, cells were co-labeled with Red CC1 and mitotracker green in the presence/absence losartan (an Ang II blocker), and a VDR agonist (VDA, EB1089). Cells treated under above mentioned conditions, were also evaluated for occurrence of apoptosis by TUNEL assay/Western blotting for caspase-3 cleavage and for proliferation by labeling for proiiferative cell nuclear antigen (PCNA).

**Results:** p53/HRPTC displayed down regulation of VDR, enhanced expression of renin and Agt and elevated levels of Ang II when compared to C/HRPTC and SCR/HRPTCs; however, EB1089 inhibited these effects of p53. p53/HRPTC showed enhanced expression of phospho-p66ShcA and increased generation of ROS. p53/HRPTC displayed cleavage of caspase-3 and increased number of TUNEL +ve cells. Since both VDA and losartan attenuated enhanced ROS generation as well as apoptosis in p53/HRPTCs, it appears that p53 mediated tubular cell apoptotic phenotype through VDR-induced activation of the RAS. On the other hand, siRNA-p53/HRPTC displayed enhanced VDR expression and down regulation of the RAS and attenuated generation of ROS. siRNA-p53/HRPTC not only showed resistance to apoptosis but also exhibited enhanced number of PCNA +ve cells.

**Conclusions:** These findings indicate that VDR expression determines p53-induced tubular cell phenotype.

*Funding:* NIDDK Support

## FR-PO188

**Novel Biological Gas $H_2S$ Regulates the Length of Primary Cilia of Kidney Tubular Epithelial Cell** Sang Jun Han,[1] Jee In Kim,[1,2] Kwon Moo Park.[1,2] *[1]Anatomy, Kyungpook National Univ School of Medicine; [2]Cardiovascular Research Institute, Kyungpook National Univ School of Medicine.*

**Background:** Malformation of primary cilia is associated with various kidney diseases such as polycystic kidney disease and ischemia/reperfusion (I/R)-induced acute kidney injury. Others have previously reported that hydrogen sulfide ($H_2S$) attenuated I/R-induced injury in hepatic, myocardial, renal I/R model. Our recent work revealed that length of primary cilia largely depends on oxidative stress and ERK activation. $H_2S$ is known to have antioxidative effect. However, the role of $H_2S$ in cilliogenesis is unknown yet.

**Methods:** To elucidate the role of $H_2S$ in cilia elongation and underlying mechanism, we investigated exogenous $H_2S$ donor NaHS and inhibitors of endogenous $H_2S$ producing enzymes propargylglycine (PAG, an inhibitor of CSE , cystathionine gamma lyase) and hydroxylamine (HA, an inhibitor of CBS , cystathionine beta synthase) in the kidney tubular epithelial cells. To determine the primary cilia length, cells were immunofluorescence stained with acetylated tubulin antibody.

**Results:** NaHS treatment increased expressions of Arl13b and Sec10 which are associated with cilia formation, and elongated primary cilia. Treatment of PAG and HA prevented the elongation of primary cilia. PAG or HA shortened the length of primary cilia. NaHS induced cilia elongation by upregulation of phopho-ERK, Sec10 and Arl13b expressions, whereas PAG and HA suppressed cilia elongation with downregulation of phopho-ERK, Sec10 and Arl13b.

**Conclusions:** Taken together, our results suggest that $H_2S$ accelerate primary cilia elongation by ERK activation, and provide a better understanding of molecular mechanism underlying cilia length regulation in the kidney.

*Funding:* Government Support - Non-U.S.

## FR-PO189

**The Mammalian Target of Rapamycin (mTOR) Complex Gets Activated during Tight Junction Biogenesis** Laurence Poma, Jean-olivier Defraigne, Jean-marie H. Krzesinski, Francois Jouret. *GIGA Cardiovascular Sciences, ULg CHU, Liege, Belgium.*

**Background:** The tight junctions (TJ) between renal tubular cells regulate paracellular diffusion and cell differentiation. TJ disruption in acute kidney injury represents one of the earliest events. Still, the signaling network involved in TJ assembly and maintenance remains poorly characterized. The AMP-activated protein kinase (AMPK) has been implicated both *in vitro* and *in vivo* in TJ regulation. Several AMPK kinases have been identified, with Liver kinase B1 (LKB1) and $Ca^{2+}$/calmodulin-dependent protein kinase kinase β (CAMKKβ) being the main ones. In turn, AMPK coordinates various housekeeping mechanisms, including the inhibition of the mammalian target of rapamycin (mTOR) complex. Here, we pharmacologically modulated the AMPK network at the time of a $Ca^{2+}$ switch in order to further characterize TJ biogenesis.

**Methods:** The $Ca^{2+}$ switch, i.e. switching cell culture conditions from low [5 µM] to high [1.8 mM] $Ca^{2+}$ concentrations, is a widely used model to study TJ assembly in Madin-Darby canine kidney (MDCK) cells.

**Results:** A $Ca^{2+}$ switch is not only associated with AMPK phosphorylation (Thr172) and activation, as stated by the phosphorylation (Ser79) of acetyl-CoA carboxylase (ACC), but also with the activation of mTOR, as indicated by an increased phosphorylation (Thr389) of p70 S6 kinase. Incubation of MDCK cells with the AMPK activator, phenformin [5mM], in low or high $Ca^{2+}$ conditions induced AMPK and ACC phosphorylation and mTOR inhibition, as previously reported. Still, phenformin treatment at the time of a $Ca^{2+}$ switch did not prevent p70 phosphorylation. By contrast, exposure to the mTOR inhibitor, rapamycin [20nM], inhibited p70 phosphorylation, even following a $Ca^{2+}$ switch. Similarly, cell loading with the $Ca^{2+}$ chelator, BAPTA-AM [50µM], before a $Ca^{2+}$ switch abolished p70 phosphorylation, with no impact on AMPK activation. Conversely, the inhibition of CAMKK by STO-609 [50µM] at the time of a $Ca^{2+}$ switch abrogated AMPK phosphorylation but did not hamper mTOR activation.

**Conclusions:** A $Ca^{2+}$ switch in MDCK cells, which ultimately leads to TJ assembly, independently involves both the AMPK and mTOR pathways, with a differential role of intracellular $Ca^{2+}$.

*Funding:* Private Foundation Support, Clinical Revenue Support, Government Support - Non-U.S.

## FR-PO190

**Hgf Stimulates Gsk3-Dependent Lrp5/6 Phosphorylation in Proximal Tubular Cells** Farrukh M. Koraishy,[1] Cynthia J. D'Alessandri,[3] Sherene Mason,[2] Lloyd G. Cantley.[1] *[1]Section of Nephrology, Yale Univ, New Haven, CT; [2]Section of Pediatric Nephrology, Yale Univ, New Haven, CT; [3]Section of Pediatric Nephrology, CT Children's Medical Center, Hartford, CT.*

**Background:** The signaling processes that promote renal repair after acute kidney injury (AKI) remain poorly defined. Two signaling cascades that are activated following AKI are hepatocyte growth factor (Hgf)/Met and Wnt/β-catenin pathways. A critical early event in Wnt signaling is phosphorylation of the co-receptors Lrp5/6, leading to nuclear translocation of β-catenin. We have previously demonstrated that Hgf stimulation of subconfluent TECs leads to increased nuclear β-catenin activity, due to inhibition of Gsk3 via S9 phosphorylation. In the current study we further investigated the cross-talk between these pathways.

**Methods:** Mouse proximal tubule (MPT) cells were stimulated with Hgf or Wnt3a followed by western blotting and immunoprepcitation experiments. For ischemia reperfusion injury (IRI), gGT-Cre;$Met^{fl/fl}$mice (lacking Met receptor in the proximal tubule) or γGT-Cre;$Met^{+/+}$controls were subjected to warm left kidney ischemia with contralateral nephrectomy, followed by reperfusion.

**Results:** In MPTs, Hgf was found to rapidly stimulate Lrp5/6 phosphorylation at 3 separate activation motifs with maximal effect in subconfluent, de-differentiated cells. The phosphorylation was dependent on Gsk3 kinase activity but independent of Wnt. Hgf treatment stimulated the selective association of active Y216/Y279 phosphorylated Gsk3 with Lrp5/6 while S9 phosphorylated inactive Gsk3 was excluded from this association. In vivo, Lrp5/6 phosphorylation was increased within 24 hours after IRI and this was significantly reduced in gGT-Cre;$Met^{fl/fl}$mice as compared to γGT-Cre;$Met^{+/+}$controls.

**Conclusions:** Our results identify a novel mechanism of Hgf mediated transactivation of Wnt signaling that involves the Met-dependent selective association of active Gsk3 with Lrp5/6. The likely relevance of this pathway for epithelial injury responses is supported by the observation that the initial increase in Lrp5/6 phosphorylation after renal IRI in mice is dependent on the Met receptor.

*Funding:* NIDDK Support, Private Foundation Support

**FR-PO191**

**Tubular Secretion of Indoxyl Sulfate and Residual Renal Function** Sachin Jhawar,[1] Prabhjot Singh,[1] Jiri Zavadil,[1,2] Jerome Lowenstein.[1] *[1]Medicine, NYU Langone Medical Center, New York, NY; [2]Medicine, NYU Langone Medical Center, New York, NY; [3]WHO International Agency for Research on Cancer, NYU Langone Medical Center, New York, NY; [4]Medicine, NYU Langone Medical Center, New York, NY.*

**Background:** Residual Renal Function (RRF), persistent urine production in patients with ESRD, is associated with better patient survival, less severe atherosclerotic vascular disease, and lower plasma concentrations of a putative protein-bound, poorly dialyzable uremic toxin, indoxyl sulfate, IS. This solute is normally secreted via Organic Anion Transporters (OATs) in the proximal renal tubule rather than filtered at the glomerulus.

**Methods:** We devised a reporter system using cultured human renal tubular cells to compare gene expression profiles (using Affymetrix gene chip) in response to incubation with pre- and post-dialysis plasma from subjects with RRF (n=5) and anuric subjects (n=5). Total IS in plasma was measured by HPLC.

**Results:** Patients with RRF had lower pre- (36.3±18.7 μg/ml) and post-dialysis (19.8±12.8 μg/ml) total IS levels than patients without RRF (pre- 51.2±24.8 μg/ml, post-dialysis 27.3±11.9 μg/ml). We examined the hypothesis that plasma from subjects with RRF might display lesser activation of inflammatory pathways than plasma from patients without RRF. Patterns of gene expression in cultured cells revealed a differential response between the two groups. Our tubular cell reporter system detected broad differential biological effects of pre- and post-dialysis plasma from patients with RRF compared to those without RRF, as determined by Gene Set Enrichment Analysis. Plasma from patients without RRF elicited greater activation of the TGF-β pathway and dysregulation of additional pathways that might underlie fibrotic changes or vasculopathy.

**Conclusions:** These findings suggest a role for poorly dialyzable uremic toxins in the pathogenesis of renal scarring and uremic vasculopathy. We suggest that Residual Renal Function may reflect an important component of fluid generated by tubular secretion rather than glomerular filtrate. If true, this paradigm would warrant close attention to the avoidance of medications that compete for transport by OATs.

*Funding:* Private Foundation Support

**FR-PO192**

**The Effect of Farnesyl ThioSalicylic Acid (FTS) on TGFb1 Signaling in Murine Proximal Tubule Epithelial Cells (PTEC)** Subash Somalanka,[1] Claire C. Sharpe,[2] Mysore Keshavmurthy Phanish,[1] Mark Edward Dockrell.[1] *[1]SWTIRR, St. Helier Hospital, London, United Kingdom; [2]Renal Medicine, Kings College, London, United Kingdom.*

**Background:** Targeting Ras-GTPases has been shown to ameliorate renal fibrosis in recent studies. Our group has published that in human PTEC, CTGF expression was upregulated by TGFβ1 in an FTS sensitive manner. Expression of CTGF is regulated by multiple signaling pathways. Hence we probed the downstream effects of TGFβ1 signaling in murine PTEC by using FTS, a Ras inhibitor.

**Methods:** Cells derived from S3 segment of murine proximal tubule of a SV40 transformed brinster transgenic mouse were used for in vitro experiments. Serum starved cells were treated with TGFβ1 [2.5ng/ml] +/- MEK inhibitor PD98059 [20uM] & FTS [5uM] for 5min & 1h for signaling studies & 24h for CTGF protein expression. Cells were pre-treated for 30min in the presence of the chemical inhibitors where appropriate. Cellular lysates were analysed by western blotting. Bio-Plex Multiplex phosphoproteins assay was used to study activation of Akt, ERK1/2, GSK-3α/β, Histone H3, JNK, MEK1 & p38MAPK.

**Results:** TGFβ1 induced 'secreted CTGF' (1.5 fold, $p<0.001$) which was inhibited by PD98059 & FTS ($p<0.001$) at 24h. TGFβ1 induced SMAD activation & phosphorylation of Histone H3 ($p<0.001$) which was inhibited in the presence of FTS [SMAD ($p<0.001$), Histone H3 ($p<0.01$)]. TGFβ1 did not induce PI3-kinase activation ($p=0.08$). The effect of FTS on ERK1/2 activation, p38 MAPK & MEK1 was significant in the presence of TGFβ1 ($p=0.032$, $p= 0.032$ & $p= 0.013$). There was no significant effect of FTS on JNK at 5 mins.

**Conclusions:** Our results show that TGFβ1-driven induction of CTGF is via the Ras/ERK pathway in murine PTECs. Investigation of intracellular signaling produced unexpected & diverse results. Surprisingly FTS inhibited canonical & non-canonical SMAD pathways including pSMAD2 linker region. Inhibition of pSmad2 linker region by FTS may be due to inhibition of GSK3β/Akt at 1h. We also demonstrate for the first time that TGFβ1 rapidly phosphorylates Histone H3 in an FTS sensitive manner. Some of the effects of FTS may not involve Ras and hence more selective Ras inhibition may require other strategies such as antisense oligonucleotides.

**FR-PO193**

**Selective Proximal Tubule Injury Causes Interstitial Fibrosis and Distal Tubule Impairment** Koji Takaori,[1] Jin Nakamura,[1] Tadashi Yamamoto,[2] Motoko Yanagita.[1] *[1]Nephrology, Kyoto Univ, Kyoto, Japan; [2]Structural Pathology, Institute of Nephrology, Niigata Univ, Niigata, Japan.*

**Background:** Recently we clarified that renal fibroblasts including erythropoietin (Epo) producing cells transdifferentiate into myofibroblast and predominantly contribute to fibrosis, with concomitant loss of Epo production in the diseased kidney. It remains unclear, however, what triggers the transdifferentiation of fibroblasts to myofibroblasts and how proximal tubule injury affects other segment of the nephron.

**Methods:** For in vitro analysis, we utilized co-culture of renal fibroblasts and tubular epithelial cells. For in vivo analysis, we utilized *N-myc downstream-regulated gene-1 (Ndrg1)CreERT2* inducible simian diphtheria toxin receptor (DTR) transgenic mice (*Ndrg1CreERT2:iDTR* mice) in which Cre-mediated excision of a STOP cassette is achieved after the administration of tamoxifen, and renders *proximal tubules* sensitive to diphtheria toxin (DT).

**Results:** First, we confirmed that DTR is expressed in almost all proximal tubules and a part of collecting duct in the kidney of *Ndrg1-CreERT2:iDTR* mice after the administration of tamoxifen. A single DT injection to these mice causes proximal tubule injury and interstitial fibrosis accompanied with the proliferation of proximal tubules and fibroblasts. We also confirmed the induction of collagen expression in fibroblasts when co-cultured with damaged tubular epithelial cells. We further demonstrated the induction of distal tubule injury after the administration of DT to *Ndrg1-CreERT2:iDTR* mice.

**Conclusions:** Our data provide the new evidence that selective proximal tubule injury induces the transdifferentiation of fibroblast as well as distal tubule impairment. These results indicate the importance of protecting tubule epithelial cells to suppress kidney disease progression. Further understanding of the crosstalk between proximal tubule and fibroblast as well as the crosstalk between proximal tubule and distal tubule will give us new insight into the mechanism of kidney disease progression.

**FR-PO194**

**Biological Properties of Klotho in the Anti-Fibrotic Process and Establishment of Cell Integrity in Coordination with Cell Signaling Pathway /Translocation of Membrane Transporters** Ken Tsuchiya,[1] Shunji Shiohira,[1] Miki Nishida,[1] Kazuhiro Okano,[2] Hidekazu Sugiura,[3] Kosaku Nitta.[1] *[1]Dept of Medicine IV, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Div of Blood Purification, Tokyo Women's Medical Univ; [3]Dept of Pathology, UT Southwestern Medical Center, Dallas.*

**Background:** Klotho, which exerts anti-aging properties, is known to be acting as an obligate co-receptor of FGF23 to regulate phosphate homeostasis, and also to be involved in Insulin/IGF1 and WNT signaling, suggesting the Klotho should play important roles in establishing specific cell functions and also more primitive cell integrity and identity.

**Methods:** We explored the biological properties of Klotho in cell differentiation and proliferation quantified by using CL-Quant software to analyze time-lapse images in a Nikon Biostation CT combining with a high-throughput cell migration assay, and cell polarity by measuring the expression of Na/K ATPase/PKD-ζ, marker of cell signaling (β-catenine) in 3D culture with collagen mixture. Internal expression of Klotho was modified by siRNA transfection.

**Results:** Recombinant Klotho suppressed the accelerated migration and expression of fibrotic markers of cultured renal fibroblast cells (NRK49F cells) in which internal Klotho expression was negligible, stimulated by TGF-β1. In addition, Quercetin, a Wnt/β-catenine inhibitor, attenuated cell migration and up-regulated E-cadherin expression, induced Klotho expression three fold of mRNA and protein in IMCD cells in which Klotho expression has been confirmed. Klotho siRNA reduced the expression of Klotho, resulting in accelerating cell migration. Moreover, addition of recombinant Klotho up-regulated adhesion molecule and Na/K ATPase/ PKC-ζ translocation to the cell membrane not association with the expression of mRNA level, and Klotho siRNA tended to suppress the effect but was not significant.

**Conclusions:** Taken together, it is likely that Klotho acts anti-fibrotic process in part medicating Wnt/β-catenine pathway and also contribute to establish cell integrity by up-regulation of cell adhesion molecules and one of possible mechanism mediating translocation of membrane transporters.

*Funding:* Government Support - Non-U.S.

**FR-PO195**

**Severe Proximal Tubular Injury after Kidney-Specific Knockdown of Stanniocalcin-1** Luping Huang, Tatiana Belousova, Jenny S. Pan, David Sheikh-Hamad. *Nephrology/Medicine, Baylor College of Medicine, Houston, TX.*

**Background:** Stanniocalcin-1 (STC1) is a paracrine/intracrine protein; it binds to the cell surface, followed by internalization to the mitochondria; it diminishes superoxide generation through induction of uncoupling proteins (UCPs). In vitro, STC1 inhibits macrophages and preserves endothelial barrier function; and transgenic mice which overexpress STC1 globally and display high serum levels of STC1 are protected from anti-GBM GN and ischemia/reperfusion (I/R) kidney injuries. To determine the phenotype following kidney-specific knockdown of STC1, we employed two novel approaches.

**Methods:** We generated STC1 shRNA Tg and scrambled shRNA Tg mice that express STC1 or scrambled shRNA, respectively, upon removal of floxed reporter [phosphoglycerate kinase (PGK)-driven EGFP]; and 2) using ultrasound microbubbles, we delivered to the kidney a plasmid that expresses Cre-recombinase directed by the endothelium specific tyrosine-protein kinase receptor-2 (Tie2) promoter – to achieve kidney endothelium-specific expression of STC1 shRNA or scrambled shRNA.

**Results:** STC1 mRNA is widely expressed in the kidney. Delivery of Tie2-Cre to STC1 shRNA Tg kidneys diminished the expression of STC1 mRNA and protein throughout the kidney 4-fold. We observed no change in STC1 mRNA or protein in similarly-treated scrambled shRNA Tg kidneys. Knockdown of STC1 in STC1 shRNA Tg kidneys was associated with: severe proximal tubular injury (segments that express AQP1) characterized by vacuolization, decreased UCP2 expression, greater generation of superoxide, activation of the unfolded protein response and initiation of autophagy and apoptosis.There was no

change in Cre protein expression in cultured and Tie2-Cre-transfected proximal tubule cells, suggesting that knockdown of STC1 expression in epithelial cells in vivo is likely related to overflow of STC1 shRNA from endothelial cells.

**Conclusions:** These novel observations suggest a critical role for STC1 in tubular epithelial survival. The above approach provides efficient tool to knockdown the expression of any gene in the kidney – avoiding confounding extra-renal effects that may be associated with global gene deletion.

*Funding:* NIDDK Support

### FR-PO196

**Protein Overload Induces Renal Proximal Tubular Epithelial Cell Apoptosis by Down-Regulating WNT/β-Catenin Signalling** Dickson W.L. Wong, Wai Han Yiu, Hao-Jia Wu, Ruixi Li, Joseph C.K. Leung, Loretta Y.Y. Chan, Kar Neng Lai, Sydney C.W. Tang. *Dept of Medicine, Queen Mary Hospital, The Univ of Hong Kong, Hong Kong.*

**Background:** Numerous studies have demonstrated a tubulotoxic role of excess proteins on renal proximal tubular epithelial cell (PTECs) via various signalling pathways. However, the role of Wnt/β-catenin signalling in PTECs during protein overload remains unknown.

**Methods:** Wnt/β-catenin expressions were measured in control and human serum albumin (HSA)-treated human kidney 2 (HK-2) cells by real-time PCR and Western blotting. Genetic knockdown of β-catenin was achieved using siRNA transfection. Apoptotic phenotypes were evaluated by real-time PCR and TUNEL assay.

**Results:** Upon the 4-day-HSA stimulation, gene expression of β-catenin, frizzled-receptor 1 and Wnt-1 in PTECs declined by 26%±2 ($p<0.05$, t-test), 65%±2 ($p<0.05$) and 57%±6 ($p<0.05$) versus control, respectively. Western blots showed that protein expression of cytosolic and nuclear active β-catenin decreased by 57%±8 ($p<0.05$) and 66%±8 ($p<0.05$) after 4-day HAS treatment, respectively. Simultaneously, Bax/Bcl-2 gene expression ratio increased by 31%±8 ($p<0.05$). Transfection of β-catenin siRNA into HK-2 cells up-regulated Bax/Bcl-2 gene expression ratio by 23%±7 ($p<0.05$) relative to mock transfection. HAS treatment and β-catenin siRNA transfection increased the number of TUNEL-positive cells by 70%±10 ($p<0.05$) and 73%±8 ($p<0.05$), respectively.

**Conclusions:** Protein-overload promotes tubular cell apoptosis via down-regulation of Wnt/β-catenin signalling in PTECs. **Funding:** Research Grants Council of Hong Kong (GRF grant number 7782/12M).

### FR-PO197

**Src Kinase Mediates Renal Interstitial Fibroblast Activation and Renal Fibrogenesis in Obstructive Nephropathy** Shougang Zhuang. *Dept of Medicine, Rhode Island Hospital and Alpert Medical School of Brown Univ, Providence, RI.*

**Background:** Renal Interstitial fibroblast activation and proliferation is central to the development and progression of renal fibrosis after various insults, however, the signaling mechanism regulating this process is incompletely clear.

**Methods:** In this study, we examined the role of Src, a non-receptor tyrosine kinase in renal interstitial fibroblast activation and proliferation in cultured rat renal interstitial fibroblasts and a murine model of obstructive nephropathy induced by unilateral ureteral obstruction.

**Results:** Exposure of cultured renal interstitial fibroblasts (NRK-49F) to PP1, a specific Src inhibitor, resulted in decreased expression of alpha-Smooth muscle actin and fibronectin, two hallmarks of fibroblast activation and type 1 collagen, a key extracellular matrix protein, in a dose-dependent manner. Silencing Src with siRNA also significantly inhibited the expression of these three proteins. Moreover, inhibition of Src kinase activity with either PP1 or siRNA, also blocked cell proliferation, decreased expression of cyclin D1, cyclin B1 and cyclin E and increased expression of p27 and p21. Finally, STAT3, AKT and ERK 1/2 were phosphorylated in cultured NRK-49 cells and presence. of PP1 or knockdown of Src with siRNA inhibited phosphorylation of all of them. In a murine model of obstructive nephropathy, administeration of PP1 attenuated abolished the expression of fibronectin, largely suppressed expression of α-SMA and type I collagen, and reduced the deposition of extracellular matrix proteins.

**Conclusions:** Collectively, our results reveal an important role of Src in mediating activation and proliferation of renal interstitial fibroblasts and renal fibrogenesis, and suggest that Src inhibition might be a potential therapeutic approach for treatment of chronic fibrotic kidney disease.

*Funding:* NIDDK Support

### FR-PO198

**20-HETE Monoglyceride Induces ERK1/2 Phosphorylation via a Cannabinoid Receptor-Mediated Signaling Pathway** Kimberly Fisher, Jianchun Chen, Jorge H. Capdevila, Raymond C. Harris. *Medicine, Vanderbilt Univ, Nashville, TN.*

**Background:** We have previously shown that glycerol derivatives of epoxyeicosatrienoic acid activate cannabinoid receptors in the kidney. The present study was designed to investigate whether monoglycerol derivatives of the cyp450 arachidonic acid metabolite, 20-HETE are present endogenously and activate cannabinoid receptors.

**Methods:** For measuring distribution of 20-HETE in mouse plasma, we identified pools by resolution of plasma extracts by TLC on $SiO_2$ plates using mixtures of hexane/ether/acetic acid as solvent. Pools with Rfs corresponding to triglycerides (TG) diglycerides (DG) and monoglycerides (MG) were collected from the plates and submitted to alkaline hydrolysis, and the released 20-HETE quantified by UPLC/MS/MS by collision induced fragmentation, and product ion monitoring. To analyze the effect of 20-HETE and 20-HETE MG on ERK1/2 phosphorylation, Chinese hamster ovary (CHO) cells were transiently transfected with plasmid vector, CB1 (cannabinoid receptor-1), or CB2 (cannabinoid receptor-2), then stimulated with 20-HETE or 20-HETE MG for 10 minutes. Cannabinoid receptor-specific inhibitors were used to determine the role of CB1 and CB2 in the phosphorylation of ERK1/2 resulting from 20-HETE MG stimulation.

**Results:** 20-HETE was prevalent in mouse plasma, and 90% of the 20-HETE was found to be esterified. Immunoblot analysis demonstrated that in CHO cells transfected with either CB1 or CB2 cannabinoid receptors, ERK1/2 phosphorylation increased 3.5-fold ($p<0.05$, $N=5$) after stimulation with 20-HETE MG, but not when treated with non-esterified 20-HETE. ERK1/2 phosphorylation by 20-HETE MG was blocked by the CB1- and CB2-specific inhibitors, AM251 or AM630, respectively.

**Conclusions:** In summary, these studies suggest that 20-HETE in plasma circulates predominantly in an esterified form, and 20-HETE monoglyceride might serve as endocannabinoid. Further studies will be required to elucidate the physiologic and/or pathophysiologic role of this novel compound.

*Funding:* NIDDK Support, Veterans Affairs Support

### FR-PO199

**Phenylbutyric Acid, an ER Chemical Chaperone, Protects against Renal Fibrogenesis In Vivo and In Vitro** Ching-chin Yang,[1] Cheng-tien Wu,[1] Shing-Hwa Liu,[1] Chih-Kang Chiang.[1,2,3] *[1]Institute of Toxicology, National Taiwan Univ, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ, Taipei, Taiwan; [3]Dept of Integrated Diagnostics & Therapeutics, National Taiwan Univ Hospital, Taipei, Taiwan.*

**Background:** Renal tubulointerstitial fibrosis is the common and final pathologic changes of end-stage renal disease (ESRD). Our recent works demonstrated that endoplasmic reticulum (ER) stress contributed to the pathophysiological mechanisms during the development of renal fibrosis. We found that up-regulation of transforming growth factor-β (TGF-β) is associated with the activation of ER stress in the rat unilateral ureteral obstruction (UUO) renal fibrotic model. ER stress-related signals might lead to renal tubular apoptosis and fibrosis. However, the detail mechanisms that ER stress involved in the renal injury and fibrosis have not been completely clarified.

**Methods:** In this study, we applied ER chemical chaperon, sodium 4-phenylbutyrate (4-PBA), in the rat UUO renal fibrosis model and the normal rat kidney epithelial cells (NRK-52E). We explored the modulation of ER-associated signals and profibrotic pathways by 4-PBA.

**Results:** By using this ER-related chemical chaperon to mimics endogenous ER chaperon, we successfully enhanced the effects of unfolded protein response (UPR) in the UUO model. We found that 4-PBA successfully attenuated UUO-induced ER stress, which associated with the suppression of profibrotic factors, including collagen, fibronectin, plasminogen activator inhibitor-1 and connective tissue growth factor. In addition, we also shown that tubulointerstitial injury, renal apoptosis and fibrosis were attenuated by the administration of 4-PBA in the UUO rats. In vitro model, we also demonstrated that 4-PBA attenuated TGF-β-induced ER stress, tubular apoptosis and profibrotic factors activation in the NRK-52E. We successfully demonstrated that the attenuation of ER stress led to lesser renal apoptosis and fibrosis.

**Conclusions:** These results indicate that 4-PBA may have therapeutic potential in renal fibrosis. It is deserve to explore the potential ER stress targets in preventing renal fibrosis.

### FR-PO200

**c-Cbl, a Ubiquitin E3 Ligase That Targets Active β-Catenin–A Novel Layer of Wnt Regulation** Sowmya Shivanna, Vipul C. Chitalia. *Dept of Medicine/ Renal Section, Boston Univ Medical Center, Boston, MA.*

**Background:** Regulation of transcriptionally active nuclear β-catenin during Wnt-on phase is crucial to ensure controlled induction of Wnt target genes. Several ubiquitin E3 ligases are known to regulate cytosolic β-catenin during Wnt-off phase, but little is known about the fate of active nuclear β-catenin in Wnt-on phase.

**Methods:** Following methods were employed: Cell culture, transfection, western blotting, immunoflorescence, cellular fractionation, luciferase assay, ELISA.etc., as per reference literature.

**Results:** We now describe ubiquitination of active β-catenin in Wnt-on phase by a RING finger ubiquitin E3 ligase, Casitas B-lineage lymphoma (c-Cbl) in endothelial cells (EC). c-Cbl binds preferentially to nuclear active β-catenin in Wnt-on phase via armadillo repeat region. Wild-type c-Cbl suppresses and E3 ligase-deficient c-Cbl-70Z increases Wnt signaling. Wnt induces nuclear translocation of c-Cbl where it ubiquitinates nuclear β-catenin. Deletion of c-Cbl UBA domain abrogates its dimerization, binding to β-catenin, Wnt-induced c-Cbl nuclear translocation and ubiquitination of nuclear β-catenin. c-Cbl activity inhibits pro-angiogenic Wnt targets IL-8 and VEGF levels and angiogenesis in β-catenin-dependent manner.

**Conclusions:** This study defines for the first time c-Cbl as an ubiquitin E3 ligase that targets nuclear active β-catenin in Wnt-on phase and uncovers a novel layer of regulation of Wnt signaling.

*Funding:* NIDDK Support

**FR-PO201**

**Cross Talk between p66ShcA and p70S6K Determines Kidney Cell Injury in HIV-Associated Nephropathy** Partab Rai, Tejinder Singh, Rivka Lederman, Andrei Plagov, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

**Background:** HIV-associated nephropathy (HIVAN) is characterized by both proliferative and apoptotic kidney cell phenotypes. Recently we demonstrated the activation of mTOR pathway leading to proliferative as well as activation of p53 pathway leading on to apoptotic phenotype in HIVAN. Thus, we hypothesize that a cross talk between down stream signaling molecules of mTOR (p70S6K) and p53 (p66ShcA) determines the fate of kidney cells in HIVAN.

**Methods:** Renal tissue lysates of sex and age matched control, Tg26/p66$^{+/+,}$ Tg26/p66$^{+/-}$ mice were evaluated for phospho-p70S6K and downstream signaling. To determine the effect of modulation of p70S6K, control and Tg26 mice were administered either normal saline or rapamycin(5mg/Kg/every other day, intraperitoneally) for 4 weeks. Renal tissue lysates were probed for phospho-p66ShcA and total p66ShcA. For *in vitro* studies, mouse proximal tubular cells (MTC) were transduced with either empty vector (EV/MTC) or NL4-3 (HIV/MTC) and transfected with either siRNAp66, siRNAp70S6K, and scrambled siRNA. Subsequently, protein blots were probed for p66ShcA, p70S6K and actin. To study molecular binding, EV/MTC and HIV/MTC lysates were immunoprecipitated with anti- p66ShcA antibody and probed for p70S6K. To display functional relationship, siRNAp66-HIV/MTC and siRNAp70S6K-HIV/MTC were assayed after co-labeling with H2AX (for DNA damage) and KU80 (DNA repair).

**Results:** TG26/p66$^{+/-}$ mice displayed attenuated renal tissue expression of phospho-p70S6K, whereas, Tg26/Rapa mice displayed attenuated expression of p66ShcA. HIV/MTC displayed enhanced expression of phospho-p66ShcA and phospho-p70S6K. MTC silenced for p66ShcA displayed attenuated expression of phospho-p70S6K, whereas, MTC silenced for p70S6K exhibited attenuated expression of p66ShcA both under control and HIV milieus. EV/MTC and HIV/MTC lysates immunoprecipitated with anti-p66 antibody demonstrated expression of p70S6k. HIV/MTC silenced for either p66 or p70S6K displayed attenuated DNA damage and enhanced DNA repair.

**Conclusions:** Cross talk beween p66 and p70S6K plays a key role in the induction of tubular cell injury in HIVAN.

**FR-PO202**

**Age-Related Nrf2-Keap1 Signaling System Change in the Kidney** Seun Deuk Hwang, Eun Nim Kim, Keun Suk Yang, Myung Hyun Lee, Ji Hyun Yu, Yul Hee Cho, Chul Woo Yang, Cheol Whee Park, Yong-Soo Kim, Bum-Soon Choi. *Div of Nephrology, Dept of Internal Medicine, College of Medicine, The Catholic Univ, Seoul, Korea.*

**Background:** Aging is a multifactorial process characterized by a progressive decline in physiological function. Therefore, increasing our insight into kidney aging by understanding the anatomic, physiologic, and pathologic changes of aging in the kidney is important to prevent disastrous outcomes in elderly people.

**Methods:** Male 2-, 12-, and 24-month-old C57/BL6 mice were used in this study. We measured histological change, oxidative stress, aging-related protein expression in the kidneys.

**Results:** Twenty-four-month-old mice displayed increased albuminuria. Creatinine clearance decreased with aging, although this was not statistically significant. There were increases in mesangial volume and tubulointerstitial fibrosis in 24-month-old mice. There were also increases in F4/80 expression ps and in apoptosis detected by TUNEL. Urine isoprostane excretion increased with aging and SOD1 and SOD2 were decreased in 24-month-old mice. In our study, expression of Nrf2 in total protein(1 ± 0.2 fold vs. 1.02 ± 0.12 fold, 1.31 ± 0.24 fold)was not decreased in 24-month-old mice. However, Nrf2 expression in nuclear (1 ± 0.44 fold vs. 1.94 ± 0.7 fold, 1.61 ± 0.46 fold; $p < 0.05$ vs. 24M) and in nuclear/total protein ratio (1 ± 0.82 fold vs. 1.83 ± 0.6 fold, 1.08 ± 0.38 fold; $p < 0.05$ vs. 24M) were decreased in 24-month-old mice. Keap1 expression (1 ± 0.16 fold vs. 0.93 ± 0.12 fold, 1.15 ± 0.35 fold) was increased in 24-month-old mice compared with 2-, 12-month-old mice. HO-1 (1 ± 0.08 fold vs. 9.39 ± 0.81 fold, 8.87 ± 0.51 fold) and NQO-1 (1 ± 0.01 fold vs. 0.87 ± 0.19 fold, 0.93 ± 0.24 fold) were decreased in 24-month-old mice compared with 12-month-old mice, although this was not statistically significant.

**Conclusions:** Nrf2 was decreased with aging and may relate to antioxidant pathway. Nrf2 suppression and Keap1 activation with aging could induce oxidative stress, leading to decrease in antioxidant gene expression such as HO-1 and NQO-1. Pharmacologically targeting these signaling molecules may reduce the pathologic changes of aging in the kidney.

**FR-PO203**

**Palladin Is Required for Proper Function of Kidney Cells** Emily H. Chang,[1,3] J. Charles Jennette,[1,2] Ronald J. Falk,[1] Carol A. Otey.[3] *[1]UNC Kidney Center, UNC-Chapel Hill, Chapel Hill, NC; [2]Dept of Pathology and Laboratory Medicine, UNC-Chapel Hill, Chapel Hill, NC; [3]Dept of Cell Biology and Physiology, UNC-Chapel Hill, Chapel Hill, NC.*

**Background:** Palladin is an actin associated protein with multiple isoforms involved in development, wound healing and tumor metastasis. Previous work localizes isoform 4 to multiple kidney cell types. We show differential expression of multiple isoforms in kidney in health and disease and that abrogation affects cell function.

**Methods:** For immunohistochemistry (IHC), mouse kidney was stained with antibodies to palladin. Cells in culture were treated with TGF-β1 or low calcium and lysed for immunoblot or fixed for immunofluorescence (IF) staining. siRNA palladin knockdown cells were used in migration assays, fixed for IF or treated with TGF-β1/low calcium.

**Results:** IHC in normal mice shows palladin in glomeruli, vessels and tubules. Antibodies to different isoforms show cell specific expression: isoform 4 to proximal tubules and glomeruli, isoform 3 to distal tubules, and isoform 6 to nuclei of tubular cells. When injured by ischemia reperfusion, palladin expression in tubular cells increases and moves to the nucleus. In an α-MPO ANCA model, in addition to tubules, cellular crescents are intensely immunopositive. To explore palladin function, we used LLC-PK1 cells where palladin is present at baseline at very low levels in the cytosol by immunoblot and IF. TGF-β1 increases palladin expression primarily on actin stress fibers. Low calcium increases palladin expression further with localization to stress fibers, actin rings and nuclei. Isoform 4 knockdown results in loss of stress fiber localization, although stress fibers still form with TGF-β1. When wounded by scratch, cell migration is impaired. Isoform 6 knockdown leads to lethality.

**Conclusions:** Palladin isoforms are differentially expressed in the kidney in health and disease. Isoform 4 is necessary for proper cell migration, and isoform 6, which localizes to the nucleus, is critical as the knockdown is not viable. Understanding the different functions of the palladin isoforms may identify targets of intervention to protect against kidney injury.

*Funding:* Other NIH Support - T32 training grant

**FR-PO204**

**Targeting the Ubiquitin-Proteasome System in Complement-Mediated Glomerular Injury** Kapil Sareen-Khanna, Simon S. Wing, Andrey V. Cybulsky. *Medicine, McGill Univ, Montreal, Canada.*

**Background:** In experimental membranous nephropathy, complement C5b-9 induces sublethal glomerular epithelial cell (GEC) injury and proteinuria. In parallel, C5b-9 activates pathways to restrict injury. GEC injury is associated with protein misfolding in the endoplasmic reticulum (ER), and blocking the ubiquitin-proteasome system (UPS) exacerbates injury and proteinuria. We tested if enhancement of UPS function may confer protection against complement-mediated GEC injury. Certain deubiquitinating enzymes, including ubiquitin specific protease-14 (USP14), retard protein degradation by the proteasome. Thus, inhibition of USP14 may potentially enhance degradation of misfolded proteins, and attenuate cell injury.

**Methods:** To assemble complement C5b-9, cultured GECs were incubated with antiserum and normal serum. GFP$^u$ (green fluorescent protein fused with the CL1 degron), a misfolded protein, and CD3δ, an ER-associated degradation (ERAD) substrate, were used as reporters of the UPS and ERAD, respectively. The two reporters, as well as human disease-associated nephrin mutants (I171N and S724C), which are misfolded and undergo ERAD, were expressed by transient transfection.

**Results:** In GECs, GFP$^u$ and CD3δ reporters undergo time-dependent proteasomal degradation. The USP14-directed inhibitor, IU1, accelerated degradation of GFP$^u$ and CD3δ. Conversely, overexpression (by transient transfection) of USP14, but not a catalytically-inactive USP14 mutant, reduced degradation of GFP$^u$ and CD3δ. IU1 did not modify global ubiquitination of proteins. In 293T cells, IU1 tended to enhance degradation of the I171N and S724C nephrin mutants, but the change was not significant. Incubation of GECs with complement did not affect expression of CD3δ significantly, but accelerated degradation of GFP$^u$, and this degradation was further accelerated by IU1. However, IU1 did not alter complement-induced cytotoxicity in GECs.

**Conclusions:** USP14 controls proteasomal degradation of some, but not all, misfolded proteins. Thus, targeting USP14 may be useful in alleviating the proteotoxic effects of specific misfolded proteins. USP14 inhibition was not effective in reducing cytotoxicity in the context of global protein misfolding.

*Funding:* Government Support - Non-U.S.

**FR-PO205**

**Lack of the Small Ribosomal Protein S6 Phosphorylation Inhibits Compensatory Renal Hypertrophy** Jinxian Xu,[1] Jianchun Chen,[2] Jian-Kang Chen.[1] *[1]Experimental Medicine, Georgia Regents Univ, Augusta, GA; [2]Medicine, Vanderbilt Univ, Nashville, TN.*

**Background:** Nephron loss stimulates increases in cell size, with minimal cell proliferation, in the residual nephrons. This phenomenon is known as compensatory renal hypertrophy (CRH). Previous studies have demonstrated that activation of the mammalian target of rapamycin complex 1 (mTORC1)-S6 kinase 1 (S6K1) pathway is a major mechanism underlying CRH. However, the downstream effector of the mTORC1-S6K1 pathway that mediates CRH remains unknown, because multiple potential downstream phosphorylation targets of S6K1 have been reported.

**Methods:** We backcrossed S6 knock-in mice to the FVB/NJ background for 10 generations and produced congenic mice expressing unphosphorylatable S6 (*S6$^{P-/-}$*). 6-week-old *S6$^{P-/-}$* mice, along with their wild type littermates (*WT*), were subjected to induction of CRH by right uninephrectomy (UNX).

**Results:** *S6$^{P-/-}$* mice and *WT* mice had similar body weight and kidney-to-body weight ratios (K/Bwt). Compared with *WT* mice, UNX-induced CRH in *S6$^{P-/-}$* mice was blocked by 50-60%, assessed by increases in K/Bwt (41.40±4.04 vs. 18.54±3.40%; $P<0.01$, $n=7$-9) and increases in protein-to-DNA ratios (29.33±5.37 vs. 12.57±6.04%; $P<0.05$, $n=5$-9). UNX markedly induced S6 phosphorylation in the remaining kidney of *WT* mice, but no S6 phosphorylation was detected in *S6$^{P-/-}$* mice. However, the phosphorylation of S6K1 and the eukaryotic translation initiation factor(eIF) 4E-binding protein 1 (4E-BP1) was stimulated to equivalent levels in *S6$^{P-/-}$* mice and *WT* mice in response to UNX, indicating that mTORC1 is still activated in both *S6$^{P-/-}$* mice and *WT* mice. There was no change in

BUN levels in *WT* mice and *S6*$^{P-/-}$ mice after UNX. Ki67 staining revealed comparable minimal increases in cell proliferation in the remaining kidney of *WT* mice and *S6*$^{P-/-}$ mice in response to contralateral nephrectomy.

**Conclusions:** This study provides the first unequivocal evidence that phosphorylated S6 is the major downstream effector of the mTORC1-S6K1 activation that mediates 50-60% of the hypertrophy seen in wild type mice. Our data also suggest the existence of additional signaling pathways that mediate 40-50% of compensatory renal hypertrophy.

*Funding:* NIDDK Support

## FR-PO206

**Role of Retinol Dehydrogenase 9 in Podocyte Injury** Xuezhu Li,[1,2] Yan Dai,[3] Peter Y. Chuang,[1] John C. He.[1] *[1]Div of Nephrology, Mount Sinai School of Medicine; [2]Div of Nephrology, Shanghai East Hospital, China; [3]Div of Nephrology, Shanghai First People's Hospital, China.*

**Background:** Intracellular concentration of retinoic acid (RA) is determined by two sequential oxidation reactions that convert retinol to retinoic acid. Kidney is a major organ for RA metabolism. Our previous work demonstrated that RA synthesis is significantly less in glomeruli of HIV-1 transgenic mice (Tg26) and the expression of retinol dehydrogenase (RDH) 9, which catalyzes the rate-limiting step in RA synthesis, is downregulated. Since it is known that RA has renal protective effects and is able to induce podocyte differentiation, we hypothesize that restoration of RA synthesis could slow the progression of renal disease.

**Methods:** We compared the expression of podocyte differentiation marker in cultured murine podocytes infected with an HIV-1 provirus with or without RDH9 overexpression. Mice with tetracycline (tet)-inducible, podocyte-specific RDH9 overexpression (POD-RDH9$^{Ovx}$) were generated. We examined the effect of podocyte-specific overexpression of RDH9 on glomerular injury in two murine models of podocyte injury: HIV-associated nephropathy (HIVAN) and Adriamycin (ADR) nephropathy. Podocyte differentiation markers and cell proliferation makers were examined by immunostaining. Proteinuria was measured by urinary albumin ELISA. PAS stained sections were used to assess glomerular histololgy.

**Results:** Overexpression of RDH9 in cultured podocytes induced podocyte differentiation. Inducible overexpression of RDH9 in podocyte of Tg26 mice decreased proteinuria, increased podocyte differentiation markers, and reduced glomerular expression of cell proliferation makers. Overexpression of podocyte RDH9 also attenuated ADR-induced proteinuria and glomerulosclerosis.

**Conclusions:** Our data suggests that restoration of RA synthesis mitigates podocyte injury.

## FR-PO207

**Telmisartan Activates Endogenous Peroxisome Proliferator-Activated Receptor-δ and May Have Anti-Fibrotic Effects in Human Mesangial Cells** Daisuke Mikami,[1] Hideki Kimura,[2] Kazuko Kamiyama,[1] Kunio Torii,[2] Seiji Yokoi,[1] Yoshinari Yokoyama,[1] Naoki Takahashi,[1] Kenji Kasuno,[1] Masayuki Iwano.[1] *[1]Div of Nephrol, Sch of Med, Univ of Fukui, Fukui, Japan; [2]Dept of Clin Nephrol, Univ of Fukui Hosp, Japan.*

**Background:** Telmisartan (Telm), an angiotensin II receptor type 1 blocker (ARB), was recently reported to promote lipolysis in mice by acting as a peroxisome proliferator-activated receptor (PPAR)-δ activator, although in clinical studies, it has also been recognized to activate PPAR-γ as a major cause of its pleiotropic actions. Whether Telm activates endogenous PPAR-δ and thereby exerts anti-fibrotic effects in human mesangial cells (HMC) remains to be investigated.

**Methods:** Presence of PPAR-δ in human renal tissues was examined by immunohistochemistry. Confluent HMC were treated for 6-24h with Transforming growth factor-β1 (TGF-β1; 10-50pg/ml) in absence or presence of Telm (10 μM). PPAR activity was determined by PPRE-luciferase assay, spcific antagonists, and PPAR gene silencing.

**Results:** PPAR-δ was detected in mesangial cells of glomeruli with moderately proliferative changes. In HMC, both GW0742, an authentic PPAR-δ agonist, and Telm enhanced PPRE-luciferase activity dose-dependently, and these increases were blunted by GSK0660, a PPAR-δ antagonist, but not GW9662, a PPAR-γ antagonist. Telm also upregulated the expression of PPAR-δ target genes related to fatty acid oxidation; i.e., fatty acid-binding protein and uncoupling protein-2. These effects were inhibited by both PPAR-δ antagonism and PPAR-δ gene silencing. TGF-β1 increased the expression of plasminogen activator inhibitor-1 (PAI-1) and TGF-β1, main profibrotic factors, at least in part via the phosphorylation of extracellular signal-regulated kinases (pERK). Telm suppressed TGF-β1-stimulated PAI-1 expression and pERK, and these effects were weakened by PPAR-δ antagonism, whereas eprosartan, a non-PPAR activating ARB, did not affect TGF-β1-stimulated PAI-1 expression.

**Conclusions:** In HMC, Telm activates endogenous PPAR-δ and may prevent TGF-β1-induced fibrotic changes by reducing ERK phosphorylation in a PPAR-δ-dependent manner, and thus, might be useful for treating hypertensive patients with renal and metabolic disorders.

## FR-PO208

**Role of Calcium-Independent Phospholipase $A_2\gamma$ in Endoplasmic Reticulum Stress** Hanan Elimam, Tomoko Takano, Andrey V. Cybulsky. *Medicine, McGill Univ, Montreal, Canada.*

**Background:** Several glomerular diseases are associated with activation of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR). In the UPR, upregulation of ER chaperones, including Grp94 and Grp78, enhances ER protein folding capacity and may limit cytotoxicity. Recently, we showed that the calcium-independent phospholipase $A_2\gamma$ (iPLA$_2\gamma$) is localized at the ER, that complement C5b-9 activates iPLA$_2\gamma$ and that the iPLA$_2\gamma$ pathway is cytoprotective. The present study addresses whether the cytoprotective effect of iPLA$_2\gamma$ involves the UPR.

**Methods:** iPLA$_2\gamma$ was overexpressed in cultured glomerular epithelial cells (GEC) by transient transfection. To activate the UPR, cells were treated with tunicamycin or thapsigargin. iPLA$_2\gamma$ activity was monitored by prostaglandin $E_2$ (PGE$_2$) production. UPR activation was monitored by immunoblotting for ER chaperones and C/EBP homologous protein (CHOP), and by an activating transcription factor 6 (ATF6) luciferase reporter assay.

**Results:** Tunicamycin and thapsigargin stimulated production of PGE$_2$ in GEC and this effect was amplified by overexpressing iPLA$_2\gamma$. Amplified PGE$_2$ production was blocked by the iPLA$_2\gamma$-specific inhibitor, R-bromoenol lactone (R-BEL). Tunicamycin and iPLA$_2\gamma$ independently stimulated ATF6 reporter activity, and iPLA$_2\gamma$ amplified the effect of tunicamycin. Amplified ATF6 activity was inhibited by R-BEL, but not indomethacin, a cycloxygenase inhibitor. Tunicamycin and thapsigargin increased Grp94 and Grp78. This effect was amplified by overexpression of iPLA$_2\gamma$, and was reduced by R-BEL, but not indomethacin. Tunicamycin stimulated induction of CHOP, but unlike ER chaperones, stimulation of CHOP was not modulated by iPLA$_2\gamma$. Tunicamycin-induced cytotoxicity (lactate dehydrogenase release) was reduced in GEC overexpressing iPLA$_2\gamma$.

**Conclusions:** In GEC, induction of ER stress activated iPLA$_2\gamma$, and overexpression of iPLA$_2\gamma$ amplified the ATF6 pathway of the UPR, resulting in upregulation of ER chaperones and cytoprotection. These effects were dependent on iPLA$_2\gamma$ catalytic activity, but not prostanoids. Thus, iPLA$_2\gamma$ may regulate the UPR via effects on ER membrane lipids. Modulating iPLA$_2\gamma$ activity may provide therapeutic opportunities for glomerular diseases.

*Funding:* Government Support - Non-U.S.

## FR-PO209

**Altered Monocyte Expression and Expansion of CD14$^+$CD16$^+$ Subset in IgA Nephropathy Patients** Sharon N. Cox,[1] Grazia Serino,[1] Fabio Sallustio,[1,2] Francesco Pesce,[1,3] Francesco Paolo Schena.[1,2,4] *[1]Emergency and Organ Transplantation Dept, Univ of Bari, Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy; [3]Genomics of Common Disease, Imperial College, London, United Kingdom; [4]Schena Foundation, Research Center of Kidney Diseases, Valenzano, Bari, Italy.*

**Background:** The basic defect of IgA nephropathy (IgAN) lies within peripheral blood mononuclear cells rather than local kidney abnormalities. Previously we showed an altered gene expression in monocytes compared to B and T cells isolated from IgAN patients (Kidney Int, 2010), thus our aim here was to study this subset more closely at genomewide level.

**Methods:** 33 IgAN patients and 33 healthy blood donors (HBD) were studied. Microarrays were used to evaluate global differences in gene expression between monocytes of IgAN patients and HBD. Bioinformatic analysis was done with GenomeStudio, Genespring. The connectivity between genes was evaluated by IPA. Aberrantly expressed genes/pathways were validated on an independent cohort by RT-PCR, Western blot and flow cytometric analysis.

**Results:** We revealed 710 deregulated probes in IgAN patients' monocytes (FDR-p <0.05). Probes were primarily involved in apoptosis signalling, mitochondrial dysfunction, tnfr2/1 and death receptor signaling canonical pathways. Representative genes belonging to these pathways (*TNF, CD83, TNFRSF1A, NDUFS3*) were validated with RT-PCR. All mitochondrial respiratory chain subunits were found up regulated in IgAN patients, in particular the protein levels of NDUFS3(p=0.003) thus confirming the aberrant mithocondrial homeostasis. The enhanced apoptotic phenotype was confirmed at protein level with a higher percentage of Annexin-V/7-AAD positive staining. In monocytes of IgAN patients the CD14$^+$CD16$^+$ subset was significantly expanded in all IgAN patients, still maintaining the same total monocyte count.

**Conclusions:** Our findings demonstrate an aberrant modulation of the mitochondrial respiratory system in monocytes isolated from IgAN patients. Furthermore, the aberrant expansion of the CD14$^+$CD16$^+$ subset in IgAN patients could explain the enhanced apoptotic function of these cells in IgAN patients.

*Funding:* Government Support - Non-U.S.

**FR-PO210**

**Deficiency in Apoptosis Inducing Factor Predisposes to Chronic Kidney Disease Independent of a Decline in Complex I Activity or Assembly**
Melinda T. Coughlan,[1] Gavin Higgins,[1] Sean McGee,[2] David Thorburn,[3] Mike Ryan,[4] Vicki Thallas,[1] Mark E. Cooper,[1] Josephine Forbes.[5] *[1]Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; [2]Metabolic Research Unit, Deakin Univ, Geelong, Victoria, Australia; [3]Murdoch Children's Research Institute, Melbourne, Victoria, Australia; [4]La Trobe Institute for Molecular Sciences, Melbourne, Victoria, Australia; [5]Glycation & Diabetes, Mater Medical Research Institute, Brisbane, Queensland, Australia.*

**Background:** Deficiency in apoptosis inducing factor (AIF) results in a syndrome likened to disorders of oxidative phosphorylation with a functional loss of complex I activity. Complex I deficiency can also manifest as chronic kidney disease. Here, we have studied Harlequin hemizygous mice (Hq/Y), which are postulated to have defects in both complex I activity and assembly as a result of reduced *Aif* expression.

**Methods:** Eight week old wild type (WT, B6CBACa-A) and Hq/Y mice were rendered diabetic with streptozotocin and followed for 10 weeks. Metabolic caging to obtain 24 hour urine collections was performed at week 10, mice were killed and renal cortical mitochondria were freshly isolated and markers of mitochondrial function were assessed.

**Results:** Hq/Y mice demonstrated progressive renal dysfunction compared with WT mice, which was worsened by diabetes. The renal phenotype was associated with alterations in mitochondrial ATP synthesis and $O_2$ consumption that were exacerbated by diabetes. However, there was no loss of complex I activity or assembly despite a lower AIF protein content. Human primary proximal tubule epithelial cells (PTECs) exposed to high glucose had loss of *AIF* expression and impaired cellular respiration, which was rescued by overexpression of *AIF*. Control and diabetic Hq/Y and WT diabetic mice demonstrated renal cortical mitochondrial swelling and fragmentation and upregulation of proteins involved in mitochondrial fission.

**Conclusions:** These studies demonstrate that AIF deficiency is a risk factor for chronic kidney disease. This risk is not dependent on complex I activity or assembly but relates to specific changes in mitochondrial function in the renal cortex.

*Funding:* Government Support - Non-U.S.

**FR-PO211**

**Human and Mouse EGF Ligand and Receptor Expression in Chronic Kidney Disease**
Shannon Marie Harlan, Derek D. Yang, Jon Wilson, Matthew D. Breyer, Josef G. Heuer. *Biotherapeutic Discovery Research, Eli Lilly and Company, Indianapolis, IN.*

**Background:** The type 1 growth factor receptor (T1GFR) family consists of EGFR, ERBB2, ERBB3, and ERBB4. Accumulating evidence implicates TGFα and other T1GFR ligands in progression of chronic kidney disease (CKD). The purpose of this study was to determine whether renal T1GFR-ligand or receptor levels are altered in human CKD and experimental renal disease.

**Methods:** Kidney tissue, serum and urine samples were obtained from normals or patients with CKD for mRNA expression by Taqman. Serum creatinine and albuminuria was also measured. TGFα protein levels were obtained by ELISA. Microarray analysis by gene chip was done to determine mRNA expression in human podocytes, glomerular endothelial, mesangial and proximal tubule cells which was confirmed by Taqman. Analysis of ligand production from cultured cells was done by specific ELISA assays. The effects of ligands on cultured cells were determined by microarray gene chip analysis as well as cell migration and proliferation assays.

**Results:** mRNA expression of EGF family member ligands including TGFα, Hb-egf and the neuregulins were upregulated in kidneys from CKD patients versus normals. These ligands were also expressed in cultured renal cells. Serum and urine TGFα protein was also significantly elevated in CKD patients and serum TGFα showed a significant positive correlation with serum creatinine. In situ hybridization showed TGFα mRNA was localized to tubules and glomerular podocytes in human and mouse kidneys. Glomerular size, urine TGFα and glomerular EGFR phosphorylation were all significantly elevated in uniNx db/db mice. TGFα induced mesangial cell proliferation and podocyte cell migration *in vitro* and induced the expression of several genes associated with proliferation, inflammation, extracellular matrix and cell migration in podocytes.

**Conclusions:** TGFα levels are elevated in both clinical and experimental CKD and the activity of TGFα on podocytes and mesangial cells is consistent with of a role in disease progression. Other members of this growth factor family are also upregulated in CKD and may also contribute to disease progression.

*Funding:* Pharmaceutical Company Support - Eli Lilly and Company

**FR-PO212**

**Mizoribine Suppresses Pyroptosis and Ameliorates Renal Inflammation in Aldosterone-Salt Treatment Treated Rats**
Toshiki Doi, Ayumu Nakashima, Shigehiro Doi, Takao Masaki. *Nephrology, Hiroshima Univ Hospital, Hiroshima, Japan.*

**Background:** It has been reported that aldosterone-salt treatment induces massive inflammation that contributes to fibrosis in the rat kidney. However, the mechanism underlying renal inflammation remains unclear. Recently, pyroptosis has been recognized as a new type of cell death that is accompanied by activation of inflammatory cytokines. We hypothesized that aldosterone-salt treatment induced pyroptosis and that mizoribine, an effective immunosuppressant, would ameliorate renal inflammation and fibrosis.

**Methods:** Six-week-old male Sprague-Dawley rats underwent left uninephrectomy under anesthesia. Ten days after recovery from surgery, rats were given 1% NaCl to drink. Rats were divided into three groups (n = 7 per group): (1) vehicle infusion, (2) 0.75 μg/hour aldosterone infusion, or (3) aldosterone infusion plus a three mg/kg/day oral dose of mizoribine. All rats were sacrificed six weeks after the start of treatment. Renal tissues were assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining and Western blot analysis of caspase-1.

**Results:** TUNEL-positive cells and expression of active caspase-1 increased in rats with aldosterone-salt treatment, indicating that pyroptosis was induced by aldosterone-salt treatment in vivo. T lymphocyte and macrophage infiltration were also observed in aldosterone-salt treated rats as well as glomerulosclerosis and interstitial fibrosis. Oral administration of mizoribine suppressed both the number of TUNEL-positive cells and expression of active caspase-1 and it ameliorated renal inflammation and fibrosis.

**Conclusions:** These results suggest that pyroptosis plays an important part in the development of renal injury induced by aldosterone-salt treatment and immunosuppressants could be a rationale therapy.

**FR-PO213**

**Calcineurin Is Involved in Pathological but Not Adaptive Hypertrophy of the Kidney**
Clintoria R. Williams,[1,2] Brandi M. Wynne,[1] Jennifer L. Gooch.[1,2] *[1]Dept of Medicine / Renal Div, Emory Univ, Atlanta, GA; [2]Atlanta Veterans Affairs Medical Center, Decatur, GA.*

**Background:** Calcineurin is well described as a mediator of hypertrophy of the heart and skeletal muscle. Interestingly, calcineurin's involvement appears to be specific for pathological forms of cardiac hypertrophy such as aortic banding and not adaptive forms of hypertrophy such as exercise-induced. Previously, our laboratory showed that calcineurin inhibition blocks whole kidney and glomerular hypertrophy in STZ-induced diabetic rats. More recently, we identified a specific role for the beta isoform of the catalytic subunit of calcineurin (CnAβ) in high glucose-mediated hypertrophy in vitro and in vivo. Activation of CnAβ, in turn, transcriptionally up-regulates Nox4 expression and oxidative stress.

**Methods:** In the current study, we investigated the role of calcineurin in adaptive renal hypertrophy of the remaining kidney following uninephrectomy (UNX). Kidneys were harvested from sham versus UNX WT, CnAα-/-, and CnAβ-/- mice. Total kidney size, renal function, reactive oxygen species (ROS) production, CnAβ activity and Nox4 expression were examined.

**Results:** As expected, UNX induced a significant increase in kidney size in WT mice. Although SHAM kidneys from CnAα-/- mice are smaller, UNX caused an increase in size consistent with adaptive hypertrophy. SHAM CnAβ-/- kidneys were comparable to WT. UNX also induced hypertrophy in CnAβ-/- mice indicating that neither isoform of calcineurin is required for adaptive renal hypertrophy. Consistent with a calcineurin-independent pathway, analyses of the remaining kidneys showed no increase in CnAβ expression or activity. In addition, ROS generation is not increased and there is no change in Nox4 expression.

**Conclusions:** Taken together, these data demonstrate that CnAβ / Nox4 are selectively involved in pathological renal hypertrophy which is associated with progression to renal disease and renal failure. In contrast, adaptive hypertrophy following UNX involves alternative mechanisms that are not associated with chronic disease. Therefore, signaling mechanisms that mediate adaptive hypertrophy could be novel therapeutic renal targets.

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO214**

**Significant Increase in Podocyte Autophagy in Association with Foot Process Effacement in Patients with Minimal Change Nephrotic Syndrome**
Ayu Ogawa, Hitoshi Sugiyama, Masashi Kitagawa, Toshio Yamanari, Akifumi Onishi, Hiroshi Morinaga, Yoko Kikumoto, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Dept of Medicine III, Okayama Univ Graduate School of Medicine, Okayama, Japan.*

**Background:** Autophagy is a cellular process involved in the bulk degradation of proteins and organelle turnover. Recent studies have demonstrated the significance of autophagy of the tubular epithelium in several renal tubulointerstitial disorders using mouse models. However, the role of autophagy in the regulation of human glomerular diseases remains unclear. This study aimed to elucidate the morphological evidence for autophagy and its association with ultrastructural alterations of podocytes and clinical parameters in patients with minimal change nephrotic syndrome (MCNS).

**Methods:** The total study population included 103 patients with glomerular diseases (MCNS: 30, IgA nephropathy, IgAN: 21, membranous nephropathy, MN: 21, lupus nephritis, LN: 10 and others: 21) who underwent renal biopsies. The study investigated the number of autophagic vacuoles and the degree of foot process effacement (FPE) in podocytes using electron microscopy. The relationships between the expression of autophagic vacuoles, the degree of FPE and the clinical parameters were determined.

**Results:** Autophagic vacuoles were particularly detected in podocytes. Overall, the number of autophagic vacuoles in podocytes was significantly correlated with age ($p = 0.009$, $n = 103$). In the patients with MCNS, the number of autophagic vacuoles in podocytes was significantly correlated with the podocyte FPE score($r = -0.416$, $p = 0.022$), the amount of proteinuria ($r = 0.367$, $p = 0.046$) and the level of serum albumin ($r = -0.428$, $p = 0.018$). The number of autophagic vacuoles in podocytes was significantly increased in the patients with MCNS and MN in comparison to that observed in the patients with IgAN and LN ($p = 0.007$).

**Conclusions:** The data indicate that the autophagy of podocytes is associated with FPE and massive proteinuria in patients with MCNS. The mechanisms underlying the activation of autophagy in association with FPE in podocytes should be further determined in order to elucidate the pathophysiology of MCNS.

## FR-PO215

### Autophagy Protects Kidney from Chronic Metabolic Stress

Tomonori Kimura,[1] Atsushi Takahashi,[1] Yoshitsugu Takabatake,[1] Tomoko Namba,[1] Takeshi Yamamoto,[1] Jun-Ya Kaimori,[2] Jun Matsuda,[1] Isao Matsui,[1] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Advanced Technology for Transplantation, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

**Background:** Autophagy plays a protective role in several organs including kidney; however, the precise function in pathological metabolic condition is only understood partially. We have demonstrated that autophagy plays a protective role against metabolic stress *in vitro*. This study aimed to determine the systemic role of autophagy against chronic mitochondrial metabolic stress *in vivo*.

**Methods:** Proximal tubule-specific autophagy-deficient mice were treated with cyclosporine A, an inducer of mitochondrial metabolic stress through the blockade of the permeability transition pore of mitochondrial inner membrane, and histological changes and biological profiles were examined. In addition, we exposed kidney for the metabolome analyses.

**Results:** Autophagy-deficiency exaggerates cyclosporine A-induced kidney injury with significant increase in apoptotic cells. This damage is associated with increase in mitochondria ballooning. Metabolome analyses revealed aberrant metabolism of cysteine and its-related pathways, while corroborating our previous findings.

**Conclusions:** These observations not only proved our hypothesis that autophagy protects kidney tubular cells against kidney diseases through quality control of mitochondria, but also added a biological significance in the role of autophagy against metabolism-related diseases.

## FR-PO216

### Cyclosporine A Attenuates Lovastatin-Induced Podocyte Apoptosis

Xue-jia Chen,[1] He-ping Ma.[2] *[1]Dept of Obstetrics and Gynecology, Ruian Maternity and Child Care Hospital, Ruian, Zhejiang Province, China; [2]Dept of Physiology, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** Our recent studies suggest that inhibition of cholesterol synthesis in cortical collecting duct (CCD) cells with a very high concentration of lovastatin causes apoptosis of CCD cells and that cyclosporine A (CsA) attenuates lovastatin-induced apoptosis. We hypothesized that lovastatin and CsA might also have contradictory effects on podocyte viability.

**Methods:** We used confocal microscopy and annexin V-propidium iodide double-staining technique to detect podocyte apoptosis.

**Results:** The data demonstrated that a very high concentration of lovastatin significantly reduced cholesterol levels in the plasma membrane of podocytes and induced apoptosis. The effects of lovastatin on cholesterol levels and apoptosis were attenuated by pretreatment of podocytes with either exogenous cholesterol or CsA. However, acute depletion of cholesterol in the outer leaflet of podocyte membrane with methyl-ß-cyclodextrin failed to induce apoptosis, indicating that cholesterol in the inner leaflet of podocyte membrane plays an important role in regulating podocyte apoptosis. Treatment of podocytes with CsA caused significant accumulation of cholesterol in podocytes. These data suggest that CsA attenuate lovastatin-induced apoptosis via a cholesterol-dependent mechanism.

**Conclusions:** Since statins have been used as a complement of chemotherapy for cancer patients at a relatively high dose, the present study may provide useful information to management of statin-induced podocyte injury in these patients.

*Funding:* NIDDK Support

## FR-PO217

### Tumorigenesis and Organprotection–Two Ends of a Continuum?

Roman-Ulrich Mueller,[1,2,3] Puneet Bharill,[1,3] Hakam Gharbi,[1,2] Torsten Kubacki,[1] Marc Johnsen,[1] Tritpi Mishra,[1] Francesca Fabretti,[1] Markus M. Rinschen,[1] Peter Frommolt,[2] Volker Rolf Burst,[1] Bernhard Schermer,[1,2,3] Thomas Benzing.[1,2,3] *[1]Dept 2 of Internal Medicine and CMMC, Univ of Cologne, Cologne, Germany; [2]CECAD, Univ of Cologne, Germany; [3]SyBACol, Univ of Cologne, Germany.*

**Background:** Not only due to its growing incidence acute kidney injury has gained increasing interest during the last decade. On the one hand a growing body of evidence shows its close association with morbidity and mortality. On the other hand no causal treatment exists and preventive measures are limited. However, organ preconditioning has become a potential tool to tackle this problem.

**Methods:** We employ the nematode *Caenorhabditis elegans* as a tool to search for keyplayers in the pathways mediating organoprotection. The major signaling pathways mediating increased stress tolerance upon preconditioning are well conserved in the worm and hypotheses generated using this model can be transferred to a mouse model of renal ischemia-reperfusion injury.

**Results:** In 2009 we had introduced hypoxia signaling as a novel longevity pathway through studies in *vhl-1* knockout worms. More recently, we identified *flcn-1* - the worm orthologue of the gene mutated in Birt-Hogg-Dubé syndrome - to regulate lifespan and stress tolerance in a manner dependent on *hif-1* and TORC1.

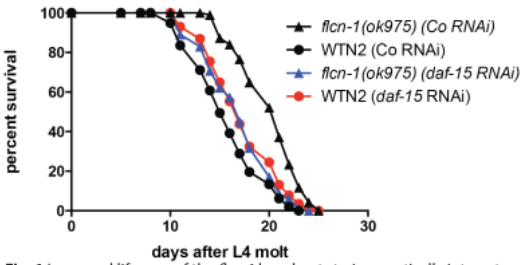



**Fig. 1** Increased lifespan of the *flcn-1* knockout strain genetically interacts with ceTORC1.

**Conclusions:** We have identified FLCN as yet another renal tumor suppressor protein regulating organismal stress resistance. On the one hand increasing knowledge about longevity pathways holds the promise to help finding novel tools to prevent AKI in a broader clinical setting. On the other hand these findings closely link cellular stress resistance, tumorigenesis, aging and organ regeneration and allow for the intriguing hypothesis of a tightly regulated balance between these processes in which reducing tumor suppressive tissue surveillance in a timely limited fashion may be a promising way to prevent AKI.

*Funding:* Government Support - Non-U.S.

## FR-PO218

### Everolimus Inhibits Invasion and Migration of Human Renal Cancer Cells by Inhibiting Epithelial-to-Mesenchymal Transition and FAK Pathway In Vitro and In Vivo

Horng-Rong Chang, Jong-Da Lian. *Div of Nephrology, Dept of Medicine, Chung Shan Medical Univ Hospital, Taichung, Taiwan.*

**Background:** Metastasis of kidney cancer, a most important cause of patient death and a multiple and intricate processes, may complicate the clinical management and lead to a poor prognosis for cancer patients and has tremendous physical or economical impact to patients or communities. The epithelial-mesenchymal transition (EMT) is implicated in converting stationary epithelial cancer cells into motile mesenchymal cells during metastasis.

**Methods:** 786-O cells were treated with everolimus at various concentrations for a defined period and then subjected to gelatin zymography, casein zymography and Western blot to investigate the impacts of everolimus on invasive, motility, and migratory potential. An *in vivo* anti-tumor studies were examined using a nude mice xenograft model and a lung metastasis model.

**Results:** In the present study, our results showed that everolimus treatment significantly inhibits invasive, motility, and migratory potential of human renal cell carcinoma 786-O cells. Molecular analyses showed that everolimus increases E-cadherin level and decreases vimentin and RhoA expression by Western blot assay, indicating the anti-EMT effect of everolimus in renal cell carcinoma. We performed western blot to find that everolimus inhibits p-Src and p-focal adhesion kinase (p-FAK) that was localized mainly at cellular membrane as evidenced by confocal analyses in 786-O cells, which might be responsible for morphologically observed shift towards epithelial character. In addition, everolimus was found to be effective in reducing the lung metastases formation via tail vein injection as compared to vehicle treated control animals. Everolimus was also evidenced by its inhibition on the growth of 786-O cells *in vivo* via a cancer cells xenograft nude mice mode.

**Conclusions:** Taken together, our results showing anti-invasive/anti-tumor effects of everolimus and associated mechanisms suggest that everolimus should be tested further in clinically relevant models towards exploiting its potential benefits against metastatic kidney cancer cells.

## FR-PO219

### DIALOGUE* Phase 2 Program for BAY85-3934 a HIF-PH Inhibitor with Daily Oral Treatment in Anemic Patients Suffering from CKD/ESRD

Iain C. Macdougall,[1] Jeffrey S. Berns,[2] Tadao Akizawa,[3] Steven Fishbane,[4] Thomas Bernhardt.[5] *[1]Kings College Hospital, London, United Kingdom; [2]Hospital of the Univ of Pennsylvania, Philadelphia; [3]Showa Univ Hospital, Tokyo, Japan; [4]Winthrop Univ Hospital, Mineola; [5]Bayer Pharma AG, Berlin, Germany.*

**Background:** Anemia, defined as a deficiency of hemoglobin (Hb), is a frequent and serious complication of severe diseases, including chronic kidney disease (CKD) and cancer. The traditional treatment of anemia due to deficient EPO synthesis has included several forms of recombinant human EPO (rhEPO), also known as erythropoiesis-stimulating agents (ESAs). BAY 85-3934, a novel inhibitor of HIF-PH, is developed for the oral treatment of anemia in patients with CKD and end stage renal disease (ESRD). Hypoxia-inducible factor (HIF) is a key transcription factor for the activation of EPO-gene expression. BAY 85-3934, a small molecule inhibitor of HIF-PH, will be evaluated for the induction of endogenous EPO by mimicking hypoxia resulting in an increase of the Hb level.

**Methods:** The BAY 85-3934 Phase 2 program consists of 3 randomized, multicenter parent studies (2 studies in Europe/Asia Pacific and 1 study in US/Japan, each lasting 16 weeks) and 2 safety extension studies (1 study in Europe/Asia Pacific and 1 study in US/Japan). Up to 400 patients suffering from anemia due to CKD or ESRD are planned

to be enrolled in the 3 parent studies to prove the efficacy and safety of BAY85-3934 in comparison to placebo, Darbepoetin alpha and Epoetin alpha/beta. Eligible patients are either treatment naïve with a Hb level below 10 g/dL or on stable ESA dose with a Hb level in the target range per regional ESA label. Different doses of BAY85-3934 will be administered and titrated when indicated. The safety of the treatment is monitored by an independent DMC. An adjudication committee supports the evaluation of reported safety events.

**Conclusions:** In this Phase 2 program BAY 85-3934, a small molecule inhibitor of HIF-PH, will be developed for the induction of endogenous EPO resulting in an increase of the Hb level. * DIALOGUE = Daily Oral Treatment Increasing Endogenous Erythropoietin.

*Funding:* Pharmaceutical Company Support - Bayer Pharma AG

## FR-PO220

**Increased Serum Hepcidin Is a Major Contributor to the Anemia of CKD in an Animal Model** Joshua Zaritsky,[1] Victoria Rivka Gabayan,[2] Grace Jung,[2] Tomas Ganz,[2] Isidro B. Salusky.[1] *[1]Pediatrics, UCLA, Los Angeles, CA; [2]Medicine, UCLA, Los Angeles, CA.*

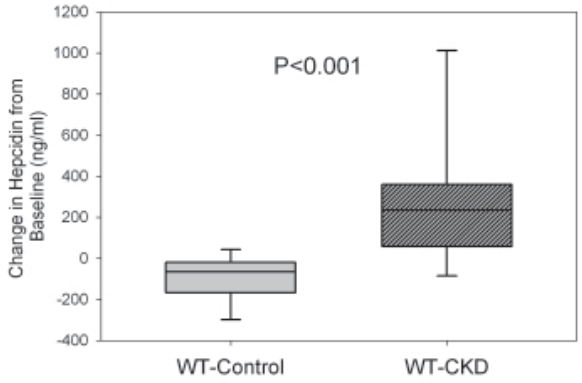
**Background:** Cross-sectional studies have shown that serum concentrations of hepcidin, a key regulator of iron metabolism, are markedly elevated in CKD patients. However it remains unknown if these elevations contribute to iron restriction and the anemia of CKD.

**Methods:** We examined changes in hepcidin levels in a mouse model of CKD, avoiding the confounding effects of ESA and iron therapy. Wild-type mice (WT-CKD) and mice with a targeted disruption of the hepcidin gene (HepKO-CKD) were placed on a 20 ppm iron diet containing 0.2% w/w adenine. Corresponding control groups (WT and HepKO-control) were placed on an identical diet without adenine. Serum hepcidin levels were measured by ELISA at diet initiation and at 2 months along with other key biochemical markers (Table).

**Results:** As expected, renal function (assessed by serum BUN) decreased in animals fed adenine. Significantly, at 2 months, Hgb levels were lower in animals fed adenine with lower MCV values consistent with iron restricted erythropoiesis. ANOVA modeling found a significant interaction between diet and genotype indicating that the HepKO-CKD group had a smaller drop in Hgb than the WT-CKD group ($p<0.04$). Serum hepcidin levels increased only in the WT-CKD group ($p<0.001$) (Figure).

| | | WT Control (n=16) | WT CKD (n=16) | HepKO Control (n=20) | HepKO CKD (n=22) |
|---|---|---|---|---|---|
| Baseline | BUN (mg/dl) | 41.5±6 | 39.8±5 | 46.8±5.2 | 46.5±8.7 |
| | HGB (mg/dl) | 14.6±0.9 | 14.9±1.2 | 12.8±2.4 | 13.1±1.9 |
| | MCV (fl/cell) | 46.7±0.6 | 46.6±0.6 | 45.8±4.3 | 45.5±4.1 |
| | IRON (uM/l) | 105±19 | 114±33 | 120±66 | 127±83 |
| | Hepcidin (ng/ml) | 315±112 | 321±87 | - | - |
| 2 Months | BUN (mg/dl) | 40±5.2 | 115±26* | 44.5±6.5 | 112±20† |
| | HGB (mg/dl) | 14±1.4 | 8.8±2.2* | 15.8±1.7 | 13.5±2.6† |
| | MCV (fl/cell) | 46.2±0.9 | 38.3±2* | 51.6±1.9 | 48.2±1.8† |
| | IRON (uM/l) | 122±51 | 99±20 | 135±76 | 116±29 |
| | Hepcidin (ng/ml) | 220±61 | 623±364* | - | - |

* p<0.01 WT-CKD vs. WT-control; †p<0.01 HepKO-CKD vs HepKO-control




**Conclusions:** We found that hepcidin levels increase as kidney function worsens in this animal model of CKD and that increased hepcidin contributes to the anemia of CKD. Future studies are warranted to examine if a targeted approach to lower serum hepcidin levels could be used to treat the anemia of CKD.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO221

**Indole Derivatives Enhance Erythropoietin Production and Have Cytoprotective Effects** Takehiro Suzuki,[1] Hisato Shima,[1] Toshihito Sahara,[1] Yusuke Suzuki,[1] Yuki Oba,[1] Eikan Mishima,[1] Yoichi Takeuchi,[1] Yasutoshi Akiyama,[1] Chitose Suzuki,[1] Ken-ichiro Hayashi,[2] Sadayoshi Ito,[1] Takaaki Abe.[1] *[1]Tohoku Univ Graduate School of Medicine, Japan; [2]Okayama Univ of Science, Japan.*

**Background:** In patients with chronic kidney disease (CKD), the accumulation of uremic toxins promotesrenal damage and anemia. During the analysis of uremic solutes on erythropoietin (Epo) production in Epo-producing Hep3B cells, we identified that some of the indole compounds have a potency of enhancing Epo production. So the aim of this study is to identify the mechanism of stimulating Epo production by indole compounds and to identify the new compounds to prevent the CKD progression and anemia.

**Methods:** Forty one indole derivatives were synthesized and analyzed by Epo production, Epo mRNA production, and the Epo promoter assay as well as ATP production in Hep3B cells. Cytoprotective activity against cytotoxic agents (cisplatin, aristolochic acid) in kidney derived HK2 cells was accessed by cytotoxicity assay kit (WST-8). Intracellular ATP contents were measured by ATP kit (TOYO INK). *In vivo* effect of the compounds were assessed with oral or intraperitoneal administration. Total HIF-1a content was measured by Cell-Based ELISA kit.

**Results:** Indole compound #2, #4, #5 at 10μM enhanced Epo production by 4 fold, Epo mRNA by 60 fold and the Epo promoter activity by 6 fold, while FG4592 at 10μM increased Epo mRNA by 3 fold. Cytotoxic effects of cisplatin (50% reduction) and aristolochic acid (30% reduction) were ameliorated to baseline by#4 at 30μM and #5 at 100μM, respectively. Administration of the compound #2, #4, #5 at 3μM augmented intracellular ATP contents by 7 fold in Hep3B. These compounds have no cytotoxic effect or ROS generation by itself. The kidney Epo mRNA was increased by 3 fold in mice administrated the compound #5 at 50mg/kg intraperitoneally. Total HIF-1a content in Hep3B was increased by compound #21 and #35.

**Conclusions:** These data suggest that the indole derivatives promote Epo production. Stabilization of HIF-1a might be the mechanism of enhancement of Epo. These compounds also have cytoprotective effect as well as increased the intracellular ATP contents without increasing oxidative stress.

*Funding:* Government Support - Non-U.S.

## FR-PO222

**Indole-3-Acetate Inhibits Erythropoietin Receptor Expression and Erythropoietin Signaling In Vitro** Yasutoshi Akiyama,[1,2] Hisato Shima,[2] Yoichi Takeuchi,[2,4] Eikan Mishima,[1,2] Chitose Suzuki,[2] Takehiro Suzuki,[2] Sadayoshi Ito,[2] Takaaki Abe.[2,3,4] *[1]Dept of Community Medical Supports, Tohoku Medical Megabank Organization (ToMMo), Tohoku Univ, Sendai, Miyagi, Japan; [2]Div of Nephrology, Endocrinology and Vascular Medicine, Tohoku Univ Hospital, Sendai, Miyagi, Japan; [3]Div of Medical Science, Tohoku Univ Graduate School of Biomedical Engineering, Sendai, Miyagi, Japan; [4]Dept of Clinical Biology and Hormonal Regulation, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan.*

**Background:** Anemia is one of the major complications of chronic kidney disease (CKD). Although many CKD patients respond to the treatment of erythropoiesis-stimulating agent (ESA), about 10% of patients have poor responsiveness to ESA. It has been known that there are several causes of ESA resistance, such as iron deficiency, inflammation, vitamin B12 deficiency and folate deficiency. However, the pathophysiology of ESA-resistant anemia has not been fully elucidated.

**Methods:** To test the hypothesis that uremic toxin(s) decreases the erythropoietin receptor (EPOR) expression, we examined the effects of uremic solutes on cell viability, EPOR expression and signal transduction through EPOR using erythropoietin-dependent human leukemic cell line, UT-7/Epo cells.

**Results:** First, we compared the cell toxicity of various uremic solutes in UT-7/Epo cells with that in kidney-derived ACHN cells by cell viability assay. Many compounds showed the same cell toxicity between the two cell lines. However, the cell toxicity of indole-3-acetate (IAA), one of the uremic toxins, was about 10-fold stronger in UT-7/Epo cells than in ACHN cells, suggesting that IAA may have the toxicity related to EPO signaling as well as the direct cellular injury. To elucidate the mechanism, we examined the effect of IAA on the expression of EPOR. As a result, the expression of EPOR was dose-dependently decreased by IAA treatment at both mRNA and protein levels. In addition, tyrosine phosphorylation of STAT5 was significantly decreased by IAA treatment, suggesting that IAA inhibited the signal transduction through EPOR.

**Conclusions:** IAA may decrease the expression of EPOR and cause ESA-resistant anemia in CKD patients.

**FR-PO223**

**Chronic Kidney Disease Increases Influx of Bone Marrow Derived Cells into the Heart and Reduces Circulating Endothelial Progenitor Cells** Yvonne Riedl,[1] Katharina Bibl,[1] Karl F. Hilgers,[2] Christoph Daniel,[1] Kerstin U. Amann.[1] *[1]Nephropathology, Univ Erlangen; [2]Nephrology and Hypertension, Univ Erlangen.*

**Background:** Cardiovascular events are common complications in patients with chronic kidney disease (CKD). CKD leads to cardiovascular functional and structural alterations with impaired angiogenesis playing an important pathophysiological role. Here we investigated if impaired cardiac angiogenesis in a CKD model is due to reduced recruitment of endothelial progenitor cells (EPCs) in the heart.

**Methods:** To evaluate the recruitment of EPCs from the bone marrow into hearts of CKD rats we transplanted bone marrow from human placental alkaline phosphatase (hPAP) transgenic F344 rats into wildtype F344. Ten weeks after lethal irradiation and bone marrow transplantation, the chimeric rats were randomly assigned to two groups, i.e. subtotal nephrectomy (SnX) or sham operation (sham). 12 weeks after SnX the experiment was terminated and cardiac pathology and bone marrow derived cell (BMDC) recruitment into the heart and circulating EPCs were analysed.

**Results:** CKD in SnX rats was accompanied by increased proteinuria as well as structural histological changes in the kidney. Blood pressure and left ventricular weight were significantly higher in rats with CKD. In addition, cardiac capillary density was about 20% lower in SnX animals as assessed by staining for CD31 positive cells (p=0.0493). In the blood EPCs were significantly reduced in CKD rats, as assessed by CD45+CXCR4+ cells (1.7±0.2 vs 3.0±0.9, p=0.03). In contrast, recruitment of BMDC into the heart was about 50% higher in the SnX group. However, in our study neither in sham animals nor in SnX rats CD31 positive BMDC could be identified, suggesting that BMDC are not an essential source for cardiac endothelial cells. Immunofluorescence double staining proved that the majority of BDMC could be characterized as CD45+ leucocytes (about 50%) and ED1+ macrophages (about 40%) cells.

**Conclusions:** Chronic kidney disease (CKD) reduced the number of circulating EPCs and simultaneously increased influx of BMDC derived inflammatory cells into the heart. However, BMDC seem not to be an important source for cardiac endothelial cells.

*Funding:* Government Support - Non-U.S.

**FR-PO224**

**Altered Macrophage Polarization Promotes Atherosclerosis in a Mouse Model of Chronic Kidney Disease** Lixia Zeng, Hye Kyong Kweon, Anna V. Mathew, Pradeep Kayampilly, Philip C. Andrews, Subramaniam Pennathur. *Univ of Michigan, Ann Arbor, MI.*

**Background:** Atherosclerotic cardiovascular disease is the leading cause of death in patients with chronic kidney disease (CKD). However, the molecular mechanisms underlying this increased risk remain poorly understood. We investigated the role of macrophages in promoting atherosclerosis in a pathophysiologically relevant mouse model of CKD.

**Methods:** Male LDL receptor deficient ($LDLr^{-/-}$) mice were subjected to sham (Control) or 5/6 nephrectomy (CKD) surgery. Subsequently, the animals were maintained in low (LFD) or high fat diet (HFD) for 24 weeks. LC/MS analysis of macrophage-derived oxidation markers and the phosphoproteomic profiling of bone marrow-derived macrophages from the subgroups were performed. Mouse macrophage cell line RAW 264.7 were used for stable isotope labeling in cell cultures (SILAC). The tryptic peptide digests were subjected to two-step phosphopeptide enrichment and analyzed by on-line nanoLC-MS/MS.

**Results:** The CKD mice had significantly higher plasma creatinine, lower hematocrit, decreased body weight and higher mortality. Quantification of lesion area revealed that both LFD and HFDCKD mice had significantly elevated aortic plaque area fraction, and greater luminal narrowing consistent with accelerated atherosclerosis. Cholesterol content and macrophage infiltration were elevated in the aortic lesions of the CKD animals. LC/MS analysis of macrophage derived myeloperoxidase oxidation markers (nitrotyrosine and dityrosine) showed marked elevation in the aortic lesions and targeted lipidomic analysis revealed inflammatory polarization of macrophages (M1) with increased long-chain fattyacyl COA accumulation. We identified 1,449 unique phosphopeptides from 434 unique phosphoproteins. Gene Ontology annotations showed selective changes in phosphoproteins involved in cellular component biological processes. 53 hyperphosphorylated and 43 hypophosphorylated domains were identified suggesting distinct phosphorylation signatures underlying inflammatory macrophage polarization.

**Conclusions:** These findings strongly implicate that altered macrophage polarization contributes to CKD-accelerated atherosclerosis.

*Funding:* NIDDK Support

**FR-PO225**

**Nuclear Translocation of β-Catenin Mediates Parathyroid Hormone-Induced Endothelial-to-Mesenchymal Transition in Human Aortic Endothelial Cells** Min Wu, Hong Liu, Bi-cheng Liu. *Institute of Nephrology, Southeast Univ, Nanjing, China.*

**Background:** Secondary hyperparathyroidism is a common complication of chronic kidney disease, which is closely correlated with the development of cardiovascular diseases. Our previous have demonstrated that PTH could induce endothelial-to-mesenchymal transition in human aortic endothelial cells (HAECs). However, the potential molecular mechanism has yet to be clarified. Thus, the present study sought to investigate whether nuclear translocation of β-catenin mediates PTH-induced EndMT in HAECs.

**Methods:** Primary HAECs were treated with PTH ($10^{-10}$ to $10^{-6}$mol/L). DKK1 (500ng/ml) was used to inhibit β-catenin nuclear translocation. The expressions of endothelial marker CD31and mesenchymal markers FSP1 and α-SMA were detected by real-time PCR and western blot. Nuclear and cytoplasmic β-catenin protein levels were examined by western blot and confocal microscopy.

**Results:** PTH treatment significantly down-regulated endothelial marker CD31 expression and up-regulated mesenchymal markers FSP1 and α-SMA expression in concentration- and time-dependent manners ($p<0.05$). Meanwhile, PTH enhanced nuclear β-catenin levels, along with decreased cytoplasmic β-catenin expression in HAECs, suggesting nuclear translocation of β-catenin. Furthermore, DKK1 treatment significantly attenuated the changes of EndMT-related markers induced by PTH ($p<0.05$).

**Conclusions:** These data demonstrated that elevated PTH promoted EndMT in HAECs. And this effect was partially mediated by nuclear translocation of β-catenin.

**FR-PO226**

**FGF-23 but Not Klotho Predicts the Presence of Aortic Valve Calcifications in Moderate Chronic Kidney Disease?** Luca Di Lullo,[1] Antonio Bellasi,[2] Fulvio Floccari,[4] Antonio Gorini,[1] Rossella Faiola,[1] Alberto Santoboni,[1] Domenico Russo.[3] *[1]Dept of Nephrology and Dialysis, L. Parodi - Delfino Hospital, Colleferro, Roma, Italy; [2]Dept of Nephrology and Dialysis, Azienda Ospedaliera S.Anna, Como, Italy; [3]Dept of Nephrology, Federico II Univ, Napoli, Italy; [4]Dept of Nephrology and Dialysis, S. Paolo Hospital - Civitavecchia, Roma, Italy.*

**Background:** Cardiovascular calcification (CVC) is a frequent complication in chronic kidney disease (CKD) patients. Abnormalities in vitamin D receptor (VDR) activation and hyperphosphoremia are all supposed to contribute to CVC. Fibroblast Growth Factor 23 (FGF-23) is a phosphaturic glycoprotein, linked to phosphate and vitamin D metabolism as well as poor outcome in CKD. We investigated association between FGF-23 and cardiac valvular calcification in moderate CKD.

**Methods:** In this cross-sectional study, 100 (60 men; mean age of 51 ± 4.6 years) CKD stage IIIB-IV patients were enrolled and underwent laboratory (25-OH Vitamin D, Klotho, FGF-23, CRP, serum calcium and phosphorus, iPTH, phosphaturia) and echocardiography testing to assess mineral metabolism such as mitral and aortic valve calcification.Parametric and non-parametric tests were used. Finally, Receiving Operating Curve (ROC) was used to test the model performance.

**Results:** Overall mean serum calcium (9,2±0,4 mg/dl), phosphorus (4,3±0,2 mg/dl) 25OH vitamin D (34,8±13,5 ng/ml) and iPTH (59,11±8,6 pg/ml) were within the reference ranges. Serum FGF-23 and Klotho mean values were 10,4±1,7 pg/ml and 887,8±110,3 pg/ml, respectively. Phosphaturia was 1,043±258 g/day. At univariable and multivariable adjusted analyses, aortic but not mitral valve calcification was associated with FGF-23 levels. Notably, Klotho, iPTH, 25OH Vitamin D, serum phosphorous, phosphaturia and CRP were not associated with either valvular calcification.

**Conclusions:** Our data suggests that FGF-23 but not Klotho is strongly and independently associated with aortic valvular calcification. Future studies should test whether therapeutic strategies aimed at lowering FGF23 (diet, phosphate binders, calcimimetics) can affect calcification progression and cardiovascular damage.

**FR-PO227**

**High Density Lipoprotein in Children with Chronic Kidney Disease Is Pro-Atherogenic** Rukshana C. Shroff,[1,2] Thimoteus Speer,[3] Sophie Colin,[4] Danilo Fliser,[3] Stephen Zewinger,[3] Francis O'Neill,[2] Marietta Charakida,[2] Ulf Landmesser,[5] John E. Deanfield.[2] *[1]Renal Unit, Great Ormond Street Hospital, London, United Kingdom; [2]Vascular Physiology Unit, Institute of Child Health, United Kingdom; [3]Dept of Internal Medicine, Saarland Univ Hospital, Germany; [4]Institut Pasteur de Lille, France; [5]Univ Hospital Zurich.*

**Background:** Patients with CKD are at increased risk for premature atherosclerosis. In adults with CKD HDL loses its vascular protective actions and promotes endothelial dysfunction, but the time course of these changes and the potential for reversibility is unknown.

**Methods:** We studied the vascular effects of HDL in 82 children with CKD stages II-V, dialysis and transplant ($HDL^{CKD}$) and compared with 12 matched controls ($HDL^{Healthy}$). Children with inflammatory diseases, diabetes or active infections were excluded. HDL was isolated by density-gradient ultracentrifugation. Endothelial nitric oxide (eNO) and superoxide (eSO) production were analyzed by electron spin resonance spectroscopy in human aortic endothelial cells after incubation with HDL.

**Results:** $HDL^{Healthy}$ stimulated eNO whereas $HDL^{CKD}$ inhibited eNO production ($p<0.0001$). $HDL^{CKD}$ promoted eSO production and reduced endothelial cell migration and cholesterol efflux (p=0.002, p=0.01, p=0.004 respectively). GFR decline was strongly associated with dysfunctional HDL ($r^2= 0.52$). $HDL^{CKD}$ correlated with increased VCAM-1 and IL6 levels suggesting increased vascular and systemic inflammation. Reduced eNO inversely correlated with pulse wave velocity.

Importantly, $HDL^{CKD}$ function was abnormal as early as CKD II with the most profound changes in dialysis and only partial recovery after transplantation. In a longitudinal follow-up of 8 children on dialysis and 3 months after transplantation HDL induced eNO and eSO production significantly improved but did not normalize.

**Conclusions:** In children with CKD and no inflammatory disease, HDL acquires pro-inflammatory and pro-oxidant properties. HDL functional changes showed an independent and graded association with severity of renal failure and clinical measures of arterial stiffness. Transplantation was associated with recovery but not normalisation of HDL properties.

**FR-PO228**

**Circulating Total Uncarboxylated Matrix Gla-Protein as Biomarker for Vascular Calcification in Chronic Kidney Disease** Elke Theuwissen,[1] Elke Magdeleyns,[1] Heather Pham,[2] Cees Vermeer.[1] *[1]Cardiovascular Research Institute, Maastricht Univ, VitaK, Maastricht, Netherlands; [2]Immunodiagnostic Systems, Boldon, United Kingdom.*

**Background:** Matrix Gla-protein (MGP) is an extra-hepatic vitamin K-dependent protein primarily synthesized by vascular smooth muscle cells. MGP is an important inhibitor of vascular calcification and its activity depends on vitamin K-dependent γ-glutamate carboxylation. Vascular calcification is not only common, but progresses aggressively in chronic kidney disease (CKD) patients. Notably, these patients are characterized by subclinical vitamin K deficiency. We have developed an assay detecting the circulating total uncarboxylated MGP (t-ucMGP), measuring both phosphorylated and desphosphorylated ucMGP fractions. We were interested to see whether t-ucMGP may serve as biomarker for vascular calcification in CKD patients.

**Methods:** Circulating t-ucMGP was measured with our lab-developed competitive mono-antibody ELISA. A monoclonal antibody against the uncarboxylated MGP sequence was used and coupled directly to the microtiter plates.

**Results:** CKD patients were characterized by extremely low circulating t-ucMGP values, suggesting high affinity for calcium. Nearly all CKD patients had values well below the healthy adult range.Lower circulating t-ucMGP levels were associatedwith higher calcification scores in these patients.

**Conclusions:** Circulating t-ucMGP levels are significantly lower in CKD patients than in the healthy population, clearly discriminating CKD patients from controls. Low serum levels of t-ucMGP were associated with the burden of vascular calcification in these patients and hence may serve as biomarker for vascular calcification.

**FR-PO229**

**Chest X-Ray May Serve as a Screening Examination for Coronary Artery Calcification in Dialysis Patients** Kyoko Watanabe, Hiraku Yoshida, Yudo Tanno, Ichiro Ohkido, Keitaro Yokoyama, Takashi Yokoo. *Div of Kidney and Hypertension, Dept of Internal Medicine, Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Coronary artery calcification (CAC) is highly prevalent among dialysis patients. Moreover, it has been reported that Coronary Artery Calcification Score (CACS) using multi-detector computed tomography (MDCT) is a good predictor of mortality in dialysis patients. However, it is not practical to perform coronary artery examination in every dialysis patient. Therefore, the aim of this study was to clarify which part of the body should be imaged with X-rays for screening of CAC in dialysis patients.

**Methods:** We examined 163 autopsy cases who had all been on dialysis (age 63.5±16.0 years, 102 male:61 female, 46 diabetic:117 non-diabetic, dialysis duration 4.4±5.3 years). Data were analyzed using the logistic model to determine the correlation between CAC and specific vascular calcification sites (aortic valve, carotid artery, thoracic aorta, and abdominal aorta).

**Results:** Logistic analyses revealed that thoracic aorta calcification had the strongest correlation with CAC. The table below shows correlation between CAC and specific vascular calcification sites.

| | Odds Ratio | P Value | 95% CI |
|---|---|---|---|
| Aortic valve calficiation | 1.50 | 0.434 | 0.544-4.136 |
| Carotid artery calficiation | 1.40 | 0.532 | 0.486-4.039 |
| Thoracic aorta calficiation | 5.04 | 0.023 | 1.252-20.26 |
| Abdominal aorta calficiation | 2.01 | 0.362 | 0.447-9.062 |

**Conclusions:** The results suggest that a chest X-ray should be performed in dialysis patients to screen for marked thoracic aorta calcification. It is those patients with marked thoracic aortic calficiation on chest X-ray who should undergo further cardiovascular assessment. Accordingly, Chest X-ray may serve as a screening examination for CAC in dialysis patients.

**FR-PO230**

**Calcified Carotid Plaque in Chronic Kidney Disease: Prevalence and Risk Factors** Angels Betriu,[1,4] Montserrat Martinez-alonso,[2,4] M. Vittoria Arcidiacono,[3,4] Merce Borras,[1,4] Jose M. Valdivielso,[3,4] Elvira Fernandez.[1,3,4] *[1]Nephrology, Univ Hospital Arnau de Vilanova; [2]Biostatistic Unit, IRBLleida; [3]Experimental Nephrology, IRBLleida; [4]NEFRONA Group, Spain.*

**Background:** Atheromatous plaque progression starts with a formation of a lipid core (type1-2) and ends with fibrosis (type3-4), and calcification (type5). The role of plaque calcification on plaque vulnerability is not clear in the general population, and also there are no reports describing which risk factors modulate plaque calcification in chronic kidney disease (CKD). The aim of the study was to define the prevalence of each plaque type in patients with CKD at stage 3(E3), 4-5(E4-5) and 5D(E5D), in comparison with subjects without CKD (C), and the risk factors responsible for plaque calcification.

**Methods:** Athermatous plaque were analyzed in 1406 CKD patients (E3:540; E4-5:460; E5D:406), and in 230 control subjects. Plaques were classified by type 1 to 5 accordingly with carotid B-mode ultrasound images. After a descriptive analysis of plaque types was done, calcified plaques were analyzed by logistic regression to identify the associated risk factors.

**Results:** CKD patients presented a 57.5% of carotid plaques: type1=14.3%; type2=20.1%; type3=51.7%; type4=43.2; and type5=23%. Control subjects presented a 41% of carotid plaque: type1=41%; type2=16.5%; type3=47%; type4=28; and type5=1.7% (p<0.0001). Calcified plaque prevalence was higher as CKD progressed (C=1.7%; E3=19%; E4-5=25%; E5D=26%). Plaque calcification in CKD was associated with dialysis treatment (p=0.05), age>55 years (p=0.01), systolic blood pressure (SBP)>180 mmHg (p=0.04), phosphorus (p=0.03), and C-reactive protein (CRP; p=0.02). Gender, smoking, lipid profile, calcium, PTH did not associate with plaque calcification.

**Conclusions:** In control subjects plaque are more lipidic than those in CKD patients that are more calcified in parallel with the severity of the disease. Within the associated risk factors, SBP, phosphorous and inflammation (CRP) are important modifiable factors for plaque calcification. A prospective study of the NEFRONA study will help to understand the role of calcification on plaque stability/vulnerability to predict cardiovascular events.

*Funding:* Pharmaceutical Company Support - ABBVIE

**FR-PO231**

**Intima Media Thickness Does Not Parallel Chronic Kidney Disease Progression in Absence of Atheromatosis** Angels Betriu,[1,4] Montserrat Martinez-alonso,[2,4] M. Vittoria Arcidiacono,[3,4] Merce Borras,[1,4] Jose M. Valdivielso,[3,4] Elvira Fernandez.[1,3,4] *[1]Nephrology, Univ Hospital Arnau de Vilanova; [2]Biostatistic Unit, IRBLleida; [3]Experimental Nephrology, IRBLleida; [4]NEFRONA Group, Spain.*

**Background:** Despite the high prevalence of atheromatosis in chronic kidney disease patients (CKD), a small population is free of atheromatosis due to unknown factors. Intima media thickness (IMT) is a subclinical marker of atheromatosis and increases as CKD progresses. The aim of the study was to evaluate if common carotid IMT (cIMT) measurements differed in CKD patients affected or not by atheromatosis.

**Methods:** We selected 2979 subjects with valid cIMT measurements (B-mode ultrasound). Subjects were then divided in two separated groups depending on presenting (WP:999) or not (W/OP:1980) atheromatosis, in carotid or femoral arteries.

**Results:** In CKD patients, W/OP group, compared to WP group, had a lower: average age (48.5 vs 62 years; p<0.0001), percentage of men (50% vs 66.6%; p<0.0001), and percentage of diabetics (15% vs 30%; p<0.0001). There were no differences about CKD stages (3:38%, 4-5:34%, 5D:36%). The same was observed in control subjects (C) not affected by CKD, with a lower: average age for the W/OP group (49.4 vs 59.4 years; p<0.0001),percentage of men (41% vs 65.4%; p<0.0001), and diabetics (5.5% vs 16.3; p=0.0001). Compared with the control group, WP patients presented the highest cIMT at CKD3 (p<0.05) (C:0.76, 3:0.80, 4-5:0.75, 5D:0.75). W/OP patients, presented a lower cIMT than in control subjects, but statistically different only in CKD5D (p<0.05) (C:0.64, 3:0.65, 4-5:0.595, 5D:0.59). When adjusted for traditional cardiovascular risk factors and CKD stages, WP patients had the highest cIMT only in CKD5D, and W/OP patients cIMT was significantly lower than that in C group at all CKD stages (p<0.01).

**Conclusions:** There are a percentage of patients that do not develop atheromatosis and whose cIMT decreases with the severity of CKD, in contrast to what is observed in patients with atheromatosis. A prospective analysis of the NEFRONA study will attempt to investigate the prognostic value of the cIMT on survival from cardiovascular events in CKD patients.

*Funding:* Pharmaceutical Company Support - ABBVIE

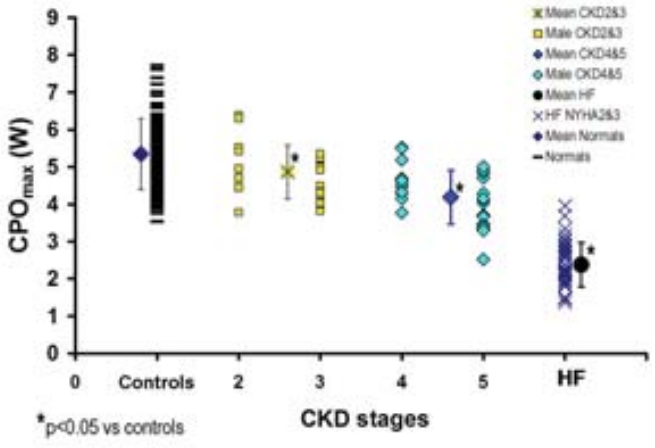
**FR-PO232**

**Evidence of Cardiomyopathy in Asymptomatic Chronic Kidney Disease Patients without Primary Cardiovascular Disease** Shanmugakumar Chinnappa,[1] Andrew F. Mooney,[2] Meguid El Nahas,[1] Lip-Bun Tan.[2] *[1]Sheffield Teaching Hospitals; [2]Leeds Teaching Hospitals, United Kingdom.*

**Background:** Evaluation of asymptomatic cardiac dysfunction in chronic kidney disease (CKD) is highlighted as a research priority by KDIGO to improve our understanding of heart failure (HF) in CKD. The present study is aimed at testing the hypothesis that asymptomatic CKD patients without any known cardiovascular disease or diabetes mellitus have impaired cardiac reserve compared to controls. We measured peak cardiac power output ($CPO_{max}$) to quantify cardiac reserve as $CPO_{max}$ is shown to be a direct indicator of cardiac dysfunction and the best predictor of survival in patients with HF.

**Methods:** We compared the cardiopulmonary exercise performance and non-invasive haemodynamics of 30 male patients with late CKD (stages 4&5) and 20 patients with early CKD (stages 2&3) against 101 healthy male volunteers. Cardiac functional reserve was measured during treadmill exercise testing with standard respiratory gas analyses and a $CO_2$-rebreathing method of non- invasively measuring cardiac output during peak exercise. As positive controls, data from 39 age- and gender-matched HF patients in NYHA class II&III were also obtained. The results are presented as mean±SD.

**Results:** The mean eGFRs of early and late CKD patients were 54.4±18.2 and 16.2±5.8 ml/min respectively. None of the CKD patients had inducible cardiac ischaemia during exercise. Compared to healthy controls, the CKD patients showed a graded reduction in cardiac reserve with 92.4±12.2% (P<0.05) of predicted $CPO_{max}$ in early CKD and 81.4±13.2% (P<0.001) of predicted $CPO_{max}$ in late CKD. In comparison, HF patients had 45.5±10.7% of predicted $CPO_{max}$ (P<0.001).




**Conclusions:** This novel study shows that CKD per se causes impaired cardiac functional reserve, thus confirming the presence of cardiomyopathy, which requires further cardio-renal collaborative research.

## FR-PO233

**High Sensitivity Troponin T in Chronic Kidney Disease** Javier Reque, Borja Quiroga, Soledad Garcia de Vinuesa, Marian Goicoechea, Ursula Verdalles, Juan Manuel Lopez Gomez, Jose Luno. *Nephrology, Hospital Gregorio Marañon, Madrid, Spain.*

**Background:** Chronic kidney disease (CKD) is an independent risk factor for developing coronary heart disease (CHD). The cardiac troponins are structural proteins predictors of (CHD). It has been demonstrated that high sensitivity Troponin T (hs-TnT) has greater predictive value than conventional Troponin T in the development of CHD in the general population. However, its usefulness in patients with CKD is unclear.

**Methods:** We conducted a prospective study including 563 patients: 58% male, 32.5% diabetics, aged 64 ± 17 years, at different stages of CKD. We collected clinical history, routine laboratory parameters and hs-TnT. 20% had CHD history and 9.2% of acute myocardial infarction (AMI). Glomerular filtration rate was 50 ± 29 ml/min/m2 (MDRD-4) and 51 ± 29 mil/min/m2 (CKD-EPI). In 408 patients an echocardiogram was performed simultaneously.

**Results:** The mean hs-TnT was 18.5 ng/ml. Plasma concentrations of as-TnT were directly relate to age (r = 0.643, p <0.001) and inversely with the MDRD 4 (r = -0,674 p <0.001). The hs -TnT is higher in men than in women (20.4 vs 15.9 ng / ml, p <0.01), is significantly higher in patients with history of CHD (p = 0.032) and especially in patients with a history of myocardial infarction ( p <0.01). The mean hs-TnT according to stages of CKD was: Stage1: 3.97 ng / ml; Stagee 5: 45.56 ng/ml, with a statistically significant difference in the variance analysis (p <0.01). When we divided the patients with and without history of CHD, the differences remained significant (p <0.01 and p <0.001 respectively). 19.1% of patients had left ventricular hypertrophy in this group, the values of hs-TnT were higher (40.4 vs 13.8 ng / ml, p <0.001). In a multivariable model, remain as predictors of high hs-TnT values the loss of renal function, history of CHD and LVH.

**Conclusions:** Hs-TnT levels increased as the severity of CKD, even without evidence of acute myocardial damage, so the value of this marker must be adjust according to the degree of renal function. hs-TnT concentrations are higher as in men, patients with history of CHD and those with LVH.

## FR-PO234

**Effect of Adaptive Servo Ventilation Treatment for Chronic Heart Failure in Patients with Chronic Kidney Disease** Akihiro Kuma, Masahito Tamura, Emi Hasegawa, Yoko Fujimoto, Kenichiro Bando, Tetsu Miyamoto, Ryota Serino, Narutoshi Kabashima, Yutaka Otsuji. *The Second of Internal Medicine, School of Medicine, Univ of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

**Background:** Many chronic kidney disease (CKD) patients also have cardiovascular diseases, and it is particularly difficult to treat heart failure (HF) accompanied by CKD. Recent studies have shown that adaptive servo ventilation (ASV) is effective for treating and managing HF. We investigated whether ASV has potential for treating HF in CKD patients.

**Methods:** We registered non-replacement CKD patients (CKD stage II~Ⅴ) with chronic HF in our hospital. We then selected patients whose HF was difficult to control using HF medication alone and who were diagnosed with sleep apnea syndrome by the polysomnography test. These patients underwent ASV therapy while sleeping every night. We then analyzed renal and cardiac function 1 and 6 months after initiating therapy.

**Results:** Patients (n = 23) comprised 16 males and 7 females. Mean age was 66.8 ± 12.2 years. CKD stage was II (33.3%), III (38.1%), IV (23.8%), and Ⅴ (4.8%). Estimated glomerular filtration rate (eGFR) was 46.5 ± 22.4 mL/min per 1.73m$^2$ before ASV therapy (0M), 53.0 ± 21.2 mL/min per 1.73m$^2$ 1month after ASV therapy (1M) (p < 0.01 vs 0M), and 51.4 ± 25.4 mL/min per 1.73m$^2$ 6months after ASV therapy (6M) (p < 0.05 vs 0M). Left ventricular ejection fraction (LVEF) was 31.2 ± 15.1 % (0M), 37.4 ± 12.9 % (1M) (p < 0.005 vs 0M), and 39.0 ± 11.9 % (6M) (p < 0.005 vs 0M). No correlation was observed between eGFR (0M) and improvement in LVEF (0M-6M) (r = 0.2192, p = 0.34). Data are expressed as mean ± standard deviation values.

**Conclusions:** ASV treatment for HF in patients with CKD improved both cardiac and renal functions in the early stage (1M), and functions were maintained for six months. Regardless of renal function, a positive effect of ASV treatment was observed. ASV therapy appeared to be useful for treating HF in CKD patients.

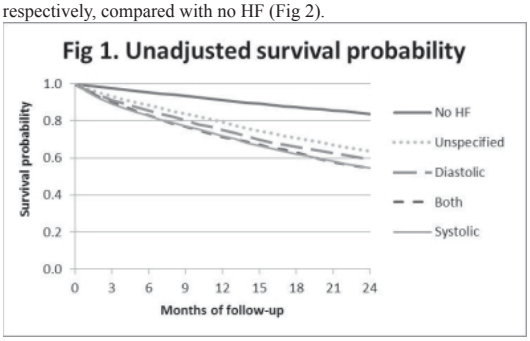
## FR-PO235

**Risk of Death for Systolic and Diastolic Heart Failure in Patients with Chronic Kidney Disease** Shuling Li,[1] Yan Hu,[1] Charles A. Herzog.[1,2] *[1]CDRG/MMRF, U.S. Renal Data System/CVSSC; [2]Univ of MN.*

**Background:** Heart failure (HF) is common in elderly pts and its prevalence increased in pts with chronic kidney disease (CKD). Few studies have examined systolic vs diastolic heart failure and their associated risk of death in elderly CKD pts.

**Methods:** We identified 101,337 CKD pts (age ≥66) prevalent on 12/31/09 and continuously enrolled with Medicare in 2009 using the Medicare 5% sample data. HF was identified from ICD-9 codes 398.91, 428.xx, 402.x1, 404.x1, and 404.x3 in claims during 2009 and included systolic (428.2x), diastolic (428.3x), both systolic and diastolic (428.4x), and unspecified. F/U began on 1/1/2010 and ended at the earlier date of death or 12/31/2011. Unadjusted survival was estimated by Kaplan-Meier method. The risk of death associated with HF types was assessed in a comorbidity-adjusted Cox model.

**Results:** Of all pts, the percent of pts with systolic, diastolic, both, and unspecified HF was 7%, 6%, 2%, and 16%, respectively. Prevalence of HF increased with age; by type, from 6% in 66-69 yrs to 9% in ≥85 yrs for systolic HF, 4% to 8% for diastolic HF, 1% to 2% for both HF, and 10% to 23% for unspecified HF. Prevalence of systolic HF was higher in male than females (9% vs. 6%), but prevalence of diastolic HF was lower in males than females (4% vs. 7%). Two-year survival probability was 54% systolic, 55% both, 59% diastolic, and 64% unspecified HF, and 84% no HF (Fig 1). The adjusted hazards ratio (95% CI) for death was 2.3 (2.2. 2.4) and 1.9 (1.8, 2.0) for systolic and diastolic HF, respectively, compared with no HF (Fig 2).




**Conclusions:** HF of any type in elderly CKD pts is associated with high long-term mortality. Systolic HF has the worst outcome. Future studies targeting this high risk population are warranted.

*Funding:* NIDDK Support

## FR-PO236

**Accumulation of Advanced Glycation End-Products Is Associated with Increased Pulse Pressure in Patients with Progressive Chronic Kidney Disease** Khai Ping Ng, Stephanie J. Stringer, Mark David Jesky, Punit Yadav, Mary Dutton, Charles Ferro, Paul Cockwell. *Renal Medicine, Queen Elizabeth Hospital, Birmingham, Birmingham, West Midlands, United Kingdom.*

**Background:** Accumulation of advanced glycation end-products (AGEs) and chronic kidney disease (CKD) are both strongly and independently associated with cardiovascular mortality. Emerging evidence indicates that both these processes are associated with an increase in arterial stiffness. We investigated whether AGEs accumulation, as measured by skin auto-fluorescence (SAF), is independently associated with pulse pressure (PP), a measure of arterial stiffness, in patients with CKD.

**Methods:** Cross-sectional observation study of the first 500 patients recruited in Renal Impairment in Secondary Care (RIISC) study.

**Results:** The mean age of participants was 64 ± 16 years with 60% of male gender and 71% of white ethnicity. Forty percent were diabetic and 34% had a previous history of cardiovascular disease (CVD). The median estimated glomerular filtration rate (eGFR) was 25 (interquartile range (IQR), 20- 32) ml/min/1.73m$^2$. The mean SAF was 3.0 ± 0.8 AU. In univariate analysis, SAF correlated with age (r=0.521, p<0.001), diabetes (r=0.134, p=0.005), CVD (r=0.171, p<0.001), use of an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker (r= -0.167, p<0.001), PP (r=0.286, p<0.001), natural log-transformed (Ln) eGFR (r= -0.243, p<0.001), Ln albumin:creatinine ratio (r= -0.128, p=0.014), uric acid (r=0.149, p=0.002) and Ln high-sensitivity C-reactive protein (r=0.141, p=0.018). SAF did not correlate with gender or smoking status. In multivariate linear regression model, only age (beta=0.460, p<0.001), PP (beta=0.251, p<0.001) and Ln eGFR (beta=0.112, p=0.034) was independently associated with SAF. The model explained 35% of the variance in SAF.

**Conclusions:** Tissue accumulation of AGEs is independently associated with increased PP in people with CKD. Interventions aimed at preventing the accumulation of AGEs might prevent the development of arterial stiffness in CKD and improve cardiovascular outcomes.

**FR-PO237**

**Skin Perfusion Pressure: An Early Predictor of Peripheral Arterial Disease**

Nia J. Jones,[3] Ian Mathieson,[2] Keith Morris,[2] Aled O. Phillips,[1] Steve Riley.[1] *[1]Institute of Nephrology, Cardiff Univ, Cardiff, United Kingdom; [2]Cardiff Metropolitan Univ; [3]Cardiff and Vale Univ Health Board.*

**Background:** CKD patients treated with haemodialysis have a high prevalence of peripheral arterial disease (PAD), an increased risk of foot ulceration and mortality. Skin perfusion pressure (SPP) assesses the microcirculation and is superior in the diagnosis of PAD compared to Ankle Brachial Pressure Index (ABPI).SPP value ≤70mm/Hg is an independent risk factor for amputation and survival of haemodialysis patients. This prospective study analysed the risk factors for foot ulceration in CKD stage 4 and measured the progression of PAD.

**Methods:** 126 CKD patients (53 with Diabetes) and 46 age matched healthy controls were recruited. PAD was investigated using a Doppler and the Sensilase System (PAD 3000) over a 2 year period.

**Results:** 33 (26%) CKD cohort had a cumulative history of foot ulceration at end point ($p<0.001$). 15 (12%) were referred for vascular intervention and three patients had amputation. Analysis demonstrated a highly significant difference in mean SPP and Tukey's pairwise confirmed the significance was associated with the CKD cohort versus the controls ($p<0.0005$). The right SPP correlated with the left SPP ($p<0.018$) and no differences were observed between SPP values of the CKD and diabetic cohorts. Mann-Whitney U test confirmed the median SPP was significantly lower in participants that developed foot ulceration (SPP 40mm/Hg v 75mm/Hg: $p<0.014$). 20 of the CKD patients at baseline had normal biphasic Doppler waveforms despite a reduced SPP ≤50mm/Hg; 10 of this cohort had developed foot ulceration at end point with 7 of these progressing to monophasic pulse waveform by study end.

**Conclusions:** The micro-circulation is often overlooked during routine foot screening. Risks associated with SPP values ≤ 70 mm/Hg are not confined to the dialysis population. These findings suggest SPP is an important early predictor of lower limb ischemia when compared to the Doppler and may allow early intervention in this patient group to improve outcome.

**FR-PO238**

**Stroke in CKD Patients; Prevalence, Subtypes and Prognosis**

Debasish Banerjee, Hugh Markus. *Div of Clinical Sciences, St. Georges, Univ of London, United Kingdom.*

**Background:** Strokes are common in patients with chronic kidney disease (CKD) and associated with poor outcome. Data on prevalence, stroke subtypes and risk factors in CKD patients within large cohorts of stroke patients are lacking. The study aimed to estimate the prevalence of CKD in stroke patients admitted to an inner-city hyper-acute stroke unit; together with associated risk factors, stroke subtypes and outcome.

**Methods:** Data collected prospectively in 2333 stroke patients were analysed using SPSS 17. Stroke subtypes were reported by clinicians using the "Trial of Org 10172" (TOAST) classification with brain imaging and clinical information. The study was approved as a clinical audit. The GFR was estimated using MDRD equation. CKD was defined as eGFR < 60 ml/min/1.73m$^2$. Short term outcome was estimated with the "Barthel index"; 20 being the best score.

**Results:** In 2333 stroke patients, 639 (27.4%) had CKD. CKD patients were older (79±11 vs. 70±14 years; $p<0.001$), less likely of Afro Caribbean or African descent (11.5% vs. 17.3%; $p<0.0.005$), with less smokers (12% vs. 23%; $p<0.0001$), more diabetes (31% vs. 21%; $p<0.0001$), less hyperlipidaemia (35% vs. 41%; $p<0.01$), lower cholesterol (4.5±1.4 vs 4.8±1.3; $p<0.001$) and lower LDL cholesterol (2.7±1.1 vs. 2.9±1.0; $p<0.001$), compared to non CKD patients.

CKD patients suffered less haemorrhagic strokes (9.2% vs. 14.6%; $p<0.001$) but more cardioembolic ischaemic strokes (36.8% vs. 23.8%; $p<0.0001$) compared to non CKD patients. Adjusted for age, gender, ethnicity, systolic BP and cholesterol; CKD was associated with more cardioembolic strokes (OR=1.31, 95%CI 1.05 to 1.64; p=0.015) and less haemorrhagic strokes (OR=1.51, 95%CI 1.07 to 2.13; p=0.020).

The Barthel Index at 1 week was lower in CKD patients (11.38±7.84 vs. 13.61±7.38; $p<0.001$). In multivariable regression age, diabetes, cholesterol, systolic BP and eGFR were all independently related to lower Barthel Index.

**Conclusions:** Among stroke patients admitted to the hyper-acute stroke unit, CKD was present in 27.4% associated with lower Barthel index at 1 week. The CKD patients were older, more diabetic, with lower cholesterol, similar blood pressure and suffered from less haemorrhagic but higher cardioembolic strokes.

**FR-PO239**

**A Novel Model of Advanced Diabetic Kidney Disease in Mice**

Jean-Francois Thibodeau,[1,2] Dylan Burger,[1] Chet E. Holterman,[1] Kevin D. Burns,[1] Rhian Touyz,[4] Chris R. Kennedy.[1] *[1]Kidney Research Center, Ottawa Hospital Research Institute, Ottawa, Canada; [2]Cellular and Molecular Medicine, Univ of Ottawa, Ottawa, Canada; [3]Institute of Cardiovascular and Medical Sciences, Univ of Glasgow, Glasgow, United Kingdom.*

**Background:** Insight into the pathogenesis of diabetic nephropathy (DN) has been limited by a lack of animal models which recapitulate key features of human DN. While the majority of currently available rodent models exhibit characteristics of early DN (hyperfiltration, mesangial expansion, albuminuria), features of advanced DN (hypertension, GFR decline, tubulointerstitial fibrosis) are absent or require a significant time investment for full phenotype development. A possible explanation for resistance to renal complications of diabetes in rodents is the lack of a concomitant increase in blood pressure. Accordingly, the aim of the present study was to develop a mouse model of advanced DN with hypertension superimposed.

**Methods:** Type 1 diabetic OVE26 mice were crossed with a mouse model of angiotensin-dependent hypertension utilizing mice transgenic for active human renin cDNA under the control of the transthyretin promoter (TtRhRen). TtRhRen mice exhibit angiotensin-dependent hypertension and progressive cardiac hypertrophy beginning at 6 weeks of age.

**Results:** At 20 weeks of age, OVE26 mice exhibited modest albuminuria as compared with wild-type and TtRhRen mice. However, albuminuria was significantly more pronounced in OVE26/TtRhRen mice. Additionally, OVE26/TtRhRen mice displayed renal and glomerular hypertrophy and evidence of tubulointerstitial fibrosis. Interestingly, beginning at 10 weeks TTRhRen/Ove26 mice were severely hypertensive, significantly exceeding that of TTRhRen littermates.

**Conclusions:** In summary, our results suggest that TtRhRen/OVE26 mice are a robust model of type I DN that recapitulates key features of human disease. Such a model may be of significant interest in the analysis of DN pathogenesis and in the assessment of putative therapeutics.

**FR-PO240**

**Hyperglycemia Triggered Inflammosomes Activation in Glomerular Cells via Mitochondrial ROS Contributes to Diabetic Nephropathy**

Madhusudhan Thati, Khurrum Shahzad, Fabian Bock, Hermann-Josef Groene, Berend Heinrich Isermann. *Institute for Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke Univ Magdeburg, Magdeburg, Germany.*

**Background:** Activation of the Nlrp3-Inflammosome has been implicated in many diseases including diabetic nephropathy (dNP). Whether inflammosome activation is causally involved in the pathogenesis of dNP and whether resident or bone marrow derived cells are involved remains to be elucidated.

**Methods:** Here, we analyzed the processing of caspase-1 and IL-1β in two different diabetes models (type 2 diabetic db/db mice and in STZ induced mice). A subset of mice was injected with anakinra, an IL-1β inhibitor. In addition we transplanted bone marrow cells from Nlrp3-/- or caspase1-/- mice into db/db or wt control mice to investigate role of hematopoietic cells. Diabetic mice deficient for the mitochondrial RedOx-enzyme p66$^{shc}$ were analyzed. Supplementary in vitro assays were performed in endothelial cells and podocytes.

**Results:** In renal cortex extracts of diabetic mice an increased expression of Nlrp3 and of mature IL-1β was observed. Nlrp3-/- or caspase1-/- mice as well as anakinra treated mice were protected against dNP. Confocal immunofluorescence analyses revealed that inflammasome activation is enhanced in glomerular cells in mice and humans with dNP. The severity of dNP was unchanged in bone marrow chimeras (Nlrp3-/- or caspase1-/- > db/db, compared with wt > db/db). Inhibition of mitochondrial ROS prevents inflammasome activation in podocytes in vitro. In diabetic p66$^{Shc}$-/- mice ROS-makers and inflammasome activation were reduced and these mice were protected against diabetic nephropathy. In vitro studies revealed activation of Nlrp3 /caspase-1/ pro-IL-1β upon glucose stimulation, while caspase-1 inhibitor/anakinra abolished IL-1β induction. Anakinra had no effect when cells were transduced with mutated Nlrp3 (Q705K) compared with Nlrp3-WT.

**Conclusions:** Taken together, these results strongly support that activation of the inflammasome in residual glomerular cells contributes to dNP. The inflammosomes may be a new therapeutic target in patients with diabetic nephropathy.

**FR-PO241**

**Baseline Characteristics of Diabetic Kidney Disease Patients Enrolled in a Phase 2 Trial of CTP-499**

LuAnn A. Sabounjian,[1] Philip B. Graham,[1] Lijun Wu,[1] Ara Aslanian,[1] Changfu Cheng,[1] David J. Turnquist,[1] James Shipley,[1] George L. Bakris.[2] *[1]Concert Pharmaceuticals, Inc., Lexington, MA; [2]The Univ of Chicago Medicine, Chicago, IL.*

**Background:** CTP-499, a novel, deuterium-containing methylxanthine derivative that selectively inhibits PDE subtypes that modulate cAMP and cGMP hydrolysis, is currently being developed as an additive treatment for delaying the progression of diabetic kidney disease. In preclinical studies, CTP-499 has been shown to suppress inflammatory, oxidative, and fibrotic processes associated with the pathophysiology of diabetic kidney disease. The objective of this double-blind, placebo-controlled, multicenter study (39 centers in the U.S.) is to assess the safety and efficacy of a minimum of 24 weeks of treatment with CTP-499 in patients with macroalbuminuric type 2 diabetic kidney disease who were maintained on a stable ACEi/ARB regimen.

**Methods:** The study randomized 182 patients 1:1 to either CTP-499 (600 mg BID, oral tablet) or placebo. Key entry criteria were: systolic blood pressure ≤ 145 mm Hg, diastolic blood pressure ≤ 90 mm Hg, macroalbuminuria with UACR from 200 for males and 300 for females to a max of 5000 mg/g, and eGFR by MDRD formula between 23 and 89 mL/min/1.73m$^2$. Additionally, patients were not expected to start dialysis in the next year.

**Results:** Based on these parameters, the characteristics of the enrolled population are shown below.

**Characteristics of Enrolled Patients**

| | | |
|---|---|---|
| **Randomized** | Total, N | 182 |
| **Gender** | Males, N (%) | 138 (75.8) |
| | Females, N (%) | 44 (24.2) |
| **Age** | Mean (range) years | 63.5 (29 to 86) |
| **HbA1c** | Mean ± SD (range) | 7.5 ± 1.2 (4.6 to 11.6) |
| **UACR** | Mean (range) mg/g | 1114.6 (173 to 3991) |
| | ≤ 1500 N (%) | 137 (75.3) |
| | > 1500 N (%) | 45 (24.7) |
| **eGFR** | Mean (range) mL/min/1.73m$^2$ | 45.8 (23 to 83) |
| | ≤ 60 N (%) | 148 (81.3) |
| | > 60 N (%) | 34 (18.7) |

The primary endpoint is the change in UACR over a minimum 24 weeks of treatment. Secondary endpoints include changes in eGFR, serum creatinine, inflammatory and fibrotic biomarkers and hematology markers, and assessment of population PK.

**Conclusions:** Phase 2 trial results are expected in the second half of 2013 and will inform the next stage of clinical development.

*Funding:* Pharmaceutical Company Support - Concert Pharmaceuticals, Inc.

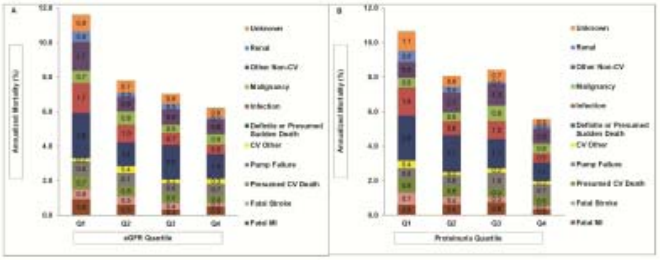
## FR-PO242

**Mode of Death in Diabetic Chronic Kidney Disease: Analysis of the Trial to Reduce Cardiovascular Events with Aranesp (Darbepoetin-Alfa) Therapy (TREAT)** David M. Charytan,[1] Eldrin F. Lewis,[1] Akshay S. Desai,[1] Larry A. Weinrauch,[1] Peter Ivanovich,[4] Robert D. Toto,[2] Jerome A. Rossert,[5] Brian Claggett,[1] Marc A. Pfeffer,[1] Scott D. Solomon.[1] *[1]Brigham & Women's Hospital, Boston, MA; [2]UT Southwestern, Dallas, TX; [3]BHF Cardiovascular Research Centre, Glasgow, United Kingdom; [4]Northwestern Univ, Chicago, IL; [5]Amgen, Thousand Oaks, CA.*

**Background:** How the incidence of specific modes of death changes across the spectrum of chronic kidney disease (CKD) is uncertain.

**Methods:** We analyzed adjudicated endpoints from 4038 subjects with anemia and diabetic CKD enrolled in TREAT, a randomized trial comparing darbepoetin alfa and placebo. Urine protein to creatinine ratio (PCR) at baseline and GFR estimated by the CKD-Epi equation were related to specific modes of death.

**Results:** Median GFR and PCR ranged from 20.6 ml/min/1.73m$^2$ and 4.1 g/g, respectively, in quartile (Q)1 to 47.0 ml/min/1.73m$^2$ and 0.1 g/g in Q4, P<0.01. Annualized mortality (AM) was higher in patients with lower GFR (6.2%, in Q4 to 11.6% in Q1) or higher PCR (5.6% in Q4 to 10.6% in Q1), P<0.01. Both CV and non-CV mortality increased from Q4 to Q1 of GFR or PCR, but there was a trend towards greater increase in non-CV compared with CV death at lower GFR. Sudden death was more frequent with lower GFR (1.4% in Q4, 2.6% in Q1, P=0.02) or higher proteinuria at baseline (1.1% in Q4, 2.6% in Q1, P<0.01). AM from infection was three-fold higher in the lowest GFR (0.6% in Q4 to 1.7% in Q1) and highest PCR quartile (0.5% in Q4 to 1.6% in Q1), P<0.01.



**Conclusions:** In diabetic CKD with anemia both lower GFR and higher PCR at baseline are associated with higher CV and non-CV mortality rates, particularly from infection and sudden death. Efforts to improve outcomes should focus on prevention of infection as well as CV disease.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO243

**PROVALID – A Multinational, Prospective Cohort Study in Patients with Type 2 Diabetes Mellitus** Susanne Eder,[1] Sara S. Roscioni,[2] Hiddo Jan Lambers Heerspink,[2] Dick de Zeeuw,[2] Andrzej Wiecek,[3] Janos Toth,[4] Laszlo Rosivall,[4] Alan G. Jardine,[5] Patrick B. Mark,[5] Gert J. Mayer.[1] *[1]Dep Int Med IV, Med Univ Innsbruck, Innsbruck, Austria; [2]Dep Clin Pharmacol, Univ Med Center Groningen, Groningen, Netherlands; [3]Dep Nephrol, Endocrinol and Metabol Diseases, Med Univ Silesia, Katowice, Poland; [4]Inst Pathophysiol, Nephrol Research and Training Center, Semmelweis Univ, Budapest, Hungary; [5]Nephrol and Transplant Lab, Univ Glasgow, Glasgow, United Kingdom.*

**Background:** One of the most devastating complications of type 2 diabetes is end stage renal disease (ESRD). Interestingly, the incidence of ESRD varies considerably within Europe. PROVALID is a prospective cohort study in 4.000 individuals with type 2 diabetes in five European countries (Austria, Netherlands, Poland, Hungary and U.K.) to determine and compare the cumulative incidence of renal and cardiovascular complications.

**Methods:** Patients aged 18 to 75 years are recruited at primary health care facilities and treated according to local practice. Medical history, incident events and laboratory parameters are entered into a web based data repository. Blood and urine samples for routine laboratory analysis are collected annually; samples for validation of biomarkers at the genome, transcriptome, proteome and metabolome level are collected during the first year of the study and analyzed during follow up. The primary endpoint is the incidence of de novo micro- and macroalbuminuria, doubling of serum creatinine, ESRD or death. Cardiovascular death, non-fatal stroke, myocardial infarction and hospitalization for heart failure will be components of the secondary endpoint.

**Results:** As of May 2013 more than 2600 subjects have been included, recruitment is estimated to be complete by the end of 2013.

**Conclusions:** PROVALID will provide information on the incidence of cardiorenal complications in type 2 diabetes patients in European countries with varying health care systems. Blood and urine samples will be available to allow validation of potential biomarkers to improve monitoring of disease in this high risk population. Funding: European Union (FP7 project no. 241544, SysKid), ABBVIE.

*Funding:* Pharmaceutical Company Support - ABBVIE research project, Government Support - Non-U.S.

## FR-PO244

**Prevalence of Metabolic Syndrome after Donor Nephrectomy** Pisut Katavetin, Yingyos Avihingsanon, Kearkiat Praditpornsilpa. *Div of Nephrology, Dept of Medicine, King Chulalongkorn Memorial Hospital, Thai Red Cross Society and Faculty of Medicine, Chulalongkorn Univ, Bangkok, Thailand.*

**Background:** The association between chronic kidney disease and metabolic syndrome has now been widely appreciated. However, the potential mechanisms for this association are still unclear. Studies mostly focused on how metabolic syndrome cause chronic kidney disease. We, therefore, examined the association in the other way around: Could reduction of nephron mass lead to metabolic syndrome?

**Methods:** Data of the donors who visited the 'Donor Clinic' from 2009-2012 were retrospectively review to examine the prevalence of metabolic syndrome after kidney donation. Data of 494 'Donor Clinic' visits (0.1 to 176.9 months after the kidney donation) from 102 kidney donors were included in the present analysis.

**Results:** The prevalence of metabolic syndrome was only 6.1% during the first year after kidney donation. The prevalence of metabolic syndrome in the second and the third year after kidney donation was 10.0 and 20.0% respectively. Prevalences of high blood pressure (≥130/85 mmHg) impaired fasting glucose (≥100 mg/dl) and elevated triglycerides (≥150 mg/dl) were also increasing during the first 3 year after kidney donation.

Prevalence of each component of metabolic syndrome

| Time after kidney donation | High blood pressure (≥ 130/85 mmHg) | Impaired fasting plasma glucose (≥100 mg/dl) | Elevated triglycerides (≥150 mg/dl) | Reduced HDL (< 40 in male or 50 in female) |
|---|---|---|---|---|
| First year | 18.8 | 3.3 | 20.0 | 23.3 |
| Second year | 17.5 | 25.0 | 33.3 | 25.0 |
| Third year | 31.5 | 27.8 | 41.7 | 25.0 |

**Conclusions:** Reduction of nephron mass after kidney donation may increase risk for metabolic syndrome. These findings would explain, at least in part, the association between metabolic syndrome and chronic kidney disease.

## FR-PO245

**Treatment of Chronic Kidney Disease in Rats Early or Late Post 5/6$^{th}$ Nephrectomy with Multiple Doses of Allogeneic, Marrow Stromal Cells Is Effective in Arresting the Progressive Loss of Renal Function** Anna Gooch,[1] Zhuma Hu,[1] Ping Zhang,[1] Christof Westenfelder.[1,2] *[1]Medicine, U. of Utah and VA Medical Centers, Salt Lake City, UT; [2]Physiology, U of Utah, Salt Lake City, UT.*

**Background:** The NIH reported that from 1980-2009 the prevalence of ESRD increased 600%. Chronic kidney disease (CKD) of all etiologies regularly progresses to ESRD, requiring dialysis or transplantation. Our pre-clinical (AJP Renal 2005) and Phase I Clinical studies (Nat Rev Neph 2010) show that allogeneic rat and human Marrow Stromal Cells (MSCs) effectively protect renal function post IRI Acute Kidney Injury (AKI) and prevent secondary development of CKD. Similar early and late renoprotective responses were observed in on-pump cardiac surgery patients at risk for post-op AKI. The current, preclinical study explores whether administration of MSCs also ameliorates or prevents worsening of CKD.

**Methods:** Groups of adult, male Sprague-Dawley (SD) rats (n=7/group) underwent 5/6$^{th}$ nephrectomy and were followed for 12 weeks. Animals were treated with 4 i.v. allogeneic MSC injections (1x10$^6$ cells/kg b.w.) early (during weeks 2 to 3) or late (during weeks 8 to 11) post nephrectomy. Serum Creatinine (SCr), systolic blood pressure (BP) and urinary albumin (U Alb) excretion were assessed weekly and compared to vehicle treated 5/6$^{th}$ nephrectomized control and sham control groups. Renal histology was performed at end of study to assess glomerular sclerosis, interstitial fibrosis and inflammation.

**Results:** At end of study animals treated with MSCs either early or late in the course of CKD showed significantly lower SCr levels and U Alb excretion vs. vehicle control animals, as well as lower scores for interstitial fibrosis and glomerular sclerosis. While BPs were significantly lower in early MSC treated animals vs. vehicle controls, they were not significantly different in late treated animals vs. their vehicle controls.

**Conclusions:** Repeated administration of allogeneic MSCs early or late in the course of CKD appears to arrest the progressive loss of renal function and rise in albuminuria in these preclinical studies, supporting the possibility that these cells may be of use clinically in treating CKD.

*Funding:* Veterans Affairs Support

**FR-PO246**

**The Enzymatic Activity of the VEGFR2-Receptor for the Biosynthesis of Dinucleoside Polyphosphates** Vera Jankowski,[1] Axel Kretschmer,[2] Mirjam Schuchardt,[1] Markus Van der Giet,[1] Joachim Jankowski.[1] *[1]Med Klinik IV, Charite, Berlin, Germany; [2]Biomarker, Bayer AG, Wupptal, Germany.*

**Background:** The group of dinucleoside polyphosphates encompasses a large number of molecules consisting of two nucleosides which are connected by a phosphate chain of variable length. Dinucleoside polyphosphate are uremic toxins influencing different physiologic and pathophysiologic processes. While the receptors activated by dinucleoside polyphosphates as well as their degradation have been studied in detail, its biosynthesis has not been elucidated so far.

**Methods:** Since endothelial cells released the dinucleoside polyphosphate uridine adenosine tetraphosphate ($Up_4A$), we tested cytosolic proteins of human endothelial cells obtained from dermal vessels elicited for enzymatic activity. When incubated with ADP and UDP these cells showed increasing concentrations of $Up_4A$.

**Results:** The underlying enzyme was isolated by chromatography and the mass-spectrometric analysis revealed that the enzymatic activity was caused by the vascular endothelial growth factor receptor 2 (VEGFR2). Since VEGFR2 but neither VEGFR1 nor VEGFR3 were capable to synthesise dinucleoside polyphosphates, Tyr-1175 is most likely essential for the enzymatic activity of interest. VEGFR2-transfected HEK 293T/17 synthesised dinucleoside polyphosphates in vivo too. The simultaneous biosynthesis of dinucleoside polyphosphates could amplify the response to VEGF, since dinucleoside polyphosphates induce cellular growth via P2Y purinergic receptors. Thus the biosynthesis of dinucleoside polyphosphates by VEGFR2 may enhance the proliferative response to VEGF. Given that VEGFR2 is primarily expressed in endothelial cells, the biosynthesis of dinucleoside polyphosphates is mainly located in the vascular system. Since the vasculature is also the main site of action of dinucleoside polyphosphates, activating vascular purinoceptors, blood vessels appear as an autocrine system with respect to dinucleoside polyphosphates.

**Conclusions:** We conclude that VEGFR2-receptor is capable of synthesizing dinucleoside polyphosphates. These mediators may modulate the effects of VEGFR2 due to their proliferative effects.

**FR-PO247**

**Modulation of NADPH Oxidase Activity by Known Uremic Retention Solutes** Vera Jankowski,[1] Joachim Jankowski,[1] Gerald Cohen.[2] *[1]Med. Klinik IV, Charite, Berlin, Germany; [2]Med. Klinik, Medical Univ of Vienna, Vienna, Austria.*

**Background:** Chronic kidney disease (CKD) and uremia are associated with increased oxidative stress. In addition, a number of diseases like cardiovascular disease are caused by increased concentration of reactive oxygen species (ROS). However, the link between uremic toxicity and enhanced oxidative stress in patients with chronic kidney disease is not well-understood until now.

NADPH oxidase is one of the keyplayer in genesis of oxidative stress by producing superoxide anion and secondarily derived reactive oxygen species (ROS) causing cardiovascular diseases. Based on experiments demonstrating an increased NADPH oxidase activity, when incubated with plasma of CKD patients compared to plasma of healthy volunteers, we investigated the effect of forty eight known and commercial available uremic retention solutes on the enzymatic activity of the NADPH oxidase.

**Methods:** Mononuclear leukocytes isolated from healthy volunteers were isolated, lysed and incubated with NADPH in the presence of plasma from healthy volunteers and CKD patients. Furthermore lysed leukocytes were incubated in presence of uremic retention solute of interest and DPI a specific inhibitor of the NADPH oxidase. The enzymatic activity of NADPH oxidase was quantified by monitoring NADPH degradation.

**Results:** Thirty nine of the tested uremic retention solutes showed a significant effect on the NADPH oxidase activity. All of the thirty nine uremic retention solutes decreased the NADPH oxidase activity. Oxalate has been characterized as the strongest inhibitor of the NADPH oxidase. Furthermore, glyoxal, kynurenic acid, cytidine and homocysteine showed strong inhibition of the NADPH oxidase. None of the investigated uremic retention solutes increased the NADPH oxidase activity.

**Conclusions:** All of the tested uremic retention solutes decreased the NADPH oxidase activity and thus the ROS formation. The results of this study demonstrate that uremic retention solutes may be strong modulators on the genesis of radicals by the NADPH oxidase and thus a cause for cardiovascular complications in CKD patients.

**FR-PO248**

**Endothelial TGF-β Signaling Contributes to Fibrosis Development during Chronic Renal Injury via Microvascular Rarefaction and EndoMT Processes** Sandhya Xavier, Radovan Vasko, Kei Matsumoto, Jun Chen, Michael S. Goligorsky. *New York Medical College, Valhalla, NY.*

**Background:** Manipulation of TGF-b signaling in a certain cell type provides information on its contribution to the progression of disease as opposed to its untargeted interruption in the whole organ. While several studies focused on the ablation of epithelial TGF-b signaling or fibroblasts on development of fibrosis there is none on its ablation in the endothelium, owing to the unavailability of a KO mouse model due to embryonic lethality. We previously reported a model with partial ablation of TbRII in the endothelium ($TbRII^{endo+/-}$ mice).

**Methods:** Chronic kidney disease was induced in these mice using a folic acid nephrotoxic (FAN) model. We isolated primary endothelial cells from kidneys of $TbRII^{endo+/+}$ and $TbRII^{endo+/-}$ mice using anti-CD31 antibody coated dynabeads and assessed the profiles of Smad signaling and expression levels of TGF-b auxiliary receptor endoglin both short (S) and long (L) isoforms.

**Results:** Chronic injury (6 weeks of FA) showed ameliorated fibrotic response in kidneys of $TbRII^{endo+/-}$ mice as compared to $TbRII^{endo+/+}$ mice. $TbRII^{endo+/-}$ mice exhibited better preserved microvasculature in kidneys and increased angiogenic potential of ex vivo cultured aortic rings following challenge with FA. Endothelial to Mesenchymal transformation was significant in kidneys of $TbRII^{endo+/+}$ mice during FAN but ameliorated in kidneys of $TbRII^{endo+/-}$ mice. Smad signaling was dysregulated. Smad2 phosphorylation was impaired in $TbRII^{+/-}$ EC's, following TGF-b treatment. Interestingly, ALK1 mediated Smad1/5 phosphorylation was reduced at baseline in $TbRII^{+/-}$ EC's, however, following TGF-b treatment phosphorylation of Smad1/5 was not affected. Endoglin S/L mRNA expression ratio was significantly lower in $TbRII^{+/-}$ EC's with and without TGF-b treatment as compared to $TbRII^{+/+}$ EC's. An increased L-endoglin mediated Smad1/5 signaling in $TbRII^{+/-}$ EC's could explain the increased angiogenic potential of these mice observed in vivo.

**Conclusions:** Our data indicate a critical role of endothelial TGF-b signaling in regulating angiogenic and fibrotic response to injury.

*Funding:* NIDDK Support

**FR-PO249**

**Endothelial Sirtuin 1-Deficiency Leads to Down-Regulation of Matrix Metalloproteinase 14 and Exaggerated Kidney Fibrotic Response to Injury: Relevance to Fibrosis of Vascular Senescence** Jun Chen,[1] Radovan Vasko,[1] Sandhya Xavier,[1] Chi Hua Sarah Lin,[1] Brian B. Ratliff,[1] May M. Rabadi,[1] Julien Maizel,[1] Tanokuchi Rina,[1] Jian Cao,[2] Michael S. Goligorsky.[1] *[1]New York Medical College, Valhalla; [2]State Univ of New York, Stony Brook, NY.*

**Background:** Sirtuin 1 (SIRT1) depletion in vascular endothelial cells mediates endothelial dysfunction and premature senescence. However, the role of endothelial senescent cells and their contribution to organ pathology remain tenuous.

**Methods:** To investigate the contribution of endothelial SIRT1 deficiency *per se* to SIPS and vasculopathy, we used cre-lox strategy to generate mice with truncated SIRT1 gene in Tie-2-expressing endothelial (EC) and endothelial progenitor cells (EPC).

**Results:** Studies of $SIRT1^{endo-/-}$ mice showed impaired endothelium-dependent vasorelaxation and angiogenesis and traces of spontaneous fibrosis already at the early age. In contrast to the mild phenotype of SIRT1endo-/- mice under basal conditions, induction of nephrotoxic stress (acute and chronic folic acid nephropathy, FAN) resulted in a robust acute renal functional deterioration and exaggerated fibrotic response as examined three months later. To disclose the link between endothelial SIRT1 deficiency and exaggerated renal fibrosis, we examined matrix metalloproteases-14 (MMP-14), which was suppressed in $SIRT1^{endo-/-}$ mice. MMP-14 suppression induced by Sirt1 inhibitor was accompanied by impaired angiogenesis of HUVEC and matrilytic activity of mouse aortic rings, as well as retention of cleavage substrates such as tissue transglutaminase and endoglin, both endowed with the pro-fibrotic activity. Restoration of MMP-14 expression via Concanavalin A (ConA) treatment in vitro and in vivo resulted in improved angiogenic and matrilytic functions of the endothelium, prevented renal dysfunction induced by SIRT1 inhibition and attenuated nephrosclerosis. Same antifibrotic effect was observed in $Sirt1^{endo-/-}$ mice with FAN treated with ConA.

**Conclusions:** Data obtained in Endo-SIRT1(-/-) mice demonstrate the pathogenetic link between defective SIRT1 expression, SIPS and fibrosis. The latter is a result of the MMP-14 deficiency that develops as a consequence of SIRT1 deficiency.

*Funding:* NIDDK Support

**FR-PO250**

**Mechanisms of Proteinuria in a Chronic Kidney Disease (CKD) Model following Ischemic Injury** Silvia B. Campos-bilderback,[1] Monique Desiree Dino,[3] Ruben M. Sandoval,[1] Sarah E. Wean,[1] Bruce A. Molitoris.[1,2] *[1]Medicine/ Nephrology, IU School of Medicine, Indianapolis, IN; [2]Roudebush VAMC, Indianapolis, IN; [3]Univ of Contestado, Mafra, Santa Catarina, Brazil.*

**Background:** The development and or acceleration of CKD progression following acute kidney injury (AKI) is now well recognized, as is the importance of proteinuria as a risk factor for progression of CKD.

**Methods:** Therefore,to further understand the pathophysiology underlying CKD progression and proteinuria we used male Munich Wistar Fromter rats and induced CKD with ischemic injury and a unilateral nephrectomy.

**Results:** Serum creatinine levels increased from 0.3 to 1.5 ± 0.3 ($P<0.01$) and urinary albumin increased from 100 ± 10 to 1150 ± 300mg/24 hr/GFR/100gm ($P<0.01$). There was progression of both proteinuria and loss of GFR in this CKD model. Histologic evaluation revealed tubular atrophy with an interstitial mononuclear infiltrate and edema, peri-glomerular fibrosis, hyaline drop formation and generalized fibrosis. Two-photon microscopy demonstrated albumin glomerular sieving coefficients (GSC) increased from 0.015 ± 0.006 to 0.03 ± 0.010 ($P<0.01$) with intra-glomerular differences also noted within a single glomerulus. In PTs uptake of fluorescently labeled albumin was reduced from 2,911.3 ± 1,815.5 AFU/um2 to 1,763.3± 932.5 AFU/um2 ($p\leq0.05$); the most noticeable difference occurred in the area devoid of albumin uptake among PT's. Untreated rats had only 18.2 ± 7.2% of PT area with no albumin uptake while CKD rats had 42.9 ±21.1% of the PT area devoid of albumin uptake ($p\leq0.05$).

**Conclusions:** These data demonstrate albuminuria in rats with ischemic CKD resulted from a combination of increased $GSC_A$ and reduced albumin uptake by PTC. The model offers a rapid reproducible model of fibrosis and progression to further study glomerular, tubular and interstitial interactions, and to utilize in developing therapeutic agents to minimize CKD and AKI-CKD interactions.

**FR-PO251**

**Abnormal Circadian Rhythm of Blood Pressure Related to Kidney Damage in IgA Nephropathy** Lirong Lin, Yani He. *Nephrology, Daping Hospital, Third Military Medical Univ, Chongqing, China.*

**Background:** Abnormal circadian rhythm of blood pressure is a risk factor of cardiovascular and cerebrovascular lesions in the patients with primary hypertension, also it is closely related with diabetic nephropathy progression and cardiovascular events in hemodialysis patients. The purpose of this study is to investigate the role of abnormal BP rhythm in IgAN.

**Methods:** 341 IgAN patients were recruited in the study. Renal biopsy and 24 hour ambulatory blood pressure monitoring were performed. The pathological scores were evaluated according to the IgAN Oxford criterion. Influencing factors were determined by Spearman and Pearson correlation analysis and logistic regression analysis.

**Results:** (1) Incidence of abnormal BP rhythm is 73.0% (249/341) in IgAN patients, 73.8% (93/126) in IgAN patients with hypertension, 72.6% (156/215) in IgAN patients without hypertension.(2) Cystatin C, urinary P/Cr, uric acid, renal histological score, TIF, hyaline degeneration of small arteries were closely associated with abnormal blood pressure rhythm in IgAN patients with or without hypertension. The decline of nocturnal BP was negatively related with cystatin C, uric acid, renal histological score.(3) EGFR levels was closely associated with abnormal blood pressure rhythm in IgAN patients with hypertension. The decline of nocturnal BP was positively related with eGFR.(4) Urinary P/Cr and night/day ratio of urinary sodium excretion (RN/DUNa)were closely associated with abnormal blood pressure rhythm in IgAN patients without hypertension, and the decline of nocturnal BP was positively related with urinary P/Cr, RN/DUNa.

**Conclusions:** Abnormal blood pressure rhythm is closely related with kidney glomerulus, renal tubulointerstitial and arteriolar lesions in IgAN patients, indicating that abnormal blood pressure rhythm is a potentialrisk factor for IgAN prognosis. Abnormal blood pressure rhythm in IgAN patients is directly related with RN/DUNa and blood uric acid level, implying the ion transport dysfunction caused by renal tubular interstitial lesions in IgAN may be an important cause of abnormal blood pressure rhythm. Abnormal blood pressure rhythm can increase the risk for the progression to ESRD in hypertensive IgAN patients.

*Funding:* Clinical Revenue Support

**FR-PO252**

**Association of Systolic Blood Pressure with Progression to Chronic Dialysis in Patients with Advanced Kidney Disease** Shyamal K. Palit,[1] Anna Jeanette Jovanovich,[1] Alfred K. Cheung,[2,3] James S. Kaufman,[4] Gerard John Smits,[1] Michel Chonchol,[1] Jessica B. Kendrick.[1] *[1]Univ of Colorado Denver, Denver, CO; [2]VASLCHCS, Salt Lake City, UT; [3]Univ of Utah, Salt Lake City, UT; [4]VA New York Harbor Healthcare System, New York, NY.*

**Background:** Hypertension is a risk factor for progression of kidney disease in patients with normal or mildly reduced kidney function. However, the relationship between systolic blood pressure (SBP) and progression to dialysis in patients with advanced kidney disease is unclear.

**Methods:** We performed an analysis on 1,099 patients with advanced kidney disease not yet on dialysis who participated in the Homocysteine in Kidney and End Stage Renal Disease study. Blood pressure was measured in a standardized fashion. SBP levels were examined in clinically defined cutoffs (<120, 120-139, 140-159 and ≥160 mmHg) and as a continuous variable. We used Cox proportional hazard models to examine the association between SBP levels and time to initiation of chronic dialysis.

**Results:** The mean eGFR was 18 ± 7 ml/min. 615 patients (56%) initiated dialysis after a median follow-up of 2.9 years. After adjustment for age, gender and race there was an increased risk of progression to dialysis with a SBP 140-159 and ≥ 160 compared to a level <120 mmHg (HR 1.33, 95% CI 1.02-1.74 and HR 1.47, 95% CI 1.11-1.94, respectively). For every 1 mmHg increase in SBP, there was a 0.7% increased risk of progression to dialysis (HR 1.007, 95% CI 1.004-1.01). After further adjustment for hypertension, diabetes, cardiovascular disease, smoking, BMI, and eGFR every 1 mmHg increase in SBP was still associated with a 0.4% increased risk of progression to dialysis (HR 1.004, 95% CI 1.001-1.008). However, there was no longer an increased risk of progression to dialysis with a SBP 140-159 or ≥ 160 compared to a level <120 mmHg (HR 1.2, 95% CI 0.94-1.62 and HR 1.28 95% CI 0.96-1.70, respectively).

**Conclusions:** Elevated SBP levels were weakly associated with progression to chronic dialysis in patients with advanced kidney disease and there was no increased risk with a SBP >140 or >160 mmHg. Hence, in advanced kidney disease, lowering SBP may not be helpful in slowing progression to dialysis initiation.

*Funding:* NIDDK Support

**FR-PO253**

**Low Serum Levels of High-Density Lipoprotein Cholesterol Are Not Associated with Progression to Dialysis in Patients with Advanced Kidney Disease** Shyamal K. Palit,[1] Anna Jeanette Jovanovich,[1] Alfred K. Cheung,[2,3] James S. Kaufman,[4] Gerard John Smits,[1] Michel Chonchol,[1] Jessica B. Kendrick.[1] *[1]Univ of Colorado Denver, Denver, CO; [2]VASLCHCS, Salt Lake City, UT; [3]Univ of Utah, Salt Lake City, UT; [4]VA New York Harbor Healthcare System, New York, NY.*

**Background:** Patients with chronic kidney disease often have low high-density lipoprotein cholesterol (HDL-C) levels. In the general population and in patients with mild kidney disease, low HDL-C levels are associated with worsening of kidney function. However, the relationship between HDL-C levels and progression to dialysis in patients with advanced kidney disease is unknown.

**Methods:** We performed an analysis on 1,099 patients with advanced kidney disease not yet on dialysis, who participated in the Homocysteine in Kidney and End Stage Renal Disease study. The study population was divided into quartiles based on serum HDL-C levels. We used Cox proportional hazard models to examine the association between HDL-C levels and time to initiation of chronic dialysis.

**Results:** The mean age and eGFR were 69 ± 11 years and 18 ± 7 ml/min/1.73m$^2$, respectively. After a median follow-up time of 2.9 years, 615 patients (56%) initiated chronic dialysis. After adjustment for age, gender and race there was no association between HDL-C levels and kidney disease progression (HR 0.99, 95% CI 0.99 to 1.004). After further adjustment for age, race, gender, diabetes, hypertension, cardiovascular disease, smoking, body mass index, eGFR, systolic blood pressure, low-density lipoprotein cholesterol and triglycerides, HDL-C levels did not predict progression of kidney disease (HR 1.03, 95% CI 0.99 to 1.01, p 0.30). There was no increase in risk of kidney disease progression in patients in the lowest quartile of HDL-C levels compared to the highest quartile (HR 1.2, 95 % CI 0.96 to 1.51, p=0.12).

**Conclusions:** Low HLD-C levels are not associated with progression to chronic dialysis in patients with advanced kidney disease. Further studies are needed to elucidate the role of HDL-C in kidney disease progression.

*Funding:* NIDDK Support

**FR-PO254**

**Gastric Hormone Ghrelin Improves Exercise Endurance of 5/6 Nephrectomized Mice through PGC1α-Dependent Activation of Muscle Mitochondria** Masanori Tamaki, Kazutoshi Miyashita, Shu Wakino, Masanori Mitsuishi, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, School of Medicine, Keio Univ, Tokyo, Japan.*

**Background:** Chronic kidney disease (CKD) decreases physical performance of the patients, and the physical inactivity constitutes an independent risk for cardiovascular diseases. We have identified a decrease in muscle mitochondria from an early phase of CKD, which lead to a reduction in exercise endurance of 5/6 nephrectomized mice, an animal model of mild CKD. These deteriorative effects on physical performance preceded a loss of muscle mass, which resulted in a decrease in muscle power. To improve the physical performance of the CKD model mice, the effect of a gastric hormone, ghrelin, was examined, whose circulating concentration is reported to increase in CKD patients.

**Methods:** C57bl/6 mice which had undergone 5/6 nephrectomy at 6 to 7 weeks old were examined for physical performance at 16 weeks. Ghrelin (0.1 μg/gBW) or insulin-like growth factor-1 (IGF-1, 0.05 μg/gBW) was administered intra-peritoneally twice a week from 8 weeks old. C2C12 cultured myocytes were treated for 24 hours with ghrelin (1 nmol/l) and were examined for the effects on mitochondrial energy metabolism, with or without siRNA for PGC1α, which promotes mitochondrial biogenesis.

**Results:** 5/6 nephrectomy decreased muscle mitochondria and exercise endurance of the mice. An increase in muscle mitochondria, expression of genes promoting mitochondrial biogenesis, and an improvement of running distance were identified in ghrelin-treated mice. Increases in mitochondrial amount and oxygen consumption were identified in ghrelin-treated C2C12 myocytes, and the effects were suppressed by knocking down of PGC1α gene.

**Conclusions:** The gastric hormone ghrelin improved exercise endurance of 5/6 nephrectomized mice through an increase in muscle mitochondrial amount. The in vitro experiments suggested that ghrelin-induced increases in mitochondria and oxygen utilization were PGC1α-dependent. The results indicate that ghrelin plays novel roles in the improvement of physical performance of CKD patients, who could not perform exercise sufficiently.

**FR-PO255**

**Role of PCSK9 (Proprotein Convertase Subtilisin Kexin Type 9) in the Pathogenesis of Hypercholesterolemia in Nephrotic Syndrome** Kyubok Jin,[1] Bongsoo Park,[1] Yang Wook Kim,[1] Nosratola D. Vaziri.[2] *[1]Dept of Medicine, Inje Univ Haeundae Paik Hospital, Busan, Korea; [2]Dept of Medicine, Div of Nephrology and Hypertension, Univ of California Irvine, Irvine, CA.*

**Background:** Nephrotic syndrome (NS) is associated with impaired LDL clearance and elevated serum total and LDL cholesterol levels. LDL receptor (LDLR) plays a key role in cholesterol metabolism by mediating endocytosis and lysosomal degradation of LDL. In a series of earlier studies we found marked reduction in LDLR despite normal LDLR mRNA expressionin the liver of nephrotic rats, denoting a post-transcriptional or post-translational phenomenon. LDLR is, in part, regulated by PCSK9 which is secreted in the blood by the

liver where it binds, internalizes, and facilitates degradation of LDLR. Thus by preventing recycling of LDLR, upregulation of PCSK9 causes LDLR deficiency.Loss-of-function mutation of PCSK9 is associated with reduced LDL-cholesterol and diminished risk of coronary disease.This has lead to emergence of PCSK9 as a novel target for treatment of hypercholesterolemia. The mechanism(s) by which NS causes LDLR deficiency is not known. This study was undertaken to test the hypothesis that impaired LDL clearance in NS may be due to increased PCSK9.

**Methods:** Study Design: Prospective cohort study. Participants: Fifteen patients with nephrotic range proteinuria and 15 age- and gender-matched healthy individuals. Outcomes: Assessment of plasma PCSK9 and its relationship with serum total and LDL cholesterol and proteinuria. Measurements: Fasting serum PCSK9, lipids, and albumin concentrations and urine protein excretion.

**Results:** Elevation of serum total and LDL cholesterol in the nephrotic group was associated with significant increase in plasma PCSK9 level. Plasma PCSK9 was directly related with total and LDL cholesterol concentrations and urinary protein excretion and inversely related with serum albumin concentration.

**Conclusions:** NS results in elevation of the circulating PCSK9 which can contribute to elevation of LDL by promoting LDLR deficiency.

*Funding:* Private Foundation Support

## FR-PO256

### Is ENPP1 Gene SNP Associated with Lower Pyrophosphate Levels in ESRD

Sean M. Connery, Dianne C. Mitchell, German T. Hernandez, Ramin Tolouian. *Texas Tech Univ HSC at El Paso, TX.*

**Background:** ENPP1 is a trans-membrane glycoprotein, cell surface enzyme, encoded by the *ENPP1* gene. ENPP1 regulates inorganic pyrophosphate (PPi) which is involved in bone mineralization & soft tissue calcification. PPi is an essential inhibitor of calcification and has been reported to be lower in hemodialysis (HD) patients. ENPP1 inhibits soft tissue calcification by generating PPi. The dbSNP rs1044498 single nucleotide polymorphism (SNP) in ENPP1 results in an amino acid change from lysine to glutamine at codon 121 (K121Q) and it has been associated with increased vascular calcification in patients with end-stage renal failure (ESRD). Therefore the purpose of this study was to examine the association between SNP variant dbSNP rs1044498 and the levels of circulating PPi in HD patients.

**Methods:** 75 HD pts studied: mean age of 61 ±12 yr, 87% Hispanic, mean HD vintage of 4 ±3.2 yr (range .12 – 21.3). 25 matched controls were also evaluated: mean age of 51 ±13 yr, 96% Hispanic. Platelet free PPi levels in circulating blood were measured by enzymatic method as previously described by Tolouian. SNPs were measured using Human Omni1-Quad BeadChips (Illumina Inc.; San Diego, CA) from gDNA purified from human PBMCs and analyzed using the Illumina iScan & the Illumina's Genome Studio.

**Results:**

| | | **Genotype** mean ±SD | | |
|---|---|---|---|---|
| **Phenotype** | **A/A** | **A/C** | **C/C** | Group |
| **Ppi** (µm) - **Hemodialysis** n=75 | 1.59 ±0.49 | 1.54 ±0.45 | 1.64 ±0.47 | 1.58 ±0.47 |
| **Ppi** (µm) - **Control** n= 25 | | | | 2.06 ±0.53* |

Eller also reported no significant difference in Ppi in 79 white (Australian) HD subjects: n=60 A/A Ppi = 2.7, n = 19 A/C Ppi = 2.4 µm/L with Minor Allele frequency (MAF) = 0.137. The MAF in our HD group was much higher (0.21) and also higher than the MAF (0.12) of the Hispanic HapMap MEX normal (n=100).

**Conclusions:** In our study of predominantly Hispanic HD patients there was a much higher MAF in rs1044498 SNP, and 5 had the putative ENPP1 risk variant C/C, but there was no significant difference between PPi levels and gene variants. There was a statistically significant difference in PPi levels between the HD group and age- & ethnicity-matched controls.

## FR-PO257

### Akt-Pathway-Selective Insulin Resistance Contributes to Decreased Angiogenic Function of Bone Marrow-Derived Mesenchymal Stem Cells under Uremic Conditions

Hyunjin Noh,[1] Mi Ra Yu,[2] Hyun Joo Kim,[2] Jin Seok Jeon,[1] Soon Hyo Kwon,[1] Dong-Cheol Han.[1] *[1]Dept of Internal Medicine, Div of Nephrology, Soon Chun Hyang Univ, Seoul, Republic of Korea; [2]Hyonam Kidney Laboratory, Seoul, Republic of Korea.*

**Background:** Chronic kidney disease (CKD) is associated with increased risk for cardiovascular disease (CVD). We recently reported a significant functional incompetence in bone marrow (BM)-derived mesenchymal stem cells (MSCs) under uremic conditions, which may contribute to the high risk for CVD in patients with CKD. To study the mechanisms of dysfunctional MSCs induced by uremia, we characterized insulin signaling in MSCs.

**Methods:** BM-derived MSCs were generated from control and subtotal nephrectomized CKD mice. MSCs' multipotency was confirmed by positive differentiation into adipocytes and osteocytes.

**Results:** In mice model of hindlimb ischemia, we confirmed that CKD MSCs show functional incompetence. Blood flow recovery, capillary density, and local production of angiogenic factors in the ischemic limb treated with CKD MSCs were significantly inferior to those promoted by control MSCs. In cultured MSCs, insulin induced HIF-1α, VEGF, and SDF-1α expressions via phospatidylinositol 3-kinase (PI3K)/Akt-dependent pathway. CKD MSCs or control MSCs treated with p-cresol exhibited altered insulin signaling in a selective manner for the activation of IRS-1/PI3K/Akt pathway, whereas the activation of ERK pathway remained active. Insulin-induced tyrosine phosphorylation of IRS-1, p85 association with IRS-1, and IRS-1-associated PI3K activity were significantly reduced by p-cresol. In parallel, p-cresol treatment was associated with basal elevation in inhibitory phosphorylation of IRS-1 at Ser 616 residues. Modifying CKD MSCs by overexpression of HIF-1α restored the impaired blood flow recovery, capillary density, and local production of proangiogenic growth factors induced by CKD MSCs in the hindlimb ischemic model.

**Conclusions:** The present study shows that insulin signaling and actions in MSCs are significantly inhibited in a selective manner for the activation of IRS-1/PI3K/Akt pathway under uremia. We suggest that this might be a biological explanation for the functional incompetence of CKD MSCs.

*Funding:* Government Support - Non-U.S.

## FR-PO258

### Carbamylation of Tissue Proteins: A Risk Factor for Complications during Chronic Kidney Disease

Laetitia Gorisse,[1] Christine Pietrement,[2,3] Stephane Jaisson,[1,3] Philippe Gillery.[1,3] *[1]Laboratory of Biochemistry and Molecular Biology, FRE CNRS/URCA n°3481, Faculty of Medicine, Reims, France; [2]Dept of Pediatrics (Nephrology Unit), American Memorial Hospital, Univ Hospital, Reims, France; [3]Laboratory of Pediatric Biology and Research, American Memorial Hospital, Univ Hospital, Reims, France.*

**Background:** Chronic kidney disease (CKD) independently increases the risk of any type of cardiovascular events and generates many other complications. Among other processes, carbamylation of proteins has been recently suggested as a novel risk factor for CKD complications. Carbamylation corresponds to the nonenzymatic binding of isocyanic acid, a urea by-product, to free amino groups of proteins. This reaction leads to the formation of carbamylation-derived products (CDPs). *In vitro* and clinical studies have suggested the potential involvement of CDPs in CKD complications, but their metabolic fate *in vivo* is still unknown, without evidences of CDPs accumulation in tissues.

**Methods:** To address this issue, we have submitted C57Bl/6 mice to sham surgery or 75% nephrectomy. Animals were sacrificed 5, 10 or 20 weeks later. Then we quantified HCit, a CDP resulting from lysine residue carbamylation, in plasma and tissues (aorta, kidney, bone, skin, liver, heart) by LC-MS/MS.

**Results:** We showed that carbamylation was a general biological process since basal plasma and tissue contents of HCit were evidenced in control mice. Carbamylated proteins accumulated to a higher extent in studied tissues after 20 weeks of CKD with a 2-fold increase of HCit content. Carbamylation rate was especially remarkable in type I collagen, a long-lived extracellular matrix protein. Carbamylated collagen accumulated progressively in CKD mice to reach a 5-fold increase after 20 weeks.

**Conclusions:** These results show that CKD increases carbamylation rate of plasma and tissue proteins, and provide new evidences for the involvement of CDPs and especially carbamylated collagen in CKD complications. They reinforce the hypotheses raised by the *in vitro* demonstrations of the deleterious effects of CDPs and by the recently evidenced relationship between plasma CDP concentrations and cardiovascular risk or mortality in CKD patients.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## FR-PO259

### Associations among Chronic Periodontitis, Vitamin D and Cathelicidin in Patients with Chronic Kidney Disease

Marcus Gomes Bastos, Jessica Do Amaral Bastos, Fernando Antonio basile Colugnati, Ana Paula Ferreira, Eduardo Machado Vilela, Patrícia de Castro Daibert, Luiz Carlos Ferreira Andrade. *Medical Clinic, Dentistry, and Institute of Biological Sciences, Federal Univ of Juiz de Fora, Minas Gerais, Brazil.*

**Background:** The occurrence of chronic periodontitis(CP) in patients with chronic kidney disease (CKD) has been associated with adverse outcomes. Vitamin D (25(OH) D), which not uncommonly is reduced in the serum of patients with CKD, directly induces expression of the antimicrobial peptide, cathelicidin (LL-37), which, if low, could explain the severity of CP frequently seen in CKD. The aim of this study was to examine the relationship among CP, 25(OH)D and LL-37 in patients with CKD.

**Methods:** A case-control study was conducted. Case and controls were defined as patients with CKD and CP (CKD+CP; n= 15), CKD without CP (CKD-CP; n= 14), CP but without systemic disease (-SD+CP; n= 14), and participants without both systemic diseases and CP (-SD-CP; n= 15). The demographic, clinical and laboratory data were obtained when the patients were seen at the outpatient clinic. CKD was defined and staged according to the NKF KDOQI™. Serum 25(OH)D was measured by chemioluminescence when evaluating the CP (defined according to the American Academy of Periodontoly). LL-37 and interleukin-6 (IL-6) were measured by ELISA.

**Results:** The data regarding the CP (clinical attachment level or CAL), IL-6, LL-37 and 25(OH)D are presented in the table. Patients with CKD and CP presented a worse CP, were more inflamed, presented lower levels of 25(OH)D and LL-37.

**Conclusions:** CKD patients have a worse CP, probably due to lower levels of LL-37 secondary to inadequate levels of vitamin D.

Table. Clinical and lab results of the 4 study groups.

| | | **GROUPS** | | | **P** |
|---|---|---|---|---|---|
| **VARIABLES** | **-SD-CP** | **-SD+CP** | **CKD-CP** | **CKD+CP** | |
| **CAL, mm** (mean±SD) | 0 | 18±16 | 0 | 28 (±20) | 0.01 |
| **IL-6, pg/mL** median (min-max) | 5 (1-10) | 5.9 (2-11) | 7.6 (2-18) | 14.3 (2-30) | 0.01 |
| **LL-37, ng/mL** median (min-max) | 8.0 (3-13) | 7.4 (3-12) | 8.0 (3-23) | 5.0 (2-6) | 0.07 |
| **25-OH-D, ng/mL** median (min-max) | 30 (15-75) | 25 (17-39) | 28 (19-37) | 20 (7-34) | 0.05 |

*Funding:* Private Foundation Support

## FR-PO260

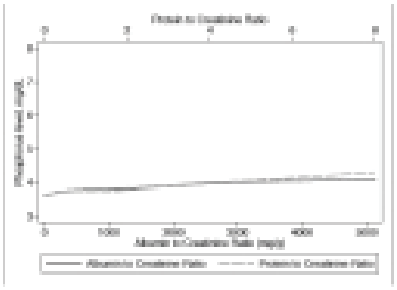
**Albumin-to-Creatinine Ratio versus Protein-to-Creatinine Ratio and Complications of CKD** Herrick Nadine Fisher, Chi-yuan Hsu, Eric Vittinghoff, Feng Lin, Nisha Bansal. *UCSF.*

**Background:** Urine albumin-to-creatinine ratio (ACR) and protein-to-creatinine ratio (PCR) are important markers of kidney damage and are utilized for prognosis in persons with chronic kidney disease (CKD). Despite how commonly these measurements are utilized in clinical practice, relatively few studies have directly compared the performance of these two measures with regard to associations with clinical outcomes. Here we studied the association of ACR and PCR with common complications of CKD.

**Methods:** This was a cross-sectional study of 3,481 participants with CKD in the Chronic Renal Sufficiency Cohort (CRIC). We examined the association between ACR and PCR with measures of common CKD complications: serum hemoglobin, bicarbonate, parathyroid hormone (PTH), phosphorus, potassium and albumin. Restricted cubic spline analyses adjusted for $eGFR_{MDRD}$ were performed to study the continuous association with our predictors with each outcome.

**Results:** Mean eGFR 43 ± 13 ml/min/1.73 $m^2$ and median levels of PCR and ACR were 140 mg and 46 mg/g, respectively. In continuous analyses adjusted for eGFR, higher ACR and PCR were comparable and both associated with lower serum hemoglobin, lower bicarbonate concentration, higher PTH, higher phosphorus, higher potassium and lower albumin (Figure). Across all outcomes, the associations of ACR and PCR were comparable with only small, absolute differences in the outcome measure. Similar associations were seen in patients with and without diabetes mellitus.

**Conclusions:** In persons with CKD, ACR and PCR are relatively comparable in their associations with common complications of CKD. Thus routine measurement of PCR may provide similar information as ACR in managing immediate complications of CKD.



*Funding:* NIDDK Support

## FR-PO261

**Factors Associated with Lower Serum Bicarbonate in Chronic Kidney Disease** Kalani L. Raphael,[1,2] Y. Zhang,[2] Jian Ying,[2] Tom Greene.[2] *[1]Salt Lake City VA Health Care System, UT; [2]Univ of Utah.*

**Background:** Acidosis is common in CKD, yet most with moderate-severe CKD do not have acidosis. The purpose of this study is to identify factors associated with lower serum bicarbonate in CKD.

**Methods:** This is a cross-sectional study using baseline data from the Chronic Renal Insufficiency Cohort (CRIC) Study. A base multivariable linear regression model included eGFR, UACR, age, gender, race, protein intake, diuretic use, and alkali use. Cause of renal disease, waist circumference, CVD, CRP, smoking, blood pressure, use of ACE-i or ARB, anemia, hyperkalemia, income, and education were individually added to the base model.

**Results:** The prevalence of acidosis was 17%. Lower eGFR had the strongest association with lower bicarbonate and higher UACR associated with lower bicarbonate. Older age and diuretic use associated with higher bicarbonate. Other base variables did not associate with variance of serum bicarbonate. Of the additional factors, ACE-I/ARB use, smoking, anemia, hyperkalemia, and larger waist circumference associated with lower bicarbonate.

| Variable | Coefficient (mM) | 95% CI (mM) |
|---|---|---|
| **eGFR (Ref 60 - <90)** | | |
| <15 | -5.7 | -9.1 to -2.4 |
| 15 - <30 | -2.4 | -2.9 to -2.0 |
| 30 - <60 | -0.5 | -0.9 to -0.2 |
| ≥90 | 1.2 | -0.9 to -3.3 |
| **Age (Ref 21 - <45)** | | |
| 45 - <65 | 0.4 | 0.1 to 0.8 |
| ≥65 | 0.5 | 0.1 to 0.9 |
| **UACR (Ref <10)** | | |
| 10 - <30 | -0.3 | -0.6 to 0.0 |
| 30 - <300 | -0.6 | -0.9 to -0.3 |
| 300 - <1000 | -0.7 | -1.1 to -0.3 |
| ≥1000 | -0.6 | -1.0 to -0.2 |
| **Use Diuretic** | 0.9 | 0.6 to 1.1 |
| **Waist circumference** | -0.5 | -0.7 to -0.2 |
| **Smoking** | -0.4 | -0.7 to -0.2 |
| **Use ACE-I or ARB** | -0.4 | -0.7 to -0.2 |
| **Anemia** | -0.4 | -0.7 to -0.2 |
| **Hyperkalemia** | -1.3 | -1.6 to -0.9 |

Other factors considered did not independently associate with a variance of bicarbonate after controlling for factors in the base model.

**Conclusions:** Low eGFR has the strongest relationship with lower bicarbonate in CKD. Albuminuria, use of ACE-i/ARB, younger age, anemia, smoking, hyperkalemia, and larger waist circumference also associate with lower bicarbonate independent of eGFR.

*Funding:* Veterans Affairs Support, Private Foundation Support

## FR-PO262

**Dose-Relativity of Calcium Acetate and Lanthanum Carbonate in the Treatment of Hyperphosphatemia** Rosamund J. Wilson,[1] J. Brian Copley,[2] Michael S. Keith,[2] Peter Preston.[2] *[1]Spica Consultants, Marlborough, United Kingdom; [2]Shire Pharmaceuticals, Wayne, PA.*

**Background:** Hyperphosphatemia can be managed with phosphate (P) binders such as lanthanum carbonate (LC) and calcium acetate (CA). Our aim was to evaluate the dose-relativity between CA and LC monotherapy and assess elemental calcium (Ca) intake.

**Methods:** We completed a *post hoc* analysis from a phase 4 study of patients with end stage renal disease (ESRD) and hyperphosphatemia. The study included a 1-week observation period in which patients remained on their existing P binder, a 12-week LC monotherapy titration phase and a 4-week LC maintenance period. Serum P and daily P binder dose were assessed at baseline for patients treated with CA and at weeks 12 and 16 after switching to LC.

**Results:** At baseline 707 patients received a prior recorded dose of CA monotherapy, 551 of whom were receiving LC at 16 weeks post-baseline. Mean dose, dose relativity, P level and elemental Ca intake overall and by prior CA dose are shown below. The recommended maximum intake of Ca from Ca-based P binders is 1.5g; ~50% of patients exceeded this. The recommended daily intake of 2.0g was exceeded in ~25% of patients based on Ca content of their P binder alone.

| Prior CA dose tab/cap | LC dose g (#tab)[a] | CA dose g (#tab/cap)[b] | Dose relativity g/g[c] | N week 16[d] | Mean P wk 0 mg/dL (*n*) | Mean P wk 16 mg/dL (*n*) | Ca g (SD) |
|---|---|---|---|---|---|---|---|
| ***Overall*** | ***2.8 (3)*** | ***5.6 (9)*** | ***2.0*** | ***551*** | ***6.03 (682)*** | ***6.21 (543)*** | ***1.4 (0.74)*** |
| 1-5 | 2.4 (3) | 2.1 (4) | 0.9 | 103 | 5.51 (122) | 5.73 (102) | **0.5 (1.26)** |
| 6-7 | 2.6 (3) | 4.0 (6) | 1.5 | 162 | 6.08 (193) | 6.15 (157) | **1.0 (0.32)** |
| 8-11 | 2.9 (3) | 6.0 (9) | 2.1 | 144 | 5.84 (193) | 5.97 (143) | **1.5 (0.86)** |
| 12+ | 3.1 (4) | 9.7 (15) | 3.1 | 142 | 6.52 (174) | 6.87 (140) | **2.4 (0.55)** |

[a]Assuming 1000mg LC tablets
[b]Assuming 667mg CA tablets/capsules
[c]CA/LC
[d]One patient had LC dose but no CA dose recorded
Tablet numbers rounded up to whole tablet.

**Conclusions:** The overall CA:LC dose-relativity was 2.0. The lower tablet burden with LC may improve adherence for patients with ESRD. The recommended elemental Ca intake was exceeded by ~50% of patients which may have vascular calcification implications.

*Funding:* Pharmaceutical Company Support - Shire Pharmaceuticals

## FR-PO263

**Statin Use and Lipid Profiles in a Predialysis Clinic** Mathilde Laferriere, Sarah Bezzaoucha, Vincent Pichette, Jean-Philippe Lafrance, Robert Zoël Bell, Michel Vallee. *Nephrology Dept, Hôpital Maisonneuve-Rosemont, Montreal, Canada.*

**Background:** Dyslipidemia and chronic kidney disease are well-recognized independent risk factors for cardiovascular disease. Large randomized-controlled studies had previously demonstrated that the treatment of dyslipidemia resulted in a substantial reduction in cardiovascular events and mortality. The SHARP trial was the first study to demonstrate that treatment of dyslipidemia resulted in a significant cardiovascular risk reduction in an advanced chronic kidney disease population. The objective of our project was to assess the lipid profile and statin use in our predialysis population, in order to determine the proportion of patients reaching target values.

**Methods:** We have retrospectively collected statin use and the lipid profile in patients at our predialysis clinic between 2010 and 2011. Demographic data as well as other risk factors for cardiovascular disease were also collected.

**Results:** At baseline, lipid profiles were available for 289 predialysis patients. Average age was 68,8 years, 40,8% were women and BMI 31 (SD ± 7). Mean glomerular filtration rate was 19,7 mL/min/1,73$m^2$ (SD ±6,5). Major comorbidities were hypertension (96,4%), diabetes (64,3%) and coronary atherosclerotic disease (49,3%). Mean LDL value was 2,2 mmol/L ±0,9 (85mg/dl ±33), with 76% patients already taking a statin and 5,4% taking ezetimibe. Despite being on atorvastatin, the most frequently prescribed statin, a subgroup analysis demonstrated that 43 % of patients did not achieve ideal target levels of LDL ≤ 2 mmol/L ( 77 mg/dl). Likewise, 27% of patients with LDL > 2 mmol/L were not on statin therapy and had significant cardiovascular risk factors.

**Conclusions:** We observed that most of our patients were already on lipid-lowering therapy. Nevertheless, the average LDL level was above target and we identified a subset of patients that were not taking a statin despite having significant cardiovascular risk factors. Implementation of a standing order for the treatment of dyslipidemia may improve the management of lipid profiles in predialysis patients.

## FR-PO264

**Warfarin Dose Requirements in Patients with Kidney Dysfunction** Sami Sakaan,[1,2] Joanna Hudson,[1,2] Carrie Oliphant,[1,2] Carolyn Cummings,[1,2] Numan Alabdan,[1] Tim Self.[1,2] *[1]The Univ of Tennessee; [2]Methodist Univ Hospital, Memphis, TN.*

**Background:** The effect of chronic kidney disease (CKD) on warfarin response remains largely unstudied despite increased risk of thromboembolism and bleeding in this population. Data suggest that patients with reduced kidney function require lower warfarin doses; however, relatively few patients with end stage renal disease (ESRD) were included in previous studies. The purpose of this study is to evaluate warfarin dosing requirements in patients with stage 3 and higher CKD compared to patients with normal kidney function.

**Methods:** A retrospective review was conducted to evaluate warfarin response in hospitalized patients with "normal" kidney function (glomerular filtration rate > 60 mL/min/1.73 m$^2$) and in patients with CKD stage 3, stage 4/5 or ESRD initiated or maintained on warfarin for ≥4 consecutive days. The average daily dose to maintain a therapeutic INR, time to achieve this INR in patients newly initiated on warfarin, and adverse effects were compared among groups.

**Results:** Of the 225 patients studied, the average daily dose to maintain a therapeutic INR was statistically lower in CKD/ESRD patients compared to patients with normal kidney function (p=0.0014). The median time to reach a therapeutic INR did not differ among groups (p=NS). Significantly more bleeding episodes during hospitalization or within 30 days of discharge were observed in patients with CKD stage 3 or higher compared to patients with normal kidney function (p = 0.0258).

| | GFR≥60 (n=50) | Stage 3 (n=48) | Stage 4/5 (n=27) | ESRD (n=100) |
|---|---|---|---|---|
| Daily Dose (mg) [p vs GFR≥60] | 5.6±1.7 | 4.4±1.6 [p=0.0003] | 4.6±1.9 [p=0.02] | 4.6±1.9 [p=0.004] |
| Days to therapeutic INR* [p vs GFR≥60] | 5.1±1.9 (n=23) | 5.3±1.4 (n=28) [p=0.696] | 3.8±1.3 (n=12) [p=0.059] | 4.7±2.3 (n=41) [p=0.431] |
| # of bleeding episodes | 0 | 3 | 3 | 10 |

*Analyzed for patients newly initiated on warfarin.

**Conclusions:** Our findings suggest that CKD and ESRD patients require 20% lower warfarin doses to maintain a therapeutic INR and warrant more monitoring for bleeding compared to patients with normal kidney function.

## FR-PO265

**Academic Achievement in Pediatric Chronic Kidney Disease** Lyndsay Harshman,[1] Douglas Russo,[1] Sheila Barron,[3] Natalie L. Denburg,[2] Patrick D. Brophy.[1] *[1]Pediatrics, Univ of Iowa Children's Hospital, Iowa City, IA; [2]Neurology, Univ of Iowa Hospitals and Clinics, Iowa City, IA; [3]Education, Univ of Iowa, Iowa City, IA.*

**Background:** Children with chronic kidney disease (CKD) are at risk for neurocognitive impairment. Interest in neurocognition has increased as medical management of CKD improves outcomes. Longitudinal, standardized achievement research is lacking. We examined academic achievement in pediatric CKD using longitudinal achievement measurements benchmarked against state normative data. The medical record was examined for medical comorbidities impacting achievement.

**Methods:** The Iowa Tests of Basic Skills & Iowa Tests of Educational Development are developed by the Iowa Testing Program and widely used achievement tests nationwide, administered annually in grades K-12 across a range of subjects. Achievement data from the Iowa Testing Program and medical data from the University of Iowa Children's Hospital were available for 47 CKD patients and analyzed to examine the association between CKD and achievement.

**Results:** Children with CKD demonstrated underachievement in all domains with significant underachievement compared to state referenced, normative data in Math, Science, and Sources of Information. Low 25OH Vitamin D was associated with underachievement in Reading ($p$ = 0.002), Language ($p$ = 0.024), and Math ($p$ = 0.005).

**Table 1**– Underachievement at mean </=50th percentile.

| Domain | N | Calculated Observations | Mean | SE | 95% CI |
|---|---|---|---|---|---|
| Core Composite | 25 | 86 | 40.6 | 5.7 | 29.5-51.8 |
| Core Total | 34 | 139 | 43.0 | 4.9 | 33.5-52.6 |
| Reading | 43 | 192 | 44.0 | 4.6 | 34.9-53.0 |
| Language | 35 | 150 | 46.9 | 4.8 | 37.5-56.4 |
| Math | 44 | 188 | **39.0*** | 4.6 | 30.0-48.1 |
| Science | 44 | 183 | **37.0*** | 3.8 | 29.6-44.5 |
| Sources of Information | 27 | 96 | **40.7*** | 5.2 | 30.5-50.0 |

**Conclusions:** The current study provides insight into academic achievement in pediatric CKD, indicating that patients demonstrate underachievement in Math and Science, and worsening metabolic disease may predispose patients to and be predictors of underachievement.

*Funding:* Other U.S. Government Support

## FR-PO266

**Contribution of Whole-Brain Gray Matter Volume to Higher Brain Function in Predialysis Patients with Chronic Kidney Disease without Overt Cognitive Impairment** Kazuhiko Tsuruya,[1] Hisako Yoshida,[1] Takanari Kitazono.[2] *[1]Dept of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

**Background:** Previous studies suggest that there are significant relationships between multiple cognitive tests and brain volume in healthy elderly, and age-related loss of gray matter volume (GMV) is involved with age-related cognitive change. However, it remains unclear whether GMV associates with cognitive function in patients with chronic kidney disease (CKD). The purpose of the present study is to examine the association of GMV with cognitive function in predialysis CKD patients without overt cognitive impairment (CI).

**Methods:** One-hundred and two predialysis CKD patients (54 men, 48 women) aged 24–80 (62 ± 11) years without overt dementia and history of cerebrovascular disease were recruited. The subjects underwent brain MRI and three kinds of cognitive tests: Mini-Mental State Examination (MMSE), Revised Hasegawa Dementia Scale (HDS-R), and Japan Stroke Scale-Higher Cortical Function Test (JSS-H). MRI images were analyzed using Statistical Parametric Mapping software (SPM8;Wellcome Department of Imaging Neurosciences, University College London, London, UK). Gray matter (GM), white matter (WM), and cerebrospinal fluid were segmented and each volume was quantified using MRI voxel-based morphometry. GMV was normalized as a percentage of intracranial volume to adjust for variations in head size. Univariate and multivariate linear regression analyses were used to examine the association of %GMV with each score of three cognitive tests.

**Results:** Percentage of GMV was significantly associated with the scores of HDS-R and JSS-H, but not MMSE. The associations between %GMV and the scores of HDS-R and JSS-H remained significant (P = 0.004 and P = 0.038, respectively) even after adjustment of potential confounding factors.

**Conclusions:** The present study demonstrates the association of %GMV with cognitive function, suggesting that brain atrophy might contribute to CI in CKD.

## FR-PO267

**The Sleep Disorder in CKD** Shinji Kitajima, Tadashi Toyama, Norihiko Sakai, Kengo Furuichi, Takashi Wada. *Div of Nephrology, Kanazawa Univ Hospital, Kanazawa, Ishikawa, Japan.*

**Background:** Various sleep disorders, such as insomnia and halfway awakening, deteriorate quality of life in patients with advanced CKD. Although uremic substances would be candidates of the cause, detailed mechanisms remain unclear so far. In this study, we evaluated sleep and awakening related gene expression in CKD patients, and brain tissue and electroencephalographic analysis in a mouse CKD model.

**Methods:** [circ1] Human analysis; Nineteen participants without diabetes were evaluated in this study. The participants were divided into three groups: HT group (8 hypertension patients with eGFR > 60 mL/min/1.73m2), CKD group (7 patients with eGFR < 60 mL/min/1.73m2), and HD group (4 patients on hemodialysis). Age and gender were matched with each group. Blood samples were collected in PaxGene RNA collection tubes at the entry of this study. Sleep and awakening related gene was evaluated by RNA microarray. [circ2] Mouse model; Activity of sleep related neuron in cerebral tissue and electroencephalogram were evaluated in a ischemia-reperfusion model in nephrecomized mice, showing advanced kidney injury.

**Results:** In the human gene expression analysis, several sleep related genes expressions were changed in accordance with renal dysfunction. A waking substance, orexin, and gene expression of its receptors were upregulated in accordance with renal dysfunction (correlation coefficient > –0.4). Similary, in a mouse model with elevated creatinine level, orexin RNA expression was downregulated in an awaking period. These findings were further confirmed by orexin neuron activity in immunostaining. Supporting this gene expression and neuron activity, electroencephalogram analysis revealed that mice with kidney injury tend to awake in sleeping period, and sleep in awaking period, suggesting similar clinical phenotypes to those observed in CKD patients.

**Conclusions:** Gene expression profiling of peripheral blood leukocytes in human CKD patients indicated that orexin would be a candidate factor for sleep disorders accompanied with kidney dysfunction. Cerebral tissue and electroencephalogram analysis of a mouse model support these findings. These results suggested that orexin might play a role in sleep disorder in CKD.

## FR-PO268

**Association between Sex and Incident Chronic Kidney Disease in a Diabetic Cohort** Margaret K. Yu,[1] Bessie A. Young.[1,2] *[1]Univ of Washington; [2]VA Puget Sound Health Care System.*

**Background:** Women with diabetes have a higher prevalence of chronic kidney disease (CKD) risk factors than men, such as hypertension, dyslipidemia, obesity, and poor glycemic control. Despite these increased CKD risk factors, whether diabetic women are at greater risk than men for incident CKD is not clear. Our objective is to evaluate the association between sex and 10-year incident CKD risk in a primary care diabetic population.

**Methods:** The Pathways Study is a prospective, observational cohort of ambulatory, diabetic patients from a large managed care population in Seattle, WA. CKD was defined as an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m$^2$ or microalbuminuria (urine albumin/creatinine ≥25 mg/g for women or ≥17 mg/g for men). Subjects without baseline CKD were followed for 10 years for incident CKD. Stepwise Cox proportional

hazards regression with mortality as a competing risk was used to analyze the associations between sex and incident CKD after adjustment for demographics (age, race/ethnicity, marital status, education, smoking), traditional CKD risk factors (baseline eGFR, hypertension, low-density lipoprotein (LDL), body mass index (BMI), hemoglobin A1c), and behavioral risk factors for CKD (depression and adherence to diet, exercise, glucose monitoring, and self-foot exam). Multiple imputation was used for covariates with missing values.

**Results:** Of the 1,468 total subjects, 766 were women (52.2%). Women were younger, less likely to be married, had lower education levels, and higher LDL, BMI and baseline eGFR compared to men. Taking into account pre-CKD death as a competing risk, women had a 33% greater risk of incident CKD at 10 years (sub-hazard ratio (SHR) 1.33, 95% CI 1.10-1.62). This association persisted after adjustment for demographics (SHR 1.44, 95% CI 1.21-1.72), traditional risk factors (SHR 1.48, 95% CI 1.23-1.77), and behavioral risk factors for CKD (SHR 1.48, 95% CI 1.23-1.77).

**Conclusions:** Compared to men, women with diabetes had an increased 10-year risk of incident CKD which was not attenuated after adjustment for traditional and behavioral risk factors for CKD. Diabetic women may represent a high risk group for the development of CKD.

*Funding:* NIDDK Support, Other NIH Support - NIMH, Veterans Affairs Support, Private Foundation Support

## FR-PO269

**Incidence of Chronic Kidney Disease in Cancer Survivors Exposed to Cisplatin** Swati Mehta, Sujata Patil, Carlos D. Flombaum, Ilya Glezerman, Sheron Latcha. *Nephrology, Memorial Sloan Kettering Cancer Center, NY.*

**Background:** Cisplatin,an important antineoplastic agent,is notoriously nephrotoxic. Reports in the literature on long term nephrotoxicity of cisplatin are sparse and have focused on pediatric population. We present data on long term (5 to 12 year follow up) renal effects of cisplatin in cohort of 859 adult patients.

**Methods:** Retrospective chart analysis was performed after obtaining approval from the Institutional review board at Memorial Sloan Kettering Cancer Center. We collected data for 859 adult patients treated with cisplatin from 1st Jan 2000 till 20th Sept 2011. Data included age,gender,race,ethnicity,primary malignancy,serum creatinine level prior to cisplatin therapy and subsequent serum creatinine levels. We calculated the estimated glomerular filtration rate (eGFR) using the 3- variable MDRD equation. Inclusion criteria for the study was age at diagnosis of malignancy>18y and patients who survived at least 5 years after their initial dose of cisplatin. Patients who received ifosfamide concomitant or subsequent to cisplatin or in whom baseline creatinine was unavailable were excluded.

**Results:** 859 patients met the inclusion criteria. 63.3% were Male and 84.6% Caucasian. Most patients were between the age of 25 and 65 when exposed to cisplatin. Head & Neck, testicular and gastrointestinal malignancies accounted for 62% cases. Most patients received a cumulative cisplatin dose from 100 to 400 mg/m$^2$ (77%). 742 patients (90%) had eGFR more than 60ml/min per 1.73 m$^2$ at baseline.93% patients maintained an eGFR >60ml/min per 1.73 m$^2$ 5 years post cisplatin exposure and per the data available; none of the patients had eGFR<15ml/min per m$^2$ or developed ESRD.

**Conclusions:** Cisplatin is an important chemotherapeutic agent with well known acute nephrotoxic effects. Long term renal effects of cisplatin in adults has not been studied well. Our study of 859 adults has limitations in being a retrospective chart review. Its strengths are the sheer sample size of 859 adult patients and a first of a kind study to show that while cisplatin is notorious for causing acute renal failure; its long term effects on renal function are not as severe with no patients developing ESRD in our study.

## FR-PO270

**Carotid Plaque Predict Rate of Renal Function Decline in Patients with Chronic Kidney Disease** Jwa-kyung Kim,[1] Sung Jin Moon,[2] Sun Ryoung Choi,[1] Jong-woo Yoon,[1] Jung-woo Noh,[1] Sung Gyun Kim.[1] *[1]Internal Medicine, Hallym Univ Sacred Heart Hospital, Kidney Research Institute, Korea; [2]Internal Medicine, Myongji Hospital, Kwandong Univ College of Medicine, Korea.*

**Background:** Carotid plaque is a surrogate marker of systemic atherosclerosis and closely associated with adverse cardiovascular outcomes. However, prospective data regarding the predictive role of carotid plaque for renal progression are limited in chronic kidney disease (CKD) patients.

**Methods:** As a prospective design, a total of 411 Stage 3 and 4 CKD patients were enrolled, and all patients underwent carotid ultrasonography at the time of CKD diagnosis. A carotid plaque was defined as a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding carotid intima-media thickness (cIMT) or a thickness >1.5mm as measured from the media-adventitia interface to the intima-lumen interface. Renal function decline was measured by estimated glomerular filtration (eGFR) slope and the renal endpoint was defined as the start of dialysis.

**Results:** Baseline eGFR was 44.5±11.6 mL/min/1.73m$^2$ and eGFR slope was -2.87±3.76 min/1.73m$^2$/yr. A carotid plaque was found in 282 (68.6%) patients, and these patients had significantly faster rates of renal function decline than those without plaque (-3.64±3.84 vs. -1.20±2.85 mL/min/1.73m$^2$/yr, p<0.001). According to multivariate analysis, statistically significant variables determining eGFR slope were diabetes (β=-0.77, p=0.033), increased pulse pressure (β=-0.02, p=0.015), elevated proteinuria (β=-0.50, p<0.001), increased cIMT (β=-4.36, p<0.001) and the presence of a carotid plaque (β=-1.48, p<0.001). During the 2.5-year follow-up, 47 (11.4%) of patients started dialysis. Patients with carotid plaque had a poorer dialysis-free survival rate than those without carotid plaque (hazard ratio 3.3: 95% confidence interval 1.01, 10.77).

**Conclusions:** Carotid plaque was closely associated with rapid decline of renal function and progression to dialysis in stage 3 and 4 CKD patients. Detecting carotid plaque may help identify patients at high-risk for progression of renal dysfunction.

## FR-PO271

**Association of Fluid Overload, Diabetes and Renal Disease Progression in Chronic Kidney Disease** Yi-chun Tsai, Mei-Chuan Kuo, Hung-Chun Chen. *Div of Nephrology, Dept of Internal Medicine, Kaohsiung Medical Univ Hospital, Kaohsiung Medical Univ, Kaohsiung, Taiwan.*

**Background:** Fluid overload is one of major presentation in patients with late stage of chronic kidney disease (CKD). Diabetes is the leading cause of renal failure and progression of diabetic nephropathy has been associated with changes in extracellular fluid volume. The aim of the study was to assess the association of the severity of fluid status, diabetes and kidney disease progression in a late CKD cohort.

**Methods:** This study analyzed the association of the severity of fluid overload measured bybioimpedance spectroscopy method, Body Composition Monitor, with maintenance dialysis and rapid renal progression (estimated GFR slope < -3ml/min/1.73 m$^2$/y) in 472 patients with stage 4-5 CKD.

**Results:** During a median 17.3-month follow-up, there were 71 (15.0%) subjects commencing maintenance dialysis, and 187 (39.6%) subjects presenting rapid renal progression. The adjusted risks of commencing dialysis increased near 3-fold (HR: 2.96, 95% CI: 1.13-7.72, P= 0.03) for non-diabetic patients with fluid overload compared with those without fluid overload. The adjusted risks of maintenance dialysis increased near 4.5-fold (HR: 4.48, 95% CI: 1.69-11.93, P= 0.003) for diabetic patients with fluid overload compared with non-diabetic patients without fluid overload. The adjusted OR for non-diabetic patients with fluid overload compared with those without fluid overload in renal function progression analysis were 4.26 (95% CI: 2.08-8.73, P<0.001). The adjusted risks of renal function progression increased more than 2.8 fold (OR: 2.82, 95% CI: 1.27-6.26, P= 0.01) for diabetic patients with fluid overload compared with non-diabetic patients without fluid overload. However, there was no significant increased risk for renal disease progression between diabetic and non-diabetic patients without fluid overload.

**Conclusions:** Fluid overload is an independent risk factor associated with maintenance dialysis and rapid renal progression. Fluid overload has a higher predictive value for an elevated risk for kidney disease progression than diabetes in late CKD.

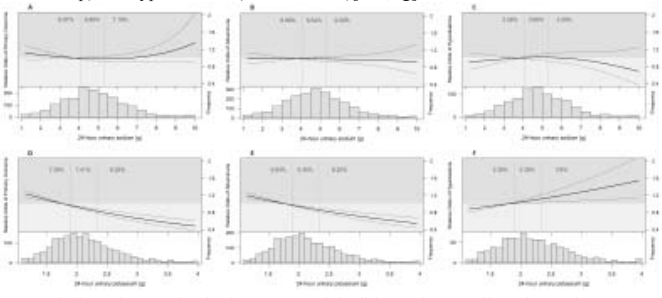
## FR-PO272

**Urinary Potassium but Not Sodium Excretion Predicts Renal Outcomes. Observational Analysis of ONTARGET and TRANSCEND** Andrew Smyth, Daniela Dunkler, Peggy Gao, Koon K. Teo, Salim Yusuf, Martin O'Donnell, Johannes F. Mann, Catherine M. Clase. *McMaster Univ, Hamilton, Canada.*

**Background:** Patients with chronic kidney disease (CKD) are frequently advised to modify sodium (Na) and potassium (K) intake, but supporting evidence is limited.

**Methods:** We studied 28,800 participants at high cardiovascular risk, 90.6% on renin-angiotensin system blockade, followed for a mean of 4.5y in the ONTARGET & TRANSCEND trials. We used the Kawasaki formula to estimate 24hr Na and K from morning fasting urine. The primary outcome was eGFR decline ≥30% or chronic dialysis (CD); secondary outcomes were eGFR decline ≥40% or CD, doubling of creatinine or CD, >5%/yr loss of eGFR, progression of albuminuria and hyperkalemia. We used multinomial logit regression with multivariable fractional polynomials, adjusted for known predictors, to determine the association between Na & K and outcomes, with death as a competing risk. We also analysed Na & K in thirds.

**Results:** At baseline, 9.8% had eGFR<45 and 22.6% eGFR 45-60 mL/min/1.73m$^2$; 3.5% had macro- and 12.5% micro-albuminuria. The primary outcome occurred in 7.0% (n=1,685). There was no significant association between Na excretion and any renal outcomes [A-C, fig] [primary outcome OR 0.97; 95% CI 0.87-1.08 for highest (median 6.1g/d) vs. lowest third (median 3.3g/d)]**.** Higher K excretion was associated with a lower risk of renal outcomes [primary outcome OR 0.71; 95% CI 0.64-0.79 for highest (median 2.7g/d) vs. lowest third (median 1.7g/d)], except doubling of creatinine or CD (no relationship) and hyperkalemia (increased risk)[D-F,fig].



Inclusion of Na and K in the same models did not change effect sizes. There was no interaction with baseline eGFR or albuminuria.

**Conclusions:** K but not Na excretion was associated with clinically important changes in risk of renal outcomes. Our findings do not support low sodium and potassium diets in CKD.

*Funding:* Pharmaceutical Company Support - Boehringer Ingelheim funded the original ONTARGET & TRANSCEND studies but had no role in this work

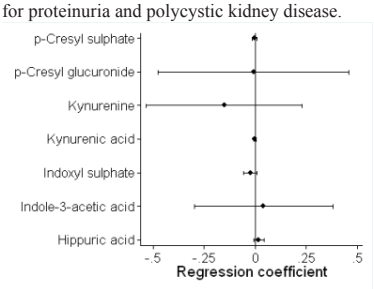
**FR-PO273**

**Uremic Toxins as Risk Factors for Progression in Chronic Kidney Disease**
Jan A.J.G. Van den Brand,[1] Henricus A.M. Mutsaers,[2] Arjan D. Van Zuilen,[3] Peter J. Blankestijn,[3] Rosalinde Masereeuw,[2] Jack F. Wetzels.[1] *[1]Nephrology, Radboud Univ Medical Centre, Nijmegen, Netherlands; [2]Toxicology and Pharmacology, Radboud Univ Medical Centre, Nijmegen, Netherlands; [3]Nephrology, Univ Medical Centre Utrecht, for the MASTERPLAN Study, Netherlands.*

**Background:** The concentration of uremic toxins is increased in patients with kidney failure. We questioned if uremic toxins may influence the rate of eGFR decrease in CKD patients.

**Methods:** Baseline serum samples of patients that participated in the MASTERPLAN trial were used (Van Zuilen *et al. Kidney Int. 2012)*. Cases were rapid progressors, i.e. patients with decline of eGFR > -4.5 ml/min/1.73m$^2$/year. Controls with lower rate of progression were selected and matched for baseline eGFR. Concentrations of uremic toxins (hippuric acid, indole-3-acetic acid, indoxyl sulphate, kynurenic acid, kynurenine, p-cresyl glucuronide, p-cresyl sulphate) were measured using in-tandem mass spectrometry and high pressure liquid chromatography.

**Results:** In total, 27 cases and 65 controls were included. Concentration of all uremic toxins was above control values. Correlations with eGFR ranged between -0.58 to 0.07. Uremic toxins correlated poorly with other known risk factors. Rapid progressors were more likely to be male, younger and have polycystic kidney disease; had higher blood pressure and proteinuria, and lower serum albumin and bicarbonate. None of the uremic toxins was statistically significantly associated to eGFR decline in univariate nor multivariate linear regression analysis. The figure shows regression coefficients and 95% confidence intervals for the association between uremic toxins and the rate of eGFR decline adjusted for proteinuria and polycystic kidney disease.




HA, IAA, IS, KYN, PCG and PCS are in μM, KYNA is in nM.

**Conclusions:** Baseline serum uremic toxin concentrations are not associated with eGFR decline in CKD patients.

*Funding:* Private Foundation Support

**FR-PO274**

**Urinary Trefoil Factor 3 Significantly Predicts the Renal Outcomes in Patients with Chronic Kidney Disease**
Toshio Yamanari,[1] Hitoshi Sugiyama,[1] Hiroshi Morinaga,[1] Masashi Kitagawa,[1] Akifumi Onishi,[1] Ayu Ogawa,[1] Yoko Kikumoto,[1] Shinji Kitamura,[1] Yohei Maeshima,[1] Daisuke Ogawa,[1,2] Kenichi Shikata,[1,3] Yasukazu Ohmoto,[4] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan; [2]Diabetic Nephropathy, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [3]Center for Innovative Clinical Medicine, Okayama Univ Hospital; [4]Otsuka Pharmaceutical Co., Ltd.*

**Background:** TFF3 plays essential roles in mucosal surface maintenance and reconstitution. A decrease in the urinary levels of TFF3 is associated with acute kidney injury in animals. However, whether the urinary levels of TFF3 are associated with renal dysfunction in patients with chronic kidney disease (CKD) is unclear.

**Methods:** We analyzed the urinary TFF (uTFF) levels in spot urine samples from 216 patients with CKD, and assessed the relationships among the uTFF, proteinuria and kidney function. Patients were prospectively followed for three years for doubling of the baseline serum creatinine concentration and the initiation of renal replacement therapy.

**Results:** The excretion of uTFF3 significantly increased with the extent of albuminuria, urinary $\alpha_1$ and $\beta_2$ microglobulin and the decline in the eGFR. A multivariate logistic regression analysis showed that the patients with higher levels of uTFF3 were more likely to have CKD stage ≥ G3b ($P < 0.01$). A longitudinal analysis demonstrated that patients with a higher uTFF3, in combination with macroalbuminuria, had a significantly worse renal prognosis (Log rank, $P < 0.0001$). The levels of urinary TFF3 in the renal end-point group were significantly higher than those in the renal survival group ($P < 0.01$). The AUC of uTFF3 for predicting the progression of CKD (0.879) was significantly higher than that of albuminuria (0.692) ($P < 0.0001$). The levels of uTFF1 and uTFF2 did not correlate with albuminuria.

**Conclusions:** The excretion of uTFF3 is therefore significantly associated with albuminuria and a decline in the renal function. Moreover, the uTFF3 level can be used as a biomarker to predict the renal outcomes in CKD patients.

**FR-PO275**

**Urinary Biomarkers as Risk Factors of End-Stage Renal Disease in the General Population: CKD Biomarkers Consortium**
Meredith C. Foster, Josef Coresh, Joseph V. Bonventre, Chi-yuan Hsu, Paul L. Kimmel, Theodore E. Mifflin, Robert G. Nelson, Vasan S. Ramachandran, Venkata Sabbisetti, Sushrut S. Waikar, Kathleen D. Liu. *CKD Biomarkers Consortium.*

**Background:** We determined the association of 3 urinary markers of tubular injury, liver fatty acid binding protein (L-FABP), kidney injury molecule 1 (KIM-1), and N-acetyl-β-D-glucosaminidase (NAG), with incident end-stage renal disease (ESRD) in the Atherosclerosis Risk in Communities Study, a community-based sample of middle-aged adults.

**Methods:** We performed a nested case-control study with Visit 4 (1996-98) as the baseline. ESRD cases were ascertained through 2008 with 2 controls frequency matched to each case on estimated glomerular filtration rate (eGFR), urinary albumin to creatinine ratio (ACR), diabetes, sex, and race. L-FABP, KIM-1, and NAG were measured and standardized to urinary creatinine. We estimated the incidence odds ratio (OR) for ESRD for an interquartile range increase in each biomarker and for an elevation (highest quartile) in any biomarker using conditional logistic regression.

**Results:** 138 cases were matched to 192 controls with urine available (mean age 64.9 vs. 64.7 years, 43.5% vs. 43.2% women). ESRD occurred a median of 6.0 years after Visit 4. L-FABP and KIM-1 were associated with an increased odds of ESRD, but associations were attenuated with adjustment (**Table**). NAG was not associated with ESRD. In multi-marker analyses, elevated levels for any marker (cases: 59%; controls: 34%) were independently associated with ESRD (**Table**).

**Table:** Adjusted OR and 95% confidence intervals (CI) of ESRD

| Model adjustment* | L-FABP° | KIM-1° | NAG° | Elevation in any biomarker |
|---|---|---|---|---|
| Age | 2.1 (1.2, 3.6)§ | 1.4 (1.0, 1.9)§ | 1.2 (0.8, 1.7) | 2.1 (1.2, 3.8)§ |
| Age, eGFR, ln(ACR) | 1.6 (0.9, 2.9) | 1.3 (0.9, 1.8) | 1.0 (0.7, 1.5) | 1.9 (1.0, 3.4)§ |

*All models account for matching factors. °Log(X+1) transformed with OR for 75$^{th}$ vs. 25$^{th}$ percentile. §P<0.05

**Conclusions:** L-FABP and KIM-1 were associated with risk of ESRD before but not after adjustment for other risk factors. Combinations of markers may have independent risk information.

*Funding:* NIDDK Support, Other NIH Support - The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C).

**FR-PO276**

**Urinary Uromodulin Excretion Predicts Progression of Chronic Kidney Disease Resulting from IgA Nephropathy**
Yuqing Chen,[1] Jingjing Zhou,[2] Xueying Li,[3] Hong Zhang.[4] *[1]Peking Univ First Hospital; [2]Peking Univ First Hospital; [3]Peking Univ First Hospital.*

**Background:** Uromodulin, or Tamm-Horsfall protein, is the most abundant urinary protein in healthy individuals. Recent studies have suggested that uromodulin may play a role in chronic kidney diseases. We examined an IgA nephropathy cohort to determine whether uromodulin plays a role in the progression of IgA nephropathy.

**Methods:** A total of 344 IgA nephropathy patients were involved in this study. Morphological changes were evaluated with the Oxford classification of IgA nephropathy. Enzyme Linked Immunosorbent Assay (ELISA) measured the urinary uromodulin level at renal biopsy day. Follow up was done regularly on 185 patients. Time-average blood pressure, time-average proteinuria, estimated glomerular filtration rate (eGFR) and eGFR decline rate were caculated. Association between the urinary uromodulin level and the eGFR decline rate was analyzed with SPSS 13.0.

**Results:** We found that lower baseline urinary uromodulin level (P=0.03) and higher time average proteinuria (P=0.04) were risk factors for rapid eGFR decline in a follow-up subgroup of the IgA nephropathy cohort. Urinary uromodulin level was correlated with tubulointerstitial lesions (P=0.016). Patients with that had more tubular atrophy/interstitial fibrosis on the surface had lower urinary uromodulin levels (P=0.02).

**Conclusions:** Urinary uromodulin level is associated with interstitial fibrosis/tubular atrophy and contributes to eGFR decline in IgA nephropathy.

**FR-PO277**

**Urine Complement C3 Cleavage Fragments Are Associated with Abnormal Kidney Function and CKD**
Erwin P. Bottinger,[1,2] Avelino Teixeira,[1,2] Girish N. Nadkarni,[1,2] Ilse S. Daehn,[1,2] Joseph V. Bonventre,[2] Kathleen D. Liu,[2] Chi-yuan Hsu,[2] Brad H. Rovin,[2] Robert G. Nelson,[2] Paul L. Kimmel,[2] Harold I. Feldman,[2] Vasan S. Ramachandran.[2] *[1]Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York City, NY; [2]The CKD Biomarker Consortium, NIDDK.*

**Background:** Urine complement C3 fragments have been associated with kidney diseases, yet little is known about urinary C3a (anaphylotactic), C5b-9 (cytotoxic), and iC3b (opsonic) fragments in individuals with abnormal kidney function.

**Methods:** We measured urine complement C3-derived fragments C3a, C5b-9, and iC3b in urine samples obtained from patients as part of routine ambulatory or inpatient clinical care using modified ELISA kits (Quidel® Corp., San Diego, CA).

**Results:** Patient groups included 96 controls, suspected abnormal kidney function without documented CKD (eGFR 60 to 90 ml/min/1.72m$^2$ and/or proteinuria)(N=230), CKDIII or higher with loss of eGFR ≤3 (N=160), or >3ml/min/1.72m2 per year for at least 3 years (N=81). Fractions (%) of samples with detectable urinary C3a, C5b-9, or iC3b were significantly increased in all kidney dysfunction groups compared with controls. In multivariable logistic regression models C3a, C5b-9, or iC3b were significantly associated with kidney dysfunction after adjusting/stratifying by proteinuria (Upro/cr) of >0.5 mg/mg, but not at lower levels.

Table 1. Multivariable logistic regression for suspected abnormal kidney function, CKD III or higher with eGFR loss ≤3, or >3ml/min/1.72m2/yr for at least 3 years, *compared with controls

| | abn. kid. function w/o CKD | | CKDIII or higher; eGFR loss ≤3ml/min/1.72m$^2$/yr | | CKDIII or higher; eGFR loss >3ml/min/1.72m$^2$/yr | |
|---|---|---|---|---|---|---|
| | *OR(95% CI) | *P* | *OR(95% CI) | *P* | *OR(95% CI) | *P* |
| Detect. C3a | 2.13(1.17-3.89) | 0.01 | 2.93(1.10-7.81) | 0.03 | 2.56(1.10-5.59) | 0.03 |
| Detect. C5b-9 | 2.58(1.15-5.78) | 0.02 | 3.41(1.08-10.8) | 0.04 | 4.02(1.14-14) | 0.03 |
| Detect. iC3b | 2.00(1.03-3.90) | 0.04 | 3.29(1.14-9.52) | 0.03 | 5.60(1.70-18) | <0.01 |

**Conclusions:** Urine complement fragments C3a, C5b-9, and iC3b were associated with kidney dysfunction. Associations were strongest between C5b-9 or iC3b and CKD with eGFR loss. We are planning validation studies in prospective CKD cohorts accessible in the CKD Biomarker Consortium.

*Funding:* NIDDK Support

## FR-PO278

**Association of Urinary LFABP, KIM-1, and NAG with Incident End-Stage Renal Disease and Mortality in Type 2 Diabetes** Gudeta D. Fufaa, E. Jennifer Weil, Robert G. Nelson, Joseph V. Bonventre, Venkata Sabbisetti, Sushrut S. Waikar, Theodore E. Mifflin, Xiaoming Zhang, Dawei Xie, Harold I. Feldman, Josef Coresh, Vasan S. Ramachandran, Paul L. Kimmel, Chi-yuan Hsu, Kathleen D. Liu. *CKD Biomarkers Consortium, NIDDK/NIH, Bethesda, MD.*

**Background:** Liver fatty acid binding protein (L-FABP), kidney injury molecule 1 (KIM-1), and N-acetyl-β-D-glucosaminidase (NAG) are urine biomarkers associated with tubular kidney injury. We determined their association with incident end-stage renal disease (ESRD) and all-cause mortality in American Indians with type 2 diabetes.

**Methods:** 258 Pima Indians (69% women, mean age 42 years, mean diabetes duration 11 years, median albumin/Cr ratio [ACR] 38 mg/g, mean glomerular filtration rate [GFR] 149 ml/min) were followed for a median of 11.9 years for ESRD (renal replacement therapy or death due to diabetic nephropathy) and 13.1 years for mortality. Biomarker concentrations were measured at baseline in urine samples stored at -80°C since collection, and associations with ESRD and mortality were evaluated by Cox proportional hazards regression. Hazard ratios (HR) were reported per interquartile range (IQR) of creatinine-normalized KIM-1 and NAG and for detectable vs. undetectable L-FABP; 106 L-FABP samples (41.1%) were below the limit of detection.

**Results:** 63 participants (24.4%) developed ESRD and 79 (30.6%) died. Median concentrations of KIM-1 and NAG were 680.9 pg/mg Cr (IQR 315.5-1377.7 pg/mg Cr) and 9.6 mU/mg Cr (IQR 5.6-14.3 mU/mg Cr), respectively. None of the biomarkers was associated with ESRD after multivariable adjustment; KIM-1 and NAG were associated with mortality. (Table).

| Table: Adjusted HR and 95% confidence intervals for ESRD and all-cause mortality | | |
|---|---|---|
| | **ESRD (n=63/258)** | **All-cause mortality (n=79/258)** |
| KIM-1/Cr | 0.85 (0.64-1.14) | 1.25 (1.05-1.50) |
| NAG/Cr | 1.21 (0.97-1.50) | 1.36 (1.13-1.65) |
| L-FABP | 0.54 (0.28-1.06) | 1.19 (0.68-2.10) |
| Adjusted for age, sex, hypertension, HbA1c, diabetes duration, ln(ACR) and GFR | | |

**Conclusions:** In Pima Indians with type 2 diabetes, urinary concentrations of the tubular injury markers NAG and KIM-1 were associated with all-cause mortality.

*Funding:* NIDDK Support

## FR-PO279

**Urine NGAL and the Risk of Cardiovascular Disease and Death in CKD: Results from the CRIC Study** Kathleen D. Liu, Wei Yang, Alan S. Go, Amanda Hyre Anderson, Harold I. Feldman, Michael J. Fischer, Jiang He, Radhakrishna Reddy Kallem, John W. Kusek, Stephen R. Master, Sylvia E. Rosas, Susan P. Steigerwalt, Kelvin Tao, Matthew R. Weir, Chi-yuan Hsu. *For the CRIC Study.*

**Background:** Patients with chronic kidney disease (CKD), identified using estimated glomerular filtration rate (eGFR) or albuminuria, have increased risk of cardiovascular disease (CVD) and death. The association of tubular injury biomarkers with CVD and death in CKD is not well understood.

**Methods:** We quantified urine neutrophil gelatinase-associated lipocalin (NGAL) (Abbott ARCHITECT) at baseline (2003-2008) among 3386 participants of the Chronic Renal Insufficiency Cohort (CRIC) Study, a large prospective CKD cohort. Time to first adjudicated hospitalization for heart failure, ischemic atherosclerotic event (myocardial infarction, ischemic stroke or peripheral arterial disease) and death were ascertained through March 2011.

**Results:** In Cox models, after adjusting for eGFR, albuminuria, demographics, traditional CVD risk factors and cardiac medications, higher urine NGAL levels were an independent risk factor for ischemic atherosclerotic events (adjusted hazard ratio [HR] highest vs. lowest quintile 1.83; 95% CI 1.20-2.81), but not heart failure events or deaths.

| | | | | Urine NGAL concentration (ng/ml) | | |
|---|---|---|---|---|---|---|
| | | *≤6.9* | *>6.9 -≤12.9* | *>12.9 - ≤22.6* | *>22.6 - ≤49.5* | *>49.5* |
| Heart failure hospitalization | Event rate (per 100 person-year) | 1.50 | 2.40 | 2.58 | 2.65 | 4.18 |
| | Adjusted HR (95%CI) | Ref | 1.45 (1.01-2.08) | 1.09 (0.75-1.59) | 1.01 (0.68-1.49) | 1.05 (0.69-1.59) |
| Ischemic atherosclerotic events | Event rate (per 100 person-year) | 1.42 | 2.03 | 1.94 | 2.19 | 3.46 |
| | Adjusted HR (95%CI) | Ref | 1.54 (1.06-2.23) | 1.26 (0.85-1.87) | 1.52 (1.02-2.27) | 1.83 (1.20-2.81) |
| Death | Event rate (per 100 person-year) | 2.22 | 2.32 | 3.20 | 2.38 | 4.32 |
| | Adjusted HR (95%CI) | Ref | 1.02 (0.74-1.39) | 1.17 (0.86-1.58) | 0.81 (0.58-1.14) | 1.15 (0.81-1.62 ) |

**Conclusions:** Urine NGAL, a biomarker of tubular injury, may measure kidney damage different than that captured by eGFR or albuminuria.

*Funding:* NIDDK Support

## FR-PO280

**Serum Lipoproteins and Progression of Chronic Kidney Disease (CKD)–A Report from the CRIC Study** Mahboob Rahman,[1] Wei Yang,[2] Sanjeev Akkina,[3] Arnold B. Alper,[4] Amanda Hyre Anderson,[2] Lawrence J. Appel,[5] Jiang He,[4] Dominic S. Raj,[6] Jeffrey R. Schelling,[1] Valerie L. Teal.[2] *[1]Case Western Reserve Univ; [2]Univ of Pennsylvania; [3]Univ of Illinois; [4]Tulane Univ; [5]Johns Hopkins Univ; [6]George Washington Univ.*

**Background:** It is unclear whether lipoprotein measurements independently predict kidney disease progression in patients with established CKD.

**Methods:** Adults (n=3939) with CKD were followed in a prospective cohort study for a median of 4.1 years. The predictors for these analyses were baseline total cholesterol, triglycerides, very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-I (apoA-I), apolipoprotein B (apoB), and lipoprotein (a) [Lp(a)]. The outcomes were a composite of end stage renal disease (ESRD) or 50% decline in estimated glomerular filtration rate (GFR) from baseline, and rate of change of GFR.

**Results:** Mean age of the study population was 58.2 years, and mean GFR was 44.9 ml/min/m$^2$; 48% had diabetes, and 55% were prescribed statin therapy. None of the lipoprotein measurements was independently associated with the risk of the composite renal endpoint, or with rate of change of GFR. However, there were significant (p=0.01) interactions by level of proteinuria. In participants with low levels of proteinuria (<0.2g total protein/day) one standard deviation higher LDL-C was associated with a 26% lower risk of the renal endpoint composite (HR 0.74, p=0.01), and one standard deviation higher total cholesterol was associated with a 23% lower risk of the renal endpoint (HR 0.77, p=0.02). In participants with levels of proteinuria >0.2g/day neither LDL-C nor total cholesterol levels were associated with renal outcomes. Results were consistent when stratified by statin therapy.

**Conclusions:** In this large cohort of patients with CKD, total cholesterol, triglycerides, VLDL-C, LDL-C, HDL-C, apoA-I, apoB, and Lp (a) were not independently associated with progression of kidney disease. However, there was an inverse relationship between LDL-C and total cholesterol levels and kidney disease outcomes in patients with low levels of proteinuria.

*Funding:* NIDDK Support

## FR-PO281

**Serum Fetuin Is Independently Associated with Protection from the Progression of Chronic Kidney Disease** Ben Caplin,[1] Markus Ketteler,[2] Willi Jahnen-dechent,[3] John Cunningham,[1] David C. Wheeler.[1] *[1]UCL Medical School, United Kingdom; [2]Klinikum Coburg, Germany; [3]Achen Univ, Germany.*

**Background:** Decline in kidney function is associated with worsening bone-mineral disorder in CKD patients. The relationship between biomarkers linked to arterial calcification and progression of CKD was investigated in the London Arterial Calcification, Kidney and Bone Outcomes study cohort.

**Methods:** 289 patients with stage 2-5 CKD were recruited. Biomarkers including fibroblast growth factor-23 (FGF23) and fetuin were measured in baseline serum samples. Follow-up data were obtained from hospital records after a median of 40 months and eGFR values over 3 years. A cox-proportional hazards method was used to examine predictors of dialysis (censored at death) and a hierarchical model of eGFR over time (censored at dialysis start) used to examine whether similar factors associated with biochemical decline in kidney function.

**Results:** In the whole cohort, baseline median MDRD eGFR was 39.7ml/min/1.73m$^2$, IQR:26.8-51.1 and median fetuin 0.40g/L, IQR:0.35-0.43. Follow-up data, fetuin, FGF and urinary protein levels were available on 189 subjects (159 with eGFR data). After adjustment for baseline eGFR, proteinuria, age, sex, ethnicity and FGF23, fetuin was associated with a reduced risk of requiring dialysis (HR:0.03 per unit log fetuin; 95%CI:0.00-0.33; Figure).

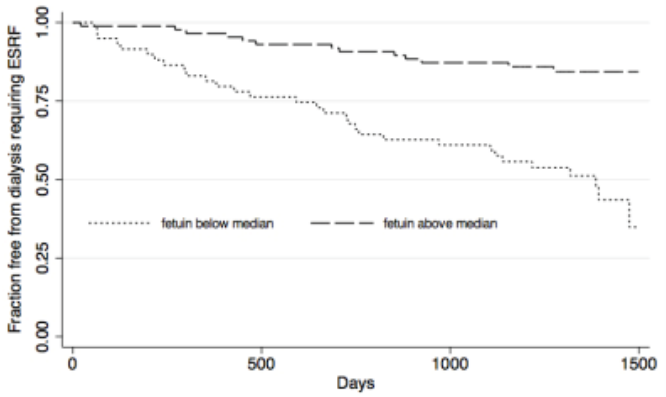



Figure: Kaplan-Meier survival estimates stratified by fetuin at baseline (unadjusted)

In the eGFR model adjusting for the same potential confounders, fetuin was associated with a 6.3mL/min/1.73m$^2$/year slower decline in eGFR per unit log fetuin (95%CI:0.03-12.5). In neither model was the association confounded by a measure of inflammation (hsCRP).

**Conclusions:** Using two independent approaches, need for dialysis and eGFR change, serum fetuin was independently associated with preservation of kidney function. Whether this association is causal and is worth exploring from a therapeutic perspective requires further study.

*Funding:* Private Foundation Support

## FR-PO282

**Associations between Osteoprotegerin, Mortality and Progression of CKD**

Helen Alderson, James Ritchie, Philip A. Kalra. *Salford Royal Hospital.*

**Background:** Elevated levels of osteoprotegerin (OPG) are observed in CKD and have been associated with vascular calcification and mortality. The relationship with cause specific mortality and rate of change in renal function is less well described.

**Methods:** Patients were selected from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS), a prospective observational study of outcome in CKD 3-5. OPG was measured in stored sera from 493 patients. Levels were analyzed in continuous and categorical forms (quartiles of OPG) in relation to all-cause and cardiovascular mortality and progression of CKD.

**Results:** At time of OPG measurement mean eGFR was 31±12ml/min/1.73m$^2$, age 64±14 years. Over a median follow up of 5.4 (3.9-7.2) years 23% of patients reached dialysis, 40% died. Median OPG level was 7.3 (5.7-9.6)pmol/L, with higher levels significantly correlated with increasing age and lower eGFR (r 0.54 and -0.26 respectively, both p<0.001). OPG levels significantly correlated with rate of change in GFR (r -0.11 p=0.009) but did not associate with increased risk for dialysis (HR 1, p=0.5). When divided by quartile of OPG there was a trend towards most rapid eGFR loss in the highest quartile, with a statistically significant difference observed between lowest and highest quartiles (-0.3±3.5 vs. -1.2±3.4 ml/min/1.73m$^2$/year p=0.04) In multivariate Cox regression (adjusted for age, eGFR, blood pressure, diabetes, smoking history and previous macrovascular events) OPG level was associated with all cause mortality, (HR 1.1 [1.04-1.1] p<0.001). The greatest risk was observed in patients in the highest quartile of OPG (HR 2.2 [1.1-4.2] p=0.03). No significant association between OPG and risk for cardiovascular mortality was observed.

**Conclusions:** OPG levels are independently associated with all-cause but not cardiovascular mortality. Patients with the highest OPG levels have a greater annual loss of eGFR but do not have increased risk for dialysis. This is most likely due to the excess mortality observed in this group. With elevated risk clustered in patients in the upper quartile of OPG, our data suggest a threshold value of clinical importance may exist.

## FR-PO283

**The Relationship of Parathyroid Hormone Levels with Cardiovascular Disease Is Stronger When Urine Fractional Excretion of Phosphorus Is Low**

Joachim H. Ix,[1] Cassianne Robinson-Cohen,[2] Michael Shlipak,[3] Andrew N. Hoofnagle,[2] Kenneth J. Mukamal,[4] Ian H. de Boer,[2] Ravi I. Thadhani,[4] S. Ananth Karumanchi,[4] Mary Whooley,[3] Bryan R. Kestenbaum.[2] *[1]UCSD; [2]U. Washington; [3]UCSF; [4]Harvard.*

**Background:** The association of FGF23 with CVD is stronger when accompanied by a low urine fractional excretion of phosphorus (FePi), perhaps due to renal resistance to FGF23 actions. However, whether renal resistance to other hormones identifies risk is unknown.

**Methods:** We tested associations of PTH, FePi, and their interaction with CVD & mortality in patients with normal kidney function (Heart and Soul [H&S]) and moderate CKD (Seattle Kidney Study [SKS]) using Cox regression.

**Results:** In H&S, 205 CVD events & 341 deaths occurred in 8 yrs follow-up. Neither PTH (HR per doubling 1.09; 0.82,1.45) nor FePi (HR 0.89; 0.73,1.09) associated with CVD evemts, but the variables interacted (p interaction=0.01). When grouped by levels above vs. below the medians, the high PTH/low FePi group represented the group at highest risk for CVD. Trends were similar for mortality. In SKS, 75 deaths occurred in 3 yrs. Neither PTH nor FePi were associated with mortality. The p-interaction was 0.09. Again, the high PTH/low FePi groups was at highest risk.

| | HR (95% CI) | P-value | P-interaction |
|---|---|---|---|
| **The Heart and Soul Study (eGFR 71±22ml/min/1.73m$^2$)** | | | |
| **CVD (MI, stroke, CVD death)*** | | | 0.01 |
| Low PTH/Low FePi | 1.00 (ref) | -- | |
| Low PTH/High FePi | 0.73 (0.45,1.17) | 0.19 | |
| High PTH/High FePi | 1.26 (0.83,1.90) | 0.27 | |
| High PTH/Low FePi | 1.61 (1.04,2.46) | 0.03 | |
| **Mortality*** | | | 0.12 |
| Low PTH/Low FePi | 1.00 (ref) | -- | |
| Low PTH/High FePi | 0.85 (0.60,1.20) | 0.36 | |
| High PTH/High FePi | 1.04 (0.76,1.42) | 0.82 | |
| High PTH/Low FePi | 1.31 (0.94,1.82) | 0.11 | |
| **Seattle Kidney Study (eGFR 44±16 ml/min/1.73m$^2$)** | | | |
| **Mortality#** | | | 0.09 |
| Low PTH/Low FePi | 1.00 (ref) | -- | |
| Low PTH/High FePi | 1.87 (0.77,4.55) | 0.17 | |
| High PTH/High FePi | 0.79 (0.36,1.73) | 0.55 | |
| High PTH/Low FePi | 1.94 (0.85,4.45) | 0.12 | |

Adjusted for *age, sex, race, eGFR, ACR, FGF23, diabetes, sbp, bmi, smoking, chol., & CRP. # age, sex, race, eGFR, ACR, & FGF23.

**Conclusions:** High PTH/Low FePi identifies risk for CVD independent eGFR and urine ACR, and may represent kidney tubule resistance to PTH's phosphaturic actions.

*Funding:* Other NIH Support - NHLBI, Private Foundation Support

## FR-PO284

**Unresolved Subclinical Hypothyroidism Is Independently Associated with Progression of Chronic Kidney Disease**

Sangju Lee,[1] Ihnsuk Lee,[2] Yoon-Kyung Chang,[1] Hye Eun Yoon,[1] Chul Woo Yang,[1] Suk Young Kim,[1] Hyeon Seok Hwang.[1] *[1]Div of Nephrology, Depts of Internal Medicine, The Catholic Univ of Korea, Daejeon, Korea; [2]Div of Endocrinology, Depts of Internal Medicine, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** Patients with chronic kidney disease (CKD) often have subclinical hypothyroidism. However, few reports have investigated changes in the status of subclinical hypothyroidism in CKD patients and its clinical significance in CKD progression.

**Methods:** We included 168 patients with nondialysis-dependent CKD stages 2–4. The normalization of subclinical hypothyroidism during follow-up was assessed, and the association between transitions in subclinical hypothyroid status and the rate of decline of the estimated glomerular filtration rate (eGFR) was investigated.

**Results:** At baseline, 127 patients were euthyroid and 41 (24.4%) patients were diagnosed with subclinical hypothyroidism. Of these 41 patients, 21 (51.2%) spontaneously resolved to euthyroid during follow-up. The rate of eGFR decline of patients with resolved subclinical hypothyroidism was similar to that of euthyroid patients. The patients with unresolved subclinical hypothyroidism showed a steeper renal function decline than patients with euthyroidism or resolved subclinical hypothyroidism (all $p < 0.05$). The proportion of CKD patients progressing to end-stage renal disease was significantly greater in those with unresolved subclinical hypothyroidism than in those who were euthyroid ($p = 0.006$). In multivariate linear regression for rate of eGFR decrease, unresolved subclinical hypothyroidism ($\beta = -0.23$, $p = 0.001$), baseline renal function ($\beta = -0.34$, $p = 0.001$) and level of proteinuria ($\beta = -0.44$, $p = 0.001$) were independently associated with the rate of renal function decline.

**Conclusions:** Half of the CKD patients with subclinical hypothyroidism did not resolve to euthyroidism, and this lack of resolution was independently associated with rapid renal function decline.

## FR-PO285

**Comparison of CKD with Modifiable Traditional Cardiovascular Risk Factors in Their Contributions to Mortality in the US**

Kunihiro Matsushita, Yingying Sang, Shoshana Ballew, M. Grams, Josef Coresh. *Dept of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

**Background:** The aim of this study was to compare chronic kidney disease (CKD) with modifiable traditional cardiovascular risk factors regarding their contributions to all-cause and cardiovascular mortality among the participants in the Third National Health and Nutrition Examination Survey (NHANES III).

**Methods:** CKD was defined as eGFR with CKD-EPI creatinine equation <60 ml/min/1.73m$^2$ or urinary albumin-creatinine ratio (ACR) ≥30 mg/g. Modifiable traditional risk factors included hypertension (blood pressure ≥140/90 mmHg or treatment), diabetes (fasting glucose ≥126 mg/dL or treatment), hypercholesterolemia (total cholesterol ≥240 mg/dL), low high-density lipoprotein cholesterol (HDL-C) (<40 mg/dl for men and <50 for women), and current smoking (yes vs. no). Prevalence, hazard ratio for mortality, and % population attributable risk (%PAR) were obtained for each risk factor.

**Results:** Among 15933 participants, the prevalence was highest for low HDL-C followed by smoking, hypertension, hypercholesterolemia, CKD, and diabetes (Table).

During a median follow-up of 8.7 years, there were 2259 deaths (987 due to cardiovascular disease). When all predictors were incorporated into Cox models accounting for sampling probability in addition to age, sex, and race, only CKD, diabetes, and smoking remained significant for both total and cardiovascular mortality (Table). %PAR of mortality outcomes for CKD was 11-14% and higher than that for hypertension, diabetes, or dyslipidemia.

**Conclusions:** In a US national cohort, the contribution of CKD to mortality in the sense of relative risk and %PAR was greater than most of the established cardiovascular risk factors, further underscoring the public health importance of CKD.

Table. Prevalence, adjusted hazard ratio, and % population attributable risk of mortality outcomes for each risk factor of interest

| Risk factors of interest | Prevalence (%) | All-cause mortality | | Cardiovascular mortality | |
|---|---|---|---|---|---|
| | | Adjusted Hazard Ratio | % population attributable risk | Adjusted Hazard Ratio | % population attributable risk |
| CKD | 11.9 | 2.09 (1.87, 2.34) | 11.5 | 2.31 (1.91, 2.81) | 13.5 |
| Hypertension | 22.8 | 1.05 (0.89, 1.23) | 1.0 | 1.32 (0.98, 1.79) | 6.8 |
| Diabetes | 7.7 | 1.46 (1.28, 1.66) | 3.4 | 1.65 (1.39, 1.96) | 4.8 |
| Hypercholesterolemia | 18.5 | 1.01 (0.90, 1.14) | 0.2 | 1.20 (0.99, 1.45) | 3.5 |
| Low HDL-C | 36.6 | 1.11 (0.99, 1.24) | 3.9 | 1.10 (0.90, 1.33) | 3.4 |
| Current smoker | 27.9 | 1.82 (1.58, 2.09) | 18.6 | 1.67 (1.32, 2.12) | 15.8 |

Models included all risk factors above together in addition to age, sex, and race.

*Funding:* Private Foundation Support

## FR-PO286

**Periodontal Disease as a Predictor of Survival in Chronic Kidney Disease: Results from the Third National Health and Nutrition Examination Survey**
Ambarish Athavale,[1] Ana C. Ricardo,[1] Jinsong Chen,[1] Hemanth N. Hampole,[2] Phillip T. Marucha,[1] James P. Lash.[1] *[1]Nephrology, Univ of Illinois at Chicago, Chicago, IL; [2]Internal Medicine, John H Stroger Jr. Hospital of Cook County, Chicago, IL.*

**Background:** Periodontal disease (PD) is more common in patients with chronic kidney disease (CKD). Although PD has also been associated with increased all cause mortality in the general population, its prognostic significance in CKD is not known.

**Methods:** We conducted a study of 10755 participants in NHANES III. CKD was defined as eGFR <60 ml/min/1.73m$^2$ or albumin/creatinine ratio ≥ 30mg/g. Moderate or severe PD (MSPD) was defined as loss of attachment ≥ 6 mm in at least 2 mesial sites and >5 mm pocket depth in at least 1 mesial site (severe PD), or loss of attachment ≥ 4 mm in at least 2 mesial sites or at least 2 mesial sites with 5 mm pocket depth not on same tooth (moderate PD). Vital status was ascertained using the NHANES III Linked Mortality File, which provides follow-up data through December 31, 2006.

**Results:** Mean age was 41.5 years. 75.7% of the study population was non-Hispanic white. MSPD was more common in participants with CKD (9.2%) than in participants with no CKD (4.8%) (P < 0.0001). There were 1773 deaths over a mean follow up period of 14 years. Results of multivariable survival analyses are summarized below (adjusted for age, gender, education, income, smoking, diabetes, hypertension and dental care use).

| MSPD by CKD status | All Cause Mortality Hazard Ratio (HR) (95% confidence interval) | P |
|---|---|---|
| No CKD, No MSPD | Reference | - |
| No CKD, MSPD | 1.42 (1.08-1.85) | 0.01 |
| CKD, No MSPD | 1.63 (1.38-1.92) | < 0.0001 |
| CKD, MSPD | 2.27 (1.77-2.92) | < 0.0001 |

When analyses were restricted to individuals with CKD, MSPD was associated with higher all- cause (HR 1.57, 95% CI 1.16-2.12, p <0.01) and cardiovascular mortality (HR 1.51, 95% CI 1.01-2.26, p<0.05) as compared to CKD and no MSPD.

**Conclusions:** MSPD and CKD are each associated with increased risk for all-cause mortality. In individuals with CKD, MSPD is associated with incremental risk for all cause and cardiovascular mortality. Future work is needed to evaluate mechanisms for this association.

## FR-PO287

**Fibroblast Growth Factor-23 Resistance, in Addition to Fibroblast Growth Factor-23, Is Associated with Clinical Outcome in CKD Patients**
Marc G. Vervloet,[1] Anneke Bech,[2] Annet Bouma-de Krijger,[1] Arjan D. Van Zuilen,[3] Michiel Bots,[4] Peter J. Blankestijn,[3] Jack F. Wetzels.[2] *[1]Nephrology, VU Univ Medical Center, Amsterdam, Netherlands; [2]Nephrology, UMCN St. Radboud, Nijmegen, Netherlands; [3]Nephrology, UMCU, Utrecht, Netherlands; [4]Julius Center for Health Sciences and Primary Care, UMCU, Utrecht, Netherlands.*

**Background:** FGF23 has emerged as an independent predictor with a high hazard ratio for clinical outcome in CKD patients. Whether FGF23 per se or tubular resistance to the actions of FGF23 is responsible for this increased risk is debated. We aimed to evaluate the effect of fractional excretion of phosphate (FePi) or TmP/GFR on the association of FGF23 with clinical in patients with moderate CKD.

**Methods:** We retrieved baseline data and available urine samples of 166 patients from the Masterplan randomized controlled trial, in two participating centers, which allowed measurement of urine calcium, phosphorus, and creatinine, and calculation of FePi and TmP/GFR. Four categories were compared, based on respective median values of FGF-23 and FePi or TmP/GFR.

**Results:** Median eGFR was 36 ml/min/1.73m$^2$ (IQR 27-44), serum phosphate 1.04 mmol/l (IQR 0.92-1.20), FGF23 140 RU/ml (IQR 81-236) FePi 0.32 (IQR 0.25-0.44) and mean TmP/GFR 0.72 mmol/l (SD 0.21). In survival analyses lnFGF23 confirmed to be a significant, independent predictor for the combined outcome of death, renal survival and cardiovascular events (HR 1.84, p<0.01). HR for the primary outcome gradually increased for the four categories of FGF-23 and TmP/GFR.

| Combined outcome death, renal survival, CV event | | | |
|---|---|---|---|
| | HR | 95% CI | p-value |
| Low FGF23/low TmP/GFR | 1 | ref | ref |
| Low FGF23/high TmP/GFR | 2.32 | 1.03-5.25 | 0.04 |
| High FGF23/low TmP/GFR | 2.81 | 1.32-5.97 | 0.01 |
| High FGF23/high TmP/GFR | 3.78 | 1.71-8.36 | <0.01 |

The results were similar when FePi was used instead of TmP/GFR (data not shown).

**Conclusions:** FGF23 predicts outcome in patients with CKD. FGF-23 resistance as defined by indices of efficacy of phosphate excretion is an additional risk factor. The tubular abnormality that causes FGF-23 resistance may be local klotho deficiency and may be causally related to clinical outcome.

*Funding:* Private Foundation Support, Clinical Revenue Support

## FR-PO288

**Incident Atrial Fibrillation and Risk of Death in Adults with Chronic Kidney Disease**
Nisha Bansal,[1] Dongjie Fan,[2] Chi-yuan Hsu,[1] Juan Daniel Ordonez,[2] Alan S. Go.[2] *[1]UCSF; [2]Kaiser Permanente.*

**Background:** Atrial fibrillation (AF) frequently occurs in patients with chronic kidney disease (CKD). Yet the long-term impact of development of AF on the risk of adverse outcomes among patients with CKD is unknown. In a large, diverse community-based population with CKD, we examined the association between incident AF and risk of all-cause death.

**Methods:** We studied adult members with CKD (glomerular filtration rate [eGFR] <60 ml/min/1.73 m$^2$ by the CKD-EPI equation) enrolled in Kaiser Permanente Northern California who were identified between 2002-2010 and did not have previous AF. Incident AF was identified using primary hospital discharge diagnoses and/or two or more outpatient visits for AF. Death was ascertained from health plan administrative databases, Social Security Administration vital status files, and California state death certificate registry. We used validated algorithms to identify demographics, comorbidity, blood pressure, eGFR, hemoglobin, proteinuria and cardiovascular medication use from health plan databases.

**Results:** Among 81,088 adults with documented CKD, a total of 6,269 (7.7%) patients developed incident AF during a mean follow-up of 4.8 ± 2.7 years. Patients who developed incident AF were more likely to be older, male, white, have other cardiovascular conditions, have higher blood pressure and lower eGFR at cohort entry. There were 2,388 deaths that occurred after development of incident AF (145 per 1000 person-years) compared with 18,865 deaths during periods without AF (51 per 1000 person-years, P<0.001). After adjustment for potential confounders, incident AF was associated with a 63% increase in rate of death.

| | Hazard Ratio (95% CI) |
|---|---|
| Unadjusted | 2.10 (2.01, 2.20) |
| Adjusted for age, gender, race | 1.61 (1.54, 1.68) |
| Adjusted for age, gender, race, socioeconomic characteristics, comorbidity, blood pressure, eGFR, proteinuria, hemoglobin and cardiovascular medication use | 1.63 (1.54, 1.73) |

**Conclusions:** Incident AF is independently associated with increased risk of death in adults with CKD. Further study is needed to identify modifiable risk factors to decrease the burden of AF and subsequent risk of death in this high-risk population.

*Funding:* NIDDK Support

## FR-PO289

**Chronic Kidney Disease and Long-Term Risk of Cardiovascular Events following CABG for Acute Coronary Syndromes**
Martin Holzmann, Ulrik Sartipy. *Karolinska Institutet, Stockholm, Sweden.*

**Background:** Renal dysfunction is related to cardiovascular events after elective coronary artery bypass grafting (CABG). We aimed to investigate the impact of renal dysfunction on long-term risk of cardiovascular events after CABG for acute coronary syndromes.

**Methods:** From the SWEDEHEART registry we included all 12,956 patients who underwent a first, isolated, non-emergent, CABG for acute coronary syndromes in Sweden between 2000 and 2008. We calculated hazard ratios with 95% confidence intervals (CI) for rehospitalization for heart failure, stroke or myocardial infarction in relation to glomerular filtration rates (eGFR) estimated by the MDRD study equation.

**Results:** During a mean follow-up of 5 years there were 2,844 events. Patients with eGFR 15-45 mL/min/1.73m$^2$ had a 24% increased risk of an adverse event compared with patients with eGFR > 60 mL/min/1.73m$^2$, even after adjustment for confounders including postoperative acute kidney injury. The hazard ratios with 95% CI were 1.08 (0.98-1.19) and 1.24 (1.08-1.43), in patients with eGFR 45-60 and 15-45 mL/min/1.73m$^2$, respectively, after adjustment for age, sex, diabetes, chronic obstructive pulmonary disease, peripheral vascular disease, left ventricular ejection function, atrial fibrillation, prior myocardial infarction, heart failure, stroke or perioperative acute kidney injury.

**Conclusions:** Renal dysfunction is related to rehospitalization for cardiovascular disease in patients undergoing CABG for acute coronary syndromes.

## FR-PO290

**Biomarkers of Inflammation Are Associated with Cardiovascular Disease in Chronic Kidney Disease (CKD)–Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study** Jayanta Gupta,[1] Elizabeth Dominic,[2] Tulsi K. Mehta,[2] Alan S. Go,[3] Marshall M. Joffe,[1] Melanie Glenn,[1] Valerie L. Teal,[1] Dawei Xie,[1] John W. Kusek,[4] Harold I. Feldman,[1] Dominic S. Raj.[2] *[1]U of Pennsylvania; [2]George Washington Univ; [3]U of California, San Francisco; [4]NIDDK.*

**Background:** We hypothesized that inflammatory pathways are important in the development of cardiovascular disease (CVD) in the setting of CKD and that inflammatory cytokines would be associated with CVD even after adjustment for traditional risk factors.

**Methods:** Plasma concentrations of IL-1β, IL-1 receptor antagonist (IL-1RA), IL-6, TNF-α, TGF-β, hs-CRP, fibrinogen and serum albumin were measured at baseline in a sub-group of 2613 CRIC study participants without any history of cardiovascular disease at enrollment. Cox proportional hazards regression was used to examine the association between biomarkers of inflammation and the risk of atherosclerotic vascular disease events (myocardial infarction, stroke, or peripheral arterial disease) (ASVD) and congestive heart failure (CHF) events. Analyses were adjusted for age, sex, race/ethnicity, diabetic status, current smoking status, estimated glomerular filtration rate, proteinuria, systolic blood pressure, body mass index, total cholesterol as well as use of ACEI/ARB and lipid lowering agents.

**Results:** During a 4 year follow-up 130 participants developed ASVD and 139 developed CHF.

| Biomarker | ASVD | | CHF | |
|---|---|---|---|---|
| | Hazard Ratio (95%CI) | p | Hazard Ratio (95%CI) | p |
| IL-6 | 1.23 (1.04, 1.44) | 0.01 | 1.27 (1.09, 1.49) | 0.003* |
| TNF-α | 1.25 (1.04, 1.5) | 0.02 | 1.14 (0.93, 1.4) | 0.21 |
| IL-1RA | 1.17 (0.98, 1.4) | 0.09 | 1.07 (0.89, 1.29) | 0.46 |
| IL-1β | 1.12 (0.93, 1.34) | 0.24 | 1.08 (0.9, 1.29) | 0.40 |
| TGF-β | 1.17 (0.96, 1.42) | 0.11 | 0.96 (0.8, 1.16) | 0.67 |
| Fibrinogen | 1.25 (0.95, 1.64) | 0.12 | 1.45 (1.11, 1.93) | 0.007 |
| hs-CRP | 1.38 (1.14, 1.66) | 0.001* | 1.22 (1.01, 1.48) | 0.04 |
| Albumin | 1.00 (0.83, 1.22) | 0.98 | 0.82 (0.68, 0.98) | 0.03 |
| *significant after Bonferroni correction | | | | |

**Conclusions:** hs-CRP and IL-6 were associated with increased risks of ASVD and CHF respectively in patients with CKD.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

## FR-PO291

**Newer Biomarkers Slightly Improve Prediction of Progression to Renal Replacement Therapy in CKD Patients–Can Predict Study Outcomes** Adeera Levin,[1] Ognjenka Djurdjev,[2] Claudio Rigatto,[3] Francois Madore,[4] Brendan J. Barrett,[5] Norman Muirhead,[6] Myles S. Wolf.[7] *[1]Univ of British Columbia; [2]BC Renal Agency; [3]Univ of Manitoba; [4]Univ de Montréal; [5]Memorial Univ of Newfoundland; [6]Western Univ; [7]Univ of Miami Miller School of Medicine.*

**Background:** Patients with chronic kidney disease (CKD) experience variable progression of kidney disease (KD). Better prediction models are needed. To assess if newer biomarkers (NBM), alone or as a panel, improve risk prediction of renal replacement therapy (RRT), over and above conventional clinical, demographic and laboratory predictors.

**Methods:** Pan-Canadian prospective cohort study of 2544 referred CKD patients, from 25 centres. NBM tests at baseline included asymmetric dimethylarginine (ADMA), high sensitivity C-reactive protein (hsCRP), interleukin 6, pro-brain natriuretic peptide (NTproBNP), troponin I, transforming growth factor β1, cystatin C and fibroblast growth factor (FGF23). Outcome: dialysis or transplantation (RRT) within 3 years. We compared discrimination (C statistic) and classification (net reclassification index (NRI)) of proportional hazards models based on conventional vs. combination of conventional and NBM predictors.

**Results:** Mean age of the cohort is 68yrs; median eGFR was 28ml/min/1.73m2 (20% <20ml/min, 38% 20-29ml/min and 41% 30-45ml/min); 62% were male. 14.4% patients initiated RRT during the 3-year follow-up. Models based on base, base+NBM and 'best' predictors are presented in the following figure:

| Variables | Base Model | Base + NBM | 'Best' Model |
|---|---|---|---|
| Age (per 5 yrs.) | 0.97 (0.93-1.01) | 0.91 (0.87-0.95) | 0.91 (0.87-0.95) |
| Male Sex | 1.88 (1.46-2.40) | 1.77 (1.38-2.27) | 1.68 (1.31-2.15) |
| eGFR (per 1 mL/min/1.73m$^2$) | 0.89 (0.87-0.91) | 0.92 (0.90-0.94) | 0.92 (0.90-0.94) |
| Hemoglobin (per 5 g/L) | 0.94 (0.91-0.98) | 0.97 (0.93-1.01) | |
| Phosphate (per 0.1 mmol/L) | 1.06 (1.02-1.11) | 1.05 (1.01-1.09) | |
| Albumin (per 1 g/L) | 0.96 (0.94-0.98) | 0.97 (0.95-0.99) | 0.97 (0.94-0.99) |
| Bicarbonate (per 1g/L) | 1.06 (1.03-1.10) | 1.06 (1.02-1.09) | 1.05 (1.02-1.09) |
| log ACR (per 1SD) | 2.07 (1.78-2.41) | 1.87 (1.61-2.17) | 1.87 (1.61-2.17) |
| Cystatin C (per 1SD) | | 1.33 (1.15-1.53) | 1.33 (1.15-1.54) |
| log NT-ProBNP (per 1SD) | | 1.36 (1.20-1.55) | 1.36 (1.20-1.55) |
| log TGF-β1 (per 1SD) | | 0.87 (0.78-0.97) | 0.86 (0.77-0.96) |
| log FGF-23 (per 1SD) | | | 1.12 (1.02-1.24) |
| *C* statistic | 85.6 (83.9-87.3) | 86.3 (85.2-87.9) | 86.4 (85.1 - 88.0) |
| NRI Categorical, % | | 6.6 (-1.6 - 10.9) | 2.9 (-6.9 - 10.6) |

**Conclusions:** This is the first analysis of CKD cohort progression that includes NBM as a panel. Although NBMs are independent predictors of RRT progression, addition of the panel of NBMs to models based on conventional clinical, demographic and laboratory predictors results in only modest improvement of RRT risk prediction.

*Funding:* Pharmaceutical Company Support - Ortho Biotech

## FR-PO292

**Newer Biomarkers Improve Prediction of Death in CKD Patients – Can Predict Study Outcomes** Adeera Levin,[1] Ognjenka Djurdjev,[2] Claudio Rigatto,[3] Francois Madore,[4] Brendan J. Barrett,[5] Norman Muirhead,[6] Myles S. Wolf.[7] *[1]Univ of British Columbia; [2]BC Renal Agency; [3]Univ of Manitoba; [4]Univ de Montréal; [5]Memorial Univ of Newfoundland; [6]Western Univ; [7]Univ of Miami Miller School of Medicine.*

**Background:** Chronic kidney disease (CKD) patients have variable risk of death. General population risk prediction tools perform poorly in this patient group. Better prediction models are needed. **Objective:** To assess if the inclusion of newer biomarkers (NBM) improves risk prediction of death in the CKD cohort, over and above conventional clinical, demographic and laboratory predictors.

**Methods:** Pan-Canadian prospective cohort study of 2544 referred CKD patients, from 25 centres. NBM tests at baseline included asymmetric dimethylarginine (ADMA), high sensitivity C-reactive protein (hsCRP), interleukin 6, pro-brain natriuretic peptide (NTproBNP), troponin I, transforming growth factor β1, cystatin C and fibroblast growth factor (FGF23). Outcome: all-cause mortality within 3 years. We compared discrimination (*C* statistic) and classification (net reclassification index (NRI)) of proportional hazards models based on conventional vs. combination of conventional and NBM predictors.

**Results:** Mean age of the cohort is 68yrs; median eGFR was 28ml/min/1.73m$^2$; 62% were male. 15.5% patients died during 3-year follow-up. Models based on base, base+NBM and 'best' predictors are presented in the following figure:

| Variables | Base Model | Base + NBM | 'Best' Model |
|---|---|---|---|
| Age (per 5 yrs.) | 1.31 (1.23 - 1.38) | 1.25 (1.18 - 1.33) | 1.30 (1.22 - 1.38) |
| Male Sex | 1.22 (0.97 - 1.53) | 1.29 (1.03 - 1.61) | |
| eGFR (per 1 mL/min/1.73m$^2$) | 0.97 (0.95 - 0.98) | 0.98 (0.96 - 0.99) | 0.98 (0.97 - 0.99) |
| History of CVD | | | |
| Ischemic HD | 1.85 (1.37 - 2.48) | 1.37 (1.01 - 1.86) | 1.46 (1.09 - 1.98) |
| Congestive HF | 2.18 (1.57 - 3.02) | 1.40 (0.99 - 1.97) | 1.48 (1.06 - 2.08) |
| Both IHD & CHF | 2.76 (2.11 - 3.63) | 1.60 (1.20 - 2.15) | 1.67 (1.25 - 2.24) |
| Diabetes | 1.30 (1.05 - 1.62) | 1.27 (1.02 - 1.58) | 1.32 (1.06 - 1.65) |
| Hemoglobin (per 5 g/L) | 0.94 (0.90 - 0.97) | 0.96 (0.92 - 0.99) | |
| Albumin (per 1 g/L) | 0.93 (0.91 - 0.95) | 0.95 (0.92 - 0.97) | 0.95 (0.93 - 0.98) |
| Bicarbonate (per 1g/L) | 1.04 (1.01 - 1.07) | 1.02 (0.99 - 1.06) | |
| log PTH (per 1SD) | 1.19 (1.06 - 1.34) | 1.09 (0.97 - 1.21) | |
| ADMA (per 1SD) | | 1.20 (1.11 - 1.29) | 1.21 (1.12 - 1.31) |
| log NT-ProBNP (per 1SD) | | 1.64 (1.44 - 1.88) | 1.59 (1.39 - 1.81) |
| log hsCRP (per 1SD) | | 1.24 (1.11 - 1.39) | 1.25 (1.12 - 1.40) |
| log FGF-23 (per 1SD) | | 1.23 (1.11 - 1.37) | 1.25 (1.12 - 1.39) |
| *C* statistic | 79.1 (77.7 - 80.8) | 82.4 (81.0 - 84.5) | 82.1 (80.4 - 84.3) |
| NRI, Categorical, % | | 13.8 (8.2 - 26.5) | 13.7 ( 8.8 - 23.1) |

After adjusting for base predictors, NTproBNP yields the highest improvement in mortality prediction (NRI=8.9;95%CI:3.3-17.4), followed by hsCRP (NRI=4.5;95%CI:1.3-9.6) and FGF23 (NRI=3.1;95%CI:0.4-11.4).

**Conclusions:** Inclusion of NBMs in risk prediction models significantly improves precision of death prediction in the cohort of CKD patients but needs to be validated in similar cohorts.

*Funding:* Pharmaceutical Company Support - Ortho Janssen

## FR-PO293

**Cystatin C Independently Predicts Change in Left Ventricular Mass Index (LVMI) in Children with Chronic Kidney Disease (CKD)** Tammy M. Brady, Kelly C. McDermott, Michael F. Schneider, Christopher Cox, Bradley A. Warady, Susan L. Furth, Mark Mitsnefes. *CKiD Study Group.*

**Background:** In the Chronic Kidney Disease in Childhood (CKiD) longitudinal cohort study of children 1-16 yrs with CKD, iohexol GFR (iGFR) is not associated with LVMI. In adults, cystatin C is more associated with cardiovascular (CV) risk factors than GFR. We investigated whether cystatin C had an independent association with LVMI over time among CKiD participants.

**Methods:** The longitudinal association of cystatin C with change in log(LVMI) was assessed using a linear mixed model adjusting for confounders and visit with random subject effects to account for repeated measurements. Log(LVMI) was converted to %change LVMI.

**Results:** 475 children (median age 11.5yrs (Interquartile range (IQR):8.3, 15.2), 39% female, median ieGFR 50.2 mL/min/1.73m$^2$ (IQR:38.3, 65.5)), contributed data at ≥1 of 766 CKiD visits with complete data. Median follow up time was 3.1 yrs (IQR:1.1, 5.0). Over time and after adjustment, each mg/L increase in cystatin C was associated with a 7.9% increase in LVMI. In addition, younger age, lower Hb and height z-scores, greater BMI and SBP z-scores and African American (AA) race were independently associated with a significant percentage increase in LVMI.

| | Percentage Change in LVMI (95% Confidence Interval) | p-value |
|---|---|---|
| Cystatin C, mg/L | 7.9 (4.6, 11.4) | <0.001 |
| Baseline age, yrs | -0.9 (-1.4, -0.4) | <0.001 |
| Race-Age-Sex Specific Hemoglobin (Hb) z-score | -1.6 (-2.8, -0.4) | 0.01 |
| Age-Sex Specific Height z-score | -2.9 (-4.6, -1.1) | 0.002 |
| Age-Sex Specific BMI z-score | 6.8 (5.0, 8.7) | <0.001 |
| log(Calcium*Phosphorus), mg/dL | -4.1 (-12.2, 4.6) | 0.3 |
| Systolic Blood Pressure z-score | 2.7 (0.6, 4.9) | 0.01 |
| Diastolic Blood Pressure z-score | 0.5 (-1.8, 2.9) | 0.7 |
| log(iPTH), pg/mL | -1.2 (-3.2, 1.0) | 0.3 |
| Female | -1.1 (-5.4, 3.3) | 0.6 |
| African American | 9.2 (2.6, 15.6) | 0.005 |

**Conclusions:** Higher cystatin C levels are independently associated with increase in LVMI among children with mild-moderate CKD. His easily obtained serum biomarker has the potential to contribute to CV risk stratification among children with CKD.

*Funding:* NIDDK Support, Other NIH Support - Eunice Kennedy Shriver National Institute of Child Health and Human Development; National Heart, Lung, and Blood Institute

## FR-PO294

**Longitudinal Association of Body Mass Index and Waist Circumference with Left Ventricular Mass in Hypertensive Predialysis Chronic Kidney Disease Patients** Vianda S. Stel,[1] Kyriakos Ioannou,[2] Katharina Brueck,[1] Evangelia Dounousi,[3] Konstantinos D. Pappas,[4] Kostas C. Siamopoulos,[3] Carmine Zoccali,[5] Kitty J. Jager,[1] Dimitrios Tsakiris.[6] *[1]ERA-EDTA Registry, Netherlands; [2]Nicosia General Hospital, Cyprus; [3]Univ of Ioannina, Greece; [4]Univ Hospital of Ioannina, Greece; [5]Ospedali Riuniti, Reggio Cal, Italy; [6]Papageorgiou General Hospital of Thessaloniki, Greece.*

**Background:** Studies with longitudinal data on left ventricular mass (LVM) in chronic kidney disease (CKD) patients are scarce. However, to prevent left ventricular hypertrophy and its major consequences in CKD patients, identification of modifiable risk factors for increased LVM is needed. This is the first study investigating the association of body mass index (BMI) and waist circumference (WC) with LVM in hypertensive predialysis CKD patients.

**Methods:** In 2004 and 2005, 206 incident adult patients from the outpatient CKD clinics of two hospitals in Greece, were included. Inclusion criteria were the presence of CKD and hypertension. BMI (kg/m²), WC (cm) and LVM (g) were assessed annually for three years.

**Results:** Mean age was 68.1 years, mean BMI was 29.1 kg/m², mean WC was 103.7 cm, and median LVM was 245.7 g. In the cross sectional data, BMI (ß=4.7; 95%ci:2.0;7.4) and WC (ß=1.2; 95%ci:0.14;2.3) were significantly associated with LVM after adjustment for age, sex, primary renal disease, smoking and history of cardiovascular disease. These associations were pronounced in CKD stage 1-3, but not in CKD stage 4-5. In the longitudinal analysis, linear mixed models adjusting for confounders showed that an increase in BMI (ß=2.9; 95%ci:0.74;5.1) and WC (ß=1.1; 95%ci:0.28;1.8) were significantly associated with an increase in LVM.

**Conclusions:** In hypertensive predialysis CKD patients, BMI and WC were associated with LVM in CKD stage 1-3, but not in CKD stage 4-5. In these later stages the relationship may affected by uremia and peripheral muscle wasting. Longitudinal analysis showed that an increase of BMI and WC was associated with an increase in LVM. These results suggest that in the early CKD stages, overweight and obese patients should be advised to lose weight to prevent cardiovascular events.

## FR-PO295

**Impaired Kidney Function after Myocardial Infarction Is a Risk Factor for 40 Months All-Cause Mortality** Ellen K. Hoogeveen,[1] Johanna M. Geleijnse,[2] Sabita Soedamah-muthu,[2] Janette De Goede,[2] Daan Kromhout,[2] Erik Giltay.[3] *[1]Nephrology, Jeroen Bosch Hospital, Den Bosch, Noord-Brabant, Netherlands; [2]Human Nutrition, Wageningen Univ, Wageningen, Gelderland, Netherlands; [3]Psychiatry, Leiden Univ Medical Center, Leiden, Zuid Holland, Netherlands.*

**Background:** Post-myocardial infarction patients have an increased risk for all-cause mortality. Chronic kidney disease (CKD) 3-5 (eGFR<60 ml/min/m²) is an important risk factor for mortality. Little is known whether CKD 2 is also a risk factor for mortality.

**Methods:** We explored the relation between kidney function at baseline and all-cause mortality during 40-months follow-up in an observational study of 4650 post-myocardial infarction patients of the Alpha Omega Trial, receiving state-of-the-art cardiovascular treatment. Creatinine-cystatin C-based eGFR was assessed with the CKD-EPI equation 2012.

**Results:** We accrued 14,957 person-years of follow-up. Of all patients 350 (7.5%) deceased. Mean (SD) age was 69y (5.7), 78.5% were men, 21% had diabetes, 60% hypertension, 85% used statins, and 17% were current smokers. At baseline the median (interquartile range) $eGFR_{creat-cyst}$ was 79 (66-91) ml/min/m². Patients were divided into three categories on the basis of their baseline kidney function: CKD 0-1: ≥90 (27%; reference), CKD 2: ≥60 to <90 (55%), and CKD: 3-5 <60 (18%) ml/min/m². Using a Cox proportional hazards model the HRs (95%-CIs) for death by CKD level, after adjustment for the intervention with n-3 fatty acids,age, sex, diabetes, smoking, statin-use, hypertension, and C-reactive protein were: 1 (reference), 1.51 (1.06 to 2.14) and 3.51 (2.41 to 5.12).

**Conclusions:** We conclude that there is a dose response relation between kidney function and all-cause mortality in post-myocardial infarction patients. Independent of classic risk factors, CKD 2 is a 1.5-fold and CKD 3-5 is a 3.5-fold stronger risk factor for death compared with CKD 0-1 in post-myocardial infarction patients.

*Funding:* Other NIH Support - Dutch Kidney Foundation, Dutch Heart Foundation, US National Institutes of Health (NIH) and Unilever R&D

## FR-PO296

**Cardiovascular Events and Mortality in a Prospective Cohort of CKD Patients in Japan: Effects of Underlying Kidney Diseases** Hiroyuki Tanaka, Soichiro Iimori, Shotaro Naito, Tomokazu Okado, Tatemitsu Rai, Shinichi Uchida, Sei Sasaki. *Nephrology, Tokyo Medical and Dental Univ, Bunkyo-ku, Tokyo, Japan.*

**Background:** Individuals with CKD were more likely to die than to reach renal replacement therapy (RRT) in Western countries. We analyzed the risk of cardiovascular (CV) events, CV-related death and initiation of RRT stratified by underlying kidney diseases.

**Methods:** New patients from 16 nephrology centers who were older than 20 years of age and who visited or were referred for the treatment of CKD stage 2-5 were recruited in this study. 1138 patients were registered and classified by underlying kidney diseases: diabetes nephropathy (DN) (n=287), nephrosclerosis (NS) (n=451), glomerulonephritis (GN) (n=216), other nephropathies (ON) (n=173). The association between underlying kidney diseases and cardiovascular events, initiation of dialysis and mortality were analyzed using Cox proportional hazards models.

**Results:** 1. During 12 months follow-up, all groups showed decreased blood pressure (p<0.001) and decreased LDL-cholesterol (p<0.001), and no change in proteinuria. Body mass index and hemoglobin decreased in DN (p<0.05) but did not change in other groups.

2. A total of 44 patients died (including 19 patients of CV-related death), 103 patients reached RRT, and 62 patients were suffered from CV events (ischemic heart disease (n=9), peripheral arterial disease (n=7), stroke (n=10), congestive heart disease (n=36)).

3. The mortality and RRT rates (per 100 patient-years) in the three groups (DN, NS, GN) were as follows: mortality, 9.51, 3.06, 3.28; RRT, 34.1, 5.81, 8.49, respectively, indicating that participants were more likely to reach RRT than to die.

4. Compared to those with GN, patients with DN had significantly higher risks for CV events (HR 3.4; 95%CI 1.3-8.9) and initiation of RRT (HR 5.0; 95%CI 2.5-9.8), but not for all-cause death or CV-related death. NS group did not show significant difference in those events and mortality compared to GN group.

**Conclusions:** This prospective cohort study suggests that new CKD patients in Japan are likely to reach RRT than to die within 12 months observation. Risks of CV events and mortality differ according to underlying kidney diseases.

## FR-PO297

**Differences in Augmentation Index between Different Etiologies of Chronic Kidney Disease** Thilini Nishani Abeygunaratne, Darren Green, James Ritchie, Diana Chiu, Philip A. Kalra. *Salford Vascular Research Group, Univ of Manchester, Manchester, United Kingdom.*

**Background:** Chronic kidney disease (CKD) is associated with increased vascular stiffness. This can be defined by pulse wave velocity and augmentation index (Aix), is independently associated with all cause mortality and cardiovascular mortality. This study aims to consider if Aix varies by cause of CKD.

**Methods:** 360 patients with AIx measured by pulse wave analysis were selected from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS) study, a prospective study of outcome in patients with CKD 3-5. Primary cause of CKD was assigned by notes review. Heart rate adjusted AIx was assessed against primary disease as a binary variable (above/below median) using the Chi-squared test and as a continuous variable using student's t-test.

**Results:** At the time of AIx measurement mean±SD age was 66±12 years, eGFR 30±14 ml/min/1.73 m². 65% of patients were male. 27% had diabetic nephropathy, 13% IgA nephropathy, 14% autosomal dominant polycystic kidney disease (ADPKD) and 46% renovascular/hypertensive renal disease. Median AIx was 24.

For each primary disease a different proportion of patients had a measured AIx above the median - diabetes 36%, IgA 55%, APKD 65% and vascular 53%, p between groups = 0.004.

When individual primary diseases were compared to the other primary disease in combination the most significant differences was observed using diabetic nephropathy as the referent (36% vs. 52% above median AIx p=0.003). This difference persisted when AIx was considered as a continuous variable (21±8 vs. 24±9 p=0.001). Patients with diabetic nephropathy were generally comparable to the remainder of the study population: age 54 vs.57 years p=0.9, systolic blood pressure, 134 ±22mmHg vs. 135±21 mmHg p=0.6; diastolic blood pressure 68±9 mmHg vs. 71±11 mmHg p=0.02, despite having a lower eGFR 26±11 ml/min/1.73 m² vs. 33±16 ml/min/1.73 m², p<0.001.

**Conclusions:** The study suggests primary disease may confound measurements of vascular stiffness in CKD. Also, a major confounder that would need to be considered is the effect of different classes of drugs. Further study is required to validate this finding.

## FR-PO298

**Prognostic Value of Pulse Pressure in Patients with Chronic Kidney Disease: A Report from the Gonryo Study**

Tae Yamamoto,[1] Mariko Miyazaki,[1] Masaaki Nakayama,[2] Hiroshi Sato,[1] Toshinobu Sato,[3] Sadayoshi Ito.[1] *[1]Tohoku Univ, Sendai, Mkyagi, Japan; [2]Fukushima Medical Univ, Fukushima, Japan; [3]Sendai Shakaihoken Hospital, Sendai, Miyagi, Japan.*

**Background:** Cardiovascular disease is high prevalent in chronic kidney disease (CKD) due to decreased kidney function. Studies suggest that pulse pressure (PP) may be a better predictor of outcome, but information about PP on the risk for cardiovascular events (CVE) or renal failure in CKD is limited.

**Methods:** 2,656 CKD patients, the mean age 60 ± 16 years and males 53%, recruited from 11 outpatient nephrology hospitals, and evaluated prospectively the effects of PP on CVE, including ischemic heart disease, congestive heart failure, strokes and death, and on progression of end-stage kidney disease (ESKD).

**Results:** During a median follow-up of 1096 (645 - 1101) days, 124 patients experienced cardiac disease or strokes, 64 died and 225 patients started renal replacement therapy (RRT). PP correlated positively with age ($\rho$= 0.378, p<.0001) and systolic blood pressure (SBP) ($\rho$= 0.735, p<.0001), and negatively with eGFR ($\rho$= -0.203, p<.0001). When patines were divided by PP level of 60 mmHg, patients with high PP were elder, and had higher SBP, higher prevalence of hypertensive renal disease and diabetic nephropathy as the underlying renal disease and a higher eGFR. CVE rate increased linearly in patients with higher PP levels than 60 mmHg. The effect of renal function on CVE modified in patients group with high PP, but did not in patients group with low PP. High PP level modified the CVE risk predicted by renal function at baseline, but did not low PP level. Such modification of high PP level was not observed for the risk of ESKD.

**Conclusions:** We concluded that PP > 60 mmHg increased a risk for CVE in Japanese CKD population, and was a modifier of CVE predicted by decreased renal function.

## FR-PO299

**Stage 4 to 5 Chronic Kidney Disease Is Associated with Coronary Artery Calcification in Patients with Atypical Chest Pain**

Jung-woo Noh, Jong-woo Yoon, Seungyeon Son, Jwa-kyung Kim, Soo Jin Kim, Sung Gyun Kim, Jieun Oh, Youngki Lee. *Dept of Internal Medicine, Hallym Kidney Research Institute, Hallym Univ College of Medicine, Seoul, Republic of Korea.*

**Background:** Coronary artery calcification (CAC) screening can predict the risk of cardiovascular event in individuals with atypical chest pain. It is well known that CACoccursin patients on maintenance dialysis commonly and severely. The increased risk of cardiovascular disease has been also reported even at moderate declines in renal function. But direct relationship between CAC and less severe renal function is still controversial.

**Methods:** To address this question, 2562 participants with atypical chest pain underwent coronary CT from March 2006 to September 2011. CAC score was quantified using the method described by Agatston. Renal function was categorized by estimated GFR (eGFR). Decreased kidney function was definedby an eGFR <60 mL/min/1.73 m2and staging of CKD was done according to GFR (G stage).

**Results:** Fifty-twopercent of subjects had CAC, mean eGFR was 76.44 mL/min/1.73 m2 and 15.3% had eGFR<60 mL/min/1.73 m2. The median CAC scores by CKD stage 3a, 3b, 4 and 5 were 25.6, 55.5, 197 and 119.5, respectively. In unadjusted analysis, eGFR was strongly associated with CAC score (r=-0.203, P <0.001). Logistic regression showed the association between reduced renal function and CAC scores >100, and >400 versus scores <10. Compared with no CKD, stage 4 to 5 CKD was associated with CAC scores >100, (odds ratio(OR), 2.64; 95% confidence interval(CI), 2.03-3.45) and >400 (OR, 2.79; 95% CI, 2.11-3.71) after adjustment for covariates. However, no valuable association was noted between stage 3a to 3b CKD and CAC scores >100 and >400. In diabetic patients, stage 4 to 5 CKD had a more than five-fold increased odds of CAC scores >400.

**Conclusions:** Stage 4 to 5 CKD is associated with increased CAC scores, and this association is more intense in diabetic populations. In future, we need further evaluations in prospective study in patients with CKD.

## FR-PO300

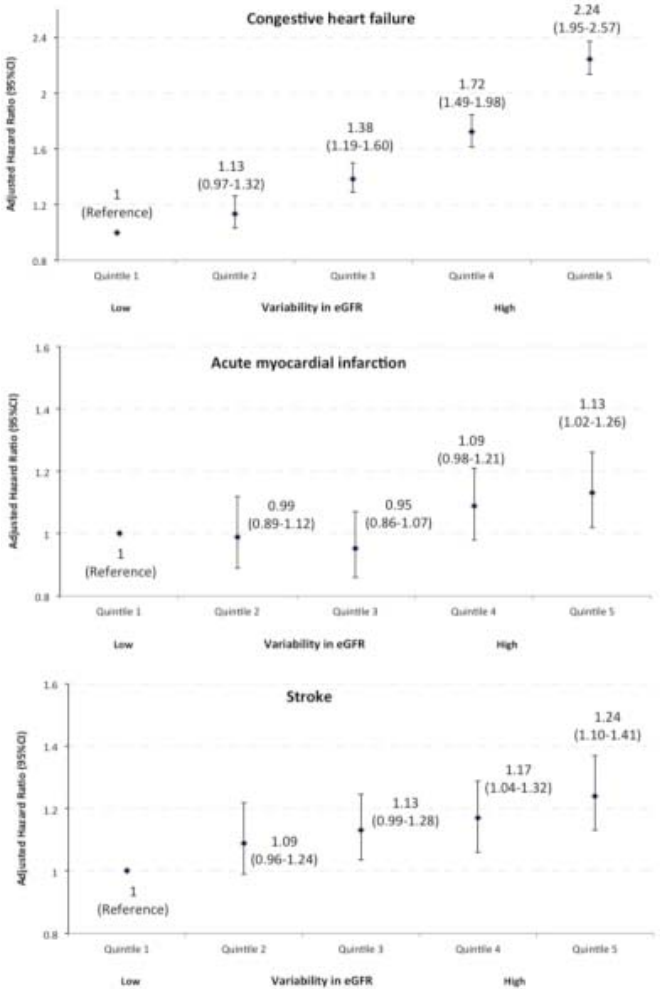
**Association between Variability in eGFR and Risk of Cardiovascular Events**

Tanvir Chowdhury Turin,[1] Min Jun,[1] Marcello Tonelli,[2] Braden J. Manns,[1] Vlado Perkovic,[3] Hiddo Jan Lambers Heerspink,[4] Mark Woodward,[3] Brenda Hemmelgarn.[1] *[1]Univ of Calgary; [2]Univ of Alberta, Canada; [3]Univ of Sydney, Asutralia; [4]Univ of Groningen, the Netherlands.*

**Background:** Relationship between dynamic changes in kidney function, specifically variability in eGFR, and risk of CVD has not been defined. We studied association between eGFR variability and risk of congestive heart failure(CHF), acute myocardial infarction(AMI), and stroke among a community-based population.

**Methods:** We studied 529,954 adults who had atleast 3 outpatient eGFR measurements over 4-year period(the accrual period) in Alberta during 2002-2008. Variability in eGFR was defined using the coefficient-of-variation(CV) from regression of measurements for each participant during the accrual period. Variability was categorized by quintile, with the lowest quintile representing patients with the least variability(referent) and increasing quintiles reflecting greater degrees of variability. Cox models (adjusting for baseline covariates, kidney function, proteinuria, and rate of change in eGFR) were used to estimate the HRs for outcomes during the subsequent follow up period, associated with increasing quintiles of eGFR variability, with follow-up to March 2009.

**Results:** Among the participants(mean age 55y, 42% male) there were 5255(1%) CHF, 4795(0.9%) AMI, and 4014(0.8%) stroke events over a median follow-up of 2.5 y. Increasing eGFR variability was associated with an increased risk of CVD events, including CHF(trend *p*:0.01), AMI(*p*:0.13) and stroke(*p*<0.01). Compared with the lowest quintile, patients in the highest quintile had more than two-fold increased risk of CHF, 13% increased AMI risk, and 24% increased risk of stroke.




**Conclusions:** We observed an independent and graded association between variability in kidney function and the risk of CVD. These results suggest that overall variation in eGFR over time may be an important prognostic marker.

## FR-PO301

**Soluble Urokinase Receptor (suPAR) Predicts Mortality and Cardiovascular Disease in Patients with Mild-to-Moderate Chronic Kidney Disease**

Bjorn Meijers,[1] Ruben Poesen,[1] Markus Storr,[2] Kathleen Claes,[1] Dirk R. Kuypers,[1] Pieter Evenepoel.[1] *[1]Nephrology, Univ Hospitals Leuven, Belgium; [2]Gambro dialysatoren GmbH, Hechingen, Germany.*

**Background:** suPAR is linked to mortality and cardiovascular disease in patients without chronic kidney disease (CKD). Studies evaluating the role of suPAR as FSGS biomarker observed an inverse association between eGFR and suPAR. Whether suPAR accumulates in patients with CKD and whether accumulation of suPAR is associated with mortality in CKD has not been studied to date.

**Methods:** We measured suPAR concentrations in patients with chronic kidney disease (CKD) from the Leuven mild-to-moderate CKD study (Clinical trials protocol NCT00441623) using the human uPAR enzyme-linked immune sorbent assay (R&D systems™). Associations with overall mortality and cardiovascular disease were explored using Kaplan-Meier estimates and multivariate Cox proportional hazards analyses.

**Results:** We determined suPAR concentrations in 486 patients with known non-FSGS CKD patients. eGFR was the strongest determinant of suPAR concentrations (P<0.0001) and suPAR accumulated in patients at low eGFR. suPAR concentrations were associated with overall mortality (P<0.0001) and cardiovascular events (P<0.0001). These associations persisted after correction for age, sex, eGFR and C-reactive protein concentration, but for mortality was lost after correction for serum albumin. suPAR remained associated with cardiovascular disease in multivariate analysis.

**Conclusions:** suPAR accumulates in patients with CKD and is directly and gradually associated with overall mortality and cardiovascular events, independent of kidney function. Given the versatile roles of uPAR as signaling orchestrator, accumulation of suPAR in patients at lower eGFR may be in the causal chain of extrarenal manifestations of CKD.

## FR-PO302

**Atrial Fibrillation Independently Predicts Mortality in Patients with Chronic Kidney Disease after Myocardial Infarction**

Hyeon Seok Hwang,[1] Hye Eun Yoon,[1] Sangju Lee,[1] Yoon-Kyung Chang,[1] Chul Woo Yang,[2] Suk Young Kim.[1] *[1]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Daejeon, Republic of Korea; [2]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

**Background:** Chronic kidney disease (CKD) is an important predictor for mortality after acute myocardial infarction (AMI), and atrial fibrillation (AF) often coexists with CKD and AMI. However, the impact of AF on mortality and morbidity in patients with CKD is not well understood after AMI.

**Methods:** This study was based on a retrospective cohort, the COREA-AMI registry. A total of 4744 patients with AMI were enrolled, and CKD was defined as estimated glomerular filtration rate <60 mL/min/1.73 m$^2$. Patients were divided into CKD (n = 2181) or non-CKD (n = 2563) groups, and were analyzed to investigate the association of AF with mortality and morbidity risk.

**Results:** Of CKD patients with AMI, 123 (5.6%) had AF and it was significantly more prevalent than those of patients without CKD (2.9%, P< 0.001). The CKD patients with AF were associated with worse in-hospital outcomes than CKD patients without AF; more need of vasopressor and renal replacement therapy and development of resuscitation (all P < 0.05). The all-cause mortality and ischemic stroke during a median follow-up of 3.53 years was significantly higher in CKD patients with AF than in CKD patients without AF (P < 0.001 and P = 0.026, respectively). However, AF had no impact on in-hospital outcomes and all-cause mortality in patients without CKD. The adjusted hazard ratio for all-cause mortality and ischemic stroke including all other risk factors was 1.74 and 2.62 for AF in patients with CKD, respectively.

**Conclusions:** AF was more prevalent in patients with CKD, and only the association of CKD and AF increased hazard for in-hospital events and long-term mortality after AMI.

## FR-PO303

**C-Reactive Protein Mediates the Association between Low Socioeconomic Status and Pulse Pressure in Japanese CKD Cohort (CKD-JAC)**

Naohiko Fujii,[1] Takayuki Hamano,[2] Yoshitaka Isaka,[2] Tsuyoshi Watanabe,[3] Kosaku Nitta,[3] Tadao Akizawa,[3] Seiichi Matsuo,[3] Enyu Imai,[3] Hirofumi Makino.[3] *[1]Dept of Biostatistics and Epidemiology, Univ of Pennsylvania, Philadelphia, PA; [2]Osaka Univ Medical School, Suita, Osaka, Japan; [3]CKD-JAC Steering Committee, Shinjuku, Tokyo, Japan.*

**Background:** High brachial pulse pressure (PP) independently associates with faster decline in renal function and worse outcome. Low socioeconomic status (SES) has been recognized as a key environmental factor of hypertension. While dietary salt intake might explain it partially, the role of CRP, which is also known as a risk factor for hypertension, remained to be elucidated. Here we explored the association between PP, SES, and CRP in CKD patients.

**Methods:** We performed a cross-sectional observational study with the baseline data of the Japanese CKD cohort study (CKD-JAC, N=2,977). SES was assessed by self-administered questionnaire followed by classification into four income groups (Q1-4). Multivariate linear regression analyses adjusting for demographic factors, renal function, commodities, medication, nutritional parameters, and facilities were performed to test the relationships between PP, SES and CRP.

**Results:** 1,854 (62%) patients answered the questionnaire. The prevalence of diabetes and prior cardiovascular disease (CVD) was significantly higher in the lowest income group (Q1). Significant step-wise decrease in eGFR and increases in PP, age, urinary Na/K ratio (uNa/K), and CRP along with the decrease in income were observed. The association between PP and SES was robust after adjustment for age, sex, eGFR, diabetes, prior CVD, Alb, BMI, diuretics, anti-hypertensive drugs, and facilities (Coefficients [95% C.I.] vs Q4, Q1:3.0[1.3, 4.8], Q2:1.6[-0.02, 3.3], Q3:1.3[-0.4, 3.0]). Although addition of uNa/K to the model did not attenuate the coefficients, introduction of CRP dramatically reduced the magnitude of correlation coefficients and even cancelled out the statistical significance of SES (Q1:1.6[-0.3, 3.6], Q2:0.7[-1.2, 2.5], Q3:0.4[-1.6, 2.3]).

**Conclusions:** There was a significant negative correlation between SES and PP in CKD patients, where CRP plays a crucial role.

*Funding:* Pharmaceutical Company Support - Kyowa Hakko Kirin

## FR-PO304

**ABCK, a Target Organ Damage Scoring System, Predicts Mortality Independently of Cardiovascular Risk Factors in a CKD Cohort**

James Ritchie,[1] Philip A. Kalra,[1] Carlo A. Gaillard,[2] Branko Braam.[3] *[1]Salford Royal Hospital; [2]VU Univ Medical Center; [3]Univ of Alberta.*

**Background:** The novel Artery Brain, Cardiac and Kidneys (ABCK) framework is a mortality and cardiovascular staging system based upon the severity of target organ damage (TOD) in an individual. This differs from traditional scoring systems that consider risk factors, not existing/incipient vascular damage. In a low-risk cohort, ABCK added predictive value to the SCORE and Framingham systems. Here we assess the utility of ABCK in predicting mortality in a higher-risk CKD population.

**Methods:** Patients were selected from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS), a prospective study of outcome in CKD 3-5. Patients were scored using ABCK, SCORE and Framingham (all cause mortality). ABCK assigns a stage for TOD from 0-4 for the 4 major target organs. Because of the cohort size 4 degrees of TOD were recognized: very mild (ABCK1-2), mild (ABCK 3), moderate (ABCK 4-6), severe (ABCK 7-9). Risk for death was assessed in a Cox proportional hazards model.

**Results:** Data on 1437 patients were analyzed. Over a median follow-up of 4.1 (1.9-6.4) years, 314 (24%) of patients died. When ABCK was considered in isolation, risk for death increased with increasing score. When considered in the same model as SCORE or Framingham risk classification, ABCK scores remained independently associated with increased risk for death.

| | ABCK alone | Adjusted for SCORE | Adjusted for Framingham |
|---|---|---|---|
| Very mild TOD | 1 | 1 | 1 |
| Mild TOD | 4.4 (1.1-17)* | 2 (0.5-9) | 3 (0.7-11) |
| Moderate TOD | 12 (3-47)** | 5 (1-19)* | 6 (2-25)** |
| Severe TOD | 20 (5-87)** | 7 (2-31)** | 9 (2-40)** |

*Results: hazard ratio (95% CI).* *p<0.05 **p<0.01. When SCORE and Framingham risk scores were weighted for ABCK score, AUC in ROC analysis increased (SCORE 0.66 to 0.71; Framingham 0.63 to 0.70).

**Conclusions:** A systematic, multi-organ TOD staging approach predicted all cause mortality in a high risk CKD cohort independent from risk factor based approaches. The ABCK framework combined with risk factors seems to reflect susceptibility for risk and predict mortality better than risk factor based methods alone.

## FR-PO305

**Relationship of Oxidized to Non-Oxidized PTH in Children with Chronic Renal Failure, Adult Patients on Hemodialysis and Kidney Transplant Recipients**

Berthold Hocher,[1] Christoph Reichetzeder,[1] Torsten Slowinski,[1] Uwe Querfeld,[1] Franz S. Schaefer,[2] Anke Doyon,[2] Martin Tepel,[3] Heinz Juergen Roth,[4] Franz Paul Armbruster.[5] *[1]Center for Cardiovascular Research, Charité, Berlin, Germany; [2]Dept of Pediatric Nephrology, Univ of Heidelberg, Heidelberg, Germany; [3]Odense Univ Hospital, Dept of Nephrology and Institute of Clinical Research, Univ of Southern Denmark, Odense, Denmark; [4]Limbach Laboratory, Heidelberg, Germany; [5]Immundiagnostik AG, Bensheim, Germany.*

**Background:** Oxidized PTH (oxPTH) loses its PTH receptor stimulating properties, whereas non-oxidized PTH (n-oxPTH) is a full agonist of the receptor. This was described in more than 20 studies in the 70ies and 80ies of the last century. However, PTH oxidation was ignored during development of PTH assays for clinical use so far.

**Methods:** We recently developed a new assay to differentiate between oxPTH and n-oxPTH. We established normal values for this assay system. Furthermore, we compared the ratio of oxPTH to n-oxPTH in different populations with chronic renal failure: 620 children with renal failure stage 2-4 of the 4C study, 342 adult patients on dialysis, and 602 kidney transplant recipients.

**Results:** Children had the highest mean as well as maximum n-oxPTH concentrations as compared to adult patients (both patients on dialysis as well as kidney transplant recipients). The relationship between oxPTH and n-oxPTH of individual patients varied substantially in all three populations. The analysis of n-oxPTH in 89 healthy control subjects revealed that n-oxPTH concentrations in patients with renal failure were higher as compared to healthy adult controls (2.25-fold in children with renal failure, 1.53-fold in adult dialysis patients, and 1.56-fold in kidney transplant recipients, respectively).

**Conclusions:** A huge proportion of circulating PTH measured by current state of the art assay systems is oxidized and not biologically active. The relationship between oxPTH and n-oxPTH of individual patients varied substantially. Non-oxidized PTH concentrations are 1.5 - 2.25 fold higher in patients with renal failure as compared to healthy controls.

## FR-PO306

**Improvements in Control of Blood Pressure and A1c among Adults with Chronic Kidney Disease, Diabetes, and Hypertension: U.S. 1999-2010**

Sharon Saydah,[1] Edward Gregg,[1] Meda E. Pavkov,[1] Neil R. Powe,[2] Rajiv Saran,[3] Nilka Rios Burrows,[1] Yi Li,[3] Desmond Williams.[1] *[1]CDC; [2]UCSF; [3]UMich.*

**Background:** Prevention of chronic kidney disease (CKD) progression includes control of blood pressure and glycemia among adults with hypertension and diabetes. Whether control of these conditions has improved among adults with CKD in the past decade is unknown.

**Methods:** NHANES from 1999-2010 was analyzed in 4 year periods to examine trends among adults ≥ 18 years with CKD and treatment for diabetes (DM) (n = 1403), or treatment of hypertension (HYTN) (n = 2817) or treatment for both (n = 985). We report percent of uncontrolled blood pressure (BP) ≥ 140/90mmHg, BP ≥ 130/80mmHg and uncontrolled A1c (A1c ≥ 9.0%). CKD was defined as eGFR < 60 ml/min/1.73 m$^2$ or elevated urinary albumin/creatinine ratio (ACR ≥30 mg/g). Estimates were adjusted for age, sex and race/ethnicity.

**Results:** Trends in uncontrolled BP and high A1c are reported.

| % (95% CI) | 1999-2002 | 2003-2006 | 2007-2010 | Relative % change |
|---|---|---|---|---|
| Treated HTN | Treated HTN | | | |
| BP ≥ 130/80 | 77.8 (73.8, 81.4) | 64.4 (60.6, 67.9) | 59.4 (55.3, 63.4) | -24% |
| BP ≥ 140/90 | 59.2 (55.3, 62.9) | 43.4 (39.7, 47.1) | 39.6 (36.2, 43.1) | -33% |
| Treated DM | | | | |
| A1c ≥ 9.0% | 25.6 (19.7, 32.7) | 12.9 (9.6, 17.1) | 16.8 (13.9, 20.2) | -34% |
| Treated HYTN & DM | Treated HYTN & DM | | | |
| BP ≥ 130/80 | 80.0 (72.7, 85.7) | 63.8 (58.6, 68.8) | 59.9 (54.9, 64.6) | -25% |
| BP ≥ 140/90 | 64.7 (54.9, 73.5) | 42.1 (35.0, 49.5) | 42.2 (36.2, 48.5) | -35% |
| A1c ≥ 9.0% | 24.2 (17.9, 31.9) | 12.6 (9.1, 17.1) | 11.6 (9.3, 14.5) | -49% |

**Conclusions:** While significant declines were observed, 2 out of 5 adults with CKD and treated HYTN have uncontrolled BP and 4 out of 25 adults with CKD and treated DM have high A1c levels. These results indicate that among adults with CKD, there is a need for health care professionals to continue to work to reduce uncontrolled blood pressure and diabetes to prevent progression of CKD.

## FR-PO307

**A New Model for CKD Care: Home Telehealth by an Interprofessional Team** Areef Ishani,[1] Juleen Christopher,[1] Sara Otterness,[1] Deirdre A. Palmer,[1] Mark E. Rosenberg.[2] *[1]Minneapolis VAHCS; [2]Univ of Minnesota.*

**Background:** Chronic kidney disease (CKD) is common and is associated with poor patient outcomes. Multidisciplinary case management with or without telemonitoring has been demonstrated to improve outcomes in non-CKD populations. The purpose of our study was to determine if a multidisciplinary case management with remote video monitoring of patients with CKD (eGFR<60mL/min) reduces a composite outcome (death, hospitalization, ER visits, admission to a skilled nursing facility) compared to usual care.

**Methods:** We recruited 601 veterans from the state of Minnesota with a last eGFR<60mL/min. At baseline all patients completed a home visit including surveys, a brief exam, and collection of blood and urine. 450 patients were randomized to the intervention consisting of a remote monitoring device (touch screen computer, BP cuff, scale, webcam, and glucometer). Patients were also provided with broadband if required. Patients in the intervention group were cared for by a multidisciplinary team of nurses, nurse practitioners, pharmD, dietician, social worker, psychologist, and a nephrologist following CKD guidelines and the chronic disease model of care. The overall goals of care were to optimize chronic conditions and to rapidly identify and resolve acute issues. Patients in the usual care group were informed of their kidney disease and invited to attend CKD education classes. The overall study duration is 12 months, with an anticipated primary event rate of 50% in the usual care group and 35% in the intervention group.

**Results:** Over an 8-month recruitment period 601 patients were enrolled in the study. Baseline characteristics of the overall study group are: age 78 years, male 98.7%, Caucasian 98.7%, eGFR 38mL/min. Patients lived on average 98 miles from the Minneapolis VA hospital, with 147 (24%) patients greater than 200 miles away. 292 (65%) patients had existing broadband, and the study provided broadband to the remaining patients.

**Conclusions:** This is a novel multifaceted intervention aimed to reduce adverse outcomes in CKD patients. The approach is feasible, connectivity can be established, and multidisciplinary care can be delivered using telehealth.

*Funding:* Veterans Affairs Support

## FR-PO308

**Implementation of CKD Care Coordinators Is Associated with Better Outcomes When Starting Dialysis** Dugan Maddux,[1] Daniel Defalco,[1] Kevin Chan,[1] Len A. Usvyat,[1] Monet M. Carnahan,[1] Koshy O. Abraham,[2] Franklin W. Maddux.[1] *[1]FMCNA, Waltham, MA; [2]Columbia Nephrology Assosciates, Columbia, SC.*

**Background:** The Renal Care Coordinator (RCC) Program places CKD case managers and analytical tools in the Nephrology Practice to co-manage late stage CKD patients. We compared outcomes in patients starting dialysis who were previously enrolled in the RCC program to CKD patients who were cared for in the Nephrology practice for 4 months or more (Timely Referral), but were not in the RCC program.

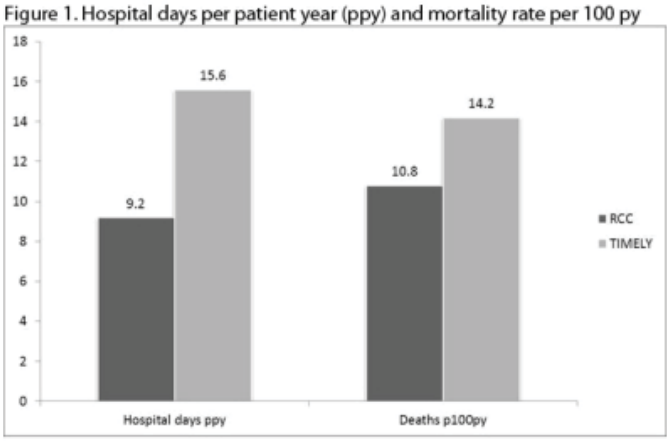
**Methods:** All CKD 5 patients who transitioned to ESRD between 1/2009 and 12/2012 in 10 nephrology practices who adopted the RCC program were included. Within this cohort, we compared outcomes in those patients who were enrolled into the RCC program [RCC] versus those who were considered to have a timely referral for dialysis but with no RCC interaction. For each patient, we noted access type, albumin, neutrophil to lymphocyte ratio [NLR] (as marker of inflammation), hgb, and EPO dose at the first outpatient dialysis treatment. We also computed the rate of hospital days and mortality in the first 120 days of dialysis.

**Results:** 4768 CKD 5 patients were identified for the study where 15% of the population was enrolled in the RCC program. Patients who were in the RCC program had a lower % of patients with catheters, higher albumin, lower NLR, non –significantly different Hgb, and lower EPO dose on the first in-center HD treatment compared to patients with no RCC care. Hospital days were 41% lower and mortality rate was 24% lower in the RCC program compared to patients with Timely Referral alone in the first 120 days.

Table 1. Comparison of parameters at the first in-center HD treatment

| Patient group | Number of patients | Catheters (%) | Albumin (g/dL) | Neutrophil to lymphocyte ratio | Hgb (g/dL) | EPO per HD treatment |
|---|---|---|---|---|---|---|
| RCC | 709 | 34.80% | 3.64 | 5.6 | 10.15 | 3116 |
| TIMELY | 4059 | 50.80% | 3.51 | 5.9 | 10.26 | 6090 |
| p-value | | <0.01 | <0.01 | <0.01 | NS | <0.01 |

Figure 1. Hospital days per patient year (ppy) and mortality rate per 100 py




**Conclusions:** This analysis demonstrates that placement of CKD coordinators in Nephrology Practices is associated with better outcomes, including mortality and hospitalizations, in the first 120 days of dialysis.

## FR-PO309

**Healthcare Resource Utilization and Costs Associated with the Treatment of Autosomal Dominant Polycystic Kidney Disease** Christopher M. Blanchette,[1,2] Serban R. Iorga,[3] Aylin A. Riedel,[3] Jerry G. Seare,[3] Ying Fan,[3] Sandro Rossetti,[1] Ben Gutierrez.[1] *[1]Otsuka America Pharmaceuticals, Inc., Princeton, NJ; [2]Univ of North Carolina, Charlotte, NC; [3]OptumInsight, Eden Prairie, MN.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disease associated with cyst growth and loss of renal function that leads to renal failure. Symptoms of ADPKD can lead to multiple complications, including most commonly hypertension, urinary tract infections, renal cyst infections, hematuria, kidney stones, rupture of intracranial aneurisms, and chronic pain. Real-world data on healthcare costs and resource utilization for ADPKD is lacking; therefore we assessed healthcare cost in a sample of commercially insured (COM) and Medicare Advantage (MAPD) patients.

**Methods:** Patients diagnosed with ADPKD (≥2 claims) with ≥30 days of continuous medical and pharmacy benefits, and no evidence of autosomal recessive polycystic kidney disease were selected from a large health plan claims dataset (1/1/06-8/31/12). Plan and patient paid costs and utilization per patient per month (PPPM) were described in total and by payer type, and estimated using generalized linear models by chronic kidney disease (CKD) stage (defined by ICD-9 or serum creatinine).

**Results:** Of 36,253,096 patients in the database during the observation period 5,051 (0.014%) were identified with ADPKD. After applying exclusion criteria, 4,356 COM and 468 MAPD remained. Patients had an average length of time-in-study of 1,299 days. Post-index complications included hypertension (88%), anemia (47%), urinary tract infections (41%), kidney stones (19%), liver disorders (35%), and kidney transplants (20%).

| | COM, N 4356 | MAPD, N 468 |
|---|---|---|
| **Mean age, years** | 46 | 70 |
| **Dialysis, %** | 41 | 59 |
| **Healthcare cost PPPM, $ (SD)** | | |
| Total | 2,219 (4,536) | 2,540 (3,259) |
| CKD stage 1 | 980 (2,143) | 1,397 (1,856) |
| CKD stage 5 | 5,542 (10,147) | 4,972 (8,915) |
| Dialysis-related (CKD stage 5) | 3,969 (9,022) | 3,547 (8,696) |

**Conclusions:** Total healthcare cost and resource utilization associated with ADPKD was high, which was driven mostly by end-stage renal disease and was similar for COM or MAPD patients.

*Funding:* Pharmaceutical Company Support - Otsuka America Pharmaceuticals, Inc.

**FR-PO310**

**Cross-Sectional and Longitudinal Associations with Creatinine Generation Rate (CGR) in the Chronic Renal Insufficiency Cohort (CRIC)** Francis P. Wilson,[1] Wei Yang,[1] Amanda Hyre Anderson,[1] Peter P. Reese,[1] Radhakrishna Reddy Kallem,[1] Sankar D. Navaneethan,[4] Akinlolu O. Ojo,[5] James H. Sondheimer,[6] Raymond R. Townsend,[1] Mary B. Leonard,[3] Harold I. Feldman.[1] *[1]Pereleman School of Medicine at the Univ of Pennsylvania; [2]Tulane Univ; [3]Children's Hospital of Philadelphia; [4]Cleveland Clinic; [5]Univ of Michigan; [6]Wayne State Univ.*

**Background:** Muscle mass may be lower in patients with more severe CKD. We examined factors associated with muscle mass and muscle loss as measured by CGR in a large national chronic kidney disease population.

**Methods:** We used data from 3725 participants in the CRIC study, a national, prospective study of chronic kidney disease and assessed their CGR, a proxy for muscle mass, from 24-hr urine collections yearly for a median of 4 yrs. We examined associations between CGR and factors assessed at study entry using mixed-effects models.

**Results:** Mean (SD) age was 58 (11) years, 55% were male, and 42% reported black race. Mean (SD) baseline CGR was 1333 (591) mg/day, and declined by 13.8 mg/year ($p<0.001$). Compared to participants in the lowest tertile of baseline CGR at study entry, those in the highest tertile were more likely to be black, male, and younger (all $p<0.001$). They also had higher BMI, larger waist circumference, larger fat mass, and lower high-density lipoprotein (all $p<0.001$), but lower rates of diabetes ($p=0.02$) and lower hemoglobin A1C ($p=0.001$). Participants with the highest CGR had lower serum creatinine and cystatin levels ($p<0.001$), greater urine urea appearance ($p<0.001$), but similar urinary protein excretion ($p=0.42$) compared to those in the lowest CGR tertile. Longitudinally, higher cystatin C levels ($p<0.001$), female sex ($p<0.001$), older age ($p<0.001$), more proteinuria ($p<0.001$), non-black race ($p=0.002$), smoking ($p=0.01$), and higher C-reactive protein ($p=0.02$) were independently associated with loss of CGR over time.

**Conclusions:** Greater protein intake is associated with greater muscle mass as measured by CGR in CKD. As well, proteinuria, anemia, and smoking are potentially modifiable factors associated with progressive muscle wasting in CKD.

*Funding:* NIDDK Support

**FR-PO311**

**Association of Creatinine Generation Rate (CGR) with Death and End-Stage Renal Disease (ESRD) in Patients with Chronic Kidney Disease (CKD) in the Chronic Renal Insufficiency Cohort (CRIC)** Francis P. Wilson,[1] Wei Yang,[1] Amanda Hyre Anderson,[1] Peter P. Reese,[1] Radhakrishna Reddy Kallem,[1] Sankar D. Navaneethan,[3] Akinlolu O. Ojo,[4] James H. Sondheimer,[6] Raymond R. Townsend,[1] Harold I. Feldman.[1] *[1]Pereleman School of Medicine at the Univ of Pennsylvania; [2]Tulane Univ; [3]Cleveland Clinic; [4]Univ of Michigan; [5]Univ of Illinois; [6]Wayne State Univ.*

**Background:** Greater severity of CKD may be associated with lower muscle mass, but the association of muscle mass to clinical outcomes in CKD is undefined.

**Methods:** We used data from 3725 participants in the CRIC study, a national prospective study of CKD and assessed their CGR, a proxy for muscle mass, from 24-hr urine collections at study entry. Proportional hazards analysis was used to assess the association between CGR and all-cause mortality or ESRD.

**Results:** Higher CGR was associated with a lower rate of all-cause mortality in unadjusted models with hazard ratio for death 0.68 (0.61 -0.75, $p<0.001$) for each standard deviation increase in CGR. After adjustment for age, sex, race, ethnicity, serum cystatin, urine protein, hemoglobin, diabetes, cardiovascular disease, smoking status, height, weight, waist circumference, and urine urea appearance, CGR remained independently associated with death - aHR 0.86 (0.76 - 0.98, $p=0.02$). CGR was associated with the development of ESRD in unadjusted - HR 0.72 (0.66 - 0.80, $p<0.001$) and fully adjusted models - aHR 0.79 (0.70 - 0.89, $p<0.001$). Accounting for death as a competing risk showed similar results; aHR 0.81 (0.69 – 0.94, $p=0.006$).

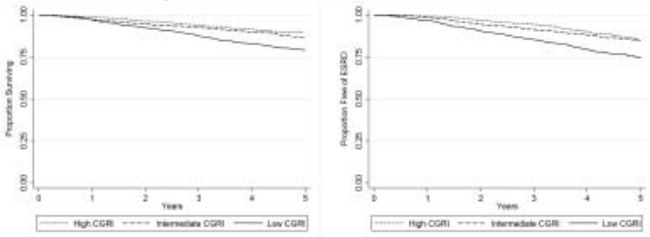



Figure 1. A) Kaplan-Meier curves of all-cause mortality for tertiles of CGR indexed to Height (CGRI). B) Kaplan-Meier curves of ESRD for tertiles of CGRI.

**Conclusions:** Lower CGR, a proxy for low muscle mass, is independently associated with death and ESRD in patients with CKD.

*Funding:* NIDDK Support

**FR-PO312**

**Low Compliance with Protein Intake Recommendations and Outcomes in Patients with Chronic Kidney Disease** Benedicte Stengel,[1] Wenlun Yuan,[1] Martin Flamant,[2] Pascal Houillier,[2,3] Francois Vrtovsnik.[2,5] *[1]Inserm U1018, Univ Paris Sud, Villejuif; [2]APHP, Paris; [3]Univ Paris Descartes, France; [4]The NephroTest Study Group.*

**Background:** Little is known about real compliance with dietary protein intake recommandations and its outcomes in patients with chronic kidney disease.

**Methods:** We measured glomerular filtration rate (mGFR) and assessed protein intake in g/kg/day based on both 24h-urinary urea nitrogen (UUN) using Maroni's formula and 7-day food record in 1,018 men and 502 women with nondialysis CKD stages 1 to 5. Cox models were used to assess hazard ratios (HR) for end-stage renal disease (ESRD) and death before ESRD over a mean follow-up of 3.5 yrs.

**Results:** Mean age was 60±15 vs 58±15 yrs in men vs women, and mean body mass index, 26.6±4.4 vs 26.2±6.0 kg/m$^2$. Baseline mean UUN-based protein intake did not significantly differ between men and women, 1.35±0.33 g/kg/d *vs* 1.32±0.39 g/kg/d; estimates based on 7- day food record were 0.27±0.44 g/kg/d lower on average in both genders. 24h-UUN significantly decreased from 1.48 to 1.14 g/kg/d with decreasing mGFR from ≥ 60 to < 15 mL/min/1.73 m$^2$ in men and from 1.45 to 1.10 g/kg/d in women. Similar decrease was observed with food record, from 1.50 to 1.15 g/kg/d in men and from 1.54 to 1.29 in women. Fifty-two percent of men and 47% of women had protein intake above the 2012 KDOQI upper limit of 1.30 g/kg/d recommended for CKD patients. Adjusted HR of ESRD associated with 1 g/kg/d UUN increase was 1.54 [0.98-2.43] and for mortality before ESRD, 0.95 [0.52-1.75]. These HR were 3.51 [2.02-6.12] and 1.84 [0.77-4.38] for the association with 7-day record-based protein intake, respectively.

**Conclusions:** Despite lower mean dietary protein intake at lower mGFR levels, compliance with recommandations remains low in patients with chronic kidney disease. High protein intake appeared to be associated with increased risk for ESRD, but not for mortality. It is worth noting that the decrease in 24h-UUN associated with kidney function decline may not be attributed to increased extrarenal urea elimination, since similar results were found for 7-day record-based protein intake.

**FR-PO313**

**Prior Statin Use in the Prevention of Kidney Outcome among Intensive Care Unit Patients** Isabelle Malbouisson,[1] Beata Marie Redublo Quinto,[1] Maria Dalboni,[1] Marcelo Costa Batista.[1,2] *[1]Medicine, Nephrology Div, UNIFESP, Sao Paulo, SP, Brazil; [2]Medicine, Hospital Israelita Albert Einstein, Sao Paulo, SP, Brazil.*

**Background:** HMG Co-A reductase inhibitors (statins) are effective in primary and secondary prevention of cardiovascular diseases, independently of their lipid lowering action. Recent data suggest that statins have pleiotropic effects which are associated with better outcomes in critical care setting. The aim of this study was to determine wheter pre hospital statin use is associated with a lower risk of dialysis therapy and/or death during Intensive Care Unit (ICU) stay.

**Methods:** Prospective cohort analysis. Patients: 670 patients admitted to the ICU of an academic tertiary-care hospital.

**Results:** Patients included in this study had a mean age of 66 years old, with a mean corporal mass index (CMI) of 26,6 kg/m$^2$ and a mean abdominal circumference of 97 cm. Patients on statin therapy (18,2%) prior to the ICU hospitalization were less likely to need dialysis therapy or die (OR: 0,41, IC: 0,18-0,93, p: 0,03). As secondary outcomes, patients using statins were also less likely to develop sepsis after infection (OR: 0,42, IC: 0,22-0,77, p: 0,006) and had a reduction in hospital length-of-stay (14,7+- 17,5 days vs 22,3 +- 48 days, p: 0,006**).** Groups were adjusted to sex, age, C-reactive protein (CRP), need of mechanical ventilation, use of vasoactive drug and presence of diabetes and/or coronariopathy, in a multivariable analysis.

**Conclusions:** Statin therapy prior to hospital admission was associated with better outcomes during ICU stay, such as lower risk for dialysis need or mortality and lower risk for the development of sepsis.

*Funding:* Government Support - Non-U.S.

**FR-PO314**

**Patterns of Serum Tumor Necrosis Factor Receptors 1 and 2 According to the Glomerular Filtration Rate in Korean Population** Su Mi Lee,[1] Ran-hui Cha,[2] Seung Hee Yang,[1] Jung Pyo Lee,[3] Dong Ki Kim,[1] Yon Su Kim.[1] *[1]Seoul National Univ Hospital; [2]National Medical Center; [3]Seoul National Univ Boramae Medical Center.*

**Background:** Tumor necrosis factor alpha(TNFα/TNF) is a central role in the inflammation. TNF signaling is mediated via two distinct receptors, TNFR1 and TNFR2. Elevated plasma concentrations of TNF receptors may contribute to pathogenesis of renal decline. We investigated how circulating TNF receptors varied according to glomerular filtration rate(GFR).

**Methods:** Study subjects were individuals who participated in study of Korean coefficients for GFR estimation by MDRD study equations [CKD(n=201) and healthy volunteers(n=43)]. GFR was measured through systemic inulin clearance. TNF receptors were measured by ELISA. We derived reference values of TNF receptors according to GFR and evaluated diagnostic power. We identified TNF receptors values in patients with early renal decline compare to healthy controls and effect on the progression of renal function. Renal function decline was defined as reduction of GFR more than 5 per year or dialysis requirement.

**Results:** TNFR1 as well as TNFR2 were correlated with measured GFR(r=-0.63andr=-0.67). Median values of TNFR1 and TNFR2 according to GFR were as follows: 594and1579 for GFR≥90, 921and2152 for 90>GFR≥60, 1691and3410 for 60>GFR≥30, 2925and6994 for 30>GFR≥15, 2267and4419 for GFR<15. TNF receptors had similar diagnostic power in patients with GFR less than 60 when compared to SCr through ROC curve. The levels of TNF receptors in CKD1 and 2 patients(n=60) were higher than healthy volunteers. Compared with stable renal function participants, TNF receptors values were elevated in individulas with renal function decline. Multivariate analysis revealed that highest quartile of TNF receptors were associated with renal function impairment compared to the other quartiles.

**Conclusions:** Our study identified that TNF receptors were correlated with measured GFR. TNF receptors showed similar increment pattern and diagnostic power compared with SCr. Even mild renal impairment was associated with higher levels of TNF receptors in comparison to healthy controls. TNF receptors were associated with progression of renal function.

## FR-PO315

**Concentration of Serum Tumor Necrosis Factor Receptors and Progression of Chronic Kidney Disease in Patients with Moderate to Severe Renal Dysfunction** Su Mi Lee,[1] Ran-hui Cha,[2] Hajeong Lee,[1] Sunhwa Lee,[1] Seung Hee Yang,[1] Jung Pyo Lee,[3] Dong Ki Kim,[1] Yon Su Kim.[1] *[1]Seoul National Univ Hospital; [2]National Medical Center; [3]Seoul National Univ Boramae Medical Center.*

**Background:** The prevalence of chronic kidney disease (CKD) has been increased steadily, and it's one of public health problems. However, there is a paucity of biomarkers for the prediction of CKD progression other than high serum creatinin (SCr), reduced glomerular filtration rate (GFR), and proteinuria. Chronic inflammation may contribute to kidney damage and interaction between tumor necrosis factor alpha (TNFα/TNF) and its receptors (TNF receptor 1 and 2) has essential role in the inflammation signaling. We aimed to evaluate the clinical significance of TNF receptors as a biomarker of CKD progression.

**Methods:** We recruited 473 patients with CKD stage 3 (n = 148) and 4 (n = 321). Primary outcome was defined as composite of SCr increase more than 2 times, GFR decrease more than 50 percent, or initiation of renal replacement therapy. We measured serum concentration of TNF receptors by ELISA (DRT100 and DRT200; R&D Systems, Minneapolis, MN).

**Results:** A total of 205 patients reached primary outcomes during 3 years of follow-up. Patients with primary outcomes showed higher baseline level of SCr, glomerular filtration rate (GFR), proteinuria, and TNF receptor 1/ 2 than patients without primary outcomes. When the levels of TNF receptors were categorized as tertiles, there was a significant association between tertiles of TNFR 1 and primary outcome after adjustment for age, gender, GFR, and proteinuria (OR (C.I.) ; 1 (reference), T2: 2.27 (1.137-4.440), T3: 4.719 (2.234-9.971)). In addition, there was a tendency to increased adjusted odds ratio of primary outcome according to increasing TNFR2 tertiles after adjusted with age, gender, GFR, and proteinuria (OR (C.I.) ; 1 (reference), T2: 1.357 (0.718-2.563), T3: 3.096 (1.618-5.926)).

**Conclusions:** This study showed higher serum level of TNF receptors (TNF receptor 1 and 2) are associated with progression of CKD in patients with moderate to severe renal dysfunction (CKD stage 3 to 4).

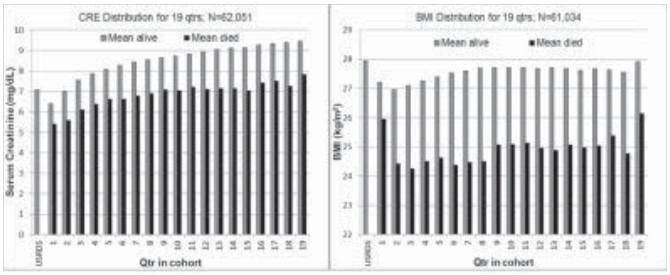
## FR-PO316

**Time Trend of Body Mass Index in Maintenance Dialysis Patients** Elani Streja,[1] Connie Rhee,[1] Lilia R. Lukowsky,[1] Allen R. Nissenson,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Davita Inc., El Segundo, CA; [3]Memphis VA Medical Center, Memphis, TN.*

**Background:** Among dialysis patients, lower body mass index (BMI) has been associated with increased mortality, a phenomenon known as the obesity paradox. This apparent paradox may be explained by protein-energy wasting and loss of muscle mass over time. We hypothesize that there are time trend associations between BMI and serum creatinine levels with dialysis vintage, and that these trends may be associated with patient survival.

**Methods:** Among 62,051 incident (vintage <90 days) dialysis patients over a 5-year period (7/2001-6/2006), repeated BMI and serum creatinine levels were averaged per quarter by patient survival.

**Results:** On average, there was a significant linear increase in the mean serum creatinine level from the first calendar quarter (6.3 mg/dL) over the subsequent seven calendar quarters. Although this trend was similar between patients who survived vs. patients who died within each calendar quarter, those who died had a lower average serum creatinine per quarter compared to those who survived. In contrast, ,whereas survivors did not experience a change in their average BMI over time, after excluding the first quarter there was a trend towards an increased BMI over time. Moreover, those who died had a lower average BMI compared to those who survived.




**Conclusions:** Incident dialysis patients were observed to have a significant increase in serum creatinine level following dialysis initiation irrespective of survival or death status, while BMI increased over time only among patients who survived. Our findings also corroborate prior reports whereby lower creatinine levels and lower BMI is associated with increased mortality. These results offer further insight into the obesity paradox and relationship between BMI, muscle mass and survival in dialysis patients.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO317

**Circulating Angiotensin Converting Enzyme 2 in Patients with Chronic Kidney Disease without History of Cardiovascular Disease** Lidia Anguiano,[1] Marta Riera,[1] Julio Pascual,[1] Clara Barrios,[1] Angels Betriu,[2] Jose M. Valdivielso,[2] Elvira Fernandez,[2] Maria Jose Soler.[1] *[1]Nephrology, IMIM-Hospital del Mar, Barcelona, Spain; [2]Nephrology, Hospital Arnau de Vilanova, Lleida, Spain.*

**Background:** Circulating ACE2 activity is increased in cardiovascular disease(CVD) and in experimental models of diabetes(DM). It has not been studied in Chronic Kidney Disease(CKD)without history of CVD.

**Methods:** Analysis of 2572 patients without history of CVD from NEFRONA study. Groups:non-dialysis CKD stage 3-5(CKD3-5,n=1458) and dialysis(CKD5D,n=546). 568 patients without CKD were controls. Variables analyzed:gender,age,DM,dyslipidemia,hypertension and ACEi or ARBs.ACE2 activity was measured by fluorimetry.

**Results:** Patients on CKD3-5 had higher levels of ACE2 activity compared to CKD5D(45.2±1.2RFU/μl/h vs 38.5±1.6,p<0.05). Control patients had higher levels of ACE2 activity(52.9±1.5)compared to CKD3-5 and CKD5D(p<0.05). In CKD3-5 ACE2 activity was increased in men compared to women(50.4±1.6 vs 36.6±1.5)and in DM(49.9±2.0 vs 43.3±1.4,p<0.05). In CKD5D,ACE2 activity was increased in men(45.6±2.5 vs 27.7±1.4),in dyslipidemia(42.6±2.8 vs 33.9±1.3,p<0.05) and in ARBs (40.3±1.9 vs 37.6±2.2,p<0.05). Direct correlation between age and ACE2 activity(p<0.05) in CKD3-5 and CKD5D. In CKD3-5 patients HbA1c directly correlated with ACE2 activity(p<0.05). By multiple regression analysis, male, old age and DM were independent predictors of ACE2 activity in CKD3-5. Predictors in CKD5D were male gender, age and ARBs. When all patients included, male gender, older age, ARBs and CKD3-5 were predictors of elevated ACE2.

| ACE2 predictors | β | p-value |
|---|---|---|
| CKD3-5 | | |
| Male | 0.22 | 0.001 |
| Age | 0.06 | 0.03 |
| DM | 0.07 | 0.01 |
| CKD5D | | |
| Male | 0.33 | 0.001 |
| Age | 0.12 | 0.002 |
| ARB | 0.09 | 0.02 |
| All included | | |
| Male | 0.25 | 0.001 |
| Age | 0.08 | 0.001 |
| ARB | 0.05 | 0.03 |
| CKD3-5 | 0.06 | 0.001 |

**Conclusions:** In CKD patients without history of CVD, old age and male gender are predictors for elevated ACE2 activity. Independent additional predictors are DM in CKD3-5 and ARBs in CKD5D. Increased ACE2 activity in CKD might indicate those patients at risk for developing CVD.

## FR-PO318

**Stroll Saves Lives and Delays Renal Replacement Therapy** I-RU Chen, Che-yi Chou, Hsin Hung Lin, I-kuan Wang, Shih-yi Lin, Yao-Lung Liu, Jiung-hsiun Liu, Chih-chia Liang, Yafei Yang, Huey-Liang Kuo, Chiu-Ching Huang. *Dept of Internal Medicine, Kidney Institute and Div of Nephrology, China Medical Univ Hospital, Taichung, Taiwan.*

**Background:** There is increasing evidence of the benefit of increasing physical activity in chronic kidney disease (CKD) patients receiving renal replacement therapy (RRT). Increasing physical activity may improve quality of life and clinical outcomes. There is little evidence for the benefit of increasing physical activity in CKD 3-5 patients. This study reported the effect of frequency, duration and types of exercise in CKD 3-5 patients on clinical outcomes.

**Methods:** We analyzed all CKD 3-5 patients in China Medical University Hospital CKD program from 2003 to 2012. The types, frequency and duration of exercise were recorded on the initiation of CKD program and all patients were followed to the date of mortality, RRT (dialysis or kidney transplant) or Dec 2012. The risk of mortality or RRT was analyzed using Cox regression with adjustments for age and CKD stage (3a,3b,4,5).

**Results:** A total of 4832 CKD 3-5 patients (stage 3a 720, stage 3b 818, stage 4 996 and stage 5 2298) including 2052 female and 2779 male with mean age of 69.1 +/- 13.6 years old were analyzed. 357 (7.4%) patients died and 2596 (53.7%) started RRT in an average 1.7 +/- 1.6 years follow-up. Diabetes and hypertension was not significantly associated with the outcomes while types, frequency, and duration of exercise were taken into consideration. The increasing frequency of exercise was linked to a decrease of overall mortality ($p<0.001$). The increasing duration of exercise was linked to a decrease of RRT risk ($p<0.001$). Stroll, independent of frequency and duration of exercise, was linked to a decreased mortality risk ($p=0.05$) and RRT risk ($p=0.04$).

**Conclusions:** Stroll is a common and easy physical activity in chronic kidney disease patients. Stroll may decrease the risk for mortality and RRT, the benefit of stroll is independent of it's' frequency and duration. More studies are needed to determine if behavior instructions can increase physical activity to improve clinical outcomes in CKD patients.

## FR-PO319

**Predictors of Renal Outcome in Nephrotic Idiopathic Membranous Nephropathy with Declined Renal Function** Xiang-Mei Chen, Yizhi Chen, Li Zhang, Guangyan Cai. *Div of Nephrology, State Key Discipline and State Key Laboratory of Kidney Diseases, Chinese People's Liberation Army (PLA) General Hospital.*

**Background:** The outcome of patients with idiopathic membranous nephropathy (IMN) who presented with nephrotic-range proteinuria and declined renal function was seldom described. The value of pathologic and clinical features in predicting the outcome has long been debated. The aim of this study was to identify factors associated with persistent renal decline and progression to end-stage renal disease (ESRD).

**Methods:** A retrospective study was conducted between 2002 and 2011 in 129 patients with biopsy-proven IMN from a single tertiary referral center in China. The primary outcomes were 20% and 50% decline in renal function and the progression to ESRD.

**Results:** All the 129 patients had nephrotic-range proteinuria (≥3.5 g/day) and declined renal function (chronic kidney disease (CKD) stages 2-4) at baseline. Of the 129 patients, 38 (30%) presented with proteinuria ≥8.0 g/day and 37 (29%) presented with CKD stages 3-4, 13 (10%) presented with focal segmental glomerulosclerosis (FSGS), and 42 (33%) presented with moderate and severe tubulointerstitial injury (T1+T2). During a median follow-up of 34 (range 12-135) months, 51 (40%) and 27 (21%) patients experienced 20% and 50% decline in renal function, respectively. Fourteen (11%) patients progressed to ESRD. Age at baseline (≥ 60 years) and the presence of FSGS and T1+T2 were independent risk factors of both20% and 50% decline in renal function. CKD stages 3-4 at baseline (hazard ratio (HR)=5.76, 95% CI 1.31-25.29, P<0.001) and the presence of FSGS (HR=4.31, 95% CI 1.15-16.08, P=0.030) and T1+T2 (HR=5.44, 95% CI 1.17-25.36, P=0.031)were independent risk factors of the progression to ESRD.

**Conclusions:** Age≥ 60 years and CKD stages 3-4 at baseline and the presence of FSGS and T1+T2 were independent risk factors of the decline in renal function and (or) the progression to ESRD in adult IMN patients with nephrotic-range proteinuria and declined renal function.

*Funding:* Government Support - Non-U.S.

## FR-PO320

**The Predictive Factors of Cisplatin-Induced Nephrotoxicity for Urothelial Cancer** Satoru Muto,[1] Syou-ichiro Sugiura,[1] Yasuhiro Noma,[1] Masahiro Inoue,[1] Kousuke Kitamura,[1] Shino Tokiwa,[1] Keisuke Saito,[1] Shuji Isotani,[1] Raizo Yamaguchi,[1] Hisamitsu Ide,[1] Shigeo Horie.[2] *[1]Urology, Teikyo Univ, Tokyo, Japan; [2]Urology, Juntendo Univ, Tokyo, Japan.*

**Background:** Though cisplastin (CDDP) shows excellent anticancer activity, its use has been restricted due to its damaging effect on the kidney. Our team designed a retrospective clinical study on renal damage in anticancer chemotherapy using CDDP.

**Methods:** This study included 63 patients with urothelial cancer who had received chemotherapy with CDDP from March 2000 to February 2012. Renal function were investigated at baseline, the first 10 days after CDDP administration, 3, 6, 12, 24 and 36 months later using the eGFR, MDRD formula, Cockcroft-Gault formula.

**Results:** Mean age was 65.5 ± 8.1 years and median follow-up period was 56 months (range; 3-146 months). In a short period of time after CDDP administration, the univariate analysis revealed the significant differences between the decreased renal function group and the non-decreased group in the renal function before CDDP administration, the volume of infusion on the day of CDDP administration, the volume of extracellular fluid administered on the day of CDDP administration and the urinary volume on the day of CDDP administration. In multivariate analysis, the renal function before CDDP administration and the urinary volume on the day of CDDP administration were significant predictive factor for the prognosis of renal function. Though GFR tended to worsen, significant change was not found . Examination of influential factors on significant reduction in GFR 36 months later revealed that in univariable analysis, the significant predictive factors were GFR during the first 10 days after CDDP administration.

**Conclusions:** Careful long-term follow-up is required after anti-cancer CDDP containing chemotherapy for urothelial cancer. Prevention of kidney function decline immediately after CDDP administration by ensuring sufficient volume of infusion and urine in concurrence with CDDP administration should be considered to prevent a decline in renal function over a prolonged period of time.

## FR-PO321

**Association of Systolic Blood Pressure with Cardiovascular Events in Patients with Advanced Kidney Disease** Shyamal K. Palit,[1] Anna Jeanette Jovanovich,[1] Alfred K. Cheung,[2,3] James S. Kaufman,[4] Gerard John Smits,[1] Michel Chonchol,[1] Jessica B. Kendrick.[1] *[1]Univ of Colorado Denver, Aurora, CO; [2]VASLCHCS, Salt Lake City, UT; [3]Univ of Utah, Salt Lake City, UT; [4]VA New York Harbor Healthcare System, New York, NY.*

**Background:** The optimal systolic blood pressure (SBP) target to reduce cardiovascular events (CVE) in patients with chronic kidney disease is unknown. The purpose of this study was to examine the relationship between SBP and CVE in patients with advanced kidney disease.

**Methods:** We performed an analysis on 1,099 patients with advanced kidney disease not yet on dialysis who participated in the Homocysteine in Kidney and End Stage Renal Disease study. Blood pressure was measured in a standardized fashion. SBP levels were examined in clinically defined cutoffs (<120, 120-139, 140-159 and ≥160 mmHg) and as a continuous variable. We used Cox proportional hazard models to examine the association between SBP levels and CVE defined as a composite of myocardial infarction, stroke and amputation.

**Results:** The mean eGFR was 18 ± 7 ml/min/1.73m$^2$. A total of 215 CVE occurred during a median follow-up of 2.9 years. After adjustment for age, gender and race there was an increased risk of CVE with a SBP ≥ 160 mmHg compared to a level <120 mmHg (HR 1.61, 95% CI 1.01-2.56). There was no increased risk of CVE with a SBP 140-159 or SBP 120-139 mmHg (HR 0.90, 95% CI 1.56-1.45 and HR 1.06, 95% CI 0.66-1.69, respectively). For every 1 mmHg increase in SBP, there was a 0.8% increased risk of CVE (HR 1.008, 95% CI 1.002-1.01). However, after further adjustment for hypertension, diabetes, history of cardiovascular disease, smoking, body mass index, eGFR and treatment arm, SBP ≥ 160 mmHg was no longer associated with an increased risk of CVE compared to a level <120 mmHg (HR 1.42, 95% CI 0.89-2.29). Similar results were obtained when SBP was modeled as a continuous variable (HR 1.005, 95% CI 1.00-1.01, per 1mmHg increase in SBP).

**Conclusions:** After adjustment for known cardiac risk factors, SBP levels were not associated with CVE in patients with advanced kidney disease. Randomized trials are needed to determine the optimal SBP in patients with advanced kidney disease.

*Funding:* NIDDK Support

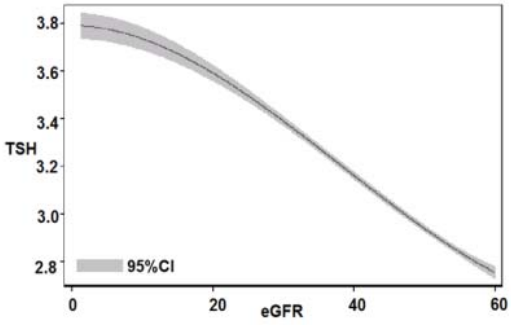
## FR-PO322

**Hypothyroidism and Impaired Kidney Function among National VA Chronic Kidney Disease Patients** Connie Rhee,[1] Kamyar Kalantar-Zadeh,[1] Elani Streja,[1] Jennie Z. Ma,[2] Jun Ling Lu,[3] Csaba P. Kovesdy.[3,4] *[1]Harold Simmons Center, Orange, CA; [2]Univ of Virginia, Charlottesville, VA; [3]Univ of Tennessee Health Science Center, Memphis, TN; [4]Memphis VA Medical Center, Memphis, TN.*

**Background:** Hypothyroidism is a common condition in the general population with pervasive effects on virtually every organ system. Studies suggest non-dialysis dependent chronic kidney disease (NDD-CKD) patients are disproportionately affected by hypothyroidism, but there is likely underrecognition of disease. We thus sought to examine the association between hypothyroidism and estimated glomerular filtration rate (eGFR) in the national VA NDD-CKD cohort.

**Methods:** We examined data from >400,000 adult patients with ≥1 thyrotropin (TSH) and ≥1 creatinine. The CKD-EPI formula was used to calculate eGFR. Using a random effects model that considered longitudinal TSH levels, we examined the association between TSH and eGFR in case-mix adjusted analyses. In analogous analyses, we examined the association between biochemical hypothyroidism (TSH>5mIU/L) and eGFR.

**Results:** Among 468,739 patients with ≥1 TSH, 8.0% had biochemical hypothyroidism, 21.0% received levothyroxine (LT-4), and 23.3% had biochemical hypothyroidism and/or LT-4 use. Among 463,219 patients with 1,560,797 TSH measurements and complete covariate data, for every 10ml/min/1.73m$^2$ increase in eGFR, there was a 10% decrease in the odds of a 1 mIU/L TSH elevation (OR 0.90 [95% CI] 0.89-0.90). For every 10ml/min/1.73m$^2$ increase in eGFR, there was a 12% decrease in the odds of biochemical hypothyroidism (OR 0.88 [95% CI] 0.87-0.90).




**Conclusions:** Among a nationally representative cohort of VA NDD-CKD patients, hypothyroidism is highly prevalent, and it is inversely associated with eGFR. Further studies are needed to determine the mechanistic link, directionality of association, and prognostic implications of hypothyroidism in CKD.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO323

**Short-Term Prognosis of Chronic Kidney Disease with Hyperuricemia Treated by Febuxostat: A Subanalysis from a Prospective Non-Controlled Safety/Tolerability Study**

Yugo Shibagaki,[1] Iwao Ohno,[2] Tatsuo Hosoya,[2] Kenjiro Kimura.[1] *[1]Div of Nephrology and Hypertension, St. Marianna Univ Hospital, Kawasaki, Japan; [2]Div of Nephrology and Hypertension, Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Hyperuricemia (HU) is a very common comorbid condition in patients with chronic kidney disease (CKD) and there is accumulating evidence that treatment of HU may reduce blood pressure (BP) level and progression of CKD.

**Methods:** We conducted a 24-week prospective, open-label, non-controlled study to investigate the safety, tolerability and efficacy of febuxostat in patients with HU and moderate to severe CKD (stage 3b to 5). 71 prevalent adult outpatients (55 males with average age of 66.3 years) at two tertially care hospitals who met the inclusion criteria [serum urate > 8 mg/dl, estimated GFR (eGFR) < 45 ml/min/1.73m$^2$, non-use of urate lowering drug in the previous month] were recruited from November 2011 to April 2012. All the participant received febuxostat. Starting dose of febuxostat was 10 mg qd, increased to 20 mg, 40mg qd in week 4, and week 8, respectively. We analyzed the data of this study to investigate change of BP, eGFR and proteinuria during treatment of HU with febuxostat.

**Results:** Serum urate level (mg/dl) at baseline/24 weeks were 9.9/5/5, 10.6/5.2, and 9.8/4.8, in stage 3b, 4, and 5, respectively. There was no significant change in BP between start and end of the study as a whole, although the diastolic BP was slight but significantly lower at week 8 and 16. eGFR at 24 weeks were not significantly different from that at baseline in CKD stage 4 and 5. However, in CKD 3b, eGFR was significantly higher at 24 weeks compared with that at baseline. There were 15 patients who had data of proteinuria. There was no significant change in level of proteinuria although proteinuria tended to decrease in CKD stage 3b. Multivariate analysis revealed that greater reduction of serum urate predicted increase in eGFR (p=0.0055) and tended to predict reduction of proteinuria (p=0.063).

**Conclusions:** Greater reduction of serum urate level by febuxostat was associated with increase in eGFR, although it did not significantly change the level of BP and proteinuria.

## FR-PO324

**Elevated Level of C-Reactive Protein Identify African Americans with Metabolic Syndrome at High Risk of Developing Albuminuria**

Satyesh K. Sinha,[1] Magda Shaheen,[1] Deyu Pan,[1] Susanne B. Nicholas.[1,2] *[1]Charles R. Drew Univ of Medicine and Science, Los Angeles, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA.*

**Background:** African Americans (AA) have a higher prevalence of the metabolic syndrome (MetS)and chronic kidney disease (CKD) than whites. Inflammation and the MetS have been shown to be associated with renal impairment; however, it is unclear whether higher levels of serum inflammatory biomarkers may account for disparities in the development of albuminuria, as a marker for CKD in AA with MetS.

**Methods:** We analyzed data from the National Health and Nutrition Examination Surveys 1999-2008 of 7,309 adults aged ≥20 years with three or more components of the MetS according to the National Cholesterol Education Program's Adult Treatment Panel III definition. Multivariate analyses were performed to test the relationship of race/ethnicity with *urinary albumin excretion (UAE)* and serum C-reactive protein (CRP), adjusting for age, gender, smoking and components of the MetS.

**Results:** CRP and UAE were significantly higher in AA (0.76±0.77mg/dl and 211.7±990.1µg/mL, respectively), compared to whites (0.54±1.05mg/dl and 48.5±344.0µg/mL, respectively), p<0.01. Adjusted logistic regression indicated that AA had higher odds for CRP ≥0.3mg/dl (adjusted odds ratio [AOR]=1.42, 95% CI 1.15-1.76) and UAE ≥30µg/ml (AOR=2.67, 95% CI=2.11-3.38), compared to whites, p<0.001. Also, adjusted linear regression showed a significant association between UAE and CRP (Adjusted Coefficient; AC=0.15, 95% CI 0.07-0.23) and that the interaction term of CRP and race/ethnicity was statistically significant, p<0.001. The adjusted change in the UAE for one unit change in CRP was higher among AA relative to whites (AC=0.63, 95% CI 0.39-0.87), suggesting a stronger relationship between CRP and UAE in AA.

**Conclusions:** We conclude that elevated CRP may predict the higher risk of albuminuria in AA with the MetS compared to white.

*Funding:* Other NIH Support - NIH-NIMHD grant U54MD007598 (formerly U54RR026138), S21MD000103 and NIH/NCRR/NCATS CDU/UCLA CTSI Grant UL1TR000124

## FR-PO325

**Dialysis at Home–Could It Be a More Frequent Choice?**

Patrik Finne,[1,2] Ilkka Helanterä,[1] Virpi Rauta,[1] Agneta V. Ekstrand,[1] Eero Honkanen,[1] Carola Gronhagen-Riska.[1] *[1]Dept of Nephrology, Helsinki Univ Central Hospital, Helsinki, Finland; [2]Finnish Registry for Kidney Diseases, Helsinki, Finland.*

**Background:** The proportion of dialysis patients on home dialysis (either peritoneal dialysis (PD) or home hemodialylsis (HD)) varies greatly both between and within countries, which indicates that there is a potential for increasing its use in many places.

**Methods:** All patients who entered dialysis in Finland in 2000 to 2010 and were still on dialysis at day 91 from start (n=4984) were included. Patient data were retrieved from the Finnish Registry for Kidney Diseases. Data on contraindications for home dialysis was collected for a randomized sample of 200 patients on in-centre HD in the healthcare district of Helsinki-Uusimaa (HUS), which has exceptionally high proportion of home dialysis (38%). A multinomial logistic regression equation with stepwise selection of explanatory variables was constructed based on the patients in HUS (n=1128) to estimate probability of home HD, PD and in-centre HD. This model was applied to the patients in the rest of the country in order to estimate whether use of home dialysis could potentially be increased.

**Results:** Out of 30 evaluated variables, 11 were selected to the final model. The most important predictors of home dialysis were young age, high hemoglobin, high albumin and normal body-mass index (for PD). Polycystic kidney disease decreased probability of PD and increased that of home HD. The model identified in-centre HD patients with large probabilities of either home HD or PD and indicated that use of home HD could potentially be increased from 0.2% to 8% and PD from 26 to 28%. Of the in-centre HD patients in HUS, 93% had a reported contraindication of home dialysis in the hospital files. Notably, the model predicted in-center HD for half of patients who in fact had entered PD in the rest of the country.

**Conclusions:** Our model identified a considerable proportion of in-center hemodialysis patients that could be suitable for either peritoneal dialysis or home hemodialysis. Conversely, the model predicted in-center HD for many patients who actually had entered PD, which shows that indications for home dialysis vary between districts in Finland.

*Funding:* Pharmaceutical Company Support - Baxter

## FR-PO326

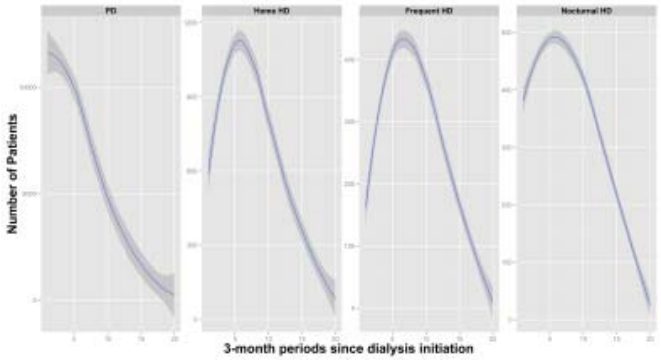
**Time Course of Utilization of Alternative Dialysis Modalities**

Sooraj Kuttykrishnan,[1] Kamyar Kalantar-Zadeh,[2] Jonathan Himmelfarb,[1] Alfred K. Cheung,[5] Onyebuchi A. Arah,[3] Elani Streja,[2] Vanessa A. Ravel,[2] Allen R. Nissenson,[4] Rajnish Mehrotra.[1] *[1]Univ of Washington; [2]Univ of California, Irvine; [3]Univ of California, Los Angeles; [4]DaVita Healthcare Partners; [5]Univ of Utah.*

**Background:** The Institute of Medicine lists studying outcomes with different dialysis therapies among the top 100 priorities for comparative effectiveness research. Understanding the time course of accrual of patients to the various dialysis therapies is needed to adjust for bias inherent in such comparisons.

**Methods:** The time course of utilization of different dialysis therapies was analyzed in three-month increments from the date of first dialysis up to five years, among the 162,671 patients who started maintenance dialysis in 2007-11 in a large dialysis organization.

**Results:** Over 5 years, 23,837 (14.7%) patients were treated with alternative dialysis modalities: PD, 18,279 (11.2%), home hemodialysis (HD), 2653 (1.6%), frequent in-center HD, 1888 (1.2%), and nocturnal in-center HD, 1452 (0.9%). Only 54% of patients ever treated with PD, started treatment with this modality compared to 18%, 7%, and 24% for home HD, frequent HD, and nocturnal HD respectively. The median interval from the time of first dialysis to first ever treatment with home HD, frequent HD, and nocturnal HD was 9, 9 and 12 months respectively (Figure). The median time of treatment with PD, home HD, frequent HD, and nocturnal HD was only 18.3, 12.1, 5.6, and 9.7 months respectively.




**Conclusions:** The actual number of patients treated with alternative dialysis therapies is significantly larger than evident from point-prevalent counts. Most patients treated with alternative modalities do so only after at least some period of treatment with in-center HD and are treated for relatively short periods of time. These are important considerations when studying comparative effectiveness of dialysis therapies.

*Funding:* NIDDK Support

## FR-PO327

**Temporal Trends in Home Dialysis Training**

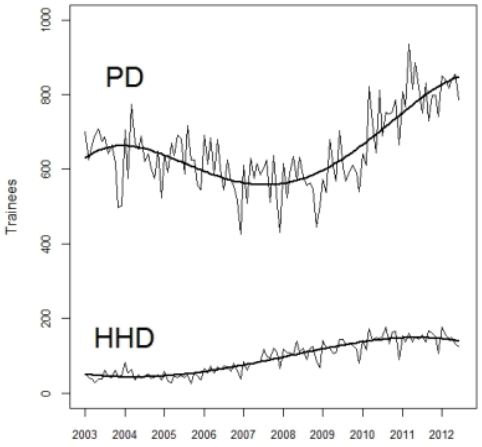
Eric D. Weinhandl,[1] Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Univ of Minnesota, Minneapolis, MN.*

**Background:** To compensate for the cost of home dialysis training, the Medicare End Stage Renal Disease (ESRD) Prospective Payment System (PPS) features a 51% increase in reimbursement for dialysis sessions during the first 120 days after dialysis initiation, as well as an add-on payment of $33.44 per training session after the first 120 days. Critics have argued that these mechanisms fail to properly compensate for the cost of home hemodialysis training. We used Medicare Quarterly Standard Analytical Files (QSAFs) to assess temporal trends in peritoneal dialysis (PD) and home hemodialysis (HHD) training between January 2003 and June 2012.

**Methods:** We searched outpatient facility claims between calendar years 2000 and 2012 for condition code 73 (self-care in training) in tandem with revenue center codes 0821 (HD), 0831 (PD), 0841 (continuous ambulatory PD), or 0851 (continuous cycling PD). For each patient with qualifying claims, we recorded the date and modality of the

first training session. For each month between January 2003 and June 2012, we tallied the number of patients who initiated either PD training or HHD training. To estimate trends, we fit fourth-degree polynomial functions to each time series of trainee counts.

**Results:** Counts of PD trainees reached their minimum in December 2006 (N = 427), and according to trend analysis, began increasing in July 2007, with sustained growth of 4 patients per month beginning in December 2008. In contrast, counts of HHD trainees tended to increase between April 2003 and April 2011, with a maximum in August 2010 (N = 177), but tended to decrease beginning in May 2011.




**Conclusions:** The introduction of the ESRD PPS coincided with sustained month-to-month increases in the number of patients in initial PD training, but was followed shortly thereafter by month-to-month decreases in the number of patients in initial HHD training.

*Funding:* NIDDK Support

## FR-PO328

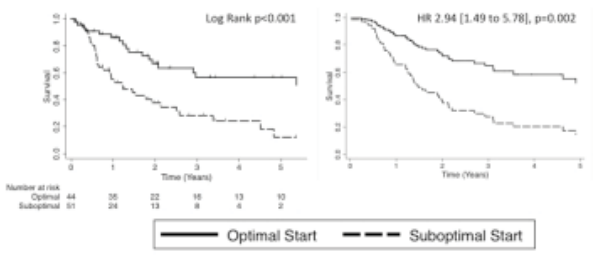
**Suboptimal Initiation of Home Hemodialysis: Determinants and Clinical Outcomes** Annie-Claire Nadeau-Fredette, Karthik K. Tennankore, Joseph Kim, Christopher T. Chan. *Div of Nephrology, Dept of Medicine, Univ of Toronto, Toronto, Canada.*

**Background:** Suboptimal initiation of conventional hemodialysis (CHD) is associated with poor clinical outcomes. In this study, we aimed to ascertain the determinants and adverse events associated with suboptimal starts in home hemodialysis (HHD).

**Methods:** We conducted a retrospective cohort study including consecutive incident HHD patients from January 1996 to December 2011. All patients had HHD as their first renal replacement therapy or returned to HHD after kidney transplantation. A suboptimal start was defined by dialysis initiation either as an inpatient or with a central venous catheter. The primary outcome was time to first hospitalization, technique failure or death. Secondary outcomes included hospitalization rate, hospital days and determinants of suboptimal starts. Suboptimal starts were further categorized as unavoidable as adjudicated by two independent observers with pre-specified criteria.

**Results:** Among 95 incident HHD patients, 44 (46%) and 51 (54%) had optimal and suboptimal starts, respectively. A suboptimal start was associated with a shorter time to the primary outcome (log rank $P$<0.001). In a multivariable Cox proportional hazards model, the hazard ratio for the composite outcome (comparing suboptimal to optimal starts) was 2.94 (95% CI: 1.49, 5.78; $P$ = 0.002).

**Figure 1: Kaplan-Meier and adjusted Cox survival curves for time to composite outcome (hospitalization, technique failure or death).**




Additional variables in the adjusted model: age, gender, Caucasian race, Charlson Comorbidity Index, previous follow-up. HR: Hazard ratio

Transplantation clinic follow-up (OR 3.18 [95% CI: 1.15, 8.79]) and the Charlson comorbidity index (OR 1.47 [95% CI: 1.09, 1.97]) were associated with higher odds of suboptimal starts.

**Conclusions:** Suboptimal initiation of HHD is associated with adverse clinical events including early hospitalization. Given the high proportion of suboptimal starts in patients returning from transplantation, better incorporation of dialysis planning and renal replacement therapy education is warranted.

## FR-PO329

**Bayesian Interpretation of the Estimated Treatment Effects on Mortality in the Daily and Nocturnal Frequent Hemodialysis Trials (FHN)** Tom Greene, John T. Daugirdas, Glenn M. Chertow, Nathan W. Levin, Michael V. Rocco, Gerald J. Beck, Alan S. Kliger. *NIDDK.*

**Background:** The FHN Daily and Nocturnal Trials were considered insufficiently powered to detect effects of frequent dialysis on mortality. However, new analyses of long-term mortality data from these trials (this meeting) suggest mortality may be reduced in patients randomized to daily vs. conventional in-center hemodialysis, but increased in patients randomized to frequent nocturnal vs. conventional home hemodialysis. Because positive results in underpowered studies incur elevated risk of false positive conclusions (Ioannidis, Plos Med, 2005), we performed Bayesian analyses to quantify the uncertainty of these results.

**Methods:** Posterior distributions of the mortality hazard ratios (HRs) were simulated under an "equipoise" prior, with a median prior HR of 1.00, and under a "mildly optimistic prior", with a median prior HR of 0.80 for frequent dialysis.

**Results:** Original and Bayesian Posterior Hazard Ratios assuming equipoise or a median prior HR=0.80. Bottom 3 rows show the original estimated mortality HR or median posterior HR, 95% confidence limits or posterior intervals for frequent HD, and posterior probabilities that HR ≤ 0.8 and that {HR ≤ 1}.

| Daily Trial | | | Nocturnal Trial | | |
|---|---|---|---|---|---|
| Original (56 deaths) | Bayes - equipoise* | Bayes - median prior HR = 0.80* | Original (19 deaths) | Bayes - equipoise* | Bayes - median prior HR = 0.80** |
| 0.54 | 0.65 | 0.63 | 3.82 | 1.71 | 1.34 |
| (0.32, 0.93) | (0.41, 1.02) | (0.41, 0.96) | (1.26, 11.63) | (0.92, 3.20) | (0.77, 2.38) |
| - | 0.82 {0.97} | 0.88 {0.99} | - | 0.01 {0.04} | 0.03 {0.15} |

**Conclusions:** The original estimated treatment effects of frequent HD on mortality were attenuated by Bayesian analysis, illustrating that large estimates of treatment effects in trials with low power may overstate the true effects. Even so, in the Daily Trial posterior probabilities that HR < 0.8 are > 0.80, suggesting that daily dialysis is reasonably likely to substantially reduce mortality. As implemented by the FHN, frequent nocturnal dialysis is unlikely to substantially reduce, and might increase, mortality compared to conventional home dialysis.

*Funding:* NIDDK Support

## FR-PO330

**The Magnitude of Phosphorus Mobilization during Dialysis in the Frequent Hemodialysis Network (FHN) Daily Trial Was Patient-Specific** John T. Daugirdas,[1] Brett Larive,[1] Thomas A. Depner,[1] Andreas Pierratos,[1] Tom Greene,[1] Robert S. Lockridge,[1] Juan Carlos Ayus,[1] Michael V. Rocco,[1] Glenn M. Chertow,[1] J. Ken Leypoldt,[2] Alp Akonur,[2] Brent W. Miller,[1] The FHN Trial Group.[1] *[1]NIH, NIDDK; [2]Baxter Healthcare.*

**Background:** In the FHN Daily Trial we tracked 3 measures of phosphorus mobilization (M) to assess constancy of M status over time.

**Methods:** Monthly values of pre and postdialysis serum phosphorus (P) were used to compute the phosphorus reduction ratio (PRR), PRR/URR (U = urea) and Km, the intercompartment clearance of P from a theoretical very large remote compartment (Agar et al, *CJASN* 2011). Low PRR or PRR/URR and high Km correspond to high M status.

**Results:** Baseline PRR (%), PRR/URR (ratio), and logKm (ml/min) were 57.9 ± 9.8 , 0.79 ± 0.13, and 4.56 ± 0.41. In baseline multivariate models, lower predialysis serum P (preP), male sex, younger age, and greater weight independently associated with higher M. Higher serum PTH associated with higher M (PRR/URR and Km) but here the effect size was small. In high or low M patient subgroups identified at baseline, preP-adjusted M indices were tracked over 12 months. Mean values (3x: n=120; 6x: n=125) are shown in the Table.

| **Baseline M status subgroup** | **Freq** | **PRR** | | **PRR/URR** | | **log Km** | |
|---|---|---|---|---|---|---|---|
| | | Baseline | Mo 10-12* | Baseline | Mo 10-12* | Baseline | Mo 10-12* |
| top 20 | 3x | 45.5 | 52.2 | 0.64 | 0.73 | 5.10 | 4.84 |
| | 6x | 47.5 | 51.0 | 0.67 | 0.81 | 5.06 | 4.87 |
| <top 20; > median | 3x | 54.7 | 57.6 | 0.76 | 0.79 | 4.71 | 4.57 |
| | 6x | 55.9 | 54.2 | 0.78 | 0.83 | 4.68 | 4.84 |
| <median; > bottom 20 | 3x | 60.9 | 58.6 | 0.83 | 0.81 | 4.46 | 4.57 |
| | 6x | 60.5 | 57.3 | 0.82 | 0.91 | 4.45 | 4.62 |
| bottom 20 | 3x | 67.2 | 61.5 | 0.89 | 0.82 | 4.12 | 4.36 |
| | 6x | 63.2 | 58.7 | 0.82 | 0.89 | 4.13 | 4.53 |

*Average values during follow-up months 10-12 for each baseline-defined subgroup.

**Conclusions:** Measures of P mobilization (M) during dialysis were positively associated with lower preP, male sex, younger age, greater weight, and somewhat with higher PTH. PreP-adjusted measures of M were patient-specific over a 12-month period, although some regression to the mean was seen.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO331

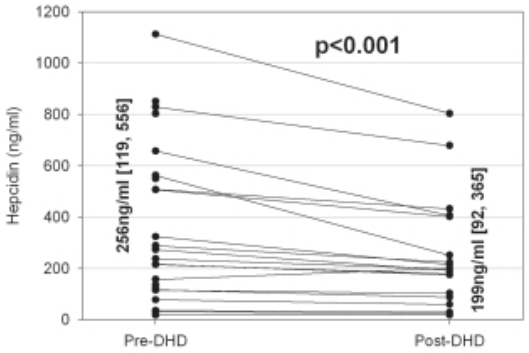
**Short Daily Hemodialysis Is Associated with Lower Serum Hepcidin Levels When Compared to Conventional Hemodialysis**

Joshua Zaritsky,[1] Anjay Rastogi,[1] George E. Fischmann,[1] Jieshi Yan,[2] Barbara Gales,[1] Tomas Ganz,[1] Mark E. Westerman,[3] Isidro B. Salusky.[1] *[1]UCLA, Los Angeles, CA; [2]CKMG, Simi Valley, CA; [3]Intrinsic Life Sciences, La Jolla, CA.*

**Background:** Hepcidin, a key regulator of iron homeostasis, may be the molecular link between inflammation and anemia in ESRD. Short daily hemodialysis (DHD) patients (pts) typically require less recombinant erythropoietin (EPO) than conventional hemodialysis (CHD) pts. Therefore, we compared serum hepcidin levels between the two dialytic modalities.

**Methods:** We compared serum hepcidin levels, indicators of anemia, inflammation and iron status between 24 pts treated with DHD using the NxStage System One® and 54 CHD pts (Table). Serum hepcidin levels were measured by competitive ELISA.

**Results:** DHD pts were younger but there were no differences in the duration of ESRD. In DHD pts, hepcidin levels were lower than in CHD pts, which corresponded to less inflammation (as assessed by high sensitivity C-reactive protein (hs-CRP)). Hepcidin levels post-DHD were lower than pre-DHD (Figure). We previously reported hepcidin was independently predicted by ferritin and hs-CRP in CHD pts by both univariate and multivariate regression analyses (MVA). In DHD pts, hepcidin levels correlated with ferritin ($r=0.7$, $p<0.001$) and hs-CRP ($r=0.4$, $p<0.05$). MVA ($R^2=0.6$) revealed that hepcidin was independently predicted by only ferritin ($p<0.001$). Additionally MVA ($R^2=0.6$) across both groups revealed that hepcidin remained predicted by ferritin ($p<0.001$) and hs-CRP ($p<0.001$).




| Biochemical Variables | DHD mean±SD or median[25,75%] | CHD mean±SD or median[25,75%] | p |
|---|---|---|---|
| Age (yrs) | 47±18 | 61±19 | <0.01 |
| ESRD duration (yrs) | 2.8 [1.1, 7.5] | 2.8 [1.2, 4.3] | NS |
| Hepcidin* (ng/ml)† | 256[119, 556] | 690[200, 905] | <0.001 |
| Hgb (g/dL) | 11.4±1.4 | 11.8±1.3 | NS |
| rhEPO (units/ week) † | 9700 [6000,18000] | 8250 [6600,13200] | NS |
| hs-CRP (mg/L) † | 0.45 [0.3, 1.35] | 3.9 [2.2, 8.5] | <0.001 |
| Ferritin (ng/mL) | 539±461 | 740±409 | NS |
| % Iron Saturation† | 26 [22, 34] | 29 [26, 47] | NS |
| Std weekly KT/V | 2.4 [2.2, 2.7] | 2.4 [2.2, 2.5] | NS |

*Normals= 91±57 ng/ml; †variables were log-transformed for MVA

**Conclusions:** We demonstrated that lower hepcidin values in DHD pts predominantly reflect a lower inflammatory state in this population. Since hepcidin appears to be cleared by DHD, future studies are needed to see if increased dialysis dosages could potentially lower serum hepcidin levels further and in turn decrease EPO requirements.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO332

**Evaluation of Change in Extracellular Fluid Status Using Calf Bioimpedance in Patients during One Year Frequent Hemodialysis**

Fansan Zhu,[1] George A. Kaysen,[2] Shubho Ranjan Sarkar,[1,3] Fredric O. Finkelstein,[4] Mary Carter,[1] Nathan W. Levin,[1] Nicholas A. Hoenich.[5] *[1]Renal Research Institute, New York, NY; [2]Univ of California, Davis, CA; [3]Nephrology & HIT Ass of Morris County, Morristown, NJ; [4]Yale Univ, New Haven, CT; [5]Newcastle Univ, Newcastle upon Tyne, United Kingdom.*

**Background:** Calf extracellular resistance ($R_E$) reflects both plasma ($R_P$) and interstitial ($R_{Int}$) fluid. The aims of this study were to investigate whether: 1) calf normalized resistivity (CNR) satisfactorily indicates change in fluid status in frequent HD (FHD) patients; 2)The extent to which $R_E$ is affected by $R_P$ at different levels of fluid status.

**Methods:** Patients were switched from 3 to 6 times/week HD. $R_E$ and extracellular fluid (ECV) was measured at baseline (BL) and monthly over one year using the Hydra 4200. Four electrodes and a blood pressure cuff were placed on the calf to separately measure $R_E$ and $R_{Int}$ at zero cuff pressure and at > than systolic blood pressure (SBP) respectively. Change in calf resistance was related to reduction of local plasma volume ($V_P$) (Fig.1). The ratio of $V_P$/ECV is calculated as $1\text{-}R_P/R_{Int}$.

**Results:** In nine available patients' body weight decreased significantly by the first month and then did not differ statistically in following months; SBP decreased continuously and CNR increased significantly from BL to the first month and remained approximately at the same level over one year (Fig 2). $V_p$/ECV did not differ significantly between BL and at any month over the one year period (Table1).

**Conclusions:** Significant improvement in extracellular volume fluid status in patients during one year of FHD was clearly indicated by CNR increase and SBP and ultrafiltration volume reduction. CNR was not affected by plasma volume changes at different levels of fluid load.

Table 1

| | BL | 1 month | 6 month | 12 month |
|---|---|---|---|---|
| $R_E$ (Ω) | 42.6±12 | 61.6±15.6# | 60.5±13 | 62.8±15** |
| $R_{Int}$ (Ω) | 45.9±12 | 65.6±16.3# | 65±13 | 66.8±17** |
| $V_p$/ECV (%) | 7.7±5 | 6.3±3.6 | 6.9±4 | 5.84±3.2 |
| BMI kg/m² | 34.8±8 | 32.5±7.1# | 33±8 | 33±7.6 |
| CNR ($10^{-2}$Ωm³/kg) | 12.2±4 | 16.7±4.2# | 15.8±4 | 15.3±3* |
| Wt (kg) | 97.3±22 | 90.9±18# | 92.3±20 | 92.6±22 |
| SBP (mmHg) | 156±21 | 140.9±16# | 130±20 | 130±24** |
| UFV (L) | 3.8±1 | 2.5±0.84# | 2.98±0.8 | 3±1.4* |
| UFR (L/h) | 1.8±0.3 | 1.2±0.35# | 1.5±0.5 | 1.5±0.6* |

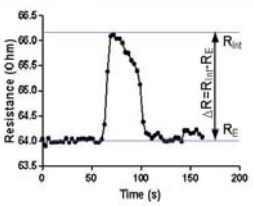


Fig.1

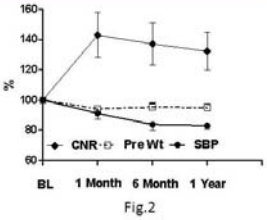


Fig.2

## FR-PO333

**Patient Survival, Treatment Survival and Hospitalization for Intensive Home Hemodialysis Patients Compared to Kidney Transplant Recipients**

Karthik K. Tennankore, Joseph Kim, Christopher T. Chan. *Div of Nephrology, Univ of Toronto/Univ Health Network, Toronto, Canada.*

**Background:** Patients receiving intensive home hemodialysis (IHHD, ≥16 hours/ week) have comparable survival to US deceased donor kidney transplant recipients (KTRs). A comparison between Canadian IHHD patients and KTRs has not been conducted. We hypothesized that IHHD patients would have better outcomes compared to expanded criteria donor (ECD) and standard criteria donor (SCD) recipients.

**Methods:** We analyzed a cohort of IHHD patients and KTRs from 2000-2011. The primary outcomes were time to first hospitalization and time to treatment failure/death for IHHD patients compared to ECD, SCD and living donor (LD) KTRs. Treatment failure was defined as a permanent switch to an alternative form of dialysis for IHHD patients, and graft failure for KTRs.

**Results:** The cohort consisted of 173 IHHD patients, 204 ECD, 643 SCD and 679 LD KTRs. There were 1036 first hospitalizations and 286 treatment failures/deaths (Figure 1). After adjusting for comorbidity, age, gender, race, cause of end-stage renal disease and dialysis vintage, hospitalization risk favoured IHHD in the short term and transplantation in the long term. Time to treatment failure/death was shorter for IHHD patients. **Table 1.Adjusted HRs for time to first hospitalization and time to treatment failure/death.**

| | 0-1 years | >1 year |
|---|---|---|
| **Time to First Hospitalization** | **HR (95% CI)** | **HR (95% CI)** |
| LD | 1 | 1 |
| IHHD | 0.54 (0.39-0.75) | 2.14 (1.52-3.00) |
| SCD | 1.06 (0.87-1.28) | 1.14 (0.86-1.50) |
| ECD | 1.05 (0.81-1.36) | 1.07 (0.71-1.60) |
| **Time to Treatment Failure/Death** | | |
| LD | 1 | 1 |
| IHHD | 2.09 (1.01-4.35) | 2.37 (1.49-3.78) |
| SCD | 1.23 (0.63-2.40) | 0.77 (0.54-1.09) |
| ECD | 1.30 (0.58-2.93) | 0.87 (0.55-1.38) |

**Conclusions:** IHHD is associated with a lower risk of short-term hospitalization compared to transplantation. Outcome-risk in the long term favors transplantation.

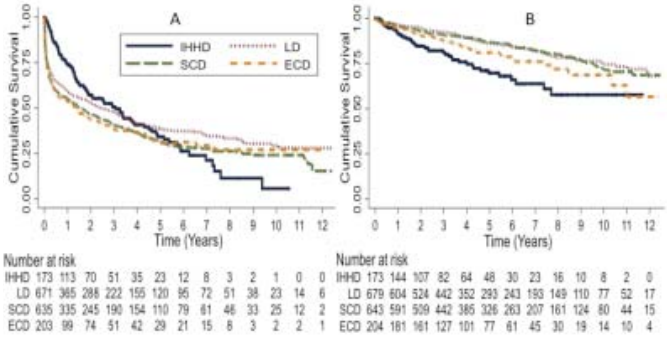



Figure 1. A: Time to first hospitalization (log rank p=0.01) and B: time to treatment failure/death (log rank p<0.001)

## FR-PO334

***Staphylococcus aureus* Bacteraemia: Evaluation of Access Techniques in Home Haemodialysis** Karen E. Brown, James A. Chess, Ashraf I. Mikhail. *Renal Dept, Morriston Hospital, Swansea, Wales, United Kingdom.*

**Background:** Vascular access-related *Staphylococcus aureus* bacteraemia is a major cause of morbidity and mortality amongst haemodialysis patients. Recent studies have reported an increased risk of fatal infective metastatic complications with home haemodialysis patients using buttonhole cannulation.

Methods: Microbiologically reported cases of MRSA/ MSSA bacteraemia were analysed in a retrospective cohort study at a renal tertiary referral centre over a 2 year period (January 2011 - December 2012).

Results: 26 episodes of MRSA/ MSSA bacteraemia were identified from a population of 317 dialysis patients. Risk factors included vascular access type (VasCath vs. AVF), immunosuppression, diabetes and warfarin use. UK Renal Association Guidelines recommend that the annual Staphylococcus aureus bacteraemia rate in the haemodialysis population should be less than 2.5 episodes per 100 HD patients and less than 1.0 for MRSA over 2 years. At our unit 0.53 episodes per 100 HD patients were identified for MSSA (3.19 including acute kidney injury) and 1.06 (2.66) for MRSA. This was in comparison to 12 *episodes of MSSA in home HD patients and 8.11 in self-care. 61% of* Staphylococcus aureus bacteraemias were directly attributable to vascular access complications (infected AVF/ AVG, line sepsis, post- fistula formation). Clinical outcomes of blood culture positive Staphylococcus aureus bacteraemia included death < 30 days, infective endocarditis and microbiologic recurrence.

Conclusions: This study highlights known concerns regarding increased infection rates in patients who self-cannulate using the buttonhole technique. As a result of this study, our centre has commenced the use of topical mupirocin in order to reduce the occurrence of MRSA/MSSA sepsis and therefore promote prolonged patency and AVF survival. The development of mupirocin resistance is being monitored. Further randomised controlled trials would assist in the evaluation of constant vs. different site cannulation in order to establish a superior technique and accurately inform the prevalent haemodialysis population.

## FR-PO335

**At-Home Short Daily Hemodialysis Attenuate Vascular Calcification** Hiromichi Suzuki, Hirokazu Okada, Tsutomu Inoue, Tsuneo Takenaka. *Dept of Nephrology, Saitama Medical Univ, Iruma Gun, Saitama, Japan.*

Background: Vascular calcification (VC) has a significant effect in cardiovascular disease on dialysis patients. However no pharmacological interventions have been demonstrated for prevention and/or attenuation of VC. Recent advances in frequent hemodialysis proves facilitation of hyperphosphatamia. It is proposed that there is a strong relation between VC and hyperphosphatamia. It is therefore likely that frequent hemodialysis reduced VC. Aim To examine whether at-home short dial hemodialysis reduces VC.

Methods: Using Volume Viewer software with a 16-detector CT scan, the total calcification volume of the aorta was calculated with a cut-of of 130 Housefield Unit. Abdominal CT scan was taken before and 2 years after the start of at-home short daily hemodialysis in 37 patients (average age 52 + 7.0 years, male 33). The underlying kidney disease were chronic glomerulonephritis (33), diabetic nephropathy (2) and congenital kidney disease (2).

Results: The base line values of calcification scores of the abdominal aorta were 5.03 + 5.85 (cm3) and at-home short daily hemodialysis reduced to 4.82 + 4.72 at the end of the study. Besides, at-home short daily hemodialysis attenuated several factors contributing to vascular calcification including hyperphosphatemia.

Conclusions: This is the first demonstration that at-home short daily hemodialysis reduces calcification of the abdominal aorta through several factors including serum levels of phosphate.

*Funding:* Pharmaceutical Company Support - Baxter 2010 Renal Discovery Grant

## FR-PO336

**Removal Water from the Legs Does Not Explain Hypotension in Short Daily Hemodialysis** Alessandra Martins Bales, Luciana Loureiro Nardotto, Rafael Sanches Humel, Ligia Costa Battaini, Bruno C. Silva, Rosa M.A. Moyses, Manuel C. Castro, Rosilene M. Elias. *HCFMUSP.*

**Background:** Previous studies on conventional hemodialysis (CHD) indicate that fluid loss during dialysis occurs from the legs. Whether the fluid loss from the legs is associated with hypotension symptoms in patients on short daily hemodialysis (SDH) is unknown.

**Methods:** Thirty-five conventional and fifteen short daily subjects on hemodialysis had segmental bioimpedance performed using the InBody10 Analyzer, pre and post dialysis. Demographic and biochemical data was also obtained.

**Results:** Baseline characteristics were similar between CHD and SDH. For both groups, from pre to post dialysis, there was a reduction in the extracellular water (ECW) for each of the segments (leg, arm, and trunk), total body water (TBW), and ECW/TBW ($p < 0.05$). The delta of legs ECW was higher on CHD than SDH [-5.4% (-7.3, -2.4) vs. -3.3% (-4.8, -0.7), p=0.009]. Hypotensive symptoms(Hypo+) were observed in 6 patients on SDH(37.5%) and 6 patients on CHD(17.1%). When comparing the subgroups of patients hypo+ and hypo-, the conventional group's leg ECW/TBW was lower in hypo+ (p=0.003) as well as the hemoglobin (p=0.009). However, there was no difference between hypo+ and hypo- in these or any other parameters in SDH.

**Conclusions:** When comparing CHD and SDH, the removal of leg ECW was higher in CHD. Patients on CHD who had hypotension presented low hemoglobin and lower ECW/TBW removal from the legs. This was not observed in SDH. Further studies are needed to better elucidate the hypotension pathogenesis on SDH, other than leg fluid removal.

| | Short Daily | | Conventional | |
|---|---|---|---|---|
| | Hypo+(n = 6) | Hypo-(n = 10) | Hypo+(n = 6) | Hypo-(n = 29) |
| Pre dialysis sBP, mmHg | 115.8±16.3 | 121.7±20.5 | 119.3±23.4 | 133.4±23.7 |
| UF rate, l/h | 0.9±0.3 | 0.9±0.3 | 0.5±0.3 | 0.6±0.2 |
| Hemoglobin, g/dl | 12.4±1.2 | 12.1±1.5 | 10.0±0.8 | 11.7±1.5* |
| Delta ECW Legs, ml | -140 (-670, 585) | -420 (-745, -285) | -690 (-970, -20) | -660 (-985, -360) |
| ECW/TBW leg, geometric mean | 11.8 | 9.4 | 2.8 | 15.1* |

UF, ultrafiltration; sBP, systolic blood pressure; *p<0.005

## FR-PO337

**Health Related Quality of Life (HRQoL) in Community House Hemodialysis (CHHD) versus Home Hemodialysis (Home HD)** Mark R. Marshall,[1] Sharen K. Supershad.[2] [1]*Renal Medicine, Counties Manukau DHB, Auckland, New Zealand;* [2]*Renal Medicine, Northland DHB, Whangarei, New Zealand.*

**Background:** CHHD is a novel way of increasing uptake of home HD for patients who have inadequate housing and/or other social circumstances. Community houses are unstaffed, and provided through a collaboration with a local patient support group. The houses provide pleasant and home-like settings. Patients share an HD machine with one other and organize their own rosters. Previously, we have shown comparable patient survival between CHHD vs. home HD (Marshall et al, AJKD. 2013;61:598-607). In this abstract, we explore whether HRQoL is also comparable.

**Methods:** All prevalent patients on CHHD or home HD in our service were invited to complete an anonymous KDQoL survey. This was done twice, firstly on 1/1/2007 and then on 1/1/2013. We compared HRQoL between CHHD vs. home HD patients, adjusting for mean vintage and age-and-albumin-adjusted Charlson comorbidity index (CCI, Beddhu et al, Am J Med 2000;108:609-13) as ecological variables.

**Results:** Results are presented as n, or means (SD). Data are pooled across era for analyses. The source population are those who received a survey, the study population those who returned it.

| Parameter (population) | CHHD 2007 | Home HD 2007 | CHHD 2013 | Home HD 2013 |
|---|---|---|---|---|
| n (source) | 15 | 40 | 21 | 54 |
| Male (source) | 9 | 30 | 11 | 33 |
| CCI (source) | 3.5 (1.6) | 4.7 (1.8) | 4.2 (2.5) | 5.2 (2.0) |
| Age, years (source) | 39.6 (12.8) | 50.9 (11.8) | 45.6 (10.9) | 49 (13.3) |
| Vintage, months (source) | 66.4 (35) | 60.2 (41.4) | 79.8 (72.3) | 81.7 (70.7) |
| n (study) | 14 | 31 | 17 | 26 |
| Effects of kidney disease (study) | 55.4 (25) | 63.5 (25.4) | 56.4 (19.2) | 59.5 (22.1) |
| Burden of kidney disease (study) | 40.6 (31.1) | 39.9 (32.8) | 36.4 (22.6) | 49.3 (32.4) |
| SF-12 Physical Composite (study) | 46.2 (9.5) | 38.8 (11.3) | 44.1 (9.4) | 41.3 (10.3) |
| SF-12 Mental Composite (study) | 51.7 (7.0) | 47.5 (10.6) | 45.7 (9.0) | 48.9 (9.3) |

Using Wilcoxon rank-sum testing, HRQoL domains were not significantly different between CHHD vs. home HD, other than SF-12 Physical Composite which was higher for CHHD (P=0.04). Adjusting for vintage and CCI by regression, there were no significant differences in any HRQoL domain between CHHD vs. home HD.

**Conclusions:** Acknowledging small sample, HRQoL is comparable between CHHD and home HD.

*Funding:* Government Support - Non-U.S.

**FR-PO338**

**Procedure-Related Serious Adverse Events among Home Hemodialysis Patients–A Quality Assurance Perspective** Ben C. Wong,[1] Deborah Lynn Zimmerman,[2] Frances D. Reintjes,[3] Mark J. Courtney,[1] Scott Klarenbach,[1] Graeme Dowling,[4] Robert P. Pauly.[1] *[1]Div of Nephrology and Transplant Immunology, Univ of Alberta, Edmonton, Canada; [2]Div of Nephrology, Univ of Ottawa, Ottawa, Canada; [3]Northern Alberta Renal Program, Alberta Health Services, Edmonton, Canada; [4]Office of the Chief Medical Examiner, Edmonton, Canada.*

**Background:** There has been resurgent interest in home hemodialysis (HD) in recent years because of the reported benefits and its excellent safety record. However, the potential for adverse events, including potentially catastrophic events, exists when patients are performing HD in their homes without supervision. There is a lack of literature on this important topic.

**Methods:** We present the experience of two adult home HD programs in Canada from 2001 to 2012, including a total of 190 patients and approximately 500 patient-years of treatments. We retrospectively reviewed all life-threatening adverse events occurring in our programs and reexamined our approach to patient training, re-training, and safety monitoring.

**Results:** We report 1 death and 6 potentially fatal adverse events yielding a crude rate of 0.060 events per 1000 dialysis treatments. Six of 7 events involved significant blood loss (including 1 exsanguination); 5 of 7 events involved human error with lapses in protocol adherence. Because such events are rare, evaluation of specific intervention strategies will require much longer follow-up.

**Conclusions:** Life-threatening adverse events in home HD are uncommon, but when one does occur, should prompt review of home HD-related policies and procedures to make this therapy even safer.

**FR-PO339**

**Cost Evaluation of In-Center Nocturnal Hemodialysis** Ben C. Wong, Robert P. Pauly, Mark J. Courtney, Scott Klarenbach. *Div of Nephrology and Transplant Immunology, Univ of Alberta, Edmonton, Canada.*

**Background:** There is interest in providing in-center nocturnal hemodialysis (ICNHD), typically conducted overnight (6-8 hours) thrice weekly. In addition to putative health benefits, this allows a dialysis unit to offer two additional shifts on alternate nights. We conducted a costing study to determine the incremental costs of ICNHD compared with in-center thrice-weekly conventional hemodialysis (CHD) from the health care perspective using data from our program that provides both ICNHD and CHD.

**Methods:** Using micro-costings methods, we identified health care resources that differ between INCHD and CHD, including staffing, dialysis materials, and utilities. The units of resource consumed for each strategy was determined, and the cost of each unit was determined from administrative data (CAN $ 2012) to determine incremental costs of ICNHD and CHD. Alternate scenarios examining nursing grade and ratio, full care (reference case being a 1:3 staft-to-patient ratio) vs. self-care (with attendant training costs), and differences in medication costs were examined.

**Results:** In the reference case, CHD was $54.89 less costly per dialysis treatment compared with ICNHD, with a difference of $8562.84 per HD patient per year, with staffing costs accounting for >80% of the difference. Incremental annual costs for staffing, dialysis materials, and utilities were $7225.92, $1193.4, and $143.52, respectively. Fully independent ICNHD, which utilizes a staff-to-patient ratio of 1:10, results in to an annual saving $15,762.24 for ICNHD patients relative to CHD patients, including patient training costs of $17821 in the first year. If previously described reduction in medication use (erythropoietin-stimulating agents, anti-hypertensives, phosphate binders) occurs, ICNHD is $5126.04 more costly than CHD.

**Conclusions:** Compared to CHD, provision of ICNHD is more expensive, largely driven by increased staffing costs. Alternate staffing models, including fully independent ICNHD, are more attractive. The incremental costs of ICNHD should be balanced with potential improvements in patient outcomes and employment, as well as capital costs associated with creating more CHD capacity if required.

**FR-PO340**

**High Dose Home Hemodialysis and Conventional In-Center Hemodialysis: A Cost Utility Analysis** Frank Xiaoqing Liu,[1] Catrin Treharne,[2] Bruce F. Culleton,[1] Murat Arici,[3] Lydia Lees.[2] *[1]Baxter Healthcare Corporation, Deerfield, IL; [2]Abacus International, Oxfordshire, United Kingdom; [3]Baxter Healthcare Ltd., Compton, United Kingdom.*

**Background:** A cost-effectiveness analysis of home hemodialysis (HD) vs. in-center HD (ICHD) from the National Health Service (NHS) Center for Evidence-based Purchasing showed that Home HD was associated with lower costs and better outcomes than ICHD. The evaluation did not address increased dialysis frequency and/or duration. We performed an analysis to estimate the cost-effectiveness of high dose (higher frequency and/or longer duration) Home HD vs. ICHD.

**Methods:** An Excel-based Markov model was constructed to compare costs and quality adjusted life years (QALYs) associated with high dose Home HD and ICHD using current dialysis reimbursement in England and various hypothetical Home HD reimbursement levels. We modeled the incident adult dialysis population over a 10-year horizon from a payer's perspective. Model inputs included published articles, UK Renal Registry annual reports, NHS Payment-by-Results, and 2010 ERA-EDTA registry report. Future costs and benefits were discounted at an annual rate of 3.5%. One-way and probabilistic sensitivity analyses explored the robustness of the results.

**Results:** Under the current reimbursement (≤458/week, irrespective of the number of HD sessions/week), high dose Home HD is associated with lower total costs (≤8,504 less per patient) and higher QALYs (0.835 higher per patient) vs. ICHD over 10 years. High dose Home HD remained more effective and less costly over ICHD in all sensitivity analyses. High dose Home HD was cost-effective up to a weekly tariff of ≤620. Assuming that the Home HD tariff was equal to 5 times the 2010 reference cost of ≤115/session (i.e., ≤575/week), the incremental cost-effectiveness ratio of high dose Home HD would be ≤12,020/QALY. At this tariff (i.e., ≤575/week), high dose Home HD has an 84-92% probability of being cost-effective at a willingness-to-pay threshold of ≤20,000-≤30,000/QALY.

**Conclusions:** In England, high dose Home HD is cost-effective vs. ICHD, even if the tariff is as high as ≤620/week.

*Funding:* Pharmaceutical Company Support - Baxter Healthcare Corporation

**FR-PO341**

**High Dose In-Center Haemodialysis and Conventional In-Center Haemodialysis: A Head-to-Head Cost-Effectiveness Analysis** Frank Xiaoqing Liu,[1] Catrin Treharne,[2] Lydia Lees,[2] Murat Arici,[3] Bruce F. Culleton.[1] *[1]Baxter Healthcare Corporation, Deerfield, IL; [2]Abacus International, Oxfordshire, United Kingdom; [3]Baxter Healthcare Ltd., Compton, United Kingdom.*

**Background:** High dose haemodialysis (HD) (i.e., short daily HD or frequent nocturnal HD) improves clinical and humanistic outcomes. However, depending upon the location of therapy, it may be associated with higher costs. This study aimed to evaluate the cost-effectiveness of high dose in-centre HD (ICHD) versus conventional ICHD.

**Methods:** An Excel-based Markov model was constructed to estimate the costs and quality adjusted life years (QALYs) associated with conventional and high dose ICHD from the payer's perspective in England. We modeled the incident dialysis patient population over a 10-year time horizon. A scenario where all patients start on conventional ICHD (3 sessions/week) was compared with 3 scenarios where all patients start on high dose ICHD (3.5, 4, or 5 sessions/week). Model inputs were from published articles, annual UK Renal Registry reports, NHS Payment-by-Results, and ERA-EDTA registry report. All future costs and benefits were discounted to their present value at an annual rate of 3.5%. One-way sensitivity and probabilistic sensitivity analyses were conducted to test the robustness of the results.

**Results:** Starting all patients on high dose ICHD is associated with higher costs (an increase of ≤40,542, ≤54,115, ≤81,259 per patient for 3.5, 4, or 5 sessions per week respectively) and higher QALYs (an increase of 0.464 per patient) versus conventional ICHD over 10 years. This gives an incremental cost-effectiveness ratio (ICER) of ≤87,293, ≤116,516, ≤174,961 per QALY for the 3.5, 4, & 5 sessions/week scenarios, indicating that high dose ICHD is not cost-effective vs. conventional ICHD at UK NICE's willingness-to-pay threshold of ≤20,000-≤30,000. Sensitivity analyses showed consistent results.

**Conclusions:** Our analysis indicates that high dose HD, while being more effective than conventional HD, is not cost-effective when performed in-center based on the current UK NICE guideline.

**FR-PO342**

**Effects of Randomization to Frequent In-Center Hemodialysis on Long-Term Mortality: Frequent Hemodialysis Daily Trial** Glenn M. Chertow,[1] Nathan W. Levin,[2] Gerald J. Beck,[3] Paul W. Eggers,[4] Tom Greene,[5] Brett Larive,[3] Michael V. Rocco,[6] Alan S. Kliger,[7] The FHN Trial Group.[4] *[1]Stanford; [2]Renal Research Institute; [3]Cleveland Clinic; [4]NIDDK; [5]U Utah; [6]Wake Forest; [7]Yale.*

**Background:** The FHN Daily Trial randomized 245 subjects to "daily" (6 times per week) versus conventional (3 times per week) in-center hemodialysis (HD) for 1 year. Daily in-center HD resulted in favorable effects on the co-primary composite outcomes of death or change in left ventricular mass and death or change in self-reported physical health, and facilitated control of hypertension and hyperphosphatemia. Here we describe effects on mortality over a mean follow-up of 3.7 years.

**Methods:** We obtained dates of deaths and transplants through July 2011 using linkage to the USRDS and queries to study centers. Long-term mortality was related to the initial randomized treatment assignment using Kaplan-Meier product limit estimates and Cox regression, without censoring transplants as the primary analysis.

**Results:** Most (90/100) surviving non-transplanted subjects randomized to daily HD reverted to 3 or 4 times per week HD within 2 months after the trial period. Numbers of deaths (deaths/patient-year) among subjects randomized to the frequent and conventional groups, respectively, were 5 (0.041) and 10 (0.087) during the 1-year intervention period, 4 (0.034) and 6 (0.057) during the first year after the trial, and 12 (0.050) and 19 (0.089) subsequently. The overall relative hazard of mortality (daily versus conventional) was 0.54, 95% CI (0.32, 0.93), p=0.024; after censoring transplants the relative hazard was slightly attenuated: 0.60, 95% CI (0.34, 1.05), p=0.07.

**Conclusions:** The current results suggest that frequent in-center HD may reduce mortality long-term. Any benefits of frequent in-center HD must be balanced against potential risks including possible adverse effects on the vascular access, and the increased burden and cost of therapy. These results should be interpreted cautiously, as most patients randomized to daily HD reverted to conventional HD after the 1-year intervention, and statistical power was limited by relatively few (56) deaths.

*Funding:* NIDDK Support, Other U.S. Government Support, Private Foundation Support

## FR-PO343

**The Impact of Home Haemodialysis on Facility-Based Growth and Total Program Costs in an Australian Dialysis Service** Hasan Falih Alkhayyat, John W. MacD. Agar, Alwie Tjipto. *Renal Unit, Barwon Health, Geelong, Victoria, Australia.*

**Background:** In Australia, as elsewhere, the ever-rising dialysis population is placing increasing strain on an already stretched healthcare system. Yet, Australian home haemodialysis (HHD) has been shown to consume fewer resources, provide better patient outcomes and cost ~AUD $25,000/patient/year less than facility haemodialysis (FHD), HHD patients still comprise only a small fraction of most Australian dialysis units.

We report how the introduction of a home nocturnal haemodialysis (HNHD) program into a single dialysis service has influenced FHD distribution patterns over a 12-year follow-up period.

**Methods:** We have retrospectively reviewed (Jan 2000 - Feb 2013) the HD modality patterns (FHD vs. HNHD) in our renal service. While peritoneal dialysis (PD) is also actively provided, PD numbers have remained stable at 15% of all dialysis throughout the study period and have therefore been excluded from further analysis. In addition, we have the assessed the global program cost savings compared to the notional costs that would have accrued had all patients on HNHD remained on FHD over the study period.

**Results:** Over the 12 years, the total HD population increased (69 to 138) but is predominantly accounted for by an increase in HNHD (3 to 36). There was a relatively smaller proportional rise in FHD (66 to 102). All increase in FHD was confined to the first 3 years (2000 to 2003) while the HNHD program was being established. After 2003, FHD numbers have not altered significantly while HNHD has continued to grow. Furthermore, the HNHD program has notionally saved AUD $6.33 million against the global costs that would have accrued had all HNHD patients been started on and stayed within the FHD program over the full 12 year period. The calculated savings = the mean HNHD program pts/yr (21.1 pts) taken over 12 years @AUD $25,000 less/HNHD pt/year than the equivalent FHD cost.

**Conclusions:** Our HNHD cohort now makes up 26.1% of our total HD population. Most HD program growth has been delivered by HNHD since 2003. HNHD has resulted in significantly lower healthcare costs while synchronously maintaining a high quality of care.

## FR-PO344

**Is Progression of Arteriosclerosis in ESRD Patients Inhibited by Nocturnal Hemodialysis or Renal Transplantation? Baseline Results from the NOCTX Study** Franka E. Van Reekum,[1] Marianne C. Verhaar,[1] Petrus F. Vos,[2] Akin Ozyilmaz,[4] Brigit C. Van Jaarsveld.[3] *[1]Nephrology, Univ Medical Center, Utrecht, Netherlands; [2]Dianet Dialysis Center, Utrecht, Netherlands; [3]Nephrology, VU Medical Center, Amsterdam, Netherlands; [4]Dialysis Center Groningen, Groningen, Netherlands.*

**Background:** Coronary artery calcification (CAC) is associated with cardiovascular morbidity and mortality in patients with ESRD. Improvement of calcium-phosphate homeostasis and uremia by nocturnal HD or kidney transplantation might favorably influence CAC compared to conventional HD and PD. The ultimate goal of this study is to assess whether nocturnal HD and transplantation are associated with less progression of CAC, compared with conventional HD and PD. Here we present our baseline data of CAC.

**Methods:** CAC score (CACS) will be measured with multislice CT of the heart in 4 different groups (4x40 pts): patients treated with conventional HD (3-4x/week, 3-4 hr), PD, incident nocturnal HD (5-7x/week, 6-8 hr) and kidney transplant after dialysis. CAC is expressed as Agatston score and is measured at baseline and repeated after 1, 2 and 3 years of treatment. Apart from clinical characteristics and biochemistry, we will evaluate inflammation markers and serum calcification inhibitors.

**Results:** So far, 93 patients underwent multi slice CT, mean age was 49.1±13.1 yr, 60.2% males. Mean duration of RRT (excluding periods with transplant) was 35.5±28.9 months. Two thirds of our patients had considerable coronary calcifications (31% CACS 0, 36% CACS 1-400, 32% CACS > 400). CACS was significantly associated with age, and not with duration of dialysis treatment, presence of DM, levels of cholesterol, phosphate, albumin or CRP. We included 31 HD, 16 PD, 28 nocturnal HD and 18 kidney transplant patients.

**Conclusions:** There is significant coronary calcification in this Dutch cohort of patients treated with renal replacement therapy. Despite the long duration of dialysis treatment there was no association between duration of dialysis and CACS. We confirm the relation of coronary calcification with age as mentioned in literature. The progression of calcification with the different treatment modalities will be followed up in the NOCTX study.

*Funding:* Pharmaceutical Company Support - Novartis, Amgen, Baxter, Shire, Fresenius, Roche, Private Foundation Support

## FR-PO345

**Effects of Randomization to Frequent Nocturnal Hemodialysis on Long-Term Mortality: Frequent Hemodialysis Network Nocturnal Trial** Michael V. Rocco, John T. Daugirdas, Tom Greene, Robert S. Lockridge, Andreas Pierratos, Brett Larive, Gerald J. Beck, Alan S. Kliger, The FHN Trial Group. *NIH, NIDDK, Bethesda, MD.*

**Background:** The Frequent Hemodialysis Network (FHN) Nocturnal trial randomized 87 subjects to either 6 times per week home nocturnal hemodialysis (NHD) or to 3 times per week home hemodialysis (CHD) for 1 year. We describe long term effects on mortality over an average follow-up of 3.7 years.

**Methods:** Dates of deaths and transplants were obtained through July 2011 using linkage to USRDS and queries of study centers. Long-term mortality was related to initial randomized treatment assignment using log-rank tests and Cox regression, without censoring transplants in the primary analysis.

**Results:** 64% vs 0%, 54% vs 24%, and 45% vs 30% of patients randomized to NHD vs CHD respectively received ≥27 hours of dialysis over ≥4.5 treatments per week during the trial, the first year after the trial, and subsequently. Numbers of deaths (deaths/patient-year) were 2/45.1 (0.047) and 1/42.6 (0.023) in the NHD and CHD groups during the intervention period, 9/37.0 (0.243) and 1/39.3 (0.025) during the first year after the trial, and 3/69.4 (0.043) and 3/85.2 (0.035) subsequently. The overall mortality hazard ratio (HR) for randomization to NHD vs CHD was 3.82, 95% CI (1.26, 11.63), p = 0.011. A similar HR was obtained after censoring transplants; HR 5.84, 95% CI (1.67, 20.40), p = 0.002.

**Conclusions:** These extended follow-up results raise the possibility that CHD may lead to lower mortality than NHD. However, some caution in interpretation is warranted because the mortality rate for patients randomized to the CHD comparison group was surprisingly low (0.03 per patient-year), a substantial portion of patients randomized to conventional home dialysis switched to a frequent hemodialysis regimen after the trial without incurring an observable increase in mortality, and because statistical power was limited by the number of patients randomized (87) and the relatively few (19) deaths.

*Funding:* NIDDK Support

## FR-PO346

**Intensive Hemodialysis Is Associated with Improved Pregnancy Outcomes: A Canadian and United States Comparison** Michelle A. Hladunewich,[1] Susan H. Hou,[2] Ayodele Odutayo,[1] Tom Cornelis,[3] Andreas Pierratos,[1] Johannes Keunen,[4] Dini Hui,[4] Christopher T. Chan.[1] *[1]Medicine, Nephrology, Univ of Toronto, Toronto, Canada; [2]Medicine, Nephrology, Loyola Univ Medical Center, Maywood, IL; [3]Medicine, Nephrology, Maastricht Univ Medical Center, Maastricht, Netherlands; [4]Obstetrics and Gynecology, Univ of Toronto, Toronto, Canada.*

**Background:** Pregnancy is rare in women with end stage renal disease (ESRD), and when it occurs is often accompanied by significant maternal and fetal morbidity, and even mortality. Preliminary data from the Toronto Nocturnal Dialysis Program suggested that increased clearance of uremic toxins by the provision of intensified hemodialysis improves pregnancy outcomes, but small numbers and absence of a comparator group limited widespread applicability of these findings.

**Methods:** We compared pregnancy outcomes from the *Toronto Pregnancy and Kidney Disease (PreKid) Clinic and Registry* to the *American Registry for Pregnancy in Dialysis Patients (ARPD)*. The primary outcome was the live birth rate.

**Results:** The live birth rate was significantly higher in the Canadian as compared to the American cohort at 85.7 compared to 61.4% (p=0.038). Among established ESRD patients, the duration of pregnancy was longer in the more intensively dialyzed *PreKid* cohort at 36 (32-37) as compared to 27 (24-32) weeks in the *ARPD* patients (p=0.001) along with a trend towards higher birth weights. Further, a dose response between dialysis intensity and pregnancy outcomes emerged with live birth rates of 48% in women receiving < 20 hours of hemodialysis, 73% in women receiving between 21 and 36 hours of hemodialysis, and finally, 85% in women dialyzed in excess of 36 hours a week (p=0.027). Pregnancy complications were few and overall manageable.

**Conclusions:** We conclude pregnancy is safe and feasible in women with ESRD receiving intensive hemodialysis.

## FR-PO347

**Conversion to In-Centre Nocturnal Hemodialysis Was Associated with Regression of Left Ventricular Mass** Ron Wald,[1] Marc B. Goldstein,[1] Ziv Harel,[1] Jeffrey Perl,[1] Niki Dacouris,[1] Darren A. Yuen,[1] Myles S. Wolf,[3] Andrew T. Yan.[4] *[1]Nephrology, St. Michael's Hospital, Toronto, Canada; [2]Nephrology, St. Paul's Hospital, Vancouver, Canada; [3]Nephrology, Univ of Miami, Miami, FL; [4]Cardiology, St. Michael's Hospital, Toronto, Canada.*

**Background:** There are limited prospective data on the cardiovascular effects of in-centre nocturnal hemodialysis (INHD), especially with the use of cardiac magnetic resonance (CMR), the current reference standard for evaluating cardiac effects of intensified dialysis schedules.

**Methods:** We conducted a prospective, 2-centre observational study of 67 prevalent recipients of conventional hemodialysis (CHD, 4 h/session, 3x/week) of whom 37 converted to in-centre nocturnal hemodialysis (INHD, 7-8 h/session, 3 x/week in the outpatient dialysis unit overnight) while 30 remained on CHD. Conversion to INHD was guided by patient preference and/or physician recommendation. CMR performed at study entry and after 1 year were independently analyzed. The outcome was the change in left ventricular mass (LVM). The primary analysis evaluated patients by the dialysis regimen chosen at enrollment; a secondary as-treated analysis reflected the predominant regimen actually used over 1 year.

**Results:** 57 (23 CHD, 34 INHD) patients had CMRs at baseline and end-of-study. As compared to patients who remained on CHD, INHD was associated with a 16.8 (95 % CI, 1.4-32.2) g reduction in LVM after 1 year (p=0.03). In the as-treated analysis, INHD was associated with a decline in LVM of 18.5 g (p=0.02).

| | CHD (n=23) | INHD (n=34) | p-value |
|---|---|---|---|
| Age,y | 51 ± 12 | 57 ± 11 | 0.07 |
| Female (%) | 39 | 44 | 0.71 |
| DM as cause of ESRD (%) | 17 | 47 | 0.02 |
| Median time on dialysis pre-enrollment, months | 51 (23, 102) | 21 (10, 56) | 0.13 |
| Mean baseline systolic BP, mmHg | 138 ± 11 | 146 ± 17 | 0.02 |
| Mean LVM, g | | | |
| Baseline | 118 ± 32 | 139 ± 42 | 0.05 |
| One-year | 126 ± 33 | 131 ± 35 | 0.65 |

**Conclusions:** Compared to continuation of CHD, conversion to INHD was associated with LVM regression at 1 year. The magnitude of LVM regression observed with INHD was comparable to the effects of short-daily and nocturnal hemodialysis in clinical trials.

*Funding:* Government Support - Non-U.S.

## FR-PO348

**Risk of Hospitalization with Home Daily Dialysis Using Slow Dialysate Flow Rates** Rita Suri,[1] Lihua Li,[2] Gihad E. Nesrallah.[3] *[1]Nephrology, CHUM, Univ of Montreal, Montreal, Canada; [2]Kidney Clinical Research Unit, Western Univ, London, Canada; [3]Nephrology, Humber River Regional Hospital, Toronto, Canada.*

**Background:** Daily hemodialysis with slow dialysate flow rates ($DHD_S$) is an emerging home treatment option for end-stage renal disease. Comparative effectiveness studies of home $DHD_S$ and peritoneal dialysis (PD) are needed to help inform patient choice between these therapies.

**Methods:** We identified 2273 consecutive patients prescribed $DHD_S$ (NxStage SystemOne in US DaVita facilitates between Jan 2004 and Dec 2009. All patients received HD ≥5 days/week for ≥4 hours/day for at least 1 month. We matched 1942 of these $DHD_S$ patients by propensity scores to 5103 contemporaneous patients receiving home PD registered in the USRDS. Comorbidities, demographics, hospitalizations, deaths, transplants, and losses to follow-up for both groups were ascertained from the USRDS. Hospitalizations were categorized using the same definitions as in the USRDS 2012 data report. We used an Andersen-Gill model to compare hospitalization rates due to the composite of the pre-specified diagnostic categories of: cardiovascular, infectious, access-related, or bleeding. The single diagnostic categories were specified as secondary outcomes.

**Results:** After matching, between-group standardized differences for all baseline characteristics were <10%. Mean age was 51 years, 32% were male, 73% were white, and 27% had diabetes. Arteriovenous fistulae were present in 64% of $DHD_S$ patients. During 11,949 patient-years, 3952/7045 patients had 11,972 hospitalizations. The composite hospitalization rate was significantly lower for patients receiving $DHD_S$ than those receiving PD ($DHD_S$: 0.69/patient-year, PD: 1.09/patient-year; HR 0.62 (95% CI 0.58-0.66); p< 0.001). The number of days spent in hospital per patient-year was 4.1 with HD and 7.5 with PD (p<0.001). Overall, 61% of $DHD_S$ vs. 37% of PD patients remained admission-free for the pre-specified diagnoses (p=<0.001). Analyses of secondary outcome yielded similar results.

**Conclusions:** Compared to PD, $DHD_S$ was associated with fewer admissions and days spent in hospital. These results may have implications for those considering home-based renal replacement therapy options.

*Funding:* Pharmaceutical Company Support - Baxter Extramural Grant Program

## FR-PO349

**Hemodynamic Profile of Patients with Left Ventricular Assist Device during Hemodialysis** Jessica McAbee, Christopher J. Areephanthu, Bennet J. George, B. Peter Sawaya. *Internal Medicine, Div of Nephrology, Bone and Mineral Metabolism, Univ of Kentucky, Lexington, KY.*

**Background:** Left ventricular assist device (LVAD) has emerged as a common treatment for patients with advanced heart failure. It is not uncommon for these patients to suffer from renal failure requiring renal replacement therapy. However, due to potential hemodynamic instability, hemodialysis (HD) has been performed mainly on an inpatient basis with intensive nursing involvement and high medical cost. The purpose of this study is to retrospectively assess hemodynamic parameters and ability to complete the prescribed HD treatment in a series of patients who underwent numerous HD sessions.

**Methods:** Ten patients with Heart Mate II LVAD received 182 intermittent HD treatments. The following parameters were retrospectively evaluated: vital signs, ultrafiltrate (UF) removed, HD duration, symptoms, adverse events and interventions during each HD session. Hemodynamically significant events were defined as systolic blood pressure (SBP, mmHg) < 80, lowest SBP > 80 with a drop > 20 mmHg from baseline, or inability to register a blood pressure using regular sphygmomanometer due to LVAD-induced reduction in pulse pressure.

**Results:** Pre- and post-HD (mean ± SD): SBP (mmHg) 96 ± 17 and 95 ± 20; heart rate (HR, bpm) 86 ± 17 and 88 ± 20, respectively. The mean lowest SBP during HD was 83 ± 19 mmHg (p < 0.001 vs. pre-SBP). The prescribed and achieved (mean ± SD): UF (liters) 2.6 ± 1.8 and 2.7 ± 1.9 (NS); HD duration (hours) 3.3 ± 0.5 and 3.3 ± 0.6 (NS), respectively. Hemodynamic events, as defined above, occurred in the following frequency: SBP < 80 = 36%; SBP > 80 with a drop > 20 = 9% and inability to register BP = 35%. However, patients during these events were rarely symptomatic. When BP could not be registered, HR assessed by telemetry was 93 ± 19. Albumin was infused in only 16% of all the HD treatments and blood was transfused in less than 4%.

**Conclusions:** Patients with LVAD can often tolerate and complete the prescribed HD treatment. These patients are likely to tolerate outpatient in-center HD.

*Funding:* Other NIH Support - National Center for Advancing Translational Sciences, UL1TR000117, Clinical Revenue Support

## FR-PO350

**Abstract Withdrawn**

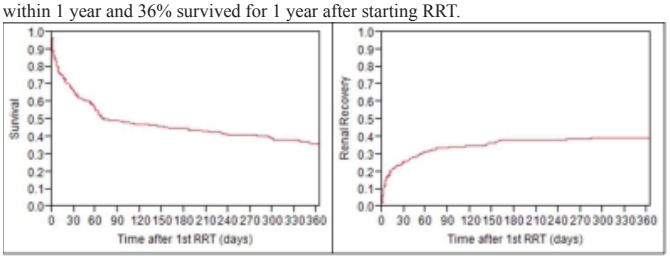
## FR-PO351

**Outcomes and Prognostic Factors for Patients Requiring Dialysis after Cardiac Surgery** Charat Thongprayoon,[2] Ishan Shah,[3] Kianoush Banaei-Kashani,[1] Rahul Kashyap,[4] Soon J. Park,[3] John J. Dillon.[1] *[1]Nephrology, Mayo Clinic; [2]Critical Care Medicine, Mayo Clinic; [3]Cardiac Surgery, Mayo Clinic; [4]Anesthesia, Mayo Clinic.*

**Background:** Acute kidney injury (AKI) requiring renal replacement therapy (RRT) may occur after cardiac surgery and is a risk factor for postoperative mortality. Long-term outcomes, particularly beyond hospital discharge, and their prognostic factors, have not been well studied.

**Methods:** This is a retrospective study of all adult patients requiring RRT for AKI within 30 days after cardiac surgery between Jan. 2007 and Dec. 2011. Patients receiving RRT preoperatively and patients receiving ventricular assist devices or cardiac transplants were excluded. The primary outcomes were all-cause mortality and renal recovery 1 year after starting RRT. We defined renal recovery as remaining alive and free of dialysis for 14 days. Outcomes were presented using Kaplan-Meier plots. Multivariate Cox proportional hazard analysis was used to assess factors associated with outcomes.

**Results:** 202 patients met the inclusion criteria. Of these, 39% recovered renal function within 1 year and 36% survived for 1 year after starting RRT.




Only 4% were both alive and requiring RRT at 1 year. In multivariate analysis, older age, heart failure, lower preoperative creatinine, longer interval between cardiac surgery and RRT start, mechanical ventilation and intra-aortic balloon pump use were independently associated with increased 1-year mortality, whereas vasopressor and extracorporeal membrane oxygenation use were independently associated with renal non-recovery within 1 year.

**Conclusions:** Mortality is high for patients requiring RRT after cardiac surgery, but few survivors require long-term dialysis. An unexpected mortality risk factor was lower preoperative creatinine. This may reflect greater injury among patients with lower preoperative creatinines who require RRT.

## FR-PO352

**Acid-Base (A-B) Changes in Acute Kidney Injury (AKI) Patients with Hypercapnic Respiratory Failure Treated with 24-Hour (h) Sustained Low Efficiency Dialysis (SLED) with Regional Citrate Anticoagulation (RCA)** Jian Li, Lenar T. Yessayan, Jerry Yee, Balazs Szamosfalvi. *Nephrology, Henry Ford Hospital, Detroit, MI.*

**Background:** Previous studies showed that hemodialysis induces transient $Pco_2$ elevation in ESRD patients, returning to baseline post therapy. Metabolic acidosis and alkalosis have been reported with RCA. Studies of A-B changes in patients with respiratory and metabolic acidosis on mechanical ventilation and 24-h SLED-RCA are lacking.

**Methods:** We conducted a retrospective chart review of 268 AKI patients on CRRT between Nov. 2009 and Oct. 2012. 32 had respiratory and metabolic acidosis. 26 had complete ABG data for over 72-h while on 24-h SLED-RCA with Qb 60ml/min, Qd 400 ml/min and Rexeed 15SX filter. Dialysate K was 4 mmol/L, Na was 140-142 mmol/L in all 26 patients, HCO3 was 32 mmol/L in 24 patients, 34 and 36 mmol/L in two. A-B parameters were collected at time(t)= 0, 24, 48 and 72-h (± 4h) on SLED-RCA. Pairwise comparison between any two time points was performed using paired t test. P-value <0.05 was considered statistically significant.

**Results:** Mean age:49.88±17.13 years, 50% male, mean serum creatinine at t=0-h: 4.18±2.16 mg/dL. A-B parameters pre/post SLED-RCA are shown in table.

| ABG | t=0 h | 24 h | 48 h | 72 h | p value |
|---|---|---|---|---|---|
| pH | 7.22±0.10 | 7.26±0.07 | 7.29±0.06 | 7.30±0.06 | 0.001 |
| $Pco_2$(mmHg) | 62.88±12.51 | 57.84±10.57 | 55.99±10.59 | 53.63±9.93 | 0.004 |
| $HCO_3$(mmol/L) | 25.28±6.77 | 25.12±2.83 | 25.59±2.22 | 25.13±3.00 | 0.47 |
| Minute Ventilation(L/Min) | 7.22±3.23 | 8.04±2.53 | 8.01±2.61 | 7.83±2.54 | 0.20 |
| Blood chemistry(mmol/L) | | | | | |
| Na | 139.81±8.28 | 139.58±3.82 | | | |
| K | 4.56±0.9 | 4.48±0.57 | | | |
| CL | 102.54±7.60 | 105.46±4.12 | | | 0.02 |
| $HCO3^-$ | 26.04±6.02 | 25.81±2.61 | | | 0.80 |
| Corrected Anion Gap | 16.96±3.00 | 13.85±3.26 | | | 0.003 |

**Conclusions:** Fixed dialysate bicarbonate delivery with SLED-RCA reduced or did not worsen pCO2; meanwhile it efficiently corrected high anion gap metabolic acidosis while approximating a normal serum bicarbonate concentration.

## FR-PO353

**Factors Associated with Dose of Intermittent Hemodialysis in Acute Kidney Injury** Gunyamol Thamniramol, Pongsathorn Gojaseni, Kolasorn Pakchotanon. *Medicine, Bhumibol Adulyadej Hospital, Bangkok, Thailand.*

**Background:** KDIGO guideline recommends delivering a Kt/V of 3.9 per week when using intermittent RRT in acute kidney injury. In Thailand, however, adequacy of hemodialysis in AKI patients is not routinely monitored. The aim of this study, therefore, was to assess the prescribed and delivered Kt/V and its associated factors in AKI patients underwent intermittent hemodialysis.

**Methods:** A cross-sectional study was performed in Bhumibol Adulyadej hospital, directorate of medical services, Royal Thai Air Force during January 2012 to January 2013. Prescribed Kt/V was calculated from in vitro dialyzer clearance and actual body weight while delivered Kt/V was calculated using natural logarithm formula. Patient clinical data and dialysis prescription were analyzed to determine their association with dialysis adequacy.

**Results:** Fifty-two AKI patients, who collectively underwent 248 dialysis treatments, were studied prospectively. Mean (± SD) age was 69.4 ± 16.9; 50% were male. At dialysis initiation, APACHE II score was 20.6 ± 6.2 and SOFA score 8.2 ± 3.6.Mean session length was 3.54 ± 0.81 h, and 78.9% used a femoral venous catheter. The results showed that the mean prescribed and delivered Kt/V were 1.28 ± 0.51 and 1.20 ± 0.58 respectively. Thirty-nine percent of the prescriptions were for a Kt/V less than 1.2 while 55.6% of treatments delivered a Kt/V less than 1.2. The independent determinants of adequate Kt/V (≥ 1.2) in a multiple logistic regression model were; blood flow rate ≥ 250 ml/min (odds ratio (OR) = 3.87, 95% confidence intervals (CI): 2.04-7.31), dialysis time ≥180 min (OR = 3.01, 95% CI: 1.03-8.81), female gender (OR = 2.80, 95% CI: 1.50-5.21), heparin used (OR = 2.45, 95% CI: 1.13-5.33) and actual body weight < 65 kg (OR = 2.38, 95% CI: 1.18-4.80).

**Conclusions:** More than half of patients with AKI are received an inadequate dose of dialysis. Barriers to adequate dose delivery were patient size, gender and inadequate dialysis prescription, i.e., blood flow rate, dialysis time and anticoagulant.

*Funding:* Government Support - Non-U.S.

## FR-PO354

**Inflammatory Cytokine Reductions with Sustained Low Efficiency Online Hemodiafiltration (Online SLED-f) Using High Cut-Off Dialyzer versus High-Flux Dialyzer in Sepsis-Related Acute Kidney Injury Patients** Khajohn Tiranathanagul, Jeeraluk Tunpornchai, Nattachai Srisawat, Wiwat Chancharoenthana, Asada Leelahavanichkul, Kearkiat Praditpornsilpa, Somchai Eiam-ong. *Div of Nephrology, Dept of Medicine, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn Univ, Thailand.*

**Background:** Hypercytokinemia plays a central role in the pathogenesis and is related to the high mortality in sepsis-related acute kidney injury (S-AKI). The reductions of these cytokines have been reported to improve clinical outcomes. Sustained low efficiency online hemodiafiltration (online SLED-f) using traditional high-flux (HF) dialyzer could remove some inflammatory cytokines. Interestingly, the potential of enhancing cytokine removal by using newly designed high cut-off (HCO) dialyzer that could theoretically remove larger solutes has never been studied in SLED-f before.

**Methods:** This prospective randomized trial was conducted in 15 S-AKI patients to compare the efficacy of cytokine removal including IL-6, IL-8, IL-10,TNF-α, and VEGF by six-hour SLED-f between using HCO dialyzer (HCO-SLED-f,n=8) and HF dialyzer (HF-SLED-f,n=7). The clinical parameters as well as the adverse effects including albumin loss were observed and compared.

**Results:** HCO-SLED-f provided significantly IL-8 and TNF-α reduction (p=0.012 for both) after treatment whereas HF-SLED-f could only demonstrate significantly TNF-α reduction (p=0.018). However, the degree of all cytokine reductions did not show significant difference between both treatment groups. There were significantly higher total albumin losses in effluent fluid in HCO-SLED-f group than HF-SLED-f group [4.72(range 3.5-5.8) vs. 0 (0-0.11) gram,p=0.010]. However, the percentage of plasma albumin reduction was not different between both groups (p=0.418). There were no significant differences in intra-dialytic blood pressure parameters during both treatments.

**Conclusions:** In S-AKI, HCO-SLED-f could be safely applied and could reduce more types of cytokines when compared with traditional HF-SLED-f despite the degree of reduction did not significant difference. However, the higher degree of albumin loss should be considered when using HCO-SLED-f.

## FR-PO355

**Sustained Low Efficiency Dialysis (SLED) versus Continuous Renal Replacement Therapy (CRRT) for AKI in Critically Ill Patients** Abhijat Kitchlu,[1] Karen E.A. Burns,[2] Jan O. Friedrich,[2] David Klein,[2] Robert M. Richardson,[1] Neill Adhikari,[2] Ron Wald.[1] *[1]Div of Nephrology, U. of Toronto; [2]Div of Critical Care, U. of Toronto, Toronto, Canada.*

**Background:** SLED, which reflects the use of conventional dialysis technology over an extended time, is increasingly used as a renal replacement modality in critically ill patients with AKI and hemodynamic instability. SLED may reduce the hemodynamic perturbations of IHD, while obviating the resource demands of CRRT. Although SLED is being increasingly used in the intensive care unit (ICU), few studies have evaluated its impact on clinical outcomes.

**Methods:** We conducted a cohort study comparing SLED (target 8 h/session, $Q_b$ 200 mL/min) to CRRT in four ICUs at an academic medical centre. The primary outcome was mortality 30 days after RRT initiation, adjusted for demographics, comorbidity, baseline kidney function, and Sequential Organ Failure Assessment (SOFA) score. Secondary outcomes were RRT dependence at 30 days and early clinical deterioration, defined as death or higher SOFA score at 48 hours.

**Results:** We identified 158 patients who initiated treatment with CRRT and 74 with SLED. Mortality at 30 days was 54% and 61% among SLED- and CRRT-treated patients, respectively [adjusted odds ratio (OR) 1.07, 95% CI 0.56–2.03, as compared with CRRT]. RRT dependence at 30 days (adjusted OR 1.36, 95% CI 0.51–3.57) and early clinical deterioration (adjusted OR 0.73, 95% CI 0.40–1.34) were similar between groups.

**Table 1: Baseline data**

| Variable | CRRT | SLED | p value |
|---|---|---|---|
| Patients | 158 | 74 | |
| Age at RRT initiation (mean±SD) | 62.1±5.3 | 60.6±17.3 | 0.50 |
| Male (%) | 59.5 | 67.6 | 0.25 |
| Mechanical ventilation (%) | 95.6 | 86.5 | 0.03 |
| Pre-morbid creatinine (mean±SD) | 130.6±91.7 | 135.3±83.8 | 0.57 |
| SOFA score at RRT initiation (mean±SD) | 16.4±3.08 | 15.4±3.65 | 0.03 |
| Urine output on day of RRT initiation, mL (mean±SD) | 328.8±580.5 | 387.6±607.0 | 0.48 |
| Vasopressors at RRT initiation (%) | 87.3 | 79.7 | 0.17 |

**Conclusions:** Notwithstanding the limitations of this small non-randomized study, our findings support similar clinical outcomes for patients treated with SLED vs CRRT. In hemodynamically unstable patients with AKI, trials to establish the non-inferiority of SLED are needed.

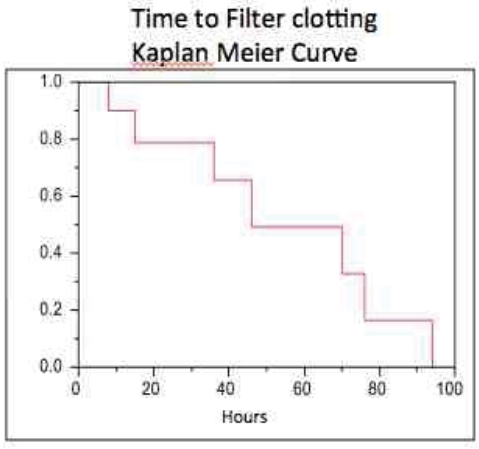
## FR-PO356

**Calcium Replacement Using Post Filter Replacement Fluid during Continuous Renal Replacement Therapy with Citrate Anticoagulation** Nithin Karakala,[1] John M. Arthur,[1] Ashita J. Tolwani.[2] *[1]Medicine, Nephrology, Medical Univ of South Carolina, Charleston, SC; [2]Medicine, Nephrology, Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** Continuous Renal Replacement Therapy (CRRT) is the choice of dialysis modality in hemodynamically unstable patients with Acute Kidney Injury (AKI). Anticoagulation is required to prevent filter clotting and is done by using regional citrate anticoagulation or systemic heparin. The recent national calcium shortage has caused major problems in using citrate anticoagulation protocols.

**Methods:** This is a retrospective analysis of data from 4 patients. Gambro Prismaflex machines, with HF 1000 filter sets were used. Commercially available PrismaSol BGK 4/2.5 (potassium 4 meq/L and calcium 2.5 mew/L) was used as replacement fluid. Acid Citrate Dextrose A(ACDA)was used as an anticoagulant. Calcium gluconate (6 grams in 250 ml of 0.9% normal saline) was used if the patients ionized calcium was less than 1 mmol/L.

**Results:** Patients were on CRRT for an average of 6±4.7 days, for a total of 24 days. During this period 4 filters clotted. The calculated Kaplan Meier Curve for time to filter clotting was 46 hours. The average delivered dose of dialysis was 39.66± 9.45 ml/kg/hr. The average systemic ionized calcium was 1.06±0.07 mmol/L. Calcium gluconate drip was needed in for a combined 13 days during this period with an average rate of 6.4±2.35 ml/hr.




**Conclusions:** Use of calcium containing post filter replacement fluid decreased the need for systemic calcium replacement during CRRT treatment. The above described protocol improved the filter patency and the delivered dose in patients who were not suitable for heparin anticoagulation and had filter clotting problem and maintained systemic calcium greater than 1 mmol/L.

## FR-PO357

**A Continuous Veno-Venous Hemofiltration Protocol with Anticoagulant Citrate Dextrose Formula A and a Calcium-Containing Replacement Fluid: An Easy Remedy for the National Calcium Shortage?** Song Ching Ong, Keith M. Wille, Ashita J. Tolwani. *Univ of Alabama at Birmingham.*

**Background:** Regional citrate anticoagulation (RCA) is used as an anticoagulant for continuous renal replacement therapy (CRRT). A systemic calcium ($Ca^{2+}$) infusion is required to replace the $Ca^{2+}$ lost in the effluent. The U.S. shortage of intravenous $Ca^{2+}$ has limited the use of RCA. We describe a continuous veno-venous hemofiltration (CVVH)

protocol with RCA using 2.2% anticoagulant citrate dextrose formula-A (ACDA) and a commercially available dialysate containing $Ca^{2+}$3.0 meq/L, $HCO_3^-$ 35 meq/L and $K^+$ 4 meq/L (NxStage) as post-filter replacement fluid (RF), without need for $Ca^{2+}$infusion.

**Methods:** We prospectively evaluated 5 patients on CRRT who had at least 3 episodes of clotting of the filter within 24 hours. Patients were switched to CVVH using ACD-A infused pre-blood pump with the Prismaflex device and titrated to achieve a post-filter ionized calcium ($iCa^{2+}$) level <0.5 mmol/L. The $Ca^{2+}$–containing dialysate was delivered post-filter as RF at rate 3.0 L/hr and titrated to maintain systemic $iCa^{2+}$ levels between 0.9-1.3mmol/L.

**Results:** Mean age was 57±14 years and mean APACHE II score was 33±3.2. Steady state mean serum chemistries were: $Na^+$: 140.8±2.3meq/L (range 136-145), $K^+$: 4.22±0.44meq/L (range 3.5–5.1) $HCO_3^-$: 30.9±3.7meq/L (range21–36), pH: 7.42 ± 0.07 (range 7.26-7.55), $CO_2$: 47.9 ± 8.3 (range 35–76), total $Ca^{2+}$: 8.08 ± 1.09 mg/dL (range 6.6–10.8). Post-filter $iCa^{2+}$ ranged 0.27-0.36mmol/L, and patient $iCa^{2+}$ ranged 0.81-1.24mmol/L. Mean post-filter RF rate: 3086±164ml/hr (range 3000-3600), mean ACDA rate: 298±21ml/hr (range 250-350). Mean blood flow rate: 200±17 ml/min (range 170–250), mean filtration fraction: 37±0.04% (range 28-48). Mean effluent flow rate: 38.6±6.7ml/kg/hr (range 28.7–55.8). Median filter survival was 24.5 hrs (95% C.I. 16.4-67.3).

**Conclusions:** CVVH using ACDA for RCA and a $Ca^{2+}$-containing RF without a continuous $Ca^{2+}$ infusion was safely and effectively used. Due to the high $HCO_3^-$ content of the RF, patients developed mild alkalemia. Despite high filtration fractions, filter survival was much improved.

## FR-PO358

### Relations of Volume Parameters in Continuous Renal Replacement Therapy

Harin Rhee,[1] Min Ji Shin,[1] Byeong Yun Yang,[1] Il Young Kim,[2] Dong Won Lee,[2] Soo Bong Lee,[2] Ihm Soo Kwak,[1] Eun Young Seong.[1] *[1]Dept of Internal Medicine, Pusan National Univ School of Medicine; [2]Dept of Internal Medicine, Pusan National Univ Yangsan Hospital.*

**Background:** In critically ill patients, acute kidney injury(AKI) is common and these patients often suffer from fluid overload. Once kidney failure occurs, renal replacement is initiated for volume overload, in this setting, successful volume management depends on an accurate assessment of fluid status. We investigated the relationship of volume parameters in AKI patients with continuous renal replacement therapy(CRRT).

**Methods:** Sixty AKI patients with CRRT ≥48 hours were enrolled. Volume status was determined by measuring central venous pressure(CVP), cardiothoracic ratio(CT ratio) in chest x-ray as traditional volume marker and extracellular water(ECW)/total body water(TBW) by bioimpedance analysis, absolute diameter of inferior vena cava(IVCd) and IVC collapsible index (IVCci) by ultrasound. In patients with CRRT ≥72 hours, follow-up volume parametes were measured and the difference between parameters at CRRT start and after 72 hours were calculated.

**Results:** Mean CVP,CT ratio,and ECW/TBW were 9.8±4.6mmHg,0.55±0.08,and 0.412±0.015. Mean IVCd and IVCci were 1.71±0.27cm and 31.8±16.1%. ECW/TBW was positively correlated with IVCd(r=0.604,p=0.001). IVCci was negatively correlated with CVP(r=-0.398,p=0.032). There was no correlation between ECW/TBW,CVP,CT ratio,and IVCci. The difference between parameters of volume status at CRRT start and after 72 hours were 3.7±6.5mmHg, 0.18±0.28, and 0.135±0.200 in CVP, CT ratio, and ECW/TBW. In IVCd and IVCci, the difference were 0.47±0.82cm, and 8.6±17.4%. ΔECW/TBW was positively correlated with ΔCVP(r=0.542,p=0.002), ΔCT ratio(r=0.705,p<0.001), and ΔIVCd(r=0.772,p<0.001). ΔIVCd was positively correlated with ΔCVP(r=0.565,p=0.001), ΔCT ratio(r=0.564,p=0.001), and ΔIVCci(r=0.560,p=0.001). In comparison of volume parametes according to hospital mortality, IVCd(1.82±0.29 vs 1.59±0.20,p=0.015), and IVCci(37.9±11.5 vs 25.2±18.1,p=0.036) was significantly higher in non-survivors.

**Conclusions:** IVCd, IVCci, and ECW/TBW was correlated with traditional volume parametes in CRRT patients.

## FR-PO359

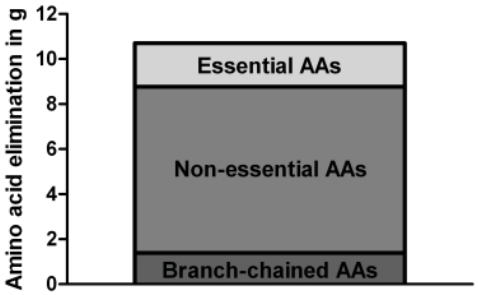
### Removal Characteristics of Glutamine and Other Amino Acids in Critically Ill Patients with Acute Kidney Injury Undergoing Extended Dialysis

Julius Schmidt, Carsten Hafer, Eva Schönenberger, Bernhard M.W. Schmidt, Jan T. Kielstein. *Dept of Internal Medicine, Div of Nephrology, Medical School Hannover, Germany.*

**Background:** Acute kidney injury in critically ill patients is associated with an activation of protein catabolism. Renal replacement therapy (RRT) with modern dialysis membranes may aggravate this problem by eliminating a substantial amount of amino acids (AAs).

**Methods:** Prospective, cross-over study in ten extended dialysis sessions using a polysulfone membrane (either EMiC2 or AV 1000S dialyzer, FMC, Germany). Blood samples for AAs were drawn before, during and after a 10 h treatment. In addition samples for calculation of dialyzer clearance and samples from the total spent dialysate were measured.

**Results:** Despite no significant difference in pre- and post -dialysis plasma AA levels we found an impressive amount of AAs in the collected spent dialysate of 10.48 g/treatment.




Dialyzer clearance is shown below.

| AA | Dialyzer clearance (ml/min) | Eliminated amount (g) |
|---|---|---|
| Lys | 86.61 | 0.74 |
| Met | 74.25 | 0.12 |
| Phe | 67.56 | 0.54 |
| Thr | 86.6 | 0.53 |
| Trp | 67.8 | 0.06 |
| Ala | 88.91 | 0.91 |
| Arg | 94.67 | 0.29 |
| Asp | 82.01 | 0.05 |
| Cys | 93.22 | 0.07 |
| Glu | 80.82 | 0.03 |
| Gln | 87.37 | 3.17 |
| Gly | 94.07 | 0.64 |
| His | 88.66 | 0.26 |
| Pro | 87.42 | 0.99 |
| Ser | 90.84 | 0.29 |
| Tyr | 72.84 | 0.52 |
| Ile | 69.85 | 0.27 |
| Leu | 69.76 | 0.44 |
| Val | 140.0 | 0.67 |
| Total AAs | | 10.48 |

**Conclusions:** Modern means of RRT eliminate AAs to an extent that has not been met by our nutritional support standards. Especially the removal of glutamine, important for immune function and cell regeneration, might have detrimental effects on the recovery of critically ill patients.

*Funding:* Pharmaceutical Company Support - Fresenius Medical Care, Germany, Clinical Revenue Support

## FR-PO360

### Recombinant Human Soluble Thrombomodulin Administration Improves Septic Shock Requiring Continuous Renal Replacement Therapy: A Retrospective Cohort Study

Naoki Kurashima,[1] Atsushi Ohkubo,[1] Naofumi Yui,[2] Tatemitsu Rai.[2] *[1]Dept of Clinical Engineering, Tokyo Medical and Dental Univ, Japan; [2]Dept of Nephrology, Tokyo Medical and Dental Univ.*

**Background:** Recombinant thrombomodulin (rTM) has emerged as a new treatment option for disseminated intravascular coagulation (DIC). In addition to its anticoagulant properties, rTM is considered to produce direct anti-inflammatory effect. The purpose of this study is to evaluate the efficacy of rTM treatment to critically ill patients with septic shock requiring continuous renal replacement therapy (CRRT).

**Methods:** A retrospective analysis of patients admitted to the intensive care unit of our hospital between June 2009 and April 2013 was performed. Patients diagnosed as septic shock requiring transient CRRT were included. They were either treated with rTM (treatment group) or without rTM (control group) during CRRT. The primary outcome was the decrease in DIC score on day 3 after initiation of CRRT. Secondary outcomes were changes in the severity of patients' conditions on day 3 as assessed by Acute Physiologic and Chronic Health Evaluation II (APACHE II) score and the period of time required for withdrawal from CRRT. Data were expressed as group means ± standard deviations. The Mann-Whitney test was used for comparison and a $p$ value under 0.05 was considered statistically significant.

**Results:** Six and nine patients were assigned to the treatment and control groups, respectively. Baseline characteristics, including DIC and APACHE scores, showed no significant differences between the two groups. A significant decrease in the DIC score was seen in the treatment group compared with that in the control group (1.1 ± 1.1 vs -1.4 ± 2.1, respectively, $p$ = 0.0184). Decrease in APACHE II score also showed significant improvement (treatment group 9.8 ± 2.9 vs control group 4.0 ± 5.8, $p$ = 0.0339). The period until CRRT withdrawal was not significantly different between the two groups (treatment group 4.5 ± 1.8 days vs control group 6.8 ± 3.1 days, $p$= 0.1407).

**Conclusions:** In critically ill patients with septic shock requiring CRRT, treatment with recombinant thrombomodulin may improve patients' severity and coagulation disorders.

*Funding:* Government Support - Non-U.S.

## FR-PO361

**Calcium and Phosphate Replacement during Continuous Renal Replacement Therapy with Regional Citrate Anticoagulation Predict Mortality in Critically Ill Patients**

Wee Kim Fong,[1] Markos George Kashiouris,[2] Venu Velagapudi,[2] Abbasali Akhoundi,[2] Kianoush Banaei-Kashani.[2]
*[1]Anesthesiology, Mayo Clinic, Rochester, MN; [2]Pulmonary and Critical Care Div, Mayo Clinic, Rochester, MN.*

**Background:** During the recovery phase, the regenerating cells consume phosphorus for cell cycle and growth. Therefore increase phosphorus requirement during CRRT may be in prognostication of critical illness recovery. On the other hand, increased calcium replacement requirement could indicate cell lysis . We hypothesized that the dosing of calcium and phosphate replacement during CRRT treatment, predicts hospital mortality.

**Methods:** We conducted a retrospective cohort study in eight intensive care units of a tertiary academic center in the United States. We collected demographic and laboratory data and constructed a database of critically ill patients who required CRRT with regional citrate anticoagulation, from December 2007 to January 2009. We performed logistic regression analysis to predict mortality, based on the amount of hourly calcium and phosphate replacement dose. We adjusted for severity of illness with the APACHE III score and comorbidities using the Charlson index score, independent of delivered dose of CRRT.

**Results:** We enrolled 595 patients into the study. The ICU and hospital mortality were 39.7% and 50.9%, respectively. The average APACHE III and Charlson score were 118 (95% CI 112.5 – 117) and 3.0 (95% CI 2.8 – 3.2), respectively. The median calcium and phosphorus replacement dose was 0.94 gr/h of CRRT (IQR 0.79 – 1.05) and 1.25 mMol/h of CRRT (IQR CI 0.8 – 1.8), respectively. In multivariate analysis, each additional incremental unit of the following was significantly associated with differential odds of death: APACHE III OR 1.014 (95% CI 1.007 – 1.02, p<0.01), calcium replacement dose OR 3.0 (95% CI 1.26 – 7.13, p=0.01) and phosphate replacement dose OR 0.67 (95% CI 0.52 – 0.86, p<0.01).

**Conclusions:** Increased calcium replacement and decreased phosphate replacement requirements during CRRT, were associated with increased mortality even after accounting for severity of illness and comorbid conditions.

*Funding:* Clinical Revenue Support

## FR-PO362

**Perspectives of Continuous Renal Replacement Therapy: A Paired Survey Study of Patients, Physicians, and Nurses**

Andrew Allegretti,[1] Gregory L. Hundemer,[1] Katherine M. Cosgrove,[3] Ednan Bajwa,[3] Ishir Bhan.[2] *[1]Medicine, Massachusetts General Hospital, Boston, MA; [2]Nephrology, Massachusetts General Hosptial, Boston, MA; [3]Critical Care, Massachusetts General Hospital, Boston, MA.*

**Background:** Recent data in suggests considerable discrepancies between patients and providers with respect assessment of hemodialysis prognosis, but such data is lacking for patients undergoing continuous renal replacement therapy (CRRT). We sought compare the prognostic assessment of patients/health care proxies (HCPs) and their providers (physicians and nurses).

**Methods:** A multiple-choice questionnaire was given to a triad of participants around an index case of CRRT. Each triad included (1) a patient (or HCP) on CRRT, (2) his/her resident or attending ICU physician, and (3) his/her ICU nurse administering CRRT. Questionnaires were performed in medical and cardiac ICUs at a single tertiary care center. We assessed knowledge of the probability of patient survival to hospital discharge and the probability of requiring lifelong dialysis if discharged.

**Results:** Twenty two survey triads were completed. All patients/HCPs (n = 22/22) correctly identified the purpose of CRRT. Fifty five percent of patients/HCPs (n = 12/22) incorrectly thought CRRT improved the speed of renal recovery. Probability of survival to hospital discharge (accepted quartile: 25-49%) was correctly identified by 5% of patients/HCPs (n = 1/22), 50% of physicians (n = 11/22), and 45% of nurses (n = 10/22). Both physicians (p = 0.001) and ICU nurses (p = 0.002) were more likely than patients/HCPs to assess survival accurately. Probability of requiring lifelong dialysis if discharged (accepted quartile: 0-24%) was correctly identified by 35% of patients/HCPs (n = 6/17), 23% of physicians (n = 5/22), and 36% of nurses (n = 8/22). Seventy three percent of patients/HCPs (n = 16/22) felt that their doctors "completely" explained the purpose of CRRT.

**Conclusions:** Understanding of CRRT and knowledge of prognosis is poor among patients/HCPs and ICU providers. Patients may overestimate their level of understanding of CRRT. Further intervention is needed to improve this knowledge gap for patients/HCPs, physicians, and ICU nurses.

## FR-PO363

**Hemodialysis Initiation Associates with Highest Stroke Risk in U.S. and European Populations**

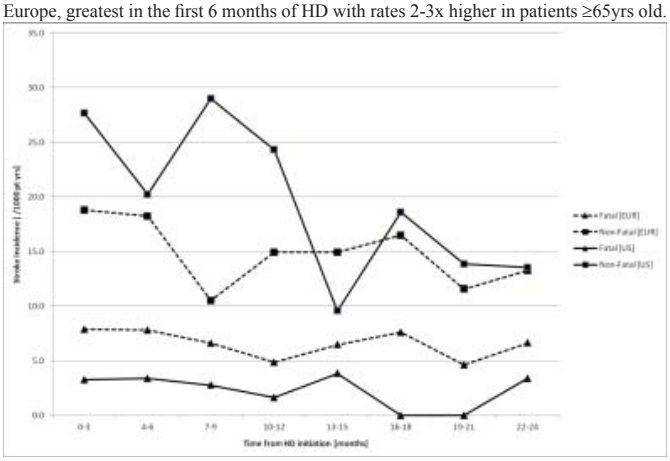
Albert J. Power,[1] Len A. Usvyat,[2] Daniele Marcelli,[3] Neill D. Duncan,[1] Charles D. Pusey,[1] Peter Kotanko,[2] Bernard Canaud,[3] Mondo Consortium.[4] *[1]Imperial College London, London, United Kingdom; [2]Renal Research Institute, New York, NY; [3]Fresenius Medical Care (FMC), Germany; [4]MONDO Research Consortium.*

**Background:** Stroke is a major cause of death in the US with hemodialysis [HD] patients particularly at risk with rates >8x higher than the general population. Initiation of HD in the elderly appears to confer much greater stroke risk in the US. In the absence of published data we examined if this applied globally especially in younger patients.

**Methods:** The MONDO research initiative consists of HD databases from the US, Europe, Asia & Latin America [Usvyat, Blood Purif 2013]. All incident patients from US Renal Research Institute [RRI] clinics & Fresenius Medical Care [FMC] Europe clinics within 30d of their first ever dialysis [Jan 2010-May 2013] were studied with follow up for a maximum of 2yrs with fatal & non-fatal hospitalized stroke defined by ICD-9 & 10 coding and excluding transient ischemic attack.

**Results:** We studied 1030 strokes in 37793 patients [FMC Europe n=32892; RRI n=4901]. Overall stroke incidence was 22.3 /1000 pt yrs [95%CI 21.0-23.8], higher in patients ≥65yrs old [13.5 vs 30.5 /1000 pt yrs, p<0.001] and similar between Europe & the US [22.1 vs 24.2 /1000 pt yrs, p=0.2].

Stroke incidence was highest within 6 months of HD initiation particularly driven by peak rates of non-fatal stroke [Fig 1]. Incidence of fatal stroke was consistently higher in Europe, greatest in the first 6 months of HD with rates 2-3x higher in patients ≥65yrs old.




**Conclusions:** The first 6 months of HD initiation are a time of maximal stroke risk in all patients, especially in older people who experience greater stroke mortality. Urgent studies are required to reduce the disproportionately high incidence of stroke in HD populations with focus on modulating stroke risk by modifying the HD prescription.

## FR-PO364

**Identical Index Hospitalization and 30-Day Readmission Discharge Diagnosis Codes**

Eduardo K. Lacson, Weiling Wang, Franklin W. Maddux.
*Fresenius Medical Care, North America, Waltham, MA.*

**Background:** Potentially preventable hospital readmissions are most likely those that have the same diagnosis between the index and 30-day readmission events. We preliminarily determined the most common diagnosis codes in hemodialysis (HD) patients.

**Methods:** All adult in-center HD patients, treated 3x/ week as of 1/1/11 in Fresenius Medical Care, North America facilities with at least one hospital discharge in 2011 were followed up to 12/31/11. Hospital readmissions within 30-days of discharge were recorded, along with the index primary discharge and readmission ICD-9 codes (also as classified into diagnosis categories). We sought to determine the most frequent pairs of similar hospital diagnoses.

**Results:** There were 164,258 hospital events in 64,128 patients in 2011. 50,380 (30.7%) 30-day readmissions occurred in 23,575 patients. There were 8,965 (17.8%) readmissions (852 valid ICD-9 codes) with the exact diagnosis as in the index admission. The 10 most common identical ICD-9 code pairs for index and readmission diagnoses are shown in the table.vFluid overload conditions that may present as shortness of breath and CHF rank #1, #2, and #7 on the list, totaling ~11.2% of readmissions. The mean time from discharge to readmission was 10.4±8.3 days (IQR: 3-16; median: 8).The mean (weighted) rate of identical diagnoses was 22.7% for 30-day readmissions. 4,255 of 50,380 (8.4%) readmissions with identical diagnoses occurred within 7 days of discharge.

| Order | ICD-9 Code & Description of Readmission | N of Readmissions | % | N of Same ICD-9 in Prior Hospitalization | % of Same ICD-9 in Prior Hospitalization |
|---|---|---|---|---|---|
| 1 | 786.05 - SHORTNESS OF BREATH | 2484 | 4.93 | 709 | **28.5** |
| 2 | 276.69 - OTHER FLUID OVERLOAD | 1975 | 3.92 | 539 | **27.3** |
| 3 | 786.5 - CHEST PAIN | 1642 | 3.26 | 451 | **27.5** |
| 4 | 780.97 - ALTERED MENTAL STATUS | 1344 | 2.67 | 328 | **24.4** |
| 5 | 780.99 - OTHER GENERAL SYMPTOMS | 634 | 1.26 | 316 | **49.8** |
| 6 | 996.1 - MECHANICALCOMPLICATIONOFIMPLANT,GRAFT | 659 | 1.31 | 255 | **38.7** |
| 7 | 428.0 - CONGESTIVEHEARTFAILURE | 1173 | 2.33 | 248 | **21.1** |
| 8 | 996.73 - OTHER COMPLICATION | 676 | 1.34 | 230 | **34.0** |
| 9 | 787.01 - NAUSEAW/VOMITING | 764 | 1.52 | 202 | **26.4** |
| 10 | 578.9 - HEMORRHAGE OF GASTRO INTESTINAL TRACT | 699 | 1.39 | 201 | **28.8** |

**Conclusions:** Among identically diagnosed readmissions, the most common potentially actionable condition is fluid related. The identical diagnosis rate of 8% of readmissions within 7 days of discharge indicate potential problems with transition to outpatient care and/or that some patients were discharged from the hospital prematurely. Further study is needed in these subgroups of patients to evaluate for actionable items to avoid readmissions.

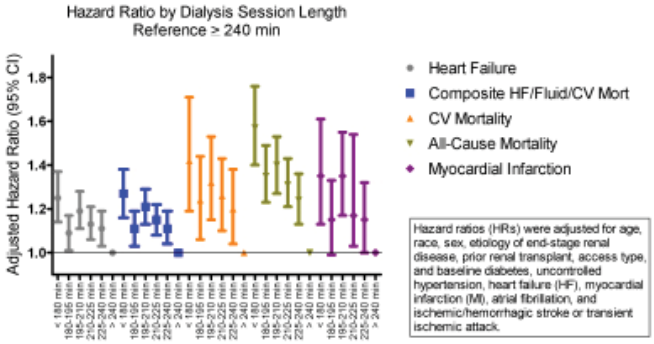
## FR-PO365

**Shorter Hemodialysis Session Length Is Strongly Associated with Higher Rates of Mortality and Hospitalization** Steven M. Brunelli,[1] Emmanuel A. Anum,[1] Karthik Ramakrishnan,[1] Donna E. Jensen,[1] Gilbert Marlowe,[1] Mahesh Krishnan,[1] Allen R. Nissenson.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]DaVita Healthcare Partners, Denver, CO.*

**Background:** Prescribing patterns of hemodialysis (HD) vary widely. Prior data demonstrate that shorter HD session length is associated with increased mortality. There is a paucity of data with respect to associations with cause-specific morbid events. The objective of the current study was to determine the relationship between duration of HD and rates of cardiovascular (CV) mortality and events.

**Methods:** The records of in-center HD patients incident to dialysis (1-Jan-07 through 31-Dec-08), treated at DaVita within 30 days of first HD and having Medicare or Medicaid as a primary insurer were studied. Mean session length was calculated over days 91-180. Patients were at-risk for outcomes beginning on day 181 of HD and continuing until death, transfer of care, modality change, or end of study (31-Dec-09). Outcomes were identified through linkage to US Renal Data Systems claims data and included hospitalization for heart failure (HF)/fluid overload, myocardial infarction (MI), all-cause mortality, CV mortality, and a composite hospitalization for HF/fluid overload or CV mortality.

**Results:** Of ~150,000 DaVita patients, 39,497 patients qualified for study. All-cause mortality was greatest for patients receiving mean sessions <180 min (HR 1.57; CI95% 1.40-1.76) and lowest for those receiving mean sessions ≥ 240 min (HR 1, reference).




A similar dose effect was observed with CV mortality, the composite outcome, and hospitalization for HF and MI.

**Conclusions:** These data demonstrate a strong association between shorter dialysis session length and CV events causing hospitalization and death.

*Funding:* Pharmaceutical Company Support - This analysis was supported by Ardelyx, Inc. This publication is supported by DaVita Healthcare Partners, Inc.

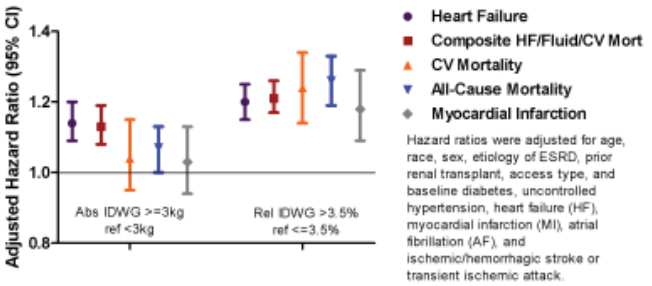
## FR-PO366

**Interdialytic Weight Gain and Cardiovascular Disease Outcomes** Steven M. Brunelli,[1] Claudia S. Cabrera,[2] David P. Rosenbaum,[3] Emmanuel A. Anum,[1] Karthik Ramakrishnan,[1] Donna E. Jensen,[1] Nils-olov Stalhammar,[2] Bergur V. Stefansson.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]AstraZeneca, Molndal, Sweden; [3]Ardelyx, Inc., Fremont, CA.*

**Background:** Patients with end-stage renal disease (ESRD) have a higher risk of cardiovascular (CV) disease-related morbidity and mortality than the general population. One putative risk factor for this is fluid accumulation between hemodialysis (HD) sessions; measured as interdialytic weight gain (IDWG). This analysis examined associative risks between IDWG with incident CV events and deaths.

**Methods:** Study patients were incident to HD (1Jan2007-31Dec2008) and were on in-center HD for at least 181 days. Exposures of interest were absolute and relative IDWG (IDWG as percentage of body weight), each considered as patients' mean value over dialysis days 91-180. Outcomes are listed below and were obtained by linkage to USRDS claims data. Patients were considered at-risk from dialysis day 181 until death, care transfer, voluntary withdrawal, modality change, or study end (31Dec2009).

**Results:** Among the study population (N=39,497) mean age was 62 years; 46% were white, 32% black, 15% Hispanic; 47% had ESRD attributed to diabetes. Absolute IDWG >3kg (26% of patients) was independently associated with greater risk of composite hospitalization and all-cause mortality; Relative IDWG >3.5% (37% of patients) was independently associated with greater risk of HF, composite HF/CV mortality, CV mortality, all-cause mortality, and MI; magnitudes of association were greater than for absolute IDWG.




Considered granularly, there was a strong incremental dose-response association between relative IDWG and all outcomes considered.

**Conclusions:** This analysis found increased risk of CV events for ESRD patients undergoing HD with greater IDWG. Mitigating IDWG may improve health and survival among ESRD patients.

*Funding:* Pharmaceutical Company Support - Ardelyx Inc.

## FR-PO367

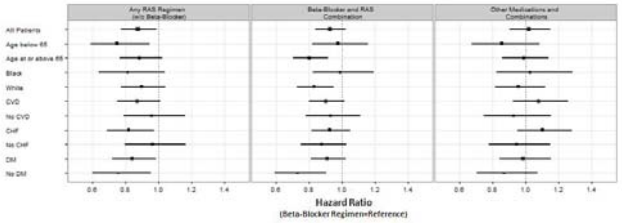
**Antihypertensive Medications and Mortality in Incident Hemodialysis Patients: A Marginal Structural Analysis of a National Cohort** Stephen M. Sozio,[1,2] Tariq Shafi,[1,2] Wendy L. St. Peter,[1,3] Karen J. Bandeen-Roche,[1,2] Patti Ephraim,[1,2] Jason Luly,[1,2] L. Ebony Boulware.[1,2] *[1]DEcIDE Network Patient Outcomes in ESRD Study Investigators; [2]Johns Hopkins Univ; [3]Chronic Disease Research Group.*

**Background:** Despite the burden of hypertension in dialysis patients, the optimal blood pressure medication (BPM) regimen has not been determined.

**Methods:** We performed a retrospective cohort study of all adult patients initiating hemodialysis at Dialysis Clinic, Inc. facilities from 2003-2008 and prescribed a BPM at 6 months. We obtained clinical parameters, BPMs, and outcomes from linked dialysis electronic medical records, USRDS data, and the National Death Index. We classified BPM regimens into 4 mutually exclusive regimens: β-blocker, renin-angiotensin system agent (RAS), β-blocker+RAS containing, or other BPMs. We also allowed discontinuation of BPMs if they were no longer prescribed in follow-up. We quantified each regimen's effect on all-cause and cardiovascular mortality using discrete time proportional hazards regression. Time-varying marginal structural models accounted for confounders and patient-level predictors of BPM prescriptions, including predialysis systolic BP and volume removal on dialysis.

**Results:** Among 11,291 hemodialysis patients, the mean age was 62 yrs, 55% male, 53% White, 56% had CVD and 3200 died in follow-up. Compared to β-blocker regimens, RAS regimens were associated with lower all-cause mortality (HR [95% CI] 0.82 [0.69, 0.97] for RAS regimens, and 0.93 [0.84, 1.02] for β-blocker+RAS regimens), and discontinuing BPMs was associated with higher mortality (HR 1.22 [1.02, 1.46]). Similar results were seen when analyzing cardiovascular mortality.

**Figure: Association of Medication Use on All-Cause Mortality in Incident HD Patients**




**Conclusions:** RAS regimens are associated with lower risk of mortality for patients receiving hemodialysis, and may be preferred in this population. Further studies are warranted to identify additional effects of these agents.

*Funding:* Other U.S. Government Support

## FR-PO368

**Lower Predialysis Blood Pressure Is Associated with Worse Physical Function in HD Patients** Adrian Paul Abreo,[1] Charles A. Herzog,[2] Janice P. Lea,[3] Nancy G. Kutner,[3] Patricia Lynn Painter,[4] Kirsten L. Johansen.[1] *[1]UCSF; [2]Hennepin County Medical Center; [3]Emory; [4]Univ of Utah.*

**Background:** New data suggests tight blood pressure (BP) control may not reduce mortality and may be associated with more side effects. The link between BP and physical function (PF) has not been examined in patients on HD.

**Methods:** We performed a cross-sectional analysis to examine the association between predialysis systolic blood pressure (PBP) and the short physical performance battery (SPPB) among 765 patients in the USRDS ACTIVE/ADIPOSE cohort. The SPPB assesses lower extremity function and includes 3 components: timed repeated chair stands, tests of balance test, and a timed 15-ft walk. PBP was grouped into the following categories: <110, 110-129, 130-159, and ≥160. We used multivariate ordinal logistic regression with PBP as the primary predictor, and SPPB categorized into three ordinal groups (≤6, 7-9, 10-12).

**Results:** Mean age was 55 yr. Participants with PBP 130-159 and ≥160 had lower odds of being in the lowest SPPB category compared to the 110-129 (reference) group (Table), even when BP medication use was added to the model. Each component of the SPPB showed the same pattern of association but results for balance were not statistically significant. **Higher PBP is associated with lower odds of worst PF.**

| | OR(95% CI) | p-value |
|---|---|---|
| *Mean Predialysis Systolic BP (mmHg)* | | |
| <110 | 0.93(0.36-2.39) | 0.88 |
| 110-129 | Reference | |
| 130-159 | 0.60(0.37-0.90) | 0.04 |
| ≥160 | 0.55(0.33-0.93) | 0.03 |
| | | |
| CVA | 2.51(1.10-5.70) | 0.03 |
| Age(10 y) | 1.76(1.55-2.01) | <0.0001 |
| Albumin(0.5 mg/dl) | 0.55(0.44-0.68) | <0.0001 |
| Black race | 1.68(1.19-2.36) | 0.003 |
| PVD | 3.42(1.91-6.13) | <0.0001 |
| Hemoglobin | 0.95(0.86-1.04) | 0.23 |
| CHF | 1.39(0.91-2.09) | 0.13 |
| CAD | 0.90(0.50-1.62) | 0.73 |
| ESRD vintage (log) | 1.11(0.96-1.28) | 0.15 |

**Conclusions:** PBP at traditionally targeted levels was associated with worse PF among patients on HD, independent of heart failure and BP medications. The risk-benefit tradeoff of aggressive BP control, particularly in low-functioning patients, should be reexamined.

*Funding:* NIDDK Support

## FR-PO369

**Characteristics and Outcomes of End-Stage Renal Disease Patients with Cardiac Rhythm Management Device Infections** Oluwaseun Opelami, Ankit Sakhuja, Xiaobo Liu, Jesse D. Schold, Sankar D. Navaneethan. *Cleveland Clinic, Cleveland, OH.*

**Background:** Sudden cardiac death due to arrhythmias remains the leading cause of death in end-stage renal disease (ESRD). This has led to increased use of cardiac rhythm management devices (CRMD) in ESRD patients, contributing to the increased rates of CRMD infection-related hospitalizations. We studied the characteristics and outcomes of ESRD patients admitted with CRMD infections in a large national inpatient database.

**Methods:** We conducted a retrospective analysis of the Nationwide Inpatient Sample (NIS) discharge records (2005-2010). We identified patients with CRMD infections using the ICD-9 codes for device-related infections or other device procedure (change and/or removal) along with bacteremia, endocarditis or systemic infection. Logistic regression and linear regressions were performed to examine inpatient mortality, length of stay and cost in ESRD patients after adjusting for patient demographics, primary payer and hospital characteristics.

**Results:** Of the 88,008 admissions for CRMD infections, 6,751 of them (7.7 %) were ESRD patients. ESRD patients were younger and had higher proportion of African Americans and Hispanics. In the multivariable model, ESRD patients had higher odds (OR = 2.58, 95% CI: 2.2, 3.1) of in-hospital mortality, compared to non-ESRD patients. Among survivors, the mean length of stay for ESRD patients was 18.1 days as compared to 11.9 days among non-ESRD patients (p<0.0001). The mean total charges during the hospitalization for the ESRD group was $158,640 as compared to $102,943 among non-ESRD group (p<0.0001). Among ESRD population, only 26.3% were discharged home (vs. 33.5% in non-ESRD group, p<0.001) and 51.1% were transferred to skilled nursing facility or other rehabilitation center (vs. 34.9% in non-ESRD group, p<0.001).

**Conclusions:** End stage renal disease patients admitted with cardiac rhythm management device-related infections have an increased risk of in-hospital mortality, length of stay and higher costs. Furthermore, ESRD patients most commonly require skilled nursing facilities following discharge, further increasing healthcare costs.

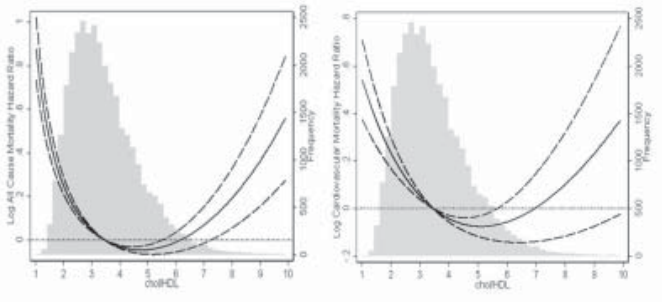
## FR-PO370

**Elevated Serum Total Cholesterol to High Density Lipoprotein (HDL) Ratio Is Paradoxically Associated with Improved Overall and Cardiovascular Mortality in Patient on Hemodialysis** Hamid Moradi,[1,2] Elani Streja,[1,2] Moti L. Kashyap,[2] Nosratola D. Vaziri,[1] Gregg C. Fonarow,[3] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Orange, CA; [2]Univ of California, Irvine; [3]Univ of California, Los Angeles.*

**Background:** In the general population, increasing serum total cholesterol to HDL ratios are associated with increased risk of cardiovascular (CV) mortality. This association is not well established in patients with end stage renal disease. We hypothesize that the association of serum total cholesterol to HDL ratio with all-cause and CV mortality in hemodialysis patients is different from the general population.

**Methods:** We examined the survival impact of serum total cholesterol to HDL ratio in a 3-year (6/2004-6/2007) cohort of 33,109 maintenance hemodialysis patients being treated in clinics of a large dialysis organization using Cox models adjusted for demographics and case-mix variables and cubic splines plots.

**Results:** In this cohort the mean age (mean + SD) was 60+15 years old and included 45% women, 33% African-Americans, and 61% diabetics. In the fully adjusted models, higher serum total cholesterol to HDL ratios were paradoxically associated with better overall and CV survival. This trend remained significant until the cholesterol/HDL ratio increased beyond 6.




**Conclusions:** Increasing serum total cholesterol to HDL ratio are paradoxically associated with improved all-cause and CV mortality in patients on maintenance hemodialysis. These associations are significant despite adjustment for demographics and case-mix variables. The underlying mechanisms responsible for these seemingly paradoxical associations await further investigation.

*Funding:* NIDDK Support

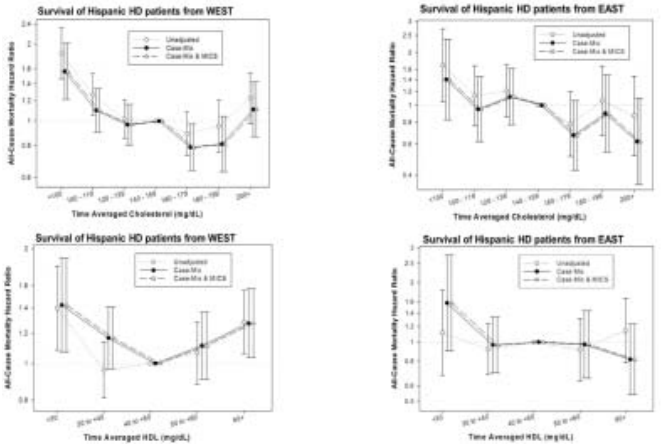
## FR-PO371

**Association of Lipid Profile with Mortality in Dialysis Patients of Hispanic Origin in the East versus West Coast** Hamid Moradi,[1,2] Elani Streja,[1,2] Nosratola D. Vaziri,[1] Moti L. Kashyap,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1,2] *[1]Univ of California, Irvine; [2]Harold Simmons Center, Orange, CA; [3]Memphis Veterans Affairs Medical Center.*

**Background:** We have previously shown that in Hispanic patients on maintenance hemodialysis (MHD) paradoxical associations exist between serum lipid levels and mortality. However, significant racial and ethnic differences exist in patients of "Hispanic" background. While the Hispanic population of the West Coast consists mainly of those from Mexico and Central America, on the East Coast there is a large Afro-Caribbean contingency. We hypothesized that major differences exist in association of dyslipidemia and survival in Hispanic MHD patients on the West versus East Coast.

**Methods:** We examined the survival impact of serum lipids in a 2-year (6/2005-6/2007) cohort of 5,185 maintenance hemodialysis patients of Hispanic origin being treated in clinics of a large dialysis organization (in California, Texas, New York, New Jersey and Florida) using Cox models adjusted for case-mix and markers of malnutrition-inflammation complex (MICS) variables.

**Results:** In this cohort there were 4,220 patients from the West and 965 from the East Coast. In the fully adjusted models, significant differences were noted in the association of lipid markers and mortality in Hispanic patients from the West when compared to the East Coast.




**Conclusions:** Significant differences exist in association of serum lipids with mortality in MHD patients of Hispanic background depending on whether they live on the West or East Coast of the United States. These geographical discrepancies most likely reflect ethnic and racial differences which usually go unnoticed. Future studies should take into account these critical variations in a population of patients who will make up a majority of our society in the future.

*Funding:* NIDDK Support

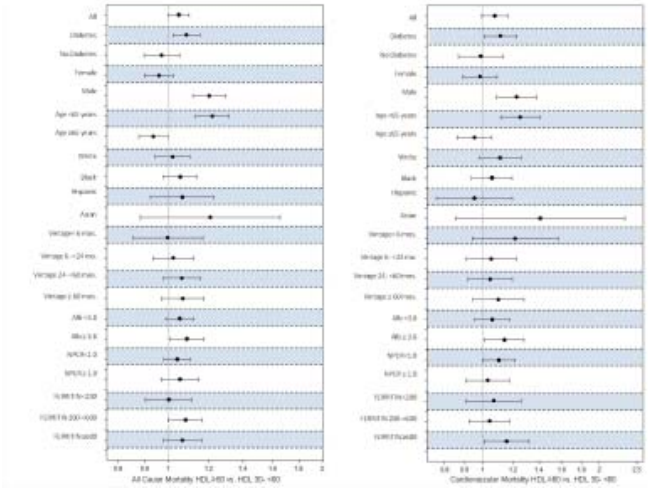
**FR-PO372**

**Subgroup Analysis of Hemodialysis Patients with Elevated Serum High Density Lipoprotein (HDL) Cholesterol Levels Demonstrates Men and Those Younger Than 65 Years of Age Have the Greatest Risk of Overall and Cardiovascular Mortality** Hamid Moradi,[1,2] Elani Streja,[1,2] Moti L. Kashyap,[2] Nosratola D. Vaziri,[2] Gregg C. Fonarow,[3] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Orange, CA; [2]Univ of California, Irvine; [3]Univ of California, Los Angeles.*

**Background:** In the general population, increasing levels of HDL are associated with reduced cardiovascular (CV) mortality. We hypothesize that the association of serum HDL concentration and mortality in hemodialysis patients is different from the general population and that in subgroups of patients on dialysis, elevated serum HDL cholesterol level may be paradoxically associated with increased mortality.

**Methods:** We examined the survival impact of serum HDL level in a 3-year (6/2004-6/2007) cohort of 33,109 maintenance hemodialysis patients being treated in clinics of a large dialysis organization using Cox models adjusted for demographics and case-mix variables. Hazard ratio of overall and cardiovascular mortality for two selected dichotomized levels of serum HDL cholesterol concentrations (left: 30- <60 mg/dl; right: ≥60 mg/dl) were plotted in selected subgroups of the study population.

**Results:** In this cohort, the mean age (mean + SD) was 60+15 years old and included 45% women and 61% diabetics. All-cause and CV mortality HR was 1.05 (1.00-1.10) and 1.08(1.00-1.16) for HDL≥60 mg/dL, respectively (reference: HDL 30-<60 mg/dL). This risk was highest in men, diabetics, those with albumin > 3.8 g/dL and in those under the age of 65.



**Conclusions:** Serum HDL cholesterol levels over 60 mg/dL are associated with increased all-cause and CV mortality in patients on hemodialysis and this risk is most pronounced in men and those younger than 65 years of age. Further studies are needed to determine the mechanisms responsible for these associations.

*Funding:* NIDDK Support

**FR-PO373**

**Intradialytic Cardiac Function and Inflammatory Cytokine Changes during Acetate-Free Online Hemodiafiltration Compared with Standard Online Hemodiafiltration in End-Stage Renal Disease Patients** Kamonwan Tangvoraphonkchai, Khajohn Tiranathanagul, Nattachai Srisawat, Paweena Susantitaphong, Kriang Tungsanga, Somchai Eiam-ong, Kearkiat Praditpornsilpa. *Div of Nephrology, Dept of Medicine, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn Univ, Thailand.*

**Background:** Acetate in standard dialysis fluid could suppress myocardial function and potentially induce cytokine production, resulting in intradialytic hypotension(IDH) in hemodialysis(HD) patients. Online hemodiafiltration(HDF) provides superior intradialytic hemodynamic stability over HD. The present study was aimed to investigate the potential additive hemodynamic benefits and cytokine changes of the novel acetate-free dialysis fluid in online HDF.

**Methods:** A randomized, double-blind, crossover clinical trial was conducted in 22 stable online HDF patients. The patients were randomly assigned to firstly receive either acetate-free or standard online HDF and then were switched to the other after one week. Clinical parameters, cardiac index(CI), cardiac output(CO), and peripheral vascular resistance(PVR) were hourly assessed as well as the cytokines(IL-2ß, IL-6, IL-8, IL-10, and TNF-α) were measured at pre- and post-dialysis in each study session.

**Results:** The baseline clinical and cardiac parameters were similar between acetate-free and standard online HDF groups. There were comparable changes of arterial pressure($p$=0.86) and the incidences of composite IDH and other adverse events between both groups. The changes of CI, CO, and PVR during dialysis were comparable($p$ = 0.53, 0.20, and 0.64,respectively). The percent reductions of NT-proBNP and troponin-T were not significantly different($p$=0.99 and $p$=0.51). The changes of IL-2ß, IL-6, IL-8, TNF-α, and IL-10 during dialysis were not significant different between both groups.

**Conclusions:** In stable online HDF patients, acetate in the standard dialysis fluid did not adversely affect clinical and cardiac parameters. The hemodynamic stability provided by online HDF might protect the adverse effects of acetate. Therefore, utilization of the costly acetate-free dialysis fluid did not offer additional cardiac benefits for stable online HDF patients.

**FR-PO374**

**Racial Differences in Intra-Dialytic Blood Pressure–Results from the HEMO Study** Finnian R. McCausland, Sushrut S. Waikar. *Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Racial differences in blood pressure control are evident in both the general and hemodialysis populations, with Blacks generally having higher blood pressure than non-blacks. We examined for differences in intra-dialytic blood pressure parameters according to race in a post-hoc analysis of hemodynamic data from the HEMO Study.

**Methods:** Data from 1825 participants were analyzed, using self-reported race (categorized as Black, Hispanic vs non-Hispanic white) as the exposure of interest. The association of race with nadir intra-dialytic systolic (SBP) and diastolic (DBP) blood pressure was analyzed with generalized linear regression models.

**Results:** The mean age was 57.8 years; 44.7% were diabetic. Blacks accounted for 62.3% and Hispanics for 5.9% of total participants. In unadjusted analyses, compared with non-Hispanic whites, Hispanic and Black participants had 2.9 (95%CI -0.3 to 6.1) and 3.2 (95%CI 1.5 to 4.9) mmHg higher nadir SBP and 2.1 (95%CI -0.1 to 4.4) and 3.1 (95%CI 2.0 to 4.2) mmHg higher nadir DBP. Upon multivariable adjustment for potentially confounding covariates (including pre-dialysis BP), Blacks remained with significantly higher nadir SBP (3.2; 95%CI 1.8 to 4.6 mmHg) and DBP (2.0; 95%CI 1.2 to 2.8 mmHg). Similar patterns were found with post-dialysis systolic and diastolic pressures. There was no evidence for effect modification according to age in all models considered (p-interaction>0.2).

**Conclusions:** We found evidence for racial differences in intra-dialytic blood pressure control, with Blacks appearing to have less marked decline in SBP and DBP during dialysis compared with non-Hispanic whites. Further study is warranted to determine if intra-dialytic blood pressure stability is causally related to lower mortality in Black hemodialysis patients.

**FR-PO375**

**A Predictive Score for Cardiovascular Morbidity and Mortality in Hemodialysis Patients: Retrospective Cohort Study** Stefan D. Anker,[1] Iain A. Gillespie,[2] Kai-Uwe Eckardt,[3] Florian Kronenberg,[4] Sharon Richards,[2] Ronald L. Pisoni,[5] Bruce M. Robinson,[5] Daniele Marcelli,[6] Marc Froissart,[7] Jürgen Floege.[8] *[1]Charite Campus Virchow-Klinikum, Greece; [2]Amgen Ltd, United Kingdom; [3]Univ of Erlangen-Nuremberg, Germany; [4]Innsbruck Medical Univ, Austria; [5]Arbor Research Collaborative for Health; [6]Fresenius Medical Care, Germany; [7]Amgen Europe GmbH, Switzerland; [8]RWTH Univ of Aachen, Germany.*

**Background:** Simple clinical tools to assess HD patients' cardiovascular disease (CVD) risk do not exist, hence clinicians often rely on the Framingham Heart Study score designed to assess ten-year CV risk in the general population. Accordingly, this study aimed to derive and validate a risk score for two-year CV morbidity and mortality (CVMM) in 2007-09 incident European adult HD patients recruited in the second Fresenius Medical Care-based ARO cohort (AROii).

**Methods:** Using a modified Framingham approach, we derived and internally validated a two-year CVMM risk score in patients randomly assigned to either a development (N=4,831) or validation dataset (N=4,796). External validation was conducted in the DOPPS III cohort (N=10,615). Additional discrimination, compared to the Framingham score, was assessed.

**Results:** The two-year CVMM event rate was 22.6 per 100 person-years (95% Confidence Interval 21.9-23.4). Major risk markers included CVD history, increasing age, underlying diabetic nephropathy, high interdialytic weight change, high calcium and low hemoglobin levels. Traditional CV risk factors (gender, blood pressure, cholesterol) were absent. Our new risk score was predictive in AROii (c-index range 0.66-0.67),albeit less so in DOPPS (0.63). In contrast, the Framingham Score was not very predictive of CVMM in AROii (AUC 0.56). Additional discrimination analysis showed that 58% of patients' outcomes were correctly reclassified using our risk model parameters.

**Conclusions:** Our risk score for CVMM in HD patients, which is easy to apply and interpret, represents a major improvement over the Framingham score, the use of which may cloud clinical judgment and should be discouraged in the HD population.

*Funding:* Pharmaceutical Company Support - Amgen (Europe) GmbH

**FR-PO376**

**Associates of Cardiopulmonary Arrest during Hemodialysis** Jennifer E. Flythe,[1] Nien-chen Li,[2] Shu-Fang Lin,[2] Steven M. Brunelli,[1,3] Jeffrey L. Hymes,[2] Eduardo K. Lacson.[2] *[1]Brigham & Womens Hospital, Boston, MA; [2]Fresenius Medical Care, North America, Waltham, MA; [3]DaVita Clinical Research, Minneapolis, MN.*

**Background:** Cardiopulmonary arrest (CPA) during and immediately prior to or after hemodialysis (HD) treatment (i.e. peridialytic period) is a rare event (~7/100,000 treatments) that has a high case fatality rate. This study was designed to elucidate patient and procedural factors associated with peridialytic CPA in the largest cohort reported to-date.

**Methods:** Data from the period prevalent HD population of Fresenius Medical Care, North America over 2010 were analyzed. 924 in-center CPA events (cases) and 75,538 eligible controls were identified. Cases and controls were 1:5 matched on age (+/- 3 years),

sex, race, and diabetes status. A comprehensive multivariate logistic regression model screening for demographic, treatment, and laboratory factors was built to identify factors predictive of peridialytic CPA.

**Results:** All 924 cases were matched to 4614 controls: age (65.7 years), sex (50% male), race (60% white), and diabetes (72%). Missed HD sessions due to hospitalization (prior 30 days), coronary artery disease, heart failure, lower serum albumin and hemoglobin, lower dialysate potassium, higher serum calcium, higher erythropoietin stimulating agent dose, and nPCR (j-shape) were associated with peridialytic CPA. The final model is shown in the table. Dialysate sodium, calcium, and buffer (bicarbonate + acetate) were not significant factors.

| Variables in the Final Model | OR (95% CI) | Variables Removed by the Stepwise Procedure | |
|---|---|---|---|
| Missed HD due to hospitalization (y/ n) | 1.44*** (1.17-1.77) | | |
| Coronary Artery Disease | 1.37** (1.13-1.66) | BMI | Ferritin (ng/ml) x 100 |
| Heart Failure | 1.24* (1.03-1.50) | Vintage (x 100 days) | Bicarbonate (mEq/L) |
| Albumin (g/dL) | 0.63**** (0.51-0.78) | % Interdialytic Weight Gain | eKt/V |
| Hemoglobin (g/dL) | 0.82**** (0.76-0.88) | Treatment shortened >10 minutes (yes vs. no) | EDW |
| Calcium (mg/dL) | 1.18* (1.04-1.33) | Missed treatment without excuse (yes vs. no) | Dialysate Sodium |
| nPCR | 0.54*** (0.38-0.76) | Vascular access: catheter vs. F/G | Dialysate Calcium |
| ESA per Treatment (per 1000u) | 1.01* (1.00-1.02) | Phosphorus (mEq/L) | Dialysate Buffer (Bicarbonate + Acetate) |
| Dialysate Potassium (mEq/L) | 0.77* (0.63-0.94) | Potassium (mEq/L) | |
| nPCR$^2$ | 2.00*** (1.25-3.21) | | |

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

**Conclusions:** Markers of patient-related health status and modifiable procedural factors (dialysate potassium and ESA dose) are significant associates of peridialytic CPA. In addition to maximizing nutritional status, it may be prudent to limit exposure to low dialysate potassium (<2K+ bath) and to use the lowest effective ESA dose. However, further study of peridialytic CPA preventive strategies is needed.

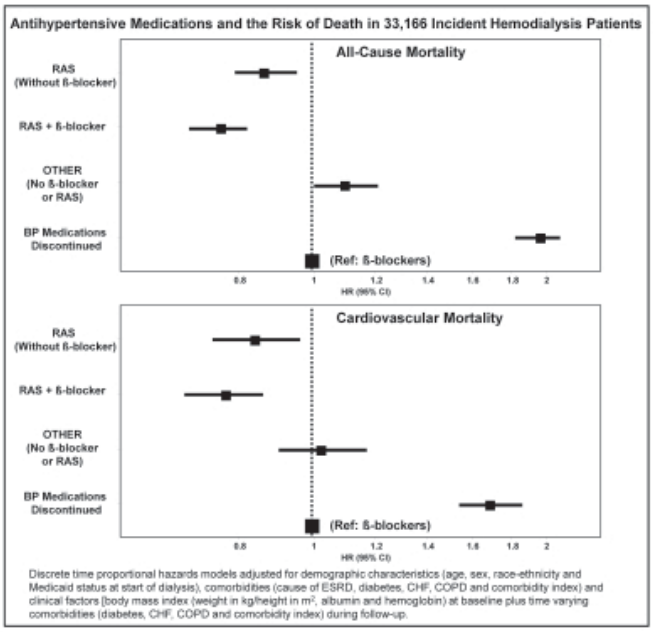
## FR-PO377

**Renin-Angiotensin System (RAS) Blocking Drugs Reduce the Risk of Death in U.S. Incident Hemodialysis (HD) Patients** Tariq Shafi,[1] Wendy L. St. Peter,[2] Stephen M. Sozio,[1] Patti Ephraim,[1] Karen J. Bandeen-roche,[1] L. Ebony Boulware.[1] *[1]Johns Hopkins Univ; [2]Univ of Minnesota.*

**Background:** The optimum blood pressure medication (BPM) regimen for patients on HD remains unclear. We quantified the association of BPM regimens with mortality in HD patients.

**Methods:** Our retrospective study cohort included 33,166 incident in-center US HD patients that initiated HD in 2006-2008 and were on HD at 6 months. We assessed BPM use from Medicare Part D prescription fill data and classified BPM use as 4 mutually exclusive regimens: β-blockers (BB), RAS, BB+RAS or OTHER regimens not including BB or RAS. Patients who discontinued BPM during follow-up constituted the 5th group. We used discrete time proportional hazards models adjusting for demographic and clinical characteristics including time-varying BPM use and comorbidities to assess the association between BPMs and death [all cause and cardiovascular (CV)]. Cause of death was assessed using Form 2746 and National Death Index.

**Results:** Patients' mean age was 65 years, 48% were male and 47% were White. At baseline (day 180), medication regimens were as follows: BB 37%, RAS 19%, BB+RAS 26% and OTHER 18%. There were 9,107 (28%) deaths during follow-up; 4,521 (50%) were due to CV causes. Patients in the BB group were more likely to have CV disease and congestive heart failure. In fully adjusted models, RAS-based regimens were associated with a lower hazard of mortality compared to BB regimens without RAS [HR (95% CI) for RAS group was 0.87 (0.79-0.95) for all-cause mortality and 0.84 (0.74-0.96) for CV mortality and for BB+RAS group was 0.75 (0.69-0.82) for all-cause mortality and 0.77 (0.68-0.86) for CV mortality].




**Conclusions:** RAS-based BPM regimens are associated with a lower risk of all-cause and CV death among HD patients. Our findings suggest that RAS agents may be preferred antihypertensive drugs for patients starting HD.

*Funding:* NIDDK Support, Other U.S. Government Support

## FR-PO378

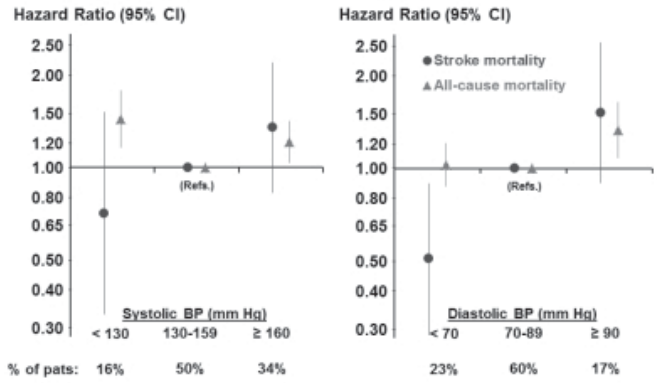
**Association of Pre-Dialysis Blood Pressure with All-Cause Mortality and Stroke in Japanese Hemodialysis Patients: The Japan DOPPS** Masaaki Inaba,[1] Angelo Karaboyas,[2] Takashi Akiba,[3] Tadao Akizawa,[4] Akira Saito,[5] Shunichi Fukuhara,[6] Christian Combe,[7] Bruce M. Robinson.[2] *[1]Osaka City Univ, Japan; [2]Arbor Research, Ann Arbor; [3]Tokyo Women's Medical Univ, Japan; [4]Showa Univ, Japan; [5]Tokai Univ, Japan; [6]Kyoto Univ, Japan; [7]Univ Bordeaux Segalen, France.*

**Background:** The association of low blood pressure (BP) with high mortality is characteristic for hemodialysis (HD) patients. In Japan, HD mortality is lower but there is a burden of high BP. This analysis clarifies the association of BP with mortality and stroke in Japanese HD patients, and examines the association separately for patients with and without antihypertensive medication (BP meds).

**Methods:** We analyzed 9,134 patients from Japan in phases 1-4 (1999-2011) of the Dialysis Outcomes and Practice Patterns Study (DOPPS), a prospective cohort study of in-center HD patients. The association of pre-dialysis systolic (SBP) and diastolic (DBP) BP with all-cause and stroke mortality was assessed using adjusted Cox regression.

**Results:** 34% had SBP ≥160 mm Hg. A U-shaped association between SBP and all-cause mortality was observed (Figure); no evidence of interaction (p=0.97) was found between SBP and use of BP meds. Both SBP and DBP were positively and monotonically associated with stroke mortality: hazard ratio (95% CI) was 1.24 (1.01-1.53) per 20 mmHg higher SBP and 1.23 (1.05-1.44) per 10 mmHg higher DBP. The association between SBP and stroke mortality was slightly stronger (p=0.09) among patients not on BP meds than on BP meds.

**Conclusions:** This analysis documents a positive and monotonic association of BP with stroke-related deaths in Japanese HD patients. High BP remains common, and while our analysis indicates that the prescription of BP meds to hypertensive patients might protect against stroke-related death, additional study is warranted.




Association of Systolic (SBP) and Diastolic (DBP) Blood Pressure with stroke and all-cause mortality; 4 separate Cox models, each stratified by DOPPS phase and adjusted for age, sex, vintage, BMI, 12 comorbidities, albumin, creatinine, phosphorus, hemoglobin, IDWG, Kt/V, and accounted for facility clustering.

*Funding:* Pharmaceutical Company Support - The DOPPS is supported by research grants from Amgen (founding sponsor, since 1996), Kyowa Hakko Kirin (since 1999, in Japan), AbbVie (since 2009), Sanofi Renal (since 2009), Baxter Healthcare (since 2011), and Vifor Fresenius Medical Care Renal Pharma (since 2012), with additional country-specific support provided in Canada by Amgen-Canada, Janssen, BHC Medical, Takeda and Kidney Foundation of Canada, and in Germany by Hexal and WiNe Institute. Support from the DOPPS sponsors is provided without restrictions on publications.

## FR-PO379

**Skin Autofluorescence Predicts Cardiovascular Mortality in Patients on Chronic Hemodialysis** Hiroshi Kimura, Kenichi Tanaka, Makoto Kanno, Kimio Watanabe, Yoshimitsu Hayashi, Koichi Asahi, Hiroyuki Terawaki, Masaaki Nakayama, Tsuyoshi Watanabe. *Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Medical Univ School of Medicine, Fukushima, Japan.*

**Background:** Tissue accumulation of advanced glycation end products (AGE) is thought to contribute to the progression of cardiovascular disease (CVD). Skin autofluorescence, a non-invasive measure of AGE accumulation using autofluorescence of the skin under ultraviolet light, has been reported to be an independent predictor of mortality and associated with CVD in Caucasian patients on chronic hemodialysis. The aim of this study was to assess the predictive value of skin autofluorescence on all-cause and cardiovascular mortality in non-Caucasian (Japanese) patients on chronic hemodialysis.

**Methods:** Baseline skin autofluorescence was measured with an autofluorescence reader in 128 non-Caucasian (Japanese) patients on chronic hemodialysis. All-cause and cardiovascular mortality was monitored prospectively during a period of 6 years.

**Results:** During the follow-up period, 42 of the 128 patients died; 19 of those patients died of CVD. Skin autofluorescence did not have a significant effect on all-cause mortality. However, age, carotid artery intima-media thickness (IMT), serum albumin, high-sensitivity C-reactive protein (hsCRP), skin autofluorescence and pre-existing CVD were significantly correlated with cardiovascular mortality. Multivariate Cox regression analysis showed skin autofluorescence [adjusted hazard ratio (HR) 3.97; 95% confidence interval (CI) 1.67-9.43], serum albumin [adjusted HR 0.05; 95% CI 0.01-0.32], and hsCRP [adjusted HR 1.55; 95% CI 1.18-2.05] to be independent predictors of cardiovascular mortality.

**Conclusions:** The present study suggests that skin autofluorescence is an independent predictor of cardiovascular mortality in non-Caucasian (Japanese) patients on chronic hemodialysis.

## FR-PO380

**Comparative Risk of Sudden Cardiac Death in Incident Hemodialysis (HD) and Peritoneal Dialysis (PD) Patients** Shuling Li,[1] Charles A. Herzog.[1,2,3] *[1]USRDS/CVSSC, MMRF, Minneapolis, MN; [2]Hennepin County Medical Center; [3]Univ of MN.*

**Background:** HD initiation is a vulnerable period with a markedly heightened risk of death. We previously reported that the hazard of sudden cardiac death (SCD) is markedly increased in incident HD pts in the first 90 days after dialysis initiation. Few published data exist regarding SCD rate in the first 90 days after PD initiation and risk of SCD in HD vs PD pts.

**Methods:** Incident HD and PD pts in 2008-2010 were identified in the USRDS database. Pts were followed from initiation to earliest of death, transplant, modality change, recovery of kidney function, loss-to-follow-up, or 1 yr after initiation. SCD was determined from CMS Form 2746. Cumulative probability of SCD was estimated by Kaplan-Meier method. The association between modality and risk of SCD was examined using piecewise Cox PH model with the cut-offs of F/U intervals ≤ 3 mos., > 3-≤ 6 mos. and > 6-≤ 12 mos. and adjusted for pt baseline characteristics.

**Results:** A total of 314,233 HD and 19,476 PD pts were available for study. More HD than PD pts were aged 65+ yrs (51% vs. 36%) and black (29% vs. 22%), had DM or HTN (74% vs. 68%) as primary cause of ESRD, and comorbid conditions at initiation. The cumulative probability of SCD (HD vs. PD) was 2.5% vs. 0.6% at 3 mos. and 7.0% vs. 2.2% at 1 yr (p<0.001). Overall, HD pts had higher SCD rate than PD pts. But the rate for HD pts decreased steadily with increasing time on dialysis, compared with a roughly stable rate for PD pts (See Table). Although risk of SCD associated with HD attenuated with increasing time on dialysis, it remained a significant 94% higher for HD pts vs PD pts even after 6 mos. of initiation.

| | Rate (SCDs/1000 pt-yrs) | | Adj. HR (95% CI) |
|---|---|---|---|
| F/U intervals | HD | PD | (Ref:PD) |
| ≤ 3 mos. | 102.1 | 23.3 | **3.20 (2.64, 3.88)** |
| > 3 - ≤ 6 mos. | 74.3 | 20.5 | **2.75 (2.21, 3.41)** |
| > 6 - ≤ 12 mos. | 57.6 | 23.3 | **1.94 (1.66, 2.26)** |

**Conclusions:** The hazard of SCD is markedly higher for incident HD pts than PD pts, especially in the first 90 days after initiation. These findings highlight the importance of early prevention of SCD for HD pts at initiation.

*Funding:* NIDDK Support

## FR-PO381

**Risk of Death for Systolic and Diastolic Heart Failure in Dialysis Patients** Shuling Li,[1] Yan Hu,[1] Charles A. Herzog.[1,2] *[1]CVSSC, U.S. Renal Data System; [2]Univ of MN.*

**Background:** Heart Failure (HF) is reportedly the most prevalent cardiac disease in dialysis pts. Few data exist on the comparative outcome of systolic vs diastolic HF in stage 5D CKD.

**Methods:** We identified 191,709 dialysis pts (age ≥20) prevalent on 1/1/09, surviving through 2009, and continuously enrolled in Medicare in 2009 using the USRDS database. HF was identified from ICD-9 codes 398.91, 428.xx, 402.x1, 404.x1, and 404.x3 in 2009 claims and included systolic (428.2x), diastolic (428.3x), both systolic and diastolic (428.4x), and unspecified. F/U began on 1/1/2010 and ended at the earliest of death, transplant, or 12/31/2011. Unadjusted survival was estimated by Kaplan-Meier method. The risk of death associated with HF types was assessed in a Cox PH model adjusting for baseline characteristics.

**Results:** The overall prevalence of systolic, diastolic, both, and unspecified HF was 7%, 6%, 2%, and 22%, respectively. Prevalence of HF increased with age; by type, from 4% in 20-44 yrs to 8% in ≥75 yrs for systolic HF, 3% to 7% for diastolic HF, 1% to 2% for both HF, and 15% to 26% for unspecified HF. Two-year survival probability was 47% systolic, 51% both, 54% diastolic, and 58% unspecified HF, and 74% no HF (Fig 1). The adjusted hazards ratio (95% CI) for death was 1.90 (1.84. 1.95) and 1.58 (1.53, 1.63) for systolic and diastolic HF, respectively, compared with no HF (Fig 2).

**Fig 1. Unadjusted survival probability**

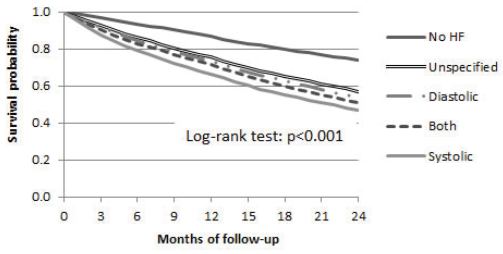
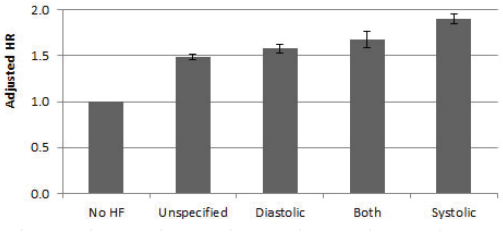



**Fig 2. Adjusted HR for Death by HF Type**




**Conclusions:** Dialysis pts with HF of any type have poor long-term survival. Systolic HF in Stage 5D CKD pts has the worst outcome. Interventions targeting this very high risk population are indicated.

*Funding:* NIDDK Support

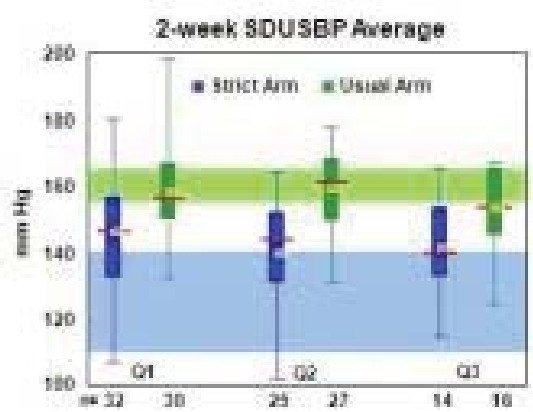
## FR-PO382

**Blood Pressure in Dialysis (BID) Study: A Pilot RCT Assessing Treatment to Two Different BP Targets** Jose L. Vega,[3] D. Miskulin,[1] Jennifer J. Gassman,[2] David W. Ploth,[4] Manisha Jhamb,[5] Christine Stidley,[3] P. Zager.[3] *[1]Tufts Medical Center; [2]Cleveland Clinic Foundation; [3]Dialysis Clinic Inc.; [4]Medical Univ of South Carolina; [5]Univ of Pittsburgh; [6]Univ of New Mexico.*

**Background:** The optimal blood pressure (BP) goal for treatment of hypertensive hemodialysis patients is unknown. More aggressive BP lowering may reduce cardiovascular events, or it may not be tolerated due to intradialytic hypotension. Which BP measure (in-center, home or ambulatory BP readings) should guide management is also unknown. In the ongoing NIH-sponsored Blood Pressure in Dialysis (BID) Study, hypertensive HD patients are randomized to a pre-dialysis in-center BP of 110-140/90 mm Hg vs. 155-165/90 mm Hg.

**Methods:** We assess adherence with prescribed in-center standardized (SDUBPM), home (HBPM) and ambulatory (ABPM) monitoring. SDUBPM was taken each treatment, after 5 minutes rest, 3 readings per sitting, per AHA guidelines. HBPM was to be done twice, 3 readings each sitting, on the day following the mid-week treatment. ABPM was done for 44 hours in one interdialytic period per quarter. We also compared the distribution of BPs across study arms over time.

**Results:** As of 05/01/13, blood pressure data were available for 51 randomized patients. Adherence with requirements for SDUBPM, HBPM and ABPM in the first 3 months (Q1) was 94, 54 and 16%, in Q2 was 84, 47, and 37%, and in Q3 was 79, 50, and 14% respectively. Boxplots of SDUBPM readings across treatment arms is shown in Figure 1. The mean (SD) separation between arms at the end of the 3rd, 6th, and 9th month was 12, 19, and 12mm Hg, respectively.




**Conclusions:** Current data show adherence to staff-measured SDUBPM was substantially better than patient-measured HBPM or ABPM. Only 50% or fewer of patients perform HBPM or ABPM at a given timepoint. Separation in BP between treatment arms was achieved at month 3 and was maintained at month 9.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Dialysis Clinic Inc.

## FR-PO383

**Blood Pressure in Dialysis (BID) Study: Differences in Blood Pressure Measured inside versus outside the Dialysis Unit** Jose L. Vega,[1] Jennifer J. Gassman,[3] D. Miskulin,[2] David W. Ploth,[4] Manisha Jhamb,[5] Christine Stidley,[1] P. Zager.[1] *[1]Dialysis Clinic Inc.; [2]Tufts Med Ctr.; [3]Cleveland Clinic Foundation; [4]Medical Univ of South Carolina; [5]Univ of Pittsburgh.*

**Background:** Although ambulatory blood pressure monitoring (ABPM) is considered the 'gold standard' for assessing BP since multiple readings are obtained while patients are performing usual activities, they may be difficult to obtain. Pre-dialysis BPs taken in the dialysis unit are used to manage patients, though these may lead to over-treatment as patients are volume expanded. In the Blood Pressure in Dialysis (BID) study we obtain 44-h ABPM, intermittent Home BP and standardized pre-dialysis dialysis unit BP (SDUBPM). We compare differences across measures at baseline.

**Methods:** SDUBPM was measured each treatment after 5 minutes rest prior to dialysis start. Home BPs were measured twice per day the day after the midweek treatment. ABPM was performed during a 44 h interdialytic period. We compared 44 h ABPM (day, night, full 44-hour) with the 2-week averaged SDUBPM (week before and during ABPM) and Home BP (average of week before and after ABPM).

**Results:** 51 patients had all 3 BP measurements within the allotted time frame. Comparisons of Systolic BP across measures are shown in the table.

| | Median (IQR) | > 10 mm Hg lower(%) | > 20 mm Hg lower(%) | > 10 mm Hg higher(%) | > 20 mm Hg higher(%) |
|---|---|---|---|---|---|
| ABPM Day - SDUBPM | -5.2 (-15.6, 7.3) | 43 | 18 | 24 | 14 |
| ABPM Night - SDUBPM | -3.9 (-16.9, 9.3) | 45 | 18 | 22 | 8 |
| ABPM Full - SDUBPM | -6.2 (-17.5, 7.5) | 39 | 2 | 24 | 12 |
| ABPM Day - Home BP | +4.5 (-8.9, 13.2) | 20 | 14 | 37 | 6 |
| ABPM Night - Home BP | +3.3 (-8.9, 14.4) | 24 | 10 | 33 | 12 |
| ABPM Full - Home BP | +4.3 (-8.9, 13.9) | 20 | 6 | 36 | 8 |

**Conclusions:** On average SDUBPM were higher than day and night ABPM. In contrast, Home BP values were lower than either day or night ABPM. However, SDUBPM were not always greater than ABPM and Home BPs were not always lower than ABPM. In a large number of patients the differences between measures were > 10 mm Hg. These results suggest that optimal control of BP in hemodialysis patients requires assessment of BP measured inside and outside the dialysis unit. The feasibility of obtaining ABPM and Home BP measurements longitudinally is being assessed in the ongoing BID Study.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Dialysis Clinic Inc.

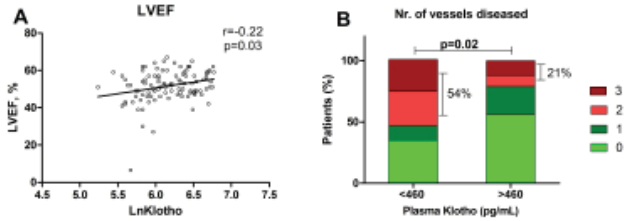
## FR-PO384

**Soluble Klotho Is Associated with Left Ventricular Function and Coronary Artery Stenosis in Dialysis Patients** Maurits S. Buiten,[1] Mihaly K. De Bie,[1] Bastiaan Van Van Dam,[3] Annet Bouma-de Krijger,[4] J.wouter Jukema,[1] Ton J. Rabelink,[2] Joris I. Rotmans.[2] *[1]Cardiology, LUMC, Leiden, Albinusdreef 2, Netherlands; [2]Nephrology, LUMC, Leiden, Albinusdreef 2, Netherlands; [3]Nephrology, MCA, Alkmaar, Netherlands; [4]Nephrology, Spaarne Ziekenhuis, Hoofdorp, Netherlands.*

**Background:** Dialysis patients suffer from a disproportionally high burden of cardiovascular disease which might be due to unique risk factors. Klotho, a protein linked to aging, has been suggested as such a factor. Recently an ELISA has become available to measure soluble Koltho (sKlotho). The purpose of the present study was to determine the relationship between sKlotho, coronary artery stenosis and left ventricular ejection fraction (LVEF) in dialysis patients.

**Methods:** Plasma levels of sKlotho were measured in dialysis patients. LVEF was measured using echocardiography. Coronary artery stenosis was determined by coronary CT scan. Coronary artery occlusion was defined as >50% luminal narrowing. Associations were analysed using linear regression.

**Results:** We included 129 patients (67±7 yrs old, 76% male, 65% HD as dialysis modality). Patients with a low sKlotho (<460pg/mL) had significantly more occluded coronary arteries compared to patients with a high sKlotho.




sKlotho was significantly associated with LVEF, however this association disappeared after adjustment for occluded coronary arteries. Furthermore, patients with high sKlotho used angiotensin receptor blockers (ARBs) and peritoneal dialysis more frequently and had significantly lower PTH.

**Conclusions:** Patients with higher sKlotho levels show significantly less occluded coronary arteries and a better cardiac function than patients with lower sKlotho. Further research is needed to show if low sKlotho levels play a role in the high cardiovascular burden of dialysis patients and if the correlation between ARBs and sKlotho represents a possible therapeutic benefit.

## FR-PO385

**Blood Pressure and Mortality in Patients with End Stage Renal Disease. Results from the CONvective TRAnsport STudy (CONTRAST)** Irina Mostovaya,[1] Michiel Bots,[2] Muriel Grooteman,[3] Marinus A. Van Den Dorpel,[4] Pieter M. Ter Wee,[3] Peter J. Blankestijn.[1] *[1]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]Julius Center for Health Sciences and Primary Care, Univ Medical Center Utrecht, Utrecht, Netherlands; [3]Nephrology, VU Medical Center, Amsterdam, Netherlands; [4]Internal Medicine, Maasstad Hospital, Rotterdam, Netherlands.*

**Background:** Contradictory data has been reported on the relation between blood pressure (BP) and clinical events in dialysis patients. The aim of this study is to determine the association between different measures of BP and all-cause mortality in this population during follow-up.

**Methods:** Data from all 714 patients from the CONvective TRAnsport STudy (CONTRAST), were used for this analysis. The primary outcome of CONTRAST was all-cause mortality. At baseline, BP was measured thrice pre-, and thrice post-dialysis. The means of these pre- and post-dialysis measurements were computed, as well as the mean of all six BP measurements. Adjusted Cox proportional hazard models were used to study the relation between systolic BP (SBP), diastolic BP (DBP) and pulse presuure (PP) and mortality.

**Results:** At baseline mean age was 64±14 years, and 445 (62%) of patients were male. The means (of pre- and post- dialysis measurements averaged) of SBP, DBP and PP were 141±19, 73±11, and 68±16 mmHg respectively. After a median follow-up of 2.9 years a total of 269 patients died. The mean of both the pre- and the post-dialysis BP measurements was found to be more predictive for all-cause mortality, than the pre- or post-measurements alone. Patients in the highest tertile (>77mmHg) of DBP had an increased risk of mortality (hazard ratio (HR): 1.44, 95%CI: 1.03 - 2.02), versus patients in the lowest tertile (DBP<66mmHg, HR:1.0; DBP 66 - 77mmHg: HR: 1.31, 95%CI: 0.93 - 1.74). A similar trend, albeit not significant was observed between mortality risk and SBP, and PP.

**Conclusions:** The mean of both the pre- and the post-dialysis BP measurements is a better predictor for mortality, than pre- or post- measurements alone. Baseline DBP is a better predictor of mortality risk than SBP and PP in ESRD patients.

## FR-PO386

**Blood Pressure Changes over Time in Patients with End Stage Renal Disease. Results from the CONvective TRAnsport STudy (CONTRAST)**
Irina Mostovaya,[1] Michiel Bots,[2] Muriel Grooteman,[3] Marinus A. Van Den Dorpel,[4] Pieter M. Ter Wee,[3] Peter J. Blankestijn.[1] *[1]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]2 Julius Center for Health Sciences and Primary Care, Univ Medical Center Utrecht, Utrecht, Netherlands; [3]Nephrology, VU Medical Center, Amsterdam, Netherlands; [4]Internal Medicine, Maasstad Hospital, Rotterdam, Netherlands.*

**Background:** Limited data is available on changes over time of blood pressure (BP) in dialysis patients. The aim of this study is to assess changes over time of BP in dialysis patients and evaluate the impact of age, gender, medical history and dialysis-treatment related characteristics on these changes.

**Methods:** Data from all 714 patients from the CONvective TRAnsport STudy (CONTRAST), a randomized trial comparing online hemodiafiltration and low-flux hemodialysis, were used for this analysis. BP measurements [systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP) and pulse pressure (PP)] were performed at baseline and every 3 months up to 6 years, three times pre-, and three times post-dialysis. The means of these pre- and post-dialysis measurements were computed, as well as the mean of all six BP measurements (7025 measurements per variable in total). The rate of change over time of these parameters was estimated using linear mixed effects models.

**Results:** At baseline mean age was 64±14 years, and 445 (62%) of patients were male. SBP, DBP, MAP and PP all declined over time. For every incremental 10 years of age at baseline, the yearly excess decline of SBP was 1.7mmHg (p=0.02), and the excess decline of PP was 1.9 mmHg (p=0.002). Patients with a dialysis vintage of >2 years experienced an excess decline in SBP of 2.0 mmHg (p=0.01) and an excess decline in DBP of 0.4mmHg (p=0.003), when compared to those with a vintage of <2 years. Males experienced an excess decline of SBP of 1.6mmHg (p=0.03) and an excess decline of PP of 1.2mmHg (p=0.02) when compared to females.

**Conclusions:** 1) In dialysis patients all parameters of BP (SBP, DBP, MAP, PP) decline over time. 2) Age, gender and dialysis vintage are important determinants of the rate of decline of BP in this patients group.

## FR-PO387

**Effect of Anticoagulation and/or Antiplatelet Drugs on Cerebrovascular Events in Hemodialysis Patients: Miyazaki Dialysis Cohort Study (MID Study)**
Tatsunori Toida,[1] Yuji Sato,[1] Masao Kikuchi,[1] Hiroyuki Komatsu,[1] Shouichi Fujimoto.[2] *[1]Dept of 1st Internal Medicine, Faculty of Medicine, Univ of Miyazaki; [2]Dept of Hemovascular Medicine and Artificial Organs, Faculty of Medicine, Univ of Miyazaki, Japan.*

**Background:** The beneficial effect of antiplatelet (AP) and anticoagulant (AC) drugs on cerebrovascular (CV) events has been shown in the general population, but has not been clarified in hemodialysis (HD) patients. The aim of this study was to examine the association between the onset of CV events and these drugs in Japanese HD patients.

**Methods:** Patients with maintenance HD (n=1,551) were enrolled into the MID Study in December 2009 and were prospectively followed up for 3 years. CV events (cerebral hemorrhage, CH; cerebral infarction, CI) were determined using head CT/MRI in addition to typical neurological symptoms. Kaplan-Meier (KM) and Cox regression (CR) analyses were used to clarify the association between AP and/or AC drugs and CV events.

**Results:** A total of 51 and 74 patients developed CH and CI, respectively, with incidences of 13.1 and 19.2/1000 patient-year. KM analysis showed that CH events were significantly more common in the patients with combination therapy of AP and AC drugs than in other groups. CR analysis revealed that combination therapy (HR 5.06, 95% C.I. 1.71-14.98) was factor contributing to CH, in addition to a past history of CH (HR 4.61, 95% C.I. 2.03-10.48). The incidence of patients with a past history of CI was remarkably higher than in those without this. CR analysis on the patients excluding those with a past history of CI revealed that the presence of atrial fibrillation (AF), diabetes mellitus and age were the predictive factors of CI, while AC and AP therapies were not significant factors. Among the patients with AF, no significant difference was observed in the frequency of CI events between warfarin users and non-users.

**Conclusions:** Combination therapy of AP and AC drugs is a risk factor for developing CH in patients with maintenance HD. HD patients with AF more frequently suffered CI than those without AF; however, preventive effects of AP/AC drugs against developing CI were not evident.

## FR-PO388

**The Subjective Feeling of Thirst and the Presence of Oral Symptoms in People on Hemodialysis and Their Association with Adverse Outcomes: Oral-D Study**
Giovanni F.M. Strippoli,[1,2,3,4] Suetonia Palmer,[5] Marinella Ruospo,[1,2] Patrizia Natale,[1] Valeria Maria Saglimbene,[1,2] Michela Sciancalepore,[1] Letizia Gargano,[1] Fabio Pellegrini,[2] David W. Johnson,[6] Pauline J. Ford,[6] Jonathan C. Craig,[4] Paul Stroumza,[1] Luc Frantzen,[1] Miguel Rodrigues Leal,[1] Marietta Torok,[1] Anna Bednarek,[1] Jan Dulawa,[1] Eduardo Jorge Celia,[1] Ruben Gelfman,[1] Jorgen B.A. Hegbrant,[1] Charlotta Wollheim,[1] Staffan Schön,[1] Michele De benedittis,[3] Massimo Petruzzi.[3] *[1]Diaverum; [2]Mario Negri Sud Consortium; [3]Univ of Bari; [4]Univ of Sydney; [5]Univ of Otago; [6]Univ of Queensland.*

**Background:** The presence of thirst and xerostomia, a subjective complaint of dry mouth, may be increased in people on hemodialysis. The prevalence of thirst and xerostomia and their association with the risk of death and adverse vascular outcomes have not been formally established in large scale cohort studies.

**Methods:** The ORAL-D study is a multinational cohort study of oral diseases in consecutive adults on hemodialysis. We administered xerostomia and thirst inventories based upon validated methodology. We analyzed prevalence data using descriptive analyses and association with death and adverse vascular outcomes is being analyzed with Cox regression.

**Results:** 4324 hemodialysis patients in the participating clinics completed a self-administered questionnaire on oral symptoms. 1274 (30%) patients used candies for dry mouth sensation, 992 (23%) had difficulties swallowing and 2243 (52%) needed to sip to aid swallowing. 1940 (45%) reported waking up during the night to drink, and 2101 (49%) had dry lips. Thirst was a reported problem for 2651 (61%) patients; 3280 (76%) were thirsty during the day and 1995 (46%) during the night. Overall, 1068 (25%) patients reported that thirst influenced their social life. Analyses of association with death and adverse vascular outcomes are in progress.

**Conclusions:** Oral symptoms are highly prevalent in hemodialysis, with marked interference with daily life. Further research will evaluate the impact of these symptoms on clinical outcomes in people in hemodialysis. Analyses of association with death and adverse vascular outcomes are in progress.

## FR-PO389

**The Risk of All Cause and Cardiovascular Mortality in Dentate and Edentulous Patients in Hemodialysis: Oral-D Study**
Giovanni F.M. Strippoli,[1,2,3,4] Suetonia Palmer,[5] Marinella Ruospo,[1,2] Patrizia Natale,[1] Valeria Maria Saglimbene,[1,2] Michela Sciancalepore,[1] Letizia Gargano,[1] Fabio Pellegrini,[2] David W. Johnson,[6] Pauline J. Ford,[6] Jonathan C. Craig,[4] Paul Stroumza,[1] Luc Frantzen,[1] Miguel Rodrigues Leal,[1] Marietta Torok,[1] Anna Bednarek,[1] Jan Dulawa,[1] Eduardo Jorge Celia,[1] Ruben Gelfman,[1] Jorgen B.A. Hegbrant,[1] Charlotta Wollheim,[1] Staffan Schön,[1] Michele De Benedittis,[3] Massimo Petruzzi.[3] *[1]Diaverum; [2]Mario Negri Sud Consortium; [3]Univ of Bari; [4]Univ of Sydney; [5]Univ of Otago; [6]Univ of Queensland.*

**Background:** Dental disease may be a potential determinant of excess death in people on hemodialysis, due to inflammation or as an indicator of general health status. We are exploring whether total teeth loss predicted mortality (total and cause-specific) in patients on hemodialysis.

**Methods: ORAL-D** is an ongoing multinational prospective cohort study of consecutive adults receiving hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Italy, Hungary, Poland, Argentina, Portugal, France and Spain. A dental surgeon evaluated the presence or absence of teeth during a standardized oral examination. We assessed survival at 12 months using centralized mortality data. We conducted analysis using a Cox regression controlling for age, gender, previous cardiovascular events, income status, clinical performance measures, dialysis prescription and performance indicators, and depressive symptoms.

**Results:** 4720 hemodialysis patients in participating clinics received a complete dental evaluation and completed follow up. Median follow up 8 (6.5 to 7.8)months. 868 patients were edentulous (20%) and 344 (10%) died during follow up. Complete loss of teeth had uncertain associations with risks of all-cause (HR 1.06 [95% CI, 0.86-1.31]) and cardiovascular mortality (HR 0.90 [95% CI 0.66-1.22]) when adjusted for potentially confounding variables. Complete 12 month follow-up data will be available by September 2013.

**Conclusions:** Dentate status has uncertain associations with all-cause or cardiovascular mortality in patients on hemodialysis. ORALD will be completed by end of 2013.

## FR-PO390

**Snoring Is a Strong Amplifier of the Risk by Heart Failure for All Cause and Cardiovascular Mortality in Chronic Kidney Disease in Patients on Dialysis (Stage 5D-CKD)**
Claudia Torino,[1] Graziella D'arrigo,[1] Maurizio Postorino,[1] Giovanni Tripepi,[1] Francesca Mallamaci,[1] Carmine Zoccali,[1] Progredire Work Group.[2] *[1]Clin. Epid. and Physiopath. of Renal Dis. and Hypertens., CNR-IBIM, Reggio Calabria, Italy; [2]PROGREDIRE Working Group, Italy.*

**Background:** Self-reported snoring, an indicator of sleep disordered breathing (SDB), may associate with mortality in the general population and in pts with heart failure (HF). SDB and HF are frequent in the stage 5D-CKD population but the hypothesis that snoring

may affect the HF-mortality link in these patients (pts) has never been tested. The issue is important because SDB has been in part attributed to reversible pharyngeal edema secondary to volume expansion in HF pts and may therefore be a modifiable risk factor.

**Methods:** We investigated this problem in a cohort of 827 stage 5D-CKD pts. HF was assessed at baseline on the basis of clinical symptoms and radiological/echocardiographic examinations. At enrolment, participants provided self-reported information about snoring and were followed up for a median time of 28 months.

**Results:** 132 pts had HF at baseline. Overall, 194 pts were classified as heavy snorers, 308 as moderate snorers and 325 as non-snorers. During the follow-up, 233 pts died (CV causes: 54%). Both on univariate (P<0.001) and multiple (P<0.02) Cox analyses, HF significantly predicted the study outcomes whereas snoring did not (P=NS). However, snoring was a strong modifier of the risk of HF for the study outcomes. In Cox models (including Framingham factors, anti-hypertensive treatment, CV comorbidities, dialysis vintage, CRP, P, Hb and albumin), the hazard ratios (HR) associated to HF for the study outcomes were highest in heavy snorers [all-cause death:HR:2.5, P<0.001;CV death:HR:3.1,P<0.001], intermediate in moderate snorers [all-cause death:HR:1.5,P=0.01); CV death:HR:1.6,P=0.009) and not significant in non-snorers [all-cause death:HR:0.9; CV death:HR:0.8].

**Conclusions:** Snoring is an effect modifier of the HF-mortality link in stage 5D-CKD pts. Clinical trials are needed to verify whether intensified surveillance and treatment (UF intensification) of HF snorers on dialysis may translate into better clinical outcomes in these pts.

*Funding:* Government Support - Non-U.S.

## FR-PO391

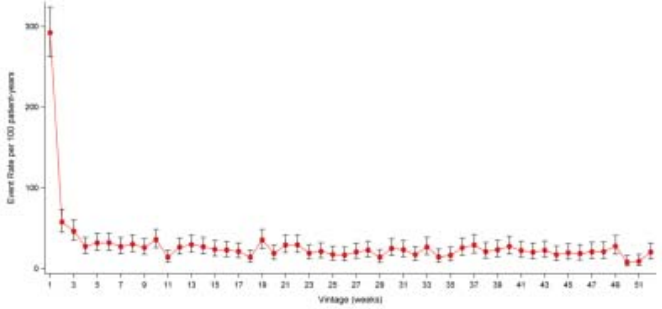
**Early Cardiovascular Events in an Incident European Hemodialysis Cohort**

Kai-Uwe Eckardt,[1] Iain A. Gillespie,[2] Stefan D. Anker,[3] Sharon Richards,[2] Daniele Marcelli,[4] Marc Froissart,[5] Jürgen Floege.[6] *[1]Univ of Erlangen-Nuremberg, Germany; [2]Amgen Ltd, United Kingdom; [3]Charite Campus Virchow-Klinikum, Germany; [4]Fresenius Medical Care (FME), Germany; [5]Amgen Europe GmbH, Switzerland; [6]RWTH Univ of Aachen, Germany.*

**Background:** Hemodialysis (HD) initiation is associated with high all-cause and cardiovascular (CV) deaths rates. However, there is a paucity of data on the occurrence and potential causes of cardiovascular events (CVE) during this crucial period. No studies have examined risk by underlying CV cause.

**Methods:** This study aimed to decipher the early CVE risk in a European Fresenius Medical Care HD patient cohort ('AROii') with a dialysis vintage of <7 days (median 4d) . Monthly rates of the composite fatal/nonfatal CVEs were calculated for the first 2 years on HD. High-risk periods were identified by comparing, with Rate Ratios (RRs), monthly first year rates with the second year rate. Constituent events were also examined.

**Results:** 2411 CVE were reported for 1454/6322 patients (23%). The first year rate (30.4/100 person-years (PY) [95% Confidence Interval (CI) 28.9-31.9]) greatly exceeded the second (19.4/100PY [CI 18.0-20.8]). Whilst incidence was generally highest in the first month and particularly in the first week (figure), the high risk period extended to month 5 (RR 2.17 [CI 1.97-2.38]). High risk periods differed by CVE category (coronary artery: months 1-2; cerebrovascular: month 1; peripheral arterial: month 1; congestive heart failure: months 1-4; sudden cardiac death: month 8). Rate of CVE by weekly dialysis vintage.




**Conclusions:** CVE incidence in early weeks after HD initiation remains very high and is not confined to specific CV disease categories. Extended high risk periods suggest the potential to prevent events in early HD based on risk factor identification, risk stratification and preventive care.

*Funding:* Pharmaceutical Company Support - Amgen (Europe) GmbH

## FR-PO392

**Challenging Physical Activity in Dialysis Patients**

Marine Panaye,[1] Anne Kolko-Labadens,[2] Catherine Deforges-lasseur,[3] Marie Paule Guillodo,[4] Martial Levannier,[5] Daniel Teta,[6] Denis Fouque.[7] *[1]Nephrology, Hopital Edouard Herriot, Lyon, France; [2]Dialysis, AURA Nord, Saint Ouen, France; [3]AURAD Aquitaine, Gradignan, France; [4]AUB Santé, Brest, France; [5]Amgen, Neuilly/Seine, France; [6]Nephrology, CHUV, Lausanne, Switzerland; [7]Nephrology, Centre Hospitalier Lyon Sud, Pierre Benite, France.*

**Background:** Physical inactivity is a risk factor for mortality in dialysis patients and is still poorly documented during maintenance dialysis (MD). The Step by Step initiative aimed at evaluating the weekly physical activity on a large cohort of dialysis patients.

**Methods:** All French nephrologists were invited to participate in this national prospective multicenter observational study. 149 investigators included 1163 adult patients who were on MD since more than 3 months. Physical activity was assessed by the number of daily steps measured by a pedometer (model NO PE317C multifunction pedometer, USA) for 7 consecutive days.

**Results:** Median age was 63 yr (Q1 51-Q3 75), 66% were male. Most patients (95%) were on hemodialysis (median 3yr). 24% were diabetic, 69% had hypertension, and 22% had peripheral arterial disease. 63% were sedentary (<5000 steps/day) with a median of 3688 steps /day. Daily steps were lower on dialysis days (DD) (2912, 1439-5232) compared to non DD (4054, 2136-7108) (p <0.001). Physical activity decreased with age with 57% being sedentary between 50 and 65 yr and above up to 83% after 80 yr. Number of steps increased with dialysis vintage from 3620 steps in patients on dialysis for <5 yr (66% pts) to 4465 for dialysis time>10 yr (18% pts).

**Conclusions:** This survey, largest ever done, highlights the low level of physical activity in MD patients. Patients are mostly sedentary with a number of daily steps less than 5000. Activity is more reduced if they are older and on MD for a shorter time (suggesting that frail patients died earlier). These results confirm the need to enforce rehabilitation programs that promote intra and inter dialytic physical exercise.

*Funding:* Pharmaceutical Company Support - Amgen

## FR-PO393

**Advanced CKD Results in Reduced Cardiovascular Reserve with Loss of Adaptive Cardiac Alterations When Compared to Hypertension**

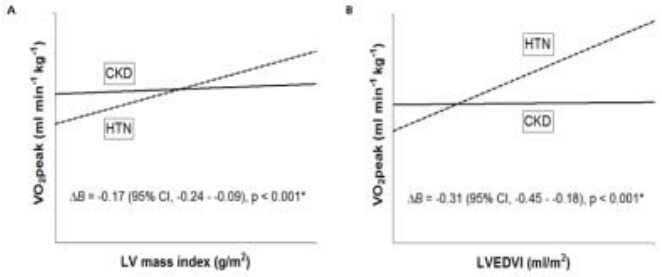
Stephen M.S. Ting,[1,2] Thomas Hamborg,[2] Gordon McGregor,[1] Kenneth Lim,[4] Nicolas Aldridge,[1] David Oxborough,[3] Sudheer Koganti,[1] Rosemary Bland,[2] Robert Higgins,[1] Prithwish Banerjee,[1] Daniel Zehnder.[1,2] *[1]Univ Hospitals Coventry&Warwickshire NHS Trust; [2]Warwick Medical School; [3]Liverpool JohnMoores Univ, United Kingdom; [4]Oregon Health & Science Univ.*

**Background:** Oxygen consumption at peak exercise ($VO_2$peak) is reduced in advanced CKD patients. To-date, no studies have examined whether the reduced functional measure of cardiovascular reserve in CKD is related to an impaired cardiovascular compliance. We also postulate that loss of adaptive cardiac alterations is a contributor to low $VO_2$peak in CKD.

**Methods:** We assessed 136 kidney transplant-waitlisted patients in parallel with 80 healthy hypertensive subjects prospectively between 2010-2012. Cycle-ergometric exercise testing, TD-echocardiography, pulse wave velocity (PWV) were performed.

**Results:** $VO_2$peak was lower in the CKD subjects compared with the hypertensives (19 vs 23 ml $min^{-1}kg^{-1}$,p<0.001). Independent predictors of $VO_2$peak for CKD were PWV(coefficient, *B*=-5.4), LV end-diastolic volume index(LVEDVI;*B*=0.1) & LV filling pressure(E/mean e';*B*=-6.8); in the hypertensives, these were LV mass index(*B*=0.4) & LVEDVI(*B*=0.4).

In demographic-adjusted multiple regression model, significant predictors in the CKD were E/mean e'(*B*=-4.7) & albumin(*B*=0.3); in the hypertensives, it was LVEDVI(*B*=0.3). Interestingly, higher LV mass index & LVEDVI were associated with greater $VO_2$peak in the hypertensives than the CKD subjects.




**Conclusions:** This is the first study to suggest that impaired LV and arterial compliance are mechanistic factors resulting in reduced cardiovascular reserve in advanced CKD. Our data is indicative of dysregulated LV function in CKD patients beyond predominantly ventricular wall changes that occur in otherwise healthy hypertensive patients.

## FR-PO394

**Exercise Anaerobic Threshold Predicts Survival Pre- and Post- Kidney Transplantation**

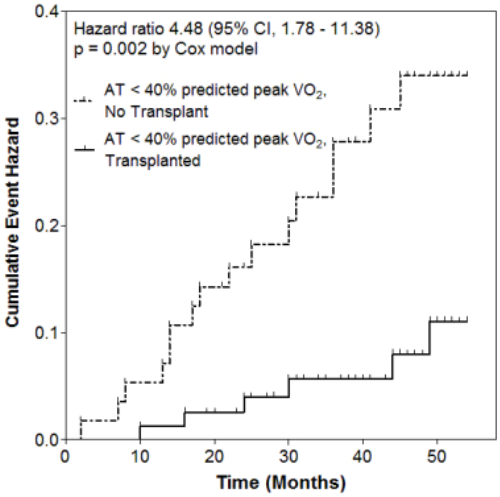
Stephen M.S. Ting,[1,4] Hasan Iqbal,[2] Thomas Hamborg,[5] Nicolas Aldridge,[1] Nithya Krishnan,[1,4] Prithwish Banerjee,[2] Rosemary Bland,[4] Robert Higgins,[1] Daniel Zehnder.[1,4] *[1]Renal Medicine & Transplantation, Univ Hospitals Coventry & Warwickshire NHS Trust; [2]Cardiology, Univ Hospitals Coventry & Warwickshire NHS Trust; [3]Vascular Surgery, Univ Hospitals Coventry & Warwickshire NHS Trust; [4]Metabolic & Vascular Health, Warwick Medical School, United Kingdom; [5]Health Sciences Statistics & Epidemiology, Warwick Medical School, United Kingdom.*

**Background:** Reduced anaerobic threshold (AT) carries a poor prognosis among patients with impaired cardiovascular reserve. This study investigated the prognostic capacity of AT on survival in CKD patients waiting for a kidney transplant and the impact of transplantation on survival in those with reduced cardiovascular reserve.

**Methods:** We performed cardiopulmonary exercise testing in 240 patients who were waitlisted for kidney transplantation between 2008 and 2010. Clinical, exercise and 5-year mortality data were compared.

**Results:** 24 patients who died had a significantly lower AT than the survivors (29.9 vs. 39.7% of predicted peak $VO_2$, p<0.001). Non-survivors were more likely to have CVD (38 vs 13%, p=0.002) and dialysis vintage >1 year (79 vs 49%, p=0.005).

According to Kaplan-Meier estimates, a significantly reduced 5-year cumulative overall survival rate (p<0.001) was observed in patients with AT<40% compared to those with AT≥40% predicted $VO_2$peak. Survival in the cohort with AT<40% was significantly different in kidney transplanted (6 deaths) vs non-transplanted patients (17 deaths) with a hazard ratio of 4.5 (95%CI, 1.8–11.4, p=0.002).




**Conclusions:** This is the first prospective study to demonstrate an association of the functional cardiovascular reserve AT with overall survival in advanced CKD patients. High risk patients with reduced cardiovascular reserve had a better survival after receiving a kidney transplant.

## FR-PO395

### Serum FGF-23 Associated with the Progression of Coronary Artery Calcification in Hemodialysis Patients

Alaattin Yildiz, Abdullah Ozkok. *Internal Medicine, Nephrology, Istanbul, Turkey.*

**Background:** Disordered mineral metabolism is implicated in the pathogenesis of vascular calcification in hemodialysis (HD) patients. Fibroblast growth factor 23 (FGF-23) is the main regulator of phosphate metabolism. In this prospective study, we aimed to investigate the association of serum FGF-23 with progression of coronary artery calcification in HD patients.

**Methods:** Seventy-four HD patients(36 male/38 female,mean age:52±14 years) were included. Serum FGF-23 levels were measured by ELISA. Coronary artery calcification score(CACS) was measured twice with one year interval. Patients were grouped as progressive (PG)(36 patients-48%) and non-progressive (NPG).

**Results:** Age, serum phosphorus, baseline and first year CACS were found to be significantly higher in the PG compared to NPG group. Serum FGF-23 levels were significantly higher in PG (468±132 vs 190±272 pg/mL, p=0.04). Patients were divided into two groups according to baseline CACS (low group, CACS≤30; high group, CACS>30). Serum FGF-23 levels were significantly correlated with the progression of CACS (ΔCACS) in the low baseline CACS group (r=0.510, p=0.006), but this association was not found in high baseline CACS group (r=0.11, p=0.44). In logistic regression analysis for predicting the PG patients; serum FGF-23, phosphorus levels and baseline CACS were retained as significant factors in the model.

**Conclusions:** Serum FGF-23 was found to be related to progression of CACS independent of serum phosphorus levels. FGF-23 may play a major role in the progression of vascular calcification especially at the early stages of calcification process in HD patients.

*Funding:* Government Support - Non-U.S.

## FR-PO396

### Impacts of Dry Weight Determined by Calf Bioimpedance Ratio on Carotid Stiffness and Left Ventricular Hypertrophy in Hemodialysis Patients

Yi-Lun Zhou, Li-jie Ma, Fang Sun, Yang Shen, T.G. Cui. *Dept of Nephrology, Chao-Yang Hospital, Capital Medical Univ, Bei-Jing, China.*

**Background:** Our previous study has shown that modification of bioimpedance technique by the measurement of bioimpedance ratio in the calf (Calf-BR = impedance at 200 kHz / impedance at 5 kHz)) was a simple and practical method in assessing fluid status in hemodialysis patients. Under physiological conditions, the Calf-BR was positively related to the ratio of extracellular volume to total body water. However, the consequences of periodical dry weight (DW) adjustment under the guidance of Calf-BR on target organ damage have not been investigated.

**Methods:** 115 hemodialysis patients were enrolled in this pilot trial. Patients were divided into bioimpedance group and control group according to their dialysis schedule. In bioimpedance group, DW was routinely adjusted under the guidance of Calf-BR every 3 month. In control group, the assessment of DW remained a clinical judgment. Carotid stiffness, left ventricular mass index(LVMI), and Calf-BR were measured at baseline and at the 12th month in both groups. Home blood pressure (BP) was monitored monthly. Dialysis-related adverse events were recorded.

**Results:** No significant differences were observed in parameters between the two groups at baseline. Compared to control group, bioimpedance group had significantly lower values in terms of the annual averages of systolic home BP (147.4±15.3mmHg vs 152.6±16.9mmHg, p=0.019), carotid stiffness index β (10.7±3.3 vs 12.2±3.1, p=0.003), LVMI (155.21±15.64 g/m$^2$ vs 165.17±16.76 g/m$^2$, p<0.001), and the percentage of individuals with Calf-BR over target range (p=0.040) at month 12, with less annual averages of antihypertensive medications used and lower frequency of intradialytic hypotension, muscle cramp, and clotted angioaccess.

**Conclusions:** Probing DW via periodical measurement of Calf-BR may have the potential to improve arterial stiffness and left ventricular hypertrophy. In addition, this intervention may also decrease incidence of intradialytic hypotension, muscle cramp, and arteriovenous fistula complications in hemodialysis patients.

*Funding:* Government Support - Non-U.S.

## FR-PO397

### The Progress in Malnutrition and Inflammatory Conditions Affect on Adverse Events and Mortality in the Patients on Maintenance Hemodialysis (MHD)

Takahiro Kuragano, Takeshi Nakanishi. *Internal Medicine, Div of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo.*

**Background:** In MHD, protein-energy malnutrition can be critical and is one of the main causes of mortality. Nutritional and inflammatory condition could progress or regress during follow-up period, and may cause the adverse events and premature mortality in MHD. However, few studies reported the influence of changes in nutritional or inflammatory status on adverse events or survival in MHD.

**Methods:** Study design: Prospective, observational multi center study. Observational period: 2 years. Subject and measurement: In 1086 MHD patients, blood levels of Hb, albumin, prealbumin, high sensitive CRP (hCRP), were measured every 3 month. And body mass index (BMI) is also evaluated.

**Results:** In logistic regression analysis, Hb (p=0.008, Exp (β)=0.797) and pre-albumin (p=0.001, Exp (β) =0.968) were selected as the significant predictors of the change in BMI during the follow up period. Moreover, age (p=0.003, Exp (β) =1.039) was selected as a significant predictor of the change in serum albumin. In the time dependent cox hazard model, patients who were under 62 years old, there were no significant changes in BMI and serum albumin levels during 2 years. On the other hand, in the patients over 62 years old, BMI (p=0.0025, from 21.4 ± 0.17 to 21.2 ± 0.17) and serum albumin (p=0.0001, from 3.72 ± 0.015 to 3.66 ± 0.017) levels significantly decreased during the follow-up period. Patients who showed the decline in BMI was associated with elevated risk for cerbro-cardiovascular disease (CCVD) (p=0.012, HR: 2.19) and hospitalization (p=0.002, HR: 1.57). Moreover, patients who showed the decline in serum albumin also associated with higher risk at infection disease (p=0.001, HR: 1.55) and hospitalization (p=0.049, HR: 1.35). On the other hand, patients who showed the increase in hCRP had a higher risk for CCVD (p=0.023, HR: 0.44) and death (p=0.023, HR: 0.32).

**Conclusions:** This study revealed that the downward trend of nutritionnal status was prominent in elderly MHD. Furthermore, the progress in malnutrition and inflammation might be associated with the several adverse events in MHD patients.

## FR-PO398

### Increased Serum Levels of GDF-15 Are Associated with Cardiovascular Death, Subclinic Atherosclerosis in Patients on Maintenance Hemodialysis

Hakki Yilmaz,[1] Huseyin tugrul Celik,[2] Ali Akcay,[1] Ozgul Malcok Gurel,[3] Ayse Mukadder Bilgic,[1] Nuket Bavbek.[1] *[1]Dept of Internal Medicine and Nephrology, Turgut Ozal Univ, School of Medicine, Ankara, Turkey; [2]Dept of Biochemistry, Turgut Ozal Univ, School of Medicine, Ankara, Turkey; [3]Dept of Cardiology, Turgut Ozal Univ, School of Medicine, Ankara, Turkey.*

**Background:** Increased carotid intima media thickness(CIMT) was shown to be independent predictor of cardiovascular(CV) mortality in dialysis and general population. Growth differentiation factor 15(GDF-15), a member of the transforming growth factor superfamily, is produced by cardiomyocytes and atherosclerotic lesions under stress conditions such as inflammation. Increased GDF‐15 levels may be suggested as powerful biomarker for cardiovascular diseases, atherosclerosis and mortality. We assessed associations between serum concentrations of GDF-15, mortality and CIMT for subclinic atherosclerosis in hemodialysis(HD) patients.

**Methods:** Eihgty-seven patients on maintenance hemodialysis and 45 sex and age-matched healthy controls were included in this prospective study. Serum GDF-15 levels were measured by ELISA. CIMT was assessed by Doppler ultrasonography. Association between serum GDF-15 level and mortality was assessed in Cox regression analysis with serum levels categorized into two groups according to median value(328.18 pg/ml). Patients were followed for 2 years and had cause-specific and all-cause mortality determined.

**Results:** The mean serum GDF-15 levels were significantly higher in HD patients than controls (376.57±216.78 vs. 176.32±43.62 p<0.01, respectively). Serum GDF-15 levels were correlated to CIMT ($\rho$=0.607, P<0.01), CRP ($\rho$=0.250, P<0.01) and serum albumin ($\rho$=-0.156, P=0.03). The multivariate analysis revealed that GDF-15 was found to be an independent variable of CIMT in HD patients. In the study, serum GDF-15 level was an independent marker of cv mortality when adjusted for age, CRP, history of diabetes mellitus.

**Conclusions:** The relationship demonstrated between serum GDF-15, mortality, and carotid artery thickening suggests that GDF-15 may be a novel marker of atherosclerosis, inflammation and malnutrition in HD patients.

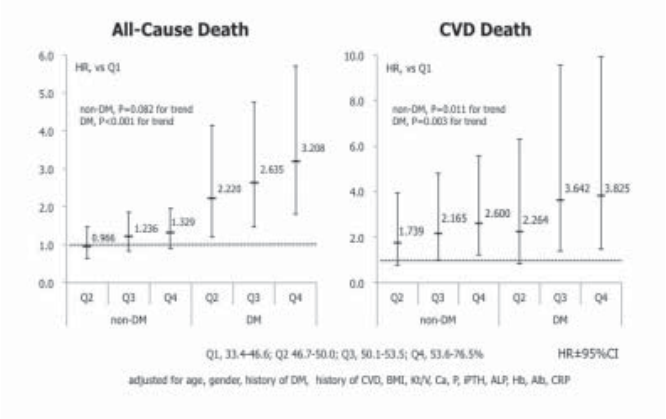
**FR-PO399**

**Cardiothoracic Ratio and All-Cause and Cardiovascular Disease Mortality in Patients on Hemodialysis: Results from the MBD-5D Study**
Hiroaki Ogata,[1] Masahide Mizobuchi,[2] Eriko Kinugasa,[1] Masafumi Fukagawa,[3] Tadao Akizawa,[2] Shunichi Fukuhara.[4] *[1]Dept of Internal Medicine, Showa Univ Northern Yokohama Hospital, Yokohama, Japan; [2]Div of Nephrology, Dept of Medicine, Showa Univ School of Medicine, Tokyo, Japan; [3]Div of Nephrology, Endocrinology and Metabolism, Tokai Univ School of Medicine, Isehara, Japan; [4]Dept of Healthcare Epidemiology, Graduate School of Medicine, Kyoto Univ, Kyoto, Japan.*

**Background:** The cardiothoracic ratio (CTR) is a non-invasive index of volume status and left ventricular hypertrophy. However, whether or not CTR is associated with cardiovascular disease (CVD) event and mortality in hemodialysis population is unclear.

**Methods:** The MBD-5D is a multicenter, prospective observational study of hemodialysis patients with secondary hyperparathyroidism (SHPT) in Japan. Data from MBD-5D study were analyzed to assess the association between CTR and all-cause, CVD mortality or CVD hospitalization in HD patients (N=3,194, Age 61.9 yrs, female 38.6%, diabetes mellitus (DM) 24.2%, HD vintage 121.7 months).

**Results:** CTR was significantly associated with age, gender, HD vintage, comorbidity, Kt/V, Hb, SCr, P, iPTH levels, and P binders use. All-cause, CVD mortality, and CVD hospitalization increased across quartiles of CTR (Log rank test, P<0.0001, P<0.0001, and P<0.0001, respectively). In time dependent Cox regression analyses, all-cause death increased across quartiles of CTR in DM patients, but in non-DM patients CVD death was significantly higher in patients with the higher quartile of CTR compared to those with the lowest quartiles of CTR in non-DM and DM patients.




Higher CTR across quartiles was associated with greater risk of CVD hospitalization in non-DM patients, but not in DM patients.

**Conclusions:** CTR is associated with all-cause, CVD mortality and hospitalization in HD patients with SHPT.

*Funding:* Pharmaceutical Company Support - Kyowa Hakko Kirin Co, Ltd.; The MBD-5D is supported by research grants from Kyowa Hakko Kirin co., Ltd., without restrictions on publications.

**FR-PO400**

**Effects of Acute Intradialytic Exercise on Blood Pressure and Circulating Cytokines**
Maurice Dungey,[1,2] Nicolette C. Bishop,[1] Hannah M.l. Young,[2] James O. Burton,[2] Alice C. Smith.[2] *[1]School of Sport, Exercise and Health Sciences, Loughborough Univ, United Kingdom; [2]Leicester Kidney Exercise Team, Univ Hospitals of Leicester, United Kingdom.*

**Background:** Regular exercise can enhance the quality of life of haemodialysis (HD) patients. Intradialytic cycling (IDC) is increasingly offered on HD units but its potential benefits and drawbacks have not been fully investigated. In the general population, exercise causes a transient increase in blood pressure (BP) which falls below resting levels after exercise. Exercise can also stimulate IL-6 production from contracting myocytes, initiating rises in circulating anti-inflammatory cytokines such as IL-1ra. Patients often experience BP fluctuations during HD and are frequently in a state of chronic systemic inflammation which can be exacerbated by HD. This study examined the effects of a bout of IDC on BP and circulating IL-6 and IL-1ra levels.

**Methods:** 10 HD patients (age 59.6 ± 8.3yr) completed two trials in a randomised crossover design. On the control trial patients rested throughout dialysis. On the exercise trial, after 60 min of dialysis, patients cycled for 30 min at a "somewhat hard" intensity (rating of perceived exertion 13) during dialysis. BP was measured before dialysis and with blood samples at pre, post, 1h post-exercise and at the end of dialysis (or equivalent times during control).

**Results:** Systolic BP (SBP) decreased after initiation of dialysis on both trials (P<0.01). ANOVA revealed a time*trial interaction (P=0.003): SBP was significantly higher immediately post-exercise (131 ± 18 vs. 117 ± 22mmHg) and lower 1h post-exercise (109 ± 26 vs. 121 ± 26mmHg) compared with control. Plasma IL-6 increased during dialysis in both trials (P=0.015). Fold-changes in IL-6 and IL-1ra did not differ between trials.

**Conclusions:** IDC does not exacerbate markers of systemic inflammation. SBP increases with exercise and falls substantially in the hour afterwards compared with a resting control trial. As intradialytic BP changes have significant implications for patient outcomes, this novel finding highlights the importance of patient monitoring during and after IDC. The longer term impact of these observations requires further investigation.

*Funding:* Government Support - Non-U.S.

**FR-PO401**

**Effects of Sleepiness on Survival in Japanese Hemodialysis Patients: J-DOPPS-Q1**
Kunitoshi Iseki,[1] Kazuhiko Tsuruya,[2] Eiichiro Kanda,[3] Takanobu Nomura,[4] Hideki N. Hirakata.[5] *[1]Dialysis Unit, Univ Hospital of the Ryukyus, Nishihara, Okinawa, Japan; [2]Internal Medicine, Kyushu Univ, Fukuoka, Japan; [3]Dept of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences,, Tokyo Kyosai Hospital, Tokyo, Japan; [4]Clinical Research Management Dept Development Div, Kyowa Hakko Kirin Co., Ltd, Tokyo, Japan; [5]Internal Medicine, Fukuoka Red-Cross Hospital, Fukuoka, Japan.*

**Background:** Sleep disorder and poor sleep quality have been claimed as a cause of morbidity and mortality. Survival of Japanese HD patients is better than any other countries, yet the reasons are not clear. Among them, the relationship between the degree of sleepiness and survival has not been studied.

**Methods:** We studied the degree of sleepiness in 1,252 adults (age 20 and over) HD patients using the Japanese version of the Epworth Sleepiness Scale (JESS) questionnaire which was developed to apply for Japanese. Demographic data were presented as mean (standard deviation) for three subgroups: low, intermediate, and high JESS score. Kruskal-wallis test or analyses of covariance were performed to compare the significance of discrete variables and with chi-square test or Fisher's exact test for categorical variables. Cox proportional Hazard regression analysis was performed to estimate the independent effect of several variables on survival.

**Results:** During the study period, there were 85 deaths and 652 hospitalizations. The survival was poor in the high JESS score group. The hazard ratio (95% confidence interval) was 2.268 (1.240-4.148, P=0.008) when the reference was those with low JESS score after adjusting for age, vintage of HD, gender, diabetes, BMI, CVD, HD treatment regimen, laboratory data, and medication. Relative risk of death was significantly higher in high JESS score groups even after multiple adjustments. Patients with elderly, 70 years and over, comorbid conditions such as congestive heart failure, stroke, and diabetes showed significantly higher JESS score, 16 and over.

**Conclusions:** Results showed that the degree of sleepiness is related to survival in Japanese HD patients, in particular elderly patients.

**FR-PO402**

**NT-proBNP Is a More Significant Prognostic Biomarker for Mortality Than Troponin T in Incident Hemodialysis Patients**
Ji Suk Han,[1] Hyung Jung Oh,[1] Hyang Mo Koo,[1] Chan Ho Kim,[1] Tae-Hyun Yoo,[1,2] Shin-Wook Kang.[1,2,3] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul; [3]On behalf of the Clinical Research Center for ESRD Investigators, Korea.*

**Background:** Numerous previous studies have demonstrated that cardiac and inflammatory biomarkers are significant predictors of cardiovascular (CV) and all-cause mortality in ESRD patients. However, these studies were limited by retrospective analyses, inclusion of small numbers of patients, mostly prevalent dialysis patients, and measurement of one or two biomarkers. In this study, we investigated the association between three biomarkers (NT-proBNP, cTnT, and hsCRP) and mortality in incident hemodialysis patients.

**Methods:** A prospective cohort of 864 incident hemodialysis patients from 36 centers of the CRC for ESRD in Korea was followed for up to 30 mo. CV and all-cause mortality were compared between 'high' and 'low' groups according to the median baseline values of biomarkers. Cox proportional hazard analysis was performed to determine the independent prognostic value of each biomarker for CV and all-cause mortality.

**Results:** CV survival rates were significantly lower in the 'high' NT-proBNP and cTnT groups compared to the corresponding 'low' groups. However, there was no significant difference in CV survival rates between the 'high' and 'low' hsCRP groups. All-cause mortality rates were significantly higher in all three 'high' groups. In multivariate Cox models, natural log value (Ln) of NT-proBNP and cTnT but not Ln hsCRP were found to be significant independent predictors of CV (Ln NT-proBNP, HR=2.228, P=0.002; and Ln cTnT, HR=1.274, P=0.015) and all-cause mortality (Ln NT-proBNP, HR=1.392, P=0.012; and Ln cTnT, HR=1.182, P=0.034). Moreover, among the three biomarkers, NT-proBNP had the highest positive predictive values for not only CV mortality (AUC=0.812, P<0.001) but also all-cause mortality (AUC=0.666, P=0.003).

**Conclusions:** Although high levels of NT-proBNP and cTnT, but not hsCRP, were independently associated with CV and all-cause mortality in incident hemodialysis patients, the prognostic value of NT-proBNP for mortality were higher than that of cTnT.

*Funding:* Government Support - Non-U.S.

**FR-PO403**

**Real-Time Monitoring of Cardiovascular Status during Routine Hemodialysis** D. Miskulin,[1] Klemens B. Meyer,[1] John E. Moran.[2,3] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Intelomed, Inc, Pittsburgh, PA; [3]Nephrology, Stanford Univ School of Medicine, Stanford, CA.*

**Background:** Intradialytic hypotension (IDH) is common among patients on maintenance hemodialysis (HD) and may contribute to the high rate of cardiovascular (CV) events and mortality. Studies have shown IDH to be associated with impaired myocardial perfusion and stunning during HD.

**Methods:** 25 patients with a history of frequent hypotensive episodes during routine HD were monitored during 4 HD treatments using the CVInsight device (Intelomed, Inc), which captures and processes the signal from a standard pulse oximeter (Nonin Medical, Inc) to display the rates of change of pulse frequency and pulse amplitude. We assessed the device's prediction of intradialytic events, but did not intervene on the basis of the CVInsight display. Events were defined as either active staff intervention because of perceived hemodynamic instability, such as reduction in ultrafiltration rate or administration of saline, OR a fall in systolic BP below 90 mm Hg.

**Results:** 22 patients (88%) experienced one or more events: 30 episodes of systolic BP < 90 mm Hg, 25 episodes of intervention for symptoms suggesting hypovolemia, including lightheadedness, cramping, and weakness. Events occurred a mean of 93 min. into treatment. A recognized event did not follow 22 false positive alerts; the device yielded 12 false negatives, failing to predict an event, for sensitivity 78.2 % and specificity 51.1 %. CVInsight device alerts occurred a mean of 53 min. into treatment, giving a mean lead time of 40 mins. to allow intervention to prevent CV events.

**Conclusions:** The CVInsight device provides non-invasive real-time monitoring of CV status during HD and predicts the majority of CV events with a lead time which would allow intervention to prevent such events.

*Funding:* Pharmaceutical Company Support - Intelomed, Inc

**FR-PO404**

**Is There an Association between Copeptin and NT-proBNP in Hemodialysis Patients?** Jae Won Yang, Jae Seok Kim, Seung-Ok Choi, Byoung Geun Han. *Nephrology, Yonsei Univ Wonju College of Medicine, Wonju, Gangwon, Republic of Korea.*

**Background:** Copeptin, precursor to vasopressin, is associated with body fluid volume and heart dysfunction. Therefore, this study was intended to investigate the level of copeptin and the relationships with fluid and heart dysfunction markers in patients with hemodialysis.

**Methods:** This study included forty-one patients with hemodialysis. At the time of their visit for hemodialysis treatment, laboratory data including NT-proBNP was collected and excessive body fluid (OH, overhydration, liter) was measured by bioimpedance spectrometry (BIS). In addition, E/Ea ratios were obtained by echocardiography for assessing left ventricular dysfunction (LVDs).

**Results:** The mean concentration of pre-dialysis copeptin was 224.67±241.91 pg/ml. Pre-dialysis copeptin had positive correlation with pre-dialysis OH (r=0.314, P=0.046), but not with NT-proBNP (r=0.163, P=0.308) and E/Ea ratios (r=-0.023, P=0.888). Body fluid markers other than copeptin, such as NT-proBNP and pre-dialysis OH, E/Ea ratio, all showed significant correlation with each other. Based on previous reports, non-LVDs and LVDs in hemodialysis patients were defined by a cut off value of NT-proBNP 5300 [pg/ml] (specificity 0.8, sensitivity 0.93). When comparing the non-LVDs group with the LVDs group (non-LVDs vs. LVDs), the results showed significant differences in pre-dialysis copeptin (141.33±209.20 vs. 259.9±255.7 pg/ml, P=0.014), NT-proBNP (2294.70±1233.53 vs. 22826.85±11739.56 pg/ml, P=0.000), pre-dialysis OH (1.75±1.03 vs. 3.15±1.90 liters, P=0.023), E/Ea ratio (4.33±1.30 vs. 6.60±2.27, P=0.002), KtV (1.32±0.13 vs. 1.47±0.25, P=0.014). In addition, the ROC curve for discriminating LVDs from non-LVDs showed that the AUC of pre-dialysis copeptin was 0.737 (P=0.023), and the cut-off value was 125.48 pg/ml (specificity 0.8, sensitivity 0.7).

**Conclusions:** The level of pre-dialysis copeptins was elevated in patients with hemodialysis. In addition, copeptin was correlated with pre-dialysis volume status. However, copeptin did not show a significant relationships with NT-proBNP, E/Ea ratio. Finally, the patients with LVDs showed higher level of pre-dialysis copeptins than the patients without LVDs.

**FR-PO405**

**Clinical Impacts of Hemocontrol Biofeedback System on Hemodialysis Patients** Jae Won Yang, Jae Seok Kim, Seung-Ok Choi, Byoung Geun Han. *Nephrology, Yonsei Univ Wonju College of Medicine, Wonju, Gangwon, Republic of Korea.*

**Background:** Hemocontrol™ biofeedback system (Gambro) is the new dialysis method for reducing IDH (Intradialytic hypotension). We investigated how this new method affects the dialysis patients in this study.

**Methods:** 7 patients undergoing hemodialysis were enrolled in this study. Study period was 26 weeks, composed of stage A, B1 and B2. 'A' stage was control period, B1 and B2 were identical period for applying hemocontrol system. In each stage, laboratory data and clinical parameters including dialysis and ambulatory blood pressure (BP), pre-post dialysis body weight (BWt), total body water (TBW) measured by bioimpedence spectroscopy (FMC) were checked.

**Results:** After applying hemocontrol system, IDH episodes in B1 period were significantly reduced. Other parameters did not show the significant changes. And hemocontrol-high responsive patients (n=3) as compared with low responsive patients (n=4), showed significant differences in IDH (B1) (1.7 vs 6.5, P=0.032), intervention (B1, B2) (4.7 vs 12.0, P=0.032, 4.7 vs 14.5, P=0.034, respectively), potassium level (A) (4.7 vs 5.8, P=0.032), phosphorus level (A) (4.5 vs 6.1, P=0.032), prothrombin time (B2) (11.1 vs 10.0, P=0.034), pre-dialysis SBP (A) (150 vs 135, P=0.034), pre-dialysis DBP (B2) (67 vs 87, P=0.034), pre-dialysis MAP (B2) (91 vs 106, P=0.032), 48 hours ambulatory DBP (B1, B2) (78 vs 95, P= 0.032, 80 vs 94, P=0.034, respectively), post-dialysis overhydration (OH, liter) by bioimpedance (B2) (1.9 vs -0.5, P=0.032), intradialytic reduced OH (B1, liter) (1.3 vs 2.6, P=0.034), intradialytic reduced Bwt (B1) (1.6 vs 2.6, P=0.032), beta-blocker potency (A, B1, B2) (1.3 vs 0.3, P=0.029, 1.3 vs 0.3, P=0.031, 1.3 vs 0.4, P=0.026, respectively).

**Conclusions:** This study showed that hemocontrol system could be benefit from reducing IDH episodes, but there were no specific changes in other parameters. In addition, hemocontrol-high responsive patients had lower interdialytic BP and less amounts of ultrafiltration. The clinical consideration of these results will be needed in the future.

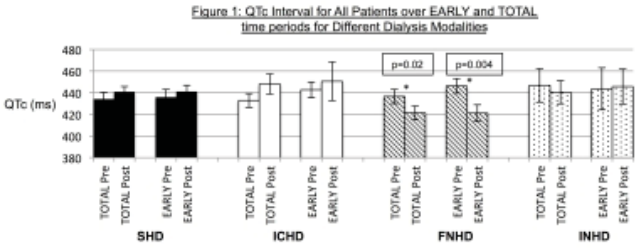
**FR-PO406**

**Frequent Nocturnal Hemodialysis Associates with Improvement of Prolonged QTc Intervals** Benjamin Ka Thomson,[1] Brad Urquhart,[2] Shih-Han S. Huang,[3] Christopher T. Chan,[4] Robert M. Lindsay.[5] *[1]Western Univ; [2]Western Univ; [3]Western Univ.*

**Background:** Sudden cardiac death remains the leading cause of death in hemodialysis (HD) patients. Prolongation of QTc intervals (as measured by the tangent method) increases sudden cardiac death risk in populations without kidney disease.

**Methods:** We performed a retrospective electrocardiograph (ECG) and chart review of HD patients. Our objectives were (1) to establish the effect of one of four different dialysis modalities on interdialytic QTc intervals, (2) to determine the effect of dialysis frequency and time on QTc interval and on the prevalence of borderline or prolonged QTc intervals, and (3) to determine if changes in QTc interval were simultaneous to changes in electrocardiographic left ventricular mass.

**Results:** Frequent nocturnal HD was associated with a decrease in QTc interval for all patients who initiated dialysis with prolonged QTc (468.2 to 438.2 ms, p=0.0134).




This change happened before changes in let ventricular mass were evident. Dialysis duration predicted a decrease in QTc better than dialysis frequency (R2 6.50 vs. 3.00%, p=0.023 vs 0.102). Prevalence of borderline or prolonged QTc increased in patients dialyzed <4hours/session (12/39 to 22/39, p-0.039).

**Conclusions:** Frequent nocturnal HD may be the ideal modality to initiate HD in end-stage kidney disease patients with prolonged QTc.

*Funding:* Government Support - Non-U.S.

**FR-PO407**

**The Effects of Study Events and Treatment on Health-Related Quality of Life (HRQoL) in the EVOLVE Trial** Andrew H. Briggs,[1,2] Patrick S. Parfrey,[3] Nasreen Khan,[4] Spring Tseng,[5] Bastian Dehmel,[5] Glenn M. Chertow,[6] Vasily Belozeroff.[5] *[1]Univ of Glasgow, United Kingdom; [2]Oxford Outcomes Inc; [3]Memorial Univ; [4]IMS Health; [5]Amgen Inc; [6]Stanford Univ School of Med.*

**Background:** EVOLVE™ (EValuation Of Cinacalcet HCl Therapy to Lower CardioVascular Events) is a randomized, controlled trial evaluating the effects of cinacalcet in patients with secondary hyperparathyroidism (sHPT) on hemodialysis. The objective of this analysis was to assess the impact of study events and treatment on HRQoL.

**Methods:** HRQoL was measured with the EQ-5D at scheduled visits and after a study event. The pre-planned analysis assessed changes in EQ-5D from baseline to scheduled visits. We also conducted a regression analysis to assess the acute (first three months) and chronic (beyond three months) effects of major sHPT-related health events (see Table) on HRQoL, and , including randomized assignment (cinacalcet vs. placebo) to assess the residual effect of cinacalcet on HRQoL not mediated by events.

**Results:** Data on HRQoL were available for 3547 of 3883 subjects with a total of 1650 events in the placebo and 1502 in the cinacalcet arm. No difference in HRQoL was observed in the direct (naïve) comparison of EQ-5D by treatment arms. The regression analysis (Table) showed significant effects of events. After accounting for events, there was a modest positive effect of cinacalcet on HRQoL.

| Effects of events and treatment on HRQoL | | | | |
|---|---|---|---|---|
| **Parameter** | **Coeff.** | **SE** | | |
| No event | 0.726 | 0.013 | | |
| Treatment | 0.019 | 0.005 | | |
| **Non-fatal events** | **Acute coeff.** | **SE** | **Chronic coeff.** | **SE** |
| Myocardial Infarction | -0.103 | 0.026 | -0.097 | 0.030 |
| Unstable Angina | -0.086 | 0.052 | -0.018 | 0.051 |
| Heart Failure | -0.102 | 0.020 | -0.097 | 0.022 |
| Peripheral Vascular Event | -0.319 | 0.029 | -0.177 | 0.028 |
| Stroke | -0.218 | 0.057 | -0.103 | 0.052 |
| Fracture | -0.310 | 0.024 | -0.118 | 0.020 |
| Parathyroidectomy | -0.049 | 0.020 | 0.013 | 0.017 |

**Conclusions:** A comparison of HRQoL using the EQ-5D score at scheduled visits in EVOLVE showed no difference between randomized groups. A regression analysis showed large decrements in HRQoL after major sHPT-related health events and a modest improvement in HRQoL with cinacalcet.

*Funding:* Pharmaceutical Company Support - Amgen

## FR-PO408

### Coronary Intervention Reduces Mortality among Dialysis Patients with Acute Coronary Syndrome: Taiwan National Cohort Study

Chih-Chiang Chien. *Dept of Nephrology, Chi-Mei Medical Center, Taiwan.*

**Background:** Patients on chronic dialysis have poor outcomes after acute coronary syndrome (ACS). Epidemiological data forAsian patients are scarce.

**Methods:** In the Taiwan National Health Insurance research Database, we examined records of ESRD patients who initiated dialysis between 1999 and 2001. Patients were followed until death, end of dialysis, or December 31, 2008. Predictors of ACS and mortality were calculated using Cox models.

**Results:** A total 19974 incident dialysis patients were examined in this study. ACS was diagnosed in 1785 patients during follow-up (1.78/100 person-years): 832 (46.6%) had acute myocardial infarction (AMI), 681 (38.2%) underwent cardiac catheterization, 398 (22.3%) percutaneous transluminal coronary angioplasty (PTCA), and 50 (2.8%) coronary artery bypass grafting. Male (HR 1.35, 95% CI: 1.23-1.49) and elderly (HR 3.289, 95% CI: 2.71-4.00) patients had a high rate of ACS. Patients with baseline comorbidities (diabetes mellitus, hypertension, congestive heart failure, coronary artery disease,dysrhythmia, other cardiac and chronic obstructive lung diseases) had a higher incidence of ACS than did those without. Overall in-hospital mortality was 9.7%. The cumulative 6-month post-hospitalization survival rate was 79.3%; the 1-year rate was 72.3%. Being elderly (≥ 65 years old), and having DM or AMI were associated with an increased risk for mortality; PTCA was associated with a decreased risk (HR0.77, 95% CI: 0.66-0.91).

**Conclusions:** ESRD dialysis patients had a high incidence of ACS and mortality. Being male, elderly and having baseline comorbidities were independent risk factors for ACS. Coronary intervention is possible benefits for dialysis patients.

## FR-PO409

### The Effect of Cincalcet-Associated Troponin Changes on Cardiovascular Event Rates in Hemodialysis Patients–A Possible Link?

Samra Abouchacra, Ahmed Chaaban, Fares Chedid, Nicole Gebran, Mohammad Budruddin, Mohamed E.O. Ahmed, Hanan Eljack, Muhy Eddin Hashem Hasan. *Tawam Hospital, Al Ain, United Arab Emirates.*

**Background:** Raised troponins in hemodialysis patients are prognostic indicators for cardiovascular (CV) risk and correlate with markers of uncontrolled SHPT. Though evidence suggests that control of the latter may reduce CV risk, however the effect of the calcimimetic agent, cinaclacet, remains uncertain.

**Methods:** This 2 year retrospective study compared trends in troponin and PTH along with CV morbidity and mortality among HD patients with severe SHPT receiving cinacalcet vs matched controls, not on this agent.

**Results:** PTH levels were lower at baseline in controls yet maintained, whereas troponin was similar and did not decrease over time. Conversely, both PTH and troponin levels decreased significantly from baseline in the cinacalcet group vs controls (table 1).

| | Cinacalcet Group (n =63) | Controls ( n =40) |
|---|---|---|
| Baseline PTH (pmol/l) | 95.45±62 | 49.76±44¶ |
| 18 mos PTH | 71.91±48* | 51.37±78¶† |
| baseline Troponin I ( ng/ml) | 0.05±0.11 | 0.04±0.1§ |
| 18 mos Troponin I | 0.02±0.03* | 0.04±0.03§† |
| CV mortality(%) | 3 (5) | 2(5)§ |
| Composite cardiac events(%) | 21(33) | 27(68)¶ |
| CVA events (%) | 1(2) | 8(20))¶ |
| PVD complications (%) | 4(6) | 7(18)§ |

P value vs baseline † NS * p < 0.05
cincalcet vs controls § NS ¶ p < 0.001

Moreover, similar CV mortality was observed in both groups, however cardiac events (composite of acute coronary events, congestive heart failure, arrhythmia ) were lower in cinacalcet group vs controls. Similarly, less cerebrovascular events and PVD complications were seen, but latter did not achieve significance.

**Conclusions:** Our study showed a decrease in troponin levels in cinacalcet -treated patients associated with lower cardiovascular events vs controls. Larger placebo-controlled trials are necessary to further explore the link between cardiac outcomes and changes in biomarker levels associated with cinacalcet use in severe SHPT.

## FR-PO410

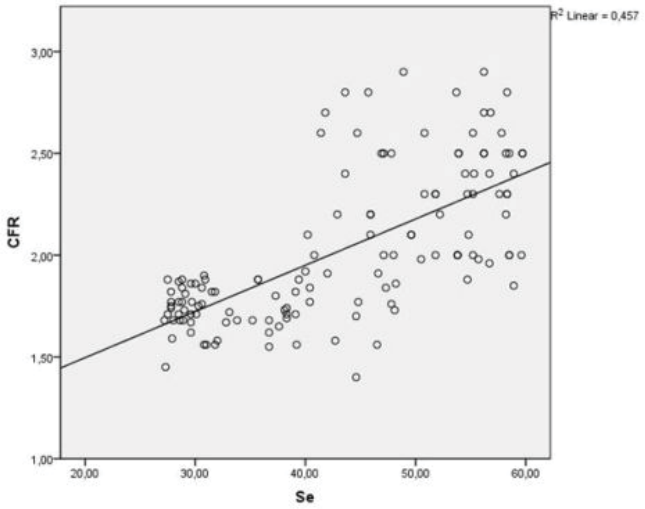
### Serum Selenium Levels Correlate with Coronary Artery Flow Reserve in Hemodialysis Patients

Elif Ari Bakir,[1] Beyza Macunluoglu.[2] *[1]Nephrology, Kartal Research and Training Hospital, Istanbul, Turkey; [2]Nephrology, Uskudar State Hospital, Istanbul, Turkey.*

**Background:** The objective of this study was to assess the correlation between serum selenium levels and coronary artery flow reserve (CFR) as an indicator of endothelial dysfunction (ED) and accelerated atherosclerosis in hemodialysis (HD) patients.

**Methods:** Seventy chronic HD patients and 69 age and sex-matched healthy controls were included in the study. Plasma selenium levels were performed by high-performance liquid chromatography measurements. CFR was assessed by transthoracic Doppler echocardiography.

**Results:** Serum levels of selenium (34,16[lrm]±6,15 vs 52,4[lrm]±5,51, p<0.001) and CFR values (1,73±0,11 vs 2,32±0,28, p<0.001) were lower in HD patients compared to controls. There was a significant positive correlation between CFR and serum levels of selenium (r = 0.676, p<0.001). A linear regression analysis showed that serum levels of selenium were independently and positively correlated with CFR (regression coefficient = 0.457, p<0.05).




**Conclusions:** This study was the first to show a positive and independent correlation between serum selenium levels and CFR as an indicator of ED and accelerated atherosclerosis in HD patients.

## FR-PO411

### Role of Monocyte Membrane Expression of Tumor Necrosis Factor Alpha (TNFα) Receptors in Altered Immune Response of Hemodialysis Patients

Nathalie Neirynck, Griet Lrl Glorieux, Eva Schepers, Annemieke Dhondt, Raymond C. Vanholder. *Nephrology Div, Ghent Univ Hospital, Belgium.*

**Background:** TNFα is elevated in hemodialysis (HD) patients and very likely plays a role in HD-related micro-inflammation. Signal transduction of TNFα depends on the membrane expression of TNFα-receptor 1 (TNFR1) and 2 (TNFR2). We hypothesized that changes in monocyte membrane expression of TNFR1 and TNFR2 could contribute to the altered monocyte function in HD patients. In this study, we compared the expression of membrane (m)TNFR1, mTNFR2 and mTNFα on monocytes in healthy controls and HD patients.

**Methods:** Whole blood samples from healthy controls (C) and predialysis samples of HD patients were labelled in vitro with fluorescent anti-mTNFR1, anti-mTNFR2 and anti-mTNFα at baseline and after stimulation with LPS (1 µg/ml, 30 min) (n = 8) and analyzed by flow cytometry. Monocytes were gated and the mean fluorescence (MFI) was measured.

**Results:** At baseline**,** there was no difference in monocyte mTNFR1, mTNFR2 or mTNFα expression between C and HD. After LPS stimulation, however, the expression of mTNFα and mTNFR2 was lower in HD compared to C, respectively 1265 vs 2266 (p < 0.05) and 840 vs 1324 (p < 0.05). A similar trend was observed for mTNFR1 (HD: 499 vs C: 782, p = 0.092). Comparing the expression after stimulation and baseline, a significant increase in mTNFα in C (2266 vs 753, p < 0.05) and decrease in mTNFR1 in HD (499 vs 681, p < 0.05) was observed. This lower expression of mTNFR1, 2 and mTNFα after stimulation in HD patients may induce a different TNFα signal transduction in HD patients compared to C.

**Conclusions:** There was no difference in baseline expression of mTNFR1, mTNFR2 and mTNFα on monocytes in dialysis patients compared to healthy controls. However, after LPS stimulation, a lower receptor expression in dialysis patients was observed compared to controls. Our findings might play a role in the patho-physiology responsible for the depressed immune response in HD patients upon infection.

*Funding:* Government Support - Non-U.S.

## FR-PO412

**Relevance of Fibroblast Growth Factor and Vascular Endothelial Growth Factor in End Stage Renal Disease and Their Relevance to Cardiovascular Events** Vinod K. Bansal, Kristiyana Kaneva, Debra Hoppensteadt, Jawed Fareed. *Pathology and Medicine, Loyola Univ Medical Center, Maywood, IL.*

**Background:** Fibroblast Growth Factors (FGF) represent families of heparin binding growth factors and Vascular Endothelial Growth Factor (VEGF) also known as Vascular Permeability Factor are a potent mediator of angiogenesis and vasculogenesis in various diseases. We hypothesized that both of these growth factors may be upregulated in End Stage Renal Disease (ESRD) and may contribute to the cardiovascular events.

**Methods:** This study included 119 ESRD patients undergoing maintenance hemodialysis after appropriate IRB approval and patient consent. Citrated blood samples were collected prior to and immediately after the dialysis session. The blood samples were centrifuged for 15 minutes at 3000 g at 4°C and platelet poor plasma (PPP) was extracted. Citrated plasma was frozen at -70°C. Samples collected from ESRD patients were analyzed for the circulating levels of FGF-23 by using a sandwich ELISA kit (Millipore, St. Charles, Missouri). The VEGF analysis was carried out using Quantikine sandwich ELISA method (R&D Systems, Minneapolis, Minnesota). Plasma samples collected from normal male and female (n = 80) comprised the normal group.

**Results:** The FGF-23 levels ranged from 0 to 5934 pg/ml (Mean = 1861 pg/ml with S.E.M = 151) in contrast to the normal levels of 18.4 + 6.1 pg/ml. Of the 119 patients, 67 (56.3%) had greater than 1000 pg/ml FGF-23 levels. The VEGF levels ranged broadly from 8.2 to 3673 pg/ml (Mean = 141 pg/ml with S.E.M = 50.1) in contrast to the normal levels of 8.7 + 4.2 pg/ml. Of the 119 patients, 16 (13.4%) had VEGF levels greater than 100 pg/ml of which 4 (3.36%) had levels greater than 1000 pg/ml. No correlation was observed between the VEGF and FGF23 levels.

**Conclusions:** End Stage Renal Disease represents a complex polypathologic disorder where growth factors such as VEGF and FGF-23 play an important role. Both of these growth factors are increased in ESRD and may contribute to the cardiovascular and cerebrovascular events. Newer targets to modulate these growth factors may be useful in the therapeutic management of these patients.

## FR-PO413

**Posterior Reversible Encephalopathy Syndrome in End Stage Kidney Disease: Not Strictly Posterior or Reversible** Mark N. Canney, Dearbhla Kelly, Michael Clarkson. *Dept of Renal Medicine, Cork Univ Hospital, Ireland.*

**Background:** Posterior Reversible Encephalopathy Syndrome (PRES) is an uncommon clinico-radiological condition that can result in severe brain injury. Little is known about PRES in kidney disease despite the presence of recognised risk factors including hypertension and immunosuppression. This study aimed to define the incidence of PRES in an End Stage Kidney Disease (ESKD) population and identify potential aetiological factors.

**Methods:** This was a retrospective observational study. The database of adult incident ESKD patients in South West Ireland was interrogated over the period 2002 to 2012 and cross-referenced with the patient database of the Department of Radiology to identify cases of PRES. Those cases were further assessed by a detailed review of their hospital record.

**Results:** 592 patients developed ESKD between 2002 and 2012. Five individuals (0.84%) developed PRES. The aetiology of their kidney disease included hypertension, bilateral nephrectomy, pyelonephritis, systemic vasculitis and lupus nephritis. Four patients were established on haemodialysis (HD) at the time of onset. One patient commenced HD during the same admission. The dominant feature at presentation was severe hypertension. The average mean arterial blood pressure was 135mmHg (range 123-159). No incident transplant recipient developed PRES (n=150). Two patients were receiving immunosuppression for active nephritis. Seizure was the most common presenting symptom. Neuroimaging showed diffuse white matter lesions not restricted to the posterior territory but including frontal, parietal, occipital and brainstem distributions. Two patients died following cerebral infarction and intracranial haemorrhage respectively. The remaining patients had complete clinical resolution.

**Conclusions:** PRES is an uncommon complication in incident ESKD patients. Marked hypertension appears to be the common aetiological factor. MRI findings were diverse involving both anterior and posterior circulation territories. Three of the five patients had started haemodialysis within weeks of presentation. This group may be particularly vulnerable to devastating hypertension-associated acute microvascular brain injury.

## FR-PO414

**Superhighflux Therapies for Hemodialysis: Ultrafiltrate Proteomic Profile and Protein Identification** Mauro Atti,[1] Aurora Cuoghi,[2] Elisa Bellei,[2] Stefania Bergamini,[2] Emanuela Monari,[2] Giuseppe Palladino,[1] Luca Corazza,[1] Marialuisa Caiazzo,[1] Luisa Sereni,[1] Aldo Tomasi.[2] *[1]Scientific Affairs, Bellco S.r.l., Mirandola, Modena, Italy; [2]Dept of Diagnostic, Clinical and Public Health Medicine, Univ of Modena and Reggio Emilia, Modena, MO, Italy.*

**Background:** End-stage renal disease is often related with the uremic syndrome that leads to an increase in the morbidity and mortality rate. Uremia pathophysiological process is not completely understood but the retention of a high number of toxic solute compounds seems to play an important role. Uremic toxins are heterogeneous group of substances. Hemodiafiltration with on-line endogenous reinfusion (HFR) dialytic method needs an optimal combination of the membrane permeability and cartridge resin bed. In this study, SELDI-TOF and ESI-QTOF Mass Spectrometries were used for protein profiles and identification of pre cartridge ultrafiltrate (UF) obtained from dialysed patients using three different membranes.

**Methods:** 25 dialysed patients were treated with three membranes: polyphenylene High Flux (pHF), polyphenylene Super High-Flux (pSHF) and Synclear 0.2 (Sync 0.2). UF samples, taken at 30, 60, and 240 min during the dialysis, were loaded on ProteinChip and analysed by SELDI-ToF. Differences in protein profiles, in terms of molecular weight and peaks intensities, were detected by statistical analysis. UF samples from Sync 02 treatment were analyzed by ESI-QTOF.

**Results:** Proteomic profiles of UF at 30 min of dialysis, showed higher cluster peaks intensities for the pHF respect to the pSHF and Sync 0.2 membranes. On the contrary, at 60 and 240 min, the cluster peaks intensities decreased for pHF and increased for pSHF and Sync 0.2. Cluster peaks in the range of 30-60 kD and 60-100 kDa, had the lower number of detected peaks and intensities, for the pHF, while the Sync 0.2 showed the higher permeability, especially for the species >60 kDa, during all treatment. Several inflammatory proteins were identified by ESI-QTof.

**Conclusions:** The results of this study demonstrate that, compared to pHF and pSHF, the Sync 0.2 membrane offers the higher permeability and efficiency, showing its potential use for the clearance of high molecular toxins.

## FR-PO415

**A Randomized Control Trial for the Best Dialyzer Choice for Elderly Dialysis Patients** Ikuto Masakane,[1] Jun Minakuchi,[2] Hideki Kawanishi.[3] *[1]Yabuki Hospital, Nephrology, Yamagata, Japan; [2]Kawashima Hospital, Nephrology, Tokushima, Japan; [3]Tsuchiya Hospital, Surgery, Hiroshima, Japan.*

**Background:** Several recent reports warned that high efficient hemodialysis with polysulfone (PS) membrane occasionally led to the deterioration of nutritional status in elder patients. Ethylene vinyl alcohol (EVAL) membrane has high biocompatibility and a broad solute removal property. In the prior studies, EVAL membrane improved the deteriolated nutritional status caused by PS membrane. The E-HOPOED-Study Group was established in 2010 to determine the best dialyzer choice for elder dialysis patients with early dialysis stage.

**Methods:** The E-HOPED-Study is a open-label, dynamically allocated, central registrated, randomized control trial being performed in 325 dialysis facilities in Japan. The patients who accept the enrollment to the study will be randomly divided into the next two groups; the Group A, treated by EVAL membrane; the Group B, treated by several high flux membrane such as PS. The entry criteria of the patients are the age more than 70 years old, the dialysis vintage less than 180 days. The target number of the entry is 800 in each Group. The 5 year-survival, changes in the nutritional status, occurrence of complications and others are the end points.By June 5th 2013, 601 patients have recruited to the study.

**Results:** The mean age of the patients was 78.2 years old (221patients for 80 years of age or older, 380 patients for less than 80 years of age), 389 (65 %) were male, 289 ( 48 %) were diabetes, and 280 patients for serum albumin level 3.5 g/dL or more. Up to now, 23 patients have died (9 patients for Group A and 14 patients for Group B. There are few deferences in clincal parameters between two groups except post-dialysis pulse pressure. At 0-month there were no diffenrecnes in pre-diaysis and post-dialysis pulse pressure between two groups but at 12-month post-dialysis purelse pressure had significantly reduced in Group B.

**Conclusions:** Based on this interim analysis there are no clinically noteworthy differences between EVAL and PS for elder dialysis patients.

*Funding:* Pharmaceutical Company Support - ASAHIKASEI MEDICAL CO., LTD.

## FR-PO416

**Serum Levels of Free Retained Organic Solutes and Outcomes in Hemodialysis Patients** Tariq Shafi,[1] Timothy W. Meyer,[2] Thomas H. Hostetter,[3] Michal L. Melamed,[4] Yang Liu,[1] Tanushree Banerjee,[5] Neil R. Powe.[5] *[1]Johns Hopkins Univ; [2]Stanford Univ; [3]Case Western Reserve Univ; [4]Albert Einstein College of Medicine; [5]Univ of California San Francisco.*

**Background:** Uremia is associated with accumulation of multiple solutes often poorly cleared by dialysis but the association of these solutes with clinical outcomes remains unclear.

**Methods:** We measured free levels of p-cresol, indican, phenylacetylglutamine and hippurate by HPLC in 396 hemodialysis patients of a national prospective cohort study of incident dialysis patients (CHOICE Study). Outcomes evaluated were mortality (all-cause and cardiovascular [CV]) and CV events analyzed using Cox proportional hazards models adjusted for potential confounders.

**Results:** Mean age was 57 years, 60% were White and 55% male. There were 241 deaths (121 CV deaths) and 203 CV events during follow-up. In fully adjusted models, p-cresol was associated with CV events whereas indican, phenylacetylglutamine and hippurate were associated with mortality. Patients with the highest tertile for all solutes had higher risk of outcomes compared with those in the lowest tertile [HR (95%CI), 2.12 (1.18-3.80) for all-cause mortality and 1.81 (1.00-3.29) for CVD mortality].

| | Clinical Outcomes | | | | | |
|---|---|---|---|---|---|---|
| | All-Cause Mortality | | CV Mortality | | CV Events | |
| Solute | HR* | p | HR* | p | HR* | p |
| P-Cresol | 1.08 (0.94-1.24) | 0.29 | 1.13 (0.96-1.32) | 0.15 | 1.25 (1.14-1.37) | <0.001 |
| Indican | 1.13 (0.98-1.30) | 0.09 | 1.15 (0.92-1.44) | 0.22 | 1.05 (0.90-1.23) | 0.50 |
| Phenylacetylglutamine | 1.13 (1.03-1.26) | 0.02 | 1.15 (0.95-1.40) | 0.15 | 1.12 (0.95-1.32) | 0.18 |
| Hippurate | 1.09 (1.01 1.16) | 0.02 | 1.02 (0.93-1.12) | 0.63 | 0.98 (0.88-1.08) | 0.67 |

* Hazard Ratio per SD increase in marker adjusted for age, sex, race, comorbidities, BMI, urine output and serum albumin

**Conclusions:** Certain uremic solutes are associated with adverse outcomes in dialysis patients. Replication of these findings in other studies may allow identification of individuals at highest risk for adverse outcomes and perhaps the choice for optimal dialysis modality.

*Funding:* NIDDK Support

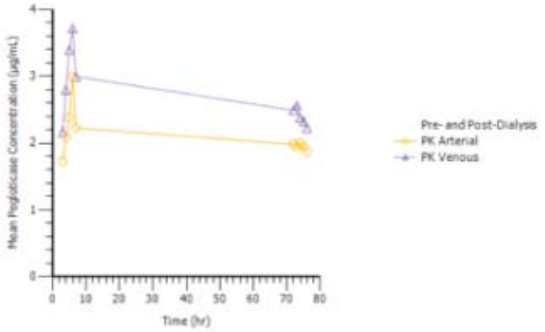
**FR-PO417**

**Pegloticase, a Recombinant Uricase for the Treatment of Advanced Gout, Maintains Therapeutic Concentrations during Dialysis** Anthony J. Bleyer,[1] David E. Wright,[2] Alan Glicklich.[2] *[1]Wake Forest Univ Sch Med, Winston-Salem, NC; [2]Savient Pharmaceuticals, Inc.*

**Background:** Patients (pts) commonly proceed to dialysis with advanced tophaceous gout and information on the use of urate-lowering medications in the dialysis population is scant. Pegloticase is reserved for pts with chronic gout who are refractory to current oral treatments and does not require dose adjustment based on renal function. Here we present results from a pharmacokinetics/pharmacodynamics study of pegloticase in hemodialysis (HD) pts.

**Methods:** A single intravenous dose of pegloticase (8mg) was administered to pts (age 18-75 years; N=12) without gout. The pegloticase infusion was completed over a 2-hour period at 3 hours prior to the Day 1 HD. A second HD was monitored on Day 4. Pts received prophylactic antihistamine and corticosteroid prior to the pegloticase infusion. Blood samples were drawn pre-HD and hourly over 4 hours post-HD during the Day 1 and Day 4 HD sessions.

**Results:** Mean age of this cohort was 44 years, 58% were male, 25% were white, 67% were black, and mean BMI was 28.6 kg/m$^2$. HD vintage was 6.4 years and 2 pts had a prior kidney transplant. Mean arterial and venous pegloticase concentrations across sampling times during the Day 1 and Day 4 HD sessions are shown in the figure.




Baseline mean serum uric acid was 5.98 mg/dL; by 3 hours post-infusion serum uric acid was undetectable and remained so through the final serum sampling at 72 hours. One AE of headache was possibly related to study drug.

**Conclusions:** In this Phase 1 study, pegloticase concentration was not affected by a single HD session and remained stable following a second HD session 3 days later. Serum uric acid levels in this cohort fell to non-detectable levels by 3 hours post-infusion. While additional research is needed, these data support the use of pegloticase in patients with chronic tophaceous gout in HD.

*Funding:* Pharmaceutical Company Support - Savient Pharmaceuticals

**FR-PO418**

**Low Hip Bone Mineral Density Predicts Mortality in Maintenance Hemodialysis Patients: A Five-Year Follow-Up Study** Sinee Disthabanchong.[1,2] *[1]Div of Nephrology, Saint Louis Univ, Saint Louis, MO; [2]Div of Nephrology, Dept of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol Univ, Phayathai, Bangkok, Thailand.*

**Background:** Bone loss is common among hemodialysis patients and may contribute to increased mortality. The degree of bone loss is related to the severity of vascular calcification which may explain the increased mortality risk. Studies that investigated the relationship between bone loss and mortality in maintenance hemodialysis patients are limited.

**Methods:** Eighty three maintenance hemodialysis patients underwent the measurements for bone mineral density (BMD) of the hip and lumbar spine by DEXA scan and coronary artery calcification (CAC) by multi-slice CT. The relationship between BMD and mortality were analyzed after adjustments for relevant cardiovascular and CKD risk factors after 5 years of follow-up.

**Results:** The average follow-up duration was 48±15 months. Mortality occurred in 26 patients (31.3%): twelve (46%) were from cardiovascular causes. Eighty percent of the patients had reduced total hip BMD (t-score<=-1) at baseline. Total hip BMD showed negative relationship with CAC scores. In univariate cox regression analyses, age, cardiovascular disease (CVD), dyslipidemia, increased c-reactive protein, increased CAC scores and decreased hip BMD (HR=0.007, 95%CI=0-0.136, *P*=0.001) were associated with mortality. There was no relationship between lumbar BMD and outcome. Low hip BMD remained independently associated with mortality after adjustments for age, gender, DM, CVD, dyslipidemia, serum albumin, c-reactive protein, CAC score, dialysis vintage, serum phosphate and alkaline phosphatase levels (HR<0.001, 95%CI=0-0.055, *P*=0.005). In Kaplan-Meier survival curve, patients with total hip BMD in the lowest tertile displayed the worst survival (*P*=0.045).

**Conclusions:** Low hip BMD is common among maintenance hemodialysis patients and has the ability to predict mortality independent of cardiovascular and CKD risk factors.

*Funding:* Private Foundation Support

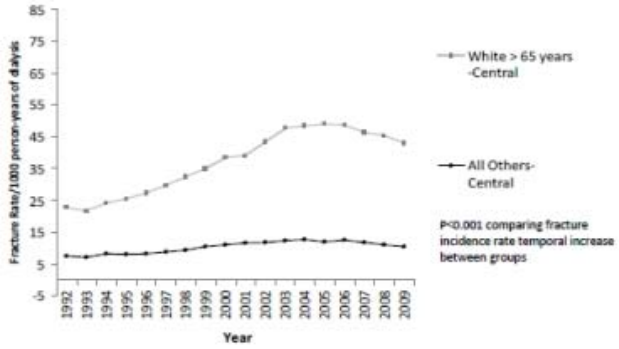
**FR-PO419**

**Increasing Fracture Rates in Elderly Dialysis Patients in the United States** Anna Mathew,[1] John D. Wagner,[1] Lisa M. Rosen,[2] Kenar D. Jhaveri,[1] Steven Fishbane.[1] *[1]Div of Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY; [2]Dept of Biostatistics, Feinstein Institute for Medical Research, Great Neck, NY.*

**Background:** Fractures cause excess morbidity in dialysis patients. New approaches for bone mineral disorders in end stage renal disease have recently evolved. This study aims to determine if rates of fractures in dialysis patients have declined.

**Methods:** Using an overdispersed Poisson regression model, we modeled fracture counts from 935,221 dialysis patients in the United States Renal Data System (USRDS) from 1992 to 2009 for the primary outcome of central fractures (hip and vertebral) requiring hospitalization. The first 90 days of dialysis were excluded. The study population was stratified by the demographic characteristics of age, race and gender.

**Results:** From 1992 to 2004, central fracture rates increased from 12.5 to 25.3 per 1,000 person-years. After 2004 fracture rates stabilized but did not decline. The overall p-value <0.0001 indicated a significant increase in central fracture rates from 1992 to 2009. With stratification by age and race, the sharp rise in central fracture rates was explained almost entirely by patients 65 year or older in age, and occurred primarily in white patients. Taken together, the striking rise in bone fractures occurred almost exclusively in elderly, white patients with 22.7 fractures per 1,000 person-years in 1992 to 48.4 fractures per 1,000 person-years in 2004.




**Conclusions:** Central fracture rates have increased from 1992 to 2004, mostly in white, elderly dialysis patients and despite availability of new treatments. Untreated loss of bone volume and density in ESRD may contribute to these findings, and a "one size fits all" treatment paradigm for bone disease may be harmful to some dialysis patients. Further studies on diagnosis and management of bone disease in elderly dialysis patients are warranted.

**FR-PO420**

**Mineral and Bone Disorder Medication Use Patterns in U.S. Dialysis Patients** Akeem Yusuf,[1,2] Wendy L. St. Peter.[1,2] *[1]USRDS Coordinating Center, MMRF; [2]Univ of Minnesota.*

**Background:** Chronic kidney disease mineral and bone disorder (CKD-MBD) management involves use of multiple therapeutic agents. We describe CKD-MBD medication use trends in US Medicare Part D enrolled dialysis patients.

**Methods:** We created annual cohorts of adult dialysis patients (2007-2010) using Centers for Medicare and Medicaid Services End-Stage Renal Disease database linked with Medicare Part D data. We included patients ≥18 years, alive on December 31 of the previous year, with Medicare Parts A, B and D coverage from January 1 to earliest of death or December 31 of the present year. Medication use was defined as ≥1 claim for an oral medication (or administration record for intravenous (IV) Vitamin D) during follow up, with stratification by low-income subsidy (LIS) status and dialysis organization. Medication groups included prescription phosphate binders, oral and IV vitamin D analogues and cinacalcet. We calculated odds of medication use for LIS vs. non-LIS patients and for patients from various dialysis organizations using logistic regression, adjusted for age, sex, race, vintage and dialysis organization, where appropriate.

**Results:** Phosphate binders (~83% of patients) and IV vitamin D (77.5%-79.3%) were most common CKD-BMD medications used from 2007-2010 followed by calcimimetics (31.0-33.3%), and oral vitamin D analogues (8.5%-9.9%). Sevelamer was the main phosphate binder used (~53%); with decreasing use of calcium acetate and lanthanum over study period. Paricalcitol and calcitriol constituted majority of IV and oral vitamin D use, respectively. The odds of using trade name phosphate binders, IV vitamin D and cinacalcet were significantly higher for LIS vs. non-LIS patients. DaVita patients were more likely to use lanthanum, sevelamer and cinacalcet and less likely to use calcium acetate, while Fresenius patients had highest use of IV Vitamin D.

**Conclusions:** LIS vs. non-LIS and DaVita vs. other provider patients were more likely to be prescribed expensive trade name oral CKD-MBD medications, after adjustment for some factors that affect prescribing. Further research is needed to see if higher use of trade name CKD-MBD agents is associated with better clinical outcomes.

*Funding:* NIDDK Support

**FR-PO421**

**Costs Associated with Mineral and Bone Disorder Medications in Dialysis Patients** Akeem Yusuf,[1,2] Wendy L. St. Peter.[1,2] *[1]USRDS Coordinating Center, MMRF; [2]Univ of Minnesota.*

**Background:** Dialysis-related chronic kidney disease mineral and bone disorder (CKD-MBD) medications (meds) represent a large portion of Medicare Part D costs in these patients (pts). This has implications for pts, providers and Medicare when these meds are included in the dialysis bundle in 2016. We describe costs associated with these meds in US Medicare Part D-insured dialysis pts.

**Methods:** Using Medicare standard analytic files linked with Medicare Part D data, we created annual cohorts of dialysis pts (2007 through 2010). We included pts >=18 years, alive on Dec 31 of each year, with Medicare Parts A, B and D coverage from Jan to Dec. Per member per month (PMPM) net Part D payment and per user per month (PUPM) out-of-pocket (OOP) meds costs for each Part D-covered CKD-MBD med and med class were computed, with stratification by low-income subsidy (LIS) status and dialysis organization.

**Results:** In 2010, PMPM net Part D costs were $555.22 and $278.65 for all Part D meds and CKD-MBD meds respectively. There was a 22% vs. 36% increase in total Part D vs. CKD-MBD Part D med costs from 2007 to 2010. CKD-MBD meds represented about 50% of overall net Part D costs in 2010 (Phosphate binders(PB) 30%, cinacalcet 19%, oral vit D 2%). Between 2007 and 2010, PMPM Part D costs were highest among DaVita pts for cinacalcet, sevelamer and lanthanum as compared to other dialysis providers. PUPM OOP costs for PB decreased from 2008 to 2009 while OOP costs for sevelamer carbonate had a sustained year-to-year increase. PUPM OOP costs for all CKD-MBD meds were much higher for pts without LIS vs. with LIS.

**Conclusions:** Part D net costs increased faster for CKD-MBD meds compared to all Part D meds in dialysis pts, despite relatively stable use within med classes from 2007-2010. This was mainly due to increases in net Part D PMPM spending for sevelamer and cinacalcet. OOP costs for PB were reduced in 2009, due to availability of generic calcium acetate. However, pts without LIS, on average, had high OOP costs for brand name CKD-MBD meds. In a bundled environment, there will be incentives to shift to generic PB and reduce use of cinacalcet, particularly with no MBD performance measures in place.

*Funding:* NIDDK Support

**FR-PO422**

**End-Stage Renal Disease due to Multiple Myeloma in the United States, 2000-2010** Scott Reule,[1,2] Donal J. Sexton,[1,2] Craig Solid,[1] Shu-cheng Chen,[1] Allan J. Collins,[1,2] Robert N. Foley.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, Univ of MN, Minneapolis, MN.*

**Background:** As management has advanced rapidly, we combined US census data with data from the USRDS patients to determine whether incidence rates and survival of ESRD from myeloma and light chain deposition disease ('MM') have improved correspondingly.

**Methods:** A total of 13,832 of 1,140,882 (1.2%) patients initiated dialysis due to MM between 2000 and 2010. Using incidence rates for 2000 to calculate expected rates for subsequent years (based on age, sex and race/ethnicity), standardized incidence ratios peaked in 2002 at 1.19, declining subsequently to 0.86 in 2010.

**Results:** Characteristics of MM patients included older age (≥ 65, 63.7% vs. 43.7%), white race (76.3% vs. 65.1%), catheter for hemodialysis (94.9% vs. 82.4%), and < 1 year of prior nephrology care (90.4% Vs. 76.3%). When adjustment was made for demography, MM was associated with a greater likelihood of death (adjusted hazards ratio [AHR] 2.32) and a lower likelihood of listing for (AHR 0.018) and receipt of (AHR 0.2) of a renal transplant. Among patients with myeloma, likelihood of death remained unchanged, transplant listing increased (AHR 1.21 for 2005-2010 Vs. 2000-2004) and transplantation decreased (AHR 0.80). Regional variation in outcomes was apparent, as patients in the South were more likely to die (AHR 1.04 Vs. the Northeast), and less likely to be listed (AHR 0.49) or received a transplant (AHR 0.41).

**Conclusions:** While ESRD due to MM has declined in the United States, outcomes on dialysis remain poor and exhibit substantial geographic variation.

*Funding:* NIDDK Support

**FR-PO423**

**Cancer Risk in Incident Dialysis Patients in the United States, 2005-2009** Robert N. Foley,[1,2] Craig Solid,[1] Shu-cheng Chen,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, Univ of MN, Minneapolis, MN.*

**Background:** Few recent large-scale epidemiological studies are available to determine whether patients starting dialysis therapy are at greater risk of cancer than the general population.

**Methods:** Hence, we set out to address this information gap among 112,699 patients aged 65 or more with Medicare insurance and no prior history of malignancy that started maintenance dialysis in the United States between 2005 and 2009 and were followed for 1.7 years. We used age, sex and race-specific incidence rates from the US general population (Statistics Epidemiology and End Results (SEER) database, (United States) National Cancer Institute) to calculate expected rates for cancer of any cause, and for the 10 most common types, based on the demographic profile of the dialysis population. Actual cancer rates were calculated from Medicare hospital admissions claims.

**Results:** All cancers studied occurred more frequently than expected ($P < 0.05$) in dialysis patients. For any cancer, actual and expected incidence rates were 14,747 and 682 per 100,000 per year, respectively, an actual-to-expected incidence ratio of 21.6. Incidence ratios for individual cancers were as follows: prostate 5.3, breast 5.1, lung and bronchus 3.0, colon and rectum 3.9, melanoma 2.8, urinary bladder 8.4, non-Hodgkin lymphoma 5.5, kidney 17.3, uterus 3.8, and leukemia 8.2.

**Conclusions:** These data suggest that patients starting dialysis patients have greater risks of the commonest types of cancer.

*Funding:* NIDDK Support

**FR-PO424**

**Effect of Frequent Nocturnal Hemodialysis (NH) or Renal Transplantation (RT) on Cognition** Bradley S. Dixon,[1] John M. Vanburen,[1] Jacob J. Oleson,[1] Robert S. Lockridge,[2] James R. Rodrigue,[3] Jane S. Paulsen,[1] John B. Stokes.[1] *[1]Univ of Iowa; [2]Univ of Virginia; [3]Harvard Med School.*

**Background:** It is unknown whether frequent hemodialysis or renal transplantation will reverse the cognitive impairments seen in patients with end stage kidney disease on conventional hemodialysis.

**Methods:** We performed a prospective cohort trial analyzing the effect of switching from conventional hemodialysis 3 days per week (HD) to either nocturnal hemodialysis (NH) 6 days per week or renal transplantation (RT) on tests of memory and learning (Rey Auditory-Verbal Learning Test, AVLT and Brief Visuospatial Memory Test, BVMT), executive function (Trails B test) and psychomotor processing speed (Buttons and Digit-Symbol tests). Participants were measured at baseline, 4 and 12 months and tested for significant change at follow-up using a linear mixed model.

**Results:** A total of 77 patients switched to nocturnal hemodialysis (n=18), received a renal transplant (n=28) or remained on conventional hemodialysis (n=31). Baseline characteristics were not significantly different between the groups. 48 patients (62%) returned for their 12-month study visit (67% NH, 46% RT and 74% HD) at a mean (± SD) follow-up of 13.4 (± 2.2) months. Patients who received a renal transplantation showed improvement in AVLT ($p<0.01$) and Digit-Symbol test ($p=0.02$) but not BVMT, Trails B or Buttons. Treatment with nocturnal hemodialysis did not improve any of the cognitive tests and BVMT declined ($p=0.02$). Surprisingly, patients remaining on conventional hemodialysis showed improvement in the Digit-Symbol test ($p<0.01$) and Buttons test ($p=0.05$) and a decline in verbal fluency ($p<0.001$) but no change in AVLT, BVMT or Trails B.

**Conclusions:** After 13 months, renal transplantation improved auditory/verbal memory and processing speed but not executive function. In this small study, no improvement in cognitive testing was seen with initiating nocturnal hemodialysis.

*Funding:* NIDDK Support

**FR-PO425**

**Cognitive Function and All-Cause Mortality in Hemodialysis Patients** Mark J. Sarnak, Hocine Tighiouart, David A. Drew, Kristina Lou, Saeed Kamran Shaffi, Tammy Scott, Li Fan, Daniel E. Weiner. *Tufts Medical Center, Boston, MA.*

**Background:** Cognitive impairment is highly prevalent in dialysis patients. Although advanced dementia is associated with mortality, it remains unknown whether milder degrees of cognitive impairment, and which components of this impairment, are associated with mortality.

**Methods:** 292 patients enrolled in the Dialysis and Cognition Study underwent a detailed battery of previously validated cognitive tests. Principal factor analysis was used to derive two summary factors representing memory and executive function. Multivariable Cox proportional hazards models were used to evaluate the association of the individual tests, as well the memory or executive component with all- cause mortality.

**Results:** Mean (SD) age was 63 (16) years, 53% were women and 23% African American. 146 patients died during a median follow up of 27.4 months. Higher executive function scores were associated with lower risk of mortality. In contrast, memory was not associated with mortality.

| | Unadjusted | | Model 1* | | Model 2** | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Executive Factor (per 1 SD higher) | 0.62 (0.53, 0.73) | <0.001 | 0.78 (0.65, 0.94), | 0.008 | 0.80 (0.66, 0.97), | 0.02 |
| Memory Factor (per 1 SD higher) | 0.81 (0.68, 0.95) | 0.01 | 0.98 (0.81, 1.18), | 0.8 | 0.98 (0.81, 1.17) | 0.8 |

Executive and memory factors both have mean (SD) of 0 (1)
*Adjusted for age, sex, race, vintage, cause of kidney disease, education, and dialysis access
**Further adjusted for cardiovascular disease, Kt/V, and serum albumin

Consistent with these results, better performance on several individual tests that assess executive function, such as the Block Design and Trail Making Tests, were associated with lower risk of all-cause mortality.

**Conclusions:** Worse executive function is associated with higher risk of mortality in hemodialysis patients. These results need to be verified in additional studies and the mechanism underlying this association evaluated.

*Funding:* NIDDK Support

## FR-PO426

**End-of-Life Inpatient Cost Trajectories for Patients with Chronic Kidney Disease Compared with Those with Other Chronic Conditions**

Kenn B. Daratha,[1] Ann M. O'Hare.[2] *[1]Washington State Univ; [2]VA Puget Sound Health Care System.*

**Background:** The cost of caring for patients receiving chronic dialysis is extremely high, due mostly to high rates of inpatient utilization. To date, little is known about cost trajectories toward the end of life in patients with kidney disease in relation to those with other chronic conditions.

**Methods:** We conducted a longitudinal study among 7,065 adults who were hospitalized at the time of death in the State of Washington in 2011 (WA-CHARS). We estimated inpatient costs during the last year of life for members of this cohort with the following chronic conditions: heart failure (HF-39% of all terminal cases with select chronic conditions), chronic obstructive pulmonary disorders (COPD-35%), metastatic cancer (METS-21%), kidney failure without renal replacement therapy (CKD-21%) and kidney failure with renal replacement therapy (ESRD-8%).

**Results:** Patients with ESRD were more likely than those with other chronic conditions to have at least one inpatient hospitalization (84%) in the year preceding the terminal hospitalization, as compared with 73% for patients with HF, 71% for CKD, 67% for COPD and 67% for METS. Total costs in the last year of life were also highest for patients with ESRD (Figure 1).

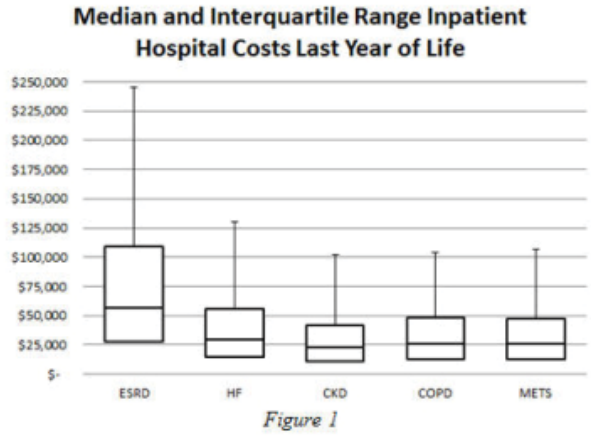



*Figure 1*

For patients with COPD and METS no relationship was found between costs in the last quarter of life and terminal hospitalization costs. A positive relationship was observed between costs in the last quarter of life and the cost of terminal hospitalization for patients with HF (r=0.12, p<0.001), CKD (r=0.15, p<0.001) and ESRD (r=0.17, p=0.002).

**Conclusions:** Among chronically ill patients who died in the hospital, the cost of the terminal hospitalization and overall inpatient costs during the final year of life were markedly higher than for other chronic conditions.

*Funding:* Private Foundation Support

## FR-PO427

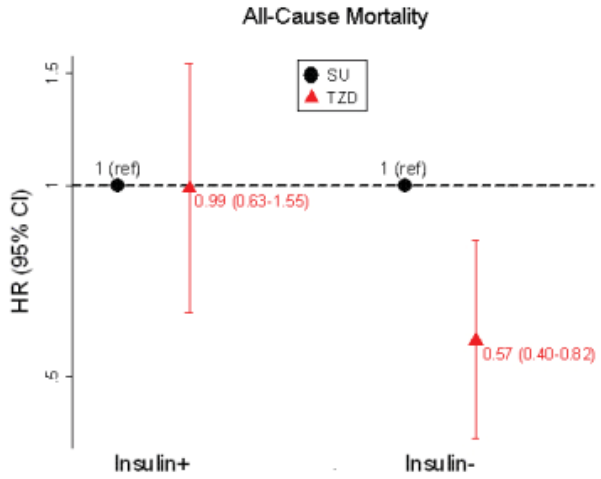
**Thiazolidinedione Use Is Associated with Improved All-Cause Mortality Compared with Sulfonylureas among Non-Insulin Dependent Diabetic Hemodialysis Patients**

Katherine E. Lynch,[1,2] Connie Rhee,[2] Steven M. Brunelli.[2,3] *[1]Beth Israel Deaconess Medical Center, Boston, MA; [2]Brigham and Women's Hospital, Boston, MA; [3]DaVita Clinical Research.*

**Background:** Among diabetics on hemodialysis (HD), there are conflicting data as to whether thiazolidinedione (TZD) use improves or worsens survival. Previous studies have compared TZD users to non-users. However, non-user control groups are known to increase risk of bias. The purpose of this study is to evaluate the comparative effectiveness of TZDs versus sulfonylureas (SU) with respect to mortality.

**Methods:** Data were taken from 14,643 prevalent adult HD patients from a large dialysis care organization. Incident TZD users were matched (1: n) to incident SU users on the basis of insulin use and a propensity score based on age, sex, race, dry weight, catheter use, heart failure, and serum albumin. Associations with mortality were estimated using Cox proportional hazard models; analyses were stratified on concurrent insulin use.

**Results:** Among diabetics on insulin, 94 TZD users were matched to 131 SU users; among diabetics not on insulin 136 TZD users were matched to 201 SU users. Cumulative at-risk time was 372 and 594 pt-yrs during which 70 and 128 deaths were observed in the insulin+ and insulin- cohorts, respectively. For the insulin using cohort, the mean age was 63 years, 49% were female, 34% were black, and mean serum albumin was 3.7 g/dL. In comparison, the non-insulin users mean age was 65 years, 49% were female, 36% were black, and mean serum albumin was 3.8 g/dL. Among diabetics not on insulin, TZD use was associated with lower risk of death than SU use; no difference was observed among diabetics on insulin (figure).




**Conclusions:** Among non-insulin dependent diabetics on HD, TZD use versus SU use was associated with 43% lower all-cause mortality. There was no difference in mortality seen for insulin users.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO428

**High Prevalence of Elevated Non-A1c Glycemic Indices in Chronic Dialysis Patients without Documented Diabetes**

Neal Mittman,[1] Lin Ma,[2] Mark E. Williams,[3] Julia I. Brennan,[4] Chinu M. Jani,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[2] Eduardo K. Lacson.[2] *[1]Long Island College Hospital, Brooklyn, NY; [2]Fresenius Medical Care, North America, Waltham, MA; [3]Joslin Diabetes Center, Boston, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

**Background:** Quarterly glycosylated hemoglobin (A1c) has been the standard of care for assessing glycemic control in the general population and in dialysis patients. However, serum fructosamine (SF) and glycated albumin (GA) have both been proposed as alternative markers in dialysis. Based on our previous work (Desiraju et al, 2005), we hypothesized that non-diabetic (NDM) ESRD pts (normal A1c, <=5.6%) may exhibit undetected hyperglycemia when assessed by alternative glycemic indices.

**Methods:** We explored simultaneous measurement of SF and GA in NDM pts by A1c criteria in a national sample of 1192 NDM pt from 26 FMCNA facilities. Residual blood from routine monthly specimens was used for analysis. We report on mean values in these pts for the period January-March 2013.

**Results:** The cohort's mean age was 56 years, 45% female, 43% white, 55% black, and 21% on PD. Fewer than 60% of these non-diabetics had all three glycemic markers within the normal non-diabetic range. More than 50% had elevated SF, 16% had elevated GA, and 15% had increased levels of both. More than 90% of pts with elevated GA had concomitantly elevated SF; however, more than 70% of pts with elevated SF had normal GA.

| All HgbA1c ≤5.6% | GA<=285 | GA>285 | Total |
|---|---|---|---|
| SF<=285 | 577 (57.4%) | 12 (6.4%) | 589 |
| SF>285 | 429 (42.6%) | 174 (93.6%) | 603 |
| Total | 1006 | 186 | 1192 |

**Conclusions:** To our knowledge, there are no published data assessing alternative glycemic markers in non-diabetic dialysis pts and the potential impact on morbidity of elevated values. We and others have reported that elevated levels of SF and/or GA are predictive of morbidity and mortality in diabetic dialysis pts. Therefore, further analyses of these NDM patient subgroups is needed to determine the significance of these elevated glycemic indices.

## FR-PO429

**Biochemical Variables and Survival of Patients with Type 1 Diabetes on Renal Replacement Therapy**

Jaakko Helve,[2] Mikko Haapio,[1] Per-Henrik Groop,[1] Carola Gronhagen-Riska,[2] Patrik Finne.[2] *[1]Dept of Nephrology, Helsinki Univ Central Hospital, Helsinki, Finland; [2]Finnish Registry for Kidney Diseases, Helsinki, Finland.*

**Background:** End-stage renal disease is one of the most serious complications of type 1 diabetes. Yet, data are limited regarding factors that predict survival of patients with this complication. Our aim was to estimate the effect of biochemical variables on survival of type 1 diabetes patients on renal replacement therapy (RRT).

**Methods:** An incident cohort of all patients with type 1 diabetes entering chronic RRT (n=834) in Finland 2000-2011 was followed up until death or end of follow-up on 31 December 2011. All data came from the Finnish Registry for Kidney Diseases. Creatinine, albumin, urea, ionized calcium, phosphorus, hemoglobin, C-reactive protein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, trigycerides, and HbA1c were measured from blood or plasma before the initiation of RRT. Main outcome measure was adjusted relative risk of death according to biochemical variables. Cox proportional hazards models were used for these analyses.

**Results:** When measured before RRT and adjusted for age and sex, low serum creatinine, albumin and hemoglobin, and high C-reactive protein concetration predicted increased mortality. Further adjustment for comorbidities, and initial treatment modality of RRT showed that the most important predictors of death are low creatinine and albumin and high C-reactive protein.

**Conclusions:** Among type 1 diabetes patients entering chronic RRT, biochemical variables independently associating with survival are creatinine, albumin and C-reactive protein. They reflect nutritional status, proteinuria, liver function, and ongoing inflammatory process. Anemia also associates with worse survival. Treatment of these might improve survival.

*Funding:* Private Foundation Support

## FR-PO430

**Propensity-Matched Comparison of New Onset Diabetes Mellitus between Incident Peritoneal Dialysis and Hemodialysis Patients** Chiu-Ching Huang,[1,2] Che-yi Chou.[1,2] *[1]Div of Nephrology, China Medical Univ Hospital, Taichung, Taiwan; [2]Graduate School, China Medical Univ, Taichung, Taiwan.*

**Background:** New-onset diabetes mellitus (NODM) is associated with poor outcome in patients(pts) treated with hemodialysis (HD) or peritoneal dialysis (PD). PD pts were exposed to extra glucose load from daily dianeal dwelling,we ask the question "Are PD pts more prone to develope NODM after dialysis?".We used a propensity score(PS) matching appoach to study this subject. We aim to compare the incidence and outcome of NODM by intention-to-treat analysis in a propensity-score matched HD and PD cohorts.

**Methods:** All HD and PD pts in Taiwan Renal Registry Database(include all pts who survived ≥ 1M after dialysis) were analyzed from 1995 to 2005. Pts were followed until death,renal transplantation or up to December 31, 2008. We attempted to correct for differences between patient charcteristics using a PS-matching method.We matched each PD pt with 4 HD pts with a similar PS. PS accounted for factors recorded in the database, including age, sex, body weight, primary renal disease and co-morbidity . We compared the incidence and survival outcomes of NODM in PD and PS-matched HD pts. Predictors of NODM and mortality were studied using Cox models.

**Results:** We analyzed 2548 non-DM incident PD pts and 10192 PS-matched HD pts with 5.9±3 years follow-up.Mean age of PD pts were 50.2± 14.7y vs. 50.2± 14.4y of matched HD pts.During follow up , 13% of PD patients and 22% of PS-matched HD pts developed NODM. The crude incidence of NODM was 2.2%/y for PD pts, lower than 3.7%/y for HD pts ($p<0.001$). The risk of NODM after starting dialysis is approximately 50% lower (HR 0.48) for pts treated with PD. Male gender, a lower serum albumin and lower hematocrit were independently linked to increased NODM risks. After adjusting for covariates,regardless of dialysis modality,patients who developed NODM had increased overall mortality (HR 3.628, $p<0.001$) than those did not.

**Conclusions:** In a PS-matched Chinese cohort, incident pts receiving HD carry a higher risk of developing NODM than those receiving PD. The pts with NODM are associated with increased overall mortality reardless of dialysis modality chosen.

*Funding:* Government Support - Non-U.S.

## FR-PO431

**Factors Associated with Higher Mortality Rates among Dialysis Facilities in the United States** Janice P. Lea,[1] Muhammad Sarmini,[2] Rachel E. Patzer.[1] *[1]Emory Univ; [2]Univ of Missouri.*

**Background:** Yearly mortality rates average over 20% in the United States (US) End Stage Renal Disease (ESRD) population. It is unclear the extent to which dialysis facility level factors influence the risk-adjusted standardized mortality ratio (SMR) across dialysis facilities in the US.

**Methods:** We analyzed data from the Centers for Medicare and Medicaid Services Dialysis Facility Reports from 2007-2010 to examine dialysis facility-level factors associated with low standardized mortality rates (SMRs) among all dialysis facilities in the United States. Multivariable-adjusted linear regression models were used to identify the facility-level characteristics associated with high SMR.

**Results:** A total of 5,277 dialysis facilities with complete data on SMR from 2007-2010 were examined. Network SMRs ranged from lower than expected (SMR=0.856; SD:0.23 ) in Network 16 (Northwest) and higher than expected (SMR=1.19, SD:0.27) in Network 4 (Pennsylvania and Delaware). In multivariable linear regression models, factors significantly associated with a higher SMR included a higher proportion of African American patients, for profit dialysis facilities, ESRD Network region, and the proportion of patients not informed of transplant options. Clinical factors such as hemoglobin and urea reduction ratio did not influence mortality rates.

**Conclusions:** Dialysis facility demographics such as race, geographic location, for-profit status, and transplant education influence ESRD mortality rates. Clinical factors tested in our study were not associated with mortality. Recent changes in operational aspects of dialysis-i.e., bundling implemented by CMS in 2011 as well as erythropoietin dosing guidelines may change these associations in future analyses.

## FR-PO432

**Epidemic of ESRD Prediabetes: Risk Factors** Mark E. Williams,[1] Neal Mittman,[2] Lin Ma,[3] Julia I. Brennan,[4] Chinu M. Jani,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[3] Eduardo K. Lacson.[3] *[1]Joslin Diabetes Center, Boston, MA; [2]Long Island College Hospital, Brooklyn, NY; [3]Fresenius Medical Care, North America, Waltham, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

**Background:** In the general population, baseline hemoglobin A1c (HbA1c) levels are an independent predictor of type 2 diabetes, even in the normal range. While the ADA supports hemoglobin A1c of 5.5-6.4% as a screening test for prediabetes, its diagnosis in ESRD patients may be more difficult. We sought to determine risk factors for prediabetes in a dialysis cohort using HbA1c and casual glucose levels as the glycemic markers.

**Methods:** Of 3,447 dialysis patients with laboratory values obtained the first quarter of 2013, 1454 (with mean age 56.6 years; 78% on hemodialysis) were without known diabetes. The distributions of glycemic markers including casual glucose and HgbA1c were determined, using the mean of all available values used in the analysis. Age and BMI subgroup analyses were performed.

**Results:** In those with known diabetes, mean HbA1c was 6.5% (SD=1.4%, CV= .05). In non-DM, mean HbA1c was 5.3% (SD 0.5%,range=3.8-11.5%,CV=.04). Glucose levels without diabetes ranged from 49 to 383mg/dL (mean 106). Initial analyses indicated that 2 percent of nondiabetic HbA1c values were >6.5%, indicating undiagnosed diabetes and that 16% of patients had prediabetes by A1c criteria. Subgroup analyses indicated that the risk of prediabetes almost doubled with body mass index>25(overweight or obese) as well as with increasing age, and in males, but not in Blacks or Hispanics.

**Conclusions:** These data confirm obesity and aging as risk factors for prediabetes in ESRD patients, particularly in males. Results do not suggest an association with race, unlike in the general population. Because HbA1c levels may be reduced in ESRD (previous studies), the prevalence of prediabetes may be even higher. Further studies are needed to determine the sensitivity and specificity of HbA1c in diagnosing prediabetes in ESRD patients.

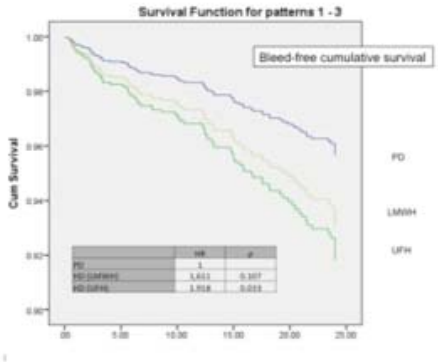
## FR-PO433

**Major Bleeding in Haemodialysis Patients Using Unfractionated or Low Molecular Weight Heparin: A Single Centre Study** Luxme Nadarajah, Stanley Fan, Neil Ashman. *Nephrology Dept, Barts Health NHS Trust, London, United Kingdom.*

**Background:** It is uncertain whether different heparins alter bleeding risk in hemodialysis (HD) patients. We report a comparison of major bleeding in HD patients receiving unfractionated heparin vs tinzaparin (LMWH), using peritoneal dialysis (PD) patients as a control. We also examined any effect of anti-platelet agents or oral anticoagulants on bleeding.

**Methods:** A single centre study conducted before/after a switch from UFH to LMWH as HD anticoagulant. Case notes identified any admission due to bleeding. Bleeding events are described using the ISTH definition of a major bleed (category A), and in more detail, extending to include all bleeds that led to an admission (category B). We report incident event rates and event-free survival calculated using multivariate analysis by Cox-proportional hazard ratio.

**Results:** 889 patients (1200 patient years) on LMWH HD, 522 patients (784 patient years) on UFH HD and 540 patients (1,103 patient years) on PD were studied. The incidence of Category A bleeds was 1.33%, 0.89% and 0.69% bleeds respectively. Incidence of category B bleeds were 3.33 %, 3.96 % and 1.78%. A significant difference in the event free survival of a Group B bleed occurred between HD UFH patients compared to PD patients, (OR 1.918, 95% CI 1.054 - 3.491 *p = 0.03*).




Warfarin usage also significantly increased the risk of bleeding when comparing patients not on any anti-platelet/coagulant (OR 2.755, 95% CI 1.06-7.123, p=*0.037)*.

**Conclusions:** Bleeding risk in our population is similar to that reported elsewhere in the USA and Canada. There is *no* significant difference in major bleeding rates between hemodialysis patients anticoagulated with either UFH or LMWH. There is significantly more bleeding in UFH HD patients compared to PD patients (but not between LMWH HD and PD patients). Warfarin use significantly increases the risk of bleeding episodes.

**FR-PO434**

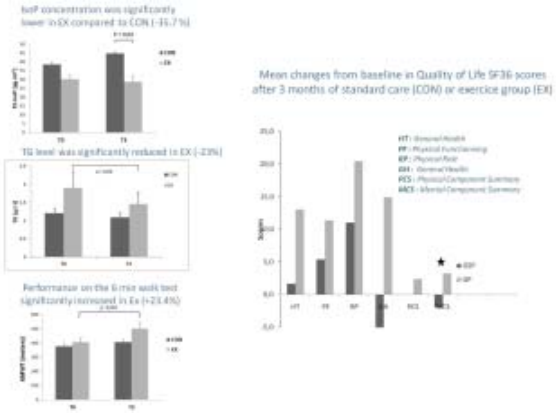
**Oxydant Stress, Quality of Life and Physical Function in Chronic Kidney Disease Patients Are Improved by Intradialytic Aerobic Cycling Program**

Myriam Rouchon Isnard,[1] Céline Coutard,[1] Céline Forte,[5] Cuenin Christine,[4] Nathalie Boisseau.[3] *[1]AURA Auvergne, Chamalières; [2]UFR STAPS, Univ BP, Aubière; [3]Cardiologie, Clinique, Durtol; [4]Biologie, Gen Bio, Clermont-Ferrand.*

**Background:** In Chronic Kidney Disease (CKD), oxidant stress plays a central role in the development of cardiovascular diseases. This study aimed to determine if an intradialytic aerobic cycling training protocol, by improving aerobic fitness, could reduce oxidant stress and others related CKD disorders as lipid profile and quality of life (QOL) of these patients.

**Methods:** Eighteen haemodialysis patients randomly were assigned to either intradialytic exercise training (cycling) group (EX; n = 8) or a control group (CON; n = 10) for 3 months. Aerobic fitness (6 min walk test), pro/antioxidant status (in plasma: 15F2-α-isoprostanes [$F_2$-IsoP], oxidized LDL [ox-LDL], in erythrocytes: SOD, GPX, GSH/GSSG), lipid profile (total cholesterol, HDL, LDL, TG), and QOL (SF36QD) were measured at baseline and 3 months later.

**Results:** Intradialytic training protocol had beneficial effects on pro/antioxidant status, aerobic fitness, lipid profile and QOL. Indeed, at 3 months: i) IsoP concentrations were significantly lower in EX compared to CON (-35.7 %) ii) performance on the 6 min walk testsignificantly increased by 23.4% in EX, but did not change in CON and iii) plasma TG were significantly reduced in EX (-23%) without modification in CON iv) QOL was clinically improved and significantly for the Mental Component Summary +2.2% in EX *vs* -2% in CON.




**Conclusions:** These results show that intradialytic aerobic cycling training protocol exerts beneficial effects in CKD patients by reducing the most sensitive and reliable marker of OS (IsoP) and by improving their lipid profile, physical function, and QOL.

**FR-PO435**

**Association of Daily Physical Activity with Bone Mass in Hemodialysis Patients**

Kei Yoneki,[1] Atsuhiko Matsunaga,[1] Jun Kitagawa,[1] Ryota Matsuzawa,[1,2] Akira Ishii,[1,2] Yoshifumi Abe,[1] Manae Harada,[1] Yasuo Takeuchi,[1] Atsushi Yoshida,[2] Kouju Kamata.[1] *[1]Kitasato Univ, Sagamihara, Japan; [2]Sagami Junkanki Clinic, Sagamihara, Japan.*

**Background:** Low bone mass is a leading cause of fracture. Although daily physical activity is recommended as a preventative measure against bone loss in healthy adults, the effects of physical activity on bone mass in hemodialysis (HD) patients are unclear. This study aimed to determine whether daily physical activity is associated with bone mass in HD patients.

**Methods:** This observational, prospective study consisted of two parts: cross-sectional Study 1 and longitudinal Study 2. At the beginning of the study, 165 HD outpatients (87 men and 78 postmenopausal women; mean age, 68±8 years) who visited our HD center 3 times a week were recruited. Clinical characteristics including age, sex, body mass index, HD duration, and medications (active vitamin D) were recorded from clinical records in both parts of the study. Biochemical parameters including serum phosphorus, corrected calcium, and intact parathyroid hormone levels were also measured. With respect to bone mass, the stiffness index was assessed by quantitative ultrasound of the calcaneus. With respect to physical activity, the number of steps walked per day was measured using an accelerometer. In Study 1, the association between physical activity and bone mass was assessed by multiple regression analysis for each sex. In Study 2, participants were divided into two subgroups according to physical activity based on results from Study 1 (≥5,000 steps per day or not), and two-way analysis of variance (ANOVA) was used to identify the effect of physical activity on bone mass for each sex after a 2-year follow-up.

**Results:** Multiple regression analysis adjusted for clinical characteristics and biochemical parameters in Study 1 indicated that physical activity was significantly associated with bone mass in HD patients of each sex ($P$<0.05, respectively). ANOVA showed that changes in bone mass were significantly different between the two HD patient subgroups for each sex ($P$<0.05, respectively) in Study 2.

**Conclusions:** Decreased daily physical activity is strongly associated with bone loss in HD patients.

**FR-PO436**

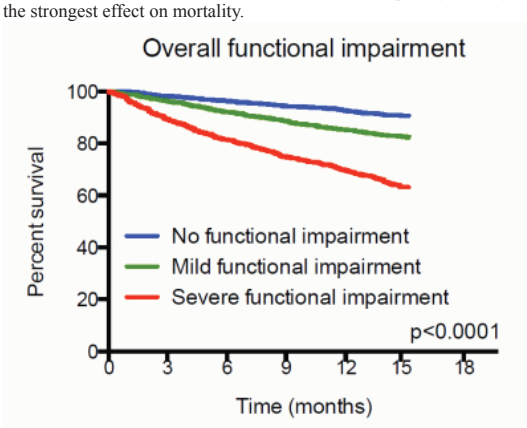
**Functional Impairment and Survival of Dialysis Patients**

Wim P.D. Lemahieu,[1] Maarten Naesens,[2] Johan M.J. De Meester.[3] *[1]Nephrology, Imelda Hospitals, Bonheiden, Belgium; [2]Nephrology, Univ Hospitals Gasthuisberg, Leuven, Belgium; [3]Nephrology, St. Nicolas Hospitals, Sint Niklaas, Belgium; [4]Nephrology, Jessa Hospitals, Hassel, Belgium.*

**Background:** This study aimed to explore functional status of dialysis patients, its possible overlap with classical medical parameters and impact on survival.

**Methods:** 3753 prevalent (95% coverage) Flemish dialysis patients completed a survey on 3 fields: medical (diabetes, wound care, amputations, hospitalization), ambulatory autonomy (ability to walk, recent falls, type of balance, means of transportation to/from dialysis facility) and care dependency (need for assistance with meals, drug management, incontinence and nursing home residency). Patients ≥ 75 years vs those younger were compared by Student's T test. Clustering of individual survey items was assessed by principal components analysis. This was complemented by follow-up on 15 months' mortality and Cox proportional hazards analysis including all survey items, gender and age.

**Results:** Only 15% of all patients was completely impairment-free versus 28% suffering from at least one impairment in each field. For those ≥ 75 years this was respectively 6% and 38% versus 25% and 19% for the younger cohort. Two distinct clusters of patients could be identified: 32% had impairments in all but one ('recent falls') items of ambulatory autonomy and care dependency, and in 11% all (except 'hospitalizations') medical field items clustered. Overall 15 months' mortality was 16 %. The number of impaired items ('no' or 0-1 vs 'mild' or 2-4 vs 'severe' or > 4) composing the major patient cluster had the strongest effect on mortality.




**Conclusions:** Diminished ambulatory autonomy and increased care dependency cluster in an important fraction of Flemish dialysis patients. This clustering does not correlate with classical medical factors but is none the less associated with increased mortality.

**FR-PO437**

**Race and Frailty Differences in a Prevalent Hemodialysis Cohort**

Nancy G. Kutner,[1] Rebecca H. Zhang,[2] William M. McClellan,[3] Quinlyn A. Soltow,[4] Janice P. Lea.[3] *[1]Rehabilitation Medicine, Emory Univ, Atlanta, GA; [2]Biostatistics & Bioinformatics, Emory Univ, Atlanta, GA; [3]Medicine, Nephrology Div, Emory Univ, Atlanta, GA; [4]Medicine, Pulmonology Div, Emory Univ, Atlanta, GA.*

**Background:** Among non-dialysis dependent persons with chronic kidney disease (CKD), blacks are more likely than whites to be identified as frail. No prior research has investigated race and frailty patterns among prevalent end-stage renal disease (ESRD) patients undergoing dialysis, a population in which blacks appear to have better survival and quality of life.

**Methods:** Setting & Participants: 745 prevalent ESRD patients undergoing hemodialysis 2009-2011 in 7 Atlanta dialysis clinics and 7 San Francisco Bay Area dialysis clinics for whom frailty indicators were assessed. Measurements: Study coordinators interviewed participants; measured grip strength, walk speed and body composition; and reviewed charts for clinical and laboratory parameters. Vintage, primary ESRD diagnosis, and receipt of pre-dialysis nephrology care were identified in United States Renal Data System files. Using the Fried frailty index (recent unintentional weight loss, reported exhaustion, low grip strength, slow walk speed, low physical activity), frailty was defined by the presence of 3 or more criteria and pre-frail by the presence of 1 or 2 criteria.

**Results:** In this prevalent hemodialysis cohort (median vintage = 3 years) with mean age = 57, almost 75% were classified as pre-frail or frail. However, the adjusted analysis showed that the risk for being classified as frail was 40% lower among blacks compared with whites ($p < .05$). Peripheral vascular disease and low serum albumin were additional significant predictors of frailty.

**Conclusions:** We observed a lower risk for frail status among black compared with white hemodialysis patients, in contrast with higher frailty risk reported for black compared with white non-dialysis CKD patients. Examination of frailty patterns among black and white CKD patients in the transition to ESRD may offer increased understanding of apparent paradoxes in survival and well-being associated with race among patients with kidney disease.

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## FR-PO438

### Frailty: The Strongest Predictor of Quality of Life in Dialysis Patients

Jun Chul Kim,[1] Ki-soo Park.[2] *[1]Div of Nephrology, CHA Gumi Medical Center, CHA Univ, Gumi-si, Gyeongsangbuk-do, Korea; [2]Dept of Preventive Medicine, Gyeongsang National Univ, Jinju, Gyeongsangnam-do, Korea.*

**Background:** Frailty is a growing concern in maintenance dialysis (MD) patients, which is significantly related to the higher incidence of disability, hospitalization and mortality. However, there is no report evaluating the relation of frailty with quality of life (QOL) in this population.

**Methods:** We only included ambulatory MD patients aged ≥20y on hemodialysis (HD) or peritoneal dialysis (PD) ≥6mo, without hospitalization for the last 3mo. Study participants completed the Korean version of Kidney Disease Quality of Life Short Form 36 (KDQOL-36) and other data were obtained by review of medical records. We adopted frailty phenotype composed of the following components; 1)unintentional weight loss >4.5 Kg or 5% of the previous body weight for the last 1y, 2)physical inactivity, 3)physical function (PF) scale <75 and vitality scale <55 as surrogates for weakness/slowness and exhaustion, respectively. Low PF scale was scored as 2 points and other components as 1 point for each. Those with ≥3 points were considered as "frail".

**Results:** 1,658 (1,255 HD, 403 PD) patients evaluated aged 55.9±12.9y (mean±SD) on MD for 5.2±4.5y; 55.7% were male. Compared to the non-frail, frail patients (34.9%) showed significantly (all P-values <0.001) lower scores in Physical Component Score (PCS) (43.7±18.7 vs. 70.4±14.7), Mental Component Score (MCS) (45.9±20.0 vs. 65.8±17.1) and kidney disease (KD) related health concerns such as burden of KD (26.5±24.3 vs. 39.9±26.3), symptom/problems (72.4±16.6 vs. 84.4±12.0) and effect of KD on daily life (63.9±20.6 vs. 77.8±16.5). In multiple regression analysis, frailty was found to be the strongest predictor (all P-values <0.001) for worse conditions of PCS (B=-25.1), MCS (B=-20.1), burden of KD (B=-14.1), symptom/problems (B=-12.7) and effect of KD on daily life (B=-15.3) after adjustment for age, gender, vintage, dialysis modality, co-morbidity, blood hemoglobin, serum albumin and creatinine.

**Conclusions:** Frailty is significantly related to poor outcomes in QOL and KD related health concerns and can be very useful predictor for QOL even in a relatively healthy MD patients.

## FR-PO439

### Predictors of Physical Performance in Patients on Maintenance Haemodialysis: A 12 Month Follow-Up Study

Ying Wang,[1] Cathie Sherrington,[1] Martin P. Gallagher,[1] Alan Cass,[2] Paul Snelling,[1] Meg J. Jardine.[1] *[1]The George Institute for Global Health, NSW, Australia; [2]Menzies School of Health Research.*

**Background:** Poorer physical quality of life is associated with worse outcomes among people with end stage kidney disease (ESKD). While muscle metabolism is known to be impaired, the associations of strength and performance in ESKD remain poorly understood.

**Methods:** We recruited 51 maintenance haemodialysis (HD) patients and 10 healthy volunteers to a longitudinal study. Physical performance (Short Battery Performance Test-SBPT) and strength (ankle dorsiflexion strength-AS) were assessed at baseline and 12 month follow-up. A subgroup of 28 dialysis participants underwent neuropathy assessment (Total Neuropathy Score-TNS). Linear regression was used for normally distributed variables and correlation for skewed variables.

**Results:** Fifty HD patients and 9 controls completed the study. At baseline, HD participants had poorer physical performance than controls (respective median SBPT scores 10 and 12, p=0.002) but similar strength (respective mean AS 13.9 and 14.2, p=0.6). Neuropathy was inversely correlated with physical performance ($r^2$=-0.46, p=0.011) but not strength (p=0.2). At 12 months, strength and physical performance were significantly reduced in the HD group, compared with baseline (mean AS 11.0 versus 13.9, p<0.001; SBPT 9 versus 10, p<0.001, respectively). Neuropathy had deteriorated over 12 months (mean TNS score 7.48 at 12 months versus 6.48 at baseline). Among HD participants, poorer physical performance at baseline was predicted by age (p<0.001), diabetes (p=0.007), strength (p<0.001) and neuropathy (p=0.011) while deterioration over 12 months was predicted by age (p<0.001), diabetes (p=0.008), and strength (p<0.001). Poor strength at baseline was predicted by age (p<0.001) and diabetes (p=0.041) with no strong predictors of deterioration (age, p=0.096; diabetes, p=0.57).

**Conclusions:** Haemodialysis patients have poor physical performance that is not explained by muscle strength alone. Physical performance and strength deteriorated over 12 months in a stable haemodialysis cohort. Better understandings of the underlying mechanisms of the poor physical health in ESKD are needed.

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## FR-PO440

### Physical Activity Energy Expenditure and Quality of Life in Maintenance Hemodialysis Patients with and without Major Comorbidities

Marina Albuquerque Dantas, Jean M. Monteiro, Clarcson Plácido Santos, Gildete Barreto Lopes, Antonio Alberto Lopes. *Federal Univ of Bahia, Salvador, Brazil.*

**Background:** Maintenance hemodialysis (MHD) patients with comorbidities have, in general, lower physical activity (PA) levels and poorer health-related quality of life (HRQOL). We investigated associations between physical activity energy expenditure (PAEE) and HRQOL in patients with > 3 mo. on MHD, separately by groups with and without major comorbidities.

**Methods:** Cross-sectional analysis of data of 640 MHD patients in the Prospective Study of the Prognosis of Chronic Hemodialysis Patients (PROHEMO) developed in 4 clinics in Brazil. The International Physical Activity Questionnaire was used for PA. The following comorbidities were considered in the analysis: diabetes, heart failure, coronary disease, cerebral vascular disease, peripheral vascular disease, pulmonary diseases and cancer; 217 patients were diagnosed and 423 were not diagnosed with any of these comorbidities. Metabolic equivalent of task (MET) was used for determining PAEE and categorized by the median (≤498 and >498 kcal/week). SF-36 was used for scores of 8 primary HRQOL scales and the physical (PCS) and mental component summaries.

**Results:** Among patients without comorbidities, HRQOL scores were higher in those with PAEE>498 kcal/week for all scales, with difference in score (DS) > 4 points for PCS (DS=4.7), vitality (DS=6.8), role emotional (DS=9.6), pain (DS=7.8), role physical (DS=14.9), and physical functioning (DS=13.6). Associations of higher PAEE with better HRQOL were not markedly changed after linear-regression adjustments for age, sex, education, economic class, marital status, and living with family. In patients with comorbidities, the associations followed the same directions and were not substantially different from those without comorbidities (interaction coefficient PAEE*comorbidity>0.2).

**Conclusions:** The results based on PAEE provide further support for potential beneficial effects of physical activity on the health of MHD patients, both for those with and without major comorbidities, but it is not possible to establish causal links or the direction of the association due to the cross-sectional design of the study.

## FR-PO441

### Differences in Health Status and Healthcare Delivery between Men and Women Starting Dialysis Therapy in the United States: 1995-2010

Ahad Abdalla,[1,2] Ailish Hannigan,[2] Liam F. Casserly,[1,2] Austin G. Stack.[1,2] *[1]Medicine and Nephrology, Univ Hospital Limerick, Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Limerick, Ireland.*

**Background:** Differences in survival between men and women may be related to their health status at the start of dialysis and differences in care delivery prior to dialysis initiation. The aim of this study was to compare health care status and healthcare delivery among men and women in the national US Renal Data System Registry.

**Methods:** We compared baseline health indicators in 1, 220, 000 incident US patients (662, 985 men and 557,015 women) who commenced dialysis from 1995-2010. Comparisons were made across 10 comorbid conditions, and 6 measures of pre-dialysis care delivery (anaemia, nutrition, pre-dialysis care, timing of dialysis initiation, dialysis modality). The timing of dialysis initiation was assessed using the CKD-EPI equation. Multivariable logistic regression was used to compare gender differences for each outcome with the generation of adjusted odds ratios (OR). SAS v9.3 was used for all statistical analysis. Ethical approval was received from the University Hospital Ethics Committee.

**Results:** The likelihood of having a major medical disease was significantly lower for women than men for most conditions listed with the exception of diabetes, heart failure and stroke. However, women were significantly more likely than men to have a low body mass index, hypoalbuminemia at dialysis initiation and were more likely to have dialysis initiated late. These differences persisted when consideration was taken into account for case mix.

Adjusted Odds Ratios (OR) and 95% CI for each Clinical Condition or Health indicator for Women versus Men

| | Age and Race Adjusted OR[1] | Multivariate Adjusted OR[1] |
|---|---|---|
| **Clinical Condition (present vs absent)** | | |
| Coronary Disease | 0.79 (0.78-0.79) | 0.75 (0.74-0.76) |
| Peripheral vascular disease | 0.82 (0.81-0.82) | 0.85 (0.83-0.86) |
| Pulmonary disease | 0.84 (0.83-0.85) | 0.90 (0.88-0.92) |
| Cancer | 0.72 (0.71-0.73) | 0.71 (0.70-0.73) |
| Alcohol use | 0.32 (0.31- 0.33) | 0.38 (0.36- 0.40) |
| Stroke | 1.01 (0.99-1.02) | 1.05 (1.03-1.07) |
| Body mass index < 18kg/m² | 1.17 (1.16-1.19) | 1.19 (1.18-1.21) |
| Heart Failure | 1.08 (1.08-1.09) | 1.27 (1.25-1.28) |
| Diabetes | 1.25 (1.24-1.26) | 1.30 (1.29-1.32) |
| **Health Care Delivery (yes vs no)** | | |
| Haemoglobin < 10g/dl | 0.91 (0.90-0.92) | 0.96 (0.95-0.97) |
| Albumin < 3mg/dl | 1.15 (1.14-1.15) | 1.21 (1.19-1.22) |
| GFR < 5 ml/min/min² | 1.70 (1.69-1.71) | 1.81 (1.79-1.84) |
| Seen by nephrologist | 1.02 (1.00-1.03) | 1.02 (1.00-1.03) |
| Seen by Dietitian | 0.91 (0.90-0.93) | 0.92 (0.90-0.94) |
| Peritoneal dialysis (vs haemodialysis) | 1.11 (1.09-1.12) | 1.09 (1.07-1.11) |

**Conclusions:** Substantial differences in health status and clinical care delivery exist between men and women at the initiation of dialysis. The interplay of these factors require further exploration and may contribute to important survival differences between men and women.

**FR-PO442**

**Fibroblast Growth Factor 23 and the Risk of Infectious Hospitalizations and Deaths in Hemodialysis Patients: Results from the HEMO Study**
Kristen L. Jablonski,[1] Jessica B. Kendrick,[1] Alfred K. Cheung,[2,3] Tom Greene,[3] Michel Chonchol.[1] *[1]Univ of Colorado Denver, Aurora, CO; [2]VASLCHCS, Salt Lake City, UT; [3]Univ of Utah, Salt Lake City, UT.*

**Background:** Fibroblast growth factor 23 (FGF23) acts as a phosphaturic factor. Recently, FGF23 has been suggested to be a regulator of innate immunity, as it has the potential to inhibit the enzyme 25-hydroxyvitamin D-1α-hydroxylase in monocytic cells, with concomitant effects on intracrine responses to 1,25-dihydroxyvitamin D.

**Methods:** The HEMO Study was a randomized multicenter study evaluating the effects of high-dose versus standard-dose and high-flux versus low-flux hemodialysis. Serum intact FGF23 levels were measured in stored serum samples obtained at baseline and annually in 1,340 HEMO participants. Quartiles of FGF23 concentrations were chosen as the primary predictor variable, with the lowest quartile serving as the reference category. Time-dependent Cox proportional-hazards models were used to examine the association between FGF23 levels with a composite of hospitalizations and death attributed to an infectious cause.

**Results:** Participants had a mean age of 57±14 years, 55% were females and 46% were white. During a median follow-up of 3.0 years, 499 (37%) participants had an event attributed to an infectious cause. Median (IQR) serum FGF23 levels was 3118 [726, 12928] pg/mL. Overall, median levels of FGF23 rose during follow-up (p=0.01). After adjustment for potential confounders available in the database, including inflammatory markers and usage of calcitriol, higher serum FGF23 levels were directly associated with higher risks of the infectious composite. Compared to the first (lowest) quartile, the HR (95% CI) for infections were as follows: second quartile, 0.95 (0.72-1.25); third quartile, 1.41 (1.06-1.89) and fourth quartile, 1.58 (1.14-2.21). Similar results were obtained when FGF-23 was examined as a continuous variable (HR; 1.10, 95% CI 1.02-1.13 per doubling of FGF23; p=0.01).

**Conclusions:** In the HEMO study, higher serum FGF23 levels were independently associated with infectious hospitalizations and death in time-dependent Cox regression models.

*Funding:* NIDDK Support

**FR-PO443**

**Associations of Serum 25-Hydroxyvitamin D and 1,25-Dihydroxyviatmin D Levels with Infectious Hospitalization and Infectious Death in the HEMO Study**
Anna Jeanette Jovanovich,[1] Jessica B. Kendrick,[1] Alfred K. Cheung,[2,3] Tom Greene,[3] Michel Chonchol.[1] *[1]Div of Renal Diseases and Hypertension, Univ of Colorado Denver, Aurora, CO; [2]VA Salt Lake City Healthcare System, Salt Lake City, UT; [3]Univ of Utah, Salt Lake City, UT.*

**Background:** Determine whether serum vitamin D levels are related to infectious hospitalization and death in subjects requiring hemodialysis.

**Methods:** The HEMO Study was a randomized multicenter study evaluating the effects of high-dose vs. standard-dose and high-flux vs. low-flux hemodialysis. 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels were measured in stored serum samples obtained at baseline and annually in 1,340 subjects. Serum 25(OH)D and $1,25(OH)_2D$ level quartiles were chosen as the primary predictor variable, the lowest quartile served as the reference category. Time-dependent Cox proportional-hazards models were used to examine the association of vitamin D levels with infectious hospitalization and infectious death.

**Results:** Mean age was 57±14 years, 55% were female, and 46% were white. During a median follow-up of 3.0 years, 499 (37%) subjects were hospitalized for or died of an infectious cause. Median [IQR] serum 25(OH)D and $1,25(OH)_2D$ levels were 19.1 [14.2, 26.6] ng/mL and 6.3 [2.9,14.5] pg/mL, respectively. After adjustment for potential confounders available in the HEMO cohort including inflammatory markers and use of calcitriol, the highest quartile of 25(OH)D was associated with a reduced risk of infectious hospitalization and death (HR 0.72, 95% CI 0.53-0.97; p=0.03) compared to the lowest quartile. When 25(OH)D was evaluated as a continuous variable, higher levels of 25(OH) D were associated with a reduced risk of infectious death and hospitalization (HR 0.84, 95% CI 0.72-0.98 per doubling of 25(OH)D; p=0.02). There was no association between $1,25(OH)_2D$ levels with infectious events (HR; 1.02, 95% CI 0.0.95-1.08 per doubling of $1,25(OH)_2D$; p=0.64).

**Conclusions:** Low serum 25(OH)D level, but not $1,25(OH)_2D$ level, is independently associated with infectious hospitalization and infectious death in subjects requiring hemodialysis.

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**FR-PO444**

**Infectious Hospital Admissions among Prevalent Hemodialysis and Peritoneal Dialysis Patients from 1993 to 2011**
Tricia L. Roberts,[1] Robert N. Foley,[1,2] David T. Gilbertson,[1] Craig Solid,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, Univ of Minnesota, Minneapolis, MN.*

**Background:** In the past two decades, infectious hospital admission rates have increased among hemodialysis (HD) patients, while admissions for peritoneal dialysis (PD) peritonitis have fallen close to those for HD vascular access infection. However, infectious admissions traditionally have been defined by principal ICD-9-CM diagnosis codes which could be susceptible to shifts in billing practices. Rates using both principal and secondary diagnosis codes have not yet been assessed.

**Methods:** We included U.S. Medicare period prevalent HD (N=305,901 in 2011) and PD (N=23,558 in 2011) patients from 1993 to 2011. Admission rates were computed for all-cause infection, bacteremia/sepsis, PD peritonitis, and HD vascular access (VA) infection. Two methods defined infectious admissions from inpatient claims using principal diagnosis codes only, method 1 (M1), and principal and secondary diagnosis codes, method 2 (M2). Rates were adjusted for age, gender, race, and primary ESRD diagnosis using model-based adjustment and a Poisson model.

**Results:** For all-cause infection, admission rates were 1.5 to 2.0 times higher with M2 than M1 (in 2011 among HD and PD, respectively, 462 and 551 admissions per 100 patient months with M1; 903 and 935 with M2). All-cause infectious admission rates among PD patients were generally stable across years and higher than HD; however, by 2011, admissions with infection (M2) increased among HD patients to levels similar to PD. Rates were 2 to 4 times higher for M2 than M1 for bacteremia/sepsis (in 2011, 123 and 82 with M1; 282 and 201 with M2) and for PD peritonitis (81 with M1; 354 with M2). With M2, PD peritonitis admissions were 2 to 4 times higher than HD VA infection (88 with M1; 118 with M2).

**Conclusions:** Rates for admissions with infection were strikingly high compared to those for the purpose of infection. Results suggest that attention is needed to address lack of improvement in the last two decades in all-cause infectious admissions among dialysis patients using both methods and high admissions with peritonitis among PD patients.

*Funding:* NIDDK Support

**FR-PO445**

**Spinal Epidural Abscess in Hemodialysis Patients**
Jennifer Marie White,[1] Chan Jin,[1] Rhonda E. Colombo,[1] Stephanie L. Baer,[1,2] Usman Afzal,[1] Kristina W. Kintziger,[1] Mufaddal F. Kheda,[1] Lu Y. Huber,[1] Puja Chebrolu,[1] N. Stanley Nahman.[1,2] *[1]Georgia Regents Univ, Augusta, GA; [2]Augusta VAMC, Augusta, GA.*

**Background:** Spinal epidural abscess (SEA) may be a serious infectious comorbidity in hemodialysis (HD) patients. SEA may be the result of bacteremia (BAC), but other factors are possible. We have previously shown that BAC occurs in over 20% of incident HD patients, suggesting a significant risk for this condition (Chebrolu, IDSA, 2012). To more fully address the role of BAC and other co-morbidities associated with SEA, we queried the United States Renal Data System (USRDS).

**Methods:** All incident HD cases from the USRDS for calendar years 2005-2008 were queried for a diagnosis of SEA, BAC and several potential clinical covariates using ICD-9 diagnosis codes submitted for Medicare billing. Descriptive statistics and log-binomial regression analysis were performed.

**Results:** For the 4-year period of study, 355,084 patients were available for analysis. The median age was 65 years. SEA was identified in 660 (0.19%) patients. Vascular access type at the initiation of HD included AVF in 47,732 (13.4%), AVG in 14,179 (4.0%) and vascular catheter in 293,173 (82.6%) patients. Of the demographic variables, black race [relative risk (RR) 1.218, 95% confidence interval (CI) 1.025- 1.447] and female sex [RR 1.179, 95% CI 1.009-1.379] carried significant risks for SEA. Infectious comorbidities, including diagnosis of BAC [RR 7.757, 95% CI 6.432-9.355], MRSA infection [RR 2.684, 95% CI 2.192-3.286] and hepatitis C [RR 1.660, 95% CI 1.223-2.253] were associated with the highest risk. Of the non-infectious factors, diabetes mellitus [RR 1.515, 95% CI 1.278-1.797] and vascular catheters [RR 1.347, 95% CI 1.008-1.800] carried an increased risk of SEA.

**Conclusions:** SEA is an uncommon but serious complication of HD. The risk of SEA is highest in patients with infectious co-morbidities, especially BAC, but diabetes and the presence of a vascular catheter are also significant risk factors.

**FR-PO446**

**Nontuberculous Mycobacterial Infections and End Stage Renal Disease in the Outpatient Setting**
Rhonda E. Colombo,[1] Puja Chebrolu,[1] Mufaddal F. Kheda,[1] N. Stanley Nahman,[1,2] Kristina W. Kintziger.[1] *[1]Georgia Regents Univ, Augusta, GA; [2]Charlie Norwood VA Medical Center, Augusta, GA.*

**Background:** Nontuberculous mycobacteria (NTM) have been implicated as an uncommon cause of peritonitis complicating peritoneal dialysis (PD) and in periodic reports of infection in hemodialysis (HD) patients. We previously characterized NTM infections among hospitalized ESRD patients on HD (Colombo, JIM, 61(2), 2013). However, NTM may have an indolent presentation and be diagnosed in the outpatient setting. We thus conducted a retrospective analysis of the USRDS to describe NTM diagnoses during outpatient visits among the incident ESRD population.

**Methods:** All patients newly diagnosed with ESRD between 2005-2007 were queried for a diagnosis of NTM infection and potential clinical covariates using ICD-9 diagnosis codes. Medicare Part B claims were the source of outpatient data. Classification of NTM disease was via querying separate codes for pulmonary (PULM), disseminated (DISS), cutaneous (CUT), and "other" (OTH) NTM. Univariable and bivariable descriptive analysis was conducted on all variables of interest.

**Results:** During the three year period, 345 individuals had an outpatient visit with an associated NTM diagnosis. Of these, 241 (69.9%) had not been identified in previous hospitalization data. Demographics included: 55.9% male, 59.4% white, and 57.7% ≥ 70 years. HD was the initial treatment modality for 90.7% of individuals. There were 110 (31.9%) PULM, 11 (3.2%) CUT, 61 (17.7%) DISS, and 163 (47.3%) OTH cases. Underlying pulmonary disease was significantly more common in patients with PULM NTM ($p = 0.02$), particularly bronchiectasis ($p < 0.0001$). HIV, documented in 19.4% of cases, was

associated with DISS disease (40.98%, p<0.001). Significant differences (p<0.05) were also seen with age distribution; DISS was more common in patients under age 50. Nearly half (49.6%) of all patients resided in the Southern US at time of diagnosis.

**Conclusions:** Although rare, NTM may infect outpatient ESRD patients. Type and presentation of NTM infection in ESRD varies depending on underlying comorbidities. NTM is most likely to be diagnosed in the Southern United States.

## FR-PO447

**Association of Dietary Sodium Restriction and Thirst in Hemodialysis Patients** Joselyn Reyes, Caroline M. Williams, Jochen G. Raimann, Penny Faith Sheppard, Stephan Thijssen, Nathan W. Levin, Peter Kotanko. *Nephrology Research, Renal Research Institue, New York, NY.*

**Background:** Control of dietary sodium (Na) intake is central to fluid management in hemodialysis (HD) patients. It is generally assumed that lowering dietary Naintake translates into reduction of thirst and interdialytic weight gain (IDWG). Here we test this hypothesis prospectively in a cohort of chronic HD patients.

**Methods:** We report interim results from an ongoing randomized controlled trial (RCT) of salt interventions in chronic HD patients [clinicaltrials.gov#NCT01015313]. Subjects were randomized 1:1 to an intervention group and a control group. The intervention consisted of a comprehensive approach to reduce salt intake, including intensified dietary counseling by a dietitian using Motivational Interviewing, abandonment of intradialytic saline and Na profiling. Dietary Naintake was assessed by a certified dietitian using 72-hours dietary recalls. Associations of monthly self-assessed thirst levels (Thirst Questionnaire; ThQ) and Na-Calorie Intake Ratios (Na/Cal) were assessed by Pearson correlation analysis and in a longitudinal fashion using linear mixed models (LMM).

**Results:** Out of 24 HD patients (age 60±13.7 years, 58% male, 46 % diabetic, 42% Blacks), 11 were randomized to receive low-salt intervention. At baseline ThQ and Na/Cal ratio were not correlated (r=0.2; P=0.4). Analysis employing a LMM in a subgroup of 8 patients who completed the study indicated that the intervention resulted in reduced thirst as quantified by the ThQ (P<0.001). In the same subgroup non-significant decreases of Na intake, Na/Cal ratio, ThQ and IDWG were observed between baseline and the average of the last three months.

| | Na intake(mg/day) | Calories intake (cal/day) | Na+/Cal (mg/cal) | Thirst Q (score) | IDWG(kg) |
|---|---|---|---|---|---|
| Baseline | 2531±1015 | 1576±635 | 1 6±0 2 | 14 2±2 4 | 2 4±1 |
| Average months (9-12) | 2002±1203 | 1323±451 | 1 4±0 5 | 13 3±5 | 2 3±0 7 |
| Delta (95%CI) | -529 ( 9.4 to - 1068) | -253 ( 92 to -598) | -0.2 ( 0.2 to -0.6) | -0.9 ( 3.4 to -5.2) | -0.1 ( 0.2 to -0.4) |

**Conclusions:** The interim analysis of our RCT of salt intervention demonstrates that changes in dietary salt intake associates to reduce thirst.

## FR-PO448

**Alignment of Dialysate (DNa) and Serum Sodium (SNa) in Chronic Hemodialysis (HD) Patients (pts): Results of a Quality Improvement Initiative** Jochen G. Raimann,[1] Linda H. Ficociello,[2] Len A. Usvyat,[1,2] Qingqing Xiao,[1] Yuedong Wang,[3] Claudy Mullon,[2] Jose A. Diaz-Buxo,[2] Paul M. Zabetakis,[1] Peter Kotanko.[1] *[1]Renal Research Institute; [2]Fresenius Medical Care NA; [3]UC Santa Barbara.*

**Background:** Small studies in HD pts indicate favorable effects of DNa to SNa alignment, however, results from larger groups are needed.

**Methods:** At 4 participating HD clinics, pts with SNa<standard DNa (137 mEq/L) received HD with DNa aligned to the average of the last 4 SNa measurements. "Aligned" were matched to "unaligned" pts (from 44 not participating clinics) based on SNa, observation period, age (<, >60 years), HD vintage (<1, 1-3.9, >4 years) and catheter presence. T-Test was employed for group comparisons of changes in weight (wt), systolic blood pressure (SBP), and saline administration. Hospitalization (Hosp) rates per pt year (pt-yr) were compared using linear mixed-effects models.

**Results:** Aligned (n=84) and unaligned (n=84) pts were similar at baseline (68±13 yrs, 50% male, 3.7±3.7 yrs vintage, 62% diabetics and 28% pts with catheters; P >0.05). Treatment effects were significant for pre-HD wt and interH wt gain (IDWG). Although not reaching statistical significance, trends in all parameters suggest alignment to be favorable. Change in all-cause hosp rates from 2.6 to 1.9/pt-yr and 2.4 to 1.6 /pt-yr were observed for aligned and unaligned pts, respectively (p=0.4). Changes in fluid-related hosp rates from 0.3 to 0.3 / pt-yr and 0.4 to 0.1/ pt-yr were observed for aligned and unaligned pts, respectively (p=0.7).

**Table 1:** Treatment effect of sodium alignment on changes in clinical parameters. Mean±SD or mean (95% CI) as appropriate.

| | Aligned patients (N=84) | | | Unaligned patients (N=84) | | | Treatment |
|---|---|---|---|---|---|---|---|
| Parameter | Baseline | Follow up | Change | Baseline | Follow up | Change | Effect |
| IDWG [kg] | 2.4±0.9 | 2.3±0.8 | -0.1 (-0.4 to 0.1) | 2.4±1.0 | 2.5±1.1 | 0.1 (-0.3 to 0.4) | -0.2 (-0.4 to 0) |
| Pre HD SBP [mmHg] | 141.6±21.7 | 138.7±22.8 | -2.9 (-9.7 to 3.9) | 151.8±20.5 | 151.2±19.7 | -0.6 (-6.7 to 5.6) | -2.4 (-5.4 to 0.7) |
| Post HD SBP [mmHg] | 133.9±18.9 | 132.2±19.0 | -1.7 (-7.5 to 4.1) | 142.1±17.6 | 139.9±19.6 | -2.3 (-8 to 3.4) | 0.6 (-2.5 to 3.6) |
| Pre HD wt [kg] | 80.4±21.1 | 78.8±19.8 | -1.6 (-7.8 to 4.6) | 75.8±24.2 | 75.5±24.2 | -0.3 (-7.7 to 7.1) | -1.3 (-2.5 to -0.1) |
| Post HD wt [kg] | 78.1±20.7 | 76.6±19.5 | -1.5 (-7.7 to 4.6) | 73.4±23.7 | 73.1±23.5 | -0.3 (-7.6 to 6.9) | -1.2 (-2.4 to -0.1) |
| Intradialytic saline [mL] | 454±127 | 447±128 | -7.0 (-46 to 32) | 488±162 | 491±163 | 5 (-48 to 54) | -12.0 (-42 to 17) |

**Conclusions:** Consistent with previous reports, results from the largest sodium alignment program to date suggest positive effects on clinical parameters. Well-matched control pts minimize confounding effects. The lack of effect on hosp and small effects on other parameters may be explained by low baseline DNas limiting the interventional change of gradient.

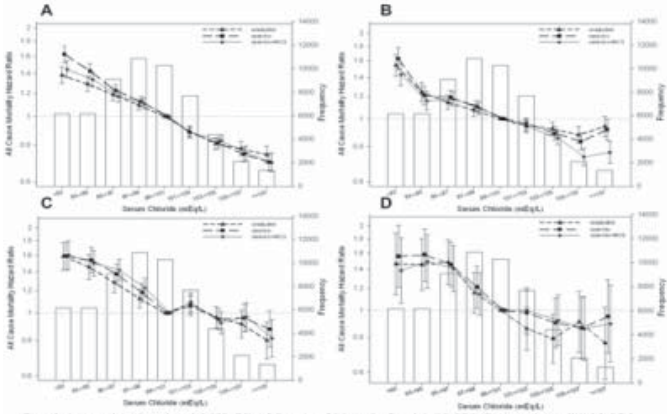
## FR-PO449

**Hypochloremia as a Novel Risk Factor of All-Cause Mortality in Hemodialysis Patients** Vanessa A. Ravel,[1] Connie Rhee,[1] Jongha Park,[1] Elani Streja,[1] Csaba P. Kovesdy,[2,3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Orange, CA; [2]Univ of Tennessee Health Science Center, Memphis, TN; [3]Memphis VA Medical Center, Memphis, TN.*

**Background:** Emerging data suggest that decreased serum chloride (Cl) levels may be associated with increased all-cause and cardiovascular death risk in the general population, but its mortality predictability has not yet been examined in hemodialysis (HD) patients.

**Methods:** Using DaVita clinical data, we identified 104,675 maintenance HD patients who underwent treatment from 2001-2007. Associations between time-averaged Cl level and all-cause mortality were estimated in the overall cohort and within subgroups of race/ethnicity using Cox regression.

**Results:** Patients were on average 61±15 years old and included 45% women and 59% diabetics. In the overall cohort, there was an inverse association between Cl levels and all-cause mortality in unadjusted, case-mix, and case-mix and malnutrition-inflammation complex syndrome adjusted analyses (reference Cl 99-<101mEq/L). In subgroup analyses of race/ethnicity adjusted for case-mix and malnutrition-inflammation complex syndrome covariates, a similar pattern of association was observed among non-Hispanic Caucasians, African-Americans, and Hispanic patients. Among Asians, lower Cl levels were associated with increased mortality, whereas higher Cl levels were not associated with greater risk.




Death hazard ratios (and 95% CI) of 8-year Cl levels for (a) 43,370 Caucasian; (b) 33,854 Black; (c) 16,156 Hispanic; and (d) 3,133 Asian HD patients.

**Conclusions:** Although our findings suggest that hypochloremia is a risk factor for mortality in HD patients, further studies are needed to confirm findings and to determine if Cl level is a direct mediator of increased mortality, or a proxy of an alternative mortality predictor in this population.

*Funding:* NIDDK Support

## FR-PO450

**Recovery of Renal Function in Patients Treated for End-Stage Renal Disease** Shari T.S. Lewis,[1] Paul L. Kimmel,[1,2] Paul W. Eggers.[2] *[1]Renal Diseases and Hypertension, George Washington Univ, Washington, DC; [2]National Institute of Diabetes Digestive and Kidney Diseases, NIH, Bethesda, MD; [3]National Institute of Diabetes Digestive and Kidney Diseases, NIH, Bethesda, MD.*

**Background:** Recovery of renal function in patients with End-Stage Renal Disease (ESRD) is thought to be uncommon. There are few data showing whether recovery rates have changed recently. Previous work has shown improved renal recovery rates in patients receiving peritoneal dialysis compared to hemodialysis, but this is not a consistent finding. Factors that predict renal recovery as well as patient outcomes after recovery remain unclear.

**Methods:** This observational cohort study included all ESRD patients enrolled in the US Renal Data System (USRDS) and Standard Information Management System (SIMS) who initiated dialysis between January 1st 1996 and December 31st 2010.

**Results:** During the study period, 1,481,248 patients were started on dialysis for ESRD. 53,553 (3.6 %) of them recovered renal function. From 1996 to 2010, there was a four-fold increase in the number of patients who achieved recovery of renal function (1152 to 5907) and a 2.5 fold increase in the proportion who recovered (2.1% to 5.2%). Recovery of renal function was almost twice as likely in White compared to Black patients (4.4% vs 2.3%, p <0.0001). A greater proportion of hemodialysis patients achieved recovery compared to peritoneal dialysis patients (2.2% vs 0.6 %, p<0.0001). 97% of patients who recovered renal function did so by 6 months. Recovery was more likely to occur in patients with baseline GFR ≥ 15 ml/min/1.73m² (6%) and in patients with acute tubular necrosis (28.5%). By 3.5 years after recovery, 50% of patients with recovered renal function had either died or returned to ESRD therapy.

**Conclusions:** We conclude that the rate of renal recovery has increased over time. Trends in early starts, an increase in AKI patients entering the ESRD program and less stringent acceptance criteria for dialysis therapy may contribute to the observed increasing recovery rates. Genetic studies may be warranted to explain the dramatic differences in rates of recovery between White patients and minority groups.

*Funding:* NIDDK Support

## FR-PO451

### Loss of Residual Renal Function in the First Year on Hemodialysis

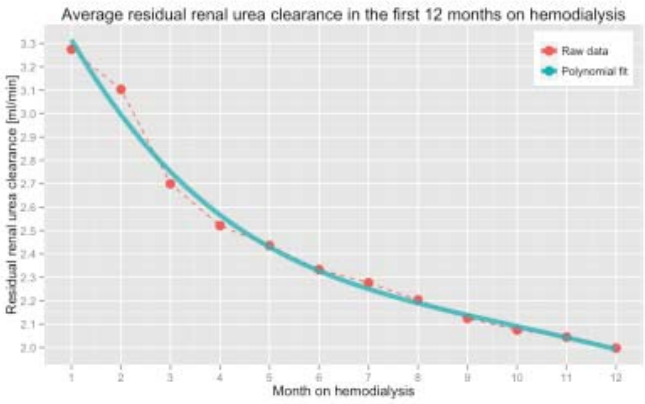
Stephan Thijssen,[1] Zachary Z. Brener,[2] Len A. Usvyat,[3] Peter Kotanko.[1] *[1]Renal Research Institute, New York, NY; [2]Beth Israel Medical Center, New York, NY; [3]Fresenius Medical Care, Waltham, MA.*

**Background:** Residual renal function (RRF) is important for several crucial aspects in kidney disease patients and is a powerful predictor of survival. RRF is generally believed to decline relatively quickly after initiation of hemodialysis (HD). We studied the time course of residual renal urea clearance (Kru) as a surrogate of RRF in the first year on HD.

**Methods:** Data for the first year on HD were extracted for incident patients who started HD between 12/2000 and 09/2009. Missing Kru values were imputed using the "next observation carried backward" method. We analyzed the subset of patients who had a Kru >0 ml/min in the first month on HD and who survived at least 18 months. The time course of Kru over the first 12 months on HD was fit using a 4th order polynomial function. The first derivative of this function was used to assess the instantaneous rate of change in Kru.

**Results:** The analysis cohort comprised 1,654 patients (age 60.4±14.5 years, 61% male, 56% diabetic, 57.3% white, 36.6% black). Average Kru in the first month was 3.3 ml/min (95% CI: 3.06-3.49). The figure shows the time course of Kru over the first 12 months on HD (top panel) and the instantaneous rate of decline in Kru (bottom panel). Kru initially declined at a rate of about 0.36 ml/min/month. This rate of loss progressively decelerated and reached a stable rate of decline of around 0.05 ml/min/month from month 9 onwards.

**Conclusions:** In this U.S. cohort of incident HD patients from Renal Research Institute clinics, Kru declined slower than we had expected. Comparisons with other North American clinics and international data will put these results into perspective and show whether Kru is unusually well-preserved in RRI clinics or whether Kru generally declines more slowly than one might expect.




*Funding:* Pharmaceutical Company Support - Renal Research Institute

## FR-PO452

### Angiotensin II Receptor Blockade Does Not Reduce Renal Function Decline in Hemodialysis Patients: A Randomized Controlled Trial

Krista D. Kjaergaard,[1] Christian D. Peters,[1] Bente Jespersen,[1] Kathrine S. Laursen,[2] Jens K. Madsen,[1] Marija K. Novosel,[4] Birgitte Pedersen,[2] I.N. Tietze,[3] Bo M. Bibby,[5] Charlotte Strandhave,[2] J. Dam Jensen.[1] *[1]Dept of Renal Medicine, Aarhus Univ Hospital, Denmark; [2]Dept of Nephrology, Aalborg Univ Hospital, Denmark; [3]Dept of Internal Medicine, Region Hospital Viborg, Denmark; [4]Dept of Internal Medicine, Fredericia Hospital, Denmark; [5]Dept of Public Health - Institute of Biostatistics, Aarhus Univ, Denmark.*

**Background:** Glomerular filtration rate (GFR) declines during chronic dialysis treatment. In peritoneal dialysis, ramipril as well as valsartan reduces GFR decline. Observational studies suggest that similar treatment may preserve renal function in hemodialysis (HD).

**Methods:** A multicenter randomized placebo-controlled double-blinded trial initiated by the investigators with one year follow-up. Primary outcome was the rate of GFR decline. Methods: Inclusion criteria were urine output >300 mL/24h, HD vintage <1 year, and cardiac ejection fraction >30%. Patients were randomized to the ARB irbesartan (300 mg daily), or placebo. Target systolic blood pressure (BP) was 140 mmHg. GFR was estimated as the mean of creatinine and urea renal clearance and measured at baseline, 1 week and 3, 6, 9, 12 months.

**Results:** Of the 82 patients randomized (placebo *n*=41/irbesartan *n*=41), 56 completed one year of treatment. Patients were similar at baseline: males 26/30, (mean±SD) age 62±14/61±16 years, HD vintage 168±95/171±93 days, HD time 10±2/11±3 hours/week, urine volume 1.31±0.71/1.45±0.79 L/24h, GFR 4.8±2.3/5.7±3.3 mL/min/1.73m$^2$. The target BP level was reached in both groups and adverse event rates were comparable. GFR declined by 1.6(1.0;2.0) (mean,95%CI) in the placebo group and 1.7(1.0;2.3) mL/min/1.73m$^2$/year in the irbesartan group, p=0.29. Urine volume decreased with 431(179;684) in the placebo group and 129(-86;144) mL/24h/year in the irbesartan group, p=0.26. In each group, four patients progressed to anuria.

**Conclusions:** In HD patients, the decline in GFR and urine volume over one year was not affected by irbesartan treatment. We found no evidence against that irbesartan could be safely used in HD patients.

*Funding:* Pharmaceutical Company Support - Sanofi A/S & NorDiaTech A/S, Private Foundation Support, Government Support - Non-U.S.

## FR-PO453

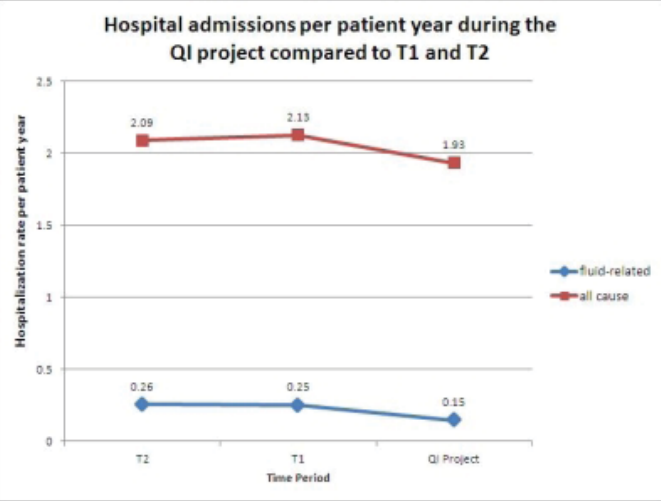
### Hospitalization Rates before and after the Initiation of a Quality Improvement Project on Fluid Management Using Crit-Line Blood Volume Monitors at 8 RRI Clinics

Linda H. Ficociello,[1] Len A. Usvyat,[1,2] Patrice B. Taylor,[2] Claudy Mullon,[1] Paul M. Zabetakis,[2] Jose A. Diaz-Buxo.[1] *[1]Fresenius Medical Care NA (FMCNA), Waltham, MA; [2]Renal Research Institute (RRI), New York, NY.*

**Background:** A quality improvement (QI) project on fluid management using FMCNA's Crit-Line Blood Volume Monitors (CLM) has been ongoing in 8 RRI Clinics. One aim of the QI project is the reduction of hospitalizations, especially fluid-related.

**Methods:** Hospitalization rates were calculated as all-cause or fluid-related hospitalizations across the 8 clinics divided by patient-time accumulated. All patients receiving hemodialysis were included (1000+ patients per time period).These ICD-9 codes were used to define fluid-related: 276.6, 276.69, 398.91, 402.01, 402.11, 402.91, 404.03, 404.11, 404.91, 428, 428.0, 428.21, 428.22, 428.23, 428.3, 428.31, 428.32, 428.33, 428.4, 428.42, 428.43, 514, 518.4, and 786.05. Repeated measures survival analysis was used to compare all-cause and fluid-related hospitalizations during the 7 months of the QI project and two comparison periods: (T2) the same 7 months in the prior calendar year and (T1) 7 months before the QI project.

**Results:** Hospitalization rates were observed to be lower during the QI project (Figure) and, in survival analysis, the difference was statistically significant for fluid-related but not for all-cause. Hazard ratios for fluid-related hospitalizations during QI project was 0.53 (p=0.05) and 0.42 (p=0.009), compared to T1 and T2, respectively. Hazard ratios for all-cause hospitalizations during the QI project was 0.86 (p=0.21) and 0.89 (p=0.30), compared to T1 and T2, respectively.




**Conclusions:** A reduction in fluid-related hospitalizations was observed during a QI project on fluid management using CLM (Hazard Ratios 0.53 and 0.42). Trends toward lower rates were observed for all-cause hospitalizations (Hazard Ratios 0.86 and 0.89).

*Funding:* Pharmaceutical Company Support - Fresenius Medical Care NA

## FR-PO454

**Patient Satisfaction of Different Aspects of Long-Term Haemodialysis Care: A Multinational Cross-Sectional Survey of Patients**

Suetonia Palmer,[1] Giorgia De berardis,[2] Jonathan C. Craig,[3] Allison Tong,[3] Marinella Ruospo,[2,4] Marcello Tonelli,[5] Jorgen B.A. Hegbrant,[4] Charlotta Wollheim,[4] Marco Angelo Murgo,[4] Eduardo Jorge Celia,[4] Ruben Gelfman,[4] Miguel Rodrigues Leal,[4] Marietta Torok,[4] Anna Bednarek,[4] Jan Dulawa,[4] Juan Nin-ferrari,[4] Staffan Schön,[4] Giovanni F.M. Strippoli.[2,3,4,6] *[1]Univ of Otago; [2]Mario Negri Sud Consortium; [3]Univ of Sydney; [4]Diaverum; [5]Univ of Alberta; [6]Univ of Bari.*

**Background:** Patients with end-stage kidney disease experience high rates of mortality, reporting profoundly impaired quality of life. Greater knowledge of how patients experience different facets of long-term dialysis could inform the design of targeted and testable strategies to improve dialysis care and quality of life. The aims of our study were to describe and quantify the satisfaction of long-term patients with their haemodialysis care.

**Methods:** This is a multinational cross-sectional survey using the 23-item CHOICE questionnaire in 2145 long-term outpatient haemodialysis patients in Europe and South America. Patients' ratings of satisfaction with overall care and specific aspects of dialysis care were evaluated.

**Results:** 2145 (78.1%) haemodialysis patients completed the survey. Fewer than half (46.5% [95% confidence interval (CI), 44.5 to 48.6%]) rated their dialysis care as excellent overall. Within countries, global perceptions of care were uninfluenced by most patient characteristics except age and depressive symptoms; older patients were less critical of their care (adjusted odds ratio for excellent rating (AOR) 1.44 [CI 1.01-2.04] for patients 70 years or older compared to those 18-49 years) and those with depressive symptoms were less satisfied (AOR for excellent rating 0.56 [CI 0.44 to 0.71] for patients with a higher depression score).

**Conclusions:** Patients treated with in-centre haemodialysis are least satisfied with the amount and reliability of information they receive during their care, particularly from nephrologists. Meeting patients' expectations of information, prognosis, the likelihood of kidney transplantation and patients' options when choosing dialysis treatment may improve patients' satisfaction with dialysis care.

## FR-PO455

**GFR for Dialysis Initiation and Patient Survival: A Physician-Based Analysis**

Jiannong Liu,[1] Haifeng Guo,[1] David T. Gilbertson,[1] Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, Univ of MN, Minneapolis, MN.*

**Background:** Although a large randomized trial (IDEAL) showed no clear difference between planned early (higher glomerular filtration rate [GFR]) and late (lower GFR) initiation groups in Australia and New Zealand, whether this is the case in the US is unknown. Hence, we linked data from the USRDS and the Medicare 5% database to examine survival associations of receiving care from physicians who typically initiate dialysis at higher GFR levels among stage 4 chronic kidney disease (CKD 4) patients.

**Methods:** Physicians who signed end-stage renal disease (ESRD) Medical Evidence forms for at least 10 patients in 2005-2007 and provided care for these patients in the 6 months before dialysis initiation were included. Patient adjusted mean eGFRs at dialysis initiation were calculated for each physician from a random effect regression model with adjustment for patient demographic and clinical characteristics. Physicians were classified as tending to initiate early (eGFR ≥ 10.9 mL/min/1.73 m$^2$, the mean) or late (eGFR< 10.9) based on the adjusted mean eGFRs. Patients first diagnosed with CKD 4 in 2008 and cared for by one of the classified physicians within 6 months before or after the first diagnosis date, identified in the 5% Medicare data, were included. Follow-up of the CKD 4 patients extended from 6 months after the first diagnosis to death, transplant, or December 31, 2011.

**Results:** 915 physicians and 2241 patients were included, with median follow-up time 33.7 months; early and late groups included 1111 and 1130 patients, respectively, defined based on their physicians. The two patient groups had very similar characteristics. Both unadjusted mortality rates (15.6 per 100 patient-years, early group, vs. 16.1, late group) and the adjusted hazard ratio for death (0.98, early vs. late, *P=0.72*) showed group similarity. For patients who developed ESRD, mean eGFRs were 13.3 and 11.8 mL/min/1.73 m$^2$ for early and late groups, respectively.

**Conclusions:** Patient survival appears to be independent of physician behavior regarding GFR levels for dialysis initiation.

*Funding:* NIDDK Support

## FR-PO456

**Comparison of Survival by Dialysis Modality: Korean Nation-Wide Prospective Cohort**

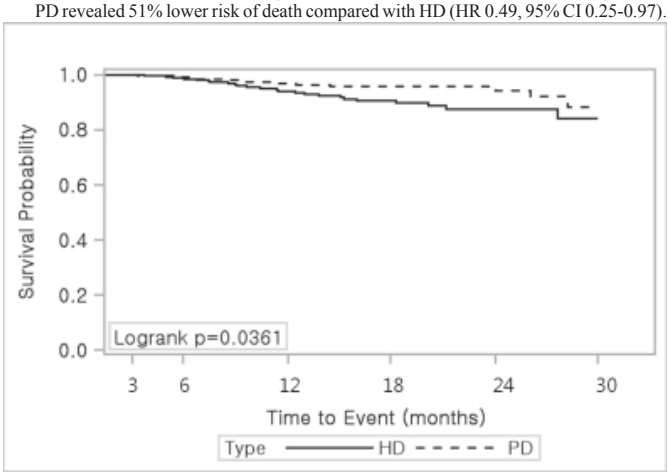
Ji-Young Choi,[1,2] Hye Min Jang,[1,2] Jongha Park,[1,3] Yon Su Kim,[1,4] Shin-Wook Kang,[1,5] Chul Woo Yang,[1,6] Nam Ho Kim,[1,7] Jang-Hee Cho,[1,2] Sun-Hee Park,[1,2] Chan-Duck Kim,[1,2] Yong-Lim Kim.[1,2] *[1]Clinical Research Center for End Stage Renal Disease (CRC for ESRD) in Korea; [2]Kyungpook National Univ; [3]Univ of Ulsan; [4]Seoul National Univ; [5]Yonsei Univ; [6]The Catholic Univ of Korea; [7]Chonnam National Univ.*

**Background:** The impact of dialysis modality on survival has still some controversies. Given possible differences in patients' characteristics, cause and rate of death among different countries, the issue needs to be further evaluated in various cohorts.

**Methods:** We compared all-cause mortality between hemodialysis (HD) and peritoneal dialysis (PD) using unmatched (n=1,060) and propensity score (PS) matched (n=556) cohorts. Data came from a Korean nation-wide prospective cohort for end-stage renal disease, in which patients starting dialysis therapy from September 1, 2008 to June 30, 2011 were enrolled.

**Results:** Patients (HD vs PD: 69.4% vs 30.6%) were followed up for 16.3±7.9 months. PD patients were significantly younger and had lower body mass index, lower proportion of diabetes, and larger urinary volume compared to HD patients. Infection was the most common cause of death (31.9% in overall, 29.2% in HD, 41.7% in PD). Multivariate Cox regression with unmatched cohort showed PD tended to be associated with 37% lower risk of death compared with HD [hazard ratio (HR) 0.63, 95% confidence interval (CI) 0.36-1.08]. In PS-matched cohort, cumulative survival probabilities from day 90 for PD and HD were 96.9% vs 94.1% at 12 months (p=0.14) and 94.3% vs 87.6% at 24 months (p<0.05).

PD revealed 51% lower risk of death compared with HD (HR 0.49, 95% CI 0.25-0.97).




**Conclusions:** PD therapy shows superior survival to HD in the early period of dialysis even after adjusting differences in patients' characteristics between two modalities.

*Funding:* Government Support - Non-U.S.

## FR-PO457

**Lower Serum Sodium Level Predicts Higher Risk of Infection-Related Hospitalization and Death in Maintenance Hemodialysis Patients**

Shintaro Mandai,[1] Michio Kuwahara,[1] Sei Sasaki.[2] *[1]Dept of Nephrology, Shuuwa General Hospital, Saitama, Japan; [2]Dept of Nephrology, Graduate School of Medicine, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** Hyponatremia is associated with increased mortality in patients with chronic kidney disease, but the specific factors contributing to mortality are unknown. This study investigated the association of serum sodium level (sNa) with risk of infectious disease in hemodialysis (HD) patients.

**Methods:** This observational cohort study included 332 patients who underwent maintenance HD in our dialysis unit in May 2009. The mean of 3 monthly measurements of glucose-corrected sNa before each dialysis session in May, June, and July 2009 was applied as baseline sNa. The primary endpoint was the first infection-related hospitalization (IRH), and secondary endpoints were infection-related death (IRD) and death of any cause.

Data were analyzed using Cox hazards modeling, adjusted for baseline demographics and characteristics, or laboratory data. Patients were followed up from May 1, 2009 until transfer, kidney transplant, death, or January 31, 2013.

**Results:** Mean sNa was 138.9 mEq/L (tertile 1: <138.0 mEq/L, n=104; tertile 2: 138.0-140.0, n=116; tertile 3: >140.0 mEq/L, n=112). During a median follow-up of 45.0 months, 57 patients experienced IRH (56.4/1,000 patient-years overall; 89.7/1,000 in tertile 1; 57.9/1,000 in tertile 2; 28.0/1,000 in tertile 3), 68 patients died, and 15 of the 68 deaths (22%) were IRDs. Higher sNa was associated with lower risk of IRH (unadjusted hazard ratio (HR) per +1 mEq/L, 0.86; 95% confidence interval (CI), 0.79-0.94; p=0.0005, and adjusted HR, 0.90; 95%CI, 0.81-0.99; p=0.036). Higher sNa was associated with lower risk of IRD (unadjusted HR, 0.80; 95%CI, 0.69-0.93; p=0.003, and adjusted HR, 0.76; 95%CI, 0.61-0.96; p=0.019), but was not associated with all-cause mortality (unadjusted HR, 0.87; 95%CI, 0.81-0.95; p=0.001, and adjusted HR, 0.92; 95%CI, 0.84-1.02; p=0.115).

**Conclusions:** Lower sNa was a predictor of higher risk for infection-related hospitalization and death in maintenance HD patients.

## FR-PO458

**Hypoalbuminemia, Renal Transplantation, and Survival in Prevalent Hemodialysis Patients** James R. Smith, Colin C. Geddes, Neal Padmanabhan. *Dept of Renal Medicine, Glasgow Renal and Transplant Unit, Western Infirmary, Glasgow, United Kingdom.*

**Background:** Hypoalbuminemia is strongly associated with increased mortality among hemodialysis (HD) patients, and guidelines suggest that renal transplantation is contraindicated in those with a predicted survival less than 5 years. The aim of this study was to assess the influence of hypoalbuminemia on mortality and subsequent transplantation in a prevalent HD cohort.

**Methods:** All prevalent patients attending 6 HD units receiving HD for >90 days on 04/06/2007 were included. Laboratory data averaged over 3-months was extracted from a prospectively maintained electronic patient record, as were demographics, hospital admissions, and transplant wait-list status. A Cox regression model was used to analyze the impact of serum albumin (sAlb) as well as phosphate and calcium on 5-year survival.

**Results:** Of 502 prevalent patients, 5-year survival was 63.3% when mean sAlb was >=3.7g/dL, falling to 38.8% if 3-3.69g/dL, and 16.5% if <3g/dL. This remained significant when corrected for age, sex, wait-list status, diabetes, HD access, alfacalcidol use, HD vintage, and weight. Pre-HD serum phosphate and calcium were not associated with survival. 117 patients were transplant wait-listed at census date; 51 had a sAlb <3.7g/dL with a 5-year survival of 70.6% compared with 86.4% for those with a sAlb >=3.7g/dL. However sAlb was not predictive in multivariate analysis. 31 (60.8%) of those wait-listed with a sAlb <3.7g/dL were transplanted, with 90.3% alive at 5 years; while 47 patients (71.2%) wait-listed with a sAlb >=3.7g/dL were transplanted, with 93.6% alive at 5 years. Of the 8 patients wait-listed with sAlb <3g/dL, 4 received a transplant, all surviving to 5 years, while only 1 of the 4 not transplanted survived.

**Conclusions:** In this large, well-defined HD cohort, mild hypoalbuminemia averaged over 3 months predicted reduced survival independent of other factors known to influence survival. However hypoalbuminemia alone may be less important in those deemed otherwise suitable for renal transplant wait-listing.

## FR-PO459

**Education Predicts Non-Compliance with Maintenance Hemodialysis** Kana Noshiro, Kobena A. Dadzie, Nijal R. Sheth, Nikolas B. Harbord, James F. Winchester. *Div of Nephrology & Hypertension, Beth Israel Medical Center, New York.*

**Background:** During a recent disaster in New York, Hurricane Sandy, stranded patients had incomplete knowledge of their hemodialysis(HD) parameters and required triage. We therefore sought to understand the depth of factual knowledge about the patient's individual dialysis prescription. It is known that missed/shortened HD times are associated with increase in hospitalization and mortality. We aimed to identify baseline characteristics which may be predictive of non-compliance with HD therapy.

**Methods:** We conducted a cross sectional study of 100 randomly selected HD patients at a HD center in Manhattan. Patients participated in a 1:1 interview about their socioeconomic status, educational background, and knowledge on hemodialysis and kidney disease. Relevant information from their medical records were retrieved. IRB approval was obtained.

**Results:** Level of education (±high school diploma) appeared to correlate with missed treatments. 27 patients were classified as "skippers" (absent for >3% of scheduled HD); and was associated with less education, less knowledge of potassium rich foods, higher serum phosphorus, more frequent shortening of HD, and more prescribed time on each HD. There were no significant differences in age, sex, number of years on HD, satisfaction, and knowledge of phosphorus rich foods, vascular access, or cause of renal failure. Though not statistically significant, there was a trend towards patients who work being less likely to skip HD.

| Characteristic | Skippers(n=27) | Nonskippers(n=73) | P |
|---|---|---|---|
| Age | 55.6±14.4 | 58.2±14.1 | 0.41 |
| >High school graduate | 21(78%) | 70(96%) | <0.01 |
| Prescribed dialysis time(hours) | 3.9±0.48 | 3.7±0.47 | <0.05 |
| On transplant waiting list | 6(22%) | 40(55%) | <0.05 |
| Know potassium rich foods | 2.26±1.43 | 3.06±2.34 | <0.05 |
| Shortened dialysis(%) | 28.1±32.5 | 8.0±13.2 | <0.01 |
| Potassium(mmol/L) | 4.8±0.73 | 4.9±0.10 | 0.43 |
| Phosphorus(mg/dL) | 5.9±1.5 | 5.3±1.2 | <0.05 |

**Conclusions:** Compliance assessment should include educational level, and efforts should be made to improve understanding and motivation for HD treatment to achieve adequate adherence. It may be necessary to direct specialized educational resources to these patients.

## FR-PO460

**Prevalence of Protective Immunoglobulin G Antibody in Maintenance Hemodialysis Patients in Korea** Jong-woo Yoon, Myung Jin Choi, Youngki Lee, Jwa-kyung Kim, Ja-Ryong Koo, Jung-woo Noh. *Internal Medicine, Hallym Kidney Research Institute, Chuncheon, Gangwon, Korea.*

**Background:** Hepatitis A is one of the most common acute infectious diseases and usually does not cause chronic disease process. But around 1% of it progress to life-threatening fulminent hepatitis. In the past, seroprevalence of anti-HAV was higher, but recently it has been decreasing in Korea. The incidence of acute hepatitis A in 2010 is 10-fold higher than 20 years before. It shows more severe clinical course and composes more than 80% of inpatient hepatitis care. ESRD is associated with increased propensity to infection, more severe clinical outcomes. DOQI guidelines suggest active immunization and having protective antibody for hepatitis A if seronegative is found. But the prevalence of protective immunoglobulin G antibody in maintenance hemodialysis patients is not known.

**Methods:** 171 ESRD maintained more than 3 months dialysis duration were evaluated for seroprevalence of protective immunoglobulin G antibody and its antibody titer for hepatitis A. Individuals with evidence of acute and chronic infection were excluded. Whole blood was centrifused and collected serum was tested by CMIA(Chemiluminescent microparticle immunoassay) to detect antibody titer.

**Results:** 164 from 171 hemodialysis patients have protective level of Immunoglobulin G antibody and prevalence of seropositivity was 95.88%. It was higher with increasing age*(58.74±11.42 vs 30.92±29.32, p=0.01)* and long dialysis duration*(51.08±51.94 vs 30.92±29.32, month, p=0.01)*. Prevalence of seropositive for hepatitis A was not different between patients living in metropolitan Seoul and ruler area Chuncheon. Anti-HAV seroprevalence was about 50% in those aged less than 40 years old and which was significantly lower than that of age over 50. Seroprevalence of anti-HAV of hemodialysis patients was not different from general population.

**Conclusions:** Prevalence of protective immunoglobulin G antibody in maintenance hemodialysis patients in Korea was about 95%. Patients of age under 40 showed about 50% seroprevalence and that was not different from general population. Active immunization against hepatitis A is more strongly recommended for ESRD patients age under 40 years.

## FR-PO461

**N-DEPTH–Nephrology DVT and Pulmonary Embolism Prophylaxis Study in Hospitalized Patients** Jule Pinter, Azim S. Gangji, Catherine M. Clase, Christine M. Ribic. *Medicine, Div of Nephrology, McMaster Univ, Hamilton, Canada.*

**Background:** The prevalence and mortality rate of venous thromboembolism (VTE) among patients on dialysis has been reported to be higher than previously thought. However, the prophylaxis prescription practice, VTE and bleeding risks in hospitalized patients with endstage renal disease (ESRD) have not been studied adequately.

**Methods:** This single centre, retrospective chart review examined the rate of VTE prophylaxis in adult patients treated with chronic dialysis (>3 months) on index admission during Sept 2008 to March 2010. Patients admitted for < 48 hours, suspected VTE, bleed or renal transplant were excluded. VTE prophylaxis was defined as administration of prophylactic doses of heparin within 24 hours of admission. We assessed 27 VTE risk factors per patient based on the 8th edition of ACCP guidelines; VTE events objectively confirmed by imaging and major bleeds were adjudicated.

**Results:** We screened 1183 consecutive admissions of which 238 patients formed the study cohort. 45 (18.9%) patients were ineligible for prophylaxis due to therapeutic anticoagulation (AC), prophylactic anticoagulation, heparin allergy or severe thrombocytopenia on admission. Of the 193 eligible patients, 71(36.8%) received VTE prophylaxis. The number of baseline VTE risk factors (range 1 to 7; p=0.59), anticoagulant or antiplatelet use and typical VTE risk factors such as Immobility (22.3 %), recent surgery (13.5%), a history of malignancy (16.1%) or of VTE (4.7%) did not predict the prescription of VTE prophylaxis.

VTE events (1 pulmonary embolism, 1 deep vein thrombosis) occurred in 2 (1.6%) eligible patients not receiving prophylaxis, no events occurred in those receiving prophylaxis (p=0.53). There was no difference in minor or major bleeds between those that did (n=5) and did not receive prophylaxis (n=12; p=0.51).

**Conclusions:** Approximately 1/3 of eligible hospitalised patients received VTE prophylaxis and prescription practices did not correlate with the quality or summation of VTE risk factors. Further study into individualized risk stratification strategies for hospitalized patients with ESRD are needed to help guide VTE prophylaxis practices.

*Funding:* Private Foundation Support

**FR-PO462**

**The Diagnostic Accuracy of the Tuberculin Skin Test, QuantiFERON-TB Gold, and T-Spot.TB in Determining Latent Tuberculosis Infection in Hemodialysis Patients: A Systematic Review and Meta-Analysis**
Thomas W. Ferguson,[1] Navdeep Tangri,[1] Kerry Macdonald,[3] Brett M. Hiebert,[1] Claudio Rigatto,[2] Manish M. Sood,[2] Salah Mahmud,[1] Souradet Y. Shaw,[1] Blake R. Lerner,[1] Paul Komenda.[2] *[1]Community Health Sciences, Univ of Manitoba, Winnipeg, Canada; [2]Medicine, Univ of Manitoba, Winnipeg, Canada; [3]Library Services, Univ of Manitoba, Winnipeg, Canada.*

**Background:** Detection and treatment of Latent Tuberculosis Infection (LTBI) is a concern in patients with kidney failure (KF) wait-listed for a kidney transplant, since post-transplant reactivation of LTBI is associated with catastrophic outcomes. There are several diagnostic tests available to determine the presence of LTBI: the Tuberculin Skin Test (TST), QuantiFERON-TB Gold (QFT-G), and ELISPOT (T-SPOT.TB). Our objective was to compare the diagnostic performance of these tests for LTBI in the KF population.

**Methods:** We conducted a systematic review of studies from PubMed, Scopus, EMBASE, and Cochrane Database of Systematic Reviews from the date of their establishment until August 2012. Studies reporting test results allowing the calculation of the sensitivity and specificity with respect to clinical risk factors (previous TB contact, history of TB, and/or suggestive chest X-ray) of the TST, TSPOT.TB, and/or QFT-G in the KF population were included. Data was extracted by two investigators with disagreements resolved by consensus.

**Results:** Our search strategy retrieved 1780 citations for screening. Of these, 92 articles were selected for full-text review and 15 met criteria for inclusion. The TST had a pooled sensitivity of 0.22 across 11 studies and a pooled specificity of 0.65 across 10 studies. The QFT-G test had a pooled sensitivity of 0.58 across 8 studies and a pooled specificity of 0.69 across 8 studies. The T-SPOT.TB test had a pooled sensitivity of 0.50 across 3 studies and a pooled specificity of 0.67 across 3 studies.

**Conclusions:** The QFT-G and the T-SPOT.TB tests were more sensitive and specific than the TST for diagnosis of LTBI in the hemodialysis population. This systematic review calls into question the current practice of using the TST to screen for LTBI due to the low sensitivity of this test.

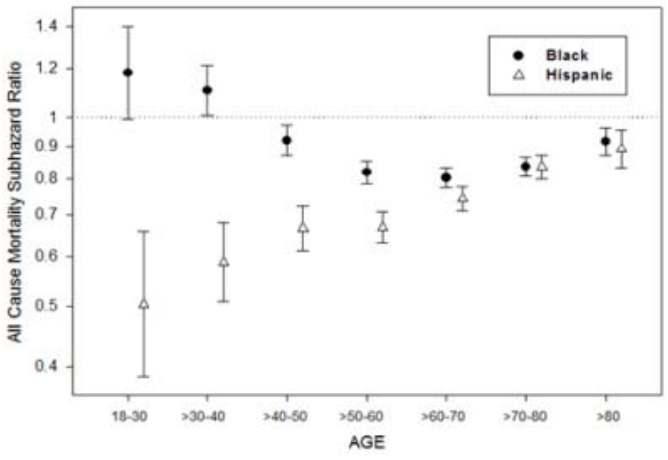
**FR-PO463**

**Age, Race-Ethnicity and Dialysis Patient Mortality Using Competing Risks Regression**
Connie Rhee,[1] Paungpaga Lertdumrongluk,[1] Elani Streja,[1] Jongha Park,[1] Hamid Moradi,[1] Wei Ling Lau,[1] Keith C. Norris,[2] Allen R. Nissenson,[3] Alpesh Amin,[4] Csaba P. Kovesdy,[5,6] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Orange, CA; [2]Drew Univ, Los Angeles, CA; [3]DaVita Inc., El Segundo, CA; [4]UCI, Orange, CA; [5]Memphis Veterans Affairs Medical Center, Memphis, TN; [6]Univ of Tennessee Health Science Center, Memphis, TN.*

**Background:** Recent studies suggest the paradoxical African-American (referred to as black) survival advantage on dialysis is restricted to older ages, but did not separately consider Hispanic ethnicity from white and black racial groups. Prior studies have not examined the impact of age and race-ethnicity on dialysis mortality using competing risks regression, which estimates death risk on dialysis while considering racial-ethnic disparities in kidney transplantation (a competing risk that prevents observing the outcome of interest). We thus re-examined the hypothesis that minorities on dialysis have survival advantages using this method.

**Methods:** We examined associations between race-ethnicity with all-cause mortality among a 2001-2009 cohort of 130,909 adult dialysis patients within 7 age groups using competing risks regression (in which transplant and end of study were considered as competing risk and censoring events, respectively) to account for differential transplant rates in non-Hispanic whites (referred to as whites), blacks, and Hispanics.

**Results:** Compared to whites, blacks had similar, slightly higher, and reduced mortality within age groups of 18-30, >30-40, and >40 years, respectively, in case-mix analyses. Hispanics had decreased mortality compared to whites across all ages.




**Conclusions:** After accounting for racial-ethnic differences in transplantation, we observed that compared to white dialysis patients, Hispanics have greater survival across all ages, while in blacks this survival advantage is limited to patients >40 years old.

*Funding:* NIDDK Support, Private Foundation Support

**FR-PO464**

**Association between Depression Symptoms and Mortality in Hemodialysis Patients**
Li Fan, Mark J. Sarnak, Hocine Tighiouart, David A. Drew, Kristina Lou, Saeed Kamran Shaffi, Tammy Scott, Daniel E. Weiner. *Tufts Medical Center.*

**Background:** Depression and symptoms of depression are associated with increased risk of mortality in the general population and in people with chronic kidney disease. We evaluated the association between depression symptoms and all-cause mortality in a cohort of hemodialysis (HD) patients.

**Methods:** In 313 maintenance HD patients, depression symptoms were assessed using the Center for Epidemiologic Studies Depression (CES-D) scale, with higher scores indicating a greater burden of depression symptoms. Cox proportional hazards models were used to evaluate the relationship between depression symptoms and mortality in univariate and multivariable analyses.

**Results:** Mean age was 63 ± 16 years, 53% were female and 22% black. Mean CES-D score was 10.6 ± 8.1, and 80 (26%) participants had CES-D scores ≥16, a threshold consistent with depression in prior studies. Participants with CES-D scores ≥16 were more likely to have a history of diabetes, heart failure and smoking and have lower Kt/V and serum albumin. During mean follow-up of 33 ± 25 months, 157 participants died; there were 16.7 and 23.4 deaths per 100-patients years in individuals with CES-D scores <16 and ≥16, respectively. There was a borderline association between CES-D score ≥16 and death in the univariate model [HR 1.40 (0.99 - 1.98)]. After adjusting for age, sex, race, cause of kidney disease, dialysis vintage, vascular access, history of cardiovascular disease, Kt/V and serum albumin, CES-D score ≥16 was associated with an increased risk of all-cause mortality [HR 1.44 (1.00- 2.08)]. In multivariable analyses examining CES-D as a continuous term, for each 1 SD higher CES-D there was a 19% increase in all-cause mortality risk [HR 1.19 (1.00-1.42)].

**Conclusions:** Symptoms of depression are associated with increased risk of mortality in hemodialysis patients. Further studies are needed to confirm these results and evaluate whether effective treatments for depression may impact mortality among HD patients.

*Funding:* NIDDK Support

**FR-PO465**

**CD4+CD25+Foxp3+ Tregs and TH-17 Cells Balance in Tumor Immunity after Kidney Transplantation**
Jianghua Chen. *Zhejiang Univ.*

**Background:** To find the relationship between tumor incidence of the kidney transplantation and Tregs expression, we analyzed the $CD4^+CD25^+Foxp3^+$ Tregs and IL17 expression in the cancer patients after the kidney transplantation.

**Methods:** Blood samples were obtained from age-matched 19 kidney transplantation patients got tumor, 19 normal kidney transplantation patients and 19 healthy controls. $CD4^+CD25^+Foxp3^+$ Tregs were analyzed by flow cytometry. Serum levels of cytokines IL-17 was measured using enzyme-linked immunosorbent assays.

**Results:** The healthy controls have the highest $CD4^+CD25^+Foxp3^+$ Tregs expression in the peripheral blood, and the normal kidney transplantation patients have the lowest expression, and the cancer group get the middle level of expression. And the healthy controls have the highest IL17 expression in the peripheral blood, and the cancer group have the lowest expression, and the normal kidney transplantation patients get the middle level of expression.

**Conclusions:** Tregs expressions are higher, while IL17 expressions are lower in the patients got tumor after the kidney transplantation, they plays an important role in tumor incidence after the kidney transplantation.

*Funding:* Government Support - Non-U.S.

**FR-PO466**

**An Anti-CD154 Domain Antibody Prolongs Graft Survival and Induces FoxP3+ iTreg in Both the Absence and Presence of CTLA4-Ig**
David F. Pinelli,[1] Anish Suri,[2] Steven Nadler,[2] Mandy L. Ford.[1] *[1]Emory Univ; [2]Bristol Myers Squibb.*

**Background:** Blockade of CD40-CD154 interactions has been shown to be highly effective in inducing long-term survival of allografts in both murine and NHP models. However, the clinical potential has yet to be realized, due to the thromboembolic complications seen during clinical trials of an anti-CD154 monoclonal antibody in humans. Thus, development of novel therapies with improved safety profiles is imperative for the field of transplantation.

**Methods:** We compared the most commonly used clone of anti-CD154 antibody, MR-1, with an anti-CD154 domain antibody with a silent Fc region (dAb). We first measured efficacy of these therapies in a fully allogeneic BALB/c to B6 model of skin transplantation. To further investigate the mechanism of action of these therapies, we used a transgenic model where OT-I CD8 and OT-II CD4 T cells were adoptively transferred to B6 mice that then received skin grafts that ubiquitously express the OVA protein.

**Results:** Mice treated with CTLA4-Ig alone quickly rejected fully allogeneic grafts (MST = 15.5 d), whereas treating with MR-1 or dAb in combination with CTLA4-Ig resulted in significant prolongation of graft survival (MST= 33 and 31 d, respectively). Treatment with either therapy led to a significant reduction in allospecific T cell expansion

in the spleen (No Rx 1.64±0.55%, MR-1 0.50±0.14%, dAb 0.62±0.23%), as well as a reduction in the number of IFN-γ-producing allospecific CD8 T cells (No Rx 31.05±5.68%, MR-1 10.91±4.59%, dAbs 12.66±7.80%). In addition, both treatments led to a significant increase in antigen-specific iTreg conversion (No Rx 3.46±0.86%, MR-1 19.06±2.41%, dAb 14.74±1.55%), and dAb-induced iTreg conversion was even preserved in the presence of CTLA4-Ig (26.35±5.05%), a therapy known to have a negative effect on Treg.

**Conclusions:** Taken together, these data demonstrate that alternative means of blocking CD40-CD154 interactions may provide a safer and equally efficacious way to attenuate alloreactive responses, and suggest the potential for clinical application of this therapy in combination with existing modes of treatment.

*Funding:* Other NIH Support - NIAID R01 AI073707-05

## FR-PO467

**Myd88 Plays a Critical Role in Acute and Chronic Rejections of Kidney Allografts** Nadine M. Lerret,[1] Ting Li,[2] Jiaojing Wang,[2] Xueqiong Wang,[2] Zheng Jenny Zhang,[2] Xun-Rong Luo.[1,2] *[1]Medicine, Northwestern Univ, Feinberg School of Medicine; [2]Surgery, Northwestern Univ Feinberg School of Medicine; [3]Microbiology and Immunology, Northwestern Univ Feinberg School of Medicine.*

**Background:** Toll-like receptors in kidney injuries have been best studied in ischemic renal dysfunction and in autoimmune GNs. Their role in kidney transplantation rejection is less well characterized.

**Methods:** In the present study, we used a stringent C57BL/6 (B6) to BALB/c mouse kidney transplant model to study the role of myeloid differentiation protein 88 (MyD88) in kidney allograft survival.

**Results:** We found that Myd88$^{-/-}$ BALB/c recipients significantly protected B6 kidney allografts, with ~50% achieving graft survival >90 days, whereas majority of wild-type (WT) recipients rejected their grafts by day30. In long-term protected Myd88$^{-/-}$ recipients, donor skin grafts were accepted indefinitely but third party skin grafts were rejected, demonstrating donor-specific tolerance. Interestingly, we observed that initial acute rejection (AR) manifested as tubulitis with infiltrating T cells was similar in both Myd88$^{-/-}$ and WT recipients at day7 post transplant, although there was trend towards a decrease in infiltrating Gr1$^{+}$ cells in Myd88$^{-/-}$ recipients and the infiltrates further decreased if Myd88$^{-/-}$ donor B6 kidneys were transplanted. However, by day14, Myd88$^{-/-}$ recipients exhibited a significant decline in both graft infiltrating CD8 T cells as well as Gr-1$^{+}$ cells, accompanied by a decrease in the levels of IL-6, IL-17, IFN-γ, and CCL2. By day90, kidney allografts in Myd88$^{-/-}$ recipients showed minimal cellular infiltration or interstitial fibrosis. T cells from Myd88-/- recipients exhibited diminished proliferation to donor stimulation that could be rescued with exogenous IL-6, providing a mechanism linking the lack of MyD88 signaling to a decrease in cellular infiltration of kidney allografts that resulted in long-term graft acceptance.

**Conclusions:** This study demonstrates that Myd88 signaling plays a critical role in both acute and chronic rejection of kidney allografts, and provides a model for studying druggable targets involving Myd88 signaling in kidney transplantation.

*Funding:* Other NIH Support - NIAID T32 training grant

## FR-PO468

**Cell-Based Therapy with S1P3 Deficient Dendritic Cells Induces Prolongation of Heart Allograft Survival and Protection from Kidney Ischemic-Reperfusion Injury** Amandeep Bajwa, Liping Huang, Hong Ye, Peter I. Lobo, Mark D. Okusa. *Dept of Med./CIIR, Univ of Virginia.*

**Background:** The plasticity of dendritic cells (DCs) permits phenotypic modulation *ex-vivo* by gene expression or pharmacological agents and these DCs exert their *in-vivo* therapeutic immunosuppressive effects through direct interactions with T-cells by either deleting or causing anergy. Sphingosine 1-phosphate (S1P), a ligand for 5 receptors (S1P1-5Rs) and S1PR agonists reduced kid ischemia-reperfusion injury (IRI) in mice. S1P3-/- (3KO) mice are protected from kid IRI and allogenic heart Tx 3KO (donors) have prolonged graft function with immature DCs. Since ischemic injury contributes to transplant outcome, we tested therapeutic advantage of using bone marrow derived DCs (BMDCs) from 3KO in kidney IRI and allogenic heart transplants (Tx) in mice.

**Methods:** WT B6DC and 3KO B6DCs from mice were adoptively transferred into Balb/c or B6 (LPS stimulated) mice 2d prior to kidney IRI and 7d prior to heart Tx (B6 heart□Balb/c).

**Results:** Naïve Balb/c mice (no DC) had significant rise in creatinine (Cr; mg/dl) compared to sham operated mice (1.2±0.17 and 0.2±0.004, p≤0.01) and mice pretreated with WT B6DC had Cr that were similar to mice receiving no DCs. However, 3KO B6DCs pretreated Balb/c mice were significantly protected from kidney IRI with Cr of 0.3±0.07, p≤0.01. Additionally, 3KO B6DC pretreated Balb/c mice prolonged allogenic heart allografts (6 vs 12d; sensation of heart beat) with less inflammatory cells compared to no DCs or WTB6 DCs injected mice. 3KO B6DCs pretreated mice had significantly higher Treg compared to naïve Balb/c mice and WT B6DC pretreated mice. 3KO B6DCs induced protection required the recipient to have an intact spleen and B/T cells, as pre-treatment of splenectomized mice or use of Rag1KO rendered 3KO B6DCs ineffective in attenuating IRI.

**Conclusions:** We therefore conclude that adoptive transfer of 3KO B6DCs attenuates kidney IRI and improves graft function through interaction with splenocytes and induction of Tregs (CD4$^{+}$FoxP3$^{+}$). Development of selective S1P3 inhibitors may lead to a new therapeutic strategy to prevent AKI and reduce incident of acute rejection.

*Funding:* NIDDK Support

## FR-PO469

**Glomerular Endothelial microRNA Expression Profiles in an In-Vitro Model of Acute Humoral Rejection** Stephanie Zell,[1] Philip Zeuschner,[1] Oliver Witzke,[2] Clemens L. Bockmeyer,[1] Jan U. Becker.[1] *[1]Institute of Pathology, Hannover Medical School, Hannover, Lower Saxony, Germany; [2]Univ Duisburg-Essen, Clinic for Nephrology, Essen, Northrhine-Westfalia, Germany.*

**Background:** Acute humoral rejection (AHR) is mediated by alloantibodies against donor endothelial cells with and without complement activation. Histological and serological diagnosis remains challenging. Therefore, identification of a diagnostically useful miRNA signature for complement-mediated and complement-independent AHR is most desirable.

**Methods:** Human renal glomerular endothelial cells (HRGEC) were HLA-typed and treated with binding (HLA-A1) or non-binding (HLA-A2) antibody with and without addition of complement. Expression of 762 microRNAs was analyzed by Taqman Low densitiy arrays in a model for 1. complement-mediated AHR, 2. complement-independent AHR and 3. the additional effects of complement in AHR. Here we defined miRNAs as up- or down-regulated when a p value of < 0.05 in two-sided t-test.

**Results:** In complement-mediated AHR several microRNAs were down-regulated (miR-29b, miR-195, miR-215, miR-641) and miR-770-5p was up-regulated. In complement-independent AHR several miRNAs were up-regulated (miR-125a-5p, miR-374b#, miR-501-3p among others) and down-regulated (miR-520c-3p, miR-1201, miR-1255b). Additional effects of complement in AHR show a signature of up-regulated miRNAs (miR-554, miR-601, miR-604, miR-886-3p, miR-1255b) and down-regulated miRNAs (miR-133a, miR-423-5p, miR-502-3p).

**Conclusions:** With microRNA profiling in an *in-vitro* model we identified a glomerular endothelial-specific microRNA signature of complement-mediated and independent forms of AHR. These miRNA signatures will be validated for the tissue-based diagnosis of complement-mediated and independent forms of AHR. Putative target mRNAs will be identified by *in-vitro* experiments.

## FR-PO470

**Protective Effect of Anti-HMGB1 Antibody against Chronic Cyclosporine Nephrotoxicity in a Mouse Model** Hoon Suk Park, Eun Nim Kim, Min Young Kim, Ji Hee Lim, Jong Hoon Lee, Keun Suk Yang, Ji Hyun Yu, Seun Deuk Hwang, Cheol Whee Park, Chul Woo Yang, Bum-Soon Choi. *Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** HMGB1 (High mobility group box1) is known to be an important mediator in inflammatory pathway. It is associated with ischemic insults in myocardial and cerebral infarction, so its blockade leads to protection from organ damages. We performed this study to see if the blocking of HMGB1 prevents chronic cyclosporine (CsA) toxicity in a mouse model.

**Methods:** Male ICR mice (25g) were used. Chronic CsA toxicity was caused by its subcutaneous (SC) injection daily for 4 weeks. Each group (n=6) was respectively control group (olive oil 1mL/kg SC injection), CsA toxicity group (CsA 30mg/kg SC injection), anti-HMGB1 group (anti HMGB1 chicken IgY antibody (600mg/mouse) intraperitoneal (IP) injection weekly for blocking HMGB1) and non-specific IgY group (polyclonal non-specific chicken IgY antibody (600mg/mouse) IP injection weekly).

**Results:** Anti- HMGB1 group showed decreased 24 hour albuminuria (23.78 ± 8.06 mcg/day vs. 62.69 ± 28.83 mcg/day, p = 0.03), increased creatinine clearance (0.12 ± 0.03 ml/min vs. 0.07 ± 0.02 ml/min, p = 0.05) and decreased serum creatinine level (0.22 ± 0.02 mg/dl vs. 0.33 ± 0.04 mg/dl, p = 0.01), compared with CsA toxicity group. Tubular interstitial fibrosis area (2.19 ± 1.97 % vs. 14.65 ± 6.54 %, p = 0.008) and TGF-beta immunohistochemical stain (11.47 ± 0.88 fold vs. 16.06 ± 4.81 fold, p = 0.05) were also decreased in anti-HMGB1 group vs. CsA toxicity group. 8 OHDG level in 24 hour urine was decreased, but was not significant (52.94 ± 15.34 mcg/day in anti HMGB1 group vs. 72.45 ± 13.77 mcg/day in CsA group, p = 0.12). RAGE (0.74 ± 0.03 fold vs. 1.27 ± 0.29 fold, p = 0.02) and TLR4 (0.41 ± 0.09 fold vs. 0.89 ± 0.14 fold, p = 0.05), which are known to interact with HMGB1, expressions were decreased in anti-HMGB1 group vs. CsA toxicity group.

**Conclusions:** The administration of anti-HMGB1 brought renal functional improvements and ameliorated fibrosis induced by CsA and it is thought to result from decrease in TLR4 & RAGE expressions.

## FR-PO471

**Analysis of the Impact of Immunosuppressive Drugs on DNA Methylation in Kidney Cells** Jessica Thalmann, Barbara Hertel, Hermann G. Haller, Annette D. Wagner. *Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** Immunosuppressive drugs from different groups, e.g. calcineurin inhibitors, IMPDH inhibitors, or mTOR inhibitors are used to prevent rejection after kidney transplantation. The desired immunosuppressive effects are mediated by distinct molecular mechanisms. Moreover, these drugs produce a variety of nonimmune adverse effects to other tissues, and the molecular mechanisms are widely unknown. Epigenetic side-effects have been described or suggested for other pharmaceuticals, and there is growing evidence that methylation modifications are linked to a number of dysfunctions and diseases. We therefore hypothesize that immunosuppressive drugs alter epigenetic methylation patterns, representing a new molecular mechanism how adverse effects of immunosuppressive drugs are mediated.

**Methods:** In *in vitro* experiments we treated several epithelial kidney cells (Vero cells, the human proximal tubular epithelial cell line HK-2, and the primary human renal proximal tubular epithelial cells RPTEC) with either cyclosporine, tacrolimus, everolimus or mycophenolic acid in various concentrations. For determination of relative DNA methylation levels we established a flow cytometry protocol using anti-5-meC antibody.

**Results:** A significant decrease of DNA methylation was observed after tacrolimus treatment in a dose dependent manner. However, neither cyclosporine, nor mycophenolic acid, nor everolimus induced significant changes in the DNA methylation signal. In order to identify affected genes, and for absolute quantification of the methylation status, a genome-wide screening of DNA methylation was carried out using Infinium HumanMethylation27 BeadChip. Significant methylation changes were found in 323 genes. Network analysis reveals that the top three network functions include hematological system development and function, renal and urological disease, drug metabolism, endocrine system development and function, and inflammatory response, among others.

**Conclusions:** Our data indicate that tacrolimus changes epigenetic patterns, and provide evidence that these changes may contribute to tacrolimus´ adverse effects (nephrotoxicity, hemolytic-uremic syndrome, post-Tx diabetes, BKV infection).

*Funding:* Government Support - Non-U.S.

## FR-PO472

**Polyoma Virus Infection Leads to Hypermethylation and Down-Regulation of Matrix Metalloproteinases in Primary Renal Tubulus Epithelial Cells** Jessica Thalmann, Barbara Hertel, Hermann G. Haller, Annette D. Wagner. *Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** BK virus is a ubiquitous polyomavirus that persistently infects the kidney. This otherwise silent infection can reactivate in transplanted and immunosuppressed individuals leading to polyomavirus-associated nephropathy (PVAN) with irreversible renal scarring and fibrosis, even when the infection is cleared. The pathomechanisms are poorly understood. We hypothesize that BK virus infection influences host cell DNA methylation that leads to ongoing alteration of gene expression contributing to the development of PVAN.

Pathological fibrosis results from an imbalance in the normal processes of synthesis and degradation of extracellular matrix components, and kidney scarring is associated with a reduction of matrix metalloproteinases (MMP) activity.

**Methods:** We infected primary human renal proximal tubule epithelial cells (RPTEC) with BK virus and analyzed the CpG island methylation status of matrix metalloproteinases by MassArray. mRNA expression levels were determined by real time PCR.

**Results:** We found that the CpG islands of MMP-1 and MMP-2 show hypermethylation in BKV infected cells as compared to noninfected cells. Moreover, DNA methyltransferase-1 that is known to induce de novo methylation was found to be up-regulated in BKV infection. The mRNA expression of both MMP-1 and MMP-2 was down-regulated.

**Conclusions:** The down-regulation of MMPs might lead to the accumulation of extracellular matrix in the progression of PVAN. These findings might reflect a novel mechanism how BKV infection contributes to the onset and maintenance of renal scarring in PVAN, and provide an explanation for the surpassing effect even in the absence of BKV when the infection is cleared.

*Funding:* Government Support - Non-U.S.

## FR-PO473

**A Novel Mouse Model for Unraveling the Mechanism behind Tacrolimus Mediated Hypertension and Hyperkalemia** Rebecca A. Lazelle, Nick Meermeier, Chao-Ling Yang, David H. Ellison. *Div of Nephrology and Hypertension, Oregon Health and Science Univ, Portland, OR.*

**Background:** Calcineurin inhibitors, such as tacrolimus (FK-506), are used to prevent organ transplant rejection. While effective, tacrolimus causes clinically limiting side effects including hypertension, hyperkalemia, and chronic kidney disease. We have shown that the sodium chloride cotransporter (NCC), located in the distal convoluted tubule (DCT) of the kidney, is necessary for the development of hypertension, but it is unclear whether tacrolimus acts directly in the DCT to cause this pathology. We have developed a unique mouse model, which takes advantage of the fact that tacrolimus requires an endogenous binding partner-FKBP12-to inhibit its target calcineurin. This model allows us to test the role of both components of the immunosuppressive pathway-FKBP12 disruption and calcineurin inhibition-in the DCT *in vivo*.

**Methods:** Using a Doxycycline inducible CRE/LOX system, we excised FKBP12 from renal epithelial cells of adult mice. We characterized these mice using immunofluorescence, Western blots, radiotelemetry and I-stat analysis.

**Results:** Immunofluorescence revealed that FKBP12- considered ubiquitous-is found predominantly in the glomeruli and the DCT of the kidney. Doxycycline treatment reduced FKBP12 by approximately 90%, with deletion occurring only along the nephron. FKBP12 KO mice appeared phenotypically normal. Mean arterial pressure was similar in WT and KO mice (WT 102 ±1 mmHg; KO 101 ±5.7). Total and phosphorylated NCC expression was also comparable (tNCC WT 100 ±11%; KO 90 ±19%, pNCC WT 100 ±10%; KO 102 ±10%), as was serum $Na^+$ (WT 145.3 ±0.42 mmol/L; KO 144.4 ±0.30) and $K^+$ (WT 3.94 ±0.1 mmol/L; KO 3.96 ±0.1).

**Conclusions:** Our data suggests that FKBP12 disruption in the DCT does not drive tacrolimus-mediated hypertension and hyperkalemia. We are now testing whether it protects against tacrolimus-mediated toxicity by preventing calcineurin inhibition in the DCT. The expression pattern of FKBP12, however, may explain the predominance of hyperkalemia in patients treated with tacrolimus compared with calcineurin inhibitors, such as cyclosporine, that have different binding partner requirements.

*Funding:* NIDDK Support

## FR-PO474

**Antiproteinuric Effects of Green Tea Extract on Tacrolimus-Induced Nephrotoxicity in Mice** Byung Chul Shin, Jong Hun Back. *Dept of Internal Medicine, Chosun Univ Hospital, Gwangju, Republic of Korea.*

**Background:** It has been reported that the proteinuria is a early useful marker in detection of tacrolimus (TAC) nephrotoxicity. Green tea extract (GTE), as an antioxidant , induces apoptosis in renal interstitial fibroblast cells in vitro studies. The aim of this study was to investigatethe anti-proteinuric effects of GTE on TAC-induced acute renal injury in mice.

**Methods:** The mice (n=20) were divided into 4groups (n=5/group); control group wereintraperitoneally (IP) injected 0.9% saline for 7 days,TAC group were IP injected TAC1 mg/kg, iNOS inhibitor group were given inaddition NG-nitro-L-arginine-methyl ester (L-NAME)12 mmol/L by subcutaneous injection. TAC-GTE group were given TAC by IP injection andGTE 100 mg/kg by subcutaneous injection.

**Results:** The 24 hours urine protein levelswere significantly increased in TAC group (36.1± 9.9mg/day) compared to control group (13.3± 5.4mg/day, $P < 0.01$) and significantly decreasedin TAC-GTE group (19.1± 6.9mg/day, $P < 0.01$) compared to TACgroup. The NO production by TAC was significantly suppressed by GTE and iNOS inhibitor ($P < 0.01$). Renal tissue MDA level was significantly increased in TAC group compared to control group andsignificantly decreased in TAC-GTE group compared than that of TAC group ($p<0.01$). The anti-oxidant enzyme activities of superoxide dismutase and catalase were significantly suppressed in TAC group compared with control group and restored in TAC-GTE group compared with CsA group ($p < 0.05$).

**Conclusions:** This study proves that proteinuriaof the TAC induced nephrotoxicity is associated withlipid peroxidation and nitric oxide production. GTE treatment has meaningful anti-proteinuric effects throughantioxidative effect in the kidney from TAC-inducedacute renal injury in mice.

## FR-PO475

**Impact of Blocking Interleukin 6 Receptor with Antibody Producing Nonviral Minicircle DNA in Skin Allograft Rejection** Jian Jin,[1] Kyoung Chan Doh,[1] Long Jin,[1] Shang Guo Piao,[1] Youngkyun Kim,[3] Jihyun Ju,[3] Sun Woo Lim,[1] Byung Ha Chung,[1,2] Chul Woo Yang.[1,2] *[1]CRCID & Transplant Research Center, The Catholic Univ of Korea; [2]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea; [3]Div of Rheumatology, Dept of Internal Medicine, The Catholic Univ of Korea.*

**Background:** Blocking of interleukin-6(IL-6) is effective in decreasing resistance to allogenic tolerance. In this study, we investigated whether anti-IL-6 receptor producing nonviral minicircle(MC)-DNA, is competent to inhibit alloresponse-induced IL-6 in highly immunogenic murine skin allograft model.

**Methods:** We designed MC-DNA producing anti-IL-6R and verified in vitro system. One day before skin allograft modeling, systemic MC-DNA exposed via hydrodynamic delivery(tail vein, single injection). Using GFP tagging MC-DNA, we confirmed its organ distribution. At day 5, we measured the amount of IL-6 and anti-IL-6R in serum. We evaluated survival rate, morphological changes of graft, immune cell infiltration, and population of Th17($CD4^+$/RoRγt) and Treg($CD4^+Foxp3^+$). We compared its alloimmunity and graft survival efficiency with the CsA-treated group.

**Results:** Hydrodynamic delivery of MC-DNA was mainly localized in hepatocytes. Serum IL-6 and IL-6R antibody detected in anti-IL-6RMC-DNA treated mice. At day 8.5, untreated mice completely rejected the graft confirming by daily observation of loss of graft and erosion. However, mice received either anti-IL-6R MC-DNA or CsA presented prolonged acceptance of graft until day 15 or 15.6, respectively. Morphological changes and immune cell infiltration in the graft were also consistent with survival rate. FACS results showed that anti-IL-6RMC-DNA treatment markedly suppressed Th17population compared with the untreated mice. However, there was no effect on Treg population. However, CsA showed the increased Th17and decreased Treg population compared with untreated group.

**Conclusions:** We found that single injection of nonviral MC-DNA targeting IL-6R is effective in both acute allograft acceptance and allogenic tolerance. This suggests that simple and effective gene therapy method using antibody producing minicircle DNA may represent a powerful tool for the transplantation.

## FR-PO476

**X-Linked Inhibitor of Apoptosis Protein Expression Is Not Associated with Increased Apoptosis in an In Vivo Model of Cold Ischemia** Daniel Keys,[1] Swati Jain,[1] Kameswaran Ravichandran,[1] Zhibin He,[1] Danica Ljubanovic,[2] Charles L. Edelstein,[1] Alkesh Jani.[1] *[1]UC Denver, Aurora, CO; [2]Dubrava Hospital, Zagreb, Croatia.*

**Background:** Delayed graft function (DGF) independently predicts reduced 5 yr kidney transplant survival. Cold ischemia (CI) contributes to the development of DGF and results in tubular cell apoptosis and brush border injury (BBI). Our published data demonstrates that mouse kidneys subjected to CI have increased caspase-3, renal tubular cell apoptosis and BBI at 48 hours of CI. XIAP is the most potent inhibitor of apoptosis currently known. The purpose of this study was to evaluate the time course of XIAP, active caspase-3, caspase-3 activity and histologic changes in a mouse model of CI.

**Methods:** C57BL/6 mice were anesthetized and right kidneys were processed at time zero as control following perfusion with cold UW solution. Left kidneys were exposed to CI for 1, 6, 12, and 24 hours. Active caspase-3 and XIAP proteins were evaluated by

immunoblot. Caspase-3 activity was measured using absorbance of cell lysates after incubation with substrate for caspase-3/7. Apoptosis and BBI were quantified by an independent nephropathologist.

**Results:** After CI, BBI occurs first and was significantly increased at 6 hours. Active caspase-3 protein levels and caspase-3 activity levels were significantly increased at 24 hours of CI. Apoptosis was significantly increased at 24 hours of CI. XIAP levels were not significantly different at any time point in this model of CI.

| * = p < 0.05 | Control | 1 hour | 6 hour | 12 hour | 24 hour |
|---|---|---|---|---|---|
| XIAP Densitometry | 0.99 | 1.18 | 1.11 | 1.22 | 1.17 |
| Caspase-3 Densitometry | 0.99 | 0.9 | 1.26 | 1.64 | 2.63 * |
| Caspase-3 Activity | 0.010 | 0.009 | 0.012 | 0.016 | 0.035* |
| Apoptosis / 10 hpf | 0.013 | 3.2 | 8.4 | 3.2 | 13.75* |
| BBI | 13 | 17 | 44* | 58* | 42* |

**Conclusions:** CI injury results in apoptosis in a time dependent manner. Increased apoptosis was associated with an increase in active caspase-3 protein levels and caspase-3 activity but no change in XIAP protein levels in a murine model of CI. Mechanistic studies of caspase-3 activation are planned.

*Funding:* NIDDK Support

## FR-PO477

**Integrated Messenger RNA and microRNA Profiles Suggest an Interferon Alpha Signature in Chronic Antibody-Mediated Rejection (CAMR)**

F. Rascio,[1] Paola Pontrelli,[1] Matteo Accetturo,[1] Margherita Gigante,[2] Giuseppe Castellano,[1] Maddalena Gigante,[2] Anna Zito,[1] Gianluigi Zaza,[3] Annarita Oranger,[1] G. Stallone,[2] E. Ranieri,[2] Loreto Gesualdo,[1] G. Grandaliano.[2] *[1]Dept Emergency and Organ Transplantation, Univ of Bari, Bari, Italy; [2]Dept of Medical and Surgical Sciences, Univ of Foggia, Foggia, Italy; [3]Nephrology, Dialysis and Transplantation Unit, Univ of Verona, Verona, Italy.*

**Background:** CAMR is the main cause of chronic graft injury but its pathogenesis is unknown. Aim of the study was to investigate the molecular mechanisms underlying CAMR pathophysiology, integrating microRNAs (miRNAs) and gene expression profiles of peripheral blood mononuclear cells (PBMCs) from CAMR patients and controls.

**Methods:** We enrolled 6 patients with biopsy-proven CAMR and 6 stable transplant recipients with normal graft histology and function (control group). miRNAs and gene expression profiles of PBMCs from both groups were assessed by Agilent technologies. Results were evaluated by statistical analysis and functional pathway analysis and validated by real-time PCR in an independent set of patients.

**Results:** Comparison between CAMR and control patients (FDR<5% and a FC≥2) revealed that18 miRNAs were differentially expressed, all down-regulated in CAMR. The principal component analysis showed the ability of these miRNAs to distinguish the two groups. When matching miRNA data with mRNAdata we identified 4 out of 18 differentially expressed miRNAs, resulted down-regulated in CAMR, as being predicted modulators of 6 mRNAs, resulted up-regulated in our gene expression data. Ingenuity Pathway Analysis (IPA) performed on all 18 miRNAs demonstrated that 9 of them were connected in the interferon-alpha (INF-alpha) network (IPA score=38). IPA assessed on the 6 target mRNAs further confirmed the involvement of INF-alpha pathway (IPA score=14). qPCR performed in an independent set of 6 CAMR and 6 controls of these miRNAs confirmed the microarray data.

**Conclusions:** Our data would suggest a key role of INF-alpha pathway during CAMR and open new perspectives for identification of therapeutic targets.

*Funding:* Government Support - Non-U.S.

## FR-PO478

**Acute Graft-versus-Host Disease in the Kidney after DA-to-Lewis Rat Bone Marrow Transplantation**

Seiichiro Higo,[1,2] Akira Shimizu,[1] Shinya Nagasaka,[1] Yusuke Kajimoto,[1] Go Kanzaki,[1] Akiko Mii,[2] Shuichi Tsuruoka.[2] *[1]Dept of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan; [2]Dept of Internal Medicine, Nephrology, Nippon Medical School, Tokyo, Japan.*

**Background:** Allogeneic hematopoietic and bone marrow cell transplantation (BMT) causes acute and choronic graft-versus-host-disease (GVHD). Liver, gut, and skin are well known to primary target sites of acute and chronic GVHD. However, kidney is still uncertain to be influenced by GVHD. In the present study, we examined the pathology of kidney in acute GVHD in DA-to-Lweis rat BMT.

**Methods:** Acute GVHD was induced in Lewis rats (RT1l) by transplantation of DA rat (RT1a) bone marrow cells ($6.0x10^7$ cells) without immunosuppression after lethal irradiation (10Gy). We examined the clinical and pathological characteristics of acute GVHD in several organs, including skin, liver, gut, and kindney after BMT.

**Results:** Almost all white blood cells were replaced with DA donor phenotype in peripheral blood. At least 21 days after BMT, severe acute GVHD was developed in all Lewis rats with body weight loss (>20%), skin rush with alopecia, and kyphosis. In laboratory findings, liver and kidney dysfunction was detected: asparate aminotransferase (AST: 382±18.0U/L), alanine aminotransferase (ALT: 75.4±6.70U/L), lactate dehydrogenase (LDH: 1306±645U/L), and blood urea nitrogen (BUN: 33.6±4.50mg/dL). In the circulation, the increase in percentage of CD8+T cells in all peripheral blood cells was detected by flow cytometry. In addition to the development of GVHD in liver, skin, and gut, infiltration of CD3+T cells including most of CD8+ T cells and CD68+ macrophages were noted in the kidney. In kidney, many CD3+T cells, CD8+ T cells, and CD68+ macrophages infiltrated that mediated the interstitial inflammation with peritubular capillaritis, tubulitis, acute glomerulitis and acute endarteritis in small arterioles. No obvious IgG, IgM, and C3 deposition was detected in the kidney.

**Conclusions:** Kidney is one of the target organs of acute GVHD after BMT. The pathology of acute GVHD in the kidney was the quiet similar findings of acute T-cell mediated rejection in kidney transplantation.

## FR-PO479

**Syndecan-1 and Dyslipidemia after Renal Transplantation**

Saritha Adepu,[1] Kirankumar Katta,[1] Arjan J. Kwakernaak,[1] Robin P.F. Dullaart,[2] Gerjan Navis,[1] Stephan J.L. Bakker,[1] Jacob Van den Born.[1] *[1]Nephrology, Univ Medical Center Groningen, Groningen, Netherlands; [2]Endocrinology, Univ Medical Center Groningen, Groningen, Netherlands.*

**Background:** Syndecan-1 is a transmembrane heparan sulfate proteoglycan involved in hepatic uptake of triglyceride-rich lipoproteins. Renal transplantation is often complicated by a currently unexplained dyslipidemia, contributing to an increased cardiovascular risk. We hypothesized that altered syndecan-1 metabolism could be involved in dyslipidemia in renal transplant recipients.

**Methods:** To test this hypothesis, in a rat model of renal transplantation we quantified liver syndecan-1 expression and measured plasma triglycerides. Anti-syndecan-1 monoclonal antibody was injected into mice to localize syndecan-1 in order to assess source of shed plasma syndecan-1. In a cross-sectional human renal transplantation recipient (RTR) cohort of 510 patients plasma syndecan-1 was measured by ELISA.

**Results:** We found syndecan-1 to be localized to the apical cell membranes of hepatocytes. Hepatic syndecan-1 mRNA expression was increased 4-5 fold after renal transplantation ($p<0.01$) and was accompanied by a significant increase in plasma triglyceride values ($p<0.05$). Upon injection of anti-syndecan-1 monoclonal antibody into mice, the antibody mainly localized to the hepatic sinusoids. In a cross-sectional human RTR cohort, multivariate regression analysis showed soluble syndecan-1 independently to be positively associated with triglycerides and inversely associated with HDL cholesterol (both $p<0.0001$).

**Conclusions:** These data suggest liver syndecan-1 to be a major source of soluble plasma syndecan-1, and that via a yet unknown mechanism renal transplantation increases liver syndecan-1 turnover. We speculate shed syndecan-1 as decoy receptor to hamper lipoprotein uptake by the liver resulting in dyslipidemia. Our data opens perspectives towards improvement of lipid profiles by targeted inhibition of syndecan-1 shedding in (renal) transplantation.

## FR-PO480

**Sirolimus (SIR) Induces Increases in Baseline Glomerular Permeability, but Partly Inhibits Puromycin Aminonucleoside (PAN) Induced Hyperpermeability in Rats**

Josefin Axelsson, Anna Rippe, Bengt Rippe. *Dept of Nephrology, Clinical Sciences, Lund, Sweden.*

**Background:** It is well established that the immunosuppressant SIR, an mTOR inhibitor, can produce de novo proteinuria in transplanted patients. On the other hand, SIR has been shown to suppress kidney disease progression in animal models. In the present study we wanted to investigate whether glomerular permeability would be altered by SIR alone and whether SIR can affect PAN induced glomerular hyperpermeability.

**Methods:** In anaesthetized Wistar rats (250-280g) the left urether was cannulated for urine collection, while simultaneously, blood access was achieved. SIR was administered as a single dose i.v. 30 min before the start of the experiments in animals infused with PAN or animals not exposed to PAN. Polydisperse FITC-Ficoll-70/400 (mol.radius 10-80Å) and $^{51}$Cr-EDTA infusion was given during the whole experiment. Measurements of Ficoll in plasma and urine were performed sequentially at start (baseline) and at 5, 15 and 60 min after the start of the experiments. Urine and plasma samples were analyzed by high performance size exclusion chromatography (HPSEC) to assess steady state glomerular sieving coefficients (θ) for $Ficoll_{10\text{-}80Å}$.

**Results:** SIR increased baseline glomerular permeability to $Ficoll_{50\text{-}80Å}$ at 15 min, but not at 5 min or at 60 min. θ for $Ficoll_{70Å}$ thus increased from $2.91 \times 10^{-5} \pm 1.18 \times 10^{-5}$ at baseline to $2.27 \times 10^{-4} \pm 5.02 \times 10^{-5}$ ($p<0.01$) at 15 min after SIR. PAN alone caused a rapid increase in glomerular permeability, peaking at 5 min, and again at ~60 min. SIR blunted the first PAN induced permeability peak and almost completely abrogated the second one.

**Conclusions:** mTOR inhibition with SIR induced rapid, reversible increases in baseline glomerular permeability, but also blunted PAN induced glomerular hyperpermeability in rats, underpinning the complex interactions of SIR with the glomerular filtration barrier.

*Funding:* Government Support - Non-U.S.

## FR-PO481

**MRI Detectable Nanoparticles to Measure the Size and Number of Glomeruli in the Human Kidney**

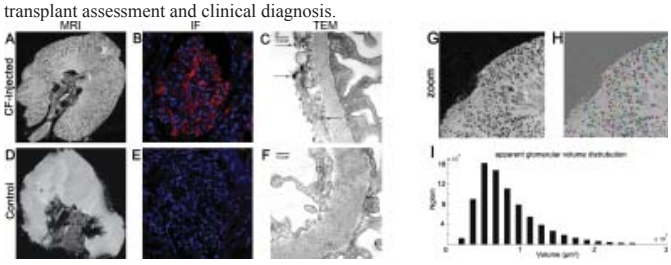
Scott Charles Beeman,[1] Edwin J. Baldelomar,[2] John F. Bertram,[3] Luise A. Cullen-McEwen,[3] Jennifer Richardson Charlton,[4] Teresa Wu,[5] Min Zhang,[5] Kevin Bennett.[2] *[1]Washington Univ in St. Louis; [2]Univ of Hawaii; [3]Monash Univ, Australia; [4]Nephrology, Univ of Virginia Medical Center, Charlottesville, VA; [5]Arizona State Univ.*

**Background:** The number (Nglom) and volume (Vglom) of kidney glomeruli may predict cardiovascular and renal health (Brenner, 1988; Puelles, 2012). There are currently no methods to make these measurements in the clinic. The cationic ferritin (CF) nanoparticle has been proposed as a glomerulus-specific MRI contrast agent (Bennett, 2008; Beeman, 2011, Heilmann, 2012). Here we use intravenous CF to measure Nglom and Vglom in intact human kidneys with MRI.

**Methods:** Four viable human kidneys (not viable for transplant but without known renal disease) were acquired within 24 hours of death though a donor network (IIAM) with IRB and informed consent. Kidneys were perfused with CF, PBS, and formalin. Control kidney received no CF. Kidneys were imaged with 3D MRI (resolution 117 $\mu m^3$). Binding of CF to glomeruli was confirmed by fluorescence (IF) and electron microscopy (TEM). We measured Nglom and Vglom from MRI with custom software.

**Results:** MRI of labeled human kidneys showed dark spots at each glomerulus (Fig. 1A,D). IF (Fig. 1B,E; red is CF) and TEM (Fig. 1C,F; arrows show CF accumulation) confirmed CF-binding to glomeruli. The software identified CF-labeled glomeruli (Fig. 1H), counted glomeruli (772,922, 328,181 and 702,506), and measured glomerular volumes ($8.0x10^6 \pm 4.28x10^6$ $\mu m^3$, $6.9x10^6 \pm 3.9x10^6$ $\mu m^3$ and $6.8x10^6 \pm 2.9x10^6$ $\mu m^3$). Results are consistent with the literature(Nyengaard, 1992). This MRI technique allows for a new metric - the intra-renal glomerular volume distribution; a single-kidney example is shown in Fig. 1I.

**Conclusions:** This work may enable quantitative renal morphology measurements for transplant assessment and clinical diagnosis.




*Funding:* Other NIH Support - Diabetic Complications Consortium

## FR-PO482

**Ultrasound Based Detection of Microbubbles Targeted to T-Lymphocytes for Non-Invasive Imaging of Acute Allograft Rejection in Different Rat Models of Renal Disease** Alexander Grabner,[1] Dominik Kentrup,[1] Mareike Muehlmeister,[2] Helga Pawelski,[1] Eberhard Schlatter,[1] Hermann Pavenstaedt,[1] Klaus Tiemann,[2] Stefan Reuter.[1] *[1]Dept of Internal Medicine D, Univ Hospital Muenster, Muenster, Germany; [2]Dept of Cardiology and Angiology, Univ Hospital Muenster, Muenster, Germany.*

**Background:** We propose anti-CD3-antibody-mediated contrast-enhanced ultrasound targeting human T-lymphocytes for image-based diagnosis and differentiation of acute allograft rejection (AR) established in a rat renal transplantation model.

**Methods:** After tail vein injection of $30x10^6$ human T-lymphocytes, microbubbles conjugated with a human anti-CD3 antibody were i.v. administered to male 10 weeks old uni-nephrectomized, allogeneically transplanted rats (Lewis-Brown Norway (LBN) to Lewis, aTX). Ultrasound was used to investigate the transplanted as well as the native kidney. Syngeneically (sTX) transplanted rats (LBN to LBN), rats with ischemia/reperfusion injury (IRI, 45 min warm ischemia), and rats subjected to acute cyclosporine A toxicity (CSA) (50 mg/kg for 2 days i.p.) served as controls. *In vivo* results were confirmed with immunohistochemical CD3-stainings.

**Results:** Accumulation of human T-lymphocytes was clearly detected by antibody-mediated ultrasonography und was significantly elevated on postoperative day 4 in allografts undergoing AR ($5.41 \pm 1.32$ A.U., $p < 0.05$ vs. all controls, $N = 4$) compared to native control kidneys ($1.09 \pm 0.18$ A.U.). Concordantly no differences were found between native kidneys and sTX ($0.99 \pm 0.30$ A.U.), CSA ($0.12 \pm 0.04$ A.U.) and kidneys with IRI ($0.46 \pm 0.29$ A.U.). A strong correlation between ultrasound signal and results obtained by immunohistochemical analysis was found.

**Conclusions:** Contrast enhanced ultrasound using CD3-antibodies for detection of infiltrating T-lymphocytes is a novel option to highly specific and non-invasively assess AR after rat renal transplantation. This method allows to discriminate between AR, acute tubular necrosis and acute Calcineurin inhibitor toxicity. Since it can easily be transferred into clinics it might potentially be useful to improve the early diagnosis of AR, to investigate its kinetics and to monitor treatment efficiency.

*Funding:* Government Support - Non-U.S.

## FR-PO483

**Non-Invasive Magnetic Resonance Imaging in Rats for Prediction of the Fate of Grafted Kidneys from Cardiac Death Donors** Jun-Ya Kaimori,[1] Satomi Iwai,[2] Masaki Hatanaka,[1] Hidetoshi Tsuda,[1] Yoshitsugu Obi,[1] Naotsugu Ichimaru,[1] Masayoshi Okumi,[1] Hiromi Rakugi,[1] Eiji Kobayashi,[3] Shiro Takahara,[1] Yoshitaka Isaka.[1] *[1]Osaka Univ, Suita, Osaka, Japan; [2]Kitasato Univ, Aomori, Japan; [3]Jichi Medical Univ, Tochigi, Japan.*

**Background:** The worldwide shortage of organs for transplantation (Tx) has highlighted donation after cardiac death (DCD) as new organ sources. But such donor organs have many problems. The main objective of this study was to assess cardiac death (CD) kidney grafts before Tx to determine whether blood oxygen level-dependent (BOLD) and diffusion MRI can predict damage to these grafts after Tx.

**Methods:** We assessed CD kidney tissue by BOLD and diffusion MRI with pathological and gene expression in Rat CD kidney grafts before and after Tx.

**Results:** Although there was significantly more red cell congestion (RCC) in the inner stripe of the outer medulla (IS) in both 1 h after cardiac death (CD1h) and CD2h kidneys destined for grafts before Tx compared with CD0h ($p < 0.05$), CD2h, but not CD1h, kidney grafts had significantly different RCC in the IS 2 days after Tx ($p < 0.05$). Consistent with these pathological findings, tissue plasminogen activator (tPA) gene expression was increased only in the cortex and medulla of CD2h kidney grafts after Tx. BOLD MRI successfully and non-invasively imaged and quantified RCC in the IS in both CD1h and CD2h kidney grafts ($p < 0.05$). Diffusion MRI also non-invasively assessed increased the apparent diffusion coefficient in the IS and decreased it in the outer stripe (OS) of CD2h grafts, in concordance with interstitial edema in the IS and tubule cellular edema in the OS. These two types of edema in the outer medulla could explain the prolonged RCC in the IS only of CD2h kidney grafts, creating part of a vicious cycle inhibiting red cells coming out of capillary vessels in the IS. Perfusion with University of Wisconsin solution before MRI measurements enhanced these differences.

**Conclusions:** BOLD and diffusion MRI, which are readily available non-invasive tools for evaluating CD kidney grafts tissue damage, can predict prolonged organ damage, and therefore the outcome, of transplanted CD kidney grafts.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## FR-PO484

**Oleanolic Acid-Induced Nrf2 Activation Attenuates Renal Oxidative Stress and Tubulointerstitial Fibrosis in Chronic Cyclosporine Nephropathy** Sungjin Chung, Sung Jun Kim, Hye Eun Yoon, Seok Joon Shin, Cheol Whee Park. *Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** Cyclosporine (CsA)-induced kidney injury is characterized by renal dysfunction with inflammatory cell infiltrations, apoptosis and fibrosis. Nuclear factor erythroid-2-related factor-2 (Nrf2) is known to confer protection against tissue injury by orchestrating antioxidant and detoxification responses to oxidative stress. This study investigated whether upregulation of Nrf2-depedent antioxidative signaling by olenoalic acid (OA) would attenuate renal inflammation and fibrosis in CsA-induced kidney injury.

**Methods:** Male ICR mice fed a low-sodium diet were divided into four treatment groups: control+vehicle, control+OA, CsA+vehicle, and CsA+OA. In the OA-treated groups, 25 mg/kg/day of OA was administrated by intraperitoneal injection for last 1 week of experimental period. Renal inflammation and fibrosis, markers of oxidative stress, and changes in Nrf2 signaling were evaluated at the end of 4 weeks.

**Results:** Following the CsA treatment, kidney function and urine osmolality were decreased, and urine volume and albuminuria were increased. However, these findings were attenuated by OA treatment. Administration of OA decreased tubulointerstitial fibrosis score and $\alpha$-SMA that were increased in CsA-treated mice. Increased apoptotic cell death and a high ratio of Bax to Bcl-2 expression in kidneys of CsA-treated mice were also significantly ameliorated with OA treatment. OA treatment was attributed with the increased levels of nuclear Nrf2 and heme oxygenase (HO)-1. Furthermore, administration of OA improved SOD1 expression and decreased urinary 8-OHdG and isoprostane concentrations. However, there were no changes in the expression of total Nrf2, Kelch-like ECH-associated protein 1 (Keap1), NQO-1 and catalase, indicating that OA enhanced nuclear translocation of Nrf2 and subsequently activated HO-1.

**Conclusions:** Our results suggest that OA has beneficial effects on oxidative stress and fibrosis through activation of Nrf2–HO-1 signaling in the chronic cyclosporine nephropathy.

## FR-PO485

**Renal Oxygenation after Renal Transplantation: Towards Long-Term Telemetry** Diana A. Papazova, Jaap A. Joles, Marianne C. Verhaar, Maarten P. Koeners. *Nephrology & Hypertension, Univ Medical Center Utrecht, Netherlands.*

**Background:** Lower renal $O_2$ is observed after ischemia/reperfusion injury (IRI). Reduced renal graft function and survival are related to IRI. Our aim is to compare the renal $O_2$ in healthy kidneys and kidneys after transplantation (TX). A critical barrier to investigate this is lack of methods to measure kidney tissue $O_2$ continuously. Recently we developed a telemetry-based carbon paste $O_2$ electrode (CPE) which can record renal $O_2$ in rats, unhindered by anaesthesia or restraint. To test this new modality we measured renal $O_2$ before, during and after ischemia.

**Methods:** In a terminal setting renal $O_2$ was measured before, during and after 20 min of ischemia induced by a vascular clamp (n=3). Syngenic renal TX (n=4) was performed using male Lewis rats, controls (n=5) were age-matched. Briefly, donor left kidney was connected to the recipient's left renal vessels and urether with end-to-end anastomoses with a period of 30 min cold ischemia and 30 to 40 min warm ischemia. Two weeks after TX renal function (glomerular filtration rate (GFR): inulin clearance, effective renal plasma flow (eRPF): PAH clearance) were measured. Tubulo-interstitial injury (TI) was scored. Oxygenation of transplanted kidneys was compared to non-transplanted kidneys using CPEs for amperometric detection of $O_2$.

**Results:** During ischemia $O_2$ levels fell to practically zero but stabilized to 1.5 times baseline during reperfusion. Renal $O_2$ ($40.5 \pm 19.2$ uM vs. $110.1 \pm 31.4$ uM) and GFR ($1.02 \pm 0.46$ vs. $1.98 \pm 0.46$ ml/min) were lower in transplanted vs. non-transplanted kidneys (both $p<0.05$), while no significant differences were found in eRPF, filtration fraction and mean arterial pressure. TI was higher in transplanted kidneys vs. controls ($2.0 \pm 0.5$ vs. $0.7 \pm 0.4$, $p<0.05$).

**Conclusions:** CPE could successfully measure renal $O_2$ before, during and after ischemia. As the CPE can be used with telemetry, this method can be applied for continuous long-term monitoring of kidney $O_2$ in, for instance, experimental renal transplantation. The CPE detected a marked decrease in $O_2$ in transplanted vs. non-transplanted kidneys. Whether this is due to delivery or consumption of $O_2$ should be elucidated.

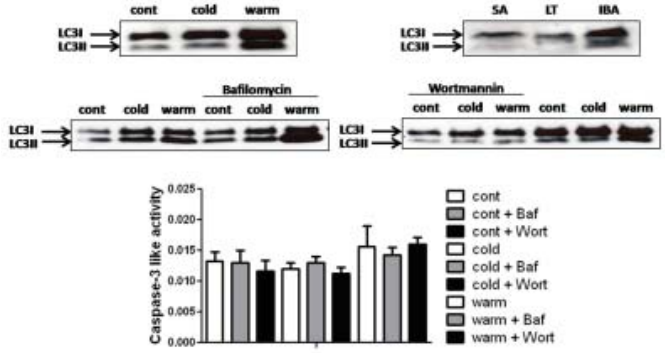
**FR-PO486**

**Increased Autophagic Flux during Extreme Cold Ischemia Followed by Warm Reperfusion** Swati Jain, Daniel Keys, Charles L. Edelstein, Alkesh Jani. *UC Denver, CO.*

**Background:** AKI post transplant DGF is associated with prolonged cold ischemia (CI) of > 30 hours followed by warm reperfusion (WR) and renal tubular cell (RTEC) apoptosis. We have shown that hibernating ground squirrel (GS) kidneys endure CI for **several days** followed by WR during Arousal (IBA) without AKI or RTEC apoptosis. Since Autophagy is a survival strategy employed by cells under stress, we hypothesised that GS kidneys employed Autophagy to survive hibernation. We examined autophagic flux in: 1) i*n vivo* hibernating GS kidneys 2) *in vitro* in GS RTECs.

**Methods:** LC3-II (an autophagosome marker) and cleaved caspase 3 (CC3) were examined: a) *In vivo* after prolonged CI of several days during torpor (LT), followed by WR during IBA; b) *In vitro* in GS RTECs subjected to cold storage (CS) in UW solution followed by rewarming (WR) in normal media to simulate *in vivo* CI/WR. Autophagic flux was examined using Bafilomycin and Wortmannin.

**Results:** *In vivo* IBA kidneys and cells subjected to CS/WR *in vitro* had significantly increased LC3-II expression. Treatment of GS RTEC with Bafilomycin and Wortmannin demonstrated:(a) increased autophagic flux under conditions of CS/WR (b) no increase in CC3 protein or its activity.




**Conclusions:** Hibernators survive WR after several days of CI during IBA that would be lethal to non-hibernators. To determine a mechanisn of tolerance to CI/WR we examined the role of Autophagy, a survival strategy employed by cells. Autopagic flux inhibition with Bafilomycin or Wortmanin did not increase CC3 protein or its activity suggesting that; (a) autophagic flux is employed by hibernators to withstand stress, but (b) is not required for cell survival during WR after CI in hibernators.

*Funding:* NIDDK Support

**FR-PO487**

**Cold Injury Is Mediated by Loss of Ezrin In Vivo** Peter Hamar,[1] Csaba Imre Szalay,[1] Anita A. Wasik,[2] Laszlo Rosivall,[1] Sanna H. Lehtonen.[2] *[1]Institute of Pathophysiology, Semmelweis Univ, Budapest, Hungary; [2]Dept of Pathology, Haartman Institute, Helsinki, Finland.*

**Background:** Early graft injury is the most important determinant of prognosis after kidney transplantation. Although organ preservation is performed by organ storage at 4°C it has been suggested, that cooling may be associated with substantial organ damage due to cytoskeletal rearrangement including the loss of a crucial cytoskeletal protein: ezrin.

**Methods:** We have investigated renal function in an orthotopic renal transplantation model in inbred male Lewis (L) and Fisher (F) rats. F-to-L allografts (A), F isografts (I) or autografts by performing cold perfusion+ischemia (P) as well as following in-situ ischemia reperfusion (IRI) without cold perfusion, uninephrectomy (U) or sham (S) operations were performed. A, I and P kidneys were cold perfused, whereas IRI, U and S were not. Renal function was measured the day before or 3, 5 and 7 days after surgery (n=8/group/time). In a separate set, histologic investigations and ezrin western blotting was performed, 1, 3 and 5 days after surgery.

**Results:** Before operations GFR was 2.41±0.52 and ClPAH: 11.5±3.5 in F animals. In cold perfused (A, I and P) rats a significantly decreased initial GFR and ClPAH improved later. In I/R initial GFR, and ClPAH decreased less, but remained constant later. Serum crea and urea paralleled these results. Sham and I/R animals did not differ significantly. An intense mononuclear infiltrate and IL-2 mRNA (RtPCR) expression indicated acute rejection only in A, but not in any other group. Light and electronmicroscopic investigations demonstrated mild glomerular and intense tubular damage in transplanted and perfused kidneys. This graft injury caused by cold perfusion was accompanied by a severe reduction of ezrin in cold perfused kidneys.

**Conclusions:** In summary, initial kidney function and histologic tubular damage was not determined by ischemia or acute rejection in this model, but it was severely impaired in cold perfused A, I, and P rats at day 3. In the background loss of the cytoskeletal protein: ezrin was detected. Our study draws attention to cold injury as a possibly deleterious factor in transplantation. Funding: OTKA, K 81972.

*Funding:* Government Support - Non-U.S.

**FR-PO488**

**Role of Ubiquitin Proteasome System on Renal Cold Storage: A Possible Link to Mitochondrial Dysfunction?** Nirmala Parajuli, Lee Ann MacMillan-Crow. *Pharmacology and Toxicology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

**Background:** Renal transplantation is the treatment of choice for patients suffering from end stage renal disease (ESRD) but, due to the shortage of donor kidneys, many patients die while on the waiting list. Kidneys procured from deceased donors are stored in cold storage (CS) solutions; however extended CS leads to renal damage and poor long-term outcome following transplantation. Our published reports show that CS induces reactive oxidant species generation (ROS), mitochondrial dysfunction, and renal damage. Exciting new studies in the liver and heart suggest that the Ubiquitin Proteasome System (UPS), a proteolytic pathway that removes modified proteins, is activated during CS and appears to contribute to organ damage. Therefore, we hypothesize that CS mediated ROS triggers activation of UPS which leads to mitochondrial dysfunction and renal damage.

**Methods:** Male rodent kidneys were isolated and cold stored for 0-24 hr. Mitochondrial function was assessed via high resolution respirometry and ATP measurements. UPS function was monitored by specific activity assays as well as ubiquitin expression.

**Results:** Our data showed that CS induces altered proteasome activity, loss of total ubiquitin protein expression, and mitochondrial dysfunction (decreased ATP). Interestingly, this CS-mediated ATP depletion is partially restored by a clinically relevant proteasome inhibitor (Bortezomib), suggesting that increased UPS activity contributes to mitochondrial dysfunction. Similar to ubiquitin expression, total protein expression of AMP-activated protein kinase (AMPK), a metabolic regulator that helps to maintain cellular ATP and mitochondrial turnover, is also reduced during renal CS. Interestingly, a cell permeable activator of AMPK (AICAR) partially restored renal ATP and ubiquitin expression, suggesting that loss of AMPK may play a critical role in linking altered mitochondrial and proteasomal function during renal CS.

**Conclusions:** These data suggest, for the first time, that renal CS leads to altered UPS and mitochondrial dysfunction. New studies designed to target the UPS with Bortezomib may have promising therapeutic implications for renal transplantation.

*Funding:* NIDDK Support

**FR-PO489**

**MitoQ Blunts Mitochondrial Damage during Renal Cold Storage via Improved Mitochondrial Biogenesis** Lee Ann MacMillan-Crow, Nirmala Parajuli, Akira Marine. *Pharmacology and Toxicology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

**Background:** A major hurdle in the field of renal transplantation is the severe shortage of suitable donor kidneys despite the increasing number of patients awaiting transplant. Renal transplantation using living-donor organs (not exposed to cold storage) perform better when compared to deceased donor kidneys that were exposed to cold storage prior to transplant. Our published reports showed that cold storage induces mitochondrial and renal damage, and we recently reported in a porcine kidney model that a mitochondrial targeted antioxidant, MitoQ, blunted mitochondrial damage (PLOS One 7:11, 2012). However, it remains unknown what mechanisms are governing the cold-mediated mitochondrial damage or protection with MitoQ.

**Methods:** Male rodent kidneys were isolated and cold stored in University of Wisconsin solution for 0-24 hr +/- MitoQ (100 μM). Mitochondrial function was assessed using fresh renal biopsies via high resolution respirometry and ATP measurements. Mitochondrial biogenesis was monitored using immunohistochemistry and mtDNA integrity assessment.

**Results:** Preliminary data showed that cold storage lead to decreased mtDNA levels, reduced respiratory complex function, and impaired mitochondrial biogenesis. Interestingly, IHC analysis suggested that mitochondrial biogenesis was extensively impaired within proximal tubules, but induced in distal nephron cell types. We also showed that adding MitoQ increased ATP and mtDNA after cold storage (24 hr) alone, and improved renal function following cold storage (4 hr) plus transplantation (24 hr) (serum creatinine: 2.9 versus 1.5 mg/dL + MitoQ).

**Conclusions:** These studies suggest that MitoQ can protect against mitochondrial and renal damage during cold storage and transplantation, possibly by restoring mitochondrial biogenesis.

Supported by NIH DK089659 (LAMC).

*Funding:* NIDDK Support

**FR-PO490**

**NOX4 Activation in Renal Ischemia-Reperfusion (I/R) Injury Is Complement-Dependent** Simona Simone,[1] F. Rascio,[1] Giuseppe Castellano,[1] C. Divella,[1] Paola Pontrelli,[1] P. Ditonno,[1] Loreto Gesualdo,[1] G. Pertosa,[1] G.Grandaliano.[2] *[1]Dept of Emergency and Organ Transplantation, Univ of Bari, Bari, Italy; [2]Dept of Medical and Surgical Sciences, Univ of Foggia, Foggia, Italy.*

**Background:** Renal I/R plays a key role in the pathogenesis of delayed graft function after renal transplantation and is characterized by an increased of reactive oxygen species generation. The complement cascade has been suggested to play a pathogenic role in I/R-induced renal damage. Aim of the study was to investigate the activation of NADPH oxidase renal isoform, NOX4, in a pig model of renal I/R injury focusing on complement system.

**Methods:** Renal I/R was induced in 5 pigs by arterial clamping. The NADPH oxidase activity was assessed by chemiluminescence on renal tissue taken before ischemia (T0) and at different time after reperfusion (T15', 30', 60'). NOX4 and 8-oxo-dG, a marker of DNA

oxidation, were evaluated by immunohistochemistry. The α-smooth muscle actin (SMA) expression was assessed by confocal microscopy (in vivo model) and immunoblotting in human proximal tubular epithelial cells (HK2) treated C3a ($5*10^{-7}$M). Chemiluminescence assay was used to evaluate NADPH oxidase activity in HK-2 cells after C3a stimulation.

**Results:** NADPH-oxidase activity was significantly increased during reperfusion in a time-dependent manner with a peak at T60 (T0 1.3±0.4, T60 5.3±2.0 ΔURL/Dt, p=0.03). We observed a significant increase in tubular NOX-4 (T0 5.1±0.8, T60' 18.6±.5, pixels/total area, p=0.03), 8-oxo-dG (T0 3.1±0.8, T60' 28.6±0.5, pixels/total area, p=0.03) and α-SMA expression at the same time point (T0 0.8±0.1, T60' 4.6±0.1, pixels/total area, p=0.02). In vitro, C3a induced both NOX4 and α-SMA protein expression in HK2 cells (p=0.02) and caused a statistically significant increase in NADPH-dependent superoxide generation compared to basal conditions (p=0.01). Interestingly, Nox4 silencing by siRNA abolished both C3a-induced NADPH oxidase activation (p=0.01) and α-SMA expression (p=.02).

**Conclusions:** NOX4 is activated during I/R injury. Complement cascade may play a role in NOX4 activation. Complement and NADPH oxidase may represent a pharmacological targets to prevent oxidative damage during I/R injury.

*Funding:* Government Support - Non-U.S.

## FR-PO491

### Renal Protection through CBS/H2S Pathway in Mammalian Hibernation–A Natural Model of Hypothermic Organ Preservation during Cold Ischemia and Reperfusion

George Johnson Dugbartey,[1] Leo E. Deelman,[1] Robert H. Henning.[1] *[1]Clinical Pharmacology, Univ Medical Center Groningen, Groningen, Netherlands; [2]Clinical Pharmacology, Univ Medical Center Groningen, Groningen, Netherlands.*

**Background:** Hibernation represents the most radical example of hypometabolism among mammalian species and is characterized by repetitive cycles of cooling and rewarming, which resembles several clinically relevant conditions such as deep hypothermia, organ storage for transplantation, major surgery and ischemia-reperfusion. Therefore mechanisms applied by hibernators to undergo hibernation without reperfusion injury or other ill effects may have potential application to human medicine. This study aimed at investigating the role of CBS enzyme and $H_2S$ in the induction of torpor and kidney preservation during hibernation.

**Methods:** Male Syrian golden hamsters (*Mesocricetus auratus*) were housed in cages in a climate controlled chamber at 5°C under dim red light to induce torpor. Movement of all animals was continuously monitored with passive infrared detectors. Osmotic mini-pumps filled with saline or AOAA (100mg/kg/day) were implanted i.p. during torpor following a bolus injection of AOAA (10mg) under 2.5% isoflurane anesthesia. At 4 days following implantation of pumps, hamsters that re-entered torpor were aroused by handling for 4 hours and euthanized under pentobarbital anesthesia . Blood samples were taken and kidney of the hamsters were obtained. Summer euthermic hamsters served as controls.

**Results:** Torpid hamsters were aroused during pump implantation. In contrast to saline infusions, infusion of AOAA prevented hamsters from re-entry into torpor. Infusion of AOAA also induced excess renal damage as indicated by high expression of kidney injury marker as well as changes in renal morphology. In contrast, renal morphology was well preserved during hibernation in the saline and non-hibernating summer control groups.

**Conclusions:** Our data show that CBS/$H_2S$ pathway is essential in entrance into torpor and preservation of kidney morphology and function during hibernation.

*Funding:* Government Support - Non-U.S.

## FR-PO492

### Association of Genetic Polymorphisms of Matrix Metalloproteinases with New-Onset Diabetes after Transplantation in Renal Transplantation

Tae Hee Kim, Sunwoo Kang, Yang Wook Kim, Miseon Kang. *Internal Medicine, Univ of Inje, Busan, Republic of Korea.*

**Background:** New-Onset Diabetes After Transplantation (NODAT) is a serious metabolic complication that may follow renal transplantation. Excess fat deposition requires space, created by adipocyte (hypertrophy and hyperplasia) and extracellular matrix (ECM) remodelling. This process is regulated by several factors, including several adipocyte-derived Matrix metalloproteinases (MMPs) and the adipokine cathepsin, which degrades fibronectin, a key ECM protein. Excess fat, also deposited in visceral organs, generates chronic low-grade inflammation that eventually triggers insulin resistance and the associated diabetes mellitus. Therefore, we examined the association between NODAT and 11 single nucleotide polymorphisms (SNPs) located within the 3 genes of Matrix metalloproteinases (MMPs) which might be related with NODAT.

**Methods:** A total of 309 renal transplants recipients were included without a history of diabetes. We analyzed the association between NODAT development and a panel of 11 SNPs within 3 genes (MMP1, MMP2, MMP3) of MMPs.

**Results:** In terms of allele frequencies, rs243849*C (MMP2) was significantly higher in patients with NODAT. Two SNPs among 11 (18.1%) were significantly associated with NODAT development after adjusting for age, sex, and tacrolimus usage. They include MMP2 (rs1132896) and MMP2 (rs243849).In multiple logistic regression analysis, these 2 SNPs were significantly associated with the development of NODAT in the codominant and recessive or, codominant and dominant models, respectively.

**Conclusions:** The data suggest that excess fat deposition and ECM remodelling might play a role in the pathogenesis of NODAT in renal transplantation recipients. In particular, significant variations of MMP2 might confer susceptibility to NODAT in patients who receive renal transplants.

*Funding:* Private Foundation Support

## FR-PO493

### Clinical Impact of an Angiotensin I Converting Enzyme Insertion/Deletion and Kinin B2 Receptor +9/-9 Polymorphisms in the Renal Graft Survival

Carlos Eduardo Neves Amorim,[1] Alvaro Pacheco-Silva,[1,3] Niels O.S. Camara,[1,2] Ronaldo Araujo.[1] *[1]Nephrology, UNIFESP, Sao Paulo, Brazil; [2]Medicine, Univ of Sao Paulo, Sao Paulo, Brazil; [3]Hospital Israelita Albert Einstein, HIAE, Sao Paulo, Brazil.*

**Background:** There is a consensus in the scientific literature that supports the importance of the kallikrein kinin and renin angiotensin systems in renal physiology, but few studies have investigated their importance after renal transplantation.

**Methods:** The aim of this study was to investigate the clinical effects of the insertion/deletion polymorphism in the angiotensin I-converting enzyme (ACE) gene and the + 9/-9 polymorphism in the kinin B2 receptor (B2R) gene in kidney-transplanted patients (n = 215 ACE, n = 203 B2R) compared with 443 healthy individuals.

**Results:** Demographic results showed that there is a higher frequency of the D allele (high plasma ACE activity) and + 9 allele (lower B2R expression) in transplant patients compared with control individuals. We also observed a higher frequency of these alleles in patients who had an elevated level of plasma creatinine. At day 7 post-transplantation, we found a higher prevalence of individuals with the DD genotype with elevated plasma creatinine level. Furthermore, individuals with the DD genotype had a higher chronic and acute rejection and graft loss compared with the II patient genotype, which showed no loss of graft. We also found that transplantation performed between a patient DD and a donor II or ID presented a higher chance of acute rejection than the opposite way. In the analyze of the prescription drug, 161 Individuals were medicated with some type of antihypertensive, and 44 were taking ACE inhibitor, 36% of transplanted group, and was observed that ACE inhibitors were renoprotective.

**Conclusions:** Taken them together, our data suggest that the genotyping of these individuals for ACE polymorphism could be clinical relevant.

*Funding:* Government Support - Non-U.S.

## FR-PO494

### Established Renal Trait SNPs and Allograft Function in a Kidney Transplant Population

Paul J. Phelan,[1] Robert P. O'Brien,[2] Gianpiero Cavalleri,[2] Peter J. Conlon.[1] *[1]Nephrology, Beaumont Hospital, Dublin, Ireland; [2]Molecular Medicine, Royal College of Surgeons of Ireland, Dublin, Ireland.*

**Background:** Recent large scale genome-wide association studies (GWAS) in individuals of European ancestry have demonstrated several SNPs associated with estimated glomerular filtration rate (eGFR) and Chronic Kidney Disease (CKD). We recently completed a GWAS in an Irish renal transplant population. Twenty of the previously associated renal-function SNPs were captured by the Illumina Bead Chip we employed for our genotyping. We tested these SNPs for association with eGFR at 5 years post transplant.

**Methods:** Patients were adult, first time, deceased donor, kidney-only transplants, on calcineurin inhibitors between 1993 and 2002. We had data on 263 patients with a functioning graft and eGFR data at 5 years post transplant (median MDRD eGFR 43.5 mls/min/1.73m²). One-third of patients were female and mean age was 47.6 years. Principal components analysis showed our cohort to cluster with other northern European populations.

**Results:** Our results show that while 2 variants approached statistical significance for eGFR in our cohort, none had a p value of <0.05. Our most significant SNP (rs6036478; p=0.05415) was in full LD with rs911119, an intergenic variant on chromosome 20 which associated strongly with eGFR (Cystatin C) in the original study. The second most significant SNP was rs6465825 (p=0.0629; chromosome 7), another intergenic variant which was associated with eGFR (creatinine) in the original study. No other SNPs approached significance. Additionally, if Bonferroni correction is applied to correct for multiple testing, these 2 borderline significant SNPs do not approach significance.

**Conclusions:** It is perhaps not surprising that genetic loci associated with renal function in native kidneys are not validated in a renal transplant cohort. Determinants of renal function in a post-transplant population are likely to be very different from a normal or CKD populations. Non-replication of renal trait SNPs in our transplant cohort may also be due to differences in population structure and our small patient sample which may be underpowered for small effect size SNPs.

*Funding:* Private Foundation Support

## FR-PO495

### Genetic Predisposition of Donors Affects the Allograft Outcome in Kidney Transplantation; Single Nucleotide Polymorphism of Aquaporin-11

Ji In Park,[1] Jung Pyo Lee,[2] Seung Hee Yang,[3] Yon Su Kim.[1,3] *[1]Dept of Internal Medicine, Seoul National Univ College of Medicine, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [3]Kidney Research Institute, Seoul National Univ, Seoul, Republic of Korea.*

**Background:** Aquaporin-11 (AQP11) is a novel aquaporin family member. Disruption of the murine AQP11 gene causes severe proximal tubular injury and renal failure. The rs2276415 (G>A) single nucleotide polymorphism (SNP) in the human AQP11 gene results in Gly102Ser substitution in a functionally important domain. In this study, we evaluated the role of the genetic predispositions of AQP11 rs2276415 (G>A) on renal allograft outcomes.

**Methods:** A total of 206 pairs of donors and recipients were enrolled. Long-term graft survival was traced and clinical parameters that could have influenced graft outcome were collected by reviewing electronic medical record system.

**Results:** Despite similar allele frequencies between donors and recipients, minor allele of AQP11 rs2276415 (GA+AA) from donor, not from recipients, has a harmful effect on the graft survival compared to wild type donor (GG) (P=0.029). This association was significant after adjusting for several risk factors including age, sex, HLA mismatch, donor type, hypertension, and diabetes mellitus (P=0.032).In immunohistochemistry staining, AQP11 was differently expressed according to genetic variations of donor rs2276415 showing higher expressions in the grafts from GG donors.

**Conclusions:** In conclusion, a donor-derived, not recipient derived, genetic AQP11 polymorphism has different effects on graft outcome. Thus, the genetic influence from donors should be carefully considered for the proper management of allografts after kidney transplantation.

## FR-PO496

**Prospective Randomized Study of Tolerability and Efficacy of Combination Therapy on Hypertensive Chronic Kidney Disease (CKD)** Matsuhiko Hayashi,[1] Shunya Uchida,[2] Tetsuya Kawamura,[3] Michio Kuwahara,[4] Masaomi Nangaku,[5] Yasuhiko Iino.[6] *[1]Keio Univ, Sch. Med., Japan; [2]Teikyo Univ, Sch. Med., Japan; [3]The Jikei Univ Sch. Med., Japan; [4]Shuwa General Hospital, Japan; [5]The Univ of Tokyo Sch. Med., Japan; [6]Nippon Med. Sch., Japan.*

**Background:** In the treatment of hypertension in CKD patients, angiotensin receptor blocker (ARB) and calcium channel blockers (CCB) are the two mainstays, although it is not clear which second-line drug is beneficial for CKD patients.

**Methods:** In a randomized, open-label trial, the patients with CKD defined by K/DOQI guideline and hypertension (systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 80mmHg) in spite of usual doses of ARB were randomly assigned to receive losartan 50mg plus either 5mg of amlodipine (CCB group, n=27), 5 mg of enalapril (ACEI group, n=25), or 25 mg hydrochlorothiazide (HCTZ group, n=30). The primary endpoints were changes in blood pressures, ratio of urinary excretion of protein to creatinine (uP/C ratio), and eGFR during 12 months study period. The secondary endpoints were changes in biochemical parameters and side effects.

**Results:** Systolic and diastolic blood pressures were not significantly different between three groups throughout the study period. The percent changes in uP/C ratio was significantly different between HCTZ group (-26.5-±9.8%, means±SE) than that in CCB group(+46.7±33.6%, p<0.05) at 12 months after the assignment to each therapy, while eGFR was significantly lower in HCTZ group than ACEI and CCB groups at 4 months but not at 12 months study period. Serum uric acid levels were significantly higher in HCTZ group than CCB and ACEI groups during the entire period of the study. By logistic analysis, 25% decrease in uP/C ratio was more likely in HCTZ group (odds ratio 5.78, CI 1.14 to 37.8, p<0.05) compared with CCB group.

**Conclusions:** Addition of diuretics to ARB is though to be beneficial combination therapy for the control of hypertension in CKD in terms of urinary excretion of protein compared with addition of CCB or ACEI, while the effects of increased serum uric acid levels in HCTZ group on the progression of CKD should be determined by long-term study in the future.

*Funding:* Pharmaceutical Company Support - MSD Co. Ltd., Government Support - Non-U.S.

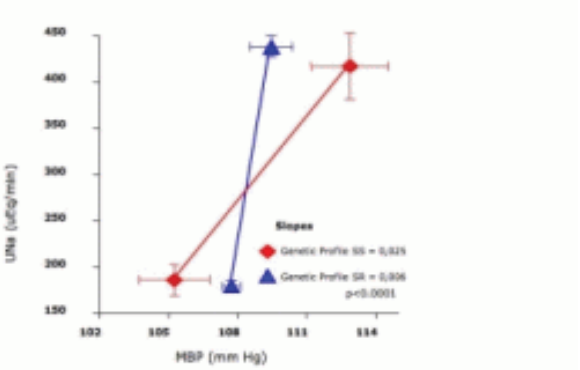
## FR-PO497

**Genetic Profile of Salt Sensitivity in Essential Hypertension** Chiara Lanzani, Marco Simonini, Elena Brioni, Simona Delli Carpini, Lorena Citterio, Laura Zagato Villa, Stefano Tentori, Paolo Manunta. *San Raffaele Scientific Institute, Milan, Italy.*

**Background:** Blood pressure (BP) is controlled primarily by Na and water balance because of the infinite gain property of the kidneys to rapidly eliminate excess fluid and salt. Up to 50% of patients with essential hypertension are salt-sensitive, as manifested by a rise in BP with salt loading.

**Methods:** 626 naive hypertensives underwent an acute Na load to monitor the simultaneous changes in BP and renal Na excretion. Genotyping was performed by Open Array (OA)technique with fluorescent probes arrays and ability to allelic discrimination of 124 SNPs in candidate genes for multiple mid-throughput genotyping. Associations with genetic markers were performed with GLM and chi-squared; logistic regression analysis for salt resistant/sensitive (SR/SS) comparison was used.

**Results:** OA study detected a strong association with variation in BP after acute Na load with 7 genes (ADD1, NCX1, NEDD4L, PRKG1, MYO16, SIK1, UMOD). In combined analyses, we found significant epistatic interactions between these SNPs. A genetic profile is a specific combination of variants in terms of SNPs or SNP interactions. Furthermore, we built a genetic profile able to identify the SS and SR hypertensive: those carrying the SS profile display changes in systolic BP of 10.4±1.25 vs 2.99±0.39 mmHg p<0.0001). SS genetic profile showed OR 6.13, and had a 96% specificity, a 72% PPV. Pressure-natriuresis curve was obtained by plotting urinary Na excretion in Y axis and mean BP in the X axis. The slope of SS pts resulted in a right shift along X axis, while was vertically steep in SR profile (GLM p<0.0001).




**Conclusions:** Our finding suggests a relationship between body Na, genetic pathways regulating the contractile state of muscular vascular cells, renal Na excretion and intracellular activity underline pressure natriuresis and salt sensitive phenotype.

## FR-PO498

**L/N-Type Calcium Channel Blocker Cilnidipine Added to Renin-Angiotensin Inhibition Improves Ambulatory Heart Rate Profile and Suppresses Cardiac Hypertrophy in Hypertension with Chronic Kidney Disease** Kouichi Tamura. *Dept of Medical Science and Cardiorenal Medicine, Yokohama City Univ Graduate School of Medicine, Yokohama, Japan.*

**Background:** Pathological alteration of ambulatory blood pressure (BP) and heart rate (HR) profile is proposed to play a critical role in the pathophysiology of renal deterioration and cardiovascular complication in hypertension and chronic kidney disease (CKD). In this study, we examined beneficial effects cilnidipine, a unique L/N-type calcium channel blocker (CCB), in addition to a renin-angiotensin system inhibitor, on ambulatory BP and HR profile as well as cardiorenal function in hypertensive CKD patients.

**Methods:** Forty-five patients were randomly assigned to the cilnidipine replacement group (n=21) or the control CCB group (n=24) after a 2-week run-in period. At baseline and after 24-week active treatment period, 24-h ambulatory BP monitoring and measurements of cardiovascular and biochemical parameters were performed.

**Results:** There was an increase in ambulatory HR in the control group and a decrease in the cilnidipine group, and differences in these changes were significant between the two groups. The ambulatory low-frequency (LF)-to-high-frequency (HF) ratio (LF/HF), an index of cardiac sympathetic nerve activity, was increased in the control group but decreased in the cilnidipine group, and differences in these changes were also significant. In addition, the left ventricular mass index (LVMI) was significantly decreased in the cilnidipine group compared to the control group after the study (LVMI, 135.3±26.4 versus 181.2±88.4, *P*=0.031), with a significant difference in the changes in the LVMI between the cilnidipine and control groups (change in LVMI, -12.4±23.7 versus 26.2±64.4, *P*=0.007). Furthermore, multivariate regression analysis showed that decrease in ambulatory LF/HF was a significant contributing factor to the improvement in LVMI.

**Conclusions:** These results indicate that cilnidipine is beneficial for the suppression of pathological cardiac remodeling through an inhibitory modulation of cardiac sympathetic nerve activity in hypertensive CKD patients.

*Funding:* Government Support - Non-U.S.

## FR-PO499

**White-Coat, Masked, and Sustained Hypertension in Chronic Kidney Disease–The CRIC Study** Paul E. Drawz,[1,2] Arnold B. Alper,[2] Amanda Hyre Anderson,[2] Denise C. Babineau,[2] Carolyn S. Brecklin,[2] Jeanne Charleston,[2] Jing Chen,[2] Yonghong Huan,[2] Susan P. Steigerwalt,[2] Jonathan J. Taliercio,[2] Raymond R. Townsend,[2] Matthew R. Weir,[2] Mahboob Rahman.[2] *[1]Univ of Minnesota; [2]CRIC Investigators.*

**Background:** The goal of this prospective study was to evaluate whether elevated proteinuria and low eGFR are associated with increased odds of white-coat (WCH), masked (MH), and sustained hypertension (SH) in a large cohort of participants with CKD.

**Methods:** In the Chronic Renal Insufficiency Cohort (CRIC) study, 24 hour ambulatory blood pressure (ABP) was measured between 2008 and 2012. Clinic blood pressure (BP) was measured in triplicate by study staff. WCH was defined by a clinic BP ≥140/90 mmHg and daytime ABP <135/85 mmHg; MH by a clinic BP <140/90 mmHg and daytime ABP ≥135/85 mmHg; and SH by a clinic BP ≥140/90 mmHg and daytime ABP ≥135/85 mmHg. Urine protein was measured in a 24 hour sample at baseline. Creatinine was measured annually.

**Results:** ABP was obtained in 1439 CRIC participants. The average time from the baseline visit to ABP was 5.1 years. The percent of participants with controlled BP, WCH, MH, and SH was 52.7%, 5.5%, 24.5%, and 17.2%. In a multivariable model, a doubling of urine protein was associated with increased odds of WCH, MH, and SH. In a separate multivariable model, a decrease in eGFR of 10ml/min/1.73m$^2$ was associated with increased odds of MH but not WCH or SH.

| | | Odds Ratio Based on Adjusted Models | |
|---|---|---|---|
| **BP category** | **N (%)** | **Urine protein** | **eGFR** |
| Controlled BP | 759 (52.7) | 1 (ref) | 1(ref) |
| White-coat HTN | 79 (5.5) | 1.14 (0.99 to 1.28) | 1.11 (0.94 to 1.36) |
| Masked HTN | 353 (24.5) | **1.16 (1.08 to 1.24)** | **1.11 (1.02 to 1.22)** |
| Sustained HTN | 248 (17.2) | **1.19 (1.10 to 1.28)** | 1.05 (0.95 to 1.17) |

**Conclusions:** In a large cohort of participants with a wide range of CKD, an increase in urine protein was associated with increased odds of white-coat, masked, and sustained hypertension. Ambulatory BP may be particularly important in patients with CKD in order to better characterize their BP control, especially among those with proteinuria.

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## FR-PO500

### Renal Denervation Halts the Decline of Renal Function in Patients with Chronic Kidney Disease and Treatment Resistant Hypertension

Christian Ott,[1] Felix Mahfoud,[2] Axel Schmid,[3] Tilmann Ditting,[1] Roland Veelken,[1] Michael Böhm,[2] Roland E. Schmieder.[1] *[1]Nephrology and Hypertension, Univ Hospital, Erlangen, Germany; [2]Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, Homburg/Saar, Germany; [3]Dept of Radiology, Univ Hospital, Erlangen, Germany.*

**Background:** Hypertension is a predominant risk factor of renal function decline over time and achieving blood pressure (BP) targets preserves renal function. Renal denervation (RDN) emerged as an interventional antihypertensive therapy in patients with treatment resistant hypertension (TRH) and we asked whether reducing BP by RDN preserves renal function by reducing BP or/and decreasing sympathetic activity.

**Methods:** 12 patients with TRH (office BP ≥140/90 mmHg and diagnosis confirmed by 24-h ABPM ≥130/80 mmHg) and chronic kidney disease (CKD) stage 3 and 4 underwent RDN using the Symplicity catheter (Medtronic Inc., Palo Alto, CA). The study was registered at http://www.clinicaltrials.gov/ (ID: NCT01442883). Renal function was evaluated up to 3 years prior and till 1 year after RDN. Estimated glomerular filtration rate (eGFR) was calculated using MDRD formula and the change in eGFR over time was calculated by regression slope individually for each patient.

**Results:** Patients (66±8 years) were treated with 5.8±1.1 antihypertensive drugs on average, and 73% had type-2 diabetes. One year after RDN, office BP was reduced by 20/6 mmHg (systolic: 156±19 vs. 137±18 mmHg, p=0.029; diastolic: 77±14 vs. 71±12 mmHg, p=0.019) and average 24-h ABPM by 13/6 mmHg (systolic: 157±13 vs. 144±12 mmHg, p=0.028; diastolic: 80±11 vs. 74±8 mmHg, p=0.028). Mean eGFR decline before RDN was -5.6±4.4 ml/min/1.73m$^2$ per year, which fits with postulated decline of renal function based on long-term clinical trials (Bakris et al, Am J Kidney Dis 2000;36:646-61). In contrast, eGFR remained stable after RDN (baseline: 47.0±11 vs. 1 year: 49.2±14 ml/min/1.73m$^2$), with a significance between eGFR change per year before and after RDN (-5.6±4.4 vs. +2.2±8.0 ml/min/1.73m$^2$ per year, p=0.021).

**Conclusions:** Our pilot study data indicate that RDN decreases BP and, most importantly, slows or even halts the decline of renal function in patients with TRH and CKD.

## FR-PO501

### Mindfulness Meditation Lowers Muscle Sympathetic Nerve Activity and Blood Pressure in Chronic Kidney Disease Patients

Jeanie Park,[1,2] Robert H. Lyles,[3] Susan Bauer-Wu.[4] *[1]Renal Div, Dept of Medicine, Emory Univ School of Medicine, Atlanta, GA; [2]Atlanta VA Medical Center, Decatur, GA; [3]Biostatistics and Bioinformatics, Emory Univ, Atlanta, GA; [4]Nursing, Univ of Virginia, Charlottesville, VA.*

**Background:** Chronic kidney disease (CKD) is characterized by chronic sympathetic nervous system (SNS) overactivity that contributes to hypertension and mortality. Prior studies have shown that mindfulness meditation (MM) lowers blood pressure (BP) and heart rate (HR) in hypertensive individuals. We hypothesized that MM acutely lowers BP and HR in hypertensive patients with chronic kidney disease (CKD), and that these hemodynamic changes are mediated by a reduction in sympathetic nerve activity (SNA).

**Methods:** 13 patients with CKD Stage III and hypertension were studied at two separate study visits to eliminate carryover effects, in a randomized, cross-over design. We measured continuous arterial BP, HR via continuous electrocardiography, respiratory rate (RR), and muscle sympathetic nerve activity (MSNA) using microneurography, at baseline, and during: 1) 14 minutes of guided MM during one study visit, and 2) 14 minutes of BP education (control condition) during the other study visit. Data were analyzed using mixed effects linear modeling.

**Results:** Linear modeling revealed that the rate of change in systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), and MSNA was significantly greater over time during MM compared to the control condition. At the14-minute time point, there was a significantly greater reduction in SBP (-10.2±2.9 vs. -0.8±1.3 mm Hg, p=0.007), DBP (-6.4±1.3 vs. -1.8±0.8, p=0.005), MAP (-7.7±1.6 vs. -1.4±0.8 mm Hg, p=0.002), during MM versus the control condition, respectively, but no difference in HR. Concomitantly, there was a significantly greater reduction in MSNA during MM compared to the control condition (-10.7±1.6 vs. +1.9±2.8 bursts/min, p=0.02).

**Conclusions:** These data are the first to demonstrate that MM may lower BP in hypertensive patients with CKD, and the reduction in BP may be mediated by an acute reduction in SNS activity. MM may have real physiological effects on autonomic control, and may be a useful complementary therapy in CKD patients.

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## FR-PO502

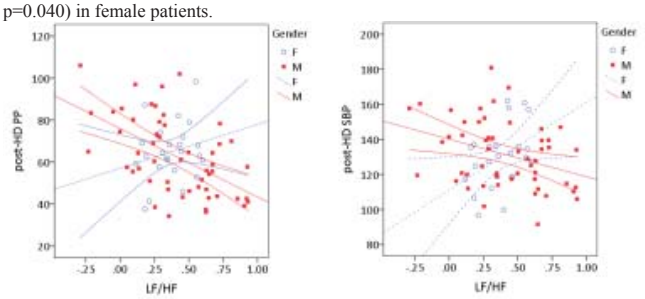
### Sex Dependent Association between Heart Rate Variability and Pulse Pressure in Hemodialyzed Patients

Dimitrios J. Poulikakos,[1,2] Debasish Banerjee,[1,2] Marek Malik.[1] *[1]Cardiovascular Sciences Research Centre, St. George's Univ of London, London, United Kingdom; [2]Renal and Transplantation, St. George's Hospital NHS Trust, London, United Kingdom.*

**Background:** Elevated Pulse Pressure (PP) is associated with increased cardiovascular mortality in hemodialysis (HD) patients. Autonomic imbalance is common in HD patients but its relationship to PP is unknown. We investigated the relationship between cardiac autonomic status assessed by heart rate variability (HRV) spectral analysis and PP in HD patients.

**Methods:** In stable HD patients, continuous electrocardiograms were obtained during HD and repeated 5 times at 2-weeks intervals. The high- (HF) and low-frequency (LF) components and the LF/HF ratio of HRV were calculated every 5 minutes in absolute values and averaged during the first and last hour of each recording. These values and the corresponding pre and post-HD systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were averaged in repeated recordings of each patient.

**Results:** We investigated 80 patients aged 60±14 years, 32% females and 37% diabetics. All blood pressure and HRV indices showed intra-subject stability (repeated measures Anova). LF and HF decreased through dialysis but LF/HF did not. LF/HF correlated inversely with pre and post-HD PP (r=-0.369, p=0.007 and r=-0.546, p=0.000 respectively), positively with pre and post-HD DBP (r=0.358, p=0.009 and r=0.306, p=0.028 respectively) and inversely with post-HD SBP in males patients (r=-0.350, p=0.011). In contrast, LF/HF did not correlate with PP or DBP and correlated positively with post-HD SBP (r=0.422, p=0.040) in female patients.




**Conclusions:** Strong association between PP and spectral HRV parameters was found in male but not in female HD patients. The sex differences in autonomic cardiovascular regulation during HD may contribute to the differences in cardiac risk between both sexes.

## FR-PO503

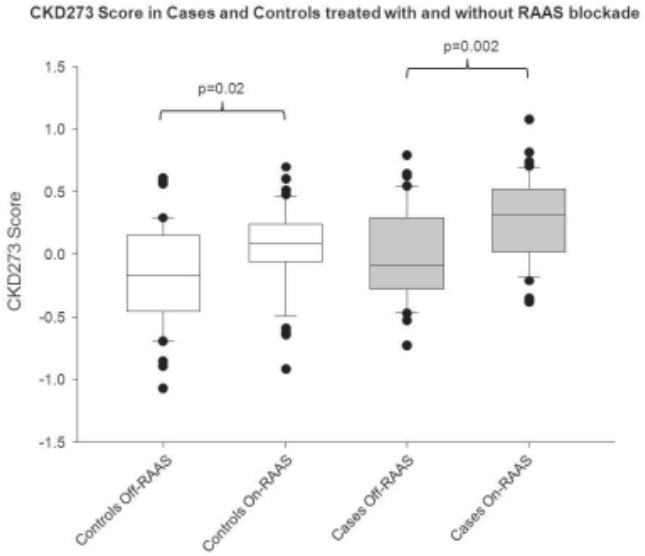
### A Urinary Peptide Score That Predicts Albuminuria Progression Differs in the Presence or Absence of RAAS Blockade

Michelle Pena,[1] Sara S. Roscioni,[1] Petra Zürbig,[2] Harald Mischak,[2] Stephan J.L. Bakker,[1] Ron T. Gansevoort,[1] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink.[1] *[1]Univ Medical Center Groningen; [2]Mosaiques Diagnostics.*

**Background:** Previous studies have shown that a urinary proteomic-based risk score (CKD273) predicts albuminuria progression. We assessed whether CKD273 scores differ in hypertensive subjects treated with or without RAAS blockade.

**Methods:** We performed a case-control study using data from the prospective, general population-based PREVEND Study (The Netherlands). We identified 75 hypertensive cases who progressed in albuminuria stage (from normo- to microalbuminuria or from micro- to macroalbuminuria). We matched these subjects for age, gender, baseline albuminuria stage, and use of RAAS blockade with hypertensive controls who had stable albuminuria during 3.0±0.9 years of follow-up. Mann-Whitney *U* test was used to compare CKD273 scores in subjects treated with or without RAAS blockade.

**Results:** RAAS blockade was used by 52% of cases and controls. CKD273 scores differed significantly between cases and controls (median [interquartile range]: 0.08 [-0.14;0.42] *vs* 0.02 [-0.28;0.22], p=0.03). Baseline characteristics such as blood pressure and albuminuria did not significantly differ between subjects with or without RAAS blockade, except for Hs-TnT, a marker of microvascular damage. CKD273 scores were higher in subjects on RAAS blockade than in subjects off RAAS blockade (cases on-RAAS 0.31 [0.01;0.53] *vs* off-RAAS -0.09 [-0.30;0.28], p=0.002; controls on-RAAS 0.09 [-0.07;0.25] *vs* off-RAAS -0.17 [0.46;0.15], p=0.02).




**Conclusions:** Higher CKD273 scores observed in hypertensive subjects treated with RAAS blockade may reflect more advanced renal/microvascular damage despite similar blood pressure and albuminuria levels. This likely reflects indication bias and suggests that RAAS blockade should be accounted for as a confounder in proteomic studies.

## FR-PO504

**Rapid Test to Identify the Salt Sensitivity** Andrea Pio de Abreu, Giovanio Vieira Da Silva, Isac De Castro, Katia Ortega, Decio Mion Junior. *Nephrology, Univ of Sao Paolo School of Medicine, Sao Paulo, Brazil.*

**Background:** Salt sensitivity, defined as the variation of blood pressure with the intake of different amounts of salt, has both clinical and prognostic implications. Nevertheless, the gold standard test for this diagnosis is hardly used in clinical practice, mainly due to the duration, at least a 2-week period of diet, with compliance difficulties in patients. The objective of study was to evaluate whether the use of fludrocortisone, wich causes sodium retention, is accurate compared to the dietary cycle. Until the moment, there is no practical test with an good accuracy.

**Methods:** A crossover design study was conducted with thirty unmedicated hypertensive patients. The dietary cycle consisted of one week with a low-sodium diet (40 mmol/day) and one week with a high-sodium diet (200 mmol/day). After one month, the fludrocortisone test was performed by administering 0.4 mg/day for 4 days, maintaining the usual food ingestion. BP was measured at the end of each dietary cycle and on the first and fourth day of fludrocortisone intake.

**Results:** The 30 volunteers (60% female), whose mean age was 53.7 ± 7.6 years, had a mean systolic blood pressure (SBP) of 146 ± 12 mmHg and diastolic of 91 ± 10 mmHg. The mean NaU values for the low and high-salt-diet cycles were, respectively, 40 ± 25 mmol/d and 212 ± 44 mmol/d. During the administration of fludrocortisone, there was a significant reduction of renin, aldosterone and potassium levels compared to the basal period. The Area Under Roc Curve (AUCROC) for SBP on the reference test was 0.89 ± 0.04 (p<0.001) and on the fourth day of fludrocortisone was 0.76 ± 0.06 (p<0.001).

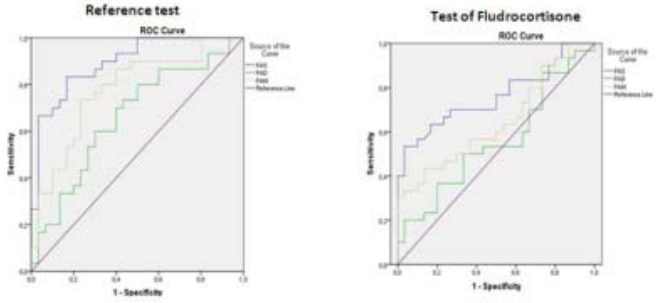



Figure 1 - Area Under Roc Curve (AUCROC) for systolic (PAS), mean (PAM) and diastolic blood pressure (PAD) for the reference and Fludrocortisone tests. AUCROC values for the reference test were 0.89 ± 0.04 (p<0.001), 0.77 ± 0.06 (p<0.001), and 0.66 ± 0.07 (p=0.032), respectively, for systolic (PAS), mean (PAM) and diastolic blood pressure (PAD). The AUCROC values on the fourth day of fludrocortisone were 0.76 ± 0.06 (p<0.001), 0.65 ± 0.07 (p < 0,05), and 0.55 ± 0.07 (p=0.46), respectively, for PAS, PAM and PAD.

**Conclusions:** The Rapid Test To Identify the Salt Sensitivity, which consists in the change in SBP after administration of 0.4 mg / day of fludrocortisone for four days, has good accuracy to identify salt-sensitive hypertensive patients.

*Funding:* Government Support - Non-U.S.

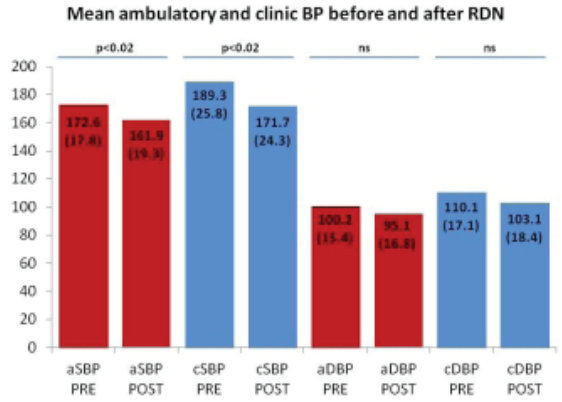
## FR-PO505

**The Impact of Renal Denervation on Ambulatory Blood Pressure in Two Specialist Hypertension Units in the United Kingdom** Alison H.M. Taylor,[1] Patrick B. Mark,[1] Alan G. Jardine,[1] Jonathan S. Freedman,[2] Indranil Dasgupta.[3] *[1]BHF Glasgow Cardiovascular Research Centre, Univ of Glasgow, Glasgow, United Kingdom; [2]Dept of Radiology, Birmingham Heartlands Hospital, Birmingham, United Kingdom; [3]Dept of Nephrology, Birmingham Heartlands Hospital, Birmingham, United Kingdom.*

**Background:** Percutaneous renal artery denervation (RDN) is a novel technique for treatment of resistant hypertension, which has been shown to have major effects on clinic blood pressure (BP). We studied ambulatory BP (ABP), a more accurate representation of BP, in patients undergoing RDN out with a specific clinical trial.

**Methods:** Patients with systolic BP ≥160/90mmHg despite compliance with ≥3 antihypertensive agents and no secondary cause for hypertension were treated with bilateral RDN. All patients underwent ABP monitoring and laboratory tests prior to and 6-months following RDN.

**Results:** 20 consecutive patients (55% female, mean age 50.1 years) were studied. At baseline, the median time since diagnosis of hypertension was 8.5 years (range 1-32 years), 20% of patients had diabetes mellitus and 35% had a history of vascular disease. The median serum creatinine was 0.83mg/dL (range 0.61-3.60). Mean clinic BP was 189/110mmHg (±26/17) and mean ABP 173/100mmHg (±18/15). The mean number of antihypertensive agents prescribed was 5.65 (±1.3). At six months following RDN there was a significant fall in both ambulatory (-9.9mmHg, p<0.02) and clinic (-18.9mmHg, p<0.02) systolic BP. There was a non-significant fall in diastolic BP using both measurements. The mean reduction in number of medications prescribed was 1.6 (±2.1, p=0.003). There was no significant change in renal function at six months.




**Conclusions:** RDN does lead to reduction in ABP in 'real world' patients with resistant hypertension, although the magnitude of effect is less than previously demonstrated.

## FR-PO506

**Comparison of Sleep Apnea Rates and Characteristics in Resistant and Non Resistant Hypertension** John J. Sim, Jiaxiao Shi, Glory Tseng. *Nephrology & Hypertension, Kaiser Permanente Los Angeles Medical Center.*

**Background:** Sleep apnea is associated with hypertension and is a risk factor for resistant hypertension (RH) but the degree to which it is more prevalent in RH is largely unknown. We sought to compare rates and characterize sleep apnea in RH and non RH within a large ethnically diverse hypertension population.

**Methods:** Cross sectional study during 1/1/2006- 12/31/2007 of hypertensive individuals within the Kaiser Permanente Southern California health system. Resistant hypertension defined as uncontrolled blood pressure on 3 meds or requiring >/=4 meds. Sleep apnea identified by ICD-9 coding and CPT coding for dispensation of cpap/bipap. Rates of sleep apnea in RH and non RH determined. Demographic, co morbidity and laboratory information extracted from the electronic medical records. Multivariable logistic regressions performed to calculate odds ratios (OR) for sleep apnea.

**Results:** Among 470,386 individuals with hypertension, sleep apnea was identified in 23,184 (4.9%). RH individuals had a sleep apnea rate of 7.1% (4,007) compared to 4.9% (19,177) in non RH (p<0.001). Among the entire hypertension cohort, adjusted OR for sleep apnea were greater in those with RH, BMI>29, male gender, and diabetes. Whites were more likely to have sleep apnea compared to blacks, Hispanics, and asians.

| **Odds Ratio for Sleep Apnea among a Hypertension Population With adjustment for age, gender, race, BMI, and co morbidities (N=470,386)** | | |
|---|---|---|
| **Variable** | **OR** | **95% CI** |
| Resistant Hypertension | 1.08 | 1.04-1.12 |
| BMI>29 | 3.42 | 3.32-3.53 |
| Male gender | 2.28 | 2.22-2.35 |
| Race Ethnicity (ref white) | | |
| Hispanic | 0.61 | 0.58-0.63 |
| Black | 0.76 | 0.73-0.79 |
| Asian | 0.59 | 0.55-0.63 |
| Diabetes | 1.27 | 1.24-1.31 |
| CHF | 2.02 | 1.94-2.11 |

**Conclusions:** Within a large ethnically diverse hypertension population, we observed a modest increase in rate and risk for sleep apnea in RH compared to non RH. Among the traditional risk factors, BMI>29 had the greatest risk for sleep apnea within our hypertension population.

*Funding:* Private Foundation Support

## FR-PO507

### Disturbed Circadian Rhythm of the Intrarenal Renin-Angiotensin System: Relevant to Nocturnal Hypertension and Renal Damage

Shinsuke Isobe,[1] Naro Ohashi,[1] Hideo Yasuda,[1] Yoshihide Fujigaki.[1,2] *[1]Internal Medicine 1, Hamamatsu Univ School of Medicine; [2]Internal Medicine, Teikyo Univ School of Medicine.*

**Background:** The intrarenal renin-angiotensin system (RAS) plays an important role in the development of hypertension and renal damage. Disruption of diurnal blood pressure (BP) variation is an additional risk factor for renal damage. However, little is known regarding whether intrarenal RAS circadian rhythm exists or if it influences the disruption of diurnal BP and renal damage.

**Methods:** We investigated the circadian rhythm of urinary angiotensinogen that reflects intrarenal RAS activity, albuminuria, proteinuria and urinary sodium excretion every hour ($Na^+$/h) in 10 healthy individuals and 33 chronic kidney disease (CKD) patients classified according to circadian BP rhythms.

**Results:** BP values were higher during the day than during the night in healthy individuals and CKD patients. Urinary angiotensinogen levels were not different between the day and night in healthy individuals, but were significantly higher in the daytime in CKD patients (log urinary angiotensinogen/creatinine: daytime, 2.48 ± 0.99; nighttime, 2.31 ± 1.08; p = 0.001). Furthermore, in CKD patients showing a riser pattern of circadian BP, urinary angiotensinogen levels did not decrease during the night compared with those in the day (log urinary angiotensinogen/creatinine: daytime, 2.50 ± 0.65; nighttime, 2.52 ± 0.71; p = 0.092). Circadian fluctuation of albuminuria and proteinuria occurred parallel to that of the urinary angiotensinogen levels. Urinary angiotensinogen levels were significantly and positively correlated with the degree of hypertension and circadian fluctuation of urinary angiotensinogen were correlated with diurnal BP changes.

**Conclusions:** These data suggest that the circadian rhythm of intrarenal RAS activation may lead to renal damage and hypertension, which are associated with diurnal BP variation.

## FR-PO508

### Variables Indicative of Kidney Injury Are Related to the BP-Lowering Effect of Renal Denervation

Eva Vink,[1] Willemien Verloop,[2] Wilko Spiering,[3] Michiel Voskuil,[2] Evert-jan Vonken,[4] Michiel Bots,[5] Peter J. Blankestijn.[1] *[1]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]Cardiology, Univ Medical Center Utrecht, Utrecht, Netherlands; [3]Vascular Medicine, Univ Medical Center Utrecht, Utrecht, Netherlands; [4]Radiology, Univ Medical Center Utrecht, Utrecht, Netherlands; [5]The Julius Center for Health Sciences and Primary Care, Univ Medical Center Utrecht, Utrecht, Netherlands.*

**Background:** Renal denervation(RDN) is a promising treatment for resistant hypertension. However, there is a wide range in the BP-lowering effect. Evidence on factors that determine BP-lowering effect after pRDN is limited. Aim of current study was to further explore these factors.

**Methods:** Patients from a prospective cohort of patients treated with RDN with available 6months follow-up data,were included in current analysis. Data collected during routine clinical standardized work-up before RDN were used in present analysis:urine collected during 24-h, blood samples, ambulatory BP monitoring and a captopril challenge test. When considered safe, anti-hypertensive drugs were stopped before these investigations.

**Results:** 54 patients were included in the present analyses. 59% was female, mean age was 61(±11)yrs. At baseline, patients used a median number of 3 antihypertensive drugs, office BP was 202(±25)/109(±14)mmHg and eGFR was 72(±19)mL/min/1.73$m^2$. A higher BP at baseline, a higher level of BP during nighttime(β:-0.53 mmHg/mmHg,P=0.03), a higher albumin-to-creatinine ratio(β:-10.58, P=0.06), higher levels of aldosterone(β:-0.020 mmHg/pmol/L,P=0.09) and catecholamines(norepinephrine(β:-0.07mmHg per nmol/24-h, P=0.03) and VMA(β:-1.04mmHg per µmol/24-h, P=0.05)) in urine as well as a greater decrease in BP after captopril(β:0.731mmHg/mmHg, P=0.53), were related to the BP-lowering effect of RDN. Kidney function was inversely related to the BP-lowering effect of RDN(β:0.597mmHg/mL/min/1.73$m^2$, P=0.003).

**Conclusions:** Present study shows that variables related to kidney injury are related to the BP-lowering effect of RDN. This very well fits within current thinking of the pathophysiology and it may give some insight in the great effect range of RDN.

*Funding:* Government Support - Non-U.S.

## FR-PO509

### BP-Lowering Effect of Renal Denervation in Patients with Multiple Renal Arteries

Eva Vink,[1] Willemien Verloop,[2] Wilko Spiering,[3] Michiel Bots,[4] Evert-jan Vonken,[5] Michiel Voskuil,[2] Peter J. Blankestijn.[1] *[1]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]Cardiology, Univ Medical Center Utrecht, Utrecht, Netherlands; [3]Vascular Medicine, Univ Medical Center Utrecht, Utrecht, Netherlands; [4]Julius Center for Health Sciences and Primary Care, Univ Medical Center Utrecht, Utrecht, Netherlands; [5]Radiology, Univ Medical Center Utrecht, Utrecht, Netherlands.*

**Background:** In contrast to previous studies investigating safety and efficacy of renal denervation (RDN), we decided not to exclude patients with multiple renal arteries from treatment with RDN. The aims of present study were: to determine the prevalence of multiple renal arteries in patients referred for RDN, to investigate the relation between presence of multiple renal arteries and blood pressure (BP)-lowering effect of RDN.

**Methods:** Patients referred for treatment with RDN who underwent non-invasive imaging of the renal arteries before treatment, were included in present analysis. Eligible patients underwent RDN. BP and renal function were evaluated 6 months after treatment.

**Results:** 179 patients underwent imaging in the work-up. Thirty-five percent had multiple renal arteries. No differences in baseline characteristics were found between those with and without multiple arteries. Out of 179 patients, 109 underwent RDN, of which 60 patients completed six months follow-up. Office BP significantly changed in these patients from 200(±23) / 108(±14) mmHg to 168(±24) / 96(±14) mmHg (P<0.001). The change in BP was not different in patients with multiple- compared to patients with solitary arteries. Renal function at 6 months did not differ from baseline in both subgroups. A classification of eligibility of the renal arteries for treatment with RDN is proposed.

**Conclusions:** Current analysis suggests that RDN is effective in patients with multiple renal arteries. Based on the results of this present study, and the high prevalence of multiple arteries, it seems reasonable not to exclude patients with multiple renal arteries from RDN.

*Funding:* Government Support - Non-U.S.

## FR-PO510

### The Impact of Sodium, Fructose Restriction and Allopurinol on Blood Pressure amongst Overweight and Prehypertensive Subjects

Magdalena Madero,[1] Francisco E. Rodríguez Castellanos,[1] Silvia Hernández-Lobato,[1] Richard J. Johnson,[2] Maria Villalobos-Martín,[1] Armando Vazquez-Rangel,[1] Jonathan Salazar,[1] L. Gabriela Sanchez-Lozada.[1] *[1]National Heart Institute Mexico; [2]U Colorado.*

**Background:** Fructose causes hypertension partly through rising intracellular and serum uric acid (UA). The objective of this study was to compare the effect of sodium and fructose restriction in addition to allopurinol on blood pressure and metabolic syndrome components.

**Methods:** Randomized trial that included overweight and prehypertensive or hypertensive adults not on antihypertensive meds. The trial consisted of two phases: 1) Wks 1-4 patients were randomized to either isocaloric (with respect to baseline caloric intake) low sodium- low fructose diet (LS-F) or isocaloric low sodium diet (LS) during 4 wks. 2) Wks 4-8) the groups continued with the same diet prescriptions and were further randomized to receive placebo (P) or allopurinol (A, 300 mg/d). Clinic and 24hr ambulatory BP, anthropometric measures, laboratory data, were determined at weeks 0, 4 and 8.

**Results:** 72 patients were included in the trial. Compared to Wk0, at Wk8 clinic SBP and DBP were significantly lower in the LS-F group but not in the LS group. Both diets were associated with a significant day and global ABPM reduction. There were no between group significant differences with diet interventions at Wk4 or 8. Compared to placebo, at Wk8 subjects in the A group had a lower clinic SBP and this was significant within and between group comparisons. The % of dippers was also significantly higher in the A group. In addition weight was significantly reduced in the LS-F and A groups.

Table1

| | LS Diet (n=38) | | LS- F Diet (n=34) | | Placebo (n=36) | | Allopuriol (n=36) | |
|---|---|---|---|---|---|---|---|---|
| | Week | | Week | | Week | | Week | |
| | 0 | 8 | 0 | 8 | 0 | 8 | 0 | 8 |
| Age (years) | 48±7 | | 44±9 | | 47±9 | | 46±8 | |
| Sex (M/F) | 22/16 | | 22/12 | | 22/14 | | 22/14 | |
| Weight (kg) | 86±16 | 87±16 | 90±16 | 89±16[a] | 85±15 | 85±15 | 91±17 | 90±17[a] |
| SBP (mmHg) | 125±11 | 120±16 | 124±13 | 116±10[a] | 125±13 | 122±15 | 124±10 | 115±12[ab] |
| DBP (mmHg) | 86±7 | 82±8[a] | 84±8 | 80±7[a] | 84±7 | 83±7 | 86±7 | 80±8[a] |
| Global SBP - ABPM | 134±10 | 130±10[a] | 132±12 | 129±10[a] | 133±12 | 129±9[a] | 133±10 | 130±10[a] |
| Global DBP - ABPM | 80±8 | 79±9 | 80±7 | 77±7[a] | 80±8 | 77±8 | 80±7 | 79±8 |
| Day SBP - ABPM | 138±10 | 135±10[a] | 136±12 | 132±10[a] | 138±12 | 132±10[a] | 137±9 | 135±10 |
| Day DBP - ABPM | 83±9 | 82±9 | 83±8 | 80±7[a] | 83±9 | 80±9[a] | 83±8 | 82±8 |
| Dippers (%) | 55.2 | 68[b] | 44.8 | 32 | 51.7 | 32 | 48.3 | 68[b] |
| Chol (mg /dl) | 199±43 | 190±56 | 194±32 | 185±31 | 200±39 | 185±51[a] | 192±37 | 190±40 |
| TGL (mg/dl) | 183±99 | 186±93 | 190±114 | 161±64 | 206±124 | 173±70[a] | 167±81 | 177±91 |
| Uric acid (mg/dl) | 6.2±1.6 | 5.2±1.4[a] | 6.1±1.7 | 5.1±1.8[a] | 6±1.7 | 5.9±1.4 | 6.2±1.6 | 4.5±1.4[ab] |

Values shown as mean ± SD. a= p<0.05 week 8 vs. week 0 b= p<0.05 week 8 between group comparisons. SBP: Systolic Blo Pressure, DBP: Diastolic Blood Pressure, ABPM: Ambulatory Blood Pressure Monitor, Chol: Cholesterol, TGL:Tryglicerides

**Conclusions:** Both LS and LS-F diets are efficient to lower BP. Allopurinol significantly reduced clinic SBP and increased the percentage of dippers. Both LS-F and A were associated with weight loss despite the absence of caloric restriction.

*Funding:* Government Support - Non-U.S.

## FR-PO511

**Type 2 Diabetic Patients with Treatment-Resistant Hypertension Display Urine Plasmin Excretion That Correlates with Blood Pressure and Activates ENaC *In Vitro*** Kristian B. Buhl,[1] Christina Stolzenburg Oxlund,[2] Ulla G. Friis,[1] Per Svenningsen,[1] Claus Bistrup,[2] Ib A. Jacobsen,[2] Boye Jensen.[1] *[1]Univ of Southern Denmark, Denmark; [2]Univ Hospital of Odense, Denmark.*

**Background:** Aberrant filtration of plasminogen from plasma and subsequent activation to plasmin in the urinary space may proteolytically activate the epithelial sodium channel, ENaC. In conditions with chronic albuminuria this may cause hypertension. It was hypothesized that patients with type 2 diabetes mellitus (T2DM) and treatment-resistant hypertension (RHTN) excrete plasmin(ogen) in urine in proportion to albumin and that plasmin confers to urine the ability to activate ENaC.

**Methods:** A Cross sectional design of 113 patients with T2DM and RHTN (systolic BP > 130mmHg and diastolic BP > 80mmHg despite use of at least three drugs with one diuretic and one renin-angiotensin system inhibitor). 24 hour blood pressure meassurements was obtained and urine was analyzed for albumin, creatinine, plasmin(ogen), protease activity and ability to activate inward current in single collecting duct cells.

**Results:** Mean ambulatory blood pressure was 143 ± 1/77±0.7 mmHg; HbA1c 7.35 %; eGFR 81.4 ml/min/1.73m$^2$ (geometric means). Micro- (39%) and macroalbuminuric (13%) T2DM patients with RHTN displayed significantly elevated levels of urinary plasmin(ogen) normalized to urine creatinine (plg/crea) compared to normo-albuminuric-T2DM-RHTN patients (48%). Urinary plg correlated significantly to urine albumin. Western immunoblotting and gelatine zymografi confirmed active plasmin in urine samples from T2DM-RHTN patients with micro- and macroalbuminuria. Single collecting duct cells showed significantly increased, amiloride-sensitive, inward current when superfused with urine from albuminuric T2DM-RHTN compared to normalbuminuric T2DM-RHTN. Urinary plg/crea correlated significantly to 24 hour ambulatory blood pressure.

**Conclusions:** Aberrant presence of plasmin in pre-urine may inappropriately activate ENaC in patients with type 2 diabetes and microalbuminuria. This may contribute to treatment-resistant hypertension.

*Funding:* Private Foundation Support

## FR-PO512

**Effects of Renal Denervation with a Standard Irrigated Cardiac Ablation Catheter on Blood Pressure and Renal Function in Patients with Chronic Kidney Disease and Resistant Hypertension** Jocemir R. Lugon,[1] Maria A. Carreira,[1] Miguel Luis Graciano,[1] George Luiz Marques Maia,[1] Marcio Galindo Kiuchi,[1,2] Tetsuaki Kiuchi.[1] *[1]Nephrology, Universidade Federal Fluminense, Niterói, RJ, Brazil; [2]Vascular Surgery, Hospital Regional Darcy Vargas, Rio Bonito, RJ, Brazil.*

**Background:** Recently, transcatheter renal sympathetic denervation (RSD) has proved effective in lowering refractory blood pressure (BP). We evaluated the safety and efficacy of this procedure using a standard irrigated cardiac ablation catheter (SICAC) in 24 chronic kidney disease patients (stages 2-4) with refractory hypertension.

**Methods:** Twenty four patients were included and treated with a SICAC. Denervation was performed by single operator following standard technique. Patients included with CKD were on stages 2 (n=16), 3(n=4) and 4(n=4). Data were obtained at baseline and monthly until 180$^{th}$ day of follow up.

**Results:** Data were obtained at baseline and monthly until180th day of follow up. Baseline values of blood pressure

(Mean±SD) were: 186±19 mmHg / 108±13 mmHg in the office, and 151±18 mmHg / 92±11 mmHg by 24 hour ambulatory blood pressure monitoring (ABPM). Office blood pressure values at 180th day after procedure were 135±13 mmHg/88±7 mmHg

(P<0.0001, for both comparisons). Mean ABPM decreased to 132±15 mmHg/ 85±11 mmHg at the 180th day after the procedure (P <0.0001 for systolic and P =0.0015 for diastolic). Estimated glomerular filtration (Mean±SD) increased from baseline (64.4±23.9 ml/min/1.73m2) to the 180th day (85.4±34.9 ml/min/1.73m2, P<0.0001) of follow-up. Median urine albumin:creatinine ratio decreased from baseline (48.5, IQR 35.8-157.2 mg/g) to the 180th day after ablation (ACR = 15.7, IQR 10.3-34.2 mg/g, P=0.0017). No major complications were seen.

**Conclusions:** The procedure using SICAC seemed to be feasible, effective and safe resulting in better control of BP, a short-term increase in eGFR, and reduced albuminuria. Although encouraging, our data are preliminary and need to be validated in the long term.

## FR-PO513

**Microvascular/Hypertensive Disease Is Increased in Patients with Obstructive Sleep Apnea** Sky K. Chew,[1] Deb J. Colville,[1] Ecosse L. Lamoureux,[2] Judith A. Savige.[1] *[1]Dept of Medicine (Royal Melbourne Hospital), The Univ of Melbourne, Melbourne, VIC, Australia; [2]Centre for Eye Research Australia, The Univ of Melbourne, Melbourne, VIC, Australia.*

**Background:** Microvascular/hypertensive abnormalities in the retina reflect systemic small vessel disease. This study used retinal examination to compare the prevalence of of microvascular/hypertensive disease (severity of changes and calibre) in patients with Obstructive sleep apnea (OSA) or Chronic obstructive pulmonary diseas disease (COPD).

**Methods:** Patients were recruited from the Respiratory clinic and wards of a metropolitan teaching hospital. All participants underwent retinal photography using a non-mydriatic camera (KOWA, KOWA Japan). Images were graded for microvascular/hypertensive retinopathy (Wong and Mitchell classification) by an ophthalmologist and a trained observer. Images were sent to the Centre for Eye Research Australia for measurement of the retinal arteriole and venular calibre by a grader using Knudtson's revised version of the Parr-Hubbard formula. Statistical analysis was performed using Stata 11.2 software (Stata Corp).

**Results:** Seventy-nine patients with OSA and 132 with COPD were recruited. Patients with OSA alone were younger (p <0.01), had a higher BMI (p <0.01), and were less likely to be smokers (p <0.01) than those with COPD.

Patients with OSA had more microvascular disease than those with COPD (OR 9.90, 95%CI 2.29 to 42.90, P <0.01). In addition, their arterioles (mean difference 18.00µm, 95%CI 12.88 to 23.08, P <0.01) and venules (mean difference 25.30µm, 95%CI 17.09 to 33.52, P <0.01) were narrower. These changes were not worse with more severe OSA and were not reversed with the use of CPAP. Microvascular/hypertensive retinopathy was still more common and the arteriolar and venular narrowing persisted in patients with OSA after adjusting for age, BMI, hypertension, smoking and dyslipidemia.

**Conclusions:** Patients with OSA have increased small vessel disease compared with patients with COPD, with worse microvascular/hypertensive retinopathy and narrower vessels. This narrowing was not related to OSA severity and was not reversed with CPAP treatment.

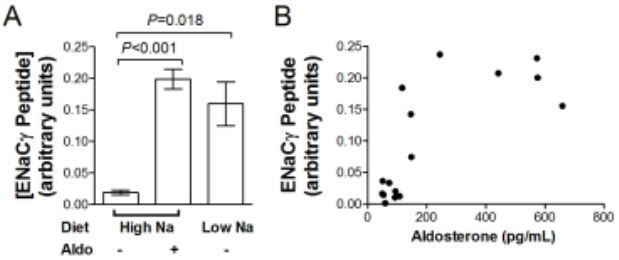
## FR-PO514

**Dietary Sodium Reduction and Aldosterone Acutely Alter the Urine Exosome Proteome in Humans** James M. Luther,[2,3] Ying Qi,[1] Bing Zhang,[1] Kristie Rose,[1] Xiaojing Wang,[1] Kevin Schey.[1] *[1]Biochemistry, Vanderbilt Univ, Nashville, TN; [2]Medicine, Vanderbilt Univ, Nashville, TN; [3]Pharmacology, Vanderbilt Univ, Nashville, TN.*

**Background:** Urinary exosomes are a potential biomarker source for human disease, but their physiologic relevance remains uncertain.

**Methods:** To test the hypothesis that the urinary proteome is regulated by endogenous and exogenous hormones, we analyzed urine exosomes in 14 healthy control subjects in a cross-over study during high (HS, 160mmol/d) and low salt (LS, 20mmol/d) diet to activate the endogenous renin-angiotensin-aldosterone system (RAAS). In a separate cohort during HS diet, we collected urinary exosomes after vehicle and aldosterone infusion (0.7 µg/kg/hr for 10 hrs).

**Results:** LS diet increased renin activity (5.9±3.1 vs 0.8±0.3 ng AngI/mL/hr; P<0.01) and aldosterone (16.4±2.7 vs 7.5±1.1 ng/dL; P<0.001), and aldosterone infusion increased plasma aldosterone (55.9±5.5 vs 7.8±1.5 ng/dL; P<0.001). Urinary exosome peptide analysis by Multidimensional Protein Identification Technology (MudPIT) detected 2,892 unique proteins, 1,342 were significantly altered during LS diet (117 up, 717 down) after correction for multiple testing. In a separate validation cohort, proteins including NCC, ENaC-alpha, and ENaC-gamma were validated using targeted multiple reaction monitoring mass spectrometry (MRM MS). Dietary sodium restriction and acute aldosterone infusion similarly increased urine exosome ENaC-gamma excretion (**Figure A**), which correlated positively with plasma aldosterone (**Figure B**; $R^2$=0.56, P<0.001). Urinary Na/K concentration during LS diet corresponded similarly.




**Conclusions:** The urinary exosome content is dynamically altered by the RAAS and corresponds with anticipated physiologic changes. Furthermore, exosomal ENaC gamma may provide a useful biomarker of sodium channel activation in clinical studies.

*Funding:* NIDDK Support, Other NIH Support - HL100016, UL1 TR000445

## FR-PO515

**Night-Time BP of <120/70 Can Improve the Renal Outcomes in Patients with CKD** Shuichi Watanabe,[1] Hiroyuki Togawa,[1] Daisuke Fuwa,[1] Yukako Isobe,[1] Yoshiaki Ogiyama,[1] Toshiyuki Miura,[1] Tadashi Ichikawa,[1] Shirasawa Yuichi,[1] Michio Fukuda,[1] Genjiro Kimura.[2] *[1]Cardio-Renal Medicine and Hypertension, Nagoya City Univ, Japan; [2]Asahi Rosai Hospital, Japan Labour Health and Welfare Organization.*

**Background:** ABPM has been already widely adopted for the diagnosis and classification of hypertension. In some reports, optimal blood pressure was proposed to prevent hypertensive organ damage especially heart diseases and cerebrovascular diseases. However, the evidence of optimal blood pressure for the renal dysfunction still unclear.

**Methods:** Two hundred and five patients with chronic kidney disease (CKD) (male 106, female 99, age 49±19 year-old, eGFR 57±35 ml/min/1.73m$^2$, mean follow-up 1,207days), who have undergone 24-h ABPM, were studied. The subjects were grouped into 2 according to the presence or absence of the night-time high blood pressure (night time systolic blood pressure>=120 mmHg or <120mmHg). The primary end-point was the composite of a doubling of the base-line serum creatinine concentration, or end-stage renal disease. Analysis was by intention to treat.

**Results:** Fifty out of 80 subjects with night time systolic hypertension subjects reached the primary endpoint, whereas 13 out of 125 subjects without night-time systolic hypertension ($p<0.0001$). Compared with the night-time BP of <120/70 mmHg, the night time BP of ≥120/70 mmHg exhibited higher risk for the primary endpoint (Crude HR 4.68, 95%CI 2.00-11.0, p=0.0004, Adjusted HR 3.68, 95%CI 1.28-10.6, p=0.02), whereas, presence of day time hypertension (≥135/85 mmHg) didn't become the major risk for the renal outcome. Interestingly, ROC analysis also indicated that the optimal night-time blood pressure value to prevent the renal dysfunction was 120/70 mmHg (positive likelihood ratio, 3.27/1.62).

**Conclusions:** The presence of night-time hypertension was a strong predictor of renal dysfunction. Since day time blood pressure was also plays an important role for hypertensive organ damages, further studies are needed to determine the optimal day time blood pressure goal for prevention of the CKD progression.

## FR-PO516

### 8-Week Study on Effects of Chlorthalidone in Hypertensives with Low eGFR

Massimo Cirillo, Fabiana Marcarelli, Alessandra Antonia Mele, Massimo Romano, Cinzia Lombardi, Giancarlo Bilancio. *Dept of Medicine, Univ of Salerno, Baronissi (SA), Italy.*

**Background:** Efficacy of chlorthalidone (CT) and thiazides is considered low in low kidney function (LKF).

**Methods:** A parallel-arm,non-inferiority study was done on CT effects in hypertensives with LKF and hypertensives without LKF (Italian Drug Agency Registry ID#671).Study design included:screening visit,baseline visit, 8-week CT treatment with visits at week 1,2,4,6 and 8.The screening visit selected patients on antihypertensive treatment with uncontrolled hypertension (SBP≥140 or DBP≥90),ages 25-74,complete diagnostic work-up.Eligible patients were prescribed lab evaluations and re-examined after 1-2 week (baseline).Exclusion criteria were treatment with diuretics,CT contraindications,refused consent,SBP≥180 or DBP≥110, severe co-morbidities.At baseline, 25 mg CT was prescribed on the top of ongoing treatments to 60 patients with LKF (eGFR by CKD-Epi equation stably <60 mL/min) and 60 patients without LKF (Control, eGFR stably ≥60 mL/min). Blood pressure was measured at each visit by blinded trained physicians according to WHO guidelines.Lab evaluations were repeated at visit 8. Study power was 80% ($\alpha$=0.01,one sided test,$\sigma$=15,difference in SBP reduction=8).

**Results:** LKF and Control were similar for men% (70.2% and 64.2%),age (mean= 57 and 53),baseline blood pressure (SBP/DBP= 150/90 and 150/91) but differed for eGFR (mean= 39 and 76, range=15-59 and 60-104).Changes over baseline were significant at week 8 in LKF and Control for SBP (mean= -20 and -23; 95%CI= -22/-18 and -26/-19) and DBP (-9 and -10; -11/-7 and -13/-8).Differences between groups were not significant for changes in SBP (-3; -7/+1) and DBP (-1; -4/+2). Baseline eGFR did not predict SBP/DBP changes in either groups (R<0.18,P>0.17). Week 8 changes were significant for eGFR (LKF and Control, mL/min= -2 and -5; -4/-1 and -7/-3), serum potassium (mmol/L= -0.2 and -0.2; -0.3/-0.1 and -0.3/-1), serum uric acid (mg/dL= +0.8 and +0.9; +0.5/+1.1 and +0.7/+1.1). Adverse events incidence was 13.3% in both groups. The commonest events were serum sodium <135 mmol/L and SBP/DBP <110/90.

**Conclusions:** Data do not support the idea of reduced CT efficacy in low kidney function.

*Funding:* Government Support - Non-U.S.

## FR-PO517

### Association of Renin and Aldosterone Concentrations with Race/Ethnicity and Blood Pressure in Community-Living Individuals: The Multi-Ethnic Study of Atherosclerosis

Dena E. Rifkin,[1] Ali Khaki,[1] Nancy Jenny,[2] Robyn L. McClelland,[3] Matthew Jay Budoff,[4] Karol E. Watson,[4] Joachim H. Ix,[1] Matthew Allison.[1] *[1]UCSD; [2]Univ of VT; [3]Univ of Washington, Seattle; [4]UCLA.*

**Background:** Though variations in plasma renin activity (PRA) and aldosterone (aldo) have been examined in Caucasians and African-Americans, little is known about the levels of these hormones and their association with blood pressure in other races/ethnicities.

**Methods:** We measured PRA and aldo in 1021 community-dwelling participants who were not taking antihypertensive medications and then examined the association between race/ethnicity (Caucasian, Chinese, African-American and Hispanic) and both PRA and aldo, as well as the association between PRA and aldosterone with systolic blood pressure (SBP) overall and by race/ethnicity.

**Results:** The average (SD) age was 62(9). Median (IQR) PRA was 0.51 (0.29 to 0.87) ng/ml/hr and aldo was 12.6 (9.1 to 17.1) ng/dl. After age and gender adjustment, compared to Caucasians, African-Americans had 27.5% lower PRA and 17.2% lower aldo, and Hispanics 19.1% higher PRA but similar aldo levels. After multivariate adjustment, compared to Caucasians, only Hispanic race/ethnicity was associated with higher PRA (0.19 ng/ml/hr [95% CI 0.07, 0.31]). African-American race/ethnicity was associated with lower aldo(-1.58 ng/dl [95% CI -2.97 to -0.18]) compared to Caucasians. After similar multivariate adjustment for demographic and clinical characteristics, PRA was associated with lower SBP in Caucasians (-3.2 mmHg [95% CI -5.2, -1.2] per standardized unit PRA), Chinese (-3.5 mmHg [95% CI -6.2, -0.80] per standardized unit) and Hispanics (-2.3 mmHg [95% CI -4.1, -0.6] per standardized unit) but not African-Americans. Aldowas associated with higher SBP only in Hispanics (2.5 mmHg [95% CI 0.4, 4.5] per SD).

**Conclusions:** Compared to community-living Caucasians, African-Americans have lower aldo and Hispanics have higher PRA, whereas levels in Chinese have no significant difference in either measure. Aldo had a significant association with higher SBP in Hispanics compared to other races/ethnicities, a finding that may suggest a different mechanism of hypertension for this race/ethnicity.

*Funding:* NIDDK Support, Other NIH Support - NHLBI supports the MESA study.

## FR-PO518

### Podocyturia Is Not Predictive of the Development of Preeclampsia

Belinda Bun Jim,[1] Swati Mehta,[2] Andi Qipo,[1] Elizabeth Ann Phipps,[1] Kwanghee Kim,[4] Shuchita Sharma.[3] *[1]Nephrology/Medicine, Jacobi Medical Center, Bronx, NY; [2]Medicine, James J. Peters Veterans Affairs Hospital, Bronx, NY; [3]Nephrology/Medicine, Mount Sinai School of Medicine, New York, NY; [4]Medicine, Trinity Health Center, Minot, ND.*

**Background:** Despite the increasing prevalence of preeclampsia (PEC) in the US, the ability to predict its occurrence is still problematic. Recent evidence suggests that podocyturia is highly predictive of PEC at 27 weeks of gestation.

**Methods:** Fresh urine samples of patients who were at high risk of developing PEC (chronic hypertension, diabetes mellitus, CKD, SLE) were collected, as were control patients in their 2nd and 3rd trimesters of gestation. To assess for podocyturia, an aliquot of urine was centrifuged to obtain the cell pellet and fixed for immunofluorescence staining. Podocytes were identified by colocalization of markers podocin and synaptopodin and counted. These counts were expressed as number of podocytes/mL/urine creatinine mg/dL (pod/creat). These subjects were followed until delivery. The unpaired parametric t-test analysis was performed to assess differences in outcome and spearman correlations were calculated to assess trend for continuous variables.

**Results:** There was no statistically significant difference in the pod/creat for high risk patients (n=13) who developed PEC vs. those who did not (n=63) (p=0.34). However, there was a statistically significant difference between control patients (n=13) and high risk patients who did develop PEC (n=13) (p=0.01). There was also a statistically significant difference between control patients (n=13) and high risk patients who did not develop PEC (n=63) (p=0.02). No significant correlation was found between pod/creat and albuminuria (rho=0.14, p=0.63) in those who developed PEC or in those who did not (rho=0.03, p=0.80).

**Conclusions:** Podocyturia does not appear to be predictive of PEC when obtained in the 2nd or 3rd trimester of gestation in high risk obstetric patients. However, there does appear to be a baseline difference between high risk patients and uncomplicated control patients in their podocyturia counts irrespective of whether PEC is the outcome.

*Funding:* Clinical Revenue Support

## FR-PO519

### Relationship between Circadian Variation of Blood Pressure and Renal Tissue Damage in Biopsy Proven Glomerulonephritis

Keiji Kono,[1,2] Hideki Fujii,[1] Kentaro Nakai,[1] Shunsuke Goto,[1] Shinichi Nishi.[1] *[1]Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Japan; [2]Div of Nephrology, Akashi Medical Center, Akashi, Japan.*

**Background:** Hypertension (HT) is a common complication in chronic kidney disease (CKD) patients. It has been reported that CKD patients often have circadian rhythm disorder of blood pressure (BP), which is known to be an independent risk factor for CKD progression. However, the relationship between 24-h ambulatory BP (24-h ABP) pattern and renal tissue damage remains unclear, and thereby we investigate their relationship in biopsy proven glomerulonephritis.

**Methods:** Thirty-seven patients with glomerulonephritis were included in this study. The patients with diabetes mellitus, malignancy, acute kidney injury and vasculitis, were excluded. Clinic BP (CBP) and 24-h ABP measurements were performed in all the study patients. HT was diagnosed from CBP and/or 24-h ABP recordings. Moreover, we calculated nocturnal BP fall (%), after which the patients with HT were divided into three groups (dipper, non-dipper and riser). For assessment of renal tissue damage, we evaluated the percent of sclerotic glomeruli and interstitial fibrosis, and intimal thickening of intra-lobular arteries and arteriolar hyalinosis were classified into four grades (none, mild, moderate and severe) in each biopsy specimen.

**Results:** Twenty-six patients (70%) have had normal blood pressure based on CBP recordings. Of these, nine patients (24%) were diagnosed as HT according to 24-h ABP recordings. When dividing into three nocturnal BP groups in twenty patients who have had HT based on CBP and/or 24-h ABP recordings, the grade of arteriolar hyalinosis was significantly higher in the non-dipper and riser groups compared to the normotensive and dipper groups ($p< 0.05$). Furthermore, the percent of interstitial fibrosis was significantly higher in the riser group compared to the normotensive and dipper groups ($p< 0.05$).

**Conclusions:** Our findings suggest that nocturnal BP elevation develops renal tissue damage potentially through the progression of arteriolosclerosis. Careful ABP control should be performed for protecting against renal tissue damage in CKD patients.

## FR-PO520

### Effects of Oral L-arginine Supplementation and Acute Resistance Exercise on Blood Pressure and Nitric Oxide in Hypertensive Patients

Elisa MS Higa,[1,2] Margaret Mouro,[1,2] Giovana R. Punaro,[1] Marco Túlio Mello,[3] Sérgio Tufik,[3] Marcos A. Nascimento.[2] *[1]Nephrology, UNIFESP; [2]Translational Medicine, UNIFESP; [3]Psychobiology, UNIFESP, Brazil.*

**Background:** High blood pressure (BP) is a world health problem and a risk factor to cardiovascular disease. This study examined the effects of oral L-arginine supplementation and acute resistance exercises on blood pressure and nitric oxide in hypertensive men.

**Methods:** Sixteen hypertensive men (45±7 yrs, 92.46±12.99 kg, body weight and 31.03±3.76 kg/m$^2$, body mass index) volunteered to be in this randomised, double-blind, and repeated-measure study: control placebo (CTL-PLA), control arginine (CTL-ARG), exercise placebo (EXE-PLA) and exercise arginine (EXE-ARG). The supplementation period was (6 g/day of placebo or L-arg for 7 days) separated by a 7-day washout. The

acute resistance exercises (ARE) comprise 8 exercises, with an intensity of 60% of 1 maximum repetition. Each session was performed at the beginning and at the end of the supplementation. The systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), double product (DP) and heart rate (HR) were determined at rest, immediately after ARE and 5, 10, 15, 30, 45 and 60 minutes after ARE; the nitric oxide (NO) was determined at rest, immediately after ARE and 1 hour after ARE.

**Results:** SBP decreased at all periods after ARE in the EXE-PLA and EXE-ARG, but not significantly (P>0.05); DBP decreased at 45' comparing CTL-ARG to EXE-PLA and EXE-ARG (83±2.31 vs 76±2.23 and 75±1.25, P<0.05, respectively), and at 60' comparing CTL-PLA to EXE-PLA and EXE-ARG (85±2.90 vs 79±1.87 and 78±1.09, P<0.05, respectively). DP was increased immediately after ARE, and at 5, 10, 15, 30, 45 and 60' comparing exercises to control groups, with P<0.05 as well as HR, which showed the same result. MBP and NO levels did not show differences (P>0.05).

**Conclusions:** In summary, ARE reduced DBP, and maintained SBP and MBP in lower levels than controls; arginine supplementation groups had lower levels than placebo groups but not significant. HR and DP increased, but into safety levels; NO did not show changes. These results suggest that ARE could be a powerful tool to control the blood pressure in hypertensive men.

*Funding:* Government Support - Non-U.S.

## FR-PO521

### T/L-Type Calcium Channel Blocker Reduces the Composite Ranking of Relative Risk According to New KDIGO Guidelines in Patients with Chronic Kidney Disease

Masanori Abe, Kazuyoshi Okada. *Nephrology, Hypertension and Endocrinology, Nihon Univ School of Medicine, Tokyo, Japan.*

**Background:** Recently, the Kidney Disease: Improving Global Outcomes (KDIGO) group recommended that patients with chronic kidney disease (CKD) be assigned according to stage and composite relative risk on the basis of glomerular filtration rate (GFR) and albuminuria criteria. The aim of this post-hoc analysis was to investigate the effects of add-on therapy with calcium channel blockers (CCBs) on changes in the composite ranking of relative risk according to KDIGO guidelines. Benidipine, an L- and T-type CCB, and amlodipine, an L-type CCB to angiotensin II receptor blocker (ARB), were examined.

**Methods:** Patients with blood pressure (BP) > 130/80 mmHg, an estimated GFR (eGFR) of 30–90 mL/min/1.73 $m^2$, and albuminuria > 30 mg/gCr, despite treatment with the maximum recommended dose of ARB, were randomly assigned to two groups. Each group received one of two treatments: 2 mg benidipine daily, increased to 8 mg daily (n=52), or 2.5 mg amlodipine daily, increased to 10 mg daily (n=52).

**Results:** After 6 months of treatment, a significant and comparable reduction in systolic and diastolic BP was observed in both groups. The eGFR was significantly decreased in the amlodipine group, but there was no significant change in the benidipine group. The decrease in albuminuria in the benidipine group was significantly lower than in the amlodipine group. The composite ranking of relative risk according to the new KDIGO guidelines was significantly improved in the benidipine group; however, no significant change was noted in the amlodipine group. Moreover, significantly fewer cases in the benidipine group than the amlodipine group showed a reduced risk category score.

**Conclusions:** The present post-hoc analysis showed that compared to amlodipine benidipine results in a greater reduction in albuminuria accompanied by an improved composite ranking of relative risk according to the KDIGO CKD severity classification.

## FR-PO522

### Azelnidipine Attenuates the Non-Gaussian Heart Rate Variability in CKD Patients with Preceding Treatment with ARB

Michio Fukuda,[1] Shuichi Watanabe,[1] Daisuke Fuwa,[1] Hiroyuki Togawa,[1] Toshiyuki Miura,[1] Junichiro Hayano.[2] *[1]Cardio-Renal Medicine and Hypertension, Nagoya City Univ, Japan; [2]Medical Education, Nagoya City Univ, Japan.*

**Background:** Sympathetic nervous system can be activated even in the early stage of CKD. However, controversy exists as to whether the frequency-domain HRV (eg, LF/HF) can indicate the sympathetic activity. Recently, we have proposed that the non-Gaussianity index (λ), which exhibited the probability of the large HR deviations from its trend, can be served not only as a marker potentially related to sympathetic cardiac over-drive, but also as a powerful predictor of mortality and morbidity in cardiovascular diseases [Kiyonoetal. 2008; Hayano et al. 2011].

**Methods:** At the interim analysis, 19 men and 3 women (57±14 year-old) were enrolled. When subjects had taken olmesartan for at least two months (10-20 mg/day), subjects underwent a 24-h hECG with a portable ECG recorder under their usual daily activities. After the baseline examinations, the participants received single daily doses of a CCB, azelnidipine (16 mg/day), in the morning to attain the daytime BP goal <130/80 mmHg (or 125/75 mmHg if proteinuria was greater than 1 g/day). During the eight week study period, change in the dosage of olmesartan or additional administration of other antihypertensives were not allowed. After 8-wk add-on treatment with azelnidipine to olmesartan, hECG was recorded again.

**Results:** At baseline, all of the traditional markers (eg, SDNN, VLF, Scaling exponent α1, DC), which represent both reduced parasympathetic and enhanced sympathetic activity, were higher than their cut-off values for cardiac events. However, azelnidipine significantly attenuated the non-Gaussianity index (λ), which was the indicator of sympathetic activity rather than parasympathetic activity.

**Conclusions:** These findings suggested that 1) in patients of post-myocardial infarction, both sympathetic and parasympathetic nervous system can be altered, 2) whereas in patients with CKD, sympathetic nervous activity is the matter of importance, and 3) azelnidipine can suppress the sympathetic overactivity.

## FR-PO523

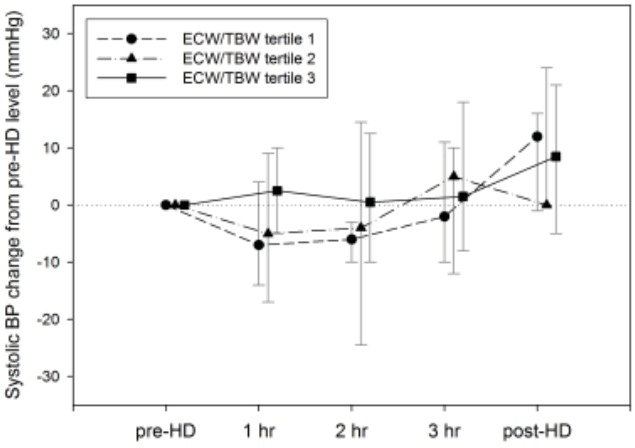
### Blood Pressure Patterns during Hemodialysis According to Volume Status and Ultrafiltration Amount

Jongha Park, Hyun Chul Chung, Jong Soo Lee. *Div of Nephrology, Ulsan Univ Hospital, Univ of Ulsan College of Medicine, Ulsan, Korea.*

**Background:** Blood pressure (BP) pattern during hemodialysis (HD) may be associated with survival. Volume status is a likely major determinant of BP change during HD along with ultrafiltration (UF) amount and/or rate. However, interrelation between body volume and UF amount hampered to evaluate an impact of volume status separately.

**Methods:** A total of 177 incident HD patients (age 56±15 yrs old, female 33.3%, diabetes 54.2%) were analyzed. BP was recorded at pre-HD, 1, 2, 3 hours after, and post-HD during the third HD session (the first 4-hr session). UF amount was calculated as pre-HD body weight *minus* post-HD body weight. Ratio of extracellular water to total body water (ECW/TBW) estimated by bioimpedance analysis (InBody S30, Seoul, Korea) just before HD, was used as an index of volume status. Higher ratio reflects volume overloaded state.

**Results:** Patients were divided based on tertiles of UF amount (≤0.7 kg, 0.7< to 1.9 kg, >1.9 kg) and ECW/TBC (≤0.39, 0.39 to 0.41, >0.41). We plotted change in systolic BP from pre-HD value (median, interquatile range) according to ECW/TBW tertiles in each UF stratum. In patients with UF ≤0.7 kg, systolic BP tended to increase similarly among ECW/TBW tertiles. In the stratum of UF >1.9 kg, systolic BP dropped at 1-hr and 2-hr then rose up in patients with ECW/TBW ≤0.41 (tertile 1 & 2). However, patients with ECW/TBW >0.41 (tertile 3) showed no drop in systolic BP at 1-hr and 2-hr points (Figure).




**Conclusions:** BP pattern during HD differs by volume status as well as UF amount. No dip in BP during HD despite of usual UF amount such as 2 - 3 kg could reflect volume overload.

## FR-PO524

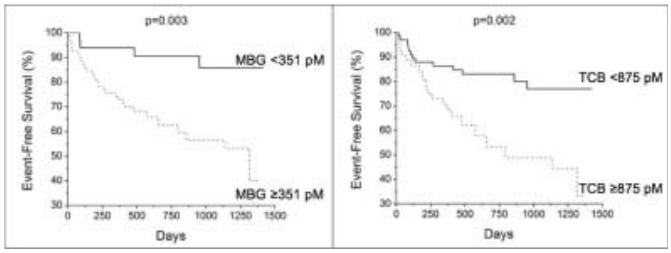
### Elevated Marinobufagenin and Telocinobufagin Levels in Chronic Heart Failure Predict Adverse Long-Term Clinical Outcomes

David Kennedy,[1] Kevin Shrestha,[1] Michael Finucan,[1] Alaa Gabi,[1] Charles M. Medert,[1] Allen Borowski,[1] Olga Fedorova,[2] Alexei Y. Bagrov,[2] W.H. Wilson Tang.[1] *[1]Cellular & Molecular Medicine, Cleveland Clinic, Cleveland, OH; [2]Laboratory of Cardiovascular Science, National Institute on Aging, Baltimore, MD.*

**Background:** Cardiotonic steroids such as marinobufagenin (MBG) and telocinobufagin (TCB) increase in response to volume expansion and induce inflammation, oxidative stress and fibrosis. In chronic heart failure (HF) patients, we hypothesized that elevated MBG and TCB levels are associated with poorer cardiac and renal function as well as increased oxidant stress and adverse long-term clinical outcomes.

**Methods:** In 116 chronic HF patients, we measured MBG and TCB levels and performed comprehensive clinical and echocardiographic assessment. All-cause mortality, cardiac transplantation, and HF hospitalization were tracked for 5 years.

**Results:** In our study cohort (age 56±13 years, LVEF 27±10%, median NTproBNP 1059 pg/mL, eGFR 74±26 mL/min/1.73m2), median MBG levels were 521 [307-734] pM, and median TCB levels were 700 [378-1272] pM. MBG and TCB were directly correlated (Spearman's r=0.55, p<0.0001). Higher MBG and TCB both were associated with higher WBC count (MBG: r=0.53, p=0.010; TCB: r=0.48, p=0.021). Higher MBG was modestly associated with worse baseline renal function (serum creatinine: r= 0.37, p=0.031), worse RV systolic function (RV s': r= -0.33, p=0.018), and worse RV diastolic function (Tricuspid E/e': r=0.39, p=0.008). Higher TCB was modestly associated with higher plasma myeloperoxidase levels (r=0.32, p=0.028). Higher MBG and TCB predicted increased risk of adverse clinical outcomes (MBG≥351 pM: HR 4.36 [1.71-14.73], p=0.001; TCB≥875 pM: HR 2.86 [1.44-5.89], p=0.002).




**Conclusions:** In chronic HF patients, elevated MBG and TCB levels are associated with worse HF disease severity and predict adverse long-term clinical outcomes.

*Funding:* Other NIH Support - R01HL103931

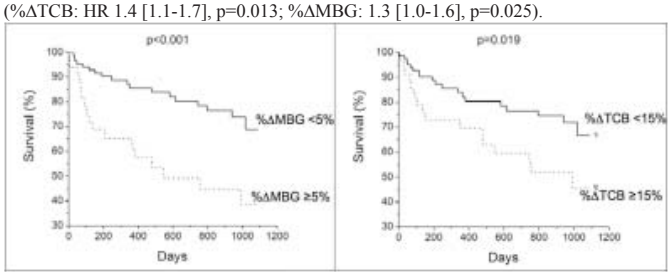
## FR-PO525

**Rising Marinobufagenin and Telocinobufagin Levels during Acute Decompensated Heart Failure Admission Predict Poor Long-Term Clinical Outcomes** David Kennedy,[1] Kevin Shrestha,[1] Allen Borowski,[1] Charles M. Medert,[1] Olga Fedorova,[2] Alexei Y. Bagrov,[2] W.H. Wilson Tang.[1] *[1]Cellular & Molecular Medicine, Cleveland Clinic, Cleveland, OH; [2]Laboratory of Cardiovascular Science, National Institute on Aging, Baltimore, MD.*

**Background:** Cardiotonic steroids such as marinobufagenin (MBG) and telocinobufagin (TCB) increase in response to volume expansion and induce oxidative stress and fibrosis. We hypothesized that elevated levels of MBG and TCB in acute decompensated heart failure (ADHF) patients are associated with poorer cardiac and renal function as well as increased inflammatory and oxidant stress, and adverse long-term events.

**Methods:** In 129 ADHF patients, we measured MBG and TCB levels and performed comprehensive clinical and echocardiographic assessment at baseline and 48-72 hours after admission. All-cause mortality was tracked for 5 years.

**Results:** In our cohort (age 61±14 years, LVEF 31±14%, median NTproBNP 4684 pg/mL, eGFR 72±38 mL/min/1.73m2), median MBG and TCB levels were 630 [438-903] pM and 823 [406-1392] pM, respectively. MBG and TCB were directly correlated (r= 0.51, p<0.0001). Higher baseline MBG and TCB both correlated with higher plasma myeloperoxidase levels (MBG: r=0.27, p=0.014, TCB: r=0.22, p=0.048) and worse RV systolic function (RV s'; MBG: r= -0.26, p=0.039; TCB: r= -0.38, p=0.003) but not LV systolic or diastolic function. In Cox proportional hazard analysis, higher baseline TCB predicted increased risk of death within 5 years (HR 1.4 [1.1-1.8], p=0.018). Rising TCB or MGB levels over the course of admission also predicted increased risk adverse outcomes (%ΔTCB: HR 1.4 [1.1-1.7], p=0.013; %ΔMBG: 1.3 [1.0-1.6], p=0.025).




**Conclusions:** In patients with ADHF, elevated MBG and TCB levels are associated with increased systemic oxidative stress and worse RV systolic function. Rising levels of TCB and MGB after admission predict worse long-term clinical outcomes.

*Funding:* Other NIH Support - R01HL103931

## FR-PO526

**Insufficient Sleep Is Associated with Higher Blood Pressure in Pediatric Nephrology Clinic Patients** Neena R. Gupta,[1] Rakesh Gupta.[2] *[1]Pediatric Nephrology, UMass Memorial Medical Center, Worcester, MA; [2]Pulmonary and Sleep Medicine, Roger Williams Medical Center, Providence, RI.*

**Background:** Hypertension (HTN) is a common concern in pediatric nephrology patients either as primary problem or an associated finding affecting outcomes in other disorders. While several risk factors and mechanisms such as obesity, salt intake, and renal dysfunction get most attention, insufficient sleep is not appreciated as a risk factor for hypertension. Data on sleep disorders and HTN in children are primarily restricted to association with sleep apnea. Some studies have shown sleep deprivation to be associated with higher BP. We have previously shown that weekday sleep time is inversely related to ambulatory BP parameters in children referred for evaluation of HTN (ASN 2012). We hypothesize that low weekday sleep time is likely to be associated with higher BP in all pediatric nephrology patients.

**Methods:** A sleep questionnaire was administered to 34 patients attending pediatric nephrology clinic. It was a sample of convenience. Demographic data, BMI, clinic BP, diagnoses were recorded. Weekday and weekend sleep times were calculated in minutes. Correlation coefficient between weekday sleep time, SBP, DBP, and BMI percentile were calculated.

**Results:** The baseline characteristics for 34 patients were: Age – 13.8±3.5 years, male-60%, BMI percentile 66.3±29.1, Weekday sleep time (WDST) 527.6±75 minutes, weekend sleep time 611±84.9 minutes, SBP 118.2±13.1 mm Hg, DBP 74.7±6.8 mm Hg. Most common diagnoses were Hypertension (61%), VUR (12%), Nephrotic syndrome (12%), Glomerulonephritis (6%), Vasculitis (18%) and others (24%). A significant correlation was found between WDST and SBP (r=-0.29, p=.047), WDST and DBP (r=-0.358, p=0.019). A trend was noticed for correlation between BMI percentile and BP parameters.

**Conclusions:** Low WDST was associated with higher clinic BP in pediatric clinic patients with a wide variety of diagnoses. The link between insufficient sleep and BP as a potential risk factor for HTN as well as therapeutic target needs to be explored further.

## FR-PO527

**Histopathological Diagnoses in Patients Presenting with Hypertension and Kidney Damage** Jennifer R. Joslin, Sarah Wood, Rebecca Suckling, Pauline A. Swift. *Renal Medicine, Epsom and St. Helier Univ Hospitals NHS Trust, London, United Kingdom.*

**Background:** Hypertension is a recognized cause of acute and chronic kidney disease (CKD). It is also a common complication of CKD from any etiology. The underlying histopathological diagnoses have not been well described in patients who present with a clinical diagnosis of hypertensive kidney disease.

**Methods:** We have conducted an observational study of consecutive renal biopsies performed in a 2 year period, in patients with a clinical diagnosis of hypertensive nephropathy (HN). Outcomes were the histological presence of HN or glomerulonephritis (GN), and clinical predictors of HN or GN.

**Results:** 28 out of the 410 native renal biopsies were performed in patients with a clinical diagnosis of HN. Mean age was 48 years, and mean eGFR was 26 mls/min/1.73m$^2$.

| Probable Underlying Diagnosis Based on Histopathological Findings | Cases |
|---|---|
| Hypertensive Nephropathy | 14 |
| Glomerulonephritis | 10 |
| *IgA Nephropathy* | *8* |
| *FSGS* | *2* |
| Diabetic Nephropathy | 1 |
| Inadequate | 1 |
| Chronic damage, no obvious cause | 2 |

21% (6/28) presented with accelerated hypertension. On biopsy 50% (3/6) had confirmed HN and 50% had GN (2/6 IgA nephropathy, 1/6 FSGS). All had at least moderate/severe chronic changes. 43% (12/28) had a documented urinary protein-creatinine ratio (uPCR) >300mg/mmol. This cohort were equally as likely to those with uPCR <300mg/mmol to have a non-hypertensive histological diagnosis. 75% (21/28) had no post-procedure complications. 14% (4/28) patients had minor bleeds, and 4% (1/28) had a major bleed requiring renal arterial embolisation. 2 of the complications (1 major, 1 minor) were in patients with accelerated hypertension.

**Conclusions:** Native renal biopsies performed in patients with a clinical diagnosis of HN showed histological evidence of HN as the primary diagnosis in only 50% of cases. Similar results were seen in the small sub-group who presented with accelerated hypertension, in whom the procedural complication rate was higher. Urinalysis and quantification of proteinuria did not predict histological diagnosis. Kidney biopsy should be considered in individuals who present with hypertension and kidney damage.

## FR-PO528

**Renal Artery Denervation in Patients with Chronic Kidney Disease and Hemodialysis, with Resistant Arterial Hypertension – North Israeli Center Experience** Farid M. Nakhoul,[1,3] Ana Roth,[1] Yonathan Hasin,[2,3] Farber Evgeny,[1] Ganem Diab.[2] *[1]Nephrology, Baruch Padeh Poriya, Lower Galilee, Israel; [2]Cardiology, Baruch Padeh Poriya, Israel; [3]Faculty of Medicine, Bar Ilan Univ, Israel.*

**Background:** A number of cardiovascular disease have been shown to be characterized by a marked increase in sympathetic drive to the heart and peripheral circulation as in essential hypertension, chronic kidney disease (CKD). CKD patients show sympathetic hyperactivity, with aggravation of HTN. In CKD and HD patients is difficult to control HTN, and need multiple drugs. Here we report our first experience on renal sympahthetic nerve ablation for treatment of severe resistant hypertension in CKD patients. Our results demonstrate high efficacy of this procedure for TX of resistant hypertensive in CKD.

**Methods:** 33 patients- aged average between 40-79 yrs old, were treated with RAD,: 66% of them were with diabetes mellitus, 64% with LVH. 24 Patients were with follow up to 3 months (Range 1-12 months) 9 patients with CKD (Two of them were on Hemodialysis and two with RAS). All patients were with systolic BP≥160 mmHg under three drugs. Mean number of antihypertensive drugs per patient were 4.5. Patients with CKD: Pl. Creatinine range was.2-3.6 mg/dl.

**Results:** At Baseline: Mean SBP of the whole group = 179±19 mmHg and Mean DBP= 83±17 mmHg. Three months after RAD: Mean systolic BP = 142±16 mmHg and Mean diastolic BP = 77±11 mmHg. There was significant reduction of the systolic blood pressure an average of 36 mmHg with (P <0.0001 ). Significant reduction in the diastolic BP by an average of 6 mmHg (P= 0.038). Subgroup analysis of patients with CKD: Mean SBP: 183±20 mmHg and Mean DBP: 79±12 mmHg. Three months after RAD: Mean SBP = 147±17 mmHg and Mean DBP = 75±10 mmHg. CKD patients had significant reduction of 36 mmHg in the systolic blood pressure (P- 0.005). There was no significant reduction in the DBP. During follow-up, renal function (eGFR) was unchanged.

**Conclusions:** 1. Bilateral RAD is associated with significant reduction in systolic and DBP in hypertensive patients resistant to multiple drug therapy.

2. CRF patients had significant reduction in systolic BP but no significant reduction in diastolic BP.

*Funding:* Government Support - Non-U.S.

**FR-PO529**

**Telmisartan Improves Blood Pressure Control, Albuminuria and Inflammation in Hypertensive Diabetic Patients Not Adequetely Controlled under ACE-Inhibitor Therapy** Juan F. Navarro-Gonzalez,[1,2] Mercedes Muros,[3] Carmen Mora,[2] Patricia Garcia Garcia,[1] María Adela Getino,[1] Ana Jarque,[1] Nieves Del Castillo Rodriguez,[1] Antonio Rivero,[1] Javier Garcia Perez.[1] *[1]Nephrology Service, Univ Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [2]Research Unit, Univ Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [3]Clinical Analysis Service, Univ Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain.*

**Background:** Renin-angiotensin system blockade is the mainstay of antihypertensive therapy in diabetic patients. The aim of this study was to evaluate the effect of the switch from angiotensin converting-enzyme inhibitors (ACEi) to angiotensin receptor blockers on blood pressure (BP), urinary albumin excretion (UAE) and inflammatory profile in diabetic patients not adequately controlled despite maximal dosage of ACEi therapy.

**Methods:** Twenty-seven patients with BP >130/80 mmHg and residual albuminuria were switched to Telmisartan (TEL, 80 mg/day). Serum concentrations of high-sensitive C-reactive protein (hsCRP), tumor necrosis factor-α (TNFa) and interleukin-6 (IL-6), as well as mRNA expression levels of TNFa and IL-6 in peripheral blood mononuclear cells (PBMC) were determined.

**Results:** After 4 months, BP decreased from 141±6/89±5 to 133±5/83±5 mmHg ($p<0.0001$), and UAE decreased by 18%. Reduction in UAE correlated with change in systolic BP ($r=0.76$, $p<0.05$). Serum hsCRP, TNFa and IL-6 reduced significantly by 15%, 9% and 16%, respectively ($p<0.001$). Urinary TNFα excretion decreased by 13% ($p<0.01$), whereas mRNA expression levels of TNFα and IL-6 in PBMC also experienced a significant reduction ($p<0.05$). A significant association was found between change in serum hsCRP ($r=0.82$) and TNFα ($r=0.59$), and urinary TNFα ($r=0.66$) with reduction in UAE. Finally, multivariate regression analysis showed that age and change in urinary TNFα were independently associated with reduction in UAE ($r=-0.29$ and $r=0.49$, $p<0.05$, respectively).

**Conclusions:** In conclusion, in hypertensive diabetic patients not adequately controlled under ACEi therapy, change to TEL resulted in a significant reduction in BP and UAE, and an improvement in inflammatory profile.

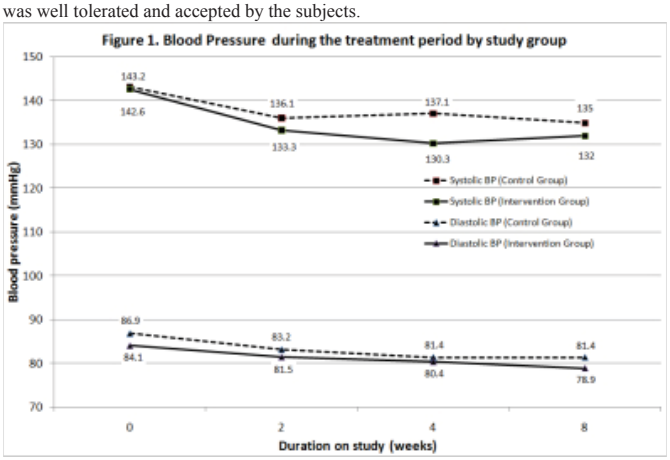
**FR-PO530**

**The Impact of Music Guided Deep Breathing Exercise on Hypertensive Control** Loke Meng Ong, Kum Keong Ng, Fei Ping Kow, Bakar Adlina, Dharminy Thurairatnam, Hadzlinda Z. *Ministy of Health, Penang, Malaysia.*

**Background:** Hypertension (HPT) remains poorly controlled despite drug therapy. Non-pharmacological intervention may enhance control of blood pressure. We conducted a single blinded, multicentre, randomized controlled trial over 8 weeks to evaluate the effects of deep breathing exercise and relaxation music on HPT.

**Methods:** Patients with Grade 1 essential HPT were recruited. They were randomized into control group (CG) or intervention group (IG) in a 1:1 ratio. Both groups listened to relaxation music CD at least 15 minutes once daily. In addition the IG also practised deep breathing exercise guided by the music. Training was conducted by qualified therapists. The primary end point was reduction in Diastolic blood pressure (DBP) at 8 weeks. The managing team was blinded to the treatment arm.

**Results:** 87 patients were recruited. The baseline characteristics were similar between groups. They had a mean age of 61 years, 51% were females and 54% had received more than one anti-HPT agents. The mean baseline BP was 143/86. 93% successfully completed the study. Compliance was good in both groups. There was a significant reduction in Systolic BP (SBP) & DBP from baseline at 8 weeks in both groups. (Fig. 1) Reduction in mean SBP was 10.5mmHg in CG compared to 8.3mmHg ($p< 0.001$) in IG while the mean DBP reduction was 5.2 mmHg ($p< 0.001$) in CG and 5.6 mmHg ($p< 0.001$) in IG. However, SBP & DBP reduction at 8 weeks did not differ significantly between groups. The absolute difference in SBP reduction from baseline in IG & CG was -2.2 (95% CI -7.8, 3.5) & DBP was -0.4 (95% CI -2.9, 3.6). Heart rate did not change significantly. Deep breathing exericse was well tolerated and accepted by the subjects.




**Conclusions:** Relaxation with music was associated with a clinically significant reduction in both SBP & DBP. Deep breathing exercise did not augment the benefit of music in BP control.

*Funding:* Government Support - Non-U.S.

**FR-PO531**

**Subclinical Aldosterone Levels Help Distinguish between Future Aldosterone Producing Adenoma and Essential Hypertension** Austin Parker,[1] Kevin C. Abbott,[2] Lisa K. Prince,[2] Stephen W. Olson.[2] *[1]Nephrology Dept, Naval Medical Center Portsmouth, Portsmouth, VA; [2]Nephrology Dept, Walter Reed National Military Medical Center, Bethesda, MD.*

**Background:** Aldosterone and aldosterone to plasma renin activity (PRA) ratios are used for the biochemical evaluation of patients with refractory hypertension and incidentally identified adrenal adenomas. However, there is no single threshold of maximum sensitivity and specificity for the diagnosis of aldosterone producing adenoma (APA). Knowledge of subclinical aldosterone trends could improve current diagnostic capabilities.

**Methods:** We performed a DoD Serum Repository (DoDSR) retrospective case-control study to compare aldosterone levels months to years before the histopathologic diagnosis of 28 APA patients versus 82 age-, race-, sex-, and age-of-serum-matched control subjects with essential hypertension. We hypothesized that aldosterone levels would be elevated and rising prior to diagnosis. PRA levels could not be tested on these stored serum samples.

**Results:** A higher percent of APA patients had a serum aldosterone level >10 ng/dL compared to matched essential hypertension disease controls at <3 years (82% vs. 6%; $p<0.001$) and 3-11 years (47% vs. 11%; $p=0.001$) as well as >15 ng/dL at <3 years (59% vs. 0%; $p<0.001$) and 3-11 years (21% vs. 4%; $p=0.03$) prior to diagnosis. A greater percent of APA patients had a serum aldosterone rate of rise of > 0.25ng/dL/year (75% vs. 27%; $p<0.001$), >0.5ng/dL/year (50% vs. 17%; $p=0.001$), and >1ng/dL/year (29% vs. 6%; $p=0.004$).

**Conclusions:** Compared to control subjects with essential hypertension, APA patients more frequently have aldosterone levels > 10 ng/dL that continue to rise over time in the 11 years leading up to diagnosis. Knowledge of the subclinical serum aldosterone trajectory could help to better differentiate between essential hypertension and APA for patients undergoing a secondary hypertension evaluation or with an incidentally discovered adrenal adenoma.

*Funding:* Other U.S. Government Support

**FR-PO532**

**Low Glomerular Density Is a Characteristic Finding in Renal Biopsies of Hypertensive Nephrosclerosis with Massive Proteinuria** Kotaro Haruhara, Nobuo Tsuboi, Go Kanzaki, Kentaro Koike, Akira Fukui, Yoichi Miyazaki, Tetsuya Kawamura, Makoto Ogura, Takashi Yokoo. *Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Hypertensive nephrosclerosis (HNS) is a secondary renal disease that may occur in individuals with hypertension. However, there are likely to be factors other than hypertension that contribute to the development of HNS since only a minority of hypertensive individuals manifest overt proteinuria. In this study, we explored the histological characteristics contributing to the development and progression of proteiuria in hypertensive patients.

**Methods:** Glomerular density (GD; glomerular number per renal cortical area) and mean glomerular volume (GV) in renal biopsies from HNS patients (eGFR>60) with mild (<1g/day) or massive (>1g/day) proteiuria were measured and compared with those in the autopsy kidneys from hypertensive patients without CKD. Renal biopsy specimens from kidney transplant donors (KTD) were also analyzed as healthy controls.

**Results:** The GD of HNS patients with massive or mild proteinuria was significantly lower than that from KTD. The GD of hypertensive patients without CKD was also significantly lower than that from KTD. Of note, the GD of HNS patients with massive proteiuria was significantly lower than those of HNS patients with mild proteiuria and hypertensive patients without CKD. These differences were still significant when global glomerulosclerosis (GGS) were included in the calculation of the GD. Moreover, the GV of both HNS with mild and massive proteinuria are significantly larger than that of KTD.

| | KTD n=34 | HNS; UPE≥1g n=15 | HNS; UPE<1g n=14 | Hypertensive patients without CKD n=35 |
|---|---|---|---|---|
| GD excluding GGS (/mm$^2$) | 3.1±1.1 | 1.5±0.8*§ | 2.5±0.8* | 2.5±0.6* |
| GD including GGS (/mm$^2$) | 3.3±1.1 | 2.1±0.7*§ | 3.2±0.7 | 2.7±0.5* |
| GV (x10$^6$μm$^3$) | 2.6±0.8 | 3.2±1.2* | 3.5±1.4* | 3.0±0.7 |

*$p<0.05$ vs KTD, §$p<0.05$ vs Hypertensive patients without CKD.

**Conclusions:** These results suggest that a low GD is a renal histological characteristic of HNS with massive proteinuria.

**FR-PO533**

**Lipoprotein(a), an Independent Predictor of ARAS in High-Risk Patients**
Yanqing Mei,[1] Peng Xia,[1] Ling Qiu,[2] Xuejun Zeng,[3] Xuemei Li,[1] Limeng Chen.[1]
*[1]Nephrology Dept, Peking Union Medical College Hospital, Beijing, China; [2]Dept of Laboratory Medicine, PUMCH, Beijing, China; [3]Internal Medicine Dept, PUMCH, Beijing, China.*

**Background:** Lipoprotein(a) [Lp(a)] has been confirmed to be a prospective cardiovascular disease risk factor through large-scale meta-analysis. In this study, we aim to explore that whether Lp(a) is associated with occurrence of atherosclerotic renal artery stenosis (ARAS) among high-risk patients.

**Methods:** We divided consecutive 453 patients who underwent renal angiography into ARAS group (≥ 50%, n=169) and non-ARAS group (n=284). A control group (n=177) was collected among people receiving health checkup. Data collection included the age, sex, lipid profiles, renal function, and liver function. Multivariate logistic regression analysis was applied to explore that whether the clinical and biomedical parameters were associated with ARAS in these high-risk patients.

**Results:** We divided consecutive 453 patients who underwent renal angiography into ARAS group (≥ 50%, n=169) and non-ARAS group (n=284). A control group (n=177) was collected among people receiving health checkup. Data collection included the age, sex, lipid profiles, renal function, and liver function. The levels of Lp(a) were higher in ARAS group in comparison with the control group (median: 172.0 vs 92.0 mg/L, p<0.001). Lp(a) was positively correlated with hsCRP and LDL ($r$ = 0.217 and 0.231, p < 0.001, respectively) among high-risk patients. In comparison with non-ARAS group, the ARAS patients were older (67.1±10.2 vs 65.0±10.4, p=0.002) with higher levels of uric acid (371.0±91.7 vs 333.6±98.3 μmol/L, p=0.001) and lower eGFR (60.0±19.7 vs 68.4±19.7 mL/min/1.73m$^2$, p=0.002). Multivariate logistic regression revealed that Lp(a) (OR = 1.806, 95%CI 1.006, 3.245, p = 0.048) and uric acid (OR = 2.098, 95%CI 1.107, 3.978, p = 0.023) were independent predictors of ARAS among high-risk patients after adjustment of age and eGFR.

**Conclusions:** Lp(a) and uric acid are independently associated with ARAS among high-risk patients. Mei, Y and Xia, P contributed equally to this work.

*Funding:* Government Support - Non-U.S.

**FR-PO534**

**Comparison of 24-Hour Blood Pressure Profile between Diabetic and Non-Diabetic Patients with Advanced Chronic Kidney Disease**
Pablo Marcos Braillard Poccard, Cesar Garcia-canton, Pilar Rossique, Ivan Chamorro, Agustin Toledo, Mar Lago, Ernesto Fernandez, Santiago Suria, Eduardo Baamonde, Dolores Checa. *Nephrology, Hospital Insular, Las Palmas, Gran Canaria, Spain.*

**Background:** Blood pressure is a factor in cardiovascular-related morbidity and in the progress of kidney failure in patients with ACKD.

**Methods:** The objective of our study was to offer a descriptive analysis of daily blood pressure profiles of incident ACKD patients through 24-hour ambulatory blood pressure monitoring (ABPM), as well as a comparison between diabetic and non-diabetic patients.

**Results:** The analysis included 547 patients, 64.3 years of age, 57.8% male, 68% diabetic, 44.6% history of cardiovascular disease (CVD). Diabetic patients were older and with worse CVD background. Diabetic patients had higher mean systolic blood pressure (MSBP) and lower mean diastolic blood pressure (MDBP), which resulted in similar mean blood pressure (MBP) in both groups, although with a significantly higher differential pulse pressure (PP) in diabetics.

| | Diabetic | Non diabetic | significance |
|---|---|---|---|
| MSBP | 137.1 | 127.9 | p<0.001 |
| MDBP | 69.7 | 74.9 | p<0.001 |
| MBP | 94.7 | 93.7 | p=0.368 |
| PP | 68.6 | 52.9 | p<0.001 |
| %dipper | 30.1 | 37.4 | P=0.09 |

The fact that diabetic patients had lower diastolic pressure could be accounted for by the use of antihypertensive drugs: 16% of non-diabetic patients used one or no drug, 31% of them used two drugs, 32% used three drugs and 21% used four or more drugs; whereas 3.5% of diabetic patients used one or no drug, 18.5% used two drugs, 41% used three drugs and 37% used four or more drugs. Noticeably, renin-angiotensin axis blockade occurred in 68% of diabetic as compared to 55% of non-diabetic patients (p<0.005).

**Conclusions:** In conclusion, this study revealed that diabetic patients had lower DBP, which could be explained by the use of antihypertensive treatments, and higher SBP, which could be explained by increased vascular stiffness due to older age, worse CVD background or vascular calcification due to diabetes, which result in reduced vascular distensibility and higher pulse pressure.

**FR-PO535**

**Percutaneous Renal Revascularization: A Long Term Follow-Up of 155 Procedures**
Daniel Kushnir, Jerome Marcusohn, Alon Antebi, Tatiana Tanasiychuk, Victor Frajewicki. *Dept of Nephrology and Hypertension, Carmel Medical Center, Haifa, Israel.*

**Background:** Arterial Hypertension and Renal Insufficiency may be improved by percutaneous intervention in patients with Renal Artery Stenosis (RAS) but the clinical benefit of such therapy is still obscure. The aim of the study is to evaluate the long term outcomes after renal artery intervention.

**Methods:** Files of patient who underwent intervention of the renal arteries at the Carmel Medical Center were retrospectively reviewed .

**Results:** Data of 143 patients (79 men and 64 women, mean age, 64±11 years) with RAS who underwent 155 interventions were reviewed. Main indications were Hypertension (70 %) and Renal Insufficiency (26.5 %). The stenosis was bilateral in 26 % and unilateral in 63 %; single kidney in 9 %. When atherosclerosis (90 %) was the cause of obstruction, a stent was placed. In 10 patients with Fibromuscular Dysplasia, an angioplasty was performed without stenting. Average stenosis was 81±15 %, which was ostial in 61 % of cases. Overall angiographic success rate was 92 %. Major complications occurred in 10 % of the procedures. Patients were followed for 96±42 months. Data were analyzed before the procedure, after 36 months and at the end of follow-up. Reduction of systolic (155±25, 145±23 and 134±21 mmHg, P=0.0013) and diastolic (84±12 mmHg, 78±11 and 72±9 mmHg, P=0.004) blood pressure was achieved. Blood pressure improved in 61 %, was unchanged in 18 % and worsened in 21% after 36 months. Serum creatinine of patients on dialysis were censored. Mean serum creatinine was unchanged (1.4±0.7, 1.5±0.8 and 1.5±0.9 mg/dl, NS). After 36 months creatinine improved in 16 %, was unchanged in 70 % and worsened in 14 % of patients. The use of anti- hypertensive drugs was reduced from 3.2±1.3 to 2.4±1.5, P=0.00001. Fifty three patients (37%) died during the observation period, of those 94 % had chronic kidney disease. Seventeen patients (12%) needed chronic renal replacement therapy.

**Conclusions:** Stenting of the renal arteries may be useful for treatment of hypertension in RAS without causing deterioration of renal function. Renal insufficiency patients require careful evaluation before intervention.

**FR-PO536**

**Prevalence of Obstetric Thrombotic Thrombocytopenic Purpura within 8,908 Consecutive Deliveries: A Not to Be Missed Diagnosis**
Yahsou Delmas,[1,2] Sebastien Helou,[1] Pierre Chabanier,[2,3] Anne Ryman,[2,4] Fanny Pelluard,[5] Dominique Carles,[5] Jacques Horovitz,[3] Paul Coppo,[6] Christian Combe.[1,2] *[1]Service de Néphrologie Transplantation Dialyse, CHU de Bordeaux, France; [2]Centre de Compétence des Microangiopathies Thrombotiques, CHU de Bordeaux, France; [3]Pôle Gynécologie-Obstétrique-et Biologie de la Reproduction, CHU de Bordeaux, France; [4]Service d'Hémostase Spécialisée, CHU de Bordeaux, France; [5]Service d'Anatomopathologie, CHU de Bordeaux, France; [6]Département d'Hématologie Clinique and Centre de Référence des Microangiopathies Thrombotiques, Hôpital Saint Antoine, Paris, France.*

**Background: Thrombotic Thrombocytopenic Purpura (TTP)** defined as A Disintegrin And Metalloprotease with ThromboSpondin type 1 domain 13 (ADAMTS13) activity <10% has an unknown prevalence among pregnant women with thrombocytopenia (Tp). Its recognition can lead to efficient prevention of maternal and fetal morbi-mortality.

**Methods:** In a tertiary obstetrical unit, TTP prevalence was assessed retrospectively among women with Tp≤75 G/L. ADAMTS13 functional activity was measured by *full von Willebrand Factor length* technique. ADAMTS13 inhibitor and anti-ADAMTS13 IgG were assessed in these patients.

**Results:** Among 8,908 deliveries over a 2-year period, 80 women had Tp≤75 G/L. Twenty had an identified etiology of Tp ( from TTP) and 11 were lost to follow-up. Among 49 remaining patients, ADAMTS13 activity was in the range of TTP in 4 patients (initially diagnosed HELLP syndrome); Tp spontaneously recovered few days after delivery in all women with TTP. Three primipara patients had sustained ADAMTS13 deficiency without inhibitor. Two of them lost their babies due to prematurity. Prophylactic plasma infusion enabled subsequent successful three pregnancies in two constitutive TTP women with improved placental histopathology.

**Conclusions:** 5% of women with Tp≤75 G/L in a tertiary obstetrical unit had TTP. Platelet normalization after delivery cannot eliminate TTP. Prophylactic plasma therapy during pregnancy in constitutive TTP allows proportional placenta pathology improvement and good mother and child outcome.

**FR-PO537**

**Angiographic Intervention for Renal Artery Stenosis – Predictors of Favourable Renal Response**
Haridian Sosa Barrios,[1] Parthipan Sivakumar,[1] Damien Ashby,[1] Wladyslaw M. Gedroyc,[2] Mohamad S. Hamady,[2] Neill D. Duncan.[1] *[1]Renal and Transplant Services, Hammersmith Hospital, Imperial College, United Kingdom; [2]Interventional Radiology, St. Mary's Hospital, Imperial College, United Kingdom.*

**Background:** Renovascular disease is increasingly recognised as a cause of renal impairment but the role of endovascular intervention in this setting is unclear. The ASTRAL (Angioplasty and Stenting for Renal Artery Lesions) trial established that revascularisation does not improve renal prognosis in the average patient, but many clinicians believe that there is a subgroup of patients who benefit.

**Methods:** In this retrospective single-centre cohort study we included all patients who had renal angioplasty during a 2 year period for whom 12 month follow-up data were available. Patient outcomes were defined according to mean GFR in the year post intervention, compared to baseline: group A improved by 5ml/min, or stabilised after a decline of 5ml/min in the previous year; group B remained the same; group C deteriorated by at least 5ml/min.

**Results:** Ninety-four patients (aged 49-86, 72% male) underwent angioplasty and stent insertion which was technically successful in all cases, and bilateral in 29%. Change in renal function post procedure was symmetrically distributed, with median(IQR) GFR change -2.0(-4.3 to 1.8)ml/min, and with 25.5, 59.6 and 14.9% of patients in groups A, B, and C respectively.

Group A patients tended towards higher GFR during the 12 months prior to intervention (45.0 vs 39.8ml/min, p=0.14), but there were no differences in age or gender between outcome groups. Favourable (group A) outcome was observed more frequently in those with GFR>30ml/min (29 vs 14%, p=0.25), and in those undergoing bilateral intervention (33 vs 22%, p=0.30).

**Conclusions:** Heterogeneity of outcome can be expected after intervention for renal artery stenosis. Patients benefitting from the procedure are not clearly distinguishable from the remainder by simple clinical criteria, but higher GFR and bilateral disease were weakly associated with a favourable response.

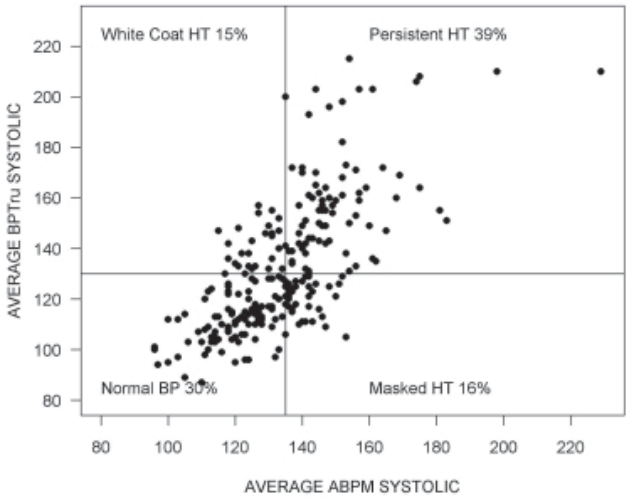
## FR-PO538

**Correlation between Automated Office Blood Pressure and Ambulatory Blood Pressure Monitoring in CKD** George Thomas, Jesse D. Schold, Susana Arrigain, Joseph V. Nally, Sankar D. Navaneethan. *Cleveland Clinic.*

**Background:** Automated office blood pressure monitors (AOBP) have previously been shown to correlate with Ambulatory blood pressure monitors (ABPM) in the general population. We examined the correlation of AOBP with ABPM in CKD patients seen at our institution.

**Methods:** We included 238 CKD patients who had both AOBP and ABPM measured within 2 weeks of each other. Automated office BP was measured using the BPTru device ( VSM MedTech Ltd, Coquitlam, BC, Canada) and is the average of five automated consecutive readings. Ambulatory BP was measured every 20 minutes during the day (6:00 AM to 10:00 PM, "awake" blood pressure) using automated BP monitors on the arm (Spacelabs, Issaquah, Washington, USA). We used Linn's concordance coefficient to assess AOBP and ABPM. We compared characteristics of patients with concordant and discordant data using t-tests and Chi-square tests.

**Results:** We identified 238 patients who fit the above criteria; mean age was 64.2 years, with more females (62%) and more Caucasians (78%). The concordance rates between BPTru and awake ABPM (systolic and diastolic pressure) were 0.65 (95% CI 0.58, 0.71) and 0.64 (95% CI 0.56, 0.71) respectively. Figure 1 shows the scatterplot of BPTru (systolic) and awake ABPM (systolic).




White coat hypertension (WHT) and masked hypertension (MHT) was seen in 15% and 16% of this population, respectively. In our analysis, patients who had WHT and MT were older (67.5 vs. 62.7 years, p=0.03) and were more likely to have associated malignancy (37.8% vs. 21.3%,p=0.008) compared to those who had concordant values. Further, BPTru values among African Americans had higher concordance with ABPM.

**Conclusions:** BPTru correlated moderately well with awake ambulatory systolic BP in patients with CKD. Our data also suggest that BPTru readings from older patients and Caucasians should be interpreted with caution.

## FR-PO539

**Human Kidney Proximal Tubule Epithelial Cells (PTEC) Modulate Autologous Immune Responses in T, B and Dendritic Cells (DC)** Sandeep Sampangi,[1,2,3] Andrew J. Kassianos,[1,2] Xiangju Wang,[1,2] Helen G. Healy,[1,2] Ray Wilkinson.[1,2,3,4] *[1]Conjoint Kidney Laboratory, Pathology Queensland; [2]Dept of Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Australia; [3]Queensland Univ of Technology, Brisbane, Australia; [4]Medical School, Univ of Queensland, Brisbane, Australia.*

**Background:** We have previously demonstrated that PTEC actively participate in the kidney disease process by down modulating autologous human T, B and DC responses within an inflammatory setting (Wilkinson et al NDT 2011, 2013). We now analyse the mechanisms of this suppressive effect further.

**Methods:** B cells, T cells and monocytes were cultured with interferon-activated PTEC, to mimic the inflammatory setting, in the presence of 1) poke weed mitogen (PWM) or toll-like receptor (TLR) agonist R848, 2) phytohaemagglutinin (PHA) or anti-CD3/28 and 3) GM-CSF and IL-4, respectively. The numbers of B cells producing Ab were analyzed using isotype specific ELISPOT. Readouts for T cells and DC included proliferation, antigen expression and cytokine secretion in contact dependent or independent (transwell) cultures.

**Results:** PTEC significantly reduced the number of B cells producing Ab in response to TLR stimulation. PTEC also significantly down modulated T cell proliferation which was restored when cell contact was blocked by a transwell membrane. Similarly, PTEC down modulation of monocyte-derived DC (MoDC) differentiation was partially ablated when the MoDC and PTEC were separated by a transwell membrane, resulting in mature MoDC as defined by loss of monocyte marker CD14 and no or low IL-10 secretion. However, the decreased expression of antigen presentation and co-stimulatory molecules HLA-DR/CD86 and increased expression of the inhibitory signaling molecule PD-L1, seen when MoDC are in contact with PTEC, was not altered upon transwell culture.

**Conclusions:** These results demonstrate for the first time that human autologous PTEC can regulate B cell Ab production and that their modulatory effects on T cells and DC are, at least in part, contact dependent. We are now analyzing the molecular signatures of cell dependent and independent contact to identify putative therapeutic targets.

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## FR-PO540

**The Reactivity of Renal Dendritic Cells for Prostaglandin E2** Shinya Nagasaka, Toru Iwahori, Seiichiro Higo, Go Kanzaki, Kayori Tsuruoka, Yusuke Kajimoto, Akira Shimizu. *The Dept of Analytic Human Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

**Background:** Prostaglandin E2 (PGE2) has been shown to mediate autoimmunity disease to expand Th17 cells via dendritic cells in animal models of multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases and allergic skin disorders. Recently, the dendritic cell specifically localized in kidney (renal dendritic cells: RDC) was identified. Despite RDCs have been recognized as the earliest producers of pro-inflammatory cytokines in renal ischemia and experimental unilateral ureter ligation, suggesting a sentinel role against kidney injury, their role in reactivity for PGE2 on renal inflammatory disease is still incompletely understood. Here we compared the reactivity between RDCs and splenic DCs (as general DCs) in response to PGE2.

**Methods:** RDCs and splenic DCs were purified by magnetic bead-labeled anti-mouse CD11c antibodies from mouse kidney, and stimulated with 1 μg/ml of LPS in the presence or absence of 100 nM of PGE2. After LPS stimulation, the expressions of PGE2 receptors (EP1, EP2, EP3, and EP4), cyclooxygenase-2 (Cox-2), membrane-associated PGE synthase-1 (mPGES-1), and pro-inflammatory cytokines were measured by real-time PCR.

**Results:** The expression of EP4, one of the PGE2 receptors and IL-12p35 in RDCs were more increased in the presence of PGE2 than in splenic DCs, whereas we obtained no change of IL-12p40 expression. Furthermore, in RDCs, the expressions of Cox-2 and mPGES-1 which associated with PGE2 synthesis were up-regulated in the presence of PGE2 upon LPS stimulation.

**Conclusions:** RDCs apparently had distinct characteristics from splenic DCs. It is suggested that RDC enhances the production of pro-inflammatory cytokine in the presence of PGE2 which is synthetized in renal disease, and therefore regulates inflammatory response in kidney via autocrine loop of PGE2 through the induction of Cox-2 and mPGES-1 expression.

*Funding:* Government Support - Non-U.S.

## FR-PO541

**Cytokines Accentuate Synthesis of Galactose-Deficient IgA1 in IgA Nephropathy by Dysregulating C1GalT1 and ST6GalNAc-II Enzymes** Hitoshi Suzuki,[1,2] Milan Raska,[3] Koshi Yamada,[1,2] Zina Moldoveanu,[1] Bruce A. Julian,[1] Robert J. Wyatt,[4] Yasuhiko Tomino,[2] Ali G. Gharavi,[5] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Juntendo Univ Faculty of Medicine, Tokyo, Japan; [3]Palacky Univ in Olomouc, Olomouc, Czech Republic; [4]Univ of Tennessee Health Science Center, Memphis, TN; [5]Columbia Univ, New York, NY.*

**Background:** IgA nephropathy (IgAN) is characterized by renal immunodeposits containing IgA1 with galactose (Gal)-deficient *O*-glycans (Gd-IgA1). IgA1-producing cell lines from patients with IgAN (IgAN cells), but not those from healthy controls (HC cells), secrete Gd-IgA1 with *O*-glycans with terminal or sialylated *N*-acetylgalactosamine (GalNAc). The aberrant glycans constitute the neoantigen that leads to formation of nephritogenic immune complexes. As clinical disease onset and activity often coincide with mucosal infections and dysregulation of cytokines was described in IgAN, we hypothesized that cytokines may affect IgA1 *O*-glycosylation.

**Methods:** We used IgAN cells and HC cells to assess whether IgA1 *O*-glycosylation is altered by cytokines. To confirm the roles of specific glycosyltransferases in the production of Gd-IgA1, we knocked-down the C1GalT1 and ST6GalNAcII genes by siRNA.

**Results:** Of the eight cytokines tested, only interleukin-6 and, to a lesser degree, interleukin-4 significantly increased production of IgA1 and the degree of galactose deficiency of IgA1; changes in IgA1 *O*-glycosylation were robust for the IgAN cells. Both cytokines reduced galactosylation of the *O*-glycan substrate directly via decreased expression of the galactosyltransferase C1GalT1, and, indirectly, via increased expression of the sialyltransferase ST6GalNAc-II (sialylation prevents subsequent galactosylation by C1GalT1). siRNA knock-down of the corresponding gene and in vitro enzyme reactions confirmed these findings.

**Conclusions:** Interleukin-6 and interleukin-4 accentuated galactose deficiency of IgA1 via coordinated modulation of key glycosyltransferases. These data provide a mechanism to explain increased immune-complex formation and disease exacerbation during mucosal infections in patients with IgAN.

*Funding:* NIDDK Support

**FR-PO542**

**Pathogenic Role of IgA1-Containing Immune Complexes in IgA Nephropathy** Hitoshi Suzuki,[1] Yusuke Suzuki,[1] Kenji Satake,[1] Bruce A. Julian,[2] Jan Novak,[2] Yasuhiko Tomino.[1] *[1]Nephrology, Juntendo Univ Faculty of Medicine, Tokyo, Japan; [2]Medicine and Microbiology, Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** IgA nephropathy (IgAN) is an autoimmune glomerulonephritis wherein immune complexes (IC) composed of galactose-deficient IgA1 (Gd-IgA1; autoantigen) and anti-glycan IgG autoantibodies deposit in the glomeruli. Serum levels of Gd-IgA1 as well as anti-glycan IgG autoantibodies, responsible for the formation of ICs with Gd-IgA1, are elevated in patients with IgAN. However, the pathogenic roles of Gd-IgA1-containing IC and mechanisms of immune deposits in the mesangium are still obscure.

**Methods:** Polymeric Gd-IgA1 myeloma protein and recombinant anti-glycan IgG were used to form IC (Gd-IgA1-IgG IC) in vitro to inject *i.v.* into nude mice. After various time intervals, mice were sacrificed; blood and urine were collected to determine serum IgA1 and IgG, urinary protein and creatinine and hematuria. Also, to assess the potential capacity of these IC to activate endothelial cells, human renal glomerular endothelial cells (HRGEC) were co-cultured with Gd-IgA1 alone or Gd-IgA1-IgG IC for 72 h. Then, transcript levels of TNF-α, TGF-β, IL-6, ICAM1 and E-selectin in HRGEC were measured by RealTime PCR.

**Results:** Gd-IgA1 and anti-glycan IgG formed IC that deposited with murine C3 in the mesangium and in small amounts in the subendothelial area of the glomerular capillaries, and induced hematuria and proteinuria. In control mice injected with only Gd-IgA1 or Gd-IgA1 with IgG from a healthy control, IgA1 deposited only transiently and did not cause tissue injury. After stimulation with Gd-IgA1-IgG IC in HRGEC, transcript levels of TNF-α, ICAM-1 and E-selectin were upregulated (P<0.01).

**Conclusions:** Our studies confirmed the role of Gd-IgA1-IgG-containing IC in the pathogenesis of IgAN and induction of proteinuria and hematuria. Furthermore, the Gd-IgA1-IgG IC may bind to glomerular endothelial cells and induce release of pathogenic cytokines and chemokines.

**FR-PO543**

**IgA Antibodies to Glomerular Endothelial Cells in Patients with Lupus Nephritis: Correlations with Renal Injury** Kazuo Takahashi, Ayako Kondo, Daisuke Hirano, Shin'ichi Akiyama, Hiroki Hayashi, Shigehisa Koide, Midori Hasegawa, Yoshiyuki Hiki, Shunji Yoshida, Keiji Miura, Yukio Yuzawa. *Fujita Health Univ, Toyoake, Aichi, Japan.*

**Background:** Although anti-endothelial cell antibodies (AECA) against human umbilical vein endothelial cells (HUVEC) have been detected in systemic lupus erythematosus (SLE), their pathological role remains unclear. Because antigens expressed on the endothelial cell (EC) surface are pivotal for autoimmune reactions, methods that detect antibodies only to EC surface molecules are required. Thus, we developed a solubilized cell surface protein capture enzyme-linked immunosorbent assay (CSP-ELISA) that is able to detect antibodies against membrane proteins. We also aimed to elucidate the clinical importance of AECA for tissue-specific EC.

**Methods:** Sera from 52 patients with biopsy-proven lupus nephritis (LN), 25 with SLE without renal involvement (non-LN SLE), 10 disease controls (DC) and 81 healthy controls (HC) were tested for IgG- and IgA-AECA to human glomerular EC (HGEC) by CSP-ELISA.

**Results:** Titers of IgG- and IgA- AECA to HGEC were significantly higher in LN and non-LN SLE patients than in the combined DC and HC ($P < 0.001$) groups. The level of IgG-AECA did not correlate with active lesions, but the level of IgA-AECA to HGEC did correlate with histological evidence of active lesions in LN patients ($P < 0.001$). Immunocytochemical analysis showed AECA recognized membrane proteins on HGEC. The significant correlation of titer of AECA to both HGEC and HUVEC (R = 0.95 for IgG-, 0.93 for IgA-AECA, respectively) indicated AECA in LN patients recognize membrane proteins expressed on HGEC and HUVEC. To identify specific antigens against AECA, biotinylated CSPs were incubated with sera from LN patients with high titers of IgG-AECA, immunoprecipitated with immobilized protein G followed by immobilized avidin, and blotted with NeutrAvidin. A 150-kDa protein band that shifted to a 55-kDa protein band under reducing conditions was detected in patients with LN, but not in HC.

**Conclusions:** IgA-AECA was observed to be associated with pathological activity in LN. These EC membrane components recognized by AECA may be linked with the pathogenesis of LN.

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**FR-PO544**

**Enzymatic Sialylation of IgA1 Hinge-Region: Pathogenic Role of Sialic Acid in IgA Nephropathy (IgAN)** Kazuo Takahashi,[1,2] Milan Raska,[1,3] Milada Stuchlova-Horynova,[1,3] Alena Kasperova,[1,3] Stacy D. Hall,[1] Yoshiyuki Hiki,[2] Yukio Yuzawa,[2] Zina Moldoveanu,[1] Bruce A. Julian,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Fujita Health Univ, Toyoake, Japan; [3]Palacky Univ in Olomouc, Olomouc, Czech Republic.*

**Background:** Patients with IgAN have high serum levels of galactose (Gal)-deficient IgA1; some hinge-region (HR) *O*-glycans consist of terminal *N*-acetylgalactosamine (GalNAc) with or without *N*-acetylneuraminic acid (NeuAc, sialic acid). Sialylation of GalNAc blocks subsequent galactosylation. IgA1-producing cells from IgAN patients have increased activity of α2,6 sialyltransferase (ST6GalNAc) that sialylates GalNAc. Thus, premature sialylation of GalNAc may be favor production of Gal-deficient IgA1.

**Methods:** We established protocols for enzymatic α2,6-sialylation or α2,3-sialylation (ST3Gal1; adds NeuAc to galactose) of IgA1 *O*-glycans of an asialo-IgA1 myeloma protein (Ale) that mimics the Gal-deficient IgA1 in IgAN patients. The products were assessed by high-resolution mass spectrometry and ELISA with the GalNAc-specific lectin from *Helix aspersa* (HAA).

**Results:** Changes in SDS-PAGE mobility of the IgA1 heavy chain indicated that both enzymes were active. Enzymatic sialylation of the myeloma protein generated sialylated IgA1 that mimics the circulating nephritogenic IgA1 in IgAN patients, characterized by α2,6-sialylated GalNAc, or the IgA1 typical for healthy controls, characterized by an α2,3-sialylated Gal attached to GalNAc. Lectin ELISA was used to assess binding to the IgA1 before and after the enzymatic reactions. α2,6- or α2,3-sialylation of IgA1 markedly decreased reactivity with the HAA lectin. Neuraminidase treatment (removes sialic acid) completely restored the level of lectin reactivity. Thus, lectin binding to GalNAc decreased after sialylation of a neighboring glycan in the cluster of *O*-glycans of the IgA1 hinge region.

**Conclusions:** Neuraminidase should be used to treat serum IgA1 before a lectin assay to assess the content of hinge-region Gal-deficient GalNAc. Our *in vitro* enzymatic sialylation model will be useful to study the biological roles of NeuAc in the IgA1 HR in the pathogenesis of IgAN.

*Funding:* NIDDK Support, Other NIH Support - NIGMS, Government Support - Non-U.S.

**FR-PO545**

**Proteomic Analysis of Engineered IgA1-IgG Immune Complexes Reveals Association with Activated Complement C3** Nicolas Maillard, Leeann Boerma, Stacy D. Hall, Zhi Qiang Huang, Michal Mrug, Zina Moldoveanu, Bruce A. Julian, Matthew B. Renfrow, Jan Novak. *Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** In IgA nephropathy (IgAN), C3 colocalizes with IgA in mesangial deposits. Deletion of CFHR1,3 genes has been recently described to protect from the occurrence of IgAN, highlighting the contribution of complement alternative pathway (AP). Using an *in vitro* model of polymeric galactose-deficient (Gd) IgA1 with recombinant antiglycan IgG derived from an IgAN patient in immune complexes (ICs) formed in the presence of serum, we identified and characterized by proteomic analyses complement C3 products associated with the complexes.

**Methods:** ICs were formed overnight at 4°C, using purified Gd- polymeric IgA1 (pAle) myeloma protein, recombinant antiglycan IgG (r1123), and IgA- and IgG-depleted serum of a healthy control. High-molecular-mass fractions (HMMFs; >700 kDa) were isolated by size-exclusion chromatography. The fractions that stimulated proliferation of cultured human mesangial cells were pooled, concentrated, and fractionated by SDS-PAGE for proteomic analysis. After silver staining, protein bands were excised, digested by trypsin, and analysed by high-resolution tandem mass spectrometry (LTQ OrbitrapVelos; MS/MS). Identification of proteins was allowed by use of MASCOT and SEQUEST algorithms.

**Results:** Proteomic analysis revealed that C3 α and β chain elements were present in the active ICs and only low amount of β chain was in the corresponding fractions in negative control (serum only). Amino-acid sequence obtained by MS/MS combined with information on the molecular mass of corresponding bands from SDS-PAGE identified iC3b, C3c, and C3dg (confirmed by anti-C3 immunoblotting). The presence of these low-molecular-mass products in HMMFs can only be explained by their binding to ICs. These results thus provide evidence that biologically active IgA1-ICs activate complement C3 and associate with C3 degradation fragments. iC3b, C3c, and C3dg are the products of the action of factor I and H, suggesting a critical role of regulators in the activation of complement AP in IgAN.

**Conclusions:** Soluble engineered IgA1 – IgG ICs can bind C3 elements *in vitro*.

*Funding:* NIDDK Support, Private Foundation Support

**FR-PO546**

**Prevention of Formation of IgA1-Containing Immune Complexes in IgA1 Nephropathy** Alena Kasperova,[1,2] Hiroyuki Ueda,[1] Milada Stuchlova-Horynova,[1,2] Tyler J. Stewart,[2] Matthew B. Renfrow,[2] Bruce A. Julian,[2] Jan Novak,[2] Jiri F. Mestecky,[2] Milan Raska.[1,2] *[1]Immunology, Faculty of Medicine, Palacky Univ, Olomouc, Czech Republic; [2]Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** IgA nephropathy (IgAN), the most common primary glomerulonephritis in the world, is characterized by mesangial deposits enriched for galactose-deficient IgA1 (Gd-IgA1). These immunodeposits likely originate from circulating immune complexes

consisting of Gd-IgA1 bound by IgG and/or IgA antibodies specific for terminal *N*-acetylgalactosamine (GalNAc) in the *O*-linked glycans in the Gd-IgA1 hinge region. Such circulating immune complexes likely activate mesangial cells upon glomerular deposition, thus inciting renal injury. We tested the hypothesis that a GalNAc-containing competitor will prevent formation of Gd-IgA1-IgG immune complexes.

**Methods:** We tested two potential competitors of immune-complex formation based on non-glycosylated IgA1 heavy chain and its CH1-CH2 fragment. The recombinant proteins were produced in *E. coli*, purified, and glycosylated (GalNAcosylated) *in vitro* using recombinant GalNAc-transferase 2. The reaction products were analyzed by lectin immunoblotting and mass spectrometry. Sera of IgAN patients containing anti-glycan IgG antibodies were supplemented with Gd-IgA1, with or without a competitor, and formation of immune complexes was assessed by ELISA.

**Results:** Supplementation of sera of IgAN patients with Gd-IgA1 induced formation of new immune complexes. GalNAcosylated competitors reduced formation of such complexes by 5-20%. The inhibition was dose-dependent and glycan-specific, because non-GalNAcosylated inhibitors did not affect formation of new immune complexes.

**Conclusions:** GalNAc-containing competitors can interfere with binding of Gd-IgA1 and anti-glycan IgG to form an immune complex *in vitro*. This finding may offer a new approach for disease-specific therapy of IgAN.

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## FR-PO547

**Clinical Impact of Tonsillar APRIL on the Severity of IgA Nephropathy** Masahiro Muto,[1] Yusuke Suzuki,[1] Hitoshi Suzuki,[1] Kensuke Joh,[2] Shozo Izui,[3] Bertrand Huard,[3] Yasuhiko Tomino.[1] *[1]Juntendo Univ Faculty of Medicine, Tokyo, Japan; [2]Sendai Shakaihoken Hospital, Sendai, Japan; [3]Univ of Geneva, Geneva, Switzerland.*

**Background:** A proliferation-inducing ligand (APRIL) is a critical mediator for antibody-producing plasma cell survival. Because immunological disorders in mucosal immunity are recently discussed in the pathogenesis of IgA nephropathy (IgAN), we investigated the clinical impact of mucosal APRIL expression in IgAN.

**Methods:** In addition to clinical background before and after tonsillectomy, the expressions of APRIL and its receptors (TACI; transmembrane activator and calcium modulator cyclophilin ligand interactor, BCMA; B-cell maturation antigen) in tonsils from IgAN patients ($n$=56) and control patients (chronic tonsillitis, $n$=12) were evaluated by real-time PCR, immunohistochemistry (IHC) and flow cytometric analysis (FCM). For IHC and FCM, polyclonal rabbit anti-APRIL antibody specifically recognizing APRIL-producing cells (Stalk-1) was used.

**Results:** Tonsillar transcript levels of APRIL and its receptors were significantly higher in IgAN than those of control ($P$<0.05). IHC revealed that Stalk-1$^+$cells in IgAN were detected not only in the subepithelial area but also germinal centers (GC) much more than those in control. Percentage of Stalk-1$^+$GC (27.4±21.3%) in IgAN patients was significantly higher than that in control (7.2±6.81%, $P$=0.0005) and correlated with amount of proteinuria ($P$=0.0017) and treatment responses, such as decrease of proteinuria ($P$=0.0003). Combined IHC and FCM analysis revealed that CD11c$^+$ dendritic cells and CD19$^+$ B cells are the major cell type of APRIL-producing cells in subepithelial area and GC, respectively in patients with IgAN. Moreover, in case of high percentage of Stalk-1$^+$GC, serum levels of IgA containing immune complexes (IC) were decreased after the tonsillectomy.

**Conclusions:** Up-regulation of tonsillar APRIL impacts on the disease activity in IgAN. This overexpression may involve long-term survival GC B cells responsible for the production of nephritogenic IgA/IgA IC.

## FR-PO548

**microRNA-155 Deficiency in Peripheral Lymphocyte Is Related to IgA Nephropathy** Xiaolei Chen, Wei Qin, Zi Li, Junming Fan. *Div of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

**Background:** MicroRNA-155 (miR-155) is an important immune regulator involves in lymphocyte homeostasis, which is key pathogenesis of IgA nephropathy (IgAN). miR-155 level in peripheral lymphocytes of IgAN patient was studied in this study.

**Methods:** Forty biopsy-proven IgAN patients and 15 unrelated healthy controls were included. Expression of miRNAs in peripheral lymphocyte was determined using Exiqon microRNA microarray. Realtime RT-PCR of miR-155 was performed. The expression level of Foxp3, a regulator of miR-155, was also measured. Treg and Th17 cell ratio was measured by flowcytometry. Correlation between miR-155, Foxp3 expression level, Treg/Th17 ratio and clinical indexes, such as proteinuria, hematuria, renal function and albumin level was analyzed.

**Results:** Microarrayindicated that in 1035 microRNAs successfully analyzed, 533 are upregulated and 499 are downregulated compared with normal controls. The expression level of miR-155 in IgAN patients was dramatically lower than normal control (fold change: -0.61), which was further confirmed by realtime RT-PCR (IgAN 0.197±0.07 vs Control 0.796±0.13, p<0.01). Significantly correlation between miR-155 and Foxp3 expression level was also noticed (R=0.681, p<0.001). A significant correlation was also observed between Treg/Th17 ratios. Further study showed that proteinuria and hematuria were significantly correlated with miR-155 level (proteinuria: r=-0.594, p<0.001, hematuria: r=-0.590, p<0.001). However, no apparent correlation was observed in serum creatinine, BUN,Cystatin-C and albumin. Moreover, miR-155 expression level is also related to the severity of renal pathology changes.

**Conclusions:** Remarkable lower expression of peripheral lymphocytes miR-155 was observed in IgAN patients, which correlates with proteinuria, hematuria and pathological grade, which suggests that miR-155 might play important roles in the pathogenesis of IgAN and might be a potential biomarker of the disease.

## FR-PO549

**Aberrant *O*-Glycosylation of IgA1 in IgA Nephropathy (IgAN) and the Role of Sialyl-Tn Antigen** Tyler J. Stewart,[1] Hitoshi Suzuki,[2] Milan Raska,[3] Kazuo Takahashi,[4] Koshi Yamada,[2] Milada Stuchlova-Horynova,[3] Bruce A. Julian,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Juntendo Univ, Tokyo, Japan; [3]Palacky Univ, Olomouc, Czech Republic; [4]Fujita Health Univ, Toyoake, Japan.*

**Background:** IgAN is associated with galactose deficiency of circulatory IgA1 hinge-region (HR) *O*-glycans. Previous data revealed enhanced α2,6 sialylation of *O*-linked GalNAc and suggested involvements of ST6GalNAc-II in aberrant IgA1 *O*-glycosylation in IgAN. Using several complementary approaches, we tested whether premature sialylation by ST6GalNAc-II can inhibit the addition of β1,3-linked galactose to IgA1 HR by C1GalT1.

**Methods:** *In vitro* reactions of purified recombinant ST6GalNAc-II and C1GalT1 with IgA1 HR synthetic glycopeptides (previously GalNAcosylated by GalNAc-T2) or desialylated IgA1 were characterized using high-resolution mass spectrometry (MS) or ELISA. Relative MS quantification of different HR glycoforms using total ion counts was performed to determine inhibition of C1GalT1 galactosylation by previous ST6GalNAcII sialylation at the molecular level. Lectin ELISA was used to determine the proportion of terminal (unoccupied) GalNAc.

**Results:** Using lysates from IgA1-secreting B cells from IgAN patients and healthy controls as the source of ST6GalNAc-II or C1GalT1, we performed *in vitro* enzymatic sialylation of desialylated IgA1 followed by galactosylation. Assessment of glycosylation by lectin ELISA revealed that sialylation of IgA1 by ST6GalNAc-II prevented effective galactosylation by C1GalT1. We also plan to confirm these findings by using recombinant enzymes. We have now produced recombinant ST6GalNAc-II and C1GalT1 and demonstrated that both enzymes are active and quantified relative amounts of various HR glycoforms after single glycosyltransferase reactions.

**Conclusions:** Our results suggest that a steric hindrance imposed by the addition of α2,6-linked sialic acid to IgA1 HR inhibited C1GalT1 enzymatic galactosylation of IgA1. Thus, overproduction of sialyl-Tn antigen prior to galactosylation could result in galactose-deficient IgA1 HR, the phenotype associated with IgAN.

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## FR-PO550

**In Patients with IgA Nephropathy (IgAN) and Healthy Controls Cells Expressing Mucosal Homing Receptors Secrete Polymeric J-Chain-Containing but Differentially *O*-Glycosylated IgA1** Hiroyuki Ueda,[1] Zina Moldoveanu,[1] Hitoshi Suzuki,[2] Koshi Yamada,[2] Bruce A. Julian,[1] Jiri F. Mestecky,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Juntendo Univ, Tokyo, Japan.*

**Background:** Patients with IgAN have elevated levels of circulatory IgA1 with some *O*-glycans that are galactose-deficient (Gd-IgA1). This IgA1 forms pathogenic immune complexes and is mostly polymeric, indicating a possible mucosal association. However, the origin and nature of the Gd-IgA1-producing cells are not well understood. To address these questions, we developed protocols for separation of cells expressing specific mucosal homing receptors and analyses of IgA1 secreted by these cells.

**Methods:** Using immortalized IgA1-secreting cells derived from the circulation of IgAN patients (IgANP) and healthy controls (HC), we isolated by FACS sorting the cell populations expressing either gut- or upper respiratory tract-homing receptors, α4β7 integrins and L-selectin, respectively. Then, we characterized the molecular forms and *O*-glycosylation of IgA1 secreted by these cells using SDS-PAGE separation under non-reducing conditions followed by Western blotting with anti-heavy chain and anti-J chain antibodies and lectin ELISA, respectively.

**Results:** IgA1 secreted by cells from HC was predominantly monomeric, whereas IgA1 from cells of IgANP was enriched for J chain-containing polymers. IgAN-derived and HC-derived cell populations expressing mucosal homing receptors (α4β7 or L-selectin) secreted predominantly polymeric IgA1. However, only the IgANP cells produced Gd-IgA1, suggesting that galactose deficiency is not an exclusive characteristic of polymeric glycoforms, but may be a disease-specific feature.

**Conclusions:** Separation of circulating IgA1-secreting cells according to specific homing receptors, combined with analyses of molecular forms and *O*-glycosylation of IgA1 at the molecular level, may reveal the origin and nature of the cells producing Gd-IgA1 that plays a key role in the pathogenesis of IgAN.

*Funding:* NIDDK Support, Other NIH Support - NIGMS, Private Foundation Support

## FR-PO551

**Production of Aberrantly Glycosylated IgA1 in IgA Nephropathy Is Associated with Abnormal Distribution of ST6GalNAc-II in Golgi Apparatus** Milada Stuchlova-Horynova,[1,4] Zora Smrzova,[2] Miroslav Ovecka,[1] Zina Moldoveanu,[4] Bruce A. Julian,[4] Hitoshi Suzuki,[3] Jiri F. Mestecky,[4] Jan Novak,[4] Milan Raska.[1,4] *[1]Palacky Univ, Olomouc, Czech Republic; [2]Veterinary Research Institute, Brno, Czech Republic; [3]Juntendo Univ, Tokyo, Japan; [4]Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** Patients with IgA nephropathy (IgAN) have IgA1 with galactose (Gal)-deficient *O*-glycans which are recognized by anti-glycan antibodies, resulting in formation of circulating immune complexes (CIC) that deposit in glomeruli. *O*-glycosylation of IgA1 in the Golgi apparatus is initiated by attachment of GalNAc residues to the hinge region by GalNAc-transferases, followed by attachment of Gal to GalNAc residues by galactosyltransferase C1GalT1. Sialic acid (SA) may be attached to Gal by α2,3-sialyltransferase and/or to GalNAc by α2,6-sialyltransferase-II (ST6GalNAc-II). Premature sialylation of IgA1 by the attachment of SA to GalNAc blocks attachment of Gal thus creating Gal-deficient GalNAc. Premature sialylation may be caused by increased concentration of ST6GalNAc-II and/or changes in the enzyme distribution within the Golgi, i.e., moving toward *cis*-Golgi. We generated ST6GalNAc-II-specific monoclonal antibody (mAb) to track this enzyme through the Golgi apparatus.

**Methods:** ST6GalNAc-II-specific mouse mAb was produced by DNA vaccination with plasmid encoding human ST6GalNAc-II. Recombinant ST6GalNAc-II produced in HEK293 FreeStyle cells was used for screening mAbs by ELISA or Western blotting. Our mAb and commercial *cis*- and *trans*-Golgi markers-specific mAbs were used for co-localization of ST6GalNAc-II in IgA1-producing cells by confocal microscopy.

**Results:** Using recombinant ST6GalNAc-II, we isolated ST6GalNAc-II-specific mAb. Confocal microscopy with image analyses revealed that in IgA1-producing cells from IgAN patients ST6GalNAc-II had about 13% more protein localized in *cis*-Golgi compared to cells from healthy controls ($P<0.05$).

**Conclusions:** Partial relocation of ST6GalNAc-II from *trans*- to *cis*-Golgi in IgA1-producing cells may represent a new mechanism contributing to production of Gal-deficient IgA1 in IgAN.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## FR-PO552

**Effect of Urine Protein on Renal Intersitienal Injury of IgA Nephropathy by Interleukin-17 Pathway** Junming Fan,[1,2] Ji Wen,[1] Nan Mao,[1] Zi Li,[1] Man Yang.[2] *[1]Dept of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China; [2]Div of Nephrology, Dept of Internal Chinese Medicine, Luzhou Medical College, Luzhou, Sichuan, China.*

**Background:** IgA nephropathy is a common glomerulonephritis throughout the world. The evidence suggested the imbalance of autoimmune affected the IgA production, transformation and clearance in IgAN. Interleukin-17(IL-17) known as a pro-inflammatory factor could promote the generation cytokines and induce T cell activation and proliferation; function in autoimmune diseases. This study is to investigate the expression of IL-17 in IgAN, and effection of IL-17 on renal interstitial injury.

**Methods:** The plasma and urine samples of 28 IgAN patients and 8 healthy controls were gathered. NRK-52E were cultured in medium and BSA. The concentration of IL-17 was detected by ELISA. Kidney tissue from 8 IgAN patients and 3 patients with renal tumor selecting remote normal tissue as control. The expression of IL-17 in kidney cells was measured by Immunohistochemistry.

**Results:** IL-17 level of urine was increased in IgAN patients compared with normal controls. The urine level of IL-17 was significant positive correlation with level of proteinuria and chronic renal injury. Compared with control group, the expression of IL-17 in renal tissue was increased in IgAN patients. The number of IL-17 positive cells is related with CD68 positive cells. FACS and Immunofluorescence showed the BSA promote renal tubular epithelia cell express IL-17 with dose-dependent and time-dependent manner. ELISA detected the level of IL-17 in supernatant was rised with the concentration of BSA. IL-17 directly promoted macrophage migration in a dose-dependent and time-dependent manner. The supernatant of BSA incubation could promote the macrophage migration.

**Conclusions:** The higher level of urine IL-17 was related with level of urine protein and the infiltration of CD68 positive cells in kidney. Urine protein could induced the secretion of IL-17 in tubular epithelial cells. IL-17 alone or collaborate with cytokine take part in pathogenesis of IgAN.

*Funding:* Government Support - Non-U.S.

## FR-PO553

**The Deposition of Secretory IgA in Patients with IgA Nephropathy Are Associated with Different Clinical, Pathology and Complement Activation Pathway** Junjun Zhang, Yan Liang, Zhangsuo Liu. *Nephrology, First Affiliated Hospital of Zhengzhou Univ, Zhengzhou, Henan Province, China.*

**Background:** Mucosal infection associated with episodic macroscopic haematuria are observed in many patients with IgAN. Secretory IgA (sIgA) is a mainly immunoglobulin involved in mucosal immunity. The aim of this work is to investigate the characteristics and complement activation in IgAN patients with or without renal sIgA deposition.

**Methods:** 186 IgAN patients with different renal pathology were enrolled. Their frozen renal sections were immunofluorescence stained and examined by confocal microscopy to detect the co-deposition of IgA and secretory component (SC),properdinandC4d,MBL and C3d.The association between deposition of sIgA and characteristics of patients, complement activation were analysed.

**Results:** In 186 patients, 59 patients (31.72%) were found sIgA deposition in kidneys. Compared with no sIgA deposition group, the incidence of infection history,gross hematuria history,macroscopic hematuria were higher, the level of serum cystatin C, serum β2 - microglobulin, blood urea nitrogen and serum triglyceride were lower, the percentages of crescents and C3 deposition in glomerulus were higher in sIgA deposition group. Meanwhile, compared with no sIgA deposition group, the co-depositions of properdin and C4d,factor B and C5b-9 were more frequently, the co-localization of MBL and C3d were rarelier in sIgA deposition group.

**Conclusions:** SIgA were deposited in kidneys of part of IgAN patients.which might be associated with different clinical manifestation and renal immunopathogenesis.Compared with IgA, sIgA may activate different renal complement pathway in IgAN.

## FR-PO554

**Overexpression of *N*-Acetylgalactosaminyltransferase-14 Contributes to Galactose-Deficient IgA1 Production: Relevance for IgA Nephropathy** Jana Novakova,[1,2] Tyler J. Stewart,[2] Koshi Yamada,[2,3] Hitoshi Suzuki,[3] Zina Moldoveanu,[2] Bruce A. Julian,[2] Jan Novak,[2] Milan Raska.[1,2] *[1]Palacky Univ in Olomouc, Olomouc, Czech Republic; [2]Univ of Alabama at Birmingham, Birmingham, AL; [3]Juntendo Univ, Tokyo, Japan.*

**Background:** IgA nephropathy (IgAN) is characterized by deposition of IgA1-containing immune complexes in the renal mesangium leading to glomerular injury; IgA1 in these deposits in enriched for galactose-deficient *O*-glycans (Gd-IgA1). IgA1 *O*-glycosylation is initiated by attachment of *N*-acetylgalactosamine (GalNAc) to serine or threonine in the hinge region and is catalyzed by GalNAc-transferases (GalNAc-Ts). Among the known human GalNAc-Ts, only GalNAc-T14 is up-regulated in IgA-producing cells from IgAN patients compared to those from healthy controls; siRNA knock-down has confirmed involvement of GalNAc-T14 in the production of Gd-IgA1. Therefore, we investigated the effect of overexpression of GalNAc-T14 in IgA1-secreting cells on Gd-IgA1 production.

**Methods:** An EBV-immortalized IgA1-secreting cell line was transfected by bicistronic plasmid pT14-EGFP expressing GalNAc-T14 and enhanced-green fluorescent protein (EGFP). EGFP-positive and EGFP-negative cells were sorted by FACS. RT-PCR analysis was used for determination of GalNAc-T14 mRNA levels. ELISA with a GalNAc-specific lectin was used to determine production of Gd-IgA1.

**Results:** Sorting of pT14-EGFP-transfected cells based on EGFP-high positivity yielded a cell population with GalNAc-T14 expression 67-fold higher than in non-transfected cells and 17-fold higher than in transfected EGFP-low positive cells. Lectin ELISA revealed that the GalNAc-T14 overexpression increased production of Gd-IgA1. Furthermore, the lectin ELISA suggested that most GalNAc attached by GalNAc-T14 was not subsequently galactosylated or sialylated.

**Conclusions:** FACS sorting of transfected cells is a suitable method for systems in which the transfection efficiency is low. Our data suggest that overexpression of GalNAc-T14 in IgA1-secreting cells may be associated with production of Gd-IgA1.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## FR-PO555

**Deep-Sequencing Reveals Class-Specific Urinary microRNAs in Human Lupus Nephritis** Beatrice Goilav,[1] Iddo Z. Ben-Dov,[2] Irene Blanco,[5] Olivier Loudig,[4] Dawn Wahezi,[3] Chaim Putterman.[5] *[1]Pediatric Nephrology, Children's Hospital at Montefiore, Bronx, NY; [2]RNA Biology Laboratory, Rockefeller Univ, NY, NY; [3]Pediatric Rheumatology, CHAM, Bronx, NY; [4]Epidemiology, AECOM, Bronx, NY; [5]Rheumatology, Albert Einstein College of Medicine, Bronx, NY.*

**Background:** Lupus nephritis (LN), particularly, class IV LN, is associated with significant morbidity. microRNAs (miRs) are small, non-coding RNAs that regulate translation. Previous studies report changes in miR expression in kidney tissue, urine and PBMCs that correlate with LN disease activity. However, LN class-specific miRs have not been described.

Using deep-sequencing, we aimed to identify class-specific miRs in urine from adult and pediatric patients with biopsy-proven LN.

**Methods:** Cell-free urine from adult (n=25) and pediatric (n=8) female patients with class III, IV (proliferative) and V LN were obtained at time of active disease and during remission. Total RNA was used to prepare small RNA cDNA libraries for sequencing. Multiplexing through sample-specific 3' adapters was applied to limit cost. Sequence reads were mapped to the human genome and small RNA databases. miRs were quantified by relative read abundance. qRT-PCR was used for quantitative validation.

**Results:** We had specimen from adult patients with distinct class III, IV, and V LN and pediatric patients with class III and IV. In a paired-sample analysis we compared miR abundance in adult and pediatric active vs inactive LN, proliferative vs non-proliferative LN, class IV vs class III LN, active class III vs inactive class III LN, active class IV vs inactive class IV LN, and adult active class V vs inactive class V LN. We found significant changes in miR-324, -320, -200c (adult), -375 (pediatric), -200c, -30a, and -671*, respectively. All changes had $p<0.001$, except for miR-30a ($p=0.0011$).

**Conclusions:** We detected significant changes in miR abundance related to specific LN classes. Given that the prognosis of class IV LN is significantly worse than other classes, identifying miRs that are associated with class IV LN is an important step in biomarker discovery of this particular aggressive form of LN.

## FR-PO556

**Tolerance Failure at the Crossroad of Innate and Adaptive Immunity in Murine Lupus Nephritis** Amy G. Clark, Melissa L. Boor, Mary H. Foster. *Medicine and Research Service, Duke Univ Medical Center and DVAMC, Durham, NC.*

**Background:** Failed immune tolerance underlies pathogenesis in lupus nephritis. To identify genetic modulators of disease, we tracked tolerance mechanisms regulating a well-characterized autoantibody (autoIg) transgene (Tg) established on each of four major inbred lupus strains, which collectively model human lupus genetic complexity. We found that Tg B cells in the NZB strain, a contributor of major susceptibility loci for fulminant nephritis, uniquely display aberrant reversible anergy: they produce high levels of autoIg after toll-like receptor 4 (TLR4) stimulation, a phenotype not observed in other strains. Herein we explore the mechanistic and genetic basis of altered tolerance in NZB lupus.

**Methods:** Purified B cells or whole splenocytes from NZB Tg mice (n=4-11/grp) were incubated +/- ligands for TLR4 (LPS), TLR7 (R848), and/or TLR9 (CpG), and 8-10 d supernatants assayed for Tg autoIg by ELISA. NZB Tg mice were bred with B6 or NZW mice to generate Tg (B6xNZB)F1 and Tg (NZBxNZW)F1 (BWF1) lupus mice, respectively.

**Results:** Multiple TLR ligands induce Tg autoIg secretion from NZB Tg B cells: autoantigen binding OD, mean±SD, 1.233±1.30 for LPS, 0.573±0.75 for R848, 0.451±0.30 for CpG, and 3.061±0.32 for LPS+R848+CpG, compared to trace autoIg produced by medium alone or by LPS-stimulated B cells from B6 mice (mean OD < 0.05). In contrast to the innate cell-mediated suppression of anergic cell autoIg secretion previously described for non-lupus strains, NZB non-B cells fail to suppress LPS-stimulated Tg autoIg secretion in whole splenocyte cultures: OD 0.429±0.64 (despite 4.6-fold fewer B cells). LPS also induces autoIg secretion by Tg B cells from (B6xNZB)F1 and >4 month old BWF1 mice: OD 0.361±0.05 and 0.854±0.45, respectively.

**Conclusions:** NZB lupus results in part from defective anergy due to genetic disruption of coordinated TLR and B cell receptor signaling, possibly exacerbated by failure of innate cell suppression. The strain variance pattern suggests high heritability of this NZB tolerance defect. These results point to a genetic basis for lupus that involves inherited failure in autoimmune regulation that intersects innate and adaptive immunity.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO557

**Loss of Anergy and Innate Marginal Zone Cell Enrichment in BXSB Lupus Nephritis** Amy G. Clark, Melissa L. Boor, Mary H. Foster. *Medicine and Research Service, Duke Univ Medical Center and DVAMC, Durham, NC.*

**Background:** BXSB mice develop lupus nephritis that is accelerated in males carrying the *yaa* locus. To identify mechanisms by which BXSB genetic susceptibility modifies disease, we established a well-characterized autoantibody (autoIg) transgene (Tg) in BXSB. In contrast to stringent tolerance observed in Tg C57BL/6 (B6) and other strains, BXSB Tg B cells lack hallmarks of anergy (reduced in vivo life span, hypoproliferative responses) and are enriched in innate-like marginal zone (MZ) B cells, a subset resistant to anti-CD20 therapy. Herein we further explore the genetic origins of altered tolerance in BXSB lupus.

**Methods:** BXSB autoIg Tg mice were bred with nonautoimmune B6 mice to generate Tg (B6xBXSB)F1 progeny. B cell subsets and ex vivo proliferation of vital dye-stained purified B cells, +/- Ig receptor crosslinking or TLR4 stimulation, were measured by flow cytometry.

**Results:** B cells from Tg+ (B6xBXSB)F1 mice (n=4) hyperproliferate to TLR4 ligand LPS relative to B cells from nonTg F1 littermates (n=2): % divided cells, mean±SD, 52.0±3.0 vs 36.7±2.5, p<0.05. These results are similar to those using Tg and nonTg cells from the parental BXSB strain as previously reported. Tg+ (B6xBXSB)F1 mice also display a marked increase in frequency of MZ B cells (%CD21hiCD1dhi) vs nonTg F1 littermates: 15.8±3.4 vs 7.5±1.2, p<0.05, although MZ expansion is less than observed in parental Tg+ BXSB mice (31.1±9.8%, n=8, p<0.05). We do not observe sex differences in these phenotypes.

**Conclusions:** Our findings suggest that BXSB lupus mice have heritable defects in B cell tolerogenic signaling that are apparent early in cell development and that divert autoimmune B cell fate from anergy to innate MZ cell enrichment. Defects are detected in F1 progeny and in both sexes, implicating susceptibility genes on BXSB autosomes. Identification of responsible genes or pathways will provide insight into molecular mechanisms regulating autoimmunity that destroys kidneys.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO558

**Innate Immune Cells Produce Interleukin-17A which Drives Autoimmunity and Lupus Nephritis** Shaun A. Summers,[1] Oliver M. Steinmetz,[2] A. Richard Kitching,[1] Stephen R. Holdsworth.[1] *[1]Dept of Nephrology and Medicine, Monash Health and Monash Univ, Melbourne, Victoria, Australia; [2]III. Med Klinik, Univ Hospital, Hamburg, Eppendorg, Germany.*

**Background:** Systemic lupus erythematosus (SLE) and lupus nephritis are significant causes of morbidity and mortality. Clinical studies have demonstrated that serum Interleukin (IL)-17A levels are increased in disease, although direct evidence of pathogenicity is lacking. We sought to define the role of IL-17A in SLE induced by pristane.

**Methods:** We injected pristane (500µl) intraperitoneally into C57BL/6 wild type (WT) mice and assessed IL-17A production after 6 days, using flow cytometry. Subsequently we treated WT and IL-17A-/- mice with pristane and assessed; cellular immunity (8 weeks), humoral autoimmunity and renal injury, both functionally and histologically, after 7 months.

**Results:** After 6 days, IL-17A production was readily detected in pristane treated WT mice, but not in control (WT) mice. The majority of IL-17A producing cells were innate cells (% of IL-17A producing cells: macrophages 41.4±6.3%, neutrophils 24.0±3.3%, natural killer cells 11.5±1.5%, gammadelta T cells 7.7±1.2%) only 13.8±1.8% were CD4+Tcells. After 8 weeks systemic IFNY (WT 591.3±188.6 vs. IL-17A-/- 176.0±15.8ng/ml, P<0.05) and TNF (WT 294.7±11.6 vs. IL-17A-/- 196.6±35.7ng/ml, P<0.05) production were decreased in IL-17A-/- mice. Systemic IL-17A was still readily detected in WT mice. After 7 months humoral autoimmunity was diminished in IL-17A-/- mice, with decreased levels of total IgG and anti-dsDNA, at serial dilutions. Glomerular IgG (WT 1.8±0.1 vs. IL-17A-/- 1.1±0.1 intensity score [0-3], P<0.0001) and complement (WT 2.0±0.1 vs. IL-17A-/- 1.3±0.1 intensity score [0-3], P<0.0001) deposition was decreased in the absence of IL-17A. Compared to WT mice, glomerular injury assessed by albuminuria (WT 271.5±55.8 vs. IL-17A-/- 87.6±15.9µg/24 hours, P<0.05) and histological injury (WT 32.0±2.1 vs. IL-17A-/- 22.9±3.1, % abnormal glomeruli, P<0.05) was diminished in IL-17A-/- mice.

**Conclusions:** These results demonstrate that innate immune cells produce IL-17A, which drives autoimmunity and glomerular injury in experimental SLE.

*Funding:* Government Support - Non-U.S.

## FR-PO559

**Mass Spectrometry Based Proteomics Highlights the Replicability of Results between Lupus Nephritis Formalin-Fixed, Paraffin Embedded and Fresh Frozen Tissues** Abhimanyu N. Amarnani, Joe Capri, Ivan Axel Lopez, Ram R. Singh, Julian Whitelegge. *David Geffen School of Medicine, Univ of California, Los Angeles, Los Angeles, CA.*

**Background:** Lupus nephritis (LN) progresses from mild focal inflammation, to diffuse proliferative nephritis, to fibrosis and end-stage renal disease. While current treatments are based on renal pathology, the molecular basis of LN and its progression remains obscure. There is a need to use global, data-driven research techniques for analysis of molecular changes correlated with renal tissue at progressive disease stages. Here, we describe a proteomics workflow applicable to archived, clinical tissue that has been formalin fixed, and paraffin embedded (FFPE). Working towards resolving the accessibility issue of fresh frozen biopsy tissue, our goal was to directly evaluate claims that the FFPE tissue can be "as good as" fresh frozen tissue (FFT) for the purpose of global LN proteomics.

**Methods:** The workflow includes comparing proteomics results from identical transverse kidney tissue cuts from NZM-2328 mice, half FFPE and the other half FFT. Tissue processing included the FASP technique, in-solution dimethyl isotope labeling, stagetip strong cation exchange filtration and the Thermo LTQ Orbitrap XL Mass Spectrometer with Eksigent NanoLC-2d HPLC. In triplicate studies, 2 FFT groups and 1 FFPE were compared.

**Results:** Data analysis using MaxQuant (v1.3.0.5) resulted in identification of 1700+ proteins from both FFT and FFPE groups. More than 91% of proteins were identified between identical FFT groups, and more than 90% of proteins were consistently found between FFPE samples and identical FFT samples. Relative quantitative data among groups also presented good correlation. Our analysis demonstrates that FFPE tissue proteomics can produce results consistent with FFT, allowing further understanding of proteome changes in LN etiopathogenesis.

**Conclusions:** Our methodology is the first to directly compare FFT and FFPE tissue in a manner that is also applicable to clinical samples. With this, we will be able to first evaluate any differences between FFPE and FFT in clinical samples, and then begin utilizing archived, FFPE patient biopsy samples for LN studies.

## FR-PO560

**STAT3 Programs Th17-Specific Regulatory T Cells to Control Glomerulonephritis** Malte A. Kluger,[1] Michael Mülleneisen,[1] Claudia Wegscheid,[3] Hans-Joachim Paust,[1] Hans-willi Mittrücker,[2] Rolf A. Stahl,[1] Ulf Panzer,[1] Oliver M. Steinmetz.[1] *[1]III. Medizinische Klinik, Hamburg Univ, Germany; [2]Immunology, Hamburg Univ, Germany; [3]Experimental Hepatology, Hamburg Univ, Germany.*

**Background:** Th17 cells are central mediators of glomerulonephritis. The mechanisms underlying their counter regulation are largely unknown. Recently, Th17 lineage specific regulatory T cells (Treg17) have been identified which depend on activation of STAT3. To date, not much is known about their role in inflammatory diseases. We therefore studied the function of Treg17 in crescentic glomerulonephritis.

**Methods:** Specific deletion of STAT3 was achieved using Foxp3Cre or CD4CrexSTAT3fl/fl mice. Nephrotoxic nephritis (NTN) was induced and at day 10 renal parameters were assessed. Immune responses were analyzed by FACS/ELISA. In vitro Treg suppression and in vivo Treg trafficking were assessed.

**Results:** Foxp3CrexSTAT3fl/fl mice lacking Treg17 cells did not develop spontaneous disease. However, NTN was significantly aggravated. Renal and systemic immune responses were skewed towards Th17. Analyses of systemic Tregs revealed enhanced activation while numbers, proliferation and suppressive function remained unchanged. Strikingly, however, renal Treg infiltration was significantly reduced. FACS analyzes of renal Tregs from Foxp3CrexSTAT3fl/fl mice revealed complete lack of the trafficking receptor CCR6 which was abundantly expressed on wildtype Tregs. In vivo trafficking was analyzed by competitive co-transfer of CD45.2 knockout and CD45.1 wildtype splenocytes into RAG2$^{-}$ recipients. Renal trafficking of CCR6 negative Tregs from Foxp3CrexSTAT3fl/fl mice was significantly impaired. Finally we confirmed that Th17 cells are the major target of Treg17 cells as aggravation of disease was reversible in the absence of Th17 responses as shown in CD4CrexSTAT3fl/fl mice lacking both Treg17 and Th17 cells.

**Conclusions:** Our data indicate the presence of a new subtype of regulatory T cells in crescentic glomerulonephritis which specifically targets Th17 cells and depends on STAT3-activation. One of their main mechanisms of action is directional migration into areas of Th17 inflammation by using the chemokine receptor CCR6.

**FR-PO561**

**B Cell Derived IL-10 Does Not Vitally Contribute to Experimental Glomerulonephritis** Malte A. Kluger,[1] Annett Ostmann,[2] Michael Mülleneisen,[1] Matthias C. Meyer,[1] Hans-Joachim Paust,[1] Rolf A. Stahl,[1] Ulf Panzer,[1] Gisa Tiegs,[2] Oliver M. Steinmetz.[1] *[1]III. Medizinische Klinik, Hamburg Univ Medical Center, Hamburg, Germany; [2]Institute for Experimental Hepatology, Hamburg Univ Medical Center, Hamburg, Germany.*

**Background:** IL-10 secreting regulatory B cells (B10) have recently been described as negative mediators of inflammation. Their modes of action and their impact on inflammatory renal diseases remain unclear. We therefore studied the function of B10 cell derived IL-10 in a model of crescentic glomerulonephritis particularly in the light of the potential risks associated with B cell depleting therapies.

**Methods:** Analyzes of systemic and renal IL-10 producing B cells during NTN were performed using IL-10 eGFP reporter mice. Specific deletion of IL-10 in B cells was achieved by generating CD19Cre x IL-10fl/fl mice. Nephrotoxic nephritis (NTN) was induced and renal histology and function were assessed at days 4 and 10. Immune responses were analyzed by FACS and ELISA.

**Results:** IL-10 producing B10 cells were present in low numbers in kidneys of healthy mice and their frequency increased during nephritis. Lack of IL-10 production by B10 cells, however, did not influence the clinical course of NTN at early and late time points in terms of renal function and histological damage. Renal leukocyte infiltration and cytokine expression were similar except for an increased infiltration of macrophages in knockout mice. Systemic immune responses, cytokine production, leukocyte composition, proliferation and activation were unaffected in nephritic knockout mice. However, we found impaired antigen specific IgG production in these animals. In line, renal immune complex deposition was slightly reduced. Last but not least, detailed analysis of systemic and renal regulatory T cells did not show any significant differences.

**Conclusions:** In summary, we here show that IL-10 producing B cells infiltrate the kidney during the course of crescentic glomerulonephritis. Nevertheless, specific deletion of B10 cell derived IL-10 did not lead to exacerbation of renal disease. This is good news for B cell directed therapies as a lack of B10 cell derived lL-10 does not seem to deteriorate glomerular injury.

**FR-PO562**

**Complement Blockade via Eculizumab Enhances Nephroprotection in a COL4A3-Knockout Mouse-Model for Alport Syndrome** Oliver Gross,[1] Diana Rubel,[1] Jakob Valk,[1] Fred G. Pluthero,[2] Christoph Licht.[2] *[1]Dept of Nephrology & Rheumatology, Univ Medicine Göttingen, Göttingen, Germany; [2]Div of Nephrology and Cell Biology Program, The Hospital for Sick Children, Toronto, Canada.*

**Background:** COL4A3 -/- mice serve as animal model for progressive renal fibrosis. Testing the hypothesis of complement contributing to the progression of proteinuric kidney disease we investigated the antifibrotic and antiinflammatory nephroprotective effects of eculizumab alone and in combination with ACEI therapy in these mice.

**Methods:** 64 COL4A3 -/- mice were treated in 4 groups: (ECU) with Eculizumab 40 mg/kg,or (PLAC) vehicle 0.1 ml s.c 3x/wk, (ACE) with Ramipril 10 mg/kg/day p.o., and (ECU+ACE) with Eculizumab plus Ramipril. Ramipril and Eculizumab monotherapy started preemptively in week 4. In the ECU+ACEI-group, Ramipril was started in week 4 and ECU was applied in 6 week old animals already showing renal damage (proteinuria >1g/l, mild renal fibrosis) and lasted for 6 weeks. Six animals were sacrificed after 7.5 and 9.5 weeks, respectively, and kidneys were further investigated using histological, immunohistological and Western blot techniques. Survival until end stage renal failure was determined in the remaining animals.

**Results:** ECU+ACEI, but not ECU-monotherapy prolonged overall lifespan by 9% compared to ACEI-monotherapy. In contrast, ECU-monotherapy had a beneficial effect on accumulation of extracellular matrix and renal scarring. Dual therapy with ACEI and ECU showed the best effect in immunehistochemistry.

**Conclusions:** In COL4A3 -/- mice, the complement-inhibitor Eculizumab in combination with ACE-inhibition reduces fibrosis and prolongs survival compared to ACEI monotherapy. Despite the late onset of therapy, this nephroprotective and antifibrotic effect may still be substantial on top of early-on ACEI – an effect possibly applicable also to other chronic progressive kidney disease.

**FR-PO563**

**T-Cell Epitope Spreading in Experimental Autoimmune Glomerulonephritis (EAG) in Rats** Jitendra K. Gautam,[1] Anjana Gevaria,[1] Reetu Mukherji,[2] Kline Bolton.[1] *[1]Dept of Medicine, Div of Nephrology/CIIR, Univ of Virginia Health System, Charlottesville, VA; [2]Temple Univ School of Medicine, Philadelphia, PA.*

**Background:** $\alpha_3$ (IV) NC1 domain causes Goodpasture's Syndrome (GPS) in man and the same disease in rats, EAG. We have shown that an immunodominant pure T cell epitope, p13, that induces EAG, is frequently associated with antibody (Ab) B cell epitope spreading along the $\alpha_3$(IV) NC1 chain (intramolecular Ab spreading), and the $\alpha_4$(IV) NC1, (intermolecular epitope spreading). However, Ab does not occur in many rats, several other peptides can induce EAG, and EAG can be transferred by T cells. We sought to determine if nephritogenic T cell epitope spreading occurred in our model as a potential mechanism to augment disease and could be used as a potential target for therapy.

**Methods:** Rats were immunized with p13. 20 mer overlapping peptides were synthesized to include the full length of $\alpha_3$(IV) NC1. Lymphocyte proliferation assays (LPA) were done weekly and the characteristics of the individual LPA stimulation index (SI) were examined.

**Results:** T cell epitope spreading from p13 occurred to multiple peptides in an ordered chronological pattern. 12 peptides with strong SIs were used to immunize rats to assess their ability to produce EAG. Two regions not previously described induced EAG. Ab was deposited on GBM in all these rats demonstrating Ab cross reactivity and/or epitope spreading associated with the nephritogenic epitope. Non-nephritogenic peptides were not associated with Ab formation to GBM, although many induced Ab to themselves.

**Conclusions:** We have shown in EAG that in addition to B cell epitope spreading, T cell epitope spreading also occurs. However, only a few peptides with positive SI induce EAG, most do not. The nephritogenic new epitopes also are associated with Ab formation. Thus EAG is associated with both T cell and B cell epitope spreading which likely augment the severity of the disease and may be therapeutic targets.

*Funding:* NIDDK Support

**FR-PO564**

**Mac-1 Deficiency Protects Mouse from Pulmonary Hemorrhage, whereas Exacerbates Glomerulonephritis in Experimental Model of Systemic Lupus Erythematosus** Yiqin Shi, Naotake Tsuboi, Kazuhiro Furuhashi, Shoichi Maruyama, Seiichi Matsuo. *Internal Medicine, Nephrology, Nagoya Univ Graduate School of Medicine.*

**Background:** Mac-1 (CD11b/CD18), a leukocyte adhesion molecule, expressed on neutrophils, eosinophils and macrophages has been shown to mediate several adhesion-dependent processes. Recently, an association of genetic variations in Mac-1 with susceptibility to SLE has been reported in several studies.

**Methods:** To determine the underlying mechanism of how Mac-1 participates in SLE, we introduced pristine (TMPD) to induce pulmonary hemorrhage and experimental lupus nephritis in Mac-$1^{-/-}$ mice on C57BL/6 background. Organ damage was histologically analyzed. Flow cytometric analysis and ELISA were performed for the evaluation of leukocyte infiltration and cytokine concentration in inflamed sites including the peritoneal cavity, lung and kidney.

**Results:** Mac-$1^{-/-}$ mice had reduced prevalence of pulmonary hemorrhage compared to wild-type (WT) mice within 1 month after TMPD injection, but after 4 months demonstrated severe proteinuria that was significantly higher than WT mice. In Mac-$1^{-/-}$ mice, lupus nephritis was evident with glomerular hypercellularity and leukocyte infiltration associated with glomerular IC deposition. The analysis of the peritoneal lavage on day 5 and 10 after pristine treatmentrevealed an increase in eosinophils and immune regulatory (M2) macrophages but lower numbers of neutrophils and classic (M1) macrophages in Mac-$1^{-/-}$ mice compared to WT. Higher expression of IL-4 and IL-13, both key mediators of macrophage polarization toward M2 macrophages, was observed in the peritoneal cavity of Mac-$1^{-/-}$ mice.

**Conclusions:** Mac-1 promotes acute inflammatory immune responses that lead to pulmonary hemorrhage but downregulates chronic immune responses to protect mice from IC-mediated renal injury in a model of experimental lupus nephritis induced by TMPD.

*Funding:* Government Support - Non-U.S.

**FR-PO565**

**The Inflammasome-Related Molecules NLRP3 and ASC Suppress Lupus Nephritis of C57BL/6lpr/lpr Mice** Georg Lorenz, Maciej Lech, Onkar Kulkarni, Roman Guenthner, Marian Grosser, Nora Stigrot, Heni Susanti, Hans J. Anders. *Medizinische Klinik und Poliklinik IV, Clinical Biochemistry, Ludwig Maximilians Universität, Munich, Germany.*

**Background:** NLRP3/ASC inflammasome signaling translates infectious and sterile dangers into the secretion of mature IL-1β and IL-18 and subsequently into IL-1R/NF-κB-dependent secretion of multiple pro-inflammatory cytokines. We hypothesized that the NLRP3/ASC inflammasome would contribute in a similar manner to autoimmune tissue inflammation, e.g. to immune complex glomerulonephritis in systemic lupus erythematosus, i.e. lupus nephritis.

**Methods:** To address this question we generated *nlrp3*- or *asc*- or *il1r*- or *il18*- deficient C57BL/6-lpr/lpr mice, the latter being a model of spontaneous SLE-like autoimmunity.

**Results:** Surprisingly, *nlrp3*- and *asc*-, but not *il1r*- or *il18*- deficient C57BL/6-lpr/lpr mice displayed an aggravated autoimmune phenotype with massive lymphoproliferation, severe crescentic immune complex glomerulonephritis, and autoimmune lung disease, which are usually absent in C57BL/6-lpr/lpr mice. Immune phenotyping revealed that both *nlrp3*- and *asc*-deficiency both shifted lymphocyte apoptosis to lymphocyte necrosis, which induced multiple pro-inflammatory elements and suppressed negative regulators of innate immunity such as NLRP1a, NLRP2, NLRP6 and NLRP12. Lymphocyte necrosis and innate immune activation were associated with the expansion and activation of spleen dendritic cells, macrophages, T cells, and B cells.

**Conclusions:** Together, *nlrp3*- or *asc*-deficiency both aggravate autoimmunity and autoimmune tissue injury in lupus-prone C57BL/6-lpr/lpr mice. This reveals an unexpected immunosuppressive role of NLRP3 and ASC in this context, which may be related to a previously unknown role of NLRP3 and ASC in preventing lymphocyte necrosis, which exposes additional lupus autoantigens and drives the autoimmune process. These data identify both Asc and Nlrp3 as previously unknown SLE susceptibility genes in mice.

*Funding:* Government Support - Non-U.S.

**FR-PO566**

**CD147 Ameliorates Lupus Nephritis through the Regulation of Helper T Cell 17 Differentiation** Kayaho Maeda, Tomoki Kosugi, Waichi Sato, Hiroshi Kojima, Yuka Sato, Mayuko Maeda, Shoichi Maruyama, Seiichi Matsuo. *Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan.*

**Background:** CD147/Basigin (*Bsg*), a glycosylated transmembrane protein, plays important roles of cell survival, invasion and metastasis. We previously demonstrated deleterious effects of CD147 in renal inflammation caused by ischemia and renal fibrosis. As CD147 identifies activated regulatory T cell (Treg), the attention has become extended to the autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus. Interleukin (IL)-17 producing T cell and Treg also serve important roles in the pathogenesis of SLE. However, the molecular mechanism involving CD147 remains unknown. We therefore investigated the role of CD147 in lupus nephritis.

**Methods:** Lupus nephritis was induced in CD147 deficient mice (***Bsg***$^{-/-}$) or wild-type mice (***Bsg***$^{+/+}$) with an intraperitoneal injection of pristane (0.5ml/each mice). They were sacrificed at 6 months after an injection for histological and biochemical analyses. Kidney, spleen and thymus were analyzed.

**Results:** There was no difference between *Bsg*$^{+/+}$ and *Bsg*$^{-/-}$ in serum anti-nuclear/anti-dsDNA antibody during the experimental period, whereas serum C3 decreased in *Bsg*$^{-/-}$. Mesangial and endothelial cells proliferations, macrophages and CD4$^+$ T cells infiltration, wire loop lesion and albuminuria were prominent in *Bsg*$^{-/-}$ mice. Consistent with these data, IgG, C3 and C1q depositions in *Bsg*$^{-/-}$glomeruli were predominantly observed. By flow cytometry analysis, no obvious difference in the number of Treg was found in both genotypes, whereas IL-17A producing CD4$^+$ T cells (Th17) were higher in *Bsg*$^{-/-}$ spleen than *Bsg*$^{+/+}$. Th17-related gene expressions were prominent in *Bsg-/-* kidney. CD4$^+$ T cells from *Bsg*$^{-/-}$ significantly increased IL-17A level more than *Bsg*$^{+/+}$ under Th17-skewing conditions. Interestingly, STAT3 activation, essential for Th17 differentiation, was enhanced by lack of CD147. Treatment with anti-CD147 antibody was downregulated the STAT3 activation and Th17 differentiation.

**Conclusions:** Lack of CD147 promotes Th17 differentiation through the STAT3 activation, eventually leading to the development of lupus nephritis.

**FR-PO567**

**Molecular Characterization of Renal Responses in Lupus Nephritis Using Serial Kidney Biopsies** Samir Parikh,[1] Ana Malvar,[2] Jay C. Vance,[1] Huijuan Song,[1] Bruno Jorge Lococo,[2] Valeria Gabriela Alberton,[2] Brad H. Rovin.[1] *[1]Ohio State Univ Wexner Medical Center, Columbus, OH; [2]Hospital Fernandez, Buenos Aires, Argentina.*

**Background:** The molecular events occurring in the kidney during treatment of LN are unknown, but understanding these could be used to improve therapy. The expression of 511 immune-response genes was examined in serial kidney biopsies done at LN flare and after treatment.

**Methods:** RNA expression was measured in clinical biopsy tissue by Nanostring technology. Differentially expressed transcripts identified by Statistical Analysis of Microarray were compared using ANOVA followed by Tukey's. Controls (n=4) were pre-implantation donor transplant kidney biopsies.

**Results:** Five patients responded well (CR) with normalization of serum creatinine (SCr) and a fall in proteinuria to <0.5g/d, while 3 patients had no response (NR) by the second biopsy. At flare NR patients had more proteinuria (6.7±1 vs 2.7±1 g/d, P=0.03) but SCr was similar. The NIH activity index was higher in NR biopsies (10.3±1.5 vs 6.4±1.7, P=0.016), but the chronicity index and other histologic features, including glomerular and interstitial leukocyte infiltration, were similar. Of the top 50 differentially-expressed transcripts, all but one (CCAAT enhancer binding protein) was higher in LN than control tissue. Twenty-eight of these transcripts were increased only in NR, while 22 increased in CR and NR. Despite a good clinical response, transcript levels in the CR group continued to increase 6 months post-flare (n=2), but after 1-3 years of therapy (n=3) fell significantly compared to 6 month biopsies (P=0.006). Flares leading to CR had higher expression of IL-10 and NO signaling, while NR flares had increased expression of T and B cell signaling pathways. Activation of certain T cell-mediated apoptosis and antigen presentation pathways were unique to NR flares.

**Conclusions:** Despite good clinical responses as early as 6 months after treatment of LN flare, the molecular changes that occur at flare persist. Flares leading to NR have, in general, more intense activation of many pathways in common with CR flares, but also have activation of unique pathways that could be exploited therapeutically.

*Funding:* Other NIH Support - National Center for Advancing Translational Sciences

**FR-PO568**

**CXCR3$^+$ Regulatory T Cells Control $T_H1$ Immune Response in Crescentic Glomerulonephritis** Hans-Joachim Paust,[1] Christian Franz Krebs,[1] Anett Peters,[1] Sabrina Bianca Bennstein,[1] Friedrich Thaiss,[1] Thorsten Wiech,[2] Oliver M. Steinmetz,[1] Jan-Eric Turner,[1] Rolf A. Stahl,[1] Ulf Panzer.[1] *[1]III. Medizinische Klinik, Univeritätsklinikum Hamburg Eppendorf, Hamburg, Germany; [2]Institut für Pathologie, Univeritätsklinikum Hamburg Eppendorf, Hamburg, Germany.*

**Background:** Regulatory T cells (Treg) mediate suppression of excessive immune responses and tissue damage in autoimmune diseases, such as crescentic glomerulonephritis. This depends on the trafficking of Tregs to sites of inflammation. Chemokines and chemokine receptors have been shown to play a pivotal role in the directional trafficking of T cells in general, however their specific function for Treg cell migration is unclear.

**Methods:** In a model of crescentic glomerulonephritis (nephrotoxic nephritis, NTN) we assessed the time-dependent chemokine receptor expression profile of renal Tregs by flow cytometry. Furthermore, we generated conditional CXCR3-deficient mice (CXCR3-flox) and crossbred these mice with FoxP3-Cre mice. Thereby we generated CXCR3-deficiency specifically in Tregs. In addition, we performed FACS analysis from renal biopsies of patients with ANCA-associated glomerulonephritis.

**Results:** Here we show in a mouse model of crescentic glomerulonephritis that Tregs expressing the $T_H1$ chemokine receptor CXCR3accumulate in the kidney during the peak of the $T_H1$ immune response. Treg cell-specific ablation of CXCR3 resulted in reduced renal Treg cell recruitment accompanied with an enhanced $T_H1$ immune response, without affecting $T_H17$ immune response, and an aggravated course of the nephritis. This suggests that CXCR3$^+$ Tregs might control renal $T_H1$ immunity. Finally, we demonstrate the presence of CXCR3$^+$ Treg cells in areas of $T_H1$ immune response in the kidneys of patients with ANCA-associated crescentic glomerulonephritis.

**Conclusions:** These findings indicate that a subset of Tregs express CXCR3 and thereby have trafficking properties of pathogenic CXCR3$^+$ $T_H1$ cells to suppress excessive $T_H1$ responses at tissue-specific sites.

*Funding:* Government Support - Non-U.S.

**FR-PO569**

**A Novel Soluble Epoxide Hydrolase Inhibitor Ameliorates Progression of Cronic Renal Dysfunction in Glomerulonephritis Rats** Yasuhito Nakadera, Yoshiji Asaoka, Mayu Mutsuga, Takashi Serizawa, Mai Yagi, Yoshitaka Yoshizawa, Shinichi Yamaguchi, Eriko Higashi, Yuko Kato, Kazuyuki Tokumaru, Shinnosuke Hayashi, Aiko Nitta, Takumi Aoki, Yutaka Nishimura, Masateru Yamada. *Toray Industries, Inc, Japan.*

**Background:** Epoxyeicosatrienoic acids (EETs) are CYP-derived arachidonic acid metabolism, which are likely to be signaling molecules to help ameliorate a wide variety of pathological conditions. EETs are known to be metabolized by soluble epoxide hydrolase (sEH), whereby the numerous beneficial actions of EETs are eliminated. Inhibition of sEH is expected to effectively provide significant renoprotective effects based on the reports of its vasodilative, anti-proliferative and anti-inflammatory effects. In this study we assessed the hypothesis that a novel sEH inhibitor might ameliorate the progression of chronic renal dysfunction in glomerulonephritis (GN) rats.

**Methods:** GN was induced to rats by injecting anti-glomerular basement membrane antibody. Two weeks after the induction, serum creatinine (sCr) levels in GN rats were significantly elevated. Then, we divided GN rats into three groups and started the administration of vehicle, sEH inhibitior (3 and 10 mg/kg, p.o., once a day) from 2 weeks after GN induction. The levels of sCre, blood urea nitrogen (BUN) and cystatin-C (Cys-C) were measured. In addition, the histopathological changes of glomerular lesion and renal tubules were also evaluated.

**Results:** At 2 weeks after GN induction, the levels of sCr and BUN in GN rats were significantly elevated compared with normal rats, and further increased at 5 weeks. In sEH inhibitor-treated GN rats, the increase in sCr and BUN was significantly prevented. Cys-C levels in GN rats were also increased at 5 weeks. The increase in Cys-C levels was blocked by sEH inhibitor. In histopathological examination, glomerulosclerosis and hyaline cast in renal tubules were markedly observed in GN rats. The severity of these histopathological changes in the kidney was improved by sEH inhibitor.

**Conclusions:** These findings indicate the sEH inhibitor leads to functional and histological improvement in chronic renal dysfunction in GN rats.

**FR-PO570**

**Humanized Models to Study Regulation of Anti-Glomerular Basement Membrane Nephritis** Inge M. Worni-Schudel,[1,2] Melissa L. Boor,[1] Mary H. Foster.[1] *[1]Medicine, Duke Univ Medical Center and DVAMC, Durham, NC; [2]Nephrology, Hypertension, and Clinical Pharmacology, Bern Univ Hospital, Switzerland.*

**Background:** Immune nephritis is a major cause of renal injury, but little is known about genetic origins, environmental triggers, or immune regulatory control in man. A paucity of suitable model systems and an inability to recover human (hu) monoclonal autoantibodies (mAb) have posed barriers to progress.

**Methods:** We assessed the suitability of humanized NOD-scid-gamma (NSG) mice for in vivo study of anti-GBM immune responses. For Hu-HSC mice, CD34+ hu hematopoietic stem cells (HSC) from cord blood were iv injected into NSG recipients (n=2), followed by immunization with α3(IV)NC1 collagen at 14, 17 and 20 weeks. Harvested B cells were EBV transformed using CpG oligos and kinase inhibitors. Stable B cell lines were electrofused with a hu heterohybridoma to generate hu mAb. For the Hu-PBL model, NSG mice were injected with peripheral blood leukocytes (PBL) purified from two subjects with anti-GBM nephritis (n=4) and from two healthy donors (n=14).

**Results:** In Hu-HSC mice 23 weeks after engraftment spleen chimerism was 93.3% (mean % of CD45+ cells); 12.5% and 39% of cells expressed hu CD19+ and hu CD3+, respectively, confirming development of a hu lymphoid system. 47.5% of B cells expressed EBV receptor CD21. One month after transformation, viable cells and antigen-reactive Ig were detected in 49.6% and 35.8% of 240 wells, respectively. A human anti-α3(IV)NC1 mAb was generated for sequence analysis. In the Hu-PBL model spleen chimerism averaged 79% at mean 35 days post engraftment, with 6.5% hu CD19+ and 47% hu CD3+ cells. Serum hu IgG and IgM levels ranged 63-1020 μg/ml, with low levels of auto-Ig detected. Unexpectedly, spleen EBV exposure yielded only rare transformed B cells. Hu-PBL mice established using healthy PBL donors revealed loss of surface CD21 after in vivo transfer: 96% of freshly isolated donor hu CD19+ cells expressed CD21 versus 10% of Hu-PBL spleen hu CD19+ cells at day 21 (n=4).

**Conclusions:** Both Hu-HSC and Hu-PBL models permit development and expansion of human nephritogenic immunity, while posing unique opportunities and challenges for study of human disease.

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support, Government Support - Non-U.S.

## FR-PO571

**Independent In Vivo Regulation of a Goodpasture Autoantibody Light Chain** Inge M. Worni-Schudel,[1,2] Amy G. Clark,[1] Melissa L. Boor,[1] Mary H. Foster.[1] *[1]Medicine, Duke Univ Medical Center and DVAMC, Durham, NC; [2]Nephrology, Hypertension, and Clinical Pharmacology, Bern Univ Hospital, Switzerland.*

**Background:** Goodpasture syndrome results from autoimmune attack on α3(IV) collagen in kidneys and lung. To gain insight into origins and structure of pathogenic autoIg, we derived mAb from mice immunized with Goodpasture antigen. Sequence analysis revealed that anti-α3(IV)NC1 mAb from different mice share a heavy chain (HC) CDR3 motif and exclusively use IGKV3-encoded kappa light chains (LC). Biased use of IGKV3 genes was previously reported among anti-collagen IgG in rheumatoid arthritis. Mice bearing transgenes (Tg) encoding anti-α3(IV)NC1 Ig demonstrate B cell tolerance. The current studies assess the role of IGKV3 LCs in determining cell fate and regulation independent of the HC.

**Methods:** Ig Tg mice were generated by insertion of a rearranged IGKV3 LC under control of the kappa enhancer. Five Tg+ founders were bred with C57BL/6 mice deficient in endogenous kappa LCs (k-KO), such that all kappa is derived from the Tg. This strategy permits identification of the Tg LC while eliminating confounding rearrangements and editing at endogenous kappa alleles. Immune cell subsets and serum Ig levels were determined by flow cytometry and ELISA, n=5-8 mice/group.

**Results:** 3 LC-Tg+ k-KO lines display excellent B cell surface expression of LC Tg (range 55-75% of B cells), and varying levels of serum LC Tg. 2 of these lines demonstrate significantly reduced spleen B cell numbers: 9.0±3.0 and 10.4±3.0 million, vs 24.0±11.1 in non-Tg kappa+ littermates, $p<0.05$. Whereas cell counts in the third line (14.9±7.6 million) are not statistically different from non-Tg kappa+ mice, this line manifests marked editing at the lambda locus: 58.3±11.3% of B cells express lambda, compared to 7.4±5.0% in non-Tg kappa+ mice, $p<0.05$. Low levels of serum anti-collagen kappa+ Ig are detected in serum of LC-Tg+ k-KO mice.

**Conclusions:** Mice bearing the IGKV3-encoded LC preferentially expressed by anti-collagen Ig demonstrate evidence of B cell regulation, including deletion and profound receptor editing. This suggests independent roles for this LC in disease and for IGKV3 genes in autoimmunity and tolerance.

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support, Government Support - Non-U.S.

## FR-PO572

**High Induction and Low Degradation Abilities on Neutrophil Extracellular Traps of Sera in Patients with MPO-ANCA-Associated Vasculitis** Daigo Nakazawa,[1] Saori Nishio,[1] Masaharu Yoshida,[2] Utano Tomaru,[1] Haruki Shida,[1] Tatsuya Atsumi,[1] Akihiro Ishizu.[3] *[1]Depts of Internal Medicine II, Hokkaido Univ Graduate School of Medicine, Sapporo, Japan; [2]Renal Unit of Internal Medicine, Hachioji Medical Center, Tokyo Medical Univ, Tokyo, Japan; [3]Faculty of Health Sciences, Hokkaido Univ, Sapporo, Japan.*

**Background:** MPO-ANCA-associated vasculitis (MPO-AAV) is a severe and chronic autoimmune disease that closely related to neutrophil extracellular traps (NETs). The disordered regulation of NETs that contain the antigen for ANCA could possibly induce the vicious circle of pathogenesis in MPO-AAV. The aim of this study is to determine the serum abilities on NETs induction and degradation in patients with MPO-AAV.

**Methods:** Patients enrolled in this study included 38 MPO-AAV and 7 SLE patients diagnosed and treated in Hokkaido University Hospital between January 2008 and May 2012. After acquirement of written informed consent, peripheral blood samples were obtained without anticoagulants, and sera were stored at -80 °C until use. NETs induction rate was evaluated by reaction of patient-IgG with healthy neutrophils primed by TNF-α. ANCA affinity was determined by the competitive inhibitory ELISA method. DNase I activity and NETs degradation ability were evaluated by ELISA and the incubation of patient serum with phorbol myristate acetate-induced NETs, respectively.

**Results:** IgGs eluted from sera of MPO-AAV patients induced NETs (NETs induction rate: 16.6±9.7 %). This rate was significantly higher than that of SLE and healthy controls (SLE; 7.2±7.2%, Healthy control; 3.2±1.4%). In addition, the NETs induction rate was correlated with vasculitis activity and ANCA affinity. On the other hand, activity of DNase I, the important regulator of NETs in the serum, was generally low in MPO-AAV patients and many patients showed impaired degradation of NETs. Furthermore, the presence of anti-NETs antibodies, which could interfere with the degradation of NETs by DNase I, was demonstrated in some MPO-AAV sera.

**Conclusions:** These findings demonstrated high induction and low degradation abilities on NETs of sera in MPO-AAV patients.

## FR-PO573

**Hypogalactosylation and Hyposialylation of Serum IgG from Patients with ANCA-Associated Systemic Vasculitis: Relation to Disease Activity** Olivier M. Lardinois,[1] Jacob Hess,[1] Leesa Deterding,[2] Lydia Aybar,[1] Caroline J. Poulton,[1] Candace Henderson,[1] JulieAnne G. McGregor,[1] Donna O. Bunch,[1] Ronald J. Falk.[1] *[1]Medicine, Univ of North Carolina Kidney Center, Chapel Hill, NC; [2]Mass Spectrometry Group, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

**Background:** There is ample evidence that ANCA are primarily and directly involved in the pathogenesis of small-vessel vasculitis. In several inflammatory autoimmune diseases, the subfamily of Fc glycans deficient in terminal sialic acid and galactose (IgG-G0 glycan form) is significantly increased. Studies have shown that this also applies to patients with active ANCA-associated vasculitis (AAV) [1] and that the high incidence of IgG-G0 glycan form in serum of PR3-ANCA patients correlates with disease activity [2]. Here we investigate whether a similar pattern of abnormal IgG glycosylation exists in the serum of MPO-ANCA patients that correlates with disease activity.

**Methods:** Serum or plasma samples were collected from 30 healthy controls, 29 patients with MPO-ANCA, and 21 patients with PR3-ANCA. IgGs from serum or plasma samples were affinity-purified using immobilized protein AG. Isolated IgGs were digested with trypsin, and the released glycopeptides were identified and quantified by liquid chromatography-electrospray tandem mass spectrometry. The disease activity of the MPO- and PR3-ANCA patients was evaluated by Birmingham Vasculitis Activity Score (BVAS).

**Results:** We found that IgG-G0 levels of MPO-ANCA patients are elevated both at the time of active disease and during disease remission and, therefore, do not correlate with disease activity. As expected, IgG-G0 levels of active PR3-ANCA patients significantly dropped during disease remission and regained glycosylation levels indiscernible from those in healthy controls.

**Conclusions:** Our results suggest that significant differences exist between MPO- and PR3-ANCA diseases regarding the association of aberrantly glycosylated IgG levels with disease activity. It is conceivable that these differences may contribute to significant clinical difference in the disease course, severity, or relapse rate observed between the two diseases.

*Funding:* NIDDK Support

## FR-PO574

**Proteinase 3 and Myeloblastin Are Two Distinct Entities** Elizabeth Alderman McInnis,[1] Anshul K. Badhwar,[1] Akhil Muthigi,[1] Samuel C. Allred,[1] Jia Jin Yang,[1] Olivier Lardinois,[1] J. Charles Jennette,[1,2] Ronald J. Falk,[1] Dominic J. Ciavatta.[1] *[1]UNC Kidney Center, Div of Nephrology and Hypertension, UNC at Chapel Hill; [2]Dept of Pathology and Laboratory Medicine, UNC at Chapel Hill.*

**Background:** Proteinase 3 (PR-3) is a serine protease present in the azurophilic granules of PMNs and one of the major targets of ANCA. There are two annotated transcripts from the PR3 gene: PR3 ($PR3_T$) and myeloblastin ($MBN_T$). Whether the PR3 gene encodes two different protein isoforms, still remains a matter of debate. PMNs isolated from ANCA patients during active disease express elevated levels of myelopoiesis related genes including PR3 and MPO. This marked dysregulation in ANCA patients may extend to the expression of other PR3 transcripts. To test the hypothesis that abnormal expression and/or translation of PR3 transcripts play a role in the pathogenesis of ANCA, the following questions were addressed in this investigation: What is the relative frequency of both $PR3_T$ and $MBN_T$ in our patient population? Is PR3 message translated in mature neutrophils?

**Methods:** Total leukocyte mRNA was isolated from patients with ANCA disease (BVAS range 0-26) and control subjects and screened for PR3 and MBN using transcript specific primers. Total PR3 transcript levels were monitored by qPCR. Potential translation of these transcripts was studied in cell lines and patient neutrophils.

**Results:** Over 30% of unique MPO ANCA patients (n=28) and over 20% of unique PR3 ANCA patients (n=40) were positive for the MBN specific transcript; while, only one healthy subject (n=19) was positive for MBN. Total PR3 expression is elevated in patients positive for MBN. Expression of MBN is not due to an influx of progenitors into the periphery. Samples from patients with "left-shift" were negative for the MBN transcript.

The exclusive transcription of MBN in MCF-7 cell lines indicates that this gene can be translated. Neutrophils isolated from patients with ANCA vasculitis synthesize both PR3 and MPO.

**Conclusions:** MBN and PR3 are not synonymous transcripts, do not encode identical proteins and the transcripts are present in ANCA patients. Also, neutrophils from ANCA patients translate a plethora of proteins, including PR3 and MPO.

*Funding:* NIDDK Support

## FR-PO575

**Gleaning B Cell Phenotype from Rituxin Panel Data: Decreased %$CD5^+$ B Cells in Patients with ANCA Vasculitis Portend a Shorter Time to Relapse after Rituximab** Donna O. Bunch, JulieAnne G. McGregor, Lydia Aybar, Carmen E. Mendoza, Caroline J. Poulton, Elizabeth Studstill, Yichun Hu, Susan L. Hogan, John Schmitz, Ronald J. Falk, Patrick H. Nachman. *UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC.*

**Background:** To avoid infections and adverse events from therapy in patients with anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV), clinicians require improved markers of disease activity and impending relapse to guide immunosuppression

strategies post-rituximab. $CD5^+$ B cells, a surrogate marker of B regulatory cells, may serve as this needed biomarker but more data is required.

**Methods:** We examined B cell phenotype in 45 patients with AAV after rituximab therapy by flow cytometry. Immunosuppression in addition to rituximab was not an exclusion criterion. Whole blood or PBMCs were stained with antibodies to CD19, CD20, CD45 and CD5. Data from research samples was supplemented with data acquired from clinical Rituxin panels.

**Results:** Patients who had <30% $CD5^+$ B cells at the time of B cell repopulation (≥1% $CD19^+/CD20^+$ lymphocytes, n=5) relapsed sooner (14±5 mos) than patients who repopulated with ≥30% $CD5^+$ B cells (n=40, 25±14 mos; p=0.025) after rituximab. Patients in the two groups were similar with respect to age, gender, PR3 positivity and lung involvement.

**Conclusions:** Our data indicate a low $\%CD5^+$ B cells correlates with a shorter time to relapse following rituximab therapy without taking immunosuppression dose into consideration. Monitoring $\%CD5^+$ B cells serves as an effective biomarker to signal impending flare and can help guide rituximab re-dosing and/or use of other remission maintenance therapy in AAV.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO576

**Genetic Regulation of CD177 – A Receptor Presenting Anti-Neutrophil Cytoplasmic Antigen Proteinase 3** Claudia Eulenberg,[1] Sylvia Bähring,[1] Friedrich C. Luft,[1] Ralph Kettritz.[1,2] *[1]Experimental and Clinical Research Center, Charité Medical Faculty and the Max-Delbrück Center for Molecular Medicine; [2]Nephrology and Intensive Care Medicine.*

**Background:** ANCA activate neutrophils leading to crescentic glomerulonephritis. CD177 presents the major ANCA antigen proteinase 3 (PR3) on the neutrophil membrane (mPR3) yielding $CD177^{neg}/mPR3^{low}$ and $CD177^{pos}/mPR3^{high}$ subsets. The percentage of $CD177^{pos}/mPR3^{high}$ neutrophils confers a risk for ANCA vasculitis, is associated with worse clinical course and a stronger response to PR3-ANCA. We studied mechanisms that control CD177 protein expression.

**Methods:** We used human neutrophils, CD34+ stem cells, DNA and cDNA sequencing.

**Results:** We detected CD177 mRNA in $CD177^{pos}/mPR3^{high}$ neutrophils, but neither full-length nor truncated mRNA in $CD177^{neg}/mPR3^{low}$ cells. We separated both cell populations and analyzed their DNA and cDNA. By Southern blot analysis of the CD177 gene we excluded genomic recombination with a neighboring pseudogene and copy number variations to be causes of different CD177 gene expression. Structural DNA aberrations and single nucleotide variations were excluded for both subsets by genome-wide human SNP array 6.0 from Affymetrix® suggesting no differences in the DNA sequences between both subsets. DNA sequences in exon-coding region and in exon-intron transitions of the CD177 gene did also not differ between both subsets. Haplotype analysis in 13 parent-offspring trios identified 12 informative heterozygous SNPs revealing monoallelic CD177 expression in 11/13 trios with no informative SNPs in the remaining 2 trios. We identified the parental-allelic origin in 8/11 trios (2x paternal, 6x maternal). Monoallelic expression was non-synonymous in 9/11 children affecting the primary CD177 protein structure. Haplotype analysis in a neutrophil-differentiated CD34+ stem cell model suggested that one allele was preferentially expressed. The second allel showed minor expression before differentiation that decreased with G-CSF treatment.

**Conclusions:** The findings provide novel insight into CD177 gene regulation and could facilitate studying CD177-related mechanisms in ANCA-associated vasculitis and additional neutrophil-mediated diseases.

*Funding:* Government Support - Non-U.S.

## FR-PO577

**Correlation between the CD4-CD8 Values and Responses to Treatment of Primary Glomerulonephritis** Hilmi Umut Unal,[1] Ali Kilinc,[2] Mahmut Ilker Yilmaz.[3] *[1]Nephrology, Gulhane Military Medical Academy, Ankara, Etlik, Turkey; [2]Internal Medicine, Gulhane Military Medical Academy, Ankara, Etlik, Turkey; [3]Nephrology, Gulhane Military Medical Academy, Ankara, Etlik, Turkey; [4]Immunology, Gulhane Military Medical Academy, Ankara, Etlik, Turkey; [5]Immunology, Gulhane Military Medical Academy, Ankara, Etlik, Turkey.*

**Background:** Focal segmental glomerulosclerosis (FSGS) is a histologic diagnosis. Primary FSGS was previously considered to be largely unresponsive to immunosuppressive therapy; however, glucocorticoids, cyclosporine, and other immunosuppressive agents have been used with considerable therapeutic success. In a study conducted in patients with idiopathic membranous glomerulonephritis, CD4 + / CD8 + ratio was found significantly lower than the control group.In this study a CD4 +, CD8 + level and the CD4 + / CD8 + ratio evaluated the importance of the response to treatment.

**Methods:** In the 6-month follow-up responses to the treatment of patients which as a result of renal bx FSGS (primary) reported CD4+ ,CD8+ values and CD4+/CD8+ ratio evaluated retrospectively. Lymphocyte subgroups were measured by flow cytometry from peripheral blood in 23 patients. In patients between two groups who do not respond and respond to treatment were compared.

**Results:** There was significant difference between the CD 8 values($p<0.05$) in two groups. The CD4+/CD8+ T-cell ratio was significantly decreased in reponse to treatment patients compared with unresponsiveness group.

**Conclusions:** Regulatory T-cells confer negative regulatory effects on the immune response mediated by T-cells, and they can inhibit the proliferation of CD4+ T lymphocytes and the secretion of cytokines (mainly IL-2). In addition, they may also suppress self-reactive T-cells (CD8+). Studies have demonstrated that regulatory T-cells inhibit the activation of helper T-cells normally induced by antigen-presenting cells, which suppresses B lymphocyte activation and reduces the production of autoantibodies by B lymphocytes, thereby resulting in the alleviation of tissue injury mediated by the immune complex.More studies need conducting in order to understand of the importance of the Lymphocyte subgroups in the primary glomerulonephritis.

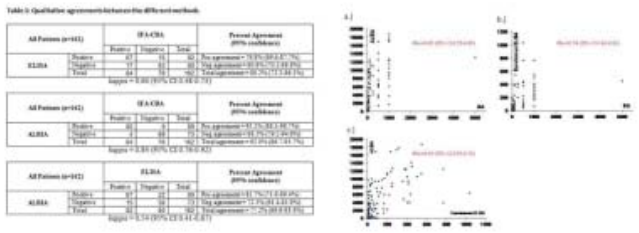
## FR-PO578

**Detection of Anti-Phospholipase $A_2$ Receptor Autoantibodies: A Comparison of Three Different Immunoassays** Astrid Behnert,[1] Mario Schiffer,[1] Laurence H. Beck,[2] Michael Mahler,[3] Marvin J. Fritzler.[4] *[1]Hannover Medical School, Hannover, Germany; [2]Boston Univ School of Medicine, Boston, MA; [3]INOVA Diagnostics, Inc., San Diego, CA; [4]Univ of Calgary, Calgary, Canada.*

**Background:** The detection of $PLA_2R$ antibodies in idiopathic membranous nephropathy (IMN), a common cause of nephrotic syndrome in adults, helps to differentiate between primary and secondary MN, it indicates whether immunosuppressive therapy is required and antibody titers are often used to monitor patients during therapy. We here compare the so far the only commercially available immunoassay, a semi-quantitative cell based assay (CBA-IIF, Euroimmun), to an observer independent, high throughput immunoassay on an addressable laser bead immunoassay (ALBIA) platform and a recently developed ELISA.

**Methods:** Serum samples of 162 patients with IMN were tested on CBA-IIF and ELISA according to the manufacturer's protocol (Euroimmun) and on ALBIA as previously described {Behnert et al., 2013}.

**Results:** Using the cut-off values established by the manufacturer for the ELISA and in our previous study for the ALBIA, good qualitative agreements were found. The overall qualitative agreements were 78.8% for CBA-IIF vs. ELISA, 91.4% for CBA-IIF vs. ALBIA, and 75.5% for ELISA vs. ALBIA. The Spearman rho values were: 0.69 for ALBIA vs. ELISA, 0.74 for ELISA vs. CBA-IIF and 0.85 for ALBIA vs. CBA-IIF.



**Conclusions:** Interestingly, our ALBIA correlated better with the Euroimmun CBA-IIF than the ELISA offered from the same company. This might be explained by the differences in the matrices utilized in these platforms. In ELISA, antigen is absorbed to plastic and reactivity of antibodies is highly dependent on sufficiently exposed epitopes. In ALBIA, the binding of antigen to spherical beads may permit binding of autoantibodies to the cognate, more stearically accessible conformational epitopes.

## FR-PO579

**Serological Profile of Idiopathic Membranous Nephropathy Cohort** Astrid Behnert,[1] Beina Teng,[1] Janina Müller-Deile,[1] Andrej Skoberne,[1] Mario Schiffer,[1] Marvin J. Fritzler.[2] *[1]Hannover Medical School, Hannover, Germany; [2]Univ of Calgary, Calgary, Canada.*

**Background:** Idiopathic membranous nephropathy (IMN), a common cause of nephrotic syndrome, has recently been identified as an autoimmune-mediated disease [Beck et al., 2009]. Autoantibodies (aab) to $PLA_2R$ are fairly specific for IMN and only found to a small percentage in secondary MN [Hofstra et al., 2012]. The clinical outcome of IMN patients is quite diverse (spontaneous remission vs. ESRD vs. proteinuria without progression). We performed serological profiles of 162 IMN patients to compare aab profiles with aab frequencies found in normal healthy individuals (NHI) and to hopefully identify factors that help to predict disease course in IMN.

**Methods:** Serum samples from 162 IMN patients were assayed for a variety of aab by ELISA (DFS70,ACA,$\beta_2$GPI), addressable laser bead immunoassay (ALBIA; Sm,U1RNP,SS-A,Ro52,SS-B,Scl-70,Jo-1,CENP-B,PMScl,PCNA) and to dsDNA by the C. lucilliae IIF assay. $PLA_2R$ aab were detected by a CBA-IIF (Euroimmun) and ALBIA [Behnert et al., 2013].

**Results:** $PLA_2R$ aab were found in ~54% of IMN patients whereas the frequency of other aab was uniformly below 2%. Anti-DFS70 were found in 16% of IMN patients.

| $PLA_2R$ CBA-IIF | $PLA_2R$ ALBIA | ACA ELISA | $\beta_2$ GPI ELISA | DFS70 ELISA | dsDNA Crithidia | Histone ALBIA | Jo-1 ALBIA | Ribosome ALBIA |
|---|---|---|---|---|---|---|---|---|
| 51,85% | 54,94% | 0,00% | 1,23% | 16,05% | 1,85% | 0,62% | 0,00% | 0,00% |

| Sm ALBIA | Sm-RNP ALBIA | Scl-70 ALBIA | PMScl ALBIA | CENP-B ALBIA | PCNA ALBIA | Ro-52 ALBIA | SSA-60 ALBIA | SSB ALBIA |
|---|---|---|---|---|---|---|---|---|
| 0,00% | 0,00% | 1,23% | 0,62% | 0,00% | 0,62% | 1,23% | 0,62% | 0,00% |

Table 1: Frequency of Autoantibodies in IMN Cohort

**Conclusions:** The frequency of anti-$PLA_2R$ aab in our IMN cohort is consistent with what has been previously published [Qin et al., 2011]. The frequency of the other aab, including anti-DFS70/LEDGF, was comparable to what has been reported in NHI and hence do not appear to serve as biomarkers to predict disease outcome or subsets. Anti-DFS70 antibodies are more prevalent in apparently NHI compared to patients with systemic autoimmune rheumatic diseases (SARD) [Mahler et al., 2012] whereas anti-Ro5/

TRIM21 reactivity is often regarded as a marker for SARD. The absence of anti-Ro52/TRIM and the high prevalence of anti-DFS70 confirms that IMN is a rather organ specific autoimmune disease.

### FR-PO580

**Invariant Natural Killer T Cells Are Depleted in Renal Impairment and Recover after Kidney Transplantation** Konrad Peukert,[1] Margret Patecki,[1] Stephan Wagner,[2] Roland Schmitt,[1] Anke Schwarz,[1] Hermann G. Haller,[1] Sibylle Von Vietinghoff.[1] *[1]Internal Medicine, Hannover Medical School, Hannover, Germany; [2]PHV Dialysis Centre, Germany.*

**Background:** Altered immune function in patients with renal failure results in both susceptibility to infection and increased inflammatory response. Invariant natural killer T (*i*NKT) cells are a conserved, immunoregulatory T lymphocyte subset that responds to lipid antigens with near-immediate cytokine production and cytotoxicity. *i*NKT cells are required for antibacterial host response. Whether chronic renal failure and renal replacement therapy alter *i*NKT cell abundance or phenotype has not been investigated.

**Methods:** NKT cells were studied by flow cytometry in peripheral blood of patients with acute renal failure, chronic hemo- and peritoneal dialysis, chronic kidney disease and after renal transplantation.

**Results:** A very marked reduction in NKT lymphocytes was found in acute renal failure before the first hemodialysis session.*i*NKT cells were depleted in peripheral blood of hemodialysis and peritoneal dialysis patients. This phenotype was accentuated after a hemodialysis session. Lesser degrees of *i*NKT cell depletion were observed in patients with chronic kidney disease. The *i*NKT cell phenotype was altered with lower levels of CD56 and CD161 expression. The decrease in *i*NKT cells and CD56 and CD161 expression were reverted within the first year after kidney transplantation.

**Conclusions:** We describe for the first time that *i*NKT lymphocytes are reduced in end-stage renal disease and further depleted by renal replacement therapy. *i*NKT cells are important for early host response including activation of other immune cells and their depletion may contribute to immune dysfunction in renal disease.

*Funding:* Government Support - Non-U.S.

### FR-PO581

**A κ-Opioid Receptor Agonist, Identified by a High-Throughput Chemical Screen, Blocks Neutrophil FcγRIIA Mediated Functions** Hiroshi Nishi,[1] Hiroaki Iwata,[2] Ralf J. Ludwig,[2] Detlef Zillikens,[2] Tanya N. Mayadas.[1] *[1]Dept of Pathology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA; [2]Dept of Dermatology, Univ of Lübeck, Lübeck, Germany.*

**Background:** Inflammation mediated by antibody-antigen immune complexes (ICs) contributes to autoimmune diseases. Studies in mice expressing the uniquely human FcγRIIA selectively on neutrophils that lack endogenous murine FcγRs (γ-chain deficient), suggest that human FcγRIIA on neutrophils represent a critical link between IgG and tissue injury in models of glomerulonephritis, arthritis and the reverse passive Arthus (RPA) reaction. Yet, there is little understanding of how this human receptor is regulated.

**Methods:** A high-throughput chemical screen was employed using 8,483 bioactive small molecules and neutrophils from mice expressing the human FcγRIIA. The effect of top-ranked compounds on IC-induced reactive oxygen species generation, phagocytosis, degranulation and adhesion was evaluated *in vitro*. Compound effect on IgG mediated tissue injury in animal models was also assessed.

**Results:** A synthetic κ-opioid receptor (KOR) agonist was identified that potently blocked FcγRIIA-mediated neutrophil cytotoxic functions in a dose dependent manner, while cell viability and responses to the phorbol-myristate-acetate were unaffected. Similar results were obtained with dynorphin A, an endogenous ligand of KOR. The KOR agonist was effective in blocking skin separation in an *ex vivo* model of epidermolysis bullosa acquisita and *in vivo*, inhibited the skin RPA reaction and K/BxN serum transfer arthritis. The effect of the KOR agonist was relatively selective for FcγRs, as it did not prevent thrombohemorrhagic vasculitis, which depends on the complement receptor Mac-1 (CR3). Current studies are evaluating the effect of this compound in glomerulonephritis.

**Conclusions:** A KOR agonist and ligand affects the proinflammatory functions of neutrophils activated with ICs. This raises the possibility that receptor targets of this class of opioids calibrate neutrophil responses to IgG and may be clinically beneficial in human neutrophil FcγRIIA-mediated inflammatory diseases.

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*Funding:* NIDDK Support, Private Foundation Support

### FR-PO582

**Development of an Immunosuppressive Adverse Effects Index: Validity and Reliability Evaluation** Calvin J. Meaney,[1,2] Ziad Arabi,[3] Rocco C. Venuto,[3] Joseph D. Consiglio,[4] Gregory E. Wilding,[4] Kathleen M. Tornatore.[1,2] *[1]Immunosuppressive Pharmacology Research Program, NYS Center of Excellence in Bioinformatics and Life Sciences; [2]Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences; [3]Medicine, Nephrology, School of Medicine and Biomedical Sciences; [4]Biostatistics, School of Public Health, Univ at Buffalo.*

**Background:** Immunosuppressive regimens (ISR) prescribed in renal transplant recipients (RTR) are complicated by interpatient variability in response and adverse drug effects (AE). Standardized, objective monitoring approaches are not available to evaluate AE of maintenance ISR consisting of calcineurin inhibitors (CNI), either cyclosporine (CYA) or tacrolimus (TAC), with mycophenolic acid (MPA).

**Methods:** An immunosuppressive AE index (index) was developed by transplant nephrologist with multi-disciplinary physician group incorporating objective AE severity in 17 clinical domains using physical exam, patient interview, laboratory and medication evaluations. This index emphasized AE associated with CNI, MPA, and steroids. Stable RTR receiving CYA (n=30) or TAC (n=28) with MPA were assessed with index and AE scores compared. Index evaluation included: face (inspection), content (subject matter expert opinion), and construct (ability to detect known AE) validities. Inter-rater reliability was evaluated with Kappa statistic and intra-class correlation (ICC).

**Results:** Multi-disciplinary index development and application to RTR verifies face validity. Nephrologists (subject matter experts) rated the 17 index domains as 3.1±0.75 of 4 (maximum) for clinical importance providing content validity. This index distinguished 1.75-fold greater gastrointestinal AE in TAC group compared to CYA (p=0.008). This AE difference is established between CNI ISR verifying construct validity. ICC was 0.81 (95% CI: 0.65-0.90) and Kappa statistic 0.68±0.25 for the overall index.

**Conclusions:** This novel immunosuppressive AE index demonstrated validity and reliability in RTR. Incorporation of this AE index into clinical evaluation post-transplantation may enhance therapeutic drug monitoring and quality of life.

*Funding:* NIDDK Support

### FR-PO583

**Urinary Xist Is a Potent Biomarker in Mouse Model of Membranous Nephropathy** Chia-chao Wu,[1,2] Yuh-feng Lin,[3] Shih-Hua P. Lin.[1] *[1]Div of Nephrology, Dept of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [2]Dept and Graduate Institute of Microbiology and Immunology, National Defense Medical Center, Taipei, Taiwan; [3]DDiv of Nephrology, Dept of Medicine, Shuang Ho Hospital, Graduate Institute of Clinical Medicine, Taipei Medical Univ, New Taipei City, Taiwan.*

**Background:** Membranous nephropathy (MN) is one of the most common forms of nephritic syndrome in adults. The key to a substantial improvement of MN treatment is the identification of reliable biomarkers and pathogenic mechanism.Recently, long noncoding RNAs (lncRNAs) are often expressed in a disease stage-specific manner making these molecules attractive biomarker or therapeutic target. Therefore, this study shows for the first time that identification of MN-related lncRNAs in the pathogenesis of experimental MN model.

**Methods:** The membrane nephritis (MN)-related protein-coding genes, long noncoding RNAs and H3 post-translation modifications during the progression of MN including in serum and urine from experimental murine MN model at different stage were collected and checked. The biological effect and molecular mechanism of MN-related long noncoding RNAs in experimental MN model were also investigated using microarray assay, quantitative RT-PCR, and checking genome-wide binding sites in murine kidney by Chromatin isolation by RNA purification-sequencing (ChIRP-seq).

**Results:** The results indicated that Xist and NEAT1 expression levels are significantly up-regulated in experimental MN kidney. Chromatin immunoprecipitation experiments showed that decreased level of H3K27me3 targeting to NEAT1 and Xist promoter region in MN kidneys. Notably, Xist is upregualted in urine from experimental MN mice using quantification RT-PCR. Moreover, Xist are upregulated in renal biopsies with acute injury using array-based databases.

**Conclusions:** In summary, we provide the first evidence that Xist appears to be a potentially useful biomarker for evaluating kidney damage in experimental MN. These results suggested the potential use of Xist urine assay can aid MN diagnosis.

### FR-PO584

**Increased Serum Soluble Urokinase Plasminogen Activator Receptor in HIV Patients Reduces Nephrin Expression in Human Podocytes** Joanna Mikulak,[1] Massimo Alfano,[2] Manuela Nebuloni,[3] Guido Giusti,[4] Pravin C. Singhal,[5] Domenico Mavilio.[1] *[1]Laboratory of Clinical and Experimental Immunology, Instituto Clinico Humanitas, Mailan, Italy; [2]AIDS Immunopathogenesis Unit, San Raffaele Scientific Institute, Milan, Italy; [3]Dept of Biomedical Clinical Sciences, Univ of Milan, Milan, Italy; [4]Dept of Urology, Humanitas Clinical and Research Center, Milan, Italy; [5]Medicine, Hofstra North Shore LIJ Medical School, New York, NY.*

**Background:** Recently increased level of serum soluble form of urokinase-type plasminogen activator receptor (suPAR) has been postulated as one of the putative causes in adults with primary FSGS. In a mouse model, circulating suPAR has been shown to cause foot process effacement, proteinuria and FSGS through the activation of podocyte β3 integrin. We investigated the possible role of suPAR in HIV-associated kidney damage.

**Methods:** The effect HIV-plasma (containing suPAR, from HIV patients) was studied on human podocyte nephrin expression. To confirm the effect of suPAR, HIV-plasma was pre-treated with anti-suPAR antibody and followed by evaluation of its effects on the expression of nephrin and other markers in podocytes. To confirm its role, the direct effect of recombinant suPAR was also studied.

**Results:** HIV-plasma down regulated only nephrin expression (no change in podocin and synaptopodin) in podocytes. This reduction of nephrin expression was specific for suPAR since it was restored after treatment of HIV conditioned plasmas with the specific anti-suPAR antibody. Similar effect was observed in human podocytes with human recombinant suPAR protein. Nephrin loss was related to nephrin mRNA downregulation and was irreversible. In addition, the effect of suPAR on nephrin reduction was specific for the full length three-domain $D_I D_{II} D_{III}$ protein and not for cleaved $D_I$ or $D_{II} D_{III}$ soluble fragments.

**Conclusions:** Both recombinant and HIV-plasma suPAR were able to downregulate the expression of nephrin in human podocytes. HIV infection may be the potential factor to link increased suPAR concentration and glomerular permeability.

*Funding:* NIDDK Support

## FR-PO585

**Identification of a Human Podocyte Subpopulation Expressing Dc-Specific ICAM-3-Grabbing Nonintegrin Receptor (Dc-Sign)** Joanna Mikulak,[1] Guido Giusti,[2] Stefano Mantero,[3] Manuela Nebuloni,[4] Pravin C. Singhal,[5] Domenico Mavilio.[6] *[1]Laboratory of Clinical and Experimental Immunology, Instituto Clinico Humanitas, Milan, Italy; [2]Dept of Urology, Humanitas Clinical and Research Center, Milan, Italy; [3]UOS/IRGB, CNR, Milan, Italy; [4]Humanita Clinical and Research Center, Milan, Italy; [5]Pathology Uni, Univ of Milan, Milan, Italy; [6]Medicine, Hofstra North Shore LIJ Medical School, New York, NY.*

**Background:** HIV-associated Nephropathy is one of the clinical manifestations of AIDS. Our previous study in human podocytes has demonstrated the contribution of Dc-sign in HIV-1 internalization and establishment of virus reservoir. Since Dc-sign receptor plays a critical role in HIV pathogenesis, we attempted to confirm these findings *in vivo* as well as in prmary podoytes.

**Methods:** Frozen human renal tissues from nephrectomized specimens were immunolabeled for Dc-sign and examined under a confocal microscope; mRNA hybridization probe was also used to localize Dc-sign. In addition, glomeruli were isolated by laser-manipulated micro-dissection and a laser pressure catapulting methods. TaqMan Q-PCR assays were carried out to identify podocyte specific molecular marker transcripts. Primary podocytes cultured from the isolated glomeruli (human renal tissues) were co-labeled for Dc-sign and podocyte specific molecular markers.

**Results:** Twenty percent of glomeruli displayed expression of Dc-sign under confocal microscopic analysis. Both in situ hybridization studies and specific anti-Dc-sign mRNA probe exhibited comparable results. TaqMan Q-PCR assays on captured glomeruli displayed podocyte specific mRNA transcripts not only for nephrin, podocin, synaptopodin, WT-1 but also for Dc-sign. In vitro studies, TaqMan Q-PCR assays on podocytes, confirmed the expression of nephrin, podocin, synaptopodin, WT-1, and DC-sign. Additionally, confocal microscopy revealed that a small percentage of cultured podocytes also express Dc-sign. The cells expressing Dc-sign receptor also co-expressed other podocyte specific markers.

**Conclusions:** These findings indicate that a small sub-population of podocytes able to express Dc-sign receptor.

*Funding:* NIDDK Support

## FR-PO586

**Angiotensinogen Copies Induce Disparate Effects during the Initiation and Progression of HIV-Associated Nephropathy (HIVAN)** Xiqian Lan,[1] Ankita Sagar,[1] Partab Rai,[1] Guohua Ding,[3] Ashwani Malhotra,[1] Praveen N. Chander,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, New York, NY; [2]Pathology, New York Medical College, Valhalla, NY; [3]Medicine, Wuhan Univ, Wuhan, Hubei, China.*

**Background:** Ang II contributes to the progression of HIVAN through its hemodynamic effects as well as direct cellular effects. We evaluated the effect of different copies of angiotensinogen (Agt) in the initiation and progression of HIVAN in genetically engineered HIVAN mice (Tg26).

**Methods:** Control and Tg26 mice with 2 (Tg26/Agt-2) and 4 (Tg26/Agt-4) copies of Agt were evaluated for severity of renal lesions, arteriosclerosis and hypertension at 8 weeks (Tg26/Agt-2/8wks and Tg26/Agt-4/8wks) and 16 weeks (Tg26/Agt-2/16wks and Tg26/Agt-4/16wks). To score collagen deposition, renal cortical sections were stained with sirus red. RNA was extracted from renal tissues and probed for AT1, AT2, PAI-1, and molecules involved in Tert and epithelial mesenchymal transition (EMT) pathways.

**Results:** Tg26/Agt-4/8wks showed lower blood pressure (110/80 mm Hg) when compared to mice with Tg26/Agt-2/8 wks (140/90 mm Hg). While Tg26/Agt-4/16wks displayed higher blood pressure compared with Tg26/Agt-2/16wks. Tg26/Agt-4/8wks displayed attenuated expression of PAI-1 vs Tg26/Agt-2/8wks; however, Tg26/Agt-4/16wks showed 3-fold greater PAI-1 expression than to Tg26/Agt-2/16wks. Tg26/Agt-2/8wks developed renal lesions which were more advanced than Tg26/Agt-4/8wks. On the other hand, Tg26/Agt-4/16wks displayed more advanced renal lesions when compared to Tg26/Agt-2/16wks. Tg26/Agt-4/8wks displayed attenuated expression of AT1 and AT2 and down regulation of Tert, TGF-β, Snail, and vimentin when compared to Tg26/Agt-2/8wks. However, all these markers were comparable between these groups at 16 wks of age.

**Conclusions:** Tg26/Agt-4 displayed slowed progression of HIVAN at early time periods because of relatively low blood pressure and attenuated expression of AT1, TGB-β, PAI-1, Tert and markers of EMT. However, Tg26/Agt-4 at 16 wks displayed accelerated growth because of high blood pressure and catching up with EMT and profibrotic molecules.

*Funding:* NIDDK Support

## FR-PO587

**Pericytes Are Critical Innate Immune Response Sentinels in the Kidney** Shunsaku Nakagawa, Julia Lichtnekert, Gabriela Campanholle, William Arthur Altemeier, Jeremy Stuart Duffield. *Dept of Medicine, Univ of Washington, Seattle, WA.*

**Background:** In recently published studies we showed kidney pericytes markedly activate innate immune pathway in response to kidney injury in vivo, that targeted therapies against pericytes are anti-inflammatory and that Toll-like receptor (TLR)-2/-4 and MyD88 signaling was involved with activation of inflammation and fibrosis after ischemia-reperfusion injury (IRI), but that MyD88 signaling in the myeloid lineage had little impact on macrophage activation or disease progression after IRI (Campanholle et al PLoS ONE 2013). In the following we have dissected innate immune responses to sterile kidney injury in pericytes.

**Methods:** Pericytes were isolated from normal kidney of wild-type, *Tlr2-4-/-* and *Myd88-/-*, *Nlrp3-/-* and *Caspase 1-/-* mice and primary cultures generated. Crude DAMPs were purified and characterized from IRI kidneys 24h after surgery. After stimulation with TLR ligands or DAMPs, pericyte cultures were analyzed for cytokine secretion and functional responses.

**Results:** Stimulation of TLR 1/2, 2/6, 3, 4, 7 and 9 in pericytes resulted in secretion of a broad array of pro-inflammatory cytokines and chemokines including IL-6 and MCP-1. Myd88 deficiency in pericytes attenuated these responses. DAMPs from IRI kidney activated TLR-2/-4, Myd88 signaling inkidney pericytes, but not primary tubular epithelial cells and vascular endothelial cells. Nuclear protein histones were identified as a major component DAMPs. DAMP-mediated pericyte activation resulted in secreted factors including IL-6, MCP-1 but also IL-1b, indicative of activation of inflammasome signaling. These responses were dependent on TLR-2/-4, Myd88 pathway, and the NLRP3 inflammasome. Finally transition of pericytes to myofibroblasts requires active MyD88 signaling.

**Conclusions:** Pericyte activation by DAMPs is a critical step in inflammation, recruitment of leukocytes, myofibroblast transition and progression of sterile kidney injury.

*Funding:* NIDDK Support

## FR-PO588

**Kidney CD103+ DCs Exacerbate Renal Injury through Activating CD8+ T Cells in Adriamycin Nephropathy** Qi Cao, Guoping Zheng, David C. Harris. *Centre for Transplant and Renal Research, Westmead Millennium Institute, The Univ of Sydney, Sydney, NSW, Australia.*

**Background:** CD103+ DCs, a newly described subset of DCs, display two distinct functions: induction of regulatory T cells and activation of CD8+ T cells by cross presentation of antigen. These have been demonstrated in diseases of lungs, intestine and skin. However, the characteristics and functions of CD103+ DCs in kidney remain unclear.

**Methods:** Adriamycin nephrosis (AN) was induced in BALB/c mice. The distribution, phenotype and *in vitro* function of kidney CD103+ DCs were assessed in normal and AN mice. CD103+ DCs were depleted by neutralizing CD103-saporin (SAP) antibody in AN mice to examine their role *in vivo*.

**Results:** CD103+ DCs were identified in kidney as CD45+/MHC-II+/CD11c+/CD103+/F4/80-/CD11b- cells. CD103+ DCs were distributed predominantly in cortex of normal and AN kidney. The number of CD103+ DCs was significantly increased in kidney of AN mice compared to that of normal mice. Depletion of kidney CD103+ DCs by CD103-SAP antibody improved renal function in AN mice, as evidenced by a decrease in proteinuria & serum creatinine and increase in creatinine clearance. AN mice treated with CD103-SAP antibody also had less glomerulosclerosis, tubular atrophy and interstitial expansion than did AN control mice. The possible mechanisms underlying the pathogenic role of CD103+ DCs were examined. Kidney CD103+ DCs expressed high levels of IL-6in AN mice, but not other inflammatory cytokines including IL-1beta, IL-12, IFN-g, TNF-α and MCP-1. The co-stimulatory molecules CD80, CD86 and B7-H1 were highly expressed in kidney CD103+ DCs in AN mice compared to those of normal mice. Kidney CD103+ DCs displayed higher capability of cross-presenting antigen to CD8+ T cells than did CD103- DCs.

**Conclusions:** CD103+ DCs are present in kidney and induce renal injury in AN mice. The mechanism underlying the pathogenic role of CD103+ DCs in AN mice may relate to their ability to activate CD8 T cells.

*Funding:* Government Support - Non-U.S.

## FR-PO589

**Characterization of Innate Lymphoid Cells in the Kidney** Jan-Eric Turner,[1] Rolf A. Stahl,[1] Ulf Panzer.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Div of Molecular Immunology, MRC National Insitute for Medical Research, London, United Kingdom.*

**Background:** Innate lymphoid cells (ILC) are a recently identified cell type that is characterized by production of large amounts of the T helper cell cytokines IL-22, IL-17A, IFN-g, as well as IL-5 and IL-13 during immune responses. As cells of the innate immune system, ILC are activated independently from antigen directly by cytokine signals. So far ILC have mainly been studied in barrier organs, such as the gut, lung and skin.

**Methods:** We used multicolor flow cytometry to identify and characterize this novel cell type in the kidney of naïve and nephritic mice (nephrotoxic nephritis) for the first time.

**Results:** Lineage-negative Thy1.2$^{+}$IL-7R$^{+}$ ILC comprised around 1% of total renal lymphocytes in naïve mice. These renal ILC displayed expression of the surface markers IL-33R, CD25 and Sca-1, and of the transcription factor GATA3, identifying them as IL-13 and IL-5-producing type 2 ILC (ILC2). Furthermore, we could show that repetitive injections

of the ILC2-activating cytokine IL-33 expanded renal ILC2 in naïve and nephritic mice, resulting in a shift to a protective type 2 immune response in the kidney during a mouse model of crescentic glomerulonephritis. In line with this, the course of the disease was ameliorated in IL-33 treated mice.

**Conclusions:** In conclusion, our data provide first evidence for the presence of ILC in the murine kidney and suggest that modulation of the renal ILC response can be a promising therapeutic strategy, warranting further investigation.

*Funding:* Government Support - Non-U.S.

## FR-PO590

**Antisense Inhibition of Renal CD40 Attenuates Adriamycin-Induced Nephropathy** Adam E. Mullick, Aaron Donner, Karen He, Huimin Li, Gene Hung, Mark Graham, Rosanne M. Crooke. *Isis Pharmaceuticals, Carlsbad, CA.*

**Background:** Adriamycin nephropathy (AN) is a common experimental model of chronic kidney disease resulting in progressive podocyte depletion and tubulointerstitial inflammation, followed by the development of glomerulosclerosis. Studies have demonstrated that blockage of CD40-CD40L via administration of a CD40L antagonistic antibody was effective at mitigating AN. The promise of such inhibitors has been stymied by thromboembolic events in patients receiving CD40L antagonists, resulting in termination of their clinical development. An alternative approach would be to inhibit CD40 via antisense oligonucleotides (ASOs).

**Methods:** To test this concept, AN was established in BALB/c mice by i.v. administration of 10.5 mg/kg adriamycin. Three days afterward treatments of 25 mg/kg/wk of CD40 ASO or a mismatch control ASO were initiated. After 6 wks, kidneys were harvested and histological and transcriptional assessments of disease severity were evaluated. Additionally, kidney function was evaluated by FITC-inulin clearance following a bolus injection.

**Results:** CD40 ASO treatment resulted in an 80% reduction in renal CD40 target mRNA expression and markers of renal injury, NGAL and KIM-1, were reduced in mice receiving CD40 ASO by 65% and 50%, respectively, relative to control ASO treatment. MCP-1 and CCL5, both downstream inflammatory targets of CD40 activation, were also reduced by >50% in renal tissue from CD40 ASO treated mice. The single dose FITC-inulin test revealed a 50% reduction in residual plasma FITC-inulin after 2hrs post-injection in CD40 ASO treated mice relative to control. Glomerular pathology, as assessed in both H&E- and PAS-stained tissues, was markedly attenuated after CD40 ASO treatment.

**Conclusions:** In summary, inhibition of CD40 in established AN mitigated functional, transcriptional and histologic assessments of disease severity suggesting that antisense inhibition of CD40 could be a therapeutically attractive modality for the treatment of renal inflammation in chronic kidney disease.

*Funding:* Pharmaceutical Company Support - Isis Pharmaceuticals

## FR-PO591

**Angiotensin II Receptor Blockers in Experimental Chronic Kidney Disease Model** Sun Moon Kim,[1] Hye-Young Kim,[1,2] Han Ro,[3] Ji Yong Jung,[3] Yon Su Kim.[4] *[1]Internal Medicine, Chungbuk National Univ Hospital; [2]Internal Medicine, Chungbuk National Univ, College of Medicine; [3]Internal Medicine, Gachon Univ of Medicine and Science; [4]Internal Medicine, Seoul National Univ, College of Medicine.*

**Background:** Activation of renin-angiotensin system has a detrimental effect on the progression of chronic kidney diseases (CKD). And blockade of RAS demonstrated clinical efficacy. To clarify the specific molecular events of angiotensin II receptor blockers in CKD, we conducted microarray analyses from subtotally nephrectomized (Nx) rats as a model of CKD.

**Methods:** Nx rats were treated early with vehicle or losartan for 8 weeks. Expression of messenger RNA (mRNA) was assessed by 'GeneChip Micorarray' technology.

**Results:** In contrast to the sham group, vehicle-treated Nx rats show increased blood pressure, albuminuria, serum creatinine, and renal lesions. Losartan therapy preserved renal function, abrogated albuminuria, and glomerular histology. Among the 171 transcripts differentially expressed between vehicle-treated Nx group and losartan group, 59 transcripts were overexpressed in vehicle-treated Nx group. These genes are significantly involved in lysosomal pathway. Two genes had the highest overexpression in Nx group: hexosaminidase A and cathepsin B. The expression of the other 109 genes were reduced in vehicle-treated Nx group compared to losartan group, these genes were significantly involved in cysteine and methionine metabolism, glutathione metabolism, selenoamino acid metabolism, and arginine and proline metabolism.

**Conclusions:** These results show that losartan therapy attenuated renal damage in CKD model and that genes implicated in lysosomal pathway, particularly hexosaminidase A and cathepsin B, may play a role.

## FR-PO592

**Effects of FTY720 on the Tubulointerstitial Inflammation in Rats with Albumin-Overload Nephropathy** Min Xu, Dan Liu, Bi-cheng Liu. *Institute of Nephrology, Southeast Univ, Nanjing, China.*

**Background:** Heavy proteinuria induces expression of inflammatory mediators resulting in tubulointerstitial inflammation. The Sphingosine-1-phosphate (S1P) analogue FTY-720 is a newly developed immunomodulatory drug with low cytotoxicity using myriocin as a lead compound, potently sequesters lymphocytes into lymph nodes by functionally antagonizing the activity of S1P receptors. This study was to investigate the effects of FTY720 on urinary protein excretion and inflammatory cells infiltration in albumin-overload rats.

**Methods:** Male Wistar rat was underwent a right-side nephrectomy, and divided into three groups: 1) saline: rats received daily intraperitoneal injections of saline solution (5g▪kg$^{-1}$▪d$^{-1}$), 2) AO (albumin-overload): induced by intraperitoneal injections of BSA 1week after surgery (5g▪kg$^{-1}$▪d$^{-1}$), and 3) AO+FTY720: FTY720 (0.5mg▪kg$^{-1}$▪d$^{-1}$) was administered concomitant to AO. All rats were killed at 9 week after nephrectomy.

**Results:** Compared with controls, urinary protein excretion increased in AO rats from the second week and increased to the highest level at 7 week, FTY720 significantly reduced these effects. In addition, routine blood test showed that FTY720 treatment attenuated inflammatory cells (including lymphocytes and macrophages) in circulation and immunohistochemical analysis showed that the number of inflammatory cells in the tubulointerstitium was decreased by FTY720. Western blot and RT-PCR analysis showed that the increase in gene and protein expression of chemokines MCP-1, TNF-α, IL-6, IL-10, arginase-1 and S1P receptors (S1P1 and S1p3 receptor) in kidney of albumin-overload rats was also decreased by FTY720. Furthermore, the expression of the S1P-synthesizing enzymes-Sphk1 was elevated in albumin-overload rats (according to the immunohistochemical analysis).

**Conclusions:** S1P may have a chemotatic effect via the S1P receptors on inflammatory cells and play a casual role in tubulointerstitial inflammation of albumin-overload nephropathy. FTY720 may function as an antagonist on S1P1 and S1P3 receptors to alleviate tubulointerstitial inflammation and proteinuria excretion.

## FR-PO593

**Cmip Is a Negative Regulator of T Cell Activation** Kelhia Sendeyo, Nahid Tabibzadeh, Djillali Sahali. *INSERM, U 955, Equipe 21, Univ Paris-Est Creteil Val-de-Marne, Créteil, France.*

**Background:** Immunopathology of acquired idiopathic nephrotic syndrome (INS) with relapse remains unclear. Active INS is associated with immune system disorders including hyporesponsiveness of lymphocytes to mitogens, decreased delayed hypersensitivity, defects in immunoglobulin switch and unclassical T helper polarization. We recently reported that cmip is overproduced in T cells of patients with INS relapse. To investigate the functional consequences of cmip induction in INS T cells, we generated transgenic mice selectively overexpressing cmip in mature lymphocytes.

**Methods:** Lck-cmip transgenic (Tg) mice were constructed by a targeting system based on reconstitution of functional X-linked hypoxanthine-guanine-phosphorybosyltransferase (HPRT) locus by homologous recombination. The full-length coding sequence of human cmip was inserted under control of the distal Lck promoter allowing cmip expression only in peripheral T-lymphocytes (TL).

**Results:** Tg mice developed an altered TL phenotype with an increase of naïve T cell subpopulation ($CD4^+$ or $CD8^+CD44^{low}CD62L^{high}$), whereas effector ($CD4^+$or $CD8^+CD44^{high}CD62L^{low}$) and memory T cell subpopulations ($CD4^+$ or $CD8^+CD44^{high}$ $CD62L^{high}$) were decreased, as compared with control littermates (CL).

Following CD3/CD28 (1μg/ml each) activation, Tg T cells displayed lower proliferation and decreased production of IFNg (Th1-like cytokine) and IL4 (Th2-like cytokine) relatively to control littermates, while IL10 production was not altered. This cytokine profile was associated with significant inactivation of ZAP70, LAT and Src kinases. Confocal fluorescence microscopy showed that these proteins colocalize in lipid rafts of activated wild-type, but not in activated Tg T cells. Dynamic videomicroscopy showed that overexpression of cmip in T cells prevents raft clustering and inhibits formation of immunological synapse in the presence of CD3 beads and soluble CD28.

**Conclusions:** Altogether, these results suggest that cmip interferes with T cell activation and may contribute to some T cell disorders including hyporesponsiveness of lymphocytes to mitogens reported in INS relapse three decades ago.

*Funding:* Government Support - Non-U.S.

## FR-PO594

**Down Regulation of Dendritic Interleukin-15 Production and Interleukin-15-Dependent Cytotoxic T-Cell Proliferation by Adenosine** Amos Douvdevani,[1] Hadar Eini,[1] Moshe Zlotnik.[2] *[1]Clinical Biochemistry and Pharmacology, Soroka Medical Center and Ben-Gurion Univ of the Negev, Beer-Sheva, Israel; [2]Nephrology, Soroka Medical Center, Beer-Sheva, Israel.*

**Background:** Dendritic cells (DCs) regulate the immune response through production of various cytokines and signaling molecules, among which is IL-15, an important NK and cytotoxic T-cell (CTL) activator and survival factor. At the site of immune reaction, adenosine is produced from ATP by CD39 and CD73, ecto-enzymes expressed on regulatory T-cells (Treg). The aim of this present study was to examine the effect of adenosine on IL-15 production by DCs and the implication of this effect on CTL proliferation.

**Methods:** Bone marrow derived DCs (BMDCs) were isolated from ICR mice. BMDCs were exposed to various adenosine receptor agonists/antagonist prior to stimulation with LPS and interferon-γ (IFN-γ). Levels of cAMP, IL-15 and IL-15 receptor α chain (IL-15Rα) were measured using QPCR and/or ELISA. To assess the effect on CTL proliferation BMDCs were gamma-radiated and co-cultured with CTLL-2, a cytotoxic T-cell line, in the presence or absence of adenosine.

**Results:** Stimulation of BMDCs with LPS and IFN-γ increased IL-15 and IL-15Rα levels. Treatment of stimulated BMDCs with adenosine significantly reduced IL-15/IL-15Rα mRNA and protein levels. This down-regulatory effect was blocked by the combination of ZM 241385, an $A_{2A}R$ antagonist, and MRS1754, an $A_{2B}R$ antagonist, while addition of only one of them separately yielded a partial inhibitory effect. Treatment of

BMDCs with adenosine receptor agonists increased cAMP levels, and di-butirilic-cAMP, a stable cAMP analog, effectively reduced IL-15Rα levels. Finally, treatment of BMDCs with adenosine, before stimulation and co-culture, significantly reduced PBMC-dependent CTLL-2 proliferation.

**Conclusions:** Our data indicate that adenosine acting through $A_{2A}R$ and $A_{2B}R$ by elevation of cAMP suppresses IL-15 and IL-15Rα levels in activated DCs and restrains CTL proliferation. These finding can set a pharmacological base for CTL dependent pathologies such as graft rejection treatment.

*Funding:* Private Foundation Support

## FR-PO595

**Delayed Treatment with Adenosine 2a Receptor Agonist Protects Kidneys from Long-Term Fibrosis** Isaah Vincent,[1] Diane L. Rosin,[2] Li Li,[1] Liping Huang,[1] Hong Ye,[1] Mark D. Okusa.[1] *[1]Dept of Nephrology, Univ of Virginia, Charlottesville, VA; [2]Dept of Pharmocology, Univ of Virginia, Charlottesville, VA.*

**Background:** Adenosine, released from inflamed tissues, accumulates and signals through its four G-protein-coupled receptor subtypes; activation of the adenosine 2a receptor (A2AR) on immune cells has potent tissue protective/anti-inflammatory effects following kidney ischemia-reperfusion injury (IRI).

**Results:** Using a folic acid (FA) -induced fibrosis model, we tested the hypothesis that A2AR signaling *after* the initial injury blocks kidney fibrosis. We see a large hematopoetic infiltrate into the kidneys after FA injection, with a large infiltrate of CD11b+CDllc-Ly6g- monocytes/macrophages and CD11b+CDllc-Ly6g+. neutrophils (determined by FACS analysis). This infiltrate is diminished by day 5. Tubulointerstitial fibrosis was evident in kidneys at five days after FA injection. Continuous treatment with A2aR agonist, ATL313 (1 ng/kg/min) beginning on day 1 or 3 attenuated fibrosis (trichrome staining or fibronectin immunoreactivity) on day 14. Continuous treatment of mice beginning on day 5, after much of the initial inflammation has subsided, reduced fibrosis by approximately 30% (by stereological analysis of trichrome staining and fibronectin deposition). Continuous treatment with the A2aR antagonist, ZM243185 (5 ng/kg/min), beginning on day 5 alone did not alter fibrosis, indicating endogenous adenosine is not sufficient to block fibrosis.

**Conclusions:** These results suggest that induction of A2aR signaling may reduce fibrosis either by limiting the initial inflammation or alter the repair process. We are currently examining mechanisms by which A2AR signaling diminishes fibrosis in response to FA and we are testing the effect of A2aR activation in 2 other models of fibrosis (postischemic fibrosis and aristolochic acid). These results extend our pharmacological approach to target fibrosis following acute kidney injury.

*Funding:* NIDDK Support

## FR-PO596

**A Pro-Inflammatory Change in Numbers of Monocyte and Dendritic Cell Subtypes Occurs Early in the Course of CKD** Eva Schepers, Griet Lrl Glorieux, Nathalie Neirynck, Annemieke Dhondt, Raymond C. Vanholder. *Internal Medicine, Nephrology, Univ Hospital Ghent, Ghent, Belgium.*

**Background:** Antigen-presenting cells play an important role in chronic inflammation associated with accelerated cardiovascular disease and immune dysfunction. Both monocytes and dendritic cells consist of 3 subpopulations: monocyte subtypes: CD14++CD16-, CD14++CD16+, and CD14+CD16++; and the subtypes of circulating DCs: myeloid DC1 (mDC1), mDC2 and plasmacytoid DC (pDC). The CD14++CD16+ monocytes are described as pro-inflammatory and for DCs the mDCs have antimicrobial activity while pDCs are mediators of antiviral immunity. No study up till now evaluated changes of these cell types in all stages of CKD.

**Methods:** This study for the first time describes the proportional distribution of monocytes and circulating DC populations in 198 patients throughout the consecutive stages of CKD and/or on renal replacement therapy in comparison to healthy controls. Flow cytometric analysis on whole blood was performed. Monocytes were identified based on the pan-monocytic CD86 and the subpopulations were distinguished based on CD14-CD16 expression. For the DCs, an enumeration kit was applied. Both absolute number and percentage of the total leukocyte population were evaluated.

**Results:** Although no difference in total monocytes was observed vs controls, a significant increase of CD14++CD16+ monocytes was found from CKD stage 3 on, which rises further up to a 3-fold rise in HD patients. Only in HD patients a significant decrease in number and percentage of CD14++CD16- cells combined with an increase in CD14+CD16++ monocytes was found. In PD patients no changes were observed. The DCs were decreased, by more than 25% from CKD1 on which was mainly due to a decrease in pDC. The PD group was the only population in which also the number and percentage mDC1 was decreased. An inverse correlation could be found between the CD14++CD16+ and the pDCs throughout the consecutive stages.

**Conclusions:** An increase in CD14++CD16+ monocytes and a decrease in the pDC suggest a pro-inflammatory role for these cells during progression of CKD. This points to the importance of studying these leukocyte subtypes more in depth in different stages of CKD.

## FR-PO597

**Determinants of Sclerostin Concentration in Hemodialysis Patients** Pierre Delanaye, Jean-marie H. Krzesinski, Xavier Warling, Nicole Simone Smelten, Etienne Cavalier. *Nephrologgy-Dialysis, Univ of Liege.*

**Background:** Sclerostin is an inhibitor of bone formation produced by osteocytes. A recent study showed that sclerostin was negatively associated with parathormone (PTH) levels and better predicted high bone turnover. Little is known about the other physiologic determinants.

**Methods:** Among the hemodialysis patients in 3 centers in the area of Liège, Belgium (n=212), we analyzed the results from 165 patients with vascular calcification score (Kauppila method). Sclerostin was available in 164 patients. The following clinical data were considered: age, gender, height, weight, dialysis vintage, hypertension status, diabetes, smoking and previous cardiovascular disease. The following laboratory data were considered: calcium, phosphorus, PTH, 25-OH vitamin D, albumin, troponin T and CRP. Different bone biomarkers were measured: bone-specific alkaline phosphatase(b-ALP), C-terminal telopeptide of collagen type I(CTX), intact amino-terminal propeptide of type I procollagen (PINP), tartrate-resistant acid phosphatase 5b(TRAP-5b) and osteoprotegerin(OPG). Sclerostin was measured with the TECO ELISA (TECO medical, Sissach, CH).

**Results:** The mean age of our population was 71± 14 y (44% of men). Mean sclerostin concentration was 1500±710 pg/mL. In univariate regression analysis, we found a significant positive relationship between sclerostin and age, height, dialysis vintage, albumin, troponin T and OPG . A significant negative relationship was found with PTH, CTX, P1NP and b-ALP. In multivariate analysis, age, height, phosphorus, albumin, troponin T, PTH and b-ALP were still associated with slerostin. Patients in the lower tertile of sclerostin were younger, smaller and had a lower dialysis vintage than those in the third tertile. They had lower levels of albumin, troponin, OPG and higher levels of PTH, CTX, TRAP-5b and b-ALP.

**Conclusions:** We confirm that the concentrations of sclerostin are high in hemodialysis patients. We did not find any association between sclerostin and calcification score. Like others, we found an inverse association between sclerostin and PTH or b-ALP levels. Interaction between sclerostin and albumin or troponin levels would deserve additional studies.

## FR-PO598

**Dephosphorylated-Uncarboxylated Matrix Gla Protein Concentration Is Predictive of Vitamin K Status and Correlated with Vascular Calcification in Hemodialysis Patients** Pierre Delanaye, Jean-marie H. Krzesinski, Xavier Warling, Nicole Simone Smelten, Etienne Cavalier. *Nephrology-Dialysis, Univ of Liege, Belgium.*

**Background:** Matrix Gla protein (MGP) is a potent local inhibitor of vascular calcification. MGP must benefit from a vitamin K-dependent carboxylation. The dosage of the inactive form (uncarboxylated and dephosphorylated: dp-ucMGP) could reflect the status of vitamin K. dp-ucMGP could also be correlated with vascular calcification.

**Methods:** Among the hemodialysis patients in 3 dialysis centers in Liège, Belgium (n=212), we analyzed the results from 165 patients with vascular calcification score (Kauppila method). 5 patients do not have MGP concentrations measured. We compared 23 patients treated with anti-vitamin K and 137 patients without this therapy. The following clinical data were considered: age, gender, BMI, dialysis vintage, hypertension status, diabetes, smoking and previous cardiovascular disease. Following laboratory data were utilized: calcium, phosphorus, PTH, 25-OH vitamin D, bone alkaline phosphatase, albumin, and CRP. dp-ucMGP was quantitated with the automated iSYS system (IDS, Boldon, UK).

**Results:** No difference was observed between patients treated or not with anti-vitamin K regarding the clinical characteristics. We observed significant differences for dp-ucMGP (5549±2230 pM vs. 2217±1134 pM, $p < 0.0001$) and albumin (40 mg / L [38, 41] versus 38 g / L [36, 40] $p = 0.048$). In 137 patients without anti-vitamin K therapy, a slight but significant correlation was found between dp-ucMGP levels and the calcification score ($r=0.17$, $p=0.04$). Considering the tertiles of dp-ucMGP (1647 et 2404 pM), subjects in the high tertile had a significant higher vascular calcification score than those in tertile 1: 9 ± 6 versus 11 ± 6 ($p=0.0414$). Tertile 3 comprised of more men whom had a higher BMI and lower albumin levels.

**Conclusions:** We confirm that the concentration of dp-ucMGP is higher in dialysis patients treated with anti-vitamin K. This confirms the interest of the dp-ucMGP as a marker of vitamin K status. We found an association between the concentration of dp-ucMGP and the level of vascular calcifications.

## FR-PO599

**Sclerostin and DKK-1 Levels across CKD Stages** Geert J. Behets,[1] Liesbeth Viaene,[2] Frank A. Blocki,[3] Patrick C. D'Haese,[1] Pieter Evenepoel.[2] *[1]Univ of Antwerp, Antwerp, Belgium; [2]Catholic Univ Leuven, Leuven, Belgium; [3]DiaSorin Inc, StillWater, MN.*

**Background:** Canonical Wnt pathway inhibitors, Sclerostin and Dickkopf-1 (DKK-1), suppress bone formation. In dialysis patients, serum sclerostin levels correlate positively with bone mineral density and bone volume, and negatively with bone turnover.

**Methods:** In a cross-sectional, observational study we evaluated 169 pre-dialysis (CKD stage 1-5), 98 hemodialysis (HD), 62 peritoneal dialysis (PD) and 78 apparently healthy subjects (HC). Serum sclerostin and DKK-1 were measured by ELISA. Other parameters included 1-84 PTH and FGF-23.

**Results:**

| | HC | CKD 1-2 | CKD 3 | CKD 4-5 | HD + PD |
|---|---|---|---|---|---|
| Age | 54±18 | 45±15 | 64±15 | 67±11 | 68±13 |
| Male (%) | 41 | 32 | 55 | 70 | 60 |
| Sclerostin (pmol/l) | 43.8±13.0° | 28.1±13.7° | 66.1±33.7° | 99.5±45.9° | 116.8±62.3* |
| PTH (pg/ml) | 48.8±29.6° | 25.4±17.7° | 38.5±21.6° | 105.4±88.9° | 231.7±218.4* |
| DKK-1 (pmol/l) | 65.9±21.3° | 41.1±14.0* | 40.7±16.0* | 33.2±13.1* | 37.8±25.8* |
| FGF-23 (pg/ml) | 46±14° | 40±17° | 75±34° | 513±1926° | 5860±5461* |
| Bone Alkaline Phosphatase (U/l) | 19.4±15.7° | 23.0±14.6 | 23.6±10.1 | 31.0±28.8 | 35.3±26.9* |
| *: p<0.001 vs HC; °: P<0.001 vs HD+PD | | | | | |

Interestingly, while the HC group displayed no significant correlation between PTH and sclerostin, the CKD group showed a positive correlation (r=0.272, p<0.001) whilst both dialysis groups showed a negative correlation (r=-0.256, p<0.01 in HD; r=-0.271, p<0.05 in PD). Furthermore, a strong negative correlation with eGFR was found in the CKD group (r=-0.616, p<0.001). DKK-1 levels correlated weakly (r=-0.129, p<0.05) in the whole study population, losing significance when parsed by sub-groups. Sclerostin correlated strongly with FGF-23 in the HC group(r=0.569, p<0.001), weaker in the CKD group (r=0.217, p<0.01), while the HD group showed no correlation.

**Conclusions:** Sclerostin levels increase along with renal failure progression and correlate positively with PTH in CKD and negatively in dialysis patients. The role and mechanism of the increased sclerostin levels in renal failure remains to be clarified.

*Funding:* Pharmaceutical Company Support - Diasorin Inc.

## FR-PO600

**Effects of a Magnesium (Mg) Based Phosphate (P) Binder on Bone Metabolism in a Rat Model of Chronic Renal Failure (CRF)** Geert J. Behets,[1] Ellen Neven,[1] Kristina Gundlach,[2] Sonja Steppan,[2] Patrick C. D'Haese.[1] *[1]Univ of Antwerp, Antwerp, Belgium; [2]Fresenius Medical Care, Bad Homburg, Germany.*

**Background:** We have previously shown that the Ca-Mg based P-binder Osvaren (CaMg) at doses of 375 and 185 mg/kg/day effectively reduces serum P levels and vascular calcification (VC) in adenine-induced CRF rats . However, due to the severe CRF and resulting hyperparathyroid bone (HPT) disease, this model is not suited to measure dynamic bone parameters. In the current study a less severe CRF model (5/6$^{th}$ Nx) was used to evaluate the possible effect of CaMg on bone.

**Methods:** Six groups of male Wistar rats (N=12 each) underwent a 5/6$^{th}$ Nx. Three groups received a normal P diet (0.8 %; NP) and 3 groups a high P (1.03%; HP) diet. This approach was used to differentiate between possible bone effects resulting from P-deficiency vs. a direct Mg-effect. CaMg (185 and 375 mg/kg/day) was administered orally for 8 weeks.

**Results:** Serum creatinine levels indicated a mild CRF developed 2 weeks after 5/6$^{th}$ Nx (0.48±0.16 vs 1.17±0.33 mg/dl, p<0.05), which remained stable throughout the treatment period (1.51±0.90 mg/dl after 8 weeks). Serum total and ionized Ca levels and serum P levels did not differ between groups. PTH levels were ± 4-5 times higher at week 8 compared to baseline (p<0.05) pointing to a mild HPT. After 8 weeks, only the NP 375 group showed a significant decrease in urinary P excretion (11.1±2.7 vs 52.6±6.7 mg/24h, p<0.05), while it remained stable in the other groups. Bone and serum Mg levels were not different between groups. The NP 375 group showed a (statistically non-significant) increase in osteoid area (OA) versus vehicle (2.80±1.76 vs 0.76±0.030%, p=0.28). In the HP groups, no differences between treatments were found. Osteoblast and osteoclast perimeters as well as mineralization parameters (bone formation rate, mineralization lag time, adjusted apposition rate) showed no differences between groups.

**Conclusions:** In this study, CaMg did not show any direct effect on bone metabolism. The observed slight increase in OA in the NP 375 group is due to a relative P depletion rather than a direct effect of Mg.

*Funding:* Pharmaceutical Company Support - Fresenius Medical Care, Germany

## FR-PO601

**PTH, FGF-23, Sclerostin and Bone Mineral Density in End-Stage Renal Disease** Liesbeth Viaene, Kathleen Claes, Pieter Evenepoel. *Nephrology, Univ Hospitals, Leuven, Belgium.*

**Background:** PTH, and the osteocyte derived hormones FGF23 and sclerostin all play an important role in bone biology. Circulating levels of these hormones increase in chronic kidney disease to reach levels that are many folds higher in patients with end-stage renal disease (ESRD) as compared to healthy controls. The relationship between PTH, FGF23, and sclerostin levels and bone mineral density (BMD) in ESRD patients is incompletely understood.

**Methods:** We performed a cross-sectional study in 268 patients (65% M; age 52±13 years) with ESRD referred for renal transplantation. Parameters of mineral metabolism, including serum biointact PTH, FGF23 (Kainos), sclerostin (Tecomedical), calcidiol, and calcitriol were determined prior to engraftment and all patients underwent dual energy X-ray absorptiometry (DEXA) at lumbar spine (LS), total hip (TH) and forearm (1/3R) within 2 weeks after the transplant procedure. DEXA results are expressed as BMD (g/cm$^2$) and T-scores.

**Results:** Serum levels of biointact PTH (130 [67-2377] ng/l), FGF23 (1853 [554-5814] pg/ml), and sclerostin (1.15 [0.79-1.51] ng/ml) were high and as expected in ESRD and did show significant inter-correlations. T-scores less than -2.5 were observed in 19, 8, 29 % at LS, TH and 1/3R respectively. In univariate analysis, biointact PTH (inversely), FGF23 (directly), and sclerostin (directly) correlated with BMD at LS (p<0.05), TH (p<0.05) and 1/3R (p =0.06, FGF23 only). Adjustment for classical BMD determinants (age, gender and vitamin D status) and dialysis vintage only attenuated associations for sclerostin and PTH. Regarding FGF23, significance was lost at TH but reached at 1/3R.

**Conclusions:** High sclerostin and FGF23 levels, as opposed to PTH, associate directly with BMD in ESRD. Whether sclerostin and FGF23 are part of a homeostatic feedback loop aimed at preventing excessive mineralization or whether these hormones merely reflect high osteocyte number remains to be clarified.

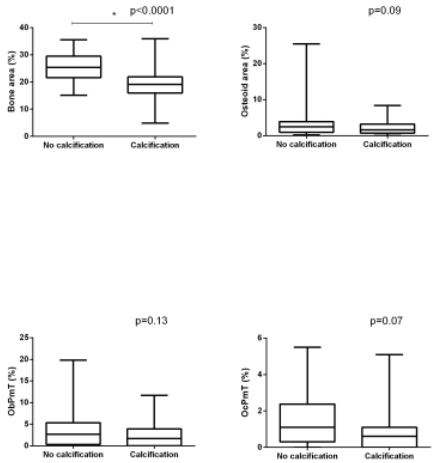
## FR-PO602

**The Bone-Vascular Axis and Inflammation in End-Stage Renal Disease** Liesbeth Viaene,[1] Geert J. Behets,[2] Sam Heye,[3] Kathleen Claes,[1] Diethard Monbaliu,[4] Jacques Pirenne,[4] Patrick C. D'Haese,[2] Pieter Evenepoel.[1] *[1]Nephrology, Univ Hospitals, Belgium; [2]Pathophysiology, Univ of Antwerp, Belgium; [3]Radiology, Univ Hospitals, Belgium; [4]Abdominal Transplantation, KU Leuven, Belgium.*

**Background:** Vascular calcification is a common complication of end stage renal disease (ESRD) and is predictive of subsequent cardiovascular disease and mortality. Mounting evidence linking bone disorders with vascular calcification has contributed to the development of the concept of the bone-vascular axis. Inflammation is involved in the pathogenesis of both disorders. The aim of the present study was to evaluate the relationship between aortic calcification (AC), inflammation, and bone disturbances in patients with ESRD.

**Methods:** Parameters of inflammation and mineral metabolism, AC and bone histomorphometry were assessed in 81 ESRD patients (55±13 year, 68% male) referred for renal transplantation. Static bone histomorphometry parameters such as bone area, and osteoblast- and osteoclast-covered perimeter to total perimeter were determined on transiliac bone biopsies performed during the transplant procedure. AC was quantified on lateral lumbar X-rays using the Kaupilla method.

**Results:** AC, low bone turnover and low bone volume was observed in 60, 37, and 21 % of patients. The association between AC and bone histomorphometric parameters are shown in Figure 1.




Inflammatory markers were found to be independently associated with aortic calcification (hsIL-6) and low bone volume (TNFα). Low bone volume was a significant risk factor for aortic calcification, independent of age, diabetes, and inflammation.

**Conclusions:** Low bone volume, as opposed to turnover, is a significant risk factor for aortic calcification, independent of traditional risk factors. Our data underscore the role of inflammation in the bone-vascular axis in CKD.

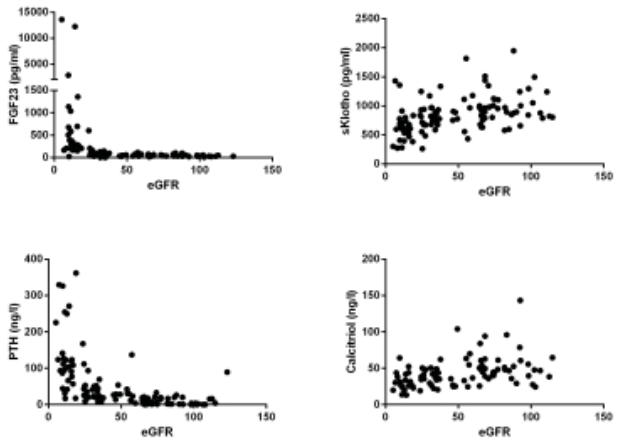
## FR-PO603

**The Protein-Bound Uremic Retention Molecule *p*-Cresyl Sulfate Inversely Associates with Soluble Klotho Levels in Patients with Chronic Kidney Disease** Liesbeth Viaene,[1] Bjorn Meijers,[1] Andreas L. Serra,[2] Pieter Evenepoel.[1] *[1]Nephrology, Univ Hospitals, Leuven, Belgium; [2]Nephrology, Institute of Physiology and Zurich Center for Integrative Human Physiology, Switzerland.*

**Background:** Klotho is a transmembrane protein that is predominantly expressed in the kidney. The extracellular domain is cleaved to generate soluble Klotho in blood, urine and cerebrospinal fluid. Membrane Klotho serves as a coreceptor for FGF23, whereas soluble Klotho functions as a hormonal factor and plays important roles in anti-aging, anti-oxidation, modulation of ion transport, and Wnt signaling. Studies exploring determinants of soluble Klotho levels in CKD patients are scanty. Recent experimental evidence indicates that the protein-bound toxins indoxyl sulfate (IndS) and p-cresyl sulfate (PCS) may suppress Klotho.

**Methods:** In order to identify determinants of soluble Klotho levels in CKD, we performed a cross-sectional observational study. In addition to soluble Klotho (ELISA), mineral metabolism parameters (including, FGF23, calcidiol and calcitriol, C-reactive protein (CRP)) and PCS and IndS were analysed in blood samples of 115 stage 1-5 CKD patients (57 men, 59±15 years, CKD-Epi eGFR 58.2±38.1 ml/min/1.72m²).

**Results:** Mineral metabolism parameters were as expected. Soluble Klotho levels (835 [263-4520] pg/ml) significantly correlated with eGFR and inversely with age, serum CRP, phosphate, FGF23, PTH, PCS and IndS.




Diabetics had significantly lower soluble Klotho levels. In multivariate analysis, PCS remained significantly correlated with soluble Klotho levels, independent of age and eGFR and explaining 13% of its variation.

**Conclusions:** Low eGFR, high age and high p-cresyl sulfate levels are independently associated with low soluble Klotho levels.

## FR-PO604

**Sclerostin: A New Player in the CKD Vascular Disease** Solenne Pelletier,[1] Cyrille B. Confavreux,[2] Julie Haesebaert,[3] Fitsum Guebre-egziabher,[4] Justine Bacchetta,[5] Marie-christine Carlier,[6] Maurice Laville,[1] Roland Chapurlat,[2] Marie-helene Lafage-proust,[7] Gerard M. London,[8] Denis Fouque.[1] *[1]Nephrology, Hopital Lyon Sud, HCL, Pierre-Benite, France; [2]INSERM 1033, Lyon, France; [3]Pôle Information Médicale Evaluation Recherche, HCL, Lyon, France; [4]Nephrology, Hopital Edouard Herriot, HCL, Lyon, France; [5]Centre de Référence des Maladies Rénales Rares, HFME, HCL, Bron, France; [6]Fédération de Biochimie, HCL, Pierre-Benite, France; [7]INSERM U890, IFR 62, Saint-Etienne, France; [8]INSERM U970, Paris, France.*

**Background:** Hemodialysis (HD) patients with adynamic bone are at higher risk of abdominal aortic calcifications (AAC), which are predictive of cardiovascular morbidity. Sclerostin, a factor mainly synthetized by osteocyte, acts on osteoblast as a potent inhibitor of bone formation. Serum sclerostin is increased in HD. We studied relationships between AAC, bone biomarkers and serum sclerostin concentration in HD patients.

**Methods:** Blood was drawn after overnight fast for PTH, calcium, phosphorus, 25OH vit D, bone specific alkaline phosphatase (bSAP). Serum sclerostin was measured by Elisa (Teco®, Switzerland). We used the Framingham risk score to estimate the cardiovascular risk based on age, gender, cholesterol (total and HDL), smoking status and systolic blood pressure. AAC was non-invasively assessed on lateral spine imaging using DXA according to the semi-quantitative Kauppila score.

**Results:** We included 53 HD patients. Median [Q1-Q3] age was 53 [35.2-62.7] and patients were on dialysis for 20 [6-44] months. There were 60% men and 24% diabetic. Framingham risk score was positively associated with the AAC score (p=0.024). Sclerostin was strongly positively associated with AAC score (R=0.48, p<0.001) whereas serum calcium, phosphorus, bSAP and PTH were not. In a multivariate analysis including Framingham risk score, diabetes, hemodialysis duration, BSAP and 25-OH vitamin D, sclerostin remained significantly associated to AAC score with an adjusted OR [95% CI] of 13.68 [2.22-84.37] (p=0.005).

**Conclusions:** Sclerostin may become an interesting cross-talk marker between bone and artery in hemodialysis patients. Whether sclerostin acts directly on arterial calcification process or indirectly through osteoblasts remains to be established.

## FR-PO605

**Association between Serun Sclerostin Levels and Serum Phosphate Levels in Patients on Hemodialysis** Yukari Asamiya,[1] Aiji Yajima,[2] Shigeru Otsubo,[3] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Dept of Medicine, Kidney Center, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Div of Renal Replacement Therapeutic Science, Akita Univ School of Medicine, Akita, Japan; [3]Dept of Blood Purification, Sangenjaya Hospital, Tokyo, Japan.*

**Background:** Osteocytes have recently been demonstrated to play highly important roles in bone remodeling and phosphate homeostasis. Sclerostin, produced by osteocytes, has been identified as a key protein that inhibits bone formation via the inhibition of the osteoblast Wnt/β-catenin signaling pathway. However, sclerostin's role in renal bone disorders is largely uncertain.

**Methods:** This study examined the association of serum sclerostin levels with clinical and laboratory parameters, including serum levels of phosphate, bone remodeling markers, intact parathyroid hormone (PTH), and estradiol, with the treatment agents for mineral and disorders in hemodialysis (HD) patients. The 102 HD patients (mean age, 66.4 ± 8.9 y) and 30 healthy age-, sex-, and menopausal-status-matched individuals were examined between April and June 2011. The HD patients underwent treatment based on the treatments guideline for patients with CKD-mineral bone disorders.

**Results:** In the HD patients, the mean intact PTH and phosphate levels were 190 ± 185 pg/mL and 5.2 ± 1.4 mg/dL, respectively. The sclerostin levels of the HD patients were extremely high and varied widely compared with those of controls (164.7 ± 77.6 vs. 12.6 ± 4.7 ng/mL, P < 0.0001). A multivariate logistic regression analysis of the HD patient data showed that the serum sclerostin levels were significantly associated with male gender (β = 0.679, P = 0.016, odds ratio [OR] = 3.89), phosphate levels (β = 0.484, P = 0.009, OR = 1.62), duration of HD (β = 0.078, P = 0.047, OR = 1.08), and use of cinacalcet hydrochloride (β = 0.705, P = 0.018, OR = 0.24). Serum sclerostin levels were negatively correlated with serum levels of bone-specific alkaline phosphatase (r = –0.254, P = 0.010) and tartrate-resistant acid phosphatase isoform type 5b (r = –0.229, P = 0.021).

**Conclusions:** Serum phosphate levels may influence serum sclerostin levels in HD patients without severe hyperparathyroidism.

## FR-PO606

**Sequential Skeletal, PTH, FGF23 and Sclerostin Changes in Rats with Renal Failure and Secondary Hyperparathyroidism** Cheryl P. Sanchez,[1,2] Subburaman Mohan.[2,3] *[1]Pediatrics, Loma Linda Univ Children's Hospital, Loma Linda, CA; [2]Musculoskeletal Disease Center, Jerry L Pettis VAHS, Loma Linda, CA; [3]Medicine, Loma Linda Univ, Loma Linda, CA.*

**Background:** Studies have shown decreases in cortical and trabecular bone in chronic kidney disease.

**Methods:** To determine the time course changes in PTH, FGF23, sclerostin and skeletal abnormalities, 39 male rats, underwent 5/6 nephrectomy (Nx=27) or sham Nx (Control, n=12). Nx rats were fed standard rodent diet (Nx-C, N=13), or high phosphorus diet (Nx-Phos, N=14). Femur and lumbar bone mineral density (BMD) were obtained at baseline, 12 and 25 days post-Nx, and micro-CT was obtained 25 days post-Nx.

**Results:** There were no differences in femur length or gain in body length. Serum creatinine increased after Nx. After 12 days post-Nx, iPTH and iFGF23 levels were higher in Nx-Phos, 1232±563 pg/mL and 0.35±0.33 ng/mL, compared to Nx-C, 771±155 pg/mL and 0.12±0.08 ng/mL, and Control, 464±126 pg/mL and 0.10±0.09 ng/mL, p<0.001. Serum sclerostin levels did not change. Lumbar BMD declined in Nx-Phos 12 days post-Nx, 0.10±0.01 g/cm$^2$, compared to Nx-C and Control, 0.11±0.01 g/cm$^2$ and 0.11±0.01 g/cm$^2$, p<0.05. The percent gain in lumbar BMD at 25 days post-Nx was lower in Nx-Phos, 79%, compared to Nx-C and Control, 99% and 112%, p<0.02. Femur BMD did not change between groups. Cortical (femur) BV/TV by micro-CT was lower in Nx-Phos, 0.50±0.03% compared to Nx-C and Control, 0.53±0.02% and 0.54±0.02%, p<0.033. Trabecular (lumbar) BV/TV declined by 20% in Nx-Phos compared to Nx-C and Control, p<0.01, likely due to lower trabecular number and increase in trabecular spacing, p<0.001. There was a 46% decrease in connectivity density and an increase in structural model index in Nx-Phos, 3.9±1.2, compared to Nx-C and Control, 2.0±1.0, p<0.0001, suggesting more rodlike trabecular bone appearance. IPTH and FGF23 negatively correlated with trabecular and cortical BV/TV, R= -0.5, p<0.01. iPTH increased with FGF23, R= 0.8, p<0.001. Compared to micro-CT, BMD did not detect alterations in cortical bone.

**Conclusions:** The findings show that changes in trabecular bone occur early in kidney failure accompanied by an increase in iPTH and FGF23.

## FR-PO607

**The Role of DMP-1, E11 and Sclerostin on High Phosphorus Induced Vascular Calcification and Early Intervention by Sodium Thiosulfate in Remnant Kidney Rats** Yi Yu. *Dept of Blood Purification, Dongfang Hospital of Fujian Province, Fuzhou, Fujian, China.*

**Background:** To observe the expression of DMP-1, E11 and Sclerostin involved in vasucular calcification in remnant kidney rats with hyperphosphatemia, and the early effect of sodium thiosulfate (STS) on the progression of vascular calcification.

**Methods:** Sprague Dawley rats (n=35) underwent 5/6 nephrectomy (n=21) or sham operation (n=14). They were fed with diet containing high phosphorus (HP) [phosphate(P)1.2%, calcium (Ca) 1.6%] or normal phosphorus (NP) [P (0.9%), Ca (1.2%)] for 16 weeks, then divided into 5 groups: (1) sham operation rats receiving NP diet (SNP, n=7), (2) sham operation rats receiving HP diet (SHP, n=7), (3) remnant kidney rats receiving NP diet (NNP, n=7), (4) remnant kidney rats receiving HP diet (NHP, n=7), (5) remnant kidney rats receiving HP diet with STS (THP, n=7). Rats in the THP group were intraperitoneally injected with STS 0.4 g/ml/kg three times a week for 16 weeks. Vascular calcification was confirmed by Von kossa staining. DMP1, E11 and Sclerostin mRNA were determined by RT-PCR. E11 expression in aorta were determined by immunohistochemistry or Western blot.

**Results:** After 16 weeks, Scr, $P^{3+}$, iPTH and uric protein were significantly higher in NHP than those in SNP group (P<0.05). With the treatment of STS, THP rats showed a marked decrease in those (P<0.05) compared with NHP group. Significant vascular calcification was found in NHP group while a little in NNP and SHP group occasionally and none in SNP group. The aorta expression of DMP1, E11 and Sclerostin mRNA were higher in NHP than in SNP group (P<0.05); their expression decreased in THP compared with NHP group (P<0.05). The aorta expression of E11 was higher in NHP compared with SNP group (P<0.05); the expression of E11 decreased in THP compared with NHP group (P<0.05).

**Conclusions:** The increase of expression of DMP1, E11 and Sclerostin in aorta shows that vascular calcification involves changes in VSMC transition into osteocyte-like phenotype. Sodium thiosulfate may delay the progression of vascular calcification by down-regulation of DMP1, E11 and Sclerostin.

*Funding:* Government Support - Non-U.S.

**FR-PO608**

**Effect of Core-Binding Factor α1 Gene Silenced by siRNA on the Calcification of Vascular Smooth Muscle Cells Induced by High Phosphate**
Yi Yu. *Dept of Blood Purification, Dongfang Hospital of Fujian Province, Fuzhou, Fujian, China.*

**Background:** To investigate the effect of Core-binding Factor α1 (Cbfα-1) gene silenced by siRNA on the osteogenic differentiation and calcification of vascular smooth muscle cells (VSMC) induced by high phosphate in vitro.

**Methods:** VSMC were cultured in vitro and passage 3 to 8 were used for the experiment. Four of the Cbfα-1 siRNA were designed and synthesized. VSMC were transfected by using cationic lipid vectors (Lipofectamine 2000). We optimized transfection condition by the FAM fluorescent labeling-siRNA and screened effective siRNA sequence by RT-PCR. VSMC were transfected with effective siRNA sequence. VSMC were divided into four groups: (1) normal phosphategroup (Pi 1.3 mmol/L); (2) high phosphate group (Pi 2.6 mmol/L); (3) siRNA transfection group: high phosphate (2.6 mmol/L) + Cbfα-1-siRNA; (4) negative transfection control group: high phosphate (2.6 mmol/L) + negative control siRNA. Cbfα-1 and osteopontin (OPN) mRNA and protein expression were detected by RT-PCR and Western Blot. Calcium deposition was visualized by Alizarin stain method.

**Results:** The transfection efficiency was about 55% at the condition of Cbfα-1 siRNA with concentration of 100 nmol/L and Lipo 8 μl/well. Cbfα-1 siRNA1952 was chosen as effective sequence with suppression ratio up to 81.8%. 24h and 48h after transfection, the expression of Cbfα-1 mRNA in siRNA transfection group was significantly lower than that in high phosphate group and negative transfection control group($P<0.01$). 48h and 72h after transfection, the expression of Cbfα-1 protein in siRNA transfection group was significantly lower than that in high phosphate group and negative transfection control group($P<0.01$). While Cbfα-1 gene was silenced by siRNA, in siRNA transfection group, the mRNA and protein expression of OPN were significantly declined($P<0.05$), and the calcium deposition in cell layers was reduced.

**Conclusions:** Cbfα-1 siRNA can effectively inhibit the expression of Cbfα-1 mRNA and protein in VSMC, thus suppress transformation of VSMC into osteoblast-like cells and calcification induced by high phosphate. Cbfα-1 may be a potential therapeutic target in vascular calcificationof chronic kidney disease.

*Funding:* Government Support - Non-U.S.

**FR-PO609**

**The Combined Therapy with Calcitriol and a Calcimimetic Agent, R568, Ameliorates Phosphorus-Induced Calcification with Altered Expression of SIRT1 and ALP in Human Vascular Smooth Muscle Cells**
Masanori Tokumoto,[1] Shunsuke Yamada,[1] Tomoe Fujino,[1] Kazuhiko Tsuruya,[2] Takanari Kitazono,[2] Hiroaki Ooboshi.[1] *[1]Dept of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan; [2]Depertmet of Medicine and Clinical Scienece, Kyushu Univ, Fukuoka, Japan.*

**Background:** Vascular calcification is life-threatening pathophysiological abnormality, especially in chronic kidney disease (CKD)-mineral bone disorder (MBD). The main contribution factor for vascular calcification is phosphorus (P), but it is difficult to control P retention completely in CKD. Therefore, we examined direct effects of calcitriol and/or a calcimimetic agent, R568, both of which are effective for treatment of secondary hyperparathyroidism, on P-induced calcification in vascular smooth muscle cells (VSMCs).

**Methods:** Human VSMCs were cultured in medium containing 2.9 mM P and treated with $10^{-10}$~$10^{-6}$M calcitriol and/or $10^{-9}$~$10^{-5}$M R568 in various combinations for two weeks.Precipitated calcium contents and expression of various factors which is involved in calcification were evaluated.

**Results:** Only $10^{-10}$ and$10^{-9}$ M calcitriol and $10^{-8}$M R568 in monotherapy significantly inhibited calcification by 10~20% ($p<0.01$), but the treatment of calcitriol combined with $10^{-8}$M R568 suppressed calcification to about 50% of monotherapy at all concentration. Calcitriol increased the mRNA expression of mammalian sirtuin 1 (SIRT1) which prevents vascular calcification, as well as alkaline phosphatase (ALP) which promote vascular calcification dose-dependently. Both increases became significant at $10^{-6}$ and $10^{-7}$M ($p<0.01$). R568 significantly inhibited ALP mRNA expression at all concentration ($p<0.01$). The combination therapy with calcitriol and $10^{-8}$M R568 decreased ALP mRNA expression with similar increase of the mRNA expression of SIRT1 to calcitriol monotherapy.

**Conclusions:** In conclusion, the combination therapy with calcitriol and a calcimimetic agent, R568, can directly ameliorate P-induced vascular calcification more effectively than monotherapy with calcitriol or R568, by reducing ALP expression and increasing SIRT1 expression, respectively.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

**FR-PO610**

**Circulating Soluble Receptor for Advanced Glycation End Product (sRAGE) Levels but Not S100A12 (EN-RAGE) Are Associated with Vascular Calcification in Patients on Hemodialysis**
Ji Yong Jung,[1] Han Ro,[1] Chungsik Lee,[2] Sun Moon Kim,[3] Ae Jin Kim,[1] Hyung Soo Kim,[1] Jae Hyun Chang,[1] Hyun Hee Lee,[1] Wookyung Chung.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Gachon Univ of Gil Medical Center, Incheon, Korea; [2]Div of Nephrology, Dept of Internal Medicine, Cheju Halla General Hospital, Jeju; [3]Div of Nephrology, Dept of Internal Medicine, Chungbuk National Univ Hospital, Cheongju.*

**Background:** The receptor for advanced glycation end products (RAGE) has emerged as a central regulator of vascular inflammation and atherosclerosis. Soluble RAGE (sRAGE) and extracellular RAGE-binding protein S100A12 (EN-RAGE) are anti-inflammatory and pro-inflammatory ligands of RAGE, respectively, in the development of vascular complications. We determined the levels of sRAGE and EN-RAGE in hemodialysis (HD) patients and evaluated their relationship with vascular calcification.

**Methods:** We performed a cross-sectional study with 199 hemodialysis patients. Plain X-ray images of the lateral lumbar spine from all subjects were studied to calculate semiquantitative vascular calcification scores (VCSs), as described by Kauppila. Commercially available ELISA kits were used to quantify the serum concentration of sRAGE and EN-RAGE.

**Results:** The patients were 57.1 ± 13.7 years of age; 54.3% were male, 49.2% were diabetic, and 36.2% had a history of cardiovascular disease. Kauppila scores revealed 40 patients (20.1%) with a high VCS (>7). In a univariate analysis, serum sRAGE was negatively associated with high VCS (log sRAGE, $P = 0.035$), whereas EN-RAGE showed a positive tendency (log EN-RAGE, $P = 0.431$). Even after adjustments for confounding risk factors, sRAGE was independently associated with a high VCS (log sRAGE, OR = 0.491, 95% CI: 0.262 – 0.918, $P = 0.026$).

**Conclusions:** This study demonstrated that the circulating sRAGE level was inversely associated with VCS in HD patients independent of the EN-RAGE level and the severity of systemic inflammation. Longitudinal observations and intervention studies are warranted to establish whether this link is causal.

**FR-PO611**

**Inorganic Pyrophosphate and Aortic Valve Calcification**
Swetha Rathan,[1] Ajit Prithviraj Yoganathan,[1,2] W. Charles O'Neill.[3] *[1]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA; [2]The Wallace H. Coulter Dept of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA; [3]Renal Div, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** Aortic valve (AV) calcification is common in chronic kidney disease but the underlying molecular mechanisms are not well understood. An *ex vivo* model of AV calcification was developed and the role of orthophosphate and inorganic pyrophosphate (PPi), factors well known to influence vascular calcification, was investigated.

**Methods:** Freshly obtained porcine AV leaflets were cultured for up to 8 days in DMEM medium containing varying concentrations of phosphate up to 3.8mM with and without inorganic pyrophosphatase (IP, an enzyme that specifically hydrolyzes PPi).

**Results:** Cell viability and structure were preserved as shown by MTT and Verhoff-Vangieson elastin stains. Calcification, measured as the incorporation of $^{45}Ca$ in the valves and confirmed with alizarin red staining, occurred only in the presence of IP and added phosphate and was prevented by etidronate, a non-hydrolyzable analog of PPi. Calcification was present on both the fibrosa and ventricularis sides of the leaflets. There was no further calcification when alkaline phosphatase, a non-selective phosphatase, was added, indicating that other phosphorylated compounds were not inhibiting calcification. Leaflets released PPi into the medium, which was enhanced by MLS38949, a specific inhibitor of tissue non-specific alkaline phosphatase (TNAP). Leaflets synthesized $^{32}PPi$ from extracellular [$^{32}P$] ATP, which was significantly decreased in the presence of β, γ-methylene-ATP, inhibitor of ectonucleotide pyrophosphorylase phosphodiesterase, (ENPP1).

**Conclusions:** An *ex vivo* model of aortic valve calcification was successfully developed. Extracellular PPi is a key endogenous inhibitor of calcification in this model and its levels appear to be controlled through synthesis by ENPP1 and hydrolysis by TNAP. This suggests that preventive therapy could be based on non-hydrolyzable PPi analogs such as bisphosphonates or on agents that enhance ENPP1 activity or inhibit TNAP action.

*Funding:* Private Foundation Support

**FR-PO612**

**L-lysine Ameliorates Vascular Calcification in Adenine-Induced Uremic Rats**
Akihiro Shimomura,[1] Isao Matsui,[1] Takayuki Hamano,[2] Kazunori Inoue,[1] Yasuo Kusunoki,[1] Chikako Nakano,[1] Yoshitsugu Obi,[1] Yoshiharu Tsubakihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Dept of Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Dept of Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

**Background:** Vascular calcification (VC) is one of the major problems in CKD. Although several studies in both animals and humans have tried to reduce the extent of VC, satisfactory therapies have not been established. Therefore, it is important to develop novel strategies. In 2006, Price *et al.* reported that low protein (LP) diet exacerbates VC in

adenine-induced uremic rats. This report suggested that amino-acid-insufficiency correlates with VC. Because L-lysine (L-Lys) is the first-limiting amino acid in most of cereal grains, we investigated the effects of L-Lys on VC.

**Methods:** Male Sprague-Dawley rats at age 13 weeks were divided randomly into four groups; LP diet (group LP), LP+0.75% adenine (group Ade), LP+Ade+2.5% glycine (group Gly), or LP+Ade+2.5% L-Lys•HCl (group Lys). Glycine served as an amino-acid-control, because it is an amino acid that has the simplest structure. We also performed *in vitro* examinations to reveal the underlying mechanisms.

**Results:** At age 18 weeks, groups LP had no VC, whereas groups Ade and Gly had comparable levels of severe VC. L-Lys supplementation almost completely ameliorated VC. Physical parameters and renal functions were not different among groups Ade, Gly, and Lys. Dietary L-Lys strongly suppressed plasma level of iPTH in adenine-induced uremic rats, and thereby protected the femora from osteoporotic changes. Using LC/ESI-MS/MS, we found that dietary L-Lys elevated plasma alanine (Ala), proline (Pro), arginine (Arg), and homoarginine (Homo-Arg). Analyses *in vitro* revealed that L-Pro inhibited apoptosis of cultured human VSMCs, whereas L-Lys, L-Arg, and L-Homo-Arg dose-dependently attenuated spontaneous precipitation of minerals in a solution of supersaturated calcium/phosphate.

**Conclusions:** Dietary L-Lys ameliorated VC in adenine-induced uremic rats. Our findings provide a novel therapeutic approach for VC.

## FR-PO613

**Association of Serum Phosphorus Variability with Coronary Artery Calcification among Hemodialysis Patients** Haiming Li, Mengjing Wang, Li You, Jing Chen. *Nephrology, Fudan Univ, Shanghai, China.*

**Background:** Coronary artery calcification (CAC) is associated with increased mortality in maintenance hemodialysis (MHD) patients, but the pathogenesis of this condition isn't well understood. We evaluated the relationship of CAC score (CACs) and variability of serum phosphorus in MHD patients.

**Methods:** Cross-sectional observational study. **Setting & participants:** 77 adults on MHD at Huashan Hospital (Shanghai). The mean dialysis vintage was 5.9 ± 4.4years. Blood chemistry was recorded every 3 months from January 2008 to July 2010. **Predictor factor:** Phosphorus variation was defined by the standard deviation (SD) and coefficient of variation (CV). **Outcomes:** Three categories of CACs (0~10, 11~400, and > 400). **Measurements:** Phosphorus variation was calculated from patient records, CAC was measured by computed tomography, and CACs were calculated by the Agatston method.

**Results:** The mean patient age was 61.7 ± 11.3 years and 51% of them were men. The mean CACs was 609.6 (± 1062.9), the median CACs was 168.5, and 78% of patients had CACs more than 0. Multivariate analysis indicated that female gender (OR = 0.20, 95% CI = 0.07-0.55), age (OR = 2.31, 95% CI = 1.32-4.04), serum fibroblast growth factor 23 (OR = 2.25, 95% CI = 1.31-3.85), SD-phosphorus calculated from the most recent 6 measurements (OR = 2.12; 95% CI = 1.23-3.63), and CV-phosphorus calculated from the most recent 6 measurements (OR = 1.90, 95% CI = 1.16-3.11) were significantly and independently associated with CACs.

**Table 1.** Multiple logistic regression analysis of factors associated with CAC score tertile in MHD patients.

| Variable | Unadjusted | | Adjusted | | | |
|---|---|---|---|---|---|---|
| | | | Model 1 | | Model 2 | |
| | OR | *P* | $OR_1$ (95% CI) | $P_1$ | $OR_2$ (95% CI) | $P_2$ |
| Female | 0.277 | <0.001 | 0.20 (0.07-0.55) | 0.002 | 0.21 (0.07-0.58) | 0.003 |
| Age (/1-y) | 1.824 | 0.012 | 2.31 (1.32-4.04) | 0.003 | 2.04 (1.18-3.53) | 0.010 |
| Laboratory measurements | | | | | | |
| Alb (/1-g/L) | 0.595 | 0.019 | | | | |
| CRP (/1-mg/L) | 2.40 | 0.04 | | | | |
| PTH (/1-ng/L) | 1.531 | 0.091 | | | | |
| FGF23 (/1-pg/mL) | 1.449 | 0.127 | 2.25 (1.31-3.85) | 0.003 | 2.50 (1.46-4.30) | <0.001 |
| HDL (/1-mmol/L) | 0.574 | 0.026 | | | | |
| Mean and variability measurements | | | | | | |
| Mean-PTH (/1-ng/L) | 1.426 | 0.164 | | | | |
| Mean-Ca (/1-mg/dL) | 1.376 | 0.171 | | | | |
| SD-Pi (/1-mg/dL) | 1.538 | 0.074 | 2.12 (1.23-3.63) | 0.007 | | |
| CV-Pi (/1) | 1.590 | 0.071 | | | 1.90 (1.16-3.11) | 0.011 |

$P_1$ adjusted for female sex, age, FGF23, and SD-phosphate.
$P_2$ adjusted for female sex, age, FGF23, and CV-phosphate.

These associations persisted for phosphorus variation calculated from past 7, 8, 9, 10, and 11 follow-up values. **Limitations:** Patients were from a single center, leading to possible selection bias. Proteins involved in bone metabolism and vascular calcification were not measured.

**Conclusions:** Variability in serum phosphorus is associated with CAC in MHD patients.

*Funding:* Government Support - Non-U.S.

## FR-PO614

**Reversibility of Uremic Vascular Calcification** Koba A. Lomashvili, Courtney A. Washington, W. Charles O'Neill. *Renal Div, Emory Univ, Atlanta, GA.*

**Background:** Research on vascular calcification has focused on formation rather than reversal. Since hydroxyapatite is insoluble under physiologic conditions, reversal requires a biologic process. Whether vascular calcification is reversible and by what mechanism are unknown and have important therapeutic implications.

**Methods:** Mice were fed 0.45 % adenine for 2 months to produce uremia and a 2% phosphorus diet + calcitriol (1000 ng/kg thrice weekly) to induce vascular calcification. After 2 months, calcified abdominal aortas were transplanted orthotopically into normal littermates and harvested after 6 and 12 weeks. The allografts were examined histologically by hematoxylin and eosin (H&E) or tartrate-resistant acid phosphatase (TRAP) staining for osteoclasts, and calcium content (measured after acid extraction by the cresopthalein method) was compared to that in the untransplanted donor aorta.

**Results:** Calcification at transplantation was variable (17 to 2360 nmoles/mg, median: 686; normal: <7) and exclusively medial. Survival after transplantation was greater than 90% and there were no signs of rejection. The reduction in calcification at 6 and 12 weeks after transplantation was 47 +/- 12 % (n=8) and 51 +/- 13 % (n=5) respectively. This reduction was 37 +/- 10% in heavily calcified (>600 nmol/mg) allografts compared with 66 +/- 13% in less calcified allografts. The allografts were histologically similar to untransplanted calcified aorta with calcium deposits readily apparent in heavily calcified allografts and no evidence of cellular infiltration or cell-mediated resorption. TRAP staining was negative. There was no calcification of normal aortas transplanted into normal mice.

**Conclusions:** Approximately half the calcification in uremic aortas is reversible by 12 weeks without histologic evidence of cell-mediated resorption. Almost all of the reversal occurs by 6 weeks and is less in heavily calcified aortas. The results suggest that vascular calcification consists of a readily reversible component that presumably represents non-apatitic calcium and a non-reversible component that likely represents apatitic calcium. The inability to resorb this latter component is likely due to the failure to recruit osteoclasts.

*Funding:* NIDDK Support

## FR-PO615

**The Role of Nrf2 in Human Aortic Smooth Muscle Cells Calcification and Mechanism Research** Li Wang. *Renal Dept, Sichuan Provincial People's Hospital, Chengdu, China.*

**Background:** To discuss the role and mechanism of Nrf2 in calcification of HASMCs.

**Methods:** HASMCs were cultured in vitro and incubated with beta-glycerophosphate to induce a diffuse mineralization and oxidative stress. Additionally, sulforaphane treatment and short interfering RNA were used to regulate the expression of Nrf2 gene. Calcium deposition was detected by the O-CPC colorimetry method and normalized with regard to protein content. Intracellular ROS levels were measured using DCFH-DA. The expression of Nrf2, HO-1, Runx2, OPN and SM22α were assessed by Western Blot.

**Results:** After 12 days of treatment, calcium deposition was significantly increased in calcification medium group than normal medium group. Within 12 hours of treatment, beta-glycerophosphate increased ROS production, this production being further enhanced after 72h. The beta-glycerophosphate-induced oxidative stress was associated with an early expression of Nrf2 and HO-1 followed by Runx2 and OPN expression at 72h. The sulforaphane treatment and short interfering RNA can up and down regulate the expression of Nrf2 as well as other factors.

**Conclusions:** Beta-glycerophosphate could induce a diffuse mineralization in HASMCs. Nrf2 protect HASMCs from oxidative stress and calcification by participating in resistance to oxidative stress.

## FR-PO616

**A Novel Method to Quantify Relationships between Vascular Calcifications and Bone in Chronic Kidney Disease Patients** Janina M. Patsch,[1] Thomas L. Nickolas.[2] *[1]Radiology, Medical Univ of Vienna, Vienna, Austria; [2]Medicine, Columbia Univ, NY, NY.*

**Background:** In chronic kidney disease (CKD) vascular calcifications (VC) are highly prevalent. They are associated with increased cardiovascular risk and fracture. However, research in osteo-vascular interactions is impeded by the high radiation dose delivered by methods used to quantify VC severity and their skeletal relationships. High-resolution peripheral QCT (HRpQCT, Scanco Medical) measures bone mass and microarchitecture at the distal radius and tibia, detects VCs and delivers low radiation dosage. We determined whether HRpQCT simultaneously quantifies relationships between VCs and bone mass and microarchitecture.

**Methods:** We enrolled patients with CKD stages 2+3 (n=18), 4+5 (n=14), hemodialysis (HD, n=16), and an age, sex, race matched reference group with normal kidney function (n=21). Tibia HRpQCT scans were reviewed for lower leg arterial calcifications (LLAC) with a semi-automated, image-processing algorithm. LLAC mass was expressed in mg hydroxyapatite (mgHA). Relationships between LLAC and eGFR, cortical (Ct) and trabecular (Tb) mass and microarchitecture, PTH, and markers of bone formation (BSAP, osteocalcin, P1NP) and resorption (CTX, TRAP5b) were assessed. Analyses were adjusted for kidney function.

**Results:** Mean±SD age was 65±9 yrs, 57% were male, 45% were Black. Compared to the reference group, LLAC was higher in patients with CKD stages 4+5 by 3.2 mgHA and on HD by 8.8 mgHA (both p<0.05). There were inverse associations between LLAC and

radius total density (r= -0.30, p<0.05) and Tb thickness (r= -0.30, p<0.03), and tibia total (r= -0.30, p<0.05) and Ct density (r= -0.30 p<0.05) and Ct thickness (r= -0.20, p<0.05). LLAC was positively associated with BSAP (r= 0.40, p<0.05).

**Conclusions:** In summary, HRpQCT simultaneously quantifies LLAC and assesses relationships between LLAC and bone mass and microarchitecture. LLAC was more severe in patients with advanced CKD and relationships between VCs and bone mass, microarchitecture and formation suggested pathogenic associations between vascular and bone biology that require further study. We conclude that HRpQCT will facilitate the elucidation of osteo-vascular biology.

*Funding:* NIDDK Support

## FR-PO617

**The Role of Adipocytes in Calciphylaxis** Nader Kassis Akl, Neal X. Chen, Kalisha O'Neill, Keith March, Dmitry O. Traktuev, Sharon M. Moe. *Indiana Univ, Indianapolis, IN.*

**Background:** Calciphylaxis is a fatal disease in dialysis patients and hyperphosphatemia and obesity are risk factors. The close proximity of the calcified arterioles to adipocytes, and the fact that osteoblasts, adipocytes, and vascular smooth muscle cells (VSMC) have a common mesenchymal origin led us to test the hypothesis that adipocytes can calcify in the presence of elevated phosphate and/or that adipocytes exposed to phosphate can induce VSMC calcification.

**Methods:** 3T3-L1 preadipocytes and human adipose stromal cells (ASC) were induced into mature adipocytes and then treated with media with high phosphorus. Calcification was assessed by biochemical assay after HCl extraction. Total RNA was isolated and real time PCR performed to determine the expression of genes for osteoblast and adipocyte differentiation. Adipocytes were also co-cultured with bovine VSMC using transwell inserts.

**Results:** The results demonstrated that both adipocytes and human ASC calcify in high phosphorus media (3.5 and 2.2 fold greater than cells without phosphate, respectively, both $p < 0.01$). In addition, compared to normal phosphorus, adipocytes treated with high phosphorus had increased expression of RUNX2 at 24 hrs (1.02± 0.40 vs. 0.68±0.07), and decreased expression of PPARPγ (0.97± 0.27 vs. 0.65±0.18, p<0.004) at day 3 and CEBPα (1.56± .57 vs 1.11 ±0.23, p<0.02) at day 7, indicating a switch to more osteoblast like phenotype. Furthermore, VSMC calcification was increased when co-cultured with adipocytes compared to BVSMC alone (2.13 ± 1.90 vs 0.04±.20, p<0.007 ) at day 3. In contrast, there was no difference in adipocyte calcification when co-cultured with or without VSMC.

**Conclusions:** Adipocytes are capable of calcifying in media that contains high phosphorus, in part by up-regulation of osteoblast genes and down-regulation of adipocyte genes. Furthermore, adipocytes exposed to elevated phosphorus can induce calcification of VSMC in a paracrine manner. The results suggest that adipocyte exposure to elevated phosphorus may be an initiating step in calciphylaxis.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO618

**High Phosphate Diet Induces Aortic Calcification in a Mouse Adenine CKD Model** Wei Ling Lau,[1] Sogol Pahlevan,[1] Kamyar Kalantar-Zadeh,[1] Cecilia M. Giachelli,[2] Nosratola D. Vaziri.[1] *[1]Nephrology, Univ of California, Irvine, School of Medicine, Orange, CA; [2]Bioengineering, Univ of Washington, Seattle, WA.*

**Background:** Hyperphosphatemia is a non-traditional risk factor for vascular calcification (VC) leading to unfavorable cardiovascular outcomes, the pathophysiologic pathway of which can be examined in rodent models of chronic kidney disease (CKD). Here we characterize biochemical and VC outcomes when calcification-prone DBA/2J mice are subjected to adenine diet to induce uremia, followed by normal vs. high phosphate (NP vs. HP) feeding.

**Methods:** Kidney failure was induced in DBA/2J mice via 0.2% adenine in food x18 days, followed by NP (0.5% phosphate) or HP (1.2% phosphate) diet x3 months. The study groups were: healthy controls on NP diet (CTL), CKD on NP diet (CKD+NP), and CKD on HP diet (CKD+HP) (n=4 per group). A subset of mice was fed adenine diet x21 days to document blood urea nitrogen (BUN) trends.

**Results:** Serum BUN was significantly elevated after 18 days on adenine diet and was 107.8+7.2 mg/dL at day 21 (Figure 1A). Kidney histology showed patchy interstitial nephritis and glomerulosclerosis. At termination of the main study (after 3 months of NP vs. HP diet), serum creatinine remained significantly elevated in CKD groups (0.34+0.04 vs. 0.18+0.03 mg/dL in CTL). The CKD+HP group had increased serum phosphorus, serum FGF23 (Figure 1B) and aortic calcium content (Figure 1C). These parameters were equivalent between the CTL and CKD+NP groups.

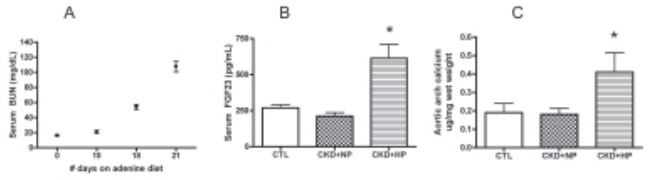



Figure 1. (A) Serum BUN values over time in mice fed a 0.2% adenine diet. (B) Serum FGF23 and © aortic calcium content in CTL, CKD+NP and CKD+HP mice. * *P*<0.05 compared to CTL and CKD+NP groups.

**Conclusions:** High phosphate diet increased serum FGF23 and aortic calcification in mice with adenine-induced CKD. This is a promising model for the study of phosphorus overload and cardiovascular outcomes.

*Funding:* Pharmaceutical Company Support - WLL funded by Sanofi fellowship award, Private Foundation Support

## FR-PO619

**Correlation of Lateral Vertebral Bone Mineral Density with Underlying Bone Histology in Patients with Chronic Kidney Disease** Maria Coco,[1] James M. Pullman.[2] *[1]Medicine, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; [2]Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.*

**Background:** Patients with renal disease have extraosseous calcifications that will give falsely elevated measurements of bone mineral density (BMD) on DEXA scans. For this reason, DEXAs, with Anteroposterior (AP) views are not routinely done in these patients. We measure lateral BMD since it may exclude non-bone calcifications and the generated vertebral BMD data more accurately reflects vertebral bone density. We also compare these lateral measurements with underlying bone histology, since it is the gold standard diagnosing bone disease.

**Methods:** BMD was measured in AP (BMD_AP) and lateral (BMD_lat) planes using Hologic QDR-4500 fan beam with C-Arm unit. Lateral and AP measurements were compared. Correlations were obtained between BMD and parameters of bone metabolism and turnover (PTH, Vit D 25OH, Urinary N-telopeptide). correlations were made with bone histology.

**Results:** 45 pts had DEXA scans. There was a significantly lower BMD in the lateral view as compared to the AP view (.718±0.16 vs .942±.16, p= 0.0001). Markers of bone turnover did not correlate with either lateral or AP BMD. There were 13 bone biopsies: Lateral BMD correlated with histology: lower BMD_lat predicted low bone turnover, while higher BMD_lat predicted higher bone turnover. This was not observed with the AP measurements.

**Conclusions:** Pts with renal disease have lower lateral BMD than AP BMD. Underlying bone histology correlates with lateral BMD. Lateral DEXAs may show the true effect of PTH on BMD: higher BMD may represent higher PTH-driven cellular activity with increased bone formation and calcification while lower BMD may represent the absence of bone stimulation with resulting decreased bone formation and decreased calcification. If confirmed with histomorphometry, this minimally invasive tool may be useful in treating renal osteodystrophy.

| | bone turnover | BMD-lateral | BMD-AP |
|---|---|---|---|
| PTH | .193 ns | -.093 ns | .101 ns |
| Vit D 25OH | .386 ns | -.065 ns | .025 ns |
| BSAP | -.437ns | .047ns | .526ns |
| OC | -.394 | -.185ns | .301ns |
| NTx | .333 | -.152 | -.301ns |
| creatinine | -.029 | .115 | -.031ns |
| Bone turnover | | -.661 p<.019 | -.562 |

*Funding:* Clinical Revenue Support

## FR-PO620

**Pathogenesis, Prevention and Treatment of the Chronic Kidney Disease-Mineral Bone Disorder** Yifu Fang,[1] Charles Ginsberg,[1] Michael E. Seifert,[1,2] Toshifumi Sugatani,[1] Hartmut H. Malluche,[3] Keith A. Hruska.[1] *[1]Pediatrics, Wash. Univ, St. Louis, MO; [2]Pediatrics, S.Ill.Sch. of Medicine, Springfield, IL; [3]Medicine, Univ of Kentucky, Lexington, KY.*

**Background:** The CKD-MBD is recognized as a major contributor to cardiovascular risk during kidney disease, but the cause of the syndrome is unknown, and there is no current therapy. At its inception, the CKD-MBD is characterized by an osteodystrophy, vascular calcification (VC) and stimulation of osteocytic protein levels in the circulation. We tested the hypothesis that the CKD-MBD is caused by increased renal production of circulating Wnt inhibitors.

**Methods:** In the background of atherosclerosis and high-fat feeding with CKD stimulated VC, we staged CKD analogous to the human clinical staging by measuring inulin clearances and BUN.

**Results:** In mice with CKD stage 2, we demonstrated increased production of Dkk-1 in remnant kidneys and increased circulating Dkk-1, sclerostin and c-klotho. There was an osteopenic low turnover osteodystrophy and stimulation of VC. Neutralization of Dkk-1 by a monoclonal antibody (mab) stimulated bone formation rates, prevented the osteodystrophy and CKD stimulated VC. The Dkk-1 mab preserved the expression of membrane α-klotho in the aorta and kidney. In addition, the aortic expression of the osteoblastic transcription factor, RUNX2 was inhibited, and vascular smooth muscle contractile protein expression was increased. Increased plasma c-klotho and FGF23 levels were not affected by the Dkk-1 mab, but FGF23 levels were reduced to normal by a Pi binder. In a treatment (not a prevention) protocol Pi binder therapy had no effect on VC or the osteodystrophy, but when combined with the Dkk-1 mab, the CKD-MBD as characterized in the CKD-2 mice was completely reversed.

**Conclusions:** The CKD-MBD is established in early CKD, characterized by the cardiovascular risk factor, VC, a renal osteodystrophy, and elevated circulating FGF23, c- klotho, and sclerostin. Neutralization of Dkk-1 was sufficient to prevent the VC and the osteodystrophy. The CKD-MBD was treated by the combination of neutralization of the Wnt inhibitor, Dkk-1, and Pi binding.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Amgen, Private Foundation Support

## FR-PO621

**Abnormal DXA Scans in Hemodialysis Patients Predict Risk of Hip Fractures** Scott A. Rasgon,[1] Richard M. Dell.[2] *[1]Nephrology, Kaiser Permanente, Los Angeles, CA; [2]Orthopedic Surgery, Kaiser Permanente, Downey, CA.*

**Background:** In 2011 and 2012 kidney week we presented data from Kaiser Permanente DEXA scan data base. There was a strong correlation between normal DXA scans in hemodialysis (HD) patients and low hip fracture( FX) risk. Last year we had 1954 HD patients with DXA scans. KDIGO makes the following recommendations for DXA scans in HD patients. " 3.2.2 In patients with CKD stages 3-5D with evidence of CKD-MBD, we suggest that BMD testing not be performed routinely, because BMD does not predict fracture risk as it does in the general population, and BMD does not predict the type of renal osteodystrophy (2B)."This is based on 13 studies with the most subjects in any of the studies was 242. 5 studies showed no correlation with FX rates and 8 showed some correlation with low BMD and increased FX rate. We now have 2387 active HD patients in our data base who have had DXA scans with scores.

**Methods:** We reviewed the HD data base of 4912 HD pateints at Kaiser Permanente SO. CA. of which 2387 had DXA scans with T-scores. The DXA scans were done between 1/1/1998 to 6/1/2013. We wanted to see if there was any correlation between DXA scan results and hip FX in this population.

**Results:** We found the following results 485 patients had a normal DXA wth only 5 hip FX with a 1.03 % FX rate. 1898 patients had an abnormal DXA (T-score <-1) with a FX rate of 5.4%. Pt. with osteoporosis (T-score<-2.5) Had a FX rate of 9.33%.

| | HIP FX | OSTEOPOROSIS T-score<-2.5 | OSTEOPENIA T-score -2.5 TO -1 | NORMAL Tscore>-1 | TOTAL |
|---|---|---|---|---|---|
| HD | N | 661 | 1140 | 485 | 2285 |
| HD | Y | 68 | 29 | 5 | 102 |
| HD TOTAL | | 729 | 1169 | 490 | 2387 |
| HD % HIP FX | | 9.33% | 2.48% | 1.02% | 4.3% |

The realtive risk of FX is 5.01 p=.0004 comparing T-scores of <-1 to >-1. Comparing T-scores of <-2.5 tp >-1 the relative risk of FX is 9.14 p=.0001.

**Conclusions:** Contrary to the KDIGO guidelines our data demonstrate that a normal DXA scan (T-score>-1) predicts a low hip FX risk in HD patients over time. Our data has continued to show that DXA scans can predict HD patient risk for hip FX and should be considered in this population and studied further.

*Funding:* Clinical Revenue Support

## FR-PO622

**Pathogenesis of Cortical Deficits and Decreased Bone Stiffness after Kidney Transplantation** Kyle K. Nishiyama, Elizabeth Shane, Thomas L. Nickolas. *Medicine, Columbia Univ, NY, NY.*

**Background:** Fractures are more than 4-fold higher after kidney transplantation (KTx) than in the general population. We reported that hyperparathyroidism (HPT) is associated with progressive cortical (Ct) losses in patients with chronic kidney disease. As HPT commonly persists after KTx we hypothesized that Ct deficits would worsen, be directly associated with HPT and elevated bone turnover markers (BTMs) and lead to impaired bone strength.

**Methods:** We scanned the distal radius and tibia by high-resolution peripheral QCT (HRpQCT, Scanco Medical) in 31 patients (22 men, 9 women; age 52±13 years) within 2 weeks of and 1 year after KTx. Ct porosity (CtPo) was quantified. Whole bone stiffness and failure load were estimated using finite element analysis. PTH and markers of bone formation (BSAP, Osteocalcin, P1NP) and resorption (CTX and Trap5b) were measured at 3, 6 and 12 months after KTx and were time-averaged. Mixed linear models were used to determine longitudinal changes.

**Results:** At 1 year, 74% of patients had HPT (PTH>65 pg/mL). At the radius, Ct density decreased by 2.2±1.8%, thickness by 2.2±0.8% and area by 2.5±0.8% (all $p<0.05$); CtPo increased by 78±9% ($p<0.01$) and trabecular area by 0.4±1.8% ($p=0.06$). Stiffness and failure decreased by 3.1±1.3% and by 3.4±1.4% (both $p<0.05$) respectively. At the tibia, Ct density decreased by 1.9±0.6% and thickness by 1.1±0.5% (both $p<0.05$); CtPo increased by 30±5% ($p<0.05$). Changes in stiffness and failure load were not significant. At 1 year, greater CtPo was significantly associated with lower Ct density, thickness and area, and stiffness and failure load (all $p<0.001$). Increases in CtPo were strongly associated with higher levels of PTH and BTMs (PTH r=0.64; BSAP r=0.62; CTX r=0.64, all $p<0.01$). Decreases in stiffness and failure load were strongly associated with higher levels of PTH (r= -0.45, $p<0.05$) and BTMs (Osteocalcin and Stiffness r= -0.70; Osteocalcin and Failure Load r= -0.64, both $p<0.001$).

**Conclusions:** In conclusion, persistent HPT and elevated bone turnover after KTx drive Ct deterioration and decreased bone stiffness. Further studies are needed to determine whether mitigating the adverse effects of HPT on Ct bone protects against fracture.

*Funding:* Clinical Revenue Support

## FR-PO623

**Altered Material Properties Are Responsible for Bone Fragility in Rats with Chronic Kidney Disease** Yoshiko Iwasaki,[1] Junichiro J. Kazama,[2] Masafumi Fukagawa.[3] *[1]Health Sciences, Oita Univ of Nursing and Health Scineces, Oita, Japan; [2]Niigata Univ Medical and Dental Hospital, Niigata, Japan; [3]Tokai Univ School of Medicine, Isehara, Japan.*

**Background:** Patients with chronic kidney disease (CKD) have considerably higher risk of fracture. Abnormalities in parathyroid function and/or bone mineral density cannot fully account for their increased bone fragility, while the role of uremia on it remains obscure.

**Methods:** CKD rats with hyperparathyroidism (HP-CKD) were underwent partial nephrectomy (Nx). Those without hyperparathyroidism (Non-HP-CKD) were made by Nx and thyroparathyrioidectomy (TPTx) followed by a continuous exogenous physiological dose of PTH supplementation. Control rats (HP-Cont or Non-HP-Cont) were prepared for each group. Femoral bone elasticity was non-invasively assessed by a direct mechanical assessment method. Bone chemical compositions were analyzed by a raman spectroscopy and a micro X-ray diffraction.

**Results:** Serum creatinine levels were significantly elevated in the CKD groups. Significantly elevated PTH levels and histomorphometrically proven high turnover bone were documented in the HP-CKD group, while PTH remained constant and bone turnover was rather decreased in the Non-HP-CKD group. Compared with each Control group, bone elasticity in femoral bone was significantly reduced in each CKD groups. Decreased BMD level was observed in the HP-CKD group whereas that in the Non-HP-CKD remained unchanged. Raman spectroscopic analyses revealed that carbonate phosphate ratio and crystallinity were altered in both the CKD groups. In addition, mineral matrix ratio and non-physical collagen crosslinks were increased in the Non-HP-CKD group. Apatite c-axis orientation was significantly decreased in both the CKD groups. Multiple regression analysis revealed that apatite orientation and mineral matrix ratio, but not BMD, were independently associated with bone elasticity.

**Conclusions:** Regardless of parathyroid function or bone turnover, altered bone material properties induced by uremia were responsible for bone fragility in CKD animals.

*Funding:* Government Support - Non-U.S.

## FR-PO624

**High Frequency of Iron Bone Deposits in a Brazilian Hemodialysis Population with Renal Osteodystrophy** Camila Barbosa L. Oliveira,[1] Alline S. A. Oliveira,[1] Carla Queiroz Neves,[1] Clarissa Jacob Barros Carvalho,[1] Vanda Jorgetti,[2] Jose Edevanilson Gueiros,[1] Ana Paula Gueiros.[1] *[1]Nephrology, UFPE, Recife, Pernambuco, Brazil; [2]Nephrology, USP, Sao Paulo, Brazil.*

**Background:** Little is known about iron bone deposits in dialysis patients. Previous studies have demonstrated the association between iron and the development of low bone turnover. The purpose of this study was to evaluate the presence of iron deposits on bone biopsy of hemodialysis patients.

**Methods:** We performed a retrospective analysis of 158 patients underwent bone biopsy in the period from March 2003 to March 2013. Clinical data were evaluated: age, sex, time on dialysis and occurrence of fractures. Laboratory tests: total calcium, phosphorus, intact parathyroid hormone (iPTH pg/mL), alkaline phosphatase (ALP U/L), iron (µg/dL) and ferritin (ng/mL). Bone biopsy was performed after double tetracycline labeling and specimens were classified as osteitis fibrosa (OF), mixed disease (MD), adynamic bone disease (ABD), osteoporosis (OS) or osteomalacia (OM). To evaluate iron bone deposits, sections were stained with Perl's Prussian blue and for aluminum, solochrome azurine was used. Aluminum intoxication (AI) or iron intoxication (IBD) were defined when more than 20% of the trabecular bone surface were covered by metal. Patients were divided into 2 groups: IBD group (patients with IBD) and NIBD group (patients free of IBD). We performed a comparative analysis between the groups to assess factors associated with IBD.

**Results:** The prevalence of IBD was 32.3%. There were no diferences between the groups regarding clinical parameters. ALP (median 138 vs 297; $p<0.001$) and iPTH (median 645 vs 1514; $p<0.001$) levels were lower in the IBD group. Ferritin levels were higher in IBD group (median 528 vs 313; $p=0.019$). ABD ($p=0.029$) and aluminum intoxication ($p<0.001$) were closely associated with IBD.

**Conclusions:** We found high prevalence of siderosis on the bone of a Brazilian hemodialysis population. IBD was associated with low bone turnover and with aluminum intoxication. More studies are needed to assess the clinical importance of IBD.

## FR-PO625

**Renal Osteodystrophy: 10-Year Experience of a Brazilian Center** Alline S. A. Oliveira,[1] Carla Queiroz Neves,[1] Clarissa Jacob Barros Carvalho,[1] Camila Barbosa L. Oliveira,[1] Vanda Jorgetti,[2] Jose Edevanilson Gueiros,[1] Ana Paula Gueiros.[1] *[1]Nephrology, UFPE, Recife, Pernambuco, Brazil; [2]Nephrology, USP, Sao Paulo, Brazil.*

**Background:** Chronic kidney disease mineral bone disorder (CKD-MBD) is highly prevalente among patients with end stage renal disease. The aim of the presente study was to evaluate the clinical and laboratory profile of patients who underwent bone biopsy.

**Methods:** We performed a retrospective analysis of 158 hemodialysis patients underwent bone biopsy from March 2003 to March 2013. Clinical data were evaluated: age, sex and time on hemodialysis. Laboratory tests consisted of total calcium (Cat, mg/dL), phosphorus (P,mg/dL), intact parathyroid hormone (iPTH,pg/mL) and alkaline phosphatase (ALP,U/L). Bone biopsy was performed after double tetracycline labeling. The specimens were classified histologically as osteitis fibrosa (OF), mixed disease (MD),

adynamic bone disease (ABD) or osteomalacia (OM). Aluminum intoxication (AI) or iron intoxication (IBD) were defined when more than 20% of the trabeluar bone surface were covered by metal. Comparative analysis was performed according to the biopsy diagnosis.

**Results:** The mean age was 49±12.5 years, 58.9% were female. The mean hemodialysis duration was 9.6±4.7 years. OF, MD, ABD and OM were present in 54.4%, 29.7%, 12.6% and 2.5% of patients, respectively. AI and IBD were found in 53.3% and 32% of patients, respectively, and were more associated with the ABD. The table shows the comparative analysis of biochemical parameters according to histological diagnosis.

| | OF | ABD | MD | OM | p= |
|---|---|---|---|---|---|
| CAt | 10.1±1.1 | 9.7±0.9 | 9.9±1.1 | 9±0.6 | 0.16 |
| P | 6.5±1.7 | 5.3±2 | 5.6±1.7 | 3.3±2 | <0.001[a,b,c] |
| ALP | 219 (129;440) | 124 (96;167) | 386 (141;680) | 175 (155;243) | 0.002[a,b,d] |
| iPTH | 1318 (750;2033) | 865 (28;207) | 1533 (827;2101) | 348 (267;657) | <0.001[a,c,d,e,f,] |

.*mean±dp; **median ($P_{25}$;$P_{75}$) [a]OFxABD,[b]OFxMD,[c]OFxOM,[d]ABDxMD,[e]ABDxOM,[f]MDxOM.

**Conclusions:** Our data demonstrate that the high turnover disease are the most prevalent and still is a high prevalence of AI. IBD emerges as a frequent complication and clinical significance undefined. The FA and iPTH were good markers of bone remodeling.

## FR-PO626

**The Skeleton as a Reservoir of Calcium in Dialysis Patients: The Less You Have, the More You Lose** Patricia T. Goldenstein, Rosilene M. Elias, Luciene dos Reis, Fabiana Graciolli, Wagner Dominguez, Gisele Antunes Lins, Hugo Abensur, Manuel Carlos Martins Castro, Vanda Jorgetti, Rosa M.A. Moyses. *Nephrology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil.*

**Background:** Calcium (Ca) balance during dialysis session is an important, but unrecognized tool for the management of CKD patients. Current mathematical models estimate dialysis Ca balance employing Ca gradient between serum and dialysate, as well as ultrafiltration (UF). The skeleton, despite being the most important reservoir of Ca, is not included in these models.

**Methods:** The aim of this study was to verify the influence of bone on Ca mass transfer during dialysis in CKD patients with severe hyperparathyroidism (SHPT) who were submitted to bone biopsy. Fourteen patients (11 males), aged 45 ± 12 yrs, on hemodialysis for a median of 75 (48-138) months were dialysed using two different (d[Ca]):1.25 and 1.75mmol/L.

**Results:** When a d[Ca] of 1.25mmol/L was used, all but 2 patients had a negative Ca mass transfer (median -283 mg (-782, +439)), total serum Ca and PTH remained relatively stable (Δ = -0.17 mg/dl and 109 pg/ml, ns). In a d[Ca] of 1.75mmol/L, all but 3 patients had positive Ca balance (median +103 mg (-291, 676)), total serum Ca increased and PTH decreased (Δ = +1.34 mg/dl and -1042 pg/ml; p≤ 0.0001 for both). On a d[Ca] of 1.25mmol/L, stepwise multiple regression analysis adjusted for Ca gradient and UF ($R^2$ = 0.55) showed that Ca balance was dependent on bone volume (p = 0.008) and osteoclast surface (p = 0.0048). On a d[Ca] of 1.75mmol/L, stepwise multiple regression analysis adjusted for Ca gradient and UF ($R^2$ = 0.77) showed that Ca balance was dependent on bone formation rate (p = 0.001) and eroded surface (p < 0.0001).

**Conclusions:** Employing bone biopsy, we could show, for the first time, that Ca balance varies widely during dialysis and the skeleton plays a key role in this acute Ca transfer. Patients with SHPT and lower bone volume lose more Ca when dialyzing with a d[Ca] of 1.25mmol/L. Conversely, in a d[Ca] of 1.75mmol/L, those with a higher bone formation rate and lower bone resorption, gain more Ca. Bone remodelling should be taken into account when choosing d[Ca] in dialysis.

*Funding:* Government Support - Non-U.S.

## FR-PO627

**Inter-Method Variability for the Measurement of Bone Alklaine Phosphatase: Implication for the Monitoring of CKD-MBD** Etienne Cavalier,[1] Pierre Delanaye.[2] *[1]Clinical Chemsitry, Univ of Liege, CHU Sart-Tilman, Liege, Belgium; [2]Nephrology, Dialysis Transplantation, Univ of Liege, CHU Sart-Tilman, Liege, Belgium.*

**Background:** KDIGO suggested measuring PTH or bone-specific alkaline phosphatase (b-ALP) to evaluate bone disease. Little information is however available regarding the variability among the b-ALP methods; we aimed to compare the values obtained by 3 different automated b-ALP in a population of hemodialyzed (HD) patients.

**Methods:** Serum from 81 HD, prior a hemodialysis session was obtained and stored at -80°C until analysis. b-ALP was determined with the Beckman Access, DiaSorin Liaison and IDS-iSYS in a single batch on the same day. Intact PINP (iSYS) and 3rd generation PTH (Liaison) were also determined.

**Results:** b-ALP median (95%CI) for Access, iSYS and Liaison were: 16.4(14.9-19.6), 20.9(18.8-25.6) and 14.7(13.4-16.9)µg/L, respectively. Passing-Bablock (PB) regressions were Access=1.16xLiaison-0.02, Access=0.78xiSYS+0.8 and iSYS=1.53xLiaison-1.4. Correlation between iSYS and PTH was weak (0.24) but significant (p<0.05) whereas correlations between Access (0.22) and Liaison (0.18) were not. There was a significant correlation (p<0.0001) between PINP and the 3 methods: 0.61(Access), 0.64(iSYS) and 0.52(Liaison). Recalibration of iSYS and Liaison to the Access according to the PB equations harmonized the results. Bland-Altman plot showed Liaison has a larger variation of the results than others.

**Conclusions:** There is a significant problem in the b-ALP determination today which has clear clinical consequences. The variations among the methods are mainly due to assay drifted and reagents stability. The kit manufacturers should be keen to improve reagents stability, lot-to-lot variation and harmonization of the calibrations. The nephrologists should be aware that, just like PTH, b-ALP results are not transposable from one laboratory to the other. Dialogs between the laboratories and nephrologists remain essential.

## FR-PO628

**Relationship between Bone Parameters on Histomorphometry and microCT** R.C. Pereira,[1] David S. Bischoff,[2] Dean T. Yamaguchi,[2] Barbara Gales,[1] Isidro B. Salusky,[1] Katherine Wesseling-Perry.[1] *[1]Pediatrics, UCLA, Los Angeles, CA; [2]Medicine, VA Healthcare System, Los Angeles, CA.*

**Background:** Biochemical markers are imperfect markers of bone histology in renal osteodystrophy (ROD); thus, bone histormophometry remains the gold standard for the assessment of bone turnover, mineralization, and volume in CKD. While newer imaging techniques, including QCT and pQCT, have promise in predicting overall bone structure and volume, the ability of CT to assess other histomorphometric parameters remains unknown.

**Methods:** We compared to bone histomorphometry variables to microCT (µCT) analysis of 68 bone biopsy cores from dialysis patients with normal to high bone turnover, adynamic bone and pure osteomalacia and 14 controls.

**Results:** As expected, osteoid accumulation as assessed by bone histomorphometry was higher in patients with osteomalacia than in those with adynamic bone, while bone mineral density as assessed by µCT was lower.

| Parameter | Dialysis: normal to high bone turnover (n=52) | Dialysis:pure osteomalacia (n=7) | Dialysis:adynamic bone (n=9) | Healthy controls (n=14) |
|---|---|---|---|---|
| **Histomorphometry** | | | | |
| Bone volume (BV/TV) (%) | 30.1 ± 1.1 | 29.1 ± 3.5 | 28.0 ± 0.9 | 21.4 ± 1.5 |
| Trabecular thickness (Tb.Th) (um) | 167 ± 5 | 145 ± 15 | 137 ± 13 | 141 ± 14 |
| Trabecular separation (Tb.Sp) (um) | 406 ± 16 | 343 ± 38 | 355 ± 29 | 459 ± 22 |
| Osteoid volume (OV/BV) (%) | 11.9 ± 0.7 | 28.4 ± 3.9 | 1.1 ± 0.4 * | 2.2 ± 0.4 |
| Osteoid surface (OS/BS) (%) | 47.0 ± 1.8 | 67.2 ± 6.5 | 10.0 ± 2.0 * | 16.6 ± 2.7 |
| Osteoid thickness (O.Th) (um) | 19.8 ± 0.6 | 27.8 ± 4.0 | 6.1 ± 0.6 * | 8.3 ± 0.8 |
| Bone formation rate (BFR/BS) (um³/um²/y) | 95.1 ± 1.4 | 11.3 v 9.1 | 21.4 ± 14.1 | 68.7 +- + 7.0 |
| **microCT** | | | | |
| Bone volume (BV/TV) (%) | 32 ± 1 | 29 ± 3 | 29 ± 2 | 20 ± 2 |
| Connectivity Density (#/mm³) | 17.4 ± 1.2 | 36.3 ± 13.4 | 21. 3 ± 2.7 | 16.5 ± 2.2 |
| Trabecular Number (#/mm) | 2.0 ± 0.1 | 2.3 ± 0.2 | 2.1 ± 0.1 | 1.8 ± 0.1 |
| Trabecular Thickness (mm) | 0.16 ± 0.01 | 0.12 ± 0.01 | 0.15 ± 0.01 | 0.14 ± 0.01 |
| Trabecular Separation (mm) | 0.50 ± 0.02 | 0.40 ± 0.05 | 0.46 ± 0.04 | 0.55 ± 0.03 |
| Bone Mineral Density (mg HA/ccm) | 814 ± 7 | 784 v 15 | 821 ± 8 * | 792 ± 9 |

*p≤0.05 between adynamic bone and osteomalacia

In dialysis patients, bone volume measurements by both methods were highly correlated (BV/TV: r=0.70, p<0.001 ; Tb.Th: r=0.77, p<0.001; Tb.Sp: r=0.53, p<0.001 ). Bone formation rate did not correlate with any µCT determinations. Osteoid accumulation correlated with bone mineral density (O.Th: r= - 0.42, p<0.001; OV/BV: r= - 0.32, p<0.01, OS/BS: r=-0.30, p<0.05).

**Conclusions:** Measures of bone volume can be accurately assessed with CT. Skeletal mineralization may also be assessed as bone mineral density is lower in patients with excessive osteoid accumulation and similar amounts of bone volume and higher in patients with adyamic, well-mineralized bone. The potential value of non-invasive assessment of bone by pQCT, CT and bone histomorphometric variables remains to be determined.

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support

## FR-PO629

**Canopy Cells in Renal Osteodystrophy (ROD): Potential Role in Dysregulated Coupling of Bone Formation and Resorption** R.C. Pereira,[1] Thomas Levin Andersen,[3] Peter A. Friedman,[2] Barbara Gales,[1] Isidro B. Salusky,[1] Katherine Wesseling-Perry.[1] *[1]Pediatrics, UCLA, Los Angeles, CA; [2]Pharmacology and Chemical Biology, Univ of Pittsburg, Pittsburg, PA; [3]Vejle - Lillebaelt Hospital, Univ of Southern Denmark, Vejle, Denmark.*

**Background:** Disruption of canopies--flat cells which are lifted from the bone surface to form an enclosed compartment over the remodeling surface—impairs bone formation (BFR) despite osteolysis in pts with multiple myeloma. Their role in ROD is unknown.

**Methods:** Canopies were assessed in 106 bone biopsies of pediatric pts with CKD stages 2-5 and quantified on a scale of 0 to 4 (0: no surfaces with canopy lift; **0.5**: 1-2 surfaces; **1**: 3-4 surfaces; **2**: 5-6 surfaces; **3**: 7-9 surfaces; **4**: > 10 surfaces). PTHR1 immunoreactivity were assessed (rabbit anti-human PTHR1 (Gramsch Labs, Schwabhausen, Germany)) and quantified on the same scale.

**Results:** PTHR1 immunoreactivity was observed in canopies and in flat cells attached to the bone surface, although not in osteoblasts, osteoclasts, or osteocytes. Circulating PTH values were lower and fewer canopies were seen in CKD than in dialysis pts. Bone PTHR1 immunoreactivity did not differ between groups. PTH levels correlated with canopies (r=0.54, p<0.01) but not with bone PTHR1. Canopies and PTHR1 immunoreactivity correlated with BFR (r=0.31, p<0.01 and r=0.30, p<0.01), osteoid accumulation (OS/BS: r=0.58, p<0.01 and r=0.43, p<0.01) and osteoclast surface (r=0.67, p<0.001 and r=0.32, p<0.01). In multivariable analysis, OS/BS predicted PTHR1 immunoreactivity ($R^2$=0.26) while PTH, OS/BS, and Oc.S/BS were independent predictors of canopies ($R^2$=0.53).

| Parameter | CKD stages 2-4 (n=18) | Dialysis (n=88) |
|---|---|---|
| **Biochemicals** | | |
| Calcium (mg/dL) | 9.2 ± 0.2 | 9.0 ± 0.1 |
| Phosphorus (mg/dL) | 4.8 ± 0.3 | 6.5 ± 0.2 * |
| Alkaline phosphatase (IU/L) | 212 ± 38 | 246 ± 25 |
| 1st generation PTH (pg/mL) | 68 (48, 137) | 508 (310, 930) * |
| 2nd generation C-terminal FGF23 (RU/ml) | 228 (101, 462) | 1989 (617, 7345) * |
| **Histomorphometry** | | |
| Bone Volume (BV/TV) (%) | 29.8 ± 2.1 | 33.3 ± 1.0 |
| Osteoid Volume (OV/BV) (%) | 5.1 ± 2.5 | 5.1 ± 0.5 |
| Osteoid Surface (OS/BS) (%) | 19.4 ± 3.8 | 31.4 ± 1.7 * |
| Osteoid Thickness (O.Th) (um) | 10.1 ± 1.8 | 11.1 ± 1.1 |
| Osteoid Maturation Time (OMT) (d) | 14.5 (8.4, 19.5) | 12.5 (9.6, 16.0) |
| Mineralization Lag Time (MLT) (d) | 24.6 (11.9, 45.2) | 35.1 (21.1, 88.7) |
| Bone Formation Rate (BFR/BS) (um²/ um²/yr) | 14.0 (5.4, 17.8) | 25.6 (7.8, 63.2) |
| Eroded Surface (ES/BS) (%) | 4.8 ± 0.8 | 9.6 ± 0.6 * |
| Osteoclast surface (Oc.s/BS) (%) | 0.4 ± 0.1 | 2.2 ± 0.3 * |
| **Canopy and PTHR1** | | |
| PTHR1 (subjective scale: 0-4) | 1.0 (0.5, 2.0) | 1.0 (0.5, 2.0) |
| Canopy (subjective scale: 0-4) | 0 (0, 0.5) | 0.75 (0.5, 2.0) * |

*p<0.01 between pre-dialysis CKD and dialysis

**Conclusions:** Canopies in CKD more closely reflects bone formation and resorption than does PTHR1 immunoreactivity. Canopies may play a role as in independent factor in the regulation of bone formation and resorption in the pathogenesis of ROD.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO630

### The Relationship between Micropetrosis and Osteocyte Number in Hemodialysis Patients with Hypoparathyroidism

Aiji Yajima,[1] Masaaki Inaba,[2] Yoshihiro Tominaga,[3] Kosaku Nitta,[4] Shigeru Satoh.[1] *[1]Akita Univ School of Medicine; [2]Osaka City Univ Graduate School of Medicine; [3]Nagoya 2nd Red Cross Hospital; [4]Tokyo Women's Medical Univ.*

**Background:** Bone microcracks are frequently found in patients with low bone turnover probably because of decreased osteocyte number in micropetrotic bone in these patients. We hypothesized that the cause or duration of low turnover may alter these findings.

**Methods:** Fifteen HD patients with low bone turnover with or without total parathyroidectomy (PTX) or cinacalcet hydrochloride were assessed by bone histomorphometry of iliac crest bone biopsy. Group I; taken before and at 1 year (yr) after PTX (n=3) and before and after cinacalcet after 1 yr of treatment (n=4). Group II; HD patients with long-term (over 3 yrs due to unknown causes) low iPTH (< 60 pg/mL; n=8). Micropetrosis volume/bone volume (Mp.V/BV; %), osteocyte number/micropetrosis volume; (N.Ot/Mp.V; N/mm$^2$) and N.Ot/bone volume excluding micropetrosis volume; (N.Ot/(BV-Mp.V); N/mm$^2$) were compared before and after the treatment in Group I. N.Ot/Mp.V was compared with N.Ot/(BV-Mp.V) in Group I (post) and Group II. All bone samples were diagnosed as having low bone turnover.

**Results:** In group I, serum iPTH decreased from 1161 to 86 pg/mL. N.Ot/Mp.V decreased at 1 yr (216.6 ± 103.1(SD) to 145.8 ± 90.0 N/mm$^2$, p=0.016), but N.Ot/(BV-Mp.V) and Mp.V/BV did not change. For group II, iPTH was 15.9 pg/mL at the time of bone biopsies. Mp.V/BV was greater in Group II compared to Group I (63.1 ± 12.4 vs. 29.6 ± 13.6 %, p=0.004). N.Ot/Mp.V was smaller than N.Ot/(BV-Mp.V) in Group I (145.8 ± 90.0 vs. 274.3 ± 66.4 N/mm$^2$, p=0.031) and Group II (94.5 ± 63.5 vs. 172.4 ± 69.4 N/mm$^2$, p=0.007).

**Conclusions:** In patients with long term low turnover not induced by PTX or cinacalcet, there is an increase of micropetrosis with a reduction in overall osteocytes. This implies that the pathophysiology of low turnover induced by cinacalcet or PTX at 1 yr may be very different than that observed in patients without a known cause of low turnover for a long term. The latter may be due to decreased osteocytes survival, perhaps caused by the micropetrosis.

*Funding:* Pharmaceutical Company Support - Kirin, Chugai, Bayer

## FR-PO631

### Synergistic Effect of Etidronate Combined with Menatetrenone for Glucocorticoid-Induced Osteoporosis in Patients with Chronic Glomerulonephritis

Yuko Makita, Hitoshi Suzuki, Masao Kihara, Hiromitsu Fukuda, Satoshi Mano, Takashi Kobayashi, Yasuhiko Kanaguchi, Tatsuya Aoki, Teruo Hidaka, Katsuhiko Asanuma, Yasuhiko Tomino. *Nephrology, Juntendo Univ Faculty of Medicine, Tokyo, Japan.*

**Background:** Glucocorticoid therapy is useful for treatment of chronic glomerulonephritis (CGN), however, glucocorticoid may induce secondary osteoporosis. Bone loss initiate just after administration of glucocorticoid, and the degree of osteoporosis depends on the cumulative dose of glucocorticoid. Although bisphosphonate treatment is well known to improve the bone quality and reduce the risk of bone fractures, recent studies have shown that vitamin K2 also maintain stable bone mineral density (BMD). Furthermore, vitamin K2 is essential for bone formation cooperate with osteocalcin. Thus, we examined the clinical efficacy of bisphosphonate alone or bisphosphonate combined with vitamin K2 for preventing glucocorticoid-induced bone loss in CGN patients using serum levels of N-terminal telopeptide of type I collagen (NTx) and uncarboxylated osteocalcin (ucOC) with BMD.

**Methods:** We conducted a 6-month prospective randomized study at Juntendo university hospital. Overall 43 patients (mean age 39.4 ± 17.0) with CGN commencing with prednisolone were recruited from 2011 to 2013. These patients were randomized to Etidronate (17.5 mg/week) alone (Etidronate group, n=19) or Etidronate (17.5 mg/week) with Menatetrenone (45 mg/day) (Combined group, n=24) treatment groups. Serum levels of NTx and ucOC as well as BMD were measured before and after 3 and 6 months of commencing with prednisolone.

**Results:** In the Etidronate group, the percentage changes of serum levels of NTx after 3 and 6 months were -6.1 % and -9.8 %, whereas those were -28.3 % and -27.0 % in the Combined group. The percentage changes of serum levels of ucOC after 3 and 6 months were -8.3 % and -10.6 % in the Etidronate group, -51.3 % and -50.0 % in the Combined group, respectively. There were no significant differences in BMD between these groups during the study.

**Conclusions:** Our data suggested that combination therapy of Etidronate with Menatetrenone may have synergistic effect to prevent glucocorticoid-induced osteoporosis in patients with CGN.

## FR-PO632

### Blood Tests Predictive of Bone Loss in CKD-5D Patients

Hartmut H. Malluche,[1] Daniel Davenport,[2] Marie-Claude M. Faugere.[1] *[1]Div of Nephrology, Univ of Kentucky; [2]Dept of Surgery, Univ of Kentucky.*

**Background:** Low bone mass is frequently seen in patients with chronic kidney disease on dialysis (CKD-5D) and abnormalities in bone volume are an integral part of renal osteodystrophy. Patients with CKD-5D have 4.4 times the risk of hip fracture compared to the general population. This prospective study was conducted to determine whether serum biochemical parameters can help to predict bone loss in CKD-5D patients.

**Methods:** In CKD-5D patients, bone mass was measured by QCT and DXA of the hip and spine at baseline and after 1 year. At time of these bone mass measurements, blood tests were done including: PTH; FGF-23; sclerostin; procollagen type 1 N-terminal propeptide (P1NP); and tartrate-resistant acid phosphatase-5b (TRAP-5b). Demographic and clinical characteristics were recorded.

**Results:** 69 patients completed the study (Table). Bone loss was observed by QCT in the hip in 52% of the patients and in the spine in 36%; by DXA in the hip in 35% and in the spine in 36%. Bone loss in the hip was lower in black patients and in patients exercising regularly (both p<.05). Bone loss was not greater in diabetics and did not correlate with either BMI or dialysis vintage. TRAP-5b, sclerostin and FGF-23 were significantly higher in losers than non-losers (p<0.05). Bone loss by QCT of the hip correlated with baseline P1NP, TRAP-5b and sclerostin (rho=+.245,-.267,-.371 rsp, all p<.05). Bone loss by DXA of the spine correlated with FGF-23 (rho=-.393, p=.001). Sclerostin and TRAP-5b were independent predictors of bone loss by QCT of the hip after adjustment for age, race and exercise (β=-8.4, p=.008 and β=-1.4, p=.011 rsp). Log FGF-23 predicted bone loss by DXA of the spine (β=-.04, p=.003).

| | |
|---|---|
| Gender | 63% M / 37% F |
| Race | 35 Black / 33 White / 1 Other |
| Age, mean y ± SD | 53.6 ± 12.4 |
| BMI, mean kg/m2 ± SD | 32.2 ± 7.3 |
| Dialysis Vintage, median mos. (range) | 39 (3 – 241) |
| Diabetics | 41% |
| Exercises 1-2 times/week | 22% |
| Exercises 3+ times/week | 41% |
| Treated with Vitamin D analogs | 86% |

**Conclusions:** The available blood tests, TRAP-5b, sclerostin, and FGF-23 can be used to assess risk for bone loss in CKD-5D. These findings are relevant for a major clinically challenging problem.

*Funding:* Other NIH Support - R01DK080770, Private Foundation Support

## FR-PO633

### A Retrospective Review of Bone Density Scans in Different Stages of Chronic Kidney Disease

Bhanu Prasad Tikkisetty,[1] Jennifer St.onge.[2] *[1]Nephrology, Regina Qu Apelle Health Region, Regina, Canada; [2]Research and Health Information Service, Regina Qu Apelle Health Region, Regina, Canada.*

**Background:** Our renal program undertook a retrospective chart review of all patients who underwent a baseline dual-energy X-ray absorptiometry (DXA) scan in our multidisciplinary CKD clinic from Jan 2001 to Jan 2010. The purpose of the study was to determine if there was a preferential site of bone loss in CKD patients across different disease stages.

**Methods:** A total of 410 patients were included in the dataset. Measures included glomerular filtration rate (eGFR) in mls/min as per the Modification of Diet in Renal Disease formula, bone density measurements including T-scores and Z-scores for the lumbar spine, both hips, and the one-third distal radius, as well as the number of fractures. We compared these measures across patients in CKD Stages 2 through 5.

**Results:** We found a significant decline in median bone density across Stages 2 through 5 for total hip ($X^2$ (3) = 16.73, $p$=.001) and femoral neck ($X^2$ (3) =12.76, $p$=.005) sites, but not lumbar spine or one-third distal radius. Increasing serum phosphorus was significantly associated with reduced bone mineral density in the total hip (r=.15, $p$<.05, Spearman's rho) and trended towards significance for femoral neck (r=.10, $p$=.056, Spearman's rho). The percentage of patients suffering from a fracture, however, did not significantly change with CKD stage using a between-groups analysis ($X^2$ (3) = 6.42, $p$=.09, Cramer's V = .13, $p$=.09).

**Conclusions:** There is a demonstrable decline in total hip and femoral neck bone density with progression of CKD staging. In contrast, lumbar spine and distal radius bone mineral density were not affected by CKD stage. DXA scans provide information on BMD, they do not comment on trabecular and cortical microarchitecture and the resolution is too

low to distinguish between cortical and trabecular bone. Future research will determine whether decreased bone density as determined by DXA scan is associated with increased fracture risk over time in the same patients. We will also examine the effect of treatment with bisphosphonates on BMD as CKD progresses.

### FR-PO634

**Prescribing Practices and Response to Cinacalcet Treatment in Secondary Hyperparathyroidism – A Single Centre Experience** Nadeeka Kumarihamy Rathnamalala, Graham Warwick. *John Walls Renal Unit, Univ Hospitals of Leicester, Leicester, United Kingdom.*

**Background:** Managing bone disease in advanced chronic kidney disease (CKD) can be challenging. With the introduction of cinacalcet in the United Kingdom, guidelines from National Institute for Health and Care Excellence (NICE) have been issued to guide its use. The aim of this audit was to determine if cinacalcet was used in line with NICE and Kidney Disease Improving Global Outcomes (KDIGO) guidelines.

**Methods:** This was a retrospective observational study of patients under the care of a large tertiary nephrology centre in the UK. Data was extracted from the electronic medical records and included prevalent dialysis ,nephrology and transplant clinic follow ups from 01.08.2012 to 31.10.2012.

**Results:** 65 subjects were identified (M:F 38:27).Mean age was 62 (+/- 17) yrs and 89.2% were Caucasians. Majority (84.6%) were receiving renal replacement therapy(RRT) while 7.7% were transplanted and 7.7% were pre-dialysis CKD. 94.5% on RRT were on unit based haemodialysis for mean of 6.1 yrs. 50.8% had contraindications for parathyroidectomy while 49.2% declined surgery.

76.9 % of patients were already on a phosphate binder and this did not change over one year on cinacalcet. Only 62.5 % of patients were on vitamin D analogues but at the end of one year this had increased to 71.1 % of patients. 82.6 % of patients were started on 30mg cinacalcet and 59% and 62.2% remained on 30 mg at 6 and 12 months respectively.

84.4% of the study population had iPTH levels above the KDIGO targets while only 40.7% and 37.5% still remained over the target at 6 and 12 months respectively.However only 16 (26.2%) of the total study population had dose adjustments during the follow up period.

**Conclusions:** All subjects were started on cinacalcet treatment due to contraindications to parathyroidectomy or preference to have medical treatment. Cinacalcet did not significantly reduce the need for a phosphate binder but there increased requirement for vitamin D analogues. In a significant number iPTH was not reduced to the target but dose escalation was often not performed according to guidelines.

### FR-PO635

**Lanthanum Carbonate or Dietary P Restriction Ameliorates Endothelial Dysfunction in Adenine-Induced Chronic Kidney Disease Rats** Haruka Ueda, Yutaka Taketani, Maerjianghan Abuduli, Hirokazu Ohminami, Hisami Okumura, Hironori Yamamoto, Eiji Takeda. *Dept of Clinical Nutrition, Univ of Tokushima, Tokushima, Japan.*

**Background:** Cardiovascular disease (CVD) is the most important cause of mortality in chronic kidney disease (CKD) patients. Hyperphosphatemia has been identified as an independent risk factor for CVD in CKD patients, as elevation of serum phosphorus (P) levels can be involved in endothelial dysfunction as well as vascular calcification. Dietary P restriction and P binders are useful for correction of hyperphosphatemia, however, it has been unknown whether these treatments have similar beneficial effect on endothelial function or not. Here, we investigated the effect of lanthanum carbonate or dietary P restriction on endothelial dysfunction in adenine-induced chronic kidney disease rats.

**Methods:** Adenine-induced CKD model rats were prepared by feeding 0.5% adenine containing diet for 35 days on male 7-wk-old Sprague-Dawley rats. The adenine-induced CKD rats were divided 5 groups and treated with either control diet (CP; 1%P), low P diet (LP; 0.2%P), 1.5% lanthanum carbonate (La1.5; 1%P, 1.5%LaCO3), 3% lanthanum carbonate (La3; 1%P, 3%LaCO3), or 6% lanthanum carbonate (La6; 1%P, 6%LaCO3) for 14 days. Plasma were collected at sacrificed day and measured P, intact-PTH, FGF23, and creatinine. Aortic rings were also prepared and measured acetylcholine-dependent vasodilation by isometric transducers.

**Results:** Plasma P, intact-PTH, and FGF23 levels were significantly decreased by similar level and plasma calcium levels were increased in LP and La6 groups compared with CP group. Acetylcholine-dependent vasodilation was impaired in adenine-induced CKD rats. This impairment was significantly and similarly improved in LP and La6 groups compared with CP group. However, correction of hyperphosphatemia was not enough in La1.5 and La3 groups so that impaired vasodilation was not significantly ameliorated.

**Conclusions:** In conclusion, both dietary P restriction and lanthanum carbonate can ameliorate endothelial dysfunction observed in adenine-induced CKD rats, but the improvement would be depended on the correction rate of serum P level.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

### FR-PO636

**β-Glycerophosphoric Acid Promotes Vascular Smooth Muscle Cells Transdifferentiation and Calcification via miR-125b Downregulation and Ets1 Induction** Ping Wen, Junwei Yang. *2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

**Background:** Vascular calcification is highly prevalent in patients with chronic kidney disease (CKD) and is associated with increased risk of cardiovascular disease and mortality. Accumulated evidences suggested that vascular smooth muscle cells (VSMCs) to osteoblast-like cells transdifferentiation (VOT) plays a crucial role in promoting vascular calcification. MicroRNAs (miRNAs) are a novel class of small RNAs that negatively regulate gene expression via repression of the target mRNAs. In the present work, we sought to determine the role of miRNAs in VSMCs phenotypic transition and calcification induced by b-glycerophosphoric acid (β-GP).

**Methods:** Primary cultured rat aortic VSMCs were treated with β-GP for different time. The protein levels of SM-22α, cbfα-1, osteocalcin, and osteopontin in VSMCs were tested by Western Blotting assay. miR-125b expression was detected by real-time RT PCR. Calcium deposition was determined by Alizarin Red S staining.

**Results:** 1) In VSMCs, after β-GP treatment, the expressions of cbfα1, osteocalcin and osteopontin were significantly increased and SM-22α expression was decreased. ALP activity was induced by β-GP in a time and dose dependent manner. Calcium deposition was detected in VSMCs incubated with calcification media; 2) miR-125b expression was significantly decreased in VSMCs after incubated with β-GP. miR-125b mimic could inhibit β-GP-induced osteogenic markers expression and calcification of VSMCs whereas miR-125b inhibitor promoted the phenotypic transition of VSMCs and calcification; 3) miR-125b targeted Ets1 and regulated its protein expression in VSMCs. Downregulating Ets1 expression by its siRNA inhibited β-GP-induced the VSMCs phenotypic transition and calcification.

**Conclusions:** Our study suggests that β-glycerophosphoric acid, a donor of phosphorus, can stimulate VSMC transdifferentiation and calcification, which is probably through miR-125b downregulation and Ets1 induction.

*Funding:* Government Support - Non-U.S.

### FR-PO637

**Phosphate Overload Induces Local and Systemic Inflammation and Malnutrition in Uremic Rats** Shunsuke Yamada,[1] Masanori Tokumoto,[1] Masatomo Taniguchi,[2] Kazuhiko Tsuruya,[3] Takanari Kitazono.[2] *[1]Div of Nephrology, Fukuoka Dental College, Fukuoka, Japan; [2]Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [3]Dept of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

**Background:** Phosphate (Pi) overload plays a pivotal role in the development of cardiovascular disease (CVD) in chronic kidney disease (CKD) patients. Inflammation and malnutrition also contribute to the high prevalence of CVD in patients with CKD. However, the impact of Pi overload on inflammation and malnutrition remains to be elucidated.

**Methods:** The present *in vivo* study determined the effects of Pi overload on inflammation, malnutrition and CVD (cardiac weight and aortic calcium content) in CKD rats fed a diet containing 0.3% adenine, 19% protein and different concentrations of Pi (ranging from 0.3% to 1.2%) for eight weeks. The effects of lanthanum carbonate on Pi-related changes were also investigated. Furthermore, we examined the direct effects of Pi overload on cultured human vascular smooth muscle cells (VSMCs) in an *in vitro* study.

**Results:** Pi loading dose-dependently induced malnutrition (decreased body weight and serum albumin) and CVD (increased cardiac weight and aortic calcium content) without aggravating the kidney function in the CKD rats. The CKD rats exhibited dietary Pi loading-dependent increases in the serum tumor necrosis factor (TNF)-α levels, the mRNA expression of TNF-α in the aorta, heart and kidneys and the levels of an oxidative stress marker (8-hydroxy-2'-deoxyguanosine) in the urine and aorta. Treatment with 6% lanthanum carbonate blunted almost all of the changes induced by Pi loading. A linear regression analysis showed that the serum Pi level was closely correlated with the extent of inflammation, malnutrition and CVD. In the cultured VSMCs, high Pi medium increased the mRNA of TNF-α and Pit-1, and decreased that of Klotho.

**Conclusions:** Dietary Pi overload induces a sequence of events, including inflammation, malnutrition and CVD in CKD rats. Pi-binders have the potential to counteract the changes induced by Pi overload.

### FR-PO638

**Therapeutic Effects of ONO-1301 on Vascular Calcification in a Rat Model of Adenine-Induced Chronic Kidney Disease** Yohei Maeshima,[1] Hiroyuki Watatani,[2] Norikazu Hinamoto,[2] Katsuyuki Tanabe,[2] Kana Masuda,[2] Haruyo Ujike,[2] Hitoshi Sugiyama,[2] Sakai Yoshiki,[3] Hirofumi Makino.[2] *[1]CKD and CVD, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [3]ONO Pharmaceutical Co., Osaka, Japan.*

**Background:** Cardiovascular disease is a leading cause of mortality in patients with CKD, and vascular calcification is a key modifier of disease progression. ONO-1301 (ONO) is a novel prostacyclin analog possessing thromboxane synthase inhibitory activity. We recently reported the renoprotective effects of ONO in experimental models of diabetic

nephropathy and obstructive uropathy. Here, we aimed to investigate the therapeutic efficacies of ONO on progressive CKD and vascular calcification in a rat model of adenine-induced CKD.

**Methods:** Male Sprague-Dawley rats at 13 weeks of age were fed with the diet containing either 0.75% (CKD) or 0% (control) adenine along with 2.5% protein. After 3 weeks, animals were divided into one of two treatment groups with equivalent kidney dysfunction. For the following 5 weeks, animals were fed with standard rat chow, and ONO (6mg/kg/ day) or vehicle buffer was orally administered. Urine, serum, kidneys and thoracic aorta were obtained and subjected to evaluation.

**Results:** Treatment with ONO did not significantly improve adenine-induced renal insufficiency and renal histological alterations. However, vascular calcification (von Kossa staining), the Ca and Pi content of the thoracic aorta and serum osteocalcin levels were significantly decreased by treatment with ONO compared with the control CKDanimals. In addition, treatment with ONO significantly recovered the levels of matrix Gla Protein and Fetuin-A suppressed by adenine-induced CKD, and suppressed the overexpression of RUNX2 in the VSMC of the thoracic aorta (immunohistochemistry).

**Conclusions:** Taken together, these results suggest the protective role of ONO on vascular calcification via regulating the levels of RUNX-2 and locally acting inhibitors of vascular calcification in the experimental CKD model.

*Funding:* Pharmaceutical Company Support - ONO pharmaceuticals

## FR-PO639

**Usefulness of Tubular Reabsorption of Phosphate as a Probable Surrogate Marker for Phosphate Regulation in Chronic Kidney Disease**

Yul Hee Cho,[1] Yu ah Hong,[2] Myung Hyun Lee,[1] Ji Hee Lim,[1] Min Young Kim,[1] Keun Suk Yang,[1] Ji Hyun Yu,[1] Seun Deuk Hwang,[1] Bum-Soon Choi,[1] Chul Woo Yang,[1] Yong-Soo Kim,[1] Cheol Whee Park.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea; [2]Div of Nephrology, Dept of Internal Medicine, Korea Univ.*

**Background:** Fibroblast growth factor 23 (FGF23) and soluble α-Klotho, which are emerging potential biomarkers of phosphorus and vitamin D metabolism, change progressively beginning in early chronic kidney disease (CKD) to maintain normal phosphorus. However, its use for an early surrogate marker of CKD-mineral and bone disorder (MBD) is limited in clinical practice. The tubular reabsorption of phosphate (TRP) has been commonly used to assess the renal tubular phosphate transport. The aim of this study investigates to find the earliest biomarker for CKD-MBD related to the estimated GFR (eGFR) in CKD patients.

**Methods:** We performed a cross-sectional study in 93 stable patients with predialysis CKD stage 1-5. TRP, eGFR, calcium, phosphate, intact PTH (iPTH), 25-hydroxyvitamin D, serum FGF23 and urine soluble α-Klotho levels were measured.

**Results:** As renal function declined, TRP significantly decreased ($P < 0.001$; $r = 0.763$) and both iPTH and serum FGF23 increased ($P < 0.001$; $r = -0.598$, $P < 0.001$; $r = -0.453$, respectively) for the augmentation of phosphaturia. Decreased eGFR and increased iPTH were found to be independently associated with decreased TRP, but did not associate with urine soluble α-Klotho and serum FGF23 in the multiple linear regression analysis. Increased serum FGF23 was only independently associated with decreased eGFR. The areas under the curve (AUC) for serum FGF23 and TRP are 0.598 and 0.739, respectively ($P < 0.05$).

**Conclusions:** TRP is a simple, useful and cost saving method compared with serum FGF23 and urine soluble α-Klotho for the assessment of the altered mineral metabolism of CKD patients.

## FR-PO640

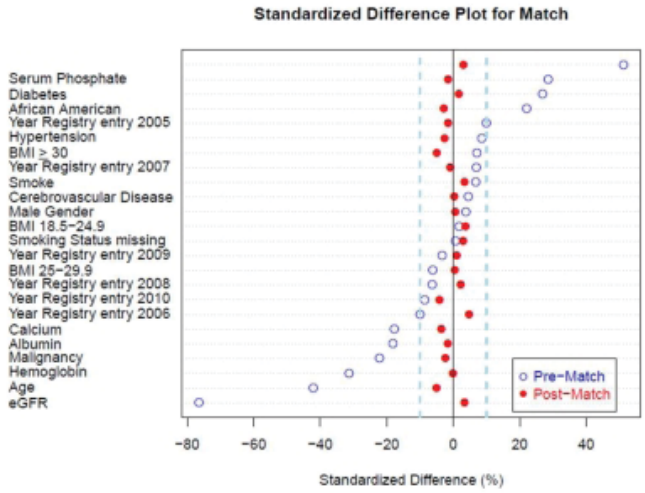
**Phosphate Binder Use and Mortality in Chronic Kidney Disease: A Propensity-Based Analysis**

Sankar D. Navaneethan,[1] Jesse D. Schold,[2] Ankit Sakhuja,[1] Susana Arrigain,[2] John W. Sharp,[2] Joseph V. Nally.[1] *[1]Nephrology; [2]Quantitative Health Sciences, Cleveland Clinic.*

**Background:** Higher serum phosphorus is associated with an increased mortality in chronic kidney disease (CKD). The practice patterns of phosphate binder use and their associations with mortality in non-dialysis dependent CKD were examined.

**Methods:** The factors associated with the use of calcium and non-calcium phosphate binders in those with stage 3 and 4 CKD were studied using logistic regression models. The associations between phosphate binder use and mortality were examined using propensity matching and Cox models.

**Results:** Out of 13,325 patients with serum phosphorus levels measured, 945 patients were on phosphate binders. 939 patients who had phosphate binder during follow up were matched using caliper width of 0.1. After matching, all of the baseline variables had standardized differences <10% between those who were and were not on binders.




238 (25%) of them were prescribed non-calcium based binders and the rest were on calcium based binders. Each 5 kg/m$^2$ higher BMI (OR 1.12, 95% CI 1.01, 1.25), 1 mg/dl higher serum phosphorus (OR 1.35, 95% CI 1.13, 1.61) and 1 mg/dl higher serum calcium (OR 1.35, 95% CI 1.06, 1.72) were associated with higher odds of being prescribed a non-calcium based binder. In the matched cohort, phosphate binder use was not significantly associated with mortality who were treated for at least 6 months. However, those who were treated for one year with a binder had a non-significant lower mortality risk (Hazard ratio: 0.85, 95% CI 0.66, 1.10). There were no differences in the risk for death between calcium and non-calcium based binders.

**Conclusions:** Short-term use of phosphate binder was not associated with lower risk for mortality in stage 3 and 4 CKD. Our results also suggest the need for studying the potential benefits of long-term phosphate binder use in this population.

*Funding:* Pharmaceutical Company Support - Genzyme

## FR-PO641

**Accelerated Progression of Vascular Calcification in Children with CKD Is Associated with Baseline Fetuin-A and Vessel Characteristics**

Rukshana C. Shroff,[1,2,4] Lesley Rees,[1] Melanie Hiorns,[3] John E. Deanfield,[2] Cathy Shanahan.[4] *[1]Renal Unit, Great Ormond Street Hospital for Children, United Kingdom; [2]Vascular Physiology Unit, Institute of Child Health, United Kingdom; [3]Radiology Unit, Great Ormond Street Hospital, United Kingdom; [4]Cardiovascular Div, King's College London, United Kingdom.*

**Background:** Vascular calcification is thought to begin early in CKD and progress rapidly on dialysis. We examined vascular changes as seen on vessel imaging with a quantitative and histological assessment of the vascular Ca load on arterial biopsy samples to study progression of vascular changes through pre-dialysis CKD, dialysis and after transplantation.

**Methods:** 48 children (16 pre-dialysis CKD 4-5 and 32 on dialysis) had vascular imaging (carotid intima-media thickness [cIMT], pulse wave velocity [PWV] and coronary artery calcification [CAC] on CT scan), biomarker analyses and an arterial biopsy (at the time of renal transplantation or PD catheter insertion). The Ca load in the vessel wall was quantitated and detailed histology performed to study hydroxyapatite deposition, vascular smooth muscle cell apoptosis and osteogenic differentiation. 43 children (22 dialysis and 21 transplants) had a second set of imaging after 14.2 ±3.9 months.

**Results:** The baseline vessel Ca load strongly correlated with cIMT in dialysis patients ($p=0.005$) whereas 11 of 16 pre-dialysis patients had normal cIMT. Dialysis patients had a significant annualised increase in cIMT and PWV ($p<0.005$ and $p=0.03$). CAC increased in 5 children with baseline CAC and was found in 3 other.

cIMT progression showed a close correlation with the vessel Ca load ($r=0.67$). Patients with cIMT progression had the highest apoptotic index implying vascular smooth muscle cell loss and greater osteogenic differentiation. The baseline cIMT ($r=0.31$) and Fetuin-A levels ($r=0.41$), but not FGF-23, 25-hydroxyvitamin D or osteopontin associated with cIMT progression. Changes in PWV and CAC did not correlate with vessel Ca load.

**Conclusions:** Calcification is rapidly progressive on dialysis and strongly correlates with baseline vessel wall characteristics and Fetuin-A levels. Fetuin-A may be a useful biomarker to predict rapid progression of vascular calcification in CKD.

## FR-PO642

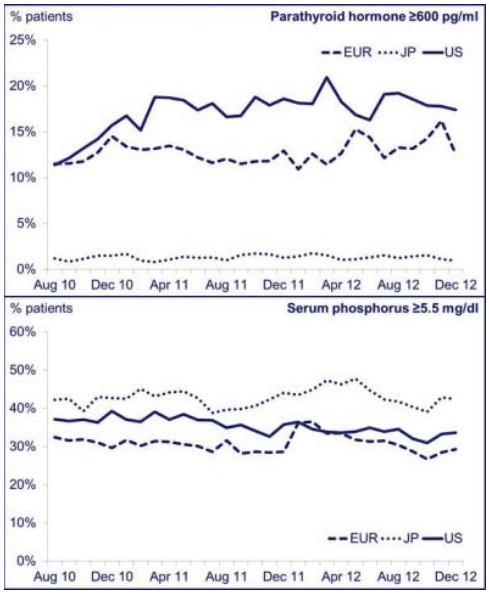
**International Trends in Markers of Mineral and Bone Disorder (MBD) among Hemodialysis Patients** Francesca Tentori,[1,2] Douglas S. Fuller,[1] Bruce M. Robinson,[1,3] Vittorio E. Andreucci,[5] Takashi Akiba,[6] Brian Bieber,[1] Yun Li,[1,3] Raymond C. Vanholder,[4] Ronald L. Pisoni.[1] *[1]Arb Res Collab Hlth, Ann Arbor; [2]Vanderbilt Univ, Nashville; [3]Univ of MI, Ann Arbor; [4]Univ Hosp, Gent; [5]Univ Federico II, Naples; [6]TWMC, Tokyo.*

**Background:** Recent MBD guidelines (KDIGO '09) and changes in reimbursement systems (e.g., Jan '11 US bundled payment) may have led to changes in clinical practice and impacted levels of serum phosphorus (P), calcium (Ca), and PTH. We tested this hypothesis within each region of the international DOPPS cohort.

**Methods:** Survey-weighted linear spline regressions adjusted for seasonality, age, black race (in US), sex, vintage, and catheter use to estimate temporal trends in frequency of PTH measurement; mean serum parathyroid hormone (PTH), P, Ca, and corrected Ca ($Ca_{alb}$); and reported target levels between Aug '10 and Dec '12.

**Results:** The % of patients with a PTH measurement in a 3-mo period declined in the US (96% to 84%) and Europe (EUR; 84% to 75%); but not in Japan (JP; 79% to 75%). There were no trends in the frequency of Ca and P measurement. Mean P fell 0.15 mg/dl (to 5.09 mg/dl) in the US and 0.08 mg/dl (to 4.84 mg/dl) in EUR; no trend was observed in JP (5.33 mg/dl). No clear trend in mean Ca or $Ca_{alb}$ levels were observed for US (mean $Ca_{alb}$ 9.10 mg/dl) and EUR (mean 9.25 mg/dl); $Ca_{alb}$ fell 0.17 mg/dl in JP (9.02 mg/dl in Dec'12). US mean PTH level rose 27% to 432 pg/ml between Aug '10 to Apr '11, stabilizing thereafter. No changes were seen in EUR (mean 333 pg/ml) or JP (mean 149 pg/ml). Reported upper limit of PTH target levels increased in the US but not EUR or JP; no changes in Ca or P upper limits were observed.

**Conclusions:** Trends towards lower P (US and EUR) and higher PTH (US) levels are strong and may reflect practice changes in response to KDIGO guidelines and US bundled payment. The potential impact of these changes on clinical outcomes remains to be determined.




*Funding:* Pharmaceutical Company Support - The DOPPS is supported by research grants from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, with additional country-specific support provided in Canada by Amgen-Canada, Janssen, BHC Medical, Takeda and Kidney Foundation of Canada, and in Germany by Hexal and WiNe Institute. Support from the DOPPS sponsors is provided without restrictions on publications.

## FR-PO643

**The PARADIGM Trial: Cinacalcet versus Vitamin D as Monotherapies for Treatment of Secondary Hyperparathyroidism in Patients on Dialysis** James B. Wetmore,[1] Konstantin Gurevich,[2] Stuart M. Sprague,[3] Gerald A. Da Roza,[4] John E. Buerkert,[5] Maureen T. Reiner,[6] William G. Goodman,[6] Kerry Cooper.[6] *[1]Hennepin County Medical Center; [2]Fresenius Medical Care Russia; [3]NorthShore U Health System; [4]U British Columbia; [5]Columbia Nephrology Associates; [6]Amgen Inc.*

**Background:** Cinacalcet (Cin) and vitamin D (vitD), often used in combination, have not been compared directly as monotherapy for the treatment of secondary hyperparathyroidism (SHPT) in patients on dialysis.

**Methods:** This study compared the efficacy of Cin to vitD as monotherapies in reducing parathyroid hormone (PTH) levels over 1 yr of treatment. The primary endpoint was mean % PTH reduction between wks 40-52. After SHPT treatment washout, patients (N=312) with PTH > 450 pg/mL were randomized to vitD (n=157) or Cin (n=155).

**Results:** Relatively severe SHPT was present at baseline, with mean (SD) PTH 846 (431) pg/mL in the Cin arm and 816 (428) pg/mL in the vitD arm. PTH reduction was modest and did not differ between study arms (12% Cin vs 7% vit D, P = 0.346). Mean (SD) dose of Cin = 83.1 (5.0) mg/day and vitD = 20.0 (12.4) mcg/wk (paricalcitol equivalents). Secondary efficacy measures also did not differ between arms. Hypercalcemia and hyperphosphatemia were more common in the vitD arm, while hypocalcemia was more common in the Cin arm. *Post hoc* analyses revealed that the use of dialysate calcium < 2.5 mEq/L or non-use of calcium-based binders negatively impacted PTH reduction during Cin monotherapy (reported separately).

**Conclusions:** Patients with relatively severe SHPT demonstrated modest reductions in PTH when using Cin or vitD as monotherapies. Combined treatment, using agents with unique mechanisms of actions and divergent effects on Ca and P levels, may be required among those with more advanced disease.

| | Cinacalcet (N = 155) | | Vitamin D (N = 157) | | p-value[1] |
|---|---|---|---|---|---|
| **Outcomes** | | | | | |
| PTH, % reduction, mean (SD) | -12.1 (4.0) | | -7.0 (4.0) | | 0.346 |
| PTH, % with ≥ 30% reduction | 42.6 | | 33.8 | | 0.110 |
| PTH, % achieving < 300 pg/mL | 19.4 | | 15.3 | | 0.346 |
| **Biochemistry, mean (SD)** | **Baseline** | **EAP** | **Baseline** | **EAP** | |
| PTH pg/mL | 846 (431) | 689 (480) | 816 (428) | 709 (561) | --- |
| Ca mg/dL | 9.6 (0.5) | 8.7 (0.6) | 9.5 (0.5) | 9.7 (0.5) | --- |
| P mg/dL | 5.7 (1.6) | 5.0 (1.3) | 5.8 (1.5) | 5.6 (1.2) | --- |

[1]Difference between treatment groups for the primary study outcomes. EAP = efficacy assessment phase.

*Funding:* Pharmaceutical Company Support - Amgen Inc.

## FR-PO644

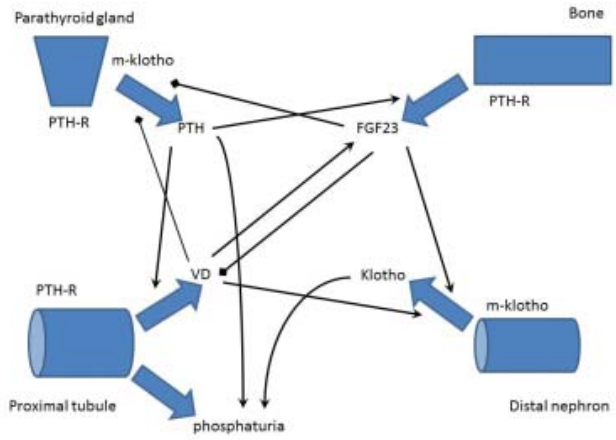
**Fibroblast Growth Factor 23 Enhances Renal Klotho Abundance** Tsuneo Takenaka, Tsutomu Inoue, Yusuke Watanabe, Takashi Miyazaki, Hiromichi Suzuki. *Saitama Medical Univ, Iruma, Saitama, Japan.*

**Background:** Klotho constitutes the receptor for fibroblast growth factor 23 (FGF23). However, the effects of FGF23 on renal and circulating klotho are not well known.

**Methods:** In vivo experiments were performed to assess the effects of FGF23 (10 μg/kg), parathyroid hormone (PTH, 10 μg/kg), and 1,25-dihydroxy-vitamin D3 (1,25VD, 1 μg/kg) on renal expression and serum concentration of klotho in Wistar rats.

**Results:** Phosphate excretion was increased at 3 hr after FGF23 administration ($p<0.05$). Renal klotho expressions and serum klotho levels were elevated at 3 hr ($p<0.01$) by FGF23. At 24 hr, phosphate excretion was still elevated ($p<0.05$), and serum phosphate, 1,25VD and PTH were reduced ($p<0.05$). However, serum and renal klotho returned to the control level at 24 hr. PTH markedly increased phosphate excretion after 24 hr ($p<0.01$). There were increases in FGF23 at 3 and 24 hr, and 1,25VD at 24 hr after PTH administration ($p<0.05$). Serum klotho concentration and renal klotho expression were elevated by PTH at 3 or 24 hr. After 24 hr exposure to 1,25VD, considerable increases in serum FGF23, calcium and phosphate were seen ($p<0.05$), but PTH was decreased ($p<0.01$). 1,25 VD elevated renal klotho expression and serum klotho ($p<0.05$) at 3 hr, but returned to control levels at 24 hr.

**Conclusions:** Our data constitute new demonstrations that FGF23 rapidly increases renal klotho expression and serum klotho. The present findings are consistent with the notion that PTH increases phosphate excretion at least in part through elevations of FGF23 and klotho . Moreover, our results indicate that 1,25VD increases klotho expression independently of FGF23. Finally, these observations suggest that free klotho may transduce the signal of FGF23 from distal to proximal tubules.




*Funding:* Government Support - Non-U.S.

## FR-PO645

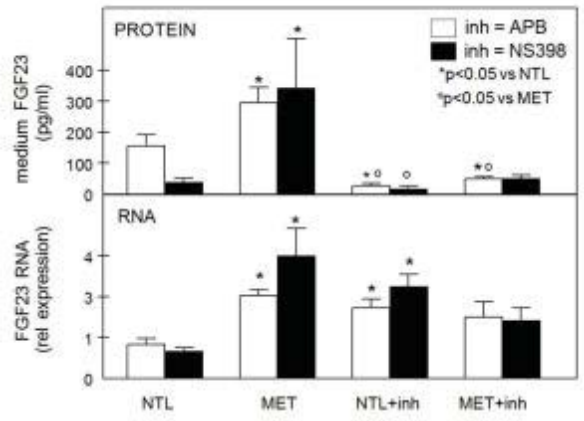
**Metabolic Acidosis Stimulation of Bone Fibroblast Growth Factor 23 Requires Intracellular Calcium Signaling and Prostaglandin Synthesis**

Nancy S. Krieger, Christopher D. Culbertson, Kelly Kyker-Snowman, David A. Bushinsky. *Medicine, Univ of Rochester, Rochester, NY.*

**Background:** Serum fibroblast growth factor 23 (FGF23) increases in chronic kidney disease (CKD) and decreases renal phosphate (Pi) reabsorption and serum $1,25(OH)_2D_3$. Patients with CKD have decreased acid excretion leading to metabolic acidosis (MET). MET is buffered by bone with release of calcium (Ca) and Pi. FGF23 is synthesized in osteoblasts and osteocytes and we have shown that MET stimulates FGF23 in mouse bone and primary osteoblasts. We hypothesize that MET increases FGF23 through intracellular Ca ($Ca_i$) signaling and prostaglandin release.

**Methods:** Neonatal mouse calvariae or primary osteoblasts were incubated in neutral (NTL, pH=7.44, $Pco_2$=38 mmHg, $[HCO_3^-]$=27 mM) or acid (MET, pH=7.18, $Pco_2$=37 mmHg, $[HCO_3^-]$=13 mM) medium ± 2-APB (50 μM), an inhibitor of $Ca_i$ signaling, or ± NS398 (1 μM), an inhibitor of prostaglandin synthesis. Medium FGF23 was measured by ELISA; calvarial or cell RNA was analyzed by realtime PCR with expression normalized to RPL13A.

**Results:** Both 2-APB and NS398 significantly inhibited MET-induced medium FGF23 protein and calvarial FGF23 RNA as well as bone resorption at 48h (not shown).




In primary cells where there is no mineral Pi present, 2-APB significantly inhibited the MET-induced increase in FGF23 RNA expression at 6h (NTL=0.9±0.2, MET=1.6±0.3, NTL+APB=1.1±0.3, MET+APB=1.1±0.2). NS398 also significantly inhibited the MET-induced increase in FGF23 RNA expression in primary osteoblasts at 6h (NTL=1.5±0.4, MET=3.2±0.6, NTL+NS398=1.8±0.3, MET+NS398=0.9±0.2).

**Conclusions:** MET stimulates FGF23 in osteoblasts by a $Ca_i$ / prostaglandin pathway, similar to the initial signaling pathways in MET-induced bone resorption. Therapeutic interventions directed toward correction of MET, especially in CKD, have the potential to lower FGF23 as well as prevent bone resorption.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO646

**Comparative Effects of FGF23 Antagonism with Calcimimetic versus FGF23 Neutralizing Antibody in Uremic Rats**

Charles M. Henley III,[1] Victoria Shalhoub,[1] Edward Shatzen,[1] Sabrina Ward,[1] James R. Davis,[1] Sean Morony,[1] William G. Richards.[1] *[1]Metabolic Disorders, Amgen, Inc, Thousand Oaks, CA; [2]Independent, Newbury Park, CA.*

**Background:** Fibroblast growth factor 23 (FGF23), a 32kD protein secreted by bone, is a phosphaturic hormone important in phosphate and vitamin D (VitD) regulation. As renal function declines serum FGF23 increases and is associated with progressive renal failure, left ventricular hypertrophy and increased mortality.

**Methods:** To determine effects of FGF23 antagonism in preclinical uremic (CKD) rats, we compared effects of decreasing serum FGF23 with calcimimetic, R-568, versus FGF23 antibody neutralization in male, S-D rats (5/6Nx or sham-operated) on a high P diet. End points: serum PTH, $Ca^{2+}$, P, bone, survivability, vascular calcification.

**Results:** Calcimimetic R-568 in 5/6Nx rats increased serum P, decreased $Ca^{2+}$, PTH, FGF23, and FGF23 mRNA and protein in bone without significant static or dynamic histomorphometric changes. A separate group of CKD rats treated with 10ng VitD had increased serum FGF-23; concomitant treatment with R-568 decreased FGF-23 and PTH in VitD-treated rats. Suppression of FGF-23 in serum/bone by R-568 is likely through activation of the parathyroid calcium-sensing receptor and resulting decrease in PTH, a regulator of FGF23. FGF23-Ab significantly increased serum P, $Ca^{2+}$, $VitD_3$, lowered PTH and improved high turnover bone disease/mineralization in CKD rats. However, FGF23-Ab promoted aortic calcification and death in CKD rats.

| FGF23 Antagonism in 5/6 Nx rats | |
|---|---|
| **Calcimimetic R-568** | **FGF23 antibody** |
| ↓ $FGF23_{serum}$ | *FGF23 neutralization* |
| ↓ PTH | ↓ PTH |
| ↓ $Ca^{2+}$, ↑ P | ↑ $Ca^{2+}$, ↑ P |
| normalizes bone | ↓ bone turnover |
| ***no vascular calcification, mortality*** | ***↑ vascular calcification, mortality*** |

**Conclusions:** These subtotal Nx rat studies support the notion that calcimimetics control PTH and significantly lower serum FGF-23 without pathological effects observed with mAb neutralization of FGF-23. However, since we do not have a preclinical model adequately reflecting absence of renal function we do not know if direct neutralization of FGF23 would be deleterious in human ESRD-dialysis patients.

## FR-PO647

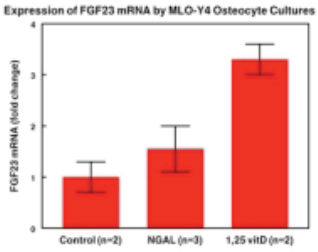
**Neutrophil Gelatinase-Associated Lipocalin (NGAL) Stimulates *In Vitro* Production of Fibroblast Growth Factor-23 (FGF23) in MLO-Y4 Osteocyte Cultures**

Shweta Bansal,[1,2] Khaled Khazim,[1] Basant Bhandari,[1] Sherry L. Werner,[1,2] Hanna E. Abboud,[1,2] Paolo Fanti.[1,2] *[1]Dept of Medicine/ Div of Nephrology, Univ of Texas Health Sciences Center at San Antonio, San Antonio, TX; [2]Renal Section, ALMVA Hospital, San Antonio, TX.*

**Background:** Regulation of FGF23 in chronic kidney disease (CKD) remains only partially defined. Besides established actions of phosphorus and $1,25(OH)_2D$ on FGF23 in CKD, positive correlation has been described described between FGF23 and interleukin-6 and tumor necrosis factor-α (TNFα) in this patient population suggesting a possible stimulatory action of inflammation on FGF23 expression. We recently found strong positive correlation between FGF23 and the acute phase reactant NGAL (r=0.72, p<0.001) in CKD patients. This association was independent of renal function, serum phosphorus and C-reactive protein, suggesting direct functional interaction between NGAL and FGF23. Since osteocytes are the only cell type producing FGF23 and they express the endocytic receptor megalin which binds NGAL with high affinity, we hypothesized that close association between NGAL and FGF23 in CKD patients is explained by direct stimulatory action of NGAL on the osteocytic synthesis and secretion of FGF23.

**Methods:** MLO-Y4 osteocytic cells were conditioned overnight with serum-free medium, incubated with 0.5-5 nM NGAL, 10 nM $1,25(OH)_2D_3$, 10 ng/ml TNFα or vehicle for 24 hours and finally tested for FGF23 mRNA expression by quantitative RT-PCR.

**Results:** FGF23 mRNA expression was increased 1.6 ± 0.5 fold by 2.5 nM NGAL (n=3), 3.2 ± 0.3 fold by $1,25(OH)_2D_3$ (n=2), and was not affected by exposure to TNFα.




**Conclusions:** NGAL directly stimulates FGF23 production in vitro although with lower strength than $1,25(OH)_2D_3$. Since circulating NGAL is very high and $1,25(OH)_2D$ is low in CKD patients, we propose NGAL may contribute to the increased production of FGF23 in CKD.

*Funding:* Veterans Affairs Support

## FR-PO648

**Effect of Standard Low-Protein Diet on Fibroblast Growth Factor 23 Levels in Patients with Early and Advanced Chronic Kidney Disease**

Shunsuke Goto, Kentaro Nakai, Keiji Kono, Hideki Fujii, Shinichi Nishi. *Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Japan.*

**Background:** High serum fibroblast growth factor 23 (FGF23) levels are associated with mortality and cardiovascular diseases in patients with chronic kidney disease (CKD). Although recent studies have demonstrated that dietary phosphorus restriction and/or phosphate binders decreased serum FGF23 levels in patients with CKD, the effects of standard low-protein diet are limited. Our study investigates the effect of standard low-protein diet on serum FGF23 levels in patients with early and advanced CKD. We also evaluate the effects on serum 1,25-dihydroxyvitamin D (1,25D) and intact parathyroid hormone (PTH) levels.

**Methods:** We enrolled 15 patients with CKD stage 1 or 2 as an early CKD group and 20 patients with CKD stage 4 or 5 as an advanced CKD group. Subjects consumed standard low-protein diets for 4 to 6 days after two-day regular diet. Serum FGF23, 1,25D, intact PTH, calcium, and phosphorus levels and 24-hr phosphorus excretion were measured at baseline and the final day of study.

**Results:** Serum FGF23 levels decreased in both groups (early CKD, 52 [36-75] to 39 [23-65], $p < 0.05$; advanced CKD, 185 [126-322] to 138 [97-258], $p < 0.05$). Changes of FGF23 levels were correlated with changes of 24-hr urinary phosphorus excretion in the advanced CKD group (early CKD, $r = -0.25$, $p = 0.37$; advanced CKD, $r = 0.46$, $p < 0.05$).

Serum 1,25 D levels increased in only the early CKD group (early CKD, 36.0 [33.0-39.0] to 47.0 [33.0-78.0], p < 0.05; advanced CKD, 14.5 [13.0-19.0] to 14.5 [9.3-17.0], p = 0.12). Serum intact PTH levels decreased in only the advanced CKD group (early CKD, 23 [19-27] to 25 [21-30], p = 0.89; advanced CKD, 124 [73-194] to 85 [61-168], p < 0.05).

**Conclusions:** Our findings suggested that standard low-protein diet decreases serum FGF23 levels with various benefits on CKD-MBD control from early to advanced CKD stages.

*Funding:* Private Foundation Support

## FR-PO649

**Serum Mineral Parameters Are Associated with Blood Cell Morphology in Non-Diabetic Chronic Hemodialysis Patients**

Basri Budak,[1] Mehmet Kanbay,[2] Ali Akcay,[3] Ali Riza Odabas,[2] Serkan Kubilay Koc.[1] *[1]Fresenius Medical Care Ankara Kecioren Dialysis Clinic, Ankara, Turkey; [2]Dept of Medicine, Div of Nephrology, Medeniyet Univ School of Medicine, Istanbul, Turkey; [3]Dept of Nephrology, Turgut Ozal Univ.*

**Background:** Parathyroid hormone (PTH) is known to induce bone marrow fibrosis in end stage renal disease patients which may influence the morphology of blood cells. We aimed to investigate the possible influence of PTH and serum mineral parameters on blood cell morphology in non-diabetic chronic hemodialysis (HD) patients.

**Methods:** Seventy non-diabetic chronic HD patients (male/female: 41/29) were enrolled in the study. Exclusion criteria were as follows; presence of diabetes mellitus, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, presence of any kind of hematological disorder including iron deficiency anemia. Serum PTH, calcium and phosphorus levels were determined together with hemoglobin, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell count, platelet count, mean platelet volume (MPV) and platelet distribution width (PDW).

**Results:** Mean age was 58 ± 14 years and mean dialysis vintage was 98 ± 58 months. Mean serum PTH was 398 ± 339 pg/mL; calcium, 8.53 ± 0.89 mg/dL and phosphorus, 4.43 ± 1.38 mg/dL. Serum PTH was significantly inversely associated with RDW (r= -0.24, p=0.05). Serum PTH was not significantly related to other blood parameters including hemoglobin concentration. Serum calcium levels were correlated with PDW (r= -0.25, p=0.03). Serum phosphorus levels were significantly associated with MCV (r= -0.24, 0.04), WBC (r= 0.30, p=0.01), platelet count (r= 0.38, p= 0.001) and MPV (r= -0.32, p=0.008).

**Conclusions:** Serum PTH was not related to parameters of blood cell morphology except for RDW. Increased serum phosphorus levels were associated with increased total leukocyte and platelet counts which may represent increased inflammation. Also high serum phosphorus levels significantly associated with low red blood cell and platelet volumes.

## FR-PO650

**Low FGF-23 Levels Suggest Malnutrition in Maintenance Hemodialysis Patients**

Sonoo Mizuiri,[1] Yoshiko Nishizawa,[1] Kazuomi Yamashita,[1] Kyoka Ono,[1] Maya Oda,[1] Touru Nakazono,[3] Kohji Usui,[2] Kenichiro Shigemoto.[1] *[1]Nephrology, Ichiyokai Harada Hospital, Hiroshima, Japan; [2]Nephrology, Ichiyokai Ichiyokai Clinic, Hiroshima, Japan; [3]Nephrology, Ichiyokai East Clinic, Hiroshima, Japan.*

**Background:** Elevated fibroblast growth factor-23 (FGF-23) level is a risk factor for mortality in incident dialysis patients. High FGF-23 levels can be attributed to hyperphosphatemia. However, poor nutritional status and hypophosphatemia have also been associated with increased mortality in maintenance HD patients (MHD).

**Methods:** Univariate and multivariate analyses into factors that relate to serum intact FGF-23 with SPSS II were performed in 332 MHD. The variables were sex, dialysis period, presence of DM, urine volume, Ccr+Curea/2, Kt/Vurea, β2-MG, cystacin C, BMI, serum albumin, nPNA, geriatric nutritional risk index (GNRI), serum phosphate, serum calcium, iPTH and dosages of activated vitamin D or phosphate binders. Clinical data were also assessed according to quartiles of FGF-23 level.

**Results:** The median age and dialysis period were 69 years and 66 months, respectively, and 83 patients had residual renal function (RRF: UV≥100 ml/day) and 249 patients did not. There were no significant differences in serum phosphate (mg/dl) [4.8 (3.9-5.8) vs. 5.0 (4.1-5.9)] and FGF-23 (pg/ml) [1120 (272-4080) vs. 1570 (357-5860)] levels in patients with and without RRF. In patients with RRF (n=83), serum phosphate levels (P<0.001) and vitamin D dose (P=0.013) were significantly associated with FGF-23 levels. In patients without RRF (n=249), serum phosphate (P<0.001), serum calcium (P<0.001) and iPTH (P=0.012) levels were significantly associated with FGF-23 levels. Patients in the highest FGF-23 group (Q4) were younger, and had longer dialysis period. BMI, nPNA, nPCR, GNRI, nPCR, serum albumin, creatinine, phosphate, dosage of vitamin D, phosphate binders and cinacalcet in Q4 were higher than in other groups and the differences between Q1 and Q4 were significant. Correlation between FGF-23 and serum creatinine levels (r=0.510), or GNRI (r=0.352) was observed (P<0.001).

**Conclusions:** Low FGF-23 levels suggest malnutrition and serum phosphate level but not RRF is the most important factor associated with FGF-23 in MHD.

*Funding:* Private Foundation Support

## FR-PO651

**The Fasting Fractional Excretion of Phosphate Is Correlated with FGF23 Levels, Mortality and Morbidity in CKD Patients Not on Dialysis**

Patricia Quadros Branco,[1] Teresa Adragao,[1] Ricardo Vizinho,[1] Maria Augusta Cabrita Silva Gaspar,[1] Andre L. Weigert,[1] Joao Faro-Viana,[2] Jose Diogo Barata.[1] *[1]Nephrology, Santa Cruz Hospital, Lisboa, Portugal; [2]Clinic Pathology, Santa Cruz Hospital, Lisboa, Portugal.*

**Background:** In CKD patients FGF23 elevation precedes the increase in PTH and phosphate (P). In advanced CKD 24h-phosphaturia is reduced and 24h-fractional excretion of P (FEP) has been inconsistently correlated with FGF23 levels. The aim of this study was to analyze, in pre-dialysis CKD patients, the correlation of the fasting FEP with FGF23 levels, biochemical markers of CKD-MBD and clinical cardiovascular events.

**Methods:** We studied 120 CKD pts (73% male, 27% diabetic, mean age 65 ± 13.7 years, 41%, 45% and 7.5% respectively in CKD stages 3, 4 and 5) during 23.9 ± 7.9 months. FGF23 (C-Terminal, ELISA Immutopics). FEP(%) was calculated with the formula:(UPO4 x PCr):(PPO4 x UCr) x 100. Blood and urine samples were taken simultaneously in a fasting steady state, after discarding overnight urine (Payne RB).

**Results:** P> 4.5 mg/dL, iPTH> normal, FEP> 20% and FGF23> 110 RU/ml, respectively were detected in 14.8%, 43.9%, 89.4% and 89.4% of pts. In a multivariate analysis, adjusted for age, eGFR and PTH, logFGF23 correlated directly with P (p<0.001) and FEP (p=0.008) and inversely with albumin (p=0.01). FEP correlated inversely with eGFR(p<0.001) and P(p=0.001) and directly with logFGF23 (p=0.008). During follow up period, 6 patients died, 26 initiated renal replacement therapy (RRT) and 31 suffered cardiovascular events. Patients with FEP> 40% had a lower survival free of cardiovascular events, death, or RRT initiation (log rank 29.11,p=0.008). In Cox regression CRP, Pulse Pressure and FEP> 40% (OR:5.32,CI:2.1-13.0,p=0.001) were predictors of cardiovascular events, RRT intiation or death (OR: 5.32,CI:2.1-13.0,p=0.001).

**Conclusions:** In summary, in this group of CKD pts, P levels were increased only in 14.8% of pts, while FEP> 20% was detected in 89.4% of pts. FEP, evaluated in fasting conditions, was an independent predictor of FGF23, CV events and RRT. FEP evaluated by this simple method may be a useful tool in the assessment of the altered mineral metabolism of CKD pts.

## FR-PO652

**Study on Correlation between Carotid Intima-Media Thickness with Serum HsCRP, FGF23 and Klotho Protein in MHD Patients**

Ying Sun, Aiqun Chen, Hua Wu. *Nephrology, Beijing Hospital of the Ministry of Health, Beijing, China.*

**Background:** To understand the clinic feature of the maintenance hemodialysis(MHD) patients with carotid artery atherosclerosis, analyze the relationship between carotid intima-media thickness(CIMT) and serum high sensitive c-reactive protein(HsCRP), fibroblast growth factor 23(FGF23), Klotho protein levels.

**Methods:** 88 MHD patients were enrolled in the blood purification center, from January to June 2012. The patients were divided into CIMT thickening group and the CIMT normal group based on their carotid color Doppler ultrasound results. FGF23 and Klotho protein were measured by ELISA. Possible risk factors of CIMT thickening, such as age, duration of dialysis, diabetes, FGF23, Klotho protein, hsCRP, lipids and other biochemical parameters were analyzed and compared between the two groups, while non-conditional logistic regression were used for multivariate analysis to determine the risk factors for CIMT thickening.

**Results:** Among the 88 MHD patients, increased CIMT was found in 53 patients(60.2%), while the remaining 35 patients (39.8%) had normal CIMT. The median of the two sets of CIMT were 1.5mm and 1.0mm respectively. Univariate analysis indicated that the average age of the CIMT thickened group was 66.64±10.61 years, and the average age of the group with normal CIMT was 58.63±11.78 years($t$=3.320, $P$=0.001). Diabetes prevalence of the CIMT thickened group was 37.7%, Diabetes prevalence of the group with normal CIMT was 17.1%($\chi^2$=4.294, $P$=0.038). The median of the two sets of FGF23 were 127.82 ng/L and 86.74 ng/L respectively, the difference was statistically significant($Z$=-3.713, $P$=0.000). The median of the two sets of HsCRP were 5.34mg/L and 2.19mg/L respectively, the difference was statistically significant($Z$=-3.547, $P$=0.000). The median of the two sets of Klotho protein were 42.48 U/L and 41.21 U/L respectively($Z$=-0.085, $P$=0.932). Non-conditional logistic regression analysis showed that age, FGF23 and hsCRP were independent risk factors for CIMT thickening in MHD patients, OR values were 1.061 (1.007,1.118), 1.016 (1.003,1.028), 1.344 (1.115,1.621), respectively.

**Conclusions:** Serum HsCRP, FGF23 and age are independent risk factors for CIMT thickening in MHD patients.

*Funding:* Private Foundation Support

## FR-PO653

**Fibroblast Growth Factor 23 Is Associated with Carotid Artery Plaque in Patients with Chronic Kidney Disease**

Sho Shimamoto,[1] Kazuhiko Tsuruya,[2] Masaru Nakayama.[3] *[1]Dept of Internal Medicine, Kyushu Kosei Nenkin Hospital, Japan; [2]Dept of Medicine and Clinical Science, Kyushu Univ, Japan; [3]Div of Nephrology, Dept of Internal Medicine, National Kyushu Medical Center Hospital, Japan.*

**Background:** Serum phosphate levels have been shown to be associated with the development of atherosclerosis in patients with chronic kidney disease (CKD). Fibroblast growth factor 23 (FGF23) is an important hormone in the regulation of phosphate metabolism. FGF23 has been suggested to play a role in vascular calcification in CKD.

However, it is unclear whether FGF23 is associated with carotid artery plaque (CAP) which is an indicator of atherosclerosis in predialysis patients. The aim of the present study was to clarify the relationship between FGF23 and CAP in patients with CKD not on dialysis.

**Methods:** Two-hundred seventy-seven predialysis CKD patients were enrolled in this cross-sectional study. CAP was assessed by B-mode Doppler ultrasound. Intact FGF23 was measured using a two-site monoclonal antibody ELISA in each patient. The risk factors for CAP were evaluated using a logistic regression model.

**Results:** We found CAP in 66.8% of the patients. The prevalence of CAP increased across CKD stages: 23.3% in CKD stages 1-2, 67.6% in stage 3, 76.2% in stage 4, and 79.8% in stage 5 ($p<0.01$). In a multivariate analysis, age, smoking, diabetes mellitus and log FGF23 (odds ratio [OR], 2.51; 95% confidence interval [CI], 1.26 to 5.36) were each identified as a risk factor for CAP. The study population was divided in quartiles of FGF23 levels. The prevalence of CAP was higher with increasing quartiles of FGF23 levels (first quartile 38.2%; second quartile 70.0%; third quartile 80.0%; fourth quartile 78.3%). Compared with the lowest FGF23 quartile, each subsequent quartile had a progressively higher OR for CAP, adjusted for confounders (OR for second quartile, 4.70 [95% CI, 1.74 to 13.42]; for third quartile, 5.83 [95% CI, 1.89 to 19.30]; and for fourth quartile, 7.72 [95% CI, 2.08 to 31.25], respectively).

**Conclusions:** The prevalence of CAP is increased with the decline in the kidney function. FGF23 was also identified as an independent factor for CAP in patients with CKD not on dialysis.

*Funding:* Private Foundation Support

## FR-PO654

### Increases in FGF23 May Not Be the Initial Trigger of Secondary Hyperparathyroidism in Early CKD

Isabel Martinez,[1] Ramon M. Saracho,[2] Yolanda Almaden Peña,[3] Adriana S. Dusso,[4] Mariano Rodriguez.[3] *[1]Nephrology, Hospital Galdakao, Spain; [2]Nephrology, HUA, Spain; [3]IMIBIC, Cordoba, Spain; [4]IRBLleida, Spain.*

**Background:** Secondary Hyperparathyroidism (SH) starts at early stages of CKD and elevations in FGF23 were shown to precede the changes in serum calcium, phosphate and calcitriol predisposing to increases in PTH. This study examined the contribution of early increases in FGF23 to the onset of SH in CKD stage 1.

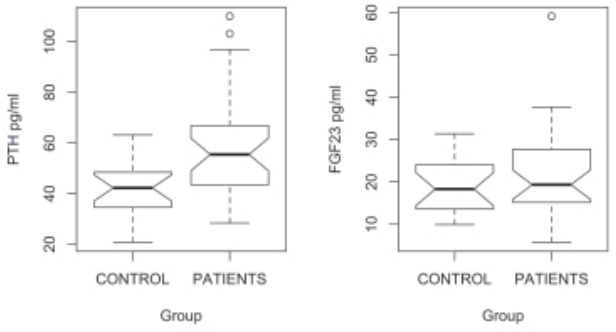
**Methods:** Thirty three patients, CKD1(creatinine clearance >90ml/ml) of different etiology, who were not receiving any vitamin D receptor activator, calcium salts, calcimimetics or bisphosphonates were compared with 17 healthy controls with no kidney disease in their total and ionized calcium, phosphorus, calcitriol, FGF23 and PTH in fasting conditions. Twenty-four hour urine was collected for calcium, phosphorus and creatinine. We estimated GFR with standard CCr corrected for 1.73 m2 of BS, using 24h urine collection. Intact PTH in serum was measured by Immunoassay(Advia Centaur XP, Siemens) and Intact FGF23 by ELISA(Kainos Laboratories Inc).

**Results:** Mean serum PTH in CKD patients was higher than controls (58.1 vs 41.4 pg/ml, p=.0003). However, mean FGF23 levels were similar in both groups, 21.8 vs 19.5 pg/ml, p=.33.

There were no differences in total and ionized calcium, phosphate and calcidiol, or calcitriol levels.

Calciuria, phosphaturia and CCr were similar in both groups, however fractional excretion of phosphate(FEP) was higher in CKD patients, 17% vs 14%, p=.03.

Linear regression analysis showed a mild positive correlation between PTH and FEP(p=.05). Instead, FGF23 and FEP were not associated. Controls were younger than patients, but differences in PTH remained significant after adjustement for age and sex.




**Conclusions:** Elevations in FGF23 is not the earliest event in the onset of SH. The FEP is associated to PTH but it is unrelated to FGF23 in early CKD.

## FR-PO655

### The Modification of the Effect of FGF23 on the Coronary Artery Calcium Score by Fractional Excretion of Phosphate in Patient with Pre-Dialysis CKD

Shin-young Ahn,[1] Seungmi Lee,[2] Kook-Hwan Oh,[2] Curie Ahn,[2] Dong Wan Chae.[1] *[1]Internal Medicine, Seoul National Univ Bundang Hospital, Seongnam-si, Republic of Korea; [2]Internal Medicine, Seoul National Univ Hospital, Seoul, Republic of Korea.*

**Background:** A recent study demonstrated that cardiovascular (CV) mortality was highest in the population having low $FEPO_4$ despite of high FGF23 level. Hence, we investigated the effect of FGF23 on the intermediate variables known to be related to CV mortality such as CACS, pulse wave velocity (PWV) and LVH according to the $FEPO_4$ level in an on-going observational cohort of CKD in Korea.

**Methods:** KNOW-CKD is an on-going, prospective, university hospital based observational cohort study under the sponsorship of Korean Center for Disease Control and Prevention. Cross-sectional analysis was performed in 702 participants. Severe CAC was defined as Ca score of ≥100 Agatston units (AU) and we regarded mean of the brachial-ankle PWV(MPWV) ≥ 1600 cm/s as a significant arterial stiffness.

**Results:** We developed four categories defined by FGF23 and $FEPO_4$ above or below their medians as follows: low FGF23/low $FEPO_4$, high FGF23/low $FEPO_4$, low FGF23/high $FEPO_4$, high FGF23/high $FEPO_4$. The participants with high FGF23/high $FEPO_4$ had more frequently diabetes, hypertension, and advanced CKD compared with the other three groups. They also had lower Hb, albumin, and 1,25 vitamin D and had higher serum P, alkaline phosphatase, and intact PTH. The mean values of CACS and MPWV were significantly higher in this group and the incidence of LVH was also highest in this group. After multivariable regression analysis, neither these groups nor FGF23 level could be an independent risk factor for severe CAC, significant arterial stiffness, or LVH. But, in subgroup analysis, high FGF23 was an independent risk factor for severe CAC in the participants with low $FEPO_4$ (OR=2.309, 95% CI=1.036-5.145, p=0.041).

**Conclusions:** The participants with high FGF23/high $FEPO_4$ demonstrated the most severe features in CAC, PWV and LVH largely due to the more frequent presence of comorbidities such as diabetes, hypertension, and advanced CKD. Only in the participants with low $FEPO_4$, high FGF23 was an independent risk factor for severe CAC.

*Funding:* Government Support - Non-U.S.

## FR-PO656

### Fibroblast Growth Factor 23 Predicts Progression of Chronic Kidney Disease in Children

Anthony A. Portale,[1] Alison G. Abraham,[2] Myles S. Wolf,[3] Isidro B. Salusky,[4] Harald Jüppner,[5] Bradley A. Warady,[6] Susan L. Furth.[7] *[1]Univ of CA San Francisco; [2]Johns Hopkins Bloomberg School of Public Health; [3]Univ of Miami Miller School of Medicine; [4]Univ of CA Los Angeles; [5]Harvard Medical School; [6]Univ of Missouri-Kansas City; [7]Univ of Pennsylvania.*

**Background:** Plasma FGF23 concentrations increase early in the course of chronic kidney disease (CKD) in children and predict disease progression in adults. Whether FGF23 associates with risk of CKD progression in children is unknown.

**Methods:** We measured plasma C-terminal FGF23 and determined GFR annually by plasma iohexol clearance or the CKiD estimating equation in 418 children with CKD stages 2-4 enrolled in the Chronic Kidney Disease in Children (CKiD) study. We analyzed the association of baseline plasma FGF23 on time to 50% decline in GFR or start of renal replacement therapy using parametric regression stratified by glomerular or non-glomerular disease, adjusting for age, gender, race, GFR, proteinuria, blood pressure, serum phosphate, and PTH.

**Results:** At baseline, median age was 12 [IQR: 8, 15] years, GFR was 46 [34, 58] ml/min/1.73 $m^2$, and plasma FGF23 was 140 [97, 216] RU/ml - 2.5-fold higher than in healthy children. During a median follow-up of 62 [40, 73] months, 31% of subjects reached the CKD progression endpoint, 35 of 80 (44%) with glomerular and 94 of 338 (28%) with non-glomerular disease. Time to the endpoint was shorter in children with glomerular compared to non-glomerular disease (17 [4, 35] vs. 29 [19, 47] months) ($P<0.001$). Compared to non-progressors, progressors had lower GFR, higher levels of FGF23 and proteinuria, and lower $1,25(OH)_2D$ ($P<0.005$). In univariate analyses, doubling of FGF23 was associated with a significant, 72% [95% CI: 53, 83] shorter time to the CKD endpoint in children with glomerular, and 53% [95% CI: 41, 63] shorter time in those with non-glomerular disease. After adjustment, doubling of FGF23 remained associated with modest reductions in time to the endpoint, 18% [95% CI: -33, 49] shorter time with glomerular, and 12% [95% CI: -6, 27] shorter time with non-glomerular disease, reductions of borderline significance.

**Conclusions:** Increased plasma FGF23 is a novel risk factor for CKD progression in children.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Abbott, Genzyme

## FR-PO657

### Evaluation of Serum FGF23 Concentration in a Small Subset of Patient-Samples from the EVOLVE Randomized Trial by Two Independent iFGF23 Assays

Marina Stolina, Denise C. Dwyer, Chun-ya Han, Bastian Dehmel, William G. Richards. *Amgen Inc.*

**Background:** Fibroblast growth factor 23 (FGF23, a phosphaturic hormone) is a regulator of calcium-phosphate metabolism. Increased FGF23 in serum from patients on dialysis is associated with increased mortality, treatment-resistant secondary

hyperparathyroidism and left ventricular hypertrophy. Growing interest in plasma/serum FGF23 as a clinical test highlights a need for a comparison of available assays in order to facilitate more meaningful interpretation of data.

**Methods:** In this study we compared the performance of 2 commercially available human intact FGF23 (iFGF23) assays: ELISA from Kainos Laboratories (Tokyo, Japan) and Luminex-based microbead assay (Millipore, Billerica, MA, USA) in a cohort of healthy adults (control, n=8) and baseline samples from patients on dialysis participating in the EVOLVE clinical trial (n=20).

**Results:** Both Millipore microbead iFGF23 and Kainos ELISA iFGF23 demonstrated 75-95% recovery of purified FGF23 standards in an inter-assay setting and acceptable (65-95%) recovery of the range of hrFGF23 concentrations spiked in to control serum. For the healthy adult group, median ($25^{th}$-$75^{th}$ percentile) serum FGF23 was 30 (24-40) pg/ml (Kainos ELISA), and 12 (8-42) pg/ml (Millipore microbead); for the dialysis group corresponding serum FGF23 were 9780 (1955-19850) and 12090 (2195-17420) pg/ml. Kainos ELISA and Millipore microbead iFGF23 assays demonstrated close numerical agreement accessed by inter-assay correlation analysis of individual results ($R^2$=0.9265, p<0.0001). Based on the range of FGF23 in serum from patients with chronic kidney disease, the broad functional concentration range of Millipore microbead assay (14-10,000 pg/ml) allows the use of a single 1:10 dilution for serum with FGF23 > 10,000 pg/ml. Due to the narrow functional dynamic range (10-800 pg/ml) of Kainos ELISA, substantial serial dilutions and repeat testing will likely be required.

**Conclusions:** Overall, the Kainos ELISA and Millipore microbead iFGF23 assays demonstrated desirable interchangeable analytical performance and may support clinical utility.

*Funding:* Pharmaceutical Company Support - Amgen Inc.

## FR-PO658

**Fibroblast Growth Factor 23 and All-Cause Mortality in Hemodialysis Patients** David A. Drew, Hocine Tighiouart, Tammy Scott, Kristina Lou, Saeed Kamran Shaffi, Li Fan, Daniel E. Weiner, Mark J. Sarnak. *Tufts Medical Center, Boston, MA.*

**Background:** Fibroblast growth factor 23 (FGF-23) levels are elevated in hemodialysis patients. Few longitudinal studies have evaluated whether high FGF-23 levels independently contribute to mortality in this population.

**Methods:** We measured FGF-23 levels at baseline in 263 maintenance hemodialysis patients who had enrolled in the Cognition and Dialysis longitudinal cohort. The cross-sectional association between baseline patient characteristics and FGF-23 level was assessed using multivariable linear regression. Multivariable Cox regression with adjustment for demographics, cardiovascular risk factors, and measures of mineral metabolism was used to explore the association between FGF-23 levels with all-cause mortality.

**Results:** Mean (SD) age was 63 (17) years, 54% were women and 22% were African American. The median FGF-23 level was 3100 RU/ml ($25^{th}$-$75^{th}$ percentile, 1100 - 8000 RU/ml). Younger age, lower prevalence of diabetes, longer dialysis vintage and higher levels of calcium and phosphorus were independently associated with higher FGF-23 levels (p <0.05 for all). There were 135 deaths over a median follow up time of 2.3 years. Higher FGF-23 was an independent predictor of mortality (adjusted HR [95%CI] = 1.17 per doubling of FGF-23 [1.01, 1.36]).

Association between baseline FGF-23 level and all-cause mortality

| | Unadjusted HR (95% CI) | Model 1 HR (95% CI) | Model 2 HR (95% CI) | Model 3 HR (95% CI) |
|---|---|---|---|---|
| FGF-23* | 1.05 (0.95, 1.16) | 1.22 (1.09, 1.36) | 1.12 (1.01, 1.27) | 1.17 (1.01, 1.36) |

*FGF-23 log transformed, represents the hazard associated with each doubling of FGF-23
Model 1: Adjusted for age, sex and race
Model 2: Adjusted for model 1 variables, vintage, cause of ESRD, access type, history of cardiovascular disease, Kt/V, and albumin
Model 3: Adjusted for model 2 variables, calcium, phosphorus, and 25OH Vitamin D

**Conclusions:** Although attenuated after adjustment for several dialysis related comorbidities, high FGF-23 levels remained an independent risk factor for mortality within prevalent hemodialysis patients. Future studies should explore the mechanisms relating FGF-23 to mortality.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO659

**Fibroblast Growth Factor 23 and Cognitive Performance in Hemodialysis Patients** David A. Drew, Hocine Tighiouart, Tammy Scott, Kristina Lou, Li Fan, Saeed Kamran Shaffi, Daniel E. Weiner, Mark J. Sarnak. *Tufts Medical Center, Boston, MA.*

**Background:** Fibroblast growth factor 23 (FGF-23) levels are elevated in hemodialysis (HD) patients and are associated with mortality and left ventricular hypertrophy. Despite FGF receptors being present throughout the brain, there are no prior studies which have assessed whether FGF-23 levels are associated with cognitive performance.

**Methods:** We measured FGF-23 levels in 263 maintenance HD patients who also underwent comprehensive neurocognitive testing. Principal factor analysis was used to derive two factors from cognitive test scores, representing memory and executive function. Multivariable linear regression adjusting for age, sex, education status as well as other relevant covariates was used to explore the relationship between FGF-23 and each cognitive factor.

**Results:** Mean age was 63 years, 46% were women and 22% African American. Median FGF-23 level was 3098 RU/ml ($25^{th}$-$75^{th}$, 1100 - 8000). Higher FGF-23 was independently associated with a lower composite memory score [beta (95%CI) = -0.42 SD (-0.82, -0.02) for highest versus lowest quartile] and a trend towards a lower composite executive function score [beta = -0.28 SD (-0.63, 0.07), for highest versus lowest quartile]. Results were similar using FGF-23 as a log transformed variable.

Table 1. Association between doubling of FGF-23 levels and cognitive function

| | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| | Beta (95% CI) | p value | Beta (95% CI) | p value | Beta (95% CI) | p value |
| Factor 1 (memory) | **-0.06 (-0.12, -0.01)** | **0.02** | **-0.08 (-0.14, -0.01)** | **0.02** | **-0.08 (-0.16, -0.01)** | **0.05** |
| Factor 2 (executive) | -0.04 (-0.10, 0.01) | 0.15 | -0.02 (-0.08, 0.04) | 0.5 | -0.03 (-0.10, 0.04) | 0.4 |

For each factor, beta represents the change per 1 standard deviation
Model 1 = Adjusted for Age, Sex, and Education Status
Model 2 = Model 1 + Adjustment for Race, Diabetes status, history of CVD, dialysis Vintage, and average pre-HD diastolic blood pressure
Model 3 = Model 2 + Adjustment for calcium, phosphorus, and 25 hydroxy vitamin D

Table 2. Association between Quartiles of FGF-23 and Cognitive Performance*

| FGF-23 level (RU/ml) | Quartile 1 134 to 1139 | Quartile 2 1139 to 3098 | Quartile 3 3098 to 7960 | Quartile 4 7960 to 15568 | |
|---|---|---|---|---|---|
| | | Beta (95% CI) | Beta (95% CI) | Beta (95% CI) | p value for trend |
| Factor 1 (memory) | ref | -0.07 (-0.38, 0.25) | -0.17 (-0.53, 0.19) | **-0.42 (-0.82, -0.02)** | **0.04** |
| Factor 2 (executive) | ref | **-0.29 (-0.57, -0.02)** | **-0.32 (-0.64, -0.01)** | -0.28 (-0.63, 0.07) | 0.13 |

Bold values indicate a p value < 0.05 or confidence intervals that do not include 0
Beta represents the mean difference between Quartile 1 and each subsequent quartile. For Factors 1 and 2, this represents a per standard deviation change.
*Fully adjusted model including age, sex, education level, race, diabetes, history of CVD, dialysis vintage, pre-HD diastolic blood pressure, calcium, phosphorus, and 25 hydroxy vitamin D

**Conclusions:** FGF-23 was independently associated with worse performance on a composite memory score, even after adjustment for potential confounders, including measures of mineral metabolism. High FGF-23 levels in HD patients may contribute to cognitive impairment.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO660

**Pretransplant FGF23 Levels Are a Good Marker of Post-Transplant Caicium and Phosphorus Metabolism** Makoto Tsujita, Daijo Inaguma. *Kidney Disease Center, Nagoya Daini Red Cross Hospital, Japan.*

**Background:** Hypercalcemia and hypophosphatemia often occur after kidney transplantation. Recently, preemptive kidney transplantation (PET) has increased in Japan; however, it is not well-known whether PET is beneficial in calcium (Ca) and phosphorus (Pi) metabolism.

**Methods:** Thirty-two consecutive patients were enrolled in this study at Nagoya Daini Red Cross Hospital in 2011. Fifteen patients were in the PET group and 17 patients were in the non PET group. Parameters of Ca and Pi metabolism including full-length fibroblast growth factor 23 (FGF23) and intact parathyroid hormone (iPTH) were measured before transplantation and 1, 3, and 24 weeks after transplantation.

**Results:** FGF23 decreased dramatically in both groups after transplantation; however, FGF23 before transplantation and at 1 and 3 weeks after transplantation was significantly lower in the PET group than in the non PET group ($p < 0.05$). In contrast, although iPTH levels were higher in the PET group than in the non PET group before transplantation, the levels were lower at 24 weeks (p < 0.05). Corrected Ca (cCa) was lower at 24 weeks in the PET group (p < 0.05), whereas Pi was lower in the non PET group at 1 and 3 weeks (p < 0.05), but not significantly different at 24 weeks.

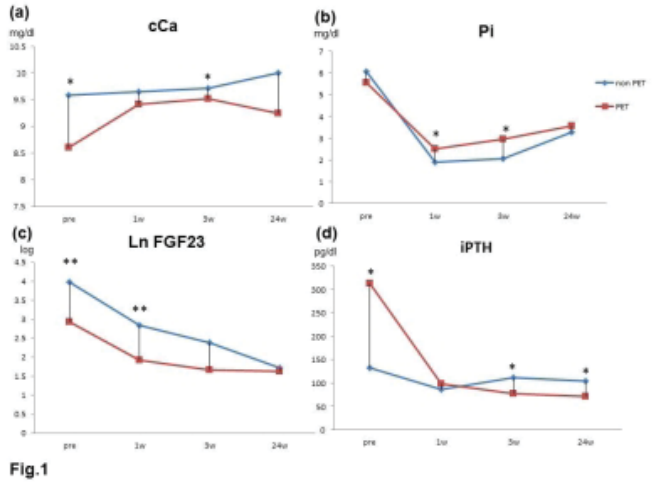



Fig.1

Multivariate linear regression analysis revealed that FGF23 before transplantation was a better predictor of Ca and Pi disorders in early post-transplant recipients.

**Conclusions:** This study suggests that PET has beneficial effects on Ca and Pi metabolism and that pretransplant FGF23 levels are a good marker of post-transplant Ca and Pi metabolism.

## FR-PO661

**Kidney Injury Increases FGF23 Independent of Reduced Glomerular Filtration Rate** Kelsey T. Smith, Tamara Isakova, Julia J. Scialla, Allison Barchi-Chung, Eva Schiavenato, Greg Lacher, Gwen Enfield, Jessica Houston, Patricia R. Wahl Pristau, Jonathan J. Suarez, Jorge Diego, Warren L. Kupin, David Roth, Myles S. Wolf. *Univ of Miami.*

**Background:** The mechanism of FGF23 elevation in CKD remains poorly understood. Decreased GFR is a primary determinant of high FGF23 in advanced CKD, but levels can be elevated in certain CKD patients despite nearly normal GFR.

**Methods:** We tested the hypothesis that kidney injury itself increases FGF23. We measured serum phosphate, calcium, FGF23, PTH, 25D, 1,25D and iohexol GFR in 3 groups: 1) CKD (N=19), 2) stable, long-term kidney transplant recipients (KT; N=8), and 3) kidney transplant donors and individuals with age-related GFR loss but no other evidence of CKD (N=11). We measured the same biochemical profile but used eGFR in healthy volunteers (N=20).

**Results:** Median FGF23 was comparable in kidney donors, individuals with age-related GFR loss and healthy volunteers. In contrast, median FGF23 was significantly higher in CKD group and median PTH was significantly higher in KT group, compared to healthy volunteers. Median FGF23 was significantly higher in CKD than KT patients (117 vs. 78 RU/mL, P=0.03) despite comparable mean iohexol GFR (53 vs. 57 mL/min/1.73 $m^2$; P=0.47). Dietary phosphorus and calcium, serum calcium, PTH, 25D and 1,25D did not significantly vary between CKD and KT groups, suggesting that differences in FGF23 were not driven by these variables. However, serum phosphate was significantly higher in CKD group than KT group (3.6 versus 3.0 mg/dl, P=0.004).

| | CKD N=19 | Transplant N=8 | Donors + Age-related N=11 | Healthy N=20 |
|---|---|---|---|---|
| Age, years | 54 ± 10.4 | 49.5 ± 17.7 | 53.6 ± 15.5 | 49.0 ± 8.9 |
| Women, n | 9 | 4 | 5 | 9 |
| Black, n | 10 | 2 | 1 | 5 |
| Hispanic, n | 5 | 1 | 5 | 12 |
| BMI, kg/$m^2$ | 31.0 ± 7.7 | 29.3 ± 9.2 | 28.0 ± 4.7 | 27.6 ± 3.4 |
| eGFR, ml/min/1.73 $m^2$ | 50.8 ± 11.4 | 62.1 ± 25.2 | 68.4 ± 10.7 | 85.6 ± 13.3 |
| iGFR, ml/min/1.73 $m^2$ | 52.6 ± 14.8 | 57.1 ± 14.0 | 72.4 ± 15.3 | --- |
| Serum Albumin, mg/dl | 4.3 ± 0.3 | 4.3 ± 0.2 | 4.6 ± 0.2 | 4.5 ± 0.2 |
| Serum Calcium, mg/dl | 9.4 ± 0.4 | 9.6 ± 0.5 | 9.5 ± 0.3 | 9.3 ± 0.2 |
| Serum Phosphate, mg/dl | 3.6 ± 0.5 | 3.0 ± 0.2 | 3.3 ± 0.5 | 3.4 ± 0.5 |
| FeCA, % | 0.45 (0.33 – 0.67) | 0.74 (0.36 – 1.28) | 0.77 (0.58 – 1.06) | 0.69 (0.53 – 1.33) |
| FePi, % | 16.3 (12.8 - 21.8) | 16.2 (11.3 – 19.6) | 10.3 (8.8 – 13.7) | 8.8 (5.1 – 11.7) |
| 24-hr Urine Pi, mg/day* | 779 (631 – 965) | 700 (354 – 1302) | 719 (566 – 904) | 800 (578 – 1030) |
| 24-hr Urine Ca, mg/day* | 93 (22 – 132) | 117 (98 – 215) | 133 (97 – 182) | 176 (136 – 366) |
| Total caloric intake, kcal/day† | 1422 (1341 – 1633) | 1473 (1213 – 1921) | 1664 (1494 – 1895) | 1895 (1554 – 2270) |
| Dietary Ca, mg/day† | 500 (388 – 578) | 596 (407 – 740) | 724 (528 – 912) | 639 (331 – 838) |
| Dietary Pi, mg/day† | 977 (842 – 1101) | 893 (731 – 1148) | 1087 (830 – 1142) | 1098 (868 – 1420) |
| FGF23, RU/ml | 117.3 (90.7 – 174.8) | 77.6 (66.9 – 104.3) | 82.0 (69.7 – 118.8) | 69.0 (61.7 – 93.9) |
| PTH, pg/ml | 49.4 (35.2 – 86.4) | 56.4 (48.2 – 74.4) | 32.3 (23.8 – 40.2) | 31.4 (23.1 – 43.4) |
| 1,25D, pg/ml | 52.1 ± 20.7 | 66.0 ± 21.0 | 58.1 ± 13.3 | 59.7 ± 17.4 |
| 25D, pg/ml | 31.1 ± 11.2 | 36.9 ± 17.8 | 38.5 ± 11.3 | 33.6 ± 10.6 |

**Conclusions:** Despite similar iohexol GFR, FGF23 is significantly higher in CKD patients compared to KT recipients. Differences in serum phosphate between the two groups may mediate these relationships. Our findings suggest that in CKD, the presence of kidney disease itself, independent of GFR reduction, could contribute to FGF23 excess.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO662

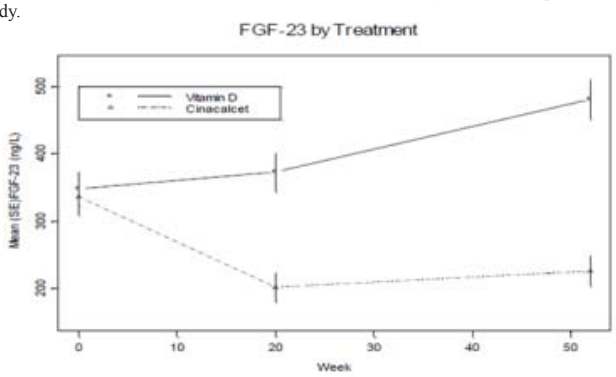
**The PARADIGM Trial: Effects of Cinacalcet and Vitamin D as Monotherapies on FGF-23 Levels during Treatment of Secondary Hyperparathyroidism in Patients on Dialysis** Stuart M. Sprague,[1] James B. Wetmore,[2] Konstantin Gurevich,[3] Gerald A. Da Roza,[4] John E. Buerkert,[5] Maureen T. Reiner,[6] William G. Goodman,[6] Kerry Cooper.[6] *[1]North Shore U Health System; [2]Hennepin County Medical Center; [3]Fresenius Medical Care Russia; [4]U British Columbia; [5]Columbia Nephrology Associates; [6]Amgen Inc.*

**Background:** Elevated levels of fibroblast growth factor-23 (FGF-23) have been associated with increased risk of death in patients on chronic hemodialysis. Previous data suggests that vitamin D (vitD) may stimulate FGF-23, whereas cinacalcet (Cin) may decrease FGF-23 levels, possibly via control of parathyroid hormone (PTH), calcium (Ca), and/or phosphorus (P). Cin and vitD were used as monotherapy in PARADIGM to treat secondary hyperparathyroidism (SHPT), providing a unique opportunity to assess their independent effects on FGF-23. The primary results of PARADIGM (reported separately) demonstrated reductions in PTH that did not differ after 1 year of monotherapy with Cin or vitD.

**Methods:** Herein we report the changes in FGF-23 observed during this trial. FGF-23 was measured by a two-site ELISA (Immunotopics, Inc.,San Clemente, CA).

**Results:** In the vitD arm, there was a median increase in FGF-23 of 47% at wk 52, compared to a median decrease of 40% in the Cin arm. The increase in FGF-23 in the vitD arm and the decrease in the Cin arm occurred despite comparable modest median PTH reductions (-20% vs –29%, respectively). This suggests that the observed changes in FGF-23 were possibly related to changes in P (-12% Cin vs 2% vitD) and/or Ca (-9% Cin vs 2% vitD) or other direct effects of Cin or vitD independent of PTH reduction.

**Conclusions:** Treatment of SHPT with Cin is associated with decreases in FGF-23, while vitD raises levels. The clinical relevance of these divergent effects requires further study.




*Funding:* Pharmaceutical Company Support - Amgen Inc.

## FR-PO663

**High Dose Intravenous Iron and Intact FGF23 in Uremic Rats** Eva Gravesen,[1] Jacob Hofman-Bang,[1] Ewa Lewin,[2,1] Klaus Olgaard.[1] *[1]Nephrological Dept P, Rigshospitalet, Univ of Copenhagen, Copenhagen, Denmark; [2]Nephrological Dept B, Herlev Hospital, Univ of Copenhagen, Copenhagen, Denmark.*

**Background:** Intravenous iron has been proposed to induce elevation of fibroblast growth factor 23 (FGF23), hypophosphatemia and osteomalacia. High FGF23 are associated with increased mortality in the CKD population. CKD patients often develop iron deficiency, anemia and need for i.v iron. As such it is important to study the possible effects of iron on FGF23. In the present study the effect of of two different iron preparations, iron (III) isomaltoside 1000 (Mono) and ferric carboxymaltose (Ferri) are examined on plasma FGF23 levels in uremic rats.

**Methods:** A single high dose of 60 mg/kg b.w of iron (III) isomaltoside 1000 (A), or ferric carboxymaltose (B), or vehicle, was given i.v. and the effects on plasma FGF23, phosphate, $Ca^{2+}$, PTH, transferrin, ferritin and iron was examined. NX rats were kept on a high phosphorus diet (0.9% calcium and 1.2% phosphorus), n=8 in each group. In **study 1** samples for determination of FGF23 were obtained at time 0, 30, 60, 120, 180 min. and after 48 hours, and in **study 2** at baseline and after one week. FGF23 was measured by the intact FGF23 assay from Kainos lab. Japan.

**Results:** The rats had significant uremia and hyperparathyroidism.**Study 1:** In the vehicle group FGF23 levels at baseline were 1354±167 pg/mL and 1551±329 at 30 minutes, and 990±155 at 48 hours. In group (A) FGF23 levels at baseline were 1191±184 and 1219±279 at 30 minutes and 890±257 pg/mL at 48 hours. In group (B) FGF23 levels at baseline were 1568±231 and 2027±267 at 30 minutes, and 1143±202 pg/mL at 48 hours. In **study 2,** FGF23 levels were 2986±780 pg/mL and 2279±475 after one week in the vehicle group, baseline FGF23 was 1711±179 in the iron (III) isomaltoside 1000 group and 1841±243 after one week. In the ferric carboxymaltose group baseline was1506±347 and 1647±327 after one week. No significant differences in FGF23 levels were observed at any time point between the groups.

**Conclusions:** A single high dose of either iron (III) isomaltoside 1000 or ferric carboxymaltose had no effect on intact FGF23 levels for up to one week after an intravenous injection in uremic rats.

*Funding:* Pharmaceutical Company Support - PharmaCosmos A/S, Denmark

## FR-PO664

**Coronary Artery Calcifications and Accelerated Atherosclerosis in Chronic Kidney Disease Patients: A Link between Fibroblast Growth Factor-23 and Diabetes?** Belda Dursun,[1] Ali Riza Aslan,[1] Baki Ya?d?,[2] Simin Rota,[3] Hande Senol.[4] *[1]Nephrology, Pamukkale Univ Medical School, Denizli, Turkey; [2]Radiology, Pamukkale Univ Medical School, Denizli, Turkey; [3]Biochemistry, Pamukkale Univ Medical School, Denizli, Turkey; [4]Biostatistics, Pamukkale Univ Medical School, Denizli, Turkey.*

**Background:** Coronary artery calcifications (CAC) are associated with atherosclerosis and increased cardiovascular mortality. Fibroblast growth factor-23 (FGF-23) is involved in regulation of mineral metabolism. Aim was to investigate the relationship between FGF-23 and coronary calcifications and accelerated atherosclerosis in diabetic and non-diabetic CKD patients.

**Methods:** The study was performed on 40 diabetic stage 3-5 CKD, 40 non-diabetic stage 3-5 CKD patients and 40 controls matched for age and gender. CAC was assessed by multislice computerised tomography and coronary artery calcium score (CACS) was quantified by Agatston score. Carotid intima-media thickness (cIMT) was measured by B-mode high resolution ultrasonography. FGF-23 (C-term) and conventional laboratory parameters were determined.

**Results:** cIMT (mm) levels were higher in diabetic (0.84 ± 0.16) and non-diabetic CKD patients (0.70 ± 0.09) than controls (0.56 ± 0.36), p = 0,001. The mean CAC score (Agatston) was higher in diabetic (443 ±993 ) and non-diabetic CKD patients (98 ± 244) than controls (1.13 ± 0.74 ), p = 0,001. FGF-23 levels (pg/ml) were higher in diabetic CKD patients (237 ± 108) and non-diabetic CKD patients (199 ± 81) than controls (93± 2), p= 0,001. There was a positive correlation between CACS and cIMT levels. Log-FGF -23 levels were found to be positively correlated with cIMT and CACS. FGF-23 levels showed positive correlations with systolic blood pressure, duration of diabetes, phosphorus, Ca × P product and PTH. Multiple regression analysis showed that blood glucose and systolic blood pressure were independent factors associated with CACS; and that log-FGF-23, blood glucose, systolic blood pressure and albumin were independent factors associated with cIMT.

**Conclusions:** Diabetes and FGF-23 is associated with CACS and cIMT in CKD and the affect of FGF-23 seems to be independent of its regulatory function on phosphorus metabolism.

*Funding:* Government Support - Non-U.S.

## FR-PO665

**Acute and Six Months Mineral Metabolism Adaptation in Living Kidney Donors: A Prospective Study** Sophie M. De Seigneux, Belen Ponte, Thomas Ernandez, Karine Hadaya, Andrea Trombetti, Pierre-Yves F. Martin. *Nephrology, Univ Hospital of Geneva, Geneva, Switzerland.*

**Background:** Mineral metabolism adaptation to loss of kidney function is debated. Living kidney donors (LKDs) present an abrupt decline in GFR. Following their evolution is important to understand renal adaptation to acute and chronic loss of function and prevent complications in LKDs. In this prospective study, we follow mineral metabolism adaptation of LKDs over six months.

**Methods:** From May 2010 to December 2012, we included and followed in a prospective study 26 adults LKDs. Their mineral parameters and renal function were repeatedly measured at day 0, 1, 2 and 3, and 6 months after donation. α-Klotho expression was also assessed in rats' kidneys after uninephrectomy.

**Results:** After nephrectomy, donors presented transient hypocalcemia and secondary hyperparathyroidism. Both circulating FGF23 and α-Klotho decreased during the first post-operative days and FGF23 decline was positively correlated to hypocalcemia. At 6 months after donation, donors had lower eGFR and $1.25(OH)_2D$ compared to predonation levels, whereas 25(OH)D was unchanged. PTH levels increased at 6 months, resulting in decreased serum phosphate levels and renal tubular reabsorption of phosphate. In comparison to pre-donation, FGF23 levels were unchanged whereas circulating α-Klotho levels were lower than pre-donation. In uninephrectomized rats, tissue Klotho expression levels did not change in the remnant kidney.

**Conclusions:** We show that acutely after nephrectomy, FGF23 is regulated by calcium levels. Six months after kidney donation, donors developed secondary hyperparathyroidism and lower phosphate levels probably related to $1.25(OH)_2D$ deficiency. FGF23 levels did not rise in this specific population, whereas α-Klotho levels were only slightly decreased compared to predonation levels. Mineral metabolism adaptation to loss of kidney function in donors includes decrease in $1.25(OH)_2D$ and increase in PTH and fractional excretion of phosphate resulting in decreased seric phosphate level, independently of FGF23. These results challenge the primary role of FGF23 in phosphaturia and decreased vitamin D hydroxylation in renal mass reduction.

*Funding:* Government Support - Non-U.S.

## FR-PO666

**Soluble Klotho Predicts Renal Phosphate Excretion in Moderate to Severe Chronic Kidney Disease** Kraiwiporn Kiattisunthorn, Chanchira Janwijit, Krongkarn Klayklung, Kriengsak Vareesangthip. *Siriraj Medical School, Mahidol Univ, Thailand.*

**Background:** Phosphate retention accelerates FGF23 and PTH secretion to keep phosphate in balance by promoting phosphaturia. Klotho is critical for FGF23-FGFR1 downstream to enhance endocytosis of NaPi2a cotransporters at proximal tubules. Despite high phosphate diet, paricalcitol induced klotho expression suppressed FGF23 but increased phosphaturia in CKD rats. In this study, we assess if soluble klotho directs tubular phosphate excretion in CKD patients.

**Methods:** Plasma, serum and urine were collected from 28 stage 3-5 CKD patients after fasting overnight. Maximal tubular reabsorption normalized by GFR (TmP/GFR) was used to determine renal threshold to filtered phosphate independent of dietary phosphorus, tissue release of phosphorus, and GFR. Serum creatinine was measured by enzymatic creatinine assays for GFR calculation by using CKD-EPI formula. Plasma klotho was measured by using human klotho ELISA kit (CUSABIO Biotech, Newark, DE) [intra- and inter-assay CV 4.9-8.1% and 8.3-13%, respectively]. Plasma c-terminal FGF23 was measured by using human FGF23 (c-term) ELISA kit (Immunotopics, Inc., CA, USA) [intra- and inter-assay CV 1.4-3% and 2.4-5.1%, respectively].

**Results:** Patient characteristics were shown in Table 1.

| Table 1 Patient characteristics | |
|---|---|
| Male [n(%)] | 18 (56%) |
| Age (years)[a] | 64 ± 14 |
| eGFR (ml/min/1.73m$^2$)[a] | 31.6 ±17.2 |
| Calcium (mg/dl)[a] | 9.2 ± 0.4 |
| Phosphate (mg/dl)[a] | 3.5 ± 0.8 |
| PTH (pg/ml)[b] | 86.1 (36.2, 397.2) |
| cFGF 23 (pg/ml)[b] | 130.6 (42.3, 738.5) |
| Klotho (pg/ml)[b] | 607.5 (48.7, 1041.5) |
| TmP/GFR (mg/dl)[a] | 2.5 ± 0.7 |
| [a] mean ± SD, [b] median (min, max) | |

TmP/GFR was negatively correlated with serum phosphate (r -0.45, p=0.048) and plasma klotho (r -0.53, p=0.02), but was not correlated with age, gender, intact PTH, GFR and plasma FGF23. After adjusted for age, GFR, serum phosphate, calcium, iPTH and FGF23, plasma klotho is independently associated with TmP/GFR (r -0.69, p=0.02).

**Conclusions:** Presence of klotho is a determinant for renal phosphate handling. In addition of dietary phosphate restriction, a rationale for klotho induction may be valuable in management of CKD-MBD.

*Funding:* Government Support - Non-U.S.

## FR-PO667

**Premature Vascular Aging Is a Major Complication in CKD and Occurs through Dysregulation of Arterial Klotho and Accumulation of Pre-Lamin A** Kenneth Lim,[1,2] Stephen M.S. Ting,[2] Guerman Molostvov,[2] Chih-Ping Chung,[1] Tzongshi Lu,[1] Arnoud Johan Groen,[3] Kathryn S. Lilley,[3] Thomas F. Hiemstra,[3] Daniel Zehnder,[2] Li-Li Hsiao.[1] *[1]Brigham and Women's Hospital; [2]Warwick Medical School, United Kingdom; [3]Univ of Cambridge, United Kingdom.*

**Background:** Accelerated vascular calcification and arteriosclerosis are age-related complications that occur in patients with Chronic Kidney Disease (CKD). We previously showed that Klotho, a 130kDa transmembrane protein is expressed in smooth muscle cells of the arterial wall and its expression is suppressed in arteries from CKD patients. Here, we describe its role as a major regular of arterial aging in CKD.

**Methods:** *In vivo:* Arteries from healthy and CKD patients. *In vitro:* Human aortic smooth muscle (HA-SMC) cells +/- Klotho siRNA. *Immuno and western blot:* Markers of HA-SMC phenotype and vascular aging.

**Results:** Immunohistochemistry analysis showed that arteries from young CKD patients and healthy elderly controls were Klotho deficient compared with arteries from young healthy patients. These arteries exhibited age-related changes, including accumulation of the smooth muscle cell aging marker, pre-lamin A and increased calcification. Molecular analysis confirmed Klotho deficiency and accumulation of pre-lamin A with the functional consequence of increased calcification, using a replicative senescence HA-SMC model. These age-related changes could be induced prematurely by circulating stress factors, including disordered mineral and pro-inflammatory conditions. Mechanistic studies revealed that Klotho knockdown directly resulted in VSMC aging with accumulation of pre-lamin A, phenotypic transformation to an osteogenic phenotype, apoptosis as assessed by caspase 3 activity and increased Poly (ADP-ribose) polymerase activity indicative of DNA damage. Both Klotho knockdown *in vitro* and arteries from CKD patients *in vivo* showed suppressed expression of FACE-1, the pre-lamin A cleavage enzyme.

**Conclusions:** Arterial aging occurs prematurely in young patients with CKD and resembles arteries from healthy elderly patients. Premature arterial aging in CKD occurs through a dysregulated Klotho - FACE-1 – prelamin-A pathway.

*Funding:* Pharmaceutical Company Support - Genzyme, Private Foundation Support

## FR-PO668

**Klotho Expression Profile in a Wide Range of Human Tissues** Kenneth Lim,[1,2] Guerman Molostvov,[2] Tzongshi Lu,[1] Arnoud Johan Groen,[3] Kathryn S. Lilley,[3] Thomas F. Hiemstra,[3] Li-Li Hsiao,[1] Daniel Zehnder.[2] *[1]Brigham and Women's Hospital; [2]Warwick Medical School, United Kingdom; [3]Univ of Cambridge, United Kingdom.*

**Background:** Klotho is a 130kDa protein that imparts anti-aging properties and functions as a co-factor for the phosphatonin, FGF-23. Previous studies have shown that it is produced in the kidney, parathyroid gland, vasculature and choroid plexus of the brain. However, no study has explored its expression profile across different tissue types in humans to-date.

**Methods:** Ethics (IRB) approval was first obtained for this study. A wide range of formalin fixed normal human tissues removed during routine therapeutic or diagnostic procedures and part of the clinical tissue bank was analyzed by immunohistochemistry. For each tissue, at least 5 different cases were analyzed. A full length Klotho protein detecting antibody (Abcam, Cat. No. ab69208) was utilized at a concentration of 1:100 to 1:150.

**Results:** As previously described, significant renal Klotho protein expression was observed in the proximal and distal part of the tubular epithelium. On extensive analysis of tissues, differential Klotho expression was found in skin, intestine, endocrine and reproductive organs, and also the central and peripheral nervous system. The epithelial layer of the skin, small bowel, colon, mammary tissue, endometrium, prostate and sertoli cells in testis showed strong Klotho expression. The same observation was made for endocrine cells of the thyroid, islet cells of the pancreas and adrenal medulla. In neuronal tissue, Klotho expression was found primarily in neuronal cells of cerebral cortex, cells of Purkinje in the cerebellum and motor neurons of the spinal cord, but also the myenteric plexus of the intestine. Smooth muscle cell (SMC) Klotho expression was confirmed in arteries of all tissues analyzed, but also observed in SMC of the intestine and myometrium. Hepatocytes did not stain for Klotho protein.

**Conclusions:** This study is the first to report the expression profile of Klotho in human tissues. Expression of Klotho may indicate a diverse function for Klotho in different tissues and cell types and render these cells a target for FGF-23 function.

*Funding:* Private Foundation Support

## FR-PO669

**Serum Soluble Klotho Level Is Associated with Abdominal Aortic Calcification in Patients on Maintenance Hemodialysis** Hong Cai, Yucheng Yan, Renhua Lu, Minfang Zhang, Mingli Zhu, Weiming Zhang, Zhaohui Ni, Jia Qi Qian. *Renal Dept, JiaoTong Univ RenJi Hospital, Shanghai, China.*

**Background:** To determine the relationship between soluble Klotho and abdominal aortic calcification in maintenance hemodialysis(MHD) patients.

**Methods:** 129 cases of MHD patients were collected prospectively. Serum soluble Klotho (sKL) level were detected by ELISA. Abdomen lateral plain were used as a criteria to determine the abdominal aortic calcification. The abdominal aorta calcification score(AAC) was calculated. The level of soluble Klotho were observed in patients with different degree of calcification. Logistic regression analysis was used to determine the risk factor of abdominal aortic calcification in MHD patients. The standard statistics were used along with ROC analysis to evaluate the diagnostic value of soluble Klotho in abdominal aortic calcification.

**Results:** 87 in 129 patients had abdominal aortic calcification. The median AAC was 4.0(0.00, 11.00). The median Klotho concentration was 616.29(378.19, 821.61)pg/ml and the soluble Klotho concentration was negatively correlated with AAC. Risk of moderate to severe abdominal aortic calcification in patients with lowest quartile of the soluble Klotho concentration was significantly higher than those in highest quartile[OR=4.004, 95%CI(1.350-11.826), P=0.012], even by adjustment for demographic data, lifestyle factors and biochemical markers [OR=4.542, 95%CI(1.368-15.081), P=0.013]. Multivariate Logistic regression analysis showed that serum soluble Klotho levels and smoking were independent risk factors for severe calcification of the abdominal aorta. ROC-AUC of serum soluble Klotho for moderate to severe abdominal aortic calcification was 0.746(cutoff:265.39pg/ml, accuracy 88.5% specificity 56.2%, P=0.001).

**Conclusions:** The lower serum soluble Klotho was independently associated with severe abdominal aorta calcification. Serum soluble klotho may have diagnostic value of MHD patients with severe abdominal aorta calcification. But still need to be further confirmed.

*Funding:* Government Support - Non-U.S.

## FR-PO670

**Soluble Klotho (sKl) and Fibroblast Growth Factor 23 (FGF23) Alterations in Pediatric (Ped) Renal Transplant (Tx) Patients (Pts) with Good Function** Rachana Srivastava, Eileen D. Brewer, Poyyapakkam Srivaths. *Ped Renal Section, Baylor College Medicine/Texas Children's Hosp, Houston, TX.*

**Background:** The phosphaturic hormone FGF23 stimulates renal production of Kl, which complexes with FGF receptors & increases binding affinity for circulating FGF23. In adult studies, FGF23 levels are an early marker for CKD (in stage 2) & may be associated with decreased sKl as CKD progresses. Very little is known about sKl & FGF23 in renal Tx or ped pts, except that sKI is higher in children than adults. **Aim:** To determine potential alterations in plasma FGF23 and sKl in ped Tx pts with early CKD & to evaluate for associations with GFR & mineral metabolism parameters.

**Methods:** X-sectional study of 63 ped Tx pts (39M); median age 15y (4-22y); 19C/26His/16B/2other; CKD stages 1-3 (Schwartz eGFR 97±24 ml/min/1.73m2); median time since Tx 1.2y (0.2-11y). Immunosuppression: 51 steroid free (SF). 12 steroid based (SB). Serum Cr, Ca, P, iPTH, 1,25vitD; urine Ca,P,Cr; plasma C-term FGF23 & sKl by ELISA were measured. 14 pts were taking P supp, 13 calciferol, & 4 calcitriol.

**Results:** Our ped Tx pts had higher plasma FGF23 (mean 136 RU/ml, range 29-752 vs normal range 1-48 RU/ml), and lower sKl (median 1429 pg/ml;IQR 906-2356 vs normal 2406;IQR 1710-3352) compared to published values for healthy children. As FGF23 increased, so did sKl (r=0.37, p=0.03). No association was found for sKl & age, eGFR, TRP, or serum Ca, P, iPTH, 1,25vitD. FGF23 & sKl were higher with SB vs SF immunosuppression.

| | Age(y) | eGFR | iPTH pg/ml | 1,25vitD pg/ml | FGF23 RU/ml | s-Klotho pg/ml |
|---|---|---|---|---|---|---|
| SF, n=51 | 14.1±4.8 | 100±24 | 99±78 | 63.4±29.9 | 100±66 | 1648±1267 |
| SB, n=12 | 14.2±3.5 | 85±20 | 85±107 | 60.8±20 | 274±252* | 2812±1932** |

Values as mean±SD;eGFR ml/min/1.73m2;*p= 0.004,**p=0.012

**Conclusions:** In our ped Tx pts with CKD stage 1-3: 1) Plasma FGF23 is higher and sKl is lower than published normals. 2) FGF23 is positively associated with sKl, likely due to intact, though possibly blunted stimulation of Kl production when the Tx kidney has good function. 3) FGF23 and sKl levels are higher with SB than SF immunosuppression, suggesting steroids may be an additional stimulus to increased FGF23 production.

*Funding:* Private Foundation Support

## FR-PO671

**Association with Fetuin-A and Ectopic Calcification in α-Klotho Mutant Mice** Nozomi Yokoyama,[1] Hironori Yamamoto,[2,3] Yutaka Taketani,[1] Masayuki Iwano,[3] Eiji Takeda.[1] *[1]Clinical Nutrition, Health Biosciences, Univ of Tokushima, Tokushima, Japan; [2]Health and Nutrition, Human Life, Jin-ai Univ, Echizen, Japan; [3]Nephrology, General Medicine, Faculty of Medical Sciences, Univ of Fukui, Fukui, Japan.*

**Background:** α-klotho mutant (kl/kl) mice exhibit hyperphosphatemia, hypercalcemia and hypervitaminosis D. These abnormalities are associated with growth retardation and ectopic calcification. Fetuin-A is a hepatic secretory protein acting as a systemic calcification inhibitor. However, it is still unknown about the role of fetuin-A in kl/kl mice.

**Methods:** Wild-type (WT) mice and kl/kl mice at 3- or 6-wk-old were used and given free access to regular chow and water. For histology using paraffin sections of kidney, aorta and heart tissues, calcification sites and fetuin-A expression were detected by von Kossa staining and immunohistochemistry (IHC), respectively. Gene expression analysis were performed using western blots (WB) and Real-time RT-PCR methods.

**Results:** Analysis of serial kidney, aorta and heart sections by IHC and von Kossa staining showed a high regional expression of fetuin-A protein in 6-wk-old kl/kl mice and its localization merged with calcifying sites. We also confirmed its co-localization with the calcified lesions in arota and heart of chronic kidney disease rats. Interestingly, the high regional expression of fetuin-A in kidney was also observed at 3-wk-old kl/kl mice without ectopic calcification. Although WB analysis observed markedly high expression of fetuin-A in kidney of kl/kl mice, its renal mRNA levels were no significant differences between WT and kl/kl mice. In contrast, fetuin-A mRNA levels in liver of kl/kl mice were significantly increased than WT mice from 3-wk-old. Moreover, we found that plasma fetuin-A levels in kl/kl mice were significantly higher than WT mice.

**Conclusions:** These results suggest that the up-regulation of hepatic futuin-A mRNA expression in kl/kl mice contributes to increase plasma fetuin-A levels and co-localize with calcified lesions as a inhibitor of hyperphosphatemia-induced ectopic calcification.

*Funding:* Government Support - Non-U.S.

## FR-PO672

**Transfer of Oral Alfacalcidol to Oral Calcitriol in the Treatment of Secondary Hyperparathyroidism in Chronic Hemodialysis Patients** Sandrine Rauscher, Jean-Philippe Lafrance, Vincent Pichette, Robert Zoël Bell, Martine Leblanc, Sarah Bezzaoucha, Michel Vallee. *Nephrology Dept, Maisonneuve-Rosemont Hospital, Univ of Montreal, Montreal, Canada.*

**Background:** There is no clear evidence in the literature regarding the optimal vitamin D therapy for the treatment of secondary hyperparathyroidism in chronic hemodialysis patients. Recent studies suggest that uremia in terminal renal failure is associated with enzymatic hepatic dysfunction altering vitamin D 25-OH hydroxylation. This may imply that calcitriol could be more effective than alfacalcidol. The goal was to compare the efficacy of calcitriol, the fully hydroxylated active form of vitamin D, to alfacalcidol, which is not hydroxylated at the 25 position, for the treatment of secondary hyperparathyroidism in hemodialysed patients.

**Methods:** We retrospectively reviewed 47 chronic hemodialysis patients to whom alfacalcidol administration was switched to calcitriol for the treatment of secondary hyperparathyroidism. We then compared the different components of mineral metabolism with paired Student's t-tests pre and post conversion.

**Results:** Before the switch, the mean dose of alfacalcidol was 3.50 mcg per week. After being transferred to calcitriol, the mean dose was decreased by 20% to 2.86 mcg ( p<0,0001). We observed a significant decrease in PTH of 11.87 pmol/L (p = 0,02), with a mean PTH of 94.47 and 82.60 pmol/L, pre and post switch respectively. The mean corrected calcium for albumin increased by 0.08 mmol/L (p = 0.0001). No significant hypercalcemia was observed during this study. There was no significant change in phosphorus levels. Also, we observed a mean decrease of PTH of 9.45 pmol/L (p = 0.07) as well as an increase of the mean corrected calcium of 0.05 pmol/L (p = 0.05) in a subgroup of patients (n=22) for whom the medication was administrated during the hemodialysis session, ensuring 100% compliance.

**Conclusions:** According to our study, calcitriol seems more effective than alfacalcidol in lowering serum PTH level in chronic hemodialysis patients. This suggests that calcitriol could be the optimalchoice for the treatment of secondary hyperparathyroidism in chronic hemodialysis patients.

## FR-PO673

**High-Dose Ergocalciferol in Patients with CKD Stage 5 on Dialysis: A Pilot Trial** Elisa Elena Del Valle,[1] Armando Luis Negri,[2] Guillermo Javier Rosa Diez,[1] Marcelo Puddu,[1] Jaime Ryba,[1] Luis Alberto Sintado,[1] Erich Fradinger.[2] *[1]Fresenius Medical Care, Argentina; [2]Instituto de Investigaciones Metabólicas, Argentina.*

**Background:** There is a growing evidence of the usefulness of vitamin D supplementation in dialysis patients who are often vitamin D deficient. There are few data on which vitamin D (D2 or D3) and which dosage scheme is the best to maintain adequate 25 OH D levels safely. **Ojective:** We aimed to determine whether high-dose vitamin D2 supplementation in HD patients is sufficient to obtain optimal vitamin D status (serum 25-hydroxyvitamin D [25(OH)D] concentration ≥30 ng/mL) without inducing hypercalcemia.

**Methods:** Open label-controlled trial.82 subjects with CKD stage 5 on dialysis not taking active vitamin D therapy were supplemented with oral vitamin D2 72.000 IU/wk for 12 wk followed by 24.000 IU wk as maintenance therapy during 36 weeks.

**Results:** By 12 wk, serum 25(OH)D increased: baseline (mean ±SD): 15.2 ± 5.4 to 42.5 ± 13.2 ng/mL; P<0.01 and remained optimal (34.7 ±12.0). iPTH at baseline was 360 ± 143pg/ml and at 48wk values were not significantly different from baseline (362 ± 193 UI; P = 0.16) as well as total alkaline phosphatase (286.7±163.1; p= 0.08). We observed a significant decrease in bone alkaline phosphatase from 54.3 ± 46.0 to 44.3 ± 25.0 (p = 0.022). Uncorrected Serum Ca was 9.03 ± 0.42 at baseline but had asignificant increase at the end of follow-up (9.14 ± 0.62; p = 0.0496); Hypercalcemia presented in the first control visit (week 12) in 2 patients, in 1 patient in the second control (week 30) and in one patient in the third control (week 48). In 222 serum calcium determinations during follow up hypercalcemia was observed in only 1.8% of cases. Serum phosphorus remained without

change (basal 4.9 ± 1.2 and 4.7 ± 1.1during the follow up). In 47 patients we were able to determine 1,25 (OH)2D serum levels: at baseline 3.1 ± 4.84 pg/ml (in 32 patients levels were not detectable); at week 48 it increased to 15.92 ± 8.32 pg/ml (not detectable in 3 patients).

**Conclusions:** This vitamin D2 oral regimen was safe and sufficient to obtain and maintain optimal serum 25(OH)D concentrations and prevent vitamin D insufficiency in CKD patients on dialysis.

## FR-PO674

**1,25-Dihydroxyvitamin $D_3$ Deficiency Induces Podocyte Injury and Proteinuria** Ramon Sonneveld,[1,2] Joost Hoenderop,[2] Marijke P.A. Baltissen,[1] Henry Dijkman,[3] Sandrine Florquin,[3] Angelique Rops,[1] Jack F. Wetzels,[1] Jo H.M. Berden,[1] Johan Van der Vlag,[1] Tom Nijenhuis.[1] *[1]Nephrology, UMC St. Radboud, Nijmegen, Netherlands; [2]Physiology, UMC St. Radboud, Nijmegen, Netherlands; [3]Pathology, UMC St. Radboud, Nijmegen, Netherlands.*

**Background:** The steroid hormone vitamin D plays an important role in renal physiology. Hydroxylation of vitamin D occurs in the kidney by 25-hydroxy-vitamin-$D_3$-1α-hydroxylase (1α-OHase), and 1,25-$D_3$ deficiency is a consequence of renal insufficiency. In animal models and patients with glomerular disease, 1,25-$D_3$ treatment ameliorates proteinuria. In this study, we determined whether 1,25-$D_3$ deficiency induces renal injury.

**Methods:** We assessed renal injury in 1,25-$D_3$-deficient 1α-OHase knockout (KO) mice. *In vitro*, we evaluated the regulation of the podocyte injury marker TRPC6 by six vitamin D analogs.

**Results:** Hypocalcemic 1α-OHase KO mice showed proteinuria and glomerular injury, characterized by partial podocyte foot process effacement and altered expression of nephrin, desmin, podocin, CD2AP and TRPC6, and activation of the calcineurin/NFAT pathway. No significant glomerular immunoglobulin or complement deposition could be shown. Minor glomerular basement membrane changes were accompanied by reduced expression of collagen IV. The 1α-OHase KO mice also showed increased urinary NGAL excretion and interstitial caspase-3 expression. *In vitro*, we found that 1α- and/or 25-hydroxylated vitamin $D_3$ analogs and paricalcitol (1,25-$D_2$) reduced TRPC6 promoter activity and its expression in injured podocytes.1,25-$D_3$ and 1,25-$D_2$ supplementation both reversed glomerular and, in part, tubular damage, and normalized calcium/phosphorus homeostasis. A low dose of 1,25-$D_3$, which did not correct serum calcium, PTH, FGF23 and phosphate, also normalized podocyte injury.

**Conclusions:** 1,25-$D_3$ deficiency is not only a consequence, but also a cause of glomerular and interstitial injury with altered expression of slit-diaphragm-associated proteins. 1,25-$D_3$ and 1,25-$D_2$ supplementation can reverse these changes. Our results are in line with the anti-proteinuric potential of vitamin D analogs in clinical practice. Currently, we are studying the renal phenotype of acquired vitamin D deficiency.

*Funding:* Private Foundation Support

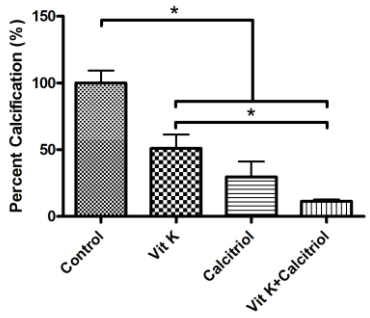
## FR-PO675

**Vitamin K and Calcitriol Attenuate the Development of Vascular Calcification in an *In Vitro* Model of Aortic Calcification** Kristin M. McCabe,[1] Bernard Burgesson,[1] Martin Kaufmann,[1] Dwight F. Newton,[1] Navid Shobeiri,[1] Glenville Jones,[1] Michael A. Adams,[1] Rachel M. Holden.[2] *[1]Biomedical and Molecular Sciences, Queen's Univ, Kingston, Canada; [2]Medicine, Queen's Univ, Kingston, Canada.*

**Background:** Non-traditional risk factors, such as increased phosphate retention, and deficiencies in vitamins D and K metabolism, likely play key roles in the development of vascular calcification (VC) during CKD progression. Matrix Gla protein (MGP) is a vitamin K dependent protein that inhibits vascular calcification and calcitriol is a key transcriptional regulator of MGP. In this study we looked at the effect of calcitriol and K individually and combined in an *in vitro* model of VC.

**Methods:** The thoracic aorta was harvested from male Sprague-Dawley rats at 14 weeks of age, cut into 3mm rings, and incubated in DMEM at 37ºC for 4 days. The media contained 3.8 mM phosphate, as well as calcitriol and vitamin K1. VC was assessed in segments of thoracic aorta using the calcium-O-cresophthalein assay. Calcitriol metabolites were analyzed in the media using LC-MSMS on a Waters BEH-C18 column (1.7 micron, 2.1x50), and a Waters Acquity-Xevo-TQ-S in MRM mode (with a MeOH/$H_2O$ gradient system).

**Results:** Calcitriol ($10^{-10}$, $10^{-8}$, and $10^{-6}$ M) inhibited the development of VC at the two higher concentrations ($p<0.05$). Both calcitriol (□ 70.4%) and vitamin K1 (□ 49.0%) alone ($10^{-6}$ M) partially inhibited VC. Added together, inhibition of VC was nearly complete (□ 88.5%) ($p<0.05$).




1,24,25-$(OH)_3D_3$, the CYP 24A1 generated metabolite of calcitriol, was found in the incubation media.

**Conclusions:** Although calcitriol and vitamin K1 alone partially inhibited VC *in vitro*, the inhibitory effect was greater in combination. Generation of the calcitriol metabolite confirmed the vascular tissue responded via CYP24A1-mediated metabolism. Optimizing the combination vitamins K and D treatment could provide a useful new therapeutic approach.

*Funding:* Government Support - Non-U.S.

## FR-PO676

**Intact PTH and FGF23 Levels of Cinacalcet Pre-Test Predict Refractory Hyperparathyroidism in Hemodialysis Patients** Atsushi Saito,[1] Hitoshi Yokoyama,[1] Hideki Yamaya,[1] Isao Ishikawa.[2] *[1]Nephrology, Kanazawa Medical Univ, Uchinada, Japan; [2]Nephrology, Asanogawa General Hospital, Kanazawa, Japan.*

**Background:** Cinacalcet hydrochloride (cinacalcet) is prescribed for secondary hyperparathyroidism (SHPT) in hemodialysis (HD) patients. However, it had been uncertain to judge the refractory SHPT before therapy. We evaluate the long-term effects of cinacalcet using the early response of intact parathyroid hormone (iPTH) and fibroblast growth factor (FGF) 23.

**Methods:** Fifty-three HD patients with mean iPTH of 720.8 pg/mL and mean corrected calcium (Ca) of 10.0mg/dL were enrolled. Primary end point was defined by iPTH levels of <240pg/mL after 48 weeks therapy. Serum corrected Ca, phosphate (P), iPTH and FGF23 were measured before and 3 hrs after oral intake of a cinacalcet 25mg tablet as pre-test. After that, 46 patients started cinacalcet intake with the daily dose between 25 and 100mg.

**Results:** Forty-two patients completed 48 weeks cinacalcet therapy. The achievement rate of primary end point was 66.7% (i.e. responders, group I). There were significantly difference on the mean levels of iPTH at 0hr (565.5 vs. 1197.5 pg/mL, p=0.001) and 3hrs (293.5vs. 593.6 pg/mL, p=0.001) on pre-test between group I and II (i.e. non-responders). These 2 groups showed quite different response patterns in FGF23 after 3hrs (ΔFGF23) on pre-test (-1493.1 vs. 2401.3 pg/mL, p=0.015). On the base line study, BAP (25.0 vs. 57.3 U/L, p=0.011) and NTx (179.7 vs. 492.6 nmolBCE/L, p=0.007) were also different in these 2 groups. Then, cut off values of cinacalcet treatment resistance were determined at 901.0 pg/mL of 0hr iPTH, 355.5 pg/mL of 3hrs iPTH, and 2500 pg/mL of ΔFGF23 by ROC analysis. A model of three factors including cut off values of ΔFGF23 and iPTH at 3hrs, and NTx >102.3 nmolBCE/L demonstrated overall accuracy of 92.3% (p=0.014) and the odds ratio of 465.6 on refractory SHPT for cinacalcet.

**Conclusions:** FGF23 levels at 3hrs showed a different response for cinacalcet intake in responders or non-responders. Cinacalcet pre-test including iPTH and FGF23 was convenient and reliable to speculate the long-term therapeutic effects of cinacalcet for SHPT.

*Funding:* Private Foundation Support

## FR-PO677

**Vitamin D Status in the Chronic Renal Insufficiency Cohort: Impact of Vitamin D-Binding Protein (DBP) Concentrations and Polymorphisms** Michelle Denburg,[1] Thomas Jemielita,[1] Jayanta Gupta,[1] Martin Hewison,[2] Myles S. Wolf,[3] Mary B. Leonard.[1] *[1]Univ of Pennsylvania; [2]UCLA Orthopaedic Hospital; [3]Univ of Miami.*

**Background:** Serum DBP concentrations influence bioavailability of 25-hydroxyvitamin D (25D). The 3 major DBP polymorphisms differ in affinity for 25D and racial distribution.

**Methods:** Serum 25D, DBP, and PTH were measured in 1280 CRIC participants (pts). Free and bioavailable (bio) 25D were calculated using total 25D, albumin (Alb) and DBP levels, and with mathematical modeling based on DBP polymorphisms (geno-free and geno-bio). Linear regression was used to determine correlates of total, free, and bio 25D.

**Results:** Median DBP was lower in black pts (10.6 vs 24.3 mg/dl, p<0.0001). 92 and 32% of black and non-black pts had ≥1 high-affinity 1F allele, respectively (p<0.001). Winter, obesity, diabetes, hypoalbuminemia, albuminuria, and lower eGFR were independently associated with lower levels of all forms of 25D, while older age, dietary vitamin D, and calciferol therapy were positively associated with all forms. Black race was associated with lower total, free and bio 25D using genotype-specific affinities, but higher free/bio if genotype was not considered.

| **Multivariable Models for Ln(25D)** expressed as % difference in 25D *p<0.05 **p<0.001 | Total | Free | Bio | Geno-Free | Geno-Bio |
|---|---|---|---|---|---|
| Age yr | 0.6** | 0.8** | 0.9** | 0.8** | 0.8** |
| Winter | -17** | -16** | -15** | -18** | -17** |
| Obese | -19** | -18** | -18** | -17** | -17** |
| Diabetes | -14** | -12* | -15** | -14** | -17** |
| DBP mg/dl | 1** | NA | | | |
| Alb <3 g/dl | -50** | NA | | | |
| Urine Alb >300 mg/d | -13** | -18** | -23** | -15** | -22** |
| eGFR <45 | -9* | -10* | -12* | -9* | -11* |
| Diet VitD mcg/d | 4** | 4** | 4** | 4** | 4** |
| On calciferol | 40** | 42** | 42** | 45** | 44** |
| Black | -23** | 24** | 23** | -16** | -17** |

Genotype-based free/bio 25D were better correlated with PTH (r=-0.35/-0.38) than traditional estimates (r=-0.26/-0.29; p<0.002) but not than total 25D (r=-0.37).

**Conclusions:** Marked differences in racial distribution of serum DBP and DBP polymorphisms must be considered when assessing vitamin D status, particularly in the estimation of free/bio 25D.

*Funding:* NIDDK Support, Private Foundation Support

**FR-PO678**

**A Novel Vitamin D Receptor Modulator, VS-105, Reduces Blood Pressure and Improves Cardiac Function in 5/6 Nephrectomized Uremic Rats**
J. Ruth Wu-Wong, Yung-wu Chen, Jerry Wessale. *Vidasym.*

**Background:** Vitamin D receptor modulators (VDRMs) are commonly used to manage hyperparathyroidism secondary to chronic kidney disease (CKD). A majority of CKD patients die from cardiovascular complications. Clinical observations demonstrate that VDRM therapy may provide cardiovascular and survival benefit for CKD patients. However, current on-market VDRMs exhibit a narrow therapeutic index (TI) of 1-4-fold (hypercalcemic side effects vs. PTH suppressing efficacy). VDRM-induced hypercalcemia remains a serious concern, which leads to the need for frequent drug dose titration and serum calcium monitoring. Significant clinical benefit may be derived from a VDRM with expanded TI and cardiovascular protective effects.

**Methods:** 5/6 nephrectomized (NX) male Sprague Dawley rat with established uremia exhibits elevated PTH, hypertension and abnormal cardiac function. Previously we have shown that after 2-weeks of i.p. or oral dosing in 5/6 NX rats, VS-105 in a non-hypercalcemic dose range suppressed PTH and reduced cardiac fibrosis (TI at ~50-fold). In this study, treatment of 5/6 NX rats by VS-105 (0.5 µg/kg, oral gavage, once daily, for 8 weeks) in the presence or absence of enalapril (30 mg/kg, p.o., via drinking water) suppressed serum PTH effectively without raising serum calcium.

**Results:** VS-105 alone reduced systolic blood pressure (from 174 ± 6 to 145 ± 9 mmHg, $p<0.05$) as effectively as enalapril (from 174 ± 6 to 144 ± 7 mmHg, $p<0.05$). Echocardiographic evaluation showed that VS-105 also improved cardiac function such as E/A ratio, ejection fraction, and fractional shortening. Enalapril or VS-105 alone reduced left ventricular hypertrophy (LVH) significantly and VS-105 plus enalapril did not exhibit further reduction in LVH. The lack of hypercalcemic toxicity of VS-105 is due to its lack of effects on stimulating intestinal calcium transport and inducing the expression of intestinal calcium transporter genes such as Calb3 and TRPV6.

**Conclusions:** These studies demonstrate that VS-105 is a novel VDRM with an overall therapeutic profile that supports clinical development for its expanded use in pre-dialysis and dialysis CKD patients to achieve cardiovascular benefits of VDR activation.

*Funding:* NIDDK Support

**FR-PO679**

**African-American and South Asian Patients with Chronic Kidney Disease and Vitamin D Deficiency Have a Blunted Response to Therapy with Standard High Dose Ergocalciferol Therapy**
Gavin Dreyer, Magdi Yaqoob. *Nephrology, Barts Health NHS Trust, United Kingdom.*

**Background:** Patients from ethnic minority (EM) groups with CKD have a higher risk of cardiovascular morbidity and mortality and a higher prevalence of vitamin D deficiency. To date, no studies have investigated the response to ergocalciferol of patients from ethnic minorities with CKD and vitamin D deficiency in the UK.

**Methods:** We prospectively analysed 93 patients with non-dialysis dependent CKD who were prescribed high dose ergocalciferol for vitamin D deficiency between 1/8/2008 and 1/8/2010. Baseline 25 hydroxy vitamin D levels were compared to 25 hydroxy vitamin D levels measured at or no more than 30 days beyond the last planned dose of ergocalciferol. Ethnicity was self reported and White ethnicity compared to a combined group containing African-American and South Asian patients. A 25 hydroxy vitamin D level of > 30 ng/ml after therapy was the main outcome measure.

**Results:** White patients (n=33) were older compared to EM patients (n=60), mean age (years) W = 64.4, EM 55.6, p=0.003. Ethnic minority patients had a higher proportion of diabetes as a cause of CKD (W = 18.2%, EM 35.0% p=0.001). There was no difference in stage of CKD at baseline. Baseline 25 hydroxy vitamin D levels were lower in EM compared to W patients (W = 15.8 ng/ml, EM = 11 ng/ml, $p<0.001$). The mean duration of ergocalciferol therapy was 7.1 months, the mean total dose of ergocalciferol prescribed was 360,655 iu and neither differed significantly between groups. In a logistic regression analysis adjusted for age, sex, baseline 25 hydroxy vitamin D level, stage of CKD and cause of kidney disease, EM status was associated with a higher odds of failing to attain the primary outcome (OR 3.75, p=0.044, 95% CI 1.04 – 13.54).

**Conclusions:** EM patients may require intensified therapy with ergocalciferol including higher doses and longer duration of treatment to achieve recommended levels of 25 hydroxy vitamin D. Given this group has a higher burden of diabetes and cardiovascular risk, particular attention is required when prescribing and monitoring ergocalciferol in ethnic minority patients in order to achieve current recommended targets of serum 25 hydroxy vitamin D.

**FR-PO680**

**Effects of 25-OH Vitamin D Levels on Hemoglobin in the Chronic Kidney Disease Population**
Jack Ellis, David Owshalimpur. *Dept of Medicine, Madigan Army Medical Center, Tacoma, WA.*

**Background:** As chronic kidney disease (CKD) progresses, so do the complications of bone mineral disease and anemia, contributing to the morbidity, mortality and overall health care expenditures in this population. 1,25-OH vitamin D has been shown to decrease serum parathyroid hormone (PTH) levels and improve anemia. 25-OH vitamin D has been shown to decrease PTH levels. One prior study found decreased erythropoietin (EPO) use in hemodialysis patients with increased 25-OH vitamin D levels after repletion with ergocalciferol. We examined the association between 25-OH vitamin D levels and anemia in the CKD 3-5 population, expecting that higher 25-OH vitamin D levels would correlate with increased hemoglobin levels.

**Methods:** A retrospective review of adult (18 years or older) patients with CKD 3-5 from January 2005 to January 2011 using the electronic health record in one Pacific Northwest center to determine the association between 25-OH vitamin D levels and hemoglobin. Secondary endpoints include reviewing data for any correlation between multiple variables to include PTH, ferritin, transferrin saturation, hemoglobin, and hematocrit.

**Results:** Three hundred and five CKD 3-5 patients were reviewed and 289 records had 25-OH vitamin D levels. There was not a significant correlation between 25-OH vitamin D level and hemoglobin. There was a statistically significant correlation between PTH and ferritin and a statistically significant inverse correlation between 25-OH vitamin D level and ferritin by Pearson correlation coefficients.

**Conclusions:** Although one prior study did show ergocalciferol use in hemodialysis patients resulted in decreased erythropoietin use, our study did not find a statistically significant correlation between hemoglobin and 25-OH vitamin D in CKD 3-5. Interestingly, an unadjusted analysis found both a direct correlation between ferritin and PTH and an inverse correlation between ferritin and 25-OH vitamin D levels. This is the first time in the literature which an inverse correlation between 25-OH vitamin D and ferritin and a direct correlation between PTH and ferritin has been reported in the CKD population.

*Funding:* Other U.S. Government Support

**FR-PO681**

**Serum 25-Hydroxy Vitamin D Status Is Predictive of Hospitalization-Free Survival in Predialysis and Dialysis Patients with Chronic Kidney Disease**
Dong Ho Yang,[1] So-young Lee,[1] Seongeun Suh,[1] Hun Jeong.[2] *[1]Internal Medicine, Bundang CHA Medical Center, CHA Univ, Seongnam, Republic of Korea; [2]Internal Medicine, Seoul Bukbu Hospital, Seoul, Republic of Korea.*

**Background:** Vitamin D has pleiotropic effects important for the proper functioning of multiple organ systems. We investigated whether serum 25-hydroxy vitamin D (25(OH) D) levels influence hospitalization-free survival in patients with chronic kidney disease.

**Methods:** In this prospective study, serum levels of 25(OH)D were obtained from 210 patients with chronic kidney disease in the winter of 2009. Data regarding hospitalizations were collected over the subsequent 3 years.

**Results:** Vitamin D deficiency, as defined by a serum 25(OH)D level below 15 ng/ml, was observed in 76.7% of the patients. The mean 25(OH)D serum level was 13.6 ± 7.8 ng/ml in predialysis patients (n=62) and 11.3 ± 6.7 ng/ml in dialysis patients (n=148). During the 3-year follow–up period, 107 patients (28 predialysis and 79 dialysis) were hospitalized because of infectious (27.4%), cardiovascular (23.6%), and cerebrovascular disease (7.5%).The predialysis and dialysis groups were divided into two subgroups based on the mean 25(OH)D serum levels: above and below average.Kaplan-Meier analysis revealed that the risk of hospitalization was significantly lower in both predialysis and dialysis patients with above average serum 25(OH)D levels (log rank test; P = 0.04 and 0.02, respectively). Multivariate Cox proportional hazards models also demonstrated that the risk of hospitalization was significantly lower for patients with above-average serum 25(OH)D levels in both the predialysis (hazard ratio [HR] 0.26, 95% confidence interval [CI] 0.12-0.57) and dialysis groups (HR 0.35, 95% CI 0.20 - 0.62).

**Conclusions:** Serum 25(OH)D status is predictive of hospitalization-free survival in patients with chronic kidney disease. The risk of hospitalization over the next 3 years is significantly reduced in predialysis and dialysis patients with an initial serum 25(OH)D level above 13.6 ng/ml and 11.3 ng/ml, respectively.

**FR-PO682**

**Continously Vitamin D Receptor Activation and Dose Adjustment According to PTH Level Directly Influence Pulse Wave Velocity among Maintenance Hemodialysis Patients**
Siren Sezer,[1] Zeynep Bal,[1] Orhan Guliyev,[1] Ugur Bal,[2] Mehtap Erkmen Uyar,[1] Emre Tutal,[1] Nurhan Özdemir Acar.[1] *[1]Dept of Nephrology, Baskent Univ Medical School, Ankara, Turkey; [2]Dept of Cardiology, Baskent Univ Medical School, Ankara, Turkey.*

**Background:** Vascular calcification (VC) represents an important contributor to the high rate of cardiovascular mortality associated to maintenance hemodialysis (MHD) patients. VC in large arteries of MHD was associated with increased pulse wave velocity (PWV). Recent studies suggest that systemic activation of VDRs may have direct effects on the cardiovascular system to decrease mortality in CKD.The aim of this study is to evaluate the effect of VDR activation on VC among MHD patients.

**Methods:** 156 patients with SHPT on chronic hemodialysis three times a week for at least 3 months were enrolled into the study. Patients were grouped according to type of VDRA: Group P (n:34) patients who recieve paricalcitol; Group C (n:71) patients who recieve calcitriol; Group PC (n:27) patients who recieve paricalcitol plus cinacalcet and Group CC (n:24) who recieve calcitriol plus cinacalcet. PWV were assessed at the beginning and end of the study.

**Results:** Mean PTH, P, Ca and ALP values were significantly higher in group PC and CC compared to group P and C ($p<0.001$). In paricalcitol based treatment groups (Group P and Group PC) percent change in PWV measurement was significantly decreased compared to baseline values ($p<0.001$). When the whole patient group was concerned, mean administerd VDRA dose / PTH ratio and mean administered VDRA dose (mcg/week) were negatively

correlated change of PWV during follow-up period (r:.-312;p<0.001 and r:.-314; p<0.001). According to logistic regression analysis being in calcitriol based treatment group and using lower doses of VDRA for PTH supression were independent risks for the PWV increment.

**Conclusions:** This observational study showed that the importance of vitamin D supplementation with VDRA intensity and maintainability in the management of cardiovascular disease in end-stage renal disease patients. Additionally higher doses of adjusted VDRA for PTH could be protective on vascular calcification.

## FR-PO683

**The PARADIGM Trial: Impact of Dialysate Calcium and Calcium Based Binders** James B. Wetmore,[1] Konstantin Gurevich,[2] Stuart M. Sprague,[3] Gerald A. Da Roza,[4] John E. Buerkert,[5] Maureen T. Reiner,[6] William G. Goodman,[6] Kerry Cooper.[6] *[1]Hennepin County Medical Center; [2]Fresenius Medical Care Russia; [3]North Shore U HealthSystem; [4]U British Columbia; [5]Columbia Nephrology Associates; [6]Amgen Inc.*

**Background:** The PARADIGM trial compared parathyroid hormone (PTH) levels during monotherapy with cinacalcet (Cin) or vitamin D (vitD) in patients on hemodialysis with secondary hyperparathyroidism (SHPT). PTH reduction did not differ between study arms (-12% Cin vs -7% vitD, P = 0.346).

**Methods:** Multivariate analysis revealed a large treatment by region interaction (US vs non-US, p <0.001). Practice patterns differed in regards to use of low (<2.5 mEq/L) dialysate calcium concentrations (DCC) and calcium containing phosphate binders (CB). Low DCC use was limited to US patients, while non US patients were more likely to be on CB. Overall, 33% of all study patients were on low DCC and 43% were on CB. In this post-hoc analysis, PTH reductions were stratified by DCC (≥2.5 vs <2.5 mEq/L) and by CB use.

**Results:** Patients treated with DCC ≥2.5 mEq/L had nominally greater PTH reduction while on Cin compared to vitD (P =0.042). Conversely, with DCC <2.5 mEq/L, there was no difference in PTH reduction. Cin dose did not vary according to DCC, while vitD dose was higher in patients on DCC<2.5 mEq/L; although, this did not result in greater PTH reduction. Cin treated patients also had greater PTH reduction than patients on vitD when on CB, but there was no difference in PTH reduction between arms when not on CB.

**Conclusions:** When used as monotherapy, the efficacy of Cin in reducing PTH levels compared to vitD may depend, in part, on the DCC and the choice of binder type.

| 1. Results Stratified by Dialysate Calcium | Dialysate Calcium ≥2.5 mEq/L | | | Dialysate Calcium <2.5 mEq/L | | |
|---|---|---|---|---|---|---|
| | Cin (N = 101) | vitD (N = 109) | Treatment Difference | Cin (N=50) | vitD (N=50) | Treatment Difference |
| Mean (SE) % change in PTH | -18.9% (4.6) | -6.0% (4.6) | -12.9% (P=0.042) | 4.8% (13.1) | -7.6% (13.1) | 12.4% (P=0.202) |
| Median dosing during EAP | 60.0[a] | 15.0[b] | — | 73.6[a] | 24.0[b] | — |
| | Calcium Based Phosphate Binder - Used | | | Calcium Based Phosphate Binder – Not Used | | |
| 2. Results Stratified by Binder Use | Cin (N = 61) | vitD (N = 73) | Treatment Difference | Cin (N = 94) | vitD (N = 84) | Treatment Difference |
| Mean (SE) % change in PTH | -16.1 (16.6) | 1.2 (6.0) | -14.8% (P = 0.045) | -9.3 (5.2) | -14.3 (5.5) | 5.0% (P = 0.45) |

a. *Cinacalcet dose in mg/day; b. Vitamin D dose in Paricalcitol equivalent units/week*

*Funding:* Pharmaceutical Company Support - Amgen Inc.

## FR-PO684

**Whole Exome Resequencing and Homozygosity Mapping Identify Mutation of ARHGAP4 as a Novel Single-Gene Cause of Nephrotic Syndrome** Carolin E. Sadowski,[1] Svjetlana Lovric,[1] Shazia Ashraf,[1] Humphrey Fang,[1] Virginia Vega-Warner,[2] Stefanie Weber,[4] Neveen Soliman,[5] Heon Yung Gee,[1] Friedhelm Hildebrandt.[1,3] *[1]Div of Nephrology, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Dept of Pediatrics, Univ of Michigan, Ann Arbor, MI; [3]Howard Hughes Medical Institute, Chevy Chase, MD; [4]Dept of Pediatrics, Univ of Essen, Essen, Germany; [5]Dept of Pediatrics, Kasr Al Ainy School of Medicine; Center of Pediatric Nephrology and Transplantation, Cairo Univ; Egyptian Group for Orphan Renal Diseases, Cairo, Egypt.*

**Background:** Idiopathic nephrotic syndrome (NS) represents a heterogeneous group of glomerular disorders occurring mainly in children. 20% percent of the cases show primary steroid resistance with a 30% risk of relapse in a kidney transplant. Identification of single-gene causes of SRNS has furthered the understanding of its pathogenesis. However, additional genes and disease mechanisms remain unknown.

**Methods:** We performed whole exome resequencing (WER) combined with homozygosity mapping in 43 sib pairs with steroid resistant nephrotic syndrome (SRNS). To identify additional families we screened our worldwide cohort of ~800 individuals with severe SRNS by an array based multiplex PCR (Fluidigm Access Array™) and next generation resequencing (NGS).

**Results:** By WER in two affected male sibs and their affected maternal grandfather, we detected a hemizygous missense mutation (p.A425V) in the gene *ARHGAP4* (Rho GTPase activating protein 4), which resides on the X chromosome. The mutation segregates in the family and was absent from 10,563 control individuals in the Exome Variant Server (EVS). After screening our worldwide cohort of 800 children with severe SRNS by multiplex PCR and NGS we identified another family with an affected son with a hemizygous missense mutation (p.R115W), which also was absent from the EVS database.

**Conclusions:** We identify mutations of *ARHGAP4* as new cause of NS. The identification of novel genes may shed light on the pathogenesis of NS.

*Funding:* Other NIH Support - DK076683

## FR-PO685

**Identification of GLCCI1 and DDX53 as Novel Genes Mutated in Steroid Resistant Nephrotic Syndrome** Shazia Ashraf,[1] Svjetlana Lovric,[1] Carolin E. Sadowski,[1] Virginia Vega-Warner,[2] Heon Yung Gee,[1] Humphrey Fang,[1] Friedhelm Hildebrandt.[1,3] *[1]Div of Nephrology, Boston Childrens' Hospital, Harvard Medical School, Boston, MA; [2]Dept of Pediatrics, Univ of Michigan, Ann Arbor, MI; [3]Howard Hughes Medical Institute, Chevy Chase, MD.*

**Background:** Steroid-resistant nephrotic syndrome (SRNS) is a malfunction of the kidney glomerular filter that leads to proteinuria, hypoalbuminemia, edema, and renal failure. Identification of single-gene causes of SRNS has furthered the understanding of its pathogenesis. However, additional genes and disease mechanisms remain obscure.

**Methods:** To identify a new causative gene for SRNS, we performed array based multiplex PCR (Fluidigm Access Array™) and next generation resequencing (1) of 25 candidate genes to screen worldwide cohort of ~800 individuals with severe SRNS as well as whole exome resequencing (WER) in 30 sib-pairs with SRNS.

**Results:** By multiplex PCR and next-generation resequencing, we detected a homozygous missense mutation (p.F453L) in the *GLCCI1* (glucocorticoid induced transcript 1) gene in a consanguineous family with two affected siblings. The amino acid residue F453 has been continually conserved since *Ciona intestinalis*. *GLCCI1* was regarded as a candidate that may cause SRNS as it has been previously shown that Glcci1 protein is highly expressed in mouse glomeruli and also the knockdown of this gene in zebrafish can result in the morphants with collapsed glomeruli with foot-process effacement (2). Furthermore, by WER of two affected male siblings, we identified a homozygous nonsense mutation (W8X) in the intronless gene *DDX53* on X chromosome. The mutations segregated with the affected status in their families. We are now screening ~800 unrelated SRNS individuals for the coding exon(s) of *GLCCI1* and *DDX53* genes to detect additional mutations in these genes.

**Conclusions:** We identified mutations of *GLCCI1* and *DDX53* genes as new causes of SRNS. The identification of novel genes for SRNS provides new steps toward our understanding of the pathomechanisms of SRNS.

(1) Halbritter J et al, J Med Genet, 2012, 49: 756-767; (2) Nishibori Y et al, J Am Soc Nephrol, 2011, 22: 2037-2046.

## FR-PO686

**Next Generation Sequencing of 37 Genes Associated with Steroid Resistant Nephrotic Syndrome** Agnieszka Bierzynska, Hugh J. McCarthy, Ian N. Day, Gavin Iain Welsh, Moin Saleem. *Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom.*

**Background:** Up to 40% of children presenting with Steroid Resistant Nephrotic Syndrome (SRNS) in early life will have a pathogenic single gene mutation in one of 37 genes currently associated with this disease. Little is known of the effect of polymorphic variants. We followed up our previous findings that the increasing reliability of Next Generation Sequencing (NGS) provides the potential for revolutionising genetic investigation of this and similar patient groups.

**Methods:** We used exome sequencing to screen 100 paediatric SRNS patients for genes known to be associated with hereditary SRNS as well as to look for novel polymorphic variants in other genes, potentially involved in the disease. Patients were collected via a national UK Renal Registry with comprehensive detail of phenotype. Significant variants detected by NGS were confirmed by conventional Sanger sequencing.

**Results:** 9% of the patients had a previously described mutation and another 15% had a variant likely to be disease causing. Analysis revealed known as well as novel disease associated variations in routinely tested genes such as *NPHS1*, *NPHS2* and *WT1* as well as in other, less common, genes such as *SMARCAL1* or *MYO1E*. A phenotypically unexpected mutation was *COL4A5* (homozygous missense) in a patient without hearing loss. Increased burden of R229Q (*NPHS2*), R310Q (*ACTN4*) as well as other rare variants were noted in known SRNS genes in cases versus controls.

**Conclusions:** Our results demonstrate the obvious clinical need for sequencing multiple genes in SRNS where genetic heterogeneity is a defining feature. We identified mutations and potentially pathogenic variants in genes that would not routinely be screened under current testing practice. Our detailed phenotypic information enabled us as well to identify potential modifier variants from this cohort.

## FR-PO687

**Renal Cysts in Thin Basement Membrane Disease, a Marker of Progression?** Angel M. Sevillano, Maria Molina, Enrique Morales, Esther Gonzalez Monte, Natalia I. Polanco fernandez, Manuel Praga. *Nephrology, H.U 12 de Octubre, Madrid, Spain.*

**Background:** Thin basement membrane disease (TBMD) is thought to be an entity with a good renal prognosis. However proteinuria is found in 50% of these patients and in some cases it is accompanied by renal function impairment. Some studies have reported simple renal cysts in proteinuric TBMD.

**Methods:** Retrospective study comparing the characteristics and evolution of three group of patients (n=16 in all of them): 1)Biopsy-proven TBMD and proteinuria >0,5g/24 h (TBMDP), 2) Biopsy-proven TBMD and no proteinuria (TBMDnoP) and 3) Biopsy-proven IgA nephropathy and proteinuria >0,5g/24 h (IgAN).

**Results:** Main results are shown in the table below.

| | TBMDP | TBMDnoP | IgAN |
|---|---|---|---|
| Age | 35,12±17,31 | 19,57±13,34 | 41,90±11,12 |
| Gender | 10♂/6♀ | 6♀/10♂ | 7♀/9♂ |
| Time of follow up | 198 months | 210 months | 206 months |
| Initial Creatinine | 1,06 ±0,35 | 0,80± 0,16 | 1,19 ± 0,38 |
| Initial Proteinuria | 0,74 (0,05-1,58) | 0,00 (0,00-0,02) | 1,08 (0,73-1,57) |
| Final Creatinine | 1,31±0,71 | 0,85±0,15 | 1,71±0,94 |
| Final Proteinuria | 0,42(0,27-1,7) | 0,00(0,00-0,02) | 0,41(0,30-0,72) |
| Cysts | 9(56,25 %) | 0 | 2(12,5%) |

Every patient with proteinuria >0,5g/24h was treated with RAAS blockers with good results. The most striking finding was the presence of simple renal cysts in 9 (56%) TBMDP patients, whereas only one IgAN patient and nobody in the TBMDnoP group presented cysts. The cysts were multiples and bilateral in TBMDP.

**Conclusions:** The presence of bilateral simple renal cysts is common among biopsy-proven TBMD patients who develop proteinuria. This finding could help to orientate the diagnosis in patients with persistent hematuria and proteinuria. The pathogenesis of cyst formation in TBMD with proteinuria is presently unknown.

## FR-PO688

**NPHS2 V260E Is a Frequent Mutation among Unrelated Black South African Children with Steroid Resistant Focal Segmental Glomerulosclerosis** Cheryl Ann Winkler,[1] Rajendra Bhimma,[2] Kareshma Asharam,[2] Sophie Limou,[1] Jeffrey B. Kopp,[3] Victor A. David,[4] George W. Nelson.[5] *[1]Basic Research Laboratory, Center for Cancer Research, Frederick National Laboratory, SAIC-Frederick, Frederick, MD; [2]Nelson Mandela School of Medicine, Univ of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa; [3]NIDDK, NIH, Bethesda, MD; [4]Basic Research Laboratory, NCI, NIH, Frederick, MD; [5]BCGC, Frederick National Laboratory, Frederick, MD.*

**Background:** The incidence and histologic patterns of nephrotic syndrome (NS) vary with geographic location and ethnicity. We sought to determine the roles of *APOL1* and *NPHS2* variants in sporadic steroid resistant (SR) and steroid sensitive (SS) NS in children from Durban, South Africa.

**Methods:** We sequenced *NPHS2* exons and genotyped *APOL1* G1 and G2 in unrelated Indian and Black African children (median age 90 months, range 1-169) with sporadic SRNS (N=60) and SSNS (n=19), and in 116 Indian and Black controls. Kidney biopsies were available for all SRNS cases. FSGS was present in 30/38 (79%) Black and 16/22 (73%) Indian children with SRNS.

**Results:** Steroid resistance was more common in Blacks (97.4%) compared to Indians (55%) with NS ($p=8 \times 10^{-6}$). Heterozygosity in SRNS cases was noted for *NPHS2* A61V, R229Q, and A242V. *NPHS2* V260E was present in the homozygous state in 7 of 23 (30%) Black SR-FSGS (FET=0.0004) and in 1/2 SRNS cases with mesangial proliferative GN, and in no controls. V260E was observed in the heterozygous state in one Black African control (allele freq.=0.8%; 95% CI [0.02-4%]), but not in Indian cases or controls. *APOL1* G1 and G2 frequencies were not significantly different between Black SRNS cases (18.5%) and controls (19.2%) (p>0.05) nor was there significant association between 2 *APOL1* risk alleles and SRNS (p=0.22). No significant associations with *NPHS2* or *APOL1* were found for SSNS in Black or Indian children.

**Conclusions:** *NPHS2* V260E is a significant risk factor for SR-FSGS in unrelated, Black African children; the variant may be polymorphic accounting for the high rate of SR-FSGS among children in this Black African population. Further data will be required to determine population frequency distributions and history of this variant in Africa.

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## FR-PO689

**Genetic and Acquired Abnormalities in the Alternative Complement Pathway in Patients with C3 Glomerulopathy** Dineke Westra,[1] Lambert Van den Heuvel,[1] Jack F. Wetzels,[2] Nicole Van De Kar.[1,3] *[1]Pediatric Nephrology, Radboud Univ Medical Centre, Nijmegen; [2]Nephrology, Radboud Univ Medical Centre, Nijmegen, Netherlands; [3]On behalf of the Dutch Pediatric C3 Glomerulopathy Working Group, Netherlands.*

**Background:** Glomerular pathologies that are characterized by the isolated deposition of C3 are nowadays called C3 glomerulopathies (C3G). These pathologies include DDD and C3 glomerulonephritis, but also immunoglobulin-negative MPGN I and III. It is thought that C3G can be caused by systemic dysregulation of the alternative and terminal complement pathway. Here we report a cohort of 29 C3G patients (all children at time of diagnosis) who have been screened for abnormalities in genes encoding (regulating) proteins of the alternative pathway.

**Methods:** In 29 biopsy proven C3G patients, mutational screening was performed of the alternative pathway genes *CFH*, *CFI*, *MCP*, *CFHR5*, *C3*, and *CFB* by means of PCR on genomic DNA and sequence analysis. For 22 of these patients, serum was available for screening for autoantibodies against CFH.

**Results:** In three patients (3/29; 10.3%) a potentially pathogenic genetic aberration was identified in *CFH*; one of these patients carried a variation in *C3* as well. All sequence variations in *CFH* are located in domains involved in heparin binding (SCR7 and SCR20); two of these have been previously described in aHUS patients. The sequence variation in *C3* is involved in properdin binding and could lead to increased affinity of CFB for C3. In two other patients (2/22; 9.1%), autoantibodies against CFH, associated with dysregulation of the complement system as well, were identified.

**Conclusions:** In 5/29 (17.2%) of the patients with C3G, abnormalities in complement genes were found, either consisting of a genetic aberration in one of the screened genes or the presence of autoantibodies against CFH. These data indicate that dysregulation of the alternative complement pathway is involved in the pathogenesis of C3 glomerulopathy. More research is needed to understand the clinical observed complement activation in C3G.

## FR-PO690

**Atypical Hemolytic Uremic Syndrome and the Application of Whole Exome Sequencing** Dineke Westra, Elena Volokhina, Lambert Van den Heuvel, Nicole Van De Kar. *Pediatric Nephrology, Radboud Univ Medical Centre, Nijmegen, Netherlands.*

**Background:** Atypical hemolytic uremic syndrome (aHUS) is a rare, but severe disease that has a heterogeneous genetic background. Mutations in several genes encoding proteins of the alternative complement pathway have been associated with the disorder. Due to the heterogeneity seen in aHUS, making the genetic diagnosis via regular methods is labor intensive and time consuming. Therefore, whole exome sequencing (WES), in which the coding regions of the entire genome can be studied, might be an option for mutation detection in aHUS.

**Methods:** Exomes of ten patients diagnosed with familial aHUS were captured and enriched using the 50MB Agilent's SureSelect human exome enrichment kit (Agilent, Santa Clara, CA, USA). One of these patients, with an identified *CFI* mutation, was taken along as a positive control.

**Results:** More than 47000 sequence variations were identified per patient. On average, ~230 of these were false substitutions located in an exon or canonical splice site, all absent in the dbSNP database or present in less than 1% of our in-house database. Overall coverage of associated genes was 86%, but SCR19 and 20 in *CFH* were not covered. The *CFI* mutation in one patient was detected. Potential pathogenicity of other identified variations will be further displayed.

**Conclusions:** The hotspot for mutations in aHUS patients (SCR19-20 in *CFH*) was not covered with the used enrichment kit. Therefore, at this moment with this specific method, WES might not be the best option for genetic screening in patients with familial aHUS.

## FR-PO691

**A Disease-Causing Mutation of INF2 Impairs the Integrity of Podocyte Slit Diaphragm in Protamine Induced Kidney Injury** Hua Sun, Victoria Charoonratana, Martin R. Pollak. *Nephrology, Beth-Israel Deaconess Medical Center, Boston, MA.*

**Background:** Rho GTPases mediated actin dynamics determine the morphology of podocyte and the integrity of slit diaphragm. Our previous work established that INF2 interacts with mDia formin protein activated by Rho GTPase and restricts the overactivation of Rho/mDia mediated actin polymerization. By doing so, INF2 regulates the cortical actin architecture responsible for the formation of lamellipodia and the trafficking of slit diaphragm proteins in cultured podocytes. R218Q, an FSGS-causing mutation in INF2, has been shown to disassociate INF2 from mDia and leads to uncontrolled Rho/mDia signaling. Here we show that this INF2-R218Q mutation impairs the morphology and integrity of slit diaphragm in protamine induced podocyte injury in a transgenic mouse model.

**Methods:** An INF2 R218Q point-mutant "knock-in" mouse model was generated. 6-month-old *INF2 wt/wt,* littermate *wt/ ki* and *ki/ki* mice were perfused with protamine sulfate (2 mg/ml in HBSS) X15 min, followed by perfusion with heparin sulfate (800 μg/ml in HBSS) X15 min. The ultra structural features of mouse kidney were examined by transmission electron microscopy. The distribution of slit diaphragm proteins was examined by immunofluorescent stain and immunogold labeling.

**Results:** Neither *wt/ki* nor *ki/ki* mice showed any significant proteinuria or altered glomerular histology up to 12 months of age compared to *wt/wt* animals. In the INF2 R218Q transgenic mouse model, protamine sulfate perfusion of the knock-in mouse (*wt/ki* and *ki/ki*) induced broadened foot process effacement with prominent intracellular actin flattening, accompanied by the mislocation of slit diaphragm proteins. The mutant podocyte (*wt/ki* and *ki/ki*) did not recover normal structure and trafficking of slit diaphragm proteins following additional perfusion with heparin sulfate, as observed in *wt/wt* mouse kidney.

**Conclusions:** Mice carrying INF2 R218Q mutation demonstrate impaired recovery from protamine induced podocyte injury, suggesting the mDia-antagonizing activity of INF2 in the maintenance of normal structure and function of podocyte. This animal model will be useful to address potential mechanisms of INF2-mediated disease *in vivo*.

*Funding:* NIDDK Support

## FR-PO692

**The Copy Number Variation of FCGR3A Affects Penetrance of Lipoprotein Glomerulopathy** Zhangxue Hu,[1] Maolu Tian,[1] Xiaoxia Liu,[1] Ye Tao,[1] Ping Fu,[1] Yuan Yang.[2] *[1]Dept of Nephrology; [2]Dept of Medical Genetics.*

**Background:** Lipoprotein glomerulopathy(LPG) is a rare hereditary disorder mostly found in east Asia. Our previous work and related literatures have demonstrated that LPG is a dominant inherited disease with incomplete penetrance. However, the mechanism of incomplete penetrance is still unclear. Fcγ receptors play an important role on the pathogenesis of LPG based on evidences from animal studies. The copy number variation (CNV) of Fcgr3 predisposes to glomerulonephritis in rats and humans. We supposed that the CNV affects penetrance of LPG.

**Methods:** LPG patients were diagnosed by renal biopsy, and the APOE mutation was detected by DNA sequencing. Asymptomatic carriers were defined as relatives carrying the identical APOE mutant, with urine protein less than 300 mg/d, eGFR over 60ml/min, and no active urine sediments. Between patients and carriers, the copy numbers of FCGR3A, FCGR3B and FCER1G were detected and analyzed by QRT-PCR ,ABI copycaller Software v2.0 and SPSS.

**Results:** 28 LPG patients and 26 asymptomatic carriers with APOE Kyoto were enrolled. All come from the same county. The average ages and gender distributions between two groups had no statistical difference. In the 54 APOE Kyoto individuals, the frequency of CNV of FCGR3A was 63%, higher than that of general population in China. The frequency of CNV of FCGR3A was higher in asymptomatic carriers than patients (76.9% vs. 50%, p=0.041). The frequency of high FCGR3A copy number(CN=3,4,5)was higher in carriers, compared with patients (73.1% vs. 42.9%, p=0.025). For FCGR3B, no CNV difference was observed between two groups(34.6% vs. 21.4%, p=0.171).

**Conclusions:** APOE Kyoto individuals with low or moderate FCGR3A copy numbers (CN=1,2) may be more susceptible to clinical onset of LPG. The high copy numbers may have renoprotective effect in APOE Kyoto carriers. In our study, the frequency of CNV of FCGR3A are higher than that of other Chinese populations. Because these families all come from the same county, which is fairly isolated, the high frequency of CNV of FCGR3A may prevail in this county.

## FR-PO693

**Exome Sequencing and In Vitro Studies Identify Podocalyxin as a Candidate FSGS Gene** Moumita Barua,[1] Eric Shieh,[2] Johannes S. Schlondorff,[1] Giulio Genovese,[3] Bernard S. Kaplan,[4] Martin R. Pollak.[1] *[1]Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Harvard Medical School, Harvard Univ, Boston, MA; [3]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; [4]Dept of Pediatrics, The Children's Hospital of Philadelphia, Philadephia, PA.*

**Background:** The integral membrane protein podocalyxin (PODXL) has been shown to play a critical role for the function of podocytes in rodents, possibly by preserving slit diaphragm patency by its negative charge repulsion. However, whether disruption of PODXL function can lead to human disease has not been previously reported. Exome sequencing in a FSGS family revealed a suspicious *PODXL* variant and we explored its effect on biochemical function.

**Methods:** Exome sequencing was performed in 2 affected cousins of a family with dominant inherited FSGS. Sequencing in 175 probands of a familial FSGS cohort revealed additional rare variants. Biochemical assays comparing expression levels, extracellular domain glycosylation, subcellular localization by immunofluorescence and confocal microscopy, and interaction with a binding partner ezrin between wildtype and rare variant forms of PODXL were performed.

**Results:** A cosegregating private variant, PODXL p.L442R, affecting the transmembrane region was identified in the index family. In comparison to wildtype, this PODXL variant p.L442R facilitates dimerization, although we were unable to establish an effect of this biochemical change on its function. Specifically, the mutation does not alter protein stability, extracellular domain glycosylation, trafficking to the cell surface, global subcellular localization, or interaction with ezrin. Comparison of these parameters between wildtype and other rare PODXL variants identified in the familial FSGS cohort revealed no differences.

**Conclusions:** A variant form of PODXL, p.L442R, is a candidate for causing disease in a FSGS family but its full effect on protein function remains unknown. Our work highlights the challenge faced in the clinical interpretation of exome data for small pedigrees with autosomal dominant diseases.

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## FR-PO694

**Natural History and Protein Expression Pattern in Autosomal Recessive Alport Syndrome Based on the Comprehensive Strategy for Genetic Analysis** Hiroshi Kaito,[1] Kandai Nozu,[1] Masafumi Oka,[2] Naoya Morisada,[1] Takeshi Ninchoji,[1] Koichi Nakanishi,[3] Norishige Yoshikawa,[3] Kazumoto Iijima.[1] *[1]Kobe Univ Graduate School of Medicine, Japan; [2]Faculty of Medicine, Saga Univ, Japan; [3]Wakayama Medical Univ, Japan.*

**Background:** Autosomal recessive Alport syndrome (ARAS) is a hereditary disorder caused by mutations in *COL4A3* or *COL4A4*. The most serious problem in the diagnosis of ARAS has been the low mutation detection rate. Moreover, some ARAS cannot be successfully-diagnosed even if detailed pathological examination is completely performed. It is the urgent problem to establish the method of genetic diagnosis and to clarify the natural history of genetically-proven ARAS.

**Methods:** We retrospectively analyzed 29 patients in 23 pedigrees suspected of ARAS with family history and/or kidney biopsy findings. We utilized and proceeded the following 3 steps for mutation detection: (1) genomic DNA analysis; (2) mRNA analysis to detect a splicing abnormality; and (3) semiquantitative PCR analysis to detect a heterozygous large deletion. Rat monoclonal antibodies that recognize type IV collagen α3, α4, and α5 were used for immunohistochemical staining.

**Results:** The mean age was 17.9±8.2 years old. Homozygous or compound heterozygous mutations could be detected in all cases. Four cases in 3 pedigrees had splicing abnormalities and one had a large deletion. Immunohistochemical staining was conducted in 19 patients, and 4 of them (21%) showed completely normal expression of α5 staining. Two with normal expression of α5 showed normal of α3 and α4, on the other hand, two with negative expression of α5 in glomerular basement membrane is also negative of α3 and α4. The median age of end-stage kidney disease was 20 years old.

**Conclusions:** We could successfully detect disease-causing mutations in all cases, and this is the largest-scaled report on patients with genetically-proven ARAS. It is of great help to use our strategy for genetic analyses of ARAS. We could also first elucidate the protein expression pattern of type IV collagen for ARAS, including α3 and α4. Moreover, prognosis for kidney function with ARAS may be severer than that of X-linked.

*Funding:* Government Support - Non-U.S.

## FR-PO695

**Urinary Connective Tissue Growth Factor in Boys with Alport Syndrome** Clifford E. Kashtan, Matthew Wroblewski, Kim Y. Nguyen, Theresa F. Cassidy, Gary L. Nelsestuen. *Univ of Minnesota, Minneapolis, MN.*

**Background:** Alport syndrome (AS) is an inherited renal disease that leads to ESRD in all affected males. Studies of murine, canine and human AS showed that tubular injury and interstitial fibrosis are important contributors to renal disease progression in AS. Connective tissue growth factor (CTGF) has been implicated as a mediator of renal fibrosis and a marker of proximal tubular dysfunction in diabetic and non-diabetic kidney disease but has not been assessed in AS. We examined urinary CTGF in boys with early untreated AS.

**Methods:** 64 AM urine samples were collected over 3 years from 35 untreated boys (< 18 yrs) with AS. Creatinine (Cr), albumin (Alb) and total protein (Pr) concentrations were measured in the UMMC Clinical Laboratory. Samples were classified as Normalbuminuric (*NA,* Alb/Cr < 30 mg/g, N=35), Microalbuminuric (*MA,* Alb/Cr > 30, Pr/Cr < 0.2, N=15) or Proteinuric (*Prot,* Pr/Cr > 0.2, N=14). CTGF concentrations were determined by ELISA (Omnikine Human CTGF ELISA Kit, Assay Biotech). CTGF/Cr (ng/mg) was calculated for each sample. The study protocol was approved by the U. of Minnesota IRB.

**Results:** CTGF/Cr was higher in *Prot* samples (mean +/- SD 0.868 +/- .833) than in *NA* (0.365 +/- 0.631) and *MA* (0.499 +/-.616) samples (ANOVA, $p = 0.037$; *Prot* vs *NA*, $p = 0.013$ [*t*-test]). Linear regression showed positive correlations between Alb/Cr and CTGF/Cr ($R = 0.254$, $p = 0.043$) and between Prot/Cr and CTGF/Cr ($R = 0.294$, $p = 0.049$). CTGF/Cr increased in 7/11 serially sampled subjects who advanced from *NA* or *MA* to a higher stage (*MA* or *Prot).*

**Conclusions:** In untreated boys with AS, urinary CTGF excretion is higher in those with microalbuminuria or overt proteinuria than in those who are normalbuminuric. In normal subjects filtered CTGF is reabsorbed in the proximal tubule by megalin-mediated endocytosis. Increased urine CTGF levels in boys with AS may be secondary to altered megalin-mediated endocytosis. It is also possible that increased urine CTGF levels result from increased renal production. Further investigation of CTGF as a marker and potential mediator of renal disease progression and as a possible target of clinical intervention in AS is warranted.

*Funding:* Private Foundation Support

## FR-PO696

**Breakdown of the GBM Ultrastructural Organization in Pierson and Alport Syndromes** Hani Suleiman,[1] Lei Zhang,[1] Adish Dani,[1] Jeffrey H. Miner,[2] Andrey S. Shaw.[1] *[1]Pathology & Immunology, Washington Univ, Saint Louis, MO; [2]Renal Div, Washington Univ, Saint Louis, MO.*

**Background:** The glomerular filtration barrier is composed of three layers: fenestrated endothelium, glomerular basement membrane (GBM), and podocyte foot processes. Numerous mutations, primarily in podocyte genes, cause a dysfunctional filtration barrier leading to proteinuria. Two such mutations, in *LAMB2* and *COL4A3*, affect the GBM and lead to proteinuria and end stage renal failure in Pierson and Alport syndrome, respectively.

**Methods:** We used sub-diffraction resolution stochastic optical reconstruction microscopy (STORM) with a GBM molecular reference frame to identify nanoscale changes in protein localization associated with a defective GBM. Two mouse models were used for this study, *Lamb2* and *Col4a3* null, and they were compared with WT as well as with the *Cd2ap* null.

**Results:** In stark contrast with WT and *Cd2ap* KO mouse kidney, the GBM in both *Lamb2* KO and *Col4a3* KO mice manifested a breakdown of the highly organized molecular architecture. Two agrin layers observed by STORM in the healthy GBM were disrupted in both disease models. In the *Lamβ2* KO, collagen α3α4α5(IV) appeared increased in irregularly thick GBM areas. On the other hand, *Col4a3* KO mice showed changes in laminin-521 positioning as well as in collagen α1α1α2(IV) localization when compared to WT. Using agrin as a reference, collagen α1α1α2(IV) shifted from its normal position near the endothelium to sites across the thickness of the GBM. Using STORM to analyze transgenic mice expressing laminin β1 in the *Lamb2* KO background and collagen a3(IV) in the Col4a3 KO background, we observed restoration of the GBM's molecular architecture to normal, which correlated with reduced proteinuria.

**Conclusions:** STORM can detect nanoscale changes in the molecular architecture of the GBM in disease models that correlated with disease progression. Further analyses of GBM architecture in Pierson, Alport, and other syndromes could reveal novel targets for therapies aimed at restoring the architecture of the GBM and the integrity of the filtration barrier.

## FR-PO697

**Identification of the Pathologic Role of miR-21 in Alport's Kidney Disease** Joseph H. Boulanger,[1] Wenping Song,[1] Jie Zhang,[1] Shweta Pandya,[2] Deidre Mackenna,[2] Lucy A. Phillips,[1] Tai-he Xia,[1] Jike Cui,[1] Lisa M. Potestio,[1] Xiaohong Cao,[1] Rachel Yabkowitz,[1] Steven R. Ledbetter,[1] Susan C. Schiavi,[1] Shiguang Liu.[1] *[1]Genzyme R &D Center, Sanofi, Framingham, MA; [2]Regulus Therapeutics, San Diego, CA.*

**Background:** Alport's syndrome (AS) is a genetic disorder resulting in progressive CKD. Anti-miR21 has been shown to mitigate CKD progression in *Col4a3-/-* mice on 129/SvJ background.The pathologic role of miR-21 in AS and the mechanism of action of anti-miR21 are currently investigated.

**Methods:** F1-hybrid (B6129SF1) *Col4a3-/-* mice generated by crossing female C57Bl6/J *Col4a3+/-* with male 129/SvJ *Col4a3+/-* were characterized by assessing urine albumin, kidney function, pathology, and gene expression at 4, 6, 9, 12 and 15 wks of age. Anti-miR21 efficacy was assessed in the F1 *Col4a3-/-*mice, dosed subcutaneously at 25 mg/kg twice a week starting at 5 wks of age. Urine albumin, renal function and kidney pathology were evaluated.

**Results:** Kidney disease progression of B6129SF1 *Col4a3-/-* mice is significantly slower than that of 129/SvJ *Col4a3-/-* mice. Proteinuria begins at 4 wks, progressive azotemia starts at 9 wks and animals die from renal failure at 17 wks. Progressive glomerular and tubular pathology, interstitial inflammation and fibrosis resembles human AS. Gene expression changes are consistent with the pathology changes. MiR-21 expression is progressively increased in the kidney of *Col4a3-/-* mice. A similar magnitude change in miR-21 expression (~ 2 fold) is observed between tubules and glomeruli at week 9, although the baseline level in tubules is 6 fold higher than that in glomeruli. Anti-miR21 treatment significantly slows the kidney disease progression measured by renal function and pathology in the B6129JF1 *Col4a3-/-* model. Gene expression profiling by RNA seq analysis in the kidney of *Col4a3-/-* mice after anti-miR-21 treatment will be discussed.

**Conclusions:** B6129SF1 *Col4a3-/-* model has a slower disease progression and therefore may be better suited to define therapeutic window. MiR-21 plays an important role in the disease progression of AS. Anti-miR-21 significantly improved both renal function and pathological changes, and therefore may be an important new therapy for AS.

## FR-PO698

**Maculopathy Is a Novel Retinal Manifestation of X-Linked and Autosomal Recessive Alport Syndrome** Judith A. Savige, Deb J. Colville. *Medicine (Royal Melbourne Hospital), The Univ of Melbourne, Melbourne, Victoria, Australia.*

**Background:** Alport syndrome is an inherited form of progressive renal failure associated with hearing loss, corneal dystrophy, lenticonus, and perimacular or peripheral retinopathy. We describe here four women with X-linked or autosomal recessive Alport syndrome and retinal maculopathy.

**Methods:** All four individuals were examined by a renal physician for clinical features and by an ophthalmologist for anterior lenticonus using a hand-held retinoscope, and for retinal changes with direct ophthalmoscopy, slit lamp biomicroscopy with a 78D lens, and by indirect ophthalmoscopy with a 20D lens. They also underwent retinal photography (KOWA non-mydriatic camera, Japan), and optical coherence tomography (OCT), and abnormalities were examined for autofluorescence and with infrared and red-free manipulation.

**Results:** The women included a mother and daughter with p.Q379X in COL4A5 and another woman with presumed X-linked disease and an unknown mutation, as well as one with autosomal recessive disease (due to p.Q131X and p.G874X in COL4A3). All patients were all aged at least 40 years, three had renal impairment or proteinuria, three had a hearing loss, and all four had a peripheral coalescing retinopathy. None had central perimacular flecks. Their macular changes were obvious on fundoscopy and retinal imaging, and confirmed on autofluorescence and infrared manipulation. These changes included depigmentation, and pigmentation at the macula. On OCT, the retinal pigment epithelium was disrupted and thinned. The worst retinal findings were associated with visual loss.

**Conclusions:** The collagen IV a3a4a5 network is found in both the internal limiting membrane and Bruch's membrane in the retina. The perimacular retinopathy results mainly from a damaged internal limiting membrane and the maculopathy described here may be due to thinning of the retinal pigment epithelium of Bruch's membrane. This feature appears to be associated with more damaging mutations and more severe renal disease.

## FR-PO699

**The Genetic Evaluation of Thirteen Israeli Families with Alport Syndrome – Our Experience from the Nephrogenetic Clinic** Idit Maya,[1] Uzi Gafter,[2,5] Miriam Davidovits.[2,4] *[1]The Raphael Recanati Genetic Institute, Rabin Medical Center, Petah Tikva, Israel; [2]The Sackler Faculty of Medicine, Tel Aviv Univ, Tel Aviv, Israel; [3]Nephrology Dept, Schneider Children's Medical Center of Israel, Petah Tikva, Israel; [4]Nephrology Dept, Rabin Medical Center, Petah Tikva, Israel.*

**Background:** Alport syndrome (AS) is a collagen IV-related nephropathy caused by a mutation in *COL4A5* in 85% of cases, resulting in X-linked APS in hemizygous males, or in *COL4A3* or *COL4A4* in autosomal recessive (AR) or autosomal dominant (AD) APS. The most severely affected organs are the kidney, inner ear, and eye. There is progressive renal impairment with a mean age for end stage renal disease of 25 years, and renal replacement is required before the age of 40 in 90% of patients. Knowledge of inheritance has allowed early molecular diagnosis of patient with AS years or even decades before renal failure. After early molecular diagnosis of AS, recent retrospective observational data show that ACE-inhibitor therapy delays renal failure and improves life expectancy in AS with proteinuria.

**Methods:** We have opened a new Nephrogenetic clinic to enable the patients and families to achieve a Molecular Diagnosis evaluated for AS in Israel. In the past year 13 families with clinical diagnosis of AS have begun the molecular workup.

**Results:** Two families were diagnosed with X-linked AS by linkage analysis to *COL4A5* gene. Two families had AR AS, in one due to a homozygous mutation in *COL4A4* and in the other as a result of two mutations in *COL4A3* (compound heterozygous). In one of the family this workup enabled a kidney donation from a family member. In nine of the other families the molecular workup has been partially completed but no definite molecular diagnosis has been reached so far.

**Conclusions:** The molecular diagnosis of AS is a crucial step in the management of patients with a clinical suspicion of AS in order to enable initiation of Pre-implantation Genetic Diagnosis (PGD), molecular diagnosis in family members prior to kidney donation and consideration of early ACE-inhibitor therapy. A Nephrogenetic clinic is a necessary step in order to make the information and molecular diagnosis accessible to patients and families.

## FR-PO700

**Whole Exome Sequencing in Familial Kidney Disease** Daniel P. Gale,[1] Thomas Michael Connor,[1] Nadia Khan,[1] Duriye Deren Oygar,[3] Fujun Fiona Lin,[1] Guy H. Neild,[1] Michael A. Simpson,[4] Patrick H. Maxwell.[2] *[1]Div of Medicine, Univ College London, London, United Kingdom; [2]School of Clinical Medicine, Cambridge Univ, Cambridge, United Kingdom; [3]Nephrology Dept, Nicosia State Hospital, Nicosia, Cyprus; [4]Medical and Molecular Genetics, King's College London, London, United Kingdom.*

**Background:** In many patients the cause of kidney failure is unknown. In some, monogenic disorders are responsible, especially where there is a family history. We used exome sequencing in patients with unexplained familial kidney disease to identify the molecular defect responsible.

**Methods:** Exomes from 48 unrelated patients with a personal and family history of unexplained kidney disease were sequenced using an Illumina Genome Analyzer IIx. Variants were filtered if non-coding, synonymous or present in >0.5% of the 1000 genomes database. This yielded 400-1200 rare, non-synonymous coding or splice site variants per person. Literature and database searches identified likely disease-causing variants. Co-segregation of candidate mutations was ascertained by Sanger sequencing in relatives.

**Results:** Likely causative mutations in genes previously associated with monogenic kidney disease were found in 11 cases. All 11 co-segregated with disease. These variants were found in 9 (33%) of the 27 families with European ancestry but only 2 (9.5%) of the 21 families with non-European ancestry (p=0.05). In 8 further cases plausible candidate variants were identified in genes not previously associated with similar human disease. In the remaining 29 cases no likely causative mutation was identified, presumably owing to one of: causative variants missed or filtered by the exome sequencing strategy used; mutations in novel or unsuspected genes; or non-monogenic disease.

**Conclusions:** Index case exome sequencing is an effective first step in investigating Mendelian kidney disease, identifying the likely causative variant in >20% of cases in this study. In the remainder, coding mutations in known genes were excluded in a single step. The higher diagnostic yield in Europeans may result from either greater representation of European variants in the literature or lower background risk of non-Mendelian kidney disease.

*Funding:* Government Support - Non-U.S.

**FR-PO701**

**Potential Role of Angiotensin II Type I Receptor in TSC Renal Angiomyolipoma Pathogenesis** Brian J. Siroky,[1] Hong Yin,[2] Ryan J. Reichert,[1] Anna R. Hellmann,[1] Marlene Amjad Bunni,[3] Joshua C. Dillon,[3] P. Darwin Bell,[3] John J. Bissler.[1] *[1]Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [3]Medicine/Nephrology, Medical Univ of South Carolina, Charleston, SC.*

**Background:** Tuberous Sclerosis Complex (TSC) is a hamartomatous disease that is genetically linked to disruptions of *TSC1* or *TSC2*. Nearly 80% of TSC patients develop renal tumors called angiomyolipomas. These lesions are highly vascularized, and associated with aberrant mammalian target of rapamycin (mTOR) signaling. About 2% of TSC patients have a severe polycystic kidney phenotype, associated with deletions in the adjacent *TSC2* and *PKD1* on chromosome 16p13, which commonly produces hypertension. Clinical observation suggested that tumor development is also decelerated when hypertensive manifestations of TSC are managed with renin-angiotensin system (RAS) blockade.

**Methods:** We reviewed renal imaging from patients with the *TSC2-PKD1* deletion syndrome for presence of renal angiomyolipomas, and noted whether or not they had been treated with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs). We evaluated angiotensin II type 1 receptor ($AT_1R$) expression by immunohistochemistry in renal angiomyolipoma tissue from TSC patients. We also measured $AT_1R$ mRNA levels by qPCR in *TSC2*-deficient human renal angiomyolipoma cells (TRI102), and cells in which *TSC2* has been re-expressed (TRI103) with and without mTOR inhibition by RAD001 (20nM, 48hrs).

**Results:** Of 56 patients studied, 4/37 (10.8%) treated with ACE inhibitors or ARBs had angiomyolipomas, while 9/19 untreated patients (47.3%) had these lesions ($P<0.03$ Fisher Exact Test). We observed robust $AT_1R$ expression in TSC-associated angiomyolipoma tissue. In TRI102 cells, $AT_1R$ levels were nearly 10-fold higher than in TRI103 cells, and were markedly reduced by RAD001 treatment.

**Conclusions:** These data suggest that $AT_1R$ may be upregulated in TSC-associated renal angiomyolipoma. In addition, these findings support a possible role of $AT_1R$ signaling in TSC pathogenesis, and suggest the potential of utilizing RAS blockade for tumor management in TSC.

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support

**FR-PO702**

**Long-Term Enzyme Replacement Therapy Is Associated with Reduced Proteinuria and Preserved Proximal Tubular Function in Women with Fabry Disease** Thaneas Prabakaran,[1] Henrik Birn,[1,2] Rikke Nielsen,[1] Erik I. Christensen.[1] *[1]Dept of Biomedicine, Aarhus Univ, Aarhus, Denmark; [2]Dept of Nephrology, Aarhus Univ Hospital, Aarhus, Denmark.*

**Background:** Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the GLA gene. Deficiency of α-galactosidase A (α-Gal A) causes intracellular accumulations of globotriaosylceramide (GL-3) and related glycosphingolipids in all organs, including the kidney, often leading to end-stage renal failure. In women with Fabry disease, accumulation of GL-3 in the glomerular podocytes and other renal cells induces progressive, proteinuric nephropathy, but not as severe as in men. Enzyme replacement therapy (ERT) with recombinant α-Gal A reduces cellular GL-3 deposits in podocytes and tubular epithelial cells. We have previously shown that α-Gal A is delivered to these cells by different pathways involving different receptors. This study investigated the long-term changes in albuminuria, eGFR and urinary markers of both glomerular and tubular dysfunction in women with Fabry disease treated with ERT.

**Methods:** A retrospective, single center, cohort study evaluating the long-term association between ERT, albuminuria and eGFR in 13 women with Fabry disease and mild renal involvement. In particular, we analyzed changes in the proteinuric profile including the glomerular marker IgG, the tubular markers $\alpha_1$-microglobulin and retinol-binding protein, and the shared tubular and glomerular markers albumin and transferrin.

**Results:** ERT was associated with a significant reduction in albuminuria and a relatively stable eGFR. The decrease in albuminuria was paralleled by a decrease in both glomerular and tubular urine protein markers.

**Conclusions:** The data indicate that long-term ERT is associated with stable renal function in women with Fabry disease and may advance both glomerular and tubular function.

*Funding:* Pharmaceutical Company Support - Genzyme Corporation - A Sanofi Company

**FR-PO703**

**Fabry Disease (FD) Is Associated with Progressive Reduction in Glomerular Podocyte Mass in Young Patients** Behzad Najafian,[1] Michael Mauer,[2] Einar Svarstad.[3] *[1]Univ of Washington; [2]Univ of Minnesota; [3]Univ of Bergen.*

**Background:** Chronic kidney disease is a major complication of Fabry nephropathy (FN). There is growing evidence that podocytes (PC) play an important role in progression of FN.We previously showed that PC GL-3 inclusion density [Vv(Inc/PC] and foot process width (FPW) progressively increase with age. We aimed to examine if these findings are associated with a change in glomerular PC mass in young FD patients.

**Methods:** Renal biopsies from 12 (M/F=8/4) FD patients, age 12 [4-19] year, median [range], urine protein/creatinine (UPCR) 40 [0-223] µg/min, glomerular filtration rate (GFR) 106 [90-183] ml/min/1.73 $m^2$ were studied. Volume of glomeruli occupied by podocytes [Vv(PC/glom)], capillaries [Vv(Cap/glom)] and mesangium [Vv(Mes/glom)], Vv(Inc/PC) and FPW were estimated by electron microscopy stereology. The results were correlated with age and renal function.

**Results:** Despite direct relationship between age and Vv(Inc/PC) (r=0.67, p=0.02), Vv(PC/glom) showed inverse relationship with age ( r=-0.65, p=0.02), while, no relationship was found between age and Vv(Cap/glom) or Vv(Mes/glom), confirming a loss in glomerular podocyte mass. 46±5% of glomerular volume was composed of podocytes, 30±5% glomerular capillaries and 14±3% mesangium and about 10% urinary space between the capillaries. UPCR directly correlated with age (r=0.61, p=0.01), FPW (r=0.66, p=0.01) and Vv(Inc/PC), and inversely correlated with Vv(PC/glom). Vv(PC/glom) inversely correlated with FPW (r=-0.59, p=0.046).

**Conclusions:** Although we did not count PC in this study, since GL-3 accumulation leads to podocyte enlargement, our observation of reduced glomerular PC mass is strongly suggestive that FD is associated with progressive PC injury (FPW increase) and loss with age in young patients, both of which correlate with UPCR. These results are confirmatory to our other studies that show urinary podocyte loss increases with age in Fabry patients and correlates with UPCR.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Genzyme, a Sanofi Company

**FR-PO704**

**Endothelial Nitric Oxide Synthase Uncoupling and Microvascular Dysfunction in the Mesentery of Fabry Mice** Liming Shu, Justin J. Kang, James A. Shayman. *Univ of Michigan, Ann Arbor, MI.*

**Background:** A defect in the gene for the lysosomal enzyme α-galactosidase A (*GLA*) results in globotriaosylceramide (Gb3) accumulation in Fabry disease and leads to premature death from cardiac and cerebrovascular events. However, gastrointestinal symptoms are often observed first during childhood and persist throught life in these patients, and are not well understood.

**Methods:** Gla knockout mice on a 129/SvJXC57BL/6 background were used. Second order mesenteric arteries were dissected and mounted in a pressure myograph system. Vessel diameter was monitored in real time. Glycosphingolipid analyses and Western blotting were performed as previously reported.

**Results:** The progressive accumulation of Gb3 in mesenteric arteries (MA) was confirmed byTLC. No endothelium-dependent dilatation was seen in MA of Gla null mice at 8 months, and suppression in acetylcholine-mediated vasodilatation was seen at 2 months. Endothelium-independent dilatation with sodium nitroprusside was normal compared with age-matched WT mice. The microvascular defect in the MA was endothelium-dependent and associated with a suppression of the active homodimer of eNOS. Phosphorylation of eNOS at the Ser1179 activation site was down-regulated while that of the inhibitory site (Thr495) was significantly enhanced in aged Gla null MA. Increased levels of nitrated proteins (3-nitrotyrosine) were seen in parallel with changes in eNOS consistent with eNOS uncoupling in the MAs.

**Conclusions:** The data provide evidence of microvascular endothelial dysfunction marked by early onset endothelial dysfunction and a total loss of acetylcholine-mediated dilatation with age in the MA of Gla deficient mice. The observed Gb3 accumulation and decrease in eNOS bioavailability in the MAs are consistent with the phenotype previously observed in macrovessels such as the carotid and aorta. In addition, the reciprocal dephosphorylation of Ser-1179 and phosphorylation of Thr-495 present in this vascular bed may contribute to the profound dysfunction. These data are consistent with Gb3 mediated eNOS uncoupling. This appears to be the first report of endothelial dysfunction in the mesenteric artery in a murine model of Fabry disease.

*Funding:* NIDDK Support

**FR-PO705**

**Fabry Disease: Clinical Characterization of the Variant Phenotype R363H Mutation** Eric L. Wallace, Leslie J. Jackson, David G. Warnock. *Div of Nephrology, UAB.*

**Background:** Fabry disease (FD) is a lysosomal storage disease caused by mutations in the α-galactosidase A (AGAL) gene and leads to globotriacylceramide (Gb3) accumulation in lysosomes.Patients with classic FD phenotype,with no AGAL activity, present in childhood with acroparesthesias, hypohidrosis, angiokeratoma, and gastrointestinal symptoms. Ultimately, renal failure, cardiac involvement, and cerebrovascular disease develop. Variant phenotypes with later-onset presentation are recognized and are caused by mutations, such as R118C and N215S, that spare some residual enzyme activity. AGAL mutations which cause no clinical manifestations of FD have also been identified.

**Methods:** We present a family with the variant phenotype R363H mutation and 5 years of clinical follow up characterizing this phenotypic variant of FD.

**Results:** The male, diagnosed at age 16, presented with acroparesthesias. He had no history of hypohidrosis,GI pain,or diarrhea. Angiokeratomas were notably absent. Laboratory studies revealed: creatinine, 0.8 mg/dL (eGFR by CKD-EPI 132 mL/min/1.73$m^2$); 24 hr urine protein, 78mg/24hr; plasma and leukocyte AGAL activity, 2 U/mL (Reference Range (RR) =12.0 +/- 4.2 U/mL) and 5.63 U/mg (RR = 34.6 +/- 14.6 U/mg), respectively, and undetectable urinary lyso-Gb3. Renal biopsy showed minimal multi-laminar inclusions in podocytes but none in the endothelium. Cardiac work up including echocardiogram and EKG showed no cardiac disease. Due to his lack of clinical manifestations, enzyme replacement therapy was not offered. After 5 yrs follow up, despite no therapy, the patient is still free of symptoms including acroparesthesias. His eGFR by CKD-EPI is 96 ml/min/1.73$m^2$, 24 hour urine protein is 162 mg/24 hr while albuminuria was unchanged. The mother had no clinical manifestations of FD, her kidney biopsy showed no endothelial and only scant inclusions in podocytes.

**Conclusions:** The R363H mutation in the AGAL gene represents a mild phenotypic variant of FD. Current guidelines recommend treatment of all 16 yo males with FD. Evidence of AGAL mutation without clinical manifestations of the disease does not suffice to initiate therapy. Better characterization of the clinical course and informative biomarkers are needed to guide treatment decisions in non-classical Fabry variants.

## FR-PO706

**Systems Genetics Identifies PDGFRA as a Candidate Driver Gene for Lupus Nephritis** Celine C. Berthier,[1] Sharon Chung,[2] Elizabeth E. Brown,[3] Carl D. Langefeld,[4] Matthias Kretzler.[1] *[1]Univ of Michigan; [2]Univ of California; [3]Univ of Alabama; [4]Wake Forest School of Medicine.*

**Background:** Lupus nephritis (LN) is a serious manifestation of systemic lupus erythematosus (SLE) and shows familial aggregation and increased morbidity and mortality. Linking transcriptional profiles with gene candidates identified from genome wide association studies (GWAS - SLEGEN) using systems genetics strategies can facilitate identification of molecular mechanisms driving LN.

**Methods:** Affymetrix based expression profiles from 47 microdissected human renal biopsies (ERCB) were used in association with a meta-analysis of 3 LN GWAS studies.

**Results:** The GWAS meta-analysis showed LN associated single nucleotide polymorphisms (SNPs) located near PDGFRA-GSX2 ($P=2.7x10^{-7}$), SLC5A11 ($P=3.0x10^{-7}$), ID4 ($P=4.3x10^{-7}$), HAS2-SNTB1 ($P=6.2x10^{-7}$), COL4A1 ($P=6.4x10^{-6}$). HLA-DR2 and HLA-DR3 (2 well known SLE susceptibility loci) were also associated with LN ($P=0.037$ and $2.2x10^{-5}$, respectively). Linked to disease phenotype, SNPs within as well as outside coding regions can impact the disease trait via associated transcript expression changes (expression quantitative trait locus or eQTL). Thus, renal gene expression profiles from LN biopsies (n=32) compared to living-donor controls (n=15) were studied for the genomic regions identified in the LN GWAS. In both glomerular and tubulointerstitial compartments, PDGFRA (fold-change 2.57 and 2.40, respectively) and COL4A1 (fold-change 2.61 and 1.89, respectively) showed significant mRNA expression changes in LN patients compared to controls. A transcriptional network was built from 961 LN regulated genes to define the functional context of PDGFRA regulation, independent of predefined canonical pathways. This co-citation literature based network highlighted PDGFRA as one of the main nodes interacting with STAT1, FN1, CCND1 .

**Conclusions:** This work is the first large-scale genome-wide investigation of LN and provides evidence of multiple biologically plausible LN susceptibility loci. Consistent with a functional role in LN, DGFRA and associated pathway members showed significant inductions in intra-renal mRNA levels in LN cases compared to controls.

*Funding:* NIDDK Support

## FR-PO707

**Mutation Analysis in 288 Individuals with Isolated Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) in 20 Known Genes** Daw-yang Hwang,[1,6] Gabriel C. Dworschak,[1,4] Stefan Kohl,[1] Radovan Bogdanovic,[2] Elijah O. Kehinde,[3] Heiko M. Reutter,[4] Velibor Tasic,[5] Friedhelm Hildebrandt.[1,7] *[1]Dept of Nephrology, Boston Children's Hospital, Boston, MA; [2]Medical Faculty, Univ of Belgrade, Belgrade, Serbia; [3]Dept of Surgery, Kuwait Univ, Safat, Kuwait; [4]Institute of Human Genetics, Univ of Bonn, Bonn, Germany; [5]Dept of Pediatric Nephrology, Univ Children's Hospital, Skopje, Macedonia, The Former Yugoslav Republic of; [6]Dept of Medicine, Kaohsiung Medical Univ Hospital, Kaohsiung, Taiwan; [7]Howard Hughes Medical Institute, Chevy Chase.*

**Background:** Congenital anomalies of the kidney and urinary tract (CAKUT) account for approximately 50% of children with end-stage renal disease, taking a high toll on affected families. Mutations in 20 genes have been identified as causing isolated or oligosyndromic CAKUT (Yosypiv, Int J Nephrol 2012:909083, 2012). Genetic diagnosis has been hampered by genetic heterogeneity and lack of genotype-phenotype correlation. To determine the percentage of cases with CAKUT that can be molecularly solved by known CAKUT genes, we analyzed the coding exons of 20 established causative genes in a cohort of 288 individuals from 244 CAKUT families.

**Methods:** We employed a newly developed method of high-throughput mutation analysis (Halbritter, J Med Genet 49:756, 2012). Coding exons of 20 genes were amplified and barcoded in multiples 48 patients using the Fluidigm 48x48 microfluidic array, followed by next-generation re-sequencing and Sanger sequencing. The most common phenotype of our CAKUT cohort is vesicoureteral reflux, followed by hypodysplasia and duplex ureter.

**Results:** In 37 individuals from 32 families we identified mutations in 10 of the 20 known CAKUT genes, including BMP4 (n=2), EYA1 (n=2), HNF1B (n=2), KAL1 (n=4), PAX2 (n=4), RET (n=8), ROBO2 (n=3), SALL1 (n=3), SIX2 (n=6), SIX5 (n=3). Sixteen of these mutations are novel.

**Conclusions:** This result indicates that 13.1% (32/244) families with isolated CAKUT in our cohort are due to monogenic causes. Our report represented one of the most in-depth diagnostic studies of monogenic causes of isolated CAKUT in children.

*Funding:* NIDDK Support

## FR-PO708

**Identification of Mutations Causing Congenital Anomalies of the Kidney and Urinary Tract through Targeted Sequencing** Nayia Nicolaou, Ies J. Nijman, Edwin Cuppen, Kirsten Y. Renkema, Nine V. Knoers. *Medical Genetics, Univ Medical Center Utrecht, Utrecht, Netherlands.*

**Background:** Congenital anomalies of the kidney and urinary tract (CAKUT) are developmental disorders that involve a spectrum of renal structural malformations. They occur in 1 out of 500 live-births and comprise the major cause of end-stage renal disease in childhood. There are 15 genes reported in the Human Gene Mutation Database with mutations responsible for CAKUT. However, a large proportion of cases remain unexplained. We aim to identify novel rare mutations in candidate genes that play a role in kidney cell differentiation and elucidate their involvement in CAKUT etiology.

**Methods:** We employ a targeted next generation sequencing approach in 500 Dutch CAKUT patients. Enrichment methods allow us to capture and analyze in parallel the protein-coding regions of 208 candidate genes and 36 miRNAs. The genes selected were previously shown to have experimental evidence for their role in human isolated or syndromic CAKUT or were demonstrated to be involved in disrupted nephrogenesis in transgenic animals or cell models. For data analysis we used our in-house mapping and variant calling methodology.

**Results:** Sequencing results from the first 60 patients with severe kidney dysplasia showed that the average read depth is 210X. Approximately 200 variants were called per individual. Further variant prioritization based on variant frequency data from dbSNP and Genome of the Netherlands, evolutionary conservation, and *in-silico* predictions resulted in a list of potentially pathogenic mutations. Variants were validated by Sanger sequencing.

**Conclusions:** This study shows that our targeted sequencing approach and variant prioritization method is efficient in identifying gene mutations in a large cohort of sporadic CAKUT cases. By this approach, previously reported causal mutations in known CAKUT genes were successfully identified. Interestingly, the majority of the novel and promising pathogenic variants identified, were unique for each patient. Hence, CAKUT might be even more heterogeneous in their etiology than expected. Functional studies to test the impact of novel mutations on protein function and kidney development are currently on-going.

## FR-PO709

**Whole Exome Sequencing in 3 Brothers with Posterior Urethral Valves** Alina Hilger,[1] Gabriel C. Dworschak,[2] Daw-yang Hwang,[2] Peter Nuernberg,[3] Stefanie Weber,[4] Goedele Beckers,[5] Michael Ludwig,[6] Friedhelm Hildebrandt,[2,8] Heiko M. Reutter.[1,7] *[1]Institute of Human Genetics, Univ of Bonn, Bonn, Germany; [2]Dept of Medicine, Boston Childrens Hospital, Havard Medical School, Boston, MA; [3]Cologne Center for Genomics, Univ of Colonge, Colonge, Germany; [4]Pediatrics II, Univ Childrens Hospital Essen, Essen, Germany; [5]Dept of Pediatric Urology, VU Univ Medical Center Amsterdam, Amsterdam, Netherlands; [6]Dept of Clinical Chemistry and Clinical Pharmacology, Univ of Bonn, Bonn, Germany; [7]Dept of Neonatology, Childrens Hospital Univ of Bonn, Bonn, Germany; [8]Howard Hughes Medical Institute, Chevy Chase, MD.*

**Background:** Posterior urethral valves (PUVs) are the most common cause of lower urinary tract obstruction leading to severely comprised renal function. The incidence is in 1 in 5,000-8,000 male infants, the etiology remains unknown.

In a family with three affected and one unaffected sons from two different fathers we performed whole exome sequencing (WES) in order to identify the genetic cause of the disease.

**Methods:** X-chromosomal inheritance was excluded by linkage analysis we followed a hypothesis of dominant inheritance, of this male limited phenotype, from the unaffected mother to her 3 affected sons. Out of a total of 78 heterozygous, non-synonymous, rare variants occurring in both of the sequenced brothers 14 variants could be filtered by the criteria of conservation (UCSC Genome Browser), expression (Gudmap, Mouse Genome Database), and data such as diseases that were described previously with the respective gene (OMIM, PubMed). 9 of these variants segregated with the disease in the family. Charactarisation of variants was done by several prediction programs (Polyphen, Mutation Taster, SIFT).

**Results:** After applying the described filter steps 5 variants in the genes COL6A5, GSTA3, MIER3, LRRC27 and PHLPP1 were chosen to be possibly disease causing.

**Conclusions:** The current screening and segregation analysis of parents and affected offspring of a larger cohort of 70 PUV patients from Germany and the US for the presence of these variants should identify the disease causing gene.

*Funding:* Government Support - Non-U.S.

## FR-PO710

**Defective Transport and Epithelial Dedifferentiation: Genesis of Key Events in Nephropathic Cystinosis** Claudia Raggi,[1] Alessandro Luciani,[2] Corinne Antignac,[3] Olivier Devuyst.[2] *[1]UCL, Brussels, Belgium; [2]UZH, Zurich, Switzerland; [3]INSERM, Paris, France.*

**Background:** Nephropathic cystinosis (NC), a lysosomal storage disease caused by mutations in the lysosomal cystine transporter cystinosin (CTNS), is characterized by generalized proximal tubule (PT) dysfunction that progresses, if untreated, to end-stage renal disease. The pathogenesis of defective PT cellular transport in NC remains unclear.

**Methods:** In order to investigate the early events involved into PT dysfunction, we performed biochemical profile on a new line of C57BL/6 *Ctns* KO mice, which accumulate cystin in the kidney and show signs of tubulopathy. Endocytic uptake and dedifferentiation

and proliferation markers were assessed on primary PT cells (mPTC) derived from aforementioned mice.

**Results:** Metabolic studies revealed that *Ctns* KO mice presented with a progressive PT dysfunction with low-molecular-weight proteinuria, glucosuria and phosphaturia, before structural damage and in absence of renal failure. These changes were related to decreased expression of multi-ligand receptor complex megalin/cubilin and to increased dedifferentiation (ZONAB transcriptional factor) and proliferation (PCNA and Cyclin D1) rates. Studies on mPTC confirmed the reduced expression of endocytic receptors, with decreased uptake of specific ligands, resulting from an abnormal transcription program involving a loss of integrity of tight junctions, modified distribution of ZO-1 and release of ZONAB. The mPTC also showed increased markers of proliferation and oxidative stress, as observed in *Ctns* KO kidneys. These changes could be rescued by overexpression of wild-type CTNS.

**Conclusions:** These data reveal that the loss of CTNS function in PT cells triggers an early chain of events leading to dedifferentiation and transport defects, before structural damage. They may provide new targets for interventions which, in turn, could alleviate the burden caused by the urinary loss of vital metabolites in patients with NC.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## FR-PO711

**Increased Urinary Loss of Podocytes and Proximal Tubular Epithelial Cells in Nephropathic Cystinosis** Ekaterina A. Ivanova,[1] Fanny Oliveira Arcolino,[1] Maria Pia Rastaldi,[2] Elena N. Levtchenko.[1] *[1]Univ Hospitals Leuven & Katholieke Universiteit Leuven, Leuven, Belgium; [2]Fondazione IRCCS Policlinico & Fondazione D'Amico, Milano, Italy.*

**Background:** Cystinosis is an autosomal recessive disorder caused by mutations in the CTNS gene that encodes a lysosomal cystine transporter and results in high cystine levels in the lysosomes. Cystinosis is associated with severe renal dysfunction progressing towards end-stage renal failure. We hypothesize that increased urinary loss of podocytes and proximal tubular epithelial cells might underlie renal dysfunction in cystinosis.

**Methods:** We analysed urine samples from healthy donors (n=9) and cystinosis patients (n=14). Urine samples were characterized for the presence of mRNA of podocyte and proximal tubular epithelial cell (PTEC) markers (CD2AP, podocalyxin, CD13 and AQP1) by qRT-PCR (mean expression values calculated as Ct normalized by creatinine). Conditionally immortalized podocyte and PTEC cell lines were generated from freshly voided urine of healthy volunteers and cystinosis patients.

**Results:** We demonstrated in cystinosis a significant increased level of podocyturia and a higher number of PTECs voided into urine. In addition, urine samples from cystinosis patients provided more viable podocytes and PTECs suitable for in vitro culture in comparison to healthy donors. As expected, cystinosis podocyte and PTEC cell lines accumulated cystine. Using viable podocytes and PTEC extracted from urine of both healthy donors and cystinosis patients, we established several conditionally immortalized cell lines and characterized them by the expression of typical markers, including CD2AP, synaptopodin, and nephrin for podocytes and CD13 and AQ1 for PTECs. Cystinosis podocytes demonstrated increased motility in wound-healing assay as compared to the control. Also, the actin cytoskeleton, as shown by phalloidin and alpha-actinin staining, and cellular adhesion sites were altered in cystinosis cells.

**Conclusions:** Our data demonstrate increased urinary loss of podocytes and PTECs in cystinosis. Decreased adhesion to the extracellular matrix might be one of the mechanisms underlying renal damage in this disorder.

*Funding:* Private Foundation Support

## FR-PO712

**Endosomal/Lysosomal Dysfunction in Kidney Proximal Tubular Epithelial Cells Deficient for Lysosomal Cystine Transporter Cystinosin** Ekaterina A. Ivanova,[1] Maria Giovanna De Leo,[2] Maria antonietta De Matteis,[2] Elena N. Levtchenko.[1] *[1]Univ Hospitals & Katholieke Universiteit, Leuven, Belgium; [2]Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; [3]Bambino Gesu Children's Hospital, Rome, Italy.*

**Background:** Nephropatic cystinosis is a lysosomal storage disorder caused by a deficiency of cystinosin (CTNS), the lysosomal cystine/H+ co-transporter. The disease is characterized by lysosomal cystine accumulation with renal Fanconi syndrome being an early kidney symptom. Treatment with cysteamine prevents cystine accumulation, but does not restore Fanconi syndrome, suggesting that cystinosin has additional important roles in controlling the reabsorptive function of renal proximal tubular cells (PTC). Here we have explored the role of cystinosin in endocytic membrane trafficking.

**Methods:** We performed a detailed characterization of endosomal compartments and endocytosis in PTC obtained from urine of a healthy volunteer and a cystinosis patient bearing a homozygous 57-kb deletion of the CTNS gene, which results in a complete absence of cystinosin. We also confirmed our observations on HK-2 cells with down-regulated CTNS using specific siRNA.

**Results:** We studied endocytosis and degradation/recycling of various cargo proteins, including a ligand of megalin RAP. Surface binding of megalin ligand GST-RAP was significantly reduced in cystinosis cells. Processing of the ligand upon internalization was slowed-down in cystinosis cells in comparison with the control. Staining for specific markers of cellular compartments revealed dramatically altered early and late endosomal compartments both in PTC cells obtained from cystinosis patients and in HK-2 cells with down-regulated cystinosin. This effect could not be restored by treatment with cysteamine (100 µM, 24h). Moreover, partial co-localization of cystinosin with a motor protein kinesin was observed, indicating a possible involvement of cystinosin in controlling endo-lysosomal motility.

**Conclusions:** We demonstrated altered endocytosis and changes in endocytic compartments in cells deficient for cystinosin.

*Funding:* Private Foundation Support

## FR-PO713

**Assessment of Cystine Stone Growth In Vivo in a Mouse Model of Cystinuria** Amrik Sahota,[1] Jaspreet Parihar,[2] Min Yang,[1] Derek Adler,[1] Wonkuk Kim,[3] Derek Gordon,[1] David S. Goldfarb,[4] Jay A. Tischfield.[1] *[1]Genetics, Rutgers Univ, Piscataway, NJ; [2]Surgery, UMDNJ-RWJMS, New Brunswick, NJ; [3]Math Stat, Univ South Florida, Tampa, FL; [4]Nephrology, NYU Langone Med Ctr, New York, NY.*

**Background:** *Slc3a1* knockout mice are a model for human cystinuria. They have cystine crystalluria from an early age and males from age 3 mo have bladder stones. Newer imaging technologies allow investigation of stone growth and the effects of pharmacologic intervention on this process in live animals. We evaluated the utility of CT for identifying the temporal pattern of cystine stone growth in cystinuria male mice and for assessing the efficacy of cystine dimethyl ester (CDME), a crystal growth inhibitor.

**Methods:** Mice (2-3 mo old) were screened for bladder stones using the Albira CT scanner. Initial bladder volume (indicator of stone volume) was calculated using InviCRO VivoQuant software. Mice with stones were randomly assigned to treatment (200 µl of 10 mg/ml CDME, n=11) or control (200 µl water, n=7), given daily by gavage for 10 wks. Volume measurements were repeated at 1-2 wk intervals. Mice were then sacrificed and stone size and number analyzed using chi-square.

**Results:** 60% of mice had bladder stones. Initial bladder volumes ($mm^3$) in both groups were similar (12-96 for control and 11-86 for CDME). In both cases, there was a linear increase in stone volume that followed the equation y=mx+c. The equation for the control group was y=0.39x+11.0 to y=1.43x+84.3, and for the treatment group it was y=0.99x+18.4 to y=2.67x+73.3. Thus, the two groups could not be distinguished by CT analysis. There was a small but significant difference in the percentage of stone sizes in the two groups (p=0.05). The treatment group had 9.4% more stones and the control group had 19.6% fewer stones in the 1.1-2.0 mm range. The treatment group had 26.1% fewer stones and the control group had 54.6% more stones in the 3.1-4.0 mm range.

**Conclusions:** CT can determine the rate of stone growth in vivo, but longitudinal measurement of stone volume is not suitable for evaluating CDME efficacy since total stone volumes in treated and untreated mice were comparable. Supported in part by Rare Kidney Stone Consortium.

*Funding:* Private Foundation Support, Clinical Revenue Support

## FR-PO714

**Mutations in HAO1 Encoding Glycolate Oxidase Cause Isolated Glycolic Aciduria** Yaacov Frishberg, Avraham Zeharia, Ruth Belostotsky. *Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel.*

**Background:** Perturbations in glyoxylate metabolism are associated with excessive oxalate synthesis, as oxalate is the product of glyoxylate oxidation. Glycolic aciduria has been regarded as a hallmark of primary hyperoxaluria type I (PH1) manifesting with kidney stones, nephrocalcinosis and kidney failure. Mutations in HAO1, encoding glycolate oxidase, the enzyme catalyzing the oxidation of glycolate to glyoxylate, have been investigated as a possible cause of non-type I/type II PH.

**Methods:** Urinary organic acid profile performed by gas chromatography-mass spectrometry. Direct DNA sequencing was used for mutation screening in the HAO1 gene.

**Results:** Two brothers, born to consanguineous healthy parents of Arab descent, were evaluated for psychomotor delay associated with anisocoria, alacrima and in one, also achalasia (triple A syndrome). 4 sisters are healthy. Urinary organic acid profile performed in the 8-year old proband, showed markedly increased urinary glycolic acid excretion (2,000 mmol/mol creatinine; normal reference for age: 18-92) with normal excretion of oxalate, citrate, glycerate and glycine. Abdominal ultrasound showed normal sized kidneys with normal echotexture. Direct sequencing of the HAO1 gene, encoding glycolate oxidase, revealed acceptor splice site mutation in the invariant AG of intron 6: c. 814-1G>C leading to skipping of exon 6 and to in-frame deletion of 53 amino acids containing the riboflavin-5'-phosphate (FMN) binding domain and adjacent to the substrate binding site. The brother affected by triple A syndrome and 4 healthy sisters, all having normal urinary glycolate excretion did not carry bi-allelic mutations in HAO1. The genetic nature of triple A in this kindred, was recently identified and is unrelated to this metabolic abnormality.

**Conclusions:** Loss-of-function mutations in the HAO1 gene, encoding glycolate oxidase, are responsible for asymptomatic isolated glycolic aciduria without hyperoxaluria.

*Funding:* Clinical Revenue Support

## FR-PO715

**Muscle Involvement in Dent Disease 2** Yo Han Ahn, Eujin Park, Jiwon M. Lee, Hee Gyung Kang, IL-Soo Ha, Hae Il Cheong. *Dept of Pediatrics, Seoul National Univ Children's Hospital, Seoul, Korea.*

**Background:** Dent disease is an X-linked recessive renal tubulopathy mostly caused by *CLCN5* mutations (type 1). However, some patients have *OCRL1* mutations (type 2), known to be associated with Lowe syndrome. It is somewhat difficult to differentiate these two diseases by clinical features only. Several studies have showed differences in serum muscle enzyme levels between Dent disease 1 and 2.

**Methods:** Patients with Dent disease 1 (Group 1, n=24), Dent disease 2 (Group 2, n=5) and Lowe syndrome (Group 3, n=16) were included. The serum levels of 3 muscle

enzymes were measured; creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST). Serum levels of alanine aminotransferase (ALT) were measured as well as a control. We compared the serum levels of the enzymes in each group to test the validity of the serum levels as a biomarker to predict the genotypes.

**Results:** All of the patients in Group 2 showed no clinical symptoms of muscle involvement except for one patient having muscle hypoplasia of both upper extremities. The serum levels of the enzymes in each group are shown in Table 1. When we compared between Groups 1 and 2, the positive and negative predictive values of the abnormal levels of the enzymes were also shown in Table 1. Serum levels of AST were normal in all of the patients. Table 1. The serum levels of the enzymes in each group and the predictive values (PV) of the levels.

| | Serum levels of the enzymes | | | Comparison between Groups 1 and 2 | | | |
|---|---|---|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | PPV | NPV | Sensitivity | Specificity |
| CK (IU/L) | 137.3±45.5 | 262.6±100.9 | 344.0±231.2 | 1.00 | 0.82 | 0.6 | 1.00 |
| LDH (IU/L) | 221.7±37.9 | 365.8±60.5 | 507.9±248.6 | 0.45 | 1.00 | 1.00 | 0.33 |
| AST (IU/L) | 26.5±5.7 | 62.8±22.1 | 77.4±21.8 | 1.00 | 0.96 | 0.8 | 1.00 |

**Conclusions:** Measurement of the serum levels of muscle enzymes in patients with Dent disease can provide useful information to predict genotypes, even though the patients do not have overt clinical signs of muscle involvement. Muscle involvement maybe a cardinal finding in patients with *OCRL1* mutations regardless of the phenotype.

*Funding:* Government Support - Non-U.S.

## FR-PO716

**Urinary 2,8-Dihydroxyadenine Excretion in Patients with APRT Deficiency: Effect of Allopurinol and Febuxostat Therapy**

Hrafnhildur Linnet Runolfsdottir,[1] Vidar O. Edvardsson,[1,2] Margret Thorsteinsdottir,[1,3] Runolfur Palsson.[1,2] *[1]Univ of Iceland; [2]Landspitali - The National Univ Hospital of Iceland; [3]ArcticMass, Reykjavik, Iceland.*

**Background:** Adenine phosphoribosyltransferase (APRT) deficiency results in excessive urinary excretion of poorly soluble 2,8-dihydroxyadenine (DHA), causing nephrolithiasis and chronic kidney disease. Treatment with allopurinol is effective but reliable methods for therapeutic monitoring are lacking. We evaluated urinary DHA excretion in patients with APRT deficiency using ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-MS/MS).

**Methods:** UPLC-MS/MS was used to measure the concentration of DHA in 24 hr and random urine samples from 28 patients with APRT deficiency, before and after treatment with allopurinol or the alternative agent febuxostat. Urine samples from healthy subjects were used as controls. The pH of the urine specimens was adjusted to 10, using 20 mM $NH_4OH$, to dissolve all precipitates before testing. The urinary excretion of DHA in random urine samples is expressed as DHA-to-creatinine ratio (ng/mmol). Data are expressed as median and range.

**Results:** In 20 healthy controls, the median DHA-to-creatinine ratio was 0.4 (0-2.3) ng/mmol. In untreated patients with APRT deficiency, the median 24 hr urinary excretion of DHA was 1750 (1045-2706) μg/24 hr (n=5) and the median DHA-to-creatinine ratio in random urine samples was 272 (85-374) ng/mmol (n=8). In patients treated with allopurinol, the median 24 hr urinary excretion of DHA was 848 (4.7-2393) μg/24 hr (n=33) and the median DHA-to-creatinine ratio was 126 (2.5-865) ng/mmol in random urine samples (n=12). In patients treated with febuxostat, the median 24 hr urinary excretion of DHA was 293 (43-319) μg/24 hr (n=3) and the median DHA-to-creatinine ratio in random urine samples was 74 (5-246) ng/mmol (n=4).

**Conclusions:** Marked reduction in urinary excretion of DHA was observed after pharmacotherapy was initiated. Febuxostat may be a more effective agent than allopurinol. These preliminary data suggest that the UPLC-MS/MS assay greatly enhances monitoring of pharmacotherapy in patients with APRT deficiency.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## FR-PO717

**Kidney Function and Chronic Kidney Disease in Patients with APRT Deficiency**

Hrafnhildur Linnet Runolfsdottir,[1] Runolfur Palsson,[1,2] Vidar O. Edvardsson.[1,2] *[1]Univ of Iceland; [2]Landspitali - The National Univ Hospital of Iceland, Reykjavik, Iceland.*

**Background:** Adenine phosphoribosyltransferase (APRT) deficiency is an autosomal recessive disorder characterized by nephrolithiasis and/or chronic kidney disease (CKD). The aim of this study was to examine kidney function and the development of CKD in patients with APRT deficiency.

**Methods:** All 46 patients listed in the APRT Deficiency Registry of the Rare Kidney Stone Consortium were included in the study. Estimates of glomerular filtration rate (eGFR) were calculated from serum creatinine, using the MDRD equation in adults and the modified Schwartz equation in children. CKD was defined as eGFR <60 ml/min/1.73 $m^2$ and the slope of eGFR per year was calculated. Data are presented as median and range.

**Results:** Of the 46 patients, 21 were males (45.6%). The median age at diagnosis was 33.9 (0.6-62.8) years and treatment with allopurinol was initiated at the age of 44.4 (7.3-62.8) years. The slope of eGFR per year was –0.17 and –8.65 ml in treated and untreated patients, respectively. At last follow-up, 9 of 32 (28%) Icelandic patients had developed CKD, of whom 3 (9.4%) had progressed to ESRD. These 3 patients received limited or no treatment prior to the onset of ESRD, which occurred at age 44.0 (36.7-53.7) years. Nine of 11 (82%) US patients had CKD, of whom 5 (44.5%) had developed ESRD at age 37.7 (21.2-62.1) years. All 5 US patients with ESRD were first diagnosed with APRT deficiency and placed on allopurinol after initiation of dialysis (n=2) or a failed kidney transplant (n=3). Two Austrian patients and one from the UK with CKD received limited or no treatment. Twenty-five patients, aged 31.9 (2.4-74.9) years, with eGFR ≥60 ml/min/1.73 $m^2$ received treatment with either allopurinol or febuxostat for 17.6 (0.1-34.8) years.

**Conclusions:** The greater decline in renal function in untreated patients compared to those who received allopurinol and/or febuxostat underscores the importance of timely diagnosis and treatment. The high proportion of US patients developing ESRD prior to the correct diagnosis is of concern and suggests lack of awareness of APRT deficiency among physicians.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## FR-PO718

**Estimated Prevalence of APRT Deficiency Based on Population Frequencies of Mutated Alleles**

Runolfur Palsson,[1] Vidar O. Edvardsson,[1] Wei Wang,[2,3] Zhi Wei,[2,3] Hakon Hakonarson.[3] *[1]Landspitali - The National Univ Hospital of Iceland, Reykjavik, Iceland; [2]New Jersey Institute of Technology, Newark, NJ; [3]The Children's Hospital of Philadelphia, Philadelphia, PA.*

**Background:** Adenine phosphoribosyltransferase (APRT) deficiency is a rare autosomal recessive disorder of purine metabolism, characterized by nephrolithiasis and/or chronic kidney disease (CKD). Despite an estimated heterozygote carrier rate of 0.4–1.2%, only a handful of cases have been identified in many countries, including the US. The aim of this study was to estimate the prevalence of APRT deficiency based on frequencies of mutated APRT alleles in genomic databases.

**Methods:** We searched for 47 reported *APRT* mutations in two large databases of genome sequences available in the public domain, the 1000 Genome Project (n=1092) and the NHLBI GO Exome Sequencing Project (n=6503). Minor allele frequencies (MAF) <0.01% were determined and the heterozygote carrier frequency defined as the sum of pathogenic alleles. The prevalence rate of homozygous genotypes was calculated using the Hardy-Weinberg principle.

**Results:** In the two databases combined, 5 mutations were detected with MAF <0.01% that were predicted to have pathogenic effects on the protein function. The rare pathogenic *APRT* alleles are shown in the Table.

| No. | Position | MAF (%) | Homozygous frequency (%) | Base change | Amino acid change | Location | Mutation |
|---|---|---|---|---|---|---|---|
| 1 | 88876549 | 0.0077 | 0.00005929 | 1759T → C | L110P | Exon 4 | Missense |
| 2 | 88876953 | 0.0077 | 0.00005929 | 1355C → T | R67X | Exon 3 | Nonsense |
| 3 | 88876886 | 0.0005 | 0.00000025 | 1422G → A | R89Q | Exon 3 | Missense |
| 4 | 88876532 | 0.0005 | 0.00000025 | 1776G → A | A116T | Exon 4 | Missense |
| 5 | 88876902 | 0.0005 | 0.00000025 | 1406G → A | V84M | Exon 3 | Missense |

Considering only rare pathogenic variants yielded a homozygous frequency of 0.00005929–0.00011858%, suggesting an overall disease prevalence in the range of 1/8,000 to 1/17,000.

**Conclusions:** These data indicate a prevalence of APRT deficiency in the range of 1 in 8,000 to 1 in 17,000. Thus, APRT deficiency appears to be a seriously underrecognized cause of kidney stones and CKD.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## FR-PO719

**Estimated Incidence of Primary Hyperoxaluria Using Population Allele Frequencies of Disease Variants**

Katharina Hopp,[1] Andrea G. Cogal,[1] Hakon Hakonarson,[2] Dawn S. Milliner,[1] Peter C. Harris.[1] *[1]Mayo Clinic; [2]Children's Hospital of Philadelphia.*

**Background:** Primary Hyperoxaluria (PH) is a rare recessive condition characterized by oxalate buildup plus ESRD and caused by mutations to *AGXT* (PH1), *GRHPR* (PH2), and *HOGA1* (PH3). Clinical prevalence is estimated at 1 to 3 per million but the true disease frequency is unknown.

**Methods:** We have used allele frequencies reported in the NHLBI Exome Sequencing and the 1000 Genome Project to better estimate the incidence rate (IR) and carrier frequency (CF) of PH. This is based on published pathogenic PH alleles and data obtained within Mayo's RKSC PH Registry. Additionally, we scored the likely pathogenicity of non-synonymous variants with a minor allele frequency (MAF) <0.01% using *in silico* tools. Variants scoring as highly likely pathogenic were considered as PH mutant alleles.

**Results:** Based on published pathogenic variants an overall PH CF of ~1:84 with an IR of ~1:28,079 was determined. Including predicted mutations the CF/IR increase to ~1:70 and ~1:19,361, respectively. Interestingly, PH is predicted to be ~69% less prevalent in African American (AA) than in European American (EA) (EA: CF ~1:72, IR ~1:20,206; AA: CF ~1:129, IR ~1:66,200), due to two common EA alleles, *AGXT* p.G170R (MAF 0.1%, CF 1:429) and *HOGA1* c.700+5G>T (MAF 0.3%, CF 1:165). The *AGXT* p.R289H variant is the most prevalent AA allele and accounts for ~41% of AA mutant alleles (MAF 0.2%, CF 1:289; EA CF 1:1,956). When separated by type, PH1 and PH3 have comparable CF's (~1:174 vs ~1:204) and IR's (~1:119,714 vs ~1:165,029), while PH2 is ~94% less prevalent (CF ~1:807, IR ~1:2,602,898). Including scored likely pathogenic variants for PH2 may provide a better estimate of CF (~1:462) and IR (~1:851,037), since its rarity limits the number of mutations seen in the clinic. Interestingly, within the RKSC PH Registry, PH1 is 8 times more prevalent than PH3, highlighting a great underdiagnosis of PH3, presumably due to its milder phenotype.

**Conclusions:** PH is more common than previously estimated, suggesting that the disease is underdiagnosed/non-penetrant, especially in the case of PH3.

*Funding:* NIDDK Support, Other NIH Support - NACTS

**FR-PO720**

**Phenotype-Genotype Correlations in Primary Hyperoxaluria**
Andrea G. Cogal, Katharina Hopp, Eric J. Bergstralh, Barbara M. Seide, Julie B. Olson, Alicia Meek, Dawn S. Milliner, Peter C. Harris. *Mayo Clinic, Rochester, MN.*

**Background:** Primary Hyperoxaluria (PH) is a recessive disease characterized by the accumulation of renal oxalate and often resulting in ESRD. Three PH loci have been identified: *AGXT* (PH1), *GRHPR* (PH2), and *HOGA1* (PH3), however, limited data is available on the extent that genetic factors dictate phenotype.

**Methods:** The Rare Kidney Stone Consortium PH Registry has 367 patients with a clinical diagnosis compatible with PH. Of these, 299 were screened for mutations in the known genes. Disease variables were evaluated to derive phenotype-genotype correlations at the genic and allelic level.

**Results:** Of the analyzed patients, 74.4% were PH1, 9.8% PH2, 9.3% PH3, and 6.5% no mutation detected (NMD) but with a characteristic PH phenotype. PH1 patients had more severe disease with a higher proportion of ESRD at diagnosis (31.1%, compared to 8.3%, 0% and 4.2% for the other PH types, respectively; P<0.0001). Also, the median 24h urine oxalate was highest in the PH1 group (2.0 mmol/1.73m$^2$,compared to 1.5, 1.3 and 1.3, respectively; P=0.0001), and median 24h urine Ca was lowest in that group (54mg/1.73m$^2$), compared to 70, 109 and 161 in the other groups (P<0.0001). Interestingly, no PH3 proband carried two truncating changes, whereas 11.3% of PH1 and 60% of PH2 probands did; suggesting lethality associated with complete loss of *HOGA1*. Differences in age at diagnosis between PH1 mutation types was highly significant (P=0.0001); cases with two likely mistargeting variants had the mildest disease (median 32.4y) compared to 6.9y with two truncating mutations, 6.5y with one truncating and one mistargeting and 10.2y with two non-mistargeting, in-frame. Urine oxalate and Ca levels were lowest and highest, respectively, in the two mistargeting mutation group. No strong allelic effects were seen in the smaller PH2 and PH3 groups.

**Conclusions:** As larger PH populations are collected, the significance of genic effects for diagnostics and prognostic becomes clearer. The combinations of mutations in PH1 are also a key indicator of the disease course and can potentially help target therapies, highlighting the value of molecular diagnostics in this disease.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

**FR-PO721**

**Sustained Pyridoxine (VB6) Response in Primary Hyperoxaluria Type 1 (PH1) Recipients of Kidney Alone Transplantation (KTx)**
Elizabeth C. Lorenz, John C. Lieske, Eric J. Bergstralh, Dawn S. Milliner. *Mayo Clinic, Rochester, MN.*

**Background:** PH1 patients homozygous for the G170R mutation develop normal or near normal urine oxalate (Uox) with VB6. The purpose of this study was to examine the efficacy of long-term VB6 in G170R homozygous patients after KTx.

**Methods:** G170R homozygous patients enrolled in the Rare Kidney Stone Consortium PH Registry who underwent KTx were included. Patients were maintained on VB6 post-KTx. Uox was monitored.

**Results:** 5 G170R homozygous patients underwent KTx between 9/99-11/11. Median age was 39 yrs (range 22-67), 80% were female and 80% received LD KTx. One patient was on VB6 prior to ESRD. Median follow-up was 8.2 yrs (range 1.6-13.4). Median VB6 dose was 7.7 mg/kg/d (range 5.4-9.4). At end of follow-up, 4 grafts were functioning and 1 graft (patient 5) failed due to rejection.

| Patient | Follow-up (yr) | Mean Uox post-tx (mmol/d) | Mean Pox post-tx (mcmol/L) | Last Uox (mmol/d) | Last eGFR (ml/min/1.73m$^2$) | Allograft biopsies |
|---|---|---|---|---|---|---|
| 1 | 11.8 | 0.5±0.2 (n=14) | 2.2±1.3 (n=14) | 0.4 | 45 | Protocol bx 60.7 mos: no CaOx |
| 2 | 1.6 | 1.2±0.6 (n=7) | 13.4±6.4 (n=16) | 0.7 | 31 | Protocol bx 0 mos: no CaOx<br>Protocol bx 1.0 mos: rare CaOx<br>Protocol bx 6.1 mos: rare CaOx<br>Protocol bx 18.8 mos: no CaOx |
| 3 | 8.2 | 0.5±0.1 (n=21) | 2.2±1.0 (n=19) | 0.4 | 69 | Protocol bxs (5 between 0-61.0 mos): no CaOx |
| 4 | 13.4 | 0.4±0.2 (n=12) | 4.0±3.2 (n=10) | 0.3 | 27 | For-cause bx 37.0 mos: no CaOx |
| 5 | 2.2 | n/a | n/a | n/a | n/a | For-cause bx 26.5 mos: rejection, moderate CaOx |

Uox remained normal or near normal in the majority of patients throughout follow-up. The Uox of 1 patient who received standard 3 times per wk hemodialysis without VB6 for 1 yr prior to KTx did not normalize until 9 months post-KTx. However, her allograft biopsy 1.6 yrs post-KTx demonstrated no CaOx and only mild fibrosis.

**Conclusions:** Patients homozygous for the G170R mutation demonstrate sustained response to VB6 following KTx. The majority of allograft biopsies showed no evidence of recurrent oxalosis and long-term graft outcomes were excellent. Thus KTx with VB6 should be considered in PH1 patients homozygous for the G170R mutation.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

**FR-PO722**

**Acute Intermittent Porphyria: An Underdiagnosed Cause of Chronic Tubulo-Interstitial Nephritis**
Alexandre Karras,[1] Nicolas Pallet,[2] Eric Thervet,[1] Hervé Puy.[3] *[1]Nephrology, Hôpital Européen Georges Pompidou, Paris, France; [2]Biochemistry, Hôpital Européen Georges Pompidou, Paris, France; [3]Centre Francais des Porphyries, Hopital L Mourrier, Colombes, France.*

**Background:** Acute Intermittent Porphyria (AIP) is a rare inherited disorder of heme biosynthesis. This autosomic dominant disease is frequent (1/1600 in Western Europe) but most cases are asymptomatic or undiagnosed. Chronic kidney disease (CKD) is frequent among patients with AIP, but there is no large epidemiological study to determine the prevalence of kidney dysfunction in this population.

**Methods:** In collaboration with the French National Reference Center for Porphyria, a large coss-sectionnal study was conducted among 415 AIP patients with proven hydroxymethylbilane synthase deficiency and available laboratory data, including a serum creatinine measurement. Renal Insufficiency (RI) was determined as an estimated GFR<60 ml/min/1.73m2 (according to MDRD).

**Results:** RI prevalence in the entire AIP study population was 26.5%. Distribution of RI patients in different CKD stages was : CKD 3a in 53%, CKD 3b in 38%, CKD 4 or 5 in 9%. RI was more frequent in female (35%) than in male (9%) patients (OR 5.44), in symptomatic (48%) than in asymptomatic (9%) patients (OR 9.33). Prevalence of kidney disease increases with age : among female AIP patients, prevalence of MDRD<45 is 0% before the age of 40 yrs, 14% between 40 and 60 yrs , 28% after the age of 60. These risk factors are cumulative, yielding a 65% prevalence of RI among the 52 female symptomatic AIP patients aged >60. Patients with RI have a mean proteinuria of 0.39±0.32 g/24h, and are hypertensive in 93% of cases. Renal biopsy, when performed, shows mainly tubulo-interstitial lesions. For the 12 patients that reached ESRD, the mean age at dialysis initiation was 58±11. Six patients underwent kidney transplantation, without recurrence of tubulointerstitial nephritis on the renal allograft.

**Conclusions:** Renal involvement is frequent in AIP, especially among female patients. As AIP symptoms are unconstant and non specific, a screening for a deficiency of heme metabolism should be performed in all patients with unexplained chronic tubulo-interstitial nephropathy.

**FR-PO723**

**Functional Modeling of Compound Heterozygous KCNJ1 (ROMK1) Mutations Identified in a Kindred with Bartter's Syndrome**
Shalabh Srivastava,[1] Ann Marie Hynes,[1] Dimin Li,[2] Paul A. Welling,[2] John Andrew Sayer.[1] *[1]Institute of Genetic Medicine, Newcastle Univ, Newcastle-Upon-Tyne, United Kingdom; [2]Dept of Physiology, Univ of Maryland Medical School.*

**Background:** We report clinical, genetic and biophysical data relating to Pakistani kindred with Bartter's Syndrome.

**Methods:** A multiplex family was identified with biochemical and clinical features suggestive of Bartter's syndrome. The eldest sibling presented with developmental delay and rickets at 3 years of age with biochemical evidence of hypercalciuria and hypokalemia. The second sibling had a similar presentation. She went on to develop gestational diabetes and advanced renal impairment by 18 years of age. The third child was born after premature delivery with a history of polyhydramnios and was initially diagnosed with hypocalcemia. Following treatment she developed hypercalciuria and a hypokalemic metabolic alkalosis. There was evidence of secondary hyperreninemia and hyperaldosteronism in all 3 siblings consistent with Bartter's syndrome. Known Bartter's syndrome genes were screened.

**Results:** We detected a compound heterozygous missense changes in KCNJ1 gene, encoding the potassium channel ROMK1. The S219R/L220F mutation segregated from father and mother, respectively. In silico modeling of the missense amino acids suggested deleterious changes. Studies in Xenopus oocytes revealed that both S219R and L220F had a deleterious effect on KCNJ1 mediated potassium currents. Co-injection to mimic the compound heterozygosity produced a synergistic decrease in channel function.

**Conclusions:** In a multiplex family with Bartter's syndrome, we identified a compound heterozygous mutation in KCNJ1. Functional studies confirmed the pathogenicity of these mutations.

*Funding:* NIDDK Support

**FR-PO724**

**A Novel Homozygous FAH R237Q Missense Associated with Chronic Form of Tyrosinaemia Type 1**
Jie Ding, Fang Wang, Yong Yao, Yanqin Zhang. *Dept of Pediatrics, Peking Univ First Hospital, Beijing, China.*

**Background:** Autosomal-recessive tyrosinaemia type I, characterized by liver and renal tubular damage and neurological crises, is caused by a defect in the enzyme fumarylacetoacetate hydrolase (FAH). The aim of this study was to characterize FAH gene mutation in a Chinese boy with chronic form of tyrosinaemia type 1 and his family.

**Methods:** An 8-year-old boy of unrelated healthy Chinese parents developed X-type legs at the age of 4. There was no history of neonatal jaundice and he had never been transfused. At age 8, physical examination showed this patient had hepatomegaly, 4 cm below the costal margin in the right midclavicular line. Moreover, the skeletal deformities were noted, such as ribs valgus, X-type legs, and thickening of the wrists. The results of the laboratory tests performed were as follows: aspartate aminotransferase was normal, alkaline phosphatase 1671 U/L (<750 U/L), and AFP 566.1 ng/ml (0–20 ng/ml). In plasma, there was normal level of tyrosine and phenylalanine, but decreased phosphorus (0.38 mmol/L; 1.45–2.1 mmol/L). Urinary succinylacetone, 4-hydroxyphenylpyruvate

and 4-hydroxyphenyllactate were elevated. Aminoaciduria, phosphaturia and distal renal tubular acidosis were also detected. Abdominal MRI examination revealed diffuse hepatic parenchymal nodular changes and enlarged bilateral kidneys. Morphology and size were normal in the spleen. In addition, skeletal X-ray showed bone changes of rickets. All exons of FAH with adjacent intron–exon junctions were analyzed using direct sequencing.

**Results:** DNA analysis in our patient and his younger brother (clinically asymptomatic at the age of 5 months) revealed a homozygous G-to-A transition at nucleotide 710 in exon 9 of the FAH gene that leads to a R237Q substitution in the resulting protein. The carrier status of both the parents was revealed by sequencing the respective PCR product. The mutation R237Q was not detected in 100 Chinese control chromosomes.

**Conclusions:** Our report extends the phenotypic and genotypic spectrum of tyrosinaemia type I.

*Funding:* Government Support - Non-U.S.

## FR-PO725

**The Efficient Use of Oxygen in the Kidney Is Impaired in Claudin-2 Deficient Mice** William J. Welch,[1] Lei Pei,[2] Glenn Solis,[1] Lynn Magenheimer,[2] Alan S.L. Yu.[2] *[1]Medicine, Georgetown Univ, Washington, DC; [2]The Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** The proximal tubule (PT) is responsible for 65-70% of reabsorption of the glomerular filtrate, much of it driven by active transcellular transport processes requiring ATP and oxygen. However, a portion of PT transport is facilitated by passive paracellular movement of ions and fluid through tight junctions. Claudin-2, an integral membrane protein expressed in tight junctions of the PT functions as a paracellular cation channel and mediates Na+ movement from the lumen to the peritubular space.

**Methods:** We hypothesized that claudin-2 enhances the efficiency of renal oxygen consumption by promoting paracellular uptake of Na+. We calculated the ratio between whole kidney Na+ reabsorption ($T_{Na}$ = GFR X PNa) and oxygen consumption (QO2 = RBF X A-VO2) in anesthetized wild type (n=8) and claudin-2 KO mice (n=9).

**Results:** GFR (WT: 845±45 vs KO: 915±48 µl/min/g kw, ns), RBF (WT: 6.9±0.6 KO: 6.7±0.7 ml/min/g, ns) and MAP (WT: 102±5 vs KO: 106±6 mmHg, ns) were not different between strains. $T_{Na}$ was similar between strains (WT: 133±15 vs KO: 142±34 µmol/min/g, ns), whereas $Q_{O2}$ was higher in KO mice (WT: 17.5±1.5 vs KO: 29.6±2.1 µmol/min/g, $p<0.01$), resulting in lower $T_{Na}/Q_{O2}$ in claudin-2 deficient mice (WT: 7.26±0.66 vs KO: 4.96±0.77 µmol/µmol, $p<0.01$). Renal $O_2$ extraction was also higher in KO mice (WT: 2.5±0.4 vs KO: 4.0±3 µmol/min, $p<0.01$).

**Conclusions:** These data show that the efficient use of oxygen to reabsorb $Na^+$ in the kidney is reduced in mice deficient of claudin-2. Further these data suggest that paracellular movement of $Na^+$ is an important pathway for PT reabsorption of $Na^+$.

*Funding:* NIDDK Support, Other NIH Support - NHLBI

## FR-PO726

**Aldosterone Upregulates Claudin-4 Expression and Decreases Paracellular $Na^+$ Permeability in the Renal Cortical Collecting Duct** Madhumitha Rajagopal, Alan S.L. Yu. *Internal Medicine, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Aldosterone upregulates $Na^+$ reabsorption in the renal cortical collecting duct by increasing transcellular $Na^+$ transport. Whether it also regulates paracellular transport pathways is largely unexplored. Paracellular permeability is known to be mediated by tight junction membrane proteins called claudins. We used the murine cortical collecting duct cell line (mpkCCDc14), to study the effect of aldosterone on paracellular $Na^+$ permeability and the role of claudins.

**Methods:** We treated mpkCCDc14 cells with aldosterone or vehicle. In the presence of apical amiloride, which blocks transcellular $Na^+$ permeability, the transepithelial resistance (TER) reflects paracellular ion permeability. We measured TER with an electronic volt/ohm meter and mounted cell sheets on an Ussing chamber to calculate paracellular $Na^+$ and $Cl^-$ permeabilities. We used immunoblotting to measure changes in claudin expression.

**Results:** Aldosterone treated cells had 51.5 ± 4.3% higher TER than vehicle treated cells (9147 ±383 Ω.cm$^2$ compared to 6564 ± 295 Ω.cm$^2$). These cells also showed a 45.5 ± 2.8 % lower paracellular $Na^+$ permeability ($P_{Na}$), than vehicle treated cells ($p < 0.005$). Of the claudins known to be expressed in the collecting duct, aldosterone caused a 3.5 fold increase in protein expression of claudin-4 (which behaves as a $Na^+$barrier and $Cl^-$ pore) but did not change expression of claudin-3, -7 -8 or -10. The aldosterone-mediated increase in TER was almost completely reversed by treatment with the steroid receptor antagonist, RU486. Treatment with SGK1 inhibitor, GSK650394 significantly decreased TER in both aldosterone- and vehicle-treated cells suggesting a role for SGK1 in modulating the decrease in $P_{Na}$.

**Conclusions:** In summary, our data suggests that aldosterone might signal via SGK1 to upregulate claudin-4 expression and thereby decrease paracellular $Na^+$ permeability in the collecting duct. A decrease in paracellular $Na^+$ permeability would reduce back-flux of actively reabsorbed $Na^+$, significantly enhancing net $Na^+$ reabsorption.

*Funding:* NIDDK Support

## FR-PO727

**AUP1 Promotes Polyubiquitination and ER-Associated Degradation of NKCC2** Elie Seaayfan, Sylvie Demaretz, Nadia Defontaine, Kamel Laghmani. *INSERM UMRS 872; CNRS ERL 7226; Univs Paris V, Paris VI, Paris, France.*

**Background:** Mutations in the apical Na-K-2Cl co-transporter, NKCC2, cause type I Bartter syndrome (BS1), a life-threatening kidney disease. Yet the mechanisms underlying the regulation of NKCC2 trafficking in renal cells are scarcely known. We have previously shown that export from the ER constitutes the limiting step in the maturation and cell surface expression of NKCC2. Indeed, the majority of newly synthesized NKCC2 proteins is trapped in the ER and destined for ER associated degradation (ERAD). The aim of the present study was to identify the protein partners specifically involved in ERAD of NKCC2.

**Methods:** Protein-protein interactions were investigated by the yeast two-hybrid system (Y2H) and co-immunoprecipitation assay (Co-IP). The expression of NKCC2 protein was monitored in transiently transfected OKP and HEK cells, using immunoblot and confocal imaging.

**Results:** Using the Y2H, we identified ancient ubiquitous protein 1(AUP1), as a specific binding partner of NKCC2. AUP1 is a member of a protein family that contains the coupling of ubiquitin conjugation to ER degradation domain (CUE)**.** Co-IP assay showed that endogenously expressed AUP1 interacts mainly with NKCC2 immature forms. Accordingly, immunocytochemistry analysis showed co-localization of the proteins mainly in the ER. Co-expression with dominant negative AUP1 decreased the cotransporter polyubiquitination and increased the amount of total NKCC2 protein. Cycloheximide chase assay demonstrated that the marked increase in the cotransporter protein levels was essentially due to decreased protein degradation of NKCC2 immature forms. Consistent with this, knockdown of endogenous AUP1 by small interfering RNA decreased the polyubiqitination of NKCC2 and increased therefore its expression. Finally, inactivation of the CUE domain of AUP1 prevented its action on NKCC2.

**Conclusions:** In summary, our results demonstrate the presence of an AUP1 mediated ERAD pathway in renal cells promoting polyubiqitination and degradation of immature NKCC2 proteins. The identification and selective modulation of ERAD components specific for NKCC2 and its disease causing mutants might provide novel therapeutic strategies for the treatment of BS1.

## FR-PO728

**Regulation of NKCC2 Activity by Inhibitory SPAK Isoforms: KS-SPAK Is a More Potent Inhibitor Than SPAK2** James A. McCormick. *Medicine (Nephrology and Hypertension), Oregon Health & Science Univ, Portland, OR.*

**Background:** The WNK-SPAK/OSR1 pathway plays an important role in the regulation of ion homeostasis, and the maintenance of extracellular fluid volume and blood pressure. SPAK and OSR1 phosphorylate and activate the cation chloride cotransporters NKCC1, NKCC2 and NCC. Two putative inhibitory SPAK isoforms have been identified, Kidney-Specific SPAK (KS-SPAK) which lacks the majority of the full-length SPAK kinase domain, and SPAK2, which only lacks the initial portion of the kinase domain. Both isoforms are highly expressed in kidney, but their functional relevance is unclear.

**Methods:** We tested the ability of KS-SPAK and SPAK2 to inhibit the activity of NKCC1 and NKCC2 in *Xenopus* oocytes. $^{86}$Rubidium uptake experiments were performed under hypotonic low chloride (160 mOSM), isotonic (210 mOSM), or hypertonic (340 mOSM) conditions.

**Results:** While KS-SPAK strongly inhibited activity of NKCC2 at all tonicities (by 60-72%), SPAK2 only inhibited its activity, mildly, under hypotonic low chloride conditions (18%). Since it was reported that SPAK2 strongly inhibits NKCC1 activity, we compared the effects of KS-SPAK and SPAK2 on NKCC1 and NKCC2 activity under hypotonic low chloride conditions, which maximally activate full length SPAK/OSR1. KS-SPAK inhibited uptake by both cotransporters to a similar degree (84% and 88% respectively). In contrast, SPAK2 exerted a significantly greater effect on NKCC1 (77% inhibition) than on NKCC2 (23% inhibition). Truncation of SPAK2 revealed that deletion of the catalytic loop resulted in an inhibitory effect on NKCC2 similar to that of KS-SPAK (61% and 65% inhibition respectively). For KS-SPAK, mutagenesis studies showed that a WNK phosphorylation site in KS-SPAK (S133) is not required for its ability to inhibit NKCC2 activity, but residues involved in its interactions with cation cotransporters (D238 and L252) are required for its inhibitory effect. Finally, coimmunoprecipitation experiments showed that both KS-SPAK and SPAK2 associated with NKCC2.

**Conclusions:** These data reveal that while both KS-SPAK and SPAK2 interact with NKCC2, SPAK2 is a relatively weak inhibitor of NKCC2 activity. This difference may be physiologically important.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO729

**Salt Loading Alters Renal Expression of Tamm-Horsfall Protein, $Na^+$, $K^+$, $2Cl^-$ Cotransporter and Cyclooxygenase-2** James M. Bates, Satish Kumar. *Dept of Medicine/Nephrology Section, Univ of Oklahoma HSC and VA Medical Center, Oklahoma City, OK.*

**Background:** The American diet contains more salt than recommended. The thick ascending limb of the loop of Henle (TAL) plays a critical role in renal sodium handling. It has constitutive expression of $Na^+$, $K^+$, $2Cl^-$ cotransporter (NKCC2) and Tamm-Horsfall Protein (THP), and inducible expression of cyclooxygenase-2 (COX-2). We investigated the effect of high salt on NKCC2, phosphorylated NKCC2 (pNKCC2) and COX-2 in THP-deficient (THP $^{-/-}$) and wild-type (THP $^{+/+}$ ) mice.

**Methods:** Six THP $^{-/-}$ and THP $^{+/+}$ mice were fed high salt diet (HS,8% NaCl) for 8 months with 6 THP $^{-/-}$ and THP $^{+/+}$ mice on normal salt diet (NS, 0.4%) as controls. Kidney homogenates were processed to obtain membrane and microsomal fractions. Western Blot analysis was performed for NKCC2, pNKCC2 , COX-2 and THP. The data were statistically compared by Student's t-test and two-way analysis of variance.

**Results:** In the THP$^{+/+}$ mice, membrane-bound THP increased on HS (NS THP$^{+/+}$ 0.233 ± 0.07 vs HS THP$^{+/+}$ 0.45 ± 0.1, P = 0.021). Membrane-bound NKCC2 was elevated in THP$^{-/-}$ mice on NS and HS (NS THP$^{+/+}$ 0.07 ± 0.06 vs THP$^{-/-}$ 0.18 ± 0.03, P = 0.005, HS THP$^{+/+}$ 0.03 ± 0.005 vs THP$^{-/-}$ 0.063 ± 0.013, P = 0.015 ) , and decreased by HS in both THP $^{-/-}$ and THP $^{+/+}$ mice (genotype factor significance, F = 15.45, P = 0.0008; salt factor significance, F = 17.62, P = 0.0004, no interaction). Membrane-bound pNKCC2 was similar between THP $^{-/-}$ and THP $^{+/+}$ mice on NS and lower in THP$^{-/-}$ mice on HS (NS THP$^{+/+}$ 0.78 ± 0.15 vs THP$^{-/-}$ 0.70 ± 0.13, P = 0.3, HS THP$^{+/+}$ 1.68 ± 0.31 vs THP$^{-/-}$ 0.84 ± 0.27, P = 0.03). It was increased by HS in THP$^{+/+}$ mice (NS 0.78 ± 0.15 vs HS 1.68 ± 0.31, P = 0.013). Microsomal COX-2 was reduced in THP$^{-/-}$ mice on NS and similar between THP $^{-/-}$ and THP $^{+/+}$ mice on HS (NS THP$^{+/+}$ 0.4 ± 0.10 vs THP$^{-/-}$ 0.2 ± 0.03, P = 0.015, HS THP$^{+/+}$ 0.4 ± 0.1 vs THP$^{-/-}$ 0.34 ± 0.04, P = 0.3). It was increased by HS in THP$^{-/-}$ mice (NS 0.2 ± 0.03 vs HS 0.34 ± 0.04, P = 0.03).

**Conclusions:** Renal expression of THP is increased with chronic salt loading suggesting a role for it in sodium handling. More studies are needed to define the interactions among THP, NKCC2 and COX-2 in TAL.

*Funding:* Clinical Revenue Support

## FR-PO730

### The Sodium Chloride Cotransporter and Epithelial Sodium Channel Associate in a Complex in the Distal Convoluted Tubule

Robert S. Hoover, Hui-fang Bao, Brandi M. Wynne, Rickta Mallick, Douglas C. Eaton, Abinash C. Mistry. *Emory Univ School of Medicine, Atlanta, GA.*

**Background:** The sodium chloride co-transporter (NCC) and the epithelial sodium channel (ENaC) are two key sodium transporting proteins in the distal tubule of the mammalian kidney. Hyperactivity of either protein results in hypertension and hypoactivity results in hypotension. These two proteins are co-expressed in the second part of the distal convoluted tubule (DCT2), and are regulated by many of the same proteins. However, associations between the two have not been investigated. Therfore we investigated whether these proteins associate.

**Methods:** We utilized confocal microscopy, immunoprecipitation, and immunoblotting to asess the interaction between NCC and the subunits of ENaC.

**Results:** Utilizing a recently defined model of the mammalian DCT (mDCT15 cells) that natively expressess DCT2 proteins, including NCC and the alpha, gamma and beta subunits of ENaC, we demonstrated this association. We immmunoprecipitated NCC utilizing our immunopurified polyclonal anti-NCC antibody. Immunoblotting the immunoprecipitated sample for alpha ENaC revealed clear presence of alpha ENaC. Lysate for mDCT15 cells also demonstrated expression of alpha ENaC. Immunoprecipitation with resin only and no antibody had no ENaC. The Na-K ATPase pump and GAPDH were present in the lysate, but not the IP sample. Immunoprecipitating with anti-alpha ENaC antibody and immunoblotting with anti-NCC antibody demonstrated presence of NCC, confirming the association. Immunoprecipitating a mouse kidney cortex sample with anti-NCC antibody pulled down alpha and gamma ENaC, confirming the association in vivo. Confocal microcospy demonstrated colocalization of NCC and gamma ENaC in DCT2 cells in mouse.

**Conclusions:** NCC and ENaC co-immunoprecipitate, both in a cell culture model and in mammalian kidney cortex. This data demonstrates that these proteins associate, likely in a sodium transporting complex. This novel association between ENaC and NCC that could alter our understanding of salt transport in the distal tubule.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO731

### Augmented Calcium-Binding Protein 39 (Cab39) Expression in Renal Tubule Contributes to Salt-Sensitive Hypertension through Activating N(K)CC

Sung-Sen Yang,[1,2] Yu-wei Fang,[2] Min-hua Tseng,[2] Chih-jen Cheng,[1] Shih-Hua P. Lin.[1,2] *[1]Div of Nephrology, Dept of Medicine, Tri-Service General Hospital, Taipei, Taiwan; [2]Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan.*

**Background:** Enhanced SPAK and OSR1 kinases phosphorylation in vivo by WNK4 may lead to salt-sensitive hypertension through activating their substrates Na-(K)-(2)Cl cotransporter [N(K)CC]. Recently, it was shown that a ubiquitously expressed Cab39 protein could stimulate N(K)CC through activating SPAK/OSR1 but independent of WNK1/4 in *in vitro* study. The purpose of this *in vio* study is to uncover the physiological role of Cab39 on the regulation of SPAK/OSR1 and N(K)CC, especially in the renal tubules.

**Methods:** We generated and analyzed the kidney tubule-specific cadherin gene promoter driven flag-tagged mouse Cab39 transgenic (Tg) mice fed with normal rodent chaw. At age of 10-12 wks, phenotype including blood pressure as well as serum electrolytes was measured with or without 0.9%NaCl in tap water ad libitum.

**Results:** We obtained 8 strains of Cab39 Tg mice. However, offspring could not be got from the Cab39 Tg mice with the abundance of flag-tagged mCab39 over 50% of endogenous wild-type (WT) Cab39. Thus we chose a strain with the mildly overexpressed abundance of the flag-Cab39 (25% ±6%) in this study. With tap water, the Cab39 Tg mice were phenotypically normal but a slightly increased p-SPAK/OSR1, p-NKCC2 and p-NCC in the kidneys was found. When these mice drunk 0.9%NaCl water for 5 days, a significantly elevated blood pressure (155 ±8% in Cab39 Tg vs 135 ±7% mmHg in WT n=6, p<0.05) and slightly hyperkalemia (4.6 ±0.5 in Cab39 Tg vs 4.1 ±0.6% mEq/l in WT n=6, p=0.16) were observed in the Cab39 Tg mice. Although the amount of WT and flag-Cab39 was not affected in the kidneys of both WT and Cab39 Tg mice, the expression of p-SPAK/OSR1, p-NKCC2 and p-NCC was suppressed in WT mice but not affected in Cab39 Tg mice.

**Conclusions:** Augmented Cab39 expression in renal tubule may lead to salt-sensitive hypertension through activating SPAK/OSR1-N(K)CC signaling. Whether WNK1/4 is independent in the activating SPAK/OSR1- N(K)CC complex by the Cab39 in vivo merits further evaluation.

## FR-PO732

### Co-Localization and Fluorescence Resonance Energy Transfer Demonstrate Interaction between the Sodium Chloride Cotransporter and the Epithelial Sodium Channel

Brandi M. Wynne,[1] Alexa L. Mattheyses,[2] Abinash C. Mistry,[1] Rickta Mallick,[1] Robert S. Hoover.[1] *[1]Medicine, Renal Div, Emory Univ, Atlanta, GA; [2]Dept of Cell Biology, Emory Univ, Atlanta, GA.*

**Background:** Regulation of systemic blood pressure occurs predominately via renal maintenance of sodium and water balance. The sodium chloride cotransporter (NCC) and the epithelial sodium channel (ENaC), are both primary mechanisms for sodium reabsorption and have been found to be expressed apically in the late distal convoluted tubule (DCT2). Hyperactivity of sodium reabsorption by either protein can produce increases in blood pressure. Classically, these transporters have been thought to work independently of each other. However, regulatory systems such as aldosterone and angiotensin II for both NCC and ENaC contain appreciable overlap. Preliminary data from this laboratory suggests an interaction as shown by co-immunoprecipitation. Thus, we hypothesized that NCC and ENaC may be co-localized intracellularly, as well as interacting with each other.

**Methods:** To test this hypothesis, COS-7 cells were co-transfected with vectors containing either: GFP-tagged NCC and/or mCherry-tagged subunits of ENaC α or β. Co-localization experiments were performed along with fluorescence resonance energy transfer (FRET) acceptor photobleaching (Nikon 1AR, Emory Integrated Cellular Imaging Core).

**Results:** Transfection of COS-7 cells with GFP-tagged NCC and one subunit of mCherry-tagged ENaC (α or β) revealed significant intracellular co-localization of NCC with each ENaC subunit. Furthermore, using FRET acceptor photobleaching technology in the dual transfected (NCC+ENaC α or β), NCC+mCherry vector only or ENaC α only transfected cells, we observed a significant increase ($p<0.05$, ANOVA) in EGFP fluorescence following mCherry photobleaching. Cells co-transfected with NCC and ENaC α or β exhibited more than a 23% increase in EGFP fluorescence, as compared to NCC+mCherry vector only expressing (9.1% ± 1.9) cells.

**Conclusions:** These novel data suggest an intimate association and possible interaction between NCC and the α and β subunits of ENaC, which may influence our understanding of salt transport in the distal nephron.

*Funding:* NIDDK Support

## FR-PO733

### Hydrochlorothiazide Lowers Blood Pressure Predominantly in Volume Depleted States via Extra Renal Mechanisms That Are Independent of Na-Cl Co-Transporter (NCC)

Saeed Alshahrani,[1] Kamyar A. Zahedi,[2] Sharon L. Barone,[2] Jie Xu,[2] Manoocher Soleimani.[2] *[1]Dept of Pharmacology, Univ of Cincinnati, Cincinnati, OH; [2]Dept of Medicine, Univ of Cincinnati, Cincinnati, OH.*

**Background:** Hydrochlorothiazide (HCTZ) is an inhibitor of NCC in the distal nephron, and the most commonly used diuretic for treatment of mild hypertension. With regard to the antihypertensive effect of thiazides some investigators suggest that the primary mechanism is extra-renal and via direct vasodilatation, whereas others emphasize that the initial diuresis and salt depletion is necessary, indicating that vasodilatation is secondary to ECF contraction. We propose that HCTZ reduces blood pressure largely via extra-renal effects that are exaggerated in volume-depleted states, and that these effects are independent of NCC inhibition.

**Methods:** Age and sex matched wild type (WT), Pendrin KO, NCC KO and NCC/Pendrin double knock out (dKO) mice, which are severely volume depleted, were treated with HCTZ (40mg/kg/day) for 4 days. Balanced studies were performed and urine samples were analyzed for salt and fluid excretion. In parallel studies the blood pressure (BP) was determined by computerized tail cuff method.

**Results:** 24hr salt and urine production did not change significantly in WT mice treated with HCTZ. In NCC/pendrin-dKO mice, HCTZ caused multiple systemic derangements, including a significant reduction in urine output and salt excretion. The reduction in urine output of dKO mice as a result of HCTZ treatment was accompanied by a significant drop in their BP. WT mice did not display any significant effect on their BP in response to HCTZ treatment. HCTZ did not have a significant effect on BP of pendrin- or NCC-KO mice, both of which have normal vascular volume, but it increased the urine output in pendrin-KO mice, indicating the important role of pendrin in compensatory salt absorption in response to NCC inhibition/ablation.

**Conclusions:** 1. HCTZ reduces BP independent of the kidney function and NCC inhibition. 2. The antihypertensive effect of HCTZ is exaggerated in volume-depleted states. 3. Current studies will test the hypothesis that thiazides activate the vasodilatory signaling pathway(s) in volume-depleted states.

*Funding:* NIDDK Support, Veterans Affairs Support, Government Support - Non-U.S.

## FR-PO734

**Regulation of NCC by Angiotensin II in Response to Low K Diet** Jie Liu,[1] Richard A. Coleman,[1] P. Richard Grimm,[1] Eric J. Delpire,[2] Paul A. Welling,[1] James B. Wade.[1] *[1]Dept of Physiology, Univ of Maryland School of Medicine, Baltimore, MD; [2]Dept of Anesthesiology, Vanderbilt Univ School of Medicine, Nashville, TN.*

**Background:** Low K diet stimulates the renal NaCl cotransporter (NCC) by an unknown mechanism. Since the STE20/SPS-1-related proline-alanine-rich protein kinase (SPAK) can function to stimulate NCC by phosphorylation of specific N-terminal sites, we investigated if the acute response of NCC to low K diet is mediated by SPAK and is associated with changes in angiotensin II.

**Methods:** Using phospho-specific antibodies in Western blot and immunolocalization studies of wild type (WT) and SPAK$^{-/-}$ mice fed a low K or control diet for 4 days, we examined the effect of K diet on expression and phosphorylation of NCC and SPAK. Levels of angiotensin II and aldosterone were measured by enzyme immunoassay.

**Results:** In WT, we found that Low K diet more than doubled total NCC expression and phosphorylation of NCC at the T58 site. This was associated with an increase in total SPAK expression in cortical homogenates and an increase in phosphorylation of SPAK at the S383 activation site. By contrast, Low K diet had no effect on NCC or pNCC in SPAK knock-out mice. The increased NCC abundance and phosphorylation with low dietary potassium occurred concomitantly with an increase in angiotensin II but not aldosterone.

**Conclusions:** These findings indicate that SPAK is a critical mediator of increased pNCC in dietary K restriction. The findings support the idea that low K increases angiotensin II to increase SPAK-dependent phosphorylation of NCC. Such a response would limit Na/K exchange in CNT/CCD segments to limit urinary K loss with Low K diet.

*Funding:* NIDDK Support

## FR-PO735

**Ovarian Steroids Recover the NaCl Cotransporter Activation/ Phosphorylation in Gonadectomized Female Rats** Lorena Leonor Rojas,[1] Luis Antonio Reyes Castro,[2] Juliette Hadchouel,[3] Elena Zambrano,[2] Gerardo Gamba.[1] *[1]Molecular Physiology Unit, INNSZ-IIB, UNAM, Mexico City; [2]Reproductive Biology Dept, INNSZ, Mexico City; [3]INSERM, Paris, France.*

**Background:** The thiazide-sensitive NaCl cotransporter NCC is subjected to sexual dimorphic regulation in rats. One study showed that response to thiazide diuretics was higher in female than in male rats (JASN, 1994) and another revealed, in the distal convoluted tubule, that NCC expression of ovariectomized rats is increased by estradiol (JCI, 1998). Little is known, however, about the mechanisms and the role of other female hormones.

**Methods:** We analyzed in rats the NCC activity by urinary response to bendroflumethiazide (BFTZ), as well as the total and phospho-T58 NCC, total and phospho-S383-SPAK expression, using specific antibodies, in proteins from kidneys of control and gonadectomized male and female rats, four weeks after sham or gonadectomy. Wild type and prolactin receptor knockout (PRLR$^{-/-}$) female and male mice were also studied. Serum radioimmunoanalysis confirmed the absence of sex hormones in gonadectomized animals. To assess if ovarian hormones or prolactin restore the activity/phosphorylation of NCC, ovariectomized rats were treated with 30 µg/kg of 17-β-estradiol or 50 mg/kg of progesterone i.p for three weeks, or one adenohypophisis was transplanted into the renal capsule to produce hyperprolactinemia.

**Results:** The urinary response to BFTZ was higher in female than in male rats. No difference was observed in total NCC expression, but phospho-T58-NCC was significantly higher in female than in male rats. This was associated with increased expression and p-S383 SPAK in female rats. The differences were eliminated by ovariectomy. No effect of orquiectomy on NCC and SPAK expression/phosphorylation was observed. NCC phosphorylation in ovariectomized rats was recovered by 17-β-estradiol and progesterone administration. Hyperprolactinemia did not recover NCC phosphorylation. In addition, the higher NCC phosphorylation in female rats persisted in PRLR$^{-/-}$ animals.

**Conclusions:** We conclude that sexual dimorphic increase of NCC phosphorylation in female rats is dependent on ovarian steroidal hormones, with little to no participation of prolactin.

*Funding:* Government Support - Non-U.S.

## FR-PO736

**Clinical Significance of Urinary Thiazide-Sensitive Na-Cl Cotransporter (NCC) Measurement by Newly Developed Enzyme-Linked Immunosorbent Assays** Kiyoshi Isobe, Takayasu Mori, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Graduate School of Medicine, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** Na-Cl cotransporter (NCC) in the distal tubules in kidney is known to be excreted in urine. However, its clinical significance has not been established because of the lack of quantitative data of urinary NCC.

**Methods:** We developed highly sensitive enzyme-linked immunosorbent assays (ELISAs) for urinary total NCC (tNCC) and its active form, phosphorylated NCC (pNCC).

**Results:** We first measured the excretion of tNCC and pNCC in urinary exosomes in pseudohypoaldosteronism type II (PHAII) patients since PHAII is caused by NCC activation. Highly increased excretion of tNCC and pNCC was observed in PHAII patients, indicating that both assays could detect the increases of urinary NCC excretion caused by NCC activation in the kidney. Then, we measured tNCC and pNCC in the urine of outpatients with chronic kidney disease (CKD). Urinary pNCC was positively correlated with the estimated glomerular filtration rate (eGFR) and became undetectable when eGFR was less than 16.8 ml/min/1.73 m$^2$, which is consistent with the clinical observation that thiazide has less effect in patients with such low eGFR. pNCC excretion was also found to be negatively correlated with the fractional excretion of sodium. These data in CKD patients further validate that the ELISA systems for urinary NCC are valuable tools for estimating NCC activity in vivo. Moreover, an link between serum phosphorus and urinary NCC was observed.

**Conclusions:** Thus, the measurements of NCC, especially pNCC, in human urinary exosomes would provide novel information about the status and the regulation of NCC in the human kidney.

*Funding:* Government Support - Non-U.S.

## FR-PO737

**Unscrambling the Molecular Mechanism of Calcineurin Inhibitors-Induced Hypertension after Kidney Transplantation** Pedro San-Cristobal,[1] Marleen L.A. Kortenoeven,[2] Robert A. Fenton,[2] Joost Hoenderop,[1] René J. Bindels.[1] *[1]Physiology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Biomedicine, Aarhus Univ, Aarhus, Denmark.*

**Background:** To survive, end stage renal disease patients must immediately undergo either: *a*) dialysis treatments or *b*) kidney transplantation. Transplantation is currently the best method of treatment. Calcineurin inhibitors (CNI) are the foundation of immunosuppressive management after transplantation. CNI diminish the graft rejection rate, but have serious side-effects leading to a phenotype similar to Pseudohypoaldosteronism II. Overactivity of the thiazide-sensitive NaCl cotransporter (NCC) is the postulated mechanism of action. Indeed, patients with CNI-induced hypertension exhibit features that fit with an augmented activity of NCC.

**Methods:** We developed a unique procedure to isolate a highly enriched preparation of fluorescently labelled distal convoluted tubules (DCT) from transgenic mice employing an advanced tubular sorter. Using primary cultures of these isolated segments, a reproducible methodology to measure NCC-mediated $^{22}Na^+$ reabsorption in DCT cells was established.

**Results:** Our data showed that incubation with 5 µM CNI (Cyclosporine A or Tacrolimus) significantly stimulates the thiazide-dependent $^{22}Na^+$ transport across the primary DCT cultures. The recorded response to CsA was higher compared to control groups treated with hypotonicity, or angiotensin II (NCC stimulatory maneuvers). In line with our hypothesis, 100 µM hydrochlorothiazide added to the media prevented the NCC enhanced response. Furthermore, time courses from 1.5 to 24 hr showed similar enhanced $^{22}Na^+$ transport. Finally, in mouse kidney lysates CsA phosphorylated NCC in a dose and time response.

**Conclusions:** This study supports the hypothesis that CNI-induced hypertension is caused by a rise in NCC activity involving a phosphorylation of the amino-terminal domain at Threonine 58. In parallel, produce a molecular hallmark of the mechanism involved in CNI-induced hypertension. This could be the first step towards designation and implementation of future clinical trials to develop new guidelines for transplanted patients.

*Funding:* Government Support - Non-U.S.

## FR-PO738

**KLHL3 Modulates Kidney-Enriched WNK Kinase Isoforms to Modulate NCC** Chong Zhang,[1] Nick Meermeier,[1] Andrew Terker,[1] Maria Chavez-Canales,[3] Gerardo Gamba,[3] Juliette Hadchouel,[2] David H. Ellison,[1] Chao-Ling Yang.[1] *[1]Nephrology & Hypertension, Oregon Health & Science Univ, Portland, OR; [2]Paris Cardiovascular Research Center, INSERM-U970, Paris, Namibia; [3]Molecular Physiology Unit, Universidad Nacional Autónoma de México,, Mexico City, Mexico.*

**Background:** Mutations of two components of the cullin-RING ligase complex, Cul3 and KLHL3, cause familial hyperkalemic hypertension (FHHt). It has been reported that WNK1 and WNK4 bind to KLHL3, fostering ubiquitylation and degradationvia the proteasome. It has been postulated that the FHHt-causing KLHL3 or Cul3 mutations impairtheir ability to ubiquitylate WNKs, leading to accumulation of WNK1 and WNK4, activating NCC.

**Methods:** We used transient transfection in HEK293 cells followed by western blotting. We also used overexpression in Xenopus oocytes, with measurement of 22Na uptake.

**Results:** Surprisingly, KLHL3 did not affect endogenous WNK1 and WNK3 in HEK293 cells, although it degraded WNK4 strongly. Alternative splicing generatesdifferent isoforms of WNK kinases in a tissue-specific manner, so we compared effects of WNK1, KS-WNK1, and a WNK1 D11, an isoform highly expressed in the kidney. We also tested forms of WNK3expressed preferentially in brain(B-WNK3) and kidney (K-WNK3). Like WNK4, K-WNK3 and WNK1 D11 were degraded more than 70% at the protein level, whereas L-WNK1 was unchanged. We have reported WNK kinase form signaling complex via protein interaction and phosphorylation to regulate NCC activity. In the mammalian or Xenopusoocyte expression system, WNK4 inhibits NCC, whereas WNK1 and WNK3 activate it. Since WNK isoforms, regulated by KLHL3, are all expressed in the DCT, mutations in KLHL3 must affect not only WNK4, but also WNK1 D11 and K-WNK3. In agreement with this hypothesis, co-expression of wild type KLHL3 reduced Na uptake in oocytes expressing NCC. In contrast, FHHt-mutant KLHL3 stimulated Na uptake to more than 150%, suggesting that the mutations are not simply loss of function.

**Conclusions:** KLHL3 exerts differential effects on renal and extrarenal WNK isoforms; FHHt-mutant KLHL3 mutations may exert dominant effects on NCC activity.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO739

**Acidic Motif in WNKs Are Crucial Components for the Binding with KLHL3** Akihito Ohta,[1] Shinichi Uchida,[1] Sei Sasaki,[1] Dario Alessi.[2] *[1]Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan; [2]MRC Protein Phosphorylation Unit, College of Life Sciences, Univ of Dundee, Dundee, United Kingdom.*

**Background:** Pseudohypoaldosteronism typeII (PHAII) is an autosomal-dominant disorder characterized by hyperkalemia, metabolic acidosis, and hypertension. WNK1 and WNK4 gene mutations were reported to cause PHAII. Lifton and another group identified KLHL3 and CUL3 mutations in PHAII, suggesting these two proteins may also form CRL E3 complex that regulates blood pressure. After these reports, we started to search for KLHL3-interacting partner and analyze how these KLHL3-CUL3 complex are related to the pathogenesis causing PHAII.

**Results:** We generated Wild-type KLHL3 and mutant KLHL3 expressing 293 cell lines and immunoprecipitated KLHL3 interacts with CUL3 and WNKs. Some KLHL3 mutations causing PHAII lost the binding with WNKs but WT- KLHL3 could bind with WNKs in 293 cell. To further analyze which parts of WNK1 interact with KLHL3, WNK1 fragments were co-transfected in WT-KLHL3 293 cell lines and found WNK1(479-667) fragment containing coiled-coil domain could bind WT-KLHL3. WNK1 fragments mutated in E633K ,D635A and Q636E which are same sites reported in PHAII WNK4 patients, also transfected in KLHL3 cell and immunoprecipitated mutated WNK1 fragments could lose binding ability of WT-KLHL3, indicating these mutated sites in not only WNK4 but WNK1 also are important for the KLHL3 interaction. To narrow the site which part interacts between WNK1 and KLHL3, small WNK1 fragments in WNK1(479-667) were transfected and found that acidic motif in WNK1 should be crucial for the bind with WT-KLHL3. With the evidence that this motif are conserved in another WNKs(WNK1-4), WT-KLHL3 or PHAII mutant (N529K) KLHL3 were transfected in WNK2, WNK3 293 cell lines and found WNK2 or WNK3 could bind WT-KLHL3 but not mutant KLHL3 (N529K).

**Conclusions:** These results indicate that acidic motif in WNKs are crucial components for the binding with KLHL3 and speculating that abolishing the ability to interact between WNKs and KLHL3 in PHAII, modulating the ubiquitilation WNK isoforms cause that the accumulation of WNKs which leads to abnormal sodium absorption in kidney causing hypertension.

*Funding:* Pharmaceutical Company Support - AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Merck KgaA, Janssen Pharmaceutica and Pfizer., Government Support - Non-U.S.

## FR-PO740

**Low Salt Intake Decreased Transcription and Protein Level of KLHL3 in Mouse Kidney** Koichiro Susa, Eisei Sohara, Moko Zeniya, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** WNK-OSR1/SPAK-NaCl cotransporter (NCC) signal cascade is important for regulating salt balance and blood pressure in the body. It is well known that low salt diet activates and high salt diet inhibits this cascade, leading to regulation of sodium reabsorption through NCC. Recently, we found that Cullin3-KLHL3 E3 ligase complex interacted with acidic motif of WNK kinase family and induced WNK4 ubiquitination, leading to reduction of the WNK4 protein level. However, physiological regulation of KLHL3 by dietary salt intake is still unclear. In this study, we sought to determine whether dietary salt intake regulates the transcription the protein level of KLHL3.

**Methods:** Male C57BL/6J mice fed high-salt, normal or low-salt diet were sacrificed, and their kidneys were dissected. The protein expression levels of KLHL3, WNK1, WNK4 were determined by immunoblots. mRNA levels of KLHL3 were determined by real-time PCR.

**Results:** Both mRNA and protein level of KLHL3 were decreased in the kidney of mice fed low-salt diet, compared to those fed normal diet. Concomitantly, WNK1 and WNK4 protein levels were increased in the kidney of mice fed low salt diet, indicating that decreased expression of KLHL3 resulted in increased protein expression of WNK1 and WNK4 under low-salt diet, leading to activation of WNK-OSR1/SPAK-NCC phosphorylation cascade. On the contrary, protein levels of KLHL3 were increased in the kidney of mice fed high salt diet, although mRNA was not increased. WNK1 and WNK4 protein levels were not decreased in the kidney of mice fed high salt diet neither.

**Conclusions:** We demonstrated that low-salt intake decreases the transcription and the protein level of KLHL3 in mouse kidney. This could be one of the physiological mechanisms how low salt diet activates WNK-OSR1/SPAK-NCC phosphorylation cascade.

*Funding:* Government Support - Non-U.S.

## FR-PO741

**Modest Increase in Plasma [$K^+$] Reduces Na-Cl Cotransporter (NCC) and STE20/SPS1-Related Proline Alanine-Rich Kinase (SPAK) Phosphorylation and Drives Kaliuresis and Natriuresis** Alicia A. McDonough,[1] Srinivas Rengarajan,[1] Donna Lee,[1] Youngtaek Oh,[2] Jang Youn.[2] *[1]Cell and Neurobiology, Keck School of Medicine of USC, Los Angeles, CA; [2]Physiology, Keck School of Medicine of USC, Los Angeles, CA.*

**Background:** Dietary potassium ($K^+$) loading results in rapid kaliuresis, natriuresis and diuresis. We aimed to test the hypothesis that the diet provoked increase in plasma [$K^+$] *per se* drives the acute kaliuresis in Sprague Dawley rats.

**Methods:** In the first protocol, after overnight fast, rats were fed a 2% $K^+$ meal for 3 hr and compared to control rats fed a 0% $K^+$ meal. In the second protocol, plasma [$K^+$] was raised by tail vein infusion over a period of 3 hr to 5.5 ± 0.12 mEq in overnight-fasted conscious rats to mimic the change measured after meal feeding; controls were also fasted and received a matched amount of saline.

**Results:** The 2% $K^+$ meal increased plasma [$K^+$] from 4.01±0.12 to 5.18±0.16 mEq, with no change in plasma [$Na^+$] or kallikrein; The 2% $K^+$ meal also decreased abundance of phosphorylated NCC (NCCp) and cortical SPAK (SPAKp) to 40% and 60% of control, respectively; abundance of cortical Na/H exchanger 3 (NHE3), Na-K-2Cl cotransporter (NKCC), NKCCp, epithelial $Na^+$ channel (ENaC) subunits and renal outer medulla $K^+$ channel (ROMK) were unchanged. Raising plasma [$K^+$] by tail vein infusion also provoked significant kaliuresis and natriuresis compared to controls, decreased NCCp and SPAKp to 40% and 60% of control, respectively, and did not alter NHE3, NKCC, NKCCp, NCC or SPAK.

**Conclusions:** In summary, a modest increase in plasma [$K^+$], equivalent to that observed in response to a single $K^+$ rich meal, triggers renal kaliuretic and natriuretic responses likely driven by decreased abundance of SPAKp and NCCp. These changes can drive tubular $Na^+$ reabsorption from the distal convoluted tubule to the collecting duct where $Na^+$ reabsorption drives $K^+$ secretion. We conclude that a modest increase in plasma [$K^+$] can drive acute homeostatic kaliuresis via depressing NCCp and activity.

*Funding:* NIDDK Support

## FR-PO742

**A Dithiothreitol-Sensitive Kidney Protease Cleaves SPAK to Regulate NaCl Co-Transporters** Nicolas Markadieu,[1] Paul A. Welling,[2] Eric J. Delpire.[1] *[1]Anesthesiology, Vanderbilt Univ, Nashville, TN; [2]Physiology, Univ of Maryland, Baltimore, MD.*

**Background:** Distal $Na^+$ transport in the mammalian kidney is mediated in part by Na-K-2Cl and Na-Cl cotransporters. The cotransporters are regulated by a variety of dietary conditions and hormones that converge to their phosphorylation and activation. SPAK and OSR1 are Ste20-like serine/threonine kinases which bind, phosphorylate, and activate NKCC2 and NCC. The molecular size of SPAK is slightly larger than the size of OSR1, due to the presence of an N-terminal proline-alanine rich domain. In kidney medulla, SPAK also exists as smaller C-terminal fragments, which identities are not fully understood.

**Methods:** Recombinant SPAK proteins were purified from bacteria and incubated with cell extracts under different conditions. Proteolytic cleavage was monitored by Western blot analysis.

**Results:** We show that protein lysates prepared from mouse kidney possess protease activity that leads to the cleavage of SPAK into several fragments. The size of these fragments is consistent with the size of the fragments observed in mouse kidney. The activity is not present in protein lysates prepared from brain, liver, and spleen. The activity is also found in rabbit and human kidney lysates. We also show that the activity is time and concentration-dependent, and occurs at temperatures ranging from 4°C to 45°C. The protease activity is resistant to a variety of protease inhibitors such as aprotinin, leupeptin, pepstatin, PMSF and EDTA. The activity is also insensitive to doxycycline and $CuSO_4$, suggesting that it is likely not due to the activity of a metalloprotease. Finally, we show that the activity is prevented by the reducing agent dithiothreitrol (DTT) and this effect can be reversed by treatment with N-ethylmaleimide or 5,5'-dithiobis-(2-nitrobenzoid acid) (DNTB). As cleavage is observed using a cystein-less SPAK protein, the DTT effect indicates the presence of key sulfhydryl groups within the protease.

**Conclusions:** Our data indicate that the SPAK fragments observed in kidney could be the product of kidney-specific protease(s). Efforts are taken to identify the sites of cleavage and the identity of the protease(s) involved.

*Funding:* NIDDK Support

## FR-PO743

**Aldosterone Modulates NCC via Altering Its Interaction with 14-3-3 Gamma** Xiuyan Feng,[1,4] Yiqian Zhang,[3] Matthew Lee,[1,4] Guangping Chen,[2] Dingying Gu,[3] Hui Cai.[1,2,4] *[1]Renal Div, Dept of Medicine, Emory Univ School of Medicine, Atlanta, GA; [2]Dept of Physiology, Emory Univ School of Medicine, Atlanta, GA; [3]Dept of Nephrology, The 2nd Affiliated Hospital, Wenzhou Medical Univ, Wenzhou, Zhejiang, China; [4]Renal Section, Atlanta VA Medical Center, Decatur, GA.*

**Background:** 14-3-3 gamma belongs to a family of multifunction regulatory proteins that mainly bind to phosphorylated Ser/Thr residues in the target proteins. Aldosterone was shown to increase NCC abundance via SGK1 and Nedd4-2 pathway. Interaction of Nedd4-2 with 14-3-3 was shown to involve the regulation of epithelial sodium channel (ENaC). Previous data have shown that 14-3-3 disrupted Nedd4-2-mediated ubiquitination of ENaC, leading to increase in ENaC activity.

**Methods:** Cell cultures, transfection, immunoprecipitation and western blot analysis were used in the study.

**Results:** To determine whether 14-3-3 is involved in aldosterone-mediated regulation of NCC, we have tested the effect of 14-3-3 gamma on NCC protein expression. We found that 14-3-3 gamma down-regulated total NCC and membrane NCC protein expressionsin a dose-dependent manner in HEK293 cells transiently transfected with GFP-NCC and 14-3-3 gamma plasmids. Aldosterone treatment (10 μM, for 3 hours) increased both total and membrane NCC protein expression in HEK293 cells transfected by GFP-NCC and 14-3-3 gamma, while decreasinginteraction of NCC with 14-3-3 gamma. We also found that aldosterone increased both total and membrane NCC expression via decreasing NCC ubiquitination.

**Conclusions:** These data suggest that aldosterone increases NCC expression through deceasing binding of 14-3-3 gamma to NCC, and therefore decreasing NCC ubiquitination.

*Funding:* Veterans Affairs Support, Private Foundation Support

## FR-PO744

**RNA Profiling Reveals Compensatory Up-Regulation of Intercalated Cell Transcriptome in SPAK-Null Mice Having a Gitleman-Like Phenotype** P. Richard Grimm,[1] Eric J. Delpire,[2] James B. Wade,[1] Paul A. Welling.[1] [1]*Physiology, Univ of Maryland School of Medicine, Baltimore, MD;* [2]*Anesthesiology, Vanderbilt School of Medicine, Nashville, TN.*

**Background:** An adaptive increase in salt reabsorption preserves salt balance when the thiazide-sensitive sodium-chloride cotransporter (NCC) is lost in Gitleman Syndrome or NCC phosphorylation is lost in Ste20/SPS-1 related proline-alaine-rich protein kinase (SPAK) null mice. To explore the mechanism of adaptive salt reabsorption, global-transcriptome analysis of the renal cortex from WT and SPAK-null animals was performed.

**Methods:** RNA was extracted from the cortex of SPAK-null and WT littermate mice, microarray analysis was performed using Affymetrix GeneChip (Mouse Gene 1.0 ST) containing probes of all mouse genes and analyzed on an Affymetrix GeneChip 3000 system. Quality control analysis, Robust Multi-array Averaging, and Linear Model for Microarray based differential expression analysis were subsequently performed. The altered expression of highlighted genes was verified by RT-PCR.

**Results:** Statistical analysis indicated that 176 genes were down-regulated and 263 genes were up-regulated significantly in the SPAK-null cohort compared to WT. Intercalated cell (ICs) markers, transporters and enzymes were especially prevalent, including pendrin, the sodium-dependent chloride bicarbonate exchanger, anion-exchanger 4, carbonic anhydrase 4 and 15, and the B1-, C2-, G3-, and d2-subunits of the V-type $H^+$-ATPase. Significantly, these transport components represent a system recently proposed to facilitate thiazide-sensitive sodium-chloride reabsorption in the β-ICs. These changes were paralleled by an increase in transcript abundance of the β- and γ-subunits of ENaC. Specific distal nephron fate factors and signaling elements were also significantly up-regulated, highlighting potential molecular pathway(s) that underlie the adaptive process.

**Conclusions:** Transcriptome-profiling reveals intercalated cell sodium-chloride transport is up-regulated to preserve sodium balance in the absence of NCC phosphorylation.

*Funding:* NIDDK Support

## FR-PO745

**Chemical Library Screening for WNK Signaling Inhibitors by Using Fluorescent Correlation Spectroscopy** Takayasu Mori, Eriko Kikuchi, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Graduate School of Medical and Dental Sciences, Bunkyo-ku, Tokyo, Japan.*

**Background:** We have shown that WNK-oxidative stress-responsive 1 (OSR1)/STE20/SPS1-related proline-alanine-rich protein kinase (SPAK)-Slc12a transporters cascade regulates blood pressure by controlling vascular tone as well as renal NaCl excretion. Therefore, agents that inhibit this signal cascade could be a new class of antihypertensive drugs. Since the binding of WNK to OSR1/SPAK kinases was postulated to be important for signal transduction, we sought to discover inhibitors of WNK-SPAK binding by screening chemical compounds that disrupt the binding. For this purpose, we established a system capable of high-throughput screening to detect the binding of two molecules.

**Methods:** Fluorescence correlation spectroscopy (FCS) was used to detect the binding. Fluorescent TAMRA-labeled small peptides of WNK4 were mixed with the GST-SPAK with different concentrations in the wells of 384-well microtiter plates.

**Results:** We succeeded in detecting the binding of SPAK to WNK in FCS assay, and we used this newly developed system to screen compounds. As a result of screening 17,000 compounds, we discovered two novel compounds that reproducibly disrupted the binding of WNK to SPAK. To improve the drug activity and toxicity, we performed derivative expansion of the hit compound. One of the 30 derivatives showed more potent inhibitory activity than the hit compound ($IC_{50}$ : 8.1 μM). Furthermore, the derivative showed a dose-dependent inhibitory effect on the phosphorylations of NCC and NKCC1 in the mouse kidney and on NKCC1 phospshorylation in the mouse aorta. These results indicate that the derivative functions in vivo, and the vasodilatory effect was also expected.

**Conclusions:** The compounds obtained in this study could be promising seeds of new types of antihypertensive drugs, and the method that we developed could be applied to any screening to identify compounds that disrupt the binding of two molecules.

*Funding:* Government Support - Non-U.S.

## FR-PO746

**Sp1 Trans-Activates and Is Required for Maximal Aldosterone Induction of the αENaC Gene in Collecting Duct Cells** Zhiyuan Yu, Qun Kong, Bruce C. Kone. *The Univ of Texas Medical School at Houston, Houston, TX.*

**Background:** The epithelial Na channel (ENaC) in distal nephron constitutes the rate-limiting step for renal Na reabsorption. Aldosterone increases αENaC transcription in the collecting duct. Af9 binds to +78/+92 of the αENaC promoter and recruits Dot1a to repress basal and aldosterone-sensitive αENaC transcription in mIMCD3 cells. Despite this epigenetic repression, basal αENaC transcription is still evident and physiologically necessary, indicating basal operation of positive regulators.

**Methods:** mIMCD3 cells were treated with vehicle or 1 μM aldosterone, with or without 1 μM spironolactone, in charcoal-stripped medium. Gel shift, antibody competition, ChIP/qPCR and re-ChIP assays were used to measure interactions of Sp1, Dot1a, and Af9 with the αENaC promoter. Af9 and Sp1 binding site mutant promoter-reporter genes were stably transfected in mIMCD3 cells and used for promoter activity and ChIP/qPCR assays. Sp1 levels were manipulated by overexpression and siRNA knockdown.

**Results:** Gel shift and antibody competition assays with a +208/+240 αENaC probe revealed DNA-Sp1-containing complexes. Mutation of +222/+229 abrogated Sp1 binding in vitro and in stably expressed promoter-reporter constructs. Compared to the wild type promoter, a +222/+229 mutant αENaC promoter-luciferase construct lower activity and impaired Sp1 trans-activation. Conversely, Sp1 knockdown inhibited endogenous αENaC mRNA and the activity of the wild type αENaC promoter but not the mutated construct. Aldosterone triggered Sp1 recruitment to the αENaC promoter, which was required for maximal promoter activity and was blocked by spironolactone. ChIP/re-ChIP assays and functional tests of +78/+92 and +222/+229 αENaC promoter mutants indicated that while Sp1, Dot1a, and Af9 co-occupy the αENaC promoter, the Sp1 effects are functionally independent from Dot1a and Af9.

**Conclusions:** Sp1 binding to +222/+229 of the αENaC promoter independently offsets Dot1a-Af9-mediated epigenetic repression to foster basal αENaC transcription in mIMCD3 cells. Moreover, aldosterone-mediated, and mineralocorticoid receptor-dependent enrichment of Sp1 at this element is required for maximal aldosterone induction of the gene.

*Funding:* NIDDK Support

## FR-PO747

**Biphasic Regulation of the Epithelial Sodium Channel by Inflammation and the IKK/NF-κ Pathway** Hui Li, Eric D. Morrell, Robert S. Edinger, Nuria M. Pastor-Soler, Kenneth R. Hallows. *Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** The kidney collecting duct epithelial sodium channel (ENaC) is critical for hormonal regulation of volume and blood pressure but also responds to pathologic conditions like sepsis-induced inflammation. We have previously shown that the inflammatory mediator IκB kinase-β (IKKβ) stimulates ENaC by phosphorylating the ubiquitin ligase Nedd4-2. Others have observed that the downstream mediator NF-κB inhibits ENaC. To define the time course and mechanisms of ENaC regulation by this pathway, we treated polarized epithelial cells with lipopolysaccharide (LPS) or TNFα, activators of this pathway, or IKK inhibitor and measured ENaC whole-cell and surface expression levels and currents.

**Methods:** Mouse polarized kidney cortical collecting duct ($mpkCCD_{c14}$) and primary airway epithelial cells were cultured on Transwells and then treated with 100 ng/ml LPS or TNFα prior to measurements. Amiloride-sensitive short-circuit currents were measured in Ussing chambers or with an epithelial volt-ohmmeter. Immunoblots, cell surface biotinylation assays and co-immunoprecipitations were performed to measure changes in ENaC whole-cell and apical surface expression and binding to Nedd4-2. Confocal microscopy was performed on immunofluorescently labeled ex vivo kidney slices to examine changes in ENaC subcellular localization in response to IKK modulation.

**Results:** ENaC activity increased at 1 h but decreased by 18 h in response to activation of the IKK/NF-κB pathway by TNFα treatment in primary airway epithelial cells. This decrease correlated with decreased α-ENaC and mineralocorticoid expression. Overnight treatment of $mpkCCD_{c14}$ cells with either TNFα or LPS similarly decreased ENaC currents and also β- and γ-ENaC apical surface expression. TNFα acutely decreased the binding of Nedd4-2 to β-ENaC, an effect that was reversed by treatment with IKK inhibitor. Finally, acute TNFα treatment of kidney slices induced apical translocation of β-ENaC, which was blocked in the presence of IKK inhibitor.

**Conclusions:** Biphasic ENaC regulation by the IKK/NF-κB pathway may help integrate the response of ENaC and epithelial salt transport to hormonal and inflammatory stimuli.

*Funding:* NIDDK Support

## FR-PO748

**ENaC Inhibition Stimulates Conductive H and Cl Secretion in the Mouse Cortical Collecting Duct** Masayoshi Nanami,[1] Hui-fang Bao,[1] Alan Mark Weinstein,[2] Takeshi Nakanishi,[3] Douglas C. Eaton,[1] Susan M. Wall.[1] [1]*Medicine and Physiology, Emory Univ School of Medicine, Atlanta, GA;* [2]*Medicine, Weill Medical College of Cornell Univ, New York, NY;* [3]*Medicine, Hyogo College of Medicine, Nishinomiya, Japan.*

**Background:** Inhibiting the epithelial $Na^+$ channel (ENaC) reduces $Cl^-$ absorption in the cortical collecting duct (CCD). Since ENaC does not transport $Cl^-$, the purpose of this study was to explore how ENaC modulates $Cl^-$ absorption in mouse CCDs perfused in vitro.

**Methods:** Wild type or collecting duct-specific ENaC null mice consumed a NaCl-replete diet or diet with aldosterone administered by minipump. CCDs were perfused in vitro in the presence and absence of an ENaC inhibitor (benzamil, 3 mM) in the luminal fluid.

**Results:** Application of an ENaC inhibitor (benzamil, 3 mM) to the luminal fluid unmasked conductive $H^+$ and $Cl^-$ secretion. Benzamil-sensitive $HCO_3^-$ and $Cl^-$ flux were reduced or abolished with the application of the non-selective $Cl^-$ channel blocker, DIDS, or the $H^+$-blocker, bafilomycin to the perfusate. Similarly, ENaC gene ablation unmasked DIDS-sensitive Cl- secretion. Single channel recordings of intercalated cell apical membrane in split open CCDs demonstrated a stilbene-sensitive $Cl^-$ channel with properties that resembles ClC-5.

**Conclusions:** 1) In CCDs from aldosterone-treated mice, most $Cl^-$ absorption is benzamil-sensitive, 2) Benzamil application stimulates stilbene- and bafilomycin-sensitive conductive secretion of $Cl^-$ and $H^+$, and 3) an intercalated cell apical membrane $Cl^-$ channel may mediate benzamil-sensitive $Cl^-$ secretion.

*Funding:* NIDDK Support

**FR-PO749**

**An Exon Deletion Activates the Epithelial Sodium Channel** Jingxin Chen, Thomas R. Kleyman, Shaohu Sheng. *Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** Epithelial $Na^+$ channels (ENaC) have essential roles in the regulation of extracellular fluid volume and blood pressure. The three ENaC subunits typically present in functional channel complexes in epithelia are encoded by their corresponding multi-exon genes that have a similar architecture. Common isoforms of all three human ENaC subunits (α, β and γ) are translated from mature mRNAs that are similarly spliced with inclusion of 13 exons. While the channel pore is formed by amino acid residues encoded by exons 2 and 13, ten exons (3 through 12) encode the large extracellular region.

**Methods:** We examined whether exons 3 through 12 were required for functional ENaC expression by generating a series of exon deletions. Human ENaCs with in-frame deletions of individual exon sequences in α subunit cDNAs were expressed in *Xenopus* oocytes and their functional properties were examined by two-electrode voltage clamp.

**Results:** Deletion of any of these exon-encoding sequences eliminated channel activity, with the exception of exon 11 that encodes the knuckle domain. Cells expressing channels with an α subunit exon 11 deletion showed 4-fold greater amiloride-sensitive currents than oocytes expressing wild type (WT) channels. The increased channel activity primarily reflected an increased channel open probability (5-fold greater than WT), as both levels of surface expression and single channel current were similar to WT. Surprisingly, deletion of exon 11 from either the β or γ subunit diminished channel activity, reflecting a reduced level of surface expression.

**Conclusions:** Our results suggest that the α subunit knuckle domain regulates channel gating.

*Funding:* NIDDK Support

**FR-PO750**

**Specific Inactivation of the Epithelial Sodium Channel (ENaC) in Connecting Tubule: Effect on Sodium and Potassium Homeostasis** Søren Brandt Poulsen,[1] Jeppe Praetorius,[1] Lance Miller,[2] Raoul D. Nelson,[2] Edith Hummler,[3] Birgitte M. Christensen.[1] *[1]Aarhus Univ; [2]Univ of Utah School of Medicine; [3]Univ of Lausanne.*

**Background:** In the kidney, ENaC is crucial for $Na^+$ reabsorption, but it is also involved in the excretion of $K^+$. ENaC is expressed in the late distal convoluted tubule, connecting tubule (CNT), and collecting duct (CD), where it is tightly regulated by the steroid hormone aldosterone. Inactivation of the αENaC subunit in the CD does not disturb $Na^+/K^+$ balance in mice (Rubera et al, JCI 2003). However, αENaC deletion in both the CNT and CD causes symptoms of pseudohypoaldosteronism type 1 as evidenced by disturbance in $Na^+$ and $K^+$ balance on standard and challenging diets (Christensen et al, JASN, 2010).

**Methods:** To further investigate the significance of αENaC in the CNT, mice with Cre-recombinase expressed under the control of the V-ATPase B1-subunit promoter (these mice also express Cre in a portion of the CNT cells, Miller et al, KI, 2009) were crossed with floxed *Scnn1a* mice (Hummler et al, Genesis, 2002). Thus, KO mice with a deletion of αENaC in part of the CNT were generated.

**Results:** Results revealed that at baseline level (standard diet), KO mice showed no difference in urinary $Na^+$ and $K^+$ excretion, urine output, water intake, blood $K^+$, osmolality or aldosterone compared to controls. On low $Na^+$ diet (7 days), however, KO mice showed increased urinary $Na^+$ excretion (on day 5 and 6) but no difference in blood $K^+$ (sampled on day 7). When challenged with high $K^+$ diet (2 days), KO mice decreased their food intake, lost weight, and excreted less $K^+$ through the urine (on day 1 and 2; $P < 0.05$). Moreover, they showed a lower water intake and urine output (on day 1; $P < 0.05$).

**Conclusions:** All baseline levels were unchanged in KO mice deficient for αENaC in part of the CNT, but on challenging diets the KO mice appeared with a phenotype. The phenotype was however less severe compared to mice with αENaC deleted in both the CNT and the CD. This may suggest that expression of αENaC both in the CNT and the CD are important for $Na^+$ and $K^+$ balance.

*Funding:* Private Foundation Support

**FR-PO751**

**Effects of Long-Term Aldosterone Administration on mRNA Expression Levels in the Renal Distal Nephron Examined by FACS and RNA Sequencing** Søren Brandt Poulsen, Jeppe Praetorius, Robert A. Fenton, Birgitte M. Christensen. *Aarhus Univ.*

**Background:** Renal sodium reabsorption and potassium excretion in the distal convoluted tubule 2 (DCT2) and connecting tubule (CNT) are tightly regulated by aldosterone. Previously, microarrays have been used to investigate the regulatory role of aldosterone. However, the emergence of next generation sequencing (RNA sequencing) offers improved sensitivity by employing sequence-based approaches directly determining the mRNA sequence, thus making it possible to generate a more complete catalog of aldosterone regulated genes. In this study, we used FACS sorting and RNA sequencing to identify genes in the DCT2 and CNT not previously known to be regulated by aldosterone.

**Methods:** We used a mouse model expressing EGFP driven by the TRPV5 gene promoter, i.e., the mice expressed EGFP in DCT2 and CNT cells. The mice were administrated saline (n = 7) or 100 μg aldosterone/kg body weight (n = 7) for six days. Following, a population of EGFP positive cells (DCT2 and CNT) was generated using FACS sorting (purity ~90%). The cell population was sequenced using an Illumina High seq 2000 (yield: ~40 mio paired end 100 bp reads.). Finally, data was analyzed using the tophat pipeline (Trapnell et al, Nature Protocols, 2012).

**Results:** Western blotting of cells isolated from whole kidney of aldosterone-administrated mice and corresponding control mice confirmed that NCC and αENaC, which are proteins classically regulated by aldosterone, were increased in abundance ($p < 0.05$). RNA sequencing showed a similar trend for mRNA levels in the DCT2/CNT cell population (NCC: $p = 0.058$; αENaC: $p = 0.147$). Furthermore, RNA sequencing showed an increase in Sgk1 ($p = 0.002$) and a decrease in Nedd4-2 ($p = 0.011$), proteins known to be involved in ENaC regulation. Based on regular *p*-values (significance level set to 0.05) and changes in abundance of ≥ 50%, the RNA sequencing identified 354 genes differentially regulated by aldosterone.

**Conclusions:** Using RNA sequencing, we have identified a high number of genes that are regulated by aldosterone. The data may contribute to a better understanding of the complex regulatory role of aldosterone.

*Funding:* Private Foundation Support

**FR-PO752**

**The Role of Epithelial Sodium Channel ENaC and the Apical $Cl^-/HCO_3^-$ Exchanger Pendrin in Compensatory Salt Absorption in the Distal Nephron in NCC (Na-Cl Cotransporter) KO Mice** Mina Patel- Chamberlin,[1] Kamyar A. Zahedi,[1,2] Sharon L. Barone,[1,2] Manoocher Soleimani.[1,2] *[1]Dept of Internal Medicine, Univ of Cincinnati; [2]Research Services, Veterans Administration.*

**Background:** Lack of NCC function does not cause significant salt wasting under basal conditions. NCC KO mice have increased expression of ENaC and pendrin, suggesting enhanced compensatory salt absorption in the distal nephron. Pendrin/NCC double knockout (KO) mice develop profound salt wasting compared to wild type (WT), NCC KO or Pendrin KO mice, demonstrating the important role of pendrin in compensatory salt absorption in NCC KO mice. We hypothesize that the inhibition of ENaC or down regulation of pendrin can cause significant salt wasting in NCC KO mice.

**Methods:** WT and NCC KO mice were treated with daily injection of amiloride, an ENaC inhibitor, or acetazolamide (ACTZ), a carbonic anhydrase inhibitor (CAI), for 6 days. Animals were subjected to balanced studies. At the end of studies, kidneys were harvested and examined for proteins and mRNAs of interest. Blood samples were collected for electrolytes and acid base analysis.

**Results:** There was an ~80% increase in urine output (UO) in NCC KO compared to WT mice after amiloride treatment (0.74ml vs. 1.29ml, $p<0.02$). Salt excretion increased and urine osmolality decreased more significantly in NCC KO vs. WT mice after amiloride treatment ($p<0.02$). Daily treatment with ACTZ resulted in ~85% reduction of pendrin expression in both WT and NCC KO mice at 6 days of treatment. However, the diuretic response was more exaggerated in NCC KO mice (1.1 ml/day before to 2.3 after ACTZ) ($p<0.01$) vs. WT mice (1.05 ml/day before to 1.30 after ACTZ).

**Conclusions:** 1. ENaC plays an important role in salt reabsorption in NCC KO mice. 2. NCC plays an important role in salt reabsorption in the setting of carbonic anhydrase inhibition, which is associated with increased delivery of salt from the proximal tubule and the down regulation of pendrin. We propose that the combined inhibition or inactivation of NCC and ENaC or NCC and carbonic anhydrase can provide a strong diuretic regimen, particularly in salt retaining conditions (e.g. congestive heart failure and nephrotic syndrome).

*Funding:* NIDDK Support, Veterans Affairs Support

**FR-PO753**

**Albumin-Induced Oxidative Stress Prevents Aldosterone-Induced Expression of ENaC in Collecting Ducts of Nephrotic Rats** Khalil Mohammad Udwan, Gaëlle Brideau, Alain Doucet. *Centre de Recherche des Cordeliers, Paris, France.*

**Background:** Puromycin aminonucleoside (PAN) nephrotic rats show high plasma aldosterone and retain sodium via stimulation of ENaC and Na,K-ATPase in the late aldosterone-sensitive distal nephron. PAN nephrotic rats with clamped aldosteronemia (adrenalectomy plus supplementation with constant infusion of aldosterone and dexamethasone) do retain sodium although ENaC is not induced. We evaluated the role of albumin in the regulation of ENaC expression.

**Methods:** Experiments were performed either in cortical collecting ducts (CCD) from control and PAN nephrotic rats or in mCCD cells (mCCDs). Production of reactive oxygen species (ROS) was evaluated through the nuclear labeling with dihydro-ethidium (DHE), and gene expression was quantified by RT-PCR.

**Results:** In mCCDs, apical addition of albumin induces its endocytosis and the production of ROS. Albumin-induced accumulation of ROS was prevented by dynasore, an inhibitor of endocytosis, by apocynin, an inhibitor of NADPH oxidase, and by N acetyl cysteine (NAC), a ROS scavenger. Addition of $H_2O_2$ (0.5-1 mM) altered the basal level of ENaC subunits mRNAs and abolished aldosterone-induced increase in aENaC mRNAs. This suggests that the balance between the plasma level of aldosterone and the cellular oxidative stress controls the expression of ENaC. In CCDs, we observed intracellular albumin and accumulation of ROS in PAN nephrotic rats but not in controls. We also observed high levels of several subunits of NADPH oxidase 2, the main isoforms expressed in CCDs.

**Conclusions:** Luminal endocytosis of albumin in collecting ducts of nephrotic rats triggers oxidative stress, via activation of NADPH oxidase, which counter balances aldosterone-induced expression of aENaC.

*Funding:* Government Support - Non-U.S.

## FR-PO754

**Apical Shear-Stress Regulates Sodium Transport in Principal Cells of the Collecting Duct** Thomas Ernandez,[1,2] Alexandra Chassot,[2] Pierre-Yves F. Martin,[1] Eric Feraille.[1,2] *[1]Service of Nephrology, Univ Hospital of Geneva, Geneva, Switzerland; [2]Dept of Physiology and Cellular Metabolism, Univ of Geneva, Geneva, Switzerland.*

**Background:** Sodium (Na) transport in renal tubules is tightly controlled and plays a central role in homeostasis of the body extracellular fluid volume. In addition to the classical neuro-endocrine regulatory inputs (incl. RAA system), other local factors such as apical shear-stress produced by tubular luminal urinary flow might participate to Na homeostasis.

**Methods:** We designed an in vitro experimental setting to explore the effect of apical flow on a cellular model of collecting duct (CD) using the well-described mouse CD cell line mCCDcl1 grown on polycarbonate filters. Directional flow was generated using an orbital shaker delivering a shear stress of 2 dyne/cm2 mimicking physiological luminal flow.

**Results:** We observed a delayed and sustained 40% decrease of the amiloride-sensitive Na current in cells subjected to flow. This was correlated with a significant decrease of ENaC subunits and SGK1 mRNA expression. The flow-mediated Na transport reduction was not prevented by PKD1 or KIF3A silencing, excluding a role of the primary cilium as a mechanosensor in this mechanism. On the other hand, whole-genome transcriptional analysis of wild-type mCCDcl1 cells subjected to flow suggested a role for PKA signaling and PKA substrates phosphorylation including CREB was increased by flow. This was further confirmed by PKA inhibition that partially prevented the flow-mediated decrease of Na transport in vitro as well as the downregulation of ENaC subunits mRNA expression.

**Conclusions:** These results are in line with physiological adaptation reported after unilateral nephrectomy in rodents in which a larger tubular fluid delivery is observed together with an increased CD fractional excretion of Na. We propose that such shear-stress mediated adaptive mechanism is involved in the homeostasis of extracellular fluid volume after nephron reduction.

## FR-PO755

**Primary Cilia Influence the Roles of TRPM3 and TRPV4 in Renal Epithelial Cell Adaptation to Acute Hyperosmolal Stress** Brian J. Siroky,[1] Bradley P. Dixon,[1] Raven Gail Comer,[1] Nancy Kleene,[2] John J. Bissler.[1] *[1]Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Cancer and Cell Biology, Univ of Cincinnati, Cincinnati, OH.*

**Background:** Ability of renal epithelial cells to adapt to changes in extracellular osmolality is critical to cell volume regulation. Primary cilia sense environmental conditions including osmolality, but whether cilia participate in osmotic response in renal epithelial cells is not known. Transient receptor potential (TRP) channels TRPV4 and TRPM3 are osmoresponsive. TRPV4 localizes to cilia in certain cell types, while cellular localization of TRPM3 is not known. We investigated the role of cilia in the adaptive response of renal epithelial cells to acute osmotic stress, and whether TRPV4 and TRPM3 are involved.

**Methods:** TRPV4 and TRPM3 expression was determined in ciliated (176-5) and nonciliated (176-5Δ) murine renal epithelial cells by RT-PCR, and TRPV4 localization was observed by immunocytochemistry. Cells were challenged with 16 hours of osmotic stress at 400 or 500mOsm/kg with NaCl, or maintained under control conditions. This stress was applied in the presence or absence of a TRPV4 agonist, 100nM GSK1016790A, or a TRPM3 agonist, 100μM pregnenolone. Expression of betaine-GABA transporter (*Bgt1*) and aldose reductase (*Akr1b*), osmotic response genes, was measured by qPCR. Aldose reductase expression was confirmed by immunoblotting.

**Results:** Ciliated and non-ciliated cells expressed TRPV4 and TRPM3. TRPV4 was observed in apical and basolateral membranes of both cell types, but was absent from cilia of 176-5 cells. Hyperosmolal stress induced *Bgt1* and *Akr1b* expression several fold in both cell lines. However, this induction was attenuated in non-ciliated cells compared to ciliated cells. Presence of TRPV4 agonist abrogated *Bgt1* and *Akr1b* induction in non-ciliated cells only. Presence of TRPM3 agonist attenuated *Bgt1* and *Akr1b* induction primarily in ciliated cells.

**Conclusions:** These findings suggest that cilia mediate osmotic response in renal epithelial cells, and that TRPM3 is involved in this mechanism. Furthermore, in the absence of cilia, TRPV4 appears to modulate the attenuated osmotic response.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO756

**Cholesterol Is a Biomechanical Regulator of Flow-Mediated Prostaglandin E2 (PGE2) Secretion in the Renal Collecting Duct (CD)** Yu Liu,[2] Daniel Armando Flores,[1,2] Rajeev Rohatgi.[1,2] *[1]Medicine, James J. Peters VAMC, Bronx, NY; [2]Medicine, Icahn School of Medicine, New York, NY.*

**Background:** Essential hypertension (HTN) affects millions of adults worldwide, the etiology of which remains obscure. Deficiency in renal prostaglandin E2 (PGE2) synthesis, which augments renal sodium (Na) avidity, is suspected to contribute to essential HTN. Moreover, dyslipidemia and, in particular, hypercholesterolemia is also associated with the development of HTN. Evidence from endothelial cells suggest that cholesterol-rich lipid rafts (LRs) act as flow sensors. Tubular flow rate in collecting ducts (CDs) mediates PGE2 release, and led us to hypothesize that cholesterol, and its incorporation into LRs, regulates mechanostimulated signaling pathways; specifically, suppressing flow-induced PGE2 release that, in turn, enhances Na absorption in the distal nephron.

**Methods:** PGE2 secretion is measured in flow-exposed CD cells and microdissected CDs before and after manipulating plasma membrane cholesterol.

**Results:** Cholesterol extraction of inner medullary CD3 (IMCD3) cells stimulates basal-(cholesterol replete vs. deplete cells; 3.8±0.9 vs. 31.5±1.5 pg/mL PGE2 per μg protein; $p<0.05$) and flow-(cholesterol replete vs. deplete cells; 24.7±2.3 vs. 83.4±16.8 pg/mL PGE2 per μg protein; $p<0.05$) mediated PGE2 release while cholesterol integration suppresses flow-(6.9±0.7 pg/mL PGE2 per μg protein; $p<0.05$) mediated PGE2 synthesis. Cholesterol extraction raised intracellular $Ca^{2+}$ ($[Ca^{2+}]i$) and chelation of $[Ca^{2+}]i$ suppressed cholesterol-dependent PGE2 release, implying that cholesterol extraction raises $[Ca^{2+}]i$ to release PGE2. To validate our *in vitro* experiments *in vivo*, mice were fed a control or high cholesterol diet, injected with isotonic saline to generate high urine flows, and CDs microdissected in media. The PGE2 secreted into the media by CDs from control mice was 221±50 vs. 113±34 pg/mL/mm tubule (n=3, $p<0.05$) from cholesterol fed mice.

**Conclusions:** Plasma membrane cholesterol regulates flow-stimulated PGE2 release in CDs, *in vitro* and *in vivo*, which presumably affects Na transport. We speculate that hypercholesterolemia leads to Na retention and HTN, by suppressing CD PGE2 release.

*Funding:* Veterans Affairs Support

## FR-PO757

**Long-Term Amiloride Therapy Partially Normalises Chronic Lithium-Induced Nephrogenic Diabetes Insipidus (NDI) in a Rat Model** Priyakshi Kalita,[1] Andrew Bahn,[1] Jennifer J. Bedford,[2] John P. Leader,[2] Robert J. Walker.[2] *[1]Physiology, Univ of Otago, Dunedin, New Zealand; [2]Medicine, Univ of Otago, Dunedin, New Zealand.*

**Background:** Lithium therapy is associated with the development of impaired water handling through to NDI in about 60% of patients prescribed lithium. Lithium down-regulates the expression and synthesis of aquaporin 2 (AQP2), reducing collecting duct water reabsorption. We have previously shown that amiloride, blocking ENaC mediated lithium uptake in the principal cells, partially attenuates NDI after 3 weeks of therapy (1). However, the long-term (5 months) effect of amiloride in this model has not been studied. **Aim:** To investigate the long-term effects of amiloride on chronic lithium induced nephrogenic diabetes insipidus in an established rat model.

**Methods:** Male wistar rats were divided into control, lithium alone and lithium plus amiloride groups (n=6 per group). Lithium was provided in rat chow (60 $mmol \cdot kg^{-1}$ food) for 6 months and amiloride was introduced in drinking water (0.2 $mmol \cdot l^{-1}$) following the establishment of NDI after one-month of lithium treatment and continued for 5 months. Following metabolic studies the rats were euthanised andkidneys processed for histology, immunohistochemistry and western blotting.

**Results:** Amiloride partially restored the urinary concentration from 199±14 $mosm \cdot kg^{-1}$ (lithium alone) to 612±72 $mosm \cdot kg^{-1}$ (lithium + amiloride) vs controls 2258±182 $mosm \cdot kg^{-1}$. This effect was accompanied by a decreased urine output (lithium alone 174±31 $\mu l \cdot min^{-1} \cdot kg^{-1}$ vs amiloride/lithium 54±8 $\mu l \cdot min^{-1} \cdot kg^{-1}$ vs control 11±4 $\mu l \cdot min^{-1} \cdot kg^{-1}$).AQP2 water channel expression examined by western blotting and immunohistochemistry showed a significant decrease (40.37± 2%; $p < 0.01$) in the lithium alone treated rats and it was restored (88.72 ±16 %; $p < 0.01$) with amiloride administration.

**Conclusions:** This study demonstrates a persistent partial recovery from NDI with amiloride over 6 months in long-term lithium treated rats. This has potential clinical significance for the management of patients with lithium-induced NDI.

1. Bedford J et al. Am J Physiol Renal 2008;294:812.

*Funding:* Government Support - Non-U.S.

## FR-PO758

**Pharmacological Blockade of $P2Y_{12}$ Receptor Reverses Lithium-Induced Downregulation of NKCC2, NCC and α-ENaC Protein Abundances in Mice** Yue Zhang,[1] Noel G. Carlson,[1] Bellamkonda K. Kishore,[1] Carolyn M. Ecelbarger.[2] *[1]Univ of Utah & VA Medical Center, Salt Lake City, UT; [2]Georgetown Unv, Washington, DC.*

**Background:** $P2Y_{12}$, is an ADP-activated G protein-coupled receptor (R) that inhibits adenylyl cyclase activity, potentially reducing cellular cAMP levels. We found that $P2Y_{12}$-R protein is expressed in the rodent kidney, and its irreversible blockade by clopidogrel (CLPD; Plavix®), significantly increased urinary concentration and vasopressin (AVP) and AQP2 protein abundance in the kidney, and ameliorated lithium (Li)-induced diabetes insipidus (DI) in rodents. Since Li also affects renal sodium transport, here we examined the effect of CLPD on Li-induced alterations in renal sodium transporters/channels in mice.

**Methods:** Groups of mice (B6D2 strain; n = 5-7/group) were fed Li-added diet (40 mmol/kg chow) with or without addition of CLPD (80 mg/kg bw/day) to drinking water. Other groups were fed regular diet with/without CLPD. After 14 days, major renal sodium transporter, channel, and exchanger expressions were measured by immunoblotting in cortex (Cx) and medullary (Md) fractions. Urinary AVP and PGE2 levels were quantified.

**Results:** The most striking finding was upregulation of the abundance of the thick ascending limb (TAL) Na-K-2Cl cotransporter (NKCC2) by CLPD treatment alone (30 and 137%, respectively in Md and Cx). In the presence of Li, CLPD restored the moderate down-regulation of NKCC2 (19 and 52%, respectively in Cx and Md) to control levels. In addition, in Li-treated mice, CLPD restored the abundance of the thiazide-sensitive cotransporter (NCC) and α-subunit of the epithelial sodium channel (ENaC) in Md. Li-induced increases in urinary AVP and PGE2 were either augmented ($P < 0.001$) or reversed ($P < 0.01$), respectively, by CLPD.

**Conclusions:** CLPD was able to reverse Li-induced down-regulation of major apical proteins involved in sodium reabsorption in the TAL through collecting duct (CD). This effect was not solely the result of increased AVP levels, but appears to be due to re-sensitization of the TAL and CD to AVP action, aided by the reversal of Li-induced increased PGE2 levels. Thus, $P2Y_{12}$-R antagonism may be a promising approach for the treatment of Li-induced DI.

*Funding:* Veterans Affairs Support, Private Foundation Support

## FR-PO759

**Physiological Roles of Moesin, a Crosslinker between Membrane Proteins and Actin Cytoskeleton in the Electrolytes and Water Reabsorption**
Ryo Hatano, Kotoku Kawaguchi, Shinji Asano. *Dept of Molecular Physiology, Ritsumeikan Univ, College of Pharmaceutical Sciences, Kusatsu, Shiga, Japan.*

**Background:** Moesin is a member of ERM (ezrin-radixin-moesin) family protein, which is known to function as a crosslinker between membrane proteins and actin cytoskeleton. Moesin is expressed in several tissues including kidney, liver, lung, and intestine. In the kidney, moesin is mainly expressed in the endothelial cells, and epithelial cells as proximal tubule cells, although the physiological importance in the kidney still remains unclear. Recently, Carmosino *et al*. reported that moesin but not radixin interacts with the intracellular C-terminal region of $Na^+$-$K^+$-$2Cl^-$ cotransporter (NKCC2) (*Biol Cell*. 104(11): 658-676, 2012). They reported that moesin regulated the exocytosis of NKCC2 in cultured cells, although it is not clear whether moesin regulates the apical surface expression of NKCC2 directly or indirectly. However, it is reported that moesin deficient mice did not exhibit apparent phenotypes in vivo (Doi Y *et al*. *J Biol Chem*. 274(4): 2315-2321, 1999).

**Methods:** In the present study, we investigated the physiological roles of moesin in the functional regulation of NKCC2 using moesin deficient mice.

**Results:** In wild type (WT) mice kidney, relatively low level of moesin was expressed in the thick ascending limb of Henle (TALH) compared to endothelial cells and brush border membrane of proximal tubules, and it co-localized with NKCC2 at apical membrane of TALH. In moesin deficient mice, the apical localization of NKCC2 was disturbed in the TALH. Furthermore, the metabolic cage studies revealed that moesin deficient mice exhibited the urinary losses of electrolytes as $Na^+$, $K^+$, and $Cl^-$ whereas daily urine volume were not different between WT and moesin deficient mice.

**Conclusions:** These results suggest that moesin plays important role in the regulation of electrolytes/water homeostasis in the kidney. Further experiments are required to reveal the detailed mechanism how moesin regulates the apical membrane localization of NKCC2.

## FR-PO760

**Relation between BK-α/β4-Mediated K Secretion and ENaC-Mediated Na Reabsorption**
Donghai Wen, Ryan J. Cornelius, Dianelys Rivero, Steven C. Sansom. *Cellular and Integrative Physiology, Univ of Nebraska Medical Center, Omaha, NE.*

**Background:** The BK-α/β4 channel is located in the intercalated cells (IC) of the distal nephron and mediates K secretion when mice are on a high K, alkaline diet. The present studies were conducted to determine whether BK-α/β4-mediated K secretion was dependent on ENaC-mediated Na reabsorption from principal cells.

**Methods:** Wild type (WT) and BK-β4 knockout mice (β4KO) were adapted to a low Na, high K, alkaline diet (LNaHK) for 7-10 days to maximize Na reabsorption-driven K secretion in the aldosterone-sensitive distal nephron. At the end of diet treatment, we gave a 12 hr bolus of vehicle, hydrochlorothiazide (HTZ; 50 mg/kg) or amiloride (5 mg/kg; ENaC inhibitor). Plasma and urine [Na], [K], [amiloride] and osmolality were measured using a flame photometer, HPLC, and osmometer, respectively. Luminal [K] and [Na] in the terminal cortical collecting duct (CCD) were calculated via the formulas: U[K] • POsm / UOsm, and U[Na] • POsm / UOsm.

**Results:** The calculated concentration of amiloride in the lumen of the terminal CCD, determined by HPLC and the plasma and urine osmolalities, was at least 20 μM, which would maximally block ENaC-mediated Na reabsorption. We found that HTZ, which enhanced Na excretion in WT on a control diet, did not affect Na handling in mice on LNaHK. However, amiloride significantly enhanced Na excretion, reduced plasma [Na], and evoked volume depletion in WT and β4KO on LNaHK. The amil-sensitive K secretion to Na reabsorption ratio (-Ks/Nar), calculated from the luminal [K] and [Na] in vehicle and amil-treated mice, was 1.5 in WT and 0.49 in β4KO, which was close to a value of 0.54 found in WT on a control diet.

**Conclusions:** We conclude that the high -Ks/Nar in WT, compared with β4KO, on LNaHK demonstrates a mechanism for BK-α/β4-mediated K secretion in intercalated cells that is driven by ENaC-mediated Na reabsorption in principle cells, by a mechanism likely involving recycling of Na from plasma to lumen.

*Funding:* NIDDK Support

## FR-PO761

**WNK1 Ativates BK Channel Activity through MAPK ERK1/2 Signaling Pathway**
Yingli Liu,[1] Xiang Song,[2] Weihui Niu,[3] Yanling Shi,[1,4] Hui-fang Bao,[2] He-ping Ma,[2] Douglas C. Eaton,[2] Jieqiu Zhuang,[3] Hui Cai.[1,2,4] *[1]Renal Div, Dept of Medicine, Emory Univ School of Medicine, Atlanta, GA; [2]Dept of Physiology, Emory Univ School of Medicine, Atlanta, GA; [3]Dept of Nephrology, The 2nd Affiliated Hospital, Wenzhou Medical Univ, Wenzhou, Zhejiang, China; [4]Renal Section, Atlanta VA Medical Center, Decatur, GA.*

**Background:** WNK (with no lysine) kinase is one of the serine/threonine kinase family. We have previously shown that WNK4 inhibits BK channel activity through enhancing its degradation via a lysosomal pathway. WNK4 was also found to inhibit BK channel activity which is kinase-dependent and via activating MAPK ERK1/2 and p-38 signaling pathways. However, little is known on the role of WNK1 in the regulation of BK activity.

**Methods:** To determine the role of WNK1 in regulation of BK channel activity, we have investigated the effect of WNK1 on BK channel activity using cell attached single channel recording technique, cell cultures, transfection, siRNA knock-down and western blot analysis.

**Results:** We have found that the knock-down of WNK1 expression in HEK 293 cells stably expressing alpha subunit of BK (HEK BKα cells) channel significantly inhibited both BKα activity (NPo, 0.024 ± 0.036, n=18, compared to the control group, 0.1316 ± 0.0573, n=36, p =0.006) and open probability (Po, 0.0094 ± 0.0098, compared to the control group, 0.0512 ± 0.0573, p<0.001). Western blot analysis showed that knock-down WNK1 significantly inhibited BKα total expression in HEK BKα cells in a dose-dependent manner (1.0 ± 0, 0.848 ± 0.213, 0.422 ± 0.297, n=3, p < 0.05). Over-expression of WNK1 was also shown to increase BKα protein expression in a dose-dependent manner (1.0 ± 0, 1.723 ± 0.136, 2.729 ± 0.385, n=3, p < 0.05) in HEK 293 cells transiently transfected with myc-BKα and HA-WNK1. We also found that WNK1 decreased ERK1/2 phosphorylation in a dose-dependent manner.

**Conclusions:** These data suggest that WNK1 enhanced BKα activity and total protein through inhibiting MAPK ERK1/2 signaling pathway.

*Funding:* Veterans Affairs Support, Private Foundation Support

## FR-PO762

**Dietary K Changes Modulate BKα Protein Abundance through MAPK Signaling Pathway**
Yanling Shi,[1,5] Yanhui Wang,[3] Matthew Lee,[1,5] Xiuyan Feng,[1,5] Yingli Liu,[1] Dingying Gu,[4] Hui Cai.[1,2,5] *[1]Renal Div, Dept of Medicine, Emory Univ School of Medicine, Atlanta, GA; [2]Dept of Physiology, Emory Univ School of Medicine, Atlanta, GA; [3]Dept of Nephrology, The First Affiliated Hospital, Wenzhou Medical Univ, Wenzhou, Zhejiang, China; [4]Dept of Nephrology, The Second Affiliated Hospital, Wenzhou Medical Univ, Wenzhou, Zhejiang, China; [5]Renal Section, Atlanta VA Medical Center, Decatur, GA.*

**Background:** Dietary K loading has been shown to increase urinary K excretion. High K diet has been shown to suppress renin-angiotensin system, whereas low K diet stimulates rennin-angiotensin system. High K diet increased BKα activity in rabbit cortical collecting duct (CCD) cells. Inhibiting ERK1/2 and p-38 was found to increase BKα channel activity in rat CCD cells. However, exact mechanism on how dietary K changes modulate BK channel activity remains not entirely clear.

**Methods:** Mice metabolic cage study, cell cultures, transfection, western blot analysis were used for the studies.

**Results:** To investigate the mechanism on how the dietary K changes affect BKα abundance, we have fed mice with low K (K deficient diet), normal K (1.0 %) and high K (10 %) diets for 10 days. After 10 days of dietary K challenges, mice fed with high K diet exhibited a high urinary K excretion, whereas mice fed with low K diet exhibited a low urinary K excretion. We have found that high K diets increased BKα protein abundance (200.03 ± 96.41, n=9, vs normal K group 100.00 ± 28.25, n=8, p=0.01), whereas low K diets decreased BKα abundance (54.39 ± 35.62, n=6, vs normal K group 100.00 ± 12.24, n=6, p=0.01). High K diet decreased p-38 phosphorylation, whereas low K diet increased p-38 phosphorylation. We also found that angiotensin II (Ang II) treatments decreased BKα protein expression in a dose-dependent manner in HEK293 cells transiently transfected with myc-BK plasmids.

**Conclusions:** These data suggest that dietary K changes modulate BKα protein abundance possibly via altering Ang II-mediated p-38 MAPK signaling pathway.

*Funding:* Veterans Affairs Support, Private Foundation Support

## FR-PO763

**The BK Channel Localizes to Lipid Rafts in the Apical Membrane of the Cortical Collecting Duct**
Rolando Carrisoza-Gaytan,[1] Carlos Schreck,[1] Marcelo D. Carattino,[2] Thomas R. Kleyman,[2] Lisa M. Satlin.[1] *[1]Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Medicine, Univ of Pittsburgh School of Medicine, Pittsburgh, PA.*

**Background:** The $Ca^{2+}$- and stretch-activated BK channel, present in principal (PC) and intercalated (IC) cells but more abundant in IC, mediates flow-induced K secretion (FIKS) and the enhanced K secretion observed in response to dietary K loading in the cortical collecting duct (CCD). BK channel activity is regulated by membrane cholesterol (Pharmacol Ther 135: 133-150, 2012). Studies by others have shown in non-renal cells that (i) BK channels localize to sphingolipid-cholesterol-rich lipid rafts (LRs) and (ii) channel activity is regulated by the composition and/or integrity of LRs. We hypothesized that BK channels in the CCD localize to LRs which provides a structural foundation for signaling complexes that regulate channel activity.

**Methods:** Single CCDs from NZW rabbits were microperfused in vitro for immunofluorescence labeling of endogenous BKα (the pore forming subunit of the BK channel) and caveolin 1 (Cav-1), a protein marker of LRs, and visualized by confocal microscopy. Monolayers of MDCK C7 and C11 cells (PC and IC models, respectively) were transfected with c-myc-tagged BKα and assayed for colabeling of c-myc and Cav-1. LRs were isolated from MDCK cells by sucrose density fractionation and analyzed by Western blotting (WB) for c-myc and Cav-1; CoIP was also performed using total membrane for c-myc-BK and Cav-1.

**Results:** Endogenous apical BKα colocalizes with Cav-1 in the native CCD. MDCK C11 cells express more recombinant BKα than C7 cells, but in both cell lines, the channel colocalizes with Cav-1. Immunodetectable recombinant BKα and Cav-1 were identified in the same sucrose fraction, corresponding to the migration pattern of LRs. Immunoprecipitation using anti-myc followed by WB demonstrated that Cav-1 and BKα are physically associated.

**Conclusions:** BKα in the CCD localizes to lipid rafts, which we speculate serve as a platform for flow-induced signal transduction leading to FIKS.

*Funding:* NIDDK Support

## FR-PO764

**Regulatory Adaptation of ROMK Channel in Thick Ascending Limb**
Yuliya Sharkovska, Alexandra Böhlick Böhlick, Kerim Mutig, Sebastian C. Bachmann. *Institute of Vegetative Anatomy, Charite Universitätsmedizin Berlin, Berlin, Germany.*

**Background:** The renal outer medullary potassium channel (ROMK) plays a critical role in the regulation of potassium recycling across the *apical membrane* in thick ascending limb (TAL) of Henle's loop, providing K availability for the action of the luminal Na-K-2Cl cotransporter (NKCC2).

*ROMK localization along rat TAL* has previously revealed a cellular heterogeneity. *Some cells* demonstrated *strong ROMK signal in the apical plasma membrane, whereas* others exhibited little or no immunoreactivity. This study aimed to characterize the expression and regulation of ROMK in the TAL after short- or long-term stimulation of transport by vasopressin (AVP).

**Methods:** Vasopressin-deficient Brattleboro rats were treated with the vasopressin V2 receptor-selective agonist desmopressin (dDAVP) *using* osmotic minipumps for 7-hour (short-term) or 72-hour periods (long-term study). Biosynthesis and cellular distribution of ROMK were studied in the kidneys.

**Results:** Double immunofluorescencelabeling in Brattleboro ratsshowed an intermittent ROMK-immunoreactivity pattern in the apical membrane of TAL, whereas the expression of NKCC2 was continuous. ROMK-deficient portions comprised TAL cells with low or absent signal. Treatment with dDAVP induced the expression of ROMK abundance in parallel with pNKCC2 expression (+130% in cortex and +73% in medulla for long-term study). Numerical evaluation of ROMK and NKCC2 double staining in TAL cells revealed that the ROMK-positive cells were differentially increased in number upon short- and long-term dDAVP treatments. In long-term treated rats, ROMK-positive cells were increased by 21% in cortical and by 35% in medullary TAL. Measuring the inner, ROMK-positive perimeter of TAL profiles, there were increases by 21% and 14%, respectively. Western blot revealed no significant difference of ROMK abundance in the cortex and an 1.7-fold-increase in the outer medulla ($p<.05$ for all values).

**Conclusions:** Our results show that activation of TAL transport by AVP involves the recruitment of ROMK-positive cells in medullary and cortical TAL. The change is considered as an adaptive, regulatory response of the loop of Henle.

## FR-PO765

**Renal Potassium Conservation Is Impaired in a Kidney-Specific Kir4.1 Knock Out Mouse**
Daniel A. Gray, Emily Lambert, Wilson Nino, Jonathan Tran, Anita de Oliveira Silva. *Nephrology, Univ of Rochester.*

**Background:** Mutations in the Kir4.1 potassium channel underlie the SeSAME/EAST syndrome, a neuro-renal condition characterized by hypokalemic, hypomagnesemic metabolic alkalosis. The generalized Kir4.1 knock out mouse lives only to 2-3 wks of age. To better understand the pathophysiology of this syndrome, we therefore developed a kidney-specific Kir4.1 knock out mouse (ksKO).

**Methods:** The ksKO was generated by first crossing a mouse expressing the cadherin 16 promoter, which drives Cre recombinase expression in the distal nephron (P. Igarashi), with a generalized Kir4.1 heterozygote. Offspring that were $Kir4.1^{(+/-)}Cre^{+}$ were then crossed with a floxed Kir4.1 mouse, $Kir4.1^{(f/f)}$ (K. McCarthy). Offspring included the ksKO ($Kir4.1^{(f/-)}Cre^{+}$) and associated controls. Blood, obtained by cardiac puncture, and urine, from bladder aspiration, were analyzed in a reference lab. Significance (*) was taken at $p<0.05$.

**Results:** ksKO mice grew normally to adulthood. Kir4.1 protein expression (by immunofluorescence) in the distal nephron was markedly reduced but not abolished. Under basal conditions, the mice showed metabolic alkalosis but not hypokalemia or hypomagnesemia. They had a severe urinary concentration defect associated with hypernatremia, grossly enlarged kidneys and dilated collecting ducts. On low K, low Mg diet x 1 wk, ksKO mice showed marked hypokalemia (K 1.5 vs 3.0 mEq/L) associated with an inappropriately elevated TTKG (11.0 vs. 1.7).

| | Na, mEq/L | K, mEq/L | HCO3, mEq/L | Mg, mEq/L | U Na, U K, mEq/L | U osm, mOsm/Kg | TTKG |
|---|---|---|---|---|---|---|---|
| **KO** (n=4) | *153±4 | *1.5±0.1 | *25±5 | *2.7±0.2 | 25±4, 27±4 | *523±91 | *11.0±0.8 |
| **WT**(n=10) | 141±1 | 3.0±0.1 | 21±1 | 1.9±0.1 | 136±23, 23±5 | 1466±163 | 1.7±0.5 |

Metabolic alkalosis, hypernatremia and a urinary concentrating defect were again seen. Serum Mg level was paradoxically increased, suggesting that Kir4.1 function in the DCT was intact or that compensation elsewhere (ie in TAL) had occurred.

**Conclusions:** Our results suggest that Kir4.1, which underlies basolateral K recycling in the distal nephron, is required for adaptation to low K diet.

*Funding:* NIDDK Support

## FR-PO766

**RNA-Seq Identification of Transcriptome of Native DCT1 Cells**
Jae Wook Lee, Chung-Lin Chou, Fahad Saeed, Mark A. Knepper. *NIH.*

**Background:** Complete transcriptomes have been identified for the inner medullary collecting duct, medullary thick ascending limb, and proximal tubule cells because they can be biochemically isolated from kidney in large enough numbers for DNA microarray analysis. However, only limited data from SAGE experiments exist for the DCT1 segment.

**Methods:** Here, we use deep-sequencing technology (RNA-seq) to identify the transcriptome of native rat DCT cells manually microdissected from collagenase-treated rat kidneys. The microdissection used a high resolution optical system capable of discriminating cell types. The DCT1 segment was carefully identified and cells from adjacent segments were trimmed away. Several DCT1 segments were pooled yielding 300-500 cells per sample. Deep sequencing was done on an Illumina HiSeq 2000 sequencer after RT-PCR amplification. Expression levels were calculated in 'reads per kilobase exons per million mapped reads' (RPKM).

**Results:** After mapping to the rat reference genome (rn4), transcripts corresponding to a total of 5171 distinct genes were identified in 6 DCT1 samples. Comparison to RNA-seq data in all 11 other tubule segments revealed that 58 transcripts were unique to the DCT1. These include 3 protein kinases (*Nek6*, *Stk17b*, and *Fgr*), 7 receptors (*Gfra1*, *Gpr37*, *Gpr39*, *Gpr137*, *P2ry1*, *Ptger4,* and *Olr1104*), 3 solute transporters (*Slc12a3*, *Slc29a2* [nucleoside transporter], and *Slc39a2* [zinc ion transporter]) and 1 transcription factor (*Zfp189*). Of more than 100 protein kinases expressed, the 10 most abundant were *Wnk1*, *Cdkl1*, *Stk16*, *Fastk*, *Map2k2*, *Pink1*, *Pdk2*, *Wnk4*, *Sgk1*, and *Stk39* (SPAK). Of G protein-coupled receptors expressed, the 10 most abundant were receptors for somatostatin (*Ssr2* and *Ssr3*), parathyroid hormone (*Pth1r*), glucagon (*Gcgr*), calcium ion (*Casr*), PGE2 (*Ptger3*), zinc ion (*Gpr39*), vasopressin (*Avpr2*), and unknown ligands (*Gpr56* and *Gprc5c*).

**Conclusions:** These data provide a new resource for the study of the DCT1 segment and its associated transporter function. Further studies are needed to address whether any of the newly identified DCT1 genes play roles in regulation of transepithelial NaCl transport.

*Funding:* Other NIH Support - NHLBI

## FR-PO767

**Use of Serum Total Protein (TP) to Correct for Colloid-Related Bias of Serum Sodium (Na) Measured by the Indirect Ion-Selective Electrode (ISE) Method**
Pratima Ghimire,[1] Isabelle Ayoub,[1] Robert H. Barth,[2] Philip Goldwasser.[2] *[1]SUNY Downstate; [2]VA NY Harbor Healthcare System, Brooklyn, NY.*

**Background:** When the serum colloid level is lower than normal, the measurement of Na by the usual indirect ISE method (iNa) tends to be artifactually high—compared with direct ISE (dNa) used in gas panels—while above normal serum colloid results in the opposite. Studies disagree on whether a linear equation using TP (as a proxy for serum colloid) can be used to predict iNa - dNa (ΔNa). Jones & Twomey ('07) found ΔNa fell by 1.2 mM per g/dL increase in TP, while Dimeski ('05) found the TP effect to be nonlinear.

**Methods:** To test this, we (i) paired chemistry and gas panels obtained < 20 minutes apart (median=5 min.) in a retrospective cohort of all patients (pts) admitted to our critical care units during a 1 year period, (ii) calculated ΔNa and the inter-panel glucose difference (ΔGlu), and (iii) examined the influence of TP on ΔNa, adjusting for ΔGlu and dNa. Limiting each pt to one pairing and excluding hemolyzed or turbid samples left 228 iNa/dNa pairs.

**Results:** Significant differences [mean ± se] were found between the panels (ΔNa: 1.8± 0.1 mM, $p<10^{-30}$; ΔGlu: -5.9±1.9 mg/dL, $p<.002$) indicating bias. TP ranged from 2.1-10.1 g/dL. ΔNa correlated significantly with TP ($r= -0.28$ $p<10^{-4}$), weakly with ΔGlu ($r= -0.11$ $p<.09$), and not at all with iNa + dNa ($r=0.05$). The trend of ΔNa means versus TP, grouped into categories, is shown.

| TP CATEGORY | N | TP, g/dL | ΔNa, mM | Range | T-test |
|---|---|---|---|---|---|
| A (< 4 g/dL) | 8 | 3.4±0.2 | 2.9±0.6 | 0 to 6 | |
| B (4-5.9 g/dL) | 64 | 5.2±0.1 | 2.3±0.2 | -4 to 8 | |
| C (6-7.9 g/dL) | 137 | 6.9±0.1 | 1.8±0.2 | -3 to 8 | |
| D (>7.9 g/dL) | 19 | 8.4±0.1 | 0.0±0.6 | -5 to 7 | p<001 vs. A, B, & C |

Trend analysis indicated only a significant linear component ($P<.001$); deviation from linearity was not quite significant ($p<0.07$). By regression, for each g/dL increase in TP, ΔNa fell by 0.49±0.11 mM ($p<10^{-5}$) [or 0.34±0.12 mM ($P<.004$) in the subset with TP<8] adjusted for ΔGlu(slope = -1.0 mM per 100 mg/dL; $p< .04$) and dNa.

**Conclusions:** In summary, the discordance between iNa and dNa varies inversely with the level of TP, and can be corrected using a linear equation based on TP.

*Funding:* Veterans Affairs Support

## FR-PO768

**Renal Effects of Caveolin-1 Deletion**
Yan Willière, Tatiana Nikitina, Andreas Patzak, Mauricio Michalak Sendeski, Sebastian C. Bachmann, Kerim Mutig. *Charité-Universitätsmedizin Berlin, Berlin, Germany.*

**Background:** Caveolin-1 (Cav1) is essential for caveolae biogenesis. These cholesterol-rich subdomains of lipid rafts microdomains are involved in signal-transduction, vesicular trafficking, and functional modulation of plasma membrane proteins. Little is known about the role of the caveolae in the kidney. In this study we tested the hypothesis that caveolae interfere with renal NaCl- and water reabsorption.

**Methods:** To evaluate the role of caveolae in the kidney, Cav1-deficient (Cav1-/-) mice were analyzed for their physiologic kidney performance, kidney morphology, biochemical profile of renal transporters, and renal vascular contractility.

**Results:** In wildtype (WT) mouse kidneys, Cav1 was strongly expressed in the endothelium and moderately expressed in the epithelial cells of the late distal convoluted tubule (DCT2) and collecting ducts. No Cav1 expression and no caveolae were detected in Cav1-/- mice. Physiologic evaluation of WT and Cav1-/- mice showed significantly increased urinary excretion of water (+124%) and electrolytes upon Cav1 disruption (+94% for $Na^{+}$). Immunoblotting evaluation of distal epithelial salt transporters and water channels revealed decreased levels of phosphorylated Na-Cl cotransporter in DCT2 (-40%) and of $Na^{+}/K^{+}$-ATPase (-33%) in the collecting duct upon Cav1 disruption. Functional analysis of renal interlobar arteries showed significantly decreased contractile response of Cav1-deficient vessels to phenylephrine. Concomitant inhibition of NO production by L-NAME has suppressed the differences between WT and Cav1-/- indicating that Cav1 disruption goes along with an increased activity of NO synthases. Indeed, abundance of the endothelial NO synthase (eNOS) was significantly increased in Cav1-/- kidneys compared to WT controls (+212%).

**Conclusions:** Our data suggest that Cav1 promotes renal reabsorption of water and electrolytes by inhibiting eNOS and facilitating the function of the distal epithelial transporters.

**FR-PO769**

**Total Body Adiposity Is Associated with Inflammation and Vitamin D in Chronic Kidney Disease** Maria Ines Barreto-Silva,[1] Márcia R.S.G. Torres,[1] Carla C.S. Lemos,[2] Simone Vargas Da Silva,[3] Rachel Bregman.[2] *[1]Nutrition, State Univ of Rio de Janeiro; [2]Nephrology, State Univ of Rio de Janeiro; [3]Pharmacology, State Univ of Rio de Janeiro, Brazil.*

**Background:** High body adiposity, inflammation and vitamin D 25(OH)D deficiency may contribute to poor outcome in chronic kidney disease (CKD) patients.

The aim was to describe vitamin D profile and its association with inflammation and body adiposity in CKD patients.

**Methods:** CKD patients under multidisciplinary treatment were evaluated. Total body adiposity was evaluated by X-ray absorptiometry- (DXA-total-BF; %); central body fat evaluated by DXA trunk fat (%) and by anthropometry (waist circumference, waist-to-hip-ratio; waist-to-height-ratio). Body mass index (BMI; kg/m2) was estimated. Serum levels analysis for: leptin, high molecular weight adiponectin (HMWAdipo), C-reactive protein, interleukin-6, tumor necrosis factor alpha (TNFα), interferon-gamma and 25(OH)D.

**Results:** One hundred patients (56% men) under treatment for at least 1 year were studied. Results as mean±SD: age=66±12 years, estimated glomerular filtration rate (eGFR)=29±13 ml/min (CKD-EPI), BMI=25±4. Overweight/obesity prevalence:52% (29±3) vs 48% normal (22±2). DXA-total-BF was positively associated with leptin (r=0.6) and negatively with HMWAdipo (r= -0.33) (p<0.0001; adjusted for confounders: age, gender, eGFR). TNFα was the only inflammatory parameter associated with DXA-total-BF (r=0.24; p<0.01; adjusted for confounders). Vitamin D profile was: 40% insufficiency (25±3), 25% deficiency (15±4), 35% normal (37±7 ng/mL) (ANOVA: p<0.001), i.e. 65% patients with low vitamin D levels. The 25(OH)D was inversely associated with DXA-total-BF (r= -0.25; p=0.02), but showed no association with central body fat parameters. TNFα was independently associated with 25(OH)D by multiple regression analysis (R2 adjusted=0.14; multiple correlation coefficient=0.42; p<0.0001; Stepwise MODEL, variables: dependent 25(OH)D; independent: DXA-total-BF, leptin,HMWAdipo, TNF-αlpha).

**Conclusions:** TNFα was the best inflammation marker in overweight CKD patients. High body adiposity may contribute with inflammation and vitamin D deficiency and should be a target in the nutritional treatment of this population.

**FR-PO770**

**Renal Inflammation and Injury Induced by Cisplatin Are Regulated by Magnesium Status** Malvika H. Solanki, Prodyot K. Chatterjee, Madhu Gupta, Xiangying Xue, Christine N. Metz. *Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, Manhasset, NY.*

**Background:** Cisplatin-induced acute kidney injury (AKI) occurs in 1/3 of patients taking cisplatin. The risk of cisplatin-AKI increases with age and is higher among females. Cisplatin impairs renal handling of magnesium (Mg) and Mg deficiency is common among US adults. Because Mg deficiency is associated with enhanced inflammation, we examined the effects of Mg deficiency and supplementation on inflammation during cisplatin-AKI.

**Methods:** Older female C57BL/6 mice (10/group, 10mo old) were fed either normal (100%Mg) or Mg-deficient (10%Mg) diets. After 2wks, saline or cisplatin (12mg/kg, ip) was injected. An additional group of Mg-deficient mice was supplemented with $MgCl_2$ in water and $MgSO_4$ s.c prior to cisplatin. Mice were euthanized 48hrs post-cisplatin. Blood urea nitrogen (BUN) levels and renal inflammatory markers (mRNA and protein) were measured. Renal neutrophil infiltration was measured by Leder staining and myeloperoxidase (MPO) levels. Renal STAT3 and ERK1/2 expression/activation were analyzed by Western blotting.

**Results:** Mg deficiency elevated BUN levels and increased markers of inflammation (*Cxcl2, Ccl2, Cxcl10* and *Tnfa*-mRNA and CXCL2, CCL2, IL-6, IL-1β and CXCL10-protein) following cisplatin compared to 100%Mg-cisplatin controls (P<0.05). Renal neutrophil infiltration and MPO levels were higher in cisplatin treated Mg-deficient mice when compared to controls (P<0.01). Mg-deficient mice treated with cisplatin exhibited enhanced renal STAT3 protein expression/phosphorylation, as well as ERK1/2 phosphorylation compared to controls (P<0.05). Mg replacement reversed cisplatin-induced renal damage and inflammation and cisplatin-mediated STAT3 and ERK1/2 phosphorylation (P<0.01).

**Conclusions:** Mg deficiency significantly exacerbates cisplatin-associated renal inflammation and AKI; Mg replacement reverses this effect. Mechanistic studies reveal the renoprotective role of Mg in controlling STAT3 and ERK1/2 activation, two pathways involved in cisplatin-mediated inflammation. These findings support investigating the effects of Mg status before and during cisplatin therapy on renal outcomes in patients.

**FR-PO771**

**Effect of Brazilian Nut Supplementation on Nrf2 and NF-kB Expression in Hemodialysis Patients** Ludmila Fmf Cardozo,[1] Liliana M. Pedruzzi,[1] Milena Barcza Stockler-pinto,[1] Julio Beltrame Daleprane,[2] Juliana M.S. Siqueira,[3] Olavo M. Cabral,[3] Maurilo Leite,[4] Denise Mafra.[1] *[1]Post Graduation Program in Cardiovascular Sciences, Federal Fluminense Univ (UFF), Niterói, Rio de Janeiro (RJ), Brazil; [2]Institute of Nutrition, State Univ of Rio de Janeiro (UERJ), RJ, Brazil; [3]Nefrológica Clinic, Niterói, RJ, Brazil; [4]Div of Nephrology, Federal Univ of Rio de Janeiro (UFRJ), RJ, Brazil.*

**Background:** Previous studies in our laboratory showed that the supplementation of 1 unit of Brazilian nut (Bertholletia excelsa, family *Lecythidaceae, t*he richest known food source of selenium-Se) a day during 3 months, is effective to improve Se status, reduce oxidative stress and inflammation markers, and increase the antioxidant levels in hemodialysis (HD) patients. However, we did not evaluate whether this nutritional compound can modulate the expression of nuclear E2-related factor 2 (Nrf2), a protein that regulates the expression of detoxifying enzymes by recognizing the human Antioxidant Response Element. This study evaluated the effect of nut supplementation on the mRNA expression of Nfr2 and NF-kB in HD patients.

**Methods:** Fourteen HD patients (50% men; 55.1±13.8yrs; BMI 24.2±3.2 kg/m$^2$; time on dialysis 80±42.7 months) from Nefrológica Clinic in Niterói, RJ, Brazil were studied. They received 1 nut (5g, courtesy: Agriculture Arauanã) a day for 3 months. Blood samples were obtained after 12h fasting and the peripheral blood mononuclear cells were isolated before and after supplementation. Quantitative Real-Time PCR analysis was performed using *7500 Real-Time PCR* System (*Applied Biosystems)* to evaluate the levels of mRNA expression encoding Nrf2 and NF-kB.

**Results:** After nut supplementation, the NF-kB expression decreased from 1.92±0.64 to 0.71±0.36 (p=0.001) and the Nrf2 expression increased from 0.60±0.39 to 1.45±0.27 (p=0.001).

**Conclusions:** These data suggest that the consumption of only one Brazilian nut per day during 3 months can activate Nrf2 and diminish inflammation by reducing NF-kB expression in HD patients. This study is the first to report and confirm the molecular beneficial effects of nuts on reducing inflammation and oxidative stress on HD patients.

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**FR-PO772**

**Nrf2/Nf-kB Equilibrium Is Altered in Hemodialysis Patients** Ludmila Fmf Cardozo,[1] Liliana M. Pedruzzi,[1] Milena Barcza Stockler-pinto,[1] Julio Beltrame Daleprane,[2] Juliana M.S. Siqueira,[3] Olavo M. Cabral,[3] Maurilo Leite,[4] Denise Mafra.[1] *[1]Post Graduation Program in Cardiovascular Sciences, Federal Fluminense Univ (UFF), Niterói, Rio de Janeiro (RJ), Brazil; [2]Institute of Nutrition, State Univ of Rio de Janeiro (UERJ), RJ, Brazil; [3]Nefrológica Clinic, Niterói, RJ, Brazil; [4]Div of Nephrology, Federal Univ of Rio de Janeiro (UFRJ), RJ, Brazil.*

**Background:** Reactive oxygen species (ROS) activate NF-kB that regulates the transcription of several genes, including pro-inflammatory cytokines. Oxidative stress and inflammation are frequent findings in patients with Chronic Kidney Disease (CKD), and considered mediators of cardiovascular disease. Several studies address the strategies to halt this process, including the activation of the transcription nuclear E2-related factor 2 (Nrf2), which regulates the expression of detoxifying enzymes. The aim of this study was to evaluate the expression of Nfr2 and NF-kB in CKD patients on hemodialysis.

**Methods:** Twenty hemodialysis patients (65% men, BMI 23.6±3.0 kg/m$^2$, time of dialysis 78±46.4 months, DM 21,4%, 54.9±15.2yrs) from Nefrológica Clinic in Niterói, RJ, Brazil, were compared to 11 healthy individuals (45.5% men, BMI 23.8±1.9 kg/m$^2$, 50.9±8.0 yrs). Blood samples were drawn after 12h fasting, and the peripheral blood mononuclear cells were isolated. Quantitative Real-Time PCR analysis was performed using *7500 Real-Time PCR* System (*Applied Biosystems)* to evaluate the levels of mRNA expression encoding Nrf2 and NF-kB.

**Results:** The HD patients had lower expression of Nrf2 (0.58±0.35) when compared to healthy individuals (1.13±0.64, p=0.005). By contrast, the NF-kB expression were 2 fold in HD patients (2.18±0.8), when compared to healthy individuals (1.04±0.22, p=0.0001). The HD patients presented NF-kB expression inversely correlated with Nrf2 levels (r=-0.54, p<0.01) and this correlation was positive for the healthy individuals (r= 0.85, p=0.001).

**Conclusions:** The results show that HD patients present an increased NF-kB and reduced Nrf2 expression, which is the opposite of healthy individuals. NF-kB may participate in the negative regulation of Nrf2 expression in these patients.

*Supported by:* CAPES, Faperj, CNPq.

**FR-PO773**

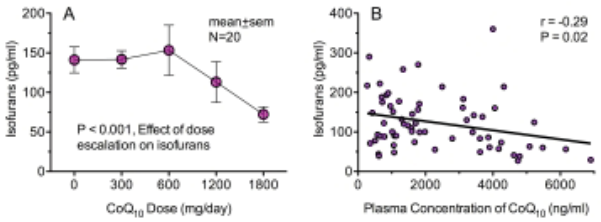
**The Effect of Coenzyme $Q_{10}$ Dose-Escalation on Oxidative Stress in Hemodialysis Patients** Frederic Tremaine Billings,[1] Catherine K. Yeung,[2] Danny D. Shen,[2] T. Alp Ikizler,[1] Jonathan Himmelfarb.[2] *[1]Vanderbilt Univ, Nashville, TN; [2]Univ of Washington, Seattle, WA.*

**Background:** Coenzyme $Q_{10}$ ($CoQ_{10}$) supplementation improves mitochondrial coupling of respiration to oxidative phosphorylation and decreases reactive oxygen species generation in endothelial cells. Oxidative stress is increased in patients with end stage renal disease and may partially account for the increased risk of cardiovascular disease in this population.

**Methods:** We performed a $CoQ_{10}$ dose escalation study (2 weeks each of 300, 600, 1200, and 1800 mg/day) in 20 chronic hemodialysis patients to test the hypothesis that $CoQ_{10}$ therapy reduces oxidative stress. $F_2$-isoprostanes, isofurans and high-density lipoprotein (HDL) apolipoprotein A1 (apoA-1) oxidation were measured to assess oxidative stress and $CoQ_{10}$ to determine dose, concentration, and response relationships.

**Results:** Plasma $CoQ_{10}$ levels increased in a linear fashion from 704 ± 286 ng/mL at baseline to 4033 ± 1637 following 1800 mg/day, and plasma isofuran concentrations, a marker of oxidative stress reported to be associated with mitochondrial dysfunction, decreased from 141 ± 67.5 pg/mL at baseline to 72.2 ± 37.5 following 1800 mg/day (P=0.003 vs. baseline and P<0.001 for the effect of dose escalation, Figure, Panel A). In addition, reduced plasma isofuran concentrations correlated with increased plasma concentrations of $CoQ_{10}$ (P=0.02, Figure, Panel B). Plasma $F_2$-isoprostane concentrations and HDL apoA-1 oxidation did not change during the study.

**Conclusions:** Short-term daily $CoQ_{10}$ supplementation significantly decreased plasma isofuran concentrations in a dose-dependent manner but did not affect HDL oxidation. This might indicate improved mitochondrial function and decreased oxidative stress in hemodialysis patients treated with CoQ10.




*Funding:* NIDDK Support, Other NIH Support - NIGMS - 1K23GM102676-01

## FR-PO774

**Targeting of Rho Kinase Protects Mice from Progressive Nephropathy by Improving Podocytes Lipophagy during Diet-Induced Hyperlipidemia**

Wenjian Wang,[1] Xinling Liang,[1] Farhad R. Danesh.[2] *[1]Div of Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China; [2]Div of Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** The trapping of lipid-laden podocytes in the glomerulus is a critical but reversible step in glomerulosclerosis. Recently, an alternative pathway of lipid metabolism through the lysosomal degradative pathway of autophagy is described and now termed lipophagy. However, whether and how this pathway is involved in the progression of lipid-induced nephropathy remains unclear.

**Methods:** In this study, we demonstrate the first evidence that Rho kinase (ROCK) pathway plays a critical role in lipophagy in podocytes treated with oxidative low density lipoprotein in vitro. Overexpression of ROCK increase of lipid retention in oxidative low density lipoprotein treated podocytes, which consequently activates, and results in a marked increases of reactive oxygen species production. Interestingly, this process is reversed by ROCK inhibition evidenced by increased lipophagy which associated to low phosphorylated Akt and actin-related protein 2 levels. Furthermore, pharmacological inhibition of ROCK in high fat diet mice exhibits a significantly ameliorated albuminuria and mesangial matrix accumulation compared to control. The renoprotective effect of fasudil, a ROCK inhibitor, on high fat diet mice is attributed to the decreased lipid retention and improved lipophagy in kidney.

**Results:** Our data suggests a previously unknown contribution of ROCK to lipid-induced nephropathy. Constitutive inhibition of ROCK may be a major protective strategy to ameliorate lipid-overload in podocyte and lipid-related loss of renal function.

**Conclusions:** Constitutive inhibition of ROCK may be a major protective strategy to ameliorate lipid-overload in podocyte and lipid-related loss of renal function.

## FR-PO775

**Docosahexaenoic Acid Counteracts Palmitate-Induced Proteolytic Signaling in Myotubes**

Myra Woodworth-Hobbs,[1,2] Matthew B. Hudson,[1] Jill Rahnert,[1] Bin Zheng,[1] Harold A. Franch,[1] Russ Price.[1,3] *[1]Dept of Medicine/Nephrology, Emory Univ, Atlanta, GA; [2]Nutrition and Health Sciences, GDBBS, Emory Univ, Atlanta, GA; [3]Atlanta VAMC, Decatur, GA.*

**Background:** Dyslipidemia is a comorbidity of illnesses such as diabetes and chronic kidney disease that contributes to insulin resistance and dysfunctional protein metabolism in skeletal muscle, resulting in debilitating muscle atrophy. The saturated fatty acid palmitate (PA) induces muscle insulin resistance and dysregulated protein metabolism in cultured myotubes, whereas the omega-3 fatty acid docosahexaenoic acid (DHA) has beneficial metabolic effects. The purpose of this study was to evaluate the effects of PA and DHA on atrophy-related signaling in skeletal muscle. We hypothesized that DHA prevents PA-induced myotube atrophy by counteracting its effects on protein degradation.

**Methods:** C2C12 myotubes were treated with 500uM PA and/or 100uM DHA for up to 28h. The rate of protein degradation was measured by monitoring the release of radiolabeled phenylalanine into the culture media, and the expression of mRNAs and proteins involved in atrophic signaling pathways were measured by quantitative real time PCR and western analysis, respectively.

**Results:** PA increased the rate of protein degradation by 31% (P<0.05 vs. all other groups), while co-treatment with DHA completely prevented the response. Akt is a key modulator of protein balance that inhibits the FoxO3 transcription factors which regulate "atrogene" expression. PA reduced the activation state of Akt (phospho:total Akt) by 24%-40% at timepoints ranging from 2h-24h (P<0.05 vs. control) and increased the nuclear protein levels of FoxO3 by 40%. PA also increased the mRNA levels of three FoxO3 atrogene targets, the E3 ubiquitin ligases MuRF-1 and atrogin-1/MAFbx and the autophagy mediator Bnip3. DHA attenuated the effects of PA on Akt, FoxO3, and all three atrogenes.

**Conclusions:** These data indicate that PA induces myotube atrophy by inducing the ubiquitin-proteasome and autophagic proteolytic systems and that DHA counters the catabolic effects of PA by improving Akt signaling.

*Funding:* NIDDK Support, Other NIH Support - NIH R01DK95610, Other U.S. Government Support, Veterans Affairs Support

## FR-PO776

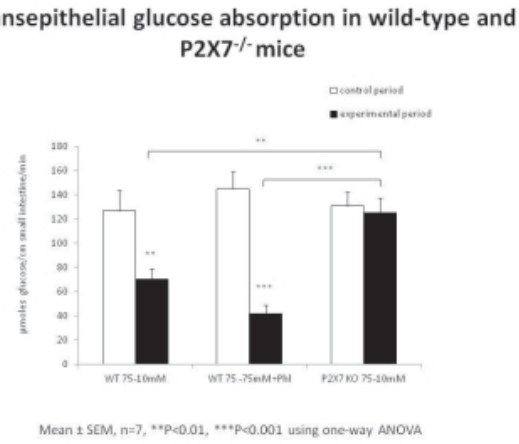
**P2X7 as a Potential Target for Improved Glycemic Control in Diabetes**

Joanne Marks,[1] Ballal Seddique,[1] Gregory Fong,[1] Frederick W.K. Tam,[2] Edward S. Debnam,[1] Robert J. Unwin.[1] *[1]Centre for Nephrology, Univ College London, London, United Kingdom; [2]Dept of Medicine, Imperial College London, London, United Kingdom.*

**Background:** Dynamic regulation of GLUT2 at the enterocyte brush border membrane (BBM) provides a high capacity route for post-prandial dietary glucose absorption; however, GLUT2 is permanently expressed at the BBM in diabetes and probably contributes to hyperglycemia and development of diabetic complications, including nephropathy. Our study investigated the putative role of the P2X7 receptor in regulating intestinal GLUT2 expression *in vivo*.

**Methods:** Wild-type (WT) and $P2X7^{-/-}$ mice were anaesthetized and 10cm segments of proximal small intestine were cannulated *in vivo*. These were perfused with Krebs buffer containing 75mM glucose, oxygenated with 95%$O_2$:5%$CO_2$ to create segmental flow at a rate of 1ml/min. The intestine was removed and placed in an organ chamber at 37°C and allowed to equilibrate for 30 mins. Serosal fluid was collected after an additional 30 mins (control period) and 30 mins after the solution was switched to one containing 10mM glucose or 1mM Phloretin (Phl), a competitive inhibitor of GLUT2 (experimental period). Serosal glucose concentration was measured using a glucose oxidase assay.

**Results:** In WT animals, glucose absorption was decreased by switching the perfusate to 10mM glucose, indicating rapid loss of GLUT2 expression after removing the high glucose stimulus. The reduction in transport induced by phloretin confirmed the contribution of GLUT2-mediated transport at 75mM glucose. In contrast, in $P2X7^{-/-}$ mice glucose absorption was unaffected by reducing glucose concentration.




**Conclusions:** These data suggest a role for P2X7 receptor in controlling GLUT2 expression at the enterocyte BBM and provide evidence that this pathway may be a novel target to improve glycemic control in diabetic patients.

*Funding:* Private Foundation Support

## FR-PO777

**Iron Metabolism in Kidney and Erythropoietin Synthesis: A Case of Hepcidin**

Karim Zoubida,[1] Boualem Moulouel,[1] Dounia Houamel,[1] Laurent Gouya.[1] *[1]Paris Diderot Univ, INSERM U773, Paris, France; [2]Paris Diderot Univ, INSERM U773, Paris; [3]Paris Diderot Univ, INSERM U773, Paris.*

**Background:** Erythropoietin (EPO) is produced exclusively in renal interstitial fibroblasts. Of interest, it has been shown that the production of EPO is regulated by iron but the mechanism of this regulation remains unclear. We have recently shown that hepcidin, the iron regulatory hormone, regulates renal handling of iron and that hepcidin knockout mice (hepc-/-) exhibited a marked iron deposition within the thick ascending limb of Henle. In this study we investigated whether EPO synthesis is monitored by the intrarenal hepcidin-dependant regulation of iron. Indeed, EPO-producing fibroblasts that are immersed in a faintly vascularized environment and their iron content may be modulated by the degree of iron reabsorption in the tubular cells.

**Methods:** Iron deposits in renal fibroblasts were analysed by electron microscopy (EM). After iron restricted diet (ID) renal and serum EPO were quantified by RT-qPCR and ELISA.

**Results:** -in hepc$^{-/-}$ mice, EM analysis showed significant iron deposits in renal interstitial fibroblasts.

-EPO mRNA was reduced in hepc$^{-/-}$ mice as compared to WT. ID induced the expression of EPO in WT but not in hepc$^{-/-}$ mice. Similar data were obtained after phlebotomy suggesting that iron deposits in interstitial fibroblasts may impair renal EPO production.

-Hepcidin mRNA level in liver was decreased in ID mice versus controls. However in kidney, hepcidin expression was slightly increased, which may probably potentiate iron depletion of interstitial fibroblasts and therefore EPO production.

-Treatment of REPC cells (obtained from J. Fandrey, Essen) with the iron chelator DFO increased EPO-mRNA level whereas iron excess (Fe-NTA) decreased EPO-mRNA. However, when cells were incubated with hepcidin, EPO synthesis remained unchanged suggesting no direct effect of the hormone on renal fibroblasts.

**Conclusions:** Our data indicate that hepcidin, by controlling renal tubular iron transport, may exert a feedback control on EPO production. Thus a crosstalk between tubular cells and interstitial fibroblasts may be required for an appropriate EPO production in normal and pathological situations.

*Funding:* Government Support - Non-U.S.

## FR-PO778

**Oxidative Balance Score (OBS) Is Associated with Chronic Kidney Disease (CKD)** Titilayo O. Ilori,[1] Young Sun Ro,[3] Orlando M. Gutierrez,[4] Soyeon Joyce Kong,[2] William M. McClellan.[1,2] *[1]Dept of Medicine, Emory Univ; [2]School of Public Health, Emory Univ; [3]School of Public Health, Seoul National Univ, Seoul, Korea; [4]Dept of Medicine, Univ of Alabama.*

**Background:** The OBS measures the overall oxidative balance status of an individual. The aim of this study was to investigate the relationship between OBS , albuminuria, eGFR <60 ml/min/1.73m$^2$ (CKD),and End Stage Renal Disease (ESRD) in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study.

**Methods:** Data was collected from 2003-2007 by phone questionnaire and in-home examination. OBS was calculated by combination of 13 *a-priori* pro- and anti-oxidant factors; polyunsaturated fatty acid, iron, selenium, vitamin C & E, α-& β -carotene, lutein lycopene, aspirin, NSAID, and alcohol use. Quartiles of OBS were the exposure, with the lowest quartile (high pro-oxidants) as the reference.The p for trend was calculated by MH chi square test for prevalence (albuminuria and CKD), poisson regression for incidence rate (ESRD), logistic regression for odds ratio OR (albuminuria and CKD), and cox-proportional hazard regression for HR (ESRD).

**Results:** 19,462 participants were analyzed, 2,519 had albuminuria and 1,957 had CKD. During a median follow-up of 3.5 years, 90 participants developed ESRD. Decreased prevalence of albuminuria and CKD were noted at higher quartiles of OBS. ESRD rates were higher at lower OBS quartiles (pro-oxidant) (p for trend <0.01), and the trend persisted, but was not statistically significant, after controlling for other covariates.

| | CKD | | ESRD | |
|---|---|---|---|---|
| | | Model 1 | | Model 1 |
| OBS | % | OR (95% CI) | IR** | HR(95%CI) |
| Q1 | 10 | 1 | 154.2 | 1 |
| Q2 | 10.2 | 0.93(0.8-1.08) | 169.8 | 1.27(0.7-2.29) |
| Q3 | 10.4 | 0.90(0.77-1.04) | 140 | 1.08(0.59-2.01) |
| Q4 | 9.6 | 0.77(0.66-0.91) | 105.5 | 0.81(0.41-1.63) |
| p for trend | 0.56 | <0.01 | <0.01 | 0.47 |

Model 1: completely adjusted model **IR = incident rate per 100,000 person-years.

**Conclusions:** These findings suggest that higher OBS is associated with decreased prevalence of kidney disease. The reason for this is unknown and warrants further study.

## FR-PO779

**In Utero Exposure to Maternal Obesity Can Affect Gene Expression within the Kidneys of Rat Offspring** Sarah J. Glastras,[1] Ibrahim Al-odat,[2] Hui Chen,[2] Carol A. Pollock,[1] Sonia Saad.[1] *[1]Kolling Insitute, Univ of Sydney, NSW, Australia; [2]Univ of Technology, Sydney, NSW, Australia.*

**Background:** Maternal obesity can 'program' the offspring to be prone to obesity, hyperglycaemia, dyslipidaemia, diabetes, hypertension and chronic kidney disease. We aimed to study the effect of maternal obesity on renal gene expression in the offspring in rats.

**Methods:** Female breeders were fed either normal or high-fat diet and the kidneys from the offspring were collected at postnatal Day 1 and Day 20 (weaning). The kidneys were weighed and snap frozen for further analysis. The anthropometric measures, plasma triglycerides and glucose/insulin levels were measured. mRNA expression of profibrotic factors (TGFß, CTGF, PAI-1), pro-inflammatory cytokines (TNF, IL-6 and MCP-1), and lipid metabolic markers (FXR, PPAR, SREBP) were measured by real time PCR.

**Results:** Offspring from obese rats displayed increased body weight, fat and kidney mass, blood triglyceride levels, and glucose intolerance compared with those from the lean rats. mRNA expression of TGFß, PAI-1 and MCP1 was significantly increased by maternal obesity at postnatal Day 1. The upregulated TGFß was sustained at Day 20. FXR expression was reduced by maternal obesity at both postnatal Day 1 and Day 20.

**Conclusions:** Maternal obesity is associated with the downregulation of renal FXR and upregulation of TGFß and MCP1 expression at Day 1 in the offspring. This effect was sustained until weaning. Given the known role of inflammatory cytokines in CKD, this suggests that maternal obesity confers an increased risk of kidney disorders in the offspring.

*Funding:* Government Support - Non-U.S.

## FR-PO780

**Sex Differences in Dietary-Fructose-Induced Renal Hypertrophy and Electrolyte Abnormalities in Mice** Nikhil Sharma, Lijun Li, Shehar-bano Awan, Allison Yunghans, Carolyn M. Ecelbarger. *Dept of Medicine, Georgetown Univ, Washington, DC.*

**Background:** Consumption of fructose has been implicated in the development of renal disease and impaired kidney function, but the mechanisms are not fully understood. Because females may be more sensitive to certain electrolyte disturbances such as hyponatremia, we tested the impact of chronic fructose feeding on electrolyte homeostatic mechanisms in the kidney.

**Methods:** Young (2-month) male (M) and female (F) mice (129/Sv background) were *offered ad libitum* access to either a corn-starch based control (C) or a high fructose (HFr, 60% dry weight) diet as pelleted chow and plain drinking water for 15 weeks (n = 6/sex/diet). Diets contained similar amounts of sodium (Na+), potassium (K+), and chloride (Cl-). Urine (24-hr) was collected for measurement of urine electrolytes. Kidneys and blood (cardiac puncture) were collected under anesthesia at euthanasia.

**Results:** HFr diet did not largely affect final body weight (bw) or weight gain, both which were higher in M; however, average kidney weight was increased by HFr in both sexes (g/30 g bw): 0.18 ± 0.01 (MC), 0.18 ± 0.01 (FC), 0.21 ± 0.01 (MHFr), 0.20 ± 0.003 (FHFr), $p < 0.005$ for diet, (2-way ANOVA, diet X sex). HFr diet also significantly increased 24-hour urine volume ($p = 0.011$) with a greater effect in F (increased 66% in M and 258% in F). Plasma Na+ and Cl- concentrations were reduced by HFr but only in F mice. Na+ (mmol/L): 151 ± 1 (MC), 151 ± 1 (FC), 153 ± 1 (MHFr), 142± 2 (FHFr); Cl- (mmol/L): 111 ± 1 (MC), 113 ± 1 (FC), 114 ± 1 (MHFr), 106± 2 (FHFr), $p = 0.008$, and 0.045 for a significant interaction between diet and sex for Na+ and Cl-, respectively. In contrast, plasma K+ was elevated by HFr in F (mmol/L): 3.7 ± 0.1 (MC), 3.2 ± 0.1 (FC), 3.9 ± 0.1 (MHFr), 4.0 ± 0.01 (FHFr), $p < 0.001$ for interaction. Plasma aldosterone, urine osmolality, and a glucose tolerance were not affected by HFr diet.

**Conclusions:** Our results demonstrate renal hypertrophy and altered plasma electrolytes in response to HFr, with F mice being more sensitive. More studies are warranted to fully elucidate the impact of specific carbohydrates including HFr on renal function, and cellular mechanisms involved.

*Funding:* Other NIH Support - NHLBI

## FR-PO781

**Effects of Kefir on Cytokines and Nitric Oxide Production, by Peritoneal Macrophages of Streptozotocin-Induced Diabetic Rats** Fabiane R. Maciel,[1] Giovana Punaro,[1] Adelson M. Rodrigues,[1] Marcelo Macedo Rogero,[2] Cristina Bogsan,[3] Marice Oliveira,[3] Thamires Fernandes,[1] Guilherme Nogueira,[1] Margaret Mouro,[1] Elisa MS Higa.[1] *[1]Medicine, UNIFESP, Sao Paulo; [2]Nutrition, School of Public Health, USP, Sao Paulo; [3]Biochemical Technology - Pharmaceuticals, USP, Sao Paulo.*

**Background:** The excessive production of reactive oxygen species in diabetes mellitus is due to hyperglycemia, and the oxidative stress decreases immune response. Careful control of gycemic status is known to minimize the diabetic complications. Kefir (K) is a fermented dairy product, which presents immunological properties.

**Methods:** Male adult Wistar rats received injection of streptozotocin (45mg/kg, I.V.). DM was defined as glycemia ≥200mg/dL. The animals were allocated in 4 groups (n=4 each): control (CTL); CTLK; DM; DMK. They received K, or its vehicle at 1.8 mL/day by gavage, started in the 5$^{th}$ day of DM, during 9 weeks. Before and after the treatment, the animals were maintained in metabolic cages for 24 hour urine collection. Retro-ocular blood was collected to determine the glycemia. The animals were sacrificed and macrophages were obtained from the peritoneal cavity, for cytokines and NO determination. The results are presented as mean±SEM, analyzed by One way ANOVA with Newman-Keuls post-test.

**Results:** DMK when compared to DM presented decreased levels of glycemia (mg/dL) (421±46 vs 567±12) accompanied by decrease in water ingestion (mL/24h) (94±14 vs 124±12), chow intake (g/24h) (31±2 vs 37±2) and diuresis (mL/24h) (71±9 vs 91±9); increased levels of IL-10 (µg/mL) (925±69 vs 555±92), TNF-α (µg/mL) (178±19 vs 109±20), IL-1β (µg/mL) (101±14 vs 70±5) as well as the NO (µM) (102±9 vs 66±5), $p<0.05$.

**Conclusions:** Probiotic supplementation resulted in attenuation of the classic symptoms of diabetes, with decrease in glycemia and increased levels of IL-10, TNF-α, IL-1β and NO. These results suggest that K could be an adjuvant therapy in DM, resulting in a better control of the metabolic parameters, with potential to modulate the immune response, improving the immunocompetence of diabetic patients.

*Funding:* Government Support - Non-U.S.

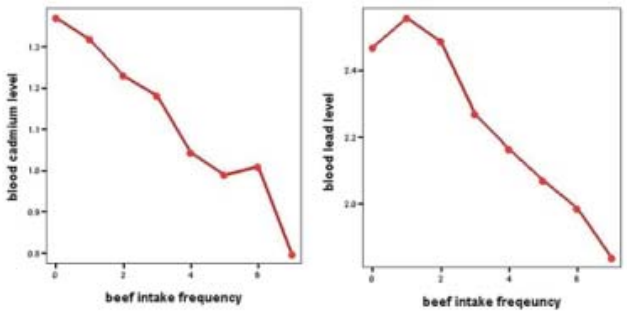
## FR-PO782

**The Levels of Blood Mercury, Cadmium and Lead Are Associated with Age, Dietary Intakes and GFR in Korean Population: KNHANES 2011** Ji Hye Ahn, Won Suk An, Ki Hyun Kim, Seong Eun Kim. *Internal Medicine, Dong-A Univ Hospital.*

**Background:** Heavy metals are widely distributed in the environment and oral exposure is a main route in the general population. There are few studies about the association between heavy metal concentration, dietary intakes and glomerular filatraton rate (GFR). The aim of this study is to investigate whether heavy metals (mercury, cadmium, lead) are associated with GFR and dietary intakes.

**Methods:** We examined the association between heavy metal concentration, dietary intakes and estimated GFR using data from the Korean national Health and Nutritional Examination Survey 2011. For the present study, the analysis was restricted to 1,753 adult participants who were aged >18 years and provided serum heavy metals levels, eGFR and diet survey.

**Results:** The mercury, cadmium and lead were negatively correlated with eGFR and positively correlated with age (p<0.001). The mercury was positively correlated with the mackerel and shellfish intake (p<0.001). The cadmium and lead were positively correlated with shellfish intake and negatively correlated with pork and beaf intake.




The mercury was independently associated with age (β=0.208;p<0.001), shellfish (β=0.138;p<0.001), mackerel intake (β=0.132;p<0.001). The cadmium was independently associated with age (β=0.327;p<0.001), shellfish intake (β=0.126;p<0.001), pork (β=-0.064;p=0.018) and beaf intake (β=-0.076;p=0.004). The lead was independently associated with age (β=0.338;p<0.001), eGFR (β=-0.048;p=0.005), shellfish (β=0.054;p=0.022), pork (β=-0.081;p=0.003) and beaf intake (β=-0.101;p<0.001).

**Conclusions:** Age, GFR and dietary intake were associated with level of heavy metals. Shellfish intake may increase heavy metal levels, beaf and pork intake may decrease cadmium and lead levels. Therefore, attention to heavy metals is necessary in elderly chronic kidney disease patients needing protein restriction.

## FR-PO783

**Altered Renal Lipid Metabolism Results in Lipotoxicity and Progression of Human Diabetic Nephropathy and Obesity Related Glomerulopathy** Michal Herman-Edelstein,[1] Moshe Levi,[2] Pnina Scherzer,[3] Ana Tobar,[1] Amalia Getsztain Bakshi,[1] Uzi Gafter.[1] *[1]Nephrology, Felsenstein Medical Research Center and Rabin Medical Center, Petah Tikva, Israel; [2]Div of Renal Diseases, Univ of Colorado, Denver; [3]Nephrology, Hadassah, Jerusalem, Israel.*

**Background:** Animal models link ectopic lipid accumulation to renal dysfunction, but whether this process occurs in the human kidney is uncertain.To this end we investigated whether there is altered renal triglyceride and cholesterol metabolism resulting renal lipid accumulation and lipotoxicity in human *diabetic nephropathy* (DN) and *obesity related glomerulopathy* (ORG).

**Methods:** Lipid staining and the expression of lipid metabolism genes were studied in kidney biopsies of patients with diagnosed DN (n=40) or ORG (n=10) comparing those with normal kidneys (n=15). We further characterized the effect of free fatty acid and ox-LDL on lipid metabolism in human podocyte cell line.

**Results:** We observed heavy lipid deposition and increased intracellular lipid droplets both in glomerular and tubular cells. Lipid deposition was associated with dysregulation of lipid metabolism genes. Fatty acid β-oxidation pathways including PPAR-α, CPT1, ACO and L-FABP were down-regulated. Down-regulation of lipid catabolism enzyme lipoprotein lipase was associated with increased expression of angiopoietin-like protein 4 (ANGPTL4). Cholesterol uptake receptors including LDL receptor , oxidized LDL receptors (OLR-1, CD36), and acetylated-LDL receptor (SR-A1) expression were all significantly increased, while there was down-regulation of genes effecting cholesterol efflux including ATP-binding cassette transporters (ABCA1, ABGC1) and ApoE. There was a highly significant correlation between eGFR, fibrosis, inflammation and lipid metabolism genes supporting the role of abnormal lipid metatabolism in the pathogenesis of DN and ORG.

**Conclusions:** These data imply that studying lipid metabolism may serve as a target for specific therapies aimed at slowing the progression of glomerulosclerosis.

*Funding:* Private Foundation Support

## FR-PO784

**Protein Intake and Kidney Function in the Population: Differences in Cross-Sectional and Longitudinal Data** Massimo Cirillo,[1] Cinzia Lombardi,[1] Daniela Chiricone,[1] Natale Gaspare De Santo,[2] Alberto Zanchetti,[3] Giancarlo Bilancio.[1] *[1]Dept of Medicine, Univ of Salerno; [2]Second Univ of Naples; [3]Istituto Auxologico Italiano.*

**Background:** Protein intake is considered a determinant of glomerular filtration rate (GFR). Urinary urea (U-urea) is an objective marker of protein intake. This population-based study investigated cross-sectionally and longitudinally the association of U-urea as index of protein intake with GFR indexed by serum creatinine (S-cr) and estimated GFR (eGFR).

**Methods:** Data were collected about overnight U-urea, S-cr, eGFR, and other variables in 1,522 men and women aged 45-64 year who participated in the Gubbio Study (baseline). Age, S-Cr, and eGFR were re-assessed after 12-year follow-up in 1,144 of the 1,425 surviving participants.

**Results:** Mean±SD of U-urea was 17.0±7.1 mmol/h corresponding to an estimated daily protein intake of 71.5±29.8 g/d. U-urea associated inversely with S-cr and directly with eGFR in cross-sectional quartile analyses (P<0.001). In multi-variable cross-sectional regression, 7 mmol/h higher U-urea (approximately one SD of U-urea corresponding to 30 g/d higher protein intake) related to 0.026 mg/dL lower S-cr (95%CI= 0.020/0.032) and 2.56 mL/min higher eGFR (2.01/3.12). At follow-up, change over baseline was an increase for S-cr (+0.054±0.100 mg/dL) and a decrease for eGFR (-11.8±9.1 mL/min x 1.73 $m^2$). Baseline U-urea associated directly with S-cr change and inversely with eGFR change in quartile analyses (P <0.001). In multi-variable regression, 7 mmol/h higher baseline U-urea related to 0.011 mg/dL more positive S-cr change (95%CI=0.006/0.016) and -0.07 mL/min more negative eGFR change per year (95%CI=-0.111/-0.038).

**Conclusions:** In middle-aged adults, higher protein intake is associated cross-sectionally with higher GFR but longitudinally with faster GFR decline over-time.

*Funding:* Government Support - Non-U.S.

## FR-PO785

**Kidney Function, Adipokines, and Quality of Life after Weight Loss Surgery in Obese Patients with Stages 3-4 Chronic Kidney Disease: A Randomized Controlled Pilot Study** Helen L. MacLaughlin,[1,2] Wendy L. Hall,[2] Ameet G. Patel,[1] Rochelle Maree Blacklock,[1] Iain C. Macdougall.[1] *[1]King's College Hospital, London, United Kingdom; [2]King's College London, United Kingdom.*

**Background:** Obesity is an independent risk factor for chronic kidney disease (CKD). Weight loss is associated with improvements in eGFR, yet few studies have examined changes in measured GFR (mGFR) in obese patients with CKD. This pilot study aimed to determine the effect of weight loss on iohexol clearance GFR in obese patients with stages 3-4 CKD.

**Methods:** 16 obese patients with stages 3-4 CKD were randomized to either laparoscopic sleeve gastrectomy (SG) weight loss surgery or best medical care (BMC), including dietary & physical activity modifications. Body weight, fat mass, adipokines, insulin resistance (HOMA-IR) & quality of life (QOL) scores were measured. Differences between groups were examined at 12 months with ANOVA corrected for baseline values or by Hodges-Lehmann median difference.

**Results:** 11 patients (9F, 2M; median age 52y, median BMI 39.5 kg/$m^2$) completed the 12-month study; 5 in the SG group & 6 in the BMC group. Significantly greater weight loss was achieved in SG at 12 months vs BMC (mean diff -29kg; 95% CI -36, -22). There was no association between eGFR and mGFR, eGFR significantly under-predicted mGFR, & there was hyperfiltration at baseline in both groups. Hyperfiltration was reversed in SG, but not in BMC, at 12 months. In SG, adiponectin increased (med diff 6.1 mg/L; 95% CI 1.0, 19.8) & HOMA-IR decreased (-8; 95% CI -29, -1) at 12 months vs BMC, & both were associated with decreased fat mass. SF-36 QOL physical domain score significantly improved in SG compared to BMC at 12 months (mean diff 20; 95% CI 4, 35). Hospital Anxiety & Depression Scale scores decreased significantly in SG compared to BMC at 12 months (Anxiety mean diff -4; 95% CI -7,-1; Depression mean diff -5; 95% CI -8,-2). There were 3 minor adverse events (2 SG; 1 BMC) & no mortality during the study.

**Conclusions:** eGFR significantly underestimated mGFR in obese patients with CKD. Weight loss surgery may reduce hyperfiltration & insulin resistance, & improve adiponectin & QOL in obese patients with eGFR determined stages 3-4 CKD.

*Funding:* Government Support - Non-U.S.

## FR-PO786

**Changes in Abdominal Fat and Thigh Muscle Masses over 1 Year after Initiation of Maintenance Hemodialysis Therapy** Akihiko Kato,[1] Yukitoshi Sakao,[1] Naoko Tsuji,[2] Shinsuke Isobe,[2] Masafumi Ono,[2] Tomoyuki Fujikura,[2] Takayuki Tsuji,[2] Naro Ohashi,[2] Hideo Yasuda.[2] *[1]Blood Purification Unit, Hamamatsu Univ Hospital, Hamamatsu, Shizuoka, Japan; [2]Internal Medicine 1, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan.*

**Background:** Initiation of hemodialysis (HD) therapy is reported to increase whole body fat mass by dual X-ray absorptiometry. However, it remains to be determined whether abdominal adiposity and skeletal muscle mass may change over time after HD initiation.

**Methods:** We enrolled 75 patients who had started HD therapy within 18 months (time on HD: 6±4 [1-18] months, age: 66±15 [29-91] years old, male/female=53/22, diabetes n=31), and measured abdominal subcutaneous fat mass area (ASFA), abdominal visceral fat mass area (AVFA), thigh muscle mass area (TMA), and thigh fat mass area (TFA) form cross-sectional CT images using the software (Fat index view, The Aquilion™ TSX-101A, TOSHIBA. Japan). We then followed all of the patients for the next 12±1 [5-15] months, and repeatedly assessed nutritional parameters.

**Results:** During the follow-up period, there was a significant increase in serum albumin (3.3±0.4 vs. 3.5±0.4 g/dL, p<0.01) and prealbumin (27±7 vs. 29±8 mg/dL, p<0.05). Body mass index (BMI) was identical, but ASFA was increased from 107.4±53.2 to 117.0±63.2 $cm^2$ (p<0.05). There was a significant decrease in TMA both in the right (84.5±25.2 vs. 80.8±26.6 cm2, p<0.01) and in the left legs (82.1±23.9 vs. 78.2±26.1 cm2, p<0.01). In patients who had gained body weight (+3.8±4.3%, n=38), a significant increase was found in ASFA (110.4±55.3 vs. 134.2±65.9 $cm^2$, p<0.01) and AVFA (99.7±51.3 vs. 116.0±56.3 $cm^2$, p<0.01), while TMA and TFA did not change. In patients who had lost their weight (-3.9±4.2%, n=37), a significant decrease was found in TMA and TFA in the both legs, but ASFA and AVFA did not alter.

**Conclusions:** These findings suggest that initiation of maintenance HD therapy increased abdominal adiposity while decreased thigh muscle volume over 1 year, indicating the accelerated sarcopenic obesity in incident HD patients.

### FR-PO787

**The Beneficial Effects of Body Weight Reduction in Overweight Patients with Proteinuric Immunoglobulin A Nephropathy: A Randomized Controlled Trial** Piyawan Kittiskulnam, Talerngsak Kanjanabuch, Wiwat Chancharoenthana, Kearkiat Praditpornsilpa, Somchai Eiam-ong, Kriang Tungsanga. *Faculty of Medicine, Chulalongkorn Univ, Bangkok, Thailand.*

**Background:** The study was conducted to examine the effects of body weight (BW) reduction on proteinuria in overweight and biopsy-proven IgA nephropathy (IgAN) patients (body mass index, BMI>23 kg/m$^2$) with persistent proteinuria ≥1 g/day.

**Methods:** 26 overweight IgAN patients were randomized into 2 groups: 1) control-usual dietary intake (n=13) and 2) low-calorie, a 500-Kcal/d subtraction from basal energy expenditure, and normal protein diet (n=13). Patients were treated with the maximal doses of ACEI/ARB and other anti-hypertensive agents to control BP at the level of less than 130/85 mmHg. At baseline and 6-month treatment, anthropometric measurement, dietary food record, urine protein nitrogen appearance (PNA), plasma adipokines (leptin, adiponectin & resistin) and interleukin (IL)-6 as well as laboratory parameters were assessed. (NCT01773382)

**Results:** There were no significant differences in baseline characteristics and demographics, including BP, BMI, renal function, 24-hour urine protein, normalized PNA (nPNA), and plasma cytokines. After 6 months, nPNA and dietary protein intake were not different, indicating comparable protein intake. Total daily calorie intake were significantly lower in the low-calorie diet group (1,307±171 *vs.* 1,772±315 Kcal/d, $p$<0.01). There were significant reductions in BW (3.9±2.6 kgs, $p$<0.001) and 24-hour urine protein (0.6±0.4 g/d, $p$<0.001) in the low-calorie diet group. BP and renal function parameters were not different. In the low-calorie diet group, patients with BW reduction of more than 5% had significantly lower plasma levels of leptin (46.5±27.6 *vs.* 1.9±37.8%, $p$=0.02) but higher adiponectin than the control group (18.6±14.7 *vs.* 3.1±21.2%, $p$=0.03).

**Conclusions:** 6-month BW reduction is independently associated with significantly decreased proteinuria in overweight IgAN patients. This effect may be partly mediated by changes in adipokine levels. Further studies are required to examine the long term effect of BW reduction in IgAN.

*Funding:* Government Support - Non-U.S.

### FR-PO788

**Increased Daily Caloric Intake Is Associated with Increased Exercise Capacity in Obese Diabetic Patients with Chronic Kidney Disease** Cheryl Cooper, Eileen Collins, Jolene Butler, Susan O'Connell, Christine Jelinek, Conor McBurney, Anne Garabedian, Holly J. Kramer, David J. Leehey. *Medicine and Research, Hines VA Hospital, Hines, IL.*

**Background:** Patients with diabetes, obesity, and chronic kidney disease (CKD) are generally physically inactive which may be an important contributor to their high mortality rate. Whether higher daily caloric intake has a beneficial or detrimental effect on ability to exercise in this population is unknown.

**Methods:** We examined baseline data from a VA funded randomized controlled trial (NCT01036490) to assess physical fitness in this population. Inclusion criteria are type 2 diabetes, obesity (body mass index > 30 kg/m$^2$), chronic kidney disease (stage 2-4) and persistent proteinuria (> 200 mg/day for at least 3 months). Data from the baseline symptom-limited treadmill test were analyzed. We calculated daily caloric intake at baseline using a 24-hour diet recall. Average daily intake in kilocalories (kcal) was calculated using Food Processor software (Version 9.5.0, ESHA Research, Salem Oregon).

**Results:** Baseline data on 30 subjects were evaluated. Mean values (± SD) were as follows: age 66 ± 8.1 yrs, body mass index 36.5 ± 4.9 kg/m$^2$, percent body fat 41.3 ± 6.6%, glycated hemoglobin 8.1 ± 1.8%, serum creatinine 2.3 ± 1.0 mg/dL, creatinine clearance 46.5 ± 35.7 mL/min, urinary albumin excretion rate 1013 ± 1073 mg/24h. Average treadmill time was 6.8 ± 3.8 minutes and peak oxygen consumption ($VO_2$ peak) was 13.1 ± 3.6 mL/kg/min (peak METS 3.7 ± 1.0; range 2.0-5.8). Average daily caloric intake was 2102 ± 591 kcals (range 1076-3266). Daily caloric intake was highly correlated with $VO_2$ peak and METS (r = 0.49, p < 0.01) and creatinine clearance (r = 0.52, p < 0.01). Interestingly there was no correlation between caloric intake and body mass index (r = - 0.06) or percent body fat (r = - 0.19).

**Conclusions:** These data indicate that obese diabetic subjects with CKD have markedly impaired baseline physical fitness (peak METS less than half of what would be expected in a healthy population). However, despite obesity, increased caloric intake was associated with a beneficial rather than a detrimental effect on treadmill exercise capacity.

*Funding:* Veterans Affairs Support

### FR-PO789

**Body Cell Mass, Measured by Electrical Body Impedance, Is Adequate to Estimate Muscle Mass in Renal Patients** Carlo Donadio. *Clinical and Experimental Medicine, Univ of Pisa, Pisa, Italy.*

**Background:** Malnutrition is particularly evident in ESRD patients treated by dialysis (MHD). Malnutrition can be found also in pre-ESRD patients. Since the nutritional status is a major determinant of long-term outcome of renal patients, there is a need for simple and accurate methods to evaluate nutritional status.

The measurement of the electrical impedance of the body (BIA) is a simple, rapid, and inexpensive method to analyze body composition and body fluid compartments. Among other parameters, BIA allows to estimate Body Cell Mass (BCM), which is the metabolically active component of fat-free mass and is mainly related to muscle mass.

Aim of this study was to validated the measurement of BCM obtained with single and multifrequency BIA as an indicator of the amount of muscle mass in pre-ESRD and in MHD patients.

**Methods:** Sixty-two adult renal patients (29 F, 33 M) aged 19-79 years, affected by different kidney disease. Their serum creatinine (Creat) ranged between 0.55 and 2.27 mg/dL and GFR (99mTc-DTPA) between 32 and 127 mL/min/1.73 m$^2$. Other 19 anuric MHD patients (7 F, 12 M) aged 28-82 years, dialysis vintage 0.6-15y, mean 7.3 years were evaluated. The values of BCM were obtained using a single frequency and a multifrequency tetrapolar impedance plethysmograph. In CKD patients 24 hour urinary creatinine excretion (24h Ucr) was calculated. In MHD patients 24h Creat Generation was calculated by the increase in serum Creat in the inter-dialytic period.

**Results:** A very high linear correlation was found between the values of BCM and 24h Ucr (r=0.807). The correlation between BCM and Creat generation was even higher (r=0.894). Similar results were obtained by single and multifrequency BIA. Since Creat is produced by muscle cells 24h UCr in CKD and Creat Gen in MHD patients equal creatinine production, and both are useful tools to assess the amount of muscle mass.

These data indicate that the value of BCM obtained with BIA is strictly related to the amount of muscle mass, estimated from 24h Ucr in CKD patients and by creatinine generation in MHD patients.

**Conclusions:** In conclusion, the measurement of body cell mass seems an adequate tool to evaluate muscle mass and nutritional status of renal patients.

*Funding:* Government Support - Non-U.S.

### FR-PO790

**Low Grade Albuminuria and Its Relationship with Metabolic Syndrome and eGFR in a Mexican Population: Results of the Salmex Study** Olynka Vega, Maria de Los Angeles Mendoza-De la Garza, Rodolfo Rincon-Pedrero, Yolanda Victoria Baeza-arias, Angeles Espinosa-cuevas, Jorge Ignacio Fonseca-Correa, Bertha Herrero-bervera, Ricardo Correa-Rotter. *Nephrology and Mineral Metabolism, National Medical Science and Nutrition Institute, Mexico, Distrito Federal, Mexico.*

**Background:** Low-grade microalbuminuria (LowACR, <30 mg/day) has previously been identified as a cardiovascular risk factor. Metabolic syndrome (MS) has also been shown to be related with microalbuminuria. We assessed the relationship among LowACR, renal function, and the present components of the MS from the Salmex Cohort Study.

**Methods:** From a cohort of 1009 subjects, we included 983 adults (62.3% women). Participants with albuminuria greater than 300 mg/g (n=26) were excluded. MS components were defined using the AHA definitions.

**Results:** A high prevalence of MS (30.3%) was observed. Table 1 shows the studied variables according to the number of components of the MS. LowACR correlated with BMI (r 0.23, p <0.0001), MS components (r0.22 p <0.0001), CKD-EPI (r0.16, p0.03), age (r0.12, p<0.001), and systolic blood pressure (SBP, r0.09, p0.005). In a stepwise multiple regression model, with LowACR as the dependent variable, the final model identified, age, CKD-EPI, and MS components as the independent variables explaining the variance of the LowACR (model R=0.36).

| | 0 N 180 | 1 N 246 | 2 N 259 | 3 N 199 | 4 N 91 | 5 N 8 | P |
|---|---|---|---|---|---|---|---|
| Age | 34.7±10.9 | 36.04±10.2 | 39.8±9.8 | 41.7±9.4 | 42.7±9.0 | 46.6±9.0 | <0.0001 |
| BMI | 23.05±3.04 | 24.8±3.9 | 27.3±4.8 | 29.6±3.9 | 32.3±4.9 | 34.5±3.2 | 0.0001 |
| **CKD-EPI** | 109.9±14.6 | 109.4±13.6 | 106.1±14.5 | 105.7±12.8 | 104.9±15.1 | 102.4±13.9 | 0.001 |
| **LowACR, mg/g** | 3.1±4.4 | 3.2±4.5 | 4.0±5.1 | 5.7±5.4 | 9.7±10.1 | 17.5±20.2 | <0.0001 |
| **PCR, mg/dL** | 0.09±0.3 | 0.17±0.05 | 0.31±0.05 | 0.61±0.45 | 0.71±0.61 | 0.86±0.75 | <0.0001 |
| **SBP** | 111.9±8.7 | 114.9±12.6 | 119.7±12.3 | 124.8±13.7 | 127.0±13.5 | 146.0±13.1 | <0.0001 |

**Conclusions:** LowACR was associated with MS and its components.

### FR-PO791

**Calibration of the Brief Food Frequency Questionnaire among Patients on Dialysis** Cynthia Delgado,[1] Patricia Ann Ward,[2] Glenn M. Chertow,[3,7] Lindsey Storer,[1] Lorien S. Dalrymple,[4] Torin Block,[5] George A. Kaysen,[4,7] John Kornak,[6,7] Barbara A. Grimes,[6,7] Nancy G. Kutner,[2] Kirsten L. Johansen.[1,7] *[1]Univ of California, San Francisco; San Francisco Dept of Veterans Affairs; [2]Emory Univ; [3]Stanford Univ School of Medicine; [4]Univ of California, Davis; [5]NutritionQuest Berkley; [6]Univ of California, San Francisco; [7]USRDS Nutrition Special Studies Center.*

**Background:** Accurately estimating dietary intake is particularly challenging in patients with chronic diseases. The aim of this study was to develop a calibrated equation based on dietary intake restricted by food items from the Block Brief 2000 food frequency questionnaire (BFFQ) using 3-day food diary records among patients on dialysis.

**Methods:** 3-day food diary data from 146 incident dialysis patients were reviewed and entered into a web-based dietary interview system. Information was re-entered omitting any foods in the diaries that were not included in the BFFQ to generate a "BFFQ-restricted" set of intakes. Major dietary components were modeled separately using linear regression. Independent variables were BFFQ-restricted food diary estimates as the average of the 3 days of diaries, restricted to items included in the BFFQ, with the unrestricted 3-day food diary averages as dependent variables.

**Results:** The BFFQ-restricted diary energy estimate of 1325 ± 545 kcal was 87% of the energy intake in the full food diary (1510.3 ± 510.4, p<0.0001). BFFQ-restricted diary carbohydrate intake was 83% of the full food diary (156.7 ± 78.7 g vs. 190.4 ± 72.7, p<0.0001). The BFFQ-restricted fat intake was 90% of full diary-reported fat intake (50.1 ± 24.1 g vs. 56.4 ± 21.6 g, p<0.0001). Daily protein intake assessments were not statistically different. Associations among BFFQ-restricted diary and unrestricted intake were linear. Final equations did not include adjustments for age, sex, or race because the patterns of associations were not significantly different.

**Conclusions:** Energy and macronutrient estimates by BFFQ are lower than estimates from 3-day food diaries, but simple calibration equations can be used to approximate total intake from BFFQ responses.

*Funding:* NIDDK Support, Other U.S. Government Support, Veterans Affairs Support

## FR-PO792

**Validating a Computer Generated Food Frequency Questionnaire in Children with Hypertension** Rossana Baracco, Nancy Fassinger, Mauricio Romero Olvera, Murty Adabala, Amrish Jain, Gaurav Kapur. *Pediatrics, Children's Hospital of Michigan/Wayne State Univ, Detroit, MI.*

**Background:** Lifestyle modifications, including a low sodium diet, are the cornerstone in the management of hypertension (HTN). VioFFQ is a computer generated food frequency questionnaire that collects detailed nutritional intake data and processes it immediately into a user friendly report that helps the healthcare team assess dietary habits and provide counseling. The aim of this study was to validate VioFFQ in children with HTN using 24 hour urine excretion of urea.

**Methods:** Prospective study of children ages 10 to 19 who were referred for evaluation and management of HTN. Twenty four hour urine was collected for estimating excretion of sodium and urea. The VioFFQ was administered on the morning after the urine collection was done. Pearson correlation was done for intake assessed by the questionnaire and 24 hour urinary measurement of sodium and urea.

**Results:** Of 50 patients enrolled, 36 completed the study and were included. Of these, 51.4% were African American, 58.3% were male and 58.3% were obese. The medians of age, weight and BMI were 15 (11 – 18), 75.2 kg (42.6 – 131.4) and 27.9 (16.1 – 47.1) respectively. Protein and sodium intake measured by VioFFQ correlated with urine excretion of urea and sodium (r=0.5, p<0.05; r=0.05, p<0.05 respectively). The median sodium intake measured by VioFFQ was 3795 grams (2077 – 12765). Obese and African American patients had higher sodium intake.

| | Obese median (range) | Non Obese median (range) | African-American median (range) | Non-African-American median (range) |
|---|---|---|---|---|
| Sodium intake (mg) | 4199 (2295 - 12765) | 3608 (2077 - 8123) | 4324 (2077 - 12765) | 3618 (2442 - 10676) |
| Sodium intake per 1000 calories (mg) | 1792 (1452 - 3159)* | 1494 (1103 - 2126)* | 1603 (666 - 2776) | 1497 (585 - 2640) |
| *p<0.01 | | | | |

**Conclusions:** VioFFQ is a reliable tool to estimate dietary patterns of sodium and protein intake in children. Obese children have higher intake of sodium as assessed by the survey. As the VioFFQ is picture based, meaningful and easy dietary recommendations can be made regarding dietary changes in children with HTN.

*Funding:* Private Foundation Support

## FR-PO793

**Increasing Dietary Fiber Reduces Plasma Levels of Colon-Derived Uremic Solutes in Hemodialysis Patients** Tammy L. Sirich,[1] Natalie Plummer,[1] Thomas H. Hostetter,[2] Timothy W. Meyer.[1] *[1]Medicine, Stanford Univ, Palo Alto, CA; [2]Medicine, Case Western Reserve Univ, Cleveland, OH.*

**Background:** Numerous uremic solutes are derived from the breakdown of amino acids by colon bacteria. Two such solutes, indoxyl sulfate (IS) and p-cresol sulfate (PCS), have been associated with adverse outcomes in renal failure. This randomized study tested whether increasing fiber intake would lower the plasma levels of IS and PCS in hemodialysis patients.

**Methods:** Forty patients on maintenance hemodialysis received either daily supplements of fiber (n=20) or a control starch (n=20) for 6 weeks. Fiber was administered as 18 g/day of resistant starch from high amylose maize. The pre-dialysis plasma levels of IS and PCS were assessed at week 0 and week 6 by liquid chromatography and tandem mass spectrometry. The free, unbound solute concentrations were measured as these are the levels to which body tissues are exposed.

**Results:** Results showed (mean±sd; [a],p<0.025 Week 0 vs Week 6; [b], p<0.025 Fiber vs Control):

| | | Fiber | Control |
|---|---|---|---|
| IS (mg/dl) | Week 0 | 0.36 ± 0.23 | 0.28 ± 0.16 |
| | Week 6 | 0.25 ± 0.17 [a] | 0.28 ± 0.15 |
| | % change | - 27 ± 39 [b] | 8 ± 39 |
| PCS (mg/dl) | Week 0 | 0.27 ± 0.15 | 0.22 ± 0.13 |
| | Week 6 | 0.21 ± 0.14 [a] | 0.23 ± 0.14 |
| | % change | -24 ± 44 | 7 ± 46 |

Increasing fiber intake reduced the plasma levels of IS while the control starch had no effect. Comparison of the % change in plasma levels of IS confirmed a statistically significant effect of increasing fiber intake. Increasing fiber intake also reduced the plasma levels of PCS but the difference between the fiber and control groups did not achieve statistical significance. There were no differences in gastrointestinal or other side effects between the two groups.

**Conclusions:** Results are in accord with the hypothesis that by providing colon microbes with carbohydrate that escapes digestion in the small intestine, increasing fiber intake reduces microbial production of uremic solutes from amino acids. Increasing fiber intake thus provides a potential means to reduce levels of colon-derived solutes without intensifying the dialysis prescription.

*Funding:* Other NIH Support - National Center for Complementary and Alternative Medicine (NCCAM)

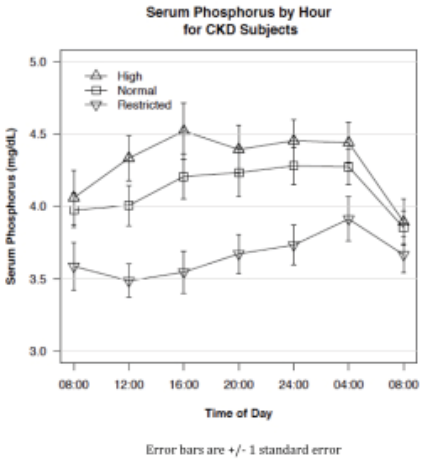
## FR-PO794

**Effects of Dietary Phosphorus on Circadian Rhythm of Serum Phosphorus in Chronic Kidney Disease** Cheryl A. Anderson,[1] Dena E. Rifkin,[1] Joachim H. Ix,[1] Gerard John Smits,[2] Geoffrey A. Block.[2] *[1]Univ of California San Diego; [2]Denver Nephrologists PC.*

**Background:** Effects of dietary phosphorus (P) on circadian rhythm of serum P have been described in healthy individuals, but not in individuals with moderate chronic kidney disease (CKD). We describe the effects of three diets with varying P on circadian rhythm of serum P.

**Methods:** We conducted a crossover trial in 11 individuals with eGFR 30 – 45 mL/min/1.73m$^2$. Participants received 3 different P diets sequentially ("restricted": 750 mg/day + Lanthanum Carbonate 1000 mg (TID), "normal": 1500 mg/day, and "high": 3000 mg/day). Participants ate only foods provided for 7 days, and were then admitted to an in-patient facility for 24-hours of observation followed by a 10-day washout. Serum P was collected every 4 hours starting at 8am during in-patient observation. Mixed effects regression models were used for analyses.

**Results:** Participants were 91% white, 64% female; with baseline mean(SD) age of 67(14) years, serum P of 3.6(0.5), and eGFR of 36.5 (8.4) mL/min/1.73m$^2$. There was a significant time*diet interaction on serum P (P< 0.05). On normal and high P diets, serum P increased from morning through the afternoon, whereas no diurnal difference was seen on the restricted P diet (Figure). Serum P was lowest and had the least difference by diet at 8am. The largest difference in serum P by diet was observed at 4-8pm.




**Conclusions:** Change in serum P was observed in CKD patients consuming normal and high P diets. There was also evidence of a circadian pattern with the nadir at 8am. Future intervention studies targeting intestinal P absorption may benefit from evaluation of afternoon/evening measurements rather than morning serum P.

*Funding:* Pharmaceutical Company Support - CM&D Pharma, Limited

## FR-PO795

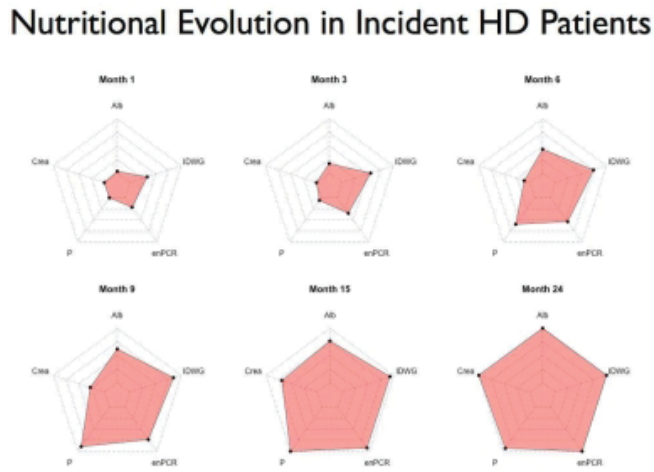
**Trajectories of Nutritional Parameters and Formulation of a Composite Nutritional Score in Hemodialysis Patients** Michelle M.Y. Wong,[1] Stephan Thijssen,[1] Len A. Usvyat,[2] Peter Kotanko,[1] Franklin W. Maddux.[2] *[1]Renal Research Institute, New York, NY; [2]Fresenius Medical Care North America, Waltham, MA.*

**Background:** Nutritional status is assessed using a combination of multiple parameters in hemodialysis (HD) patients. However, most existing nutritional scores are derived from cross-sectional data and do not consider trajectories of nutritional parameters. Therefore, a cohort study was performed 1) to assess temporal patterns of nutritional parameters using radar plots and 2) to generate a composite nutritional score.

**Methods:** The patient cohort was derived from the Fresenius Medical Care North America database, and included incident patients who started HD between 2007 and 2011. Serum albumin (Alb), creatinine (Crea), phosphate (P), equilibrated normalized protein catabolic rate (enPCR) and interdialytic weight gain (IDWG) were graphed on radar plots at months 1,3,6,9,15,24 after initiating HD. A composite nutritional score was formulated by converting these five component variables to z scores and generating an additive composite, which was then converted to percentiles. The nutritional score was tracked over two years.

**Results:** The full dataset comprised 47,238 patients, but not every patient had data available for every parameter each month. Early after HD initiation, albumin, creatinine, phosphate, enPCR and IDWG values indicated poorer nutritional status. Over the first 2

years of HD, each of these nutritional parameters improved, as illustrated on radar plots. The composite nutritional score also improved from the 26th percentile at the start of HD to the 57th percentile at 2 years.




**Conclusions:** Serial radar plots are useful tools to assess overall nutritional status by incorporating multiple nutritional parameters and their trajectories over time. In future analyses, trajectories of the composite nutritional score can be applied to predict outcomes in HD patients.

## FR-PO796

**Does a Low Salt Diet Affect Protein Intake in Chronic Kidney Disease Patients?** Eiichiro Kanda,[1] Toshitaka Muneyuki,[2] Kaori Sakamoto,[3] Tomoya Hirayama,[4] Kei Nakajima,[5] Yoshihiro Matsumoto,[6] Sanae Watanabe,[3] Yoshihiko Kanno.[7] *[1]Dept of Nephrology, Tokyo Kyosai Hospital; [2]Saitama Citizens Medical Center; [3]Kagawa Nutrition Univ; [4]Kitasaito Hospital; [5]Josai Univ; [6]Shizuoka City Hospital; [7]Tokyo Medical Univ.*

**Background:** Among chronic kidney disease (CKD) patients with severe diet restriction, their compliance to diet restrictions declines. We have reported that protein intake can be estimated on the basis of creatinine-correlated urinary urea nitrogen (UNC) level of spot urine. In this study, we investigated the relationship between salt intake and UNC as an alternative index of protein intake.

**Methods:** 132 CKD patients (80 males), who were administered a low salt diet, were enrolled in this prospective cohort study in Japan. Their UCN and urinary sodium (UNa) levels were measured three times at separate outpatient clinics. Salt intake was estimated using the salt intake formula of The Japanese Society of Hypertension Guidelines 2009. The relationship between salt intake and UNC was evaluated on the basis of Pearson's correlation coefficient (r) with the initial measurement, and generalized estimating equation (GEE) and generalized linear mixed model (GLMM) with repeated measurements. GEE and GLMM were adjusted for age, gender, estimated glomerular filtration rate, proteinuria level, and diuretic use.

**Results:** The mean age (SD) was 64.6(13.0) years; eGFR 64.1(25.9) ml/min/1.73m$^2$; UPro/Cr 4.5(11.1) mg/gCr; UNC 6.97(2.51) g/gCr; estimated dietary protein intake 48.5(8.6) g/day; and salt intake 10.02(3.0) g/day. Salt intake correlated with UNC at the initial measurement (r=0.341, p=0.0005). After adjustment for baseline patient characteristics, a positive longitudinal relationship was indicated by GEE between the salt intake and UNC (beta=0.345, p=0.0001). The GLMM showed that the increase in salt intake can predict the increase in UNC (beta=0.346, p=0.0001).

**Conclusions:** This study showed that UNC is associated with salt intake in CKD patients administered a low salt diet. Because UNC is positively associated with protein intake, it is suggested that a protein intake restriction may be achieved with only salt restriction.

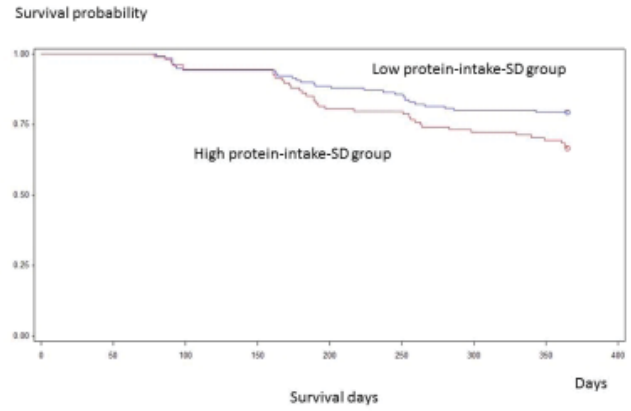
## FR-PO797

**Fluctuating Protein Intake Is an Independent Risk Factor for the Progression of Chronic Kidney Disease** Eiichiro Kanda,[1] Masumi Ai,[2] Renjiro Kuriyama,[3] Masayuki Yoshida,[2] Tatsuo Shiigai.[4] *[1]Tokyo Kyosai Hospital; [2]Tokyo Medical and Dental Univ; [3]Kokubunji Minamiguchi Clinic; [4]Shiigai Clinic.*

**Background:** A low-protein diet prevents the progression of chronic kidney disease (CKD). However, the consistent adherence to a low-protein diet is difficult. We investigated the longitudinal effects of changes in protein intake on the CKD progression in elderly CKD patients.

**Methods:** CKD patients (n=249, 190 males) administered a low-protein (0.6 to 0.8 g/kg/day) were enrolled in this retrospective cohort study in Japan. Their protein intake was measured every three months. The longitudinal change in protein intake was evaluated by the standard deviation (SD) of their protein intake (protein-intake SD). The endpoint was 25% decline in estimated glomerular filtration rate (eGFR) or starting dialysis. The outcome was evaluated using a time-dependent Cox proportional hazard model adjusted for demographics.

**Results:** The mean age (SD) was 70.6 (7.0) years; eGFR 22.2 (14.1) ml/min/1.73m$^2$; protein intake 0.85 (0.22) g/day; protein-intake SD 0.12 (0.07) (median, 0.10; Q1, 0.07; Q3, 0.15). The primary endpoint was observed in 65 patients (26.1%). The patients with high protein-intake SD (>0.10) had a high risk of the renal death: hazard ratio (HR), 1.712 (95%CI 1.050, 2.792); adjusted HR, 2.144 (1.046, 4.391).




An increase in protein-intake SD by 0.1 increased the risk of renal death; adjusted HR, 2.386 (1.293, 4.405). A generalized estimating equation model showed a longitudinal relationship between protein intake and serum albumin level [beta=0.0011 (0.0001), p=0.0043].

**Conclusions:** This study showed that protein-intake SD is associated with CKD progression. Because protein intake is associated serum albumin level, a stable protein intake and nutritional monitoring are necessary to prevent CKD progression and malnutrition.

## FR-PO798

**Efficacy of Eicosapentaenoic Acid (EPA) on Chronic Kidney Disease (CKD) due to Benign Nephrosclerosis** Yoshihiko Inoue, Tadahide Maezumi, Daisuke Komukai, Kiyoko Inui, Ashio Yoshimura. *Div of Nephrology, Showa Univ Fujigaoka Hospital, Yokohama, Kanagawa, Japan.*

**Background:** We studied the efficacy of EPA to prevent the progression of both renal impairment and atherosclerosis in CKD stage 3-4 patients due to benign nephrosclerosis (BNS).

**Methods:** 31 CKD stage 3-4 patients due to BNS with dyslipidemia were followed for 1 year after the start of EPA treatment. The dosage of 1800 mg/day was newly started. T-cho, LDL-cho, Triglycerides (TG), EPA, arachidonic acid (AA), Dihomo-gamma-linolenic acid (DGLA), docosa hexaenoic acid (DHA), right and left (RL) brachial-ankle pulse wave velocity (baPWV), RL maximum carotid intima-media thickness (max IMT), RL maximum carotid plaque thickness and RL ankle-brachial index (ABI) were studied at before treatment (baseline) and at the end of the study. eGFR and the amount of proteinuria were studied at 1year before the start of treatment, baseline and at the end of the study.

**Results:** EPA treatment elicited statistically significant increase in EPA (1-year of EPA: 242.2±100.7 vs. baseline: 73.0±39.3 μg/ml, P<0.01), EPA/AA ratio and decrease in TG. RL baPWV and RL max IMT were significantly improved (1-year of right baPWV: 1564.3±333.4 vs. baseline: 1686.1±369.2, 1-year of left: 1597.1±332.0 vs. baseline: 1723.4±378.8 cm/s, 1 year of right max IMT: 0.85±0.26 vs. baseline 0.96 ± 0.35, 1-year of left: 0.80±0.24 vs. baseline 0.92±0.31, P<0.05). eGFR and amount of proteinuria also showed significant improvement at the end of the study (1-year of eGFR: 46.9±13.0 vs. baseline: 43.8±11.1 ml/min/1.73m$^2$, P<0.05). Both EPA and DHA levels were significantly low in eGFR exacerbation patients group (n=7) compared with eGFR improvement patients group (n=24) at both baseline and the end of the study. The highest EPA level patients at baseline (EPA: 127.2±16.3 μg/ml, n=8) showed significant improvement changes in both eGFR and baPWV compared with the lowest EPA level patients (EPA: 30.1±8.1 μg/ml, n=8). There was no difference on both plaque thickness and ABI between baseline and the end of the study.

**Conclusions:** EPA may prevent both the development of renal dysfunction and the atherosclerotic change in CKD stage 3-4 patients due to BNS.

## FR-PO799

**Selenium Supplementation and Antioxidant Outcome Markers in Hemodialysis Patients** Elizabeth J. Sussman,[1] Carol S. Johnston,[2] Kenneth R. Boren,[3] Bhupinder Singh.[3] *[1]Family and Consumer Sciences, California State Univ, Northridge, Northridge, CA; [2]School of Nutrition and Health Promotion, Arizona State Univ, Phoenix, AZ; [3]Southwest Kidney Institute/DaVita, Inc., Tempe, AZ.*

**Background:** Hemodialysis (HD) patients elicit an oxidant-antioxidant imbalance in addition to a selenium deficiency, possibly contributing to cardiovascular disease mortality. The objective of this study was to evaluate the effect of selenium supplementation on antioxidant status in HD patients.

**Methods:** In this intervention trial, 27 maintenance (≥90days) HD patients (61.1±17.5y, 14M, 13F) receiving treatment in the greater Phoenix, AZ area were randomized to receive either a tablet of selenium (200µg/day [purported] of selenium as selenomethionine), or placebo daily for three months. Antioxidant status outcome measures included total antioxidant capacity, vitamin C, and RBC and plasma glutathione peroxidase (GSH-Px). Repeated measures ANOVA analyzed changes over time and between groups at months 0 and 2 and months 0 and 3.

**Results:** Independent analysis showed the pill provided 266µg of selenium/day. 21 patients completed 2 months of the study and 17 patients completed the study in its entirety. Data were analyzed for months 0, 1 and 2. No significant differences were noted for antioxidant status outcome measures with the exception of plasma GSH-Px. Patients receiving the selenium pill had a significant increase in plasma GSH-Px compared to the placebo group (6.0±11 and -4.0±7.6, respectively, p=0.023 for change between month 0 and month 2). In addition, vitamin C concentrations were well below normal ranges at all time points for both groups throughout the study (group averages ranged from 0.24 to 0.38 mg/dL and there were no differences between groups at any time point).

**Conclusions:** These data indicate that selenium supplementation increased plasma GSH-Px concentration in HD patients. Moreover, the low vitamin C status of HD patients warrants further research, specifically in conjunction with selenium supplementation to improve antioxidant status.

*Funding:* Pharmaceutical Company Support - Academy of Nutrition and Dietetics

## FR-PO800

### Association of Height with Death Risk in End-Stage Kidney Disease

Austin G. Stack,[1,2] Lookman Olalekan Abdul,[1] Ailish Hannigan,[2] Bhamidipati V.R. Murthy,[3] Liam F. Casserly.[1,2] *[1]Nephrology and Internal Medicine, Univ Hospital Limerick, Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Ireland; [3]Dept of Transplantation, Baylor College of Medicine.*

**Background:** Studies in the general population have identified an inverse association between height and the risk of death with tall individuals having lower mortality over shorter individuals. The aim of this study was to explore the associations of height with mortality among patients with end stage kidney disease.

**Methods:** We identified 989, 994 incident US dialysis patients who began dialysis treatment between 1995 and 2008 and followed until December 2010. Individual height measurements were recorded prior to the onset of first dialysis therapy. The association of height with 2-year mortality was explored in quintile groups of Q1 < 157.5 cm, Q2 157.5 to <165, Q3 165 to <170.2, Q4 170.2 to <177.8 cm and Q5 > 177.8 cm with Q1 as the referent, stratified by race, and with adjustment for age, sex, weight, clinical conditions, laboratory variables and lifestyle indicators These relationships were expressed as adjusted Hazard Ratios (HR). All analyses were conducted using SAS v 9.3 (Cary NY).

**Results:** The average age of the population was 62.8 years, 65 % were white, 30% Black, 3.6 % Asian and 1.1% Native American/Alaskan Native. Race-specific heights for White, Black, Asian and Native Americans were 174, 175, 167 and 174 cm respectively for men and were 160, 163 , 155, and 160cm respectively for women. Increasing height was associated with significantly higher mortality although this relationship did not extend to all race groups. **P<0.001

**Adjusted Hazard Ratios for Death by Height among US Dialysis Patients**

| | | Adjusted HR for Height in Quintiles (cm) | | | | |
|---|---|---|---|---|---|---|
| | | Q1 | Q2 | Q3 | Q4 | Q5 |
| Race | Patients | 122-158 | 158-165 | 165-170 | 170-178 | >178 |
| Native American | n=11, 089 | 0.97 | 1.00 | 1.02 | 1.03 | 1.05 ** |
| Asian | n=41,564 | 0.99 | 1.00 | 1.00 | 1.02 | 1.02 |
| Black | n=296, 365 | 0.99 | 1.00 | 1.01 | 1.00 | 1.02 |
| White | n=640, 976 | 0.96 ** | 1.00 | 1.02 | 1.01** | 1.02 ** |

| | | Adjusted HR for Height (cm) | | |
|---|---|---|---|---|
| Race | Patients | Per increasing quintile | | P-trend |
| Native American | n=11, 089 | 1.07 | (1.01-1.12) | < 0.01 |
| Asian | n=41,564 | 1.01 | (0.98-1.05) | NS |
| Black | n=296, 365 | 1.01 | (0.99-1.02) | NS |
| White | n=640, 976 | 1.04 | (1.03-1.04) | <0.0001 |

**Conclusions:** Unlike the general population, tallness is associated with higher mortality in patents who reach end stage kidney disease. This reverse epidemiological phenomenon requires further study to uncover putative mechanisms.

## FR-PO801

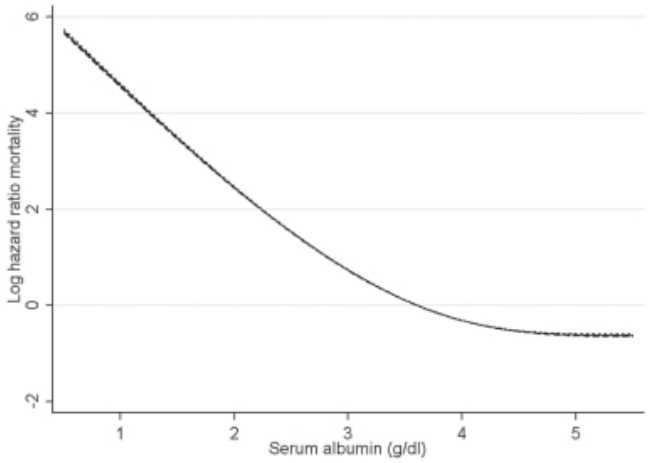
### Changes in Serum Albumin Associated with Decreased Kidney Function, and Its Effects on Mortality in a Nationally Representative Cohort of U.S. Veterans with Non-Dialysis Dependent CKD

Csaba P. Kovesdy,[1,2] Jun Ling Lu,[2] Miklos Zsolt Molnar,[3] Jennie Z. Ma,[4] Joel D. Kopple,[5] Kamyar Kalantar-Zadeh.[6] *[1]Memphis VA Medical Center; [2]Univ of Tennessee Health Science Center; [3]Univ of Toronto; [4]Univ of Virginia; [5]Harbor-UCLA Medical Center; [6]Univ of California-Irvine.*

**Background:** Serum albumin (ALB) level is a marker of protein-energy wasting and is associated with increased mortality. It is unclear if worsening CKD is associated with progressive decrease in ALB, and if intra-individual changes in ALB affect its association with mortality in patients with non-dialysis dependent (NDD) CKD.

**Methods:** We examined longitudinally ALB and its association with all-cause mortality in a cohort of 484,157 US veterans with NDD-CKD stages 1-5 and at least 2 ALB measurements, using mixed effects regression models, time-dependent Cox models and joint modeling, adjusted for demographic characteristics, comorbidities and medication use.

**Results:** Patients had 3,285,026 ALB measurements (median (IQR): 5/patient (3-9)) over 5.9 years median follow-up. The adjusted odds ratio (95%CI) of ALB<3.6 g/dl associated with a 10 ml/min/1.73m$^2$ lower eGFR was 1.09 (1.08-1.10, p<0.001), and individuals experienced an ALB decline at 0.047 g/dl/year (95%CI: 0.046-0.048, p<0.001). 159,761patients died (mortality rate: 61.4/1000 patient-years (95%CI: 61.1-61.7)). The adjusted mortality hazard ratio (95%CI) associated with each 1 g/dl lower ALB was 3.24 (3.21-3.27, p<0.001, Figure 1).




**Conclusions:** Thus, hypoalbuminemia worsens over time in patients with NDD-CKD, and progressively lower ALB is associated with increased mortality. The effect of therapeutic interventions to prevent or treat hypoalbuminemia will need to be examined in clinical trials.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO802

### Acute Kidney Injury in Shoshin Beriberi – A Case Series

Shashidhar Baikunje,[1] Rama S. Prakasha,[2] Glaxon Alex.[2] *[1]Dept of Nephrology, K. S. Hegde Medical Academy, Mangalore, India; [2]Dept of Medicine, K. S. Hegde Medical Academy, Mangalore, India.*

**Background:** Shoshin Beriberi is a rare and unusual metabolic cause of acute kidney injury and literature in this regard is sparse worldwide. Patients often present with evidence of right heart failure, severe shock and metabolic acidosis in addition to renal failure.

**Methods:** Retrospective analysis of eight cases with above manifestations.

**Results:** The mean age was 34.8 years and five out of eight were males. Three patients were alcoholics. The most common presenting features were edema, breathlessness and oliguria. Mean creatinine on admission was 2.54 mg/dl and mean MDRD eGFR was 36.6 ml/min/1.73 m$^2$. Mean haemoglobin was 11.9 g/dl, total count 13,412/cu.mm, mild proteinuria was present in seven, RBCs in urine in two and granular casts in four. Abnormal liver enzymes were noted in six of the eight patients, CPK and CK-MB were elevated in four. Echocardiographic evidence of right heart dilatation and pulmonary hypertension were seen in all the cases. Severe metabolic acidosis was noted with mean pH of 7.06 and mean arterial bicarbonate of 5.74mmol/l. Three patients required renal replacement therapy, two needed ventilatory support and four were treated with inotropes. Six patients were treated with broad spectrum antibiotics but the cultures were negative in all except one. IV thiamine was used in seven patients with excellent clinical response and one patient who did not receive thiamine died. There was improvement in acidosis, blood pressure and renal function within 48-72 hours and all the seven of them had normalization of creatinine levels.

**Conclusions:** Presentation with multiorgan involvement, severe metabolic acidosis, evidence of right heart dilatation with pulmonary hypertension on echocardiogram and dramatic response to IV thiamine were the basis of the diagnosis since the diagnostic tests for thiamine deficiency are rarely used in acute setting. Renal dysfunction has excellent prognosis and the recovery is complete if the condition is suspected and treated promptly.

**FR-PO803**

**Investigation of Carnitine Deficiency in Children Receiving Continuous Renal Replacement Therapy** Kristen Sgambat, Kirtida Mistry, Shamir Tuchman, Asha Moudgil. *Nephrology, Children's National, Washington, DC.*

**Background:** Carnitine deficiency is common in patients receiving chronic hemodialysis (HD) due to inadequate intake, decreased production, and removal during dialysis. The effect of continuous renal replacement therapy (CRRT) on carnitine homeostasis has not been previously studied. We hypothesized that children receiving CRRT may be at risk for carnitine deficiency due to continuous removal, absent intake, and comorbidities related to underlying critical illness.

**Methods:** Medical records of all patients recieving CRRT at Children's National between 2011 and 2013 were reviewed for total carnitine (TC)and free carnitine (FC) levels. Patients on carnitine supplementation were excluded. Prevalence and progression of carnitine deficiency at baseline, 0.5, 1, 2, and 3 weeks duration on CRRT was assessed. Mean TC and FC levels of children on CRRT were compared to those of a prospective comparison group of children on chronic HD for > 3 months by Student's t test.

**Results:** The study group included 27 CRRT patients with mean age 9.9±7.4 years. At initiation of CRRT,14.3% of children were TC deficient. Prevalence of deficiency increased with duration of CRRT to 54.5%, 72.7%, 75%, and 100% at 0.5,1,2,and 3 week time points, respectively. The prevalence of FC deficiency similarly increased wtih duration of CRRT from 42.9% at baseline to 70%, 72%, 75%, and 100% at 0.5,1,2,and 3 weeks. Mean carnitine levels after 1 week on CRRT were significantly lower compared with baseline levels (TC decreased from 41.7 to 20.9 and FC from 27.1 to 14.1 µmol/L), p<0.01. Mean carnitine levels of children on CRRT for 0.5 week was lower compared with levels of 9 children on chronic HD for mean duration of 9.3±6 months (TC 27.1±17.5 vs 49±5.1 and FC 17.5±13.0 vs 29±3.6 µmol/L), p<0.03.

**Conclusions:** Carnitine levels significantly decrease with longer time on CRRT, and the majority of children on CRRT for 1 week are carnitine deficient. Deficiency is more severe after only 0.5 week on CRRT in comparison to children on chronic dialysis for a mean of 9.3 months. Consequences of carnitine deficiency and possible benefits of supplementation in the critically ill pediatric CRRT population should be investigated.

**FR-PO804**

**The Effect of Serum Zinc on Salt Taste Acuity, Preference and Dietary Sodium Intake in Hemodialysis Patients** Hyun woo Kim,[1] So Mi Kim,[1] Eun Kyoung Lee,[2] Ji Eun Lee.[2] *[1]Div of Nephrology, Jeju National Univ Hospital, Jeju, Korea; [2]Div of Nephrology, Dankook Univ Hospital, Cheonan, Korea.*

**Background:** High sodium intake has been known for the major cause of fluid overload in hemodialysis patients, leading to increased cardiovascular mortality. High sodium intake is related to low salt taste acuity and high salt preference and zinc status is known to influence taste acuity. We tried to analyze the effect of serum zinc status on salt taste acuity, preference and dietary sodium intake in hemodialysis patients.

**Methods:** This cross-sectional study enrolled 77 patients, who underwent hemodialysis in the Jeju national university hospital or Dankook university hospital. The patients were divided into two groups based on the level of serum zinc : 26 patients showed normal range and 51 patients showed zinc deficiency. 1-day dietary recall was used to estimate the dietary sodium intake and Salt taste acuity and preference were determined by sensory test using variable concentration of NaCl solution.

**Results:** Baseline characteristic, mean age, sex, cause of ESRD, HTN medication, BP were not different between the two groups. The NaCl solution concentration of both salt taste acuity and preference showed higher tendency in the Zn deficient group than that in the Non- Zn deficient group ( mean NaCl % of taste acuity : 0.13±0.05 vs 0.16±0.57 p=0.04, mean NaCl % of salt taste preference : 0.48 ±0.13 vs 0.54±014, *p*=0.07). Although the sodium intake showed high tendency in zinc deficient group, there was no significant difference ( 2134 ± 937 vs 2546 ± 297 mg/day, *p*=0.056). But the correlation between the sodium intake and the serum zinc showed the significant negative linear relationship ( r=-0. 313 , *p* <0.05 ). Interdialytic weight gain was significantly higher in the zinc deficient group than that in non-zinc deficient group (2.68± 1.02 *vs.* 3.18 ± 1.02 kg, *p*=0.04).

**Conclusions:** We conclude that zinc deficiency may lead to high sodium intake in hemodialysis paitents, affecting on the salt taste acuity and preference.

**FR-PO805**

**Inadequate Dietary Intake Correlates with Thiol Markers of Oxidative Stress in Maintenance Hemodialysis Patients (HD)** Paolo Fanti,[1] Daniela Giustarini,[2] Ranieri Rossi,[2] Franco Folli,[1] Khaled Khazim,[3] Padam Hirachan,[1] Sue Cunningham,[1] Georgiana Gross,[1] Shweta Bansal.[1] *[1]Div of Nephrology, UT Health Science Center San Antonio, San Antonio, TX; [2]Univ of Siena, Italy; [3]Western Galilee Hosp., Israel.*

**Background:** Anorexia and poor nutrition are the most important immediate causes of protein-energy wasting (PEW) in uremia. Oxidative stress (OS) is believed to participate in the pathogenesis of uremic PEW, but it is unclear if direct association exists between poor nutrition and OS in this disease state.

**Methods:** To test the hypothesis that poor nutrition and OS are linked in uremia, we analyzed the 3-day diet record and the plasma thiols redox of 76 clinically stable HD and 24 age-matched healthy controls (C). 3-day diet record was analyzed with The Food Processor software (ESHA, Salem OR). Analysis of thiol redox included plasma total cysteine (CYS), cystine (CYSS) and protein thiolation index (PTI) a new marker of OS *[Giustarini D, et al. Free Radic Biol Med 53:907, 2012]*.

**Results:** As expected, HD showed very low intake of Calories (18 ± 7 kcal/kg/day) and Protein (0.8 ± 0.4 g/kg/day) and they displayed profound abnormalities of circulating thiols as compared to C, including high PTI (0.77 ± 0.21 vs. 0.46 ± 0.09; *p<0.001*), CYS (448 ± 101 vs. 316 ± 48 µM; *p<0.001*) and CYSS (101 ± 29 vs. 66.9 ± 11.6 µM; *p<0.001*). In multiple linear regression of appropriately transformed variables, HD showed negative association of PTI with Protein/kg/day (β=-0.360; *p=0.007*) and positive with age (β=0.274; *p=0.038*) while controlling for HD clinic, sex, ethnicity, cause of ESRD, body mass index and intake of Calories, Carbohydrates and Fat. Moreover, CYS was negatively associated with Calories/kg/day (β=-0.480; *p<0.001*), and CYSS was negatively associated with Calories/kg/day (β=-0.292; *p=0.021*) and positively with age (β=0.306; *p=0.015*) while controlling for the same co-variables as above.

**Conclusions:** Markers of thiol redox correlate negatively with Protein and Calorie intake in HD, raising the possibility that poor nutrition may contribute to OS in HD. Prospective studies are needed to determine whether cause-and-effect relation exist between low dietary intake and OS in CKD.

*Funding:* Other NIH Support - NCCAM, Veterans Affairs Support

**FR-PO806**

**The Effect of Oral Vitamin D Supplementation in Non-Dialytic CKD Patients** Sun Moon Kim,[1] Soon Kil Kwon,[1] Hye-Young Kim,[1] Han Ro,[2] Ji Yong Jung.[2] *[1]Internal Medicine, Chungbuk National Univ, College of Medicine; [2]Internal Medicine, Gachon Univ of Medicine and Science.*

**Background:** The anti-inflammatory, antifibrotic, and antiproteinuric properties of vitamin D have been defined in studies using active vitamin D analogs. Here, we study the effects of nutritional vitamin D supplementation on bone mineral metabolism and progression of renal disease in chronic kidney disease (CKD) patients.

**Methods:** We conducted the study in adult, vitamin D deficient [25(OH) D < 30 ng/mL], non-dialytic CKD patients. The patients were classified into oral vitamin D supplementation (cholecalciferol 1,000 IU/day) or not for 6 months. The serum level of 25(OH) D, parathyroid hormone (PTH), calcium, phosphate, alkaline phosphatase, creatinine, and urinary protein excretion were measured serially.

**Results:** Ninety seven patients were enrolled. The baseline characteristics were not different between vitamin D group vs. control group. Vit D supplementation achieved the improvement in serum 25(OH) D level (vitamin D vs. control, 28.01 ± 12.82 ng/mL vs. 13.50 ± 7.29 at 3 month, p <0.001; 29.49 ± 16.19 vs. 14.17 ± 8.17 at 6 month, p < 0.001). This difference was persistent in the subgroup analysis of baseline GFR <30 mL/min/1.73m$^2$ vs. GFR 30-60 or 25(OH) D <15 ng/mL vs. 25(OH) D 15-30. PTH level was decreased in the vitamin D group compared to control group (vitamin D vs. control; 40.2 (26.8 – 72.0) pg/mL vs. 54.2 (38.2 – 101.1) at 3 month, p = 0.013; 38.8 (19.2 – 65.3) vs. 58.1 (39.5 – 137.0) at 6 month, p = 0.014). Vitamin D supplementation increased corrected calcium level compared to control at 3 month, whereas the difference was not significant at 6 month. The level of phosphate, estimated GFR, and random urine protein-to-creatinine ratio were not different. During the study period, four patients in vitamin D group and five in control group started renal replacement therapy. The most common adverse effect was gastrointestinal irritation. There was no case of symptomatic hypercalcemia.

**Conclusions:** Oral vitamin D supplementation was effective in replenishing vitamin D store in CKD patients. However, we could not find any beneficial effect on renal disease progression.

**FR-PO807**

**The On-Line Hemodiafiltration Improves the Nutritional Status in Hemodialysis Prevalent Patients: A 1-Year, Prospective, Controlled Study** Pablo Molina,[1] Belen Vizcaino,[1] Angela Maria Serrato,[1] Daniel A. Molina,[1] Sandra Beltrán,[1] Avila Ana,[1] Julia Kanter,[1] Mariola D. Molina,[2] Jose L. Gorriz,[1] Luis M. Pallardo.[1] *[1]Dept of Nephrology, Hospital Universitari Dr Peset, Valencia, Spain; [2]Dept of Statistics and Operational Research, Universidad de Alicante, Spain.*

**Background:** Compared to conventional haemodialysis, on-line hemodiafiltration (OL-HDF) removes more efficiently uremic toxins, which could attenuate the hypercatabolism and increase the protein anabolism of hemodialysis patients. The present study evaluated the 1-year effect of OL-HDF on nutritional status in prevalent haemodialysis patients.

**Methods:** This single-center, controlled, intervention study enrolled all high-flux hemodialysis (HF-HD) subjects attending our Unit from 2012 to 2013 (n=33; mean age: 60.2±17.5 years; women: 42%; median time on dialysis:37(8-133) months). Patients were randomized to receive posdilution OL-HDF (n=17) or to remain on HF-HD (n=16). Changes in multifrequency bioimpedance parameters (primary outcomes) and other biochemical and anthropometric parameters (secondary outcomes) were used to assess the effect of OL-HDF in nutritional status. A repeated-measures analysis between baseline to 12-month follow-up was conducted.

**Results:** OL-HF treatment group showed no changes in body composition.

| Effect parameter | OL-HF | HF-HD | *p* |
|---|---|---|---|
| LTM (kg), mean | 0.3 | -7.5 | 0,001 |
| | -2.8 to 3.8 | -11.1 to -3.8 | |
| 95% CI | | | |
| Fat (kg), mean | 0.3 | 7.3 | 0,005 |
| | -1.9 to 2.4 | 2.8 to 11.8 | |
| 95% CI | | | |
| TBW (L), mean | -0.8 | -3.5 | 0,014 |
| | -2.2 to 0.7 | -5.6 to -1.5 | |
| 95% CI | | | |
| ICW (L), mean | -0.1 | -2.3 | 0,005 |
| | -1.0 to 0.8 | -3.8 to -0.9 | |
| 95% CI | | | |
| ECW (L), mean | -0.6 | -1.2 | 0,249 |
| | -1.5 to 0.2 | -2.0 to -0.4 | |
| 95% CI | | | |

A significant decrease in lean-tissue mass and intracellular water was observed only in the HF-HD in absence of changes in protein intake, with an increase in fat mass. Creatinine levels decreased only in HF-HD group (9,96±3.0 mg/dl vs. 9.5±3.5; p<0.001). Transferrin levels were reduced in both groups. No other changes were observed.

**Conclusions:** 1-year after its implementation, OL-HDF preserved protein nutritional status better than HF-HD. These results support the hypothesis that the convective dialysis treatment could ameliorate the hypercatabolic state of dialysis patients.

## FR-PO808

**The Effect of Lanthanum Carbonate on Metabolic Acidosis in Stage IV-V Chronic Kidney Disease Patients** William Beaubien-souligny, Jean-Philippe Lafrance, Denis Ouimet, Vincent Pichette, Robert Zoël Bell, Michel Vallee. *Dept of Medicine, Div of Nephrology, Maisonneuve-Rosemont Hospital, Montreal, Canada.*

**Background:** Hyperphosphatemia and metabolic acidosis are two frequently encountered problems in advanced CKD patients that are associated with significant mortality and morbidity. Lanthanum carbonate is a potent phosphate binder frequently used for control of hyperphosphatemia. Some studies have showed an increase in bicarbonate concentration in renal replacement patients but this effect have never been demonstrated in predialysis patients.

**Methods:** Retrospective data was collected from 2009 to 2012 on 62 consecutive CKD IV-V patients (46 hemodialysis, 3 peritoneal dialysis patients and 13 pre-dialysis patients) who start using lanthanum carbonate in a single center. Laboratory assays for phosphorus, calcium, parathormone (PTH) and serum bicarbonates were analyzed. The primary outcome identified was the serum bicarbonate change after introduction of lanthanum carbonate.

**Results:** Compare to baseline, introduction of lanthanum carbonate led to a mean increase in serum bicarbonate concentration of 2.33 mmol/L (p = 0.03) at one month. No significant increase was found after the initial month. The initial increase at one month tended to be greater in the pre-dialysis patients (3.33 mmol/L p = 0.13) compared with the renal replacement groups. A decrease in the phosphorus level of 1.11 mg/dL (0.36 mmol/L, p = 0.001) was found after the introduction of lanthanum carbonate, and was maintained over time. There was no significant change in calcium and PTH concentration compared with baseline.

**Conclusions:** Lanthanum carbonate introduction temporarily increased serum bicarbonate concentration in CKD IV-V patients. Further studies are needed to explore the role of lanthanum carbonate on the control of metabolic acidosis in predialysis patients.

*Funding:* Government Support - Non-U.S.

## FR-PO809

**Kidney Failure Is Associated with an Imbalance between Pro- and Anti-Oxidant Agents: Relationship with Uremic Solutes** Bertrand Gondouin,[1] Noemie Jourde,[1] Philippe Brunet,[1] Stephane Burtey,[1] Guieu Regis,[2] Bertrand Dussol.[1] *[1]Centre de Nephrologie et Transplantation Renale, Hôpital Conception, Assistance Publique des Hopitaux de Marseille, Marseille, France; [2]Service de Biochimie et Biologie Moleculaire, Hôpital la Timone, Assistance Publique des Hopitaux de Marseille, Marseille, France.*

**Background:** Patients with chronic kidney disease (CKD) are at high risk for cardiovascular events.This is related to non-classical risk factors, including oxydative stress. We evaluated a wide range of parameters involved in oxidative stress in CKD population and their relationship to different uremic solutes.

**Methods:** We performed an observational study on 50 hemodialysis (CKD HD) and 50 patients with CKD stage 3 to 5 not on dialysis (CKD NOD) and 38 healthy subjects (control). We performed dosages of (i) anti-oxidant agents: super oxide dismutase (SOD), vitamin C, A, E, zinc (Zn), copper (Cu) and selenium (Se), (ii) pro-oxidant agents: malondialdehyde (MDA), ischemia modified albumin (IMA), (iii) enzymes: xanthine oxidase (XO), red blood cells glutathione reductase (RBC-GR). and (iiii) 5 uremic solutes: indoxyl sulfate (IS), indole acetic acid (IAA), para cresyl sulfate (PCS), homocysteine (HCYS), Beta 2 microglobulin (B2M).

**Results:** Se and Zn were decreased (p<0.001 respectively) in CKD HD and CKD NOD compared to controls. Vitamin C was dramatically decreased in CKD HD and CKD NOD compared to control (both p <0.001). SOD activity was decreased in CKD HD compared to control (p<0.01). RBC-GR was increased in CKD HD and CKD NOD when compared to control (both p<0.001).

IMA levels were increased in CKD NOD and CKD HD compared to controls (both p<0.001). MDA and XO activity levels were increased in CKD HD compared to controls (p>0.001).

MDA was inversely correlated with eGFR in CKD NOD (r=-0.288; p <0.05). In CKD HD, IAA was positively correlated with IMA (r= 0.487; p=0.01), para cresyl sulphate (PCS) was inversely correlated with RBC-GR (r= -0.33; p <0.05), B2M was positively correlated with MDA (r= 0.51; p=0.001).

**Conclusions:** These results showed an imbalance in favour of an oxidative stress in CKD patients and a clear relationship between markers of oxidative stress and different uremic solutes.

## FR-PO810

**Treatment of IgA Nephropathy Using a Chinese Herbal Medicine Prescription (Long Hua-1)** Lin Wang,[1] Bing Xu,[2] Yueyi Deng,[1] Zhi Qiang Huang,[3] Yiping Chen.[1] *[1]Longhua Hospital, Shanghai Univ of Traditional Chinese Medicine, China; [2]Yue Yang Hospital, Shanghai Univ of Traditional Chinese Medicine, China; [3]Univ of Alabama at Birmingham.*

**Background:** Long Hua-1 (LH-1) is a Chinese herbal medicine prescription used to treat IgA nephropathy (IgAN) in Long Hua Hospital for decades. To evaluate its efficacy, we compared treatment responses to LH-1 with those to LH-1 plus steroids, cytotoxic agents and/or ARB (LH-1+).

**Methods:** Fifty-eight biopsy-proven IgAN patients with normal renal function and blood pressure were included in this retrospective study. Average age was 32±10 and 34±11 years respectively. Urinary protein (UP) ranged from 0.5 to 2 g/24 h in both groups, with or without microscopic hematuria. Thirty patients were treated with LH-1 only, 160 g bid, and 28 patients with LH-1+. Median follow-up time was around 13 mo. In each group, patients were divided into two sub-groups according to biopsy results: group 1: major changes were mesangial cell proliferation; group 2: mesangial cell proliferation with focal segmental sclerosis.

**Results:** There were no differences in blood pressure and renal function changes among the groups with different treatments. In group 1 treated with LH-1, UP dropped from 1.04±0.34 to 0.35±0.21 g/24 h after the treatment; in group 1 treated with LH-1+, UP dropped from 1.08±0.48 to 0.48±0.27 g/24 h. In group 2 treated with LH-1, UP dropped from 1.08±0.44 to 0.55±0.43 g/24 h after the treatment; in group 2 treated with LH-1+, UP dropped from 1.13±0.39 to 0.33±0.22 g/24 h. There was no statistically significant difference between UPs in group 2 after LH-1 vs. LH-1+. Among patients treated with LH-1+, 6 patients developed upper-respiratory infection followed by exacerbated proteinuria. Five other patients developed fungal and/or skin infections. Another patient showed liver function changes with increased GPT. No side effects were reported for patients treated with LH-1 only.

**Conclusions:** LH-1 improved proteinuria in IgAN with similar efficacy as LH-1+. Significant side effects were found in LH-1+ group but not in LH-1. These data justify future randomized clinical trials to assess the clinical impact of LH-1 in IgAN treatment.

*Funding:* Private Foundation Support

## FR-PO811

**Brachial–Ankle Pulse Wave Velocity Predicts Decline in Renal Function and Cardiovascular Events in Early Stages of Chronic Kidney Disease** Hye Eun Yoon, Sung Jun Kim, Hyeon Seok Hwang, Seok Joon Shin. *Internal Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

**Background:** In this study, we investigated the predictive capacity of the brachial–ankle aortic pulse wave velocity (baPWV), a marker of arterial stiffness, for the decline in renal function and for cardiovascular events in the early stages of chronic kidney disease (CKD).

**Methods:** Two hundred forty-one patients who underwent a comprehensive check-up were included and were divided into two groups according to their estimated glomerular filtration rates (eGFR): patients with CKD stages 2 and 3 (30 £ eGFR < 90 ml/min/1.73m$^2$, the eGFR < 90 group; n=117) and those with eGFR ≥ 90 ml/min/1.73 m$^2$ (eGFR ≥ 90 group; n=124). The change in renal function, the eGFR change, was determined by the slope of eGFR against time. We analysed whether baPWV was associated with eGFR change or predicted cardiovascular events.

**Results:** baPWV was independently associated with eGFR change in a multivariate analysis of the total patients (b=–0.011, p=0.011) and remained significantly associated with eGFR change in a subgroup analysis of the eGFR < 90 group (b=–0.008, p=0.028). baPWV was independently associated with cardiovascular events (odds ratio=1.002, p=0.048) in the eGFR < 90 group, but not in the eGFR ≥ 90 group. The receiver operative characteristic curve analysis showed that 1,568 cm/sec was the cut-off value of baPWV for predicting CV events in the eGFR < 90 group (area under curve=0.691, p=0.03).

**Conclusions:** In patients with early stages of CKD, baPWV was independently associated with the decline in renal function and short-term cardiovascular events.

*Funding:* Government Support - Non-U.S.

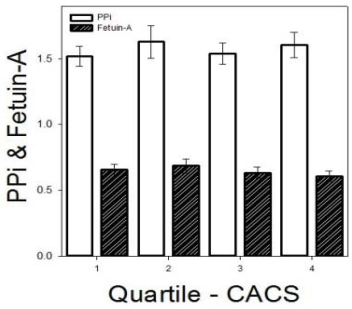
**FR-PO812**

**Inhibitors of Vascular Calcification and Coronary Artery Calcification in ESRD** Ramin Tolouian, Sean M. Connery, German T. Hernandez. *Texas Tech Univ HSC at El Paso, TX.*

**Background:** Vascular calcification (VC) is an important predictor of cardiovascular mortality in ESRD. Disturbances in mineral metabolism, especially Ca and $PO_4$, have been linked to enhanced calcification of blood vessels but the Ca & PO4 levels do not adequately explain this pathology. □ levels of inhibitors of the calcification process, such as Fetuin-A and inorganic pyrophosphate (PPi), are thought to VC. Therefore, we evaluated the association between Fetuin-A and PPi, and coronary calcification scores in maintenance HD.

**Methods:** 106 maintenance HD patients were studied (mean ±SD) : Age (yr.) 58 ±11.2, HD vintage (yr.) 4.0 ±3.2, Ca*P (mg/dl) 43 ±11.6, Male 63%, Hispanic 89%, diabetic 72%. Platelet free plasma Ppi was measured by radiometric, enzymatic method. Serum Fetuin-A was measured with the Ultrasensitive ELISA kit (Alpco 43-FETHUU-E01) on a Beckman DTX 800 microplate reader. Coronary arterial calcification score (CACS) was measured by sub-second gated helical computed tomography with an Imatron C-150 XL ultra fast CT scanner using a standard CACS protocol: slice thickness 3mm, Density Threshold (HU) 130, Pixel threshold 3, Algorithm Discrete, read by a single, experienced cardiologist.

**Results:**




There was no statistically significant difference between PPi and Fetuin-A when comparing low-risk CACS (<300) and high risk CACS (≥300), Ppi (mM) 1.56 ±0.49 vs 1.57 ±0.46, Fetuin-A (g/L) 0.67 ±0.24 vs 0.65 ±0.25. Furthermore, there was no significant difference in PPi and Fetuin-A levels between CACS quartiles (p=ns Kruskal-Wallis test). Mean CASC for each quartile were 0.80, 66, 391, and 2,957 respectively. Error bars indicate error of the mean.

**Conclusions:** There does not appear to be an unadjusted inverse relationship between inhibitors of vascular calcification and CACS our study of HD patients. The possibility of confounding by age, HD vintage, and diabetes needs to be further evaluated.

*Funding:* Private Foundation Support

**FR-PO813**

**Dietary Habits Are Associated with Proteinuria Independent of Major Cardiovascular Risk** Makoto Kanno,[1] Koichi Asahi,[1,2] Kenichi Tanaka,[1] Yoshimitsu Hayashi,[1] Masaaki Nakayama,[1] Kunitoshi Iseki,[2] Toshiki Moriyama,[2] Kunihiro Yamagata,[2] Kazuhiko Tsuruya,[2] Hideaki Yoshida,[2] Shouichi Fujimoto,[2] Tsuyoshi Watanabe.[1,2] *[1]Dept of Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Medical Univ School of Medicine, Fukushima, Japan; [2]Steering Committee for "Research on the Positioning of Chronic Kidney Disease (CKD) in the Specific Health Check and Guidance System of Japan", Fukushima, Japan.*

**Background:** Irregular dietary habits are associated with a number of risk factors for cardiovascular disease (CVD), including obesity, insulin resistance, and dyslipidemia. However, few studies have examined the relationship between these habits and proteinuria or chronic kidney disease (CKD).

**Methods:** This cross-sectional cohort study examined 181,524 subjects (73,753 men; 107,771 women; mean age, 63.8 years; age range, 40-74 years) who participated in an annual nationwide health checkup system in 2008. We compared prevalence of proteinuria (≥1+ on dipstick test) among participants according to the number of irregular dietary habits (skipping breakfast (SB), dinner within 2 h before bedtime (DbB), snacking after dinner (SaD) and eating quickly (EQ)) obtained by questionnaire response. Multivariate logistic regression models were used to adjust for possible confounding factors.

**Results:** Overall prevalence of proteinuria was 5.9%, and increased significantly from 5.2% to 8.6% with increases in the number of irregular dietary habits. The odds ratio (95% confidence interval) for proteinuria was 1.24 (1.17-1.32) with SB, 1.14 (1.08-1.20) with DbB, 1.08 (1.02-1.15) with SaD and 0.97 (0.93-1.01) with EQ, after adjusting for sex, age, body mass index, metabolic syndrome, hypertension, diabetes, dyslipidemia, estimated glomerular filtration rate, history of stroke, cardiac disease and kidney disease, smoking and exercise habit.

**Conclusions:** Some irregular dietary habits are associated with proteinuria independent of major CVD risk factors. Future research should investigate whether aggressive intervention regarding daily dietary habits can prevent the development of CKD.

**FR-PO814**

**Outcomes Associated with Oral Protein Supplementation during Maintenance Hemodialysis: A Quality Improvement Prospective Interventional Study** Buthayna Dinary, Keyvan Ravakhah, Fazel Dinary. *Internal Medicine/Nephrology, St. Vincent Medical Center, Cleveland, OH.*

**Background:** Low serum albumin levels in dialysis patients are associated with increased morbidity & mortality. Dietary protein intake is a cheap and equally effective way of achieving an increase in serum albumin and improve quality of life (QOL).

**Methods:** A multicenter, prospective, Interventional study included patients from three HD units affiliated with academic and tertiary-care hospitals in Cleveland OH. Patients were adult patients aged > 18 YO and have been treated by HD for at least 6 months. An inclusion criterion was: serum albumin concentration < 3.8 mg/dl in HD patients. Exclusion criteria were documented malignancies and any acute infectious diseases within the last 2 months. From a cohort of 106 patients who were enrolled, 87 were eligible for the analysis. LiquaCel, which provides 16 gm protein and 2.5 gm L-Arginine per 1 oz. serving, was given to patients with albumin level < 3.8. Blood samples for serum albumin were drawn on the day of midweek HD session. On the same day, the Quality of life Health Survey (QOLHS) Score was evaluated. Patients were evaluated for Serum albumin concentration which was measured monthly for 6 months as primary outcome and they were assessed for QOLHS score obtained before supplementation and at the end of project.

**Results:** A total of 87 patients were included in the analysis. The mean Baseline QOLHS score was 16.2 and the mean Baseline Albumin was 3.37, when the pre and post protein supplementation data were analyzed using paired *t*-tests, significant differences were present; the mean increase in serum albumin concentration of 0.9 mg/dL observed ($P < .05$).The QOLHS score correlated significantly with the serum albumin level at all measurements (baseline: r = -0.37, P = .0001; and 6 months: r = -0.46, P < .0001).

**Conclusions:** Following oral protein supplementation, paired comparisons (i.e., paired pre to post supplementation for individual patients) showed that there was significant improvement in the malnutrition parameter (represented by hypoalbuminemia). This correlated with a significant improvement in the responses to the overall QOL. (P value < 0.5).

*Funding:* Pharmaceutical Company Support - Sanofi (Genzyme)

**FR-PO815**

**Effects of Intravenous Ascorbic Acid on Vascular Function and Oxidative Stress in Chronic Kidney Disease** Keith Gillis,[1] Kathryn K. Stevens,[1] Markus P. Schneider,[3] Scott Morris,[1] Christian Delles,[1] Alan G. Jardine,[1] Patrick B. Mark.[1] *[1]Univ of Glasgow, Glasgow, United Kingdom; [2]Glasgow Renal and Transplant Unit, Glasgow, United Kingdom; [3]Univ of Erlangen-Nuremberg, Erlangen.*

**Background:** Endothelial dysfunction and arterial stiffness may explain premature cardiovascular disease seen in chronic kidney disease (CKD). Antioxidant therapy may improve endothelial dysfunction and reduce cardiovascular risk. We studied the effect of intravenous ascorbic acid (VitC) on oxidative stress and arterial stiffness in hypertensive patients with normal renal function (HTN) and with CKD.

**Methods:** This was a single-blind crossover study. Patients received both VitC and normal saline (NaCl). At baseline and following each intervention, vascular function, (pulse wave analysis, augmentation index (Aix) and pulse wave velocity) was measured and bloods were drawn for markers of oxidative stress. Rate of reactive oxygen species (ROS) production was measured by electron paramagnetic resonance and total antioxidant capacity (TAC) by colorimetric assay.

**Results:** 12 CKD and 12 HTN patients were recruited with a mean age of 56 (SD 12) and a mean blood pressure (BP) 142/88mmHg (SD 15/13). There were no significant differences between the groups in gender, age or BP. The CKD group had an eGFR of 28.3 ml/min (SD 10.5) compared with 98.2 ml/min (SD 10.9) in the HTN group ($p<0.05$). VitC led to a significant reduction in AIx in both goups: from 26% (SD 8%) after NaCl to 16% (SD 11%) after VitC ($p<0.05$) in CKD patients and 24% (SD 12%) to 19% (SD 11%) ($p<0.05$) in HTN. There was no significant difference between the groups. Rate of ROS production was transiently increased in both CKD (43% $p<0.05$) and HTN (37% $p<0.05$) at 10 minutes, before falling to a baseline level at 60 minutes. TAC was initially increased in both CKD (57%, $p<0.05$) and HTN (50%, $p<0.05$) before falling to baseline at 60 minutes.

**Conclusions:** Ascorbic acid ameliorates arterial stiffness and paradoxically increases ROS production in both CKD in HTN. Whilst the pro oxidant effect of ascorbic acid is acknowledged, this is the first in vivo evidence of increased ROS production and further research is required to elucidate the mechanisms of this.

*Funding:* Private Foundation Support

**FR-PO816**

**Fruits and Vegetables or Oral $NaHCO_3$ Prevent Progression of Kidney Injury in Stage 1 CKD due to Hypertensive Nephropathy** Nimrit Goraya,[1,2] Chanhee Jo,[4] Jan Simoni,[3] Donald E. Wesson.[1,2] *[1]Internal Medicine, College of Medicine, Texas A and M, Temple, TX; [2]Internal Medicine, Scott and White Healthcare, Temple, TX; [3]Surgery, Texas Tech Univ Health Sciences Center, Lubbock, TX; [4]Biostatistics, Scott and White Healthcare, Temple, TX.*

**Background:** Dietary alkali reduces kidney injury and slows GFR decline in patients with reduced GFR due to hypertensive nephropathy (HN). Reduced GFR further increases cardiovascular risk of HN and kidney injury typically precedes GFR reduction. Consequently, preventing further kidney injury appears to benefit hypertensives with

presently normal GFR. We explored if dietary alkali as base-producing fruits and vegetables (F+V) or oral $NaHCO_3$ ($HCO_3$) prevents kidney injury progression in hypertensives with normal MDRD-estimated GFR (eGFR).

**Methods:** Macroalbuminuric HN subjects with eGFR>90 ml/min (n=71) had systolic blood pressure (SBP) reduced to <150 mm Hg with regimens including ACE inhibition, then randomized to receive F+V, (n=23) in amounts to reduce dietary potential renal acid load 50%, oral $NaHCO_3$ ($HCO_3$, n=23) 0.4 meq/Kg/day, or no added alkali (Time Control, n=25). Urine excretion of albumin (Ualb), N-acetyl-D-glucosaminidase (UNAG), an index of tubule-interstitial kidney injury, and urine angiotensinogen (UATG), an index of kidney levels of angiotensin II, a possible mediator of progressive nephropathy, were measured at time zero and at one year.

**Results:** Ualb (347±82 to 387±73 mg/g cr, p<0.01) and UNAG (2.5±0.5 to 2.7±0.4 U/g cr, p<0.01) increased at one year in Time Control but were not different at one year for F+V (Ualb: 350±75 to 344±53 mg/g cr, p=0.46; UNAG: 2.5 ±0.4 to 2.4±0.4 U/g cr, p=0.23) or $HCO_3$ (Ualb: 341± 83 to 327±73 mg/g cr, p=0.10; UNAG: 2.5±0.3 to 2.5±0.3 U/g cr, p=0.33). UATG increased in Time Control (21.3±3.1 to 22.7±3.0 ug/g cr, p<0.01) but decreased in F+V (21.2±3.4 to 20.1±2.9 ug/g cr, p<0.01) and $HCO_3$ (21.4±3.6 to 20.7±3.0 ug/g cr, p<0.01).

**Conclusions:** Kidney injury progressed in macroalbuminuric HN despite SBP reduction and ACE inhibition. By contrast, dietary alkali as added F+V or oral $NaHCO_3$ prevented progression of kidney injury, possibly through reduced kidney angiotensin II activity.

*Funding:* Private Foundation Support, Clinical Revenue Support

## FR-PO817

### Epoetin Beta Pegol (C.E.R.A.) Augmented Dietary Iron Absorption by Decrease in Serum Hepcidin Levels and Increase in Expression of Duodenal Iron Transporters

Yusuke Sasaki, Mitsue Kurasawa, Keigo Yorozu, Yasushi Shimonaka. *Chugai Pharmaceutical Co., Ltd., Japan.*

**Background:** Epoetin beta pegol (C.E.R.A.) is a novel long-acting erythropoiesis stimulating agent. We previously reported C.E.R.A. promotes mobilization of iron storage from reticuloendothelial cells through intensive suppression of serum hepcidin levels. The aim of this study is to evaluate the contribution of dietary iron utilization for erythropoiesis after C.E.R.A. treatment in mice.

**Methods:** Hematological and iron parameters including serum hepcidin levels were analyzed in C57BL/6N (B6) mice intravenously treated with 10 μg/kg of C.E.R.A. or vehicle. The expression of duodenal iron transporters, ferroportin (FPN) which is internalized by hepcidin binding and divalent metal transporter (DMT) -1, were assessed by immunohistochemical staining. B6 mice intravenously treated with 10 μg/kg of C.E.R.A. or vehicle were fed iron deficiency diet to inhibit dietary iron absorption or control diet, and transitions of hematological parameters were analyzed.

**Results:** C.E.R.A.-treated mice showed significantly higher hemoglobin (Hb) levels than vehicle-treated mice for 14 days after treatment, while serum hepcidin levels were continuously suppressed and serum iron levels were markedly decreased on the fifth day in C.E.R.A.-treated mice. FPN expression on the basolateral membrane of intestinal enterocytes and DMT-1 expression on luminal surface of them were increased after C.E.R.A. treatment. The inhibition of dietary iron absorption resulted in impaired Hb elevation and decrease in mean corpuscular volume (MCV) after C.E.R.A. injection.

**Conclusions:** C.E.R.A. augmented dietary iron absorption by the suppression of serum hepcidin levels and thereby increasing duodenal expression of FPN and DMT-1, and absorbed iron was beneficially utilized for erythropoiesis. C.E.R.A. has a strong power to promote iron utilization for erythropoiesis through enhancement of dietary iron absorption as well as mobilization of iron storage from reticuloendothelial cells.

## FR-PO818

### Calcitriol Regulates Monocyte Hepcidin and Ferritin: A Role for Vitamin D in Iron Homeostasis

Anjali B. Nayak,[1] Justine Bacchetta,[1] Joshua Zaritsky,[1] Isidro B. Salusky,[1] Martin Hewison.[2] *[1]Dept of Pediatric Nephrology, Univ of California Los Angeles, Los Angeles, CA; [2]Dept of Orthopaedic Surgery, Univ of California Los Angeles, Los Angeles, CA.*

**Background:** Calcitriol (1,25OHD), is an important component of chronic kidney disease(CKD)therapy, and its diverse effects on immune function may have additional benefits for CKD patients. We have shown that hepatocytes and monocytes treated with calcitriol show decreased expression of hepcidin, a key iron-regulatory protein that targets ferroportin, the only known exporter of intracellular iron. To investigate further the iron-regulatory function of calcitriol, we carried out studies using human monocytes exposed to varying levels of iron.

**Methods:** THP-1 human monocytic cells were cultured in regular medium with or without added ferric nitrate ($FeNO_3$) at (5-45 μM) and 1,25OHD for 24 hrs. Cells were then lysed to generate RNA and the expression of various target genes analyzed using qRT-PCR.

**Results:** THP-1 cultured with 5, 15 or 45 μM $FeNO_3$ in the absence of calcitriol showed increased mRNA for hepcidin (4.35-, 2.81- and 4.26-fold respectively) relative to cells in regular medium. Conversely, mRNA for ferritin was decreased with increasing $FeNO_3$ (0.27-, 0.07-, 0.17-fold respectively. In the absence of $FeNO_3$, THP-1 treated with 1,25OHD(1,10 and 100 nM) showed decreased expression of hepcidin (0.25-, 0.14-, and 0.21-fold), and ferritin (0.17-, 0.15-, and 0.11-fold). This effect was also observed in THP-1 cultured with 10nM calcitriol in the presence of low dose(5μM) $FeNO_3$. 10 nM calcitriol in 5 μM $FeNO_3$ decreased expression of hepcidin (0.26-fold) and ferritin (0.25-fold) relative to vehicle. However, 10 nM calcitriol had no effect on expression of mRNA for hepcidin or ferritin at higher(15 or 45 μM) $FeNO_3$ concentrations.

**Conclusions:** These data indicate that active vitamin D, calcitriol, is a potent suppressor of hepcidin and also suppresses ferritin, a surrogate marker for intracellular iron concentrations. These effects are lost under conditions of enhanced hepcidin expression following exposure to high dose exogenous iron, suggesting a selective role for vitamin D in regulating intracellular iron concentrations.

*Funding:* NIDDK Support

## FR-PO819

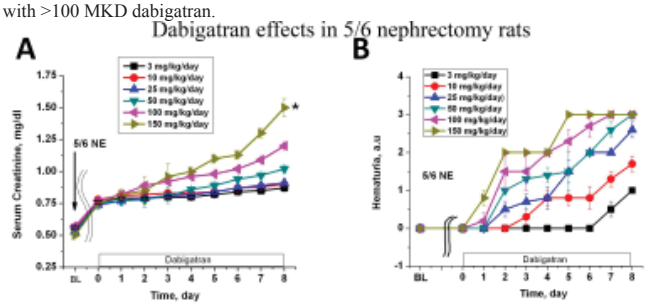
### Direct Thrombin Inhibitor Dabigatran Induces Glomerular Hemorrhage with Acute Kidney Injury Similar to Warfarin Related Nephropathy

Kyle M. Ware, Zahida Qamri, Anjali A. Satoskar, Omar Sameer Hassanain, Lee A. Hebert, Brad H. Rovin, Tibor Nadasdy, Sergey V. Brodsky. *The Ohio State Univ, Columbus, OH.*

**Background:** Excessive anticoagulation with warfarin can result in acute kidney injury (AKI) by causing glomerular hemorrhage and renal tubular obstruction by red blood cell (RBC) casts in some patients, especially in those with chronic kidney disease (CKD). This condition was described early as warfarin related nephropathy (WRN). An animal model of WRN is developed. Recent evidence suggests that WRN-like syndromes are not confined to anticoagulation with warfarin, but may be seen with novel anticoagulants, such as dabigatran. The aim of this study was to investigate dabigatran effects on kidney function in an animal model of CKD.

**Methods:** Sham-operated (control) and 5/6 nephrectomy rats (5/6NE) were treated with different doses of dabigatran for 1 week. Changes in serum creatinine (Scr), hematuria and renal pathology were studied.

**Results:** Dabigatran resulted in 2-fold increase in aPTT in rats at 50 mg/kg/day (MKD). Dabigatran in a dose-depended manner increased Scr and hematuria in both control and 5/6NE (Figure 1, A). Increase in Scr were noted in both groups when >100 MKD dabigatran were used. Hematuria increase and numerous RBC tubular casts in the kidney were seen in 5/6NE treated with >25 MKD dabigatran (Figure 1, B) and in control with >100 MKD dabigatran.




**Conclusions:** Our data indicate that WRN represents part of a broader syndrome, which is anticoagulant related nephropathy. Dabigatran resulted in AKI not only in CKD, but in control rats as well, which was not the case in WRN. Our findings suggest that not only CKD patients, but other patients as well are at high risk of developing AKI if the therapeutic range of anticoagulation with dabigatran is exceeded. Close monitoring of kidney function in patients on dabigatran therapy is warranted.

## FR-PO820

### Intravital Multiphoton Microscopy Can Visualize Oxidative Damage and Tubulointerstitial Fibrosis after Kidney Ischemia-Reperfusion Injury

David M. Small,[1] Washington Yamandu Sanchez,[2] Glenda C. Gobe,[1] Sandrine F. Roy.[3] *[1]Centre for Kidney Disease Research; [2]Therapeatics Research Centre, Sch Medicine, Univ Queensland; [3]Diamantina Institute, Brisbane, Australia.*

**Background:** Acute kidney ischemia-reperfusion (IR) injury is common, serious, and may progress to chronic kidney disease (CKD). Mediators of oxidative stress exacerbate acute injury but their role in CKD is unclear. The aim of this project was to use in vivo multiphoton microscopy (MPM) to visualize IR-induced tubulointerstitial damage in real time, and correlate these with histology.

**Methods:** Bilateral IR (20min) was performed in male 4-6 week old C57BL6 mice followed by reperfusion. Groups compared with MPM were: control kidneys; 10min ischemia; immediately at reperfusion after 20min ischemia; and 21 days post-IR. A Lavision Biotec MPM, was used to excite NAD(P)H fluorescence (740nm) and collagen (900nm) in kidney microstructure. Z-stack images were acquired from three separate areas of cortex in each kidney and the average weighted lifetime ($\tau_m$) of NAD(P)H was determined. H&E staining was performed on fixed kidney post-MPM. Oxidative stress markers were determined by Western blots and immunohistochemistry.

**Results:** NAD(P)H and collagen fluorescence in control kidneys clearly showed structure of proximal and distal tubules, and sparse interstitial space with little collagen. With ischemia and immediately after reperfusion, tubules were swollen and NAD(P)H fluorescence increased, indicating oxidative stress. At 21 days post-IR, there was focal tubular atrophy, interstitial space expansion, intense endogenous fluorescence in remaining normal-looking tubules, and the $\tau_m$ of NAD(P)H increased significantly. Intense cytoplasmic autofluorescent "hot spots" in atrophic tubules were seen, and histology indicated these may be evidence of apoptosis. Histology confirmed other in vivo changes identified with MPM. Markers of mitochondrial destabilisation, apoptosis and autophagy were increased.

**Conclusions:** Oxidative stress mediates acute injury and CKD after renal IR. MPM is a powerful tool to visualize the cellular, subcellular, and metabolic profile of the kidney *in vivo*.

*Funding:* Government Support - Non-U.S.

## FR-PO821

**Targeting Connexin 43 Protects against Chronic Kidney Disease in Experimental Nephropathy in Mice** Christos E. Chadjichristos, Ahmed Abed, Carlo M. Alfieri, Christos Chatziantoniou. *UMR S702, INSERM, Paris, France.*

**Background:** Excessive recruitment of leukocytes and progression of renal fibrosis are hallmarks of chronic kidney disease (CKD). We have recently reported that the expression of connexin 43 (Cx43) was upregulated within kidney in experimental nephropathy. The aim of our study is to investigate the role of Cx43 in the progression of CKD.

**Methods:** We interbred RenTg mice, a genetic model of hypertension-induced CKD, with Cx43+/- mice. RenTgCx43+/- and littermates were sacrificed at 5 months (n=10 mice/group). Moreover, 11 month-old RenTg mice received during 1 month a Cx43 antisense (AS) or scrambled sequence (SCR) via minipump infusion (n=8 per group). After sacrifice kidneys were assessed for morphometry, inflammation and interstitial fibrosis.

**Results:** Renal cortex of RenTgCx43+/- mice presented lesser upregulation of VCAM-1 (2.1 fold) mRNAs ($p<0.05$), leading to reduced monocyte infiltration compared to RenTgCx43+/+ mice (quantification of F4-80 immunostainings showed 4.3±1% and 1.8±0.2% of cortex surface respectively, $p<0.05$). In addition, Sirius Red colorations shown limited renal fibrosis in RenTgCx43+/- mice (0.8% versus 1.3% of cortex surface for RenTgCx43+/+, $p<0.05$). Furthermore, functional and histological parameters such as albuminuria ($p<0.05$) and glomerulosclerosis ($p<0.05$) were both reduced by half in RenTgCx43+/- mice. Interestingly, administration of a Cx43AS produced remarkable regression of the F4-80 signal (4.5±0.5% of cortex surface for SCR versus 1.9±0.4% for Cx43AS, $p<0.01$) and fibrosis (8±1% and 4±0.5% of cortex surface for SCR and Cx43AS respectively, $p<0.001$) in one year-old RenTg mice and consequently improved renal function (125±12mg/mmol versus 45±5mg/mmol of microalbuminuria for SCR and Cx43AS respectively, $p<0.001$). Moreover Cx43 was highly increased in renal biopsies of patients suffering from neproangiosclerosis.

**Conclusions:** Our results show for the first time the importance of Cx in renal diseases and may open towards new therapeutic strategies targeting Cx43 to protect against the progression of CKD.

## FR-PO822

**miR-205 Expression Correlates with Severity of Renal Involvement in a Mouse Model of Congenital Obstructive Nephropathy** Susan E. Ingraham, Michael Wilhide, Ashley R. Carpenter, Kirk M. McHugh. *The Research Institute at Nationwide Children's Hospital, Columbus, OH.*

**Background:** Congenital obstructive nephropathy (CON) is the leading cause of CKD in children. CON is a complex disease process involving pathological changes in kidney development and function resulting from obstructed urine flow beginning *in utero*. The megabladder (*mgb-/-)* mouse is an animal model of CON that develops kidney disease secondary to a bladder-specific defect in smooth muscle development.

**Methods:** Expression levels of specific microRNAs were compared by microarray analysis on the Agilent platform and by qPCR of kidney samples from wild type and *mgb-/-* mice. *In situ* hybridization and immunohistochemistry demonstrated the sublocalization of mir-205 and tubular markers within the nephron.

**Results:** There is increased expression of miR-205 across a panel of *mgb-/-* kidneys compared to wild-type controls (2.94-fold, $P=0.01$). Furthermore, upon stratification of the *mgb-/-* population by severity of kidney involvement, relative miR-205 expression levels rise with increasing severity of hydroureteronephrosis (Mild: 1.35-fold, $P=0.02$; Moderate: 2.30-fold, $P<0.001$; Severe: 5.32-fold, $P<0.001$; all values are relative to wild-type control). Thus, miR-205 expression correlates with the severity of CON in this animal model. *In situ* hybridization studies show miR-205 localizes to the renal urothelium in wild-type and *mgb-/-* kidneys, and to dilated collecting ducts in *mgb-/-* kidneys. In collecting ducts, miR-205 expression shows an inverse relationship with the presence of aquaporin-2 (Aqp2), suggesting a possible role for miR-205 in the physiological response to obstruction via Aqp2 expression or trafficking.

**Conclusions:** MiR-205 in the kidney increases proportionally to the degree of hydronephrosis in the *megabladder* mouse kidney. An inverse pattern of collecting duct expression relative to Aqp2 indicates a possible role for miR-205 in regulation of water transport. Future studies will explore the target molecules and pathways affected by miR-205 in the pathogenesis and physiology of CON, as well as the potential of miR-205 as a noninvasive biomarker of renal maldevelopment or injury in congenital urological obstruction.

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## FR-PO823

**Renal Epithelial Toxicity Is an Important Mechanism of Shigatoxin 2-Mediated Kidney Failure. Clinical and Experimental Evidence** Stefan Porubsky,[1] Giuseppina Federico,[1] Stefan Büttner,[4] Nicholas Obermueller,[4] Oliver Jung,[4] Ingeborg A. Hauser,[4] Helmut Geiger,[4] Hermann-Josef Groene,[1] Christoph Betz.[4] *[1]Cellular and Molecular Pathology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Institute for Hygiene, Univ of Muenster, Muenster, Germany; [3]Medical Research Center, Univ of Heidelberg, Mannheim, Germany; [4]Dept of Nephrology, Univ Hospital Frankfurt/Main, Frankfurt/Main, Germany.*

**Background:** The pathogenesis and optimal therapy of Shigatoxin (Stx) mediated kidney failure remain controversial.

**Methods:** Long-term outcome was investigated in patients with Stx-associated hemolytic uremic syndrome at the University Hospital in Frankfurt/Main, Germany. Patients (n=11) had kidney biopsies and were treated with supportive therapy (without complement inhibiting antibodies). To corroborate clinical and histological data *in vivo*, murine models with global and tissue specific (renal tubular cells, endothelial cells and platelets) deficiencies for the Stx-receptor Gb3 were analyzed.

**Results:** Despite a severe affection [LDH 1944U/l (753-2792), platelets 33/nl (19-124) and hemoglobin 6.2g/dl (5.2–7.8); median (range)], all patients were discharged after 33 days (19-43) with no neurological symptoms and no dialysis requirement [creatinine 1.39mg/dl (0.84-2.86)]. After a 12-month follow-up, the creatinine decreased to 0.90mg/dl (0.82-1.52). Analysis of kidney biopsies revealed a dominant acute tubular damage but not thrombotic microangiopathy as the leading cause of the renal failure. To identify the tubular compartment as the determinant of the acute renal damage, mouse models were implemented. In wild-type mice, which -like humans- express Gb3 in renal tubular epithelium, Stx-mediated tubular damage led to lethal electrolyte disturbance. Tubule-specific depletion of Gb3 protected mice from acute renal failure, but favored development of cerebral purpura.

**Conclusions:** Acute tubular damage is a clinically important and currently underestimated mechanism of Stx-mediated acute kidney failure. An excellent renal outcome can be achieved by supportive therapy only.

*Funding:* Government Support - Non-U.S.

## FR-PO824

**RAGE Deletion Enhances Lymphoproliferative Syndrome and Lupus Nephritis in B6-MRL-Lpr-J fas$^{-/-}$ Mice** Antoine Goury,[1] Karim Belmokhtar,[1] Vincent Vuiblet,[1] Aida Meghraoui,[3] Jerome Devy,[1] Thierry Tabary,[2] Jacques Cohen,[2] Annmarie Schmidt,[4] Philippe Rieu,[1] Fatouma Toure.[1] *[1]Nephrology, CHU Reims Hopital Maison Blanche - CNRS FRE3481, Reims, France; [2]Immunology, CHU Reims Hopital Maison Blanche, Reims, France; [3]Immunology, EA 4683 IMAB - Reims, Reims, France; [4]Diabetes Research Program, NYU Medical Center.*

**Background:** The Receptor for Advanced Glycation End products is a multiligand receptor able to interact not only, with AGEs, but also with S100 proteins, Amyloid fibrils and nuclear proteins such as HMGB1. Following engagement RAGE induces a pro-inflammatory signal involved in a number of disease characterized by vascular pathology. Systemic Lupus Erythematosus is associated with increased expression of HMGB1 and RAGE. The exact role of RAGE in this disease is unknown.

**Methods:** To analyze the potential role of RAGE in SLE we generated mice invalidated for RAGE in the lupus prone genetic background B6-MRL-Fas-lpr/J. We compared these mice to littermates B6-MRL-lpr-J-Fas$^{-/-}$ and WT mice.

**Results:** Mice invalidated for RAGE and carrying the Fas mutation (n=18) had a greater lymphoproliferative syndrome and increased size of the spleen compared to B6-MRL-lpr-J-Fas$^{-/-}$ mice (n=21). RAGE deletion in the B6-MRL-lpr-J-Fas$^{-/-}$ background was associated with more severe lupus nephritis (III and IV) and more frequent cutaneous lesions (75%) compared to the B6-MRL-lpr-J-Fas$^{-/-}$(25%) or WT(0%). INFγ levels were higher in the serum of the 2 groups of lupus prone mice compared to WT. In peripheral blood and in the spleen we found a significantly higher proportion of abnormal CD3+CD45R+ lymphocytes and activated T cells in B6-MRL-lpr-J-Fas$^{-/-}$ compared to WT. These subpopulations were further increased in B6-MRL-lpr-J-Fas$^{-/-}$RAGE$^{-/-}$ mice. Lastly we found an increased expression of TLR9 in the B6-MRL-lpr-J-Fas$^{-/-}$RAGE$^{-/-}$ splenocytes.

**Conclusions:** Our results suggest a critical role for RAGE in regulation of lymphoproliferation in the B6-MRL-lpr-J-Fas$^{-/-}$ backgroung. Deletion of RAGE in this genetic strain exacerbates lymphoproliferation, lupus nephritis and cutaneous lesions. Mechanisms involved could be related to a dysregulation of apoptosis and a greater involvement of TLRs.

*Funding:* Private Foundation Support

## FR-PO825

**An Antifibrotic Role of Hypoxia-Inducible Factor 3 in Renal Tubular Cells** Tetsuhiro Tanaka, Kumi Shoji, Junna Yamaguchi, Masaomi Nangaku. *Div of Nephrology and Endocrinology, Univ of Tokyo School of Medicine, Tokyo, Japan.*

**Background:** Chronic hypoxia in the tubulointerstitium is a final common pathway in CKD. Accumulating evidence suggests both pathogenic and protective roles of a family of hypoxia-iducible transcription factors (HIF), mainly HIF-1 and HIF-2. However, the function of a third member, HIF-3, remains elusive. In this study, we characterized the expression of HIF-3α in the ischemic kidney and investigated its functional role in tubular epithelial cells.

**Methods:** The expression of HIF-3α protein was characterized in models of renal fibrosis by immunohistochemistry. In vitro, the effect of HIF-3 on the induction of HIF-1 target genes was examined by luciferase reporter assays and real-time PCR. Hypoxia-responsive enhancers were identified in the lysyl oxidase (LOX) promoter by luciferase reporter assays and site-directed mutagenesis. Migration of tubular epithelial cells in hypoxia was evaluated by scratch assays, using human proximal tubular cells (HK-2) transduced with HIF-3α or MOCK, or in Caki-1 renal cancer cells transfected with HIF-3α siRNA or its control. The effect of HIF-3α on E-cadherin and fibronectin expression was examined by promoter assays and immunoblotting, respectively.

**Results:** In immunohistochemistry of renal fibrosis models, HIF-3α protein was observed in the nuclei of ischemic tubules located in the outer medulla. In vitro, overexpression of HIF-3α suppressed the hypoxic induction of LOX most consistently and strongly among other HIF-1 target genes. Promoter analysis of the human LOX gene revealed multiple hypoxia-responsive enhancers, against each of which HIF-3 had

an inhibitory effect. Functionally, overexpression of HIF-3α in human proximal tubular cells (HK-2) suppressed cell migration under hypoxia, which was phenocopied by pharmacological inhibition of LOX and was offset by LOX overexpression. Furthermore, HIF-3 decreased the expression of fibronectin and counteracted the loss of E-cadherin in hypoxic renal carcinoma cells (RCC).

**Conclusions:** Results of these studies identify HIF-3α as a novel antifibrotic gene which potentially plays a role in ischemic renal diseases.

*Funding:* Government Support - Non-U.S.

### FR-PO826

**$P2Y_2$ Receptor Deficiency Aggravates Progression in a Model of Chronic Renal Failure** Sebastian Alexander Potthoff, Johannes Stegbauer, Lars C. Rump, Oliver Vonend. *Dept of Nephrology, Medical Faculty, Univ Düsseldorf, Düsseldorf, Germany.*

**Background:** In chronic kidney disease, sympathetic overactivity, endothelial dysfunction and increase in shear stress contribute to a higher extracellular abundance of ATP. $P2Y_2$ receptors play an important role in renal tubule function, inflammation and proliferation. Knockout mice ($P2Y_2$-R KO) were used in order to investigate the impact of $P2Y_2$ receptors in a model of chronic kidney failure.

**Methods:** Wildtype (WT) and $P2Y_2$-R KO mice underwent subtotal nephrectomy (SNX) with a follow up of 56±2 days. Sham surgery mice served as controls. Survival, kidney function, kidney size, blood pressure (BP), glomerulosclereosis index were assessed. To examine the effect of extracellular ATP on cultured glomerular epithelium cells, a [3H]-thyimidine based proliferation assay was performed.

**Results:** During the observation period, survival was inferior in $P2Y_2$-R KO SNX mice. After 56 days, SNX caused an increase in serum creatinine and serum urea with a significant decrease in creatinine clearance. The decline in creatinine clearance was significantly more pronounced in $P2Y_2$-R KO than in WT mice (day 56, $P2Y_2$-R KO vs. WT: 53.9±7.7vs.84.3±8.7µl/min, $p<0.05$). BP increased after SNX to a higher extent in $P2Y_2$-R KO mice compared to WT mice (day 56, $P2Y_2$-R KO vs. WT: 177±2vs.156±7mmHg). After SNX, $P2Y_2$-R KO showed a 2.5-fold higher urinary albumin-creatinine ratio compared to WT (day 56, $p<0.05$). 56 days after SNX, in contrast to $P2Y_2$-R KO mice, WT remnant kidneys showed significant hypertrophy (increase of kidney weight compared to day 0: $P2Y_2$-R vs. WT: 113±6vs.150±6%). Markers for tissue damage (TGF-β1, PAI-1) and proinflammatory target genes (MCP1) were significantly upregulated in $P2Y_2$-R KO compared to WT SNX mice.In cultured glomerular epithelial cells, ATP induced a significant, dose dependent increase in DNA synthesis up to 180.1±20.5%.

**Conclusions:** $P2Y_2$-R KO has a detrimental impact on outcome after SNX compared to WT mice. Higher BP, increased UACR and absence of compensatory hypertrophy are the likely cause for the observed findings. The $P2Y_2$ receptor is crucial for blood pressure regulation and tissue adaptation after induction of chronic kidney disease.

*Funding:* Government Support - Non-U.S.

### FR-PO827

**Dickkopf Related Protein-1 Attenuates Connective Tissue Growth Factor Induced Fibrogenic Responses in Pericytes by Binding to LRP-6** Shuyu Ren, Cécile Olivia Fligny, Jeremy Stuart Duffield. *Univ of Washington.*

**Background:** Pericytes are mesenchymal cells thatattached to peritubular capillaries. Recent genetic fate-mapping studies have identified pericytes and closely related fibroblasts as the major progenitors of scar-forming myofibroblasts. Understanding mechanisms by which pericytes become myofibroblasts is critical in development of therapeutics to treat kidney disease. We have recently reported a central role for WNT signaling pathways in this process. CTGF has been shown to be an important regulator of fibrogenic responses. Here we investigate the role of CTGF in pericyte functions and its capacity to signal via the WNT pathway.

**Methods:** Col1a1-GFP, TCF/LEF:H2B-GFP, LRP6flox/LRP5flox mice were used to generate primary cell cultures. Synthesis and purification of recombinant DKK-1.

**Results:** CTGF induces myofibroblast gene activation, migration, morphological changes and stress fiber formation in pericytes and these effects are abolished by rDKK-1, LRP6 gene silencing, blockade of WNT blockade. CTGF rapidly phosphorylates the WNT co-receptor, LRP6. Although CTGF activates WNT/bcatenin signaling in kidney pericyte cultures and this is inhibited by recombinant rDKK-1, the functional changes in response to CTGF are predominantly independent of b-catenin translocation. CTGF rapidly induces JNK and MAP kinase activity, that are critical in CTGF responses in pericytes. rDKK-1 blocks these responses.

**Conclusions:** rDKK-1 is a candidate therapeutic protein that potently inhibits fibrogenesis by mechanisms including blockade of CTGF induced fibrotic responses.

### FR-PO828

**Deletion of Extrarenal NADPH-Cytochrome P450 Reductase Causes Vacuolization in Renal Proximal Tubular Epithelial Cells** Senyan Liu,[1,2] Changlin Mei,[1] Jun Gu.[2] *[1]Div of Nephrology, Shanghai Changzheng Hospital, China; [2]Wadsworth Center.*

**Background:** Vacuolization is a nonspecific histologic change for cells. It can be observed in many conditions. The NADPH-cytochrome P450 reductase (CPR) is needed for activity of microsomal cytochrome P450 (P450) monooxygenases. Cpr -low mouse model was generated to study the function of P450s . In previous study we found the vacuolization in renal proximal tubular epithelial cells (PTECs) of 6-month-old male Cpr -low mice.

**Methods:** Oil red O staining and transmission electron microscopy (TEM) were performed to figure out the origin of vacuoles. Serum creatinine levels were determined. Then kidney sections from proximal tubule-Cpr-null mice (PTCN), extra-proximal tubule-Cpr-low mice (XPT-CL), liver-Cpr-null mice (LCN) and extrahepatic-Cpr-low (XH-CL) mice were examined. Immunofluorescence double stainging was done to study the relation between CPR expression and vacuoles in proximal tubules.

**Results:** Our results showed these vacuoles were Oil red O and PAS negative. TEM examination showed these vacuoles were lysosome origin. The mice with proximal vacuolization had normal serum creatinine levels. Beside *Cpr*-low mice, the vacuolization was seen in kidney of XPT-CL mice and XH-CL mice, not in KCN mice and LCN mice. Double staining showed vacuoles had no relation to CPR expression in proximal tubules.

**Conclusions:** These results suggested that vacuolization in proximal tubular tubules of *Cpr*-low mice was caused by deletion of extra-proximal tubular or extra-hepatic *>Cpr* gene. The significance of this vacuolization need further study.

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### FR-PO829

**Feedback Loops between the Kidney and Peripheral Organs Link Proteinuria and Hypertriglyceridemia in Nephrotic Syndrome** Camille E. Mace,[1] Lionel C. Clement,[1] C. Avila-Casado,[2] Sumant S. Chugh.[1] *[1]Medicine / Nephrology, Univ of Alabama at Birmingham; [2]Pathology, UHN-Totonto General Hospital.*

**Background:** Circulating levels of Angiopoietin-like-4, a PPAR target gene, are increased in patients with nephrotic syndrome when proteinuria reaches nephrotic levels. Unlike podocyte secreted hyposialylated Angptl4, circulating Angptl4 is fully sialylated and does not induce proteinuria.

**Methods:** We conducted Angptl4 mRNA expression studies in animal models of nephrotic syndrome, and noted Angptl4 upregulation in heart, skeletal muscle, liver and adipose tissue.

**Results:** Circulating Angptl4 had 2 major effects (a) Inhibiting lipoprotein lipase (LPL), reducing hydrolysis of triglycerides to free fatty acids (FFA), reducing FFA uptake into heart, skeletal muscle and adipose tissue, and causing hypertriglyceridemia. Wefound significantly elevated plasma FFA/albumin ratio in nephrotic patients and in animal models of MCD and FSGS. Loss of urinary albumin with low fatty acid levels in nephrotic syndrome and progressive hypoalbuminemia were the principal determinants of increased plasma FFA/albumin ratio. (b) Circulating Angptl4 binds to the glomerular endothelium and reduces proteinuria. Using recombinant Angptl4, transgenic rats and beta 5 integrin - / - mice, we showed that reduction in proteinuria was mediated by binding to alphav beta5 integrin. Injection of four human Angptl4 mutants with reduced LPL interaction reduced proteinuria without inducing hypertriglyceridemia in Buffalo Mna rats (FSGS) and Zucker Diabetic Fatty rats.

**Conclusions:** In summary, there are two feedback loops in nephrotic syndrome mediated by FFA and Angptl4. First, proteinuria results in selective loss of albumin with low FFA levels, raising plasma FFA/albumin ratio, increasing FFA entry in peripheral organs, which activates PPARs, increases Angptl4 expression and secretion, that inhibits hydrolysis of triglycerides, thereby reducing FFA uptake, but inducing hypertriglyceridemia. Second, the increased circulating Angptl4 reduces proteinuria by binding to glomerular endothelial alphav beta5 integrin, which, reduces urinary loss of low FFA bound albumin, and decreases the plasma FFA/albumin ratio.

*Funding:* NIDDK Support

### FR-PO830

**The Effect of GSK-3β in the Glomerular Injury of db/db Mutant Diabetic-Nephropathy Mice** Zhangsuo Liu.[1,2] *[1]Nephrology, The First Affiliated Hospital of Zhengzhou Univ, Zhengzhou, Henan, China; [2]Key-Disciplines Laboratory Clinical-Medicine Henan, Zhengzhou, Henan, China.*

**Background:** To observe the effect of BIO and diabetic nephropathy on EMT, GSK-3β expression and glomerular injury in the kidney of db/db mice, and to explore the possible mechanism of proteinuria and glomerular injury in DM, by determining whether EMT is caused by high glucose.

**Methods:** Male db/+m mice were served as normal control. The age-matched db/db mice were randomly divided into BIO treated group and untreated group. Each group were sacrificed 8 mice every 3 weeks. On the day before sacrifice, mice were individually housed in metabolic cages for urine collection over 24 hours. Sections of renal tissues were used to observe structural changes with electron and light microscope. Expression of nephrin, α-SMA, GSK-3β, β-catenin, VDR, etc. were quantified by Western blot, RT-PCR or immunofluorescence.And the activities of GSK-3β were assayed by TRAPEZE enzyme detection kit.

**Results:** 1. Diabetic db/db mice had heavy albuminuria and remained hyperglycemic. BIO administration attenuated albuminuria, but had no significant effect to blood glucose. 2. Compared with NC group,the expressions of epithelial phenotype markers and VDR were downregulated time-dependently. And the expressions of β-catenin, snail and mesenchymal phenotype markers did the opposite. 3. The expression and activity of GSK-3β in cortex of db/db mice were time-dependently upregulated, compared with db/+m mice. 4. BIO administration to db/db mice suppressed the trends mentioned in result 2 and 3, and alleviated the pathologic changes caused by diabetic nephropathy.

**Conclusions:** 1. DM could lead renal cortex of db/db mice undergo EMT, BIO could partly inhibit it. 2. In high glucose conditions, GSK-3β could probably regulate EMT through VDR and other factors, besides Wnt/β-catenin signaling. 3. In diabetic kidney, BIO could protect renal function through reducing the urine albumin excretion, but not regulating the blood sugar.

*Funding:* Government Support - Non-U.S.

## FR-PO831

**Extracellular Sulfatases Regulate PDGF Signaling and Promote Nephropathy in Diabetes** Yasutoshi Takashima,[1] Hiroshi Yashiro,[1] Kumi Ohashi,[1] Hanako Yamashita,[1] Satoshi Hara,[1] Namiko Kobayashi,[1] Toshiharu Ueno,[1] Masayuki Masu,[2] Kazuko Keino-masu,[2] Michio Nagata.[1] *[1]Renal Pathology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Molecular Neurobiology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan.*

**Background:** Growth factors have important roles to regulate glomerular homeostasis. Extracelluar sulfatase (Sulf) 1 and 2 are known to regulate growth factors by controlling growth factor binding with intrinsic 6-O sulfation in HSPGs. Last ASN, we demonstrated that Sulf1 and Sulf2 double knockout (DKO) mice showed mesangiolysis, mesangial hyperplasia accompanied by up-regulation of PDGF-B, PDGF-D and its receptor. In addition significant increase of GBM out pockets suggesting PDGF-mediated GBM synthesis by podocytes. Because mesangial lesions are hallmark of diabetic nephropathy and it was partly promoted by PDGF signaling, the present study examined the role of sulfatases in the development of glomerulopathy in diabetes.

**Methods:** STZ-induced diabetes in Sulf DKO and Wild type(WT) was examined (100 mg/Kg BW, n=6 each). Glomerulopathy was estimated by morphometrical counting of mesangial lesion as previously shown. mRNA expressions for PDGF-B, PDGF-D and its receptor and mesangial phenotype change's marker (a-SMA) were done by real time PCR. Protein expression and location of growth factors, those receptor and protein phosphorylated by PDGF signaling were estimated by western blotting, and immunohistochemistry.

**Results:** STZ-treated Sulf DKO mice with showed significantly milder mesangial changes compared to those of STZ treated WT mice at 12 weeks. STZ-treated WT mice revealed significant increase in PDGF-B, PDGF-D, PDGFR-β and a-SMA mRNA, whereas Sulf DKO mice showed no increase in those parameters at 8 weeks after STZ injection. Immunohistochemistry showed that increase of PDGF-B expression in podocyte and endothelial cells and PDGFR-β expression in mesangial cell in STZ-treated WT and the expression was suppresed in Sulf DKO mice.

**Conclusions:** Sulfs may modulate PDGF signaling in glomeruli and promote mesangial lesions under diabetic mellitus.

## FR-PO832

**Pharmacological Stabilization of Hypoxia Inducible Factors Ameliorates Adenine-Induced Tubulointerstitial Nephritis** Gunnar Schley,[1] Bernd Klanke,[1] Kerstin U. Amann,[2] Kai-Uwe Eckardt,[1] Carsten Willam.[1] *[1]Nephrology and Hypertension, Univ Hospital Erlangen, Erlangen, Germany; [2]Nephropathology, Univ Hospital Erlangen, Erlangen, Germany.*

**Background:** Chronic inflammation, vascular rarefaction and extensive fibrosis of the tubulointerstitium are common hallmarks of progressive chronic kidney diseases (CKD). All three processes impair the oxygen supply to the renal tissue and can thus lead to a vicious circle. The Hypoxia Inducible Factors (HIF) are transcription factors which have a key position in regulating gene transcription under hypoxia and ischemia in order to adapt cells and organs to low oxygen tensions. In experimental models of acute kidney injury, genetic or pharmacological stabilization of HIF led to better preserved kidney structure and function. We therefore aimed to investigate the effects of HIF stabilization in an experimental CKD model.

**Methods:** Male C57BL/6 mice were fed with an adenine-supplemented diet for 3 weeks to induce chronic tubulointerstitial nephritis. Mice were treated with either two HIF stabilizers (pyridine-2,4-dicarboxylate, PDCA; 2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido) acetic acid, ICA) or vehicle for 3 weeks starting concomitantly with the adenine-containing diet.

**Results:** In control animals creatinine levels rose from 0.09±0.01 mg/dl to 0.39±0.11 after 3 weeks and kidneys showed extensive tubulointerstitial fibrosis. Pharmacological HIF stabilization reduced serum creatinine by 21% (PDCA) and 36% (ICA) as well as proteinuria. Interstitial fibrosis (assessed by Sirius Red, fibronectin and collagen IV immunohistochemistry, tissue collagen content, and real-time PCR for fibrosis-related genes) and peritubular capillary rarefaction (MECA-32 immunohistochemistry, real-time PCRs for endothelial markers) were not significantly improved by both substances. But both HIF stabilizers markedly reduced the infiltration with F4/80 positive macrophages suggesting a reduced inflammatory reaction due to HIF stabilizer treatment.

**Conclusions:** HIF stabilizers ameliorated kidney function in a model of chronic tubulointerstial nephritis induced by an adenine-containing diet by reducing inflammatory cell infiltration.

*Funding:* Government Support - Non-U.S.

## FR-PO833

**Preconditional Activation of Hypoxia-Induced Factors Improves the Impaired Angiogenic Response to Ischemia in Chronic Kidney Disease in Rats** Karl F. Hilgers, Isabel Schellinger, Nada Cordasic, Bernd Klanke, Johannes Jacobi, Rafael Heiss, Christoph Daniel, Andrea Hartner, Kai-Uwe Eckardt, Carsten Willam, Kerstin U. Amann. *Univ of Erlangen-Nuremberg.*

**Background:** Improving capillary supply in response to ischemia is an important mechanism for adaptation to macrovascular disease. This mechanism is impaired in chronic kidney disease (CKD). We tested the hypothesis that preconditional activation of hypoxia-inducible factors (HIF) improves the impaired capillary angiogenesis in response to ischemia.

**Methods:** CKD was induced in male rats by 5/6 nephrectomy; control rats were sham operated. Eight weeks later, ischemia of the right limb was induced by ligation and resection of the femoral artery. Preconditional activation of HIF target genes was performed by adding 0.1% CO to the rats' breathing air for 6 h, beginning 24 h before the onset of ischemia. Rats were sacrificed 24 h or two weeks after the onset of ischemia to analyze gene expression or capillary density, respectively. Capillary area was assessed after immunostaining for CD31 of the gastrocnemius muscle.

**Results:** In control rats, capillary area increased in the ischemic hindlimb by 44±3 % after 2 weeks, compared to the non-ischemic limb ($p< 0.001$, N=10). In contrast, there was no significant increase in the ischemic over the opposite limb in CKD rats (N=10). Pretreatment with CO did not affect the capillary density in non-ischemic limbs of CKD and control rats, nor in ischemic limbs of control rats. However, CO significantly augmented the increase of capillary area after ischemia in CKD rats (80±22% over the opposite limb, $p<0.004$, N=6). RT-PCR analysis showed a 2.4fold increase of VEGF after 24 h in non-CKD rats which was blunted in CKD animals (1.7fold, $p<0.05$). CO treatment increased VEGF expression 4.2fold in the non-ischemic and 7.2fold in the ischemic limb in CKD rats ($p<0.001$). The expression of other known HIF target genes, including VEGFR1, IGFBP-3, PAI-1 and HIG-2, was also induced by CO.

**Conclusions:** Our data provide proof of principle that HIF activation can correct the impaired angiogenic response to ischemia in rats with chronic kidney disease.

*Funding:* Government Support - Non-U.S.

## FR-PO834

**Podocyte-Specific VEGF Overexpression Stimulates Glomerular Angiogenesis but Is Insufficient Alone to Prevent Progression of Existing Sclerosis** Shaojun Liu,[1,3] Anne P. Wilson,[1] Ji Ma,[2] Haichun Yang,[1] Agnes B. Fogo.[1] *[1]Pathology, Microbiology and Immunology, Vanderbilt Univ Medical Center, Nashville, TN; [2]Pediatric Nephrology, Vanderbilt Univ Medical Center, Nashville, TN; [3]Div of Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China.*

**Background:** Glomerulosclerosis is characterized by increased matrix, obliteration of capillary lumens and loss of podocytes. VEGF is a secreted by podocytes and induces endothelial tip cell formation and new vessel branches. VEGF is decreased in sclerotic glomeruli. In this study, we investigated whether up-regulation of human VEGF in podocytes after development of glomerulosclerosis could induce angiogenesis and interrupt progression of sclerosis.

**Methods:** Subtotal nephrectomy (5/6Nx) was performed in 129/sv TetO-podocin-Cre /human VEGF loxp (RV, n=9) and TetO-podocin-Cre mice (R, n=11). At 8 weeks after 5/6 Nx, renal biopsy was done and sclerosis assessed (0-4 scale). VEGF was induced by doxycycline in drinking water. Mice were sacrificed four weeks later. Change in blood pressure, proteinuria, and glomerular morphology from biopsy to autopsy were assessed. DLL4, a marker of endothelial tip cells, was detected by immunostaining. VEGF was quantitated in isolated glomeruli.

**Results:** Human VEGF expression in RV glomeruli was significantly induced after doxycycline (44.9 ± 14.6 pg/mg),and mouse VEGF was also increased. Thus total glomerular VEGF in RV mice was 3.2 folds higher than R mice (276.0 ± 88.1 vs. 85.9 ± 83.6pg/mg , $P<0.05$). RV mice had significantly reduced blood pressure,but there were no effects on proteinuria and glomerulosclerosis. Glomerular volume was not affected by VEGF induction. However, there were more DLL4 positive endothelial cells (double staining of DLL4 and CD31) in glomeruli of RV induced mice than R control (RV 0.73±0.15 vs. R 0.27±0.28/glom, $P<0.05$).

**Conclusions:** Our data indicate that overexpression of VEGF in podocytes stimulates angiogenesis, but is not sufficient alone to prevent glomerulosclerosis progression. Thus, we speculate that coordinated interventions to augment angiogenesis and decrease matrix are necessary to optimize effects on progressive glomerulosclerosis.

*Funding:* NIDDK Support

## FR-PO835

**Klotho Reduces Kidney Injury and Fibrosis by Targeting Renin-Angiotensin System** Li Li Zhou,[1] Dong Zhou,[1] Roderick J. Tan,[2] Youhua Liu.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** Activation of the renin-angiotensin system (RAS) plays a pivotal role in the pathogenesis of kidney disorders in both animal models and human subjects. We recently demonstrated that Klotho, an anti-aging hormone and an endogenous antagonist of Wnt/β-catenin signaling, is lost in renal tubular epithelia in multiple CKD models. As Wnt/β-catenin controls the expression of multiple RAS genes, we hypothesized that Klotho may protect kidneys by targeted inhibition of RAS activation in diseased kidneys.

**Methods:** To test this, a remnant kidney model after 5/6 nephrectomy was utilized in adult CD-1 male mice. Groups of mice were intravenously administered the expression vector encoding the secreted form of Klotho (pV5-sKlotho) through hydrodynamic-based gene delivery. At 5 weeks after 5/6 nephrectomy, mice were killed and kidneys collected for renal pathology and various analyses.

**Results:** We found that kidney injury was evident, characterized by elevated albuminuria and serum creatinine, and excessive deposition of interstitial matrix proteins. These lesions were accompanied by loss of kidney Klotho, and upregulated expression of RAS components including angiotensinogen (AGT), renin, angiotensin-converting enzyme (ACE) and angiotensin II receptor type 1 (AT1). *In vivo* expression of exogenous Klotho inhibited renal β-catenin activation, abolished the induction of multiple RAS components including AGT, ACE, renin and AT1, and ameliorated morphological lesions and fibrosis. In cultured human proximal tubular epithelial cells (HKC-8), ectopic expression of Wnt1 or Wnt4 induced the expression of all major components of RAS, and co-transfection of Klotho expression vector dose-dependently blocked Wnt-triggered RAS activation.

**Conclusions:** Taken together, these results suggest that Klotho exerts its renal protection by targeted inhibition of RAS, a key pathogenic pathway in the evolution and progression of kidney and cardiovascular diseases.

*Funding:* NIDDK Support

## FR-PO836

**Exploring the Mechanistic Pathways in Developing Renal Injury Using the Rat UUO Model and Next Generation Sequencing (NGS)** Steven M. Weldon, Thomas B. Freeman, William Loging, Peng Sun, Glenn Gibson, Xiaojun Ren, Hu Sheng Qian, Glenn A. Reinhart. *CardioMetabolic Diseases and Scientific Knowledge Discovery, Boehringer Ingelheim Pharm Inc, Ridgefield, CT.*

**Background:** Unilateral ureteral obstruction (UUO) is a well established model used to mimic the pathophysiology involved in tubulointerstitial fibrosis (TIF). Key pathological features of UUO include oxidative stress, inflammation, apoptosis, vascular/tubular loss and TIF. NGS can generate large, reproducible data sets in a timely manner and is now relatively low cost.

**Methods:** We used UUO+NGS to further explore the transcriptional regulation of molecular pathways and biological processes (BP) associated with developing TIF in rats. Rats received sham or UUO±enalapril (ENA;30 mpk) for up to 10d post-UUO. mRNA from cortical tissue was subjected to NGS and computational analysis to explore BP and differentially regulated genes across key pathological pathways in UUO. TIF was assessed by sirius red morphometry (SRM) for cortical collagen.

**Results:** UUO induced robust fibrosis in controls (+65% vs sham) that was inhibited 73% by ENA. We found 417 specific genes associated with the key mechanistic features of UUO pathology; 27 genes were present in 3 or more key features. Gene Ontology (GO) pathway analysis identified >2300 specific BPs that were significantly enriched and temporally regulated over 10d post-UUO. Extracellular matrix signaling and Ub-dependent proliferation are examples of BPs regulated in early (D1-4); oxidative stress and macrophage cytokine production are examples of BPs regulated in late (D5-10), phases of renal damage in UUO. ENA showed most significant effects on inflammation and cell proliferative BPs.

**Conclusions:** These are the first data using NGS to describe quantitative transcriptional regulation of the pathology associated temporal renal injury in rats following UUO. We show that temporal changes in the transcriptome of key pathological pathways in UUO correlate with TIF and are differentially regulated by time and treatment with ENA. These data extend our previous findings using NGS in UUO as a powerful approach to identify new target pathways and potential biomarkers of fibrotic renal disease.

*Funding:* Pharmaceutical Company Support - Boehringer Ingelheim Pharm. Inc.

## FR-PO837

**Renoprotective Effect of Metformin in Mice with Unilateral Ureteral Obstruction** Rita de Cassia Cavaglieri, Denis Feliers, Hanna E. Abboud. *Dept of Medicine, Univ of Texas Health Science Center, San Antonio, TX.*

**Background:** Metformin, an indirect activator of adenosine monophosphate-activated kinase (AMPK) is widely used in patients with type 2 diabetes. Metformin has been shown to have renoprotective effects in early diabetic nephropathy. We studied the effect of metformin on inflammation and fibrosis in obstructive nephropathy.

**Methods:** Adult C57Bl/6 mice were subjected to unilateral ureteral obstruction (UUO) or sham operated and subdivided into 3 groups: **S (n=6),** sham-operated mice, **UUO (n=6),** mice subjected to unilateral ureter obstruction model; and **UUO+Met (n=6),** UUO mice receiving metformin by gavage (200mg/kg/day). Animals were followed for 7 days and injury of the obstructed kidney was studied. Markers for inflammation (F4/80-positive macrophages; VCAM-1; TNF-alpha); tubular injury (E-cadherin), myofibroblasts (alpha-SMA); and fibrosis (Fibronectin, Collagen and TGF-beta) were analyzed. AMPK activity was measured by in vitro kinase assay. Reactive oxygen species production was measured and correlated with expression of NADPH oxidases.

**Results:** Metformin significantly reduced inflammation (macrophage infiltration, TNF-alpha and VCAM-1 expression), tubular injury, interstitial fibroblast activation and tubulointerstitial fibrosis (collagen and fibronectin expression and deposition) in obstructed kidneys. AMPK activity was unchanged in obstructed kidneys and increased by metformin, suggesting that metformin acts through activation of AMPK. Superoxide anion production and Nox4 and Nox2 expression were significantly increased in obstructed kidneys and were inhibited by metformin.

**Conclusions:** Our data show for the first time that metformin reduces inflammation and tubulointerstitial fibrosis in obstructed kidneys. This effect appears to be mediated by AMPK and could involve inhibition of oxidative stress. Our study suggests that metformin could be use to accelerate renal healing in patients with obstructive nephropathy.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO838

**The Macula Densa Controls Glomerular Cell Remodeling** James L. Burford,[1] Karie G. Villanueva,[1] Anne Riquier-brison,[1] Sanjeev Kumar,[2] Jing Liu,[2] Andrew P. McMahon,[2] Janos Peti-Peterdi.[1] *[1]Physiology and Biophysics, Univ of Southern California, Los Angeles, CA; [2]Stem Cell Biology and Regenerative Medicine, Univ of Southern California, Los Angeles, CA.*

**Background:** Macula densa (MD) cells are strategically positioned at the vascular entrance of the glomerulus and control renal hemodynamics and renin. Here we addressed if MD cells play non-traditional roles in glomerular cell plasticity/remodeling.

**Methods:** Serial multiphoton microscopy of the intact kidney *in vivo* was performed in *NG2-DsRed* mice to track chondroitin sulfate proteoglycan (NG2, an established progenitor cell marker) expressing pericytes over several days. In established conditions of strong MD stimulation (low salt diet/ACE inhibition) and unilateral ureteral ligation (UUO). MD cells were FACS sorted from freshly digested kidneys of mice with MD-specific expression of GFP and their RNA isolated.

**Results:** In established conditions of strong MD stimulation (low salt diet/ACE inhibition) and unilateral ureteral ligation (UUO), increased renal interstitial density and migration of NG2+ cells to the MD area and then into the glomerular parietal layer and mesangium were observed. NG2, Ki67, and renin immunofluorescence found a significantly increased number of NG2+ proliferating cells in the MD area with partial co-localization with renin in response to low salt diet/ACEI for one week. The increased density of NG2+ cells was 70% inhibited by the administration of the selective COX-2 inhibitor SC58236. GUDMAP-based microarrays confirmed high expression of known MD-specific genes and identified new MD-enriched genes including Car15, Emb, Klk1b22, Wnt10a, Bmp3, Thsd4, Angptl7, Slit2 that play roles in cell growth, development, guidance, and extracellular matrix interactions.

**Conclusions:** Our results suggest that NG2+ pericytes maybe an important progenitor cell population in the kidney. Also, via MD-derived paracrine factors MD cells play new important roles in the maintenance and remodeling of the glomerulus and the renal interstitium in health and disease.

*Funding:* NIDDK Support

## FR-PO839

**Renin Angiotensin System Activates mTOR Pathway in HIV-Associated Nephropathy (HIVAN)** Partab Rai, Rivka Lederman, Tejinder Singh, Viki Kumar, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, New York, NY.*

**Background:** Both renin angiotensin system (RAS) and mTOR pathways have been reported to play a critical role in the development and progression of HIVAN. However the role of the RAS pathway in the activation of mTOR pathway has not been investigated so far. We hypothesize that the RAS activates mTOR pathway in HIVAN.

**Methods:** Renal cortical sections and tissues of age and sex matched control and HIV transgenic mice (Vpr) were evaluated for the expression of renin, angiotensinogen (Agt), phospho-mTOR and phospho-p70S6K. To determine the role of renin, control and Vpr mice were administered either normal saline or aliskiren (50 mg/kg/day by miniosmotic pump, an inhibitor or renin) for 4 weeks followed by renal tissue probing for the expression of phospho-mTOR, phospho-p70S6K, and actin. To determine the effect of HIV on kidney cell renin expression, mouse proximal tubular cells (MTC) were transduced with either empty vector (EV/MTC) or NL4-3(HIV/MTC) and evaluated for renin expression. To determine the effect of renin and Ang II, MTCs were treated with variable concentrations of renin or Ang II for 24 h followed by Western blot analysis for the expression of phospho-p70S6K and actin. To determine the role of pro-renin receptor (PRR), control and siRNA-PRR transfected MTCs were treated with renin and then evaluated for phospho-p70S6K.

**Results:** Renal tissues of Vpr mice displayed enhanced expression of renin, phospho-mTOR and phospho-p70S6K. Vpr mice receiving aliskiren displayed attenuated expression of phospho-mTOR and phospho-pS706K when compared to saline receiving Vpr mice. HIV/MTC displayed enhanced expression of renin, phsopho-mTOR and phospho-p70S6K. However, this effect of HIV was inhibited by aliskiren. Both renin and Ang II enhanced tubular cell expression of phospho-p70S6K in a dose dependent manner. Since MTC silenced for PRR displayed attenuated expression of phospho-p70S6K in response to renin, it appears that the RAS contributes to the activation of mTOR pathway both by renin as well as by Ang II.

**Conclusions:** HIV enhances activation of mTOR pathway in HIVAN via generation of both renin and Ang II.

*Funding:* NIDDK Support

**FR-PO840**

**Sclerotic Glomerular Phenotype in HIV-Associated Nephropathy Is Dependent on the Activation of the Renin Angiotensin System** Andrei Plagov, Partab Rai, Rivka Lederman, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical Center, New York, NY.*

**Background:** HIV-associated nephropathy (HIVAN) is a common complication of HIV-1 infection in patients with African ancestry in general and with APOL1 gene risk variants in particular. Although collapsing glomerulopathy is considered a hallmark of HIVAN, significant numbers of glomeruli in patients with HIVAN also display other variants of FSGS. We propose that collapsed glomeruli as well as glomeruli with other variants of FSGS are manifestations of HIVAN and their prevalence depends on associated host factors. We explored whether renin-angiotensin system (RAS) contributes to sclerotic, collapsing or both types of lesions in HIVAN. We hypothesized that activation of the RAS will contribute predominantly to sclerotic lesions rather than to collapsing ones.

**Methods:** To evaluate the role of the RAS we have used a genetically engineered mouse model of HIVAN (Tg26) with two and four copies of angiotensinogen (Agt) gene (Tg26/Agt2 and Tg26/Agt4). Severity of renal lesions was scored in 8 weeks old control and Tg26 mice with 2 and 4 copies. To confirm the role of the RAS on the development of glomerular phenotype, Tg26 mice in groups of 4, were administered either normal saline, aliskiren (50 mg/Kg, a renin inhibitor), aliskiren + captopril (20 mg/Kg, an angiotensin converting enzyme inhibitor), aliskiren + telmisartan (300 microgram/kg, an Ang II receptor blocker) for 4 weeks followed by scoring of severity of renal lesions.

**Results:** In Tg26/Agt2, 1 out of 6 glomeruli exhibited sclerosed phenotype, whereas 1 out of 25 glomeruli displayed collapsed phenotype; on the other hand, in Tg26/Agt4, 1 out of 3 glomeruli exhibited sclerotic phenotype and only 1 out of 7 glomeruli showed collapsed phenotype. In all experimental groups there was significant reduction of percentage of sclerosed glomeruli and only minimal reduction in collapsed glomeruli comparing to normal saline receiving Tg26/Agt2.

**Conclusions:** These findings suggest that manifestation of sclerosed phenotype in HIVAN is predominantly dependent on the activation of the RAS.

*Funding:* NIDDK Support

**FR-PO841**

**HIV Induces Podocyte Vitamin D Receptor Downregulation through Epigenetic Factors** Nirupama Chandel, Tejinder Singh, Noopur Goel, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

**Background:** Recent reports indicate that HIV induces podocyte injury through down regulation of vitamin D receptor (VDR) and associated activation of renin angiotensin system (Am J Physiol, 2013). However, the involved mechanism of HIV-induced down regulation of VDR is not clear. We hypothesized that HIV down regulated VDR through hypermethylation of cytosine phospho gunaosine (cpg) islands.

**Methods:** Renal tissues of age and sex matched control Tg26 (n=4) were assayed for VDR expression and DNA methyltransferases (Dnmt) 1, 2, and 3 and reprobed for actin. Conditionally immortalized human podocytes (CIHP) were transduced with either empty vector (EV/CIHP) or NL4-3 construct (HIV/CIHP). Protein blots of EV/CIHP and HIV/CHP were probed for Dnmt1, 2, and 3. Additionally, cpg methylation status of EV/CIHP and HIV/CIHP was evaluated by Epitecht mRNA assay and bisulphite pyrosequencing. To determine the potential of VDR agonists on normalization of VDR in HIV/CIHPs; EV/CIHPs and HIV/CIHPs were incubated in medium containing EB1089 (1nM, a VDR agonist) for 24h (n=3) followed by protein blots preparation and subsequent their probing for VDR and actin expression . To confirm the role of hypermethylation in VDR expression, effect of 5-azacytiidine (AZAC), a demethylating agent was evaluated on VDR expression in HIV/CIHP in the presence/absence of a VDR agonist.

**Results:** Renal tissues of Tg26 mice displayed attenuatd expression of VDR but enhanced expression of Dnmt. HIV/CIDHPs displayed down regulation of VDR and enhanced expression of Dnmt3b. Epitecht assay displayed more than 70% VDR cpg methylation in HIV/CIHPs. Bysulphite pyrosequencing studies in HIV/CIHPs confirmed enhanced methylation of cpg islands when compared to EV/CIHPs. Both AZAC alone and EB1089 alone increased VDR expression in HIV/CIHPs but only sub-optimally. However, AZAC exhibited an additive effect on EB1089 in enhancing VDR expression by HIV/CIHPs.

**Conclusions:** HIV induced podocyte VDR expression through hypermethylation of VDR. Optimal expressoin of VDR could be achieved only by combining VDR agonist with a demethylating agent.

*Funding:* NIDDK Support

**FR-PO842**

**Nicorandil Protects Podocyte from Puromycin Aminonucleoside-Induced Injury in the Rats** Yoshifuru Tamura,[1] Takeshi Shiraishi,[1] Takahiko Nakagawa,[2] Shunya Uchida.[1] *[1]Dept of Internal Medicine, Teikyo Univ School of Medicine, Tokyo, Japan; [2]TMK Project, Medical Innovation Center, Kyoto Univ, Kyoto, Japan.*

**Background:** Nicorandil causes vasodilatation by opening ATP-dependent potassium channels and donating nitric oxide. Recently, we reported the protective effect of nicorandil in the rat remnant kidney model, in which such beneficial effect was associated with protection of podocytes (Tamura, et al Am J Physiol 2012). In order to further confirm such podocyte protection, we here examined the effect of nicorandil in another model with podocyte injury, the puromycin aminonucleoside (PAN) induced nephrosis model.

**Methods:** PAN nephrosis was induced by a single intraperitoneal injection of PAN (10 mg/100 g body weight). Rats were divided into three groups: Normal control rats, PAN model group, PAN rats treated with nicorandil 30 mg/kg/day. 9 days later, the rats were sacrificed.

**Results:** Proteinuria was significantly ameliorated by nicorandil compared with that with no treatment at 9 days. Interestingly, puromycin significantly lowered number of WT-1-positive cells and reduced podocin immunoreactivity while both were prevented by nicorandil in PAN rats. In addition, electron microscopy documented thatthe number of filtration slits in podocyte was reduced in PAN rats whereas such reduction was significantly rescued by nicorandil treatment.

| | Serum creatinine | Proteinuria | Podocyte | Podocyte | Podocyte |
|---|---|---|---|---|---|
| | mg/dl | mg/day | podocin positive area /glomeruli | WT-1 positive cell number/glomeruli | The number of filtration slits/10 μm of GBM |
| Control (n = 4) | 0.22 ± 0.02*** | 15.3 ± 4.6*** | 50.3 ± 3.4* | 14.0 ± 1.2*** | 21.2 ± 1.3*** |
| PAN (n = 4) | 0.31 ± 0.02 | 267.7 ± 54.8 | 20.8 ± 6.1 | 7.2 ± 0.3 | 1.8 ± 0.2 |
| PAN + Nicorandil (n = 4) | 0.37 ± 0.06 | 165.8 ± 10.9** | 40.1 ± 2.7* | 10.5 ± 0.3* | 10.7 ± 1.1** |
| ***P<0.001. **P<0.01. *P<0.05 vs. PAN. | | | | | |

**Conclusions:** These results suggest that, nicorandil, a K channel opener, reduces proteinuria and ameliorates podocyte injury seen in PAN nephrosis rats. Nicorandil may serve as a novel strategy for kidney of diseases involving podocyte injury.

**FR-PO843**

**A Single Low Dose of Lithium Protects against Proteinuria by Improving Podocyte Focal Adhesion and Actin Cytoskeleton Integrity** Weiwei Xu,[1] Yan Ge,[1] Zhi-hong Liu,[2] Rujun Gong.[1] *[1]Nephrology, Brown Univ, Providence, RI; [2]Research Institute of Nephrology, Nanjing, China.*

**Background:** Evidence suggests that glycogen synthase kinase (GSK) 3β plays a detrimental role in acute and chronic renal diseases. This study examined the effect of lithium, a selective inhibitor of GSK3β, on podocytopathy.

**Methods:** Adriamycin induced podocytopathy was employed in mice and in conditionally immortalized mouse podocytes and podocyte dysfunction evaluated.

**Results:** In cultured podocytes, adriamycin elicited immediate cellular shrinkage and actin cytoskeleton disorganization as evidenced by increased cortical F-actin and diminished stress fibers and lamellipodia. Focal adhesion turnover was also promoted and podocyte motility amplified, concomitant with an increase in the number of focal adhesions and a reduction in their size. These changes were largely obliterated by lithium. Mechanistically, adriamycin reduced cofilin phosphorylation, congruent with an amplified actin-serving activity of cofilin that accounts for the loss of long bundled F-actin. Lithium counteracted adriamycin-induced cofilin hypophosphorylation by suppressing the activity of a cofilin phosphotase Slingshot (SSH)2. In addition, adriamycin-induced hyperphosphorylation of paxillin, a focal adhesion-associated adaptor protein, was abrogated by lithium. Inhibitory phosphorylation of GSK3β seems to be essential and sufficient for lithium's effects because ectopic expression of an uninhibitable mutant of GSK3β abrogated, whereas overexpression of a kinase dead GSK3β mimicked lithium's activities in podocytes. Of note, both paxillin and SSH2 interacted with GSK3β in podocytes and served as putative substrates for GSK3β. In adriamycin injured mice, a single low dose of lithium attenuated proteinuria by more than 70%, associated with an early prevention of foot process effacement. Consistently, lithium therapy inhibited GSK3β activity in injured glomeruli and blunted cofilin and paxillin activities in podocytes.

**Conclusions:** Inhibition of GSK3β by lithium protected against podocyte injury and proteinuria by reinstating podocyte actin cytoskeleton stability *via* SSH2/cofilin pathway and regulating focal adhesion-associated paxillin.

*Funding:* NIDDK Support

**FR-PO844**

**Deficient Autophagy Results in Mitochondrial Dysfunction and the Development of Focal and Segmental Glomerulosclerosis of the Kidney** Takahisa Kawakami,[1,2] Shuyu Ren,[2] Kelly L. Hudkins,[2] Ivan G. Gomez,[2] Allie M. Roach,[2] Charles E. Alpers,[2] Jeremy Stuart Duffield.[2] *[1]Univ of Tokyo; [2]Univ of Washington, Seattle.*

**Background:** Focal and Segmental Glomerulosclerosis (FSGS) is a heterogeneous fibrosing disease whose etiology remains poorly understood, and for many, lacks an effective therapy. It is also recognized as a component of other kidney diseases. Autophagy is the process by which cells degrade damaged organelles, cell membranes and proteins. The failure of autophagy may be one reason for the accumulation of cell damage and disturbance to cell homeostasis. Our Lab has recently implicated mitochondrial dysfunction, abnormal b-oxidation of triglycerides, and the aberrant production of reactive oxygen species (ROS) in the pathogenesis of tubulointerstitial kidney disease. Because of the central role of autophagy in cell homeostasis processes related to metabolism, mitochondrial and endoplasmic reticulum turnover, we explored autophagy deficiency in the kidney epithelium.

**Methods:** We mutated critical autophagy genes *ATG5* or *ATG7* during nephrogenesis, using Cre recombinase expressed in the progenitors of kidney epithelium, under regulation of the transcription factor SIX2.

**Results:** Mice with *ATG5* or *ATG7* mutations develop effacement of podocyte foot processes and albuminuria with minimal histological changes by 2 months of age. By 4 months they exhibit profound glomerular and tubular changes, which bear remarkable similarity to human FSGS, developing severe albuminuria, advanced renal failure and death by six months. Ultra-structurally, in addition to the characteristic features of FSGS, podocytes and tubular cells show vacuolization, smaller damaged mitochondria, and

changes to the endoplasmic reticulum. At these early time points, ATG5 and ATG7 deficient tubules and podocytes show evidence of ER and mitochondrial distress, reduced ATP, and increased ROS levels.

**Conclusions:** Mitochondrial dysfunction and ER stress are the result of impaired autophagy organelle turnover in podocytes and kidney tubular epithelium. The impairment in autophagy is sufficient to cause all the manifestations of FSGS.

### FR-PO845

**Calcineurin Is Mainly Localized at the Slit Diaphragm Area, and Its Altered Expression Precedes Proteinuria in Rat Nephrotic Syndrome Models** Ayako Wakamatsu,[1] Asami Takasaki,[1] Yuichi Takahashi,[1] Yoshiyasu Fukusumi,[1] Masayuki Tomita,[1] Ichiei Narita,[2] Hiroshi Kawachi.[1] *[1]Cell Biology, Inst Nephrol, Niigata Univ Medicine, Niigata, Japan; [2]Medicine II, Niigata Univ Medicine, Niigata, Japan.*

**Background:** Calcineurin (CaN) is the target molecule of cyclosporine A, which is used for the treatment of proteinuric kidney diseases. Recently, it was reported that cyclosporine A blocked CaN-mediated dephosphorylation of synaptopodin, and stabilized the actin cytoskeleton of the podocyte. However, pharmacological mechanism of cyclosporine A, and the function of CaN in podocyte are not fully understood yet.

**Methods:** The glomerular expressions of CaN-A and cyclophilin A (CyA), a binding protein of cyclosporine A were analyzed in normal rat glomeruli, and in glomeruli of puromycin aminonucleoside nephropathy (PAN) and adriamycin nephropathy (ADR). The expression of these molecules were analyzed by dual labeling IF with podocyte markers. mRNA expression of two isoforms of CaN-A -α and -β and CyA, was analyzed with RT-PCR.

**Results:** Clear 58kDa band was detected in glomerular lysate with anti-CaN-A antibody recognizing both isoforms of CaN-A (MILLIPORE). Clear immno-staining of CaN-A along capillary loop was observed in normal rat glomeruli. CaN-A was co-stained with nephrin, and was slightly apart from synaptopodin. CyA was also observed as a similar pattern as CaN-A. The staining of CaN-A shifted to discontinuous granular pattern, and its intensity decreased on day 5 of PAN, when proteinuria was not observed yet. The decrease in the staining became more remarkable on day 10. Such an alteration was also observed in CyA staining. The decrease of CaN-A staining was observed also in ADR. mRNA expression of CaN-A α was decreased already at 1h of PAN (14% to normal) and the decrease was also observed on day 28 of ADR (5%), whereas the decrease of CaN-A β was not detected at any time points of these models.

**Conclusions:** CaN-A and CyA were mainly localized at the SD area. Their altered expression was detected before the onset of proteinuria, suggesting that these molecules play a role in maintaining the integrity of the SD, and that their altered expression is involved in the development of proteinuria.

*Funding:* Government Support - Non-U.S.

### FR-PO846

**The Reduction and Morphological Alterations of Podocyte Slits Cause Acute Kidney Injury in Minimal Change Nephrotic Syndrome** Satoshi Kinugasa, Kensuke Asaba, Masaomi Nangaku, Akihiro Tojo. *Dept of Nephrology and Endocrinology, Univ of Tokyo, Tokyo, Japan.*

**Background:** Some cases of minimal change nephrotic syndrome (MCNS) present with acute kidney injury (AKI). The decrease in the number of podocyte slits due to foot process effacement, and tight junction-like alterations may contribute to the reduction of eGFR in these patients. We verified this hypothesis in human MCNS patients.

**Methods:** All biopsy-proven MCNS patients in our institute in the past 20 years who had electron microscopy images available for analysis were included in the study (n=38). We compared clinical indices at the time of renal biopsy, and several ultrastructural morphological parameters between AKI (n=13) and non-AKI patients (n=25). We also made similar evaluations in puromycin aminonucleoside (PAN) nephrotic rats.

**Results:** The rate of AKI in MCNS was 34% (13/38). The AKI group showed a signihicant reduction in podocyte slit number density (383 ± 26/mm vs 549 ± 47/mm, $p < 0.05$), and the width of the slits were significantly narrowed (20.5±2.3 nm vs 35.8±6.0 nm, $p < 0.05$). Multiple logistic regression analysis showed a significant association between the onset of AKI and slit number density. Spearman's rank correlation analysis revealed that both slit density (rS=-0.489) and slit width (rS=-0.528) was strongly correlated with AKIN stage. Also, the ratio of foot process effacement along the glomerular capillaries was significantly correlated with the amount of proteinuria (R=0.475). Between PAN nephrotic rats treated with the usual dose of PAN (n=3), and those which were administered a two-fold dose (n=3), the latter had a greater effacement ratio (69.6 ± 4.9 % vs 90.4 ± 2.0 %, $p < 0.01$) and increased proteinuria (27.1 ± 3.4 % vs 79.1 ± 1.2 %, $p < 0.01$), and presented with AKI, which was in accordance with the human biopsy study. Immunogold staining showed increased expression of the tight junction protein claudin 5 in PAN rats compared to controls.

**Conclusions:** AKI is a common complication of MCNS. In MCNS with concurrent AKI, a reduction in podocyte slit number and narrowing of slits was observed. The degree of foot process effacement correlated with urinary protein.

### FR-PO847

**Mitochondrial Dysfunction in Podocyte Induces Proteinuria via Decrease of Alpha Actinin-4 and Synaptopodin** Dae Eun Choi,[1] Jin Young Jeong,[1] Sarah Chung,[1] Yoon-Kyung Chang,[2] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, Chungnam National Univ Hospital, Daejeon, Republic of Korea; [2]Internal Medicine, Daejeon Saint Mary Hospital, Daejeon, Republic of Korea.*

**Background:** Our previous report showed that Crif1 deletion induce severe mitochondrial destruction in mice podocyte. There results induce massive albuminuria and effacement of foot process in mice. Actin cytoskeleton architecture and dynamics in podocyte are important constituents of the glomerular filtration barrier. There have been few studies about relation of mitochondria and actin cytoskeleton in podocytes of glomerulus. We evaluated the changes of actin cytoskeletal proteins and artchitecture in mitochondrial injured podocyte.

**Methods:** We used immortalized mouse podocyte cell line. . Crif1 silencing(si) RNA treatment was used for inducing mitochondrial injury. We divided 3 groups; control podocytes, scrumble(sc) RNA treated podocytes, Crif1 siRNA treated podocytes. We checked the expression of mitochondrial respiratory complex I~V, WT-1, and Crif1 for mitochondrial dysfunction in immortalized podocyte. We evaluated the expression of alpha actinin 4, synaptopodin, nephrin, ZO-1, and colfillin using western blot. Using confocal microscopy, we examined actin cytoskeleton architecture and mitochondria of podocyte. For evaluation of cell migration, we performed scratch assay.

**Results:** Crif1 siRNA treatment reduced the expressions of mitochondrial respiratory complex IV and $O_2$ consumption in cultured podocytes. Alpha actinin-4 and synaptopodin were decreased in Crif1 siRNA treated podocyte compared to control and Crif1 scRNA treated podocytes. There were no differences in nephrin and ZO-1. Crif1 siRNA treated podocyte showed an enhanced formation of dot-like alpha actinin-4 and an increase of fragment mitochondria in confocal microscopy compared to scRNA treated podocyte. Podocyte migration was increased in Crif1 siRNA treated podocyte.

**Conclusions:** With the above results, it is speculated that mitochondrial dysfunction induced by crif1 inhibition reduces alpha actinin-4 and synaptopodin in podocyte.

*Funding:* Government Support - Non-U.S.

### FR-PO848

**ApoL1 Protein in Non-Diseased Human Podocytes: Endogenous Synthesis versus Uptake?** Lijun Ma,[1] James A. Snipes,[1] Mariana Murea,[1] Peter A. Antinozzi,[1] Gregory S. Shelness,[1] Moin Saleem,[2] Simon C. Satchell,[2] Bernhard Banas,[3] Peter W. Mathieson,[2] Matthias Kretzler,[4] Snezana Petrovic,[1] Michael D. Ross,[5] Martin R. Pollak,[5] Lawrence Rudel,[1] John S. Parks,[1] Barry I. Freedman.[1] *[1]Wake Forest School of Medicine; [2]Univ of Bristol, United Kingdom; [3]Univ of Regensburg, GA; [4]Univ of Michigan at Ann Arbor; [5]Harvard Medical School.*

**Background:** Based on heat-induced epitope retrieval on paraffin-embedded kidney sections, ApoL1 protein is found localized to human proximal tubule cells (PTCs) and podocytes; however, it is unknown the extent to which this reflects local synthesis versus uptake.

**Methods:** Immunofluorescence microscopy (IF) was performed on non-diseased nephrectomy cryo-sections from subjects with normal kidney function and in various cell lines using an antibody specific for native human ApoL1. RNA *in situ* hybridization (ISH), Western blot (WB), and RT-PCR studies were also performed.

**Results:** IF revealed that ApoL1 co-localized with synaptopodin and Wilm's tumor repressor in podocytes and was present in renal tubule cell cytoplasm, albeit with lower signal intensity than podocytes. ISH detected *APOL1* mRNA in both glomeruli and tubules, consistent with endogenous synthesis in these cell types. Based on RT-PCR, *APOL1* mRNA was absent in mesangial cell lines and HEK293 cells; however, comparable expression was observed in all other cell types analyzed, including: immortalized human podocytes, glomerular endothelial cells (GEC) and PTC; primary GECs and podocytes; and HepG2 cells. Based on IF and WB, ApoL1 protein was also detected in these cell lines but not in mesangial or HEK293 cells. Based on IF, ApoL1 signal intensity was lower in both the immortalized and primary podocytes, as compared to the kidney cryo-sections. To explore the basis for this apparent discrepancy, we observed that ApoL1 protein (reference sequence) was readily taken up by human podocyte and PTC lines *in vitro*, but not by mesangial or GEC lines.

**Conclusions:** We speculate that the higher *in vivo* levels of ApoL1 observed in human podocytes on cryo-section may reflect endogenous protein synthesis as well as uptake of additional ApoL1 from the circulation or glomerular filtrate.

*Funding:* NIDDK Support

### FR-PO849

**Rituximab Ameliorates Proteinuria in Rat Adriamycin Nephropathy Independently with Its Action to B Lymphocyte** Yuichi Takahashi, Hisashi Kamada, Asami Takasaki, Ayako Wakamatsu, Yoshiyasu Fukusumi, Masayuki Tomita, Hiroshi Kawachi. *Dept of Cell Biology, Insutitute of Nephrology, Niigata, Japan.*

**Background:** Rituximab, a monoclonal antibody against CD20 on B lymphocytes, is reported to reduce proteinuria in some kidney diseases. It is postulated that Rituximab has cross-reactivity to sphingomyelin phosphodiesterase acid-like 3b (SMPDL3b), and plays a protective role for podocyte by preserving the SMPDL3b function. However, the direct action of Rituximab in podocyte is not proved yet, and the pharmacological mechanism of rituximab and the function of SMPDL3b in podocyte are still uncertain.

**Methods:** Because it is reported that rituximab has no reactivity to rat CD20, we analyzed the effect of rituximab on proteinuria in rat experimental model. Adriamycin (ADR) nephropathy was induced in rats by the single injection of 6 mg/kg BW of ADR, and the rats were intravenously injected with rituximab (10 mg/kg BW/week) from day 0. The kinetics of proteinuria were analyzed, and the kidney materials were removed on day 28 after disease induction. To elucidate the pharmacological mechanism of rituximab, the expression of nephrin and podocin, the functional molecules of the slit diaphragm, and SMPDL3b were analyzed.

**Results:** The rituximab treatment clearly reduced the amount of proteinuria in ADR nephropathy (day 18, 25.62 mg/day vs. 84.56, $p<0.005$: day 28, 52.99 vs. 138.06 $p<0.05$). The rituximab treatment rescued the decrease in the staining of nephrin and podocin on day 28. The binding of the injected rituximab to glomeruli was confirmed in rat kidney sample removed 1 h after the rituximab injection. The injected rituximab is observed at the discontinuous linear pattern along the capillary loop of glomeruli, and the staining pattern is very similar to that of SMPDL3b detected by the specific antibody against SMPDL3b. SMPDL3b staining clearly decreased at very early phase of ADR nephropathy.

**Conclusions:** The findings suggested that the decreased expression of SMPDL3b was involved in the initiation event of ADR nephropathy. It is conceivable that the injected rituximab bound SMPDL3b in podocyte and ameliorated proteinuria independently with its action to B lymphocyte.

*Funding:* Government Support - Non-U.S.

## FR-PO850

**Repopulation of Denuded GBM by Visceralized Parietal Epithelial Cells (vPECs) Is a Transient Mechanism to Limit Proteinuria**

Samy Hakroush,[1] Angelika Cebulla,[1] Thomas Schaldecker,[1] Peter H. Mundel,[1] Astrid Weins.[2] *[1]Div of Nephrology, Massachusetts General Hospital, Charlestown, MA; [2]Dept of Pathology, Brigham and Women's Hospital, Boston, MA.*

**Background:** Damage to podocytes is a central pathomechanism of proteinuric kidney disease. However, it is not fully understood how podocyte injury evolves to irreversible glomerulopathies such as Focal Segmental Glomerulosclerosis or Collapsing Glomerulopathy. In particular, the role of PECs remains controversial. Here, we provide novel insights into the role of PECs in the development of these lesions following podocyte injury.

**Methods:** Mice on a mixed *C57Bl6/Balb/c* background were analyzed at baseline and at several timepoints after *i.v.* injection of various concentrations of adriamycin (ADR) or vehicle, using clinical parameters, light and electron microscopy as well as immunohistochemistry.

**Results:** We found that ADR induces podocyte lysis through DNA damage. In contrast to other models, autophagy, ER stress and necroptosis are not involved in the pathogenesis of ADR nephropathy. Following podocyte loss, PECs are activated in two pathways: In the majority of glomeruli, PECs undergo striking vacuolization to attach to denuded glomerular basement membrane (GBM), which is followed by detachment of PECs from the parietal basement membrane (PBM). This results in repopulation of denuded GBM by PECs, ultimately leading to glomerulosclerosis. Less frequently, PECs do not undergo vacuolization, but cover the denuded GBM via formation of proliferative pseudocrescents. Of note, all "visceralized" PECs (vPECs) covering the GBM do not express VEGF, but instead show high expression of HIF1. The clinical relevance of our findings was underscored by detection of vPECs in biopsies of human glomerulopathies.

**Conclusions:** In conclusion, we propose that repopulation of the glomerular tuft by vPECs represents a compensatory response following podocyte loss in sclerotic and proliferative glomerular lesions, which is triggered by denuded GBM and is aimed at diminishing urinary protein loss. However, as vPECs do not express VEGF, they fail to induce revascularization of the hyalinized tuft, which results in hypoxic cell death.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## FR-PO851

**Rac1 in Podocytes Protects Glomeruli against the Formation of Sclerosis**

Rin Asao,[1] Katsuhiko Asanuma,[1,2] Fumiko Kodama,[1] Miyuki Takagi,[1] Yoshiko Hosoe,[1] Eriko Tanaka,[3] Takuto Seki,[1,2] Kanae Nonaka,[1,2] Yu Sasaki,[1] Teruo Hidaka,[1] Lawrence B. Holzman,[4] Yasuhiko Tomino.[1] *[1]Div of Nephrology, Juntendo Univ Faculty of Medicine, Tokyo, Japan; [2]Medical Innovation Research, TMK Project, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [3]Dept of Pediatrics, Tokyo Medical and Dental Univ, Tokyo, Japan; [4]Dept of Internal Medicine, Renal-Electrolyte and Hypertension Div, Univ of Pennsylvania, Philadelphia.*

**Background:** Rac1, one of the Rho family of GTPases, is ubiquitously expressed and plays a crucial role in various events like cell motility. In this study, we investigated the role of Rac1 in podocytes under pathological conditions.

**Methods:** Mice with podocyte-specific Rac1 conditional knockout (Rac1 cKO) were generated using Cre-lox technology and injected adriamycin, which causes nephrosis and glomerulosclerosis. Rac1-constitutively active (CA) podocytes and Rac1- dominant negative (DN) podocytes were generated for in vitro study. To evaluate the morphological variation and cell motility, immunofluorescence and cell migrating assay were performed.

**Results:** Under physiological conditions, Rac1 cKO mice didn't develop proteinuria and showed no deterioration. Histologic alteration of kidneys from Rac1 cKO after injection of ADR demonstrated a higher ratio of sclerotic glomeruli than in control at day 28 (19.12±3.85 % versus 0.56±0.23 %; $p<0.001$). However, there was no difference between Rac1 cKO and control in the number of podocytes and in the levels of urinary protein at day 28. By electron microscopy, areas of denuded GBM in Rac1 cKO were observed more frequently than in control. In in vitro study, the formation of actin stress fiber and lamellipodia were suppressed more in Rac1 DN than in WT and Rac1 CA. In wound healing assay, Rac1 CA significantly promoted directional podocyte migration compared with WT and Rac1 DN after 6 and 12 hours. Moreover, Rac1 DN is significantly less motile than WT and Rac1 CA in transwell cell migration assay.

**Conclusions:** Rac1 regulates actin organization and controls cell motility in podocytes. Loss of Rac1 in podocytes might play an important role in the formation of glomerulosclerosis.

## FR-PO852

**Urine Podocyte and TGF-β1 mRNAs as Progression Markers in Crescentic Glomerulonephritis**

Akihiro Fukuda,[1] Yuji Sato,[1] Takashi Iwakiri,[1] Masao Kikuchi,[1] Kazuo Kitamura,[1] Roger C. Wiggins,[2] Shouichi Fujimoto.[1] *[1]First Dept of Internal Medicine, Univ of Miyazaki, Miyazaki, Japan; [2]Nephrology Div, Dept of Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

**Background:** Glomerulosclerosis (GS) and interstitial fibrosis (IF) are the major pathlogic processes causing progression to end stage kidney disease (ESKD). Podocyte depletion causes GS leading to IF, and we have demonstrated that persistent podocyte loss is a major factor driving the progression process (podocyte depletion hypothesis: Wiggins R. KI 2007). We have also demonstrated that urine podocyte mRNA could be a useful for diagnostic and monitoring for progression process in two models of controlled podocyte depletion (Sato Y et al. JASN 2009, Fukuda A et al. KI 2012).

**Methods:** To determine whether tha same principles apply to crescentic glomerular injury we used the crescentic anti-GBM glomerulonephritis rat model and in parallel experiments we examined urine samples of patients with crescentic glomerulonephritis. Podocyte loss was measured by WT1 positive nuclear counting, the GLEPP1 positive podocyte tuft area, and by urine podocyte mRNAs excresion. GS and IF were evaluated by AZAN staining, and also measured urine TGF-β1 mRNA.

**Results:** In the model system, sequential kidney biopsies at 1, 2, and 4 weeks revealed that glomerular podocytes continued to decrease over time until global podocyte depletion (ESKD) by estimations using these three different methods. Urine podocyte and TGF-β1 mRNAs were excreted persistently at high levels throughout the time course of progression in experimental but not in control rats. In addition, methylprednisolone (MP) therapy slowed podocyte depletion and progression process, and also decreased urine TGF-β1 mRNA excretion. Furthermore, in patients with crescentic glomerulonephritis, urine podocyte and TGF-β1 mRNA excretion were significantly increased compared with healthy control.

**Conclusions:** These results emphasize the podocyte depletion hypothesis, and show that urine podocyte and TGFβ1 mRNAs could serve to identify and monitor the progression process in crescentic glomerulonephritis.

## FR-PO853

**A Protective Role of Indoleamine 2, 3-Dioxygenase (IDO)-General Control Non-Repressed-2 (GCN2) Kinase Axis in Glomerulonephritis**

Kapil Chaudhary,[1] Lei Huang,[1] Maggie McMenamin,[2] Michael P. Madaio,[2] Tracy L. McGaha.[1] *[1]Cancer Immunology, Inflammation and Tolerance Program, Georgia Regents Univ, Augusta, GA; [2]Dept of Medicine, Georgia Regents Univ, Augusta, GA.*

**Background:** Indoleamine 2, 3-dioxygenase **(IDO)**, a tryptophan (Trp) catabolizing enzyme rapidly induced in dendritic cells and macrophages by interferons (type I and II), depletes Trp at the site of inflammation. **G**eneral **C**ontrol **N**on-repressed **(GCN2)** kinase senses amino acid depletion (uncharged t-RNAs) and activates cellular stress response by affecting global as well as specific m-RNA translation. Including in kidney much is unknown about the functional role of IDO during inflammation in stromal cells.

**Methods:** Nephrotoxic serum nephritis (NTN) was induced in IDO-KO, GCN2-KO and WT (C57B6) mice by passive administration (i.p.) of low and high dose sheep nephrotoxic serum (NTS). Multiple i.v. injections of CpG free DNA coated nano-particles (DNPs) were used to induce IDO expression in-vivo. Enzymatic activity of IDO (kynurenines production) was measured by HPLC. Autophagy response in differentiated podocytes was measured by staining for LC3B.

**Results:** Compared to WT, IDO-KO and GCN2-KO mice developed more severe kidney damage by 3rd week of NTN as evident by significantly higher BUN, proteinuria levels and histopathological scores. IDO1 expression was induced at mRNA and protein levels in podocytes by day 10 of NTN. Interferons (IFN-γ and IFN-β1) induced IDO activity in podocytes in-vitro. Systemic administration of DNPs induced IDO activity in kidneys and significantly reduced BUN and proteinuria levels, and NGAL expression by day 21 of NTN, while IDO-KO mice did not show any protection. Expression of CHOP, a marker of GCN2 kinase activation was significantly higher in kidney cortex of NTN mice and differentiated podocytes cultured in tryptophan-depleted conditions. Tryptophan depletion suppresses IL-1β and CCL-2 production upon LPS treatment and induces autophagy in podocytes in-vitro.

**Conclusions:** IDO induces cellular stress response and autophagy in podocytes, and limits progression of immune mediated kidney injury.

*Funding:* Private Foundation Support

## FR-PO854

**Role of miRNA let-7b in the Deregulation Process of IgA1 Glycosylation in IgA Nephropathy** Grazia Serino,[1,2] Fabio Sallustio,[1,2] Claudia Curci,[2] Sharon N. Cox,[1] Francesco Pesce,[3] Giuseppe De Palma,[2] Francesco Paolo Schena.[1,2,4] *[1]Dept of Emergency and Organ Transplantation, Univ of Bari, Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy; [3]Dept of Genomics of Common Disease, Imperial College, London, United Kingdom; [4]Schena Foundation, Research Center for Kidney Diseases, Valenzano, Bari, Italy.*

**Background:** IgA Nephropathy (IgAN) is characterized by aberrant O-glycosylation in the hinge region of IgA1. The initiation step in O-glycan formation is attachment of N-acetylgalactosamine (GalNAc) to the IgA1 hinge region, catalyzed by GalNAc-transferase 2 (GALNT2). In our previous work (JASN 23: 814-24,2012) we found that let-7b is one of the miRNAs, significantly upregulated in IgAN patients.

**Methods:** To investigate the molecular mechanisms in which let-7b is involved, we performed a bioinformatic analysis to predict the target genes which were biologically validated by transient transfection experiments *ex vivo* using peripheral blood mononuclear cells (PBMCs) of 3 IgAN patients and 4 HBD. Then, we measured by Real-time PCR the content of let-7b in PBMCs of 60 Caucasian IgAN patients and 60 healthy blood donors (HBD).

**Results:** We identified let-7b gene targets using the overlapping results of at least two different algorithms (miRBase 19.0, TargetScan 5.2, PicTar and RNA22 1.0). Bioinformatic analysis revealed that GALNT2, the enzyme initiating O-Glycosylation of IgA1, was the potential target of let-7b. Then, we demonstrated that let-7b decreased GALNT2 levels in PBMCs of IgAN patients ($p=0.01$) whereas the loss of let-7b function in PBMCs of HBD led to an increase of GALNT2 mRNA and protein levels ($p= 0.02$). Let-7b was found significantly upregulated in PBMCs of 60 IgAN patients ($p< 0.0001$) and positively correlated with high serum levels of the galactose-deficient (Gal-deficient) IgA1 ($r=0.3$, $p=0.007$) supporting that abnormal increase of serum Gal-deficient IgA1 is consequent to higher let-7b content in PBMCs of IgAN patients.

**Conclusions:** We speculate that the deregulation of let-7b causes GALNT2 reduction that could lead to aberrant O-glycosylation of IgA1 in IgAN patients. Our results give novel additional information on the abnormal O-glycosylation process of IgA1 in IgAN patients.

*Funding:* Government Support - Non-U.S.

## FR-PO855

**Recombinant Pentraxin-2 Therapy Attenuates the Progression of Alport Nephropathy in Col4a3 Deficient Mice** Naoki Nakagawa,[1] Allie M. Roach,[1] Bryce Gordon Johnson,[1] Ivan G. Gomez,[1] Mark L. Lupher,[2] Jeremy Stuart Duffield.[1] *[1]Renal Div, Kidney Research Institute, Univ of Washington, Seattle, WA; [2]Promedior Inc, Boston, MA.*

**Background:** Alport syndrome in humans is an inherited form of kidney disease caused by a mutation in the gene coding the capillary basement membrane collagen IV. The disorder is characterized by progressive glomerulonephritis, leading to glomerulosclerosis, tubulointerstitial disease and organ failure. Col4a3$^{-/-}$ mice also spontaneously develop severe kidney disease highly similar to human disease. Pentraxin-2 is a naturally produced circulating plasma protein involved in innate immunity. Recent studies have shown that pentraxin-2 inhibits interstitial kidney disease with fibrosis by acting as a macrophage differentiation factor, promoting anti-inflammatory/reparative Mreg macrophages in response to tissue injury. We hypothesized that treating Col4a3$^{-/-}$ mice with recombinant Pentraxin-2 would prevent progression of disease.

**Methods:** Col4a3$^{-/-}$ mice were given pentraxin-2 (10 mg/kg, d29-31 then q2w, IP) or vehicle from wk4 to wk9. Urine, plasma and kidneys were harvested at the end of wk9. All samples were analyzed to determine changes in kidney function and fibrosis.

**Results:** Pentraxin-2 treatment significantly attenuated the loss of kidney function and development of albuminuria. Glomerulosclerosis and interstitial fibrosis were also attenuated. Proximal tubules and Podocyte number were preserved. Furthermore, Pentraxin-2 reduced infiltrating macrophage and myofibroblast appearance. Pentraxin-2 stimulates Mreg macrophages in tissue injury and phenotypic characterization of macrophage subsets in this model will be presented.

**Conclusions:** Recombinant Pentraxin-2 prevents progressive loss of kidney function in the Col4a3$^{-/-}$ mouse, attenuates both glomerular and tubulointerstitial disease. Pentraxin-2 is a potential new therapy for human Alport Nephropathy and other chronic kidney diseases.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO856

**Epigallocatechin-3-Gallate (EGCG) Inhibits Amyloid Formation in Amyloiodgenic Light Chain Perfused Kidney** Jiamin Teng,[1] Elba Turbat-herrera,[1] Takahito Moriyama,[2] Guillermo A. Herrera.[1] *[1]Pathology, LSU Health, Shreveport, LA; [2]Medicine, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** In our previous studies, we have shown amyloid formation after treatment with glomerulopathic amyloidogenic light chains (AL-GLC) purified from the urine of patients with renal biopsy-proven AL-amyloidosis. C-fos plays an important role in the generation of signals resulting in amyloidogenesis. Other studies have shown that EGCG, found in green tea and red wine, is able to ameliorate amyloid-beta protein –associated amyloidosis in Alzheimer's disease.

**Methods:** Human mesangial cells (HMC) were grown until confluent, made quiescent for 2 days, and then incubated with AL-LC and EGCG for 4 hours. Western blots and immunofluorescence stains were used to assess the effect on c-fos. Rat kidneys were isolated and mounted on an ex-vivo kidney perfusion platform. AL-LC and EGCG were perfused through the renal artery for 24 hours, then the kidneys were fixed and examined using light, immunofluorescence, and electronic microscopy (transmission and scanning). Proper controls were used to compare with experimental data.

**Results:** After incubation with AL-LC, amyloidogenic light chains were noted in mesangial areas and c-fos translocated from HMC cytoplasm into nuclei. In EGCG treated group, cytoplasmic to nuclear translocation of c-fos was inhibited and amyloid formation was significantly decreased.

**Conclusions:** EGCG down-regulates translocation of c-fos into MC nuclei when MCs are incubated with AL-LCs. In MCs co-incubated with EGCG, nuclear c-fos translocation is significantly decreased and amyloid formation is decreased. The inhibitory effect of EGCG on amyloid fibril formation occurs concomitantly with inhibition of translocation of c-fos from cytoplasm to nuclei. EGCG has an important effect in regulating amyloid formation in MCs.

## FR-PO857

**TLR4-Deficient Mice Are Protected from Chronic Kidney Disease Progression and Spleen Apoptosis in a Remnant Kidney Model** Ana C. Souza, Takayuki Tsuji, Alejandro Alvarez-Prats, Xuzhen Hu, Yuning George Huang, Robert A. Star, Peter S.T. Yuen. *NIDDK, NIH, Bethesda.*

**Background:** TLR4, the LPS receptor, recognizes HMGB1, a danger associated molecular pattern (DAMP) protein. HMGB1 is increased in patients with CKD, and after 5/6 nephrectomy (5/6Nx) in mice. We showed that CKD worsens sepsis-AKI by releasing HMGB1, and that apoptotic splenocytes are a major source of HMGB1 (KI 2011). Now we test if the HMGB1-TLR4 pathway participates in the progression of CKD.

**Methods:** C3H/HeOuJ (TLR4+) vs. C3H/HeJ (TLR4-) mice were subjected to 5/6Nx then a continuous infusion of low dose (0.0625 µg/Kg/min) Angiotensin II (AngII) by osmotic minipump for 4 weeks (N=10/group). Mice subjected to 5/6Nx without AngII infusion did not progress to CKD and were used as controls (N=8/group). BUN was measured by colorimetry, creatinine by HPLC, albumin and serum HMGB1 by ELISA. ACR was analyzed weekly. Histological analyses of kidney sections were performed. Spleen apoptosis was measured by anti-caspase-3 immunohistochemistry. Statistical analysis was performed by ANOVA.

**Results:**

| | Control TLR4+ (5/6Nx) | Control TLR4- (5/6Nx) | 5/6Nx + AngII TLR4+ | 5/6Nx + AngII TLR4- | p value (ANOVA) |
|---|---|---|---|---|---|
| BUN (mg/dl) | 73±2 | 83±11 | 223±41 | 70±5 | <0.0001 |
| SCr (mg/dl) | 0.2±0.0 | 0.2±0.0 | 0.6±0.1 | 0.2±0.0 | <0.0001 |
| ACR at 4 wks (µg/mg) | 114±26 | 113±25 | 4730±1120 | 73±9 | <0.0001 |
| Glomerulosclerosis Score | 1.3±0.1 | 1.4±0.3 | 3.1±0.3 | 1.6±0.1 | <0.0001 |
| Interstitial Fibrosis Score | 1.3±0.0 | 1.5±0.2 | 2.3±0.2 | 1.3±0.0 | <0.001 |
| Serum HMGB1 (ng/ml) | 7±1 | 7±0.4 | 64±23 | 19±5 | <0.01 |
| Spleen apoptosis (+cells/HPF) | 4±0.3 | 4±0.2 | 18±2 | 2±0.2 | <0.0001 |

**Conclusions:** TLR4 plays an essential role in CKD progression in mice. Serum creatinine and HMGB1, albuminuria, and spleen apoptosis are increased in TLR4+ but not TLR4- mice after 5/6 Nx+AngII. Both 5/6Nx and AngII are necessary, but not sufficient, for TLR4-dependent increases in spleen apoptosis, serum HMGB1, and CKD progression.

*Funding:* NIDDK Support

## FR-PO858

**CD36 Receptor Blockade Protects against Chronic Kidney Disease Progression in a Remnant Kidney Model** Ana C. Souza,[1] Alexander V. Bocharov,[2] Alejandro Alvarez-Prats,[1] Yuning George Huang,[1] Xuzhen Hu,[1] Erik H. Koritzinsky,[1] Thomas Eggerman,[1] Robert A. Star,[1] Peter S.T. Yuen.[1] *[1]NIDDK, NIH; [2]CC, NIH, Bethesda.*

**Background:** Scavenger receptor CD36 is a widely expressed cell surface receptor important in lipid metabolism, inflammation and atherosclerosis. We have demonstrated that CD36KO mice are protected from CKD progression after 5/6 nephrectomy (5/6Nx) + Angiotensin II (AngII) infusion (ASN 2012). Now we test the effect of a synthetic peptide, L37pA, a potent receptor antagonist for CD36, on CKD progression.

**Methods:** CD36KO and WT (C57BL6 background) mice were subjected to 5/6Nx plus AngII infusion (0.75 µg/Kg/min) for 4 wks by osmotic minipump (N=12/group). WT 5/6Nx mice without AngII infusion did not develop progressive CKD and served as controls (N=12). Another group of WT mice subjected to 5/6Nx+AngII received L37pA peptide (5mg/Kg/day) by osmotic mini-pump (N=9). All mice were followed for 4 wks. Albuminuria was measured weekly by ELISA; creatinine by HPLC. A blood chemistry panel was performed by an auto-analyzer (N=6/group). Masson's and PAS staining of kidney sections were performed. Statistical analysis was done by ANOVA.

**Results:** At 4 wks Scr was increased in WT mice subjected to 5/6Nx+AngII, while KO and WT+L37pA had values similar to control (control 0.28±0.02, WT 0.59±0.13, KO 0.33±0.03, WT+L37pA 0.20±0.04 mg/dl, $p=0.01$). This protection in kidney function was accompanied by reduced levels of albuminuria (ACR at 4 wks control 74.2±22.6, WT 3510±805, KO 1830±570, WT+L37pA 2220±542 µg/mg, $p<0.0001$), glomerulosclerosis (control 1.4±0.1, WT 3.2±0.2, KO 2.3±0.1, WT+L37pA 2.1±0.2, $p<0.0001$), and interstitial fibrosis scores (control 1.3±0.1, WT 3.0±0.2, KO 2.2±0.2, WT+L37pA 2.0±0.1, $p<0.0001$). Serum calcium, phosphorus and magnesium were elevated in the WT group ($p<0.05$), and not in KO and L37pA groups. Serum AST, ALT, uric acid, albumin, amylase and CK were similar in all groups.

**Conclusions:** CD36KO protection is not due to a developmental abnormality, since adult WT mice treated with L37pA are also protected from CKD progression. At this dose and route of administration, L37pA did not cause any detectable side effects.

*Funding:* NIDDK Support

### FR-PO859

**The Role of the Intestinal Microbiota in the Development of Diabetes in BTBR and BTBR ob/ob Mice** Kelly D. Smith, Tomasz Wietecha, Kelly L. Hudkins, Xiaodan Zhao, Charles E. Alpers. *Pathology, Univ of Washington, Seattle, WA.*

**Background:** Obesity and diabetes are associated with an altered gut microbiota that further aggravates metabolic syndrome, and may promote inflammation and contribute to diabetic complications. The BTBR$^{ob/ob}$ mouse is leptin-deficient, resulting in progressive obesity and type II diabetes. A novel aspect of this mouse model of type II diabetes is that these mice also develop clinical and morphologic features that closely mimic diabetic nephropathy in humans (J Am Soc Nephrol. 2010;21(9):1533-42). In this study we analyzed the role of the microbiome in modulating the BTBR and BTBR$^{ob/ob}$ mouse models of diabetes.

**Methods:** Starting at age 4 weeks, BTBR and BTBR$^{ob/ob}$ mice were fed normal mouse chow, and either water or water with an antibiotic cocktail (Ampicillin, Vancomycin, Metronidazole and Neomycin). Mice were analyzed for weight, blood glucose, glucose tolerance, urine protein, and histopathology. Fecal bacteria were analyzed by culture and 16S rDNA sequencing.

**Results:** Antibiotic treatment suppressed intestinal microbiota in BTBR mice, which was associated with a significant improvement in fasting glucose levels and glucose tolerance. In contrast, antibiotic treatment altered but did not suppress microbiota in BTBR$^{ob/ob}$ mice, resulted in an overgrowth of fungal organisms within the gut, and led to decreased survival (50% at 16 weeks).

**Conclusions:** Antibiotic mediated suppression of the intestinal microbiota improved diabetic parameters in non-obese type II diabetic BTBR mice. Antibiotics altered but failed to suppress microbiota in obese type II diabetic BTBRob/ob mice, and failed to suppress the development of diabetes.

*Funding:* Other NIH Support - DCC Pilot & Feasibility Funding Program 12GHSU185

### FR-PO860

**Timing Is Critical in Determining Intervention Effectiveness. Moderate Calorie Restriction Enhances Angiotensin II Blockade Effectiveness in Preventing Progression** Ryuzoh Nishizono, Su Qing Wang, Mahboob A. Chowdhury, Yan Yang, Larysa T. Wickman, Roger C. Wiggins. *Div of Nephrology, Dept of Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

**Background:** Obesity is and independent risk factor for progression, and increasing obesity may account for increasing prevalence of FSGS reported worldwide. We previously demonstrated that 40% calorie restriction (CR) prevented FSGS, proteinuria, podocyte loss, glomerulosclerosis and progression to ESKD in the AA-4EBP1 rat model system (Fukuda et al, JASN, 2013).

**Methods:** Transgenic AA-4EBP1 Fischer 344 rat podocytes express a dominant negative AA-4E-BP1 transgene driven by the human podocin promoter. Homozygous 4EBP1 transgenic rats were uni-nephrectomized (NX) at 100g body weight ("time 0"). Interventions (20% calorie restriction or angiotensin converting enzyme inhibition by enalapril [A2B at 0.1mg/ml in drinking water] or the combination of these two treatments were started either immediately after nephrectomy ("early start") or three weeks later when the urine protein:creatinine ratio had reached 10 ("later start"). Outcomes measurements assessed were proteinuria, urine podocyte mRNAs, glomerulosclerosis, podocyte number per glomerulus, and serum creatinine.

**Results:** Homozygous NX 4EBP1 rats reached ESKD by 12 weeks.(% glomerulosclerosis was 85.1±5.5 at week 11). Both 20%CR and A2B started immediately after NX prevented progression. However neither treatment alone prevented progression when started later after injury had become established (3 weeks after NX). % glomerulosclerosis in 20%CR was 62.3±5.4 and 52.5±8.5 in A2B at week 12. In contrast the combination of moderate CR and A2B did prevent progression when started after injury had been established(% glomerulosclerosis was 26.2±7.0 at week 12).

**Conclusions:** Timing of intervention is critical in determining its effectiveness. The combination of moderate calorie restriction together with A2B was significantly more effective at preventing progression of established injury than was either treatment alone. The impact of moderate calorie reduction is an under-recognized variable in clinical trials for prevention of progression of glomerular diseases.

*Funding:* NIDDK Support

### FR-PO861

**Dipeptidyl Peptidase 4 Deficiency Reduces Renal Injury in the Remnant Kidney Model in the Rat** Christoph Daniel,[1] Stephan Von Hoersten,[2] Kerstin U. Amann.[1] *[1]Nephropathology, Univ Erlangen; [2]Experimental Biomedicine, Univ Erlangen.*

**Background:** Dipeptidyl peptidase 4 (DPP4) is an exopeptidase inactivating incretins that promote insulin secretion and were therefore used in the treatment of type II diabetes. Beside its ability to cleave incretins, several chemokines like RANTES and CCL2 were also regulated by DPP4. In lung as well as in renal ischemia/reperfusion injury (IRI) and in acute heart rejection, treatment with DPP-4 inhibitors were protective, but the effect of DPP4 inhibition in chronic kidney disease is unknown.

**Methods:** Wildtype Dark Agouti (DA) rats (n=21) and congenic rats who did not express DPP4 (DPP4-/-) (n=20) were divided in SHAM-operated controls (n=7+8) and animals who underwent 5/6-nephrectomy (SnX; n=14+12). Kidney function was monitored every 4 weeks. Twelve weeks after model induction we measured intraarterial blood pressure and renal inflammation, fibrosis and morphologic changes.

**Results:** In both SnX groups proteinuria and serum urea raised up starting on week 4 and remained on high levels during the whole experiment showing lower levels in DPP4-/- rats. Twelve weeks after SnX mean arterial blood pressure was increased in DA rats (135±4 vs. 172±19 mmHg), but was significantly lower in DPP4-/- rats (151±23 mmHg). Kidney morphology from DPP4-/- rats was significantly preserved after SnX as shown by lower glomerular sclerosis (GSI score: 1.1±0.5 vs. 1.7±0.7) and tubulointerstitial injury (TSI score: 1.0±0.5 vs. 1.6 ±0.5). In addition, renal fibrosis was markedly lower in DPP4-/- SNx rats (sirius red score: 1.0±0.6 vs. 2.0±0.4). Of note, renal macrophages (ED1 positive cells/mm2: 30.1±8.1 vs. 65.5±17.4) and T-cell infiltrates (CD3 positive cells/mm2: 22.4±7 vs. 46.3±12.6) were also significantly decreased in DPP4-/- SnX. Interestingly, the number of renal CD163 positive M2 macrophages was also lower in DPP4-/- Snx rats but the difference did not reach significance level.

**Conclusions:** DPP4 deficiency ameliorates renal injury in rats with 5/6-nephrectomy as shown by preserved renal function, lower inflammation and fibrosis. Thus, DPP-4 inhibitors might be a therapeutic option also in the treatment of progressive chronic kidney disease.

*Funding:* Government Support - Non-U.S.

### FR-PO862

**Erlotinib Attenuates the Progression of Chronic Renal Failure in 5/6 Nephrectomized Rats** Yasutaka Yamamoto, Masayuki Iyoda, Yukihiro Wada, Kei Matsumoto, Yuki Shindo-Hirai, Yoshihiro Kuno, Taihei Suzuki, Tomohiro Saitou, Ken Iseri, Takanori Shibata. *Div of Nephrology, Dept of Medicine, Showa Univ School of Medicine, Tokyo, Japan.*

**Background:** The effects of blocking the epidermal growth factor receptor (EGFR) in chronic renal failure are unknown. In the current study, we investigated the renoprotective effect of erlotinib, a tyrosine kinase inhibitor that can block EGFR activity, in the progression of established renal failure.

**Methods:** Adult male Sprague Dawley rats were subjected to 5/6 nephrectomy (n=34) or laparotomy (sham-operated, n=9). Rats with 5/6 nephrectomy were then administered either erlotinib (20 mg/kg, n=18) or vehicle (n=16) via daily oral gavage from 2 weeks after surgery, and for a period of 8 weeks. Blood pressure (BP), proteinuria (U-P), serum creatinine (Cr) and body weight (BW) were measured periodically. Renal morphological investigations were performed at sacrifice.

**Results:** Systolic BP (8 weeks: 164.30 ± 8.20 vs. 161.50 ± 7.64 mmHg, NS) and BW (8 weeks: 404.42 ± 14.40 vs. 402.93 ± 6.94 g, NS) were comparable between the two treatment groups throughout the study. The serum Cr levels in the erlotinib-treated rats were significantly lower than that of the vehicle-treated rats at each time point (8 weeks: 1.07 ± 0.17 vs. 0.75 ± 0.05 mg/dL, $p < 0.05$; 0.28 ± 0.01 mg/dL in sham-operated rats). When compared to vehicle treatment, erlotinib-treated rats demonstrated reduced U-P at 2 weeks after treatment (62.58 ± 9.70 vs. 35.64 ± 2.34 mg/day, $p < 0.01$). This reduction was maintained over the course of the study (8 weeks: 154.08 ± 28.66 vs. 101.09 ± 14.13 mg/day, $p < 0.05$; 36.08 ± 1.75 mg/day in sham-operated rats). Erlotinib treatment reduced glomerular tuft area (11574.07 ± 368.11 vs. 10409.65 ± 252.03 mm$^2$, $p < 0.01$), and the scores of glomerulosclerosis (semiquantitative score (SEMQ-S) (0-4): 2.18 ± 0.20 vs. 1.63 ± 0.09, $p < 0.01$) and tubulointerstitial damage (SEMQ-S (0-5): 3.28 ± 0.29 vs. 2.71 ± 0.16, $p < 0.05$).

**Conclusions:** Erlotinib treatment significantly attenuates renal injury following subtotal renal ablation in rats. Our results suggest that erlotinib may prove useful in limiting the progression of chronic renal disease to end-stage renal failure.

### FR-PO863

**Implantation of a Femoral Catheter in 5/6-Nephrectomized Rats Induces Heart Fibrosis: Intravenous or Oral Treatment with PBI-4050 Reduces Both Kidney and Heart Fibrosis** Lyne Gagnon, Lilianne Geerts, François Sarra-Bournet, Kathy Hince, Mikaël Tremblay, Liette Gervais, Alexandra Felton, Martin Leduc, Pierre Laurin, Brigitte Grouix. *ProMetic BioSciences Inc., Laval, Canada.*

**Background:** PBI-4050 is a first-in-class novel orally active compound which displays anti-inflammatory/antifibrotic activities via a novel mechanism of action. The aims of this study were to determine the effect of a "permanent" vascular catheter on heart fibrosis, and to investigate the potential protective effect of PBI-4050 on kidney and heart in 5/6-nephrectomized (NX) catheterized rats.

**Methods:** Sprague-Dawley rats were partially nephrectomized (2/3 of the left kidney) on day 0. On day 7, the right kidney was removed and a catheter was implanted via the femoral vein. On day 21, rats were randomized based on glomerular filtration rate (GFR) results. NX animals were divided in five groups (n=10). Two groups were not catheterized and received vehicle or PBI-4050 (oral, 200 mg/kg). Three groups were catheterized, of which one group received vehicle, another group was treated with daily oral administration of PBI-4050 (200 mg/kg) and the other group was treated three times a week with intravenous administration of PBI-4050 (10 mg/kg).

**Results:** Non-catheterized NX rats were found to have few heart lesions (low grade). However, implantation of a catheter induced a significant increase (four fold) in heart lesions (fibrosis, necrosis and inflammation). Treatment with PBI-4050 significantly reduced

($p<0.05$) total heart lesions. Furthermore, there was a marked increase in hydroxyproline level (collagen) observed in catheterized-NX rat hearts which was significantly reduced by both IV and PO treatments with PBI-4050.This reduction in hydroxyproline level correlated with the histological heart sections (Masson's trichrome stain). Moreover, GFR was increased and inflammation of the kidney (as measured by urinary MCP-1 level) was decreased in PBI-4050-treated catheterized-NX rats.

**Conclusions:** Taken together, our results suggest that implantation of a catheter may induce heart fibrosis, and PBI-4050 offers the potential as a novel therapy for the treatment of kidney and heart fibrosis.

## FR-PO864

**Galacto-Oligosaccharides Modified Gut Microbiota and Attenuated Renal Injury** Satoshi Unuma,[1] Takamoto Ohse,[1] Airi Jo,[1] Akira Shigehisa,[2] Koji Kawakami,[2] Takahiro Matsuki,[2] Osamu Chonan,[2] Masaomi Nangaku.[1] *[1]Nephrology and Endocrinology, The Univ of Tokyo, Tokyo, Japan; [2]Yakult Central Institute, Kunitachi, Japan.*

**Background:** Tubulointerstitial injury is a final common pathway to end stage renal disease. We have previously reported that uremic toxins cause tubulointerstitial injury through oxidative stress and endoplasmic reticulum (ER) stress. Galacto-oligosaccharides (GOS) are utilized by beneficial microbes. Since the precursor of indoxyl sulfate, indole, is synthesized by gut microbiota, we focused on the effects of GOS on indoxyl sulfate synthesis by gut microbiota and renal function in kidney disease.

**Methods:** Two weeks after induction of the 5□6 nephrectomized (Nx) model, the rats were divided into two groups, control diet group (ConNx) and GOS-fed group (GOSNx). After two weeks administration of GOS, we measured serum indoxyl sulfate and examined the gut microbiota with pyrosequencing method, the cecal contents, and the renal injury.

**Results:** A comprehensive analysis of gut microbiota revealed a significant increase in Bifidobacteriaceae and decrease in Ruminococcaceae by GOS. While no significant changes were detected in blood pressure, body weight and food consumption of the rats, cecal indole and serum indoxyl sulfate were significantly reduced in GOSNx. Renal injury was improved and infiltrating macrophages were significantly decreased in GOSNx. We next examined ER stress as a pathway of renal injury caused by indoxyl sulfate. Immunohistochemistry and quantitative PCR revealed that GRP78, CHOP, and ORP150 were significantly increased in ConNx and significantly decreased in GOSNx compared with ConNx. TUNEL positive cells were also significantly increased in ConNx and significantly decreased in GOSNx compared with ConNx.

**Conclusions:** GOS administration to 5/6 nephrectomized rats modified gut microbiota, reduced serum indoxyl sulfate, and attenuated renal injury. ER stress was reduced by GOS administration and amelioration of ER stress was implicated in a mechanism of kidney protection. GOS can be a novel therapeutic agent against kidney injury.

*Funding:* Pharmaceutical Company Support - Yakult Honsha Co., Ltd.

## FR-PO865

**Integrin β6 in Macula Densa Cells Modulates Potential Tubuloglomerular Feedback** Beom Jin Lim, Haichun Yang, Agnes B. Fogo. *Pathology, Microbiology and Immunology, Vanderbilt Univ Medical Center, Nashville, TN.*

**Background:** The heterodimeric integrin αvβ6 is expressed in tubular epithelial cells and the juxtaglomerular apparatuses (JGA) in the kidney.It binds and activates latent transforming growth factor-β (TGF-β), and thus has profibrotic effect. We previously observed that$\beta6^{-/-}$ mice showed increased glomerulosclerosis vs.wild type (WT) mice with disproportionally milder interstitial fibrosis after 5/6 nephrectomy (Nx). The development of glomerulosclerosis was TGF-β-independent, but was related to increased renin activity in JGA. These results suggested that$\beta6^{-/-}$ mice may have dysregulated tubuloglomerular feedback. We therefore evaluated the effect of integrin β6 knock down in isolated mouse macula densa cells (MMDD1).

**Methods:** 5/6 Nx kidneys of $\beta6^{-/-}$ *vs.* WT mice were analyzed for nNOS and COX-2. Integrin β6-specific gene knock down was performed by shRNA transduction in MMDD1 cells,and assessed by real time RT-PCR and immunofluorescence microscopy. Cells were then treated with isoosmolar normal salt or low salt solution for 8 hours. nNOS and COX-2 were measured by western blot.

**Results:** Kidneys from $\beta6^{-/-}$ mice showed decreased nNOS expression and similar COX-2 expression vs WT mice, but immunohistochemistry showed decreased nNOS and increased COX-2 intensity in $\beta6^{-/-}$ mice.β6 mRNA assessed by real time RT-PCR in shRNA-transfected MMDD1 cells was decreased to 41.5% of WT cells. Control scrambled shRNA had no effect. Integrin β6 knock down cells showed higher baseline expression *vs.* WT cells of both COX-2 (COX-2/α-tubulin 0.15±0.02 *vs.* 0.01±0.00, $p<0.05$) and nNOS (nNOS/α-tubulin 0.10±0.01 *vs.* 0.02±0.01, $p<0.05$). After stimulating with low salt, COX-2 expression was similarly increased in β6 knock down and WT cells (0.83±0.01 *vs.* 0.78±0.01, pNS), but nNOS expression was increased only in β6 knock down cells (0.20±0.02 *vs.* 0.06±0.01, $p<0.05$).

**Conclusions:** We conclude that the absence of integrin β6 in macula densa dells results in altered expression of tubuloglomerular feedback-related molecules. The findings support our hypothesis that the absence of integrin β6 may affect progression of glomerulosclerosis by dysregulated tubuloglomerular feedback mechanisms.

*Funding:* NIDDK Support

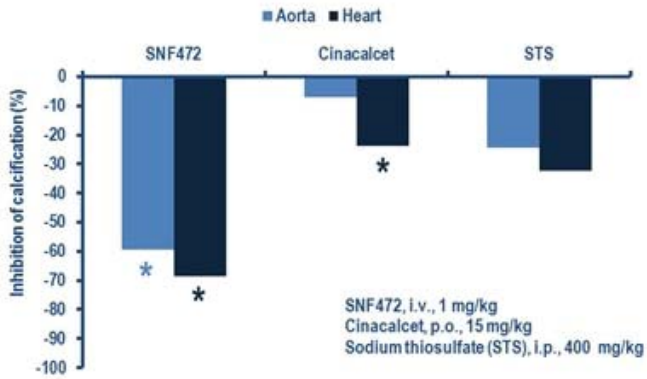
## FR-PO866

**SNF472 Inhibits Vitamin D Induced Cardiovascular Calcification in Rats** Markus Ketteler,[1] Miquel D. Ferrer,[2] Fernando Tur,[3] Bernat Isern,[2] Carolina Salcedo,[2] Joan S. Perelló,[2,3] Pieter H. Joubert.[2] *[1]Div of Nephrology, Klinikum Coburg GmbH, Coburg, Germany; [2]Research and Development Dept, Laboratoris Sanifit SL., Palma de Mallorca, Spain; [3]Laboratory of Renal Lithiasis Research, Institute of Health Sciences Research (IUNICS), Univ of the Balearic Islands, Palma de Mallorca, Spain.*

**Background:** Cardiovascular calcification has been shown to be an independent predictor of cardiovascular events in CKD patients. This study investigated the effects of SNF472, an intravenous formulation of myo-inositol hexaphosphate, on vitamin D induced vascular calcification, and compare its effects with cinacalcet and sodium thiosulfate (STS).

**Methods:** The study was done in 40 male Sprague Dawley rats divided into 3 groups of 10-16 animals. Group 1 received daily intravenous vehicle or 1 mg/kg SNF472. Group 2 received vehicle or 15 mg/kg of cinacalcet p.o. Group 3 received vehicle or 400 mg/kg of sodium thiosulfate i.p. Calcification was induced by 5 daily oral administrations of 75000 IU/kg of vitamin D starting on day 3 of treatment. Five sham treated animals served as control. Serum samples for the determination of calcium and phosphorus concentrations were collected on days 8 and 14. Rats were sacrificed on day 14 and aortas and hearts removed for calcium analyses.

**Results:** The administration of vitamin D induced a marked increase in aortic and heart calcium levels. Calcium and phosphorus from control serums increased significantly compared to the sham group, and were not affected by any of the treatments. The intravenous administration of SN472 at 1 mg/kg resulted in reductions by 60% in aortic and 68% in heart tissues. Animals treated with Cinacalcet and STS showed small reductions. The only significant reduction occurred with cinacalcet in the heart (24% reduction).




**Conclusions:** SNF472 might be an alternative therapeutic principle for cardiovascular calcification treatment.

*Funding:* Pharmaceutical Company Support - Sanifit, Government Support - Non-U.S.

## FR-PO867

**Vitamin D Inhibition of the Proteases ADAM17 and Cathepsin L Contributes to Attenuate High Phosphate-Induced Renal and Cardiovasular Damage** Petya Valcheva,[1,2] Yolanda Almaden Peña,[2] Mariano Rodriguez,[2] Adriana S. Dusso.[1] *[1]Experimental Nephrology, IRBLleida, Lleida; [2]Research Unit, Hospital Reina Sofia, Cordoba, Spain.*

**Background:** High serum phosphate (HP) is a recognized inducer of accelerated renal and cardiovascular (CV) aging through mechanisms unrelated to its potency to worsen the severity of secondary hyperparathyroidism or the osteoblast-like phenotype in vascular smooth muscle cells (VSMC). Based upon the critical impact of ADAM17 and cathepsin L (CTSL) in renal and CV damage through excessive inflammation and DNA damage, this study examined the contribution of these mechanisms, and of ADAM17 cleavage of klotho, to the potent adverse effects of HP on renal and cardiovascular function.

**Methods:** Western blots and immunohistochemical analysis of ADAM17, CTSL, and klotho was performed in VSMC, rat kidneys and renal human arteries.

**Results:** In VSMC, an 8 hour exposure to HP (3.3 mM) suffices to enhance ADAM17 expression at the cell membrane, and nuclear CTSL content. Levels of both proteases increased further in VSMC from the vitamin D receptor (VDR) null mice. Importantly, in slices of normal rat kidneys, exposure to HP results not only in similar increases in ADAM17 and nuclear CTSL, but also in a reduction in klotho content, essential to maintain the phosphaturic response to FGF23. All of these rapid adverse effects of HP in a normal kidney are markedly attenuated by co-incubation with HP and the combination of 25-hydroxyvitamin D ($10^{-10}$ M) + paricalcitol ($10^{-8}$ M). In addition, also in slices of normal human renal arteries, the nutritional+active vitamin D combination effectively counteracts the rapid HP induction of ADAM17 and nuclear CTSL with a potency for the former as that of the ADAM17 inhibitor TAPI-2 (10 and 20 μM).

**Conclusions:** Thus, maintenance of normal serum levels of nutritional and active vitamin D in kidney disease patients could prevent/attenuate HP-induced progression of either renal or cardiovascular aging through the inhibition of ADAM17 and CTSL activation and klotho loss.

## FR-PO868

**Coagulation Protease-Activated Protein C Selectively Trans Activates XBP1 via p85α and p85β to Restore Endoplasmic Reticulum Homeostasis in Diabetic Nephropathy** Madhusudhan Thati, Hongjie Wang, Hermann-Josef Groene, Jochen Reiser, Berend Heinrich Isermann. *Institute for Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke-Univ Magdeburg, Magdeburg, Germany.*

**Background:** A growing body of recent work suggests that endoplasmic reticulum (ER) stress response is causally linked to diabetes complications. However the specific physiological stimuli and molecular mechanism through which hyperglycemia modulates ER stress are not known.

**Methods:** In an attempt to determine the role of hyperglycemia in regulating ER-stress we have employed mouse models of both type-1(streptozotocin) and type-2 (db/db) diabetic nephropathy (DN).

**Results:** In DN hyperglycemia selectively impairs nuclear translocation of the highly conserved transcription factor spliced X-box binding protein-1 (sXBP1). This result in severe ER-stress associated with increased nuclear levels of the active form of activating transcription factor-6 (ATF6) and ATF6 mediated transcriptional activation of CHOP. In addition inducible expression of ATF6 specifically in podocytes aggravates ER-stress and DN. We next show that treatment with activated protein C, a coagulation protease with well-established nephroprotective properties, selectively trans activates sXBP1 via PI3K regulatory subunits p85α and p85β and restores ER-homeostasis in DN. In a mice model with impaired thrombomodulin (TM) -dependent PC activation ($TM^{Pro/Pro}$) ER-stress and DN is aggravated. Conversely in a mouse model with constitutively higher plasma levels of aPC ($APC^{high}$) ER-stress is inhibited, highlighting the physiological role of aPC in regulating ER-stress and DN. While deletion of CHOP or inhibition of ER-stress with tauroursodeoxycholic acid protected against DN in $TM^{Pro/Pro}$ and wild type mice, Conditional deletion of XBP1 ($XBP1^{flox/flox}$ x $Pod^{Cre}$) abolished the protective effect in $APC^{high}$ mice. In podocytes and endothelial cells sXBP1 is selectively, but independently of IRE1, activated by aPC in trans via p85α and p85β, which is required to inhibit hyperglycemia induced ER-stress.

**Conclusions:** These studies revise our understanding of regulation of ER-stress in DN and might have major implications for treatment of DN in humans.

## FR-PO869

**Glycogen Synthase Kinase 3 and the Podocyte: A Tale of Two Isoforms** Jenny Hurcombe,[1] Abigail Charlotte Lay,[1] Gavin Iain Welsh,[1] C. Avila-Casado,[3] Peter W. Mathieson,[1] Satish Patel,[4] James R. Woodgett,[5] Susan E. Quaggin,[2] Richard Coward.[1] *[1]Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom; [2]Cardiovascular Research Institute, Div of Nephrology, North Western Univ, Chicago; [3]Univ Health Network, Toronto General Hospital, Toronto, Canada; [4]Lunenfeld Institute Univ of Toronto; [5]Lunenfeld Univ of Toronto.*

**Background:** Glycogen synthase kinase 3 (GSK3) is a multi-functional serine/threonine kinase existing as two distinct but related isoforms (α and β) with an established role in the modulation of insulin signaling. There is evidence that the GSK3 isoforms have different functions: GSK3β null mice die during late embryogenesisis due to hepatocyte apoptosis whereas total GSK3 α knockout mice are viable and interestingly, exhibit enhanced insulin sensitivity. A number of cell-specific GSK3 knockout mouse models have been described and indicate that the functions of the GSK3 isoforms are cell-type dependent.

**Methods:** In order to investigate the role of the GSK3 isoforms in podocyte development, we have used Cre/ LoxP technology to generate mice with podocyte-specific ablation of GSK3 α and/or GSK3β.

**Results:** Using an *in vitro* and *in vivo* approach we have shown that in the glomerulus, GSK3 is preferentially expressed in the podocyte and that both of its isoforms are phosphorylated and thus deactivated in response to insulin. Mice lacking either GSK3α or GSK3β specifically in the podocyte are viable with normal life span (up to 2 years), no evidence of proteinuria and normal renal histology indicating a degree of redundancy between the isoforms in the podocyte. In contrast, mice null for both GSK3 isoforms (13 of 107 mice studied), although born at normal Mendelian frequency, die predictably at 12-14 days at which time they are proteinuric, with grossly abnormal vacuolated glomeruli on histological analysis.

**Conclusions:** This work reveals a fundamental role of GSK3 in the podocyte and its importance in glomerular development and survival.

## FR-PO870

**APOL1 Encodes a Novel miRNA That Induces PKR, and miRNA Levels Are Increased in Plasma Exosomes from G1 Homozygous Individuals** Koji Okamoto,[1] Cheryl Ann Winkler,[2] Jeffrey B. Kopp.[1] *[1]Kidney Section, NIDDK, NIH, Bethesda, MD; [2]Molecular Genetics Epidemiology Section, NCI, NIH, Frederick, MD.*

**Background:** Coding variants in *APOL1* (S342G and I384M termed G1) and NYK388K (termed G2), compared to ancestral G0) are strongly associated with FSGS among African Americans. We hypothesized that ApoL1 variants might harbor an miRNA that would alter mRNA expression.

**Methods:** We used MFOLD software to analyze APOL1 mRNA structure. We amplified miRNA from cultured human podocytes using stem loop primers and 5' RACE, from either total cell RNA or AGO2 protein immununoprecipated RNA. We transfected into HEK293 cells cDNA vectors encoding recombinant FLAG-tagged APOL1 or 400 bp of APOL1 RNA including the predicted miRNA sequence.

**Results:** RNA folding prediction revealed a stem loop construct located in the APOL1 mRNA region that contains the G1 S342G polymorphism. Using 5-RACE, we identified a 21-nucleotide miRNA, which is also RNA precipitated with AGO2. S342G is located on the antisense strand of a mature miRNA. In exosomes isolated from human plasma samples, more miRNA was detected in G1 homozygotes (n=6) compared with G0 homozygotes (n=5, p = 0.025). The novel miRNA formed dimers and long dsRNA, suggesting that it may trigger phosphorylation (activation) of interferon-induced, double-stranded RNA-activated protein kinase (PKR). Both transfection of APOL1 cDNA and APOL1 miRNA induced PKR phosphorylation; these effects were seen with G1 but not G0. Human podocyte-like urine-derived epithelial cells (G1/G2 genotype, compared to G0/G0 genotype) manifested PKR activation, and downstream effects including increased type 1 interferon production, phosphorylation of eIF2a and inhibition of protein translation, all of which were reduced following APOL1 knock down.

**Conclusions:** We report that APOL1 mRNA generates a miRNA whose expression is higher in G1 genotype cells. APOL1 G1 cells exhibit an activated PKR-related host defense pathway compared to G0 cells. Up-regulation of APOL1 derived miRNA provides a new pathogenic mechanism for APOL1 nephroapthy associated with the G1 variant and suggests that possible therapeutic targets include APOL1 miRNA and PKR.

*Funding:* NIDDK Support

## FR-PO871

***ARHGDIA*: A Novel Gene Implicated in Nephrotic Syndrome** David Auguste,[1] Richard Robins,[1] Jasmine El andalousi,[2] Lamine Aoudjit,[1] Cindy Baldwin,[1] Indra R. Gupta,[2] Tomoko Takano.[1] *[1]Medicine, McGill Univ, Montreal, Canada; [2]Nephrology, Montreal Children's Hospital, Montreal, Canada.*

**Background:** RhoGDIα (GDIα, encoded by *ARHGDIA*) is a negative regulator of Rho-GTPases that play a critical role in cytoskeleton and cell motility. We previously reported that a single amino acid deletion, D185Del, is a loss-of-function mutation of GDIα and causes congenital nephrotic syndrome. We hypothesize that the loss of the inhibitory action of GDIα leads to hyper-activation of Rac1 in podocytes and nephrotic syndrome.

**Methods:** GDIα was knocked down (KD) by an shRNA in mouse podocytes. KD cells were rescued with wild-type or D185Del GDIα resistant to the shRNA. Rac1 activity was quantified by pull-down assay and immunocytochemistry for active Rac1 (aRac1). Cell motility was studied by wound healing. GDIα and aRac1 in the mouse kidney were detected by immunofluorescence staining. The constitutively active Rac1 (CA-Rac1) was induced in podocytes *in vivo* by treating double transgenic mice (carrying a tetracycline-inducible CA-Rac1 and a reverse tetracycline transactivator driven by the podocin promoter) with doxycycline.

**Results:** The KD cells showed a significantly higher Rac1 activity and aRac1 was localized at the plasma membrane more prominently, as compared with controls. The motility of KD cells was significantly reduced, as compared with controls and this reduction was rescued by wild-type but not by the mutant GDIα. GDIα protein was detected in glomerular precursor structures such as the S-shaped body and was strongly expressed in podocytes within the mature glomerulus. In contrast, aRac1 protein was most prominent in comma and S-shaped structures, and was not detected in podocytes after the late capillary stage. When CA-Rac1 was induced in podocytes in adult mice, 3/7 double transgenic mice demonstrated heavy proteinuria after 3-5 days of doxycycline treatment, while none of the control mice, 0/17, developed proteinuria.

**Conclusions:** D185Del mutation of GDIα induced Rac1 hyper-activation and nephrotic syndrome. In the normal mature glomerulus, Rac1 is inactive in podocytes. Aberrant Rac1 activation in podocytes, possibly combined with dysregulation of other Rho-GTPases, may lead to podocyte dysfunction.

*Funding:* Government Support - Non-U.S.

## FR-PO872

**Ezrin Is Downregulated in Diabetic Kidney Glomeruli and Regulates Cortical Actin Dynamics and Glucose Transport in Cultured Podocytes** Anita A. Wasik,[1] Susanna Koskelainen,[1] Mervi E. Hyvonen,[1,2] Luca Musante,[1] Eero Lehtonen,[1] Kerttu Koskenniemi,[3] Jukka Pekka Tienari,[4] Antti Vaheri,[1] Pekka Varmanen,[5] Tuula A. Nyman,[6] Peter Hamar,[7] Harry B. Holthofer,[1] Sanna H. Lehtonen.[1] *[1]Haartman Institute, Univ Helsinki, Finland; [2]Children's Hospital, Univ Helsinki, Finland; [3]Dept Veterinary Biosciences, Univ Helsinki, Finland; [4]Dept Pathology, HUSLAB and Helsinki Univ Hospital, Helsinki and Hyvinkää, Finland; [5]Dept Food and Environmental Sciences, Univ Helsinki, Finland; [6]Institute of Biotechnology, Univ Helsinki, Finland; [7]Institute of Pathophysiology, Semmelweis Univ, Budapest, Hungary.*

**Background:** Diabetic nephropathy (DN) is a major cause of end-stage renal disease but the pathophysiological mechanisms associated with its development are poorly characterized.

**Methods:** To characterize the early pathophysiological mechanisms leading to podocyte injury in DN we performed quantitative proteomic profiling of glomeruli isolated from rats with streptozotocin-induced diabetes and controls using fluorescence-based two-dimensional difference gel electrophoresis coupled with mass spectrometry.

**Results:** We identified 29 differentially expressed spots, including ezrin and its interaction partner NHERF2 that were downregulated in the streptozotocin group. Ezrin and NHERF2 link plasma membrane proteins to the actin cytoskeleton. Quantitative Western blot and immunofluorescence analyses confirmed downregulation of ezrin and NHERF2 in the glomeruli of streptozotocin-treated rats. Knockdown of ezrin by siRNA in cultured podocytes induced cortical actin remodeling and glucose uptake at basal conditions, but reduced insulin-stimulated actin remodeling and glucose uptake as compared to

control siRNA-transfected cells. The ezrin-dependent actin remodeling involved an actin depolymerizing protein cofilin that is essential for actin filament turnover. Furthermore, immunohistochemical analysis revealed reduced expression of ezrin in the podocytes of human patients with diabetes.

**Conclusions:** Our findings suggest that ezrin may play a role in the development of the renal complication in diabetes by regulating actin cytoskeleton organization and glucose transport in podocytes.

*Funding:* Private Foundation Support

## FR-PO873

**Nck1 but Not Nck2 Regulates Podocyte Actin Dynamics through Activation of RhoA** Lisa Maria Buvall,[1] Priyanka Rashmi,[1] Astrid Weins,[2] Anna Greka,[1] Peter H. Mundel.[1] *[1]Nephrology, Harvard Medical School and Massachusetts General Hospital, Boston, MA; [2]Pathology, Harvard Medical School and Brigham and Women's Hospital, Boston, MA.*

**Background:** Podocyte dysfunction, represented by foot process effacement, disruption of the slit diaphragm and proteinuria, is often the initial insult leading to progressive kidney disease. Nck1/2 are widely expressed adapter proteins that recruit proline rich proteins via their SH3 domains. Ncks are essential for the development and maintenance of the glomerular filtration barrier and stabilize the podocyte actin cytoskeleton by phosphorylating the slit diaphragm protein nephrin.

**Methods:** To study Nck actin-regulatory pathways in cultured podocytes we use cell biological and biochemistry assays in combination with lentiviral gene silencing and over expression approaches.

**Results:** Here we identify Nck1 but not Nck2 as regulators of RhoA signaling in podocytes. We show that Nck1, but not Nck2, is a substrate of the ubiquitin ligase c-Cbl, which mediates ubiquitination and proteasomal degradation of Nck1. We uncover lysine 178 in Nck1 as the ubiquitin acceptor site, which is conserved from Xenopus to human. We have previously reported that synaptopodin, a proline rich actin binding protein, promotes stress fiber formation by blocking the Smurf1 mediated ubiquitination of RhoA. Now we find that synaptopodin competes with c-Cbl for binding to Nck1, thereby blocking the ubiquitination of Nck1 by c-Cbl. Gene silencing of c-Cbl in synaptopodin depleted podocytes restores Nck1 abundance, confirming c-Cbl as the specific ubiquitin ligase responsible for ubiquitination of Nck1. Expression of c-Cbl resistant Nck1(K178R), but not Nck2, restores stress fibres in synaptopodin depleted podocytes through activation of RhoA signaling.

**Conclusions:** These findings highlight the functional difference between Nck1 and Nck2 and reveal proteasomal regulation as a key to distinct and non-redundant Nck effects on RhoA signaling.

*Funding:* NIDDK Support

## FR-PO874

**Synaptic Vesicle Protein 2B Is Essential for Maintaining the Integrity of the Podocyte Slit Diaphragm; SV2B KO Mice Are Vulnerable to the Podocyte Injury** Yoshiyasu Fukusumi, Asami Takasaki, Ayako Wakamatsu, Yuichi Takahashi, Naoko Miyauchi, Masayuki Tomita, Hiroshi Kawachi. *Dept of Cell Biology, Institute of Nephrology, Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan.*

**Background:** We have previously reported synaptic vesicle protein 2B (SV2B) was expressed in podocyte, and the cultured podocyte of which SV2B was knock-downed with siRNA failed to arrange the proper localization of CD2AP, which is one of the component of slit diaphragm (SD) (JASN, 2006). SV2B is expressed on vesicle surface and plays a role in vesicle trafficking. However, synaptic vesicle like trafficking system in podocyte and the role of SV2B are not well understood.

**Methods:** To elucidate the function of SV2B in vivo, we generated SV2B knockout (KO) mice. The expression of CD2AP and other critical molecules of the SD (nephrin, NEPH1, podocin and ZO-1) in SV2B KO mice, and their sensitivity to podocyte injury were analyzed.

**Results:** In SV2B KO mice without any treatment, a slight increase in the amount of proteinuria (KO 1.39 mg/24 h vs. wild type (WT) 0.64, $p<0.02$), and the alteration in the staining pattern of CD2AP, nephrin and NEPH1 were detected. The KO mice overloaded with BSA (15 mg/kg/day) showed higher amount of proteinuria than WT mice with same treatment (KO 19.88 mg/24h vs. WT 2.39). The staining of CD2AP, nephrin, NEPH1, podocin and ZO-1 was clearly decreased in KO mice overloaded with BSA (nephrin: score KO 2.64 vs. WT 3.26, $p<0.005$, podocin: 2.98 vs. 3.40, $p<0.01$). No WT mice died after uninephrectomy, whereas about 50% of SV2B KO mice died after the treatment. Next, to identify the molecules related with SV2B in podocyte, the expression of other synaptic vesicle associated proteins were analyzed in SV2B KO mice. mRNA expression of neurexin, a molecule expressed on the presynaptic membrane, was decreased, whereas the expression of vesicular glutamate transporter, a molecule expressed on synaptic vesicle membrane, was increased.

**Conclusions:** These results indicated that SV2B is involved in maintaining the integrity of the SD molecules, and that the mice with defect of SV2B were vulnerable to injury in podocyte.

*Funding:* Government Support - Non-U.S.

## FR-PO875

**CD2AP Phosphorylation on Tyrosines Y10 and Y273 Has a Physiological Relevance for Slit Diaphragm Stability** Irini Schaefer, Beina Teng, Kirstin Worthmann, Hermann G. Haller, Mario Schiffer. *Nephrology, Medical School Hannover, Hannover, Germany.*

**Background:** CD2AP is an adaptor protein that can transmit intracellular signals involved in survival and cytoskeletal regulation of the cell. We have shown that CD2AP tyrosine phosphorylation determines binding to nephrin. Until now it is unknown if phosphorylation of CD2AP has a physiological relevance for slit diaphraghm stability. The aim of these studies was to analyze the involvement of CD2AP phosphorylation for slit diaphragm stability in vivo.

**Methods:** Evolutionary highly conserved tyrosine residues were identified in every SH3 domain of CD2AP on positions Y4/8/10, Y119 and Y273/280. By site directed mutagenesis we established for every tyrosine a single mutant or for close-by tyrosines double or triple mutants. We furthermore generated mRNA from WT- and mutated CD2AP DNA. By cross-species rescue experiments in CD2AP-knockdown zebrafish we analyzed the physiological relevance of every single tyrosine for slit diaphragm stability. Analyzation was performed by morphology, mortality rate and filtration of GFP-labeled fatty acid-binding protein.

**Results:** Knockdown of CD2AP by morpholino injection in zebrafish leads to edema and foot process effacement. Most of the larval fishes died after 5 days. Cross-species rescue experiments by injection of CD2AP mRNA after morpholino injection showed a rescue of the CD2AP-knockdown phenotype. To analyze the importance of the tyrosines we injected mRNA of the single tyrosine mutants. The absence of tyrosines Y10 or Y273 showed no rescue of the CD2AP-knockdown phenotype. These zebrafish larvals developed big edema, showed a high mortality rate and GFP-labelled fatty acid-binding protein was filtrated through the damaged glomerular filtration barrier. Larvals with rescued phenotypes remain healthy with intact filtration barrier.

**Conclusions:** These results suggest that CD2AP phosphorylation on tyrosines Y10 and Y273 have a physiological importance for stability of the slit diaphragm in vivo.

*Funding:* Government Support - Non-U.S.

## FR-PO876

**Role of Podocytes in Endocytosis and Glomerular Filter Integrity** Madhusudan M. Venkatareddy, Rakesh Verma, Puneet Garg. *Div of Nephrology, Univ of Michigan, Ann Arbor, MI.*

**Background:** Vesicular trafficking and autophagy is a vital part of cell development and growth and to maintain a balance between synthesis, degradation and recycling of cellular components. We generated a Podocyte specific deletion of vps34, a phosphoinositol kinase that plays a critical role in both vesicular trafficking as well as autophagy. Careful characterization of the vesicles showed differences from what was reported in recently published podocyte specific deletion of vps34. Furthermore, using this model we examined the role of podocyte in preventing filter clogging as endocytosed protein processing is altered in the absence of vps34.

**Methods:** Kidneys for IHC and Immunogold EM (IEM) were harvested from wild type and podocyte specific vps34 deleted mice (*vps34KO*). Cell culture studies used stable podocyte cell line generated from the $vps34^{fl/fl}$ mouse infected with adenovirus carrying *cre* ($Pod^{vps34-}$).

**Results:** As previously reported, *vps34KO* mice are normal at birth, develop massive proteinuria and accumulation of vesicles by 3 weeks of age. IHC and IEM studies from kidney section and $Pod^{vps34-}$Cells reveal two distinct populations of vesicles, early and late endosomes that are Rab5 and Lamp1 positive and syntaxin 6 positive vesicles that are in flux with the trans-golgi network (TGN). Autophagic vesicles were not seen. Immunogold EM shows Nephrin in vesicles suggesting either endocytosis of Nephrin or aberrant trafficking of newly synthesized Nephrin from the TGN. To study the role of podocytes in enodcytosis of filtered proteins we perfused mice with FITC-human albumin (FITC-*h*alb) as wells as stained for endogenous IgG and albumin in wild type and *vps34KO* mice. There was no leak of mouse IgG, albumin or infused FITC-*h*alb in normal wild type mouse using IF and IEM studies. There was accumulation of mouse IgG and albumin in Podocyte vesicles of non-proteinuric 2 weeks old *vps34KO* mice.

**Conclusions:** Perturbing the endocytic machinery suggests the improper targeting of slit diaphragm proteins is in part due to failure of trafficking of vesicles to and from the golgi network. This model also provides evidence for the role of podocytes in maintaing a healthy filter and prevent clogging.

*Funding:* NIDDK Support

## FR-PO877

**AMPK Activation by AICAR Is Protective against Podocyte Damage and Glomerulosclerosis** Stephen O'Brien, John N. Vassiliadis, Mandy M. Smith, Hong Ling, Steven R. Ledbetter, Cynthia M. Arbeeny, Stefan Wawersik. *Tissue Protection and Repair Unit, Genzyme/Sanofi R&D Center, Framingham, MA.*

**Background:** Podocyte injury is a key step in the onset and progression of glomerulosclerosis. Cellular functions that counteract cell stress, such as autophagy and mitochondrial biogenesis, are critical for podocyte damage resistance. AMP-activated Protein Kinase (AMPK) regulates multiple stress-protective responses, and we therefore hypothesized that AMPK activation can protect podocytes from injury.

**Results:** To functionally test the role of AMPK in podocyte health, puromycin aminonucleoside (PAN) was used to cause oxidative stress in cultured immortalized podocytes.PAN treatment reduced $AMPK^{Thr172}$ phosphorylation in podocytes, indicating decreased AMPK activation. Furthermore, PAN induced apoptotic markers, mitochondrial

membrane depolarization, and expression of the ER stress marker CHOP. Co-treatment with 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an AMP analog capable of activating AMPK, increased phospho-AMPK$^{Thr172}$ and blocked mitochondrial damage, CHOP induction, and apoptosis. To test whether in vivo AMPK activation can reduce glomerular injury, we examined the effects of AICAR treatment in adriamycin-induced nephropathy (AN), a mouse model of glomerulosclerosis.Vehicle-treated AN mice were heavily albuminuric, and kidney histology revealed extensive glomerular and tubular injury. Immunostaining with the proliferation marker Ki67 revealed an increase in dividing cells in the glomerular, tubular, and interstitial compartments. In contrast, AN mice treated with 500/mg/kg/day AICAR showed a marked reduction in albuminuria ($p<0.05$ vs. AN + Veh.). Scoring of glomerular pathology ($p<0.001$ vs AN + Veh.) and of the number of Ki67+ cells/glomerulus ($p<0.001$ vs. AN + Veh.) also indicate reduced glomerular injury in AICAR-treated AN mice.

**Conclusions:** Taken together, these data suggest that AICAR treatment prevents glomerular damage by reducing podocyte injury, and that the mechanism of this protection is through AMPK activation.

*Funding:* Pharmaceutical Company Support - Genzyme/Sanofi

## FR-PO878

**Expression of Cleavage Resistant CD2AP in a Model of Inflammatory Glomerular Disease** Mehmet M. Altintas,[1] Changli Wei,[1] Jing Li,[1] Phillip Ruiz,[2] Jochen Reiser.[1] *[1]Medicine, Rush Univ, Chicago, IL; [2]Surgery, Univ of Miami, Miami, FL.*

**Background:** We recently showed that a cytosolic variant of the cysteine protease cathepsin L (CatL) was a common downstream effector in many glomerular diseases. The GTPase dynamin, synaptopodin and CD2AP were shown to be the key targets of this enzyme. These findings emphasized the need for the integrity of these target proteins and thus podocyte foot process (FP) structure and function.

**Methods:** We utilized the serum nephritis (SN) mouse model in which injection of an antibody that reacts with the glomerular basement membrane (GBM) causes glomerular disease. We deleted CatL cleavage site from CD2AP aminoacid sequence by site directed mutagenesis. We analyzed proteinuria, podocyte apoptosis, crescent formation, glomerular sclerosis and glomerular hypercellularity.

**Results:** SN is associated with cytosolic CatL induction in podocytes that leads to proteolysis of CD2AP N-terminus but a stable C-terminal fragment (p32) and the release of dendrin to podocyte nucleus, a response that was largely absent in CatL KO mice.

Both, wild type (WT) and CatL KO mice developed strong and comparable levels of proteinuria in response to anti-GBM antibody. Interestingly, the expression of the CatL cleavage targets synaptopodin and dynamin remained the same in WT mice suggesting that development of proteinuria is independent of CatL during SN. In contrast, kidney histology after SN revealed that only WT animals developed features of disease progression.

To further analyze the effects of stable CD2AP on progressive kidney disease, we expressed WT CD2AP and CD2AP that is cleavage resistant against CatL. Mice received WT CD2AP showed a significant reduction of N-terminal CD2AP but not the mice expressed cleavage resistant CD2AP after SN. The expression of cleavage resistant CD2AP directly impacted on the severity of disease progression.

**Conclusions:** The stabilization of CD2AP by removing CatL or protecting CD2AP alters the course of a glomerular disease shifting progression into a more benign phenotype. This finding will allow us to develop additional strategies for renal protection that are in addition to anti-proteinuric modalities focusing on podocyte survival.

*Funding:* NIDDK Support

## FR-PO879

**Akt Recruits Dab2 to Albumin Endocytosis** Elif Erkan, Hui Li, Kenneth R. Hallows. *Univ of Pittsburgh.*

**Background:** Proximal tubule epithelial cells encompass a highly sophisticated endocytic machinery to retrieve albumin in the glomerular filtrate. Megalin-cubilin complex and the endocytic adaptor disabled-2 (Dab2) play a pivotal role in albumin endocytosis. We demonstrated that protein kinase B (Akt) mediates albumin endocytosis in the proximal tubule through an interaction with Dab2. Our goal was to examine the nature of Akt-Dab2 interaction. We proposed that Akt phosphorylate Dab2.

**Methods:** We utilized GST-pulldown and yeast two hybrid experiments to examine the interaction sites between Akt and Dab2. Phosphorylation of Dab2 and the phosphorylation sites by Akt were examined by in-vitro phosphorylation experiments and site-directed mutagenesis. Physiological relevance of this phosphorylation event was investigated by albumin uptake studies. The effect of Akt on membrane expression of megalin-cubilin was validated in-vivo in Akt1 and Akt2 knock-out mice.

**Results:** Pleckstrin homology and catalytic domain of Akt had an interaction with Dab2-proline-rich domain (PRD) and the initial eleven amino acids of Dab2-PRD were sufficient to establish this interaction. Experiments to delineate the Akt isoform that was involved in Akt-Dab2 interaction revealed that Akt1 and Akt2 were both involved in albumin endocytosis. Furthermore Akt phosphorylated Dab2 at 448-449 serine residues. Mutation of Dab2- 448-9 serine to alanine inhibited albumin endocytosis revealing the physiological relevance of this novel phosphorylation event. To confirm the effect of Dab2 phosphorylation by Akt on expression of megalin and cubilin, we examined the Akt1 and Akt2 knock-out (KO) mice. Akt1 and Akt2 KO resulted in a significant decrease in expression of renal megalin and cubilin.

**Conclusions:** We concluded that Akt is an important mediator of albumin endocytosis in the proximal tubule and phosphorylation of Dab2 by Akt is essential in recruitment of Dab2 to the endocytic pathway and trafficking of megalin- cubilin. We propose that understanding the link between cell signaling events and albumin endocytosis may decipher the mechanism of tubulointerstitial injury that is inflicted by proteinuria. Further research is warranted to investigate other potential Akt targets in the endocytic machinery.

*Funding:* NIDDK Support

## FR-PO880

**Cyclic AMP Signaling Prevented against Podocyte Apoptosis via Activation of Protein Kinase A and Mitochondrial Fusion** Zhaohui Ni,[1] Xiaoying Li,[1] Hua Tao,[1] Yucheng Yan,[1] Peter Y. Chuang,[2] John C. He,[2] Leyi Gu.[1] *[1]Renal Div, Renji Hospital, Shanghai Jiaotong Univ School of Medicine, Shanghai, China; [2]Div of Nephrology, Mount Sinai School of Medicine, New York, NY.*

**Background:** Our previous in vitro studies suggest that cyclic AMP (cAMP) signaling protects against doxorubicin (ADR) and puromycin aminonucleoside (PAN) induced apoptosis in podocytes. Since cAMP is an important second messenger and plays a key role in cell survival and differentiation via protein kinase A (PKA) or exchange protein directly activated by cAMP (Epac) pathways, we sought to determine the mechanism in cAMP-mediated protection of podocytes.

**Methods:** Doxorubicin was used to induce nephrosis in mice. Conditional immortalized mouse podocytes were used in vitro studies.

**Results:** In the doxorubicin nephrosis model, we found that forskolin—a selective activator of adenylate cyclase—attenuated albuminuria and improved the expression of WT-1. We failed to find 8-pCPT-2-O-Me-cAMP, a selective cAMP/Epac activator, improved PAN-induced apoptosis of cultured podocytes. When podocytes were treated with pCPT-cAMP (a selective cAMP/PKA activator), PKA activation were increased in a time-dependent manner and prevented against PAN-induced podocytes loss and caspase 3 activation, as well as mitochondrial membrane potential reduction.We found that PAN and ADR resulted in a decrease in Mfn1 expression and mitochondrial fission in podocytes. Both PAN and ADR had no effect on Drp1 phosphorylation and Fis and Opa expression. pCPT-cAMP restored the Mfn1 expression in puromycin or ADR-treated podocytes and induced Drp1 phosphorylation, as well as mitochondrial fusion. Treatment podocytes with arachidonic acid resulted in mitochondrial fission, podocytes loss and cleaved caspase 3 expression. Arachidonic acid abolished pCPT-cAMP protective effects on PAN-treated podocytes. Mdivi, a mitochondrial division inhibitor, prevented PAN-induced cleaved caspase 3 expression in podocytes.

**Conclusions:** We concluded that activation of cAMP alleviated doxorubicin mice podocyte injury. PKA signaling resulted in mitochondrial fusion in podocytes, at least partially mediated the effects of cAMP.

*Funding:* Government Support - Non-U.S.

## FR-PO881

**Twisted Gastrulation, a BMP Antagonist, Exacerbates Podocyte Injury** Motoko Yanagita,[1] Sachiko Yamada,[1] Jin Nakamura,[1] Misako Asada,[1] Masayuki Takase,[1] Taiji Matsusaka,[2] Taku Iguchi,[1] Ryo Yamada,[1] Hiroshi Kawachi,[3] Eri Muso,[4] Aris N. Economides.[5] *[1]Nephrology, Graduate School of Medicine, Kyoto Univ, Kyoto, Japan; [2]Internal Medicine, Tokai Univ School of Medicine, Kanagawa, Japan; [3]Cell Biology, Nephrology, Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan; [4]Nephrology and Dialysis, Kitano Hospital, Osaka, Japan; [5]Regeneron Pharmaceuticals, Inc., NY.*

**Background:** Podocyte injury is the first step in the progression of glomerulosclerosis. Previous studies have demonstrated the beneficial effect of bone morphogenetic protein 7 (Bmp7) in podocyte injury and the existence of native Bmp signaling in podocytes. Because the local activity of Bmp7 is controlled by cell-type specific Bmp antagonists that inhibit the binding of Bmp7 to its receptors, we screened for Bmp antagonist expressed by podocytes, and analyzed the function.

**Methods:** We utilized conditionally immortalized murine podocytes, and *Twsg1-lacZ* reporter mice.

**Results:** The product of Twisted gastrulation (Twsg1), a Bmp antagonist, was the most abundant Bmp antagonist in murine cultured podocytes. The administration of Bmp induced podocyte differentiation through Smad signaling, whereas the simultaneous administration of Twsg1 antagonized the effect. Twsg1 was expressed in the glomerular parietal cells (PECs) and distal nephron of the healthy kidney, and additionally in damaged glomerular cells in a murine model of podocyte injury. Twsg1 null mice exhibited milder hypoalbuminemia and hyperlipidemia, and milder histological changes while maintaining the expression of podocyte markers during podocyte injury model.

**Conclusions:** Twsg1 plays a critical role in the modulation of protective action of Bmp7 on podocytes, and inhibition of Twsg1 is a promising means of development of novel treatment for podocyte injury.

*Funding:* Government Support - Non-U.S.

## FR-PO882

**Nephrin Activation Induces Endosomal Signaling Necessary for Coordinated Focal Adhesion and Lamellipodial Dynamics** Qingfeng Fan, Lawrence B. Holzman. *Renal Div, Univ of Pennsylvania, Phila., PA.*

**Background:** Nephrin ligation induces Nephrin tyrosine phosphorylation-dependent endocytosis. Nephrin endocytosis might be a mechanism by which podocyte junctions are disassembled and by which Nephrin protein and signaling is degraded in disease

states. Alternatively, receptor-mediated endocytic trafficking can have signal-propagating functions, wherein "signaling endosome" complexes signal to distinct subcellular compartments. We hypothesized that Nephrin signals from a signaling endosome.

**Methods:** We evaluated the dynamics of Nephrin endosomal trafficking, focal adhesion (FA) turnover, and lamellipodial activity in cultured podocytes following chimeric CD16-nephrin cytoplasmic domain (CD16NCD) ligation focusing on the necessity of Nephrin-induced endosomal signaling in these integrated processes.

**Results:** Ligation of CD16NCD results in synchronized cellular events that culminate in lamellipodial induction peaking at 20 min. By 1 min, CD16NCD forms fine clusters on the cell surface, becomes tyrosine phosphorylated and is associated with Fyn, FAK, Cas, and caveolin. Soon thereafter, CD16NCD is dephosphorylated and undergoes endocytosis, disappearing from the cell surface and co-localizing with EEA1. By 5 min, FA proteins (e.g., FAK, Cas, paxillin, vinculin) disassemble and are transiently found associated with CD16NCD positive early endosomes between 5-10 min. By 20 min, FA proteins reassemble into linear FA when lamellipodial activity is first observed. At 40 min, CD16NCD endocytosis results in only limited CD16NCD degradation by endosomal trafficking via the late endosomal pathway. All aspects of this process require initiation by nephrin tyr phosphorylation, since Y-F mutation of all 10 CD16NCD tyr residues blocks endocytosis, FA turnover, and lamellipodial activity. Results using a mouse protamine sulfate model suggest a similar process in vivo.

**Conclusions:** Mechanisms governing lamellipodial activity in culture may be similar to those employed in vivo during foot process effacement. We suggest that podocyte injury-induced Nephrin tyr phosphorylation initiates Nephrin endosomal signaling that in turn is necessary for FA turnover, actin remodeling and foot process spreading.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO883

### GSK3β Controls Podocyte Process Elongation via Tau and CRMP2 Mediated Regulation of Microtubule Dynamics: Role in Compensatory Adaptation to Podocyte Loss

Weiwei Xu,[1] Yan Ge,[1] Zhi-hong Liu,[2] Rujun Gong.[1] *[1]Nephrology, Brown Univ, Providence, RI; [2]Research Institute of Nephrology, Nanjing, China.*

**Background:** Upon podocyte loss, remnant intact podocytes exhibit a limited adaptive capacity to extend podocyte processes and cover the denuded glomerular base membrane. Microtubules, a major cytoskeletal component of podocyte primary processes, are necessary for process outgrowth, branching and elongation. This study explored the role of glycogen synthase kinase (GSK)3β regulated microtubule dynamics in podocyte injury.

**Methods:** Adriamycin induced podocytopathy was employed *in vivo* and *in vitro* in conditionally immortalized mouse podocytes and microtubule dynamics examined.

**Results:** GSK3β physically interacted with microtubule associated proteins tau and CRMP2 in podocytes *in vivo* and *in vitro*. Of note, both tau and CRMP2 possess multiple GSK3β phosphorylation consensus motifs, denoting a GSK3β directed phosphorylation and activity control. Ectopic expression of a constitutively active mutant of GSK3β induced hyperphosphorylation of tau and CRMP2, concomitant with microtubule depolymerization, cell body shrinkage and shortening of podocyte projections, reminiscent of the effect of adriamycin injury, which amplified GSK3β activity. Conversely, overexpression of a kinase dead mutant of GSK3β suppressed phosphorylation of tau and CRMP2, accompanied with enhanced microtubule polymerization, cell body extension and thickening and lengthening of podocyte processes. Lithium, a selective inhibitor of GSK3β, abrogated adrimycin-induced hyperphosphorylation of tau and CRMP2, improved microtubule stability and normalized podocyte shape. In adriamycin injured mice, delayed lithium therapy prominently attenuated proteinuria and glomerulosclerosis, associated with thickening and elongation of podocyte primary processes but no podocyte replenishment. Mechanistically, lithium inhibited GSK3β activity in injured glomeruli and abrogated adriamycin induced hyperphosphorylation of tau and CRMP2 in podocytes.

**Conclusions:** Therapeutic targeting of GSK3β improves podocyte microtubule integrity, reinforces the compensatory adaptation to podocyte loss and attenuates proteinuria and glomerulosclerosis.

*Funding:* NIDDK Support

## FR-PO884

### Podocytes Exhibit a Specialized Protein Quality Control Employing Derlin-2

Guohui Ren, Mehmet M. Altintas, Jochen Reiser. *Dept of Medicine, Rush Univ Medical Center, Chicago, IL.*

**Background:** Protein quality control in endoplasmic reticulum (ER) may be particularly important for podocytes, highly differentiated cells, upon ER stress induction caused by substances passing the filtration barrier. Derlin-2, a target of the inositol-requiring enzyme 1 (IRE1) branch of unfolded protein response (UPR), is a key player in protein quality control.

**Methods:** Derlin-2 floxed mice were crossed with Podocin-Cre mice to generate podocyte-specific Derlin-2 knockout mice; Podocin-Cre$^-$ littermates were served as controls. Adriamycin (ADR, 20 mg/kg body wt) was injected via tail vein. Lentivirus containing a shRNA targeting Derlin-2 gene was transduced to cultured conditionally immortalized mouse podocytes. A virus containing a scrambled shRNA construct served as a control. Lentiviral transduced, differentiated podocytes were treated with ADR (0.1 μg/ml).

**Results:** Derlin-2 is expressed in podocytes in vivo and in vitro. Podocyte-specific Derlin-2 knockout mice developed significant albuminuria, peak at Day 21 (4836.9±2055.0 vs. 49.3±28.5 mg/g creatinine in control mice) after ADR administration, and foot process effacement.

Cultured podocytes with lentiviral knock down of Derlin-2 were indistinguishable from control cells in cell morphology and survival, however, were highly sensitized to ADR. Derlin-2 knockdown podocytes were increasingly retracted and detached after ADR treatment for 24 hours, and at day 5 only 61.4±6.4% cells were alive compared with control cells ($P<0.001$). After 24-72 hours of exposure to ADR, the expression of ER stress sensors (IRE1, GRP 94) was upregulated by 28~201%, while podocyte markers including synaptopodin, nephrin and CD2AP were downregulated by 21~72% in Derlin-2 knockdown podocytes compared to control cells.

**Conclusions:** Derlin-2 is expressed in podocytes. Derlin-2 is required in maintaining resistance of mouse podocytes to ER stress inducers such as ADR, and is hypothesized to provide essential protection to podocytes under stress by regulating UPR.

*Funding:* NIDDK Support

## FR-PO885

### SMPDL3b Expression Determines Differential suPAR-Mediated Podocyte Injury in FSGS and DKD

Tae-Hyun Yoo,[1,2] Johanna Guzman,[1] Alla Mitrofanova,[1] Changli Wei,[3] Rodrigo Villarreal,[1] Christopher E. Pedigo,[1] Mayrin Correa-medina,[1] Christian Faul,[1] Jochen Reiser,[3] George William Burke,[1] Matthias Kretzler,[4] Markku Lehto,[5] Per-Henrik Groop,[6] Sandra M. Merscher-Gomez,[1] Alessia Fornoni.[1] *[1]Univ of Miami; [2]Yonsei Univ College of Medicine; [3]Rush Univ; [4]Univ of Michigan; [5]Helsinki Univ Central Hospital.*

**Background:** Podocyte injury is an important feature of diabetic kidney disease(DKD) and focal segmental glomerulosclerosis(FSGS). Increased soluble urokinase receptor(suPAR), lipid-dependent podocyte αvβ3 integrin activation, and decreased glomerular sphingomyelinphosphodiesterase like 3b(SMPDL3b)/acid sphingomyelinase(ASMase) occur in patients with recurrent FSGS.

**Methods:** We hypothesized that both increased suPAR and decreased SMPDL3b are necessary to cause podocyte injury in FSGS, and we aimed to determine if suPAR and SMPDL3b are similarly modulated in DKD.

**Results:** Our data indicate that serum suPAR levels are equally elevated in two cohorts of primary FSGS and DKD. However, while SMPDL3b expression was down-regulated in glomeruli from patients with FSGS and in FSGS sera treated human podocytes, the opposite was found for DKD. Treatment of human podocytes with FSGS sera resulted in suPAR-mediated β3 integrin activation,which was prevented by overexpression of SMPDL3b. On the contrary, treatment of human podocytes with DKD sera did not result in suPAR-mediated β3 integrin activation but caused podocyte apoptosis, which was prevented by SMPDL3b knockdown. To elucidate the role of SMPDL3b expression in β3 integrin activation, we performed competitive co-immunoprecipitation experiments. We were able to show that SMPDL3b interacts with suPAR/UPAR and prevents suPAR/β3 integrin interaction and activation. Furthermore, suPAR treatment increased cell migration and Rac 1 activity in SMPDL3b knockdown cells, but it increased apoptosis and RhoA activity in SMPDL3b overexpressing podocytes. Consistent with these in vitro data, the use of an ASMase inhibitor reduced proteinuria in diabetic animals but worsened proteinuria in animal models of FSGS.

**Conclusions:** In conclusion, the degree of SMPDL3b expression determines differential suPAR-mediated podocyte injury in FSGS and DKD.

*Funding:* NIDDK Support

## FR-PO886

### Serum Albumin and Associated Factors Induce Inflammatory and Stress Responses in Podocytes and Glomeruli

Shipra Agrawal,[1] Adam J. Guess,[1] Melinda A. Chanley,[1] Rainer Benndorf,[1,2] William E. Smoyer.[1,2] *[1]Center for Clinical & Translational Research, The Research Institute at Nationwide Childrens Hospital; [2]Dept of Pediatrics College of Medicine, The Ohio State Univ.*

**Background:** Albuminuria is both a hallmark and a risk factor for progressive glomerular disease and leads to increased podocyte exposure to serum albumin (SA). We hypothesized that increased SA exposure induces podocyte pro-inflammatory and stress responses with potential pathogenic roles in progressive glomerular injury.

**Methods:** Male Wistar rats received low endotoxin BSA (5 mg/g IP) daily for 5 days and proteinuria and glomerular mRNA analyzed. Also, mRNA and protein were analyzed from mouse podocytes exposed to 40 mg/ml BSA, human SA (HSA), recombinant HSA (rHSA), charcoal-treated SA and SA preparations specifically devoid of globulins, endotoxins, glycoproteins or fatty acids. Podocytes and alveolar macrophages were also treated with 1 μg/ml LPS.

**Results:** BSA-injected rats developed marked albuminuria and enhanced glomerular gene expression of COX-2, MCP-1, CXCL1 and HSP25. In podocytes, physiological BSA levels induced B7-1 and HSP70i, as well as COX-2, MCP-1, CXCL1 and HSP25. No induction of ICAM-1, MIP-2, TNFα or CXCL5 was seen in podocytes or glomeruli upon SA-overload. LPS treatment of podocytes elicited a broad pro-inflammatory response, but did not induce COX-2, MIP-2 or TNF-a, despite strongly inducing COX-2, MCP-1 and TNF-a in macrophages. COX-2, which renders podocytes susceptible to further injury, was markedly induced (~30 fold) by SA in podocytes. While BSA, HSA and globulin-, endotoxin- and glycoprotein-free SA preparations all strongly induced COX-2, rHSA, charcoal-treated SA and fatty acid-free SA only moderately induced COX-2, indicating that SA-associated factors contributed partially to COX-2 induction.

**Conclusions:** Induction of proteinuria in SA-overload rats and SA exposure of podocytes are both associated with induction of COX-2 and pro-inflammatory and stress response genes. This response appears due in part to SA-associated factors such as fatty acids that may be removable by charcoal treatment, suggesting this as a novel potential treatment to reduce progressive glomerular injury.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO887

**Exploring the Role of Annexin A2 in the Glomerulus** Biao Li,[1] Tuncer Onay,[2] Chengjin Li,[3] Susan E. Quaggin.[4] *[1]Dept of Nephrology, Feinberg Cardiovascular Research Institute, Chicago, IL; [2]Dept of Nephrology, Feinberg Cardiovascular Research Institute, Chicago, IL; [3]Dept of Nephrology, Feinberg Cardiovascular Research Institute, Chicago, IL; [4]Dept of Nephrology, Feinberg Cardiovascular Research Institute, Chicago, IL.*

**Background:** Anxa2 (Annexin A2) is a phospholipid binding protein, which participates in a number of critical cellular processes that include specific binding to plasminogen, formation of filopodia and lamellipodia through interaction with F-actin, and acting as a capping protein for actin. Our previous studies identified Anxa2 to be involved in podocyte attachment and foot process (FP) formation triggered by soluble Flt1 (sFlt1) binding to the podocyte surface. We hypothesize that Anxa2 cooperates with sFlt1 to guide cytoskeletal changes in podocyte FP formation and maintenance.

**Methods:** We generated an Anxa2 floxed mouse line and used it to develop whole body and podocyte specific knockout (KO) mice. The stability of the FP cytoskeleton was tested by protamine sulphate perfusion of KO and WT mice followed by heparin recovery. In vitro, cell cycle and motility of human podocytes was examined, following knockdown (KD) of Anxa2 by siRNA.

**Results:** Histologic analysis did not demonstrate any major differences between Anxa2 global or podocyte -KO and control kidneys at 4 weeks of age; however, diffuse fibrin deposition was observed in glomerular capillaries of KO kidneys. Following perfusion with protamine sulphate, KO mice showed a trend towards protection. In vitro, KD of Anxa2 in human podocytes resulted in a dramatic increase in podocyte cell size, development of cytosolic stress fibers, and formation of focal adhesions. Furthermore, Anxa2 KD podocytes demonstrated a marked decrease in proliferation (Day 5, 1.33±0.12 X $10^5$ vs 2.53±0.29 X$10^5$, p=0.029). Cell cycle was arrested at G1 determined by FACS (0.7921±0.047 vs 0.7097±0.048, p=0.001). Cell motility was also decreased.

**Conclusions:** Loss of Anxa2 appears to confer stability to podocyte FPs when acutely challenged. Further studies are ongoing to determine how Anxa2 regulates podocyte adhesion, FP formation and function.

*Funding:* Government Support - Non-U.S.

## FR-PO888

**Mutual Antagonism of WT1 and β-Catenin Dictates Podocyte Health and Disease** Li Li Zhou,[1] Roderick J. Tan,[2] Dong Zhou,[1] Youhua Liu.[1] *[1]Dept of Pathology, Univ of PIttsburgh, Pittsburgh, PA; [2]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** Activation of β-catenin, the principal mediator of canonical Wnt signaling, plays a critical role in mediating podocyte dedifferentiation and dysfunction in the evolution of proteinuric kidney diseases. However, the mechanism by which β-catenin induces podocyte injury remains poorly understood. Here, we demonstrate that β-catenin directly antagonizes Wilms tumor 1 (WT1), a key transcription factor exclusively expressed in podocytes and required for the maintenance of podocyte differentiation and glomerular filtration barrier function.

**Methods:** BALB/c mice and cultured podocytes were used. Expression of various genes was analyzed by qRT-PCR, Western blot and immunostaining, respectively.

**Results:** In BALB/c mice injected with adriamycin, WT1 protein was gradually lost in glomerular podocytes at 1, 3 and 5 weeks. Interestingly, loss of WT1 was apparently not due to podocyte depletion in this model, as other podocyte-specific markers including synaptopodin and α-actinin-4 were preserved. Loss of WT1 was closely associated with up-regulation of β-cateninn in podocytes, which was accompanied by loss of nephrin and acquisition of Snail1, PAI-1, Fsp1 and MMP-7. In vitro, over-expression of β-catenin suppressed WT1-mediated podocalyxin expression. Conversely, over-expression of WT1 in podocytes inhibited β-catenin-mediated Snail1 and PAI-1 expression, suggesting that WT1 and β-catenin mutually antagonize each other. We found that β-catenin induced WT1 protein degradation through the ubiquitin-proteasomal pathway, and incubation with MG-132 preserved WT1. In vivo, either inhibition of β-catenin signaling by small molecule inhibitor ICG-001 or administration of Wnt antagonist Klotho restored WT1 protein and attenuated podocyte injury and proteinuria in adriamycin nephropathy.

**Conclusions:** These findings suggest that β-catenin signaling targets WT1 degradation, leading to podocyte dedifferentiation and dysfunction. Our results also indicate that WT1 and β-catenin play an opposing role in podocyte biology and their ratio dictates podocyte health and disease in vivo.

*Funding:* NIDDK Support

## FR-PO889

**CLIC5A Deficiency Accentuates Glomerular Damage in DOCA/Salt Hypertensive Mice** Mahtab Tavasoli, Laiji Li, Lin-fu Zhu, Benjamin Alexander Adam, Barbara J. Ballermann. *Medicine, Univ of Alberta, Edmonton, Canada.*

**Background:** Glomerular capillary (GC) hypertension is a risk factor for glomerular injury, including diabetic nephropathy. Podocytes extend inter-digitating actin-based foot processes around the exterior of glomerular capillaries, buttressing the vessel wall against the prevailing high intra-capillary pressure. CLIC5A is highly and uniquely expressed at the apical plasma membrane (PM) of podocyte foot processes, where it is part of the Ezrin-NHERF2 complex that links podocalyxin to cortical actin, and deletion of CLIC5A in mice leads to ezrin dephosphorylation and disorganization of podocyte foot processes (*Am.J.Physiol 298:F1492, 2010*). This study explored the hypothesis that lack of CLIC5A is detrimental to glomerular integrity under conditions of increased mechanical strain.

**Methods:** Hypertension was induced in wild-type (WT) and CLIC5A deficient ($CLIC5A^{-/-}$) mice using subcutaneous slow-release deoxycorticosterone (DOCA) pellets and 1% saline drinking water (DOCA/Salt), starting 14 days after uninephrectomy (UNX). Control mice were subjected to UNX only. 20 days after initiation of DOCA/Salt treatment, blood pressure, urinary albumin (Albumin:Creatinine ratio; ACR) were determined, and kidney histology was evaluated in a blinded fashion. Data are presented as the mean ± SD.

**Results:** Compared to UNX alone, systolic blood pressure increased to a similar degree in DOCA/Salt treated WT (119±6 vs. 98.9±8 mmHg; p<0.001) and $CLIC5A^{-/-}$ (121±11.5 vs. 97±8 mmHg; p<0.001) mice. All DOCA/Salt treated mice developed a hypokalemic metabolic alkalosis. In WT and $CLIC5A^{-/-}$ mice urine albumin excretion was unaffected by UNX alone. DOCA/Salt induced albuminuria in WT and $CLICA^{-/-}$ mice. The urine albumin excretion rate was significantly higher in $CLIC5A^{-/-}$ compared to WT mice (ACR mg/g: 1,720±960 vs. 780±235; p < 0.05). Histological analysis revealed microaneurysms in a much larger proportion of glomeruli from $CLIC5^{-/-}$ mice than in WT mice (57±17.9% vs. 31±17.1%; p<0.05).

**Conclusions:** These findings suggest that CLIC5A serves to stabilize the extracapillary podocyte buttress that protects against the deleterious effects of glomerular capillary hypertension in mice.

*Funding:* Government Support - Non-U.S.

## FR-PO890

**Lysosomal Processing of Albumin in Podocytes** John M. Carson,[1] Kayo Okamura,[1] Hidefumi Wakashiu,[2] Evgenia Dobrinskikh,[1] Jeffrey B. Kopp,[2] Judith Blaine.[1] *[1]Univ of Colorado Denver, Aurora, CO; [2]NIDDK, NIH, Bethesda, MD.*

**Background:** Albuminuria is a strong, independent predictor of CKD progression. The effect of albuminuria on podocytes is unknown. In collapsing FSGS, where albumin vacuoles in podocytes are a prominent feature, podocyte loss and glomerulosclerosis occur rapidly. In minimal change disease, albumin accumulation in podocytes is nominal and decline in renal function is gradual. We hypothesize that podocyte processing of albumin, via the lysosome, may be an important determinant of podocyte injury and loss.

**Methods:** Human urine derived podocyte-like epithelial cells (HUPEC) were used for all *in vitro* experiments. Albumin uptake was quantified by Western blot after loading HUPECs with FITC-albumin. Co-localization of albumin with lysosomes was determined by confocal microscopy. Albumin degradation was measured by quantifying FITC-albumin abundance in HUPEC lysates by Western blot. Degradation experiments were repeated in HUPECs treated with chloroquine, a lysosome inhibitor, or MG-132, a proteasome inhibitor. Lysosome activity was measured by fluorescence recovery after photobleaching. Cytokine production was measured by ELISA. Cell death was determined by Trypan blue staining. *In vivo*, staining with albumin and LAMP-1, a lysosome marker, was performed on tissue from a Denys-Drash mouse model of albuminuria.

**Results:** HUPECs endocytosed albumin which co-localized with LAMP-1. Chloroquine, but not MG-132, inhibited albumin degradation in HUPECs, indicating degradation occurs in lysosomes. Cathepsin B activity significantly decreased in HUPECs treated with albumin (12.5% of activity in controls) and chloroquine (12.8%) alone, and declined further when treated with albumin plus chloroquine combined (8.2%). Cytokine production and cell death were significantly increased in HUPECs treated with albumin and chloroquine alone, and these effects were potentiated by treatment with albumin plus chloroquine. Compared to wild-type mice, glomerular staining of albumin and LAMP-1 was increased in Denys-Drash mice and was most prominent in podocytes.

**Conclusions:** These data suggest lysosome dysfunction may contribute to podocyte injury and glomerulosclerosis in albuminuric diseases.

*Funding:* NIDDK Support

## FR-PO891

**Molecular Characterization of Podocyte-Endothelial Cell Signaling Crosstalk Identifies New Endothelial Cell Targets for Prevention of Glomerular Diseases** Ilse S. Daehn,[1] Gabriella Casalena,[1] Liping Yu,[1] Kerstin Ebefors,[2] Detlef O. Schlondorff,[1] Borje Haraldsson,[2] Erwin P. Bottinger.[1] *[1]Dept of Medicine - Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Molecular and Clinical Medicine - Nephrology, Institute of Medicine, Univ of Gothenburg, Gothenburg, Sweden.*

**Background:** Focal segmental glomerular sclerosis (FSGS) is characterized by progressive loss of glomerular function with proteinuria due to podocyte injury and depletion, collapse and sclerosis of glomerular capillary segments. We have previously shown that podocyte-specific TGFβ signaling in mice (PodTbrI mice) induces endothelin-1(Edn1)-mediated mitochondrial oxidative stress and dysfunction selectively in adjacent endothelial cells via Ednar activation, which precedes subsequent podocyte loss and proteinuria [Daehn et al., ASN 2011]. We have further characterized endothelial-podocyte crosstalk and confirmed the involvement of Edn1-Ednra in other models of podocyte injury.

**Results:** In the podocyte injury models: podocyte-specific dicerKO and Adriamycin-induced glomerulosclerosis we confirmed activation of TGFβ signaling in podocytes associated with mitochondrial oxidative stress and increased Ednra specifically in glomerular endothelial cells. Adriamycin treatment rapidly induced Edn1 release by podocytes, there was loss of Isolectin B4 staining associated with apoptosis and loss of fenestrae by glomerular endothelial cells. Endothelial injury provoked podocyte apoptosis and proteinuria. Selective Ednra blockade or mitochondrial-targeted ROS scavenging prevented endothelial damage and subsequent podocyte loss, albuminuria and glomerulosclerosis. Ednra knockdown in mGEC prevented mtDNA damage and mitochondrial dysfunction mediated by conditioned media with increased Edn1 from PodTbr1 podocytes. Edn1 knockdown in PodTbr1 podocytes prevented mGEC dysfunction and subsequent podocyte apoptosis in co-cultures.

**Conclusions:** Podocyte injury induces Edn1 as an essential mediator of endothelial mitochondrial stress and dysfunction that underlies proteinuric podocyte-initiated segmental glomerulosclerosis. Prevention of endothelial damage may be considered a new therapeutic approach for FSGS.

*Funding:* NIDDK Support

## FR-PO892

**$PLA_2R$ Tightly Associates with Integrin $\alpha_3\beta_1$ in the Podocyte Membrane: A New Insight into IMN Pathogenesis** Quansheng Zhu, Liyo Kao. *Medicine, Univ of California, Los Angeles, Los Angeles, CA.*

**Background:** $PLA_2R$ has been firmly established to serve as the major antigen on the podocyte surface targeted by circulating autoimmune antibodies in patients with idiopathic membranous nephropathy (IMN), however, the pathobiology of how the antibodies binding to the extracellular domain of $PLA_2R$ then cause IMN remains largely unclear. Other than serving as a scavenger receptor for inactivation and removal of the secreted phospholipase $A_2$, $PLA_2R$ was found to carry signaling functions that triggers cellular responses with an undefined mechanism. Integrin $\alpha_3\beta_1$ is a membrane adhesion receptor that is highly expressed on the basal surface of podocytes along the glomerular basement membrane (GBM), where it is critically involved in GBM matrix arrangement and podocyte intracellular cytoskeleton organization. We hypothesize that $PLA_2R$ may interact with integrin $\alpha_3\beta_1$ in the human podocytes and that autoimmune antibodies binding to $PLA_2R$ perturbs the downstream signaling pathways of both $PLA_2R$ and integrin $\alpha_3\beta_1$ resulting in thickened GBM and altered podocytes cytoskeleton organization in IMN.

**Methods:** In the present study, we tested $PLA_2R$ and integrin $\alpha_3\beta_1$ interaction in the differentiated human podocytes, the human kidney cortex and HEK 293 cells co-expressing $PLA_2R$ and integrin $\alpha_3\beta_1$ by using immunocytochemistry, co-immunoprecipitation and biochemical analysis.

**Results:** We discovered that two populations of $PLA_2R$ exist in the human podocytes: one co-localizes with integrin $\alpha_3\beta_1$ in both of the endoplasmic reticulum and the plasma membranes, and the other is free. Immunoprecipitation study using an anti-integrin $\alpha_3\beta_1$ antibody revealed that $PLA_2R$ associates with integrin $\alpha_3\beta_1$ not only in the differentiated human podocytes, but also in the human kidney cortex and the HEK 293 cells co-expressing $PLA_2R$ and integrin $\alpha_3\beta_1$ heterologously. Further analysis showed this interaction occurs in the extracellular domains between the two receptors.

**Conclusions:** Our findings demonstrated for the first time that $PLA_2R$ associates tightly with integrin $\alpha_3\beta_1$ on the podocyte surface, which may provide new insights into the pathogenic mechanism of IMN mediated by auto-immune antibodies binding to $PLA_2R$.

*Funding:* Private Foundation Support

## FR-PO893

**Glepp1 Deficency Promotes GBM Humps** Eva Koenigshausen,[1] Laura Lennartz,[1] Ivo Quack,[1] Thorsten Wiech,[2] Lars C. Rump,[1] Lorenz Sellin.[1] *[1]Nephrology, Heinrich Heine Univ, Duesseldorf, Germany; [2]Pathology, Univ Hospital Eppendorf, Hamburg, Germany.*

**Background:** Proteinuria is one of the symptoms of inherited and acquired glomerular kidney diseases. Proteinuria evolves out of the damage of the glomerular filter. One major components of the glomerular slit diaphram is nephrin, which is phosphorylated by src kinases. A loss of nephrin out of the glomerular silt, for example by endocytosis, results in proteinuria. GLEPP1 is a receptor tyrosine phosphatase in the podocyte foot process. Mutations in the GLEPP1 gene were shown to be associated with FSGS in childhhood. The precise function of GLEPP1 is not fully understood.

**Methods:** Perfused kidneys from mice were fixed in 4% paraformaldehyde or in RNA stabilization reagent. For light microscopy kidneys were embedded in paraffin for further PAS staining, or were embedded in Epon and processed for electron microscopy. RNA was isolated and qPCR for Col4 alpha1 and alpha2 was performed. Mouse urine was analyzed for albuminuria by Coomassie stain and by ELISA. Urine creatinine was measured via an ELISA kit.

**Results:** In PAS stained sections of Glepp1 deficient glomeruli in aged mice an elevated count for PAS positive thickening of the GBM is seen. In TEM sections of glomeruli the GBM of Glepp1 deficient mice presents significantly more GBM humps than the age machted wilde type control. These GBM changes in the absence of Glepp1 is observed in two Independent genetic backgrounds (129P3, Balb/c). qPCR of renal RNA shows an increased signal for type IV collagen alpha1 and alpha2 in Glepp1 deficient mice. Interestingly, the aged Glepp1 deficient mice of both strains present proteinuria.

**Conclusions:** Glepp1 deficiency mediates GBM humps. These GBM humps seem to be formed by type IV collagen alpha1 and alpha2. It can be speculated that Glepp1 deficiency switches the type IV collagen expression von alpha 3,4,5 to alpha 1 and 2. Functionally, the Glepp1 deficient aged mice with increased numbers of GBM humps present proteinuria. These findings stress the protective and beneficial role of Glepp1 in podocytes.

## FR-PO894

**Patterns of Circulating Autoantibodies in Patients with Lupus Nephritis** Dawn J. Caster,[1] Daniel J. Birmingham,[2] Ami S. Joglekar,[1] Jon B. Klein,[1,3] John Barker Harley,[4] Erik Korte,[1] Brad H. Rovin,[2] Kenneth R. McLeish,[1,3] David W. Powell.[1] *[1]Medicine, Univ of Louisville, Louisville, KY; [2]Medicine, The Ohio State Univ, Columbus, OH; [3]VAMC, Louisville, KY; [4]Pediatrics, Cincinnati Children's Hospital, Cincinnati, OH.*

**Background:** Lupus nephritis (LN) occurs in at least 50% of SLE patients, but the nephritigenic autoantibodies responsible have yet to be identified. Recently, an autoantibody to the M-type phospholipase $A_2$ receptor on podocytes was identified as causing primary membranous nephropathy using immunoblot analysis of patient sera. This study used sera from SLE patients with and without LN to test the hypothesis that patients with different classes of LN express unique patterns of autoantibodies against native glomerular proteins.

**Methods:** Human glomerular extracts were IgG depleted and separated by SDS-PAGE and immunoblotted with 1:200 dilution of sera from 16 Class V LN patients, 10 Class III/IV LN patients, 10 SLE patients without nephritis, and 5 normal controls (NC).

**Results:** A reactive band between 43-46 kDa was present in 14/16 patients with class V LN and 9/10 patients with class III/IV LN, but was seen in only 1/10 SLE patients without nephritis and 2/5 NC. A reactive band between 36-40 kDa was present in 11/16 patients with class V LN, 3/10 patients with class III/IV LN, 2/10 SLE patients without nephritis, and 1/5 NC. A reactive band between 23-28 kDa was present in 10/16 patients with class V LN, 8/10 patients with class III/IV LN, 4/10 SLE patients without nephritis, and 2/5 NC. A reactive band between 98-105 kDa was present in the majority of subjects in all groups, including NC. Using LC-MS/MS we identified 14 membrane-associated podocyte proteins, including AAMP and NCEH1 in the 43-46 kDA molecular weight range and ANXA2 in the 36-40 kDa range.

**Conclusions:** We conclude that LN patients express unique patterns of autoantibodies against native glomerular proteins that will allow identification of the targets of nephritigenic autoantibodies that cause various forms of LN.

*Funding:* Other NIH Support - NIAID; NIAMS

## FR-PO895

**Neph1 Signaling, a Novel Therapeutic Target for Protection of Podocytes against Glomerular Injury** Deepak Nihalani, Ehtesham Arif, Babita Kumari, Lawrence B. Holzman. *Medicine, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** Podocytes are specialized epithelial cells that are critical components of the glomerular filtration barrier and their dysfunction leads to proteinuria and renal failure. Thus preserving podocyte function in the event of a glomerular injury is therapeutically significant.

**Methods:** We used zebrafish as an in vivo model and poocyte cell culture as an in vitro model to demonstrate that inhibiting Neph1 signaling prevents podocyte from glomerular injury.

**Results:** In this study, we show that inhibiting the intracellular signaling of Neph1 by transducing the cytoplasmic domain of Neph1 (Neph1CD) in cultured podocytes protects podocytes from PAN induced injury. In addition to inhibiting the PAN induced Neph1 phosphorylation, the Neph1CD transduced cells were resistant to PAN induced cytoskeletal damage. In an in vivo assay using zebrafish model system, we demonstrated that transduction of Neph1CD in zebrafish prevented them from PAN induced injury. Further biochemical analysis using subfractionation of the transduced and control podocyte cells showed that unlike the control cells Neph1 was retained in the lipid raft fractions in the transduced cells following treatment with PAN. In accordance, the immunofluorescence analysis suggested that Neph1CD transduced cells had increased ability to retain endogenous Neph1 at the membrane in response to PAN induced injury. Consistent with these observations, maintaining high levels of Neph1 at the membrane using a podocyte cell line overexpressing chimeric Neph1 had a similar protective effect from PAN induced injury with increased propensity to maintain tight junctions in podocytes. The albumin filtration assay further suggested that these cells were resistant to PAN induced albumin leakage.

**Conclusions:** Collectively, these results identify Neph1 signaling as a novel therapeutic target for the prevention of podocyte damage that is commonly observed in various glomerular diseases.

*Funding:* NIDDK Support, Private Foundation Support

## FR-PO896

**Involvement of Planar Cell Polarity Pathway in Glomerular Development and Function** Brittany L. Rocque, Elena Torban. *Depts of Physiology and Medicine, McGill Univ, Montreal, Canada.*

**Background:** The Planar Cell Polarity (PCP) signaling pathway is crucial for tissue morphogenesis. Vangl2 protein is central in the PCP pathway: its loss is embryonic lethal causing neural tube defects in mice and humans. In the kidney, PCP signaling is thought to be important for the organization of glomerular epithelial cells, podocytes, along the glomerular basement membrane. Podocyte cell protrusions, foot processes (FP), are critical for kidney filtration functions; loss of FP architecture results in proteinuria and, in severe cases, focal segmental glomerulosclerosis. In our previous studies, we demonstrated an effect of PCP pathway on podocyte shape, actin rearrangement and cell motility (Babayeva, 2011, AJP). We, therefore, hypothesized that the PCP pathway is involved in glomerualar development and function.

**Methods:** Mouse embryonic kidneys were used to examine Vangl2 expression in developing glomeruli. Kidneys from *Looptail* (Vangl2) embryos and from newly generated conditional podocyte-specific Vangl2 knockout mice (Vangl2 cKO) were analyzed by histological and immunofluorescent methods. In Vangl2 cKO, ELISA was used to measure urine albumin/creatinine ratio.

**Results:** Vangl2 is dynamically expressed in developing glomeruli: in podocyte precursors, Vangl2 is expressed at the plasma membrane; as podocytes mature, it migrates to the basal side of cells where FPs extend. In mature glomeruli, Vangl2 levels are decreased. Quantification of glomeruli at various developmental stages in *Looptail* mutants vs wildtype embryos revealed a significant developmental delay in glomerular maturation in *Looptail* embryos suggesting that Vangl2 may be required for podocyte function. To examine whether loss of Vangl2 affects glomerular functions in adult animals, we created conditional podocyte-specific Vangl2 cKO mice. At 12 months, Vangl2 cKO animals do not exhibit proteinuria or signs of altered kidney morphology. An effect of podocyte injury in Vangl2 cKO vs. control mice is being currently analyzed.

**Conclusions:** During development, loss of PCP gene Vangl2 significantly affects glomerular maturation. However, it appears that Vangl2 is dispensable for the functions and maintenance of podocytes in adult mice.

*Funding:* Government Support - Non-U.S.

## FR-PO897

**Thioredoxin-Interacting Protein Mediates hHcys-Induced NLRP3 Inflammasome Activation in Glomerular Sclerosis** Justine M. Abais,[1] Min Xia,[1] Krishna M. Boini,[1] Todd W. Gehr,[2] Pin-lan Li.[1] *[1]Pharmacology & Toxicology, Virginia Commonwealth Univ, Richmond, VA; [2]Internal Medicine, Virginia Commonwealth Univ, Richmond, VA.*

**Background:** Our studies have demonstrated that NADPH oxidase-derived reactive oxygen species activates NLRP3 inflammasomes causing hyperhomocysteinemia (hHcys)-induced podocyte and glomerular injury, but the mechanism regarding how the inflammasome senses changes in oxidative stress is still unknown. Thioredoxin-interacting protein (TXNIP) inhibits the antioxidant thioredoxin (TRX) and has been shown to dissociate from TRX, bind to inflammasome protein NLRP3, and stimulate formation of the inflammasome complex. The current study explored whether TXNIP mediates hHcys-induced NLRP3 inflammasome activation *in vivo*.

**Methods:** Adult, C57BL/6J male mice were fed a folate-free diet for 4 weeks to induce hHcys. TXNIP was inhibited by verapamil (1 mg/mL, drinking water) or by local kidney microbubble-ultrasound TXNIP shRNA transfection. *In vivo* inflammasome formation and activation was assessed by confocal microscopy, coimmunoprecipitation, and measurement of caspase-1 activity and IL-1β production. Glomerular morphology and function was determined by Periodic acid-Schiff (PAS) staining and measurement of urinary protein and albumin.

**Results:** Evidenced by immunofluorescence and coimmunoprecipitation studies, mice with hHcys had significantly increased inflammasome formation and TXNIP binding to NLRP3, which were not observed in TXNIP shRNA-transfected mice or those receiving verapamil. Importantly, hHcys increased caspase-1 activity 1.5-fold and IL-1β production 2.0-fold, signifying increased inflammasome activation, which was substantially blocked after TXNIP inhibition. *In vivo* TXNIP blockade demonstrated glomerular protective effects shown by normalized expression of podocyte markers podocin and desmin, reduced proteinuria and albuminuria, and preserved glomerular morphology through reduced mesangial cell expansion, fibrosis, and capillary collapse.

**Conclusions:** These results show that TXNIP binding to NLRP3 is a key signaling mechanism necessary for hHcys-induced NLRP3 inflammasome formation and activation, and subsequent glomerular injury.

*Funding:* NIDDK Support, Other NIH Support - 1F31AG043289

## FR-PO898

**Dynamin Oligomerization Regulates Actin and Represents a Novel Therapeutic Target in Proteinuric Kidney Diseases** Mario Schiffer,[1,3] Changkyu Gu,[2] Beina Teng,[1] Nils Hanke,[1,3] Hermann G. Haller,[1,3] Sanja Sever.[2] *[1]Nephrology, Hannover Medical School, Hannover, Germany; [2]Medicine, Harvard Medical School, Charlestown; [3]Mount Desert Island Biolab, Salsbury Cove.*

**Background:** Misregulation of the actin cytoskeleton in podocytes represents a common pathway in the pathogenesis of proteinuric diseases in general and FSGS in particular. We previously showed that the GTPase dynamin maintains the actin cytoskeleton in podocytes by displacing the actin capping protein gelsolin from barbed ends and that dynamin downregulation underlies proteinuria.

**Methods:** We used in vitro and in vivo approaches to show that the small molecule Bis-T-23 promotes dynamin's natural propensity to oligomerize into higher order assemblies. Bis-T-23 is used in different rodent models of proteinuria and in different zebrafishmodels with gene knockdowns of known genes affecting the actin cytoskeletton of podocytes.

**Results:** We found that Bis-T-23 stimulates the actin polymerization in podocytes independently of RhoA, Rac1, and Cdc42 signaling pathways. Administration of Bis-T-23 in diverse animal models of kidney disease reverses foot process effacement, ameliorates proteinuria, attenuates glomerular scarring, and increases survival through an effect on actin dynamics.

**Conclusions:** Our results establish the dynamin oligomerization cycle as an important *in vitro* and *n vivo* regulator of actin and a therapeutic target in genetic or acquired proteinuric kidney diseases.

## FR-PO899

**Activation of XBP1 following Endoplasmic Reticulum Stress Is Essential for Podocyte Function in the Kidney** Hossam Hassan,[1] Xuefei Tian,[2] Shuta Ishibe.[2] *[1]Pediatrics, Yale Univ School of Medicine, New Haven, CT; [2]Internal Medicine, Yale Univ School of Medicine, New Haven, CT; [3]Internal Medicine, Yale Univ School of Medicine, New Haven, CT.*

**Background:** Protein misfolding in the endoplasmic reticulum (ER) leads to ER stress. To handle misfolded proteins, the ER has in place quality control mechanisms, including the unfolded protein response and ER-associated degradation (ERAD). Induction of ER stress in glomerular cells has been described in experimental models of membranous nephropathy and membranoproliferative glomerulonephritis but the physiologic relevance remains unknown. We have identified that loss of Sec63, an ER transport protein, in podocytes results in the activation of the unfolded protein response protein, IRE1α-XBP.

**Methods:** Wild type and podocyte specific SEC63 / XBP1 double knock out (DKO) mice were analyzed to determine the role of SEC63 /XBP1 in vivo and primary podocytes were isolated for in-vitro studies.

**Results:** Loss of Sec63 or XBP1 alone in podocytes did not elicit an overt phenotype. However, the loss of Sec63 and XBP1 specifically in podocytes demonstrated severe albuminuria, which began at 2-3 months of age and continued to progress when compared to wild type(84.59±3.78 vs. 66.23±2.3), 2 month (470.37±70.15vs. 79.96±2.45), 6 month (622.21±45.27 vs.71.39±1.80), 1 year (1988.79±224.98 vs.74.22±5.54). Histological examination of the podocyte specific *Sec63/XBP1 DKO* mice kidney cortex demonstrated mesangial matrix accumulation in the glomeruli, dilated tubules with proteinaceous casts, and interstitial fibrosis. Ultrastructural analysis demonstrated severe foot process effacement with a thickened glomerular basement membrane.

**Conclusions:** Our results emphasize that the loss of Sec63 in podocyte induces ER stress and activates the unfolded protein response arm, IRE1a-XBP1. Inability to respond to the ER stress in this arm, by concomitant genetic inactivation of XBP1 appears fundamentally required for the maintenance of the kidney glomerular filtration barrier.

*Funding:* NIDDK Support

## FR-PO900

**SIRT1 Maintains Podocyte Homeostasis via Regulation of Actin Fiber Formation** Shuta Motonishi,[1] Reiko Inagi,[1] Takehiko Wada,[1] Takamoto Ohse,[1] Akira Shimizu,[2] Masaomi Nangaku.[1] *[1]Div of Nephrology and Endocrinology, The Univ of Tokyo School of Medicine, Tokyo, Japan; [2]Dept of Pathology, Nippon Medical School, Tokyo, Japan.*

**Background:** Recent studies highlighted the renoprotective effect of SIRT1, a deacetylase that contributes to cellular regulation. However, its molecular mechanism in podocytes is still unclear. The actin cytoskeleton plays a critical role in supporting the structure and function of podocytes. We thus investigated the effect of SIRT1 on the maintenance of actin fiber in podocytes.

**Methods:** We made podocyte specific SIRT1 knockout ($SIRT1^{-/-}$) mice by crossing $SIRT1^{flox/flox}$ mice with podocin-Cre mice. Then, we induced glomerular damage by injection of sheep anti-GBM antibody in $SIRT1^{-/-}$ or wild-type mice. Measurement of urinary albumin/creatinine ratio (U-alb/cre), blood urea nitrogen (BUN), and histological analyses by PAS staining and electron microscopy were performed. In in vitro study using conditionally immortalized murine podocytes, we assessed the structural or functional changes in podocytes treated with SIRT1 inhibitor, by phalloidin staining, scratch assay, and Western blotting (WB) for actin fiber-related proteins.

**Results:** Seven days after the disease induction, both U-alb/cre and BUN in $SIRT1^{-/-}$ mice were significantly higher than those in wild-type mice (93.5±73.9 vs. 28.3±20.5 mg/mg, 26.9±3.7 vs. 22.3±3.2 mg/dl, respectively, $P<0.05$) and glomerular injury, such as crescent formation, was significantly increased in $SIRT1^{-/-}$ mice. By electron microscopy, we found that foot process effacement and actin fiber derangement were markedly exacerbated in $SIRT1^{-/-}$ mice compared with wild-type mice. Similarly, mild actin fiber derangement in $H_2O_2$-treated cultured podocytes became prominent when the cells were pretreated with SIRT1 inhibitor. Cellular motility was also reduced by SIRT1 inhibition. In WB analysis, the level of acetylated cortactin, which decays actin fiber formation, was increased in SIRT1 inhibitor-treated cells.

**Conclusions:** SIRT1 maintains podocyte homeostasis and prevents glomerular injury by deacetylated cortactin and the subsequent actin fiber formation.

## FR-PO901

**Functional Role of Sirt1 in Kidney Podocytes** Peter Y. Chuang,[1] Yan Dai,[2] Leyi Gu,[3] John C. He.[1,4] *[1]Medicine, Div of Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Medicine, Div of Nephrology, Shanghai First Affiliated Hospital, Shanghai, China; [3]Renal Div and Molecular Cell Laboratory for Kidney Disease, Renji Hospital, Shanghai Jiatong Univ School of Medicine, Shanghai, China; [4]Renal Div, James J Peters Veterans Affairs Medical Center, Bronx, NY.*

**Background:** The silent mating type information regulation 2 homolog (SIRT) 1 gene encodes for a $NAD^+$-dependent lysine deacetylase. *SIRT1* is expressed in kidney podocytes, but Sirt1's function in the podocyte has not been examined in detail.

**Methods:** *Sirt1* knockout mice exhibit developmental defects in multiple organs and a shorten lifespan, which hinder the examination of *Sirt1* in adult mice and could potentially confound the interpretation of *Sirt1* function. To study *Sirt1* function in podocytes we created two lines of mice with doxycyline (DOX)-inducible and reversible knockdown of

*Sirt1* by adapting a novel *in vivo* RNA interference (RNAi) model. *Sirt1* protein and mRNA levels were assessed by western blotting and quantitative realtime PCR. Urinary protein excretion was quantified by urinary albumin to creatinine ratio and SDS gel electrophoresis followed by Coomasie Blue staining. Serum creatinine was measured by HPLC. PAS-stained kidney sections and transmission electron microscopy were used to assess renal histology and podocyte morphology.

**Results:** We achieved temporal and spatial control over *Sirt1* expression in kidney podocytes and tubular cells.We confirmed that up to ~80% reduction of Sirt1 in podocytes is dispensable for normal kidney function. Mice with global *Sirt1* knockdown developed more proteinuria compared to those without *Sirt1* knockdown when both were injected with Adriamycin (ADR). Furthermore, ADR-treated mice with podocyte-specific *Sirt1* knockdown developed more severe glomerulosclerosis, podocyte foot process effacement and proteinuria, which were partially reversed upon reversal of *Sirt1* knockdown, whereas mice with tubular-specific *Sirt1* knockdown were not susceptible to ADR-induced nephropathy.

**Conclusions:** Podocyte Sirt1 serves a protective role in ADR-induced nephropathy.

*Funding:* NIDDK Support, Veterans Affairs Support

## FR-PO902

### PPARγ in Podocytes Promotes Resistance to Crescentic Glomerulonephritis

Carole Hénique-Gréciet,[1,2] Guillaume Bollee,[1,2] Marine Milon,[1,2] Pierre-Louis F. Tharaux.[1,2] *[1]Paris Cardiovascular Research Centre - PARCC, INSERM, Paris, France; [2]Univ Paris Descartes, Sorbonne Paris Cité, Paris, France.*

**Background:** The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) agonists have beneficial effects on renal structure and function in models of diabetes and chronic kidney diseases. In this study, we have explored the involvement of PPARγ in crescentic rapidly progressive glomerulonephritis (RPGN).

**Methods:** RPGN was induced by injection of anti–glomerular basement membrane antiserum (nephrotoxic serum, NTS).

**Results:** Gain-of-function approach: Pioglitazone, a PPARγ agonist, limited renal injury in RPGN. Pioglitazone ameliorated podocyte damage and reduced proteinuria. Pioglitazone administration was effective when performed from the first day of NTS injection or when started in a delayed manner (4 days after NTS injection). Loss-of-function approach: We generated mice with specific deletion of PPARγ alleles in podocytes (Pod-PPARγ mice). Pod-PPARγ mice developed more severe glomerular injury and RPGN upon NTS administration. Pod-PPARγ mice displayed a 2-fold increase in Albuminuria to Creatinuria ratio at day 10 (2843 +/- 329 vs. 1664 +/- 207 g/mol, $p<0.001$) than wild-type mice and higher crescent incidence (35.6 +/- 5.4 vs. 15.6 +/- 3.7 %, $p<0.01$). Accordingly, PPARγ deletion in podocytes promoted worsening of blood urea nitrogen levels (119.9 +/- 11.6 vs. 56.7 +/- 9.0 mg/dl, $p<0.001$). Such aggravation was not due to an effect on proliferation and migration of primary podocytes.

**Conclusions:** In conclusion, PPARγ activity is an essential pathway protecting podocyte phenotype and the glomerular architecture during experimental RPGN. PPARγ is involved in mitochondrial biogenesis and function. Moreover, it is known that mitochondria are the main site of reactive oxygen species production. In cells exposed to oxidative stress, NRF2 activity is increased, further driving the transcriptional activation of genes whose expression is essential to control cellular redox homeostasis. Thus, we have also further explored the role of oxidative stress via NRF2 anti-oxidant pathway in this model.

## FR-PO903

### Angiotensin II and Diacylglycerol Cause Activation of Stat-3 in Podocytes through a Pathway That Includes TRPC6 Channels, Calcineurin, and CaM Kinase

Stuart E. Dryer,[1,2] Mousa Abkhezr.[1] *[1]Dept of Biology and Biochemistry, Univ of Houston, Houston, TX; [2]Div of Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** Previous studies have shown that the transcription factor signal transducer and activator of transcription-3 (Stat-3) plays a role in progression of glomerular diseases, especially in HIV nephropathy[1,2].

**Methods:** Immunoblot analysis of Stat-3 tyrosine phosphorylation and confocal microscopy in mouse podocyte cell lines.

**Results:** Application of 100 nM angiotensin II (Ang II) for 15 min-1 hr caused a marked increase in the phosphorylation of Stat-3 on tyrosine Y705. A similar effect was seen after treatment with 100 μM of the diacylglycerol analog 1-oleoyl-2-acetyl-sn-glycerol (OAG). In confocal microscopy, we observed that both treatments led to accumulation of Stat-3 in the cell nucleus. Stat-3 phosphorylation and nuclear localization were reduced by pretreatment with the pan-TRP channel inhibitor SKF-96365, and by siRNA knockdown of TRPC6, which also reduced basal phosphorylation of Stat-3. The effects of OAG persisted in cells treated with the PKC inhibitor chelerythrine. We used several agents to examine pathways downstream of TRPC6 that could lead to Stat-3. We observed that Ang II-evoked phosphorylation of Stat-3 was blocked by pretreatment with the CaM Kinase inhibitor KN-93, by the calcineurin inhibitor cyclosporine-A, and by the JAK inhibitor AG-490.

**Conclusions:** Stat-3 is a downstream effector of TRPC6 signaling, but Ang II may activate more than one cascade leading to tyrosine phosphorylation of this transcription factor. It remains to be determined if calcineurin, CaM kinase and JAK comprise parallel pathways feeding into Stat-3.

1. Feng et al. (2009). J Am Soc Nephrol 20: 2138-46. 2. Gu et al. (2013) AIDS in press.

*Funding:* Pharmaceutical Company Support - Pfizer

## FR-PO904

### Large-Scale Analysis of the Glomerular Phosphoproteome Reveals Novel Regulatory Mechanisms at the Kidney Filtration Barrier

Markus M. Rinschen,[1] Tim König,[2] Trairak Pisitkun,[3] Henning Hagmann,[1] Stuart E. Dryer,[4] Paul T. Brinkkoetter,[1] Thomas Benzing.[1] *[1]Dept II of Internal Medicine and Center for Molecular Medicine, Univ of Cologne, Cologne, Germany; [2]Institute for Genetics, Univ of Cologne, Cologne, Germany; [3]Univ of Aarhus, Aarhus, Denmark; [4]Biology and Biochemistry, Univ of Houston, Houston, TX.*

**Background:** Diseases of the kidney filtration barrier are a leading cause of endstage renal failure. Most disorders affect the podocytes, polarized cells that are connected by a unique cell junctional complex, the slit diaphragm. Podocytes require tightly controlled signaling to maintain their integrity, viability and function.

**Methods:** We use state-of-the-art tandem mass spectrometry to provide an atlas of *in vivo* phosphorylated, glomerulus-expressed proteins including podocyte-specific gene products.

**Results:** We discovered 2,449 phosphorylated proteins corresponding to 4,079 identified high-confident phosphorylated residues. We performed a systematic bioinformatics analysis of this dataset, revealing distinct phosphorylation motifs and roles for tyrosin-phosphorylation sites. Among the 146 phosphorylation sites found on proteins abundantly expressed in podocytes, synaptopodin was a dominant phosphoprotein with 18 identified proline-directed phosphorylation sites. Several sites resided close to homologous human residues known to be mutated in human genetic forms of proteinuria. One such site discovered on the slit diaphragm protein podocin, threonine-234 (T234), is a potential aPKC site. Using extensive molecular dynamics simulations and biochemistry, we show that phosphorylation critically regulates podocin dimer formation and multimerization and that this represents a general principle for the assembly of the large family of PHB-domain containing proteins.

**Conclusions:** We show that integration of phosphoproteomics with genetic, biochemical and structural data allows a powerful approach to understand regulatory processes at the slit diaphragm and its alterations in health and disease.

*Funding:* Government Support - Non-U.S.

## FR-PO905

### Self-Regulation of HO-1 in GEC

Maria Detsika,[1] Pu Duann,[2] Elias A. Lianos.[2] *[1]Medicine, National and Kapodistrian Univ of Athens, Greece; [2]Div of Nephrology, Univ of Medicine and Dentistry of New Jersey.*

**Background:** Athough Heme oxygenase (HO)-1 is renoprotective, glomerular epithelial cells (GEC) have a limited ability to upregulate HO-1 as shown in HO-1 inducing forms of GEC injury. This raises the question of whether HO-1 induction in GEC is subject to tight regulation and was addressed in normal rat glomeruli.

**Methods:** Glomeruli were isolated from male, wild type (WT), Sprague-Dawley rats (SD), hmox1$^{+/-}$ SD, hmox1$^{-/-}$ SD, and SD with (GEC)-targeted HO-1 overexpression (GEC$^{HO-1}$). hmox1$^{+/-}$ and hmox1$^{-/-}$ SD were obtained using Zinc Finger Nuclease (ZFN) technology designed to generate a 10-bp deletion in a specific HO-1 sequence within Exon 3. GEC$^{HO-1}$ SD were obtained by Sleeping Beauty Transposon mediated transgenesis using a nephrin promoter. Glomeruli from WT, hmox1$^{+/-}$, hmox1$^{-/-}$ and GEC$^{HO-1}$ SD were treated with defined Heme concentrations for 18 h. Glomerular HO-1 protein and mRNA levels were assessed by western blotting and Real-time PCR amplification.

**Results:** A 60-70% reduction in constitutive HO-1 levels and complete HO-1 absence was observed in hmox1+/- and hmox1-/- glomeruli, respectively. In WT glomeruli, low Heme (6-200 mM) concentrations increased HO-1 synthesis (mRNA and protein) in a dose dependent manner. At higher Heme concentrations (400 mM), HO-1 synthesis (mRNA and protein) was reduced to constitutive levels while at 800 mM there was an even higher reduction to sub-constitutive levels. In hmox1$^{+/-}$ glomeruli, Heme (400 mM) failed to reduce HO-1 synthesis. In GEC$^{HO-1}$ glomeruli, there was a shift of the inhibitory effect of Heme on HO-1 synthesis to lower Heme concentrations (200 mM) capable of inducing HO-1 in WT glomeruli. This effect was mimicked in WT glomeruli co-incubated with Heme and MG132, (proteasome inhibitor) at concentrations that increased endogenous HO-1 levels.

**Conclusions:** Heme-mediated HO-1 synthesis in GEC is negatively regulated by HO-1 protein levels achieved, pointing towards a negative feed-back regulatory mechanism. This may serve as a HO-1 switch-off mechanism, upon injury-mediated HO-1 induction, preventing HO-1 from reaching potentially cytotoxic levels.

*Funding:* Pharmaceutical Company Support - ELPEN Pharmaceutical Industry

## FR-PO906

### Podocytes Lacking Cell Surface Heparan Sulfate Show Enhanced Production/Shedding of Nephrin Positive Microparticles/Exosomes

Kevin J. McCarthy, Deborah J. McCarthy. *Pathology, LSU Health Sciences Center-Shreveport, Shreveport, LA.*

**Background:** Several recent reports have shown that one cell surface proteoglycan family, syndecans (SDCs), play a key role in the regulation of exosome production/release. Since heparan sulfate (HS) is found covalently attached to SDCs and mediate SDC interactions and clustering at the cell surface, we hypothesized that loss of HS from SDC core proteins on podocyte (podo) pedicels would affect the production of podo urinary exosomes.

**Methods:** Mutant mice having podo lacking the ability to assemble heparan sulfate (HS) on SDC proteins were previously described (PEXTKO mice, Kid Int 74:289-299). Kidneys from wild type (WT), unilateral nephrectomized (UNX) WT, PEXTKO, and PEXTKO UNX

mice were processed for frozen sections and transmission electron microscopy (TEM). Sections were immunostained for SDC-4, SDC-1, ALIX (exosome marker),nephrin (also found in urinary exosomes), and synaptopodin.

**Results:** Podo in PEXTKO mice showed mislocalization of SDC core proteins from the pedicel basal surface. A disruption in synaptopodin staining pattern was seen in PEXTKO podocytes compared to WT. TEM micrographs showed the presence of microvilli on the apical surfaces of podo, the greatest number seen in PEXTKO and PEXTKO UNX podo. Coincident with the former observation, glomerular nephrin immunoreactivity showed a gradation of intensity, with PEXTKO UNX>PEXTKO>WT UNX>WT. Immunostaining for ALIX showed a pattern of intensity gradation similar to nephrin. Nephrin positive immunoreactivity, having the appearance of small particles, was also seen within the cytoplasm of proximal tubule cells (PTC). Double-label immunostaining of PTC for nephrin and ALIX showed little overlap between the two populations of particles. The number of nephrin positive particles/tubule profile was determined to be PEXTKO UNX>PEXTKO>WT UNX>WT.

**Conclusions:** We believe that SDC HS plays an integral role in regulating the genesis/release of microparticles/exosomes from podo. The microparticles/exosomes fall into two populations, ALIX or nephrin positive. This may result from differences in the mechanism of their genesis and may deliver different types of cargo to PTE cells.

*Funding:* NIDDK Support

## FR-PO907

**Morphological Change of Podocytes 1 Hour after Exposure to Serum of Recipients with Recurrence of Focal Segmental Glomerulosclerosis after Kidney Transplantation**

Kiyonobu Ishizuka,[1] Yutaka Harita,[2] Haruko Tsurumi,[2] Tatsuo Asano,[1] Kei Nishiyama,[1] Noriko Sugawara,[1] Hiroko Chikamoto,[1] Yuko Akioka,[1] Yutaka Yamaguchi,[3] Motoshi Hattori.[1] *[1]Dept of Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Dept of Pediatrics, Graduate School of Medicine, Univ of Tokyo, Tokyo, Japan; [3]Yamaguchi's Pathology Laboratory, Matsudo, Chiba, Japan.*

**Background:** The pathogenesis of recurrent FSGS after renal transplantation remains unclear. To clarify the sequential events in the glomeruli after exposure of FSGS plasma in situ, we analyzed the morphological and molecular change of podocytes in transplanted kidney.

**Methods:** Five sets of renal graft specimens were studied in three time frames, before reperfusion (0 hour), one hour after reperfusion, and several days after reperfusion. FSGS recurred in three of all five cases after transplant, with massive proteinuria within 72 hours from reperfusion. We analyzed the degree of foot process (FP) effacement, intracellular localization of various functional proteins of podocytes by confocal microscopy, and podocyte number in glomeruli through these periods of time.

**Results:** Within one hour after reperfusion, FP effacement was observed only in all the post-transplant recurrent cases. Staining pattern of Neph1, SIRP alpha, Zo-1, Podocalyxin, Ezrin, Synaptopodin, Vimentin, and Myo1E did not change in any specimens of all cases. However, in all the recurrent cases, staining pattern of Nephrin and Podocin altered from linear pattern to granular pattern in cytoplasm as early as one hour after reperfusion. These cytoplasmic Podocin and Nephrin were partially localized in Golgi apparatus, but not in ER. Coarse granular staining of CD2AP, which is distinct from that of Nephrin or Podocin, was also observed in 1 hour and later specimen only in recurrent cases.Podocyte number did not change during the study period.

**Conclusions:** Exposure to recurrent FSGS sera for one hour results in dissociation and partial translocation of slit diaphragm component to cytoplasm and simultaneous FP effacement. These hyperacute changes which precede proteinuria represent fundamental mechanism which underlie the pathogenesis of FSGS, and may hold predictive value in FSGS recurrurence.

## FR-PO908

**Aristolochic Acid Causes Albuminuria by Promoting Mitochondrial DNA Damage and Dysfunction in Podocyte**

Yang Zhou, Xueqin Bian, Chunsun Dai, Junwei Yang. *Center of Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, Jiangsu, China.*

**Background:** Aristolochic acid nephropathy (AAN), initially found in patients intaking slimming herbs containing aristolochic acid, was previously considered as a progressive renal interstitial fibrosis and urothelial malignancy. However, the presence of albuminuria in some patients with AAN suggests that aristolochic acid may also damage the glomerular filtration barrier.

**Methods:** In this study, mice AAN model was generated by daily administration of aristolochic acid I sodium salt (AA) intraperitoneally at a dose of 10mg/kg body weight for 3 days. Mice were killed 3 or 7 days later with their urine and kidney harvested for further examination. Cultured mice podocytes were incubated with AA at a dose of 10μmol/L for various time periods. Mitochondria were isolated and mitochondrial DNA (mtDNA) damage was evaluated by single cell gel electrophoresis assay (comet assay). Q-PCR assay was used to quantify the copy number of mtDNA. Western blot analysis was applied to examine the expression level of mitochondrial protein. Mitochondrial function was determined by ATP content, oxygen consumption rate, membrane potential and reactive oxygen species.

**Results:** All of the mice developed heavy albuminuria at day 3 and 7 after AA administration. In the mice received AA, morphologic change of glomeruli was minor under light microscopy but podocyte foot-process effacement was evident under electron microscopy. In mitochondria from isolated glomeruli, prominent mtDNA damage was accompanied with marked decrease of mtDNA copy number and mtDNA-encoded mitochondrial protein expression. Similar to those in vivo results, AA treatment could impair the filtration barrier function of cultured podocytes. AA promoted mtDNA damage, decreased mtDNA copy number and mitochondrial protein expression in podocytes. In addition, AA treatment could also decrease ATP content, oxygen consumption rate and mitochondrial membrane potential as well as increase cellular reactive oxygen species in cultured podocytes.

**Conclusions:** Aristolochic acid can induce podocyte damage and dysfunction, which may be mediated by promoting mitochondrial DNA damage and dysfunction in podocytes.

*Funding:* Government Support - Non-U.S.

## FR-PO909

**Assessment of Glomerular Changes in a Passive Mouse Model of IgA Nephropathy**

Lea Novak,[1] Zina Moldoveanu,[1] Zhi Qiang Huang,[1] Colleen J. Winstead,[1] Stacy D. Hall,[1] Rhubell T. Brown,[1] Bruce A. Julian,[1] Casey T. Weaver,[1] Jiri F. Mestecky,[1] Hitoshi Suzuki,[2] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Juntendo Univ, Tokyo, Japan.*

**Background:** IgA nephropathy (IgAN) is an autoimmune glomerulonephritis wherein immune complexes (IC) composed of galactose-deficient IgA1 (Gd-IgA1) and anti-glycan autoantibodies deposit in the glomeruli. We developed an animal model by using *in vitro*-formed IC from human Gd-IgA1 myeloma protein and recombinant Gd-IgA1-specific IgG for induction of IgAN-like disease in mice. The purpose of this study was to quantify mesangial proliferation in this model of IgAN.

**Methods:** Severe-combined-immunodeficiency (SCID) mice were repeatedly injected every other day (total 3 injections) with Gd-IgA1-IgG IC (IC-group) or with Gd-IgA1 only (control group). Kidneys from IC-group mice were removed 1, 4, 7, and 14 days after the last IC administration and from the control group mice 1 day after the last Gd-IgA1 administration. Kidneys were fixed in formalin, processed, and embedded in paraffin. Tissue sections, 5 μm thick, were stained with periodic-acid Schiff. Using IP Lab software, multiple glomeruli for each animal were examined and average cellularity per unit of glomerular area was calculated.

**Results:** Mesangial cellularity in the control group was 34.6±1.1 x $10^5$ nuclei per $\mu m^2$ with normal histology at 1 day after the last injection of Gd-IgA1. In the IC-group, significantly increased cellularity of mesangial cells was observed at 1, 4, and 7 days after the last injection: 42.6±1.6, 39.9±0.7 and 42.8±1.8 x $10^5$ nuclei per $\mu m^2$, respectively. At 14 days after the last injection, glomerular cellularity declined to baseline, 38±1.3 x $10^5$ nuclei per $\mu m^2$. The glomerular changes were consistent with those of human IgAN as they included glomerular hypercellularity and mesangial matrix expansion.

**Conclusions:** Our passive mouse model of IgAN confirms the importance of circulating Gd-IgA1-IgG IC in the pathogenesis IgAN and induction of histopathologic changes typical of human IgAN. Morphometric evaluation of the glomerular cellularity is an objective method for assessment of pathological changes in this model.

*Funding:* NIDDK Support

## FR-PO910

**Global Analysis of Glucocorticoid Action in Human Podocytes**

James McCaffrey,[1,2] Hellyeh Hamidi,[1] Nicholas J. Webb,[2] David W. Ray,[1] Rachel Lennon.[1,2] *[1]Institute of Human Development, Univ of Manchester, United Kingdom; [2]Dept of Nephrology, Royal Manchester Children's Hospital, United Kingdom.*

**Background:** Children presenting with nephrotic syndrome (NS) typically receive an empiric course of glucocorticoid (Gc) therapy. Glucocorticoids activate ligand-dependent transcription factors. Those who do not respond to Gc treatment are at high risk of developing end stage renal disease. The clinically-relevant, podocyte-specific mechanisms of action of glucocorticoids in NS have not been determined. Elucidating relevant pathways will guide novel therapeutic targeting. We undertook a global analysis of Gc-regulated genes in human podocytes to generate hypotheses for Gc mechanisms of action.

**Methods:** The transcriptional profile of prednisolone-treated and prednisolone-naïve wild type human podocytes were compared using the Affymetrix U133 Plus 2.0 Array. Comparing whole genome expression data from the two cell groups allowed the creation of a list of Gc-regulated transcripts. This list underwent enrichment analysis for gene ontology terms with two independent software packages, and further experimental validation with live cell imaging and immunofluorescence.

**Results:** 606 Gc-regulated transcripts were identified (fold change with treatment ±1.5, q value ≤0.05). Enriched gene ontology terms included those associated with regulation of apoptosis, cell signalling and cell motility. Live cell imaging analysis showed that Gc exposure significantly reduced podocyte motility (0.0034 μm/sec in untreated cells Vs. 0.0025 μm/sec in treated cells, p-value <0.0001), and altered sub-cellular localisation of synaptopodin, which has already been implicated in both regulation of podocyte motility and recovery of the filtration barrier from proteinuria-inducing agents.

**Conclusions:** Whole genome transcriptional profiling of human podocytes allowed identification of Gc-regulated transcripts. Selected follow-up validation demonstrated that glucocorticoids affect podocyte motility in vitro, and alter the localisation of synaptopodin, a key regulator of cytoskeletal dynamics. These findings may relate to the therapeutic effect of glucocorticoids in NS.

*Funding:* Pharmaceutical Company Support - GlaxoSmithKline, AstraZeneca, ICON, Government Support - Non-U.S.

**FR-PO911**

**Localization of SGLT2 in Bowman's Capsule of Mouse Kidney** Niloofar M. Tabatabai,[1] Paula North,[2] Kevin R. Regner,[1] Suresh Kumar,[2] Christine Duris,[2] Amy B. Blodgett.[1] *[1]Medicine, Medical College of Wisconsin; [2]Pathology, Medical College of Wisconsin.*

**Background:** SGLT2 is a sodium-dependent glucose transporter. Genetic mutations in human and gene knock out studies in mouse have shown that SGLT2 is responsible for most of the glucose reabsorbed from the renal glomerular filtrate. Treatment of diabetic patients with inhibitors of SGLT2 increases the urinary excretion of glucose and lowers the serum concentration of glucose. It is known that SGLT2 is expressed in early proximal tubule. In this study, we re-examined SGLT2 localization by immunohistological analyses on kidneys from wild-type mice using our custom SGLT2 antibody.

**Methods:** Male C57BL/6 mice at 4, 12, and 22 weeks of age were used. Western blot was performed with SGLT2 antibody (0.77 µg/ml) on proteins from whole kidney lysate (25 µg) or cortical brush border membrane (BB) (2 µg), and detection was carried out by chemiluminescence. For immunohistology, formalin-fixed paraffin-embedded tissues were sectioned (4 µm) and stained with SGLT2 (1 µg/ml) or nephrin antibody, and staining was detected with horseradish peroxidase chromogen method (IHC). Slides were counterstained with hematoxylin and then scanned to make digital images. Fluorescence detection (IF) of SGLT2 and nephrin staining was done with using Cy3 and Alexa-647 coupled secondary antibodies, respectively, and nuclei were stained with 4',6-Diamidino-2-Phenylindole. Fluorescence imaging was done with a Zeiss confocal microscope. SGLT2-stained glomeruli were counted in IHC slides and expressed as percent total glomeruli.

**Results:** In Western blot, SGLT2 antibody hybridized to a ~60 kDa protein in whole kidney lysate and in brush border membrane. As expected, SGLT2 antibody stained cortical region and apical side of the proximal tubule cells in kidneys from three age groups. SGLT2 staining was also observed in Bowman's capsule, and IF double staining showed that it was not co-localized with nephrin. SGLT2-positive glomeruli were 14%, 67%, and 87% in tissue sections from 4, 12, and 22 week old mice, respectively.

**Conclusions:** Our results suggest that SGLT2 is expressed in glomerular parietal epithelial cells and that this expression may be regulated by age.

*Funding:* NIDDK Support, Other NIH Support - NIEHS

**FR-PO912**

**Shiga Toxin 1 Induces ICAM-1 Protein Expression in Human Glomerular Microvascular Endothelial Cells** Elena Volokhina,[1] Thea J. Van der Velden,[1] Dineke Westra,[1] Nicole Van De Kar.[1] *[1]Dept of Pediatric Nephrology, Radboud Univ Nijmegen Medical Centre (RUNMC), Nijmegen, Netherlands; [2]Dept of Laboratory Medicine, Radboud Univ Nijmegen Medical Centre (RUNMC), Nijmegen, Netherlands; [3]Dept of Pediatrics, Univ Hospitals Leuven, Leuven, Belgium.*

**Background:** Hemolytic uremic syndrome (HUS) is often preceded by infection with Shiga toxin (Stx) producing *Escherichia coli* (*STEC*). The exact mechanism of how Stx exposure causes HUS is still poorly understood. Intercellular Adhesion Molecule 1 (ICAM-1) is an adhesive molecule, which is important in Stx-mediated endothelial inflammation. In this work we studied Stx1 effect on ICAM-1 protein expression.

**Methods:** HUVEC and human glomerular microvascular endothelial cells (GMVEC) from three donors were treated for 6, 12, 24 and 48 hours with 0, 0.1, and 1.0 nM of Stx1 with or without prestimulation with TNFα. The culture supernatant and cells were collected for ELISA and qPCR analyses to assess the expression of ICAM-1 on protein and mRNA levels.

**Results:** Stx1 upregulated levels of soluble ICAM-1 in culture supernatant of GMVEC when cells were preincubated with TNFα in time- and dose-dependent manner for up to 20 times ($p<0.001$). This upregulation on the protein level was consistent with enhanced ICAM-1 mRNA expression. No effect of Stx1 on HUVEC was observed. Incubation with TNFα only enhanced release of soluble ICAM-1 in both cell lines for up to 10 fold ($p<0.001$).

**Conclusions:** Our results indicate that increased expression of ICAM-1 protein in GMVEC is directly triggered by Stx1. This feature might contribute to pathophysiology of *STEC*-HUS.

*Funding:* Clinical Revenue Support, Government Support - Non-U.S.

**FR-PO913**

**A Common ß3 Integrin Polymorphism L33P Renders Enhanced Reactivity to Soluble Urokinase Receptor: Relevance to Focal Segmental Glomerulosclerosis** Changli Wei, Jing Li, Jochen Reiser. *Dept of Internal Medicine, Rush Univ Medical Center, Chicago, IL.*

**Background:** Our recent findings suggest soluble urokinase receptor (suPAR) as a circulating focal segmental glomerulosclerosis (FSGS) factor. Induction of suPAR activates podocyte □vß3 integrin both *in vivo* and *in vitro*, thus contributing to the development of proteinuria and an FSGS-like nephropathy. ß3 integrin activity is enhanced in platelets with the ß3 integrin genetic polymorphism L33P ($PI^{A2}$). We tested several well defined cohorts of native and transplant FSGS patients and found the prevalence of L33P to be around 20%. Thus we hypothesized that L33P renders enhanced reactivity to suPAR, so that comparably lower or normal suPAR levels might already cause increased ß3 integrin activity in podocytes and glomerular kidney disease.

**Methods:** ß3 integrin mutation L33P was generated by QuickChange site-mutagenesis and cloned into Lentiviral system. Fully differentiated cultured human podocytes were infected with lentivirus expressing either L33P, or wild type ß3 integrin or same amount of control virus. 48 h after infection, cells were treated with different amount of recombinant suPAR protein or saline. 24 h later, the cells were harvested to look at ß3 integrin activity.

**Results:** Compared to human podocytes infected with mock virus, cells received ß3 integrin showed significant higher baseline ß3 integrin activity, with highest signal observed in $PI^{A2}$-ß3 integrin infected cells. Simultaneous suPAR treatment even at low level further enhanced ß3 integrin activity and the highest ß3 integrin activation signal was again found in podocytes infected with $PI^{A2}$-ß3 integrin.

**Conclusions:** In summary, ß3 integrin genetic polymorphism $PI^{A2}$ allows for enhanced ß3 integrin activity in human podocytes (lowered activation threshold), and generates increased sensitivity to suPAR. While *in vivo* work is warranted, these data suggest that ß3 integrin polymorphism $PI^{A2}$ may play a role in FSGS where serum suPAR level is not significantly increased.

*Funding:* NIDDK Support

**FR-PO914**

**VEGF165b Protects against Increases in Glomerular Water Permeability in the Pod-DTR Model of Glomerular Disease** Megan Stevens, Andy Salmon, David O. Bates, Steve Harper, Sebastian Oltean. *Physiology and Pharmacology, Univ of Bristol, Bristol, United Kingdom.*

**Background:** The pod-DTR transgenic mouse model (podocin promoter – diphteria toxin receptor) is an inducible model of glomerular disease triggered by podocyte injury after treatment with diphtheria toxin (DT). The vascular endothelial growth factor splice isoform $VEGF_{165}b$ has been shown to decrease glomerular water permeability ($L_pA/V_i$) in kidneys of transgenic mice over-expressing $VEGF_{165}b$ under a nephrin promoter (neph-$VEGF_{165}b$). This study investigates whether $VEGF_{165}b$ over-expression can rescue the glomerular injury phenotype of the pod-DTR model.

**Methods:** WT, Pod-DTR and double-transgenics (Pod-DTR x neph- $VEGF_{165}b$) mice were given a single dose of DT (1, 5 or 100ng/g body weight). Urinary albumin creatinine ratio (uACR) was measured from urine collected post toxin administration on days 3, 5, 7, 10 and 14. Glomerular $L_pA/V_i$ was measured on day 14. Nephrin immunofluorescence was used as an indicator of podocyte number in WT and pod-DTR mice 14 days after toxin induction.

**Results:** Proteinuria is seen in pod-DTR mice 5 days after 1ng/g DT administration ($n=5$, $p<0.05$) and albuminuria is seen in pod-DTR mice 5 days after 100ng/g DT administration ($n=4$, $p<0.05$) in comparison with WT controls. This normalized by day 14 at both doses. Glomerular $L_pA/V_i$ is significantly increased in pod-DTR mice treated with 1 and 5ng/g DT compared to WT DT-treated controls ($n=5$, $p<0.05$). When pod-DTR x neph-$VEGF_{165}b$ mice were treated with the same doses, $L_pA/V_i$ was significantly lowered ($n=5$, $p<0.05$). There is a reduction in nephrin staining in pod-DTR mice treated with 1ng/g DT, as well as an increase in glomerular area ($n=3$, $p<0.05$) in comparison to WT mice.

**Conclusions:** An increase in glomerular permeability to water is observed in pod-DTR transgenic mice after 1 or 5 ng/g DT administration. This is rescued by over-expression of $VEGF_{165}b$ in podocytes, suggesting a protective role in this model of glomerular injury.

*Funding:* Other NIH Support - Medical Research Council

**FR-PO915**

**High Content Screening for the Discovery of Podocyte Targeted Therapeutics** Tristan Hays, Mehmet M. Altintas, Mohd Hafeez Faridi, Jochen Reiser, Vineet Gupta. *Dept of Internal Medicine, Rush Univ Medical Center, Chicago, IL.*

**Background:** Podocyte dysfunction and loss are commonly associated with proteinuric kidney diseases and adversely effect glomerular filtration. Currently, a majority of therapeutics approved for proteinuric kidney diseases focus on improving systemic hemodynamics and metabolite levels but do not directly address harmful changes seen in podocytes. Therefore, new therapeutics to prevent podocyte dysfunction in glomerular diseases are urgently needed. However, current methods are inadequate for podocyte targeted drug discovery. Here we describe a high content approach for the discovery of novel podocyte targeted therapeutics.

**Methods:** Automated high-throughput imaging and analysis was used as an unbiased approach to quantitate phenotypic changes of podocytes. Conditionally immortalized podocytes were treated with increasing doses of the podocyte damaging agent puromycin aminonucleoside to mimic in vivo podocyte damage. Cells were stained for the nucleus, cytoplasm, F-actin, and focal adhesions to visualize podocyte changes. Cells were then linearly classified using parameters based on cell morphology and the actin cytoskeleton to separate damaged and healthy cell populations. These populations were then compared to generate a percent healthy statistic per well.

**Results:** Our assay showed quantitative, dose-dependent changes in podocyte health upon treatment with damaging agents, without loss of cell viability. Our assay was also reproducible and robust, with a Z-prime factor > 0.5, which makes it suitable for high-throughput screening. Treatment with known podocyte protective agents showed a dose-dependent protection of podocytes from damage in this assay. This approach was then used to screen an FDA approved compound library for the identification of novel podocyte protective drugs.

**Conclusions:** We have developed a novel, high-throughput assay for unbiased scoring of changes in podocyte health in vitro. Application of our assay resulted in identification of novel podocyte protective agents as potential therapeutics for the treatment of proteinuric kidney diseases.

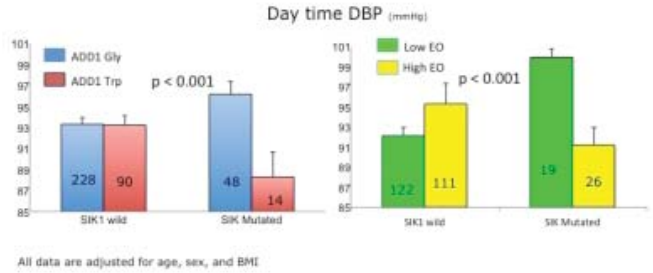
**FR-PO916**

**SIK1 Localizes in Glomerular Podocytes Affecting ADD1 and Endogenous Ouabain Control of Blood Pressure** Mara Ferrandi,[1] Isabella Molinari,[1] Marco Simonini,[1] Simona Delli Carpini,[1] Gianpaolo Zerbini,[1] Chiara Lanzani,[1] Lino Merlino,[1] John Hamlyn,[2] Paolo Manunta.[1] *[1]San Raffaele Scientific Institute, Milan, Italy; [2]Univ of Maryland, Baltimore.*

**Background:** Many genetic alterations of renal ion transport predispose to Na accumulation and may progress to hypertension and organ complications. In the kidneys, the active tubular transport of Na is driven by the Na-K ATPase (NK). ADD1 polymorphisms and increased levels of endogenous ouabain (EO) activate NK and lead to hypertension. ADD1 mutations and high EO, via downregulation of glomerular podocyte nephrin, are implicated in AKI. As the salt-inducible kinase 1 (SIK1) targets the renal NK and is activated by mutant ADD1 in proximal tubular cells, it is of potential relevance to hypertension development.

**Methods:** We investigated the influence of SIK1on the association between BP levels and ADD1 variant, EO levels in 372 naive hypertensive patients and studied the localisation of SIK1in renal glomeruli.

**Results:** In the presence of the wild-type SIK1 no difference of Diastolic BP was observed among patients carrying the wild-type or the mutant ADD1 (93±1.2 mmHg). In contrast patients with high EO (>200 pM) had increased DBP (97.5±1.1 mmHg) vs those with low EO (92.5±0.8 mmHg). However, in the presence of the mutant SIK1 variants, patients carrying the mutant ADD1 or elevated EO showed a reduced DBP vs those with the wild-type ADD1, or low EO, respectively as shown in figure 1.




SIK1 colocalized with nephrin in human glomerular podocytes and SIK1 and nephrin protein expression were highly correlated (r=0.78, p<0.001).

**Conclusions:** The present findings show that SIK1 polymorphism modulate BP control together with ADD1 and EO possibly by modulation of the tubular NK activity. Direct relationship between SIK1 and nephrin expression suggests that SIK1 may be also involved in podocyte damage and kidney injury.

**FR-PO917**

**Angiotensin II Induces Podocyte Apoptosis by the Modulation of CD2AP: Role of ROS, AMPK, and PI3-K** Tae-Sun Ha. *Pediatrics, Chungbuk National Univ, Cheongju, Korea.*

**Background:** Angiotensin II (Ang II) induces dysfunction of glomerular podocytes, which play a crucial role in establishing glomerular filtration barrier permselectivity, thereafter, proteinuria. The glomerular slit diaphragm (SD), a slit between interdigitating foot processes of podocytes, serves as a size-selective barrier and is linked to the actin-based cytoskeleton by adaptor proteins, including CD2-associated protein (CD2AP). Therefore, damages to CD2AP affect not only function of the slit diaphragm, but also directly disrupt the podocyte cytoskeleton, leading to pathological changes inducing proteinuria. In addition, CD2AP can facilitate the nephrin-induced phosphoinositide 3-kinase (PI3-K)/Akt signaling, which protects podocytes form apoptosis. We investigated the changes of CD2AP and podocyte apoptosis by Ang II, a major vascular injury inducer.

**Methods:** Mouse podocytes were incubated in media containing various concentrations of Ang II and signal-related agents. The changes of CD2AP were observed by confocal imaging and Western blotting and podocyte apoptosis by TUNEL assay according to the presence of Ang II.

**Results:** CD2AP were located diffusely but predominantly in peripheral cytoplasm and co-localized with nephrin. However, Ang II decreased CD2AP stainings diffusely and induced spatial separation from concentrated nephrin, similar to those of compound C-treated condition. AICAR and metformin, AMPK activators, ameliorated the abnormal distributional changes of CD2AP. In Western blot analysis, Ang II notably reduced CD2AP in time- and concentration-dependent manners, which were recovered significantly by AMPK activators and Ang II type 1 receptor antagonist, losartan. Ang II increases the intracellular ROS level of podocytes via Nox4 activation and also increased apoptosis in time- and concentration-dependent manners in TUNEL assay, which were also recovered significantly by LY294002, a PI3-K inhibitor.

**Conclusions:** We suggest that Ang II induces the relocalization and reduction of CD2AP protein in podocytes via AT1R, which would cause podocyte apoptosis by the oxidative stress and the suppression of AMPK and PI3-K signalings.

*Funding:* Government Support - Non-U.S.

**FR-PO918**

**JAK/STAT-Associated Inflammatory Markers and the Benefits of JAK/STAT Signaling Inhibition in Peritoneal Dialysis** Tiane Dai,[1] Ying Wang,[1] Cynthia C. Nast,[2] Janine A. La Page,[1] Sharon G. Adler.[1] *[1]Nephrology, LABiomed at Harbor-UCLA, Torrance, CA; [2]Pathology, Cedars-Sinai Medical Center, Los Angeles, CA.*

**Background:** Peritoneal barrier (Pbarrier) pathology limits long-term peritoneal dialysis (PD). Inflammation induces Pbarrier failure. JAK/STAT signaling is implicated;direct evidence is sparse.

**Methods:** Mesothelial cells were cultured in heat-sterilized, filter-sterilized, or filter-sterilized peritoneal dialysis fluid (PDF) with glucose degradation products (GDP) added with or without the pan-JAK inhibitor (JAKi) pyridone (P6). Phospho-JAK1/ 2, STAT1/ 3, periostin, and cleaved caspase-3 (CC-3) were measured by immunoblotting, and CC-3 by immunohistochemistry (IH). Ex vivo, JAK-activators IL6 and IL15 were measured by electrochemiluminescence (ECL, Meso Scale Discovery, Gaithersburg, MD) and proteins with STAT binding sites in or near their promoter regions (IL6, MCP-1, and periostin) were measured by ECL, ELISA, and/or immunoblot in 20 peritoneal dialysis effluent (PDE) samples from 8 new (N) and 7 long-term (LT) PD patients. PDE cell pellet total and phospho-JAK1/2 were measured in 4 N and 4 LT patients. In vivo, PD was performed in rats with saline (PDC, N=3), 4.25% dextrose PDF (PDF4.25%, N=3), or PDF4.25%+5mg/kg JAK1/2i (N=4) x10 days. Pbarrier was stained with trichrome & anti-phospho-JAK1 ab.

**Results:** In vitro, all PDFs induced phospho-STAT1/3 and CC-3. P6 attenuated STAT1/3 phosphorylation, periostin secretion, and CC-3 activation. Ex vivo, cell pellet JAK was phosphorylated in LT but not N patients. In PDE, the STAT-regulated proteins MCP-1, IL-6, and periostin (normalized to CA125) were higher in LT vs N patients. In rats, PDF4.25%, but not saline, activated JAK, and induced mesothelial cell hyperplasia, inflammation, and fibrosis. JAK1/2i abrogated these changes.

**Conclusions:** JAK/STAT signaling directly mediates Pbarrier pathology, which is attenuated by JAK1/2i. Therapy to preserve residual renal function is implemented in PD. Fewer therapeutic strategies target Pbarrier function preservation. Studies coupling inflammatory biomarkers with Pbarrier function may provide support for testing JAK/STATi to address this unmet clinical need.

*Funding:* Pharmaceutical Company Support - Meso Scale Discovery, Rockland, MD; DaVita Clinical Research

**FR-PO919**

**Effect of Adipose Tissue-Derived Mesenchymal Stem Cells in Peritoneal Fibrosis** Amanda Pires, Filipe M. Silva, Irene L. Noronha. *Nephrology, Univ Sao Paulo, Brazil.*

**Background:** Adipose tissue-derived mesenchymal stem cells (ADMSC) display immunomodulatory effects and may represent a strategy to block peritoneal fibrosis (PF), a serious complication of long term peritoneal dialysis. The aim of the study was to analyze the effect of ADMSC in an experimental model of PF developed in uremic rats.

**Methods:** CKD was induced in Wistar rats with a 0.75% adenine-containing diet, during 30 days. PF was induced with intraperitoneal injections of chlorhexidine gluconate. ADMSC were injected IV at days 15 and 21. Rats were divided into 5 groups (n=10/group): **Control,** normal rats; **CKD,** rats receiving adenine diet; **PF,** rats receiving chlorhexidine gluconate to induce PF; **CKD+PF**, CKD rats with PF; **CKD+PF+ADMSC**, CKD rats with PF treated with ADMSC. Peritoneal membrane thickness was measured by masson thrichrome. Macrophages (MØ), T-cells and α-smooth muscle actin (α-SMA) were analyzed by immunohistochemistry, and cytokine expression was analyzed by qPCR at day 30.

**Results:** Infusion of ADMSC significantly reduced the peritoneal membrane thickness, α-SMA expression, MØ and T-cell infiltration, and mRNA cytokine expression in the peritoneal membrane.

| | Control | CKD | PF | CKD+PF | CKD+PF+ ADMSC |
|---|---|---|---|---|---|
| **BUN** | 21±2 | 134±20*§ | 16±1 | 124±19*§ | 163±15*§ |
| **Creatinine** (mg/dL) | 0.4±0.1 | 1.8±0.2*§ | 0.6±0.1 | 2.1±0.2*§ | 1.8±0.1*§ |
| **PM thickness** (μm) | 36±1 | 27±6 | 133±69*# | 165±26*# | 36±4ω§ |
| **α-SMA** (%) | 0.1±0 | 27±6 | 5±0*# | 7±1*# | 3±0ω§ |
| **MØ** (cells/mm²) | 348±180 | 305±92 | 574±198 | 1221±223*#§ | 320±191ω |
| **T-cells** (cells/mm²) | 0±0 | 11±4 | 22±14 | 182±53*#§ | 53±19ω |
| **TNF-α** (RE) | 1±0.1 | 2±1 | 3±0.4 | 10±0.5*#§ | 5±0.1*ω |
| **IL-1β** (RE) | 1±0.4 | 5±0.2 | 4±1 | 22±3*#§ | 5±1ω |
| **IL-6** (RE) | 1±0.5 | 2±0.1 | 3±0.4 | 11±1*#§ | 0.5±0.1ω |

*p<0.05 vs Control, #p<0.05 vs CKD, §p<0.05 vs PF, ωp<0.05 vs CKD+PF. RE: relative expression.

**Conclusions:** ADMSC were effective in protecting the development of peritoneal fibrosis in the experimental model of PF, probably due to their immunomodulatory properties.

**FR-PO920**

**Effect of DNA Demethylation in Experimental Encapsulating Peritoneal Sclerosis** Sun-Hee Park, Hye-Myung Ryu, Eun-Joo Oh, Sehyun Oh, Ji-Sun Ahn, Soon Youn Choi, Ji-Young Choi, Jang-Hee Cho, Chan-Duck Kim, Yong-Lim Kim. *Div of Nephrology and Dept of Internal Medicine, Kyungpook National Univ Hospital, Daegu, Republic of Korea.*

**Background:** Encapsulating peritoneal sclerosis (EPS) is characterized by progressive inflammation and excessive fibrosis of peritoneum in patients on peritoneal dialysis, and eventually leads to constriction of viscera and bowel obstruction in the later stages. This study aims to evaluate the therapeutic effect of DNA demethylation in experimental EPS.

**Methods:** Experimental EPS was induced by intraperitoneal injection of 0.1% chlorhexidine gluconate (CG) and 15% ethanol to non-uremic male SD rats (n= 19) which were divided into 3 groups: control group (C, n=5) with normal saline injection, EPS group (CG, n=7) with CG injection for 4 weeks and treatment group (CGA, n=7) with 5'-azacytidine injection for the last 2 weeks during CG injection. Morphometric analysis of peritoneum was performed with immunohistochemical staining for type 1 collagen and α-SMA. The expression of TGF-β, fibroblast-specific protein (FSP)-1 and DNA methyltransferase (DNMT)-1 were analyzed by western blot. Methylation specific PCR for Ras GTPase activating-like protein 1 (RASAL1) was performed with measurement of RASAL1 protein expression.

**Results:** The thickness of parietal peritoneum and the number of vessels in omental tissue were significantly decreased in CGA group compared to CG group. The expression of type 1 collagen, α-SMA, TGF-β and FSP-1 were significantly attenuated in CGA group compared to CG group. DNMT1 was significantly increased in CG group, whereas it was reduced in CGA group. Hypermethylation of RASAL1 was associated with decreased expression of RASAL1 protein in CG group, whereas it was reversed in CGA group.

**Conclusions:** DNA demethylation by 5'-azacytidine treatment improved pathologic changes of peritoneum in experimental EPS and it was associated with reversal of increased expression of DNMT1 and hypermethylation of RASAL1.

*Funding:* Private Foundation Support

**FR-PO921**

**Statin Inhibits Peritoneal Dialysis-Related Epithelial-Mesenchymal Transition** Hye-Young Kang,[1] Hyung Jung Oh,[2] Seung Hyeok Han,[2] Shin-Wook Kang.[1,2] *[1]BK21; [2]Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea.*

**Background:** Small GTPases are demonstrated to be activated through the mevalonate pathway and to be involved in epithelial-mesenchymal transition(EMT). However, little is known about the effect of statins, which inhibit isoprenylation, on peritoneal dialysis(PD)-related EMT.

**Methods:** In vitro, human peritoneal mesothelial cells(HPMCs) were exposed to normal glucose(NG,5.6mM), NG+mannitol, or high glucose(HG,100mM) with or without simvastatin(SV,1μM). In addition, isoprenoid product(GGPP,5μM)-induced EMT and HG-induced small GTPases were evaluated. In vivo, PD catheters were inserted into 32 Sprague-Dawley rats, and saline(C,n=16) or 4.25% PD fluid(PDF,n=16) was infused for 4 wks. 8 rats from each group were treated with 5mg/kg/day of SV intraperitoneally. EMT markers in HPMCs and peritoneum were evaluated by Western blot analysis, and immunofluorescence or immunohistochemical staining.

**Results:** Compared to NG group, E-cadherin expression was significantly decreased, while α-SMA, snail, and fibronectin expression were significantly increased in HPMCs exposed to HG, and these changes were significantly abrogated by SV($p<0.05$). In addition, cobblestone-like appearance of HPMCs was converted into a fibroblast-like morphology after HG treatment, which was reversed by SV. Similar changes of EMT in HPMCs were also induced by GGPP($p<0.05$). HG significantly increased the expression of RhoA and Rac1 in the membrane fractions of cultured HPMCs, and these increases were ameliorated by SV($p<0.05$). Moreover, HG-induced EMT was attenuated by RhoA or Rac1 inhibitor($p<0.05$). Compared to C rats, the thickness of the mesothelial layer and the intensity of Masson's trichrome staining in the peritoneum were significantly greater in the PDF group($p<0.05$). Furthermore, E-cadherin expression in the peritoneum was significantly decreased, whereas α-SMA, snail, and fibronectin expression were significantly increased in PDF rats, and these changes were significantly abrogated by SV($p<0.05$).

**Conclusions:** PD-related EMT was significantly inhibited by statin treatment, suggesting that PD-related EMT was mediated by the activation of small GTPases through the mevalonate pathway.

**FR-PO922**

**The Predictive Value of Matrix Metalloproteinase-2 and Plasminogen Activator Inhibitor-1 in Peritoneal Dialysis Patients Who Develop Encapsulating Peritoneal Sclerosis** Deirisa Lopes Barreto, Dirk Gijsbert Struijk, Raymond T. Krediet. *Internal Medicine, Div of Nephrology, Academic Medical Center, Univ of Amsterdam, Amsterdam, Netherlands.*

**Background:** Encapsulating peritoneal sclerosis (EPS) is the most severe morphological complication that may occur in 3% of peritoneal dialysis (PD) patients. Recently the use of effluent matrix metalloproteinase-2 (MMP-2) and plasminogen activator inhibitor-1 (PAI-1) as potential biomarkers of peritoneal fibrosis has been demonstrated during longitudinal follow-up of incident PD patients. This study focuses on effluent MMP-2 and PAI-1 as early markers in the preceding years of patients who develop EPS.

**Methods:** In this nested case-control study, patients who developed EPS were compared to controls with a PD duration of at least 57months. Levels of effluent MMP-2 and PAI-1 were determined by an ELISA and appearance rates (AR) were calculated. The time courses of AR MMP-2 and PAI-1 were studied by means of a linear repeated-measures model in the years prior to the diagnosis of EPS adjusted for age. Furthermore, time-specific ROC analyses were executed to assess the predictive value of the markers in the years prior to diagnosis of EPS.

**Results:** In total 11 patients developed EPS and 34 controls were assembled. No difference in the time course of AR MMP-2 was present between controls and EPS patients. In contrast, higher AR of PAI-1 were found in EPS patients as compared to the controls ($p=0.002$). The time-specific ROC analyses indicated a discriminative ability for PAI-1 ($AUC=0.78$, $p=0.01$), whereas this was absent for AR of MMP-2.

**Conclusions:** It is unlikely that MMP-2 can be used as a biomarker of EPS as no distinction between patients who develop EPS and controls can be made. The time course confirms a persistent contribution of effluent MMP-2 in peritoneal tissue remodeling. Throughout, elevated levels of AR PAI-1 are present in patients who develop EPS, pointing to progressive peritoneal fibrosis and sclerosis. AR PAI-1 has a fair discriminative capacity from 3 years prior to EPS diagnosis. Therefore, PAI-1 can possibly be used to monitor peritoneal fibrosis and serve as biomarker of EPS.

**FR-PO923**

**Over-Expression of E-cadherin Attenuated Epithelial-Mesenchymal-Transition Induced by TGF-β in Human Peritoneal Mesothelial Cells** Hao Zhang, Ke Zhang, Liu Yan, Bin Yi. *Nephrology, The Third Xiangya Hospital, Changsha, Hunan, China.*

**Background:** Uptodate the investigation shows that E-cadherin(ECAD) might not only the effector but also regulator in Epithelial mesenchymal transition(EMT). We aimed to over express the ECAD with plasmid transfection into human peritoneal mesothelial cell lines (HMrSV5) and to investigate the effects of ECAD over-expression on the epithelial-mesenchymal transition HMrSV5 cells.

**Methods:** 1. Using E. coli DH5α to amplify the ECAD cDNA plasmid and blank plasmid, the plasmid was identified by enzyme digestion and RT-PCR; 2. HMrSV5 cells were divided into 3 groups: control group, ECAD group (transfected with ECAD plasmid) and blank plasmid group (transfected with blank plasmid). The transfection efficiency was detected by Flow cytometry. The level of ECAD mRNA and protein in 12h, 24h, 36h, 48h and 72h were detected by RT PCR and western blot; 3. HMrSV5 cells were divided into groups: normal group, TGF-beta group and E-cadherin +TGF-beta group (transfected with E-cadherin and then treated by TGF-beta with 12h, 24h, 36h and 48h, respectively). The level of E-cadherin mRNA was detected by RT PCR. The level of α-SMA and Vimentin protein were detected by western Blot. Immunofluorescence was used to detect the ECAD and α-SMA protein level.

**Results:** 1. The recombinant plasmids are verified by sequencing analysis, with the sequence identified in gene bank.

2. The transfection efficiency with ECAD transfection was peaked on 36h(74.12%). Level of ECAD mRNA is increased with ECAD transfection after 12h ($p<0.05$), and peaked on 36h ($p<0.01$).

3. In TGF-β group, the protein level of α-SMA and Vimentin are increased by TGF-β in a time-dependent manner ($p<0.01$, compared with control group), and ECAD mRNA level is decreased ($p<0.01$, compared with control group). Otherwise, the effect is attenuated in ECAD +TGF-beta group ($p<0.01$, compared with TGF-β group).

**Conclusions:** 1. ECAD cDNA plasmid transfection does over express ECAD in HMrSV5 cells; 2. Over-expression ECAD with ECAD cDNA plasmid can attenuate the EMT induced by TGF-β in HMrSV5cells.

*Funding:* Government Support - Non-U.S.

**FR-PO924**

**Paricalcitol Ameliorate Epithelial to Mesenchymal Transition of Peritoneal Mesothelial Cells** Seokhui Kang,[1] Tae woo Kim,[2] Kyu-hyang Cho,[1] Jong-Won Park,[1] Kyung woo Yoon,[1] Jun-Young Do.[1] *[1]Internal Medicine, Yeungnam Univ Hospital, Daegu, Republic of Korea; [2]Internal Medicine, Soonchunhyang Univ Gumi Hospital, Gumi, Republic of Korea.*

**Background:** Paricalcitol plays an important role not only in the establishment and maintenance of calcium metabolism but also in direct effects on renal and/or extra-renal tissues. The aim of this study is to evaluate the effect of paricalcitol on epithelial to mesenchymal transition (EMT) of peritoneal mesothelial cells.

**Methods:** The effects of paricalcitol on EMT in both human peritoneal mesothelial cells (HPMCs) and peritoneal membranes were evaluated. HPMCs were incubated with TGF-β and/or paricalcitol. EMT was assessed with α-smooth muscle actin (α-SMA) and E-cadherin. Furthermore, 36 male Sprague-Dawley rats were equally divided into three groups: C (control), PD (peritoneal dialysis), and PD + Pari (peritoneal dialysis and paricalcitol). Peritoneal dialysis were given twice daily for 8 weeks. The morphometric analyses performed on the peritoneal membranes of tissue specimens obtained at the end of the study.

**Results:** In vitro, exposure of HPMCs to TGF-β resulted in an increase of the expression of mesenchymal markers such as α-SMA and was associated with a decrease in the expression of epithelial markers, E-cadherin. Treatment of HPMCs with paricalcitol showed an amelioration of TGF-β induced changes in markers of EMT. In addition, paricalcitol decreased the expression of phosphorylated-Smad2/3 and increased the expression of Smad4. The cobblestone-like appearance of HPMCs were converted to a fibroblast-like morphology after treatment with TGF–β. Treatment with paricalcitol blocked this morphologic transformation. In vivo, Trichrome-stained parietal peritoneum

of abdominal wall showed a marked increase in submesothelial matrix in PD group, which is ameliorated by paricalcitol (7.42 ± 1.35 μm in C group, 49.26 ± 7.89 μm in PD group, and 22.04 ± 3.64 μm in PD + Pari group).

**Conclusions:** Our results suggest that paricalcitol has a protective effect on EMT in peritoneal mesothelial cells. It was mediated by TGF-β/Smad pathway.

## FR-PO925

**Impact of Alanyl-Glutamine on Peritoneal Health – Results from the Clinical Phase I/II of PD-protec™** Klaus Kratochwill,[1] Michael Boehm,[1] Rebecca Herzog,[2] Katharina Gruber,[1] Anton Lichtenauer,[2] Lilian Kuster,[2] Andreas Gleiss,[1] Christoph Aufricht,[1] Andreas Vychytil.[1] *[1]Medical Univ of Vienna, Austria; [2]Zytoprotec GmbH, Austria.*

**Background:** Peritoneal dialysis fluids (PDF) impair peritoneal health via their bio-incompatible composition, resulting in clinical complications such as deterioration of membrane function and infections. Supplementation of parenteral nutrition with alanyl-glutamine dipeptide has been shown to improve clinical outcome e.g. in critically ill patients.

**Methods:** In a phase I/II trial (EudraCT 2010-022804-29) at the Medical University of Vienna 20 stable patients (6 with a history of prior peritonitis) on PD underwent a single 4 h dwell of standard PDF (Dianeal® PD4 3.86%, Baxter) or PD-protec™ (Dianeal® with 8 mM alanyl-glutamine (Dipeptiven®, Fresenius-Kabi)) in an open label, randomized, two-period, cross-over design. Following a peritoneal equilibration test (PET) PD effluent was tested for oxidative stress, sterile inflammation and reduced immunocompetence.

**Results:** Safety of PD-protec™ was demonstrated by stable laboratory and clinical data and by absence of any drug-related adverse event. PD-protec™ also demonstrated efficacy in the sub-group of 'peritonitis positive' patients with attenuated peritoneal levels of advanced oxidized protein products (AOPP) and significantly reduced interleukin 8 (IL-8). In addition, highly sensitive fluorescent cyanine dyes and 2D-DIGE were used to quantify the oxidative stress proteome of cell pellets from PD effluents (5 μg) yielding 2D gels containing more than 1300 spots. *Ex-vivo* assay exposure of PBMC to PD effluent from the PD-protec™ group significantly improved LPS-induced cytokine release, associated with significantly increased intracellular glutamine levels.

**Conclusions:** The obtained data clearly show the potential of PD-protec™ for improving peritoneal health by counteracting PD related pathomechanisms at least in the clinically highly relevant risk population that has previously suffered from peritonitis. These results will form the basis for a trial investigating the effects of more extended treatment in a larger number of PD patients.

*Funding:* Pharmaceutical Company Support - Zytoprotec GmbH

## FR-PO926

**The Peritoneal Endothelial Glycocalyx in Uremic Rats – The Effect of Dialysis Solutions and Relationships with Peritoneal Transport** Carmen A. Vlahu,[1] Dirk Gijsbert Struijk,[1,2] Raymond T. Krediet.[1] *[1]Dept of Nephrology, Academic Medical Center, Amsterdam, Netherlands; [2]Dianet Foundation, Utrecht, Netherlands.*

**Background:** Continuous peritoneal dialysis (PD) leads to continuous exposure of the peritoneal membrane to high concentrations of glucose and its degradation products, causing inflammation and angiogenesis, which may all alter the endothelial glycocalyx. We investigated the changes in peritoneal endothelial glycocalyx induced by chronic kidney failure (CKF) and by long-term PD, using a conventional or a 'biocompatible' dialysis solution. These changes were related to peritoneal transport and morphological alterations of the peritoneal membrane.

**Methods:** Forty-four Wistar rats were divided in 4groups: normal kidney function (NKF), CKF, CKF exposed to Dianeal 4.25% (CKDD), or Physioneal 3.86% (CKDP). CKF was induced by 70% nephrectomy. At 16 weeks, the rats underwent Sidestream Darkfield (SDF) imaging of the peritoneal blood vessels. The status of endothelial glycocalyx was assessed by measuring the perfused boundary region (PBR) near the vessel wall. A standard peritoneal permeability test adapted for the rat and light microscopy were performed.

**Results:**

| | NKF | CKF | CKDD | CKDP |
|---|---|---|---|---|
| Renal creat cl (ml/min) | 4.3±0.7 | 1.8±0.5* | 2.3±0.5* | 1.8±0.6* |
| D/P creat | 0.4±0.06 | 0.5±0.03 | 0.6±0.08* | 0.6±0.05* |
| Glucose abs (%) | 62.2±8.6 | 61.8±1.5 | 69.9±1.4 | 66.7±3.3 |
| Fibrosis score | 0(0-1) | 2(0-3)* | 5(4-8)* | 4(3-6)* |
| Vessel density | 8.6±1.9 | 13.1± 4.9* | 30.4±5.4* | 18.9±5.2* |
| PBR(μm) | 2.2±0.2 | 2.1±0.4 | 2.1±0.2 | 2.1±0.1 |

*p<0.05 versus NKF.

**Conclusions:** Although we could reproduce the functional and morphological peritoneal abnormalities we described previously, this was not associated with a difference in the state of the endothelial glycocalyx as measured by SDF imaging, between the groups. Also no relationship with parameters of light microscopy and peritoneal transport could be established. This suggests that the damage induced by dialysis solutions is independent of the endothelial damage from the circulation.

## FR-PO927

**Systemic Microvascular Endothelial Glycocalyx and Peritoneal Transport in Peritoneal Dialysis Patients** Carmen A. Vlahu,[1] Dirk Gijsbert Struijk,[1,2] Hans Vink,[3] Raymond T. Krediet.[1] *[1]Dept of Nephrology, Academic Medical Center, Amsterdam, Netherlands; [2]Dianet Foundation, Utrecht, Netherlands; [3]Cardiovascular Research Institute Maastricht, Netherlands.*

**Background:** During peritoneal dialysis (PD), the capillary wall, coated at the luminal side by the endothelial glycocalyx, represents the main transport barrier between blood and the peritoneal cavity, and could thereby influence peritoneal transport. However, the magnitude of peritoneal solute transport is also dependent on the number of perfused peritoneal capillaries. In this study we investigated the relationship between the systemic endothelial glycocalyx and parameters of peritoneal transport in PD patients.

**Methods:** Investigations were performed in 17 stable PD patients. We performed Sidestream Darkfield imaging of the sublingual blood vessels. The state of the endothelial glycocalyx was assessed by measuring the red blood cells column width (RBCW) and the perfused boundary region (PBR) near the vessel wall. All patients underwent a Standard Peritoneal permeability Analysis.

**Results:** PBR and RBCW were 3.6±0.4μm and 10.2±0.6μm. Fast transport status was defined as the presence of two of the following parameters: glucose absorption >75%, MTACcreatinine>13 ml/min, MTACurate>11 ml/min. In the patients with a non fast transport status, PBR positively correlated with net ultrafiltration ($p<0.01$, $r=0.6$) and negatively correlated with MTACcreatinine ($p=0.04$, $r=-0.4$) MTACurea ($p=0.02$, $r=-0.5$) and MTACurate ($p=0.02$, $r=-0.5$), glucose absorption ($p<0.01$, $r=-0.7$). Such relationships were absent in those with a fast transport status.

**Conclusions:** The negative correlation between the state of the systemic endothelial glycocalyx and the transport of small solutes, suggests that a thicker permeable fase of the glycocalyx delays the access of small solutes from blood to the small inter-endothelial pores, probably by decreasing their diffusion velocity. The above mentioned correlation was absent in patients with fast transport status, suggesting that in this group, the increase in the number of perfused vessels, probably induced by locally produced substances, leading to an increased number of functional small pores, prevails.

## FR-PO928

**High Glucose Promotes TGF-β1 Production by Inducing FOS Expression in Human Peritoneal Mesothelial Cells** Shigehiro Doi,[1] Ayumu Nakashima,[2] Takao Masaki.[3] *[1]Blood Purification, Hiroshima Univ Hospital, Hiroshima, Japan; [2]Regeneration and Medicine, Hiroshima Univ Hospital, Hiroshima, Japan; [3]Nephrology, Hiroshima Univ Hospital, Hiroshima, Japan.*

**Background:** Peritoneal fibrosis is a serious complication of peritoneal dialysis (PD). A high concentration of glucose in PD fluid is a major factor in the development and pathology of peritoneal fibrosis. Although it is known that high glucose induces production of transforming growth factor-beta1 (TGF-β1), the mechanism remains elusive. The aim of this study was to determine the gene(s) involved in high glucose-induced TGF-β1 production in human peritoneal mesothelial cells (HPMCs).

**Methods:** Microarray analysis was performed following 3-hour preincubation of HPMCs in 4% glucose medium. Amang of the up-regulated genes, the transcriptional genes were selected by Gene Ontology analysis for biological processes, including regulation of transcription and DNA-dependent. In the selected genes, mRNA amplication were confirmed. Effects of the small interfering RNA (siRNA) treatments of the increased genes on the up-regulation of TGF-β1 mRNA were assessed by Real time PCR. Finally, enzyme-linked immunosorbent assays (ELISA) was performed to determine the gene(s) that contributes to production of TGF-β1 protein in the medium.

**Results:** Microarray analysis revealed that the expression of 51 genes increased by more than 3-fold. By Gene Ontology analysis, 13 were selected for further study. Real time PCR confirmed mRNA amplification for 9/13 genes. Furthermore, the high glucose-induced up-regulation of TGF-β1 mRNA was attenuated by the siRNA of 4 genes: MDS1 and EVI1 complex locus (MECOM), FBJ murine osteosarcoma viral oncogene homolog B (FOSB), FBJ murine osteosarcoma viral oncogene homolog (FOS) and activating transcription factor 3 (ATF3). ELISA showed that siRNA treatment of FOS, but not MECOM, FOSB or ATF3, suppressed the increase of TGF-β1 protein in the medium.

**Conclusions:** FOS is a downstream effector of high glucose stimulation in HPMCs that contributes to TGF-β1 production, and suggest that blocking FOS expression may be a therapeutic target for peritoneal fibrosis.

*Funding:* Private Foundation Support

## FR-PO929

**Peritoneal Fibrosis: A Study Comparing Histological Assessment with Abdominal Wall UltraSonography** Alferso C. Abrahams,[1] Amelie Dendooven,[2] Jan Willem Van der Veer,[1] Gerard Stapper,[3] Paul Berger,[4] Tri Q. Nguyen,[2] Marianne C. Verhaar,[1] Walther H. Boer.[1] *[1]Nephrology; [2]Pathology; [3]Radiology; [4]Surgery, UMC, Utrecht, Netherlands.*

**Background:** Long-term peritoneal dialysis (PD) causes peritoneal thickening (fibrosis). Longitudinal measurements of the thickness of the peritoneum could be useful to screen PD patients for evolving encapsulating peritoneal sclerosis. Abdominal wall ultrasonography (US) has been used to measure peritoneal thickness. However, no studies have directly related measurement of the peritoneal thickness by US with histologic examination of peritoneal biopsies. We hypothesized that US provides a reliable non-invasive method to measure peritoneal thickness.

**Methods:** We obtained biopsies of the parietal peritoneum of 50 patients during kidney transplantation: 5 uremic patients, 13 HD patients, and 32 PD patients with different vintage (<2yrs n=10; 2-4yrs n=14; >4yrs n=8). The extent of peritoneal fibrosis was determined by measuring the thickness of the submesothelial compact zone on H&E-stained formalin-fixed tissue sections. In 32 of these patients the parietal peritoneum was examined by US using a high resolution 2D real-time scanner with a linear 17 mHz probe.

**Results:** No agreement between the methods was observed when the difference between the paired measurements was plotted against the mean value (bias=257μm, 95% limit of agreement 4.6-511μm) with US measuring higher results compared to biopsy assessments (371±95μm vs 113±62μm, p<0.0001). Increased peritoneal thickness as assessed in biopsies was observed in patients who had undergone PD for >4yrs (160±102μm) compared to uremic patients (94±41μm, β=83μm, p=0.011) and patients who had undergone PD for <2yrs (94±41μm, β=83μm, p=0.008). US showed no differences in thickness between groups.

**Conclusions:** This is the first study that directly compares measurements of peritoneal thickness by US to microscopic examination. We observed no agreement between the methods. This may be due to the possibility that US measurements include other components of the parietal peritoneum than the submesothelial compact zone. Prospective longitudinal studies are needed to determine whether US can be used for routine assessment of PD treatment.

## FR-PO930

**Suppressive Effects of Intraperitoneal L-carnitine on Peritoneal Fibrosis Caused by Chronic Dialysate Exposure in Rats** Yoshihiro Matsumoto, Yasushi Shimada, Youichi Nojima. *Dept of Nephrology, Shizuoka City Hospital, Japan.*

**Background:** Conventional glucose-containing peritoneal dialysates are reportedly not biocompatible and may be associated with the development of peritoneal sclerosis. L-carnitine is a natural substance (molecular weight, 161 Da) that plays a critical role in fatty acid oxidation and metabolic homeostasis. In the present study, we aimed to evaluate the beneficial effects of L-carnitine on peritoneal fibrosis that develops due to chronic exposure to conventional glucose-containing dialysates.

**Methods:** We prepared dialysis fluids containing 188 mM glucose (GluPD) as well as dialysis fluids containing 169 mM glucose and 19 mM L-carnitine (10% of glucose replaced with L-carnitine to a concentration of 0.3% L-carnitine; CarPD). Sprague-Dawley rats (n = 7 for each treatment) were intraperitoneally administered GluPD or CarPD twice a day for 6 days per week. After 13 weeks, we histologically examined the parietal peritoneum of the rats and measured peritoneal thickness. Mesothelial cells on the liver surface were enumerated using imprint technique, and the numbers of mesothelial cells were compared between the treatment groups.

**Results:** The peritoneal thickness in rats treated with GluPD and CarPD was significantly greater than that in non-treated rats. The thickening of the peritoneum in rats treated with CarPD was significantly suppressed by 50–70% compared to that in rats treated with GluPD. However, the number of mesothelial cells on the liver surface was greater in rats treated with GluPD and CarPD as compared to that in non-treated rats; the difference in this value between rats treated with GluPD and CarPD was not significant.

**Conclusions:** Considering the suppression of peritoneal thickening in rats treated with CarPD, the use of the L-carnitine-containing dialysates might be effective in improving or preventing peritoneal sclerosis in dialysis patients.

## FR-PO931

**Expression of microRNA-200c and Its Role in the Process of High Glucose Induced EMT in Human Peritoneal Meosthelial Cells** Hong Liu, Xiang Zhou, Youming Peng, Fu-You Liu. *Dept of Nephrology, The Second Xiangya Hospital of Central South Univ, Changsha, Hunan, China.*

**Background:** To investigate the effect of miRNA-200c in the process of high glucose induced EMT and ECM accumulation in HMrSV5 cell lines.

**Methods:** HMrSV5 cells were exposed to 60mM D-glucose.The expressions of E-cadherin, vimentin ,FN and COL-1 were examined by realtime PCR and western blo, meanwhile realtime PCR for miRNA-200c.Furthermore HMrSV5 cells were transfected with miRNA-200c mimic or miRNA mimics negative control before exposing to 60mM D-glucose.Then the expressions of E-cadherin, col-1, vimentin and FN were detected by immunofluorescence, realtime PCR and western blot.

**Results:** Stimulation of HMrSV5 cells with high gluose resulted in a significant decrease of E-cadherin and increase of vimentin, COL-1 and FN, all in time-dependent manner. High glouse also repressed miRNA-200c.Compared to high glouse group, overexpression of miRNA-200c in HMrSV5 cells upregulated the mRNA and protein expression of E-cadherin, and downregulated the mRNA and protein expression of vimentin,COL-1 and FN.However, miRNA mimics negative control had no significant effect on the expression of E-cadherin, vimentin ,COL-1and FN.

**Conclusions:** Our data suggest that microRNA-200c plays a significant role in the progression of high glucose induced peritoneal fibrosis.

## FR-PO932

**Interferon-Gamma Alters Mesothelial Cell Response to Transforming Growth Factor Beta-1, Promoting Peritoneal Fibrosis** Donald Fraser,[1] Tanya Jayne Bodenham,[1] Timothy Bowen,[1] Nicholas Topley.[2] *[1]Insitute of Molecular and Experimental Medicine, Cardiff Univ, Cardiff, Wales, United Kingdom; [2]Institute of Infection, Immunity and Biochemistry, Cardiff Univ, Cardiff, Wales, United Kingdom.*

**Background:** Peritonitis infections are a serious complication for patients receiving peritoneal dialysis (PD) leading to progressive fibrosis of the peritoneal membrane, one of the major reasons for treatment failure in PD patients. TGF-β1 is a key regulator of tissue repair and the fibrotic process and the interaction between TGF-β1 and pro-inflammatory signalling may affect how cells respond to this cytokine either by repair and resolution, or by fibrosis and disease.

**Methods:** In vivo and in vitro systems have been used to characterise the interplay between TGF-β1 signalling and Signal Transducer and Activator of Transcription (STAT) activation by Interleukin 6 (IL6) and Interferon gamma (IFN-y). The in vivo system consists of a murine model of inflammation driven fibrosis, where mice are injected with repeated inflammatory stimulation resulting in scarring of the peritoneum. The in vitro system comprises culture of primary human peritoneal mesothelial cells (HPMC) in the presence of TGF-β1 +/- IFN-y.

**Results:** Stimulation of HPMC with TGF-β1 results in induction of matrix metalloproteinase 3 (MMP3) at the mRNA and protein level, which is specifically inhibited in the presence of IFN-y. IFN-y did not alter other TGF-β1 responses. Increased matrix production has been shown in the in vivo system thus supporting the in vitro findings. Chemical inhibition of mitogen activated kinase pathway (MAPK) through blockade of ERK 1/2 and p38 signalling prevented the TGF-β1 dependent induction of MMP3. This induction was also blocked via inhibition of SMAD 3 phosphorylation, thus suggesting that MMP3 induction via TGF-β1 requires both SMAD dependent and SMAD independent signalling pathways.

**Conclusions:** Within HPMC, IFN-y appears to promote fibrosis by favouring matrix accumulation over remodelling through specific inhibition of MMP3. Current investigations are directed at the mechanism by which IFN-y regulates MMP3 expression.

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## FR-PO933

**Heme Oxygenase-1 Attenuates Lipopolysaccharide (LPS)-Induced TLR4 and Proinflammatory Signaling in Human Peritoneal Mesothelial Cells (HPMCs)** Jongho Shin, Jeongho Kim, Jinuk Jeong, Kitae Bang. *Nephrology, Eulji Univ Hospital, Dae-jeon, Republic of Korea.*

**Background:** Bacterial peritonitis is a major complication of PD and a leading cause of technique failure. Recognition of bacterial pathogens by the peritoneum is mediated in part by toll-like receptors. Heme oxygenase-1 gene expression by LPS in macrophages is not only induced via a TLR-4-dependent mechanism, but also increased HO-1 activity has also been shown to have inhibitory effects on intracellular signaling, that is initiated by TLR-4 activation. This regulatory interplay between TLR-4 and HO-1 appears to form a negative feedback loop which might inhibit excessive activation of macrophage by LPS. However, The regulatory effects of HO-1 overexpression on LPS-induced inflammation, which plays a leading cause of technique failure, are unknown in HPMCs, yet. So, the objectives of this study are examine the effects of overexpression of human HO-1 on LPS-induced inflammation in HPMCs.

**Methods:** We treated HPMCs with LPS(1μmol/l) and HO-1 inducer(hemin,10μmol/l). To further investigate the pure effect of HO-1 on LPS-induced inflammation, Gene transfer of recombinant Adenovirus-harboring human HO-1 to HPMCs was done. The involvement of MAP kinase family and nuclear factor(NF)-κB in these processes was also studied.

**Results:** HPMCs constitutively expressed TLR4 and HO-1. LPS increased the expression of TLR4 and HO-1. A prominent induction of IL-8 was detected after LPS stimulation. Treatment of HPMCs with HO-1 inducer, hemin showed a amelioration of LPS- induced changes in expression of TLR4 and IL-8 with increase of expression of HO-1. Human HO-1 gene transfection resulted in a significant increase in HO-1 expression and ameliorated LPS- induced changes. NF-κB expression was also detected but ERK and JNK were not detected in our study like other previous reports.

**Conclusions:** Our study suggest that HO-1 pathway is involved in LPS-induced TLR4 responsiveness and HO-1 may regulate LPS-induced inflammation in HPMCs. This study has implications for improving treatment of infection in PD patients and is the first to show the beneficial effect of HO-1 on attenuating LPS-induced inflammation in HPMCs.

## FR-PO934

**Competing Risks of Encapsulating Peritoneal Sclerosis: Results from PD-CRAFT** Mark Lambie,[1] David W. Johnson,[2] Simon J. Davies.[1] *[1]Health Services Research Unit, Keele Univ, Stoke on Trent, United Kingdom; [2]Queensland Univ, Brisbane, Australia.*

**Background:** Encapsulating peritoneal sclerosis (EPS) is an uncommon complication of peritoneal dialysis (PD), where the risk increases significantly with increasing time on therapy. We hypothesised that, at the start of PD, risk factors for death would decrease the risk of developing EPS as death will act as a competing risk for EPS.

**Methods:** We combined 3 large datasets (AnzData, Global Fluid Study, Scottish Renal Registry(SRR)) with complete data on EPS occurrence and the denominator population. All incident patients aged ≥15 years were included and a competing risks survival analysis used with outcomes of censored, EPS (prior to death) or death and robust standard errors.

Comorbidity data was classified by either primary renal diagnosis (low comorbidity = glomerulonephritis, polycystic kidney disease, chronic pyelonephritis, high comorbidity = other) and diabetic status (all 3 datasets) or by Stoke comorbidity score (AnzData and Global).

**Results:** There were 112 cases of EPS out of 17,912 patients. The cumulative incidence at 10 years varied from 0.04 in AnzData, to 0.25 in SRR. Competing risks models showed age (SHR 0.79 per decade, 95% CI 0.5-0.83) and high comorbidity renal disease (SHR 0.54, 95% CI 0.41-0.73) decreased the risk of EPS which Cox models failed to demonstrate. The SRR had a SHR of 5.62 (95% CI 5.28-6.21) relative to AnzData but this was not through a decreased mortality (HR for mortality in SRR vs AnzData in adjusted Cox model 1.14, 95% CI 1.05-1.42) or through longer periods of PD (median months on PD, SRR 22.6, AnzData 21.1, p=0.2). The Global dataset had an intermediate risk (SHR relative to AnzData 2.11, 95% CI 1.78-2.49) but the numbers were small so no further analysis was performed on this.

**Conclusions:** For patients commencing PD, factors that increase the risk of death decrease the risk of developing EPS. Competing risks regression is an appropriate model for analysis of dialysis outcomes. The Scottish Renal Registry has a significantly higher rate of EPS than found in AnzData, possibly due to ascertainment bias or genetic factors.

*Funding:* Pharmaceutical Company Support - Baxter

## FR-PO935

**Peritoneal Membranes Are More Inflamed in Patients with Subsequent Encapsulating Peritoneal Sclerosis: Results from the GLOBAL Fluid Study**

Mark Lambie,[1] Nicholas Topley,[2] Simon J. Davies.[1] *[1]Health Services Research Unit, Keele Univ, Stoke on Trent, United Kingdom; [2]Cardiff Univ School of Medicine, Cardiff, United Kingdom.*

**Background:** Risk factors for Encapsulating Peritoneal Sclerosis (EPS), a serious, uncommon complication of Peritoneal Dialysis (PD), include a fast peritoneal solute transport rate (PSTR) and a fast PSTR is associated with increased peritoneal inflammation. Whether inflammation is present in patients with subsequent EPS is not known.

**Methods:** We performed a nested case control study on UK patients from a multi-centre, international, cohort study (the GLOBAL Fluid Study). All definite cases of EPS (n=11) were matched on centre, age and duration of completed PD with 3 controls each. All 4-hour dialysate effluent and plasma samples from cases and controls collected by repeated sampling during routine peritoneal equilibration tests prior to PD cessation were assayed by electrochemiluminescence for IL-6, IFN-γ, TNF-α and IL-1β. Analysis was by multilevel modelling of log-transformed cytokine levels accounting for clustering by person (level 2) and case-control strata (level 3) with EPS and time till PD end as predictors. A skewed distribution of IL-1β necessitated a logistic model.

**Results:** Dialysate IL-6 (β=0.78 of one $\log_{10}$ order of cytokine concentrations, 95%CI 0.13, 1.44, p=0.02), IFN-γ (β=0.63, 95%CI 0.02, 1.24, p=0.04) and TNF-α (β=0.67, 95%CI 0.30, 1.03, p<0.001) were all significantly higher in cases with subsequent EPS. Dialysate IL-1β was not significantly different, possibly due to loss of statistical power through conversion to a binary variable. Plasma IL-6 (β=0.31, 95%CI -0.01, 0.63, p=0.06) tended to run slightly higher, whereas plasma IFN-γ was significantly lower in EPS patients (β=-0.51, 95%CI -0.81, -0.22, p=0.001) and there was no difference in plasma TNF-α. Dialysate and plasma IL-6 rose significantly towards the end of PD, while plasma IFN-γ fell.

**Conclusions:** Peritoneal membranes are more inflamed in PD patients who subsequently develop EPS.

*Funding:* Pharmaceutical Company Support - Baxter

## FR-PO936

**Detection of Pericytes in a Peritoneal Dialysis Rat Model and Its Functional Study**

Nan Chen, Hong Fu Yan, Jingyuan Xie. *Dept of Nephrology, Ruijin Hospital, Shanghai Jiaotong Univ, School of Medicine, Shanghai, Shanghai, China.*

**Background:** Angiogenesis and vascular remodeling of peritoneum are associated closely with ultrafiltration failure(UF) in peritoneal dialysis (PD) patients. Pericytes may relate to angiogenesis and contribute to UF. Although it is still unavailable, the detection of angiogenesis may helpful to the early diagnosis of UF. The purpose of this study is to determine the existence of pericytes and to develop an approach to measure the peritoneum vessels and neovascularization (NV).

**Methods:** 48 SD rats were divided into 7 groups; Dialysate (100 ml/kg/d ) was injected into peritoneal cavity through the PD catheters. Rats in Group N (n=6) were subjected to sham operation; Group U (n=6) which were conducted 5/6 nephrectomy; Rats in Group PD1 (N=6), Group PD2(N=6) and Group PD3(N=6) were injected by 1.5% , 2.5%, 4.25% PD fluid for one month 12w after the 5/6 nephrectomy; Group PD4 used 4.25% PD fluid injection for two months 12w after the 5/6 nephrectomy; Rats in Group N+P (n=6) were performed 4.25% PD fluid injection for one month.

**Results:** We detected pericytes in normal peritoneum, the markers of pericyte were various in different part of peritoneum, in the superficial part, marked by CD248/PDGF-ß, while Desmin/PDGF-ß was detected in deep peritoneum. However, during PD process, the DESMIN marker appears in the superficial part. We found obvious NV in the peritoneum, the degree was obviously associated with the glucose concentration. Spatial distribution of NV present obvious lateral and vertical inhomogeneity. By electron microscopy, we observed two manners of angiogenesis: sprout new blood vessels and the angiogenesis of endothelial like tumor cells. The concentrations of VEGF secreted by the pericyte and VEGF-R 2 secreted by the endothelial cells were both rised, associated with the glucose concentration and the PD duration.

**Conclusions:** We found the pericytes are exist in the peritoneum of PD rat and the surface markers of pericyte changes to match the pathophysiological circumstance. The function of pericytes is related to angiogenesis of peritoneum and has potential values in early prediction of the development of UF.

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## FR-PO937

**Tissue Advance Glycation End Products Deposits Are Reduced in Peritoneal Dialysis Patients Treated with Biocompatible Peritoneal Fluids**

Arkom Nongnuch,[1,2] Stanley Fan,[3] Andrew Davenport.[2] *[1]Renal Unit, Dept of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol Univ, Bangkok, Thailand; [2]Centre for Nephrology, Royal Free Hospital, Univ College London, London, United Kingdom; [3]Dept of Nephrology, Barts & the London National Health Service Trust, Royal London Hospital, London, United Kingdom.*

**Background:** Advance Glycation End Products (AGEs) are a novel biomarker for cardiovascular disease (CVD) and may be absorbed during the intraperitoneal dwell in peritoneal dialysis patients. The amount of AGEs present in standard acidic lactate PD fluids is greater than those found in neutral PD fluid. The objective of this study was to compare skin autofluorescence as a measurement of AGEs between standard and bicarbonate base PD fluid.

**Methods:** We measured tissue AGEs using skin autofluorescence (sAF) (DiagnOptics, Groningen, Netherland) on 145 peritoneal dialysis patients, 78 on standard PD solution and 61 on neutral PD solution.

**Results:** The results are shown in the table.

| Parameters | Standard Solution | Neutral solution | P value |
|---|---|---|---|
| Skin autofluorescence, AU (mean ± SD) | 3.15±1.23 | 3.08±0.9 | NS |
| Age, years (mean ± SD) | 63±16 | 57±16 | 0.03 |
| Hypertension (%) | 55(70) | 33 (54) | 0.04 |
| Diabetes (%) | 30 (38) | 24 (39) | NS |
| Smoking (%) | 42 (53) | 24 (39) | NS |
| History of CVD | 19 (24) | 12 (20) | NS |
| History of PVD | 5 (6) | 4 (6) | NS |
| Dialysis Vintage, median (range), years | 10 (1-156) | 23 (2-82) | 0.004 |
| Urine volume, (median, range), ml | 944 (0-3042) | 850 (0-3324) | NS |
| B2 microglobulin, (median, range), mg/dL | 15.7 (5.2-50.5) | 23.4 (7.8-59.5) | 0.018 |
| Total Kt/V (mean ± SD) | 2.41±1.01 | 2.3±0.83 | NS |

After correcting for multiple statistical analyses, those patients treated with "biocompatible" peritoneal dialysis fluids had similar sAF results despite a longer dialysis vintage and exposure to peritoneal dialysis fluids, and a greater B2 microglobulin, a marker of inflammation and residual renal function.

**Conclusions:** The more biocompatible neutral pH peritoneal dialysis solutions may have beneficial effect on reducing tissue AGE accumulating in PD patients. Further prospective studies are warranted to confirm these encouraging findings.

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## FR-PO938

**IL6 and Peritoneal Dialysis Adequancy**

Sabrina Milan Manani, Grazia Maria Virzì, Alessandra Brocca, Giacomo Mason, Ilaria Tantillo, Carlo Crepaldi, Claudio Ronco. *Nephrology Dep-IRRIV, Italy.*

**Background:** Inflammation is a predictor of all-cause mortality in the general population and in CKD patients. In these patients several factors can contribute to inflammation. Furthermore, in peritoneal dialysis (PD) patients, many systemic and local inflammatory mediators have been associated with membrane failure and increased mortality risk. Inflammation often coexists with malnutrition and there is a relationship between nutritional indices, as serum Albumin (Alb), and mortality. C-Reactive Protein (CRP) is an index of inflammatory activity. IL6 is a proinflammatory cytokine and modulates inflammation. The aim of the study was to investigate the systemic inflammatory biomarkers and assess their relationship with PD adequacy.

**Methods:** Serum levels of Alb, CRP and IL6 were measured in 46 PD patients (31CAPD/15APD). We evaluated IL6 concentration by ELISA. We used weekly $Kt/V_{urea}$ and Creatinine Clearance (wCc) as estimates of PD adequacy. PD patients were divided into 2 groups based on $Kt/V_{urea}$ value: 1.7 was the cut-off value as recommended by K/DOKI guidelines. Statistical analysis was performed by STATA Software. A p<.01 was considered statistically significant.

**Results:** The median values of Alb, CPR and IL6 showed no significant differences between CAPD and APD. IL6 levels showed a positive correlation with CPR (p<.001). IL6 correlated negatively with Alb (p=.01). PD patients with Kt/V <1.7 had significantly higher IL6 compared to PD patients with Kt/V>1.7 (median 62; IQR 33-162 vs 12; IQR 8-59 ng/ml, p=.015).

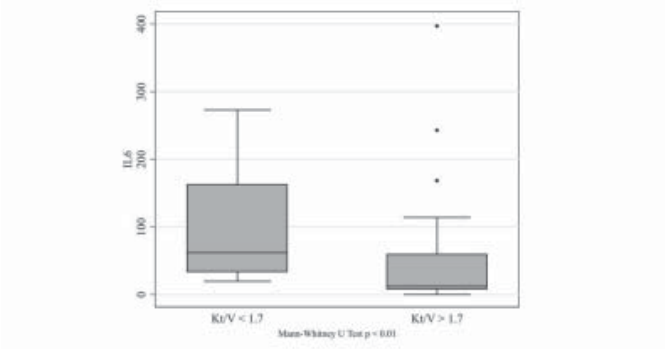


Figure1: PD patients with Kt/V <1.7 had significantly higher IL6 compared to PD patients with Kt/V>1.7 (median 62pg/mL; IQR 33-162 *vs* 12; IQR 8-59 pg/ml)

No statistically significant relationship between IL6 and the wCc was observed, but a positive trend was evident.

**Conclusions:** In conclusion, this study suggested that a low grade of IL6, as a marker of inflammation state, may be considered an index of PD adequacy. It is necessary to increase the sample size of PD subjects enrolled to validate our hypothesis.

## FR-PO939

**The Effect of Bicarbonate Containing Peritoneal Dialysis Solution on Epithelial-to Mesenchymal Transition in Omentum-Derived Mesothelial Cells** Kyu-hyang Cho,[1] Jun-Young Do,[1] Seokhui Kang,[1] Jong-Won Park,[1] Kyung woo Yoon,[1] Tae woo Kim.[2] *[1]Dept of Internal Medicine, Yeungnam Univ Hospital, Daegu, Republic of Korea; [2]Dept of Internal Medicine, Soonchunhyang Univ Gumi Hospital, Gumi, Republic of Korea.*

**Background:** The purpose of this study is to investigate the effect of bicarbonate containing PD (peritoneal dialysis) solution on EMT (epithelial-to mesenchymal transition) in omentum-derived mesothelial cells.

**Methods:** Omentum-derived mesothelial cells from 20 nonuremic patients undergoing abdominal surgery were incubated with lactate-buffered standard PD solutions (L group; Dianeal® and Stay-safe®), bicarbonate/lactate buffered PD solutions (LB group; Physioneal® and Balance®) or bicarbonate buffered PD solution (B group; BicaVera®) diluted 1:1 with culture medium. E-cadherin was measured as standard mesothelial marker by quantitative RT-PCR (real time-polymerase chain reaction) analysis. Snail and alpha-SMA (alpha-smooth muscle actin) were also measured as fibroblast marker by quantitative RT-PCR analysis. The number of CD54 (cluster of differentiation 54) positive cells (indicative mesothelial cells) was counted among confluent omentum-derived mesothelial cells by FACS (fluorescence-activated cell sorter).

**Results:** There were no significant differences in level of E-cadherin, snail and alpha-SMA among three groups.The number of CD54 positive cells among confluent omentum-derived mesothelial cells was B group, BL group and L group in order but the difference did not reach the statistical significance.

**Conclusions:** Bicarbonate containing PD solution groups showed a trend of higher number of CD54 positive cells among confluent omentum-derived mesothelial cells in vitro but without significant statistical differences in this study. We need further studies to clarify the impact of bicarbonate containing peritoneal dialysis solution on EMT in mesothelial cells.

## FR-PO940

**Hepatocyte Growth Factor Signalizes Ultrafiltration Failure in Peritoneal Dialysis Patients** Ana Paula Bernardo,[1,2] Olivia Santos,[1] Maria João Carvalho,[1] António Manuel Nunes Cabrita,[1] Anabela Rodrigues.[1,2] *[1]Nephrology, CHP, Porto, Portugal; [2]UMIB/ICBAS/Porto Univ.*

**Background:** Ultrafiltration failure (UFF) is a leading cause of peritoneal dialysis (PD) drop-out. Increased submesothelial fibrosis is a key process linked with such complication. Hepatocyte growth factor (HGF) ameliorates peritoneal fibrosis both in animal models and in-vitro studies, but no study explored dialysate HGF concentration in PD patients with UFF. Our aim was to assess the relation of dialysate HGF concentration with the UF profile, free water transport quantification (FWT), and small-solute transport ($MTAC_{creatinine}$) in a group of prevalent PD patients.

**Methods:** We performed a 4-hour, 3.86% glucose PET with additional measurement of UF at 60 minutes in 68 PD patients (age 50±14 years, 52% male, on PD for 19±23 months). We calculated Dialysate (D)/Plasma (P) ratio of creatinine, $MTAC_{creatinine,}$ FWT and small-pore ultrafiltration. We measured HGF and CA125 in the effluent.

**Results:** Effluent HGF strongly correlated with UF measured in a 4h, 3.86% glucose PET (r=-0,80, p=0,009), with FWT (r=-0,69, p=0,04) and with $MTAC_{creatinine}$ (r=0,75, p=0,02). Patients with UFF had higher dialysate HGF concentration and HGF/CA125 ratio than patients without UFF (103 pg/mL vs 77 pg/mL, p=0,018 and 6,2 pg/U vs 4,6 pg/U, p= 0,68, respectively). Moreover, patients with severe UFF forms, namely with FWT compromise, had higher dialysate HGF concentration compared with patients with UFF but no FWT compromise (104 pg/mL vs 88 pg/mL, p=0.08). FWT≤45% without UFF was documented in some patients: these also had a higher effluent HGF concentration, but a significantly lower HGF/CA125 ratio compared with those with preserved FWT (3,6 pg/U vs 5,1 pg/U, p=0,014).

**Conclusions:** Our study establishes that dialysate HGF concentration is significantly higher among patients with UFF, specially, if FWT is compromised. This increase possibly signalizes an ongoing counteracting process facing peritoneal membrane deterioration.

## FR-PO941

**Glucose Degradation Product Methylglyoxal Induces Immature Angiogenesis and Peritoneal Dysfunction in Peritoneal Membrane in Patients Undergoing Peritoneal Dialysis** Toshiaki Nakano,[1] Tohru Mizumasa,[2] Hisako Yoshida,[1] Masahiro Eriguchi,[1] Masatomo Taniguchi,[1] Kosuke Masutani,[1] Hideki N. Hirakata,[2] Kazuhiko Tsuruya,[1] Takanari Kitazono.[1] *[1]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [2]First Dept of Internal Medicine, Fukuoka Red Cross Hospital, Fukuoka, Japan.*

**Background:** Angiogenesis in peritoneal membrane has been recognized to induce ultrafiltration failure and possibly lead to encapsulated peritoneal sclerosis in patients on CAPD. We investigated whether glucose degradation product methylglyoxal (MGO) is associated with angiogenesis in peritoneal membrane and induce a disturbance of angiogenesis in vitro.

**Methods:** Peritoneum obtained when CAPD catheters were removed from 61 CAPD patients was analyzed. Peritoneum was immunohistochemically stained with anti-CD34 (for endothelial cells), anti-αSMA (for pericytes) and anti-MGO antibodies. We evaluated the associations between the number of capillary vessels, peritoneal function (D/PCr) and the degree of MGO deposition. In addition, we measured the levels of VEGF and PDGF-BB mRNA in culture endothelial cells (human umbilical vein endothelial cells) and culture smooth muscle cells (human aortic smooth muscle cells) after the administration of MGO 50μM in vitro.

**Results:** We defined CD34-positive and αSMA-negative vessels as capillary vessels in peritoneal membrane using serial sections. The number of capillary vessels was associated significantly with the value of D/PCr and the degree of MGO depositions (both P<0.01). In vitro, the expression of PDGF-BB mRNA and protein in culture endothelial cells significantly decreased after the administration of MGO, though the expression of VEGF mRNA increased (both P<0.01). The expression of PDGF-Rβ mRNA in culture smooth muscle cells did not change after the administration of MGO, though the expression of VEGF mRNA increased (P<0.01).

**Conclusions:** Glucose degradation product MGO enhanced the production of VEGF and suppressed the production of PDGF-BB, suggesting it may lead to a disturbance of angiogenesis in peritoneal membrane. The accumulation of MGO in peritoneum may cause immature angiogenesis and peritoneal dysfunction.

## FR-PO942

**Fetuin Calciprotein Particles May Be Formed in Peritoneal Dialysis Fluid and May Contribute to Peritoneal Inflammation** Edward Robert Smith,[1] Stephen G. Holt.[3] *[1]Dept Renal Medicine, Eastern Health Clinical School, Monash Univ, Melbourne, VIC, Australia; [2]Eastern Health Intergrated Renal Services, Monash Univ, Melbourne, VIC, Australia; [3]Royal Melbourne Hospital, Melbourne Univ, Melbourne, VIC, Australia.*

**Background:** Fetuin-A (Fet-A) is a protein synthesised in the liver circulating at high concentrations. Fet-A forms high molecular weight complexes with calcium phosphate nanocrystals called calciprotein particles (CPP). CPP are detectable in the serum of patients with chronic kidney disease and/or inflammation, but not normal controls. CPP may be cleared by macrophages uptake via scavenger receptor-A, but in doing so cause inflammatory cytokine and oxidant species generation. CPP have been reported in the PD fluid of patients with calcific peritonitis. Since free FetA has a similar molecular weight to albumin and CPP are too big to pass through large peritoneal membrane pores, we tested the hypothesis that CPP might be formed in situ in peritoneal fluid (PDF).

**Methods:** We measured Free FetA, CPP and albumin in spent dialysate and in the serum of 20 patients undergoing PD without obvious complication.

**Results:** Mean (SD) total Fet-A concentration was 187 (30) and 88 (46) mg/L in serum and PDF respectively. The mean (SD) CPP-associated Fet-A concentration was significantly higher in serum than in PDF [45 (13) vs. 24 (16) mg/L, P=0.012]. The proportion of Fet-A present as CPP was not significantly different to serum [24 (7) vs. 23 (6) %, P>0.1]. Mean (SD) albumin concentrations in serum and PDF were markedly different [31 (4) vs.1.2 (0.6) g/L P<0.0001]. Thus, the ratio of Fet-A to albumin was significantly higher in PDF fluid than in serum [81(44) vs 5(1) mg/g, P<0.0001]. PDF CPP Fet-A concentration was significantly correlated with phosphate (r=0.54, P=0.001), total protein (r=0.48, P=0.031) and 8-iso-PGF2α (r=0.48, P=0.032), but not with calcium (P=0.37) or albumin concentrations (P=0.40).

**Conclusions:** CPP formation may occur within the peritoneal cavity. CPP may interact with peritoneal macrophages and contribute to inflammation and oxidant stress.

*Funding:* Pharmaceutical Company Support - Amgen Australia, Private Foundation Support

## FR-PO943

**The Mutual Relationship between Peritonitis and Peritoneal Transport** Sadie Van Esch, Anouk Van diepen, Raymond T. Krediet, Dirk Gijsbert Struijk. *Nephrology, Academic Medical Centre, Amsterdam, Netherlands.*

**Background:** Preservation of the peritoneal membrane is required to achieve long-term PD. We investigated the effect of multiple peritonitis episodes on peritoneal transport.

**Methods:** We prospectively collected transport parameters from 709 incident adult PD patients treated between 1990 and 2010. Strict inclusion criteria were used to select patients: follow-up of at least 3 years with the availability of a standard peritoneal permeability analysis (SPA) in the first year after start of PD and a SPA after the third year of PD, without peritonitis preceding the first SPA. Patients either had to remain peritonitis-free (group 0, n=30) or experienced ≥3 peritonitis episodes (group ≥3, n=16) during follow-up.

**Results:** At baseline the groups were similar with regard to the transport of low molecular weight solutes and fluid. However, group ≥3 had lower peritoneal protein clearances and a higher restriction coefficient compared to group 0. This resulted in lower dialysate concentrations in group ≥3 vs group 0: albumin: 222 mg/L vs 416 mg/L, $p<0.01$, IgG: 36 mg/L vs 69 mg/L, $p<0.01$, $\alpha$2-macroglobulin: 2 mg/L vs 4 mg/L, $p<0.01$. Plasma concentrations were not different. After 3 years, group ≥3 showed a significant increase in the mass transfer area coefficient (MTAC) of creatinine and glucose absorption (GA) with a concomitant decrease in transcapillary ultrafiltration (TCUF) compared with group 0 (MTACcreat: p=0.03, GA: p=0.04, TCUF: p=0.03). No changes in free water transport were found.

**Conclusions:** Slow initial peritoneal transport rates of serum proteins result in lower dialysate concentrations, and likely a lower opsonic activity, which is a risk factor for peritonitis. This disappears during follow-up. Patients with frequent episodes of peritonitis show an increase in the transport of low-molecular weight solutes and a concomitant decrease of ultrafiltration, but no changes in free water transport; this was not found in long-term peritonitis-free PD patients. These findings suggest that frequent peritonitis leads to the development of an increase of the effective vascular peritoneal surface area without the structural membrane alterations that may develop after long-term PD.

## FR-PO944

**Characteristics and Outcomes of Fungal Peritonitis in a Modern North American Cohort** Annie-Claire Nadeau-Fredette, Joanne M. Bargman. *Div of Nephrology, Dept of Medicine, Toronto General Hospital - Univ Health Network, Univ of Toronto, Toronto, Canada.*

**Background:** Peritonitis remains a common complication of peritoneal dialysis (PD). Although representing only 1-12% of overall peritonitis in dialysis patients, fungal peritonitis (FP) is associated with serious complications, including technique failure (15-85%) and death (5-55%). Only scarce data has been published regarding FP outcomes in modern cohorts in North America. In this study, we aim to evaluate rates, characteristics and outcomes of FP in a major North American PD center.

**Methods:** We conducted a retrospective cohort study including all fungal peritonitis among peritoneal dialysis patients followed in a large PD center between January 2000 and February 2013. Our pre-specified end-points included rates of FP, characteristics, outcomes and determinants of death.

**Results:** Thirty-six episodes of FP were identified during the follow-up period (one episode per 671 patient-months), representing 4.5% of the total peritonitis events. Patients' mean age and peritoneal dialysis vintage were 61.3 ± 15.5 and 2.9 (1.5-4.8) years, respectively. Of the 36 episodes of FP, seven (19%) resulted in death and 17 (47%) led to technique failure with permanent transfer to hemodialysis. Surprisingly, PD was eventually resumed in 33% of cases with a median delay of 15 weeks (interquartile range 8-23) between FP and catheter reinsertion. In a univariable analysis, a higher Charlson comorbidity index (Odds ratio [OR] 3.25 per unit increase, 95% confidence interval [CI] 1.23-8.58) and PD fluid white blood cell (WBC) count greater than 3000/ $mm^3$ at presentation (OR 6.56, 95%CI 1.05-40.95) predicted death.

**Conclusions:** While fungal peritonitis is still associated with a high frequency of death and technique failure, one-third of our patients eventually returned to PD. Patients with a high burden of comorbidity and presenting with markedly elevated PD effluent WBC count appear at higher risk of death. We postulate that the high mortality associated with FP is partially related to the severity of comorbidity among patients with FP, rather than the infection per se. Importantly, PD can be resumed in a significant proportion of cases.

## FR-PO945

**Glucose-Based Peritoneal Dialysis Fluids Inhibit Complement-Mediated Host Defenses against *Staphylococcus aureus*** Parvathi S. Kumar,[1] Clifford T. Mauriello,[1] Pamela S. Hair,[3] Reem Raafat,[2] Kenji M. Cunnion.[1] *[1]Dept of Pediatrics, Eastern Virginia Medical Center/ Children's Hospital of the Kings Daughters, Norfolk, VA; [2]Dept of Pediatrics, Children's Hospital of the Kings Daughters, Norfolk, VA; [3]Dept of Pediatrics, Eastern Virginia Medical Center, Norfolk, VA.*

**Background:** Staphylococcus aureus peritonitis is a serious complication of Peritoneal Dialysis (CPD).Complement-mediated effectors of innate host defense are essential for optimal opsonophagocytosis of S.aureus.Hyperglycemic conditions impair optimal C3-mediated opsonization of S.aureus in vitro and in vivo.Since most peritoneal dialysis(PD) fluids are glucose-based,we hypothesized that glucose-based(GB)PD fluids likely inhibit S.aureus-induced complement host defenses compared to amino acid or dextran based PD fluids.

**Methods:** Commercial glucose-based(Dianeal) or dextran-based(Extraneal) PD fluids were purchased.Amino acid-based(Nutrineal) PD fluid was donated by Baxter International. Control PD fluid, excluding glucose,was generated with the same electrolyte composition as Dianeal. Dianeal supplemented with amino acids was also used.Human serum and $10^9$CFU *S.aureus* was incubated in each PD fluid for 1hour at 37°C.The samples were sedimented and the supernatant was assayed for C5a and C3a by ELISA and quantitative Western blot, respectively. Pelleted *S.aureus* was stripped off of covalently-bound C3-fragments and assayed by ELISA and Western blot.

**Results:** GB PD fluids inhibited *S.aureus*-initiated generation of complement anaphylatoxins C3a and C5a.GB PD fluids inhibited C3-fragment(C3b/iC3b) opsonization of *S.aureus* compared to non-glucose containing PD fluids.

**Conclusions:** *S.aureus* activation of complement was severely inhibited by GB PD fluids compared with amino acid or dextran-based PD fluids. Generation of analyphylatoxins critical for neutrophil recruitment and activation was inhibited. Opsonization of *S. aureus* with complement C3b/iC3b,vital for efficient phagocytosis, was also inhibited. The results suggest that GB PD fluids may inhibit critical complement-mediated host defenses increasing the risk of *S.aureus* peritonitis. Non glucose based PD fluids might reduce risk of infections.

## FR-PO946

**Calprotectin: A Novel Diagnostic and Prognostic Marker in PD-Related Peritonitis?** Francesco Iannuzzella,[1] Mattia Corradini,[1] Maria Parmeggiani,[2] Lucia Belloni,[2] Alfredo Stefani,[1] Sonia Pasquali.[1] *[1]Nephrology and Dialysis Unit, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; [2]Laboratory and Molecular Biology Unit, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.*

**Background:** The diagnosis of PD related peritonitis is relatively easy, but clinicians have limited tools to determine which patients will progress to more severe forms of infection. Calprotectin is a novel inflammatory biomarker widely used in the diagnosis of inflammatory bowel disease. The aim of this study was to assess the utility of peritoneal fluid calprotectin as a novel diagnostic and prognostic marker in PD-related peritonitis.

**Methods:** A total of 44 consecutive peritoneal fluid samples from 11 patients were collected at the peritonitis onset and on days 3, 7 and 30. All samples were examined for cell count, bedside culture and calprotectin concentration. Moreover we evaluated C reactive protein and blood leucocytes on the same days of PD effluent collection.

**Results:** We investigated 48 patients: 28 men, 61±18 yrs, 24.3% diabetic, mean PD vintage 30±16 months. During the follow-up, peritonitis was diagnosed in 11 patients (8 men, 59±16 yrs). The PD effluent culture was positive in 9 patients. The mean calprotectin concentration was 263.7±81.4 on day 0 and 35.7±66.8 on day 3. Calprotectin was undetectable in PD samples in 6 patients on day 3 and in 9 patients on day 7, and in all patients on day 30. The only 2 patients with persistence of calprotectin on day 7 were those who presented with a worse clinical course, a long in-hospital stay and who underwent peritonitis recurrence after treatment suspension. In all patients, both PD white cells count and calprotectin levels decreased significantly after the start of treatment ($p<0.001$). At the time of peritonitis onset, calprotectin concentration correlated well with both the neuthrophil count in the PD effluent ($r=0.68$) and in the circulation ($r=0.62$).

**Conclusions:** Calprotectin was detected in PD fluids of all patients at the peritonitis onset and then slowly disappeared within 7 days. Persistence of calprotectin on day 7 or its reappearance after a previous disappearance should be regarded as a risk factor for a worse clinical course.

## FR-PO947

**Eosinophilic Peritonitis: Possible Association with Laparoscopic Peritoneal Dialysis Catheter Insertion** Yuko Akioka, Tatsuo Asano, Kei Nishiyama, Noriko Sugawara, Kiyonobu Ishizuka, Masataka Hisano, Hiroko Chikamoto, Motoshi Hattori. *Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** The methods for peritoneal dialysis (PD) catheter insertion include the open technique and the laparoscopic technique. A major advantage of laparoscopy is the complete visualization of the peritoneal cavity with diagnosis of some potential problems such as inguinal hernias, which can be repaired at the same time. However, the specific complications of the laparoscopic insertion were not sufficiently reported. The purpose of this study was to examine the association of eosinophilic peritonitis with laparoscopic insertion.

**Methods:** We examined 14 pediatric PD patients who underwent laparoscopic catheter insertion. Age-matched 12 patients who underwent open insertion were also examined. The cell counts of the peritoneal fluid were routinely studied during the post-operative period. Eosinophilic peritonitis was defined as showing >100 leukocytes/$mm^3$ effluent, of which >10% was eosinophils in the differential cell count.

**Results:** The incidence of eosinophilic peritonitis in the laparoscopic group was significantly higher than that of open group (64.3% vs. 16.7%, $p<0.01$). Eosinophilic peritonitis developed asymptomatically at 2.4±2.0 days, and resolved spontaneously in 14.0±7.6 days after laparoscopic insertion. The peak count of leukocytes was 798.0±748.6 cells/$mm^3$ (ranging from 275 to 2100) with a mean eosinophil percentage of 72.0±20.5. In patients with laparoscopic insertion, there were no differences in the duration of pneumoperitoneum, serum IgE level, eosinophilia in peripheral blood, and the presence of allergic diseases or immunosuppressive therapy between those with or without eosinophilic peritonitis.

**Conclusions:** Although the larger numbers of study will be needed, this study suggests the possible association of eosinophilic peritonitis with laparoscopic PD catheter insertion.

**FR-PO948**

**Predictors and Clinical Course of Culture-Negative Peritonitis in Peritoneal Dialysis Patients: Experience of a Center** Daniela Lopes, Clara Santos, Ana Marta Gomes. *Serviço Nefrologia, CHVNG/E, VNGaia, Portugal.*

**Background:** Peritonitis is the main infectious complication related to peritoneal dialysis (PD) and is a major reason for hospitalization and technique failure. Up to 20% of cases are culture-negative peritonitis (CNP). The aim of the study is to examine the frequency, predictors and clinical outcome of CNP in PD patients.

**Methods:** Retrospective cohort study of all episodes of PD-related peritonitis between 1998 and 2012. To determine the predictors and clinical outcome were compared 2 groups: positive-culture or CNP. Predictors:socio-demographic and clinical variables. Outcome analysis: resolution, hospitalization, catheter removal and hemodialysis transfer. Tests were conducted multilevel logistic regression to estimate the associations between the variables in analysis. The significance level for the models were determined as $P < 0.05$.

**Results:** We studied 163 episodes of peritonitis in 67 patients with a mean age of 55 ± 11 years, 70.5% male, yielding an overall rate of 0.59episodes/patient.year. There was 40 episodes (24.5%) of CNP. When comparing the 2 groups there were no differences regarding age, gender, etiology of kidney disease and major co-morbidities between the groups. A history of previous antibiotic treatment (past 30 days) was significantly more common with CNP than culture-positive (42.5% vs 7.3%, $p < 0.05$). The reasons for the administration of antibiotics were: infection of the outlet orifice (2 cases), peritonitis (1 case) and other medical reasons (14 cases). No association was found between the need for hospitalization ($p = 0.33$), cure ($p = 0.46$) and transfer technique ($p = 0.95$) with CNP. There was no association between previous antibiotic treatment and clinical outcome ($p = 0.88$).

**Conclusions:** In our study a history of previous antibiotic treatment was an important risk factor, this can be due to the fact that antibiotic can promote the change of the local flora and cause peritonitis with fastidious microorganisms or impairing the ability of cultures to detect the infectious organism.Thus, most cases of CNP may be explained by recent antibiotic treatment but there is no differences in clinical outcome between groups.

**FR-PO949**

**Global Pediatric Perspective on Peritonitis: A Report from the IPPN** Vimal Chadha,[1] Anja Christine Sander,[2] Franz S. Schaefer,[2] Bradley A. Warady.[1] *[1]Children's Mercy Hospital and Clinics; [2]Univ of Heidelberg.*

**Background:** Peritonitis remains a frequent complication of peritoneal dialysis (PD) and is the most common reason for technique failure. Our previous published registry experience revealed world-wide variation in causative organisms. To build upon that experience, the International Pediatric PD Network (IPPN) has collected data pertaining to peritonitis and other PD related issues in children receiving PD since 2007.

**Methods:** Data on children receiving maintenance PD, enrolled in the IPPN on-line registry was analyzed for this report.

**Results:** To date, 1991 children receiving PD have been enrolled in the registry from 95 centers in 35 countries. One-third of the patients receiving PD have experienced > 1 episodes of peritonitis with an overall annualized rate of 0.47 ± 0.29. 42% of the episodes were caused by Gm pos organisms, 19% by Gm neg organisms, 2.6% by fungus and 33% were culture neg. Staphylococcal and enterococcal infections were more common in Europe, whereas MRSA and pseudomonas episodes were more common in Latin American countries. Latin America and Turkey had the highest rate of culture neg results. Exit-site/tunnel infections (8%) and touch contamination (7%) were the most common identifiable sources of infection. Of 508 episodes of exit-site infection, 20% were associated with peritonitis. Presence of ostomy (RR 1.28), hypoalbuminemia (< 3.0 g/dL) (RR 1.34) and Staph aureus carrier state (RR 1.27) were all significant risk factors for occurrence of peritonitis. Dialysis modality, catheter configuration, number of cuffs, or exit-site orientation had no impact on the peritonitis rate. Relapse of peritonitis occurred in 60 patients; 73% of these occurred due to Gm pos organisms and 70% followed intermittent antibiotic therapy. There were 32 episodes of fungal peritonitis; 59% were preceded by bacterial peritonitis.

**Conclusions:** Despite published guidelines on the prevention of infection, peritonitis continues to complicate PD in a substantial percentage of pediatric patients. Efforts to further decrease the rate of infection and enhance treatment should focus on eliminating modifiable risk factors and improving culture techniques worldwide.

**FR-PO950**

**Astragalus Membranaceus Based Chinese Medicine Formula Might Better Preserve Residual Renal Function in Patients Undergoing Continuous Ambulatory Peritoneal Dialysis** Sing-Leung Lui, Terence Yip, Wai Kei Lo. *Medicine, Tung Wah Hospital, Hong Kong SAR, China.*

**Background:** Astragalus membranaceus is a Traditional Chinese Medicine which has been shown to possess renal protective effects in animal studies. The aim of this study was to examine the effect of Astragalus membranaceus based Chinese Medicine formula on preservation of residual renal function (RRF) and daily urine volume in patients newly started on continuous ambulatory peritoneal dialysis (CAPD).

**Methods:** The RRF and daily urine volume of 8 CAPD patients who have been receiving Astragalus membranaceus based Chinese Medicine formula (CM Group) and 16 Controls matched for age, sex, diabetes status and duration of CAPD (Control Group) were compared at the commencement of CAPD and after 2 years of CAPD.

**Results:** The mean age of the patients in the CM and Control Groups was 66.3±11.6 and 64.0±11.6 years respectively (p=0.659). Half of the patients in both Groups were diabetic. The RRF and daily urine volume of the CM and Control Groups were similar at baseline (3.52±2.0 ml/min/1.73m$^2$ vs. 4.19±1.8 ml/min/1.73m$^2$, p=0.412 and 883±342 ml vs. 838±347 ml, p=0.452 respectively). After 2 years of CAPD, patients in the CM Group had significantly higher RRF (2.92±1.43 ml/min/1.73m$^2$ vs. 1.36±1.54 ml/min/1.73m$^2$, p=0.026) and daily urine volume (769±576 ml vs. 238±268 ml, p=0.005) than the Controls. Their rate of decline of RRF and daily urine volume were significantly lower than those of the Controls (-0.30±0.40ml/min/1.73m$^2$/year vs. -1.42±0.30ml/min/1.73m$^2$/year, p=0.004 and -56.9±108.5 ml/day/year vs. -299.8±59.7ml/day/year, p=0.007 respectively). A significantly higher proportion of the Controls had developed anuria (daily urine <100ml) after 2 years of CAPD (56.3% vs. 0%, p=0.009). KT/V values and D/P Cr ratio at 4 hours of the two Groups at baseline and after 2 years of CAPD were similar. The two Groups did not differ in their diuretic and ACEI/ARB usage.

**Conclusions:** The use of Astragalus membranaceus based Chinese Medicine formula might be beneficial in preserving RRF in CAPD patients during the first 2 years of dialysis. Further prospective randomized control trials are warranted to confirm these preliminary observations.

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**FR-PO951**

**Evaluation of Protein Energy Wasting and Inflammation on Continuous Ambulatory Peritoneal Dialysis Patients and Its Correlation** Sham Sunder, Venkataramanan K, Himanshu Verma, Satyanand Sathi, Rajesh Padmalatha Jayaraman, Prabhu K, Anurag Gupta. *Nephrology, PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, India.*

**Background:** Protein energy wasting and heightened inflammation are highly prevalent and is a strong risk factor for morbidity and Mortality in CAPD patients. Evaluation of malnutrition, prevalence of inflammation as well as inter-relationship between various nutritional indices and inflammation has not been studied in much detail.

**Methods:** 63 CAPD patients (M=28, F=35) were assessed for their nutritional status and inflammation. Nutritional status was assessed by dietary diary, Anthropometry, Subjective global assessment, multi- frequency BIA and serology . Inflammation was assessed by High-sensitivity C - reactive and Interleukin-6. Diagnosis of malnutrition was made. Correlation between inflammation and various other nutritional assessment indices were analyzed.

**Results:** Mean age of the patients was 57.6 ± 11.6 years. The average calorie and protein intake / Kg/ day were 25.5±4.6 Kcal and 0.81±0.2 gm resp. The mean and standard deviation of BMI(23.7±5),MAC(26.3±4.5)Cm,TST(1.624±0.4)Cm MAMC(25.6±4.5),cMA MA(45.7±19.7), were respectively .The mean values of S.protein, S.Albumin S.Pre-albumin. S.Transferrin, S.Cholesterol, S. Triglyceride, hs-CRP and IL-6 were 5.9 gm/dl, 3.0 gm/dl, and 21.11 mg/dl, 130.6 mg/dl, 155.9 mg/dl, 136.1 mg/dl, and 8.8±7.6 mg/l and 8.4±12.2 pg/dl respectively. Based on SGA, 17.4%; 54%;28.6% ; S.albumin 21%; 62%:17%: BMI 52%; 37%; 11% of CAPD patients had normal, moderate, severe malnutrition status resp. Based on hs-CRP and IL-6, 70 % and 71.8% of CAPD patients were high inflammation resp. hsCRP co-relates negatively ($p<0.05$) with S. Albumin, S.pre-albumin and S.transferrin: IL-6 co-relates negatively ($p<0.05$) with protein /kg/day, MAC, MAMA, S.albumin, S. cholesterol and S.transferrin.

**Conclusions:** Protein energy wasting (80-85%) by various methods and inflammation (70%) was very highly prevalent . Inflammatory markers shown significant negative correlation with Anthropometry and serological markers.Inflammatory markers should be included in the regular assessment of CAPD patients, for the better management of protein energy wasting.

**FR-PO952**

**Peritoneal Permeability Is Not Associated with Patient Survival** Hironori Nakamura,[1] Makoto Higuchi.[2] *[1]Dept of Nephrology, Shinonoi General Hospital, Nagano, Japan; [2]Dept of Nephrology, Shinsyu Univ, Matsumoto, Japan.*

**Background:** Several studies have suggested a relationship between high peritoneal transport and adverse outcomes in patients with peritoneal dialysis (PD). However, there have been no adequate studies comparing the outcomes of high and low peritoneal permeabilitis.

**Methods:** we investigated longitudinal changes in peritoneal permeability and patient survival, including transfer to hemodialysis after a dropout of peritoneal dialysis therapy, related to differences between PET categories at baseline. We performed a retrospective, single-hospital study of patients with PD treated from 1998 to 2009. Based on baseline dialysate-to-plasma creatinine (D/P Cr), the 76 patients were divided into two categories: L-LA (D/P Cr, <0.65) and HA-H (D/P Cr, ≥0.65). Patient demographics and clinical features were compared. The changes in D/P Cr, ultrafiltration, Kt/V, dialysate glucose load, total protein loss, and urine volume were followed for 5 years. The overall mortality rate was evaluated using the Kaplan–Meier method.

**Results:** 1) A comparison with baseline showed no significant differences between the L-LA and HA-H groups: age (57 vs. 61years), gender (64 vs. 77% male), diabetes (38 vs. 31%), APD (54 vs. 60%), icodextrin (9 vs. 26%), serum albumin (3.5 vs. 3.4 g/dL), and serum creatinine (9.1 vs. 7.9 mg/dL). 2) The D/P Cr value increased to 0.63 and 0.68 at years 2 and 3 from 0.51 in the L-LA group and decreased to 0.65 and 0.68 at years 2 and 3, respectively from 0.79 in the HA-H group. In the HA-H group, ultrafiltration increased to 521 mL at year 3 from 215 mL at baseline. No significant changes were observed in total Kt/V, dialysate glucose load or total protein loss between the two groups over the 5-year period. 3) There were no significant differences in mean PD duration (53 vs. 51 months) or mean survival time from PD initiation (71 vs. 62 months) in the L-LA vs. HA-H groups, respectively. Kaplan–Meier curves showed no survival differences between groups (log-rank test; $p = 0.362$).

**Conclusions:** Both high and low peritoneal permeabilities at baseline were rated as regression to average status in years 2 and 3, but not were associated with overall patient survival.

## FR-PO953

**Free Water Transport (FWT) and Sodium Sieving in Different Clinical Settings** Javier De Arteaga,[1] Fabian Ledesma,[1] Bengt Rippe.[2] *[1]Nephrology, Hospital Privado, Catholic Univ, Córdoba, Argentina; [2]Nephrology, Lund Univ, Sweden.*

**Background:** Peritoneal sodium sieving(Ss), an indirect measure of FWT, is mainly the consequence of aquaporin function. A high transport state and/or a plasma/ dialysate Na gradient >5 mmol/lt, may influence this sieving effect with sodium diffusion from plasma. A diffusion correction may apply for assessing FWT. Upregulated peritoneal aquaporins increasing FWT is possible and described in the postransplant state, due mostly to steroids (NDT2011vol 26). Ascitic pts on PD have a high UF and fast transport **Objective:** evaluate Ss and FWT in our pts in different clinical settings.

**Methods:** Minipets (lamilia) done yearly and in any altered clinical circumstances: ie: UFF. Patients are all consecutive chronic PD from 2007 to end 2012. N = 53 pts.

**Results:** Solute transport, FWT and Na dip

| | controls | cirrhotics | Postransplant | P | P | P |
|---|---|---|---|---|---|---|
| N | A: 44 | B: 5 | C: 4 | A/B | B/C | A/C |
| D/P Cr 60m | 0.48 ± 0.12 | 0.61 ± 0.06 | 0.44 ± 0.02 | **0.03** | **0.001** | 0.52 |
| P Na | 139.6 ± 3.9 | 141,2 ± 2,9 | 139.2 ± 1.5 | 0,38 | 0.26 | 0,85 |
| D/P Na 3m | 0,94 ± 0,02 | 0,94 ± 0,04 | 0.94 ± 0.004 | 0,69 | 0.88 | 0,51 |
| D/P Na 60m | 0,88 ± 0,03 | 0,92 ± 0,02 | 0.82 ± 0.01 | **0,004** | **0.0002** | **0,0004** |
| Na Dial 3m | 130,3 ± 2,7 | 132,8 ± 4,76 | 131.1 ± 0.9 | 0,07 | 0.52 | 0,53 |
| Na Dial 60m | 122,4 ± 4,2 | 129,8 ± 2,9 | 114.2 ± 2.2 | **0,0004** | **0.0001** | **0.0004** |
| DIP Na | 7,9 ± 3,7 | 3,0 ± 3,8 | 17.0 ± 1.6 | **0,007** | **0.0001** | **0.0001** |
| % FWT | 43,4 ± 19,7 | 15,6 ± 6,5 | 68.5 ± 19.2 | **0.006** | **0.0006** | **0.01** |
| UF60m ml | 445,7 ± 171,4 | 636 ± 132,2 | 245 ± 94.3 | **0,02** | **0.001** | **0.02** |

**Conclusions:** As compared to controls, Ss is blunted in cirrhotic pts and enhanced in the postransplant state. The cause of this in the former, we don't know, but we can hypothesize that it could be due to ascitic fluid generated "per se", a high Na P/D gradient and transport state. In the latter, an upregulation of aquaporins for steroid use is the most probable case. UF and NAR is significantly higher in cirrhotics.

## FR-PO954

**Leptin Impact in Peritonitis Events of a Peritoneal Dialysis Population** Silvia Coelho,[1] Ana Paula Bernardo,[2] Maria João Carvalho,[2] Olivia Santos,[2] Guilherme Rocha,[2] António Manuel Nunes Cabrita,[2] Anabela Rodrigues.[2] *[1]Nephrology, Hospital Fernando Fonseca, Lisboa, Portugal; [2]Nephrology, CHP Hospital Santo António, Porto, Portugal.*

**Background:** Leptin is a key link between nutritional status and immunity function. Hypoleptinemic states have been associated with increased risk of infection. In peritoneal dialysis-PD this link has not yet been studied.

We aimed to explore the clinical association between leptin levels and peritonitis events.

**Methods:** A cross sectional measurement of leptin was performed in a prevalent PD population. Peritonitis episodes occurring one year before and two years after were documented. Corporal composition was evaluated with BCM®.

**Results:** Patients (n=66) had a mean age of 50.3 years (SD 14.3), 48.5% female, 56.2% were on APD and 44% used icodextrin. Total kt/v was 2.2 (1.8-2.9) and renal kt/v 0.9 (0.4-1.3). Nutrionally, they had a mean body mass index-BMI of 25.2 (SD 4.4), normalized protein catabolic rate-nPCR 0.8 (SD 0.6) and serum albumin-sAlb 3.8 (SD 0.4). Peritonitis was observed in 36.4% with a median of 2 (1.0-2.75) episodes/patient during the study period.

Patients with peritonitis were predominantly women (p=0.006) and older (p=0.034) but did not differ in respect to BMI, LBM, FBM, nPCR and sAlb levels (p>0.05). Leptin and IL6 levels were similar in patients with or without peritonitis, p=0.178 and p=0.805, respectively.

Considering the median value of leptin in our population (17.3ng/ml), patients were divided in high leptin (HL) and low leptin (LL) groups. The HL had higher BMI (27.3 vs 23.4) and FBM (14.0 vs 8.5), lower LBM (12.5 vs 14.3) and lower nPCR (0.7 vs 0.9), all p<0.05. There were no statistical differences (p>0.05) between the HL and LL groups with respect to the presence or number of peritonitis episodes, recruited leucocytes in the peritoneal effluent or the level of IL6.

**Conclusions:** Hyperleptinemia was associated with obesity but not with systemic inflammation. On the other hand, hypoleptinemia in the absence of other signs of undernutrition, was not associated with peritonitis events or outcome. The link between leptin and immunity in PD deserves further investigation.

## FR-PO955

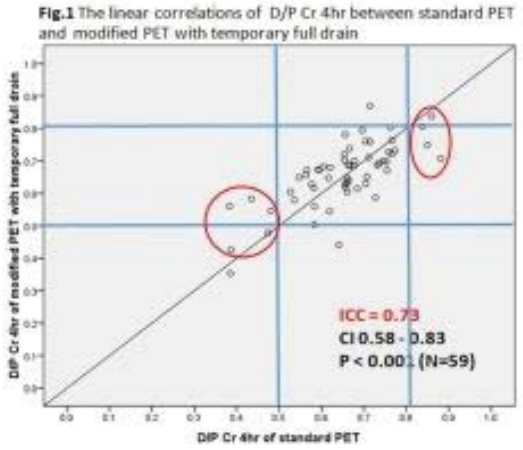
**Comparison of Small Solute Transport between Two Different Peritoneal Equilibration Tests** Suchai Sritippayawan, Kanjana Tianprasertkij, Thatsaphan Srithongkul, Nipa Aiyasanon, Sukit Raksasuk. *Renal Unit, Internal Medicine, Siriraj Hospital, Mahidol Univ, Bangkok, Thailand.*

**Background:** Measurement of small solute transport have been developed and modified to obtain more information of solute and fluid kinetics in peritoneal dialysis(PD). However, the dialysate to plasma creatinine ratio at 4 hours(D/PCr4hr) of the original standard peritoneal equilibration test(PET) has still been used in clinical practice. The aim of this study is to compare the D/PCr4hr between the standard PET and the new modified PET with temporary drainage.

**Methods:** Two PETs(2.5%Dextrose(D)PET and 4.25%D PET with temporary full drainage at 1 hr) were performed consecutively in 59 stable chronic PD patients(sample size was calculated from a pilot study of 10 patients). The D/PCr4hr at the end of 4 hr was measured and dialysate Cr was corrected for the glucose concentration. Small solute transport was classified as fast, average and slow by the D/PCr4hr of >0.81, 0.81-0.50 and <0.50 respectively. Intra-class correlation coefficients was used to assess the agreement of D/PCr4hr and weighted kappa was used to evaluate the agreement of transport categories between the 2 PETs.

**Results:** The mean age of the patients was 68 years and median time of PD duration was 11.4 months. Intra-class correlation of D/PCr4hr between the 2 PETs was 0.73(CI 0.58-0.83,p<0.001). The agreement of peritoneal transport categories among the 2 PETs was moderate(weighted kappa=0.46,p<0.001). Most agreement(88.5%) were found in average group. The D/PCr4hr of standard PET tended to be higher in fast transporter but lower in slow transporter than D/PCr4hr of modified PET with temporary drainage.

**Conclusions:** The D/PCr4hr of the modified PET with temporary drainage well correlated with the standard PET in average transporter but may overestimated and underestimated in slow transporter and fast transporter, respectively in PD patients.




*Funding:* Government Support - Non-U.S.

## FR-PO956

**Clinical Investigation of Relationship between Peritoneal Dialysis Effluent Cytokine Profiles and Peritoneal Solute Transport Rate** Guochun Chen, Feng Wen, Hong Liu, Xiang Zhou, Jing Liu, Fu-You Liu. *Dept of Nephrology, The Second Xiangya Hospital of Central South Univ, Changsha, Hunan, China.*

**Background:** Fast peritoneal solute transport rate (PSTR) is reportedly correlated with high mortality in peritoneal dialysis(PD) patients. The immune dysfunction provoked by incompatible solutions may contribute to variability of PSTR in peritoneal cavity.

**Methods:** This study was to apply the Luminex IMax technology to detect the cytokine profiles in overnight dwelled PD effluent of stable continuous ambulatory peritoneal dialysis(CAPD) patients, including inflammatory cytokines(IL-6, MCP-1, TNFα, IL-17A, IL-17F, IL-21, IL-22, IL-23), angiogenic factor (VEGF), pro-EMT/fibrosis factor(TGF-β1). The clinical data were analyzed from 30 CAPD patients in the Second Xiangya Hospital from September 2012 to February 2013.

**Results:** According to the peritoneal transport characteristics, all patients were divided into low and low-average transport (L/A) (D/P Cr <0.64) group, high and high average transport (H/A) (D/P Cr ≥0.65) group, respectively.Dialysate IL-6,MCP-1 ,TNFα, VEGF,and TGF-β1 level were above the detected limit.Dialysate IL-17A, IL-17F, IL-21, IL-22, IL-23 could not be detected. Dialysate IL-6 in H/A group were higher than L/A group(P<0.05). Among PDE inflammatory profiles, IL-6 levels (r = 0.656, P<0.001), MCP-1 level (r=0.620, P<0.001) was significantly associated with D/P Cr ;Dialysate VEGF(r=0.425, P=0.019) and TGF-β1(r=0.425, P=0.019) were also correlated with D/P Cr. Among systemic markers, serum albumin but not CRP showed a negative correlation with D/P Cr (r=-0.369, P=0.045). Multivariate regression analysis showed that D/P Cr was independently correlated with dialysate IL-6. Besides, dialysate IL-6, MCP-1, VEGF and TGF-β1 correlated with each other.TGF-β1 was negtively correlated with eGFR(r=-0.370, P=0.048), and for these patients whose eGFR≥2ml/min/1.73m$^2$, TGF-β1 level is much lower than the rest(P=0.018).

**Conclusions:** Our study indicated that these dialysate cytokines contributing to chronic inflammation, angiogenesis and fibrosis maybe contribute to high PSTR in stable CAPD.

## FR-PO957

**Decreased Circulating Klotho Levels Is Associated with Oxidative Stress in Patients on Peritoneal Dialysis** Ji Sun Paeng,[1] Hye-Young Kang,[1] Hyung Jung Oh,[2] Sung Jin Moon,[3] Tae-Hyun Yoo,[1,2] Shin-Wook Kang,[1,2] Seung Hyeok Han.[2] *[1]Brain Korea 21; [2]Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul; [3]College of Medicine, Kwandong Univ, Gyeonggi-do, Korea.*

**Background:** Klotho is known as an anti-aging gene, which is predominantly expressed in the distal convoluted tubules of the kidney. Previous studies have shown that circulating klotho levels are decreased in experimental kidney disease models and that klotho exerts anti-oxidative and anti-inflammatory effects. Meanwhile, patients with end-stage renal disease (ESRD) are particularly characterized by increased inflammation and oxidative stress. However, little is known about the relationship between these features and klotho in these patients.

**Methods:** We conducted a cross-sectional single-center study in 78 ESRD patients on peritoneal dialysis (PD). Serum concentrations of klotho, interleukin-6 (IL-6), and 8-isoprostane were measured by enzyme-linked immunosorbent assay. To identify independent factors associated with klotho, Spearman's correlation coefficients were determined between co-variates andmultiple linear regression analyses were conducted.

**Results:** When patients were classified according to the median value of serum klotho (329.6 pg/ml), serum 8-isoprostane and IL-6 levels were significantly higher in the 'high' klotho group compared to patients with 'low' klotho. In correlation analyses, log 8-isoprostane ($\gamma$=-0.310, P=0.006) and log IL-6 ($\gamma$=-0.343, P=0.002) were inversely correlated with log klotho. After adjustment for age, gender, MAP, log iPTH, and log IL-6, log 8-isoprostane was independently associated with log klotho ($\beta$=-0.158, P=0.040). In contrast, the significant relationship between klotho and IL-6 disappeared in an adjusted model.

**Conclusions:** Circulating klotho concentrations were significantly associated with 8-isoprostane levels in ESRD patients on PD, suggesting a potential link between klotho deficiency and enhanced oxidative stress in these patients. Further studies with a large number of patients are required to confirm the relationship between klotho, oxidative stress, and inflammation in ESRD patients.

## FR-PO958

**Peritoneal Protein Clearance Rather Than Faster Transport Status Determines Outcomes in Peritoneal Dialysis Patients** Gayathri K. Rajakaruna, Ben Caplin, Andrew Davenport. *UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom.*

**Background:** Fast peritoneal transport status was shown to be associated with increased mortality risk for peritoneal dialysis (PD) patients. However fast transport could either be due to increased peritoneal capillary surface area or increased permeability. Peritoneal protein clearance (PPC) has been proposed to differentiate increased permeability from increased capillary surface area.

**Methods:** Patients underwent bioimpedance measurements of extracellular water (ECW) and total body water (TBW) attending for their first peritoneal transport assessment. We also measured PPC in PD patients and grouped them into high (top quartile) or low PPC groups and followed patient outcomes.

**Results:** 300 patients, median age 57.0 (44-66) years, 47.7% male, 29.0% diabetic and 56.3% Caucasoid were studied. Mean PPC 87.7±2.8 ml/day. During a median follow up of 62 (29.3-86) months 24% died, with a median duration of peritoneal dialysis of 28 (15.3-50) months, 37.3% transplanted and 30.3% transferring to haemodialysis. Overall mortality was greater for those in the high PPC group (log rank test p=0.01). On Cox proportional hazards modeling the high PPC group was associated with an increased risk of death (hazard ratio HR 1.81, p=0.018), as was age (HR 1.04, p=0.000), increased ECW/TBW (HR 2.43, p=0.04) and Davies co-morbidity score (HR 1.55, p=0.01) independent of serum albumin and C-reactive protein.

**Conclusions:** We found an independent association between a high PPC and mortality. Mortality risk was also increased with age, co morbidity and ECW/TBW. Peritoneal protein clearance may be a surrogate for capillary permeability, and can be relatively easily measured, and as such could be used to identify patients at risk of increased mortality to allow future interventional trials.

## FR-PO959

**Protein Binding of P Cresol Sulphate and Indoxyl Sulphate in Peritoneal Dialysis Effluent** John F. Collins,[1] David B. Lee,[2] Martin Roberts.[2] *[1]Renal Medicine, Auckland City Hospital, Auckland, New Zealand; [2]VAGLA Healthcare System, David Geffen School of Medicine, UCLA, Los Angeles, CA.*

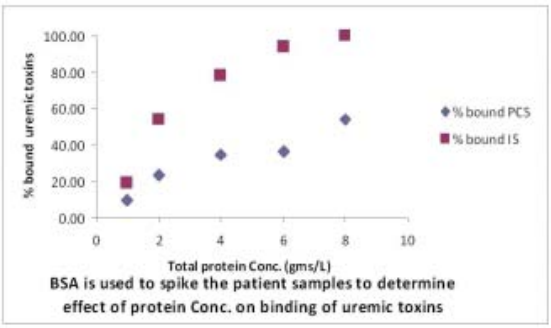
**Background:** The small MW highly protein-bound solutes P Cresol Sulphate (PCS) and Indoxyl Sulphate (IS) are recognized uremic toxins. Hemodialysis removes a tiny fraction of the total toxins as a consequence of high plasma protein binding (> 90%). In The PD-based Automated Wearable Artificial Kidney (AWAK) peritoneal proteins are not discarded but are continuously regenerated and re-turned to the patient Thus, the protein-content of the dialysate is anticipated to rise to levels higher than those seen in conventional PD effluent (PDE). Potentially the removal of protein bound toxins could be enhanced. However there is no data on PDE protein binding of PCS and IS.

**Methods:** 24 hour PDE was collected from 10 patients on CAPD/APD and total, free and protein-bound PCS/IS were measured with an HPLC method. One 24 hour PDE was spiked with BSA at concentrations ranging from 1gm/L-8gms/L to mimic the anticipated effect of increasing protein concentrations achieved with AWAK.

**Results:** The mean +/-SD concentration of total PCS in PDE was 1.73 +/-1.32 mg/L ( range 0.57-4.48 mgs/L) in 6 samples(below detectable range of 0.47 mgs/L in 4 samples). Mean protein-binding of PCS was 34 +/-29%.

The mean concentration of total IS in PDE was 1.03 +/-0.68 mg/L ( range 0.37-2.74 mgs/L) in 10 samples. Mean protein-binding of IS was 32 +/-19%.

The mean protein concentration in PDE was 0.825 gms/L (range 0.73-2.88 gms/L).




**Conclusions:** We have shown that the protein binding of PCS and IS in PDE were 34% and 32% respectively - levels substantially lower than those reported in plasma. However when the PD protein concentrations were artificially raised to mimic those aniticipated with the use of AWAK, binding was enhanced substantially for both PCS and IS. Clinical use of the AWAK may result in improved removal of these toxins.

*Funding:* Private Foundation Support

## FR-PO960

**Inflammatory Markers among Diabetic Women with End-Stage Renal Disease: A Role in Higher Cardiovascular Mortality?** Sudha P. Chennasamudram,[1] Essam N. Nakhla,[2] Tarek H. Naguib,[2] Tetyana L. Vasylyeva.[1] *[1]Pediatrics, TTUHSC; [2]Internal Medicine, TTUHSC, Amarillo, TX.*

**Background:** Cardiovascular disease (CVD) mortality is higher for diabetic women, women under 65 years of age, and those under 70 years with end-stage renal disease (ESRD) than for comparable men. CVD also worsens mortality of persons with diabetes mellitus (DM) among ESRD population. Due to the implication of interleukin 1 beta (IL-1β) and endothelin 1 (ET-1) in CVD, we hypothesized that sex differences relative to the proinflammatory and endothelial markers may exist and play a role in the higher CVD mortality of women in this population. The study objective is to investigate whether endothelial function (EF) and proinflammatory markers vary by sex among persons younger than 65 years with DM and ESRD.

**Methods:** This is a post-hoc analysis of 15 subjects (average age of 54±9 years, 8 females), on peritoneal dialysis (PD), from an earlier study done for phosphate binder comparison. We evaluated EF by reactive hyperemia index (RHI). We measured serum levels of EF biomarkers: ET-1, plasminogen activator inhibitor 1 (PAI-1), soluble vascular adhesion molecule 1 (sVCAM) and soluble intercellular adhesion molecule 1 (sICAM); and proinflammatory markers: interleukin 6 (IL-6), IL-1β, tumor necrosis factor alpha (TNF-α) and C- reactive protein (CRP). Wilcoxon test was performed for all the study parameters.

**Results:** Proinflammatory markers in males vs females were: TNF-α was 49.4±7.6 vs 36.8±3.0 pg/mL (P=0.054), IL-6 was 11.6±1.1 vs 14.0±2.1 pg/mL (P=0.158), IL-1β was 2.1±0.07 vs 2.8±0.3 pg/mL (P=0.028), CRP 25.8±9.7 vs 34.7±5.6 ng/mL (P=0.251). RHI was not significantly different between sexes. For serum markers of EF, see Table 1.

| Markers (ng/mL) | Males | Females | P Value |
|---|---|---|---|
| PAI-1 | 2.47±0.28 | 2.99±0.37 | 0.157 |
| ET-1 | 1.98±0.21 | 2.38±0.44 | **0.035** |
| sICAM | 51.14±8.2 | 35.87±4.9 | 0.327 |
| sVCAM | 53.37±18.22 | 101.06±27.26 | 0.190 |

**Conclusions:** Diabetic women aged under 65 years on PD due to ESRD have higher levels of IL-1β and ET-1, than comparable men. These markers may have a role in the higher CVD mortality in women of this population.

*Funding:* Private Foundation Support

## FR-PO961

**Competing Risk Analysis of First Peritonitis for Technique Failure and Death in Peritoneal Dialysis** Jinn-Yang Chen. *Div of Nephrology, Taipei Veterans General Hospital.*

**Background:** Competing risk outcomes may be encountered in peritoneal dialysis (PD) treatment. Identifying the differential effects of risk factors on different outcomes may improve initial assessment and treatment of PD patients.

**Methods:** Records for patients aged 20 years and older, started dialysis therapy between January 2006 and December 2009, and had received peritoneal dialysis as their initial dialysis modality (modality used on 90th day after initiating dialysis) were reviewed for our study. Cause-specific hazard and subdistribution hazard models were performed. Technique failure and death during PD were defined as censoring events or competing events for each other. Demographics, comorbidities and first peritonitis episode as a time-dependent covariate were included in the model.

**Results:** Among 4553 incident PD patients, 665 (14.6%) patients experienced technique failure and 632 (13.9%) patients died during PD. Patients who experienced technique failure had higher peritonitis rate (0.68 episode/patient-year). Patients who died during PD were older (67.7 ± 13.1 years) and had higher prevalence of comorbidities. Using the subdistribution model, male sex (HR: 1.42; 95% CI: 1.21-1.66), diabetes (HR: 1.6; 95% CI: 1.34-1.91) and the first peritonitis episode (HR: 1.76; 95% CI: 1.5-2.06) increased the risks of technique failure. By contrast, age, diabetes, CHF, CVA/TIA, GI bleeding, liver disease and cancer significantly increased risks for death during PD. The first peritonitis episode was associated with death during PD in the cause-specific model (HR: 2.44; 95% CI: 2.06-2.88), but the risk became insignificant in the subdistribution model. Time-varying risk of technique failure after the first peritonitis was highest during the first 49 days (HR: 5.01, 95% CI: 3.93-6.39) and patients remained at increased risk of technique failure for another 6 months after the episode.

**Conclusions:** After the first PD peritonitis, the risk for technique failure was higher than that for death. Patients of advanced age or multiple comorbidities had higher risks of death than technique failure. Male diabetic PD patients need the most aggressive care for peritonitis prevention.

*Funding:* Government Support - Non-U.S.

## FR-PO962

**Vascular Calcification Is Associated with Lower Weekly Phosphate Clearance in Peritoneal Dialysis** Juan Carlos Ramirez-Sandoval, I.E. Casanova, Jorge A. Villar-Tapia, Ricardo Correa-Rotter. *Dept Nephrology and Mineral Metabolism, National Medical Science and Nutrition Institute Salvador Zubirán, Mexico City, DF, Mexico.*

**Background:** Vascular calcification contributes to increase cardiovascular risk in patients on dialysis and is commonly associated with hyperphosphatemia. Lower phosphate clearance in hemodialysis might be associated with vascular calcification yet there is no data on phosphate clearance and its relation to vascular calcification in patients in peritoneal dialysis (PD).The aim of this study was to evaluate the association between phosphate removal in patients treated with PD and vascular calcification assessed by radiographic studies (KDIGO guidelines).

**Methods:** We included 47 patients >18 years with at least 6 months on PD and stable clinical course for at least 2 mo. We measured 2 vascular calcification scores: based on radiographic films (hands and pelvis or lateral abdominal). Phosphate was measured in 24-hour dialysate. In patients with residual renal function (RRF), phosphate was determined in 24-hour urine collection. Weekly total phosphate clearance was calculated (L/week). Other collected variables: age, time on dialysis, diabetes, body mass index (BMI, kg/m$^2$), RRF (mL/day), and serum lipids, phosphorus, calcium, iPTH, and alkaline phosphatase.

**Results:** Forty-seven patients aged 38 ± 12.5 y (range 18-66) were included, 29 female (62%). Negative correlations were observed between vascular calcification scores and weekly total phosphate clearance (hands and pelvis: r=-0.36, p<0.001 and abdominal lateral column r=-0.33, p<0.001). Relevant correlations were observed between vascular calcification scores and age (r=0.54, p<0.001), time on dialysis (r=0.36, p<0.01), BMI (r=0.30, p <0.04) and RRF (r=-0.26, p<0.04). Serum phosphorus, calcium, iPTH were not associated with vascular calcification. In a multiple regression analysis, weekly total phosphate clearance (p<0.01), age (p<0.001), and RRF(p<0.03) remained independently associated with vascular calcification scores.

**Conclusions:** Lower weekly total phosphate clearance is associated with increased vascular calcification and may be useful as a prognostic marker and treatment objective in patients on PD.

*Funding:* Government Support - Non-U.S.

## FR-PO963

**An Easy Way to Estimate Total Kt/V in Patients on Peritoneal Dialysis between Mandatory Routine Measurements** Fernando Sales, Rosilene M. Elias, Adriano Sanjuan, Benedito J. Pereira, Manuel C. Castro, Hugo Abensur. *Div of Nephrology, Univ of Sao Paulo, School of Medicine, Sao Paulo, Brazil.*

**Background:** For patients on peritoneal dialysis (PD), all guidelines recommend measurements of dialysis dose by calculating renal Kt/V every 2 months and peritoneal Kt/V every 4 months, in order to achieve a total Kt/V minimal of 1.7/week. However, patients can lose their residual renal function between these intervals, while the physicians are blinded to the offered dialysis dose. In this meantime an estimated Kt/V is welcome. The purpose of our study was to compare measured total Kt/V (mKt/V) with estimated Kt/V (eKt/V) based on equations.

**Methods:** From May 2006 to August 2012, 21 patients on chronic PD (age 51.5 ± 18.3 years, male sex 51%) were included in this study. To estimate renal Kt/V, we assumed urea U/P ratio as 4. To estimate peritoneal Kt/V, we assumed urea D/P ratio as 0.9 and 0.5 for CAPD and NIPD, respectively. For CCPD, we assumed urea D/P as 0.5 during night time and 1 during day time.

**Results:** Table 1 shows values as mean ± SD, as well as Pearson correlation between measured and estimated Kt/V. ROC determined that total eKt/V can predict a total mKt/V of 1.7 with sensibility of 89% and specificity of 86% (AUC= 0.872; p=0.002).

| | ***Measured*** | ***Estimated*** | *Correlation* |
|---|---|---|---|
| **Renal Kt/V (n=110)** | 0.92 ± 0.43 | 1.01 ± 0.53 | r=0.683; p<0.0001 |
| **Peritoneal Kt/V (n=51)** | 1.27 ± 0.41 | 1.08 ± 0.32 | r=0.751; p<0.0001 |
| **Total Kt/V (n=51)** | 2.19 ± 0.48 | 2.00 ± 0.43 | r=0.828; p<0.0001 |

**Conclusions:** Our data showed how to estimate PD Kt/V. We are not proposing a replacement of the well validated method to calculate Kt/V. However, as a simple tool, this estimation might help to adjust the dialysis dose in the intervals we do not have measured Kt/V.

## FR-PO964

**Comparison of Various Antiseptics Impact on Torque Required for Transfer Set Disconnection and Implications for Safety** Catherine Firanek, Ira D. Davis, James A. Sloand, Dorota Wolpiuk, Audrey M. Hutchcraft, Mary E. Gellens. *Medical Affairs, Baxter Healthcare Corporation, Deerfield, IL.*

**Background:** To evaluate the security of the attachment of the Peritoneal Dialysis (PD) transfer set (TS) to the Baxter (B) titanium catheter adapter (TCA) following the submerged contact of the TCA threads using 5 antiseptics and measuring removal torque (T).

**Methods:** The Baxter TCA was soaked separately in Amuchina, Octenisept, Braunol Povidone Iodine 10%, Chlorhexamed Forte 0.2% and Purdue 10% Povidone Iodine (PI) before connectingtothe B-TS with a clockwise torque range of 2.5-3.0 inch-lbs. A test without antiseptic application (dry) was used as a control. One lot of TCAs was used and 28 units tested in each of the five antiseptic groups. Counter-clockwise torque off assessment of the security of the connection was evaluated with a hand torque gauge at a connection time of 24 hrs prior to disconnection. Connection integrity was also measured using the tensile pull test, leak test, vacuum test and torque test.

**Results:** Connection integrity was measured. All antiseptic-containing and dry connections sustained a 0.2 in-lbf force for 10 seconds and endured a pull force of 7lbf. No leaks were found with the leak and vacuum tests.The removal torque (in-lbf) *mean* resultsare as follows:

| **Antiseptic** | **Mean Removal Torque (in-lbf) ± Standard Deviation (SD)** |
|---|---|
| Dry (no antiseptic) | 1.79 (±0.25) |
| Purdue PI 10% | 4.08 (±1.24) |
| Amuchina | 2.89 (±0.62) |
| Octenisept | 2.13 (±0.73) |
| Braunol PI 10% | 2.36 (±0.83) |
| Chlorhexamed | 1.59 (±0.50) |

**Conclusions:** Our data suggests that the use of Purdue 10% PI at the connection site is associated with a tighter TCA-TS connection compared to four other antiseptics and compared with a dry connection. Enhanced tightness of the TCA-TS connection would be expected to reduce the probability of a partial or complete TCA-TS disconnection and the possibility of peritonitis.

*Funding:* Pharmaceutical Company Support - Baxter Healthcare Corporation

## FR-PO965

**Greater Omentum Folding in Open Surgical Placement of PD Catheters: A Randomized Controlled Study and Systemic Review** Guochun Chen, Hong Liu, Linshan Zhou, Puzhang Wang, Youming Peng, Fu-You Liu. *Dept of Nephrology, The Second Xiangya Hospital of Central South Univ, Changsha, Hunan, China.*

**Background:** Mechanical catheter dysfunction caused by omentum entrapment remains a major complication of PD therapy. The purpose of this study was to determine the outcomes of omentum folding at the time of primary open catheter insertion.

**Methods:** From March 2008 to December 2012, total 67 PD subjects were enrolled in the study and randomly assigned to receive either Regular Open Insertion (ROI group, n=33) or Open Insertion with Omentum Folding (OIOF group, n=34). The primary outcome was defined as PD catheter tip migration with dysfunction. A systematic review was performed to analyze the outcomes of omentum management in PD catheter implantation, based on published data from 1990 to 2013.

**Results:** There was no statistical difference of baseline patient characteristics between ROI and OIOF groups. 9 (27.3%) patients in the ROI group presented with the catheter malposition in the late stage (>60 days) of the study, significantly higher than 2 (5.9%) of the OIOF group (P=0.049). Significant differences of catheter survival rate between 2 groups were observed in the late stage (P=0.030) and the full time of the study (P=0.028). A higher incidence of irreversible catheter dysfunction was shown in the ROI group (15.2%), whereas none occurred in OIOF group (P=0.031). No statistical difference was determined in other catheter-related complications or patient survival rate. There were no statistical differences of peritoneal transport characteristics or dialysis adequacy between 2 groups upon evaluation of 3-, 6- and 12-month. Systemic review of current publications suggested that PD catheter placement with omentum managements could lead to less irreversible catheter dysfunction and improved outcome of catheter survival.

**Conclusions:** Our data suggest that ometum folding at the initial time of open catheter placement can significantly reduce the risk of catheter tip migration with dysfunction and improve the outcome of PD technique.

## FR-PO966

**Which Fluid Space Is Affected by Ultrafiltration?** Mihaly B. Tapolyai,[1,3] Maria Faludi,[1] Tibor Fulop,[2] Klara Berta.[1] *[1]Fresenius Medical Care, Semmelweis Univ, Budapest, Hungary; [2]Medicine, Div of Nephrology, Univ of Mississippi, Jackson, MS; [3]Medicine, Div of Nephrology, WJB Dorn VA Medical Center, Columbia, SC.*

**Background:** Ultrafiltration (UF) is a common and everyday procedure performed with almost all dialysis session. During UF several liters of fluid are removed, however, what proportion of this fluid is removed from which fluid space could not be easily and clinically measured until now; we designed this study to evaluate the fluid spaces most affected by UF.

**Methods:** This is a prospective cohort study of 40 prevalent chronic dialysis (HD) patients in receiving thrice weekly hemodiafiltration. We measured the patients' fluid spaces using a whole body bioimpedence apparatus to evaluate the changes of fluid spaces right from the beginning of the HD session and immediately after. We recorded the fluid spaces, UF volumes, and blood pressures.

**Results:** 40 prevalent HD patients (mean age: 60 ±52 year; 15 male; 11 diabetic; mean weight: 71.03 ±15.48 kg had bioimpedence (BCM, Fresenius) measurements before and after HD. On the average 2.38 ±0.98 L of UF was achieved (measured overload: 2.35±1.44L). The Extracellular Volume decreased from 16.84 ±3.52 to 14.89 ±3.06 Liters (p: <<0.0001); Intracellular Volume from 16.88 ±4.40 to 16.55 ±4.48 Liters (p: 0.45); the Volume of Distribution of Urea from 31.38 ±7.28 to 30.70 ±7.32 Liters (p: 0.45) and the degree of volume overload from 13.60 ±7.30 to 3.83 ±8.32 % (p: <<0.0001) of the extracellular space. The Mean Arterial Pressures also decreased from 122.95 ±19.02 to 108.50 ±13.91 mmHg (p: <<0.0001).

**Conclusions:** We conclude that UF almost exclusively reaches the extracellular fluid space and the fluid in the intracellular fluid space is not significant affected when fluid spaces are measured immediately after a 4 hour dialysis session.

## FR-PO967

**Dialysability and Safety of Gadoterate Meglumine (Dotarem®) in Hemodialysis Patients** Eric E. Gheuens,[1] Ronald Daelemans,[1] Sofie Mesens.[2] *[1]Dept Nephrology-Hypertension, Ziekenhuis Netwerk Antwerpen, Antwerpen, Belgium; [2]Clinical Pharmacology Unit, SGS, Antwerpen, Belgium.*

**Background:** To evaluate the dialysability and safety of gadoterate meglumine (a macrocyclic gadolinium based contrast agent) in hemodialysis patients.

**Methods:** Phase I, monocentric, non-comparative, non-randomized, open-label clinical trial, including 10 patients, requiring hemodialysis 3 times a week for 4 hours. Gadoterate was injected intravenously (0.1 mmol/kg). The primary evaluation criterion was the decrease in serum gadoterate concentration (SGC) after each hemodialysis session. To calculate the dialysability, blood samples were drawn simultaneously from the inflow and outflow lines of the circuit during the first hemodialysis session, and from the vascular access just before and after each of the 3 hemodialysis sessions. These started 1 to 2 hours, 2 days and 4 days, following the gadoterate injection. The total SGC was measured by inductively coupled plasma mass spectrometry. The secondary evaluation criteria were the clinical safety (vital signs, injection site tolerance) and laboratory assessments which were evaluated during a 4-day follow-up after gadoterate injection. Adverse events (AEs) and serious AEs were evaluated after 3 weeks, 3 months and 1 year.

**Results:** All 10 subjects were Caucasian, 50.0% were female. Median age was 64.0 (31-79) years. Median weight was 70.6 (61-116) kg. During the first hemodialysis, the mean gadolinium clearance (mL/min) was 224.6 at 0.5h and 225.9 at 1.5h and the SGC decreased over time by 88% to 93% and 97% at 0.5h, 1.5h, and 4h after start of dialysis. After the third hemodialysis session the SGC dropped by 99.7% compared to the pre-dialysis value of the first session. No Aes related to gadoterate were reported. No Aes occurred at the injection site during the observation period. There were no clinically relevant changes in mean laboratory values and vital signs. No cases of Nephrogenic Systemic Fibrosis have been reported so far.

**Conclusions:** This is the first study to document the excellent dialysability of gadoterate (recently FDA approved). After 3 hemodialysis sessions the serum concentration dropped by 99.7%. Furthermore we confirm the safety in hemodialysis patients.

*Funding:* Pharmaceutical Company Support - Guerbet-group

## FR-PO968

**Dialysis Hypotension: A Role for Inadequate Arginine Vasopressin Release? A Systematic Literature Review** Esmee M. Ettema,[1] Debbie Zittema,[1] Johanna J. Kuipers,[2] Ron T. Gansevoort,[1] Paul E. de Jong,[1] Ralf Westerhuis,[2] Casper F.M. Franssen.[1] *[1]Nephrology, UMC Groningen, Groningen, Netherlands; [2]Nephrology, Dialyse Centrum Groningen, Groningen, Netherlands.*

**Background:** Intradialytic hypotension (IDH) is one of the most common complications of hemodialysis (HD) treatment. Some studies have suggested that inadequate AVP secretion could play an important role in the pathogenesis of IDH. However, AVP levels during HD and its relation to IDH has never been systematically studied.

**Methods:** We performed searches on PubMed and Embase (1970-2013, search terms "hemodialysis" and "vasopressin"). Eligible were studies reporting on AVP levels during standard HD or other dialysis techniques. Observational studies reporting on AVP levels pre- and post-HD were additionally included in a meta-analysis.

**Results:** Thirty-seven studies, including 681 patients, were finally included in the systematic literature review of which 26 studies, including 496 patients, were eligible for meta-analysis. The main findings are that pre-treatment AVP levels were more than twice as high in dialysis patients compared with healthy controls (6.4±3.5 vs. 2.5±1.3 pg/ml) and that plasma AVP levels showed little or no increase during standard HD (7.0±4.9 vs. 8.8±9.3 pre- vs. post-HD, p=NS). Significant heterogeneity was found between studies. Meta-regression analysis revealed no significant associations between the change in AVP and age, gender, treatment duration, dialysate sodium, change in blood pressure or plasma osmolality. Studies on other dialysis techniques showed mixed results with regard to the plasma AVP level course. Seven studies addressed the relation between IDH and AVP levels and showed inconsistent results with some studies reporting a rise in plasma AVP during IDH whereas others found no difference in the AVP response between patients with and without IDH.

**Conclusions:** Plasma AVP levels are higher in HD patients compared with healthy controls, but show little or no increase during standard HD. The lack of a rise in AVP levels during HD may therefore be pathophysiologically involved in the onset of IDH.

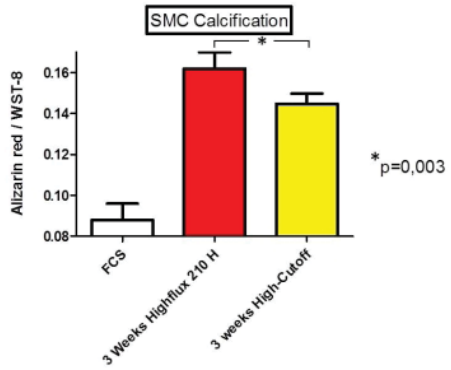
## FR-PO969

**Hemodialysis with High-Cutoff Membranes Reduces In Vitro Calcification of Human Vascular Smooth Muscle Cells** Daniel Zickler,[1] Achim Joerres,[1] Nahid Hakiy,[1] Markus Storr,[3] Roman Fiedler,[2] Matthias Girndt,[2] Ralf Schindler.[1] *[1]Nephrology, Charité Univ Medicine Berlin, Berlin, Germany; [2]Nephrology, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany; [3]Gambro Dialysatoren GmbH, Germany.*

**Background:** Vascular calcification and subsequent cardiovascular events are a major problem in chronic hemodialysis patients. One possible cause is increased chronic microinflammation related to insufficient elimination of proinflammatory mediators. A newly developed, highly permeable High-cutoff (HCO) membrane allows elimination of proteins with a molecular size of up to 45 kd.

**Methods:** In a randomized controlled trial 40 chronic hemodialysis patients were dialysed with HCO or regular Highflux membranes for three weeks following a cross-over-design. Weekly serum samples were drawn before dialysis. Afterwards, vascular smooth muscle cells were incubated with calcification media and these serum probes (10%). After ten days calcification was measured using alizarin red staining and set in relation to the number of proliferating cells using WST-8.

**Results:** After ten days of incubation, SMCs incubated with serum obtained after 3 weeks dialysis with HCO showed significantly reduced calcification compared to SMC incubated with serum during Highflux dialysis ( 0,1448±0,0052 vs. 0,1618 ±0,008, p=0,003). SMCs incubated with FCS showed a very low degree of calcification. (0,09 ± 0,007).




**Conclusions:** Calcification of SMC in vitro is lower using serum during dialysis with HCO compared to serum during Highflux dialysis. This effect may be caused by improved elimination of pro-calcifying mediators with HCO membranes.

*Funding:* Pharmaceutical Company Support - Gambro Dialysatoren GmbH, Government Support - Non-U.S.

## FR-PO970

**The Impact of Low Triiodothyronine Levels on Mortality Is Mediated by Malnutrition and Cardiac Dysfunction in Incident Hemodialysis Patients** Hyang Mo Koo,[1] Fa Mee Doh,[1] Ji Sun Paeng,[2] Hyung Jung Oh,[1] Tae-Hyun Yoo,[1,2] Shin-Wook Kang.[1,2] *[1]Dept of Internal Medicine, Yonsei Univ College of Medicine; [2]Severance Biomedical Science Institute, Brain Korea 21, Yonsei Univ, Seoul, Korea.*

**Background:** Accumulating evidence has indicated that a reduced triiodothyronine (T3) level in prevalent hemodialysis (HD) patients is a prognostic factor for adverse clinical outcome. However, little is known about the association between low T3 and mortality in end-stage renal disease (ESRD) patients starting HD and whether the impact of T3 on mortality is mediated by malnutrition, inflammation, or cardiac dysfunction in these patients.

**Methods:** A prospective cohort of 471 incident HD patients from 36 dialysis centers of the Clinical Research Center for ESRD in Korea was selected for this study. Based on the median value of T3, patients were divided into 'higher' and 'lower' groups. All-cause and cardiovascular (CV) mortality rates were compared between the two groups. The independent prognostic values of T3 levels for all-cause and CV mortality were also determined.

**Results:** Compared to the 'higher' T3 group, albumin, total cholesterol, triglyceride, lean body mass (LBM-Cr), and normalized protein catabolic rate (nPCR) were significantly lower in the 'lower' T3 group. The 'lower' T3 group also had higher left ventricular mass

index (LVMI) and lower LV ejection fraction (LVEF). All-cause (113.4 vs. 18.2 events/1000 patient-years, P < 0.001) and CV mortality rates (49.8 vs. 9.1 events/1000 patient-years, P = 0.001) were significantly higher in the 'lower' T3 group compared to the 'higher' T3 group. Kaplan-Meier analysis also showed significantly worse cumulative survival rates in the 'lower' T3 group (P < 0.001). In Cox regression analysis, low T3 was a significant independent predictor of all-cause mortality, even after adjusting for traditional risk factors (HR, 3.76; P = 0.021). However, this impact was attenuated when LBM-Cr, nPCR, LVMI, or LVEF was incorporated into the successive Cox model.

**Conclusions:** Low T3 was a significant independent risk factor for all-cause mortality in incident HD patients, which was partly attributed to malnutrition and cardiac dysfunction.

## FR-PO971

**Prominent Accumulation in Hemodialysis Patients of Solutes Normally Cleared by Tubular Secretion** Tammy L. Sirich,[1] Natalie Plummer,[1] Thomas H. Hostetter,[2] Timothy W. Meyer.[1] *[1]Medicine, Stanford Univ; [2]Medicine, Case Western Reserve Univ.*

**Background:** The native kidney clears many solutes efficiently by secretion while hemodialysis clears solutes by passive diffusion. We tested whether concentrations of solutes which the kidney clears by secretion therefore rise to high levels in dialysis patients.

**Methods:** We compared the plasma concentrations and clearances of solutes normally cleared by secretion - p-cresol sulfate (PCS), indoxyl sulfate (IS), and hippurate (HIPP) - with those of urea in patients maintained on hemodialysis (HD; n=9) and in normal subjects (Nl; n=8). Values were expressed in terms of the free, unbound solute concentrations as these are the levels to which body tissues are exposed.

**Results:** Results showed (mean±sd; [a], p<0.05 dialytic versus native kidney clearance; [b], p<0.05 secreted solutes vs urea):

| Solute | $k_{Nl}$ (ml/min) | $k_{HD}$ (ml/min) | $k_{HD}/k_{Nl}$ | HD/Nl |
|---|---|---|---|---|
| Urea | 70 ± 18 | 292 ± 52 [a] | 4.2 | 4 |
| PCS | 1319 ± 597 [b] | 517 ± 68 [a,b] | 0.39 | 45 |
| IS | 2776 ± 1190 [b] | 583 ± 94 [a,b] | 0.21 | 79 |
| HIPP | 1758 ± 629 [b] | 287 ± 39 [a] | 0.16 | 89 |

Urea undergoes partial tubular reabsorption and its native kidney clearance $k_{Nl}$ is lower than its dialytic clearance $k_{HD}$. Thus even with intermittent treatment the ratio of the average pretreatment urea concentration in dialysis patients to the average concentration in normal subjects (HD/Nl) was only 4. The native kidney obtains much higher clearances for PCS, IS, and HIPP by tubular secretion and values for the ratio of the dialytic clearance to the native kidney clearance, $k_{HD}/k_{Nl}$, for these solutes were thus much lower. Their pretreatment plasma concentrations relative to the concentrations in normal subjects were correspondingly much higher than those of urea. Mathematical modeling showed that the elevation of HD/Nl is explained by the ratio $k_{HD}/k_{Nl}$ and the concentration reduction ratio during intermittent treatment.

**Conclusions:** These results show that plasma concentrations of solutes normally cleared by tubular secretion remain very high in hemodialysis patients. Adoption of urea as our index solute for dialysis efficacy has had the unintended effect of concealing how poorly conventional treatment replaces the native kidney's secretory function.

*Funding:* NIDDK Support

## FR-PO972

**Dialysate Containing Nitric Oxide Suppresses Blood Coagulation during Hemodialysis in a Rat Hemodialysis Model** Shunichiro Urabe,[1] Yuki Kariya,[2] Kenichi Kokubo,[1,2] Hiroshi Tsukao,[1,3] Hirosuke Kobayashi.[1,2] *[1]Kitasato Univ Graduate School of Medical Science, Sagamihara, Kanagawa, Japan; [2]Kitasato Univ School of Allied Health Sciences, Sagamihara, Kanagawa, Japan; [3]Tokyo Univ of Technology School of Health Sciences, Tokyo, Japan.*

**Background:** In blood vessels, nitric oxide (NO) generated by NO synthase on endothelial cells suppresses the aggregation and activation of platelets. Therefore, the biocompatibility of dialysis membranes could likely be improved using an NO-containing dialysate capable of delivering NO to blood through the membrane. The present study examined whether an NO-containing dialysate suppressed blood coagulation during hemodialysis without any effects on blood pressure in a rat hemodialysis model.

**Methods:** Hemodialysis was performed using 10-12-week-old male rats (Sprague Dawley) for 4 h. Blood was removed from the carotid artery of a rat, forced to flow through a miniaturized dialyzer (membrane area: 100 $cm^2$) made of polysulfone using a peristaltic pump, and returned to the tail vein. NO was added using gas exchange membranes (membrane area: 56 $cm^2$) placed just before the dialysate inlet of the dialyzer. Heparin was injected once before dialysis. Pentobarbital was continuously injected to anesthetize the rats. The arterial-side and venous-side blood pressures in the blood circuit were measured. All coagulation events were recorded.

**Results:** The arterial-side blood pressures during hemodialysis were stable and similar between the control (without NO) and NO-addition groups, indicating that NO did not affect the blood pressure during hemodialysis. However, the venous-side pressures in the control group were unstable and in some cases had increased at 150 to 240 min after the start of dialysis, whereas the pressures in the NO-addition group were stable throughout hemodialysis. Coagulation events (such as blood clotting in the cannula or blood circuit) or an increase in the venous-side pressure occurred in the control group (3/5 cases) but not in the NO-addition group (0/5 cases).

**Conclusions:** The addition of NO to the dialysate suppressed blood coagulation during hemodialysis in a rat model without having any effect on the blood pressure during hemodialysis.

*Funding:* Government Support - Non-U.S.

## FR-PO973

**Vascular Refilling Is Independent of Fluid Overload in Hemodialysis Patients** Mauro Pietribiasi,[1] Krassimir Katzarski,[2,3] Magda Galach,[1] Joanna Stachowska-Pietka,[1] Daniel Schneditz,[4] Bengt Lindholm,[2] Jacek Waniewski.[1] *[1]Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland; [2]Baxter Novum and Renal Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Diaverum, Stockholm, Sweden; [4]Institute of Physiology, Medical Univ of Graz, Graz, Austria.*

**Background:** Fluid removal by ultrafiltration during a hemodialysis (HD) session is balanced by vascular refilling from the interstitium, driven by the increase in plasma oncotic pressure ($\pi p$). Inadequate refilling rate (Qr) is suspected to contribute to hypotensive crises. The refilling process was quantified by the *refilling coefficient (Kr)* expressing Qr driven by a unit increase in $\pi p$. We calculated Kr for two groups of HD patients differing by pre-treatment fluid status and session time, but similar in ultrafiltration rate (Qu).

**Methods:** Nine stable patients underwent two HD sessions: short session of 3.5 h (SH, after 2 day break) and long session of 4.5 h (LH, after 3 day break). Relative blood volume changes were measured online with Gambro AK 200 Ultra HD machine module, and solute concentrations were measured before and after HD, and regularly during the session. The volume of body fluid compartments was measured by bioimpedance analysis.

**Results:** Patients were more hydrated before LH session: initial body weight, total body water (31.7±5 l for SH vs. 32.5±5 l for LH) and extracellular water (16.6±2 l for SH vs. 17±2 l for LH) were higher. $\pi p$ was similar for both sessions. Qr was higher for LH. Kr decreased with dialysis time, but showed no statistically significant difference between sessions both at the beginning and at the end of HD (Kr at 1h: 268±127 ml/h/mmHg for SH and 396±198 ml/h/mmHg for LH, NS). The final, stable value of Kr was reached at similar times (3 h) and was similar for both sessions (137±55 ml/h/mmHg for SH and 166±94 ml/h/mmHg for LH).

**Conclusions:** Patients undergoing LH, despite being more volume expanded, did not have higher initial Kr, even with higher initial Qr. Final Kr values stabilized on similar levels at similar times, notwithstanding differences in initial fluid status or Qr in the two groups, suggesting that Kr is relatively insensitive to the initial fluid status of the patient.

## FR-PO974

**Prevalence and Risk Factors of MRSA Infections and Effect of Program for MRSA Eradication in ESRD Patients on Hemodialysis: Single Center Study** Young-Il Jo,[1] Jung-Hwan Park,[1] Jong-Ho Lee,[1] Eun Hye Seo,[1] Jeong-hwa Choi,[2] Hyun-kyun Ki.[2] *[1]Div of Nephrology, Konkuk Univ School of Medicine, Seoul, Republic of Korea; [2]Dept of Infectious Disease, Konkuk Univ Medical Center, Seoul.*

**Background:** Hemodialysis (HD) patients with meticillin-resistant Staphylococcus aureus (MRSA) infections face high morbidity and mortality. This study was designed to estimate the prevalence of MRSA nasal carriage, to define the risk factors of MRSA nasal carriage, and to investigate the effect of anti-infective program on the prevalence of MRSA nasal carriage among HD patients.

**Methods:** A total of 126 HD patients without signs of overt clinical infection (M:F 59:67, duration of dialysis 48.9±47.8 months) were enrolled in 2011. Nasal carriers for MRSA received standardized mupirocin therapy. Anti-infective program including education and hand washing has been taken place for all patients. All patients were followed up for elimination and infections for 1 year.

**Results:** The prevalence of nasal carriage for MRSA was 16.4% including 4.0% for the outpatients and 69.6% for inpatients. In the univariate analysis, a statistically significant difference was found for the number and duration of hospitalization, duration of dialysis, history of antibiotics use, history of central catheterization and history of urinary indwelling catheterization between the carriage of MRSA. In the multivariate analysis, duration of hospitalization (p=0.029, Exp(B) 1.027, 95% confidence interval 1.003-1.053) and length of stay in ICU (p=0.044, Exp(B) 1.508, 95% confidence interval 1.012-2.247) were independently associated with MRSA carriage . After anti-infective program for 1-year, the prevalence of nasal carriage for MRSA was 4.5%. Mupirocin eliminated MRSA in 75.0% (3/4) of patients. The 5.1% (4/77) patients without nasal carriage for MRSA showed nasal colonization of MRSA in follow-up screening in 2012.

**Conclusions:** Compared with previous reports, the prevalence of MRSA nasal carriage in outpatient was markedly lower. However, the prevalence in inpatients was high, and the long duration of hospitalization and ICU care was risk factor of nasal carriage for MRSA. Anti-infective program appears to be an effective preventive strategy.

## FR-PO975

**Free P-Cresol Sulfate Is Associated with Septicemia in Hemodialysis Patients** Tanushree Banerjee,[1] Timothy W. Meyer,[2] Tariq Shafi,[3] Michal L. Melamed,[4] Thomas H. Hostetter,[5] Yang Liu,[3] Neil R. Powe.[1] *[1]Univ of California, San Francisco; [2]Stanford Univ; [3]Johns Hopkins Univ; [4]Albert Einstein College of Medicine; [5]Case Western Reserve Univ.*

**Background:** The uremic syndrome is attributed to progressive retention of compounds which, under normal conditions, are excreted by the healthy kidneys. P-cresol sulfate (PCS), a prototype protein-bound uremic retention solute, has been shown to exert toxic effects in *vitro*. Recent studies have identified relations between increased levels of PCS and adverse clinical outcomes in hemodialysis (HD) patients. We explored the relationship between free PCS with septicemia in HD patients.

**Methods:** In a U.S. cohort of 294 incident HD patients enrolled in 1995-1998 and followed for an average of 3.4 years, we measured free PCS using mass spectroscopy. We linked USRDS Medicare billing records to ascertain septicemia over follow-up. We used Poisson regression to calculate incidence rate ratios (IRRs) for septicemia.

**Results:** Mean age was 57 years, 59% White and 44% female. The incidence of septicemia per 1000 patient-years was 43 in the lowest tertile, 87 in the middle tertile and 120 in the highest tertile of free PCS level. Unadjusted analysis showed a 68% higher risk of septicemia in the middle and 89% in the highest tertiles of PCS levels when compared with the lowest tertile (p=0.07, 0.002, respectively). After adjustment for age, sex, race, body mass index, comorbid disease, albumin, creatinine, Kt/V, and residual kidney function; those with levels in the middle tertile had 5% higher risk of septicemia [IRR (95% CI) =1.05 (0.65-1.69)] and those with highest tertiles had a 31% higher risk of septicemia [IRR (95% CI) = 1.31 (0.83-2.07)] when compared with the lowest tertile of PCS levels.

**Conclusions:** These results suggest an association between higher levels of free PCS and septicemia in HD patients. Better methods of dialysis should be developed to evaluate the utility of removing PCS and its effect on the outcome.

*Funding:* NIDDK Support

## FR-PO976

**Will Haemodialysis Patients Previously Immunized with a Hepatitis B Vaccine Respond to a Booster Dose Using a Different Product? Single Centre Experience on 115 Patients** Rosa M. Montero, Raja Mohammed Kaja Kamal, Khandaker Jubair Islam, Maggi Steele, David Makanjuola. *Epsom & St. Helier Univ Hospitals NHS Trust.*

**Background:** Maintaining immunity to hepatitis B in the haemodialysis (HD) population is important in order to reduce the chances of patients contracting this infection. Little evidence is available to determine whether those vaccinated with a particular type of hepatitis B vaccine will continue to maintain immunity if given a booster with a different type of hepatitis B vaccine.

**Aim:** To determine the effectiveness of a single booster dose of the recombinant Hep B DNA vaccine, Fendrix in a cohort of HD patients previously immunised with HBVaxPro.

**Methods:** All patients with established immune response to HBVaxPro had annual hepatitis B surface antibody titres measured. A titre< 10mIU/ml required a booster. All patients from October 2010- May 2013 were given booster doses using Fendrix. Data were collected through computerised systems. Davies co-morbidity scores were calculated for all patients to determine whether this affected response rates.

**Results:** 115 HD patients required boosters over this period. 62 male, 44 female. Median age 71.9 years with a median time of 2228 days on HD. 106 (92.9%) responded to a booster. The majority were Caucasian and 34% had type 2 diabetes. The non-responders had similar Davies co-morbidity score to the responders. 9 that showed no response went on to have a course of Fendrix; of these 100% responded to a full course.

**Conclusions:** Those with previous immunity to HBVaxPro showed a good response to a booster dose of Fendrix. Those that did not respond to a single booster went onto develop full immunity following a full course. Gender, age, median length on HD, type 2 diabetes or Davis co-morbidity score were not independent predictors of response. Our data show that in patients who previously responded to vaccination with the Hep B vaccine, this immunity is likely to be maintained despite changing to a different Hep B vaccine.

## FR-PO977

**Does It Matter which Type of Hepatitis B Vaccine Is Given to Haemodialysis Patients?** Rosa M. Montero, Khandaker Jubair Islam, Raja Mohammed Kaja kamal, Maggi Steele, David Makanjuola. *Epsom & St. Helier Univ Hospitals NHS Trust.*

**Background:** Maintaining immunity to hepatitis B in the haemodialysis (HD) population is important in order to reduce the chances of patients contracting this infection. We are not aware of any reports of the efficacy of switching to a different vaccine in HD patients when immunity has already been established with a different product.

**Aim:** To compare the response (R) and Non response (NR) between Hep B vaccine naive HD patients vaccinated with Fendrix compared with a historical cohort who had HBVaxPro.

**Methods:** A single centre retrospective study. Data were collected on all patients from October 2010 until May 2013. Fendrix was administered at 0, 1, 2 and 6 months. Patients with post-vaccination titres >10mIU/ml were classified as R, those with titres <10mIU/ml received a second course. Those that did not respond following 2 courses were classified as NR. Comparison was made with a historical cohort vaccinated with HBVaxPro from 1999-September 2010. The HBVaxPro course consisted of vaccinations given at 0, 1 and 6 months.

**Results:** 432 patients (3 were excluded for missing data) were in the historical cohort. 353/429 (82.3%) were immune following a single course with HBVaxPro. 212 male; 144 female, median age 66.8 years with median length of HD days 1690. 72 (17.7%) were given a second course and only 4 (0.9%) were NR. NRs had median age of 80.7 years with median length of 1544 days on HD. In contrast, the Fendrix cohort was 428 patients. 363 (84.8%) were immunised after. 258 male; 105 female with median age 70.8 years. 65 (15.2%) had a second course, of which 9 had no response (2.1% of the total cohort). The median age of NRs was 74.3 years and median time on HD was 1004 days. There were no significant correlations between response rates and ethinicity, diabetes, or the Davies co-morbidity scores in either group.

**Conclusions:** Response rates were similar between the two vaccines in our HD population. NR rates were slightly higher with Fendrix but this did not reach statistical significance. There was a trend towards non-response to the vaccines with male gender and increasing age. Overall, we found that both vaccines provided good levels of immunity.

## FR-PO978

**Discrepancy between Serological and Virological Analysis of Viral Hepatitis in Hemodialysis Patients** Jer-Ming Chang.[1,2,3] *[1]Dept of Internal Medicine, Kaohsiung Municipal Hsaio-Kang Hospital, Kaohsiung, Taiwan; [2]Div of Nephrology, Kaohsiung Medical Univ Hospital, Kaohsiung, Taiwan; [3]Falculty of Renal Care, College of Medicine, Kaohsiung Medical Univ, Kaohsiung, Taiwan.*

**Background:** The aim is to reveal the discrepancy between serological and virological analysis of viral hepatitis in hemodialysis (HD) patients, and review the suggestion of dedicated HD bed and machine.

**Methods:** 1681 HD patients (for at least six months) from 15 institutes were recruited. Hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (anti-HCV) were detected by a chemiluminescent microparticle immunoassay. HBV DNA was quantitatively measured with COBAS® AmpliPrep/COBAS® TaqMan® HBV test v2.0 (Roche Molecular System) kit. HCV RNA was measured by reverse transcription-polymerase chain reaction with Abbott RealTime™ HCV kit. HBsAg and anti-HCV were examined for every patient. HBV DNA was detected in HBsAg (+) samples and HCV RNA in anti-HCV (+) samples. HBVDNA/HCVRNA detection was done in a subset of HBsAg(-)/anti-HCV(-) patients.

**Results:** 230 (13.7%) patients are HBsAg (+) and 190 (17.3%) patients are anti-HCV (+).We are unable to detect HBV DNA in 97 of 230 (42.2%) HBsAg (+) patients, and HCV RNA cannot be found in 76 of 290 (26.2%) anti-HCV (+) patients. In 167 HBsAg (-) patients, only one shows a trace amount of HBV DNA (repeated examination showed negative result). None of 151 anti-HCV (-) patients shows detectable HCV RNA. Liver enzyme levels are significantly higher in patients with either positive serological or positive virological tests then those with negative tests.

**Conclusions:** Serological tests over-estimates the virological evidence. False positive rate for HBV is 42.2%, and for HCV is 26.2%. This study cannot provide sensitivity or specificity. But, at least in a subset of patients, false negative rate for hepatitis (negative HBsAg/anti-HCV but positive DNA/RNA) approaches zero. The annual sero-conversion rate (from negative to positive) of viral hepatitis has been very low in Taiwan (0.25% for HBV, 0.72% for HCV). Our findings might challenge the current suggestion of dedicated machine/bed and the diagnosis of viral hepatitis based solely on the serological examination.

*Funding:* Clinical Revenue Support

## FR-PO979

**Engaging Patients in the Consumer Assessment of Heathcare Providers and Systems 2012 Survey: Social Workers as Care Advocates** Deborah A. Benner, Duane V. Dunn, Shelley Murphy, Paul Asper, Andrew Lee, Andrew Barba. *DaVita HealthCare Partners, Denver, CO.*

**Background:** In 2012, the Centers for Medicare and Medicaid Services (CMS) required dialysis providers to administer the 58-question Consumer Assessment of Healthcare Providers and Systems (CAHPS) patient-experience survey to chronic in-center hemodialysis (ICH) patients (pts). Providers were directed to use a third-party vendor, applying the survey via mail or phone, but were permitted to operationalize survey administration as best suited to each provider. The administration of such an industry-wide tool had never been tested, nor were response rates easily predictable for such a tool.

**Methods:** DaVita HealthCare Partners and a vendor administered the ICH CAHPS survey between Sep-Nov 2012. Dialysis center staff received pre-education about the survey process. Social Workers and care givers provided education to each ICH patient, encouraging participation. The vendor mailed a prenotification letter then survey to patient addresses. Nonresponders received a postcard then second survey via mail. Continuing nonresponders were contacted by phone. From center-specific results, improvement plans were generated, and pts received education about center results and planned improvements.

**Results:** From 113,583 administered surveys, 52,410 (46.1%) were completed (39.0% mail, 7.2% phone), achieving an overall response rate of 46.9%. Response rate by geographic region ranged from 45%-50% with the highest rate in the Midwest. Reasons for nonresponse included no response to phone messages (14.1%), refusals (9.0%), no response (5.8%), language barriers (2.3%), callbacks (2.3%), and partial responses (0.9%). Some pts died during the survey period. Low responses came from homeless and nursing home pts. Center staff reported problems with health literacy and pts reading skills.

**Conclusions:** Intense patient advocacy by social workers resulted in high participation of pts dialyzed in DaVita HealthCare Partners centers. Achieving greater participation will require developing more effective approaches to specific patient groups. Utility of the survey would be enhanced by including home modalities and addressing challenges in health literacy.

*Funding:* Pharmaceutical Company Support - DaVita HealthCare Partners

## FR-PO980

**Removing the Two Day Gap in Three Times a Week Hemodialysis: A Cost Effectiveness Model** James Fotheringham,[1] Damian G. Fogarty,[2] Meguid El Nahas,[3] Michael J. Campbell,[1] John W. Stevens,[1] Andrew C. Rawdin.[1] *[1]School of Health and Related Research, Univ of Sheffield, Sheffield, United Kingdom; [2]The UK Renal Registry, Bristol, United Kingdom; [3]Global Kidney Academy, Sheffield, United Kingdom.*

**Background:** Excess admission and mortality rates exist after the 2 day gap in 3 times a week hemodialysis (HD). Existing comparative observational studies greatly increase HD frequency or are confounded by indication. The cost and potential impact of alternate day HD have not been evaluated.

**Methods:** Incident renal replacement therapy patients starting between 2002 and 2006 in England with available data until 2009 from the UK Renal Registry were analysed. Hospitalisation data was used to identify HD attendance patterns, comorbidity, admissions and associated cost. The model factored the cost of an additional HD session per fortnight, and a saving and survival benefit with the reduction in excess hospitalisation and mortality rates over the 2 day gap to that of the rest of the week. Estimated survival was calculated using a Weibull parametric model. Quality of life for patients on HD and time hospitalized were obtained from existing literature.

**Results:** 5790 patients were analysed. Compared to the rest of the week excess emergency admissions (2.29 vs 1.36 per patient year, P<0.001) and mortality (21.3 vs 15.7 deaths per 100 patient years, P<0.001) were identified after the 2 day gap, with greater differences for heart failure patients. For all patients mean survival was predicted at 7.7 years. Additional HD sessions cost $34,328 USD, generated a hospitalization cost saving of $2,411 and accrued 0.40 quality adjusted life years (QALYs). The cost per QALY was $79,067. For heart failure patients surviving a mean of 4.4 years, additional HD cost $20,722, saved $1,768 in hospitalisations and accrued 0.26 QALYs. The cost per QALY was $73,539. If survival and hospitalisation advantages were increased from approximately 10% to 16% the cost per QALY was $49,496 in all patients.

**Conclusions:** Alternate day dialysis would not be deemed cost effective based on a reduction in admissions and mortality after the two day gap to the rest of the week, but may be cost effective with a more global reduction in rates.

## FR-PO981

**Association of Initial Twice-Weekly Hemodialysis Treatment with Preservation of Residual Kidney Function in ESRD Patients** Minmin Zhang,[1] Mengjing Wang,[1] Haiming Li,[1] Ping Yu,[1] Jing Chen,[1] Kamyar Kalantar-Zadeh.[2] *[1]Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China; [2]Nephrology & Hypertension, Univ of California Irvine Medical Center, Orange, CA.*

**Background:** The relationship between hemodialysis(HD) and residual kidney function(RKF) has not been well examined. We hypothesized that twice-weekly HD during the first several months of HD maintains RKF longer.

**Methods:** A historical cohort study was performed in 85maintenance HD patients to compare RKF loss and clinical outcomes, in which 30 patients on twice-weekly HD for more than 6 months initially, the other 55 patients starting and maintaining thrice-weekly HD. Then, a subcohort study was implemented in 48 incident HD patients whose residual GFR was examined serially, to assess the potential predictors of the decline of RKF.

**Results:** The main cohort study showed that the HD adequacy and the clinic outcomes were same in 85 patients receiving HD treatment for more than 5 years. However, the rate of RKF loss(defined as urine volume <200 ml/day) was significantly lower in twice-weekly group(60%) compared to thrice-weekly(82%), especially during the first year of HD initiation(10% versus 40%, p=0.03). In the subcohort study, the residual GFR of 48 patients, treated with 2.69±0.43 times per week HD for 10.27±3.53 months, declined from baseline 6.95±2.66 to 2.12±1.97 ml/min/1.73m$^2$ and 40% of patients lost their RKF over an average period of 7.6±4.5 months of HD initiation. Multivariate logistical regression analysis showed that odds ratio of RKF loss for each additional HD treatment per week was 7.2 in HD patients.

| Factor | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | OR | 95%CI | *P* | OR | 95%CI | *P* |
| Sex(male to female) | 0.34 | 0.10-1.16 | 0.09 | 0.08 | 0.01-0.64 | 0.018 |
| HD frequency(time/week) | 3.38 | 0.83-13.75 | 0.09 | 7.20 | 1.10-47.09 | 0.039 |
| Urea reduction ratio(%) | 0.94 | 0.87-1.02 | 0.15 | 0.85 | 0.72-0.998 | 0.047 |
| Intradialytic hypotension episode(each episode) | 2.04 | 0.92-4.54 | 0.08 | 2.51 | 1.06-5.95 | 0.036 |

**Conclusions:** Initial twice-weekly HD during the first year of dialysis therapy appears associated with better RKF preservation.

*Funding:* Government Support - Non-U.S.

## FR-PO982

**The Returns of the MACs: A Natural Experiment Relating More Than Thrice Weekly Home Hemodialysis and Patient Outcomes** Richard Hirth, Adam S. Wilk, Wei Zhang, John R.C. Wheeler, Tammie A. Nahra, Kathryn Sleeman, Joseph M. Messana. *Univ of Michigan, Ann Arbor.*

**Background:** Fiscal intermediaries (FIs) and Medicare Administrative Contractors (MACs) that pay Medicare claims can authorize payment for >3 weekly treatments. In prior work we show FIs/MACs may contribute significantly to variation in HHD practice and a HHD patient's expected treatment frequency may be a function of the FI/MAC that processes the facility's claims. The plausibly random exposure of similar patients to different HHD treatment frequencies across FIs/MACs offers a natural experiment for assessing differences in patient (pt) outcomes due to more and less frequent HHD: we look at measures of inpatient days and drug costs per outpatient month.

**Methods:** We identified all Medicare HHD patients in 2009-12 by FI/MAC, and as average monthly HHD pt treatments (adjusted for inpatient days to reflect HHD treatments per outpatient month), average counts of inpatient days, and average amounts paid for erythropoietin-stimulating agents (ESAs) and other ESRD-related drugs per patient-month by FI/MAC.We used regression models to define the association between HHD pt treatments per month and individual FIs/MACs active by year, adjusting for pt age, race, BMI, and 23 Medicare claims-derived comorbidities. We plotted average predicted HHD treatments against each outcome measure by FI/MAC by year.

**Results:** Evidence suggests that higher HHD treatment frequency is associated with fewer inpatient days (slope=-0.27, p<.0001) and lower non-ESA drug costs per month, such as for iron (slope=-5.1, p<.0001) and vitamin D (slope=-2.9, p<.0001) was found. (Effects for ESA costs are not significant).

**Conclusions:** These cost savings offset only a fraction of the increased spending on extra HHD sessions. We find similar results in two-stage least squares analyses of effects of increased HHD treatment frequency on pt outcomes, instrumenting for HHD treatment frequency with average HHD authorizations by FI/MAC. While increased HHD treatment frequency may improve some pt outcomes, effects on other important outcomes like survival and quality of life remain to be examined.

*Funding:* Other U.S. Government Support

## FR-PO983

**Characteristics of Patients Receiving Twice Weekly Hemodialysis** Menaka Sarav, Neenoo Khosla. *Dept of Nephrology and Hypertension, NorthShore Univ HealthSystem, Evanston, IL.*

**Background:** In developing countries, large numbers of patients with ESRD receive hemodialysis (HD) less than three times a week. In the United States, this practice is uncommon. However, patients who are elderly and have residual renal function (RRF) may do well with twice weekly dialysis. We evaluated characteristics of incident HD patients at 4 dialysis units who receive twice weekly HD and compared this group's hospital rate to the United States Renal Data System (USRDS) rate.

**Methods:** All patients receiving HD, more than 3 months, twice weekly were included. Demographics, pre-dialysis and recent lab and clinical status were evaluated.

**Results:** Eighteen patients out of a total of 283 patients were included. Data evaluated is presented in the table below.

| **Demographics** | **Data** |
|---|---|
| Total HD patients | 283 |
| Total 2x week HD patients | 18 (6.3% of the total) |
| Average age | 75 +/- 15 years |
| Male | 80% |
| Caucasian | 55% |
| African American | 20% |
| Diabetics | 30% |
| Pre-CKD care with nephrologist | 70% |
| Pre-ESRD BUN | 71 +/- 27 mg/dl |
| Pre-ESRD Cr | 5.2 +/- 2 mg/dl |
| Uremia as indication for start of HD | 70% |
| Vintage | 2.9 +/- 2.2 years |
| Access at start of HD- catheter | 70% |
| Current BUN | 61 +/- 17 mg/dl |
| Current Cr | 6.7 +/- 3 mg/dl |
| URR (Urea reduction ratio) | 71.9 +/- 5 |
| Current albumin | 3.68 +/- 0.5 gm/dl |
| Average weight | 70 +/- 14 kg |
| IDW (Inter-dialytic weight gain) | 1.5 +/- 0.8 kg |
| Hospital days / pt year | 2.27 |

Patients have been maintained on twice weekly HD for an average of 2.9 years +/- 2.2 years. The majority of patients were non- diabetic males and Caucasians aged 75+/-15. Seventy percent were seen in a dedicated CKD clinic prior to HD. Uremia was the main indication for initiation. The average inter-dialytic weight gain was low (1.5 +/-0.8 kg) suggesting preserved RRF. The serum albumin was maintained at an average of 3.68 +/-0.5 mg/dl. The hospital rate was lower at only 2.27 days / patient year when compared to the USRDS rate of 12 days / patient year.

**Conclusions:** Patients can be maintained for extended periods of time on twice weekly HD with a low rate of hospitalization and preserved volume and nutritional status. Further studies comparing outcomes to patients on thrice weekly dialysis are needed.

## FR-PO984

**Outcomes of Patients with End-Stage Renal Disease Undergoing Twice-Weekly and Thrice-Weekly Hemodialysis** Thanachai Panaput, Dhavee Sirivongs, Bandit Thinkhamrop, Cholatip Pongsakul, Sirirat Ruengjui, Surapong Narenpitak, Pisith Intarawongchot, Laksamon Praderm, Sajja Tatiyanupanwong, Chavasak Kanokkanapong, Pakorn Tungkasereerak, Thatsalang Keonounma, Amarit Suwattanasin. *The Hemodialysis Study Group (HENNET), Thailand.*

**Background:** Most ESRD patients in a resource-limited setting were treated with twice-weekly haemodialysis (HD). We aimed to analyze outcomes between patients on twice HD per week (2-HD/wk) and thrice HD per week (3-HD/wk).

**Methods:** A prospective multi-center cohort study was conducted in 11 HD centers. The participants aged of 18 to 80 years, undertaken twice-weekly or thrice-weekly HD for at least 3 months were recruited. Baseline characteristics were recorded at recruitment. Outcomes including death and first hospitalization were observed. Kaplan-Meier method and cox regression analysis was computed.

**Results:** A total of 673 participants, 504 in 2-HD/wk and 169 in 3-HD/wk group were recruited. They were male in 59% with mean age of 56 years and 39.8% were DM. Median follow up time was 13.7 months. During the total follow-up time, 9,254 patient-months,45 patients died, 33 in 2-HD/wk group (6.5%), and 12 in 3-HD/wk group (7.1%). The one year survival probability of 2-HD/wk group, 0.94 (95% confidence interval (CI); 0.92 to 0.96) was comparable to that of 3-HD/wk group, 0.94 (95% CI; 0.89 to 0.96), hazard ratio = 0.99, 95% CI; 0.5 to 2.0, p = 0.99.

**Figure 1.** Kaplan Meier survival estimate among patients treated with twice-weekly and thrice-weekly HD.

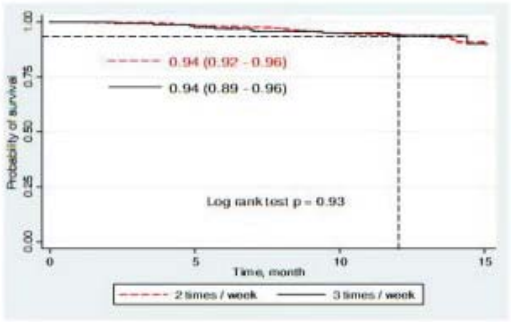
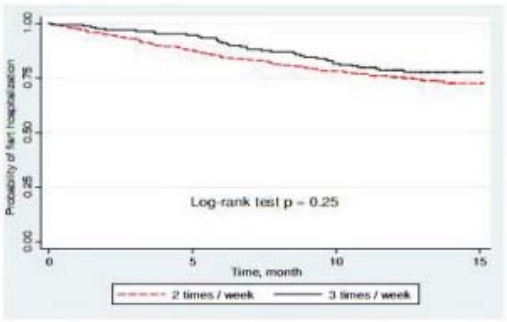



**Figure 2.** Kaplan-Meier survival curves of first hospitalization among patients treated with twice-weekly and thrice-weekly HD.




There was no significant difference in incidence of first admission between 2-HD/wk (24.2 / 100 patient-years) and 3-HD/wk group (19.7 / 100 patient-years), log rank test p-value = 0.25.

**Conclusions:** Participants who were treated with 2-HD/wk had a similar one year survival rate but tended to associate with higher incidence of hospitalization when compared to those with 3-HD/wk. Twice-weekly HD is an alternative treatment scheme in resource-limited settings.

*Funding:* Private Foundation Support

## FR-PO985

**Challenges Recruiting to a Study Investigating Extended Treatment Time on Haemodialysis Outcomes** Seema Singh, Albert J. Power, Charles D. Pusey, Neill D. Duncan, Edwina A. Brown. *Imperial College Renal & Transplant Center, United Kingdom.*

**Background:** Outcomes on conventional haemodialysis (HD) remain suboptimal with observational data suggesting better survival with extended treatment times (TT). In the absence of prior trial data we studied the effect of extended TT on HD outcomes.

**Methods:** Randomised cross-over study of in-centre HD patients with TT of either 6 hrs or 4 hrs for a period of 24 wks with a 4wk washout period between the two arms (NCT01721421). Assessments of nutritional status (malnutrition inflammation score, bioimpedance analysis, hand grip), inflammation and atherosclerosis (hs-CRP, MCP-1, BNP) are made before and after each treatment phase. Time to recovery is assessed and quality of life domains (KDQoL).

**Results:** In total 634/725 (87%) patients screened met inclusion criteria 23 of these consented to date (21 male, mean age 58.7±12.9yrs), a recruitment rate of 4%. Reasons cited by patients for refusing consent include inability to sit for long periods and commitments as carers to partners & family and anxiety that the shorter control TT (for those whose pre study TT>4) may be detrimental. 60% of nursing staff questioned felt extended TT was beneficial with 36% reporting it well tolerated. Nonetheless just 38% would recommend extended TT to patients with 66% stating it affected service provision.

**Conclusions:** Enrolled patients tolerate extended treatment times well but there is a significant impact on in-centre HD provision. Staff attitudes may impact on enrolment in similar trials and must be addressed. Extended TT maybe more pragmatic in domiciliary or nocturnal HD services.

*Funding:* Government Support - Non-U.S.

## FR-PO986

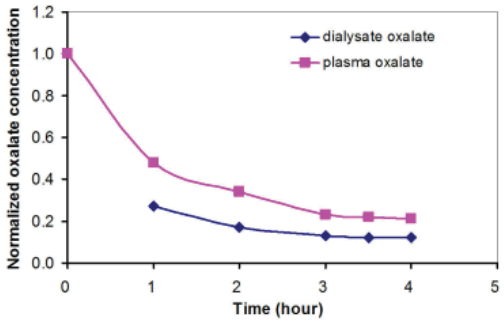
**Oxalate Quantification in Hemodialysate to Assess Dialysis Adequacy for Primary Hyperoxaluria** Xiaojing Tang, Nick Voskoboev, Stacie L. Wannarka, Julie B. Olson, Dawn S. Milliner, John C. Lieske. *Mayo Clinic, Rochester, MN.*

**Background:** Aggressive hemodialysis (HD) is required to prevent systemic oxalosis in primary hyperoxaluria (PH) patients with renal failure, and the required amount of HD increases as residual renal function declines. Due to extravascular oxalate deposition, plasma oxalate (Pox) does not reflect systemic oxalate loads. This study validated a method to accurately measure oxalate removal via HD.

**Methods:** 14 stable PH1 patients treated with HD by physicians in the Rare Kidney Stone Consortium were included. A high flux dialyzer (Polyflux Revaclear) was used 3-4 h per session. Dialysate (Dox) and Pox were measured hourly via enzymatic oxalate oxidase. Oxalate production was estimated from historical Uox measured when GFR was > 50 mL/min/1.73m$^2$. HD regimens were adjusted according to historical Uox, amount of oxalate removal at HD, residual Uox, and predialysis Pox.

**Results:** Mean reduction in Pox was 79±7% (Figure), with a mean pre and post dialysis Pox of 68.9±37.5 μM/L and 13.0±5.8 μM/L. 12 patients had urine output at the time of study with an average Uox of 1.1±0.8 mM/24h. Based upon HD oxalate removal and Uox clearance, 7 patients required HD 6 times a week, 2 patients 5 times a week, and 5 patients 3 times a week. Combined renal and HD oxalate clearance was 16.8±7.6 mM/wk, which exceeded endogenous oxalate production (13.4±3.9mM/wk). 8 patients received a kidney or combined liver-kidney transplant after a mean period of 4 mos on HD. The final pre-dialysis Pox before transplantation was significantly lower than the initial pre-dialysis Pox before individualizing HD (45.4±23.1 vs 78.8±38.0 μM/L, P=0.002).

**Oxalate kinetics on Hemodialysis averaged from 14 patients.**




**Conclusions:** In conclusion, a method to measure Dox was validated that can be used to individualize the HD prescription of PH patients to prevent systemic oxalosis, and to reduce the risk of oxalate nephropathy in the transplanted kidney.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## FR-PO987

**Calcium Equivalent Citrate Dialysis** Jan P. Sternby, Anders Nilsson, Gunilla Grundstrom, Maria Alquist. *Research Dept, Gambro.*

**Background:** Citrate has in recent years been introduced as a more physiological alternative to acetate in bicarbonate hemodialysis. Citrate entering the blood complex binds calcium (Ca). Some protein bound Ca is then released to maintain the equilibrium between protein bound and free ionized Ca. Both free and citrate bound Ca can pass the dialysis membrane which increases the Ca transport from the blood. We developed an algorithm to calculate the mass balance of Ca transfer over the membrane. The objective was to establish which Ca concentration in a citrate containing dialysis fluid gives the same total Ca mass transfer as a citrate-free dialysis fluid. The same was also done for magnesium (Mg).

**Methods:** The mass transfer area coefficients for ions and complexes were assumed proportional to their diffusive mobility. The electrical membrane potential was considered by requiring electroneutrality. As transfer of complexes across the membrane affects concentration gradients for both complexes and individual ions, we included in the calculations the (pH dependent) binding of ions like hydrogen, Ca and Mg to albumin and of Ca and Mg to bicarbonate and citrate. The dialyzer was considered being composed of serial subsegments. For each segment the transport of each solute and complex were calculated separately. With the given inlet concentrations for each solute the outlet concentrations for a dialyzer segment were calculated from the transports. From total outlet concentrations new distributions between free concentrations and complexes were calculated and used as input to the next subsegment. Parameters used: Plasma concentration of total Ca 2.2-2.4 mM (mmol/l), plasma citrate 0.1-0.3 mM, blood flow rate 200-400 ml/min, dialysis fluid flow rate 500 and 800 ml/min, urea KoA 700 and 1000 ml/min, Ca concentrations in dialysis fluid without citrate 1.0, 1.25, 1.5 and 1.75 mM, and citrate levels in the dialysis fluid 0.25 – 2 mM.

**Results:** In all cases the need for extra Ca and Mg in the dialysis fluid increases almost linearly with the citrate level. Each mM of citrate requires 0.15 mM additional Ca to maintain the same Ca mass balance.

**Conclusions:** Calcium equivalent citrate dialysis requires 0.15 mM more calcium in the dialysis fluid for each mM of citrate.

*Funding:* Pharmaceutical Company Support - Gambro

**FR-PO988**

**Extracorporeal Phosphate Removal with a Packed Bed Adsorber**

Shada M. Salem,[1] Rebecca J. Desch,[1] Vadim Guliants,[1] Stephen W. Thiel,[1] Heather Duncan,[2] Kotagal Shashi Kant.[2] *[1]Biochemical, Chemical and Environmental Engineering, Univ of Cincinnati, Cincinnati, OH; [2]Internal Medicine, Univ of Cincinnati College of Medicine, Cincinnati, OH.*

**Background:** Phosphate (PO4) continues to be a complex and vexing problem for dialysis patients. PO4 dialysance with conventional dialysis is suboptimal largely because of the multicompartment distribution of phosphate. Oral PO4 binder effectivenss is limited by cost, efficacy, and adherence. Our approach incorporates a packed bed for PO4 adsorption into the dialysis circuit to remove excess PO4 during hemodialysis. This will address the multicompartment nature of PO4 distribution and increase total PO4 removal.

**Methods:** The efficacy of adsorbents incorporating aluminum, lanthanum, and zirconium were evaluated with batch adsorption (0-40 mg/dL PO4), kinetic studies (10 mg/dL PO4), and column studies (50 mg/dL) in water at pH 8.2 and 37°C.

**Results:** PO4 adsorption to resin-based adsorbents was slow. Lanthanum-activated carbon and aluminum oxide showed relatively rapid adsorption with a PO4 capacity of 30 mg/g adsorbent at a PO4 concentration of 5 mg/dL. PO4 adsorption kinetics were often not first order, showing rapid initial uptake followed by slower equilibration after 30 min. Type I adsorption isotherms demonstrated strongly favorable adsorption at low PO4 concentrations. The interference of key blood components, including salts and proteins, was tested for the adsorption of 20 mg/dL PO4. Most components enhanced or slightly reduced PO4 loadings; however, bovine serum albumin (BSA) reduced PO4 capacity significantly. Column tests showed significant phosphate removal but more rapid PO4 breakthrough and lower adsorbent utilization than expected.

PO4 desorption using potential regenerants was measured. Concentrated acid or base effectively desorbed PO4 but damaged the adsorbents.

**Conclusions:** Lanthanum activated carbon and aluminum oxide show promise as selective, high-capacity, low-cost materials for extracorporeal phosphate removal in dialysis. Key issues moving forward are increasing the capacity of the adsorbent materials and improved column design to capitalize on the inherent equilibrium and kinetic properties of these materials.

**FR-PO989**

**Biofeedback Controlled Haemodialysis Increases Ionic Mass Removal**

Isabelle Kazes,[1] Coralie Barbe,[2] Khaled Gaha,[1] Herve Maheut,[1] Philippe Rieu.[1] *[1]Nephrology Dept, Univ Hospital, Reims, France; [2]Clinical Research Unit, CHU, Reims, France.*

**Background:** The Hemocontrol® system controls **blood volume changes** by acting on the actual ultrafiltration rate and the actual dialysate conductivity during the whole dialysis session. It has been shown that biofeedback haemodialysis can improve intradialytic hypotension.

We have studied the effect of Hemocontrol® (HC) on ionic mass balance, weight loss and dialysis quality parameters, compared to standard haemodialysis (HD).

**Methods:** Between 2007 and 2011, part of chronic dialysis patients at the University Hospital of Reims (France) were dialysed using both HD and HC haemodialysis. One or the other mode of dialysis was used with each patient in an occasional manner, only depending on organizational obligations. 35 439 dialysis sessions were analyzed. In total 2101 pairs, concerning 155 patients (i.e. 4202 sessions) were matched on 6 different criteria: the patient him/herself (the patient being his own control), the dialysis membrane, the blood pump speed (±15ml/min), patient weight before dialysis (± 100 gr), prescribed dialysate conductivity and the period (±6 months). The two groups HC and HD were compared using conditional logistic regression.

**Results:** With the same duration of dialysis sessions in the two groups (3:56±0:14 [3:00; 4:59]h:min for HC and 3:57±0:14 [3:00; 4:59]h:min for HD), we observed in the **HC group:** a **higher depurated volume** Kt (measured by ionic dialysance: 43.38±5.35 [30.0; 65.5]L *vs* 42.95±5.33 [30.0; 62.4]L; p<0.0001), and Kt/V (measured by ionic dialysance: 1,25±0,22 [0.58; 2.01] *vs* 1,24±0,21 [0.60; 2.08]; p<0.0001), a **significant increase** of **total fluid removal** (2.71±0.83 [0.47; 5.47]L *vs* 2,47±0,69 [0.50; 5.0]L; p<0.0001) and **ionic mass balance** (391.34±169 [-299; 953]mmol *vs* 286,13±116 [-91; 709]mmol; p<0,0001). No difference was observed on plasma conductivity at the end of treatment . Dry weight was obtained more often in the HC group (80,4% *vs* 78,1%; p=0,048).

**Conclusions:** This study shows that the **Hemocontrol®** System makes it possible to reach target dialysis dose with higher extraction of salt (105 mmol on average). 1/3 of this is linked to higher fluid removal (240 mL on average).

**FR-PO990**

**Individualized Dialysate Bicarbonate Concentration in Hemodialysis Patients**

Rapeepat Lekkham, Rasib Raja. *Nephrology, Einstein Medical Center, Philadelphia, PA.*

**Background:** Metabolic alkalosis is associated with adverse outcomes in hemodialysis patients. Prescription of high dialysate bicarbonate concentration was common in the United States but the optimal concentration remains uncertain. We studied the different variables among patients treated with different dialysate bicarbonate concentrations to find the optimal dialysate bicarbonate.

**Methods:** We conducted a retrospective data analysis on 90 patients who received HD 3 times a week from a single dialysis unit. Total study duration was 6 months. Data was collected retrospectively for first 3 months, when we were using 38 mEq/L bicarbonate dialysate concentration with acetate 8 mEq/L. Then we individualized the bicarbonate concentration to maintain the serum pre-dialysis bicarbonate 22±2 mEq/L and collected data for the following 3 months.We compared the different variables in the both study groups.

**Results:** The mean serum pre-dialysis bicarbonate before and after individualized bicarbonate bath was 25.44±2.09 mEq/L and 22.82±2.36mEq/L respectively (P <0.001). After individualizing dialysate, 67.8% of patients required 35mEq/L, 14.4%>35mEq/L and 17.8 %< 35mEq/L of bicarbonate concentration. Most patients before individualized dialysate had high serum pre-dialysis bicarbonate ( >25meq/L in 65.6% and >29meq/L in 5.6%). After individualizing, serum pre-dialysis bicarbonate was >25meq/L in only 8.8% and >29meq/L in 1%. There was no statistically significant difference in the other variables like Kt/V, Albumin, phosphate, iPTH, ESA, sensipar, and vitamin D requirements in both groups. In subgroup analysis, there was no statistically significant difference in those variables in patients who required dialysate bicarbonate concentration 35 mEq/L, less than 35 mEq/L or more than 35 mEq/L.

**Conclusions:** Our data suggests that there is no standard dialysate bicarbonate bath in hemodialysis patients. We need to individualize the dialysate bicarbonate concentration to maintain serum pre-dialysis bicarbonate 22±2 mEq/L. Monthly serum pre-dialysis bicarbonate was the only laboratory parameter which influence the choice of dialysate bicarbonate concentration.

**FR-PO991**

**Removal of Protein-Bound, Hydrophobic Uremic Toxins by a Combined Fractionated Plasma Separation and Adsorption Technique**

Falko Boehringer,[1] Markus Toelle,[1] Markus Van der Giet,[1] Sonja Steppan,[2] Vera Jankowski,[1] Joachim Jankowski.[1] *[1]Med. Klinik IV, Charite, Berlin, Germany; [2]Fresenius Medical Care, Germany.*

**Background:** Patients with chronic kidney disease show an increased cardiovascular mortality and morbidity due to several conservative risk factors and uremic retention solutes toxicity. Protein-bound uremic toxins, such as phenylacetic acid, indoxyl sulfate and p-cresylsulfate contribute substantially to the progression of chronic kidney disease and cardiovascular disease. However, based on their protein-binding these hydrophobic uremic toxins are poorly cleared during conventional dialysis and thus accumulate in CKD-5D-patients.Therefore, we investigated whether hydrophobic and cationic adsorbers are more effective for removal of protein-bound, hydrophobic uremic toxins than conventional high-flux hemodialyzer.

**Methods:** Five CKD-5D patients were treated using the fractionated plasma separation, adsorption and dialysis system (FPAD) for 5 h; for anticoagulation. A control group of five CKD patients was treated with conventional high-flux hemodialysis. Plasma concentration of phenylacetic acid, indoxyl sulfate and p-cresylsulfate were quantified at different time points and different sites, using gradient ion-pair reversed phase chromatography.

**Results:** The removal rates of FPAD treatment in comparison to conventional high-flux hemodialysis were increased by 130 % for phenylacetic acid, 187 % for indoxyl sulfate and 127 % for p-cresol.The plasma concentration of phenylacetic acid, indoxyl sulfate and p-cresylsulfate decreased by 1.15±0.17 mmol $L^{-1}$, 0.21 ± 0.05 mmol $L^{-1}$ and 0.24 ± 0.06 mmol $L^{-1}$ using the FPAD and 0.75 ± 0.40 mmol $L^{-1}$, 0.09 ± 0.04 mmol $L^{-1}$ and 0.23 ± 0.11 mmol $L^{-1}$ using conventional high-flux hemodialysis (p<0.05).

**Conclusions:** Due to the high impact of protein-bound, hydrophobic uremic toxins on progression of CKD and CVD in CKD-5D patients, the use of an adsorber in combination with dialysis membranes may be a new therapeutic option to increase the removal rate of these uremic toxins. However, larger, long-term prospective clinical trials are needed to demonstrate the impact on clinical outcome.

**FR-PO992**

**Elimination of the Middle Size Molecules by the FXCorDiax 60 in Relation to the FX 60-Dialyzer**

Francisco Maduell,[1] Paul Wieneke,[2] Marta Arias,[1] Manel Vera,[1] Josep M. Campistol.[1] *[1]Nephrology and Renal Transplantation, Hospital Clinic Barcelona, Spain; [2]Fresenius Medical Care Deutschland GmbH, Germany.*

**Background:** The incorporation of nano-controlled spinning technology in the design of dialyzers allowed to create a new generation of dialyzers with the goal of moving the sieving coefficient curve to the right and increase the steepness in order to get an optimal cut-off point. Theaim of this study was to compare two 1.4$m^2$ polysulfone dialyzers, current and new generation with respect to the efficiency in the elimination of a broader spectrum of uremic toxins.

**Methods:** In an open, randomized, cross-over, monocentric, controlled, prospective clinical study 30 hemodialysis patients were treated by post-dilution on-line hemodiafiltration once with the FX 60 or FX CorDiax60 dialyzer type. The remaining dialysis parameters did not vary: dialysis time 292±17 minutes, blood flow 408±32 ml/min and dialysate flow 500 ml/min. Intraindividually the removal ratio (RR) of urea, phosphate, $ß_2$-microglobulin ($ß_2$-m), myoglobin, prolactin, $a_1$-microglobulin, $a_1$-acid glycoprotein, albumin and total protein was compared.

**Results:** A significantly higher RR of myoglobin (75.2 ± 11 vs 58.6 ± 12, p<0.001), BUN (86.2 ± 4 vs 84.9 ± 5, p<0.015), $ß_2$-m (84.7 ± 4 vs 81.3 ± 5, p<0.001), Prolactin (73.0 ± 10 vs 56.9 ± 13, p<0.001) and $a_1$-microglobulin 20.9 ± 18 vs 13.6 ± 12.5, p<0.001) was detected for FX CorDiax 60 in comparison to FX 60. There were no significant differences inthe RR for phosphate, $a_1$-acid glycoprotein, albumin and total protein. On the dialysate side, a significantly increased eliminated mass was observed for myoglobin (1.83 ± 0.9 vs 1.51 ± 0.8 mg, p<0.002), $ß_2$-m (0.26 ± 0.09 vs 0.24 ± 0.09 g, p<0.001), prolactin (0.17 ± 0.13 vs 0.14 ± 0.08 mg, p<0.02) and albumin (4.25 ± 3.5 vs 3.01 ± 2.4 g, p<0.03), respectively.

**Conclusions:** In this study it could be shown that treating patients with FX CorDiax 60 dialyzers instead of FX 60 results in significantly increased reduction ratios of middle sized molecules without clinically relevant changes in albumin loss. This change could represent an improvement in the removal capacity that could be transferred to potential clinical benefits.

## FR-PO993

**Intra-Dialyzer Oxidative Stress (OS) Is Ameliorated by Molecular Hydrogen-Enriched Hemodialysis (HD) Solution ($H_2$-HD)** Wan-jun Zhu,[1,2] Hiroyuki Terawaki,[1] Masaaki Nakayama,[1,2] Yasuhito Takahashi,[1] Shigeru Kabayama,[2] Kaoru Sakurai,[1] Sadayoshi Ito.[2] *[1]Dialysis Center, Fukushima Medical Univ, Fukushima, Japan; [2]Center for Advanced and Integrated Renal Science, Graduate School of Medicine, Tohoku Univ, Sendai, Japan.*

**Background:** OS is the leading cause of HD patients' mortality. HD exacerbates OS via removing antioxidants and excess oxidative burst of neutrophils in the dialyzer. We recently revealed that $H_2$ could act as an anti-OS mediator using $H_2$-HD in the clinical setting. This study aimed to clarify $H_2$-HD's possible anti-OS effect during HD session.

**Methods:** A HD cross-over study was performed using standard solution (S-HD) and $H_2$-HD in the mid-week (n=8, 4 hours each). Blood samples of inlet and outlet were obtained from the dialyzer at the start and end. The redox state of human serum albumin (HSA) were examined as an OS marker; non-oxidized human mercapto-albumin (HMA), reversibly-oxidized human non-mercaptoalbumin-1 (HNA-1) and irreversibly-oxidized form (HNA-2) by HPLC.

**Results:** $H_2$ level of $H_2$-HD in blood and solution were 50 times of S-HD. In $H_2$-HD, f(HMA) increased and f(HNA-1) decreased significantly ($p<0.05$, start of HD, respectively), furthermore, f(HNA-1) decreased significantly ($p<0.05$, end of HD), while no changes in S-HD, suggesting $H_2$-HD's anti-OS effect.

| The albumin redox of each serum | | | | | |
|---|---|---|---|---|---|
| | S-HD | | | H2-HD | |
| | | f (HMA) % | f (HNA-1) % | f (HMA) % | f (HNA-1) % |
| Pre | | | | | |
| | Inlet | 62.2±14.6 | 34.2±14.0 | 60.5±13.3 | 35.6±12.5 |
| | Outlet | 62.9±13.2 | 33.4±13.0 | 63.3±13.8* | 32.8±13.0* |
| Post | | | | | |
| | Inlet | 77.1±9.5 | 20.0±9.0 | 74.5±10.8 | 22.7±10.5 |
| | Outlet | 76.1±9.6 | 21.2±11.0 | 75.9±11.0 | 20.9±9.5¶ |

*; P < 0.05 vs. Pre-Inlet, ¶; P < 0.05 vs. Post-Inlet, f;fraction

**Conclusions:** $H_2$-HD could suppress intra-dialyzer OS induced by HD session.

*Funding:* Private Foundation Support

## FR-PO994

**Different Impact of Membrane Flux on Mortality between Incident and Prevalent Hemodialysis Patients** Yong Kyun Kim,[1] Hyung Wook Kim,[1] Su Hyun Kim,[2] Su Jin Choi,[1] Young ok Kim,[1] Ho Cheol Song,[1] Yong-Lim Kim,[3] Yon Su Kim,[4] Shin-Wook Kang,[5] Chul Woo Yang.[1] *[1]Dept of Internal Medicine, College of Medicine The Catholic Univ of Korea, Seoul, Korea; [2]Dept of Internal Medicine, College of Medicine Chung-Ang Univ, Seoul, Korea; [3]Dept of Internal Medicine, School of Medicine Kyungpook National Univ, Daegu, Korea; [4]Dept of Internal Medicine, College of Medicine Seoul National Univ, Seoul, Korea; [5]Dept of Internal Medicine, College of Medicine Yonsei Univ, Seoul, Korea.*

**Background:** The effect of flux membrane on mortality has been controversial. Previous study reported that the effect of high-flux dialysis on mortality may vary depending on the duration of dialysis. We evaluated the effects of dialyzer membrane flux on mortality in incident hemodialysis (HD) patients and prevalent HD patients.

**Methods:** In this prospective observational study, 817 incident and 1,353 prevalent patients with HD were included. In incident HD patients, 589 patients were dialyzed using low-flux dialysis membrane and 228 were dialyzed using high-flux dialysis membrane. In prevalent HD patients, 801 patients were dialyzed using low-flux dialysis membrane and 552 patients were dialyzed using high-flux dialysis membrane. All-cause mortality was primary outcome.

**Results:** After a median 20 months of follow-up, mortality was not significantly different between the high-flux group and low-flux group in incident HD patients (HR 1.45, 95% CI, 0.86-2.58, P = 0.155). In prevalent HD patients, HD using high-flux dialysis membrane was associated with decreased mortality compared to HD using low-flux dialysis membrane in univariate (HR 0.43, 95% CI, 0.25-0.74, P = 0.002), multivariate (HR 0.40, 95% CI, 0.21-0.77, P = 0.006) or propensity score-matched (HR 0.35, 95% CI, 0.18-0.67, P < 0.001) analyses.

**Conclusions:** Our data showed that HD using high-flux dialysis membrane had survival benefit in prevalent HD patients, but not in incident HD patients. These findings suggest that the effect of flux membrane on mortality may be different between incident and prevalent HD patients and high-flux dialysis might be recommended for the prevalent patients with HD treatment.

*Funding:* Government Support - Non-U.S.

## FR-PO995

**Correlation of Ionic Dialysance Kt/V with Urea-Based Kt/V Is Better in Men Than in Women for All Estimations of V** Tuan A. Nguyen, Vincent Paracuelles, Niti Madan, Andrew I. Chin. *Div of Nephrology, Univ of California, Davis School of Medicine, Sacramento, CA.*

**Background:** Use of ionic dialysance (ID) based measures of Kt/V in HD patients requires input of an estimated urea distribution volume (V). We examined gender-stratified correlation of ID Kt/V, using various calculations of V, against traditional urea-based Kt/V.

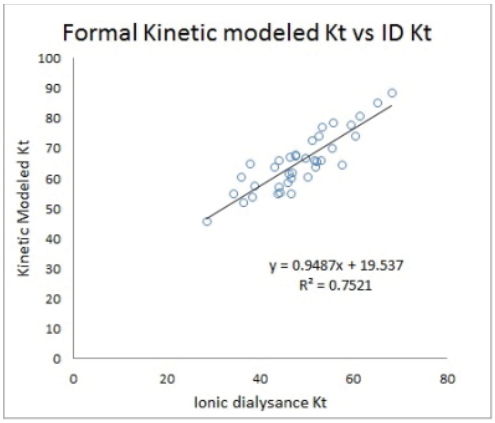
**Methods:** This was a retrospective review of CKD 5 on HD patients who had ID Kt/V measures with simultaneous pre and post HD BUN levels done. We compared ID Kt/V, using an array of calculations for V, against urea-based Kt/V by the Daugirdas equation.

**Results:** Anthropometric V methods and correlation of ID Kt/V to urea-based Kt/V are shown in the table.

| Method of calculated V | Correlation of ID Kt/V with Daugirdas Kt/V ($R^2$ value)* | | |
|---|---|---|---|
| | All subjects | Men | Women |
| | *n=68* | *n=31* | *n=37* |
| Watson | 0.43 | 0.63 | 0.29 |
| Surface Area Normalized Watson | 0.41 | 0.59 | 0.31 |
| Chertow | 0.43 | 0.68 | 0.25 |
| HEMO | 0.42 | 0.64 | 0.27 |
| Lee | 0.45 | 0.72 | 0.27 |

**all correlations statistically significant p<0.01*

There was a consistent underestimation of Kt/V by ID. While statistically significant in both genders, the correlation of ID Kt/V with Daugirdas Kt/V was much lower in women than men, regardless of V estimation method. We found a reasonable correlation between kinetically modeled Kt and ID Kt (n=38, Figure), though consistent undervaluation by ID was noted.




**Conclusions:** The usefulness of ID Kt/V as a tool depends on its correlation to established clinical measures. ID Kt/V does not account for ultrafiltration which may, in part, account for the lower values. Using all methods of estimating V, the correlation of ID Kt/V with urea based Kt/V was worse in women than in men. Given the reasonable correlation between modeled Kt and ID Kt, the problem resides in the calculation of V, especially in women.

*Funding:* Clinical Revenue Support

## FR-PO996

**No Discrepancy between Relative Plasma Volume Change and Absolute Plasma Volume during Ultrafiltration and Hemodialysis** Werner Ribitsch,[1] Gernot Schilcher,[1] Joerg H. Horina,[1] Vanessa Stadlbauer,[3] Alexander R. Rosenkranz,[1] Daniel Schneditz.[2] *[1]Clinical Div of Nephrology, Medical Univ of Graz (MUG), Austria; [2]Institute of Physiology, MUG; [3]Div of Gastroenterology and Hepatology, MUG.*

**Background:** One explanation for the poor prediction of intradialytic adverse events by excessive hemoconcentration measured by on-line techniques is assumed to reside with a systematic underestimation of the true blood volume drop induced by ultrafiltration (UF). The purpose of this study was to compare relative plasma volume changes calculated from on-line data with absolute plasma volumes determined in chronic and stable hemodialysis (HD) patients.

**Methods:** Intradialytic plasma volume (Vp) was determined in hourly intervals at 4 time points (t=1, 2, 3, 4) by indocyanine-green (ICG) dilution (modified from Mitra et al., JASN 2003). Hematocrit (H) as well as ICG concentrations were continuously measured by optical technique (CritLine, Fresenius Medical Care, USA).

**Results:** Twelve patients (6 female, 69.8±14.8 kg) were studied twice, the mean UF volume was 1.82±0.7 L. H significantly increased from 35.3±2.8% at t=1 to 37.1±3.1% at t=4 ($p<0.001$), corresponding to a drop of relative plasma volume ($\Delta Vp,H$) to 92.5%. Mean absolute plasma volume (Vp) was reproducibly measured as 3.48±0.21 L and slightly different between time points (p = 0.123). The plasma volume dropped to 94.2% of the initial value ($\Delta Vp$) and was not different from $\Delta Vp,H$ determined from on-line measurements.

| t | H | Vp | ΔVp,H | ΔVp |
|---|---|---|---|---|
| | % | L | % | % |
| 1 | 35.3±2.77 | 3.6±1.03 | 100 | 100 |
| 2 | 35.8±2.9 | 3.5±1.05 | 97.8±2.7 | 97.7±9.7 |
| 3 | 36.5±2.8 | 3.5±1.10 | 94.9±3.9 | 97.6±9.6 |
| 4 | 37.1±3.1 | 3.4±1.02 | 92.5±4.5 | 94.2±9.2 |
| | p < 0.001 | p = 0.123 | p < 0.001 | p = 0.123 |

**Conclusions:** These results indicate that on-line hemoconcentration captures the true plasma volume changes during HD in patients with moderate UF volumes. Failure of on-line H monitoring to predict intradialytic morbid events is therefore unlikely to depend on systematic overestimation of intravascular volume.

## FR-PO997

**Determinants of Aortic Stiffness by Cardiovascular Magnetic Resonance in Patients New to Haemodialysis** Aghogho Odudu,[1,2] Mohamed Tarek Eldehni,[1,2] Mark A. Horsfield,[3] Gerry P. McCann,[3] Chris W. McIntyre.[1,2] *[1]Div of Medical Sciences, Univ of Nottingham; [2]Dept of Renal Medicine, Royal Derby Hospital; [3]NIHR Cardiovascular Biomedical Research Unit, Leicester, United Kingdom.*

**Background:** Aortic stiffness is a novel cardiovascular risk factor in HD patients. Prior prevalent studies estimated stiffness by pulse wave velocity (PWV) with external devices. These are limited by inaccuracy of aortic length & determinants are unclear. Cardiac Magnetic Resonance (CMR) can accurately measure cardiac structure & function & directly measure aortic distensibility (AD) & PWV. We aimed to investigate the determinants of directly-measured AD by CMR & the utility of MR-determined PWV (MR-PWV) in a cohort of patients within 6 months of commencing HD.

**Methods:** 30 HD patients with 19 age & sex-matched controls (NC, n=19) were studied. AD and MR-PWV were determined from velocity-encoded imaging of the aortic arch using standard techniques. Carotid-femoral PWV was measured with an external oscillometric device (Ex-PWV). Skin autofluorescence (SAF), a marker of advanced-glycation end-products, high-sensitivity troponin-T & NT-Pro-BNP were measured in the HD cohort.

**Results:** Mean±IQR of data are presented. AD was reduced in HD compared to NCs, despite similar blood pressure & Ex-PWV (2.0±1.8mmHg-1×10-3 vs 4.0±4.8mmHg-1×10-3, p<0.001). Univariate analysis showed significant inverse correlations of AD to age (r=-0.67, P<0.001), MR-PWV (r=-0.72, p<0.001), Ex-PWV (r=-0.41 p=0.005) & left ventricular mass(r=-0.34, p=0.016). AD was positively correlated to ejection fraction (r=0.43, p=0.002) & strain (r=0.33, p=0.02). In HD patients, AD was inversely correlated to high sensitivity troponin-T(r=-0.53, p=0.014) & SAF (r=-0.57, p=0.013). There was no relation to anthropometric measures, NT-Pro-BNP or Calcium-Phosphate product. Multiple regression analysis identified MR-PWV & age as the strongest independent determinants of AD (adjusted $R^2$=0.70 p<0.001).

**Conclusions:** In a cohort of incident HD patients, age & PWV by CMR were independent determinants of directly-measured aortic stiffness. Long-term follow-up will investigate aortic stiffness as an independent risk factor for HD-associated cardiomyopathy.

*Funding:* Government Support - Non-U.S.

## FR-PO998

**Overhydration Index Measured with BCM Is Correlated with Mean Arterial Pressure in Dialysis Patients** Eun Kyoung Lee,[1] Ji Eun Lee,[1] So Mi Kim,[2] Hyun woo Kim.[2] *[1]Dankook Univ Hospital; [2]Jeju National Univ Hospital.*

**Background:** It is important to maintain euvolemia to prevent further cardiovascular events and to reduce mortality in dialysis patients.

But in daily practice fluid status is assessed by using clinical methods such as body weight, clinical sign (blood pressure, edema, dyspnea), laboratory data (hemoconcentration or hemodilution) or chest X-ray because there is rare objective method to assess fluid status.

We evaluated fluid status in dialysis patients using the portable whole-body multifrequency BIA device, Body Composition Monitor BCM (Fresenius Medical Care, Germany).

**Methods:** We measured the body composition in 48 dialysis patients (M:F=29:19, age 52±12.6, CAPD:HD=32:16, DM:HTN:CGN:etc=19:14:12:3) from Dankook University Hospital using the BCM. We compared the overhydration index (OH) which was measured by BCM with blood pressure and cardiothoracic ratio (CTR).

**Results:** Mean overhydration index was 3.8±2.9 Liter and mean value of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and CTR were 147±22 mmHg, 82±12 mmHg, 104±14mmHg and 0.54±0.08, respectively.

Correlation coefficient between overhydration index and SBP was 0.608 (p=0.001). It was 0.494 (p=0.08) with MAP but there was no significant correlation between overhydration index with DBP(r=0.343, p=0.109).

**Conclusions:** Overhydration index which was measured by BCM could be used as a relatively objective guide to decide ultrafiltration volume in dialysis patients.

## FR-PO999

**Plasma Sodium Levels and Cardiovascular Stability in Hemodyalisis Patients** Eduardo Baamonde,[1] Elvira Bosch,[1] German Perez Suarez,[1] Cesar Garcia-canton,[2] Mar Lago alonso,[2] Dolores Checa.[2] *[1]Nephrology, AVERICUM, Telde, Las Palmas, Spain; [2]Nephrology, Hospital Insular de Gran Canaria, Las Palmas de Gran Canaria, Las Palmas, Spain.*

**Background:** A relationship between pre-hemodialysis (HD) plasma sodium and mortality has been described. Lower sodium levels are associated with higher mortality.We analyze the relationship between pre-HD plasma sodium levels and intra-HD hemodynamic stability in a group of patients on standard HD and constant dialysate sodium.

**Methods:** Retrospective analysis of 231 prevalent patients in HD classified into three groups (**G**) according to their pre-HD sodium levels corrected for glucose (cNa): **G0**: cNa < 137 mEq/L (n=59); **G1**: cNa:137 to 139.9 mEq/L (n=126); **G2**: cNa > 140 mEq/L (n=46). Patients were followed up for two years. Mean blood pressure (MBP), inter-dialysis weigth gain (HDWG), ultrafiltration rate (UF), dry weight (DW) and dialysis hypotension (DH) was calculated in every single session. Laboratory tests, HD adequacy and antihypertensive treatment (AT) were analyzed.

**Results:**

| | G0 | G1 | G2 | p-value |
|---|---|---|---|---|
| **Average cNa mEq/L** | 135.4±1.45 | 138.5±0.78 | 140.95±0.91 | 0.000 |
| **Age (years)** | 61.73±15.2 | 60.44±13.84 | 63.39±12.08 | ns |
| **DM(%)** | 62.7 | 50.8 | 52.2 | ns |
| **DW(kg)** | 71.01±17.11 | 77.23±15.33 | 80.28±15.8 | 0.008 |
| **HDWG %** | 2.95±0.72 | 2.76±0.75 | 2.34±0.76 | 0.000 |
| **UF rate (ml/Kg/h)** | 8.13±2.18 | 7.38±2.03 | 6.33±1.76 | 0.000 |
| **% DH** | 17.33±15.56 | 11.46±13.25 | 11.76±13.7 | 0.024 |
| **Albumin (gr/dl)** | 3.57±0.29 | 3.72±0.25 | 3.71±0.25 | 0.00 |

cNa level was negatively correlated with UF rate (r: 0.325; p:0.000), percent weight gain (r: 0.279; p: 0.000) and % DH: (r: 0.146; p: 0.027).

**Conclusions:** Pre-dialysis plasma sodium was inversely related with cardiovascular stability. Patients with lower sodium levels showed higher tendency to hypotension may be related with higher inter-dialysis gain and UF rates.

## FR-PO1000

**Hemoglobin Levels, Cardiovascular and All Cause Hospitalization Rates, and Mortality in Pediatric Chronic Hemodialysis Patients** Blanche M. Chavers,[1,2] Julia T. Molony,[2] Craig Solid,[2] Michelle N. Rheault,[1] Thomas Nevins,[1] Charles A. Herzog.[2,3] *[1]Univ of MN, Pediatrics, Minneapolis, MN; [2]U.S. Renal Data System/CVSSC; [3]Univ of MN, Medicine.*

**Background:** Our objective was to describe the association of pt hemoglobin (Hgb) levels with risk of all cause and cardiovascular disease (CVD) hospitalization and mortality in pediatric chronic hemodialysis pts.

**Methods:** Pediatric pts (ages <18) from the CMS ESRD Clinical Performance Measures (CPM) project, from 2000 to 2008, were merged to the USRDS database. Prior Hgb and Epogen (EPO) use were determined from CPM data, and beginning annually on Jan. 1st, pts were followed for up to 1 yr and censored at the first date of death, lost to follow-up, or Dec. 31st. Outcomes included mortality and hospitalizations (all cause and CVD). We calculated the rates of outcome events per time at risk and stratified by pt Hgb levels.

**Results:** Pts with an average Hgb<10g/dL prior to follow-up had the highest unadjusted 1-year rates of mortality and all cause hospitalization (5.85 and 278.89, respectively, per 100 pt yrs), and both rates declined with increasing entry period Hgb levels. In contrast, the unadjusted rates of CVD hospitalizations were relatively constant across Hgb groups except for pts with Hgb>13g/dL who had the lowest rate. EPO use also differed by Hgb level with the lowest Hgb group having the fewest EPO users [at least 2 out of 3 months (Oct-Dec) with an EPO prescription] and the highest average dose per prescription.

| Hgb Group | N patients | EPO users (%) | Avg. prescribed EPO (units/wk.) | 1-year unadjusted rates per 100 pt yrs | | |
|---|---|---|---|---|---|---|
| | | | | mortality | hospitalizations (all cause) | hospitalizations (CVD) |
| Hb<10 | 461 | 76.85 | 20,418 | 5.85 | 278.89 | 41.42 |
| 10≤Hb<11 | 432 | 86.30 | 16,992 | 4.52 | 236.24 | 39.45 |
| 11≤Hb<12 | 798 | 92.67 | 12,727 | 3.19 | 203.40 | 38.41 |
| 12≤Hb<13 | 682 | 92.94 | 13,922 | 1.63 | 196.63 | 41.11 |
| Hb>=13 | 323 | 86.56 | 13,789 | 2.82 | 162.95 | 33.84 |

**Conclusions:** There is an association between a pt's Hgb levels and their risks of hospitalizations and mortality in the year following Hgb measure. Future analyses will adjust for pt characteristics and clinical profile.

*Funding:* NIDDK Support

## FR-PO1001

**Association between Blood Pressure Levels and Adverse Clinical Outcomes in Korean Incident Hemodialysis Patients** Fa Mee Doh,[1] Hyang Mo Koo,[1] Hyung Jung Oh,[1] Tae-Hyun Yoo,[1,2] Shin-Wook Kang.[1,2,3] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul, Korea; [3]On behalf of the Clinical Research Center for ESRD Investigators.*

**Background:** BP control is an important issue in ESRD patients on HD. In general, it is recommended that target pre- and post-dialysis BP should be <140/90 mmHg and <130/80 mmHg, respectively. However, there is much controversy about the optimal BP in these patients.

**Methods:** A prospective cohort of 807 incident HD patients from 36 dialysis centers of the CRC for ESRD in Korea was included in this study. Time-averaged BPs were expressed as means of each BP measured at 6-mo intervals. The impact of BP levels on a composite of all-cause mortality or cardiovascular (CV) events was clarified by multivariate Cox regression analysis.

**Results:** The mean time-averaged BPs were as follows: pre- and post-dialysis SBP, 146±19 mmHg and 143±18 mmHg; and pre-and post-dialysis DBP, 77±12 mmHg and 77±12 mmHg. During a mean follow-up duration of 20.5 mo, 65 patients died and 49 CV events occurred. Compared to patients with pre-dialysis SBP of 140-149 mmHg, HRs for the composite outcome were 2.16 (p=0.044), 1.90 (p=0.073), 2.28 (p=0.021), and 2.42 (p=0.010) in the pre-dialysis SBP groups of <130, 130-139, 150-159, and ≥160 mmHg, respectively, after adjustment for potential confounders. Similar relationship was also observed across post-dialysis SBP categories, where post-dialysis SBP ≥150 mmHg was associated with a significantly higher risk for the composite outcome. In terms of DBP, HRs for the composite outcome in the pre-dialysis DBP groups of <60, 60-69, 80-89, and ≥90 mmHg were 0.56 (p=0.171), 0.50 (p=0.011), 0.87 (p=0.596), and 2.55 (p=0.024), respectively, when compared to patients with pre-dialysis DBP of 70-79 mmHg. Similarly, post-dialysis DBP ≥90 mmHg exhibited a significantly higher risk for the composite outcome.

**Conclusions:** Pre-dialysis SBP of 140-149 mmHg and post-dialysis SBP of 130-149 mmHg were revealed to be the optimal BP levels associated with a favorable clinical outcome in incident HD patients. In addition, maintaining pre- and post-dialysis DBP <90 mmHg may provide a more beneficial effect in these patients.

*Funding:* Government Support - Non-U.S.

## FR-PO1002

**Outcome of ESRD Patients Three Months after Initiation of Hemodialysis at a Tertiary Care Hospital of a Developing Country** Syed Rizwan Bokhari,[1] Hafiz I. Ahmad,[1] Shoaib Islam,[1] Nouman Hameed,[1] Khalid Tahir,[1] Arif Asif.[2] *[1]Dept of Nephrology, Allama Iqbal Medical College, Lahore, Punjab, Pakistan; [2]Div of Nephrology and Hypertension, Albany Medical College, Albany, NY.*

**Background:** Patients with advanced CKD 5 mostly present to tertiary care hospitals with uremic complications and dialysis treatment is initiated to ameliorate acute symptoms. After a few inpatient dialysis sessions,due to paucity of dialysis services and lack of financial resources, they are unable to continue treatment or if they continue, the treatment is less than optimal. The fate of these patients is generally unknown.

**Methods:** 123 consecutive patients admitted with advanced CKD 5 (11-2012 - 03-2013) were included in this prospective study. Patients were interviewed according to a standardized questionnaire and were followed on monthly basis via telephone for a period of three months after initiation of dialysis. Eighteen patients died during hospital stay due to inter-current illnesses and 13 patients were lost to follow-up and as such were excluded from study.

**Results:** Ninety two patients completed three month follow up, of which 54(58%) were males with mean age of 38±5 years. Forty five (49%) patients were alive at the end of 3 months. Of these, only 4(4%) patients were getting thrice weekly dialysis. Thirty two (35%) were getting twice and 5 (5%) patients were getting once weekly hemodialysis. Four (4%) patients were not getting any dialysis. Forty seven (51%) patients died during follow up. Of these, 20 (21%), 38 (41%) and 47 (51%) had expired within one, two and three months of the initiation of Hemodialysis.

**Conclusions:** Majority of ESRD patients who had initiated hemodialysis due to acute uremic manifestations in a tertiary care hospital died within 3 months and those who had survived were receiving sub optimal treatment. This is an alarming situation that needs major efforts to improve pre ESRD care, timely initiation of dialysis and more resource allocation for provision of optimal dialysis services.

## FR-PO1003

**Prevalence and Management of the Dialysis Disequilibrium Syndrome: A Survey of U.S. Nephrologists** Rajeev Raghavan, Charles G. Minard, Flordeliza Lilagan. *Medicine, Baylor College of Medicine, Houston, TX.*

**Background:** The dialysis disequilibrium syndrome (DDS) presents as neurological symptoms that develop during or immediately following dialysis. Recommendations to reduce its incidence include a 50% reduction in blood flow rate and treatment time, intravenous mannitol, and repeating dialysis on two consecutive days. Despite widespread acceptance, these interventions have never been proven to reduce the incidence of DDS and are associated with substantial expense and time. This cost may be as high as $3000 extra per patient. In our institution, patients who rely on emergent dialysis seldom receive preventative measures, and we observe very few cases of the DDS. The purpose of our study was to ascertain the prevalence of DDS and assess how U.S. nephrologists manage at-risk patients.

**Methods:** Survey recipients were selected from a national sample of practicing nephrologists listed in a database licensee company for the AMA. The 18-question survey was electronically mailed to 5537 nephrologists, ages 28-80 years old, and took approximately 10 minutes to complete.

**Results:** Two hundred fifty-two (252) nephrologists responded (4.5%). Over 50% of the sample had encountered at least one case of the DDS within the past year. The number of years of experience as an attending nephrologist was not significantly associated with or predictive of using interventions to prevent/treat the DDS. However, nephrologists with less than 10 years experience had 3.9 times higher odds of using osmotically active agents (e.g. mannitol) than those practicing with >25 years experience (95% CI: 1.3, 12.0). The less seasoned nephrologist and nephrologists' practicing in the South were three times more likely to send at-risk patients to a more closely monitored setting (e.g. hospital) for dialysis (95% CI: 1.2, 4.2; 1.5, 5.4, respectively).

**Conclusions:** The dialysis disequilibruim syndrome remains highly prevalent throughout the U.S. and choice of interventions used to mitigate its occurence vary among US nephrologists, by age and practice location. This lack of concordance questions whether time-consuming and costly interventions, such as hospitalization, should be used for patients at-risk for the DDS.

## FR-PO1004

**Global Pediatric Hemodialysis Dialysis Experience: The PICCOLO MONDO Cohort** Maria E. Ferris,[1] Keisha L. Gibson,[1] Debbie S. Gipson,[2] Brett W. Plattner,[3] Peter Kotanko,[3] Daniele Marcelli,[4] Mondo Consortium,[5] Len A. Usvyat.[5] *[1]Medicine and Pediatrics, Univ of North Carolina, Chapel Hill, NC; [2]Pediatrics, Univ of Michigan, Ann Arbor, MI; [3]The Renal Research Institute, New York, NY; [4]Fresenius Medical Care, Bad Homburg, Germany; [5]MONDO Consortium, New York.*

**Background:** Pediatric hemodialysis (HD) patients worldwide have not been characterized. We describe the Global **PICCOLO MONDO (P**ediatric **I**nvestigation and **C**lose **C**ollaboration to examine **O**ngoing **L**ife **O**utcomes in the **MON**itoring **D**ialysis **O**utcomes) Cohort.

**Methods:** The MONDO consortium consists of HD data from US Renal Research Institute clinics; dialysis facilities of Fresenius Medical Care in Europe, Asia Pacific, Latin America; KfH (Germany); Imperial College (UK), Hadassah Medical Center (Israel); and University of Maastricht (The Netherlands) (Usvyat, Blood Purif 2013) with 62, 345 incident patients from 1,052 units in 26 countries. The **PICCOLO MONDO Cohort** includes incident 1 to 18 year old patients between 2000 and 2012.

**Results:** Analysis of 494 patients (0.8% of the population), revealed that 17 patients were from the Asia Pacific (41% males, mean age 13.35); 43 from the USA (49% males, mean age 15.13); 87 from Europe (64% males, mean age 15.91); and 347 from Latin America (52% males, mean age 14.3). The Asia Pacific units reported the youngest incident patients (mean age 10.83) and diabetes in 18%, whereas the Europeans had the oldest patients (mean age 15.35). The use of HD catheters is greatest in the Americas (75%), followed by Europe (52%) while Asia Pacific had 0%. The USA cohort had the greatest prevalence of hypertension (58%) and cardiovascular comorbidities (58%), followed by the European cohort (26 & 18% respectively).

**Conclusions:** The **PICCOLO MONDO Cohort** provides an opportunity to compare pediatric hemodialysis populations and outcomes at an international level. The prevalence of diabetes in the Asia Pacific units is unexpectedly high and requires additional investigation. The units in the Americas have the greatest use of HD catheters and the USA has the greatest prevalence of cardiovascular disease.

*Funding:* Private Foundation Support

## FR-PO1005

**US Dialysis Facilities Target Hemoglobin (Hb) outside of Guidelines and Conduct Frequent Hb Measures and ESA Titrations Despite Lack of Belief in Effectiveness of These Anemia Practices** Alex Yang, Jennifer Vavrinchik. *NCS Research.*

**Background:** With the recent implementation of the CMS ESRD bundle, an updated FDA label for ESAs, and shifting QIP measures, changes in the approaches to anemia management have implications to the quality of care and management of patients on dialysis. Szczech et al (2012) demonstrated a lack of association between more frequent Hb measures and decreased Hb excursions and between more frequent ESA titrations and decreased Hb excursions. In this study, we evaluated current anemia management practices.

**Methods:** In this study, >2000 nephrology professionals were screened against inclusion criteria: US nephrology professional, dialysis practice setting, >1 yr of nephrology experience, and consent. 367 eligible study candidates were invited with eventual 175 study participants (48%). Hb targets, Hb threshold for ESA holds, and frequency of Hb measures and ESA titrations were evaluated. Analyses presented here were conducted across all study participants with no excluded data for any reason.

**Results:** 30% of study participant facilities use a Hb <10g/dL as lower range for target Hb, and 36% use a Hb >11g/dL as upper range for target Hb. 22% hold ESA dose at Hb of 11g/dL or less. 88% make ESA dose adjustments on average once a month or more frequently for each dialysis patient. Respectively, 14% and 15% of study participants believe that more frequent Hb measures and more frequent ESA titrations reduce Hb excursions.

**Conclusions:** Many US dialysis facilities seem to have Hb targets outside of the recommended ranges. Despite guidance to not make frequent ESA dose adjustments, nearly all facilities report frequent Hb testing and frequent ESA dose adjustment. Furthermore, nearly all participants do not believe these frequent Hb measurements and ESA dose titrations reduce Hb excursions, the purported reason to measure Hb and titrate an ESA. These apparent disconnects may represent the struggle between clinical beliefs, regulatory guidance, reimbursement constraints, and corporate directives.

**FR-PO1006**

**Early versus Late Initiation of Dialysis on Clinical Outcomes of Quality of Life and Survival: A Propensity-Matched Analysis** Jeonghwan Lee,[1,6] Jung-woo Noh,[2,6] Yong-Lim Kim,[3,6] Yon Su Kim,[4,6] Chun Soo Lim,[5,6] Jung Pyo Lee.[5,6] *[1]Dept of Internal Medicine, Hallym Univ Hangang Sacred Heart Hospital, Seoul, Korea; [2]Dept of Internal Medicine, Hallym Univ Gangnam Sacred Heart Hospital, Seoul, Korea; [3]Dept of Internal Medicine, Kyungpook National Univ School of Medicine, Daegu, Korea; [4]Dept of Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [5]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea; [6]CRC ESRD Investigators Group.*

**Background:** There is controversy on the adequate timing of the initiation of renal replacement therapy for the patients with end-stage renal disease.

**Methods:** We enrolled a total of 1779 adult patients who start dialysis from 2007 to2012 in a multi-center, prospective cohort of the Clinical Research Center for End Stage Renal Disease in Korea. All patients were interviewed and answered the questionnaire about Karnofsky performance score (KPS), Beck depression inventory (BDI), and kidney disease quality of life (KDQOL). Patients were classified, according to the mean values of estimated glomerular filtration rate (GFR) which is 7.372 ml/min/1.73 $m^2$, as early start group or late start group when GFR was above or below the mean values at the time of dialysis start.

**Results:** Before propensity-score matching, early start group showed poor score of KDQOL (56.8 ± 13.4 vs. 61.7 ± 14.6, P < 0.001), KPS (75.5 ± 15.4 vs. 78.8 ± 14.9, P = 0.002), and survival (P = 0.002). 1554 patients were eligible for matching and 395 patients were selected in each group. KPS (76.1 ± 15.0 vs. 78.6 ± 14.5, P = 0.086), BDI (16.4 ± 10.4 vs. 15.3 ± 10.4, P = 0.290), and KDQOL (57.8 ± 15.0 vs. 60.1 ± 14.5, P = 0.113) were similar between early start group and late start group. Overall survival was not different between groups (P = 0.259). In the subgroup analysis of patients with age above 65 years old, KPS (72.7 ± 15.2 vs. 73.2 ± 14.9, P = 0.869), BDI (16.8 ± 9.3 vs. 18.1 ± 11.0, P = 0.467), KDQOL (55.3 ± 12.4 vs. 54.6 ± 15.0, P = 0.791), and survival (P = 0.845) was comparable between groups.

**Conclusions:** Early initiation of dialysis was not associated with poor survival or quality of life even in the old age group.

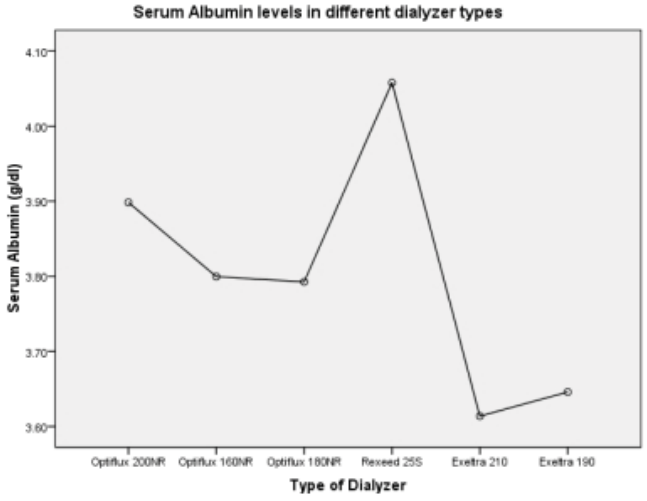
**FR-PO1007**

**Impact of Dialyzer on Serum Albumin Levels in End Stage Renal Disease Undergoing Chronic Hemodialysis** Alla Goldberg,[1] Hafiz Hussain,[2] Aruna Ray,[1] Farhanah Yousaf,[1] Bruce S. Spinowitz,[1] Chaim Charytan.[1] *[1]New York Hospital Queens, Flushing, NY; [2]Lutheran Medical Center, Brooklyn, NY.*

**Background:** Serum albumin level is an important predictor of morbidity and mortality in end stage renal disease. However, very little is known about impact of different dialyzers on filtration of albumin molecules which may affect serum albumin. The purpose of our study was to evaluate the effect of different dialyzers on serum albumin levels.

**Methods:** Patients undergoing chronic hemodialysis at a single dialysis unit between January 1st to December 31st 2012 were included in present study. Patients who were hospitalized during year 2012 were excluded from current analysis. Medical records and lab values were reviewed. Patients were divided into 6 groups based on dialyzer; Optiflux 200NR, Optiflux 160NR, Optiflux 180NR, Rexeed 25S, Exeltra 210, and Exeltra 190. Univariate analysis of variance was used to analyze the data.

**Results:** 179 patients met the inclusion criteria with 46 % females. Mean age of patients was 67 years. Mean serum albumin was found to be statistically significant different ($p<0.05$) except Optiflux 160NR versus Optiflux 180NR and Exeltra 210 versus Exeltra 190. Age, gender, and cholesterol levels were independent predictors of serum albumin levels. However, serum albumin levels in different dialyzer groups were still significantly different even after controlling for age, gender, and cholesterol.




**Conclusions:** Our study suggests that the choice of dialyzer may affect serum albumin levels in end stage renal disease. Dialyzer may be selected based on patients serum albumin levels and malnourished patients may benefit from specific dialyzers such as Rexeed 25S. Further studies are warranted to confirm these results.

**FR-PO1008**

**Correlation between Dt/V Derived from Ionic Dialysance and the Kt/V of Urea Using Daugirdas Formula in African-American Hemodialysis Patients** Wihib A. Gebregeorgis, Nishi Pradhan, Stephen D. Migdal, Lakshminarayanan Nandagopal, Reddy Singasani, Tehmina Mushtaq, Yahya M. Osman Malik. *Internal Medicine, Div of Nephrology, Wayne State Univ/Detroit Medical Center, Detroit, MI.*

**Background:** Dt/V obtained by using ionic dialysance (D) as a surrogate for urea clearance has been suggested as an adjunct measure of hemodialysis adequacy & has been shown to have variable correlation with the Kt/V of urea. We investigated the correlation between Dt/V & Kt/V of urea in African American HD patients & evaluated the impact of body size & ultrafiltration (UF) volume on the correlation.

**Methods:** A prospective single center observational study was conducted over a period of 3 months in 81 African-American HD patients. Each patient had 1-3 sessions of HD during which simultaneous evaluations of online Dt/V & spKt/V-urea were made. SpKt/V-urea was estimated using the second generation Daugirdas formula. The 'D' was evaluated using conductivity probes at the dialysate inlet & outlet and 'V' was estimated using the Watson's formula. Patients were categorized into three groups based on their body size & UF volume. The correlation between Dt/V & Kt/V was evaluated for the entire cohort & each pre-specified subgroup.

**Results:** A total of 81 patients had 186 HD sessions. The mean Kt/V was greater than the mean Dt/V (1.72 vs 1.50). There was an overall moderate correlation between Dt/V & Kt/V with an r2 of 0.602. The correlation was stronger in the medium BMI group compared to those in the lower & higher cohorts (r2 of 0.604, 0.653, 0.545 for those with BMI < 25, 25-30 & > 30kg/m2 respectively). The same trend was seen with the 3 weight subgroups. The correlation between Dt/V & Kt/V had inverse relationship to the UF volume; the lower the UF volume, the better the correlation between Dt/V & Kt/V (r2 of 0.698, 0.621 & 0.558 for those with UF volume of < 1.5, 1.5-3.0 & > 3 L respectively). 99.3% of the patients with Dt/V > 1.3 also had Kt/V > 1.3.

**Conclusions:** There is a moderate degree of correlation between Dt/V & Kt/V in African American chronic HD patients. Body size & UF volume affect the correlation between Dt/V & Kt/V. A Dt/V of > 1.3 strongly predicts adequate dialysis as defined by Kt/V of > 1.3.

*Funding:* Private Foundation Support

**FR-PO1009**

**Association of Smoking Status with Cotinine Concentration Identified via Metabolic Profiling in Plasma Samples from Hemodialysis Patients** Quinlyn A. Soltow,[1] Dean P. Jones,[2] Rebecca H. Zhang,[1] Douglas I. Walker,[2] Nancy G. Kutner.[1] *[1]USRDS Rehabilitation/QoL Special Studies Ctr, Emory Univ, Atlanta, GA; [2]Medicine, Clinical Biomarkers Laboratory, Emory Univ, Atlanta, GA.*

**Background:** Smoking is associated with increased morbidity and mortality risks in chronic hemodialysis (HD) patients, but the evidence for higher incidence of cardiovascular events in smokers vs. nonsmokers is inconsistent (Liebman et al. 2011; McCausland et al. 2012). Patient reports suggest that smoking prevalence is higher than that identified in provider-generated data such as CMS Form 2728 (Longenecker et al. 2000), but the true prevalence in this population is difficult to determine. Using an objective biomarker of nicotine exposure, cotinine, in plasma samples from a subset of patients participating in a USRDS HD cohort study, we sought to validate patient-reported active cigarette smoking.

**Methods:** A multi-center cohort of over 750 prevalent HD patients was enrolled 2009-2011 in the ACTIVE/ADIPOSE study conducted jointly by the Rehabilitation/QoL and Nutrition USRDS Special Studies Centers. In a subset of 114 patients (113 African-American, 1 white) enrolled in Atlanta, GA, plasma samples were obtained at 6-month intervals over a 2-year period. Cotinine levels were measured using reverse phase chromatography-LTQ-Velos Orbitrap mass spectrometry-based metabolic profiling. Cotinine concentrations were confirmed by MS/MS using an authentic standard. Values exceeding 10 ng/ml indicate at least light active smoking.

**Results:** 27.5% of patients in the analysis subset reported current smoking, and cotinine values were consistent with these reports. An additional 11.0% of the subset had cotinine values consistent with active smoking but did not report being smokers; the ages of these patients ranged from 36 to 74, and two-thirds were male.

**Conclusions:** The contribution of smoking to outcomes such as hospitalization and mortality may be underestimated due to misclassification bias from underreported smoking. Metabolic profiling offers a window on the true prevalence of smoking exposure among patients undergoing HD, which may be relevant for the issue of ascertaining cardiovascular risk associated with active smoking.

*Funding:* NIDDK Support, Other NIH Support - NCRR, NIA, NIEHS

**FR-PO1010**

**Comparison Study on Optimal Timing of Hemoperfusion Combined with Maintenance Hemodialysis** Chen Yu. *Dept of Nephrology, Tongji Hospital, Shanghai, China.*

**Background:** Hemodialysis (HD) combined with hemoperfusion (HP) was superior to HD in regularly eliminating middle and large molecule uremic toxins accumulated in the body. According to the different combining time of HD with HP, there were two types: Early type (HP was operated in the first two hours of HD) and Late type (HP was operated in the last two hours of HD). The purpose of this study is to compare the eliminating efficiency between these two types.

**Methods:** Twenty HD patients(4 hours each time, 3 times each week for more than 3 months treatment)were enrolled. All patients were alternately given Early type and Late type of HD + HP. Serum concentration of creatinine (sCr), BUN, immunoreactive parathyroid hormone (iPTH), ß (2) microglobulin (ß (2)-MG), interleukin-1, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined before and after each treatment. Adverse events (low blood pressure, blood coagulation and so on) were observed.

**Results:** 1) Serum concentrations of all above mentioned were significantly declined after each type of treatment ($p<0.01$). 2) The eliminating efficiency of serum iPTH, ß (2)-MG , TNF-α, IL-1 and IL-6 was better in the Late type of HD+HP ($p<0.05$). 3) There were no significant differences of the eliminating efficiency of Scr,BUN between the two types of HD+HP. 4) There was no significant difference in the incidence of adverse events (low blood pressure, blood coagulation and so on) between these two types.

**Conclusions:** Both two types of HD+HP are efficient in eliminating uremic toxins for MHD patient. Comparatively, the Late type of HD+HP ((HP was operated in the last two hours of HD) has a better efficiency on elimination of middle and large molecule uremic toxins, like iPTH, ß (2)-MG, IL-1, IL-6 and TNF-α. However, there were no significant differences on elimination of small molecules, like sCr and BUN, between these two type of treatment. There was no significant difference of incidence of adverse events in the two types of HD+HP.

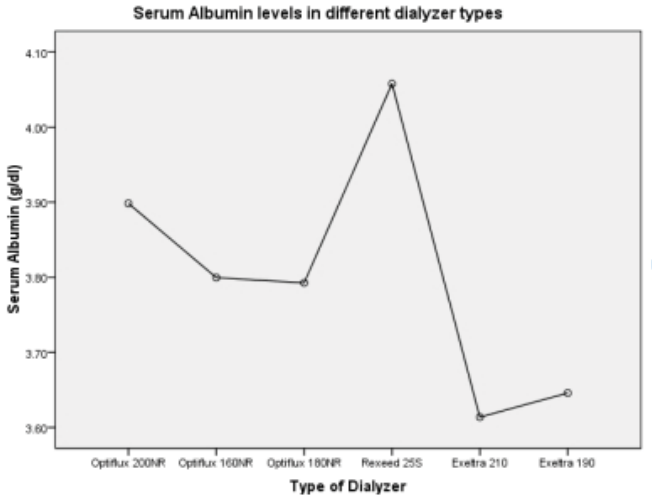
**FR-PO1011**

**Markers of Financial Efficiency and Patient Quality Outcomes Are Interrelated in Dialysis Clinic Operations** Len A. Usvyat, John Long, Norma J. Ofsthun, Franklin W. Maddux. *FMCNA, Waltham, MA.*

**Background:** Dialysis providers face an increased pressure to reduce cost while trying to provide highest quality service to its patients. With ESRD Seamless Care Organizations (ESCO) and other commercial integrated care arrangements, this pressure is even further accentuated whereby dialysis providers want to provide best care possible in the most efficient manner. We aim to understand whether patient quality measures are related to dialysis clinic financial and operational efficiency measures.

**Methods:** We used data from 1700 FMCNA dialysis clinics. For calendar year 2012, we computed measures of financial and operational efficiency: employee retention ratio (max of 100%) and EBIT per treatment as well as measures of quality such as percent of patients with albumin >4.0 g/dL and standardized mortality ratio (SMR). We divided measures of financial efficiency into quartiles and computed mean quality related measures in those groups. Comparisons between quartiles are performed by using independent t-tests.

**Results:** We found that clinics with higher employee retention ratios had lower SMR (ns) and higher percent of patients with albumin ≥4.0 (ns). Further, clinics with higher EBIT per treatment also had lower SMR ($p<0.05$) and higher percent of patients with albumin ≥4.0 ($p<0.05$).




**Conclusions:** This analysis suggests that dialysis operational efficiency is tied to patient outcomes: clinics with higher employee retention ratios and highest EBIT/treatment also have the lowest SMR and highest albumin levels. While these relationships are not always significant in this analysis, they are performed on a clinic-wide basis with much variability in other clinic related characteristics, but highlight the positive association between quality outcomes and financial performance.

**FR-PO1012**

**Dialysis Modality Decision Making** Suma Prakash,[1,2] Steven A. Lewis,[1,2] Anna McGrail,[1] Jesse D. Schold,[3] Mary Ellen Lawless,[1,2] Ashwini R. Sehgal,[1,2] Adam T. Perzynski.[1,2] *[1]MetroHealth Medical Center, Cleveland, OH; [2]Case Western Reserve Univ, Cleveland, OH; [3]Cleveland Clinic Foundation, OH.*

**Background:** Preparing patients for dialysis is part of patient centered care for late stage chronic kidney disease. Stage of change (SoC) algorithms can be useful tools to assess patient's readiness to make decisions and take health care actions. SoC algorithms classify patient readiness as pre-contemplation, contemplation (thinking SoC) preparation and action (acting SoC). The aims of this study were: 1) to develop a SoC tool for dialysis modality decision making, 2) determine associations between SoC and dialysis modality decision making and 3) determine additional factors associated with SoC and dialysis modality decision making.

**Methods:** Cross-sectional, observational study of adult patients with eGFR≤20 mL/min/1.73m$^2$. Qualitative interviews were conducted to support development and refinement of the survey. Survey responses were compared by SoC and by whether a dialysis decision was made using Fisher exact tests (dichotomous variables) and Wilcoxon's two sample tests (continuous measures).

**Results:** 49 patients are enrolled. 69%-female, 38%-White, 60%-Black, 92%-high school graduates and 82% had annual income<$25,000. 10% were in pre-contemplation, 55%-contemplation, 8%-preparation and 27%-action. 63% made a dialysis modality decision. Thinking versus acting SoC patients were less likely to have made modality decisions (50% vs. 88%, $p=0.01$). More patients made modality decisions with higher knowledge scores (1.4-decided vs. 0.2-undecided) and with physicians explaining modality options (41% vs. 74%) (both $p<0.05$). Modality decision barriers included: feeling home dialysis too difficult, others' opinions, body image with peritoneal dialysis catheter, fears about dialysis and feeling overwhelmed (all $p<0.05$). Composite barrier score was higher in the thinking compared to acting SoC (means of 1.1 vs. 0.7) ($p=0.01$).

**Conclusions:** Modality decision making is associated with SoC. Findings from this measurement development study suggest that helping patients overcome self-identified barriers may allow them to feel ready and make dialysis modality decisions.

**FR-PO1013**

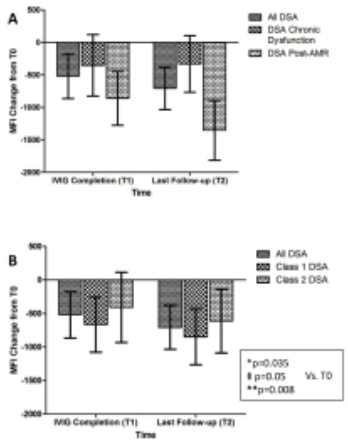
**Post-Transplant High Dose Intravenous Immunoglobulin (IVIG) for De Novo Donor Specific Antibodies in Kidney Transplant Recipients with Graft Dysfunction** James E. Cooper, Jane Gralla, Alexander C. Wiseman. *Kidney Transplant Center, Univ of Colorado, Denver, Aurora, CO.*

**Background:** Post-kidney transplant donor specific antibodies (DSA) are important contributors to graft loss and no effective therapeutic options exist. In the largest reported series of post-transplant DSA IVIG treatment to date, we describe outcomes in patients with de novo DSA and graft damage treated with a long-term high dose IVIG protocol.

**Methods:** Retrospective analysis of 28 kidney transplant recipients with DSA and graft damage in the form of either a recent prior acute antibody-mediated rejection (AMR) episode (n=8, group 1) or chronic graft dysfunction (n=20, group 2) prescribed a standard regimen of high dose (5 gm/kg) IVIG dosed over 6 months at our center**.**

**Results:** Mean fluorescence intensity (MFI) of 70 total DSA decreased by 520 at the end of treatment (T1, $p=0.14$) and by 708 at last follow up (T2, $p=0.035$) compared to treatment initiation (T0) MFI. The most robust treatment effect was seen in group 1 DSA (-1354 at T2 vs. T0, $p=0.008$) (figure 1a) and in class I DSA (-849 at T2 vs. T0, $p=0.05$) (figure 1b). Group 1, class I DSA underwent the largest MFI reduction (-1636 at T2 vs. T0, $p=0.01$). Antibody clearance, defined as MFI falling below 500, occurred in 68% of class I DSA vs. 16 % of class II ($p<0.001$) and in 63% group 1 vs. 24% of group 2 DSA ($p<0.001$). Graft function stabilized in group 1 patients but continued to decline in those in group 2.

Figure 1




**Conclusions:** High dose IVIG resulted in modest DSA MFI reductions in patients with previous graft damage, while graft function either stabilized or continued to decline despite treatment. The most robust MFI effect was seen in Class I DSA in patients with recent acute AMR. This already-at-risk cohort may benefit from IVIG therapy in order to reduce the degree of future chronic DSA-mediated graft damage.

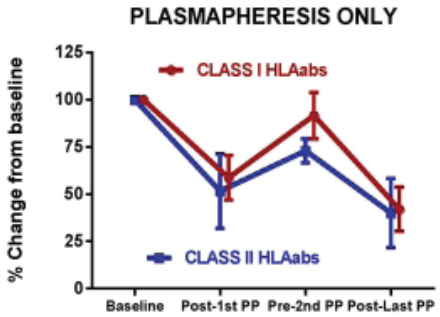
**FR-PO1014**

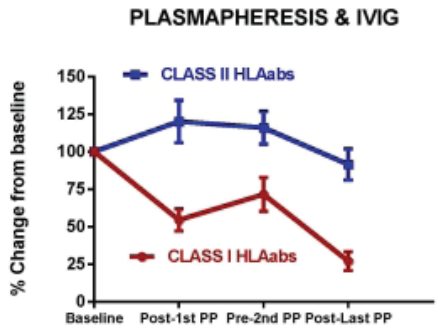
**Plasmapheresis Effect on the HLA-Antibodies in Kidney Transplantation and Desensitization** Lluvia A. Marino-vazquez,[1,2] Araminta Guichard,[1] Anh Nguyen,[2] Josefina Alberú,[1] Luis E. Morales-Buenrostro.[1] *[1]Nephrology and Transplant, INCMNSZ, Mexico, DF, Mexico; [2]Terasaki Foundation Laboratory, Los Angeles, CA.*

**Background:** Anti-HLA donor-specific antibodies (DSA) increase the risk of graft loss. Protocols for elimination/inhibition of these are heterogeneous. The cornerstone and oldest maneuvers to eliminate them is plasmapheresis (PP) with/without IVIG, but there is little information about the percentage removal of DSA with each session of PP. This report examine the behavior of DSA under PP treatment.

**Methods:** We included 33 kidney transplant recipients with humoral rejection and 4 patients who received PP for desensitization (Aug/11-Jul/13); divided in two groups: 1) Patients treated with PP (n=8) and 2) Patients treated with PP+IVIG (n=29). Sera samples were taken at baseline, after 1st PP, before 2nd PP, and after the last PP. HLA-DSA level were determined by Luminex single antigen bead assay (One Lambda, Canoga Park, CA). All samples were tested at the same time.

**Results:** Mean age was 34.39 ± 8.97 years; 64.1% were males. The 82.1% of patients received a living donor graft and the most common cause of CKD was unknown. For Group 1: 8 patients developed DSA Class I, decreasing to 42% and 6 Class II decreasing to 44%. In Group 2: 19 had Class I, decreasing to 27% and 25 Class II decreasing only to 91%. The figures depict the behavior of DSA.




**Conclusions:** PP was effective to remove DSA. We were able to identify an expected rebound after PP, due to exchange of DSA from interstitial fluid to plasma. The final reduction of DSA was similar in both groups, except for Class II when IVIG was used, maybe due to luminex measure issue. It is well known the possible interference of IVIG on DSA measure, but in this study it was only evident for Class II DSA. Each center must to compare different treatment schemes to define the best cost-benefit option for eliminate the HLA-DSA.

*Funding:* Government Support - Non-U.S.

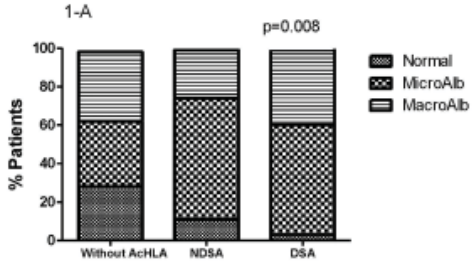
**FR-PO1015**

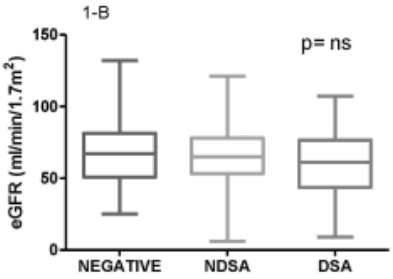
**HLA Antibodies and Albuminuria Precede Decline in Glomerular Filtration Rate Post-Transplantation** Lluvia A. Marino-vazquez,[1,2] Erick Hernández,[1] Araminta Guichard,[1] Norma O. Uribe-uribe,[1] Nubia Banuelos,[2] Josefina Alberú,[1] Luis E. Morales-Buenrostro.[1] *[1]Nephrology, Transplant and Pathology, INCMNSZ, Mexico, DF, Mexico; [2]Terasaki Foundation Laboratory, Los Angeles, CA.*

**Background:** HLA antibodies have been shown to be a predictive marker of long-term graft loss mainly due to endothelial damage. Albuminuria, a recognized marker of glomerular injury, is also considered a risk factor for graft failure. This report examine the association between post-transplant HLA antibodies and the key markers of renal injury: albuminuria and glomerular filtration rate (eGFR).

**Methods:** We included 266 renal transplant recipients, transplanted between 1984 and 2012, and at least one-year follow-up. HLA antibodies were determined by Luminex single antigen bead assay (One Lambda, Canoga Park, CA). Albuminuria was determined using turbidometry measured in urine collected over a 24 hr period. The result was normalized to the creatinine content in the same sample (albuminuria/creatinuria index). eGFR was calculated from serum creatinine values using MDRD equation.

**Results:** Of the 266 patients, 33.5% developed donor-specific HLA antibodies (DSA), and 45.1% had non donor-specific HLA antibodies (NDSA). 35 of the DSA patients had HLA Class I antibodies, while 64 had HLA Class II DSA. Patients with DSA had significantly higher albuminuria compared to patients with no antibodies (p=0.008). Interestingly, NDSA also showed higher proteinuria than the negative group. There was no significant difference between eGFR among the antibody and control groups at this point of follow-up.




**Conclusions:** Albuminuria is highly associated with the presence of HLA-DSA, and mildly correlated with NDSA. The fact that DSA and albuminuria were detected while eGFR remained at normal range, suggests that antibodies and albuminuria are earlier markers of graft injury than eGFR or serum creatinine.

*Funding:* Government Support - Non-U.S.

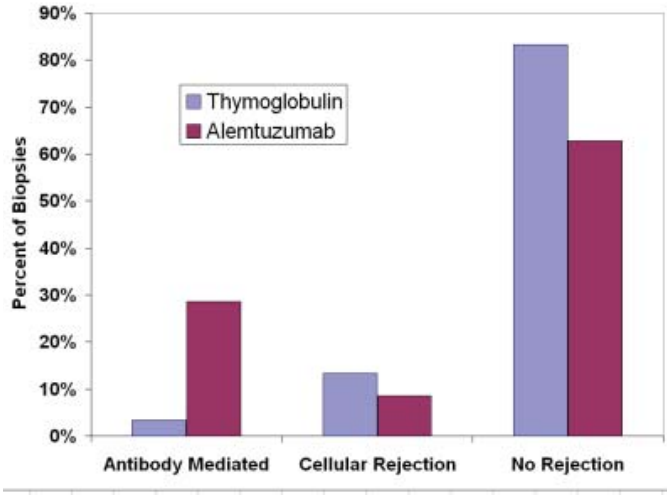
**FR-PO1016**

**Incidence of Antibody Mediated Rejection with Alemtuzumab Induction in Kidney Transplantation** Zurab Albekioni, Neeharika Muddana, Tarik Noureldeen, Leonardo P. Machado, Richard J. Marcus, Sabiha M. Hussain, Kalathil K. Sureshkumar. *Div of Nephrology, Allegheny General Hospital, Pittsburgh, PA.*

**Background:** Induction with lymphocyte-depleting antibodies is commonly used in kidney transplantation to prevent rejection. Little is known about the rate of antibody mediated rejection (AMR) with alemtuzumab(ALE) vs. rabbit-antithymocyte globulin (r-ATG) induction.

**Methods:** We conducted a retrospective cohort study of patients who underwent kidney transplants between Jan 2009 and Nov 2011. Patients were divided into 2 groups based on the induction received (ALE versus r-ATG). Primary end-points of the study were rates of AMR and acute cellular rejection (ACR) within 12 months of transplantation. Diagnostic criteria for AMR included peritubular capillary C4d deposition along with either histological changes on allograft biopsy or a positive donor specific antibody titer.

**Results:** Among 108 patients, 40 received r-ATG and 68 ALE induction. There were 30 allograft biopsies done in r-ATG and 35 in ALE groups. A significantly higher AMR (15% VS. 2%, P=0.008) but similar ACR (5% vs. 6%, p=0.69) rates were observed in ALE vs. r-ATG groups respectively. There were no differences in the incidence of delayed graft function (9% vs. 18%, p=0.30), as well as one year serum creatinine (1.7mg/dl vs. 1.8mg/dl, p=0.63), graft (91.1±3.5% vs.94.5%±3.8%, p=0.48) and patient (93.8±3.0% vs.92.7%±5.0%, p=0.92) survival rates.




**Conclusions:** A higher rate of AMR observed in the ALE group did not translate into adverse short term graft and patient outcomes. Increased AMR incidence associated with ALE induction could be related to lesser suppression of humoral immune response when compared to r-ATG. Similar rates of ACR between the groups imply adequate suppression of T cell mediated immunity by ALE. Long-term impact of these observations is less clear.

## FR-PO1017

### Rituximab with Prophylactic Intravenous Immunoglobulin Lowers Antibody Mediated Rejection in Flow Crossmatch Positive Kidney Transplant Recipients

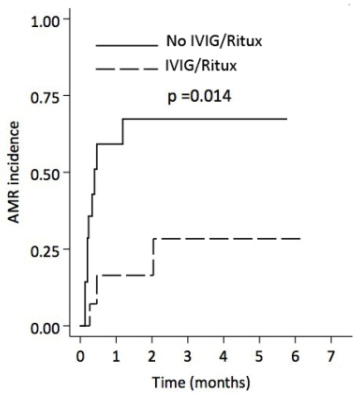
Shefali Patel,[1] Russell J. Crew,[2] Sumit Mohan,[2] Lloyd Ratner.[1] *[1]Surgery, Columbia Univ; [2]Nephrology, Columbia Univ.*

**Background:** Successful kidney transplantation in sensitized patients remains a challenge with high rates of antibody mediated rejection (AMR).

**Methods:** Our protocol for live donor kidney transplantatation with a flow positive crossmatch is pre- and post-transplant plasmapheresis (PP) with IL2 receptor antagonist induction, IV methylprednisolone tapered to prednisone 20 mg on postop day 4, with tacrolimus/mycophenolate maintenance therapy. To reduce AMR rates, in 2008, we added intraoperative Rituximab (375 mg/m$^2$) and intravenous immunoglobulin (IVIG) 2gm/Kg in divided doses at months 0, 2, 4, 6. This study compares the 17 patients (grp 1) who received this AMR prophylaxis to a historical cohort of 14 pts (grp 2) that received the original regimen. Kidney biopsies were done at weeks 1, 2, 4, 12, 26, 52, then yearly.

**Results:** There were no significant differences between groups in terms of age (46.4±12.5 vs 45±9.8 yrs, *p=0.92*), gender (females 53% vs 79%, *p=0.26*), or prior transplant (76% vs 38%, *p=0.06*). Overall biopsy proven rejection rates were similar (82 v 86%, p=0.99). However, in the Rituximab/IVIG arm, rates of AMR were lowered significantly (see figure, Log Rank = 12, *p= 0.015*), cases of concomitant ACR and AMR (0% vs 36%, *p=0.012*) were eliminated, and likely contributed to a small improvement in rejections with a complete response to therapy (85% vs 58%, *p=0.2*). Creatinine at one year was lower in grp 1, but not significantly different (1.5±0.6 vs 1.8±0.6 mg/dL, *p*=0.2). There were no significant differences in hospitalizations for infectious complication (4 vs 6) or rates of BK virus nephropathy (2 vs 0).

**Conclusions:** The addition of IVIG and Rituximab post-transplant appears to lower rates of AMR in patients receiving live donor positive flow crossmatch kidneys and should be considered in all such patients.




## FR-PO1018

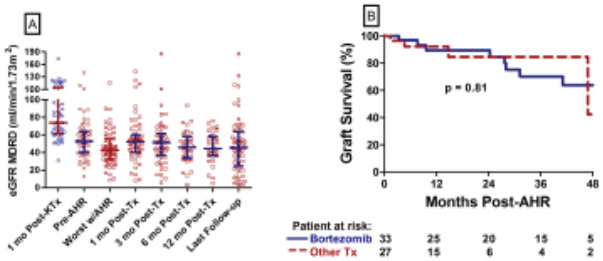
### Adding Bortezomib or Rituximab to PP Does Not Offer Advantage in the Treatment of Acute Humoral Rejection. A Single Center Experience in Mexico

Luis E. Morales-Buenrostro, Lluvia A. Marino-vazquez, Araminta Guichard, Cristhian R. Arias-delgadillo, Norma O. Uribe-uribe, Josefina Alberú. *Nephrology, Transplantation & Pathology, National Institute of Medical Sciences and Nutrition SZ, Mexico City, DF, Mexico.*

**Background:** Treatment for acute humoral rejection (AHR) has been an adaptation of the desensitization schemes. Includes removal of circulating HLA-antibodies (HLAabs) by plasmapheresis (PP), neutralization of HLAabs with IVIG, rituximab (RTX), and Bortezomib (Borte) intended to decrease HLAabs-producing plasma cells derived from B-cells, as well as use of rATG looking to control the cellular component (or interaction between T- and B-cells). This study compare different treatment protocols in order to guide future decisions.

**Methods:** Between May/08 and Apr/13, we prospectively recruited all 60 patients who had AHR. The histological description was made according to modified Banff 97 classification. DS-HLAabs were determined by Luminex single antigen bead assay (One Lambda). Kaplan-Meier survival curve with Log Rank test were used for compare graft survival.

**Results:** We included 60 KTR. Mean age 33.2±11.9; male gender 70%; first transplant 85%; living donor 76.7%; induction therapy 56.7%; maintenance triple therapy with CI 89.9%. The most used treatment schemes were PP+IVIG+RTX(36.7%), PP+IVIG+RTX+BORTE(35%), PF+IVIG+BORTE(6.7%), others (21.6%). A 41.7% received more than one treatment (2-7). A 95% of the patients used PP, and 55% received Borte. There were not differences in graft survival when Borte, RTX, or IVIG were added to PP. Also, receive more than one treatment cycle, or the time after KT did not showed difference. The figure shows the eGFR behavior, and graft survival according to Borte use.




**Conclusions:** At 4 years of follow-up, an important global graft survival reduction after AHR was found (55%) despite treatment. Adding Borte, RTX, or IVIG to PP did not showed any advantage.

*Funding:* Government Support - Non-U.S.

## FR-PO1019

### Five-Year Patient and Graft Survival in Desensitized Patients with Positive Complement-Dependent Cytotoxicity Crossmatch Is Similar to Unsensitized Patients

Kassem Safa,[1] Jude A. Yagan,[2] Helen Mah,[3] Jamil R. Azzi,[1] Edgar L. Milford,[3] Anil K. Chandraker,[1] Leonardo V. Riella.[1] *[1]Renal Div/Transplantation Research Center, Brigham and Women's Hospital, Boston, MA; [2]International Society of Nephrology; [3]Tissue Typing Laboratory, Brigham and Women's Hospital, Boston, MA.*

**Background:** More than 30% of potential kidney transplant recipients have pre-existing anti-HLA antibodies. Desensitization has been widely used and is accepted as an important strategy for overcoming this barrier. However, limited data is available regarding long-term outcomes, in particular for the highest risk group with a positive complement-dependent cytotoxicity crossmatch (CDC-XM) prior to desensitization.

**Methods:** Between 2002 and 2010, 39 patients underwent living-kidney transplantation across a positive CDC-XM against their donors at our center. The desensitization protocol involved pre-transplant immunosuppression, plasmapheresis, low-dose intravenous immunoglobulin ± rituximab. Measured outcomes included patient and graft survival, renal function and rates of rejection, infection and malignancy.

**Results:** The mean and median follow up was 5.2 years. Patient survival was 95% at 1 year, 95% at 3 years and 86% at 5 years. Graft survival was 87% at 1 year, 79% at 3 years and 72% at 5 years. Death-censored graft survival was 94% at 1 year, 88% at 3 years and 84% at 5 years. Chronic antibody-mediated rejection (AMR) caused 4 out of 6 graft failures; 24 subjects (61%) developed acute AMR with the majority occurring in the first month after transplan; one patient lost their graft due to hyperacute rejection. Chronic rejection/transplant glomerulopathy developed in 15 patients (38.5%). Acute cellular rejection occurred in 9 patients (24%). Infectious complications included pneumonia (17%), BK nephropathy (10%), and CMV disease (5%). 10% of patients developed skin cancer; there were no cases of lymphoproliferative disorder.

**Conclusions:** Desensitization in living-donor kidney transplantation results in acceptable 5-year patient and graft survival, despite high acute rejection rates. This approach represents a reasonable strategy for sensitized patients with no access to other alternatives such as paired kidney donation.

**FR-PO1020**

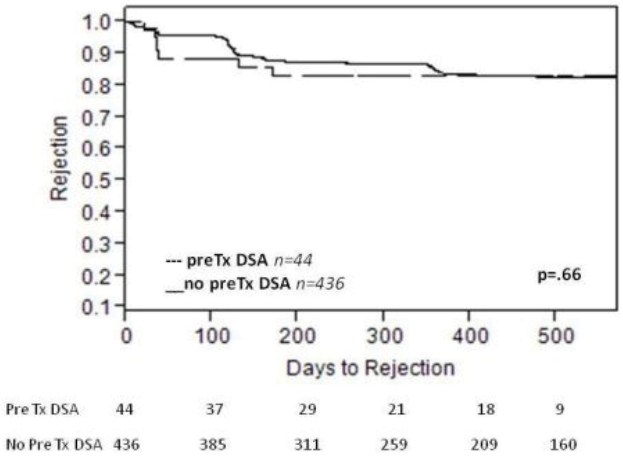
**Donor Specific Antibodies at Time of Transplant with a Negative or Weakly Positive Crossmatch Have Little Impact on Post Kidney Transplant Outcomes** Hasan Khamash, Kunam Sudhakar Reddy, Ala Nijim, Harini A. Chakkera, Janna Huskey, Raymond L. Heilman. *Mayo Clinic - Arizona.*

**Background:** We wanted to retrospectively study the impact of pretransplant donor specific antibodies DSAs on kidney allograft rejection and graft survival.

**Methods:** We included all recipients of kidneys from July 2010 to January 2013 excluding multiorgans. HLA and crossmatch (CXM) data was reviewed including the presence of DSAs at time transplant. A DSA was considered if the MFI >1000. HLA Luminex testing and protocol biopsies were obtained at 4 months and 1 year in addition to indication biopsies. Follow up data was available till May 2013. We also included transplants with weakly positive CXM in the analysis.

**Results:** 44/506 recipients identified to have at least one DSA at transplant, 26 recipient with no available pretransplant HLA data and 436 with no DSAs. The group with DSAs were more likely to have a kidney from a deceased donor, receive rATG for induction and be on steroid maintenance. Also more likely to have a previous transplant and a higher PRA. There was no difference in rejection or graft failure. AMR was increased in the DSA group but not significant.

| | Pre-Tx DSA n=44 | No DSA n=436 | p |
|---|---|---|---|
| Deceased Donor | 31(70%) | 219(50%) | .01 |
| Retransplant | 12(27%) | 38(9%) | <.01 |
| PRA (mean) | 39% | 10% | <.01 |
| Positive crossmatch | 5(11%) | 37(8%) | NS |
| rATG induction | 41(93%) | 119(27%) | <.01 |
| Steroid Maintenance | 43(98%) | 199(46%) | <.01 |
| 1 year GFR | 59±21 | 62±20 | NS |
| Antibody mediated rejection | 3(7%) | 12(3%) | NS |
| Graft failure | 2(5%) | 13(3%) | NS |




The DSAs were class I in 14/44, class II in 33/44 with a median MFI 1650. 11/44 had a DSA with and MFI>3000. By 4 month post transplant 18/40 had no DSA.

**Conclusions:** Employing rATG induction and steroid maintenance, recipients with pretransplant DSAs and negative/weakly positive CXM can be safely and successfully transplanted with at least similar short term outcomes compared to recipient with no DSAs.

**FR-PO1021**

**Bacterial and Fungal Infections Do Not Increase Sensitization in Kidney Transplant Recipients** Maria Ajaimy, Enver Akalin. *Kidney Transplant, Montefiore-Einstein Kidney Transplant Center, Bronx, NY.*

**Background:** Infections might increase sensitization in kidney transplant recipients due to interaction between innate and adaptive immunity or decreased immunosuppression during infection.

**Methods:** Patients transplanted between March 2009-2012 were included. Anti-HLA antibody testing using Luminex SAG was performed at 3, 12 months, and annually after transplantation. Increased sensitization was defined as increased panel reactive antibody and/or mean fluorescence intensity values of donor-specific anti-HLA antibodies (DSA) or development of new DSAs.

**Results:** Of the 267 patients studied, 64 (24%) demonstrated increased sensitization during a median follow-up of 25 months (3-48) after transplantation. 68 patients (26%) developed infection; 39 urinary tract, 13 pneumonia, 8 sepsis and 9 other infections. The risk factors for increased sensitization after transplantation were summarized in Table. The only statistically significant factor for increased sensitization was history of rejection prior to or within 2 months after Luminex. There was no association with the other factors studied including infection.

**Conclusions:** Acute rejection but not infection is associated with development of increased sensitization after kidney transplantation in our cohort of patients.

| Characteristic (%)or mean standard deviation | No increased sensitization N=203 | Increased sensitization N=64 | P-value |
|---|---|---|---|
| Infection prior to or within 2 months after sensitization date | 22.6 | 28.1 | 0.22 |
| Infection prior to sensitization date | 20.2 | 20.3 | 0.99 |
| Graft rejection prior to or within 2 months after sensitization date | 4.9 | 15.6 | 0.005 |
| Blood transfusion given prior to sensitization date | 37.0 | 31.0 | 0.41 |
| Mean, Age | 52.4+12.1 | 49.4+13.0 | 0.09 |
| Race, African-American | 34.5 | 25.0 | 0.16 |
| Gender, female | 40.9 | 53.1 | 0.09 |
| History of previous transplant | 14.9 | 12.5 | 0.64 |
| Donor type,( Deceased-donor) | 64.0 | 64.1 | 0.99 |
| Pretransplant class 1 PRA>79% | 11.6 | 19.2 | 0.17 |
| Pretransplant class 2 PRA >79% | 10.4 | 14.8 | 0.33 |
| Pretransplant DSA present | 16.8 | 26.6 | 0.08 |
| Induction with basiliximab | 39.1 | 35.9 | 0.61 |

**FR-PO1022**

**Safety of Transplanting Patients with Pre-Transplant Donor-Specific (DSA) Anti-HLA Antibodies without Pre-Transplant Desensitization Therapy** Maria Ajaimy, Kwaku Marfo, Adriana Colovai, Enver Akalin. *Kidney Transplantation, Montefiore-Einstein Center for Transplantation, Bronx, NY.*

**Background:** Patients with strong DSAs develop higher rates of acute and chronic antibody-mediated rejection (AMR) despite pretransplant desensitization treatment. We created a pre-transplant immunologic risk assessment protocol based on mean fluorescence intensity (MFI) of DSAs and channel shift of values of flow-cytometry cross-match.

**Methods:** Patients with DSA MFI values between 1,000-5,000 and flow-cytometry T or B cross-match channel shift values of 50-150 and 100-250, respectively were accepted for transplantation. All patients with DSA received anti-thymocyte globulin (total 6 g) and IVIG (2 g/kg) induction treatment.

**Results:** Between May 2009 and December 2012, 66 kidney transplant recipients with a mean number of DSAs (1.63 ± 0.82), MFI (2904 ± 2776), Flow T (136 ± 50), and B cell channel shift (162 ± 60) were compared to 308 patients without DSAs (Table). During a median 2.3 years follow-up, there was no difference in terms of patient and graft survival and graft function between 2 groups. Acute rejection was only observed in 8% of the DSA+ patients. Total of 73 DSAs were lost, and mean number of DSAs (0.54 ± 0.79) and MFI (2077 ± 3483) values significantly decreased posttransplant.

**Conclusions:** Successful kidney transplantation with a low acute rejection rate could be achieved in DSA+ patients with an immunologic risk assessment based on MFI and flow channel shift values and without pretransplant desensitization therapy.

| | DSA(N=66) | No-DSA(n=308) | P-value |
|---|---|---|---|
| Mean Age | 54 ±10 | 54 ±13 | 1.00 |
| Sex, %female | 61 | 41 | 0.004 |
| Race, % AA | 36 | 26 | 0.13 |
| Mean Class I PRA | 57 ± 41 | 21 ± 30 | 0.0001 |
| Mean Class II PRA | 61 ± 36 | 24 ± 30 | 0.0001 |
| Transplant type, %LT | 16 | 31 | 0.03 |
| Mean CIT | 24 ± 12 | 24 ± 12 | 1.00 |
| Previous Transplant, % | 24 | 5 | 0.0001 |
| Graft Survival | 89 | 95 | 0.15 |
| Patient Survival | 97 | 97 | 1.00 |
| Acute Rejection/AMR | 8 | 10 | 0.81 |
| BKV-viremia | 14 | 16 | 0.85 |
| CMV-viremia | 5 | 9 | 0.33 |

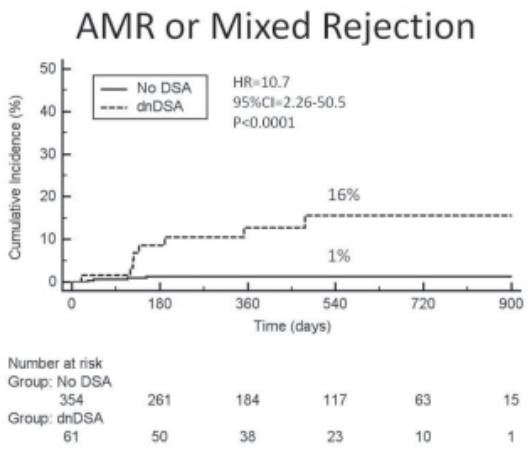
**FR-PO1023**

**Early De Novo Donor Specific Antibodies (dnDSA) after Kidney Transplantation: Impact on Rejection and Histology Findings** Raymond L. Heilman, Ala Nijim, Yvonne M. Desmarteau, Hasan Khamash, Marcelo Pando, Harini A. Chakkera, Janna Huskey, Riccardo Valdez, Kunam Sudhakar Reddy. *Mayo Clinic, Phoenix, AZ.*

**Background:** Our aim was to study (1) the frequency of dnDSA during the first year post-transplant and (2) the impact of dnDSA on protocol biopsy findings and rejection.

**Methods:** All patients receiving a solitary kidney transplant between 7/2010 and 1/2013 were included. Luminex Single Antigen Bead (SAB) assay was performed at 1, 4 and 12 months post-transplant. Only DSA's with MFI≥1000 were included. The cohort was grouped by the presence of a dnDSA at any time point during the first 12 months. Protocol kidney biopsy (Bx) was done at 12 months.

**Results:** 506 patients were transplanted during the study period. Exclusions: 51 with pre-transplant DSA, 26 without pre transplant testing, 9 with early non-immune related graft loss. 415 of the remaining 420 patients had at least one Luminex SAB assay during the first 12 months. 61 (15%) had dnDSA: 24 (6%) class I, 44 (11%) class II, 7 (2%) both. The dnDSA group was younger (50.4±13.5 vs. 55.4±13.1 yrs, p=0.007). Otherwise baseline patient characteristics were similar: female 39%, Black race 7%, deceased donor 50%, previous transplant 9%, 9% had pre transplant PRA>50%, and mean HLA mismatch 3.7±1.7. The control group was more likely to receive induction with a non depleting agent (18% vs. 3%, p=0.003). eGFR at 1 year was not different. The dnDSA group had more glomerulitis, tubulitis, interstitial inflammation and ptc>0 on the 1 yr Bx. Banff cg and C4d staining were not different. There was more antibody mediated rejection (AMR) in the dnDSA group.




**Conclusions:** The incidence of dnDSA during the first post-transplant year was 15%. Patients with dnDSA are more likely to have AMR and have changes on 1 year Bx compatible with endothelial injury. Further study of the impact of dnDSA on graft survival is needed.

## FR-PO1024

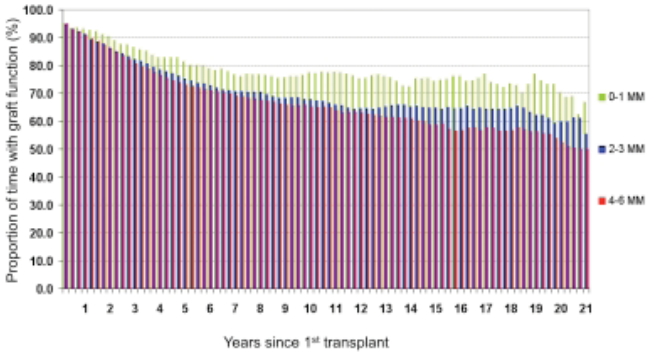
**Impact of HLA Mismatch at First Kidney Transplant on Lifetime with Graft Function in Young Recipients** Bethany J. Foster,[1] Xun Zhang,[1] Mourad Dahhou,[1] Robert Platt.[2] *[1]Pediatrics, McGill Univ, Montreal, Canada; [2]Epidemiology and Biostatistics, McGill Univ, Montreal, Canada.*

**Background:** Concerns have been raised that poor matching at 1st transplant may lead to greater sensitization and difficulty finding an acceptable donor for a 2nd transplant should the 1st transplant fail. We compared proportion of total lifetime with graft function after first transplant, and waiting times for a 2nd transplant between individuals with different levels of HLA mismatch (MM) at 1st transplant.

**Methods:** We studied patients recorded in the USRDS (1988-2009) who received a 1st DD transplant at ≤21 years old (n=8433), and the subgroup who were listed for a 2nd DD transplant following 1st graft failure (n=2498). We used logistic regression, with generalized estimating equations (GEE), to estimate the association between HLA ABDR MM at 1st transplant and proportion of total observed lifetime with graft function, and Cox models to compare the waiting times for a 2nd transplant between patients with different levels of HLA MM at the 1st transplant. All models were adjusted for sex, race, recipient age, socioeconomic status, transplant era, donor source, duration of dialysis before transplant, blood group, panel reactive antibody, and an index of HLA matchability (based on HLA profile compared with the donor pool).

**Results:** Compared with recipients of 2-3 MM 1st grafts, 4-6 MM graft recipients spent 15% [95%CI 13%, 18%] less of their time with a functioning graft after the 1st transplant (p<0.0001), and were significantly less likely to receive a 2nd transplant after listing (HR 0.87 [95% CI 0.78, 0.98]).

Figure 1




**Conclusions:** The benefits of better HLA matching at first transplant on lifetime with graft function are relatively small, and may be overshadowed by longer waits for a first graft if HLA matching were to be emphasized in allocation.

*Funding:* Government Support - Non-U.S.

## FR-PO1025

**Impact of Transplant Nephrectomy on HLA-Sensitization and Re-Transplantation** Saad Ajmal,[1] George P. Bayliss,[2] Jason T. Machan,[3] Claire Kassakian,[2] Paul E. Morrissey.[1] *[1]Surgery, Alpert Medical School, Providence, RI; [2]Medicine, Alpert Medical School, Providence, RI; [3]Biostatistics, Alpert Medical School, Providence, RI.*

**Background:** The management of a failed renal allograft is controversial. Transplant nephrectomy can lead to anti-HLA antibody formation (allo-sensitization). We evaluated the effect of transplant nephrectomy on allo-sensitization and the rate of subsequent transplantation.

**Methods:** We conducted a retrospective review of data from prospectively collected institutional and UNOS databases of kidney transplant recipients from 1997-2012 at a single academic transplant unit: some 933 renal transplants were performed out of which 183 failed (19.6%). Patients with failed transplants were divided into two groups: transplant nephrectomy for-cause (TN=53/172) and those who retained allograft (RA=119/172). Panel Reactive Antibody (PRA) values of patients who were re-listed, the main outcome measure, were collected at selected time points after graft failure.

**Results:** Age at transplantation, race, gender, living vs. deceased transplant, etiology of ESRD and time to graft failure were similar in both groups. No differences in co-morbidities were observed except for more patients with diabetes mellitus in the RA group (47.4% vs. 26.4%; p= 0.0097). TN group demonstrated a significant increase in PRA values immediately following TN (30.5 % vs. 43.8 %; p= 0.027). Patients with a failed allograft did not have significant change in PRA values (p= 0.67) over 1 year post-failure. 5/28 (17%) patients in TN group became highly sensitized (PRA>60) compared to 3/52 (5.7%) in RA group (p=0.08). Re-transplantation rates were similar at 4 years from graft failure in both groups (TN= 9/53, RA= 20/119; p=0.30). There was no statistically significant survival advantage offered by transplant nephectomy; but peri-operative morbity was high, and there were two deaths within 30 days of nephrectomy.

**Conclusions:** Although a mild increase in PRA was observed immediately following transplant nephrectomy, TN does not decrease the opportunity for re-transplantation. TN should only be performed for cause.

*Funding:* Clinical Revenue Support

## FR-PO1026

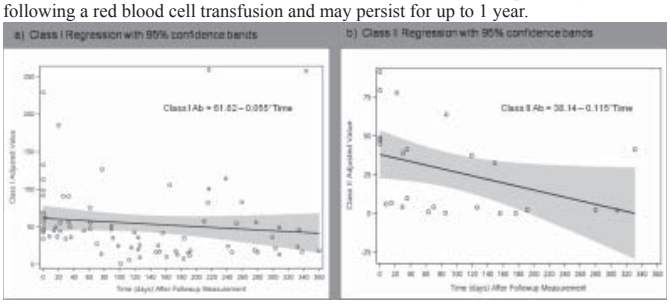
**Duration of HLA Antibody Elevation following a Transfusion Event in Patients awaiting Kidney Transplantation** Mary S. Leffell,[1] Deborah Kim,[2] Jeffrey Petersen,[3] John M. Hart,[1] Renato Vega,[1] Brian D. Bradbury,[2] Andrea A. Zachary.[1] *[1]Johns Hopkins Univ Immunogenetics Laboratory, Johns Hopkins Univ School of Medicine, Baltimore, MD; [2]Center for Observational Research, Amgen, Thousand Oaks, CA; [3]Clinical Development, Amgen, Thousand Oaks, CA.*

**Background:** HLA sensitization remains a major barrier to successful kidney transplantation. This study examines the association of transfusions with the occurrence, strength, specificity and duration of HLA antibody formation using highly sensitive/specific methods for testing HLA antibody.

**Methods:** We linked USRDS data with HLA antibody (Ab) data obtained from a pooled antigen assay comprised of HLA class I only and class II only antigens on the Luminex® platform for patients (pts) on dialysis awaiting primary kidney transplant at Johns Hopkins Transplant Center. We individually matched pts who had a transfusion event between 2 Ab measurements with ≤4 non-transfused pts on 6 demographic characteristics. We compared changes in adjusted, normalized values between treatment groups. Among pts with an increase in HLA antibodies following a transfusion, we examined changes in HLA class I and II Ab levels for up to 1 year using generalized estimating equations.

**Results:** We identified 89 transfused and 251 matched non-transfused pts. A greater proportion of pts experienced changes in adjusted values ≥25 for class I and ≥30 for class II antigens for transfused (20.4%) than non-transfused pts (2.4 %) (OR 9.6, 95% CI: 3.0-30.7). Following transfusion, Ab levels declined gradually over the following year (Fig 1).

**Conclusions:** Significant increases in HLA Ab strength and specificity can occur following a red blood cell transfusion and may persist for up to 1 year.




*Funding:* Pharmaceutical Company Support - Amgen

## FR-PO1027

**Some Renal Transplants Diagnosed as Glomerulonephritis Probably Have Antibody-Mediated Rejection** Andre Barreto Pereira,[1,3] Jessica Chang,[1] Konrad S. Famulski,[1] Philip F. Halloran.[1,2] *[1]Alberta Transplant Applied Genomics Centre, Edmonton, Canada; [2]Dept of Medicine, Div of Nephrology and Transplant Immunology, Univ of Alberta, Edmonton, Canada; [3]Dept of Nephrology, Santa Casa de Misericordia de Belo Horizonte, Belo Horizonte, Minas Gerais, Brazil.*

**Background:** In renal transplants recurrent and de novo glomerulonephritis (GN) is common, but distinguishing GN from antibody-mediated rejection (ABMR) is difficult. We hypothesized that a recently developed test for ABMR would reveal that some GN causing failure in renal transplants is actually ABMR.

**Methods:** From a prospective study of transplant indication biopsies taken >1year post transplant, we selected assessed 64 diagnosed as GN and assessed their microarray ABMR score (S) and conventional features (C).The underlying renal disease was identified as biopsy-proven GN in 24/64 (38%).

**Results:** 9/64 (16%) of transplants diagnosed as GN had positive ABMR scores (Table 1). In microarray analysis, S+ biopsies were later and had higher expression of DSA associated (DSAST), IFNG-induced (GRIT), and NK cell (NK) transcripts, typical of ABMR, and had higher AKI scores, than S-. S+ biopsies also had higher histologic cg and ci scores, and a higher frequency of failures than the 55 S- biopsies (Table 1). However, the TCMR scores were not different.

**Conclusions:** 16% of transplants diagnosed as GN in late biopsies have positive ABMR scores. These have an increased frequency of molecular features of ABMR, glomerular double contours and glomerulitis, and increased risk of graft failure. With treatment options emerging for ABMR, microarray diagnosis may aid in correctly distinguishing ABMR from GN.

Table 1 - GN in late combined biopsies (1 random biopsy per patient)

| GN variables | ABMR score<0.2(Failures) | ABMR score>0.2(Failures) | p value |
|---|---|---|---|
| n | 55(10) | 9(5) | 0.042 |
| DSA | 9(2) | 2(2) | 0.20 |
| Time post transplant (days) | 2740±2148 | 5068±2477 | 0.0017 |
| cg (glomerular double contours) | 0.35±0.70 | 1.37±1.06 | 0.002 |
| g (glomerulitis) | 0.22±0.60 | 0.62±1.06 | 0.12 |
| ptc (peritubular capillaritis) | 0.06±0.31 | 0.12±0.35 | 0.34 |
| mm (mesangial matrix increase) | 1.32±0.91 | 1.62±1.18 | 0.40 |
| ci (interstital fibrosis) | 1.15±0.81 | 2.37±0.74 | 0.0008 |
| ABMR score | 0.06±0.05 | 0.43±0.16 | 1.84e-06 |
| TCMR score | 0.007±0.029 | 0.0004± 0.0007 | 0.37 |
| AKI score (IRRAT) | 0.38±0.77 | 1.19±0.65 | 0.003 |
| DSA associated transcripts (DSAST) | 0.21±0.33 | 0.63±0.27 | 0.0008 |
| IFNG-induced transcripts (GRIT) | 0.44±0.40 | 0.80±0.27 | 0.012 |
| NK transcripts | 0.64±0.55 | 1.33±0.55 | 0.002 |

## FR-PO1028

**The Role of Medication Nonadherence in the Generation of De Novo Donor Specific Antibodies** Julia W. Tzeng,[1] Robert B. Ettenger,[1] Elaine F. Reed,[1] David W. Gjertson,[1] Ting-yan Chan,[1] Dechu P. Puliyanda,[2] Eileen W. Tsai.[1] *[1]Pediatrics, Mattel Children's Hospital UCLA, LA, CA; [2]Pediatrics, Cedars-Sinai Medical Center, LA, CA.*

**Background:** Medication nonadherence (MNA) has been associated with development of de novo donor specific antibodies (DSA) and rejection (rej) in kidney transplant (Tx) patients (pts). However, these studies linking MNA and DSA were limited, since the technique of identifying MNA was not rigorously defined. We recently showed that the percent coefficient of variation (CV%) of tacrolimus (TAC) is significantly associated with MNA and rej. Since DSA worsens Tx outcome and predicts antibody mediated rejection (ABMR), we sought to establish a precise relationship between DSA and MNA determined by pt self-report and CV% of TAC.

**Methods:** We studied 125 pediatric pts txed from Jan 2005 to Dec 2011. Pts were induced with anti-CD 25 antibody or anti-thymocyte globulin and maintained on TAC and anti-metabolite ± steroids. Biopsies and DSA were done per protocol and for clinical suspicion of rej and MNA. Rej was classified by Banff 09. DSA, using Luminex single antigen beads, were identified when mean fluorescent intensity >1000. MNA was determined by pt self-report using a standardized interview and by CV% of TAC (standard deviation divided by mean multiplied by 100%).

**Results:** Nonadherent pts defined by self-report were at higher risk of developing DSA (p <0.001, Fig 1a). Pts with DSA had a higher median CV% (p=0.002, Fig 1b). ROC analysis showed that CV% cut-off point of 42% was associated with an increased risk of developing DSA (AUC=0.71). We found an association between MNA as defined by either pt self-report or CV% and ABMR (p<0.001 and p=0.02, respectively).

Figure 1a: DSA is associated with MNA by pt self-report

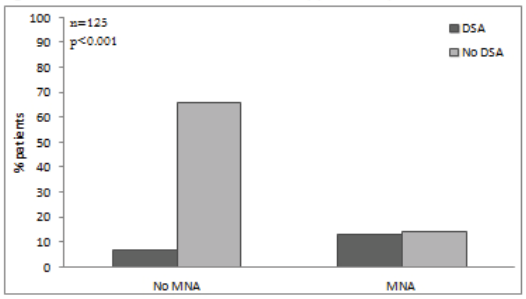
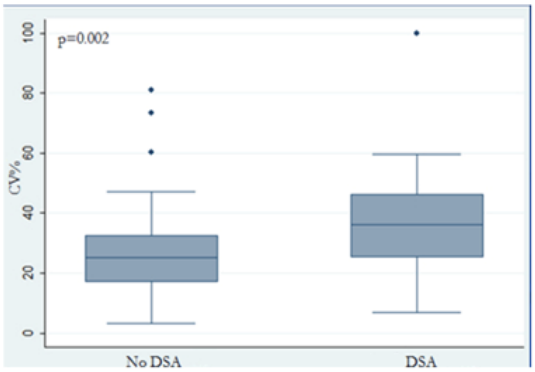



Figure 1b: DSA is associated with CV%




**Conclusions:** Rigorously defined MNA, DSA, and ABMR are conclusively associated. Thus, DSA can potentially be used as a novel marker to monitor MNA.

*Funding:* Private Foundation Support

## FR-PO1029

**Is There an Increased Risk of Posttransplant De Novo Donor-Specific HLA Antibodies in Calcineurin-Inhibitor Sparing Immunosuppression?** Claudia Sommerer,[1] Christian Morath,[1] Matthias Schaier,[1] Petra Gombos,[2] Caner Süsal,[2] Martin G. Zeier.[1] *[1]Nephrology, Univ, Heidelberg, Germany; [2]Immunology, Univ, Heidelberg, Germany.*

**Background:** The important role of humoral immunity in the pathogenesis of chronic allograft nephropathy has prompted research assessing the role of anti-HLA antibody (Ab) monitoring to predict allograft outcome. Data on the prevalence of donorspecific antibodies (DSA) depending on the immunosuppressive regimen are limited.

**Methods:** We evaluated 106 immunologically low risk renal allograft recipients for de novo Ab occurrence. DSA were assessed by Luminex technology (One Lambda; Canoga Park, CA, USA). Initial immunosuppression consisted of basiliximab as induction therapy, CsA, MPA and steroids.

**Results:** Altogether, sera of 106 immunologically low-risk patients were included in the present analysis. At month 3 after transplantation, 86 patient were eligible for randomization either to continue standard immunosuppression (S: CsA+MPA, n=28) or to be converted to a CNI-free regimen with the mTOR inhibitor everolimus (CNI-free: EVE+MPA, n=29) or to a CNI-low regimen (CNI-low: CsA+EVE, n=29). At 4-year follow up, 17/79 patients (22%) had developed de novo DSA with 4/24 (17%), 5/28 (18%) and 8/27 (30%) in the standard, CNI-free and CNI-low group, respectively (n.s.). DQ de novo DSA was much more likely to appear compared with other loci antibodies. Patients with DR mismatch had an increased risk for developing of de novo DSA. In the follow-up period, four patients developed active C4d+ antibody-mediated rejection (AMR) (2 CNI-low, 2 CNI-free). In all four patients classical de novo DSA directed to HLA-A, -B or –DR antigens were observed and all patients demonstrated low CsA and/or EVE trough levels.

**Conclusions:** In summary, in the 4-year follow-up period, de novo DSA occurred in 22% of the patients. Incidence of de novo DSA was higher in the CNI-low group without reaching statistical significance. Most of the DSA were directed to the HLA-antigen DQ. DR HLA mismatch and low immunosuppressive exposure were identified as risk factors for development of de novo DSA. AMR was only detected in patients demonstrating de novo DSA directed to "classical" HLA-antigen A, B or DR.

**FR-PO1030**

**Impact of Donor-Specific Antibodies and Biopsies of the Grafts on the Therapy of Pancreas-Kidney Transplantation Patients**
Luis Eduardo Becker,[1] Sebastian Schäfer,[1] Ruediger Waldherr,[2] Martin G. Zeier,[1] Caner Süsal,[3] Christian Morath.[1] *[1]Nephrology, Univ of Heidelberg, Germany; [2]Pathology, Univ of Heidelberg, Germany; [3]Immunology, Univ of Heidelberg, Germany.*

**Background:** While the role of donor specific antibodies (DSA) in simultaneous pancreas kidney transplantation (SPKT) is still unclear, the histological evaluation of the pancreas allograft remains essential to judge immunological processes in the graft. Because of the growing clinical significance of DSA in the kidney-alone transplant setting, we performed a critical retrospective analysis of the indications and the further therapeutic consequences of these diagnostic tools on SPKT patients from our program.

**Methods:** All 17 cadaveric SPKT (from a total of 29) patients who underwent one or more biopsies of the pancreas allograft between Oct 2009 and Jan 2012 at our facility were included in the analysis. A total of 51 percutaneous biopsies (22 of the pancreas, 29 of the kidney) was performed in a median follow-up of 24 months. Analysis of DSA IgG against HLA antigens occurred prior to transplantation and by the time of the pancreas or kidney allograft biopsies.

**Results:** Adequate samples according to Banff were obtained in 15 of 22 procedures (68%). Successful pancreas biopsy led to a significant change in the immunosuppressive therapy in eleven episodes (73%) and justified the employment of antibody directed therapy in six (40%). In the remaining six patients, the therapy was oriented by the result of a concomitant kidney biopsy or the presence of DSA; antibody directed therapy was employed in one patient. Nine patients (60%) had at least one DSA positivity, eight of them with concomitant pancreas or kidney allograft rejection (88%), three with c4d positivity (33%). Six patients had negative DSA, four of them (67%) having sings of rejection in the kidney or pancreas histology, but none with antibody mediated rejection (AMR).

**Conclusions:** Even with the employment of Luminex tests in the routine and a possible association of DSA and the presence of AMR in our patient collective, the result of pancreas histology remained pivotal for the employment of a targeted, often more aggressive immunosuppression.

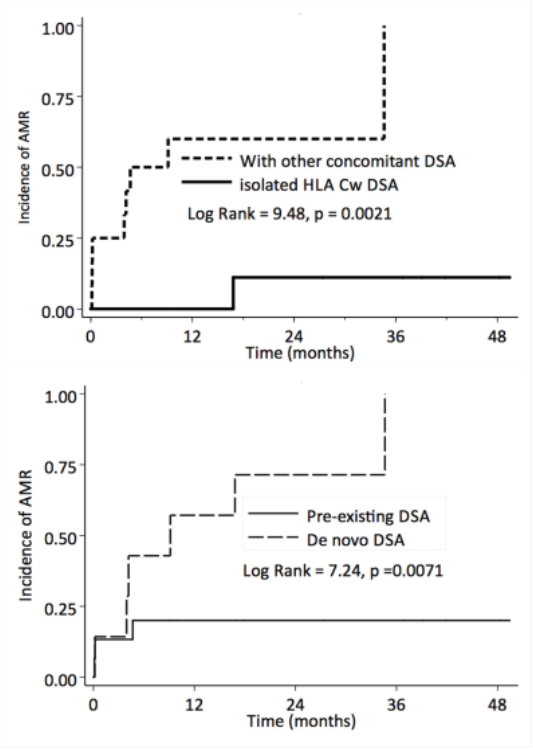
**FR-PO1031**

**Antibody Mediated Kidney Rejection Associated with HLA-Cw Specific Alloantibodies**
Ziad S. Zaky, Demetra Tsapepas, Elena Rodica Vasilescu, Geoffrey K. Dube, Russell J. Crew, Lloyd Ratner, David J. Cohen, Sumit Mohan. *Columbia Univ Medical Center.*

**Background:** It is unclear whether donor-specific antibodies (DSA) directed against HLA-Cw increase risk for antibody-mediated rejection (AMR) after kidney transplant.

**Methods:** Since 2008, kidney transplant recipients (KTR) at Columbia University Medical Center are screened for DSA against HLA-Cw pretransplant and at the time of graft dysfunction. We identified 84 KTR with Cw alloantibodies including 22 KTR with HLA-Cw DSA, either in isolation (45.5%) or combined with other class I or class II DSA (54.5%).

**Results:** Our cohort was 68.2% male, with a median age of 56.7yrs (IQR 46.5–67.6 yrs); the majority (68.2%) had no previous organ transplant. Nine KTR had an AMR (3 with pre-existing Cw DSA vs 6 with *de novo* Cw DSA, *p=0.007*). KTR with isolated Cw DSA were less likely to experience AMR (10% vs 66.7%, *p=0.03* and OR=0.56, *p=0.02*) and have a longer time to AMR (*p=0.002*) despite similar MFI (Cw DSA MFI median 1600 vs 3600, *p=0.08*) to patients with additional DSA. No KTR with isolated pre-existing Cw DSA (n=9) experienced AMR while 50% (3/6) with other pre-existing DSA had AMR (*p=0.04*). Seven KTR developed *de novo* Cw DSA at a median of 126 days (IQR 53-909 days) post transplant but only 1 developed Cw DSA in isolation; 85.7% (6/7) had AMR. KTR with *de novo* Cw DSA had significantly shorter time to rejection than pts with pre-existing Cw DSA at transplant (*p=0.007*) despite similar MFI (median 2000 vs 2200, *p=0.32*). After adjustment for the presence of other HLA DSA, KTR with *de novo* Cw DSA were still more likely to develop AMR than those with pre-existing Cw DSA.




**Conclusions:** Our results suggest a lower incidence of AMR among KTR with isolated pre-existing HLA-Cw DSA compared to KTR who have multiple DSA. However, *de novo* HLA-Cw DSA even in isolation is associated with a higher risk of AMR than preformed HLA-Cw DSA.

**FR-PO1032**

**Significance of Donor Specific Anti-HLA Antibodies Identified Using Recombinant HLA Coated Single Antigen Beads in Kidney Graft Recipients with a Negative Donor Flow Cytometry Crossmatch**
Darshana Dadhania, Dinesh Kannabhiran, John R. Lee, Jun B. Lee, Thangamani Muthukumar, Manikkam Suthanthiran. *Nephrology, Weill Cornell Medical Center, New York, NY.*

**Background:** Flow cytometry crossmatch (FCXM) and recombinant HLA coated single antigen bead assay (SAB assay) are highly sensitive assays for detection of donor specific antibodies (DSA). Many transplant centers consider a positive FCXM as a contraindication to kidney transplantation. We investigated the clinical significance of DSA using SAB assay, in the context of negative FCXM.

**Methods:** We evaluated 204 patients who underwent prospective FCXM and SAB testing prior to kidney transplantation. Sera were profiled using LabScreen SAB assay & DSA with mean fluorescence (MFI) value >1000 MFI were scored as positive. Donor FCXM was scored as positive when the mean channel displacement was >40 for donor T cells and/or >150 for donor B cells.

**Results:** Of the 204 renal transplant recipients tested, 43 had a positive FCXM with donor T and/or B cells. In the remaining 161 recipients with a negative FCXM result, 50 were positive for DSA. Seven of 50 patients with the positive DSA by SAB assay experienced AMR and four experienced graft loss. In a multivariable logistic regression analysis, DSA detected by SAB assay in patients with a negative flow cross match result was an independent risk factor for AMR. Risk of graft loss was also increased (OR=4, 0.9, 17; P=0.07).

| Multivariable Analysis | Risk of AMR Odds Ratio (95% CI) | P value |
|---|---|---|
| DSA | 11.7 (1.3, 106) | 0.03 |
| DGF | 6.2 (0.9, 43) | 0.06 |
| Class I Mismatch | 1.76 (0.69, 4.5) | 0.23 |
| Class II Mismatch | 0.86 (0.44, 1.7) | 0.66 |

**Conclusions:** Donor specific antibodies, not detected by flow cytometry but identifiable by SAB assay, is associated with an increased risk of antibody mediated rejection and with a trend toward increased graft loss in kidney graft recipients. Standardized screening of sera using SAB assay, in addition to identification of patients at risk for AMR, offers opportunities for personalized immunosuppressive therapy in kidney graft recipients.

*Funding:* Clinical Revenue Support

**FR-PO1033**

**Lower Rejection Rates with Class 1 versus Class 2 Donor Specific Antibodies in Sensitized Kidney Transplant Recipients** Russell J. Crew,[1] Shefali Patel,[2] Sumit Mohan,[1] Joan Kelly,[2] Mary Jane Samuels,[2] Lloyd Ratner.[2] *[1]Div of Nephrology, Columbia Univ; [2]Surgery, Columbia Univ.*

**Background:** Renal transplant recipients with donor specific antibodies (DSA) have high rejection rates. Determination of which class of DSA are associated with worse outcomes will aid in donor selection and paired kidney exchange participation.

**Methods:** Since 2004, we have performed 57 live donor positive cytotoxic or flow crossmatch (+XM) renal transplants. All patients received pre- and post- transplant plasmapheresis(PP) with intravenous immunoglobulin (IVIG) replacement (100mg/kg) for a minimum of 2 treatments and until CDC crossmatch was negative. Induction therapy varied with time but included either an IL-2 receptor blocker or Thymoglobulin® with or without intra-operative Rituximab. Maintenance therapy is tacrolimus and mycophenolate. All patients received IV methylprednisolone with taper to prednisone 20 mg daily by post-operative day 4. Protocol biopsies are done at 1, 2, 4, 13, 26 and 52 weeks; then annually.

**Results:** Of the 57 patients, 40 had results of DSA measurement by single antigen beads available. Among the 18 patients with cytoxic +XM, rejections rates were high regardless of DSA type (Class 1- 9/9, class 2- 3/4, class 1&2- 5/5). Analysis was then restricted to the 22 patients with only flow +XM. Patients with class 2 and class 1&2 antibodies behaved similarly and were grouped together for analysis. There were significant differences in outcomes between patients with class 1 compared to those with only class 2 or both class 1 and 2 DSA.

| FLOW CROSSMATCH RECIPIENTS ONLY | | | |
|---|---|---|---|
| | Class 1 DSA (n=5) | Class 2 and 1&2 DSA (n=17) | p value |
| Age (yrs) | 54.9±9 | 45±10 | 0.12 |
| Women | 4/5 | 8/17 | 0.32 |
| Prior txp | 2/5 | 12/17 | 0.3 |
| Creatinine 1 yr (mg/dL) | 1.26±0.47 | 1.77±0.74 | 0.28 |
| Recent creatinine (mg/dL) | 1.16±0.26 | 2.07±1.48 | 0.19 |
| Overall rejection | 2/5 | 16/17 | 0.02 |
| First rejection type | | | |
| AMR | 0/5 | 5/17 | 0.29 |
| ACR | 2/5 | 8/17 | 1.0 |
| Combined AMR/ACR | 0/5 | 3/17 | 1.0 |
| Recurrent rejection | 0/2 | 7/16 | 0.49 |

**Conclusions:** Among sensitized patients, those with only flow +XM donor specific antibodies restricted to class 1 MHC have fewer rejections and less recurrent rejections than those with class 2 or class 1 and 2 antibodies.

**FR-PO1034**

**Elevated Urinary CCL2: Cr at 6 Months Is Associated with the Development of Renal Allograft Interstitial Fibrosis and Inflammation at 24 Months** Julie Ho,[1] Chris J. Wiebe,[1] Ian W. Gibson,[2] Ang Gao,[1] Claudio Rigatto,[1] Martin Karpinski,[1] Leroy J. Storsley,[1] Peter W. Nickerson,[1] David N. Rush.[1] *[1]Internal Medicine, Section of Nephrology, Univ of Manitoba, Winnipeg, Canada; [2]Pathology, Univ of Manitoba, Winnipeg, Canada.*

**Background:** We have previously demonstrated that 6 month urinary CCL2: Cr is an independent predictor of interstitial fibrosis and tubular atrophy on 24 month biopsy and subsequent death-censored graft loss. Patients with graft loss frequently have a combination of graft interstitial fibrosis plus inflammation (GIF & "i", i>0 & ci>0). The goal of this study was to determine if 6 month urinary CCL2: Cr is an independent predictor for the development of GIF &"i".

**Methods:** The patients were from a prospective, multi-centre adult renal transplant study (n=111) that had 6 month urines and 24 month protocol biopsies. CCL2 was measured with ELISA, corrected for urinary creatinine. Renal histopathology was reported using the Banff schema. Stepwise logistic regression was performed with the potential covariates: 6 month urinary CCL2: Cr, donor age >50 years, delayed graft function, living vs. deceased donor, ACEi/ARB exposure, PRA and HLA match.

**Results:** Six-month urinary CCL2: Cr was significantly higher in GIF & "i" [26.8 ± 17 ng/mmol, p=0.003, n=28] and transplant glomerulopathy patients [36.3 ± 36 ng/mmol, p=0.004, n=15] compared to normal histology [15.4 ± 10ng/mmol, n=30] at 24 months. By multivariate analysis, 6 month urinary CCL2: Cr was an independent predictor for developing GIF & "i" at 24 months [OR 1.04, 95% CI 1.013-1.082, p=0.01]. Finally, 6-month urinary CCL2: Cr had an AUC 0.695 [95% CI 0.571-0.819], with a sensitivity/specificity of 0.71/0.62 at a cut-off value of 15ng/mmol for identifying 24 month GIF & "i".

**Conclusions:** This study demonstrates that 6-month urinary CCL2: Cr is an independent predictor for developing 24 month GIF & "i". Urinary CCL2: Cr may be used to stratify patients at risk for GIF & "i", who may require more intensive post-transplant surveillance and avoidance of drug minimization/withdrawal protocols. Finally urinary CCL2: Cr may help identify patients for novel interventional trials targeting GIF & "i".

*Funding:* Pharmaceutical Company Support - Astellas Canada, Private Foundation Support

**FR-PO1035**

**Protocol Biopsy at 0, 14 and 90 Days to Identify Renal Allograft Dysfunction – Single Center Study** Muhammad Rafiqul Alam, Md. Morshed Ul Islam, Asia Khanam, Syed fazlul Islam. *Dept of Nephrology, Bangabandhu Sheikh Mujib Medical Univ, Dhaka, Bangladesh.*

**Background:** To identify histological changes of renal allograft by protocol biopsy for detection of early graft dysfunction.

**Methods:** Thirty five kidney transplant recipients were included in this study over a period of two years in the Department of Nephrology, BSMMU, Dhaka. All received cyclosporine based immunosuppressive treatment. Serum creatinine was done daily for first 14 days and then weekly for three months. Cyclosporine blood ($C_2$) level was done 7th and 14th POD and monthly for three months. Protocol transplant kidney biopsy was done at day 0, day 14 and day 90. For histopathology haemotoxilyne and eosin (H&E) and periodic acid Schiff (PAS) stain was done.

**Results:** Over 35 recipients 23(66%) show normal graft function and 12(34%) showed early graft dysfunction. Among 35 biopsies, per operative 26 showed normal histology (no sclerotic glomeruli), 6 showed 10% sclerotic glomeruli and 3 showed 16% sclerotic glomeruli. Protocol biopsy at 14th post operative day showed 57.1% (n=20) normal histology 14.3% (n=5) had clinical rejection (elevated serum creatinine along with histological features of rejections). 14.3% (n=5) had sub clinical rejection (normal serum creatinine with histological features of rejections), cyclosporine toxicity 5.7% (n=2), ATN 5.7% (n=2) and 2.9% (n=1) had recurrent GN. Rejection episodes are further categorized by Banff-97 classification. Banff grade I (20%) (n=1) grade II (60%) (n=3) and grade III (20%) (n=1) were found in clinical rejection group and Banff grade I (80%) (n=4) and grade II (20%) (n=1) in sub clinical rejection group.

**Conclusions:** Adequate histological evaluation is helpful for detection of clinical and subclinical rejection. So routine allograft biopsy should be practiced in different transplant center of Bangladesh.

*Funding:* Clinical Revenue Support

**FR-PO1036**

**Complications Associated with Renal Graft Biopsy in Transplant Patients** Claudia Grisel Tapia Canelas, Rosa Del Carmen Zometa Estrada, Carlos Jimenez, Rafael Selgas. *Nephrology, Hospital Universitario La Paz, Madrid, Spain.*

**Background:** Percutaneous biopsy of renal grafts is a diagnostic method for the assessment, management and clinical monitoring of renal transplant, so it is necessary to know its complications. There are few data in the literature regarding complications from biopsies in renal grafts.

**Methods:** We performed a retrospective observational study, including data from 256 patients who underwent renal graft biopsies from January 2000 to September 2012. Major complications were defined as: anemia requiring blood transfusion, intraparenchymal arteriovenous fistula or arterial bleeding requiring embolization, nephrectomy, other surgery and exitus. And as minor complications: anemia without blood transfusion, intraparenchymal arteriovenous fistula without embolization, hematomas or perirenal collections, hematuria and fever. Kidney biopsies were performed with ultrasound guidance and automatic devices.

**Results:** We performed 390 kidney graft biopsies. Total complications in renal graft biopsies were 49, which corresponds to an overall rate of 12.6%, of which 22 were major complications (5.6%) and 27 minor complications (6.9%). Among the 22 biopsies with major complications, 12 presented anemia which required blood transfusions (3.1%), 6 embolizations, of which one was for arterial bleeding and 5 were for intrarenal fistulas (1.5%), 1 trasplantectomy secondary to important bleeding for laceration of the renal lower pole post-graft biopsy (0.3%), 1 required surgery for perirenal hematoma evacuation (0.3%) and two finally died (0.5 %). Among the other 27 biopsies with minor complications, 12 were perirenal collections (3.1%), 9 were arteriovenous fistula not requiring embolization (2.3%), 3 haematuria (0.8%), 2 presented anemia who did not require blood transfusion (0.5%) and 1 episode of fever (0.3%). The success rate of tissue obtained for histopathological study was 97.9%.

**Conclusions:** The rate of complications related to renal graft biopsy observed in our center are similar to those described for native kidneys, and therefore we believe it remains a safe and effective technique, and an important diagnostic tool in transplant patients.

**FR-PO1037**

**Banff Lesion Scores in Renal Allogarft Biopsies Correlate with Graft Function and Survival** Ramandeep S. Banga,[1] David J. Taber,[1] Sally Self,[1] Stuart M. Flechner,[2] Nicole A. Pilch,[1] Emilio D. Poggio,[2] Kenneth Chavin,[1] Prabhakar Baliga,[1] Titte Srinivas.[1] *[1]MUSC; [2]Cleveland Clinic.*

**Background:** The renal allograft biopsy is pivotal in establishing causes of graft dysfunction. Banff lesion scores (BLS) may allow the capture of the balance between acute and chronic pathologic lesions in the allograft and help refine both diganosis and prognosis. However, data on the correlation between individual Banff lesion scores with clinical outcomes are sparse.

**Methods:** We examined the relationship between BLS and graft outcomes in a retrospective review of 84 biopsies (all for cause, 1st biopsies) performed between 2008 & '11 The primary outcome measures were serum creatinine absolute values and changes from baseline, at biopsy and up to 2 years post-biopsy. Secondary outcomes were graft loss or death. We examined the association between BLS and outcomes in multivariate models.

**Results:** Of all the BLS, only "cg" and "t" scores were independently associated with creatinine trajectory, even after controlling for baseline clinical and demographic data in a multiple linear regression model (Table 1). The "ci" score was the only BLS independently

associate with graft loss in a multivariate logistic regression model (Table 2). Patients with moderate to severe t-scores had an improvement in renal function that was transient compared to those with mild t-scores (Figure 1).

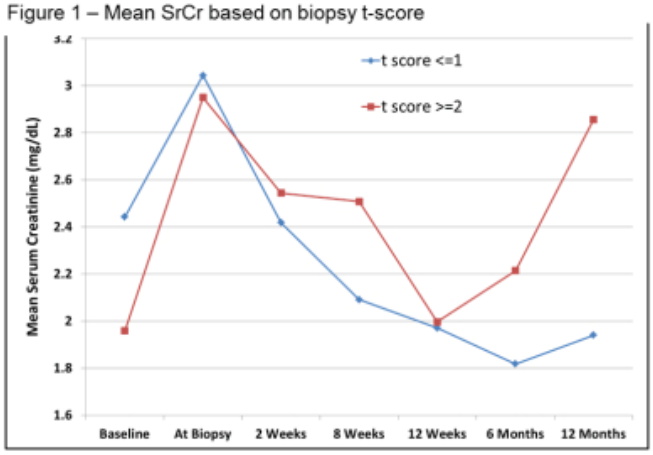
**Table 1 - Linear Regression Model for Proportion of Final SrCr to Baseline SrCr**

| Covariate | Reference Value | Beta Coefficient | p-Value |
|---|---|---|---|
| CG score | 0 | 0.231 | 0.033 |
| T score | 0 | 0.320 | 0.006 |
| Male Gender | Female | 0.248 | 0.021 |
| Age at Transplant | 15 | -0.212 | 0.053 |
| Pulse Dose Steroids for Biopsy Treatment | No Treatment | -0.187 | 0.102 |

**Table 2 - Binary Logistic Regression for Graft Loss**

| Covariate | Reference Value | Hazard-Ratio | p-Value |
|---|---|---|---|
| CI score | 0 | 2.65 | 0.020 |
| Male Gender | Female | 2.89 | 0.069 |
| African-American | Non-AA | 2.21 | 0.192 |
| Age at Transplant | 15 | 0.96 | 0.061 |
| Pulse Dose Steroids for Biopsy Treatment | No Treatment | 0.43 | 0.206 |
| SrCr at time of biopsy | 1.0 | 1.29 | 0.073 |

Figure 1 – Mean SrCr based on biopsy t-score




**Conclusions:** Biopsy derived indices provide valuable short and long-term prognostic information. The "t" score likely reflects reversible short term injury and the "cg" score likely informs short term creatinine trajectory. Post-biopsy creatinine improvement was not sustained with higher "t" scores. The "ci" score informs prognosis regarding long term graft survival. The balance between acute and chronic lesion scores determines the magnitude and sustainability of functional improvement after treatment of allograft rejection.

## FR-PO1038

**Revisiting the Natural History of IF/TA in Renal Transplantation Based on Protocol Graft Biopsies** Ken Sakai,[1] Tatsuru Matsukiyo,[1] Kiyoto Koibuchi,[1] Hiroki Hase,[3] Sonoo Mizuiri.[2] *[1]Nephrology, Toho Univ, Tokyo, Japan; [2]Nephrology, Harada Hospital, Hiroshima, Japan; [3]Nephrology, Toho Univ Ohashi Hospital, Tokyo, Japan.*

**Background:** The landmark study by Nankivell et al.in 2003, suggested two distinct phases of injury involved in IF/TA: an early tubulo-interstitial damage from allograft rejection and, beyond 1 year, an interstitial injury interpreted as secondary CsA toxicity. According to the improvement of current immunosuppression, we re-examined the natural history of IF/TA in this era.

**Methods:** 1hour, 1year, 3year and 5year protocol biopsy was performed after kidney transplant (KTx) for all recipients in this institution. From 2000 to 2003, 56 recipients underwent protocol biopsies up to 5 years after KTx. They took protocol biopsy completely and analyzed in this retrospective study. Anti CD25 antibody was used as an initial immunosuppressant for all recipients. Biopsy tissue was evaluated by Banff classification 1997. Newly appearance of Ah (arteriolar hyalinosis) score was defined as CNI toxicity.

**Results:** In this group (21% for ABO incompatible KTx, 7% for secondary KTx, 26 cases using ciclosporin and other for tacrolimus, mean age 32.8±18.4yrs, donor 52.1±12.6yrs), Cv score remained constant (0.25±0.59, 0.22±0.48, 0.09±0.39, 0.16±0.25 at 1hour, 1y, 3y, 5y respectively=NS) and Ah also remained constant (0, 0.08±0.28, 0.31±0.54, 0.17±0.31 at 1hour, 1y, 3y, 5y respectively, P=NS), while Ci and Ct (IF/TA) increased significantly (Ci:0.1±0.2 to 0.86±0.8, p=0.007, Ct:0.125±0.3 to 0.95±0.61, p=0.005) during 5 years. Inflammation score (i and t) also elevated followed by IF/TA (i: 0.35±0.73 to 0.83±0.68, p=0.005, t: 0.45±0.74 to 0.76±0.81, p=NS) during 5 years. Serum creatinine (mg/dl) remained stable during 5 years (1.04±0.33 to 1.18±0.52, P=NS).

**Conclusions:** Contemporary use of CNI with CD25 antibody significantly stabilized CNI toxicity defined as newly appearance of Ah. Interstitial inflammation and tubulitis resulted in increasing IF/TA not caused by CNI toxicity in this era. Such graft damage have an identifiable cause that is not idiopathic IF/TA or CNI toxicity and that alloimmunity remains the most common mechanism.

## FR-PO1039

**Molecular Acute Kidney Injury Score in Deceased Donor Kidney Predicts Post-Transplant Function of Both Kidneys** Konrad S. Famulski,[1] Chatchai Kreepala,[2] Declan De freitas,[3] Philip F. Halloran.[1] *[1]Univ of Alberta, Edmonton, Canada; [2]Srinakarinwirot Univ, Nakornayok, Thailand; [3]Beaumont Hospital, Dublin, Ireland.*

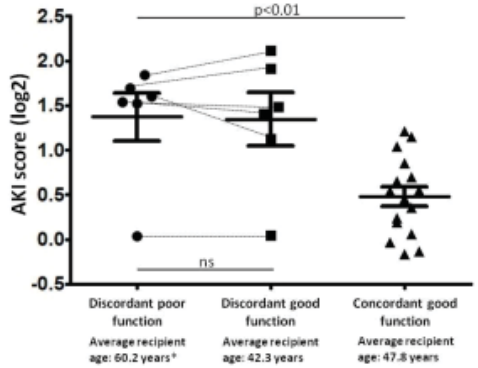
**Background:** Shortage of deceased donor (DD) kidneys for transplantation prompts clinicians to use more old and injured kidneys. Molecular acute kidney injury (AKI) score in DD kidneys can be measured by expression of injury-repair transcripts and at transplantation predicts impaired early function. We hypothesized that AKI score in each kidney predicts future function (Cr at day 7) of the mate kidney.

**Methods:** We analyzed microarray data, histology and clinical data of 15 kidney pairs from brain dead DD.

**Results:** The AKI score was highly similar within pairs r=0.95, while histology score (Remuzzi) was much more variable r=0.65. AKI score correlated with the future function of the opposite kidney,but histology did not.

| | Correlation with function | | | |
|---|---|---|---|---|
| Molecular assessment | same kidney | opposite kidney | Fold increase in DD vs LD | Correlation between pre and post-implant biopsies |
| CDH6 | 0.35 | 0.43 | 2.2 | 0.95 |
| OLFM4 | 0.40 | 0.44 | 3.4 | 0.75 |
| MEGF11 | 0.51 | 0.42 | 2.1 | 0.89 |
| OSMR | 0.42 | 0.37 | 2.7 | 0.79 |
| NNMT | 0.38 | 0.37 | 3.3 | 0.54 |
| AKI score | 0.51 | 0.47 | 2.7 | 0.92 |

AKI score reflected the effect of brain death, it was higher in DD compared to living donors (LD). In pairs with discordant outcomes - one recipient with poor, another with good function, AKI score was high in both kidneys.




Thus the discordant kidneys were more injured and actual function was influenced by other factors e.g. recipient age. AKI score was similar in pre- and 1h post implant biopsies, indicating that it is established before kidney removal.

**Conclusions:** Thus the molecular measurements in either kidney can be used in the future to predict function of both and may prove useful in organ utilization decisions.

*Funding:* Government Support - Non-U.S.

## FR-PO1040

**Glomerular Abnormalities in Cirrhotic Patients: Immune Mediated?** Anjali Gupta, Aws Aljanabi, James M. Pullman, Paul Joseph Gaglio, Enver Akalin, Graciela De boccardo. *Montefiore Medical Center.*

**Background:** We previously demonstrated universal glomerular abnormalities in kidney biopsies after orthotopic liver transplantation (OLT). We hypothesize that these changes exist prior to OLT and may play a role in the development of renal failure after OLT. We investigate the mechanism of kidney disease in patients listed for OLT by microarrays.

**Methods:** The clinical and pathological data of 25 cirrhotic patients listed for OLT who underwent kidney biopsy was analyzed. Gene expression profile of biopsies (n=8) was studied by Affymetrix HuGene 1.0 ST expression assay and compared with pre implantation living donor biopsies (n=9).

**Results:** Etiology of liver disease was hepatitis C (72%) and alcoholic hepatitis (24 %). Mean MELD, serum creatinine, GFR and proteinuria were 17+ 6, 1.8 ±0.7 mg/dl, 42±13 ml/min and 0.85±0.7 gm/day, respectively. Low complement was seen in 70%. Glomerular abnormality was seen in 92% of the biopsies, most commonly increase in mesangial matrix (88%) and focal interstitial inflammation (88%). 5 patients had nodular glomerulosclerosis (3 had DM), 2 FSGS and 1 MPGN. Immunofluorescence staining showed mesangial, capillary wall or tubular basement-membrane deposition of IgM, IgG and complement deposition in 83% and IgA in 78% of the biopsies. Electron microscope showed effacement of podocytes (93%) and duplication (35%) and widening of glomerular basement membranes (GBM) (45%). Gene expression profiles by Gene Ontology revealed significant up-regulation of

genes implicated in immune response, including T-cell, leucocyte and platelet activation and differentiation. Pathogenesis-based transcripts revealed significantly increased expression of cytotoxic T-cell, macrophage, B-cell, natural killer cell, and endothelial cell associated transcripts, indicating an ongoing inflammatory immune response.

**Conclusions:** Our study demonstrates universal presence of glomerular abnormalities in biopsies of cirrhotic patients. Majority had increase in mesangial matrix, podocyte effacement, and widening and duplication of GBM. Increased gene expression profiles related to immune activity indicates immune-mediated mechanisms in development of kidney disease.

## FR-PO1041

**Increased Rejection-Associated Gene Transcripts in Biopsies of DSA+ Patients without Histopathologic Findings of Rejection** Nicole A. Hayde, Yi Bao, James M. Pullman, Michelle L. Lubetzky, Graciela De boccardo, Enver Akalin. *Montefiore/Einstein Transplant Center, Bronx, NY.*

**Background:** The presence of donor specific antibodies (DSA) without allograft injury questions whether accommodation or other preventative mechanisms exist or if this is a state of pre-rejection. A set of genes was shown to be upregulated in the blood of tolerant patients by microarrays. We investigated the gene expression of kidney and whole blood samples of DSA+ patients with antibody-mediated rejection (AMR) and without rejection, including the tolerance genes.

**Methods:** DSA were measured by Luminex. The biopsy and whole blood samples of 28 DSA+/AMR+, 14 DSA+/AMR- and 20 DSA negative patients with normal histopathology (control) were analyzed by Affymetrix HuGene 1.0 ST expression arrays.

**Results:** Both DSA+/AMR+ and DSA+/AMR- biopsies showed increased cytotoxic T (CAT), constitutive macrophage (CMAT), natural killer cell (NKAT), and endothelial cell associated (ENDAT) pathogenesis based transcripts, and donor specific antibody selective transcripts seen in AMR (DSAST) compared to DSA- biopsies.

However, there were no significant differences in the whole blood gene expression profiles of DSA+/AMR- and DSA- patients. DSA+/AMR+ patients had increased CAT, CMAT and interferon-γ and rejection induced transcripts (GRIT) in their blood. There was also no difference in expression of tolerance-associated genes in whole blood and biopsy samples of DSA+/AMR- compared to DSA-.

**Table 1: Pathogenesis Based Transcript Comparisons**

| | BIOPSY | | BLOOD | |
|---|---|---|---|---|
| PBT | DSA+/AMR+ to DSA- | DSA+/AMR- to DSA- | DSA+/AMR+ to DSA- | DSA+/AMR- to DSA- |
| GRIT | **0.004** | 0.05 | 0.05 | 0.22 |
| CAT | **0.001** | **0.03** | **0.007** | 0.17 |
| BAT | 0.05 | **0.03** | 0.38 | 0.11 |
| CMAT | **0.02** | **0.03** | **0.03** | 0.17 |
| NKAT | **0.03** | **0.02** | 0.23 | 0.48 |
| ENDAT | **0.03** | **0.04** | 0.45 | 0.45 |
| DSAST | **0.003** | **0.02** | 0.52 | 0.91 |

GRIT: IFN-Gamma and rejection induced transcripts, CAT: Cytotoxic T cell associated transcripts, BAT: B cell associated transcripts, CMAT: Quantitative constitutive macrophage-associated transcripts NKAT: Natural killer cell-associated transcripts ENDAT: Endothelial cell associated transcripts, DSAST: Transcripts differentially expressed between rejection-classified biopsies from DSA+ patients compared to DSA negative patients

**Table 2: Tolerance Genes**

| | BIOPSY | | BLOOD | |
|---|---|---|---|---|
| | DSA+/AMR+ to DSA- | DSA+/AMR- to DSA- | DSA+/AMR+ to DSA- | DSA+/AMR- to DSA- |
| TNFAIP3 | ***0.94** | 0.43 | ***0.40** | 0.23 |
| BCL2 | ***0.15** | 0.09 | ***0.35** | 0.20 |
| BCL2L1 | 0.03 | 0.06 | -0.12 | 0.03 |
| CD79B | ***0.25** | 0.02 | -0.06 | 0.18 |
| FCRL1 | ***0.29** | 0.12 | 0.03 | 0.48 |
| HMOX1 | -0.12 | 0.05 | 0.10 | 0.25 |
| HS3ST1 | 0.12 | -0.08 | -0.08 | 0.03 |
| MS4A1 | ***1.23** | 0.65 | -0.18 | 0.05 |
| PNOC | 0.13 | 0.02 | 0.00 | 0.02 |
| SH2D1B | ***1.35** | 0.29 | 0.52 | 0.17 |
| SLC8A1 | 0.16 | 0.25 | 0.15 | 0.04 |
| STAP1 | 0.29 | 0.58 | -0.13 | 0.27 |
| TCL1A | 0.16 | 0.02 | -0.18 | 0.52 |
| TLR5 | 0.16 | 0.12 | 0.18 | 0.17 |

Values showed are log2 fold change. * denotes adjusted p-value <0.05.
**TNFAIP3:** Tumor necrosis factor, alpha-induced protein 3, **BCL2:** B-cell CLL/lymphoma 2, nuclear gene encoding mitochondrial protein, transcript variant alpha, **BCL2L1:** BCL2-like 1, nuclear gene encoding mitochondrial protein, transcript variant 1, **CD79b:** Molecule, immunoglobulin-associated beta, transcript variant 1, **FCRL1:** Fc receptor-like 1, transcript variant 1, **HMOX1:** Heme oxygenase (decycling) 1, **HS3ST1:** Heparan sulfate (glucosamine) 3-O-sulfotransferase 1, **MS4A1:** Membrane-spanning 4-domains, subfamily A, member 1, transcript variant 1, **PNOC:** Prepronociceptin, **SH2D1BL:** SH2 domain containing 1B, **SLC8A1:** Solute carrier family 8 (sodium/calcium exchanger), member 1, transcript variant A, **STAP1:** Signal transducing adaptor family member 1, **TCL1A:** T-cell leukemia/lymphoma 1A, transcript variant 1, **TLR5:** Toll-like receptor 5

In contrast, TNFAIP3, BCL2 were upregulated in biopsy and blood samples of DSA+/AMR+ patients. CD79B, FCRL1, MS4A1 and SH2D1B were upregulated in DSA+/AMR+ biopsies.

**Conclusions:** DSA+ patients without rejection showed increased immune activity in their allografts but not in their blood indicating an ongoing local alloimmune response but not accommodation/ignorance.

## FR-PO1042

**Uncontrolled Complement after Renal Transplantation and C5b9 Deposits on Graft Predict Graft Outcome in Adult Renal Transplant with C3 Glomerulopathy** Moglie Le Quintrec,[1] Marion Rabant,[2] Maria Chiara Marinozzi,[3] Frank Bridoux,[4] Michel Delahousse,[1] Christophe M. Legendre,[5] Veronique Fremeaux-bacchi.[6] *[1]Nephrology and Renal Transplantation, Hopital Foch, Suresnes, France; [2]Anatomopathologie, Hopital Necker, Paris, France; [3]Inserm UMR 872, Cordeliers Research Center, Paris, France; [4]Nephrology and Renal Transplantation, CHU de Poitiers, Poitiers, France; [5]Nephrology and Renal Transplantation, Necker Hospital, Paris, France; [6]Laboratoire d'Immunologie, Hôpital Européen Georges Pompidou, Paris, France.*

**Background:** C3 glomerulopathy (C3G) is a severe disease strongly associated with abnormal control of complement alternative pathway activation. Few data are available on recurrence risk and graft outcome.

**Methods:** We studied 47 patients with DDD (n=15), GNC3 (17) and MPGN type I (n=15) who received renal transplantation. Plasma C3 and sMAC were performed by Elisa, C3 and C5b9 staining by immunochemistry.

**Results:** Graft survival was 98% and 77% at one and five years respectively. 48% of patient (23/47) had a clinical graft recurrence biopsy proven; 60% (14/23) of them occurred in the first year after transplantation. The recurrence occurred in 33% (5/15), 47% (8/17) and 60% (9/15) of patients with DDD, GNC3 and MPGN type I respectively. At five years, 54% of graft lost was due to recurrence (n=6/11). Before transplantation, a low C3, positive C3 Nef were documented in 63 % of patients. After renal transplantation, a low C3, positive C3 Nef and high sMAC were present in 20% (7/34), 33%(11/33) and 37%(6/14) of patient respectively. Only 34%(7/17) of patients with low C3 before transplantation had a recurrence. At time of recurrence, 60%(11/18) of patients had a low C3 or/and high sMAC and 8% (2/24) if no reccurence occured . All patients who lost graft before 5 years due to recurrence had C3 low and/or sMAC high. All patients with recurrence had positive C3 staining, C5b9 staining (6/34) was positive only in lost graft due to early recurrence from patients who.

**Conclusions:** Uncontrolled pathways activation and C5b9 deposits were associated with severe recurrence and poor graft outcome.

## FR-PO1043

**Clinicopathological Correlation of Transplant-Associated Thrombotic Microangiopathy** Miriam Berry,[1] Victoria Bardsley,[2] Meryl Helen Griffiths,[2] Nicholas Torpey,[1] Verena Broecker.[2] *[1]Transplant Unit, Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Dept of Histopathology, Addenbrooke's Hospital, Cambridge, United Kingdom.*

**Background:** Transplant associated thrombotic microangiopathy (TA-TMA) is found in 1% of renal transplant biopsies. Clinicopathological correlation is required to identify the cause. However there are often discrepancies between the histological, clinical and serological results at the time of diagnosis. This uncertainty has significant therapeutic implications.

**Methods:** We retrospectively re-evaluated 69 renal transplant biopsies from 16 patients with TA-TMA over 5 years, identified by a database search. Biopsies were graded according to Banff criteria. Clinical parameters were collected and correlated with histology.

**Results:** TA-TMA was diagnosed at median 0.6 months (range 1 week to 3 years) post transplant. Donor specific antibodies (DSA) were present in 50%.

Histological diagnoses were: rejection with a humoral component (microcirculation injury) (n=9); consistent with CNI toxicity (n=6): pure cellular rejection (Banff II) (n=1). Overall the prevalence of endothelialitis (v-lesion) was high (n=9). 2/6 patients with probable CNI toxicity had DSA at the time of TMA. In 6/9 cases showing rejection with a humoral component, TMA preceded the presence of microcirculation injury. This prodrome of microthrombi with subsequent development of rejection could not be attributed to reduced immunosuppression.

4 patients lost their graft and 1 died with a functioning graft. There was no significant reduction in eGFR from baseline (45 vs 47 ml/min) in the remaining grafts.

**Conclusions:** We identified a complex overlap between microcirculation injury, DSA and endothelialitis. The absence of microcirculation injury at the time of biopsy did not exclude a humoral process as the potential cause of TMA. The high prevalence of endothelialitis suggests an endothelial insult which may be triggered by DSA in some cases, however TMA may arise in the absence of humoral pathology. The outcome was bimodal, ie graft loss or recovery to baseline renal function. TA-TMA is a histological finding rather than a diagnosis; it may be an early sign of humoral pathology and should prompt serological investigations.

## FR-PO1044

**Web-Based Virtual Microscopy to Validate Histologic Classification Schemes of Renal Diseases: Are We There Yet?** A. Gasim,[1] Harsharan Kaur Singh,[1] Surya V. Seshan,[2] Jennifer Melton,[1] Volker Nickeleit.[1] *[1]Div of Nephropathology, Univ of North Carolina, Chapel Hill, NC; [2]Pathology, Weill Cornell Medical Center, New York, NY.*

**Background:** Attempts are made to stage polyomavirus nephropathy (PVN) (A: early disease; B: florid; C: sclerosed). During the validation phase of the proposed PVN classification scheme, web-based recruitment of pathologists world-wide and virtual slide microscopy was used for interobserver reproducibility studies. How good are the results?

**Methods:** A) Web-based study on 19 PVN biopsies. 110 pathologists from 6 continents participated and used virtual microscopy to stage PVN. Four web-based mandatory study modules were designed: 1) introduction to PVN, 2) demographic data collection, 3) training module on PVN staging, 4) test set with 19 PVN cases. B) Conventional approach with exchange of glass slides on 190 cases: PVN disease staging on conventional glass slides from 9 centers was conducted. Outside results were compared to central review UNC data that served as "gold standard diagnoses" in both study groups.

**Results:** Evaluation of conventional glass slides versus web-based virtual microscopy generated similar scoring results. Only 2 significant differences were noted estimating mild degrees of intra renal polyomavirus load levels and mild degrees (ci-1) of Banff fibrosis scores. These differences do not appear to play a major role in a validation study. In the web-based study group (A) results did not significantly differ among demographic groups or according to the level of expertise in pathology.

| Diagnosis | Web-Based Study with Virtual Slides (Study Group A) Agreement Rate: Outside Pathologist versus UNC Central Review | Conventional Study with Glass Slides (Study Group B) Agreement Rate: Outside Pathologist versus UNC Central Review | p-value |
|---|---|---|---|
| PVN Stage A | 84% | 94% | ns |
| PVN Stage B | 63% | 63% | ns |
| PVN Stage C | 80% | 71% | ns |
| **PV Intrarenal Load Level** | | | |
| 1 | 79% | 84% | ns |
| 2 | 59% | 73% | **p=0.01** |
| 3 | 70% | 66% | ns |
| **BANFF Fibrosis ci-score** | | | |
| 0 | 55% | 48% | ns |
| 1 | 43% | 58% | **p=0.04** |
| 2 | 53% | 62% | ns |
| 3 | 89% | 45% | na |

**Conclusions:** Web-based virtual microscopy is well suited for validation studies of histologic classification systems of renal diseases. Yes, we are there, and things will likely improve further!

## FR-PO1045

### miRNA and mRNA Regulation in Donor and Follow-Up Kidney Biopsies Diagnosed with Acute Rejection and Acute Renal Failure

Julia Wilflingseder,[1,2] Alexander Kainz,[1,2] Judith Sunzenauer,[1,2] Eva Toronyi,[3] Robert M. Langer,[3] Rainer Oberbauer.[1,2] *[1]Nephrology, Medical Univ of Vienna, Austria; [2]Nephrology, KH der Elisabethinen, Linz, Austria; [3]Transplantation and Surgery, Semmelweis Univ Budapest, Hungary.*

**Background:** We previously showed that miRNAs are involved in the regulation of post-transplant events such as biopsy confirmed acute rejection (BCAR) and acute renal transplant failure (ARF). However, only few studies examined the concomitant regulation of miRNAs and mRNAs in the same biopsy samples.

**Methods:** We prospectively collected zero-hour and follow-up kidney biopsies of renal allograft recipients. Of these 242 donor kidney biopsies, we identified ten biopsy sample pairs of each clinical condition (1) BCAR (2) ARF, and (3) management biopsies without pathology within ten days after TX and subjected to miRNA and mRNA genome wide analyses. ARF was defined as acute tubular necrosis. An integrative bioinformatics approach was chosen to combine differentially regulated miRNA and miRNA targets before and after transplantation. miRNA target prediction was verified by correlation analysis and experimental data. Target genes were further evaluated based on their functional annotation.

**Results:** Preliminary results show that allografts with acute renal failure exhibit a distinct molecular pattern compare to acute rejection and management biopsies on the mRNA and miRNA levels. T-cell and B-cell activation (p=0.002 and p=0.03, respectively) in the zero-hour biopsies, Integrin signalling pathway (p=0.008) in the follow-up biopsies and p53 pathway (p=0.02) and Ras pathway (p=0.04) in the development are significantly enriched pathways associated with ARF. A detailed analysis of the miRNAs and target genes will be presented at the meeting.

**Conclusions:** Based on these data we identified miRNAs as major molecular regulators of post-transplant events. miRNA-182 is mainly involved in the development of ARF and miRNA-155 was identified as a classifier of BCAR. These two miRNAs will be further investigated as potential therapeutic and/or prophylactic targets towards the prevention of ARF and BCAR.

*Funding:* Government Support - Non-U.S.

## FR-PO1046

### Spleen Tyrosine Kinase (SYK) Expression in Renal Allograft Rejection

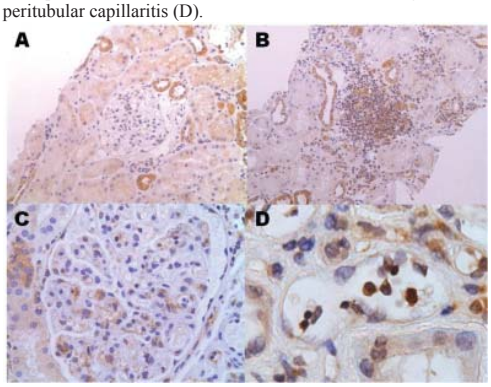
Stephen Paul McAdoo, Gurjeet Bhangal, Jennifer Smith, David Taube, Charles D. Pusey, Frederick W.K. Tam, H. Terence Cook. *Imperial College London.*

**Background:** Rejection is a leading cause of renal allograft failure. Spleen tyrosine kinase (SYK) has in important role in BCR and FcR immunoreceptor signalling, and may therefore represent a treatment target in rejection. In work previously reported by our group, SYK inhibition has shown efficacy in a rat model of renal allograft rejection. SYK expression, however, in experimental and clinical renal tissue has not been characterised.

**Methods:** We conducted immunohistochemical analysis for total and phosphorylated SYK in experimental renal allograft rejection (Brown Norway to Lewis rats) and in clinical renal allograft biopsies: normal surveillance biopsies, n=3; acute cellular rejection (ACR), 4; antibody-mediated rejection (AMR), 12.

**Results: Experimental Samples**: Normal rat tissue was positive for total SYK in distal tubules only. Rejecting kidney tissue showed positive staining for both total SYK and phophorylated SYK, localised to cellular infiltrates in the interstitium.

**Clinical Samples:** Staining for total SYK in normal surveillance biopsies (Fig A) was positive in distal tubules only, similar to that seen in normal rat tissue. Tubular infiltrates in ACR were positive for T-SYK in 4/4 patients (B). In AMR (total n=12), total SYK positive cells were identified in 5/10 cases with glomerulitis (C) and 7/10 cases of peritubular capillaritis (D).




**Conclusions:** We believe this is the first report that SYK is expressed in various types of renal allograft rejection in patients, and localises to pathological lesions (tubulitis, glomerulitis, peritubular capillaritis). SYK is also expressed and activated (i.e. phosphorylated) in a rat model of renal allograft rejection that responds to SYK inhibitor treatment. We therefore believe that clinical studies targeting SYK in allograft rejection are warranted.

## FR-PO1047

### Decreased Expression of Mitochondrial Energy Generation and Antioxidant Response Genes in One Year Protocol Biopsies Is Associated with Poor Allograft Outcome

Diana Zepeda-Orozco,[1] Richard H. Scheuermann.[2] *[1]Pediatrics, Univ of Iowa Carver College of Medicine, Iowa City, IA; [2]Informatics, J. Craig Venter Institude, San Diego, CA.*

**Background:** Progression of chronic histological damage associated with subclinical inflammation are factors associated with poor allograft survival. The role of non-immunological pathways in the activation of this chronic injury-repair response have not been assessed.

**Methods:** To identify non-immunological genes and pathways that might influence long-term allograft outcome, we analyzed a public microarray dataset that included low risk kidney transplant recipients without rejection episodes who underwent a 1-year protocol biopsy. Three patient/sample groups were defined based on their histological findings: normal histology (n=25), interstitial fibrosis alone (IF; n=24), and interstitial fibrosis with inflammation (IF+I; n=16). IF+I was associated with lower death-censored graft survival and lower renal function compared to the IF and normal histology groups. We performed data normalization, data filtering, and statistical testing with ANOVA on the gene expression microarray data to discover differentially-expressed genes across the sample groups.

**Results:** Clustering and gene ontology (GO) enrichment analysis identified significant co-clustering of genes with similar functional properties. The analysis showed downregulation of *mitochondrion* (p=6.52E-17) and mitochondrial part (p=1.75E-11) genes in the IF+I group compared to the other two groups, including genes involved in *generation of precursor metabolites and energy*, and *response to oxidative stress*, as the most significant biological processes.

**Conclusions:** Allografts with histological evidence of inflammation and interstitial fibrosis are associated with lower graft survival and poor renal function. Through gene expression analysis, we have found evidence that inadequate mitochondria function, and an inappropriate antioxidant response may be important non-immune factors involved in chronic allograft injury that could serve as novel targets for therapeutic intervention.

## FR-PO1048

### IgA Nephropathy Recurrence after Transplantation: Altered IgA Glycosylation and Its CD89 Receptor as Biomarkers of the Disease and Effectiveness of Steroid Pulse Therapy for Recurrence

Laureline Berthelot,[1] Thomas Robert,[2] Thierry Tabary,[2] Vincent Vuiblet,[2] Moustapha Dramé,[2] Philippe Rieu,[2] Renato C. Monteiro,[1] Fatouma Toure,[2] Olivier Toupance.[2] *[1]INSERM U699, INSERM, Paris, France; [2]CHU Reims, CHU, Reims, France.*

**Background:** IgA nephropathy (IgAN) is a leading cause of end-stage renal disease and kidney transplantation. However, recurrence of the disease is frequent after transplantation. We investigated the efficacy of steroid treatment and predictive value of three markers for IgAN recurrence: galactose deficient (Gd) IgA1, IgG anti-IgA autoantibodies and IgA-soluble (s) CD89 complexes.

**Methods:** Kidney transplanted recipients treated with IV pulse steroids therapy for IgAN recurrence (R group, n=11) were compared to those without recurrence (NR group, n=13). Gd-IgA1 and IgA complexes containing IgG and sCD89 levels were determined in serum collected before and after transplantation.

**Results:** Proteinuria was markedly reduced by steroids treatment in R group ($p< 0.001$). Before transplantation, serum levels of Gd-IgA1 were significantly higher in R group compared to NR group or healthy controls ($p< 0.01$). Serum IgA-IgG complexes were also elevated in R group ($p< 0.001$). Both groups had enhanced values of IgA-sCD89 complexes compared to healthy controls, but values were lower in R group compared to NR ($p<0.01$). The receiver-operating-characteristic (ROC) curve showed predictive power of each biomarker for IgAN recurrence ($p=0.003$; $p=0.0002$; $p=0.001$). In the R group, diseaserecurrence was associated with decreased serum Gd-IgA1 and IgA-IgG complexes while IgA-sCD89 complexes were significantly increased. Treatment with steroids restored levels of all 3 markers back to pre-transplantation level.

**Conclusions:** Pulse IV steroid therapy was an efficient and safe option for treatment of IgAN recurrence. Gd-IgA1 and IgA complexes containing IgG or sCD89 were found as biomarkers of the disease recurrence.

*Funding:* Government Support - Non-U.S.

## FR-PO1049

**Prognosis of Transplant Glomerulopathy with Complete versus Incomplete Criteria for Chronic Active Antibody-Mediated Rejection** Julie Lesage, Real Noel, Jean-guy Lachance, Isabelle Lapointe, Eric Wagner, Olivier Desy, Isabelle Houde, Sacha A. De Serres. *Renal Div, CHUQ L'Hotel-Dieu de Quebec, Univ Laval, Quebec, Canada.*

**Background:** Transplant glomerulopathy (TG) is currently included in the Banff classification as a criterion in the diagnosis of chronic active antibody-mediated rejection (CAABMR), with C4d, antidonor antibodies (DSA) and other lesions of chronic tissue injury. However, TG often present without complete criteria for CAABMR, making the pathological results difficult to translate clinically. Here we studied the prognosis of a large cohort of TG patients in relation with C4d and DSA.

**Methods:** 106 consecutive recipients with TG diagnosed between 1998 and 2012 were compared to controls matched 1:1 on age, donor type and year of transplant from a single-center cohort. We studied the association between a composite endpoint of death-censored graft failure or doubling of serum creatinine (Scr) and the presence of TG with incomplete or complete criteria for CAABMR. We used Cox models adjusted for SCr, recipient gender and donor characteristics.

**Results:** Median time post transplant was 108mo. Compared to controls, patients with TG had a higher Scr ($1.3\pm0.1$ vs. $2.1\pm0.1$) and a higher HLA-DR mismatch ($0.5\pm0.1$ vs. $0.7\pm0.1$; both $p<0.01$). 61 patients with TG (58%) vs. 6 controls (6%) reached the endpoint ($p<0.001$). The proportion of patients with isolated TG (DSA-C4d-), TG but incomplete CAABMR (DSA- and/or C4d-), and CAABMR (DSA+C4d+) were 59, 33 and 8% respectively, whereas their adjusted HR for the endpoint were 7.0, 9.7, and 18.5 (all $p<0.01$). TG score (cg) was also associated with the endpoint (HR 1.8, 95% CI 1.4-3.4, $p=0.01$; adjusted for the above and C4d, DSA, i and t scores). A higher proportion of patients with TG had their maintenance immunosuppression reduced or withdrawn (13 vs. 30%, $p<0.01$), mostly CNIs (8 vs. 20%, $p=0.01$).

**Conclusions:** The prognosis of TG with incomplete criteria for CAABMR, which represents a majority of cases, is dismal, however it is not considered in the current Banff nomenclature. These results are particularly important, because they provide a rationale to include these patients in coming trials studying ABMR.

## FR-PO1050

**Clinical Phenotypes of Chronic Antibody-Mediated Rejection after Kidney Transplantation** Sunhwa Lee, Ran-hui Cha, Jung Pyo Lee, Dong Ki Kim, Yon Su Kim, Hajeong Lee. *Dept of Internal Medicine, Seoul National Univ Hospital.*

**Background:** Chronic antibody-mediated rejection (CAMR) is a significant challenge for long-term graft survival over the last decade. The prognosis of CAMR is poor, but the clinical and immunological phenotype of CAMR remains unclear.

**Methods:** From 2005 to 2012, 915 kidney transplantations were performed at Seoul National University Hospital. Among them, twenty-six (2.8%) patients were diagnosed as CAMR according to Banff '05 criteria. We reviewed the clinical and immunological findings at the time of transplantation and diagnosis of CAMR. In addition, the responses to the anti-rejection therapy were classified according to the change of serum creatinine level, donor specific antibody (DSA) titer, and proteinuria amount.

**Results:** The increases of serum creatinine, DSA titer, and proteinuria were the main indications for the allograft biopsy. The median time to the development of CAMR after kidney transplantation was $8.8 \pm 5.8$ years. The mean number of human leukocyte antigen (HLA) mismatch was $2.5 \pm 1.5$. Twenty-six patients showed a progressive decline of estimated GFR of 1.6 ml/min/1.73m$^2$ over 6 months using simple regression analysis. Nine patients were non-responders, 6 patients were stable disease, and 8 patients were responders, respectively. Seven patients had reached to end stage renal disease in 24 months after biopsy. The time interval from transplantation to CAMR diagnosis was longer in patients who showed poor response. In addition, histological analysis revealed that the grade of interstitial fibrosis and tubular atrophy (IFTA) ($P = 0.04$) and vascular fibrointimal thickening score ($P = 0.03$) were lower in recipients who showed better response to anti-rejection therapy. The MFI of DSA did not predict the effect of anti-rejection therapy.

**Conclusions:** This retrospective observation revealed the basic characteristics of CAMR after kidney transplantation although it was a single center experience. For the better long-term graft survival, prognostic indicators for the specific treatment and surrogate markers for disease progression should be developed in near future utilizing multi-center registration.

## FR-PO1051

**Incidence and Clinical Course of Antibody-Mediated Rejection in Kidney Allografts Associated with Non-HLA-Antibodies Targeting Angiotensin Type 1 Receptor** Gaurav Gupta,[1] Julie Ann T. Linatoc,[1] David Donghyung Lee,[2] Marc P. Posner,[2] Qing Ren,[1] Anne L. King.[1] *[1]Nephrology, Virginia Commonwealth Univ, Richmond, VA; [2]Transplant Surgery, Virginia Commonwealth Univ, Richmond, VA.*

**Background:** Non-human leukocyte antigen (HLA) non-complement-fixing autoantibodies reacting to endothelial artery-specific antigens like the angiotensin II type 1 receptor (AT1R-AA)have been linked to antibody-mediated rejection (AbMR) and malignant hypertension in kidney allograft recipients. There is very little data about the incidence, clinical course or therapy of rejection associated with the AT1R-AA.

**Methods:** AbMR was diagnosed based upon the Banff criteria including peritubular capillaritis, glomerulitis, arteritis, and/or positive c4d staining. Eight consecutive KTx patients with 9 episodes of AbMR and no detectible anti-HLA alloantibodies at the time of rejection were retrospectively screened for the presence of AT1R-AA. A commercial ELISA assay was used for detection of antibodies. A result of >17U/ml was considered positive based upon prior published data.

**Results:** Four of nine (44%) episodes of AbMR were associated with a positive AT1R-AA. None of the patients had malignant HTN. All four episodes were treated with pulse steroids and six sessions of plasmapheresis and low-dose CMVIg (150mg/kg/session). All patients had a response to therapy with a decline in mean serum creatinine from 3.6mg/dL to 1.6mg/dL at a median follow-up of 3 months. Table 1 shows the trend in AT1R-AA titer.

| Patient Number | AT1R-AA titer at diagnosis (U/ml) | AT1R-AA titer at end of therapy (U/ml) |
|---|---|---|
| 1 | 30 | 10 |
| 2 | 75 | 14 |
| 3 (1st episode) | 28 | 18 |
| 3 (2nd episode) | 24 | 9 |

Patient 3 who had recurrent AbMR had a positive AT1-R-AA titer at the end of therapy for his first episode. He was started on losartan after his second episode and has now remained rejection free.

**Conclusions:** Kidney transplant patients with biopsy findings concerning for AbMR and undetectible HLA alloantibodies should be screened for anti-AT1R-AA. An excellent response with plasmapheresis was noted though AbMR recurred in one patient. The addition of angiotensin receptor blocker therapy might be helpful to prevent further episodes.

## FR-PO1052

**Peripheral Blood Mononuclear Cells (PBMCs) Phosphoproteome Analysis to Investigate Antibody-Mediated Chronic Rejection (AMCR)** Maria Teresa Rocchetti,[2] G. Stallone,[2] Paola Pontrelli,[1] F. Rascio,[1] Marco Fiorentino,[1] Anna Zito,[1] Giuseppe Castellano,[2] Loreto Gesualdo,[1] G. Grandaliano.[2] *[1]Dept Emergency and Organ Transplantation, Univ of Bari, Bari, Italy; [2]Dept of Medical and Surgical Sciences, Univ of Foggia, Foggia, Italy.*

**Background:** AMCR represents one of the main causes of kidney transplant failure. Specific markers for an early diagnosis of AMCR are currently missing. In the attempt to identify potential diagnostic markers and to elucidate the signaling pathways involved in AMCR pathogenesis, we analyzed the PBMCs phosphoproteome profile to identify cellular signaling networks differentially activated in AMCR patients.

**Methods:** PBMCs were harvested from 6 biopsy-proven AMCR according to Banff 2009 consensus, 7 renal transplant recipients with normal graft function and histology (tx-CTRL), and 6 healthy subjects (CTRL). Phosphoproteins were isolated by precipitation with lanthanum ions, separated by 2-D gel electrophoresis and stained by Sypro Red. Image Master Software was used to standardize the PBMCs phosphoproteome map and to list the differentially expressed protein spots among the 3 groups. MALDI-TOF-MS/MS analysis was used to identify the phosphoproteins.

**Results:** 2-D gel electrophoresis of PBMC detected $554\pm68$ (mean±SD) protein spots (CV=26%) differentially expressed in CTRL, $418\pm119$ protein spots (CV=35%) in AMCR and $475\pm75$ protein spots (CV=20%) in tx-CTRL. We recognized, by densitometric analysis, a protein signature of 10 protein spots, corresponding to 4 proteins, which discriminated AMCR patients from CTRL and tx-CTRL and 4 protein spots whose density was increased in all transplant recipients compared to CTRL. Finally, 5 protein spots, corresponding to 2 proteins, distinguished tx-CTRL from AMCR and CTRL.

**Conclusions:** Our preliminary results suggest that PBMC phosphoproteome might help to distinguish biopsy-proven AMCR patients from healthy subjects and stable renal transplant recipients. In addition, the differentially phosphorylated protein spots may indicate potential therapeutic targets for AMCR.

*Funding:* Government Support - Non-U.S.

## FR-PO1053

**Medical Complication after Acute Humoral Rejection Treatment** Araminta Guichard, Lluvia A. Marino-vazquez, Cristhian R. Arias-delgadillo, Norma O. Uribe-uribe, Josefina Alberú, Luis E. Morales-Buenrostro. *Nephrology, Pathology, and Transplantation, National Institute of Medical Sciences and Nutrition SZ, Mexico City, DF, Mexico.*

**Background:** Treatment for acute humoral rejection (AHR) includes removal of circulating HLA-antibodies (HLAabs) by plasmapheresis (PP), neutralization of HLAabs with IVIG, rituximab (RTX), and Bortezomib (Borte) intended to decrease HLAabs-producing plasma cells derived from B-cells, as well as use of rATG looking to control the

cellular component (or interaction between T- and B-cells). Some of them potentially could increased the complication rates. This study compare the rate of complications between different treatment protocols.

**Methods:** Between May/08 and Apr/13, we prospectively recruited all 60 patients who had AHR. Each event was handled as a separate treatment with a total of 97 treatments cycles in 60 patients. The histological description was made according to modified Banff 97 classification. DS-HLAabs were determined by Luminex single antigen bead assay (One Lambda).

**Results:** We included 60 KTR. Mean age 33.2±11.9; male gender 70%; first transplant 85%; living donor 76.7%; induction therapy 56.7%; maintenance triple therapy with CI 89.9%. The most used treatment schemes were PP+IVIG+RTX(36.7%), PP+IVIG+RTX+BORTE(35%), PF+IVIG+BORTE(6.7%), others (21.6%). A 41.7% received more than one treatment (2-7). A 95% of the patients used PP, and 55% received Borte. There were not differences in graft survival when Borte, RTX, or IVIG were added to PP. Also, receive more than one treatment cycle, or the time after KT did not showed difference. There was 30 complication in 26 treatments. The most frequent complication were infections (11 events), hematologyc (10 events), neuropathy (4 events), metabolic (2 events), and other (3 events). There was no relationship between type of treatment and kind of complications.

**Conclusions:** Most of the complications were like we expected. Only few of them were severe. Intensification of treatment after AHR is safe.

*Funding:* Government Support - Non-U.S.

## FR-PO1054

**ABO Antibody Removal of Plasmapheresis (PP) with Intravenous Immunoglobulin (IVIG) before ABO-Incompatible (ABOI) Kidney Transplantation: Single Center Experience**

Jeongmyung Ahn,[1] Dong Ryeol Lee,[1] Byung Chang Kim.[2] *[1]Medicine, Maryknoll General Hospital, Busan Catholic Univ, Busan, Republic of Korea; [2]Laboratory, Medicine, Maryknoll General Hospital, Busan Catholic Univ, Busan, Republic of Korea.*

**Background:** ABOI kidney transplantation is an inevitable option to overcome serious organ shortage. There are few data on success rates of ABO antibody removal or relating to patients in who antibody removal fails. The purpose of this study was to evaluate the likelihood of achieving transplantation depending on ABO antibody titers.

**Methods:** 55 patients were enrolled between 2007-2012. We perform ABOI kidney transplantation using anti-CD20 antibody, tacrolimus and PP with IVIG. The median antibody titer was 1:64 (Range 8-4096). Transplantation was preceded when the ABO titer reached ≤ 1:8. To determine the likelihood of achieving transplantation, the number of PP required to proceed transplantation and baseline ABO titer were analyzed.

**Results:** All 55 patients(100%) successfully completed transplantation after 5.75±4.3 PP with IVIG. Three patients did not reach target ABO titer and their achieved ABO titers at the time of transplantation were 1:16. (Initial ABO titers were 1:256 in one patient, 1:2048 in one patient, and 1:1024 in one patient). The median follow-up duration was 18.1 month (Range 0.9-71.8). The mean age was 45.8±9.9 year and 65.6% were female. The median ABO titer was 2 (Range 1-16) at the time of transplantation, 4(Range 1-64) at 1month posttransplantation and 4 (Range 4-128) at the last follow-up, respectively. The number of PP to reach an ABO titer of ≤ 1:8 was significantly correlated with baseline ABO IgG titers ($r^2$=0.829, P < 0.001).

**Conclusions:** All 55 patients successfully preformed ABOI kidney transplantation without failure to achieve transplantation. Three patients even failed to reach target titer at the time of transplantation, however, all of them were successfully transplanted. Though optimal ABO titer at the time of transplantation remains debatable, we carefully need to tailor our protocol with target ABO titer of greater than or equal to1:16 at the time of transplantation to expand kidney donor pool.

## FR-PO1055

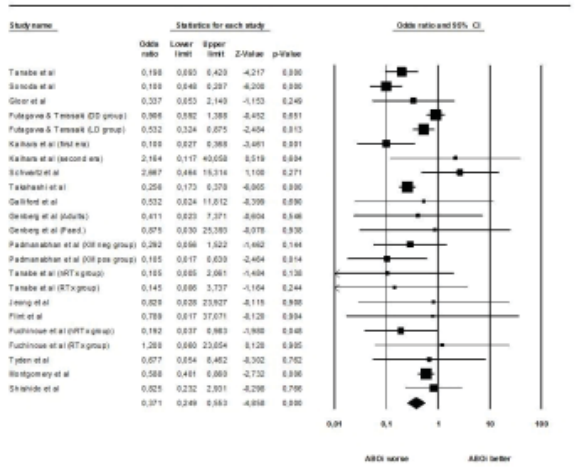
**Graft Survival after ABO Incompatible Renal Transplantation–A Metaanalysis of Available Evidence**

Bernhard M.W. Schmidt,[1] Anette Melk,[2] Daniel Kayser.[1] *[1]Dept of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Dept of Pediatric Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** ABO incompatible (ABOi) renal transplantation (rTx) is increasingly used to overcome the organ shortage. Smaller studies evaluating the outcome of ABOi rTx showed no difference in outcome compared to controls, but may have been underpowered. Our aim was to provide a broader data pool by meta-analyzing the available studies.

**Methods:** We identified studies by searching Medline and the Cochrane library. Additional studies were identified by review of cited literature. Primary efficacy endpoint was probability of 1-year-graft survival. Random effects model was used. Subgroup analysis was performed for studies using rituximab or not, meta-regression analysis was used to evaluate the impact of continuous variables.

**Results:**




From 536 articles 17 reports including 23 patient groups with ABOi rTx could be used for data retrieval. Analysis of all 23 patient groups revealed significantly worse 1-year-graft survival in recipients of an ABOi graft (OR 0.37, 95% CI 0.25-0.56, p<0.001) than in controls. The studies showed relevant heterogeneity ($I^2$=62.3, Q test p<0.001). In studies using rituximab 1-year-graft survival was also worse in ABOi rTx (OR 0.34, 95% CI 0.16-0.77, p=0.01). Within these studies we detected no heterogeneity ($I^2$=0, Q test p=.89). However, we detected signs of publication bias suggesting that studies showing worse outcome of ABOi rTx have not been published.

**Conclusions:** Metaanalysis of the available evidence showed worse 1-year-graft survival for recipients of an ABOi graft. This information should be acknowledged when informing patients about an ABOi rTx. However, we should also acknowledge that in real life the right comparator is not ABO compatible living related donorship (as used in most studies) but waiting for a deceased donation for several years.

## FR-PO1056

**Immunochemical Characterizaion of Light Chains in Posttransplant IgA Nephropathy**

Yasuhiro Otsuka, Asami Takeda, Shinichi Sueta, Keiji Horike, Daijo Inaguma, Yoshihiko Watarai, Kunio Morozumi. *Kidney Center, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan.*

**Background:** IgA nephropathy (IgAN) is a clinically and histologically diverse glomerular disease characterized by mesangial IgA deposits. The nature of the light chains in immunofluorescence (IF) studies in IgAN remains obscure, although there are a few reports that lambda light chains were found predominantly in the mesangial deposits in IgAN. In particular in posttransplant IgAN, some cases display poor prognosis, some cases can be diagnosed only by IgA deposits in the mesangial area, and the nature of the light chains is unknown.

**Methods:** In order to examine the immunochemical characterization of light chains in posttransplant IgAN, direct IF studies using polyclonal rabbit anti-human kappa and lambda light chains were performed from consecutive 30 native renal biopsies of IgAN (native IgAN) and 18 cases of posttransplant IgAN, excluding Henoch-Schönlein Purpura.

**Results:** The age at the biopsy of native IgAN and posttransplant IgAN were 28.0±21.3 and 35.9±12.2, respectively. Posttransplant IgAN were diagnosed from 3 weeks to 15 years after transplantation. In native IgAN, both kappa and lambda light chains were detected in 28 of 30 cases, and 11 of 28 were strongly positive for lambda light chains. 2 of 30 native IgAN were negative for kappa and lambda light chains. In posttransplant IgAN, both kappa and lambda light chains were detected in 11 of 18 cases, and 8 of 11 were strongly positive for lambda light chains. 7 of 18 posttransplant IgAN were negative for kappa and lambda light chains. The percentage of both kappa and lambda light chains negative cases were significantly higher in posttransplant IgAN (p=0.0091, Fisher's exact test).

**Conclusions:** The nature of the light chains in posttransplant IgAN is not the same as the one in native IgAN. Our results suggest that the mechanism responsible for the development of IgAN is different from native IgAN and posttransplant IgAN.

## FR-PO1057

**Decreased Total Interstitial Inflammation with Higher Mycophenolate Mofetil Dose after Kidney Transplantation**

Karlo K. Mihovilovic,[1] Petar Senjug,[2] Bojana Maksimovic,[1] Danica Galessic Ljubanovic,[2] Renata Zunec,[3] Biserka Palfi,[3] Mladen Knotek.[1] *[1]Clinical Hospital Merkur, Zagreb, Croatia; [2]Clinical Hospital Dubrava, Zagreb, Croatia; [3]Clinical Hospital Zagreb, Zagreb, Croatia.*

**Background:** We have recently found that higher average mycophenolate mofetil (MMF) dose during first posttransplant year was associated with decreased progression of interstitial fibrosis and tubular atrophy during first posttransplant year in kidney transplant recipients. In the present study we sought to evaluate effect of MMF dose on total interstitial inflammationscore at one year.

**Methods:** This is a single-center retrospective study in which 67 kidney, or kidney-pancreas recipients with 12 month protocol biopsy were included.Patients with recurrence of glomerulonephritis and with BKV nephropathy were excluded from the analysis. Immunosuppression consisted of anti-IL2 induction, with tacrolimus or cyclosporine and MMF ± steroid maintenance.During 2007-2010 MMF was dosed in our center according to C0 monitoring, which led to its dose dispersion (1000-4000 mg/day). Total interstitial inflammation (ti score) and interstitial fibrosis (ci score) were determined according to Banff classification. Creatinine clearence (eClcr) was estimated by the Cockroft-Gault formula.

**Results:** Recipients, were 44.75 ± 12.34 y/o, 65 percent of them were male. There were 20 living donor transplantations. At 12 months postttransplant mean MMF dose was 2205 ± 627 mg/d, eClcr 62.1 ± 16.4 ml/min, ci score 0.87 ± 0.78 and ti score 0.72 ± 0.88. Average MMF dose during first posttransplant year was 2238 ± 571 mg. There was positive correlation between 12 months ci and ti score (r=0.716, p<0.001). Both, ci (r=-0.401, p<0.001) and ti score (r=-0.319, p=0.01) were negatively correlated with MMF dose at 12 months.Similarly, there was a negative correlation of average MMF dose over first posttransplant year with ci (r=-0.337, p<0.01) and ti score (r=-0.250, p<0.05). MPA concentration (C0) at either 12 months, or 1 year average, as well as respective tacrolimus concentrations did not correlate with 12 month ci or ct scores.

**Conclusions:** Higher MMF dose during first year posttransplant may be associated with better kidney allograft histology and less inflammation.

## FR-PO1058

**Basliximab versus Low Doses of Thymoglobulin as Induction Therapies in Kidney Transplant Recipients** Daniel Perez-Vega,[1] Benjamin Gomez-Navarro,[1] Enrique Rojas-Campos.[2] *[1]Dept of Nephrology and Organ Transplant Unit, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jalisco, Mexico; [2]Medical Research Unit in Renal Diseases, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jalisco, Mexico.*

**Background:** Induction therapies such as Basiliximab (BSX) and Thymoglobulin (rATG) are associated with lower incidence of acute rejection in kidney transplant recipients (KR) but there is still conflicting data regarding the outcomes of these agents specially comparing low dose schemes of rATG versus an Interleukin 2 receptor antagonist.

**Methods:** The aim of the study is to show the incidence of acute rejection (AR), adverse effects and the graft survival at 12 months with the use of two different induction therapies in a cohort of 225 KR that were prospectively analyzedin 2 groups according to the induction agent: group 1 with 125 KR that received BSX ( 20mg in day 0 and day 4), group 2 with 100 KR that received rATG in a low dose scheme ( 0.6 to 1.2mg/kg in days 0 through 3). The primary end point was the incidence of biopsy proven rejection and graft survival. The secondary end points were renal function, adverse effects and infections.

**Results:** In the BSX group we found a significantly higher incidence of biopsy proven rejection at 12 months after transplant ( 23 % vs 10% in group of rATG) . No difference was found between groups in graft survival, adverse effects, infections and global survival as seen in Table 1.

**Conclusions:** In our population induction therapy with rATG at low doses was associated with a lower incidence of acute rejection compared with BSX in kidney transplant recipients but with a similar incidence of infections, graft loss and global survival.

**Table 1. 12 month outcomes according to induction agent.**

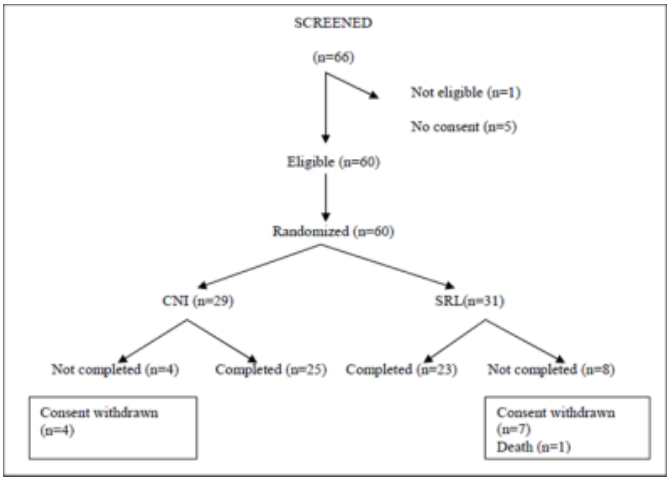
| Variable | All (n=225) | Basiliximab (n=125) | Thymoglobulin ( n=100) |
|---|---|---|---|
| Biopsy proven rejection n (%) | 39 (17) | 29 (23)** | 10 (10)** |
| Antibody treated rejection n (%) | 22 (10) | 16 (13) | 6(6) |
| Infections n (%) | 127 (56) | 72(57.6) | 55 ( 55) |
| Urinary tract infections n ( %) | 109 (48) | 56(45) | 43( 43) |
| Cytomegalovirus n (%) | 6( 2.5) | 4(3) | 2(2) |
| BK Virus n (%) | 3(1.3) | 2(1.5) | 1(1) |
| Death n (%) | 11 (5) | 4 (3) | 7 (7) |
| Graft loss n (%) | 9(4) | 5 (4) | 4(4) |
| Serum Creatinine (mg/dl) | 1.35±0.55 | 1.41±0.43 | 1.27±0.32 |
| ** p=<0.05 | | | |

## FR-PO1059

**Deferred Pre-Emptive Switch from Calcineurin Inhibitor to Sirolimus Leads to Improvement in GFR and Expansion of T Regulatory Cell Population** Dinesh Bansal,[1] Vivekanand Jha,[1] Vinay Sakhuja,[1] Mukut Minz,[2] Ashok Kumar Yadav.[1] *[1]Nephrology, PGIMER, Chandigarh, India; [2]Transplant Surgery, PGIMER, Chandigarh, India.*

**Background:** Measures to prevent chronic calcineurin inhibitor (CNI) toxicity have included limiting exposure by switching to sirolimus (SRL). SRL may favorably influence T regulatory cell (Treg) population. This randomized controlled trial compares the effect of switching from CNI to SRL on GFR and Treg frequency.

**Methods:** In this prospective open label randomized trial, primary living donor kidney transplant recipients on CNI-based immunosuppression were randomized to continue CNI or switched to SRL 2 months after surgery; 29 were randomized to receive CNI and 31 to SRL.




All patients received MMF and steroids. The main outcome parameter was eGFR at 180 days by CKD EPI equation. Treg population was estimated by flow cytometry.

**Results:** Baseline characteristics in the two groups were similar. Forty-eight patients completed the trial. At six months, patients in SRL group had significantly higher eGFR as compared to CNI group (96.00 ± 13.75 vs 81.80 ± 22.38mL/min, p = 0.012). Patients on SRL had a mean 13 mL/min gain of eGFR at the end of six months. Patients in SRL group showed significant increase in Treg population at 30 days, which persisted till day 180. There was no difference in the adverse events in terms of number of acute rejection episodes, death, infections, proteinuria, lipid profile, blood pressure control and hematological parameters between the two groups. Four patients taking SRL developed enthesitis. No patient left the study or switched treatment because of adverse event.

**Conclusions:** A deferred pre-emptive switch over from CNI to SRL safely improves renal function and Treg population at 6 months in living donor kidney transplant recipients.

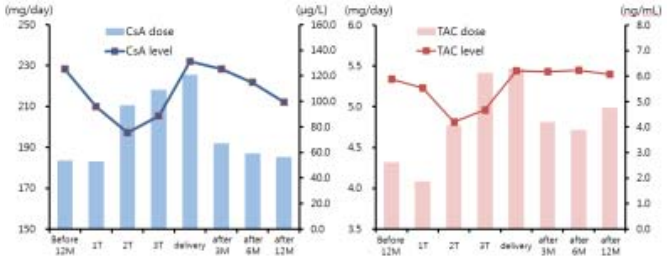
## FR-PO1060

**Tacrolimus also Needs to Be Increased during Pregnancy for Transplant Recipients** Hyosang Kim,[1] Jong Cheol Jeong,[2] Jaeseok Yang,[2] Wonseok Yang,[1] Curie Ahn,[2] Duck Jong Han,[3] Su-Kil Park.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Asan Medical Center, Univ of Ulsan College of Medicine, Seoul, Republic of Korea; [2]Transplantation Center, Seoul National Univ Hospital, Seoul, Republic of Korea; [3]Dept of Surgery, Asan Medical Center, Univ of Ulsan College of Medicine, Seoul, Republic of Korea.*

**Background:** To investigate the effects of pregnancy to renal function of women with allograft kidney and the relationship between immunosuppressant dose and blood drug level during pregnancy and postpartum.

**Methods:** The study population consisted of 75 women with 88 deliveries after kidney transplantation at Asan Medical Center and Seoul National University Hospital from 1991 to 2012.

**Results:** The mean age at the time of transplantation and delivery were 26.7±4.2 and 31.7±3.6 years old, respectively. Significant serum creatinine elevation, defined as more than 0.5 mg/dL, was found in 8 (9.1%) deliveries. In other 80 (90.9%) deliveries which maintained relatively stable creatinine levels, mean serum creatinine was slightly reduced by 0.14 mg/dL on average during gestation and returned to pre-pregnant level after delivery. As for calcineurin inhibitor (CNI), tacrolimus was used in 28 (31.8%) deliveries and cyclosporine in other cases. Neither gestational period and body weight at birth was different in both CNI groups, and the changing pattern of serum creatinine during pregnancy was similar to each other. Figure shows blood CNI levels and their doses at each time point. Other immunosuppressant doses, azathioprine and steroid, were not changed during pregnancy.




**Conclusions:** In most women with allograft kidney, renal function was slightly improved during pregnancy and returned to previous level after delivery. To maintain therapeutic blood level, about 20~25% dose elevation of tacrolimus and cyclosporine should be considered.

**FR-PO1061**

**Intensified Dosing of Mycophenolate in African American Renal Transplant Recipients Does Not Reduce Rejection or Graft Loss**
Jillian Leigh Descourouez,[1] David Hager,[1] Glen E. Leverson,[2] Arjang Djamali,[2] Luis A. Fernandez.[2] *[1]Pharmacy, Univ of Wisconsin Hospital and Clinics, Madison, WI; [2]Surgery, Univ of Wisconsin Hospital and Clinics, Madison, WI.*

**Background:** Intensified mycophenolate dosing strategies have been proposed in African American (AA) renal transplant recipients to reduce rates of acute rejection, graft loss and to improve survival. This study evaluated the efficacy of intensified dosing of mycophenolate in a cohort of AA renal transplant recipients.

**Methods:** Adult AA renal allograft recipients (n=247) transplanted between 1/1/2002 - 12/31/2011 were divided as follows; group A (n=128) had a dose reduction in mycophenolate mofetil (MMF) to <3 g/day or mycophenolate sodium (MPS) to <2160 mg/day within 3 months post-transplant; group B (n=119) remained on ≥MMF 3 g/day or ≥MPS 2160 mg/day at 3 months post-transplant. The primary outcome was a composite of suspected rejection or treatment failure. Secondary endpoints included patient (PS) and graft survival (GS); death censored graft survival; graft function as measured by estimated glomerular filtration rate (eGFR) at 1 month, 6 months, 1, 3 and 5 years; and overall infection.

**Results:** Baseline characteristics did not vary between groups except the number of primary renal allografts was greater in group B (88.2% vs. 76.6%, p=0.03). A MMF or MPS dose reduction by 3 months post-transplant occurred in 51.8% of patients (n=128). No difference was observed for the primary end point (group A: n=59, group B: n=51, p=0.38). PS and death-censored GS at 1, 3 and 5 years were not different (97, 88, and 82% PS, and 94, 85 and 75% GS respectively for group A, and 100, 92.7 and 91% PS and 95, 76.8 and 71% GS for group B). eGFR did not differ at 1 month, 6 months, 1, 3, or 5 years (p=0.43, 0.70, 0.98, 1.00, and 1.00 eGFR). Rates of rejection or infection at 1, 3 and 5 years were not significantly different between groups (p=0.86 and p=0.24).

**Conclusions:** African American renal transplant recipients remaining on intensified dosing of mycophenolate at 3 months post-transplant did not have improved efficacy or an increased risk of infectious complications. These results do not support the use of MMF > 2000 mg/day or MPS >1440 mg/day.

**FR-PO1062**

**Effect of Basiliximab Induction Therapy in Living Donor Renal Transplantation**
Flávia Oliveira, Irene L. Noronha. *Nephrology, Univ of Sao Paulo, Brazil.*

**Background:** The beneficial effects of induction therapy with basiliximab in kidney transplantation with deceased donors has been extensively confirmed, but limited data are available for transplantation with living donors. The aim of this study was to analyze the impact of basiliximab in patients with HLA-haploidentical living donor kidney transplantation compared with no induction.

**Methods:** Primary kidney transplantation (n=74) receiving calcineurin inhibitor+mycophenolate+steroids were divided into 2 groups: basiliximab (n=34) and no induction (n=40). The primary endpoint was the occurrence of biopsy proven acute rejection (BPAR) at 1 year post-transplantation (post-Tx). Secondary endpoints included patient and graft survival at the 1st and 5th year, renal function and adverse events at 1 year pos-Tx.

**Results:** The incidence of BPAR was significantly lower in the basiliximab group (2 cases Banff II) compared with the no induction group (4 cases Banff I, 3 cases Banff II, and 1 antibody-mediated). The incidence of CMV infection and cancer was not diferent between the groups. The results are shown in table 1.

| | Basiliximab | No Induction |
|---|---|---|
| **Receptor's age** | 34.8±13.4 | 39.3±14.5 |
| **Gender (male)** | 19/34 **(55.9%)** | 25/40 **(62.5%)** |
| **Race (caucasian)** | 24/34 **(70.6%)** | 29/40 **(72.5%)** |
| **Time on dialysis** (months) | 16 (10.2-33.5) | 16 (9-24.7) |
| **Donor's age** | 43.3±10 | 42.6±11.3 |
| **BPAR 1 year** | 2/33 **(6.1%)** | 9/37 **(24.3%)*** |
| **Time to BPAR** (days) | 39±35.3 | 24±45.7 |
| **Patient survival** | | |
| 1 year | 97% | 94.6% |
| 5 years | 94.1% | 92.5% |
| **Graft survival** | | |
| 1 year | 94.1% | 87.5% |
| 5 years | 82.4% | 82.5% |
| **Serum creatinine (mg/dL)** | 1.5±0.6 | 1.9±0.7* |
| 6 months | 1.4±0.4 | 1.5±0.6 |
| 12 months | 1.4±0.5 | 1.4±0.5 |
| **CMV infection** | 6/32 **(18.8%)** | 9/36 **(25%)** |
| **Cancer** | 1/32 **(3.1%)** | 2/35 **(5.7%)** |

*p<0,05.

**Conclusions:** Induction therapy with basiliximab in living donor renal transplantation was associated with lower rate of BPAR, without increasing adverse effects.

**FR-PO1063**

**Predictors of Diabetes after Kidney Transplant**
Maria P. Martinez Cantarin,[1] Scott W. Keith,[2] Bonita E. Falkner.[1] *[1]Medicine-Nephrology, Thomas Jefferson Univ Hospital, Philadelphia, PA; [2]Pharmacology, Thomas Jefferson Univ, Philadelphia, PA.*

**Background:** New Onset Diabetes After Kidney Transplantation (NODAT) is a highly prevalent complication after solid organ transplantation and is associated with worse transplant outcomes including graft loss and patient death. The aim of this study was to determine if an inflammatory phenotype is associated with development of diabetes post-transplantation.

**Methods:** End stage kidney disease participants that did not have diabetes were recruited from Thomas Jefferson University Transplant program before kidney transplantation. Participant's plasma was obtained pre-kidney transplantation and three to six months post-transplantation. Inflammatory and anti-inflammatory cytokines including IL-8, IL-6, TNF alpha, CRP, MCP-1, total adiponectin and high molecular weight (HMW) adiponectin were assayed by ELISA. Participants were followed prospectively for 1 year and monitored for development of NODAT. Unadjusted comparisons of the distributions of variables between study groups were made by the Kruskal-Wallis test or the Fisher's exact test. Logistic regression was used to determine a model of the risk of NODAT.

**Results:** Of the 32 participants enrolled in the study, 11 developed NODAT using ADA criteria. Participants that develop NODAT had the same median age, BMI and proportion of male/females than participants that did not develop NODAT. Transplant participants that develop NODAT had lower total adiponectin, lower HMW to total adiponectin ratio and higher TNF alpha levels pre-transplant. After transplant, inflammatory cytokines and adiponectin decreased in both groups but transplant patients that developed NODAT maintained higher IL-6, IL-8 and CRP and lower HMW to adiponectin ratio. The odds of NODAT were significantly increased by almost 5-fold for each half-unit increase in log TNF-$\alpha$ (OR = 4.90, p = 0.04) pretransplant after adjusting for age in years (OR = 1.10, p = 0.07).

**Conclusions:** Our data suggest that a phenotype of increased inflammation with lower adiponectin levels pre-transplant could be associated with the development of diabetes after kidney transplant and that TNF alpha may be a predictor of NODAT.

*Funding:* Private Foundation Support

**FR-PO1064**

**Low Vitamin D and Adverse Allograft Outcomes in Kidney Transplant Recipients**
Yoshitsugu Obi,[1] Takayuki Hamano,[2] Naotsugu Ichimaru,[3] Kodo Tomida,[4] Naohiko Fujii,[5] Isao Matsui,[1] Hiromi Rakugi,[1] Shiro Takahara,[3] Yoshitaka Isaka,[1] Yoshiharu Tsubakihara.[2] *[1]Dept of Geriatric Medicine & Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Dept of Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [3]Dept of Advanced Technology for Transplantation, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [4]Dept of Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [5]Dept of Internal Medicine, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Hyogo, Japan.*

**Background:** Vitamin D, which is often deficient in kidney transplant (KTx) recipients, has potential immunomodulatory effects.

**Methods:** In a prospective cohort of 264 ambulatory KTx recipients, we measured the baseline 25-hydroxyvitamin D (25D) levels and examined its association with (i) annual decline in estimated glomerular filtration rate (eGFR); (ii) the composite event of a 50% increase in serum creatinine, end-stage renal disease, or death; and (iii) rejection episodes, with intravenous methylprednisolone ≥250 mg (IV-MP) as the index.

**Results:** Multivariate cubic spline analysis showed that the serum 25D levels had an almost linear relationship with annual eGFR decline. With vitamin D sufficiency (≥20 ng/mL) as the reference, inadequacy (≥12 and <20 ng/mL) and deficiency (<12 ng/mL) showed a significant dose-dependent associations with a higher risk of the composite event and IV-MP in the multivariate Cox regression analyses. These findings were confirmed in the propensity-matched cohort independent of 30 potential confounders. Further, time after KTx was a significant effect modifier for the association of serum 25D levels with annual eGFR change and IV-MP. Stratified analyses showed pronounced relationships in patients with <10 years since KTx (1.04 [95% CI, 0.26–1.83] mL/min/1.73 m$^2$ per 10 ng/mL of serum 25D levels in the analysis for annual eGFR decline), but no significant associations in patients with ≥10 years since KTx.

**Conclusions:** Low vitamin D predicts adverse allograft outcomes, and vitamin D supplementation early after KTx may improve patient outcome.

*Funding:* Private Foundation Support

**FR-PO1065**

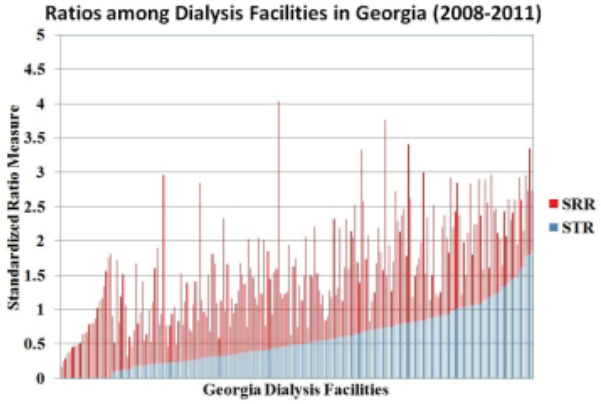
**Correlation of Kidney Transplant Referral with Transplant Rates at the Dialysis Facility Level**
Rachel E. Patzer,[1] M. Ahinee Amamoo,[2] Jenna Krisher,[2] Laura L. Mulloy,[3] Carlos F. Zayas,[4] Stephen O. Pastan.[1] *[1]Emory Transplant Center, Atlanta, GA; [2]Southeastern Kidney Council, Raleigh, NC; [3]Georgia Regents, Augusta, GA; [4]Piedmont Transplant Institute, Atlanta, GA.*

**Background:** Variability in dialysis facility-level kidney transplant (KTx) rates have been reported, but little is known about whether KTx referral rates vary by facilities and the extent to which referral rates are correlated with transplant rates.

**Methods:** Through the Southeastern KTx Coalition of End Stage Renal Disease Network 6, we collected data on all patient referrals to the 3 adult Ktx centers in Georgia (GA) from 2008-2011 and calculated crude referral rates and standardized referral ratios (SRR) -- the number of observed divided by the number of expected KTx referrals -- for each GA dialysis facility using a 2-stage Cox model. Pearson correlation coefficients (r) were calculated to examine correlations between referral and transplant rates, and the SRR and the standardized transplant ratio (STR).

**Results:** 276 dialysis facilities referred 7,180 ESRD patients for transplant in GA from 2008-2011. The median age was 52 years (IQR: 41, 61), 56.6% were male, and 68.5% were African American. The median annual KTx referral rate at the dialysis facility level was 16.6% (IQR: 9.7%, 22.2%) over 4 years. We found a weak correlation between the SRR and STR (r=0.16, p=0.0175).

**Figure. Standardized Referral Ratios and Standardized Transplant Ratios among Dialysis Facilities in Georgia (2008-2011)**




There was a moderate correlation between crude referral and transplant rates (r = 0.25, p<0.0001) that was higher among facilities with 25+ patients (r=0.33, p<0.0001).

**Conclusions:** Crude referral rates and SRR are correlated with KTx rates. Quality improvement interventions targeting facilities with lower than expected KTx referrals may improve patient access to KTx. SRR may be used to help launch a surveillance system throughout ESRD Network 6 to regularly measure KTx referral.

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## FR-PO1066

**Renin Gene Mutation Causing Anemia and Kidney Injury in Childhood**

Faris Q. Hashim,[1] Anthony J. Bleyer,[2] Stanislav Kmoch,[3] Richard E. Neiberger.[1]
*[1]Pediatric Nephrology, Univ of Florida, Gainesville, FL; [2]Internal Medicine-Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; [3]Institute of Inherited Metabolic Disorders, Charles Univ, Prague, Czech Republic.*

**Background:** *REN* mutations produce rare but treatable conditions. There have been only five reported cases of mutations in the *REN* gene encoding renin causing chronic kidney disease (CKD) [1, 2]. Here we report a new family with this condition.

**Methods:** A 5 year old girl presented for evaluation of a hemoglobin of 8.8 g/d and a serum creatinine (Cr) level of 0.5 mg/dl (eGFR 86 ml/min/1.73m$^2$). The patient's mother had undergone a kidney biopsy showing medullary cystic kidney disease. On physical examination, blood pressure was 87/36, height 101 cm (10$^{th}$ percentile) and weight 14.3 kg (3rd percentile). Her examination was unremarkable. Urinalysis was normal. The serum uric acid (SUA) was 9.2 mg/dl (normal for age <6 mg/dl) with a 24 h urate excretion of 125 mg/day (normal <175mg/day) and fractional urate excretion 6.5 %(normal 18±5 %). Serum K was mildly elevated 5.1 meq/l. SUA increased to 11 mg/dl despite strict diet control. A renal ultrasound showed normal size kidneys. A mutational analysis of the *REN* gene revealed an L16R mutation. The patient was started on allopurinol 5 mg/kg bid which decreased her UAC to 7.4 mg/dl. The mother developed acute kidney injury at 17 years after running in a race. A kidney biopsy revealed medullary cystic kidney disease. She had minimal proteinuria and a SUA concentration of 11 mg/dl. Her eGFR was 38ml/min (Cr 2 mg/dl) at age 32 years.

**Conclusions:** This child presented with a classic case of a REN mutation, anemia, mild hyperkalemia, mild hypotension, and CKD. The acute kidney injury that occurred in her mother has also been noted to occur in patients with this condition. Recognition of REN Gene Mutation allows prompt intervention to treat anemia, hyperkalemia, hyperuricemia, and avoid acute kidney injury.

1. Zivna, M., et al., Dominant renin gene mutations associated with early-onset hyperuricemia, anemia, and chronic kidney failure. Am J Hum Genet, 2009.
2. Bleyer, A., et al., Hereditary interstitial kidney disease. Semin Nephrol, 2010.

## FR-PO1067

**Implications of an Unusual Diagnosis: Uromodulin-Associated Kidney Disease**

Jonathan A. Bolanos,[1] Anthony J. Bleyer,[2] Christina M. Yuan,[1] John Stephen Thurlow.[1] *[1]Walter Reed National Military Medical Center, Bethesda, MD; [2]Wake Forest Univ School of Medicine, Winston Salem, NC.*

**Background:** Uromodulin-Associated Kidney Disease (UAKD), synonymous with medullary cystic kidney disease type 2 and familial juvenile hyperuricemic nephropathy, is a rare autosomal dominant interstitial kidney disease caused by mutations in the *UMOD* gene encoding uromodulin (Tam-Horsfall protein). These mutations result in defective assembly and subsequent accumulation of mutated uromodulin in tubular cells, leading to cell atrophy and death.

**Methods:** A 35 y/o white male presented for transplant evaluation. He had suffered from slowly progressive chronic kidney disease (CKD) for over a decade and had a 4 year history of recurrent gout. His father and paternal uncle had gout in their 30s, followed years later by ESRD attributed to glomerulonephritis. His paternal grandfather died in his 30s from an uncertain cause. Urinalysis was bland with no proteinuria or hematuria, and renal imaging was unremarkable. Mutational analysis of exons 4 and 5 of the *UMOD* gene (Athena Diagnostics) by PCR amplification identified a missense mutation resulting in a substitution of tyrosine for cysteine at codon 148, a common mutation associated with UAKD. Due to this finding, family members were contacted, and three living family members have since been diagnosed and others are undergoing evaluation.

**Conclusions:** The diagnosis of UAKD requires a careful family history in patients with a bland urine sediment, gout, and slowly progressive kidney disease. Correct diagnosis of an individual often leads to diagnosis for many family members. As in the current case, *UMOD* analysis can then be performed in potential living-related kidney donors to improve safety and quality of donation. Genetic counseling, gout treatment, and CKD management, including renal transplant evaluation, are the cornerstones of therapy for UAKD. *The views expressed are those of the authors and do not necessarily reflect the official policy or position of the Department of the Army, the Department of the Navy, the Department of Defense, nor the US Government.*

## FR-PO1068

**Tri-Allelic Inheritance of NPHS1 Gene in Infantile-Onset Steroid-Resistant Nephrotic Syndrome**

Yusuke Kumagai,[1] Hiroaki Ueda,[1] Koichi Nakanishi,[2] Norishige Yoshikawa,[2] Ryota Kurayama,[3] Kunimasa Yan,[3] Akira Ashida,[4] Daisuke Yamamoto,[5] Michio Nagata,[6] Rika Fujimaru.[1] *[1]Pediatrics, Osaka City General Hospital, Osaka, Japan; [2]Pediatrics, Wakayama Medical Univ, Wakayama, Japan; [3]Pediatrics, Kyorin Univ School of Medicine, Tokyo, Japan; [4]Pediatrics, Osaka Medical College, Osaka, Japan; [5]Biomedical Computation Center, Osaka Medical College, Osaka, Japan; [6]Kidney and Vascular Pathology, Graduate School of Comprehensive Human Sciences, Univ of Tsukuba, Ibaraki, Japan.*

**Background:** Recently, NPHS1 mutations have been identified as a cause of steroid-resistant nephrotic syndrome (SRNS) in all age groups and milder courses of the diseases. We report a case of infantile-onset SRNS associated with a severe mutation and two mild variants, which resulted in the tri-allelic hit of the NPHS1 gene.

**Methods:** A girl was diagnosed with SRNS at 9 months of age. A renal biopsy showed glomerular hypertrophy and severe interstitial fibrosis without focal glomerulosclerosis. Electron microscopy revealed effacement and fusion of podocyte foot processes. In immunohistochemistry, the expression of nephrin protein had partially decreased, while podocin was found along the glomerular capillary wall. Genetic analysis of the NPHS1 gene revealed three different amino-acid changes. One was a novel stop codon (p.E189X), and the others (p.P206T and p.R800C) had previously been reported on either missense variants or polymorphisms. No mutation of NPHS2 was detected. Mutation screening of non-consanguineous healthy parents revealed that the E189X and R800C were paternal origin and that the P206T was maternal which was predicted to a disease-causing mutation. Based on our molecular model analysis, the R800C substitution also could be classified as a mild missense variant, probably leading to conformational changes in nephrin structure. After a year of follow-up, she had a mild clinical course because of the disappearance of edema but persistent proteinuria with administration of an angiotensin receptor blocker.

**Conclusions:** The tri-allelic inheritance of the *NPHS1* gene observed in the present case might be responsible for atypical and milder form of SRNS.

## FR-PO1069

**Very Early Onset of Alport Syndrome in a Two Year Old Lithuanian Boy with Additional Polymorphisms in Nephrin- and Podocin-Genes**

Jenny Kruegel,[1] Matthias Kettwig,[2] Hermann-Josef Groene,[3] Mato P. Nagel,[4] Hildegard F. Zappel,[2] Oliver Gross.[1] *[1]Nephrology & Rheumatology, Univ Medicine Goettingen, Goettingen, Germany; [2]Pediatrics, Univ Medicine Goettingen, Goettingen, Germany; [3]Cellular & Molecular Pathology, German Cancer Research Center, Heidelberg, Germany; [4]Nephrology & Metabolic Disorders, Molecular Diagnostics, Weißwasser, Germany.*

**Background:** Some patients with familial benign hematuria develop early onset of renal failure despite a "benign" heterozygous mutation in type IV collagen genes (COL4A3/4/5). According to our hypothesis, polymorphisms in other genes of the glomerular filtration barrier can aggravate the clinical course of glomerular diseases such as Alport syndrome.

**Methods:** A two year old boy with macrohematuria and 1.2 g/gCrea proteinuria was referred to our clinic for further evaluation. His non-consanguineous parents originate from Lithuania and had no history of renal diseases. Renal biopsy revealed ultrastructural changes typical for Alport syndrome. However, referred to the patient's age, an unusual severe splitting and thickening of the glomerular basement membrane (GBM) with prominent focal segmental glomerular sclerosis was found. Additional defects in podocyte-genes were suspected. The diagnosis of X-chromosomal Alport syndrome was confirmed by sequencing. Direct sequencing of Nphs1 (Nephrin) and Nphs2 (Podocin) resulted in additional Nphs1- and Nphs2-polymorphisms. Interestingly, the COL4A5 mutation and Nphs2 polymorphism were de novo in the boy, with a heterozygous Nphs1 polymorphism in the mother. Ramipril therapy was started and proteinuria gradually dropped within 12 months below 0.5g/gCrea.

**Conclusions:** In conclusion, Podocin-polymorphisms can aggravate heterozygous COL4 mutations – as described previously in patients with familial benign hematuria. In our patient, polymorphisms of Nephrin and Podocin might exacerbate Alport-pathogenesis. This points towards a possible synergistic role of genes coding for GBM- and slit diaphragm-proteins in the pathogenesis of glomerular kidney diseases. The podocyte-interaction between GBM and slit diaphragm is currently investigated by our group using a $COL4A3^{+/-}$// $Nphs2^{+/R140Q}$ mouse model.

## FR-PO1070

**Diagnostic Difficulty in Alport Syndrome** Jennifer H. Adam,[1] Katrina M. Wood,[1] John Andrew Sayer.[1,2] *[1]Dept of Renal Medicine, Freeman Hospital, Newcastle upon Tyne, Tyne and Wear, United Kingdom; [2]Institute of Genetic Medicine, Newcastle Univ, Newcastle upon Tyne, Tyne and Wear, United Kingdom.*

**Background:** Alport syndrome is an inherited disease that causes progressive glomerular damage, often in association with sensorineural hearing loss. The histological diagnosis of Alport syndrome can present a challenge as features on light microscopy can be non-specific and ultrastructural lesions may not be apparent until later in life.

**Methods: Family 1** A 26 year old man presented with hypertension, haematuria, proteinuria and sensorineural deafness. His renal biopsy revealed light microscopy features consistent with FSGS. Electron microscopy (EM) revealed pathognomonic splitting of the glomerular basement membrane suggesting Alport syndrome. His mother had undergone a renal transplant eleven years previously. A review of her native renal biopsy revealed mesangial proliferative changes on light microscopy. There were no glomeruli in EM or IMF specimens. These mesangial changes on light microscopy had, over time, been mistranslated into a label of MCGN. Genetic testing confirmed that both mother and son had a mutation in the *COL4A5* gene causing X-linked Alport syndrome. The proband developed progressive CKD. Had his mother's initial diagnosis been accurate, his renal biopsy could have been avoided. **Family 2** A 47 year old woman presented with haematuria, proteinuria and abnormal renal function. Her renal biopsy showed mild mesangial proliferative changes. EM of glomeruli was not available. She also had high frequency hearing loss and a family history of renal disease. Genetic analysis confirmed that the proband and two of her daughters with microscopic haematuria were heterozygous for a pathogenic mutation in *COL4A3* gene consistent with autosomal dominant Alport syndrome.

**Conclusions:** These cases demonstrate that a diagnosis of Alport syndrome can elude nephrologists in patients with non-specific histological changes on light microscopy, particularly when EM is not available. It also highlights the important role of molecular genetics in confirming a diagnosis of Alport syndrome. We stress the importance of eliciting a family history of renal and extra-renal disease in all patients.

## FR-PO1071

**Early Bilateral Nephrectomy as Rescue Therapy for Autosomal Recessive Polycystic Kidney Disease with Progressive Massive Nephromegaly in Early Infancy** Tamara Mallett, Emma O'Hagan, Karl McKeever. *Dept of Paediatric Nephrology, Royal Belfast Hospital for Sick Children, Belfast, Co Antrim, United Kingdom.*

**Background:** The management of neonatal Autosomal Recessive Polycystic Kidney Disease (ARPKD) complicated by severe pulmonary insufficiency presents complex clinical challenges. Where massive nephromegaly exists, early bilateral nephrectomy, supportive peritoneal dialysis (PD) and early aggressive nutrition can improve survival. Consensus is lacking on the optimal timing and indeed role of nephrectomy, with decision-making driven by the patient's clinical condition and expertise of centre. We report an infant with ARPKD requiring PD and survival at 9 months following early bilateral nephrectomy.

**Methods:** A male infant diagnosed antenatally with ARPKD and third trimester olioghydramnios, was born at term with pulmonary insufficiency requiring high-pressure ventilation. He had massive bilateral nephromegaly with renal insufficiency and hypertension. A PD catheter was inserted on day 15 and dialysis commenced in view of progressive renal impairment, oliguria and respiratory failure. Unilateral nephrectomy was performed at 8 weeks of age in view of progressive massive nephromegaly, high-frequency oscillatory ventilator-dependence and failure to tolerate enteral nutrition. Recovery was complicated by respiratory compromise secondary to increasing nephromegaly. Contralateral nephrectomy was performed 11 days later allowing withdrawal of ventilatory support and establishment of enteral nutrition and PD. The infant is now 9 months old on nocturnal PD, complicated by a solitary episode of bacterial peritonitis. He has no respiratory support and growth is optimised by nasogastric feeding. Development is moderately delayed and there is evidence of early hepatic involvement.

**Conclusions:** Early bilateral nephrectomy should be considered as a potential rescue therapy for infants with ARPKD where progressive massive nephromegaly results in ventilator-dependent pulmonary insufficiency and failure to establish enteral nutrition.

## FR-PO1072

**Longterm Survival in an Anephric Twin** James G. Atherton,[1] Sabahat Afshan,[1] Margaret Colleen Hastings,[1,2] Bettina H. Ault.[1] *[1]Pediatric Nephrology, Univ of Tennessee Health Science Center, Memphis, TN; [2]Nephrology, Univ of Tennessee Health Science Center, Memphis, TN.*

**Background:** Bilateral renal agenesis occurs in 1 in 4,000 neonates. Live births occur but mortality from pulmonary hypoplasia usually ensues soon after birth. In monoamniotic monochorionic twins, an "experiment of nature" may arise: a twin with normal kidneys provides amniotic fluid neccessary for lung development of a twin with renal agenesis. Heretofore such infants have survived no more than 2 months. We describe an infant who has survived past 1 year of life and is near transplant.

**Methods:** Monochorionic monoamniotic twins of gestational age 34.2 wks and prenatally known congenital abnormalities were born to a 31 year old G3P5 female. Twin A had bilateral renal agenesis, hemivertebrae, 2-vessel unbilical cord, ambiguous genitalia, and imperforate anus. Twin B had omphalocele, bladder exstrophy, patent ductus ateriosis, and ambiguous genitalia. Both twins had normal lungs at birth. Both were karyotype 46 XX with normal chromosomal microarray. Twin B died on day of life (DOL) 95 from respiratory failure. Twin A started peritoneal dialysis on DOL 13. She had bacterial peritonitis three times in her first 12 months. She had complications similar to other infants with end stage renal disease: hypertension, electrolyte abnormalities, anemia, and secondary hyperparathyroidism. Her development has been near normal: at 6 months old corrected age, her gross motor skills were at a 5 month level, social at 6-9 months, and language at 3 to 6 months.

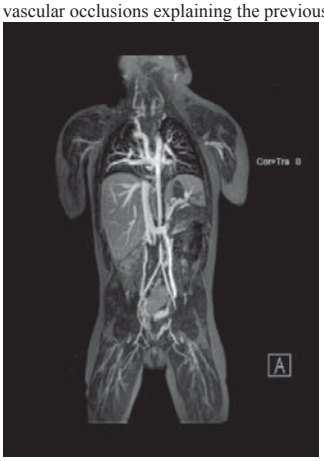
**Conclusions:** To our knowledge, our patient is the longest surviving child with bilateral renal agenesis. She has grown and developed well on peritoneal dialysis. A literature search showed 6 cases of a live born twin with bilateral renal agenesis and healthy lungs; all of these died before 2 months of age, most near the time of birth. Despite setbacks including peritonitis and secondary hyperparathryoidism, her development has been excellent and she continues advancing toward transplant.

## FR-PO1073

**Ferumoxytyl as an Alternative to Gadolinium Based Contrast Agents in a Pediatric Chronic Kidney Disease Patient** Anjali B. Nayak,[1] Aarti Luhar,[2] Mark Hanudel,[1] Theodore R. Hall,[2] Joshua Zaritsky.[1] *[1]Pediatric Nephrology, UCLA, Los Angeles, CA; [2]Dept of Radiology, UCLA, Los Angeles, CA.*

**Background:** Gadolinium based contrast agent (GBCA) exposure in ESRD patients is associated with the development of a potentially fatal disorder, Nephrogenic Systemic Fibrosis (NSF). Although contrast enhanced computed tomography(CT) is an alternative it carries the risk of radiation exposure and further reduction of residual renal function Therefore we sought to assess the efficacy of ferumoxytyl, a parenteral iron supplement that contains superparamagnetic iron oxide, as an alternative contrast agent.

**Methods:** We describe the case of a 5 year old, 13 kg female ESRD patient in whom we were unable to place vascular access despite multiple surgical attempts. Previous Doppler ultrasound imaging was inconclusive. Ferumoxytyl (4mg/kg, IV) was administered for a magnetic resonance venogram (MRV) using a Siemens scanner at 3T according to standard department protocol.The patient tolerated the procedure without difficulty. Resulting image quality was comparable to GBCA enhanced MRV studies and was notable for multiple vascular occlusions explaining the previous failures to obtain vascular access.




**Conclusions:** Given the serious safety concerns associated with GBCA, ferumoxytyl is an excellent alternative contrast agent in pediatric ESRD patients who need detailed vascular mapping via MRV. Future studies are needed in order to further evaluate safety and efficacy in this patient population and to asses whether ferumoxytyl can be used as a contrast agent for other MRI studies.

**FR-PO1074**

**ABO Incompatible Renal Transplantation Can Improve HLA Matching in Children** Anjali B. Nayak,[1] Robert B. Ettenger,[1] Gerald S. Lipshutz,[3] Eileen W. Tsai.[1] *[1]Pediatric Nephrology, Mattel Children's Hospital, UCLA, Los Angeles, CA; [2]Pediatric Radiology, UCLA, Los Angeles, CA; [3]Dept of Surgery, UCLA, Los Angeles, CA.*

**Background:** It is important for pediatric (Ped) patients (pt) to get expedited kidney (Kid) transplants (Tx) to resolve uremic symptoms and grow. Also,due to long projected life-years, it is optimal for them to get well-matched Tx to minimize the chances of long-term sensitization. Such sensitization has been reported to make second Txs difficult in these pts. In North America ped kid Tx is largely dependent upon the use of deceased donor organs making it challenging to identify timely well matched Txs. One solution is to utilize ABO incompatible (ABOi) Tx and, if necessary, paired donation.

**Methods:** In the past year, we adopted this approach for 3 pts. (age 2 -13 yrs) who got transplanted across the ABO barrier with Txs well matched for HLA A, B, DR and DQ.

Table 1. Donor and Patient HLA Typing

| | HLA | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | | B | | DR | | DQ | |
| Patient 1 | 2 | 3 | 13 | 35 | 7 | 13 | 2 | 6 |
| Donor 1 | 2 | 3 | 13 | 65 | 7 | 13 | 2 | 6 |
| Patient 2 | 2 | 4 | 18 | 41 | 11 | 13 | 7 | 6 |
| Donor 2 | 24 | 26 | 13 | 41 | 7 | 13 | 2 | 6 |
| Patient 3 | 11 | 29 | 27 | 44 | 7 | 15 | 2 | 6 |
| Donor 3 | 1 | 11 | 7 | 27 | 15 | 15 | 6 | 6 |

Pts received pre-Tx immunomodulation(anti-CD20 antibody, intravenous immunoglobulin, and plasmapheresis) until an acceptable isoagglutinin(IG) titer <1:8 was reached at Tx. IG titers were monitored, and postoperative plasmapheresis was initiated if titers increased. We utilized standard immunosuppression with anti thymocyte globulin induction, prednisone, MMF, and tacrolimus. All grafts functioned well 3-12 mos post-Tx, without development of DSAs. Particularly notable was a 13 yo male with > 90% CPRA due to a previous failed transplant, and had been waiting on the deceased donor list for 2 years. He received an ABOi paired exchange Tx donor with 5/6 HLA match; the Tx has an eGFR of >90ml/min/1.73 m2 at 1 yr post Tx. Both pt and renal allograft survival to date is 100%.

**Conclusions:** These results highlight the safety and efficacy of ABOi Tx in Ped Kid Txs. Combining the paired exchange program with ABOi Tx can improve HLA matching, minimize wait times, and enhance allograft survival.

**FR-PO1075**

**Perinephric Fluid Collection in a Girl with Dandy Walker Malformation: Mystery Revealed** David M. Shafran,[1] Rupesh Raina,[1] Katherine MacRae Dell,[1] Jonathan Ross.[2] *[1]Dept of Pediatrics, Nephrology, Univ Hospitals, Rainbow Babies and Children's Hospital, Cleveland, OH; [2]Dept of Pediatric Surgery, Univ Hospitals, Rainbow Babies and Children's Hospital, Cleveland, OH.*

**Background:** Renal lymphangiectasia, a developmental malformation of the renal lymphatics, is a rarely reported clinical entity. Hematuria, flank pain and abdominal pain are usually the presenting symptoms.It is described in the limited literature as "self-resolving".

**Methods:** 11year old girl with a distant history of Dandy Walker malformation and placement of a VP shunt at birth presented with a one week history of increasing abdominal distension and discomfort. Physical examination was remarkable for mild hypertension and a markedly distended abdomen. A shunt series was normal and an abdominal CT demonstrated large bilateral perinephric fluid collections. The diagnosis of renal lymphangiectasia was suspected (and other diagnoses, such as lymphoma were excluded) when with needle aspiration of fluid from the perinephric collections demonstrated no cells, isotonic fluid and absence of chyle, all consistent with renal lymph. Percutaneous drains were placed into the perinephric spaces. Drain output increased steadily to approximately 3 liters from each side, requiring continuous intravenous fluids to prevent volume depletion. Furosemide, HCTZ,lisinopril, octreotide , and sclerosing agents (doxycycline and bleomycin) were all unsuccessfull to dicrease the lymphatic output. The patient developed several drain infections requiring multiple courses of intravenous and oral antibiotics. Two months later, a left renal capsulectomy was performed followed by a right capsulectomy with transaction of the lymphatics approximately 4 months after initial presentation. Lymphatic output gradually decreased permitting the removal of both drains.

**Conclusions:** Renal lymphangectasia is a rare, poorly understood condition, which typically resolves without intervention. Several medical treatments, including those used in our patient, have been described in case reports in the literature. However, none were successful in this case. Whether surgical intervention resulted in resolution or was coincidental is unknown.

*Funding:* Private Foundation Support

**FR-PO1076**

**Successful Treatment for Short Stature in a Pubertal Kidney Transplant Recipient Using a Combination of Gonadotropin Releasing Hormone Agonist and Growth Hormone** Hideki Matsumura,[1] Akihiko Shirasu,[1] Hyogo Nkakura,[1] Akira Ashida,[1] Motoshi Hattori,[2] Hiroshi Tamai.[1] *[1]Pediatrics, Osaka Medical College, Takatsuki, Osaka, Japan; [2]Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** Despite various developments in the management of children with CKD, including the use of recombinant human growth hormone (rhGH), achieving an adequate final body height remains a challenge for such children, especially around the time of puberty. Here, we report a pubertal kidney transplant recipient with short stature who was treated with a combination of gonadotropin releasing hormone analog (GnRHa) and rhGH.

**Methods:** We treated an 18-year-old girl with ESRD due to Senior-Loken syndrome. Peritoneal dialysis had been started at the age of 8 years. Her height standard deviation score (SDS) had deteriorated, becoming -3.5 at the age of 12 years 5 months, and we started rhGH therapy. Although her height velocity improved from 0.5 cm/year to 6 cm/year within one year after starting rhGH therapy, her height SDS did not improve. At the start of rhGH therapy, the patient has been at Tanner stage II; therefore we decided to add GnRHa therapy in order to prolong the pre-pubertal growth phase and obtain catch-up growth. At the age of 13 years 9 months, 4 months after initiation of the combined therapy, the patient underwent living-donor kidney transplantation. Immunosuppressive agents included cyclosporine, mycophenolate mofetil, and methylprednisolone. The methylprednisolone was reduced quickly to 0.3 mg/kg/day at 4 weeks after transplantation, and to 4 mg on alternate days from 4 months. At the age of 15 years 8 months, her height SDS had improved to -2.3, and we terminated the GnRHa therapy. At the age of 16 years 6 months, the patient had her first menstrual period, and as her height SDS had reached -2.1, we completed the rhGH therapy. Finally, by the age of 18 years, her height SDS had improved to -1.8. Since then, her transplanted kidney function has been stable and her menstruation cycles have been regular.

**Conclusions:** Combination therapy with rhGH and GnRHa may be safe and effective option for the treatment of short stature in pubertal kidney transplant recipients.

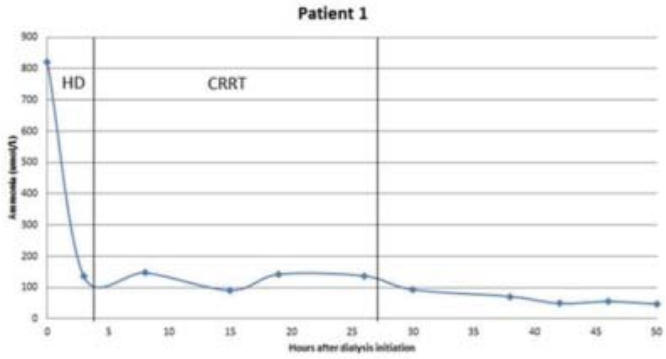
**FR-PO1077**

**A Novel Biphasic Dialytic Strategy for the Treatment of Neonatal Hyperammonemia** Mark Hanudel, Sonal Avasare, Eileen W. Tsai, Joshua Zaritsky. *Dept of Pediatrics, UCLA, Los Angeles, CA.*

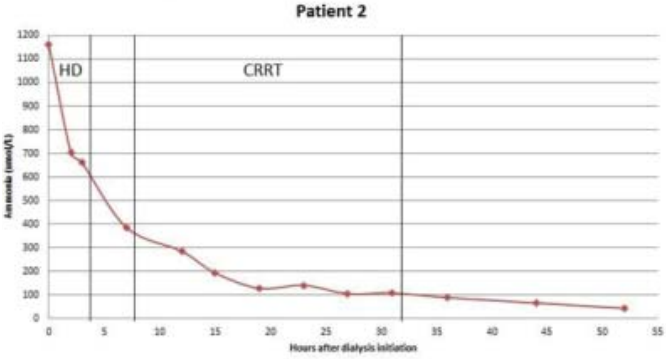
**Background:** Neonates with inborn errors of metabolism often develop hyperammonemia which, if not corrected quickly, may result in poor neurologic outcomes. As pharmacologic therapy cannot rapidly lower ammonia levels, dialysis is frequently required. Both intermittent hemodialysis (IHD) and standard-dose continuous renal replacement therapy (CRRT) are effective; however, IHD may be followed by post-dialytic ammonia rebound, and standard-dose CRRT may not effect a rapid enough decrease in ammonia levels.

**Methods:** We present two cases of IEM-associated neonatal hyperammonemia. The first patient, eventually diagnosed with citrullinemia, presented with lethargy on the fourth day of life. The second patient, eventually diagnosed with methylmalonic acidemia, presented with poor feeding and abnormal newborn screening results on the fourth day of life. Both patients were found to be hyperammonemic (peak ammonia levels 841 umol/L and 1830 umol/L, respectively). For both patients, we employed a biphasic dialytic treatment strategy, initially using dialysis parameters closer to those of IHD (blood flow rate 20-30 mL/min and dialysate flow rate 5000 mL/hr) to rapidly decrease ammonia levels to <400 umol/L, then transitioning to treatment parameters closer to those of standard CRRT (blood flow rate 30 mL/min and dialysate flow rate 500 mL/hr, approximately 4000 mL/hr/1.73m$^2$) to maintain ammonia levels <200 umol/L without rebound.

**Conclusions:** These cases provide an example of a novel biphasic dialytic treatment strategy for neonatal hyperammonemia, effecting rapid ammonia reduction without rebound, employing a seamless transition between two dialytic modalities without equipment changes.




HD: Qb 30 mL/min, Qd 5000 mL/hr; CRRT: Qb 30 mL/min, Qd 500 mL/hr




HD: Qb 20 mL/min, Qd 5000 mL/hr; CRRT: Qb 30 mL/min, Qd 500 mL/hr

**FR-PO1078**

**What Looks like Fabry Disease, May Not Be Fabry!** Reem Daloul, Francis Dumler, Ping L. Zhang. *William Beaumont Hospital, Royal Oak, MI.*

**Background:** Fabry disease is a rare x-linked lysosomal storage disorder caused by deficiency of lysosomal hydrolase alpha-galactosidase A (alpha-Gal A). This results in the accumulation of globotriaosylceramide within the lysosomes of different cell types with eventual organ dysfunction. Renal involvement is characterized by proteinuria and progressive renal failure.

**Methods:** We report a 38 year old previously healthy African American male who presented with intermittent gross hematuria. The patient was otherwise asymptomatic. Family history was strongly positive for chronic kidney disease in both parents, sister, maternal and paternal grandmothers. Urinalysis showed +3 blood, 8 RBCs, 4 WBCs and no protein. Creatinine and eGFR were 1.02 mg/dL and 99 mL/min/1.73m2. Urine protein/creatinine ratio was 0.1. Abdominal CT, renal ultrasound, and cystoscopy were all normal. Renal biopsy showed slight glomerular congestion with occasional foamy cells along the glomerular capillary wall and no proliferation, necrosis, or glomerular sclerosis. CD68 and lysozyme stained sections revealed granular aggregates in the podocytes. Electron microscopy disclosed normal basal membrane thickness without splitting or immune complex deposition and preserved foot processes. Circular and laminated materials were identified in the cytoplasm of the podocytes. These structures correlated with the CD68 positive aggregates identified by light microscopy. The pathological findings are characteristics of Fabry disease. The patient denied any history of neuropathic limb pain, cerebrovascular accidents, heart disease, or skin rash suggestive of telangiectasias or angiokeratomas. Echocardiogram showed normal ventricular wall thickness and valves. Leukocyte Alpha-Gal A was found to be within normal limits.

**Conclusions:** The sensitivity and specificity of the leukocytes alpha-Gal A assay in males approaches 100%. Normal values in our patient excludes the diagnosis of Fabry disease, particularly in the presence of a negative history and a normal physical examination. It raises the possibility of another rare lysosomal disorder being responsible for this patient's renal involvement. A question that we will turn to genetic testing for further answers.

**FR-PO1079**

**Acute Kidney Injury with Hydronephrosis, Eosinophilc Tubolinterstitial Nephritis and Extracapsular Perirenal Eosinophil Infiltrates. A Rare Presentation of Idiopathic Hypereosinophilic Syndrome** Marat Abdullin,[1] Kiran M. Goli,[1] Jonathan Freeman,[2] Syed S. Ali,[1] Jonathan Bayuk,[1] Benjamin J. Freda.[1] *[1]Medicine, Baystate Medical Center; [2]Pathology, Baystate Medical Center.*

**Background:** We describe a patient with Acute Kidney Injury (AKI), hydronephrosis and eosinophilia. To our knowledge, this is the first reported case of Idiopathic Hypereosinophilic Syndrome (IHES) with renal involvement characterized by both obstruction and renal parenchymal injury.

**Methods:** A 55-year-old male presented with 6 months of malaise, night sweats and weight loss. He had bilateral cervical and axillary lymphadenopathy. His white blood cell count (WBC) was 5600/mm$^3$, with an absolute eosinophil count (AEC) of 2000 cells/mm$^3$. The serum creatinine was 1.9 mg/dL and the urinalysis showed 1+ albuminuria, without WBC or red blood cells. A CT scan revealed multiple enlarged mesenteric and retroperitoneal lymph nodes. There was diffuse enlargement of both kidneys with bilateral hydronephrosis and perinephric inflammatory changes. There was thickening of both renal pelvises and proximal ureters with involvement of the periureteral fat. A kidney biopsy showed chronic and active tubulointerstitial nephritis with eosinophils and involvement of the outer renal cortex and extrarenal fibroadipose tissue. The glomeruli were normal. Bone marrow biopsy was normocellular with eosinophilia. Immunophenotyping did not show a B-cell clone or aberrant T-cell population. There was no evidence of eosinophilic leukemia. He was diagnosed with IHES and treated with 40 mg prednisone daily. His symptoms resolved in 1 week. After 3 weeks, his AEC was 0 cells/mm$^3$ and the creatinine decreased to 1.2 mg/dL. Six months later, a renal ultrasound revealed mild residual dilatation of the right kidney collecting system, and complete resolution of the left-sided hydronephrosis.

**Conclusions:** IHES should be considered in patients presenting with AKI and unexplained eosinophilia. Renal involvement is rarely described in IHES. Our case highlights a novel renal manifestation of IHES: obstructive AKI due to perirenal and periureteral eosinophilic infiltration and lymphadenopathy. The obstruction and AKI resolved with prednisone.

**FR-PO1080**

**Case Report: Positive Immunoreactivity for Serum Amyloid A Protein in a Case of Leukocyte Cell-Derived Chemotaxin 2-Type Associated Renal Amyloidosis Confirmed by Tandem Mass Spectrometry** Vanessa Moreno, William F. Glass. *Pathology and Laboratory Medicine, The Univ of Texas - Health Science Center at Houston, Houston, TX.*

**Background:** Leukocyte cell-derived chemotaxin 2 (LECT2) has been recently identified as an amyloidogenic protein affecting the kidney with extensive mesangial, interstitial and vascular involvement by congophilic deposits "non-reactive" for conventional amyloid immunostaining. This amyloid subtype may be identified using an anti-LECT2 antibody or by liquid chromatography tandem mass spectrometry (LC MS/MS).

**Methods:** A 61 year-old Hispanic male undergoing hemodialysis for chronic kidney disease of unknown etiology was referred for evaluation. Kidney biopsy showed extensive mesangial expansion, and diffuse interstitial and vascular involvement by congophilic deposits with typical ultrastructural features of amyloid. Immunofluorescence stains against IgA, IgG, IgM, C3, C1q, fibrinogen, albumin, and κ/λ light-chains were negative. Immunohistochemistry for serum amyloid A (SAA) protein was interpreted as positive and AA-type amyloidosis diagnosed. One year later, the case was reviewed for pre-transplant evaluation, and AA-IHC reinterpreted as positive but less so than AA amyloid control tissue. LC MS/MS was ordered to confirm the nature of the amyloid protein; however, the results demonstrated a peptide profile consistent with LECT2-type amyloid.

**Conclusions:** We present a case of LECT2-type-associated renal amyloidosis with partial immunoreactivity for AA-type amyloid. Immunohistochemistry is the usual method used for typing renal amyloid in more than 90% of cases. In the last decade, at least 12 cases of LECT2-type renal amyloidosis have been reported and described as negative for κ/λ light-chains, AA protein, fibrinogen and transthyretin. However, our experience in this case raises concern that in some cases, LECT2-type renal amyloid may react with partial positivity with antibody to SAA protein. Confirmation by LC MS/MS is crucial in such cases for accurate diagnosis.

**FR-PO1081**

**Karyomegalic Interstitial Nephritis: An Unusual Cause of Progressive Chronic Kidney Disease** Victoria Brocklebank,[1] Alison Brown,[1] John Andrew Sayer.[1,2] *[1]Renal Services Centre, Freeman Hospital, Newcastle upon Tyne, Tyne and Wear, United Kingdom; [2]Institute of Genetic Medicine, International Centre For Life, Newcastle Univ, Newcastle upon Tyne, Tyne and Wear, United Kingdom.*

**Background:** In many patients with progressive chronic kidney disease (CKD) the aetiology is not identified. Karyomegalic Interstitial Nephritis (KIN) may cause insidious CKD. KIN was first described in 1974 and since then only twelve families with KIN have been reported[1], thus it remains rare or under-diagnosed. KIN is characterised by chronic tubulointerstitial nephritis with large abnormal hyperchromatic nuclei. In 2012, recessive mutations in *FAN1* were identified as the cause of KIN[1]. *FAN1*, encoding Fanconi anemia–associated nuclease 1, is involved in DNA damage repair[1].

**Methods:** A 40 year old woman was referred to the nephrology clinic with progressive CKD (eGFR 34ml/min/1.73m$^2$). She was normotensive with negative urinalysis, and immunological tests were negative except for a weakly positive ANA. Renal ultrasound showed slightly small kidneys and cortical thinning. Renal biopsy was performed and histopathological analysis demonstrated characteristic features of KIN. We undertook a molecular genetic analysis of the *FAN1* gene and identified a homozygous missense mutation, affecting a highly conserved residue and predicted to be pathogenic.

**Conclusions:** This patient developed progressive CKD in the absence of the usual risk factors, thus an interstitial nephritis was suspected. Following a renal biopsy, a histological and molecular genetic diagnosis were obtained. These allow some insight into her disease pathogenesis, provide prognostic information, and allows genetic screening of other family members. We speculate that other cases labelled as progressive CKD of unknown aetiology may have underlying genetic abnormalities. KIN secondary to *FAN1* mutations remains rare but because *FAN1* is involved in DNA damage repair, mutations in this gene links the accumulation of DNA damage with the slow and late onset of KIN and the associated CKD.

**Reference**

1. Zhou W *et al*. *FAN1* mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair. *Nat. Genet*. **44**(8),910-917 (2012).

## FR-PO1082

**Using Fresh Frozen Plasma with Therapeutic Plasma Exchange in a Patient with Acquired C1 Esterase Iinhibitor Deficiency Without Triggering Angioedema** Sajan K. Eapen, Andre A. Kaplan, Wilner Samson. *Nephrology, Univ of Connecticut Health Center.*

**Background:** Acquired C1 esterase inhibitor deficiency (acquired angioedema [AAE]) is a rare syndrome affecting the skin, gastrointestinal (GI) and respiratory tracts. The syndrome's acquired form appears after 40 years of age and is associated with lymphoproliferative B cell disorders. In acute attacks, lack of C1 esterase leads to uninhibited bradykinin generation, causing angioedema presenting as cutaneous swelling, severe GI symptoms (colic, nausea, and vomiting) and severe laryngeal edema. The acute attack is unaffected by conventional treatments for *allergic* angioedema (steroids, epinephrine). First line pharmacologic therapies are seldom readily available. Plasma, with natural C1 esterase inhibitor, has been used for acute attacks but there are concerns that plasma could potentially trigger or worsen angioedema through bradykinin substrate replenishment. We present a case of a patient with AAE who developed thrombotic thrombocytopenic purpura (TTP), successfully treated with a series of therapeutic plasma exchange (TPE) using fresh frozen plasma (FFP).

**Methods:** A 39 year old female with B cell lymphoproliferative disease and AAE had malaise and headache with a platelet count of 20,000 k/μl, LDH 701 U/L, reticulocyte count 2.7%, haptoglobin <15 mg/dl and 10 -15 schistocytes per high power field. TTP was suspected as the underlying disease process and emergent TPE was ordered. Aware of a potentially fatal reaction with plasma use, Ecallantide (kallikrein inhibitor) , a first line therapy for AAE angioedema, was secured but not administered and TPE was performed. The patient underwent 6 treatments with a total of 18 liters of FFP. The patient tolerated therapy without angioedema.

**Conclusions:** First line therapies for AAE are not often readily available. Prematta et al (Ann Allergy Asthma Immunol. 2007; 98:383.) have described therapeutic use of FFP for management of acute AAE without incident. This is the first report of TPE using such a large volume of FFP (18 liters) in a patient with AAE and TTP, further substantiating that FFP is tolerated and potentially favorable for AAE in settings where its use is necessitated.

## FR-PO1083

**A Case of Nephritic-Nephrotic Syndrome in a Man with NF** Suzanne Boyle, Rebecca Kurnik Seshasai, Iram Aqeel, Michael Chaknos, Sidney M. Kobrin. *Renal, Hypertension, and Electrolyte Div, Hospital of the Univ of Pennsylvania, Philadelphia, PA.*

**Background:** This is a patient with nephritic-nephrotic syndrome with biopsy-proven MPGN secondary to IgG lambda monoclonal gammopathy (MG) and type I cryoglobulinemia. Bone marrow biopsy confirmed a diagnosis of malignant lymphoplasmacytic lymphoma.

**Methods:** A 26 year-old man with a history of NF type I, hypertension, and possible Noonan's Syndrome (NS) with pulmonic valve disease presented with a rising creatinine (Cr) over the course of 6 months (baseline 1.0 mg/dl). Physical examination: BP 150/70 mm Hg and anasarca. No lymphadenopathy, rash, fever, or arthritis. Lab values: Cr 3.5 mg/dl, BUN 48 mg/dl, urine protein:Cr 5.0, serum albumin 2.4 g/dl, low C3 of 52 mg/dl , normal C4 of 20 mg/dl, negative anti-nuclear antibody. Hepatitis serologies and HIV unremarkable. Urinalysis: 5-10 white cells/high-powered field (HPF), 50-100 red cells per HPF with dysmorphic features, no cellular casts. Kidney biopsy: MPGN pattern on light microscopy. Immunofluorescence demonstrated 3+ C3 and IgG with a lambda-light chain restriction. Electron microscopy revealed subendothelial deposits, pseudomthrombi, and diffuse foot process effacement. Serum and urine protein electrophoresis showed hypogammaglobulinemia with an IgG lambda band on immunofixation. Cryoglobulin screen positive for monoclonal IgG. Bone marrow biopsy: expanded lymphoid aggregate (15 to 25% of cellularity) and a lambda-biased plasma cell population (5 to 10% of cellularity). Flow cytometry negative. A diagnosis of malignant lymphoplasmacytic lymphoma was made.

**Conclusions:** Monoclonal gammopathy of renal significance (MGRS) is an increasingly recognized entity in immunoglobulin complex-mediated MPGN. We describe a case in a patient with a history of NF type I and possible NS. Both of these autosomal-dominant genetic disorders involve loss-of-function mutations in a common pathway which regulates cellular proliferation. Disruption of this pathway predisposes these patients to various malignancies. The above presentation underscores the importance of maintaining a high index of suspicion for MGRS in young patients with these disorders who present with glomerular disease.

## FR-PO1084

**Complete Remission of Nephrotic Syndrome and Acute Kidney Injury in Crescentic IgA Nephropathy: Role of Mycophenolate Sodium** Ezra Israel, Hitesh H. Shah, Louis R. Spiegel, Kenar D. Jhaveri. *Medicine, Hofstra Northshore-LIJ School of Medicine, Great Neck, NY.*

**Background:** Optimal therapy and prognosis of crescentic IgA nephropathy (C-IgAN) is not known. Reported treatment options for C-IgAN include: combination of corticosteroids and cyclophosphamide for six months. Role of mycophenolate sodium in C-IgAN is unknown. We report a case of C-IgAN that was successfully treated with combination immunosuppressive therapy with follow-up.

**Methods:** Case report: A 54 year old man presents with acute kidney injury (serum creatinine: 3.8mg/dl), microscopic hematuria and massive proteinuria (18 grams/day). Kidney biopsy revealed C-IgAN. The patient was initially started on IV pulse corticosteroids for 3 days followed oral corticosteroids and monthly IV cyclophosphamide for six months. Serum creatinine subsequently improved to 2.2 mg/dl. In addition, pt. was initiated on angiotensin receptor blocker. After 6 months of above therapy, 24 hour urine protein decreased to 2 grams and serum creatinine improved to 1.6mg/dl. Patient was subsequently started on mycophenolate sodium 360 mg twice daily for six additional months as maintenance therapy and oral steroids were slowly tapered off. At follow-up after 2.5 years of initial presentation, patient maintains normal renal function and has no proteinuria.

**Conclusions:** C-IgAN has poor renal prognosis without therapy. A combination of corticosteroid and cyclophosphamide-based induction therapy followed by mycophenolate-based maintenance therapy resulted in complete remission of nephrotic syndrome and acute kidney injury in our patient. Our patient continues to be in complete clinical remission with normal renal function, 1.5 years after completing immunosuppressive therapy. Based on our experience, one can consider mycophenolate sodium for maintenance therapy in C-IgAN.

## FR-PO1085

**Early Ultrafiltration Failure in a Patient with Familial FSGS Secondary to a Mutation in α-Actinin 4: A Postulated Extrarenal Manifestation of Hereditary Focal Sclerosis** Yael Einbinder,[1] Joanne M. Bargman.[2] *[1]Nephrology and Hypertension, Meir Medical Center, Kfar Saba, Center, Israel; [2]Nephrology, Toronto General Hospital, Toronto, Canada.*

**Background:** One variant of hereditary focal segmental glomerulosclerosis has been linked to a mutation in alpha-actinin 4. Alpha actinins are present in epithelial podocytes and endothelial cells and are highly conserved, cross-linking proteins that function in the connection between the actin filaments in the cytoskeleton and the transmembrane anchoring proteins such as cadherin and integrin (cell to cell and cell to matrix adhesion molecules). Previous studies have demonstrated that alpha actinins play a role in maintaining and enhancing the endothelial cell barrier.

**Methods:** We present a 48 year old female with this mutation who progressed to end stage kidney disease requiring dialysis. She also had a diagnosis of papillary thyroid carcinoma, history of midline laparotomy due to major motor vehicle accident and Cesarean section. An uneventful PD catheter insertion was done by advanced laparoscopy. Her continuous ambulatory peritoneal dialysis (CAPD) prescription was three daily exchanges of 1.5 liters, 2 with 2.5% dextrose and one exchange with icodextrin. Absence of ultrafiltration was noted during training. Results of the peritoneal equilibration test (PET) have demonstrated calculated D/P ratio for creatinine and urea was 0.95 and 1.04 respectively, suggesting an extremely high small solute transport rate. Ultrafiltration failure was the result of rapid dissipation of the osmotic gradient across the membrane. The patient eventually became persistently volume overloaded and was transitioned to hemodialysis.

**Conclusions:** In the peritoneal membrane, the endothelial cells of the perfusing capillaries are believed to be the most important structure determining transmembrane transport. We postulate that the mutant alpha actinin 4 altered intercellular anchorage, leading to an increase in endothelial permeability and rapid dissipation of the glucose osmotic gradient. This abnormality would be the first described extra-renal manifestation of the AD mutation of alpha-actinin 4.

## FR-PO1086

**An Unusual Association of Autosomal Dominant Polycystic Kidney Disease with Secondary Focal Segmental Glomerulosclerosis** Olga Kuchmak,[1] William L. Clapp,[2] I. David Weiner.[3] *[1]Div of Nephrology, Hypertension and Transplantation, Univ of Florida, Gainesville, FL; [2]Dept of Pathology, Univ of Florida, Gainesville, FL; [3]Div of Nephrology, Hypertension and Transplantation, Univ of Florida, Gainesville, FL.*

**Background:** In patients with autosomal dominant polycystic kidney disease (ADPKD), urinary protein excretion usually less than 1 g/24 hours. Nephrotic range proteinuria, with or without an accompanying decline in renal function, is unusual and should warrant renal biopsy to exclude coexisting glomerular disease.

**Methods:** A 39-year-old WM with a history of ADPKD diagnosed 5 years ago was referred to our clinic for further evaluation of nephrotic range proteinuria. The laboratory evaluation revealed serum creatinine 3.8 mg/dl, BUN 39 mg/dl, eGFR 18mL/min, albumin 4.3 g/dl, hemoglobin 14.8 g/dl, random UPCr 5.2 g. Based on the records his baseline Cr was 2.-2.7 mg/dl. On exam the patient was found to have elevated BP at 149/96 mmHg. Otherwise physical exam was unremarkable. To establish the etiology of this rare presentation we elected to proceed with renal biopsy. In addition to moderate to severe arterial and arteriolar nephrosclerosis, the renal biopsy also revealed presence of segmental sclerosis and hyalinosis. An additional glomerulus showed global sclerosis with prominent

hyperplastic cells present within Bowman's Space. Electron microscopy demonstrated definite segmental sclerosis with ultrastructural analysis confirming the segmental sclerosis with collapsed glomerular capillary loops accompanied by hyalinosis in the subendothelial regions. There was also fairly prominent podocyte foot process effacement present. No definitive electron dense "immune-type" deposits were identified.

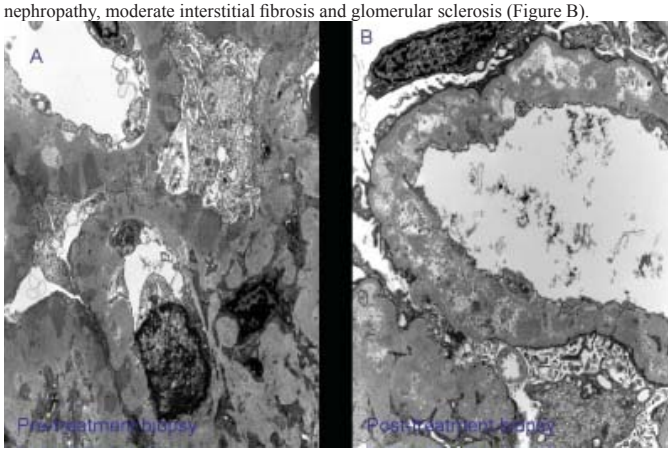
**Conclusions:** We report a rare case of FSGS in patient with ADPKD. Our case emphasizes the importance of renal biopsy in patients with ADPKD with nephrotic-range proteinuria which can provide further guidance in terms of the prognosis and treatment options.

## FR-PO1087

**A Novel Approach to Treating Membranous Nephropathy Secondary to Chronic Lymphocytic Leukemia** David Levy,[1] Wajid M. Choudhry.[2] *[1]Dept of Internal Medicine, Unity Health System, Rochester, NY; [2]Dept of Nephrology, Univ of Rochester Medical Center, Rochester, NY.*

**Background:** Although uncommon, there have been several case reports of biopsy proven membranous nephropathy (MN) caused by chronic lymphocytic leukemia (CLL). Many of these reports have demonstrated clinical improvement following CLL treatment with combination chemotherapy. However, few if any reports have shown biopsy proven disease regression following Rituximab monotherapy.

**Methods:** 62 year old male with a past medical history of diabetes, hypertension, proteinuria (2.6 grams/day) and CLL (not on treatment). Presented with worsening renal function (serum Cr increased from 0.6 mg/dl to 2 mg/dl) and increasing proteinuria (6.3 grams/day). A kidney biopsy was performed which showed lymphoid aggregates predominantly CD20. PLA2 receptor antibodies where negative and the staining pattern was consistent with secondary membranous nephropathy (Figure A). Due to worsening renal function he was initially started on Pentostatin, Cyclophosphamide, and Rituximab. Therapy was discontinued after two cycles since his serum creatinine had increased (3.88 mg/dl). Six months later treatment was restarted with Rituximab dosed at 3.75grams/$m^2$ weekly. After four cycles of treatment his kidney function gradually improved (serum Cr 2.66) and his proteinuria returned to baseline (1.7 grams/day). A repeat kidney biopsy showed clearance of intramembranous deposits consistent with resolution of membranous nephropathy, moderate interstitial fibrosis and glomerular sclerosis (Figure B).




**Conclusions:** To our knowledge we are presenting the first documented case of CLL induced membranous nephropathy with clinical and biopsy proven resolution after Rituximab treatment. This case demonstrates the potential clinical utility of Rituximab as monotherapy for CLL induced membranous nephropathy.

## FR-PO1088

**Use of Adrenocorticotropic Hormone in Managing Proteinuria and Hypoalbuminemia Associated with Focal Segmental Glomerulosclerosis Leading to End Stage Renal Disease** Pallavi D. Shirsat, Sabeen Y. Habib. *Dept of Pediatrics, LSU Health Shreveport, Shreveport, LA.*

**Background:** 14 year old African American female with history of End stage renal disease (ESRD) secondary to Focal Segmental Glomerulosclerosis (FSGS), persisted to have proteinuria and hypoalbuminemia. She was tried on RAAS blockade with no resolution. In order to improve her albumin in preparation for transplant, we tried treatment with ACTH gel (Acthar: a repository corticotrophin injection) to improve her baseline albumin of 0.5 g/dl.

**Methods:** Patient received Acthar 80 units subcutaneously twice a week x 6 months. She was followed closely at the dialysis unit. Then she was weaned off over the next one month.

**Results:** Patient responded to ACTH gel and showed significant improvement in her serum albumin level. Serum albumin improved from 0.5 g/dl at the beginning of treatment to 3.7 g/dl over a period of 6 months. She did not have any significant adverse effects except for cushingoid facies. She is now off Acthar with sustained normal albumin level.

**Conclusions:** ACTH therapy has shown to benefit patients with Idiopathic Membranous Nephropathy in a study conducted in Europe by Ponticelli et al and few observational retrospective studies conducted in the United States. Based on the encouraging results, we decided to use it in our ESRD patient, who has biopsy proven FSGS with proteinuria resistant to conventional treatment with corticosteroids and tacrolimus. After starting ACTH therapy, patient showed significant improvement in her serum albumin level; without having any serious adverse effects. The mechanism of action of ACTH remains unclear at this time; the proposed theories are it may work at the melanocortin receptor in podocytes to reduce proteinuria, or by stimulating endogenous cortisol secretion. ACTH therapy may be a promising treatment for patients with nephrotic syndrome resistant to conventional therapies like corticosteroids and immune suppression. Results available from the limited studies using ACTH therapy are encouraging. Further studies in this area using large sample size and long term follow up are warranted.

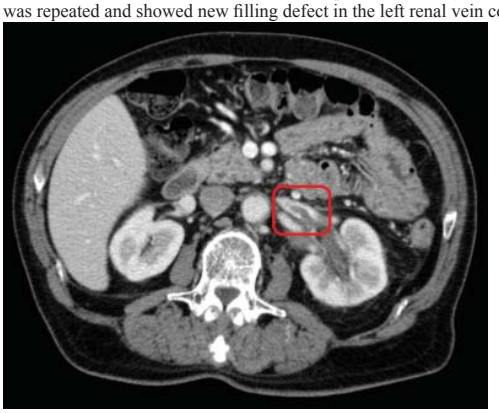
*Funding:* Pharmaceutical Company Support - Questcor Pharmaceuticals

## FR-PO1089

**Unusual Cause of Renal Vein Thrombosis** Mohammad Sharif,[1] Navin Jaipaul,[2] Seyed-ali Sadjadi.[2] *[1]Nephrology, Loma Linda Univ Medical Center, Loma Linda, CA; [2]Nephrology, Veterans Affairs Loma Linda Healthcare System, Loma Linda, CA.*

**Background:** Renal vein thrombosis (RVT) has numerous etiologies; such as, nephrotic syndrome, malignancy, hypercoagulable state, oral contraceptive pills use, steroids therapy, trauma, and kidney transplant. We present a case of an elderly gentleman who developed RVT after laser lithotripsy for obstructive nephrolithiasis.

**Methods:** A 76-year-old male, known to have hypertension, coronary artery disease, stroke and dyslipidemia presented with left flank pain, hematuria and left renal angle tenderness, was found to have left ureteric stone with hydronephrosis which was diagnosed by abdomen computed tomography (CT) scan. He underwent left ureteroscopic laser lithotripsy with stent placement. Pain and acute kidney injury improved and he was discharged home. A few days later, he pulled the string of the stent and he was able to remove it. Then two days after, he started to complain of left flank pain. Laboratory evaluation showed Creatinine decreased compared to discharge value. Abdominal CT scan was repeated and showed new filling defect in the left renal vein consistent with RVT.



He was started on anticoagulation. Extensive work up showed no evidence of glomerular disease, malignancy or thrombophilia. At 3-month follow up, the pain had subsided and a repeat CT scan showed resolution of the thrombosis.

**Conclusions:** Laser lithotripsy is not a recognized etiology for RVT. In this case, common causes were ruled out and the proximity of the procedure to his presentation made RVT likely induced by laser lithotripsy, which could be explained by edema and pressure induced by instrumentation of the ureter or by pressure induced when he self-retracted the stent or combination of both. This case could be an eye opener for unknown etiology of RVT or possibly underdiagnosed etiology of flank pain post laser lithotripsy.

## FR-PO1090

**Novel Mutations in CLDN16 Gene in a Belgian Patient with Hypomagnesemia, Hypercalciuria and Nephrocalcinosis** Pauline Erpicum,[1] Laurent E. Weekers,[1] Emilie Castermans,[2] Vincent Bours,[2] Jean-marie H. Krzesinski,[1] Francois Jouret.[1] *[1]Nephrology, ULg CHU, Liege; [2]Genetics, ULg CHU, Liege.*

**Background:** Hypomagnesemia, hypercalciuria and nephrocalcinosis (HHN) is a rare autosomal recessive tubular disorder characterized by urinary losses of $Mg^{2+}$ and $Ca^{2+}$. HHN leads to end-stage renal disease (ESRD) in early adulthood. HHN is genetically heterogeneous, with mutations in *CLDN16* or *CLDN19* genes.

**Methods:** At the age of 19, our patient presented with a loss of consciousness secondary to dehydration. His medical history included dehydration at birth, arrhythmia and polyuria/polydipsia with nycturia. His father and uncle had kidney stones. Physical examination was unremarkable. Blood parameters: creatinine [17.5mg/l], ionized $Ca^{2+}$ [1.12mM], $Mg^{2+}$ [0.61mM], uric acid [87mg/l], PTH [167pg/ml]. Urine analyses: increased fasting $Ca^{2+}$/creat. ratio (0.21), proteinuria (1.3 g/g creat.), hypocitraturia. Uroscan evidenced bilateral medullary calcinosis. Oral $Ca^{2+}$-loading test induced an appropriate inhibition of PTH secretion. Kidney biopsy showed tubular atrophy with von kossa-positive deposits, as well as glomerular segmental and focal hyalinosis. Genetic analyses identified 2 novel mutations in the *CLDN16* gene: c.340C>T (p.R114*) and c.427+5G>A. Treatment included ACE inhibitors and thiazides, as well allopurinol, statins and $Mg^{2+}$-citrate. Proteinuria got normalized in contrast to serum $Mg^{2+}$ levels. Five years later, the patient reached ESRD requiring kidney transplantation (KTx) from a deceased donor. Immunosuppression included FK506, MMF and methylprednisolone. Serum $Mg^{2+}$ levels remained low at 7 months post KTx.

**Conclusions:** The pathogenesis of ESRD in HHN remains debated. *CLDN16* genotype predicts renal decline. Our patient met the criteria for accelerated loss of renal function, with dehydration at birth and GFR decline estimated at 8.2 ml/min per 1.73$m^2$/year. The

apparent compound heterozygosity includes one nonsense mutation truncating the protein in the first extracellular domain and one intronic mutation of a highly conserved nucleotide close to exon 2 donor splice site. Their impact on claudin 16 expression, function and distribution remains to be determined.

*Funding:* Clinical Revenue Support

**FR-PO1091**

**Renal Cell Carcinoma Associated with Xp 11.2 Translocation in Autosomal Dominant Polycystic Kidney Disease** Hyuk Huh,[1] Hayne C. Park,[1] Yongjin Yi,[1] Miyeun Han,[1] Young-Hwan Hwang,[2] Curie Ahn.[1] *[1]Dept of Internal Medicine, Seoul National Univ Hospital; [2]Dept of Internal Medicine, Eulji General Hospital.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary cystic disease. The prevalence of renal cell carcinoma (RCC) has been reported to be higher in ADPKD patients with advanced renal failure. Xp11.2 translocation RCC is related to translocation of several genes involving the transcription factor E3 (TFE3) gene. Xp11.2 translocation RCC have an aggressive clinical course with tendency to spread to perirenal lymph nodes in adults. This is the first case report of Xp11.2 translocation RCC in an ADPKD patient.

**Results:** A 38-year-old woman was diagnosed of ADPKD in 1996. She had multiple cysts in kidney and liver. 10 x 11cm sized cystic lesion with inner septation and well-enhancing solid portion was incidentally detected in her right kidney upper pole on 2004. The cystic mass was followed up by biennial abdominal CT or ultrasonography and found to be stable without change in morphology or size until January 2011. Then, the size of cystic mass began to increase from 10 x 11 to 10 x 12 cm, and lymph node enlargement was newly found in the retrocaval and aortocaval areas. Cyst aspiration and cytologic examination did not show malignant cells. After 6 months, the size of cystic mass further increased to 10.7 x 12.7cm. In October 2011, we decided to perform diagnostic and therapeutic right radical nephrectomy. Microscopic examination of the right kidney revealed RCC containing clear cell components with strong TFE3 expression. The FISH confirmed the diagnosis of Xp 11.2 translocation by showing break-apart signal in TFE3 gene. Shortly after the diagnosis, multiple metastatic lesions were detected in lung and she has been treated by systemic chemotherapy.

**Conclusions:** In general, Xp 11.2 translocation RCC is reported to appear in the childhood and shows female preponderance. It accounts for approximately 5% to 20% of RCCs in pediatric patients, but is much less common in adults RCCs (1.6%-5%). This case suggests that Xp 11.2 translocation RCC change the clinical manifestation of renal mass dynamically in ADPKD patients.

**FR-PO1092**

**Ezetimibe, Hyperoxaluria and Nephrolithiasis** Ivan E. Porter, William E. Haley. *Nephrology/Hypertension, Mayo Clinic, Jacksonville, FL.*

**Background:** Excessive urinary excretion of oxalate can manifest as recurrent urolithiasis, nephrocalcinosis and progression to ESRD. This is described in primary hyperoxaluria and also with use of the lipase inhibitor orlistat. I present two cases of hyperoxaluria associated with the use of ezetimibe, a selective cholesterol absorption inhibitor.

**Methods:** Case1: 74-year-old Caucasian female was evaluated for renal insufficiency. She had no history of kidney stones, but hyperlipidemia, intolerant of statins, treated with ezetimibe starting about 10 years prior. Her urinalysis was unremarkable and 24 hr urine showed supersaturation of calcium oxalate and U oxalate of 0.47 mmol/spec. Renal US showed a 6mm nonobstructing stone in the right lower pole. Following discontinuation of ezetimibe, 24 hr urine testing showed reduction of U oxalate. Case2: 53-year-old male presented with a history of nephrolithiasis since age 18. He had passed about 50 stones in his lifetime prior to a subacute kidney injury requiring a kidney biopsy revealing calcium oxalate crystals in tubules and intratubular deposition consistent with hyperoxaluria and oxalosis. Genetic testing confirmed primary hyperoxaluria type 1 with mutations in both AGXT and GRHPR. His history of hyperlipidemia was controlled with Vytorin 10/20 for many years. Vytorin was discontinued with subjective decrease in urinary tract symptoms and passage of stones.

**Conclusions:** These cases raise the possibility of a role of ezetimibe in hyperoxaluria and calcium oxalate stone formation. Previous studies suggest that the use of lipase inhibitors is associated with an increase in urinary oxalate along with a decrease in urine calcium and urine magnesium. Fat and bile acids react with calcium in the gut preventing binding with free oxalate, raising intestinal oxalate and thus urinary excretion of oxalate and a predisposition to calcium oxalate stones. This same association has not been described with the selective cholesterol absorption inhibitor ezetimibe. Based on the mechanism of action of ezetimibe and the findings of a relationship between stone formation and the use of orlistat, ezetimibe use should be considered in the evaluation and management of patients with nephrolithiasis.

**FR-PO1093**

**Use of Cinacalcet for Treatment of Hypercalcemia of Malignancy Refractory to Conventional Therapies** Sean R. Campbell,[1] Carlos D. Flombaum,[2] Ilya Glezerman.[2] *[1]Dept of Nephrology and Hypertension, Weill Cornell Medical College, New York, NY; [2]Renal Div, Memorial Sloan-Kettering Cancer Center, New York, NY.*

**Background:** Hypercalcemia of malignancy (HCM) occurs either due to tumor bone invasion causing increased osteoclastic activity or in humoral HCM due to tumor secreted parathyroid hormone related peptide (PTHrP) direct effect on the bone. Initial treatment of hypercalcemia is intravenous (IV) hydration, IV bisphosphonates and, recently, denosumab. Cinacalcet is a calcimimetic agent that binds tissue calcium sensing receptor (CaSR). It has been used to treat hyperparathyroidism and parathyroid carcinoma. We present a case of a patient with HCM, which was resistant to standard therapy but responded to cinacalcet.

**Methods:** The patient was a 66 years old male with history of metastatic renal cell carcinoma. He had developed hypercalcemia with serum Ca (sCa) of 11.4 (8.5-10.5) mg/dl and was started on bisphosphonates and hydration. CT scan of the pelvis was significant for iliac lytic lesions, PTH was 10 (12-88) pg/dl and PTHrP 114 (14-27) pg/dl. Due to ongoing hypercalcemia two doses of denosumab (120mg) were given with no response. After 4 months of worsening hypercalcemia patient was hospitalized with sCa level of 14.2 mg/dl. He was started on cinacalcet 30mg daily and at discharge sCa was 11.0 mg/dl. One week later sCa was 11.4 mg/dl and cinacalcet was increased to 60 mg daily. Ten weeks after initiation of cinacalcet sCa was 10.1 mg/dl and PTHrP level was 159 pg/ml.

**Conclusions:** CaSR is expressed in many tissues including bone, intestine, renal tubules and in malignant tissue secreting PTHrP. Cinacalcet may act by binding CaSR and lowering PTHrP level, increasing calciuria, decreasing intestinal calcium absorption and decreasing calcium efflux from bone. Recently cinacalcet was used in a patient with non small cell lung cancer and humoral HCM resulting in lowering of SCa and PTHrP levels. In our patient the PTHrP levels did not decrease and cinacalcet therapeutic effect was likely mediated via its effect on non-malignant tissues. Cinacalcet is a well-tolerated drug with a low side effect profile which warrants further investigation as treatment of HCM.

**FR-PO1094**

**Hypercalcemia Induced by Ectopic Overproduction of Calcitriol in a Hemodialysis Patient with Rosai-Dorfman Disease** Mai Uemura,[1] Shunsuke Yamada,[1,2] Masanori Tokumoto,[2] Kazuhiko Tsuruya,[1] Takanari Kitazono.[1] *[1]Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Div of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan.*

**Background:** Hypercalcemia is usually associated with the excessive use of Ca-containing Pi-binders or VDRA in dialysis patients. Extra-renal overproduction of calcitriol occasionally leads to hypercalcemia. We report the case of a hemodialysis patient with Rosai-Dorfman disease, who developed hypercalcemia as a result of the ectopic overproduction of calcitriol.

**Methods:** A 65-year-old male receiving hemodialysis for 33 years was admitted because of declining consciousness and fever. His laboratory data showed an increase in his corrected serum Ca (12.8 mg/dL) and CRP levels, while his serum PTH and PTHrP levels were normal. His serum calcitriol level (26 pg/mL) was much higher than the average value in hemodialysis patients. Computed tomography showed enlargement of the systemic lymph nodes. A lymph node biopsy showed massive proliferation of histiocytes with the phagocytosis of intact blood cells (emperipolesis) in the dilated sinuses. The final diagnosis was “Rosai-Dorfman disease”. Interestingly, an immunohistochemical analysis of the affected lymph nodes disclosed many histiocytes (Rosai-Dorfman cells) positive for 1α-hydroxylase, provided evidence for the extra-renal overproduction of calcitriol. Oral prednisolone therapy attenuated inflammation and decreased the serum calcitriol level (9 pg/mL), followed by the normalization of hypercalcemia (8.6 mg/dL) and consciousness, and also a remission of the lymph node swelling.

**Conclusions:** Rosai-Dorfman disease is a benign lymphadenopathy characterized by the massive proliferation of histiocytes with emperipolesis in the dilated sinuses of lymph nodes (Rosai J et al. Arch Pathol 1969). The etiology is unknown, but an association with immunological disorders and the effectiveness of corticosteroid therapy are assumed. Hypercalcemia induced by Rosai-Dorfman disease has been rarely reported. This case is worth reporting, because we have demonstrated that the ectopic overproduction of calcitriol in the lymph node led to hypercalcemia.

**FR-PO1095**

**Proteomics in Guaifenesin Induced Nephrolithiasis: A Pseudomatrix Stone** Faheemuddin A. Ahmed,[1,2] Ann M. Kolbach,[2] Samuel R. Cohen,[2] Neil S. Mandel,[1,2] Jeffrey Wesson.[1,2] *[1]Medical College of Wisconsin, Milwaukee; [2]Zablocki VA Medical Center, Milwaukee, WI.*

**Background:** A number of drugs, including Guaifenesin, have been reported to cause kidney stones, but there are no proteomic reports on these stones. We present a case that was referred for evaluation of matrix stones, which turned out to be a guaifenesin-related kidney stone with a matrix-like component.

**Methods:** A 30 year-old Caucasian woman with no medical history presented at age 27 with renal colic and was found to have acute kidney injury. Work-up revealed bilateral nephrolithiasis with hydronephrosis and had subsequent stent placement. Urine sediment analysis was reported as 100% mucin and no other known stone material. Her symptoms resolved and creatinine returned to baseline. Her medications included potassium citrate

and allopurinol. Her OTC medications included mucinex and claritin for allergy symptoms. The patient was referred to our clinic and enrolled in our IRB approved study. Laboratory evaluation showed normal blood tests. Her urorisk panel revealed low urine volume, low citrate, high sodium, high oxalate, high uric acid and pH 5.5. The patient experienced bilateral flank pain similar to prior episodes and collected a urine sample that showed three discrete phases: urine, mucus strand and sediment. Microscopy of the urine sediment showed spherical, birefringent crystals. FTIR analysis of the sediment revealed 40% guaifenesin and 60% protein. The sediment was fractionated into the drug and protein isolate using ultrafiltration. Standard gel electrophoresis performed on the urine proteins as compared to the sediment proteins revealed increased concentrations of Tamm Horsfall glycoprotein, transferrin and osteopontin and decreased concentrations of albumin and zinc alpha 2 glycoprotein. These findings were further confirmed with mass spectroscopy.

**Conclusions:** Drug-induced kidney stones can mimic matrix stones and can be misdiagnosed with insufficient or inaccurate stone analysis. The guaifenesin associated proteome was found to be different from that reported in the literature for both calcium and matrix stones.

*Funding:* NIDDK Support

## FR-PO1096

### Calciphylaxis Presenting as Progressive Gangrene of the Digits and Penis

Vasil Peev, Sharad Virmani, Ali Nayer. *Div of Nephrology and Hypertension, Univ of Miami, Miami, FL.*

**Background:** Calciphylaxis, known as calcific uremic arteriolopathy, is characterized by slowly progressive necrosis of skin and subcutaneous tissue secondary to calcification and thrombosis of small- and medium-sized arteries. It manifests most commonly in Middle-aged individuals with renal disease involving their abdomen, legs, and breasts. The digits and penis are rarely affected.

**Methods:** A 49-year-old man presented with gangrene of the fingers and penis. Past medical history included diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, peripheral vascular disease, and end-stage renal disease on hemodialysis for 5 years. Physical examination revealed dry gangrene of the fingers (A, B), penis, and scrotum. Dusky red discoloration of right lateral great toe was also noted (C). Serum concentration of albumin was 1.7 g/dL, calcium 7.7 mg/dL, phosphorus 5.0 mg/dL, and parathyroid hormone 162 pg/mL. Laboratory tests revealed no cryoglobulins, cold agglutinins, cryofibrinogen, lupus anticoagulant or Prothrombin G20210A mutation. Antibodies directed against cardiolipin, beta2 glycoprotein I, myeloperoxidase, proteinase 3, platelet factor 4, nuclear antigens, hepatitis B and C were negative. Serum concentrations of antithrombin, protein S, protein C and complement C3 and C4 were normal. Gangrene of the affected areas worsened (D-F) during work-up. Radiographs of hands and feet revealed vascular calcification (G-J). Skin biopsy revealed calcified subcutaneous small blood vessels consistent with calciphylaxis (Von Kossa stain) (K). Microthrombi were noted in subcutaneous small blood vessels (L, M).


**Conclusions:** This case illustrates a striking presentation of a rare and life-threatening disorder. Similarly presenting conditions like antiphospholipid syndrome, cryoglobulinemia, Waldenstrom's macroglobulinemia, thrombophilia, and scleroderma were excluded.

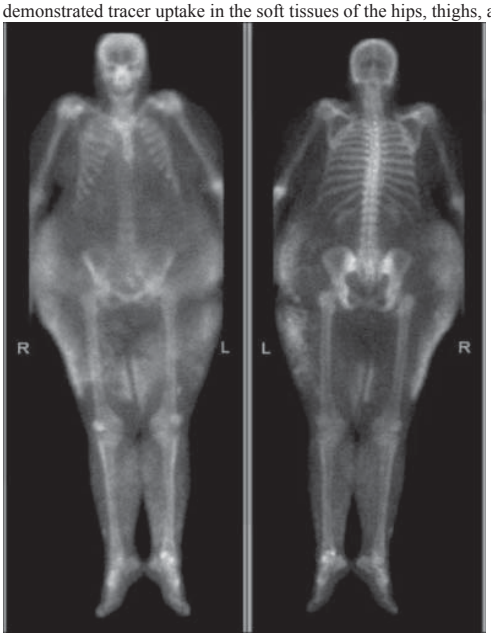
## FR-PO1097

### Calciphylaxis Presenting as Myopathy

Eric S. Kerns, Jose F. Rueda. *Div of Nephrology and Hypertension, Dept of Medicine, Oregon Health and Science Univ, Portland, OR.*

**Background:** Calciphylaxis typically presents with necrotic, painful skin ulcers. We describe the case of a woman who developed severe bilateral leg pain in the absence of visible lesions. Technetium-labeled bone scintigraphy was used to make the diagnosis.

**Methods:** A 63 year old woman with a history of obesity, diabetes, pulmonary embolism on coumadin, and end-stage renal disease (ESRD) due to lupus nephritis was admitted with leg pain. On exam, there were subcutaneous nodules along the thighs without overlying skin changes. Serum calcium was 12.6 mg/dl (normal <10.2), phosphorus was 6.0 mg/dl (normal <4.7), and calcium-phosphate product was 75.6. Erythrocyte sedimentation rate was >120 mm/hr (normal <30). A nerve conduction study showed myopathy. A bone scan demonstrated tracer uptake in the soft tissues of the hips, thighs, and calves.




The diagnosis of calciphylaxis was confirmed with a biopsy of the thigh showing medial calcification in small blood vessels.

**Conclusions:** Calciphylaxis is a disease caused by arteriolar calcification leading to tissue ischemia and necrosis. The mortality rate approaches 50% at one year. Most patients with calciphylaxis have ESRD or kidney transplant. Additional risk factors include hyperparathyroidism, an elevated calcium-phosphate product, female sex, diabetes, obesity, warfarin use, and chronic inflammation. Classic skin lesions are livedo reticularis and necrotic ulcers, though skeletal muscle myopathy has been described in the absence of cutaneous findings. Imaging modalities that aid in the diagnosis are mammography and bone scintigraphy. Catching the diagnosis at an early stage, prior to skin ulceration, confers a greater chance of treatment response. Thus, in patients with ESRD, calciphylaxis should be considered in the differential diagnosis for myopathy, and a reasonable non-invasive diagnostic modality is bone scintigraphy.

## FR-PO1098

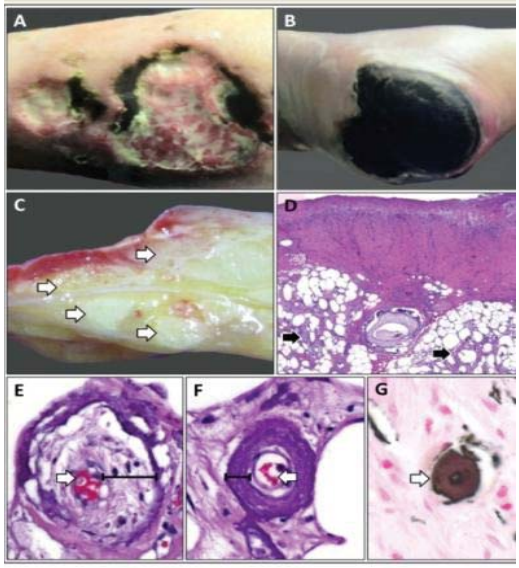
### Pre-Uremic Calciphylaxis

Sharad Virmani, Loay H. Salman, Ali Nayer. *Div of Nephrology and Hypertension, Univ of Miami Miller School of Medicine, Miami, FL.*

**Background:** Calciphylaxis, also known as calcific uremic arteriolopathy, is a life-threatening disorder characterized by calcification and thrombosis of small- and medium-sized arteries resulting in ischemic necrosis of skin and subcutaneous tissue. The pathogenesis is incompletely understood. Primarily diagnosed in dialysis patients, calciphylaxis is rarely diagnosed in patients with CKD in the pre-uremic phase.

**Methods:** A 72-year-old obese Caucasian woman with CKD presented with worsening left lower leg pain and purplish skin discoloration with ulcerations on the heel and dorsal foot.Past medical history was significant for long-standing diabetes mellitus, hypertension, peripheral vascular disease, secondary hyperparathyroidism, and deep vein thrombosis. Physical exam revealed a 10-cm calf ulcer and a violaceous eschar of the heel (Figure1). Significant tenderness to palpation was present in the left upper thigh, calf and foot. Serum creatinine was 3.1mg/dl, calcium of 7.9mg/dl,phosphorous of 6.6mg/dl, and albumin of 1.7g/dl.Angiography revealed occluded left superficial femoral artery,popliteal artery and distal tibial artery.A left below-the-knee amputation was performed.Gross examination revealed calciphylaxis within adipose tissue and severe calcific atherosclerosis (Figure1). Histologic examination disclosed calciphylaxis among large and small vasculature with Van Kossa staining.

FIGURE




**Conclusions:** We highlight calciphylaxis in a patient with chronic kidney disease before starting dialysis. When affecting distal limbs, calciphylaxis can clinically resemble atherosclerotic peripheral vascular disease. This case also emphasizes that calciphylaxis and peripheral vascular disease share many risk factors and can coexist in the same patient. This case increases the awareness to a disorder that could be disguised as peripheral vascular disease.

## FR-PO1099

**Successful Treatment of Recurrent Calciphylaxis Using Sodium Thiosulfate in a Patient with Normal Renal Function** Ankur Sharma, Burl R. Don. *Div of Nephrology, Univ of California, Davis, Sacramento, CA.*

**Background:** Calciphylaxis (CPX), also known as calcific uremic arteriolopathy, is a clinical condition of accelerated dermal arterial medial calcification resulting in severe cutaneous ulcerations and eshcar formation. It is seen generally in patients with advanced chronic kidney disease (CKD) and carries a poor prognosis. Sodium thiosulfate (STS) has been used with varying degress of success in the treatment of CPX in patients with CKD, but its use for patients with recurrent CPX and normal renal function has not been described.

**Methods:** A 53 year old woman with alcoholic liver disease and normal renal function was admitted to the hospital with preliminary diagnosis of severe bilateral lower extremity cellulitis refractory to outpatient antibiotics and cutaneous thigh ulcers. She underwent debridement of the non healing ulcers and a subsequent skin biopsy noted vascular calcification consistent with CPX. The patient had normal creatinine, calcium, phosphorus, parathyroid hormone, 25-OH and 1,25-OH vitamin D blood levels. Treatment with IV STS, 25 g thrice weekly was intiated and after two months of therapy, there was dramatic improvement of the ulcers and reduction of the cutaneous inflammation. The STS was discontinued, but after one month. She had a relapse with recurrent violaceous inflammation with the development of plaque-like nodular densities and early ulceration in her thighs. Additional lab work noted normal FGF-23, complement, PTH related peptide, protein S, protein C blood levels and a negative rheumatologic panel. STS was restarted and she had marked improvement in her cutaneous lesions within 1 month.

**Conclusions:** CPX in the setting of normal renal function is unusual and has been described in association with primary hyperparathyrodism, chronic liver disease, Crohn's disease, warfarin use, systemic steroids and malignancy. The use of STS in the treatment of CPX and normal renal function has been noted, but there are no randomized trials assessing its efficacy. This case establishes that STS is effective therapy for CPX inasmuch as discontinuation of STS resulted in relapse and retreatment resulted in remission. STS is an effective therapy for recurrent CPX.

## FR-PO1100

**Secondary Hyperparathyroidism in Association with Combined Use of Proton Pump Inhibitors and Intravenous Magnesium** Sami Safadi, Qi Qian. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** The interplay between magnesium and calcium is complex and crucially influences calcium homeostasis. We describe a unique case of secondary hyperparathyroidism associated with combined use of PPI and IV magnesium.

**Methods:** 49-year old woman with ulcerative colitis that required total colectomy and ileostomy was diagnosed with severe magnesium deficiency. Her initial magnesium level was near 0 mg/dL. Oral magnesium was started, but due to intolerance, it was switched to IV magnesium sulfate. The dose was titrated up to 4 grams of magnesium sulfate twice weekly. This was continued for almost a year, but her blood magnesium level remained low between 1.0 to 1.2 mg/dL. She remained quite weak and unable to work. The patient was then referred to our clinic. Her examination revealed a magnesium IV pump imbedded subcutaneously in the mid-abdomen, and a colostomy bag in the right lower quadrant. Labs revealed a creatinine of 0.7 mg/dL (ref. 0.6-1.1), Calcium of 10.7 mg/dL (ref. 8.9-10.1), and magnesium of 1.5 mg/dL (ref. 1.7-2.3). PTH was 105 pg/ml (ref. 15-65). A parathyroid scan showed two foci of increased sestamibi radiotracer uptake suspicious for adenomas. Her urine calcium excretion was normal. The patient's IV magnesium was stopped, and she was converted to an oral magnesium regimen. Oral PPI was discontinued as it likely had interfered with magnesium absorption from the GI tract. Her magnesium normalized to 1.7-2.0 mg/dL. Her PTH normalized as well (as per her local MD records) and a repeat scan one year later showed stable appearance.

**Conclusions:** We present here an unusual case of severe hypomagnesemia that's likely caused by oral PPI intake, and secondary hyperparathyroidism likely triggered by repeat IV magnesium. We hypothesize that severe hypomagnesemia diminishes PTH secretion. However, episodic IV magnesium infusions release the block on PTH secretion. Repeated parathyroid stimulation by high magnesium levels results in secondary hyperparathyroidism. This case cautions us to avoid using chronic IV magnesium as a treatment for total body magnesium depletion as it would not restore magnesium homeostasis, and can induce abnormalities in the PTH-Ca axis.

## FR-PO1101

**Normocalcemic Hyperparathyroidism Presenting with Multiple Brown Tumors Mimicking Malignancy in a Kidney Transplant Recipient** Shuang Ying Bao,[1] Alan Mark Weinstein,[1] Sheron Latcha,[1,2] Ilya Glezerman.[1,2] *[1]Dept of Nephrology and Hypertension, Weil Cornell Medical College, New York, NY; [2]Dept of Nephrology, Memorial Sloan-Kettering Cancer Center, New York, NY.*

**Background:** Although secondary hyperparathyroidism is common in dialysis patients, it resolves after renal transplantation in parallel with improvement in renal function. Some patients develop tertiary hyperparathyroidism and hypercalcemia which persists even after the transplantation. We present a case of normocalcemic hyperparathyroidism in a transplant patient.

**Methods:** Case Description: 36 years old female patient with history of renal transplant in 2005 presented with left arm pain. Plain X-ray showed 7 cm area of lytic bone lesion. PET scan showed multiple bone lesions involving cervical and lumbar spine, humerus, femoral neck, iliac and clavicle bone. Patient underwent fixation of the left humerus and right hip hemiarthroplasty. Bone biopsy showed giant cell rich lesion consistent with brown tumor. Parathyroid hormone (PTH) was 1947 pg/ml (12-88), serum creatinine 1.3 mg/dl (0.6-1.3), ionized calcium 4.7 mg/dl (4.8-5.3), Phos 2.9 mg/dl (2.4-4.7). Review of labs showed that patient's calcium and phosphate levels have been normal since the transplantation. No data prior to the transplantation was available for review. Thyroid ultrasound and parathyroid nuclear scan revealed possible left parathyroid adenoma.

**Conclusions:** Brown tumor is a rare manifestation of tertiary hyperparathyroidism in kidney transplant recipients and is associated with hypercalcemia. Our case shows that the calcium level may be normal in that setting. It is prudent to obtain baseline PTH level in all transplant patients to screen for hyperparathyroidism.

## FR-PO1102

**Brown Tumor: A Serious Complication of an Old Disease** Rodrigo Amblard Wanderley, Márcia Avelino, Jose Edevanilson Gueiros, Ana Paula Gueiros. *Nephrology Div, IMIP, Recife, Pernambuco, Brazil.*

**Background:** Brown Tumor (BT) is a serious consequence of secondary hyperparathyroidism (SHPT). The majority of cases report the maxilla and mandible as the main sites of occurrence. Symptoms result from considerable dimensions of the tumor and its localization. When BT involves the face and has progressive growth, it may cause severe deformities, discomfort, and alteration of the masticatory apparatus.

**Methods:** The objective of this study was to describe the case of a patient with BT in the face provoked by severe SHPT. Case: Patient of 32 years, male and on hemodialysis for 10 years. He has developed in six months a tumor located in the fast growing region of the face, affecting the palate. The lesion expanded so severely that destroyed the entire base of the nose, prevented the closure of the mouth and dramatically reduced the space between the palate and tongue. As a result, the patient presented with difficulty breathing and swallowing and also reported intense and generalized bone pain, muscle weakness, loss of height, and weight loss. Patient reported unsuccessful attempts SHPT treatment with calcitriol and sevelamer. Laboratory tests evidenced iPTH 2527pg/mL, calcium 8.1 mg/dL, phosphorus 4.0 mg/dL, alkaline phosphatase 2024 IU/L, 25OH vitamin D 58.9 pg/mL and hemoglobin 5.2 g%. The CT scan demonstrated change in bone volume and texture, highlighting a greater impairment of mandible and maxillary bones, identifying numerous erosions linear aspect, characterized as an aspect known as uremic bone leonine facies. On June 12, 2012, patient underwent parathyroidectomy (PTX) with full self-implantation. Improvement of bone pain, in addition to decreased tumor mass in the face, allowing him to better breathing and swallowing. One year after PTX, patients had weight gain of 13 pounds and his iPTH was 81pgmL.

**Conclusions:** BT represent a serious presentation of cystic fibrosis osteitis caused by SHPT. Although benign, can grow aggressively, causing destructive lesions and morbidity. Surgical PTX is the most adequate therapeutic approach in patients with unsatisfactory response to medical treatment.

## FR-PO1103

**Multiple Bone Lesions in Cinacalcet Era. A Case Report** Natacha Rodrigues,[1] Estela Nogueira,[1] Maria Alice Gonçalves Fortes,[1] Tiago Rodrigues,[3] Antonio Gomes da Costa,[1] Andre L. Weigert,[2] Sofia C.A. Jorge.[1] *[1]Nephrology, CHLN, Portugal; [2]Nephrology, CHLO, Portugal; [3]Radiology, CHLN, Portugal.*

**Background:** Chronic Kidney Disease (CKD) commonly evolves with disturbances in mineral and bone metabolism, currently defined as Chronic Kidney Disease - mineral and bone disorder (CKD-MBD). Although, management strategies have changed and progressed along the years, our knowledge is still sparse and consensus in treatment has not been achieved. Severe complications of high turnover bone disease have been significantly reduced after the emergence of calcimimetic agents. Nevertheless, sporadic cases of brown tumors in patients with controlled hyperparathyroidism have been reported in the literature. They result from increased osteoclast activity and if left untread can have disastrous consequences.

**Methods:** The authors describe a case of a 42-years-old female, undergoing hemodialysis in the last 13 years, that developed a scull lesion that was submitted to surgery and several weeks later was admitted in the hospital with severe low back pain with radiation to the left limb. CT scan revealed several lytic lesions in the jaw, dorsal vertebrae, right acetabulum, pubis and ilium (with extraosseous component of 7,5 cm) and full body X ray demonstrated lesions in the right metacarpal bones.

A biopsy of the iliac bone was compatible with brown tumor. Retrospectively, PTH levels in the last 6 months were 300 to 600 ng/l, under treatment with vitamin D analogs and cinacalcet.The gravity of this case, with multiple bone lesions, severe pain and possible neurologic impairment lead to the decision to parathyroidectomy. Following the intervention, bone pain disappeared and the lesions regressed.

**Conclusions:** This case enphasizes the importance of considering brown tumors when approaching a CKD patient with bone lesions even if PTH levels appear fairly controlled. Treatment generally requires parathyroidectomy.

## FR-PO1104

**The Formation Process of "White Kidney" in a Patient with Late Onset Primary Hyperoxaluria Type I** Emiko Kono, Junichiro J. Kazama, Michihiro Hosojima, Ichiei Narita. *Clinical Nephrology and Rheumatology, Niigata Univ, Niigata, Japan.*

**Background:** Primary hyperoxaluria type I (PHO-I) is a peroxisome disease in which glyoxlate metabolism is congenitally impaired. A characteristic whole kidney calcification called "white kidney" is seen in its advanced stage, while its formation process remains obscure.

**Methods:** A 62-year-old man with no particular personal or family medical histories visited Niigata University Medical and Dental Hospital complaining of fatigue. Although he had received regular medical check up every year, kidney dysfunction was not detected. Fever persisted for 1 week, and thereafter his appetite decreased for 2 months untill his visit. His serum urea nitrogen level was 52 mg/dl and the creatinine level was 7.39 mg/dl. The first abdominal computed tomography displayed a thin faint calcified lesion at the cortico-medullary border of bilateral swollen kidneys. Biopsied kidney tissue demonstrated scattered intratubular crystal depositions around the cortico-medullary border. Both the serine/pyruvate aminotransferase and the serine/glyoxylate aminotransferase activities in his biopsied liver samples were below detectable levels. He was initiated into a maintenance hemodialysis therapy. The second abdominal CT was performed 4 months later. The thickness and intensity of the cortico-medullary border calcification increased, and the 3-dimensional image reconstruction revealed the extension of the calcified lesion to renal columns. The third abdominal CT scan was performed 1 year later. Bilateral whole kidney cortex was calcified, which showed a typical "white kidney" appearance. A 3-dimensional image revealed that neither renal papillae nor renal pelvises were calcified even at this disease stage.

**Conclusions:** Most cases of late onset PHO-I have clinical episodes that would cause acute kidney injury before its onset. The cortico-medullary border is the most sensitive area to acute kidney injury related ischemia. We concluded that calcium oxalate crystals are initially deposited in the damaged tubular lumen, then calcified deposits extend into the upstream direction, and finally form white kidney.

## FR-PO1105

**Peritoneal Dialysis Peritonitis Associated with Beta Cap Adapters: Quality Improvement Project** Pavan Devulapally, Sudhir R. Thaduri, Dumitru Rotaru. *Dept of Nephrology, Univ Arakansas for Medical Sciences, Little Rock.*

**Background:** Puncture holes and catheter disconnection of the PD catheter is an uncommon occurrence.

**Methods:** Between Dec 2012 and Apr 2013 we identified 7 patients in our Davita PD clinic with puncture holes at the junction of PD catheter and the proximal end of the Beta cap adapter. 1 patient also had an episode of disconnection of the transfer set from the distal end of the adapter. As a consequence PD peritonitis occurred in 2 patients associated with puncture hole events and in the patient with the disconnection. 2 patients needed hospitalization and 1 attended the ER.One of the catheters was removed due to relapsed PD peritonitis.

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Puncture hole | Dec 1 | Feb 1 | Feb 19 | Mar 19 | Mar 20 | Mar 22 | Apr 19 |
| Disconnection | | | Mar 20 | | | | |
| Peritonitis | MSSE Dec2<br>MRSE Dec 28 | | Pepto streptococcus | MSSE | | | |
| Catheter removed | Yes | | | | | | |
| Hospitalised | Yes | | ER visit | | | Yes | |

All events were seen in 35 DaVita PD clinic patients using the beta cap adapters. No events were found in our 27 VA PD clinic patients where the titanium adapters are used.

**Results:** A similar experience with catheter holes associated with beta cap adapters was reported in 2012. These authors felt that these events were related to a recent change in the design of the proximal end of adapter from a rounded edge to a sharper edge. We also found an FDA class II recall in 2011 and an ECRI alert in 2012 both reporting a reduction in thread engagement which may lead to a risk of separation of transfer set from the distal end of the adapters.

**Conclusions:** We undertook a QI project with goals to decrease the risk of PD peritonitis, improve quality of patient care in our clinic and increase the awareness of this complication. We reported our findings to the UHC Patient Safety Net, DaVita Dialysis management, company representative and at our QA Faculty Meeting. Based on the experience with the titanium adapter in our VA PD clinic and also from previous literature reports we decided to use the titanium adapters for all new and existing PD catheters in our clinic.

## FR-PO1106

**Continuous Renal Replacement Therapy (CRRT) in Times of Calcium Shortage, Do We Have Options?** Nitin Relia, Yusra R. Cheema, Rupal Mehta, Cybele Ghossein. *Div of Nephrology and Hypertension, Northwestern Univ, Chicago, IL.*

**Background:** The use of citrate as regional anticoagulation in patients on continuous renal replacement therapy (CRRT) requires IV calcium to maintain therapeutic systemic ionized calcium levels. The shortage of IV calcium has limited the use of citrate. We present a case of a patient on CRRT managed with the use of citrate and only commercially available calcium containing replacement solution.

**Methods:** The patient is an 80 year old male admitted to the Surgical Intensive Care Unit for repair of a leaking abdominal aortic aneurysm. Postoperatively, he developed Acute Kidney Injury (AKI) necessitating CRRT. Continuous Veno Venous Hemofiltration (CVVH) was initiated on Prismaflex machine using a HF 1400 filter but was limited by multiple clotting episodes. Citrate, in the form of Acid Citrate Dextrose (ACD), was initiated as an anticoagulant at a rate of 300 ml/hr pre-filter and rates adjusted in units of 10 ml/hr to target circuit ionized calcium of 0.25-0.35 mmol/L. The blood flow rate was set at 200 ml/hr and post-filter replacement solution containing 3.5 mEq/L of calcium chloride was started at a rate of 2L/hr giving a total of 3.5 mmol of calcium/hr.

Figure 1

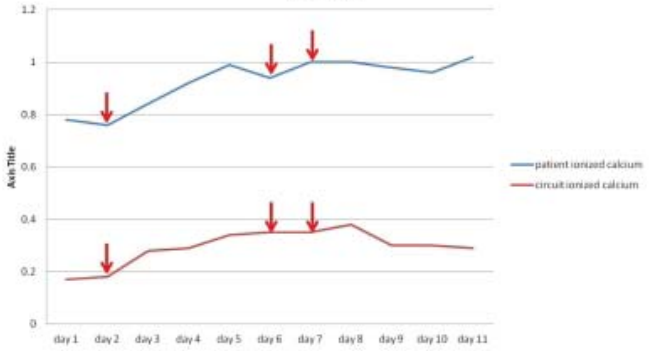


Figure 1 shows the trends of ionized calcium of the patient and circuit over the next 11 days. The post filter replacement fluid was adjusted/increased at marked time points (arrows in figure 1) to achieve near therapeutic serum ionized calcium levels (0.9-1.0 mmol/L). CVVH was able to run for next 11 days without further episodes of clotting.

**Conclusions:** We present a protocol for the use of citrate for anti coagulation in CRRT without the need of separate IV calcium drip but with the use of calcium containing replacement fluid.

**FR-PO1107**

**Peritoneal Fibrosis Caused by Short-Term Peritoneal Dialysis** Leslie J. Padrnos,[1] Nirav Patel,[1] Maxwell L. Smith,[2] Leslie F. Thomas.[3] *[1]Internal Medicine Residency, Mayo Clinic Arizona, Phoenix, AZ; [2]Div of Laboratory Medicine & Pathology, Mayo Clinic Arizona, Scottsdale, AZ; [3]Div of Nephrology & Hypertension, Mayo Clinic Arizona, Phoenix, AZ.*

**Background:** Complex changes to the peritoneum have been described in patients treated with peritoneal dialysis (PD). A rare complication of PD is encapsulating peritoneal sclerosis (EPS), which may lead to bowel obstruction due to a progressive fibrosis of peritoneal tissue. Previously described cases of EPS typically occurred after years of treatment with PD.

**Methods:** A 40-year-old woman with a three month history of PD use was hospitalized with a one month history of progressive anorexia, nausea, vomiting, and epigastric pain. The patient was found to have resolving esophagitis on endoscopy and discharged. She was hospitalized one month later with a similar presentation and PD was stopped. Six months later, she was hospitalized with severe malnutrition. She demonstrated ascites which eventually was attributed to her severe hypoproteinemia. Tube feeds were attempted, but they were not tolerated. A small bowl follow-thru was normal. Trials of anti-emetics and pro-kinetics were unsuccessful. After 30 days of multiple studies and therapeutic interventions, an exploratory laparoscopy was performed. The appearance of the organs and peritoneum was normal. Peritoneal biopsy showed marked fibrohistiocytic thickening with scattered hemosiderin laden macrophages and reactive mesothelial cells. IgG4 staining was negative. Overall, the histologic changes were strikingly similar to those found in EPS. The patient was treated with prednisone 50 mg daily for 12 weeks and tapered off over the next 12 weeks with a prolonged improvement in symptoms.

**Conclusions:** Symptomatic peritoneal fibrosis is typically associated with PD after several years of therapy. We now demonstrate similar changes in a patient who received PD for four months. Glucocorticoid therapy led to a swift resolution of her symptoms, which had previously been resistant to multiple other treatments.

**FR-PO1108**

**Initiation of Peritoneal Dialysis in a Patient with Liver Disease and Refractory Ascites** Satyam Arora, Oleg Grapp, Alden Michael Doyle. *Dept of Medicine, Div of Nephrology, Drexel Univ College of Medicine, Philadelphia, PA.*

**Background:** Patients with end-stage liver disease (ESLD) complicated by refractory ascites often have concomitant ESKD. The dialysis modality of choice has been difficult to determine for these patients. Hemodialysis is more commonly employed but is challenging because of intradialytic hypotension that limits ultrafiltration, a difficult to assess dry weight because of fluctuating amounts of ascites, and the requirement for a salt restrictive diet in a population of patients who are often nutritionally compromised. PD can be advantageous in this situation because of slow continuous UF and direct removal of ascitic fluid. There are several non-randomized case-control studies showing similar outcomes in patients receiving HD vs. PD but despite these, there remains reluctance to recommend PD because of fears about infection.

**Methods:** We report a 55 year old male with a past medical history of hepatitis C cirrhosis s/p an orthotopic liver transplant in 2005 with recurrent HCV and allograft cirrhosis, Stage V CKD secondary to HRS, CMML, and HTN. Patient was suffering from refractory ascites being managed with intensive diuretics combined with weekly paracentesis requiring pre-procedure platelet transfusions. Dialysis was recommended when the patient developed signs of uremia and became 20 lbs over his dry weight despite diuretics and salt restriction. A PD catheter was inserted laparoscopically without complications. Over the following 4 months, the patient has not had peritonitis, no episodes of bleeding, and required no additional platelet transfusions. He has been able to maintain his dry weight without a salt restrictive diet or paracentesis and has not been hospitalized. His RR KT/V was 1.76 and KT/V PD was 0.91 with a total KT/V of 2.67. He required a total of 20 L, 1.5% 6 L bags with 2 L prime, and 3 exchanges nightly on a cycler.

**Conclusions:** Our case highlights a successful outcome of initiating PD in a patient with ESLD-associated ascites and concomitant ESKD. We suggest that PD is not only safe but offers marked advantages for the patient's wellbeing when compared with the use of HD in this challenging population of patients.

**FR-PO1109**

**Hemodialysis in a Patient with Eisenmenger's Syndrome** Akinwande A. Akinfolarin,[1] Anitra W. Romfh,[2] Dirk M. Hentschel,[1] Finnian R. McCausland.[1] *[1]Renal Div, Brigham & Women's Hospital; [2]Dept of Cardiology, Children's Hospital, Boston.*

**Background:** Adults with cyanotic congenital heart disease (CCHD) present a unique set of challenges for hemodialysis. We present a patient with unrepaired patent ductus arteriosus (PDA), hypoplastic right lung and resultant Eisenmenger's syndrome referred for dialysis initiation.

**Methods:** A 49yo woman with CCHD and Eisenmenger's syndrome experienced progressive proteinuric kidney disease, presumed secondary to chronic glomerulosclerosis. Her baseline $O_2$ saturations displayed differential cyanosis, with upper extremities in the low 90's and lower extremities in the 70-80's. A left radial-cephalic autogenous access was created nine months prior without change in symptoms. Significant laboratory values included a creatinine of 4.7 mg/dL, albumin of 2.3 g/dL and hemoglobin of 23.4 g/dL. At the end of her first session (Qb 200mL/min, Qd 300mL/min, F160 membrane, no UF) she experienced increasing dyspnea and a decline in upper extremity $O_2$ sats, requiring initiation of non-invasive support. These changes were felt to be due to a hemodialysis-induced decline in systemic vascular resistance, resulting in increased right-to-left shunting and decline in systemic oxygenation. Her subsequent treatments were notable for high trans-membrane pressures, thought to be related to hyperviscosity. She clotted several filters and thus auto-phlebotomized to a hemoglobin of 20.1 g/dL. These concerns necessitated manipulations in the dialysis prescription: 1) saline infusion pre-filter with UF post-filter in an effort to dilute blood and prevent clotting; 2) slower titration in Qb, Qd and session length; 3) heparin to minimize filter clotting; 4) close attention to hemoglobin goals (iron repletion as needed; avoidance of EPO where possible; but higher target hemoglobin in setting of chronic erythrocytosis).

**Conclusions:** Hemodialysis in patients with CCHD presents several challenges. Close attention must be paid to volume status, hemodynamics, target hemoglobin, residual renal function and nutritional status. In our case we successfully manipulated the hemodialysis prescription to minimize hemodynamic perturbations and clotting.

**FR-PO1110**

**Treatment of Life Threatening Hyperkalemia with Peritoneal Dialysis. A Slower but Effective Option** D.A. Roseman, Jasvinder S. Bhatia. *Renal Section, Boston Univ Medical Center, Boston, MA.*

**Background:** Life threatening hyperkalemia is an uncommon scenario in maintenance peritoneal dialysis (PD) and because potassium clearance rates are significantly lower in PD compared to hemodialysis (HD), its use for this condition is generally discouraged. There is limited data regarding the use of PD for patients at imminent risk of death due to hyperkalemia.

**Methods:** A 48 year old man with history of hypertension, anuria and ESRD on automated peritoneal dialysis for five years presented directly to the emergency department (ED) with two days of weakness and non-productive cough after returning from a two week Caribbean vacation. The patient had discontinued dialysis for one week prior to presentation due to a perceived lack of sterility in the PD unit and fear of infection. On presentation, the patient was conversant, hypertensive, tachycardic, and hypoxic. His respiratory rate progressively increased with associated lethargy requiring non-invasive mechanical ventilation. An EKG showed sinus tachycardia with loss of p-waves and early peaked T-waves that quickly deteriorated into a wide complex tachycardia. Laboratory values showed Na 140 mmol/L, K 8.1 mmol/L, Cl 103 mmol/L, CO2 13.2 mmol/L, BUN 179 mg/dL, and creatinine 31.86 mg/dL. The patient was given calcium gluconate, insulin, dextrose, sodium bicarbonate, and albuterol. A manual PD exchange was initiated in the ED and the patient was transferred to the intensive care unit where he subsequently underwent manual 2 liter exchanges every 2 hours. After 10 hours serum potassium decreased to 6.6 mmol/L and by 16 hours was 5.4 mmol/L with no concerning events on telemetry. Exchanges were then extended to every 4 hours. The patient was discharged 3 days after admission with complete resolution of hyperkalemia.

**Conclusions:** This case illustrates that PD, when combined with standard medical therapy, can be an effective treatment modality for life-threatening hyperkalemia if initiated quickly by experienced staff. The slower potassium clearance rates of PD compared to HD should not limit its consideration. This may be particularly relevant for patients with indwelling PD catheters and centers with limited or delayed resources.

**FR-PO1111**

**Severe Mycobacterium Fortuitum Infection due to Inappropriate Exit-Site Care by Using Mountain Spring Water in a Patient Undergoing Continuous Ambulatory Peritoneal Dialysis** Yasuhiro Yoshimura,[1] Yoshikazu Miyasato,[2] Masataka Adachi,[2] Yasuyuki Fujie,[2] Naoki Shiraishi,[2] Kenichiro Kitamura.[2] *[1]Dept of Nephrology, Arao Municipal Hospital, Arao, Japan; [2]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan.*

**Background:** Exit-site infections are common complications among peritoneal dialysis (PD) patients. Nontuberculous mycobacterium (NTM) species which are usually derived from environmental sources account for less than 1% of exit-site infections. These organisms are more resistant to the antimicrobial agents that are typically used as an initial therapy for the exit-site infections and they often cause the loss of PD catheter and the interruption of PD. Here, we report a case of severe Mycobacterium fortuitum exit-site infection due to the inappropriate exit-site care by using mountain spring water.

**Methods:** A 54-year-old woman with end-stage kidney disease due to IgA nephropathy had been treated with continuous ambulatory peritoneal dialysis (CAPD) for 3 years when she presented with purulent discharge from the exit-site. Although we introduced the standard exit-site care procedure by using normal saline as a cleaning solution, she had been using mountain spring water for 1 year. M.fortuitum grew on the culture of the purulent discharge. Acid-fast staining of the mountain spring water revealed acid-fast bacilli (Gaffky 2) and M.fortuitum was confirmed by the sequencing of 16S rRNA. Then, she was treated with oral levofloxacin and clarithromycin. However, since the exit-site infection was not improved during the 6 weeks course of therapy, her PD catheter was removed and her dialysis modality was switched to hemodialysis.

**Conclusions:** Diagnosis of NTM species should be considered in PD patients with exit-site infections that are refractory to initial antimicrobial therapy. To our knowledge, this is the first report demonstrating that mountain spring water was responsible for the source of M.fortuitum exit-site infection. Routine evaluation and repeated education of the exit-site care would have prevented the M.fortuitum exit-site infection and the additional catheter surgery.

**FR-PO1112**

**A Case Report of *Staphylococcus aureus* Non-Valvular Endocarditis in a Chronic Hemodialysis Patient** Jennifer C. Rodrigues,[1] Paul E. Barre,[1] Richard Fraser,[2] Ahsan Alam.[1] *[1]Div of Nephrology, McGill Univ, Montreal, Canada; [2]Pathology, McGill Univ, Montreal, Canada.*

**Background:** Staphylococcus aureus bacteremia is a common infectious complication of central venous catheter (CVC) access. Non-valvular endocarditis occurs infrequently and may be an under recognized issue in hemodialysis. Here we present a case of right papillary muscle endocarditis diagnosed at autopsy in a hemodialysis patient treated for S. aureus sepsis.

**Methods:** A 54-year-old man was on chronic hemodialysis via a tunneled CVC. His ESRD was from reflux nephropathy and he had a failed kidney transplant. He was also known for congestive heart failure, chronic obstructive pulmonary disease, and bilateral below knee amputations secondary to thromboangiitis obliterans. He presented with progressive dyspnea, cough, nausea, vomiting, and diarrhea. He presented with a fever of 101.3ºF, bibasilar crackles, and an elevated jugular venous pressure. He did not have any stigmata of endocarditis. His CVC had been changed three months prior. His chest x-ray showed mild pulmonary edema, and thus was treated with moxifloxacin, inhaled bronchodilators, intravenous steroids, and additional dialysis treatments. Due to a lack of improvement within 48 hours, broad-spectrum antibiotics (piperacillin-tazobactam and vancomycin) were initiated. Blood cultures returned positive for S. aureus in four of four bottles. The patient subsequently deteriorated and died 2 days later with multi-organ failure secondary to sepsis. An echocardiogram was not performed; however, post mortem demonstrated endocarditis (gram positive cocci) localized to a right ventricular papillary muscle. There was no involvement of the heart valves and, although the CVC was thrombosed, there was no evidence of infection.

**Conclusions:** Non-valvular endocarditis is a rare diagnosis. It has been shown in patients with congenital heart defects, hypertrophic cardiomyopathy, and associated with implantable cardiac defibrillators. This is, to our knowledge, the first report in a hemodialysis patient. Non-valvular endocarditis should be considered in a hemodialysis patient with unexplained sepsis, particularly when a CVC is present.

**FR-PO1113**

**Prolonged Systemic Anticoagulation following Heparin Locking of Tunneled Hemodialysis Catheters** Liliana E. Rios Rojas, Ingrid Calliste, Nobuyuki (Bill) Miyawaki, Shayan Shirazian, Joseph Mattana. *Medicine, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Tunneled hemodialysis catheters (THDCs) continue to be used for hemodialysis (HD). There have been several reports of prolonged activated partial thromboplastin times (aPTTs) following HD in which heparin (H) locking of the THDC is carried out. What has been less well recognized is the danger of prolonged anticoagulation many hours after HD is completed. Here we describe three illustrative cases in which HD patients were found to have markedly prolonged aPTTs hours after THDC H locking was performed.

**Methods:** The first patient was a 68 year old man who underwent THDC locking with a 5,000 U/mL H solution into each lumen. He presented to the emergency department (ED) 8.5 h later with bleeding at the THDC site. His aPTT (repeated) was >200 sec and progressively normalized over the next 24h. The second patient was an 84 year old woman who underwent identical THDC locking with H. She presented to the ED 6 h later after a fall and had an aPTT of >200 sec which normalized over the next 24h. The third patient was a 78 year old woman who underwent HD with one needle placed into an underdeveloped AVF and with one lumen of her THDC used to complete the blood circuit. The THDC was locked with H as above. The patient was seen later in the ED with persistent bleeding from the AVF needle puncture site. Five hours later an aPTT was found to be >200 sec. As in the first two patients the aPTT also progressively normalized. In all cases the H lock volume administered was based on the volume printed on the THDC by the manufacturer. The H locking solution was subsequently decreased to 1,000 U/mL and no further bleeding episodes occurred.

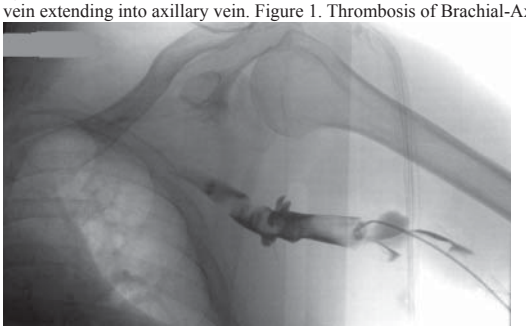
**Conclusions:** HD patients who undergo H locking may be fully anticoagulated hours after HD is completed and this may be due to ongoing diffusion of H from the THDC lumens. Among the many hazards this creates is the risk for bleeding in invasive procedures, a risk which may be unknown to the physician performing the procedure. Greater awareness of this danger might lead to strategies to help prevent the development of hemorrhagic events and enhance the safety of procedures in these patients.

**FR-PO1114**

**Fatal Pulmory Embolism after Hemodialysis Vascular Access Declotting** Maryam Sharif-Hassanabadi, Seyed-ali Sadjadi. *Nephrology, Jerry L Pettis VA Medical Center, Loma Linda, CA.*

**Background:** Thrombosis and failure to maturation are the common causes of vascular access failure. Thrombosis usually occurs because of anatomical problems of the access, trauma or sometimes infection. It can be treated by declotting that is usually uneventful. Herein we present a case of access thrombectomy that was complicated by pulmonary embolism(PE).

**Methods:** 59 years old male with history of diabetes, Hypertension, coronary artery disease, hyperlipidemia, hepatitis C and end stage renal disease on hemodialysis for 5 years presented with a clotted left upper extremity arteriovenous graft(AVG). He had multiple dialysis access placements; the most recent a brachial-axillary graft fistula created 2 years ago and clotted 3 times since its creation. Ultrasound confirmed thrombosis in the outflow vein extending into axillary vein. Figure 1. Thrombosis of Brachial-Axillary AVG.



Mechanical thrombectomy was performed by an experienced interventional radiologist who had performed all previous successful thrombectomies. With Heparin injection, balloon angioplasty was performed and rotational thrombectomy device was used. During the same session a tight arterial narrowing was also treated with balloon angioplasty. Shortly after flow restoration, the patient complained of chest pain, developed dyspnea and hypoxemia that deteriorated rapidly culminating in. Resuscitation was unsuccessful and he expired. Postmortem examination showed extensive PE.

**Conclusions:** Access thrombosis is frequently associated with subclinical PE that is usually inconsequential but occasionally it may be fatal. In cases of access clotting with extension to the central vein, it is advisable to forego the existing access and use a central vein catheter or a new access to dialyze the patient.

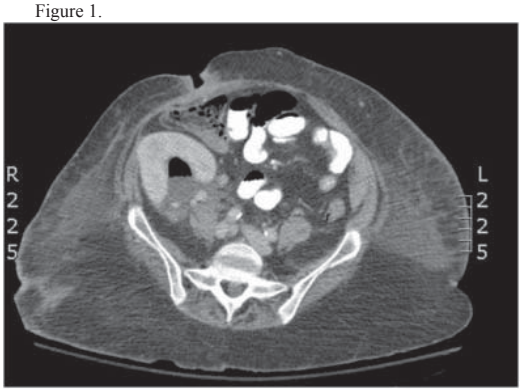
**FR-PO1115**

**Transplant Nephrectomy due to Persistent Bacteremia** Maryam Sharif- Hassanabadi, Alfred C. Cottrell. *Nephrology, Loma Linda Univ Medical Center, Loma Linda, CA.*

**Background:** Bacteremia after kidney transplant is a significant cause of morbidity and mortality. Here we report a complicated case of early post-transplant infection.

**Methods:** A 57 years old female with history of diabetes, hypertension, breast cancer and end stage renal disease on hemodialysis for 11 years was admitted for deceased donor renal transplant. She received Cefazolin as surgical prophylaxis. During the surgery, urinary bladder was found to be small with friable mucosa, making implantation of ureter difficult with ureteral stent placed. On post-op day 3 she developed fever and all cultures grew multidrug resistant Enterobacter Aerogenes(sensitive to Imipenem). Despite Meropenem, she developed necrotizing fasciitis of the right flank, for which she required agressive tissue debridement and a wound vac. Wound, urine and blood cultures grew the same organism. 3 weeks after transplant she was discharged with excellent graft function and apparent resolution of infection. 12 weeks after transplant she was readmitted with fever and persistent bacteremia with the same organism and sensitivities. Abdominal CT angiogram revealed infected pseudoaneurysm of the transplant renal artery anastomosis (Figure 1).

Figure 1.



The fully functional kidney was removed to allow vascular repair of the large mycotic aneurysm as there was no safe way to salvage the transplant organ. The likely initial source of infection was the friable bladder colonized with organisms resistant to Cefazolin. Early bacteremia at the time of transplant, failure of initial antibiotics to cover the organism and immunosuppression led to the aneurysm.

**Conclusions:** Anuric patients can have low level bladder infections and may harbor organisms with multidrug resistance. In certain instances intraoperative flushing of the bladder with extended spectrum antibiotics or perioperative use of a broad spectrum antibiotic to cover resistant bacteria may be prudent.

## FR-PO1116

**Acute Right Sided Heart Failure as a Complication of AVG Creation in a Dialysis Patient** Mohsen M. Elramah, Ahmed I. Al-Absi, Sarguni Singh, Micah R. Chan. *Nephrology Div, Univ of Wisconsin, Madison, WI.*

**Background:** Heart failure is an important source of mortality in dialysis patients. Predisposing factors that might contribute to pump failure in dialysis patients include high output hemodynamics due to dialysis access including arteriovenous fistulae (AVF) and grafts (AVG). In this case report, we describe a dialysis patient presenting with right sided heart failure and high output cardiac failure precipitated by AVG creation.

**Methods:** Case Description: A 68 year old male dialysis patient with history of hypertension, cardiomyopathy and orthotropic heart transplant presented with new onset hypotension and tachycardia. Few days prior to presentation, he had a new axillary artery to axillary vein AVG creation. Physical examination revealed a blood pressure of 66/40 and a regular pulse rate of 140/minute. Initial work up showed a severely concentric left sided heart with an estimated ejection fraction of 80%. His right ventricular chambers were hypertrophied with severely reduced systolic function . Other diagnostic tests were unremarkable. Trans-sonic flow of the AVG revealed a flow rate of 2.4 L/minute. The patient's hemodynamics improved significantly with bedside manual occlusion of his AVG. AVG fistulogram and ligation of the AVG was done by Interventional Nephrology team. The patient's hemodynamic profile improved significantly and he was discharged with normal vital signs.

**Conclusions:** To our knowledge, this is the first reported case of high cardiac output failure in a patient with AVG with axillary-axillary anastomosis. The exact prevalence of high output failure in dialysis population is unknown; however several factors are associated with an increased risk of fistula-induced high-output cardiacfailure. These include location of the dialysis access (proximalmore than distal), male sex, high blood flow through the fistula. Our patient was considered to be at high risk given his comorbid conditions. This case illustrates the importance of weighing risk factors (particularly proximal location of AV access) when considering placement of AVF/AVG in dialysis patients.

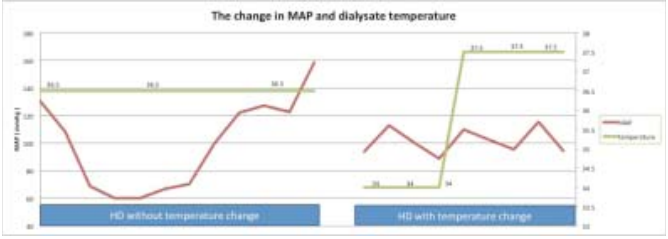
## FR-PO1117

**Dialysate Temperature Adjustment as an Effective Treatment for Baroreflex Failure Syndrome in Hemodialysis Patient** Natsumi Tanabe, Koki Takane, Yudo Tanno, Keitaro Yokoyama, Izumi Yamamoto, Ichiro Ohkido, Takashi Yokoo. *Div of Nephrology and Hypertension, Dept of Internal Medicine, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Baroreflex failure syndrome is a rare disorder which causes labile blood pressure, headache, flushing, diaphoresis and emotional lability. It is caused by history of trauma or radiotherapy around neck, bilateral carotid-body tumor or section of glossopharyngeal nerve. We experienced a case of hemodialysis patient who had difficulty in controlling blood pressure during hemodialysis because of his baroreflex failure syndrome.

**Methods:** We report a case of a 68-year-old CKD5 patient who had difficulties of hemodialysis treatment because of severe fluctuations in blood pressure with hypertensive attacks or hypotensive episodes which caused him a severe discomfort. His dialysis treatment was started in 2010 and since that time baroreflex failure syndrome has been suspected because of his clinical manifestations and history of radiotherapy around his neck for his cancer of tongue in 1994. Baroreflex failure syndrome is diagnosed by cold stressor test. So we found from the mouse experimental vagotomy model of baroreflex failure syndrome, cold stimulation of mouse tail caused signinficant elevation of blood pressure.From this experimental finding of mouse model, we changed the patients dialysate temperature between 34-38° according to his change in blood pressure through 80-240 mmHg. From this attempt, his blood pressure was successfully controlled between 100-180 mmHg and he was able to continue hemodialysis without any discomfort.

**Conclusions:** In our case, environmental stimulation such as temperature change modified the patients fluctuating blood pressure. Change of dialysate temperature could be an option for controlling the unstable blood pressure due to baroreflex failure syndrome.




## FR-PO1118

**A Rare Case of Upper Gastrointestinal Bleeding in a Hemodialysis Patient** Deepak K. Nandikanti, Saba Farooq, Elvira Gosmanova. *Nephrology, Univ of Tennessee Health Science Center, Memphis, TN.*

**Background:** Upper esophageal varices (UEV), a rare cause of upper gastrointestinal bleeding (UGIB), typically occur with superior vena cava (SVC) obstruction. Here we describe a hemodialysis (HD) patient with UEV resulting from right innominate vein (RIV) occlusion with a patent SVC, complicated by UGIB.

**Methods:** 31-year old male with ESRD on HD via right (R) brachiobasilic AV fistula (AVF) presented with hematemesis and tarry stools for 2 days. He also reported progressive worsening of R upper extremity (RUE), neck and facial swelling. Prior to AVF placement in 2011, he had bilateral internal jugular (IJ) tunneled catheters, but no arm swelling. On exam, BP was 128/80 mmHg. Facial swelling, nasal congestion, and a hoarse voice were present. RUE had pitting edema. AVF had a normal thrill. Multiple collateral veins were noted on R side of the neck and upper chest. His hemoglobin was 9.1g/dL (2 weeks prior it was 11.6g/dL) and stool hemoccult positive. EGD showed downhill UEV but no active bleeding. CT chest showed severe RIV stenosis with extensive R chest wall collaterals, bilateral IJ narrowing but no evidence of intrathoracic masses. SVC appeared patent. RUE venogram confirmed the severe RIV occlusion that was not amenable to recanalization. Facial and arm swelling, nasal congestion and mild stridor were concerning for SVC-like syndrome, related to RIV obstruction in combination with RUE AVF. The blood coming from AVF was likely overwhelming venous return from the RUE and the head, causing blood shunting into right thyroid vein that in turn, led to the development of UEV and UGIB. Since RIV could not be recanalized, the AVF was ligated and the patient was switched to peritoneal dialysis. Complete resolution of stridor, facial, and RUE swelling occurred within 10 day of AVF ligation. No further episodes of UGIB were noted.

**Conclusions:** Development of arm and facial swelling after AVF construction warrants evaluation for central vein stenosis, which untreated can lead to development of UEV. UEV are usually asymptomatic, but can lead to life threatening UGIB. Repair of the stenosis or AVF ligation leads to resolution of both SVC syndrome and UEV.

## FR-PO1119

**Pulmonary Nocardiosis and Pneumocystis Jiroveci Pneumonia: Dual Opportunistic Lung Infection in a Kidney Transplant Recipient** Ekamol Tantisattamo, Ashtar Chami, Stephen O. Pastan. *Renal Div, Dept of Medicine, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** Pulmonary infections are a common complication after kidney transplantation. We report a case of a kidney transplant recipient who presented with a mixed pulmonary infection with both *Nocardia pseudobrasiliensis* and *Pneumocystis jiroveci*.

**Methods: Case Description:** A 60-year-old man with history of ESRD received a deceased donor renal transplant and was treated with tacrolimus, mycophenolate mofetil and prednisone. He presented 1 year post transplantation with a six week history of fever, night sweats and a 14 kg weight loss. For three weeks he complained of dyspnea and fatigue. Serum creatinine was elevated to 3 mg/dL from a baseline of 2.4 mg/dL. Chest x-ray was unremarkable; however, a chest CT scan revealed a 2.8x2.6 cm right lower lobe mass. Initially, he was empirically treated for a fungal infection with amphotericin B and posaconazole. He underwent bronchoscopy with transpbronchial biopsies, but pathology revealed no evidence of malignancy and tissue culture was negative for bacteria and legionella. Acid fast bacilli and fungal smear were also negative. As he continued having fevers and hypoxemia, video-assisted thoracic surgery (VATS) with biopsy was performed and frozen section revealed evidence of granulomatous disease. Gram stain suggested *Nocardia spp.* and trimethoprim/sulfamethoxazole was started. Finally, tissue culture grew *Nocardia pseudobrasiliensis* and pathology revealed *Pneumocystis jiroveci* by GMS stain. He responded well to treatment with trimethoprim/sulfamethoxazole. Tacrolimus and mycophenolate mofetil doses were reduced during the hospitalization, and renal function returned to his baseline.

**Conclusions:** Bacterial infection is the most common cause of pneumonia in kidney transplant recipients, although opportunistic infections are also commonly seen. Immunocompromised patients are also at risk for infection with multiple organisms. Early use of invasive diagnostic measures, such as bronchoscopy or VATS, should be pursued for patients who present with nonspecific symptoms and who do not respond to initial empiric treatment.

## FR-PO1120

**Eculizumab in the Treatment of De Novo Post-Transplant Thrombotic Microangiopathy** Federico Calaf, Juan M. Gonzalez, Wadi N. Suki. *Dept of Internal Medicine, The Methodist Hospital, Houston, TX.*

**Background:** Eculizumab is an anti C5 monoclonal antibody currently approved for the treatment of Paroxysmal Nocturnal Hemoglobinuria and atypical Hemolytic Uremic Syndrome. Experimental data suggests complement activation is involved in Anti Phospholipid Syndrome (APS) pathogenesis, yet the mechanism remains unclear. We added eculizumab as a salvage therapy for treatment of acute de novo allograft thrombotic microangiopathy. Our case suggests that activation of the complement cascade plays a crucial role in graft thrombosis, even though currently there is no complement target therapy approved for APS.

**Methods:** A 42 y/o Caucasian male with history of End Stage Renal disease secondary to Anti Phospholipid Syndrome (APS) and SLE nephritis on hemodialysis for 10 months underwent living related donor renal transplantation. The patient had slow graft function post operatively requiring no dialysis. His immune suppression regimen consisted of

antithymocyte globulin, solumedrol, and mycophenolate. Due to the lack of improvement in renal function, a renal allograft percutaneous biopsy was performed three days post operatively. The biopsy showed thrombotic microangiopathy with disseminated glomerular thrombosis, focal vascular thrombosis and endothelial injury. The c4d stainings were negative. No signs of hemolytic anemia or thrombocytopenia were evident and haptoglobin remained normal. Laboratory data showed a weakly positive antinuclear antibody, elevated anticardiolipin antibodies and undetectable double stranded DNA. C3 and C4 complement levels were low, but had been within normal levels prior to surgery. An ADAMS 13 activity level was unremarkable. On post-operative day 4, eculizumab 900mg IV-weekly infusion was added to his immune suppression regimen. After three weeks, eculizumab was increased to 1200mgIV every other week for five additional doses. The serum creatinine improved from 6.7mg/dL before eculizumab to 3.4mg/dL at the end of the treatment. A repeat kidney biopsy showed tubular atrophy and interstitial fibrosis but no features of acute thrombotic microangiopathy. Seven months after his last eculizumab treatment his serum creatinine is 2.5mg/dl.

## FR-PO1121

**Eculizumab for Treatment of Acute Antibody Mediated Rejection** Manmeet Brar, Youshay Humayun, Iasmina Craici, Kenneth E. Kokko, Steven Wagner. *Internal Medicine Nephrology, Univ of Mississippi Medical Center, Jackson, MS.*

**Background:** Antibody mediated rejection (AMR) complicates about 5% of all kidney transplants. AMR is relatively refractory to treatment and leads to reduced graft survival. Current therapy is often insufficient. Here we present a patient who was successfully treated for AMR with Eculizumab, an inhibitor of the terminal complement cascade.

**Methods:** A 24 year old male with a failed living related donor kidney transplant 6 years ago received a deceased donor SCD transplant with thymoglobulin induction. Pre-transplant PRA was 16% class I and 70% class II, with negative flow and CDC crossmatches. He was discharged on post-operative day 4 with prograf, cellcept, and a prednisone taper; creatinine at discharge was 1.6. He was readmitted after one week with anuria and a creatinine of 5.1. Allograft biopsy showed diffuse 3+ C4d staining and diffuse peritubular capillaritis with luminal neutrophils, consistent with Banff grade II acute antibody mediated rejection (AMR). Donor specific antibodies were detected to A29 (1:16), B45 (1:16), DR4 (1:64), and DR15 (1:64). In addition to solu-medrol, two sessions of plasma exchange, and 4 doses of Bortezomib (1.3 mg/m2), he received a single 900 mg dose of Eculizumab. Urine output returned within 12 hours of Eculizumab infusion, and he was discharged with a creatinine of 2.2. Class I donor specific antibody (DSA) titers fell to 1:4 by 2 weeks post treatment, but class II titers remained unchanged at 1:64. Renal function continues to improve, with a creatinine of 1.15 about 6 months after treatment. To date, there have been no infectious or surgical complications.

**Conclusions:** AMR is often difficult to treat and is associated with a poor prognosis. Therapy with IV immunoglobulin, bortezomib, and plasma exchange is often inadequate. Eculuzimab, a humanized antibody specific for the C5 component of the complement cascade, has been shown to be effective for prevention of antibody mediated rejection in positive crossmatch transplant. Its usefulness in the treatment of AMR remains to be determined. Our case illustrates that Eculizumab may be a useful adjunct to standard therapy in the treatment of acute AMR.

## FR-PO1122

**Alemtuzumab for the Treatment of Acute Humoral Renal Allograft Rejection** Mohit Agarwal, Iasmina Craici, Kenneth E. Kokko, Steven Wagner. *Internal Medicine Nephrology, Univ of Mississippi Medical Center, Jackson, MS.*

**Background:** Alemtuzumab is a recombinant DNA-derived humanized, rat immunoglobulin G1 (IgG1) monoclonal antibody directed against CD52, a cell surface glycoprotein expressed at high levels by T and B lymphocytes. It was originally approved as Campath® in 2001 for B-cell chronic lymphocytic leukemia (B-CLL) but was subsequently withdrawn in 2012 in preparation for relaunch as Lemtrada® for the management of Multiple Sclerosis. It has also found use in kidney transplantation and is increasingly being utilized for induction therapy and for the management of acute cellular rejection (ACR). We report the 1st case series of its use in the management of acute humoral rejection (AHR).

**Methods:** We retrospectively evaluated the efficacy of Alemtuzumab in three patients with biopsy confirmed acute humoral rejection of renal allografts. Two patients also had prominent acute cellular rejection. All patients were on triple maintenance immunosuppression with prednisone, tacrolimus and mycophenolate and were treated with Alemtuzumab 30 mg IV in conjunction with three day course of high dose IV methylprednisolone. All patients exhibited depletion of peripheral lymphocytes. Of the two patients with mixed cellular and humoral rejections, one patient (Grade AHR:II, ACR:IA) had a drop in serum creatinine (sCr) from 3.3 to 1.8 in two weeks and the other patient (Grade AHR:II, ACR:IIA) had a sudden rise in sCr from 2.1 to 2.4 next day followed by a progressive fall to 1.8 over the next week. The third patient had predominantly humoral rejection (Grade AHR:II, ACR:Questionable) with coexisting transplant glomerulitis and chronic transplant glomerulopathy. She exhibited minimal initial response followed by a progressive sustained rise in sCr from 1.8 to 4.8. A repeat biopsy three and half month later again revealed the above, though, this time accompanied by calcineurin inhibitor acute tubular toxicity.

**Conclusions:** Alemtuzumab destroys mature lymphocytes expressing CD52. Our experience suggests that it can be a viable treatment option in the management of acute humoral rejection (AHR) of renal allografts.

## FR-PO1123

**Sirolimus-Induced Diffuse Alveolar Hemorrhage in a Kidney Transplant Recipient** Manpreet Singh, Sandeep Ghai, Moshe Shashar, Steven C. Borkan, Jean M. Francis. *Renal Section, Boston Univ School of Medicine, Boston, MA.*

**Background:** Sirolimus, a mammalian target of rapamycin inhibitor, is commonly used in solid organ transplant recipients. Diffuse alveolar hemorrhage related to sirolimus has been reported in 9 cases in the literature. The case herein describes an additional kidney transplant recipient who developed diffuse alveolar hemorrhage while on sirolimus.

**Methods:** The patient is a 45 year-old Ethiopian female with a past medical history significant for end stage renal disease secondary to uncontrolled hypertension and treated latent tuberculosis. She underwent a cadaveric renal transplant 10 months prior to her admission to our hospital. She received tacrolimus, prednisone and mycophenolate mofetil acid for maintenance immunosuppression. 9 months after kidney transplantation, sirolimus was substituted for tacrolimus due to calcineurin inhibitors toxicity. 4 weeks after the switch, she presented to our hospital with productive cough, fever and hemoptysis. Although clinically stable on admission, 24 hours later, the patient acutely decompensated and required ventilator support. Computed Tomography of her thorax demonstrated bilateral multilobular infiltrates. Bronchoscopy confirmed the presence of diffuse alveolar hemorrhage. Extensive workup was negative for infectious, autoimmune or neoplastic processes. Sirolimus induced diffuse alveolar hemorrhage was the likely etiology. Sirolimus was discontinued on admission and high-dose steroids were administered. The patient developed severe kidney graft failure secondary to acute tubular necrosis that was confirmed by allograft biopsy. She improved clinically on high dose steroids and was successfully weaned off the ventilator, but continued to require dialysis after discharge.

**Conclusions:** We describe a case of sirolimus induced diffuse alveolar hemorrhage in a kidney transplant recipient. Extensive workup to rule out other etiologies, early withdrawal of sirolimus and high dose steroids seem to be effective in the treatment of this near fatal complication. This case substantiates the need for close monitoring of pulmonary symptoms while taking Sirolimus.

## FR-PO1124

**Back to Square One: Graft Irradiation for the Treatment of Kidney Transplant Rejection** Paramveer Singh Saluja, Marwan M. Abu Minshar, Ann Kathleen N. Gamilla-Crudo, Pradeep V. Kadambi. *Internal Medicine, UTMB, Glaveston, TX.*

**Background:** Kidney transplant is the preferred option to treat end stage kidney disease (ESKD). Allograft rejection continues to be a major cause of transplant failure in spite of ongoing advancement in immunosuppression (IS). We are presenting a case series of patients who underwent local graft irradiation (RT) for acute allograft rejection refractory to conventional therapies.

**Methods:** A 40 year old male with ESKD due to Membranous Nephropathy received a kidney transplant in 2005. His clinical course within the first year was complicated by 2 episodes of acute cellular rejection (ACR) that led to loss of allograft function, necessitating nephrectomy. He received a second kidney transplant in 2009 with a baseline creatinine of 1.9 mg/dl. Three years after transplant, he developed ACR (Banff 2b) and humoral rejection, and was treated with IVIG, anti-thymocyte globulin (ATG), and steroids. His allograft function continued to worsen (peak creatinine of 4.6 mg/dl) and repeat biopsy 3 weeks later showed persistent Banff 2b ACR. Since he had refractory rejection, he was treated with 4 doses of RT combined with IS. Over 1 month following treatment, his creatinine improved to 1.9 mg/dl. A 50 year old male with ESKD due to DM type 1 received a SPK transplant (CMV D+/R-) in 2012 with new creatinine level of 1.2 mg/dl. Six months after his transplant, he developed CMV Viremia due to non-adherence to valganciclovir. His IS was reduced and valganciclovir was reinitiated. Subsequently, his creatinine rose to 5.0 mg/dl and allograft biopsy revealed ACR (Banff 2b) and humoral rejection. He was treated with IVIG, plasmapheresis, ATG and steroids. His allograft function continued to worsen (peak creatinine level of 6.9 mg/dl) and repeat biopsy 2 weeks later showed persistent ACR (Banff 1b). Further augmentation of IS was not possible because of concomitant CMV infection hence, 5 doses of RT were given and creatinine improved to 2.5 mg/dl.

**Conclusions:** Based on our experience and available literature, patients who have acute rejection refractory to IS or those who have concomitant infection, local radiation of the allograft would be a viable adjunct to the overall management.

## FR-PO1125

**Pulmonary Phaeohyphomycosis Caused by Phaeoacremonium and Dactylaria Constricta in a Renal Transplant Recipient: Successful Treatment with Posaconazole** Saivaralaxmi Monaganti, Carlos Q. Santos, Daniel C. Brennan. *Internal Medicine, Washington Univ School of Medicine, St. Louis, MO.*

**Background:** Phaeoacremonium and Dactylaria are dematiaceous fungi. Over 70 genera and 150 species cause human and animal disease. They are widely distributed in soil, wood and decomposing plant debris and characterized by melanin or melanin-like pigments in their cell wall. Phaeohyphomycosis is a collective term for cutaneous, subcutaneous and systemic disease caused by dematiaceous fungi. It is most frequently an opportunistic infection in immunosuppressed patients, and has been rarely reported to affect organ transplant recipients. The overall mortality rate with skin, soft-tissue or joint infections is 7% and 57% with systemic disease. To our knowledge, this is the first case of pulmonary infection by multiple dematiaceous fungi and only the third reported case of lung infection caused by Phaeoacremonium.

**Methods:** A 49 year old white female, 6 years post-renal transplantation, presented to the transplant clinic with a history of worsening dyspnea, cough and fatigue over 6 months after failure to respond to several courses of antibiotics prescribed by her primary physician. Physical exam was unremarkable. CT of the chest revealed nodular opacities in the right upper lobe. Culture of a fine needle aspiration yielded Phaeoacremonium species. Culture of BAL yielded Dactylaria constricta and a few Mycobacterium avium-intracellulare. The patient was started on posaconazole 200 mg PO QID and her tacrolimus dose was reduced. A repeat CT after 1 month showed improvement. Posaconazole was changed to 400 mg PO BID for 4 months with complete resolution.

**Conclusions:** There is no standard antifungal regimen for Dactylaria or Phaeoacremonium reported in the literature. Posaconazole is the most recently approved triazole with an extended spectrum of activity. Posaconazole was chosen over other azoles because it is well tolerated, has a favorable side effect profile, and low potential of drug interactions with only a moderate interaction with tacrolimus compared to other azoles. Our patient responded well to treatment with no relapse of symptoms during 2.5 years of follow up.

*Funding:* NIDDK Support, Clinical Revenue Support

## FR-PO1126

**The Story of Having Subclavian Venous Catheter and the AV Fistula on Same Side** Mohd. Majid Mohd. Ayyub Momin, Anup Chaudhari, Hemant J. Mehta, Zaheer Amin Virani, Suyash Sharma. *Nephrology, Lilavati Hospital, Mumbai, Maharashtra, India.*

**Background:** The use of subclavian veins (SCV) as hemodialysis vascular access is not recommended. Patients who had SCV catheter in the past can present with a problem years later.

**Methods:** This is the story of a 33 years old man who was initiated on hemodialysis (HD) in the year 2000 through right internal jugular vein (IJV) non-cuffed catheter, for his ESRD due to CTID. Soon, he had left wrist native AV fistula (AVF) and the right IJV catheter was removed after 4 weeks. The left AVF had spontaneous closure after 3 months following an episode of post HD hypotension. He had right SCV non-cuffed HD catheter as right IJV could not be cannulated. He agreed for a second AVF at right wrist, 8 months later. This fistula matured well and his right SCV catheter was removed after 9 months. In 2005, he developed right upper limb (UL) swelling and was diagnosed to have right SCV stenosis. He underwent right SC venoplasty with stenting and the AVF worked well till 2009, when he underwent cadaver renal transplantation. The AVF was not used since 2009. He developed swelling of right UL over past 1 year.

He was detected to have fracture of right SCV stent with obstruction. He underwent stent plasty with new covered stent in the SCV. 1 month later, his swelling disappeared, and his right wrist AVF was surgically ligated. Hand returned to near normal shape.

**Conclusions:** SCV HD catheters are associated with high risk of central venous stenosis and hence it is recommended not to use SCV for HD catheters. This patient had a SCV in the era when such recommendation did not exist. Also, stenting of central vein stenosis is not preferred at present. This patient had a stent in the right SCV at a time when it was the usual practice. The stent fractured and led to severe venous congestion of right UL which could have resulted in the loss of right UL.

## FR-PO1127

**De Novo Renal Cell Carcinoma in Renal Graft 22 Years following Cadaveric Transplantation with Atypical Presentation of Refractory Metabolic Acidosis** Zaid Brifkani,[1] Kavita Pal,[2] Raafat Farag Makary,[3] Andreea Poenariu.[1] *[1]Div of Nephrology and Hypertension, Dept of Medicine, Univ of Florida, Jacksonville, Jacksonville, FL; [2]Dept of Medicine, Univ of Florida, Jacksonville, Jacksonville, FL; [3]Pathology and Laboratory Medicine, Univ of Florida, Jacksonville, Jacksonville, FL; [4]Div of Nephrology and Hypertension, Dept of Medicine, Univ of Florida, Jacksonville, Jacksonville, FL.*

**Background:** Post-Transplant graft Renal Cell Carcinoma (RCC) has rarely been reported. The longest post-transplant time at the diagnosis of graft RCC reported has been 13 years.

**Methods:** We present the case of a fifty-two year old African American female who received a cadaveric right kidney transplantation in 1990 secondary to End State Renal Disease (ESRD) from Systemic Lupus Erythematosus (SLE). She had stable chronic allograft dysfunction and was being maintained on Cyclosporine, Mycophenolate Mofetil , in addition to Prednisone. Regular follow up revealed no complaints of gross hematuria or flank pain. Ultrasonography, performed due to refractory metabolic acidosis, revealed a 4.5 cm heterogenous hypoechoic lesion within the midportion of the transplanted allograft with increased vascularity. MRI confirmed the presence of a higly suspicious mass for renal cell carcinoma with no distant metastases. A renal allograft biopsy revealed renal cell carcinoma (RCC), conventional clear cell type. The patient subsequently underwent successful Radical Transplant Nephrectomy and was placed on Hemodialysis.

**Conclusions:** This case demonstrates a rare presentation of allograft RCC 22 years following transplantation with refractory metabolic acidosis. It also highlights the importance of regular close follow up with ultrasonography and the need for more clear guidelines for post-transplant ultrasonographic follow up, especially in cases of long post-transplant period.

## FR-PO1128

**Polyomavirus-Associated Nephropathy: Not Just BK-Virus** Sandra Barrow, Juan M. Gonzalez, Wadi N. Suki. *Internal Medicine/ Nephrology, Methodist Hospital, Houston, TX.*

**Background:** Over the past decade, polyomavirus associated nephropathy (PVAN) emerged as a major cause of allograft dysfunction and graft loss. The vast majority of PVAN is associated with BK virus infection but this case illustrates that other polyomaviridae need to be considered in the differential diagnosis.

**Methods:** A 61 year old man with end stage renal disease received a living nonrelated donor kidney transplant in 2004. He had stable renal allograft function with serum creatinine ranging from 1.9- 2.5 mg/dl. He received maintenance immunosuppressive therapy with prednisone, mycophenolate mofetil and tacrolimus. Eight years after transplantation, his serum creatinine increased to 3.5 mg/dl. Kidney biopsy was performed. Light microscopy revealed several foci of tubular lymphocytic infiltration along with viral nuclear changes. Calbiochem® Anti-SV40 T Antigen stain was strongly positive suggesting a diagnosis of BK virus-associated nephropathy. Surprisingly, BK viral loads measured by PCR assay in the blood and urine remained undetectable. However, a positive Anti-SV40 T Antigen stain may also represent the presence of other human polyomaviruses. Therefore, urinary and serologic samples were analyzed for JC virus (JCV) and PCR assays revealed markedly elevated urinary levels as well as JCV viremia. A diagnosis of JC virus associated nephropathy was established and reduction of immunosuppression was initiated. Additionally, the patient received intravenous immunoglobulin every two weeks. Treatment is currently in progress and creatinine levels have remained stable.

**Conclusions:** BKV, JCV and the monkey virus SV40 belong to the family of polyomaviridae. They share 69-75% of their DNA sequences and immunohistochemical staining using Calbiochem® Anti-SV40 T Antigen does not distinguish between them. JCV associated nephropathy is an exceptionally rare cause of PVAN. Therapy primarily targets a reduction of immunosuppression but successful treatment with immunoglobulins, cidofovir and leflunomide have been reported. Since timely treatment of PVAN is crucial it is important to recognize that a positive Anti-SV40 T Antigen stain in the absence of BKV viremia may represent JCV associated nephropathy.

## FR-PO1129

**Intrarenal Urothelial Carcinoma Staining Positive for BKV Years after Resolution of BKV Nephropathy** Patricia M. Myers-Gurevitch, Darshana Dadhania, Steven Salvatore, Jun B. Lee, Surya V. Seshan. *Nephrology and Hypertension, New York Presbyterian Hospital - Weill Cornell, New York, NY.*

**Background:** Reactivation of BKV is common in renal allograft recipients, placing them at risk for nephropathy and graft loss. Recent literature suggests BKV infection may be associated with certain malignancies. We report a case of intra-graft malignant urothelial carcinoma (UC) associated with positive staining for BKV presenting years after resolution of BK viremia/nephropathy.

**Methods:** A 53 year old sensitized woman received a living unrelated renal transplant following pre-transplant immunomodulation for a positive donor flow cytometry crossmatch. Post-transplant, baseline creatinine (crt) was 1.5mg/dL. 8 months later, her crt rose to 2.2 mg/dL in the setting of high BK viremia. Renal allograft biopsy was positive for BKV associated nephropathy. Immunosuppression was reduced, and repeat was negative for BKV. 7 months later BK viremia cleared and crt normalized. 5 years post-transplant, the patient still free of BK viremia, developed de novo DSA to DR53. Immunosuppression was intensified. 7 years post-transplant, the patient was admitted for gastrointestinal symptoms and rising crt. She underwent a repeat renal biopsy which revealed intratubular UC in situ associated with positive BKV and p53 stain. The benign tubules were negative for BKV. Subsequent transplant nephrectomy revealed microinvasive high grade UC, extensively involving renal tubules, with multifocal lymphatic invasion.

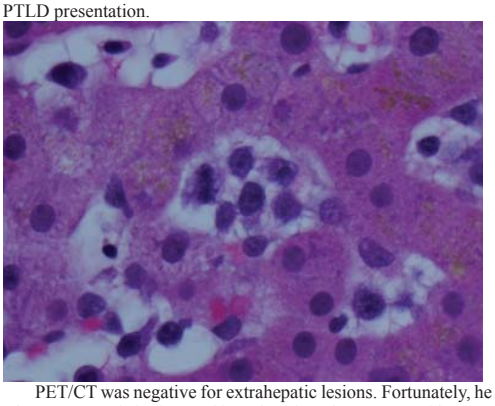
**Conclusions:** Immunosuppression places transplant recipients at higher risk for malignancy. The incidence of UC in this population is low (2%). Most UC arises in the native bladder or ureter, and is very rarely restricted to the allograft. Although there is a known association with BK virus infection and urothelial malignancy, intrarenal UC staining positive for BKV has not been reported. The positive BKV stain within the tumor nuclei may signify incorporation of BKV DNA transcripts into the genome of neoplastic cells. With negative C4d staining and no evidence of antibody mediated rejection, the rising DSA could be an indirect sign of local immune activation to control tumor growth.

## FR-PO1130

**Fever of Unknown Origin in a Renal Transplant Patient** Karl Pembaur, Grant Springman, Joseph Kremer, Blake Nestok. *The Christ Hospital, Cincinnati, OH.*

**Background:** PTLD is a complication following organ transplantation that occurs in 1-2% of patients. Current research has concluded that the degree of immunosuppression along with positive EBV serotype leads to triggering of monoclonal activation of B cells resulting in lymphoma.

**Methods:** A 72 year-old white male was 17 years post living related renal transplantation for Focal Segmental Glomerulosclerosis who presented with 3 weeks of fevers, night sweats, diarrhea, and 15lb weight loss. He had been adherent to immunosuppressive therapy with stable renal function. Review of systems was negative except for persistent dry cough. Vital signs and physical examination were within normal limits. Admission lab results demonstrated elevated bilirubin, alkaline phosphatase, and liver transaminases with stable creatinine. Ultrasound of the RUQ was obtained and revealed calcified hepatic granulomas. Autoimmune biomarkers and infectious serologies were negative. CT of the Chest/Abdomen/Pelvis was negative. A bone marrow biopsy was negative for malignant cells. Due to the persistent elevated liver profile and symptoms a liver biopsy was performed. Histopathology revealed a rare extranodal, EBV negative, large B cell lymphoma as his PTLD presentation.



PET/CT was negative for extrahepatic lesions. Fortunately, he responded to six cycles of R-CHOP and six doses of intrathecal methotrexate and is currently in remission.

**Conclusions:** Despite most PTLD arising within the first year post-operatively, multiple studies demonstrated a bimodal distribution, with a late-onset presentation occurring 5+ years later. Additionally, when comparing early versus late onset PTLD, late-onset PTLD seems to be less associated with seropositive EBV status and more likely to be extranodal than early-onset PTLD. In our case report, the patient presented 17 years post transplant with extranodal PTLD in the liver only and a seronegative EBV status.

## FR-PO1131

**Emphysematous Pyelonephritis in a Renal Allograft** Kashif J. Piracha, Edward P. Nord, Nand K. Wadhwa. *Nephrology & Hypertension, Stony Brook Medicine, Stony Brook, NY.*

**Background:** Emphysematous pyelonephritis (EPN) is a fulminant necrotizing bacterial infection of the kidney caused by gas-forming organisms, which requires prompt evaluation and treatment because of high mortality.

**Methods:** A 58 year old man with ESRD due to diabetic nephropathy received a deceased donor renal allograft in 2007, and presented with a one week history of nausea, vomiting and right lower quadrant abdominal pain. Physical examination revealed a temperature of 37.2°C, BP of 139/69 mmHg and HR of 121 beats/min. His abdominal examination revealed right lower quadrant tenderness over the allograft. Urinalysis was positive for leukocyte esterase and >182 WBC/hpf. He received empiric piperacillin/tazobactam on admission. Over the next 24 hours, the patient developed septic shock with acute respiratory failure requiring intubation and norepinephrine. His urine and blood cultures grew klebsiella pneumoniae. A noncontrast CT scan of the abdomen and pelvis showed heterogeneous gas containing collection in the allograft. Transplant kidney nephrectomy was performed. The gross pathology revealed an acutely inflamed, friable, necrotic, gas filled kidney with extensive hemorrhage with yellow exudates. Microscopic examination revealed acute severe pyelonephritis with abscesses. He postoperatively completed two weeks of antibiotic therapy and was discharged home in stable condition.

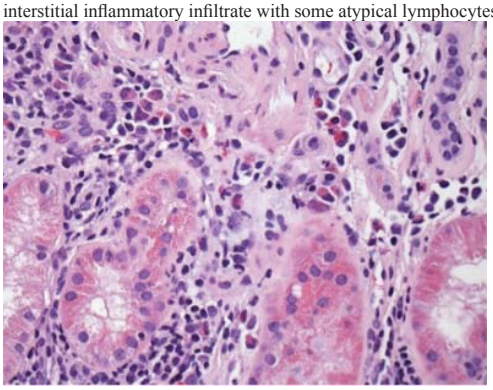
**Conclusions:** EPN is an acute, severe, necrotizing bacterial or fungal infection of the renal parenchyma, collecting system or perinephric tissue. The grade of severity of EPN guides therapy and Al-Geizawi et al have classified EPN of the renal allograft into 3 stages that is determined by the extent of gas collection. Our patient with multi-organ failure and uncontrolled sepsis not responding to medical management falls into the severest category (stage 3) of his classification. Our patient underwent emergent nephrectomy and rapidly improved thereafter. Recognition of the severity of EPN guides therapy.

## FR-PO1132

**Fever and Pancytopenia in Kidney Transplant Recipient-Malignancy Is Always a Possibility** Jie Cui, Francesca Cardarelli, Vanesa Bijol, Martina M. McGrath. *Nephrology Dept, Brigham and Women's Hospital, Boston, MA.*

**Background:** Single or multi-lineage cytopenia is very common in renal transplant patients. While infection and immunosuppression are the most common causes of pancytopenia in transplant recipients, it is crucial to maintain a high suspicion of other relevant etiologies including thrombotic microangiopathy, graft-versus-host disease, autoimmunity and malignancy.

**Methods:** A 33-year-old male with a history of ESRD due to primary FSGS, living unrelated kidney transplant (CMV+/CMV-) 9 years previously, maintained on rapamune and cellcept, presented with a 2-week history of fever and left buccal ulceration. Clinical exam was remarkable for temperature 102F and 1cm induration on the left buccal mucosa. His labs showed creatinine was up to 4.8 mg/dl from baseline 2.4mg/dl, urine protein/creatinine was 1.2, WBC count 2.06/mm$^3$ with 12% neutrophils, Hg 7.8 g/dl, HCT 24% and platelet 54,000/mm$^3$. He was treated with broad-spectrum antimicrobial and anti-viral medications. However, extensive infectious work up including HSV, EBV, CMV, parvovirus, HIV, blood culture and urine culture were negative and he remained intermittently febrile. His AKI failed to resolve with volume resuscitation and subsequent renal biopsy showed marked interstitial inflammatory infiltrate with some atypical lymphocytes.



Bone marrow biopsy showed 25% blasts and flow cytometry was consistent with acute myeloid leukemia. Cytogenetics demonstrated no clonal abnormalities. He was treated with cytarabine and daunorubicin. Bone marrow biopsy on day 29 post chemotherapy showed <5% blasts.

**Conclusions:** Hematological malignancy should be suspected in transplant recipients presenting with pancytopenia, especially in those with long term exposure to immunosuppressive medications. Hematological malignancy is also a rare cause of inflammatory infiltration in a transplanted kidney.

## FR-PO1133

**Recurrent Idiopathic Nodular Glomerulosclerosis after Kidney Transplantation** Mamoun Elsir Bashir,[1] Irfan Warraich,[2] Melvin E. Laski.[1] *[1]Internal Medicine, Texas Tech Univ Health Sciences Center, Lubbock, TX; [2]Pathology, Texas Tech Univ Health Sciences Center, Lubbock, TX.*

**Background:** Idiopathic nodular glomerulosclerosis is a rare condition that closely resembles diabetic nodular glomerulosclerosis but occurs in non-diabetic individuals. It differs from the nodular glomerulosclerosis that develops in fibrillary diseases and membranoproliferative glomerulonephritis by the absence of fibrils or tubular structures on electron microscopy and negative immunofluorescence respectively. The cause of idiopathic glomerulosclerosis remains unknown but it has been associated with tobacco use in some studies.

**Methods:** Patient is 64 year old non-diabetic, non-smoking Caucasian male with history of well controlled hypertension who developed end stage renal disease secondary to idiopathic nodular glomerulosclerosis diagnosed by renal biopsy. The biopsy tissue revealed typical nodular glomerulosclerosis and was negative for amyloid material, and negative for fibrils or other deposits on electron microscopy. The patient underwent deceased donor kidney transplant and did well in regard to his renal transplant with creatinine between 1.0 and 1.1mg/dl and no significant proteinuria. Three years later worsening allograft renal function (creatinine 1.6 mg/dl) and progressive proteinuria (up to 4 gm/d) were noted. Subsequent transplant biopsy diagnosed recurrent idiopathic nodular glomerulosclerosis, and also was negative for amyloid or other fibrillar structures. There was no sign of humoral or cell mediated rejection. The patient remained non-diabetic throughout the pre- and post-transplant course.

**Results:** .

**Conclusions:** The pathology seen in the transplant biopsy duplicated the findings of the native disease in this patient. The disease recurred approximately three years after transplant, which is much more rapid than expected in the case of recurrent diabetic nodular glomerulosclerosis. We believe this to be the first case of recurrent idiopathic glomerulosclerosis post-kidney transplantation. It establishes that the condition can redevelop and that the lag time for recurrence may be shorter than that seen with diabetic nodular glomerulosclerosis.

*Funding:* Clinical Revenue Support

## FR-PO1134

**Iron Metabolism and Rapamycin (Sirolimus)-Induced Microcytic Anemia in Renal Transplant Patients** Shivani Upadhyay, Joshua Zaritsky, Tomas Ganz. *UCLA.*

**Background:** Sirolimus is a macrocyclic antibiotic, which binds to the FK binding protein and modulates the activity of mTOR (mammalian target of rapamycin). This drug is widely used for preventing rejection of solid organ transplants and graft vs host disease following bone marrow transplantation. A unique feature of sirolimus is that it also induces profound microcytosis, which is poorly understood. Previous studies in renal transplant patients have demonstrated that oral iron supplementation in patients who received sirolimus did not improve their microcytic anemia. However, anemia improved once patients discontinued sirolimus.

**Methods:** First a retrospective chart review of pediatric renal transplant patients was done to determine the prevalence and characteristics of sirolimus-induced anemia. Then a prospective study was designed to evaluate functional iron deficiency in pediatric

and adult patients with solid organ transplants treated with sirolimus. The study design includes evaluation of serum iron, total iron binding capacity, serum hepcidin, ferritin, mean corpuscular hemoglobin concentration, reticulocyte count,hemoglobin content and CRP prior to initiation of sirolimus therapy and at four time points post initiation of therapy.

**Results:** The retrospective chart review of 21 renal transplant patients (10 pediatric, 11 adult) was completed. The hemoglobin and MCV were compared one month prior to the of start of sirolimus and 6 months after. MCV was decreased in sirolimus-treated patients compared to baseline (0.0001, paired t-test). We currently have enrolled 5 pediatric patients in the prospective study showing microcytosis and iron deficiency after the start of sirolimus. The hepcidin appropriately responded to iron deficiency showing decreasing levels from baseline to 2 months after the start of sirolimus. However, they continue to remain iron deficient. Ferritin also is low in these patients.

**Conclusions:** Hepcidin does have appropriate response to iron deficiency in patients on sirolimus. Therefore, sirolimus must work downstream of the hepcidin pathway. There is evidence to suggest that sirolimus does affect the iron pathway, however, it is not through hepcidin.

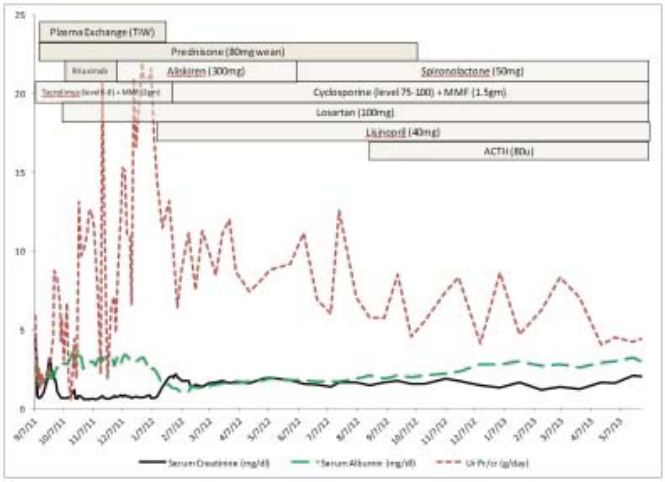
## FR-PO1135

### Treatment of Post-Transplant Immunosuppression Resistant Focal Segmental Glomerulosclerosis with Adrenocorticotropic Gel

Patrick P. McHugh, Muhammad Ahmad Mujtaba, Tim E. Taber, Muhammad S. Yaqub, Asif A. Sharfuddin. *Nephrology/Transplant, Medicine, Indiana Univ, Indianapolis, IN.*

**Background:** Post-transplant recurrence of focal segmental glomerulosclerosis (FSGS) is associated with poor outcomes and early graft failure. Adrenocorticotropic Hormone (ACTH) has shown efficacy in some cases of native kidney nephrotic syndrome including FSGS. Its actions include reducing oxidative stress and diminishing podocyte apoptosis and podocyte loss through binding to melanocortin receptors. We report a case of immunosuppression resistant FSGS after transplant treated with ACTH with resultant decrease in proteinuria.

**Methods:** A 60-year-old white female with a history of FSGS since 2003 (chronic kidney disease stage 5) underwent pre-dialysis living related kidney transplant. She was induced with Thymoglobulin®, early corticosteroid (4-day) withdrawal, and was maintained on tacrolimus and mycophenolate mofetil. Her serum creatinine (Cr) at postoperative (POD) 7 was 0.9mg/dL. On POD11 she was readmitted with Cr of 3.1mg/dL and worsening proteinuria. Biopsy showed no evidence of rejection and negative immunofluorescence staining, but electron microscopy showed widespread podocyte effacement consistent with recurrent FSGS. Plasma exchange and high dose steroids were given. Conventional antiproteinuric treatments included ACEi/ARB and statin, plus those shown below.




Nephrotic syndrome persisted, so ACTH was initiated at 10 months post-transplant. Subsequently she demonstrated continued partial remission of proteinuria, sustained rise in serum albumin, and stable graft function.

**Conclusions:** To our knowledge this is the first case of post-transplant recurrent FSGS where ACTH has been used. Our case suggests there may be an additional effect of ACTH in immunosuppression resistant FSGS patients, to durably reduce proteinuria and preserve graft function.

## FR-PO1136

### A Rare Case of Bovine Ketoacidosis in a Lactating Woman

Rekha Durairaj,[1] Viswanathan S. Iyer,[1] Pramil Cheriyath.[1] *[1]Internal Medicine, Pinnacle Health Harrisburg Hospital, Harrisburg, PA; [2]Nephrology, Pinnacle Health Harrisburg Hospital, Harrisburg, PA; [3]Internal Medicine, Pinnacle Health Harrisburg Hospital, Harrisburg, PA.*

**Background:** Lactation ketoacidosis, first described by Stinson in 1929, is a common disorder among cows. The basis is an imbalance between energy demands of milk production and the energy generating capacity of the animal. We report a rare case of bovine ketoacidosis in a lactating woman secondary to stress from influenza.

**Methods:** A 33 year old. 5 month postpartum woman presented to the emergency room with flu-like symptoms for 3 days. Patient also reported decrease in appetite with poor intake for 5 days. Patient stated that she has been actively breast feeding since delivery. She denied any alcohol or illicit substance use. Initial vital signs were unremarkable except for hypotension, which responded to fluid administration. Initial labs included: sodium 137, potassium 4.7, chloride 108, bicarbonate 11, BUN 12, creatinine 0.6, glucose 80 and anion gap was 18. Serum lactate was within normal limits. Serum acetone was 2+ with serum osmolar gap of 12. Arterial blood gas analysis at the time of presentation showed pH 7.23, PCO2 22.6, PO2 135 and HCO3 12.7. CBC was unremarkable except for WBC at 1.9K/µl. Urinalysis showed specific gravity of 1.020, 3+ acetone and no active sediments. A comprehensive drug screen was negative including salicylates and acetaminophen. Chest radiograph, blood and urine cultures were within normal limits. Patient tested positive for influenza B. Patient was volume resuscitated, given dextrose infusion and started on tamiflu. With cessation of lactation and high caloric intake, patient's symptoms rapidly improved.

**Conclusions:** The patient who was apparently doing well developed an increased anion gap metabolic acidosis secondary to physiological stress of lactation ketoacidosis. The purpose of this case report is to bring awareness among clinicians about lactation ketoacidosis in patients with similar presentation with stressors such as infection. This will aid in expediting treatment and improving the high mortality and morbidity associated with lactation ketoacidosis.

## FR-PO1137

### Pregnancy or Uremia? – A Case of Pregnancy Symptoms Mimicking Uremia in a Woman with Advanced Chronic Kidney Disease (CKD)

Anil Regmi,[1] Antonios Tzamaloukas,[2] David Buchwald,[1] Amarpreet S. Sandhu.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Univ of New Mexico Health Science Center, Albuquerque, NM; [2]Div of Nephrology, Dept of Internal Medicine, VA Hospital Albuquerque, Albuquerque, NM.*

**Background:** Pregnancy is rare in women undergoing renal replacement therapy (RRT) and may lead to maternal and fetal complications. Due to negligible rates of conception, early pregnancy symptoms can be confused with uremic symptoms in patients with CKD stage V. We present a case that illustrates the diagnostic and management challenges created by pregnancy in women on peritoneal dialysis (PD).

**Methods:** A 25-year-old-woman with CKD stage V (CrCl < 15 ml/min) due to autosomal dominant polycystic kidney disease developed nausea, vomiting, and anorexia. A PD catheter was placed for presumed uremic symptoms. Two weeks after placement of the PD catheter, she presented with persistent nausea, vomiting, and abdominal cramps. CT showed ascending colitis, scattered free intra-abdominal fluid, polycystic kidneys, and no mention of uterus. She was diagnosed with gastroenteritis. Serum β-HCG, obtained 2 weeks later due to persistent symptoms, was positive. US revealed a 20-week pregnancy. At 24 weeks of gestation, PD prescription was changed. Fill volume was decreased to 1.5 L and exchanges increased to 5 cycles. Dialysis adequacy was maintained with Kt/V urea of> 3.5/week and BUN< 50 mg/dl throughout the pregnancy. She received increased doses of iron and epoetin. Labor was induced after she developed abdominal discomfort during infusion of the dialysate at 38 weeks. She delivered a healthy baby boy. During labor PD was continued with a decreased fill volume dwell time. Regular PD prescription was resumed 12 hours after delivery.

**Conclusions:** A combination of low expectation of pregnancy as well as gastrointestinal symptoms resulted in a delayed diagnosis of pregnancy in this case. The CT scan did not show the pregnancy, probably because of the early stage. Coordinated care between a nephrologist, obstetrician, and nutritionist is essential for better outcomes. Women on PD require special care towards the end of pregnancy.

## FR-PO1138

### Pauci Immune ANCA Associated Vasculitis De Novo in Pregnancy

Hasan Riaz,[1] Quarrat-Ul-Ain Shamim,[1] Raafat Farag Makary,[2] Leighton R. James,[1] Andreea Poenariu.[1] *[1]Dept of Medicine, Div of Nephrology, Univ of Florida College of Medicine, Jacksonville, FL; [2]Dept of Pathology, Univ of Florida College of Medicine, Jacksonville, FL.*

**Background:** Antineutrophil cytoplasmic antibody (ANCA) - associated vasculitis (AAV) is infrequently seen in women of childbearing age. Pregnancies occurring in active disease have a documented unfavorable outcome for both the mother and the child. There is a dearth of literature on the de novo occurrence of AAV in pregnancy and on the best approach to management.

**Methods:** We present a case of a young woman who was admitted to hospital with hyperemesis gravidarum, volume depletion and acute kidney injury (plasma creatinine 4.46 mg/dL). Urinalysis demonstrated hematuria, pyuria and urine sedimentation showed leukocytes, erythrocytes and no casts. Despite adequate volume repletion, the patient became increasing oliguric with further deterioration in renal functional parameters. Serologic testing revealed elevated c-ANCA titer and kidney biopsy was consistent with pauci-immune crescentic glomerulonephritis. Despite steroids, intravenous immunoglobulin (x5 at 400mg/kg) and plasma exchange (1.5 plasma volumes every other day for 7 days) the patient required hemodialysis. Following pregnancy termination, intravenous cyclophosphamide therapy (1mg/m$^2$ once a month) was initiated. After two months, renal function recovered and hemodialysis was discontinued. She continues on tapering prednisone and monthly cyclophosphamide (1 mg/m$^2$) without relapse.

**Conclusions:** This case highlights the challenges related to management of pregnant patients with new onset Pauci-immune ANCA positive, rapidly progressive glomerulonephritis (ANCA-RPGN). Given limited guidelines, management of affected individuals requires mutli-disciplinary teams that have developed skills in managing pregnant patients with rare autoimmune disorders.

**FR-PO1139**

**De Novo Collapsing Glomerulopathy Associated with Pregnancy** Ederson Vidal Moura,[1] Carla Queiroz Neves,[1] Alline S. A. Oliveira,[1] Camila Barbosa L. Oliveira,[1] Luis H.B.C. Sette,[1] Gisele Vajgel Fernandes,[1] Filipe Carrilho,[1] Lucila Maria Valente.[1] *[1]Nefrologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil; [2]Nefrologia, UFPE, Recife, Pernambuco, Brazil.*

**Background:** Collapsing glomerulopathy (CG) was initialy described as idiopatic or related to HIV. Currently is associated to other clinical conditions such as: autoimmune diseases, drugs, infections and trombotic microangiopathy. In renal allografts, CG may be recurrent or rarely *de novo*.

**Methods:** A 39 years old female patient diagnosed with SLE in 1993 developed ESRD. She underwent hemodialysis for 7 years and kidney transplant (deceased donor) in 2001. During follow-up serum creatinine (Scr) levels remained around 1.0 mg/dL. She had IgG + for CMV and HTLV since pre-transplant period, without clinical evidence of infection. Immunosuppressive drugs were prednisone, FK and azathioprin. In march 2013, during the 27th week of pregnancy, she developed proteinuria (3.19 g/24 h), edema, dyslipidemia, hypertension and worsening SCr, whithout symptoms of lupus flare or increased FK levels. Pregnancy was interrupted and blood pressure levels improved, but both renal function (SCr 2.3) and proteinuria (11.4 g/24 h) worsened. Renal biopsy, performed a month after pregnancy interruption, revealing: 9 glomeruli, 4 presenting with collapse of glomerular capillary surrounded by hypertrophic podocytes consistent with CG. Moderate chronic transplant nephropathy and arteriolar hyaline deposits were also found. Immunofluorescence revealed traces of IgM in glomeruli. During follow-up the patient showed stabilization of renal function (SCr: 2.0), lower proteinuria (2.3 g/24 h) and a proper blood pressure control.

**Conclusions:** CG is associated with multiple trigger factors includding hemodynamic disturbance and endothelial lesion. In this case repport, no other factor such as: HTLV, CMV or Parvovirus B19 activity were found in histologic and sorologic investigation. Histological findings were absent for endotheliosis, however the biopsy was performed lately enough for regression of the lesions. The association with pregnancy was performed since it was the most obvious precipitating factor for the onset of nephrotic syndrome and renal allograft dysfunction.

**FR-PO1140**

**Post-Partum Hypopituitarism Presenting with Symptomatic Hyponatremia** Richa A. Pandey, Quynh N. Lam, Didier Portilla. *Div of Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

**Background:** Post partum anterior pituitary insufficiency from pituitary necrosis is rare and can manifest as SIADH causing hyponatremia.

**Methods:** 29 year old Egyptian lady presented with acute onset dizzines 7 days post-vaginal delivery of a term pregnancy, complicated by profuse vaginal bleeding, hypotension and severe anemia which responded to PRBC transfusion. She subsequently developed severe headache, salt craving and inability to lactate. She was normotensive on admission, laboratory work up revealed serum sodium and potassium (mEq/L) to be 117 and 3.5 with normal renal function. Serum and urine osmolalities (mOsm/kg) were 249 and 506, urine sodium of 191 mEq/L. Given her history of severe post partum hemorrhage Sheehan's syndrome was considered. Brain MRI revealed enlarged pituitary with features concerning for recent hemorrhage. Serum hormone panel showed partial hypopituitarism : random PM cortisol of 1 ng/dL (2-16), ACTH 6 pg/ml (6-58), TSH 0.54 uIu/mL (0.34-5.60), Free T4 0.57 ng/dL (0.58-1.68), FSH 0.59 mIU/mL, LH 0.20 mIU/mL, Prolactin 14 ng/mL. Hyponatremia was thought to be multifactorial including, hypocortisolism, hypothyroidism vs SIADH. For the first 12 hours serum sodium levels did not respond to 3% Saline and stress dose steroids. She was given a dose of conivaptan leading to a rapid rise in serum sodium from 118 to 132 mEq/lt requiring the use of hypotonic solutions, and one dose of desmopressin to dampen the rise. At this point all fluid replacement was held. The patient was discharged home on oral hormone replacements and serum sodium 10 days after discharge was 139 mEq/L.

**Conclusions:** Our patient did not have all the clinical features of post partum panhypopituitarism, however, the diagnosis of SIADH is likely based on laboratory data sugesting inappropiate excess of ADH. This case further confirms a previous report of the presence of SIADH causing hyponatremia during the early phase of post-partum hypopituitarism.

**FR-PO1141**

**Hypertension and Hypokalemia during Pregnancy with Complete Aldosterone Suppression** Louis R. Spiegel, Anna Mathew, Alessandro Bellucci. *Div of Kidney Diseases & Hypertension, Hofstra North Shore - LIJ School of Medicine, Great Neck, NY.*

**Background:** Common causes of hypertension during pregnancy include pre-existing hypertension, gestational hypertension, and pre eclampsia. If hypokalemia and metabolic alkalosis are present then disorders of the renin-angiotensin-aldosterone system must be considered. We present a case of a woman with hypertension and hypokalemia during the third trimester of her first pregnancy, likely due to a rare gain-of-function mutation of the mineralocorticoid receptor.

**Methods:** A 21 year old female with no prior medical history was admitted to the hospital from her obstetrician's office at 32 weeks of gestation with hypertension to 170/100. Upon admission serum potassium was 2.2 mEq/L and serum bicarbonate was 27mEq/L. She denied history of hypertension, or intake of licorice. Workup for secondary hypertension, including 24 hour urine for cortisol and catecholamines, plasma metanephrines and serum TSH, was negative. Transtubular potassium gradient (TTKG) was elevated indicating renal potassium wasting. She had no edema and 24 hour urine protein was 180 mg/day. Plasma renin activity was 0.97 ng/mL/hr and plasma aldosterone was suppressed to <1 ng/dL. Frequent intravenous and oral potassium supplementation normalized the serum potassium. Adequate blood pressure control was obtained via up-titration of oral labetalol.

**Conclusions:** We present a unique case of de novo onset hypertension and hypokalemia during the third trimester of pregnancy. Suppressed levels of aldosterone and elevated TTKG are most consistent with a rare gain-of-function mutation of the mineralocorticoid receptor (Geller's syndrome), causing progesterone to act as an agonist. High levels of progesterone result in hypertension during the second or third trimester. Follow-up genetic testing for this autosomal dominant point mutation of the mineralocorticoid receptor will be conducted to confirm the diagnosis.

**FR-PO1142**

**Syphilis in a Patient with Recently Acquired HIV-Infection: A Reversible Cause of Nephrotic Syndrome** Oleh M. Akchurin, Aracelis D. Fernandez, Linda Wesp, Daniel Schwartz, James M. Pullman, Frederick J. Kaskel, Amy L. Skversky. *Montefiore Medical Center.*

**Background:** Secondary syphilis is a rare but described cause of nephrotic syndrome (NS), usually with a good prognosis. Much less is known about NS in patients with syphilis and HIV co-infection.

**Methods:** A 20 year-old Hispanic male presented with pitting edema, 7 kg weight gain, macular rash to the face, trunk and extremities, and exudative tonsillitis. Patient has a history of HSP and penicillin allergy. Two months prior, patient had negative syphilis test, normal serum chemistries, creatinine and albumin. Family history: grandmother had ESRD secondary to SLE-nephritis. Social history: patient is sexually active with men, and reported negative HIV test 4 months prior. Laboratory evaluation confirmed the diagnosis of NS (urine protein-to-creatinine ratio 7.1, serum albumin 1.5 g/dL), and AKI (peak serum creatinine 1.5 mg/dL). His C3/C4 complement levels were normal, ANA, ASLO, and streptozyme were negative. A throat swab was positive for group A *streptococcus*. Urine DNA-probe test for gonorrhea and Chlamydia was negative. HIV-1 Western blot was positive and viral load was 113,011 copies/ml. Syphilis serology was positive and RPR was 1:256. Kidney biopsy showed no glomerular, tubulointerstitial or vascular abnormalities on light microscopy. Immunofluorescence revealed 2+ IgG, trace IgM, 1+ IgA, trace C3, 1+ C1q, 1+ Kappa and Lambda light chains in granular pattern. Electron microscopy showed scattered subendothelial, mesangial, and subepithelial electron dense deposits and subtotal foot processes effacement. Treatment of syphilis with Doxycycline resulted in resolution of the NS and normalization of kidney function within 2 weeks. Subsequently, treatment for HIV was initiated. Urinalysis remained protein-free and kidney function was normal 6 months later.

**Conclusions:** To our knowledge, this is the first report with the availability of renal histology describing syphilis as a reversible cause of the NS in an adolescent patient with HIV-infection. The pathology showed IgG/IgA immune complex disease as the probable cause, likely related to IgA, membranous or post infectious glomerulopathy.

## SA-PO001

**Design of a Bioartificial Renal Cell System (BRECS) for Mass Production**
H.D. Humes,[1,2] C. Pino,[1] T. DeLandsheer,[1] A. Westover,[1] D. Buffington.[1]
*[1]Innovative BioTherapies; [2]Univ of Michigan.*

**Background:** The bioartificial renal epithelial cell system (BRECS), is a cell-based device to treat acute kidney injury. This technology is based on a Phase I/II clinically proven cell-therapy approach, previously demonstrated by our group: renal assist device (RAD). To enable widespread implementation of cell therapy, the BRECS was designed to be cryopreserved as a complete device, cryostored, cryoshipped to an end-use site, thawed as a complete device, and employed in a therapeutic extracorporeal hemofiltration circuit. This strategy overcomes the storage and distribution issues that have been previous barriers to cell therapy.

**Methods:** Following computer aided design (CAD) to simulate flow through the device, prototype BRECS were produced by stereolithography (SLA-BRECS) and tested to determine the design to be mass-produced by injection molding (IM-BRECS). Cell viability and functionality were assessed by lactate production, oxygen consumption rate (OCR) and glutathione metabolism during perfusion culture. Impact of cryopreservation on SLA-BRECS and IM-BRECS were assessed by metabolic function after thaw, and viability stains upon study termination.

**Results:** The SLA-BRECS design with the best cell performance was chosen to be mass-produced by injection molding. The resulting IM-BRECS design was palm-size, had a fill volume of ~10 mL, and exhibited near plug-flow conditions without major stagnation points or areas of recirculation. This design was capable of supporting ~1 x $10^8$ cells, with a mean OCR of ~200 nmol/min, lactate production of ~700 µmol/day, and glutathione degradation of ~800 nmol/hr over extended culture periods up to 2 months. ZO-1 (epithelial tight junctions), AT-1 (central cilia) and brush border enzymes were evident in cell populations, demonstrating renal cell specific phenotypes. Cell viability was well retained throughout developed cryopreservation and thaw procedures.

**Conclusions:** IM-BRECS can be mass-produced for potential use in AKI and related indications which have been preliminarily assessed in pre-clinical large animal models. This innovative, tissue engineered device overcomes many of the hurdles to clinical implementation of cell therapy.

*Funding:* NIDDK Support, Other U.S. Government Support

## SA-PO002

**Comparison of Kidney Injury between Two Stage Revision Arthroplasty with Antibiotic Loaded Spacers and Other Revision Orthopaedic Procedures**
Anshul Bhalla,[1] Madhumathi Rao.[2] *[1]Dept of Medicine, Tufts Medical Center, Boston, MA; [2]Div of Nephrology, Tufts Medical Center, Boston, MA.*

**Background:** Two-stage revision arthroplasty using antibiotic loaded spacers (ALS) is the standard of care for prosthetic joint infections (PJI). Although systemic toxicity is believed to be rare, acute kidney injury (AKI) has been reported. We examined the attributable risk (AR) for AKI from arthroplasty with spacers, compared with other revision orthopedic procedures.

**Methods:** Between August 2007 and December 2012 we identified all patients who underwent two-stage revision arthroplasty using antibiotic loaded spacers (Group 1, N=75), revision surgeries with prophylactic antibiotic in cement (Group 2, N=33) and revision surgeries with no antibiotic in cement (Group 3, N=80) at a single Referral Center. Tobramycin and vancomycin were uniformly used in the cement. We compared the incidence of AKI (by AKIN and RIFLE definitions) and examined risk factor relationships across the three groups.

**Results:** Table 1 shows the incidence of AKI across the three groups and was significantly higher among Group 1 patients. The AR for AKI was 15.4% (95% CI 3% to 28%) for spacers compared to revision arthroplasty without antibiotic cement. Other risk factors for AKI included baseline CKD (p=0.05), cardiovascular disease (p = 0.03), hypertension (p<0.01), use of NSAIDS (p=0.02), intra operative hypotension and/or use of pressors (p<0.01) and need for transfusion (p<0.01).

| | Group 1, N=75 | Group 2, N=33 | Group 3, N=80 | P-value |
|---|---|---|---|---|
| Age (years) | 63.2 (SD 12.9) | 64.6 (SD 10.5) | 66.4 (SD 13.8) | 0.3 |
| Female (%) | 46.7% | 78.8% | 62.5% | <0.01 |
| Incidence of AKI | 26.7% | 15.2% | 11.3% | 0.04 |

**Conclusions:** AKI is significantly associated with two stage arthroplasty with antibiotic loaded spacers for PJI compared to other revisions and is accentuated by comorbidities and intra-operative complications. A better understanding of the risk benefit ratio of this procedure is needed.

## SA-PO003

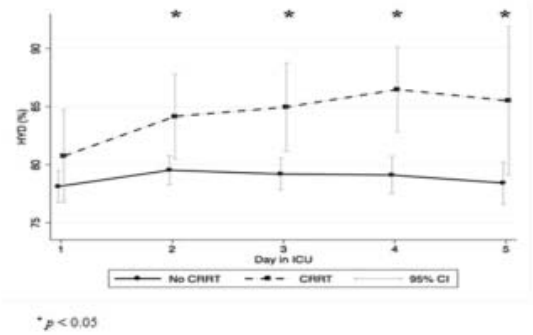
**Prospective Hydration Status Evaluation by Bioelectric Impedance Analysis in Patients Admitted to ICU**
Flavio Basso, Giacomo Mason, Sonya Day, Alessandra Brendolan, Claudio Ronco. *International Renal Research Institute of Vicenza, Vicenza, Italy.*

**Background:** Fluid imbalance is an important risk factor for morbidity and mortality in ICU, whether or not they require CRRT. The aim of the study is the prospective evaluation of hydration status, measured by Bioelectric Impedance Analysis(BIVA), in patients at the admission to the ICU and in following days.

**Methods:** This is a prospective cohort study in critically ill patients, observed daily, for a period of 72 to 120 hrs from admission. Patients were considered normohydrated if BIVA was between 72.7% and 74.3% of body water, fluid overloaded if BIVA>74.3% and dehydrated if BIVA<72.7%.

**Results:** 280 BIVA measurements were taken in 64 pts. 14 subjects were started on CRRT during ICU stay. More than 70% of pts were admitted to the ICU in a hyperhydration state, and remained overhydrated during the observation period. Pts who underwent CRRT showed significant increase in hydration during ICU stay (p<0.05). While no difference in hydration at admission was seen between CRRT and non-CRRT pts (Fig. 1), the former show a higher hydration status in the remaining 4 days of observation (p<0.05).

Fig. 1: Median Cummulative Hydration Status during the 5 days observational period for subjects receiving or not receiving CRRT




*$p < 0.05$

Univariate logistic regression shows a significant correlation between ICU mortality and maximum hydration(p<0.05) and mean hydration(p<0.05), respectively. Even in a bivariate analysis with both hydration and severity of prognosis, hydration remains significant regardless of which of the 3 ICU scoring systems (APACHE II,SAPS II,SOFA) is used to stratify the model(p<0.05).

**Conclusions:** Fluid overload is a frequent condition in critically ill patients. Hyperhydration persists during ICU stay, independently from the need of CRRT. Both mean hydration and maximum hydration reached represent a significant risk factor for mortality in the ICU. These findings suggest the need to develop adequate protocols to monitor hydration status in ICU patients.

## SA-PO004

**Early Goal-Directed Therapy in the Management of Type 1 Hepatorenal Syndrome: A Combined Retrospective and Pilot Study**
Zhiwei Zhang,[1] Geetha S. Maddukuri.[2] *[1]Nephrology, VA Loma Linda Healthcare System and Loma Linda Univ, Yorba Linda, CA; [2]Nephrology, St. Louis Univ, St. Louis, MO.*

**Background:** Appreciation of the central role for arterial vasodilatation in the pathogenesis of hepatorenal syndrome (HRS) has led to routine use of vasoconstrictors in combination with albumin as a medical therapy for HRS. Various vasoconstrictors have been explored but the optimal approach for such therapies has not yet been established. The aim of current study was to examine the role of early goal-directed therapy (EGDT) in the management of type 1 HRS, a condition associated with very poor prognosis.

**Methods:** A total of 59 patients with type 1 HRS who received a combination therapy of vasoconstrictors and albumin were enrolled into a retrospective cohort study. Subjects having a sustained increase of more than 10 mmHg in mean arterial pressure (MAP) by day 3 after initiation of therapy were categorized as EGDT and the rest as controls. In addition, five patients were enrolled into a prospective pilot study in which a titration protocol of vasoconstrictors was utilized to achieve EGDT.

**Results:** Patients in the EGDT group achieved significantly higher incidence of treatment success or total response, less requirement of dialysis and more incidence of liver transplantation. More importantly, EGDT is associated with better short-term and long-term overall survival as well as transplant-free survival. The effectiveness of such an approach was further confirmed in the pilot study.

**Conclusions:** Management of HRS with EGDT is associated with favorable clinical outcomes. Targeting an early and sustained increase in MAP is clinically feasible and physiologically sound, although the optimal target has yet to be determined by prospective studies.

*Funding:* Veterans Affairs Support

## SA-PO005

**Body Composition Monitor in Contrast-Induced AKI: A New Tool for an Old Enemy**
Paolo Lentini,[1] Luca Zanoli,[2] Stefania Rastelli,[2] Massimo de Cal,[1] Anna Basso,[1] Andrea Contestabile,[1] Valentina Pellanda,[1] Roberto Dell'Aquila.[1] *[1]Nephrology, San Bassiano Hospital, Bassano del Grappa (VI), Italy; [2]Internal Medicine, Catania.*

**Background:** Most radiological procedures require administration of contrast media. Contrast-induced acute kidney injury (CI-AKI) is the third cause of hospital acquired AKI. Extracellular volume expansion reduced contrast-induced tubulo-toxic effects.We aimed to test if control of hydration by bioimpedance is useful to reduce incidence of CI-AKI.

**Methods:** 50 patients undergoing contrast medium infusion of 100-150 ml of iopamidol for abdominal or thoracic computer tomography were enrolled and randomly assigned to receive standard CI-AKI protocol prevention or Bioimpedance-based hydration protocol. Standard CI-AKI protocol consisted in administration,1day before and 1day after the administration of contrast media,at least 1 ml/kg/h of saline solution,1 mg/kg/h of sodium

bicarbonate and N-acetyl-cysteine twice a day.In the bioimpedance-based protocol,a mild over-hydration (within +1 and +3)was obtained the day of the procedure and the next 3 days with the infusion of saline solution plus 1 mg/kg/h of sodium bicarbonate and N-acetyl-cysteine twice a day.Bioimpedance was performed daily with Body Composition Monitor(BCM -Fresenius Medical Care®)to evaluate the hydration status.Laboratory and clinical examination were performed daily after the procedure for 3 days.CI-AKI was defined as a rise in sCr of 0.5 mg/dl 48hrs after a radiological procedure.

**Results:** 49 patients were enrolled(63±14yrs;GFR64±31ml/min/1.73m2):29 patients were included in the bioimpedance-based protocol harm and 20 patients in standard protocol harm.For a comparable age,sCr and GFR at baseline,incidence of AKI was significantly lower in bioimpedance-based than in standard protocol(14%vs40%,respectively;P=0.04 ).In multivariate analysis adjusted for age,the bioimpedance-based protocol(OR0.17 if performed;95%CI 0.03-0.99;P<0.05)and high baseline GFR(OR0.92 for 1 ml/min/1.73m2 increase;95%CI0.87-0.98;P=0.005)reduced the risk of CI-AKI.

**Conclusions:** The bioimpedance-based control of hydration is useful to reduce CI-AKI incidence in patients undergoing abdominal or thoracic computer tomography.

## SA-PO006

**Acute Creatinine Improvement after CT with and without Intravenous Iodinated Contrast Use** Masahiko Nagahama,[1] Goki Eriguchi,[2] Keita Hirano,[1] Fumika Taki,[1] Takuya Fujimaru,[1] Kenichiro Koitabashi,[1] Kumiko Shimasaki,[1] Yasuhiro Komatsu.[1] *[1]Nephrology, St. Luke's International Hospital, Tokyo, Japan; [2]Biostatistics, Kurume Univ Graduate School of Medicine, Fukuoka, Japan.*

**Background:** Administration of intravenous (IV) iodinated contrast medium has been associated with acute kidney injury (AKI), known as contrast induced nephropathy (CIN). Of interest, there are some subjects whose serum creatinine (sCr) is decreased and kidney function seems to be improved after contrast use. We have termed this condition "Acute Creatinine Improvement (ACI)". The purpose of this study was to clarify the incidence and prognosis of "ACI" as well as CIN in patients undergoing CT with and without IV contrast.

**Methods:** sCr was evaluated for 7039 patients who had had, either enhanced CT (Exposure, n=3671) or unenhanced CT (Comparison, n=3368), at St. Luke's international hospital in Tokyo between 2003 and 2010. CIN is defined as a 0.5 mg/dL increase in sCr or a 25% or greater decrease in eGFR within 7 days after CT, while "ACI" is defined as a 0.5 mg/dL decrease in sCr or a 25% or greater increase in eGFR as opposed to CIN. The survival rate was compared in "ACI" and Non-"ACI "subjects as well as CIN and Non-CIN subjects using Kaplan-Meier curves.

**Results:** In exposure group, 7.2% of patients developed CIN and 7.9% of patients developed "ACI", while in comparison group, 14% of patients developed "ACI". The prognosis of patients with CIN was significantly worse than that without CIN (P<0.0001 by log-rank). Although the prognosis of patients with "ACI" and Non-"ACI" in exposure group were not significantly different (P=0.32 by log-rank), the prognosis of patients with "ACI" was significantly worse than that without "ACI" in comparison group (P<0.0001 by log-rank).

**Conclusions:** We identified a high incidence of "ACI" in both exposure group and comparison group. Remarkably, development of "ACI" has poor impact on prognosis as CIN does. This suggests that any fluctuation of sCr, either rise or fall, in one week period is related to worse prognosis.

## SA-PO007

**Hydration prior to CT-Pulmonary Angiography Is Not Required for Prevention of Contrast Induced-Acute Kidney Injury: The Randomized Nefros Trial** Judith Kooiman,[1] Yvo W.J. Sijpkens,[2] Marjolyn Van Buren,[3] Aart J. Molen, Van der,[1] Nicolas Jm Aarts,[2] Cornelis J. Van Rooden,[3] Suzanne Cannegieter,[1] Hein Putter,[1] Ton J. Rabelink,[1] Menno V. Huisman.[1] *[1]Leiden Univ Medical Center; [2]Bronovo Hospital; [3]Haga Teaching Hospital; [4]Medical Center Haaglanden.*

**Background:** Hydration to prevent contrast induced-acute kidney injury (CI-AKI) results in a diagnostic delay when performing CT-pulmonary angiography (CTPA) in patients presenting with clinically suspected acute pulmonary embolism (PE). The aim of our study was to analyze whether withholding hydration is non-inferior to one hour 250ml 1.4% sodium bicarbonate (Na-bic) hydration prior to intravenous contrast administration for CTPA in patients with a GFR<60 ml/min.

**Methods:** Primary outcome of this randomized trial was the increase in serum creatinine 48-96 hours post CT. Secondary outcomes were the incidence of CI-AKI (increase in serum creatinine >25%/>0.5mg/dl), recovery of renal function, and the need for dialysis. Withholding hydration was considered non-inferior if the mean relative serum creatinine increase was at most 15% higher compared with Na-bic.

**Results:** From 2009-2013, 135 patients with clinically suspected PE undergoing CTPA (mean age 70.4years range 69, mean GFR 41.9 range 51) were randomized. Mean relative serum creatinine increase for no hydration was -3.3%(SD20.5) and -3.0%(SD17.2) for Na-bic (mean difference -0.4%,95%CI-7.0 to 6.3, P non-inferiority<0.001). CI-AKI occurred in 9(7.0%) patients; 4(6.5%) did not receive hydration, 5(7.5%) were treated with Na-bic (p=0.82). Two patients with CI-AKI in the no hydration arm died of causes other than renal failure and one CI-AKI patient in the Na-bic arm started pre-planned dialysis within two months post CTPA. Renal function recovered in all other CI-AKI patients within two months.

**Conclusions:** Withholding hydration was non-inferior to Na-bic hydration prior to CT-PA, with a similar risk of CI-AKI in both groups. Therefore, our study results demonstrate that preventive hydration can be safely withheld in daily practice of chronic kidney disease patients undergoing acute CTPA for symptomatic PE.

*Funding:* Private Foundation Support

## SA-PO008

**Evalution of the Risk Factors for Contrast Induced Nephropathy in Hypertensive Patients with Normal Renal Function** Osman Z. Sahin,[1] Fatih Sumer,[2] Mehmet Bostan,[3] Teslime Ayaz,[2] Yavuz Ugurlu.[3] *[1]Nephrology, Recep Tayyip Erdogan Univ Faculty of Medicine, Rize, Turkey; [2]Internal Medicine, Recep Tayyip Erdogan Univ Faculty of Medicine, Rize, Turkey; [3]Cardiology, Recep Tayyip Erdogan Univ Faculty of Medicine, Rize, Turkey.*

**Background:** Coronary angiography which requires intravascular administration of iodinated contrast media (CM) is a leading cause of contrast induced nephropathy (CIN). The aim of this study was to assess the risk factors on the development of CIN in hypertensive patients who have normal renal function undergoing coronary angiography.

**Methods:** This retrospective study included all consecutive patients who underwent elective coronary angiography at our center between September 2011 and May 2012. Coronary angiography was performed 254 hypertensive patients. Before the procedure, IV saline infusion and N-acetylcysteine were given to the all patients. ACE-I or ARB was discontinued 2 days before the procedure.CIN was defined as >0.5 mg/dL increase or >25% rise in serum creatinine (SCr) concentration within 48 hours of contrast exposure compared to baseline.

**Results:** The study population consisted of 254 patients, aged 19-84 years (mean: 57.67 ± 9.7) and 57% of them were male. 29.1% of the patients had type 2 diabetes. CIN occurred in 7% of the patients. Clinical data of the patients are presented in table 1.

| | Patients with CIN (n=18) | Patients without CIN (n=236) | *p* |
|---|---|---|---|
| **Age (years)** | 61.33±9.71 | 57.39±9.72 | 0.098 |
| **Gender (% male)** | 72.2% | 56.8% | 0.201 |
| **Fasting plasma glucose (mg/dl)** | 95.61±9.96 | 116.80±40.85 | **0.029** |
| **Proteinuria (mg/day)** | 0.282±0.127 | 0.205±0.020 | 0.344 |
| **Presence of type 2 diabetes (%)** | 16.7% | 30.1% | 0.227 |
| **Contrast volume (ml)** | 138.05±50.96 | 133.26±61.151 | 0.748 |
| **Uric acid (mg/dl)** | 6.22±1.16 | 5.52±1.45 | **0.048** |

**Conclusions:** Hyperuricemia can be a predictor of contrast induced nephropathy in hypertensive patients with normal renal function.

## SA-PO009

**Sodium Bicarbonate, N-Acetylcysteine, Both, or Saline Alone for Prevention of Contrast-Induced Nephropathy: A Network Meta-Analysis** Seth Wright, Katherine H. Michener, Amy Earley, Raveendhara R. Bannuru, Katrin Uhlig. *Tufts Medical Center, Boston, MA.*

**Background:** Trials have shown inconsistent results on whether contrast-induced nephropathy (CIN) can be prevented by giving IV sodium bicarbonate (Bic) instead of IV saline or by adding oral N-acetylcysteine (NAC) to IV saline. Furthermore, it is uncertain which intervention is superior or whether they should be combined.

**Methods:** We conducted a systematic review in MEDLINE and the Cochrane Central Register of Controlled Trials (1995-4/2013) of trials using these interventions and reporting outcomes of CIN (based on creatinine rise ≥25% or 0.5 mg/dL) or dialysis for a network meta-analysis. Analysis was by a Bayesian random effects multiple treatment comparison model with non-informative priors, using Markov Chain Monte Carlo implement through OpenBUGS software.

**Results:** 38 studies (n=6466) provided data on CIN. Mean patient age ranged from 59-78 years. Most patients had CKD stage 3 (range 1-4) and received intra-arterial contrast (predominantly for coronary angiography). The Figure shows the odds ratios for CIN for the different comparisons by network meta-analysis. There was consistency between direct and indirect effect estimates. For the dialysis outcome, odds ratios showed the same order but with wide, non-statistically significant credibility intervals due to fewer events and comparisons (12 studies, 2587 patients).

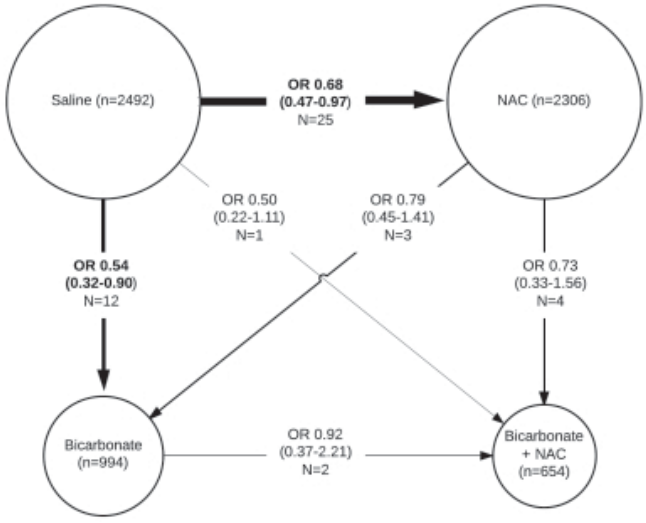



*Figure:* Odds ratios for CIN (with 95% credibility interval). Arrow tail = reference intervention. Circle size reflects the n of subjects; line width reflects the N of direct comparisons.

**Conclusions:** By network meta-analysis, Bic and NAC are both superior to saline with a 46% and 32% reduction in odds of CIN, respectively. Bic or NAC should be given rather than saline alone. These data also suggest combination therapy may have additional benefit.

*Funding:* NIDDK Support, Other U.S. Government Support

## SA-PO010

**Risk Factors and Renal Outcomes for Cisplatin Nephrotoxicity** Zeenat Yousuf Bhat, Kevin B. Ginsburg, Milani Sivagnanam, Pravit Cadnapaphornchai, Mona D. Doshi. *Internal Medicine, Wayne State Univ, Detroit, MI.*

**Background:** Nephrotoxicity is a well-known complication of Cisplatin-based chemotherapy. Our goal was to determine the risk factors for development of Cisplatin nephrotoxicity (CN) and to assess renal function at 12 months thereafter, in the current era of aggressive hydration and careful patient selection.

**Methods:** We identified 131 patients who received 317 cycles of high-dose Cisplatin for treatment of head and neck cancer. All patients were hydrated per protocol and none had baseline glomerular filtration rate via CKD-EPI (GFR) < 60mL/min/1.732m$^2$. The patients were grouped based on development of acute kidney injury (AKI) and graded for levels of injury, per RIFLE criteria. Patients with AKI were screened for the absence of nephrotoxin use and a temporal decline in serum potassium and magnesium levels to labeled as CN.

**Results:** Of the 131 patients included in the study, 77 (58.7%) developed CN -25 (33%) grade 1, 40 (52%) grade 2, and 12(15%) grade 3 level of injury. Their potassium and magnesium levels negatively correlated with level of injury (p<0.05). In the univariate analysis, African Americans (AA), males, smokers, and hypertensives were more likely to develop CN (p<0.05). In the multivariate analysis, AA race and history of hypertension remained to be significant risk factors for CN, OR 6.5 (95% CI 2.1 to 19.9) and 3.2 (95% CI 1.1 to 9.6) respectively, and having a PEG tube was protective, OR 0.25 (0.09 to 0.7). Table 1 shows the GFR at 12 month after therapy was lower than baseline in patients with CN.

| | GFR, mL/min/1.732m$^2$ | | | |
|---|---|---|---|---|
| | Baseline | During Treatment | 1-month follow-up | 12-month follow-up† |
| No AKI | 110±16 | 94±21 | 109±19 | 107±22 |
| AKI, grade 1 | 109±20 | 66±22 | 94±28 | 92±30* |
| AKI, grade 2 | 114±20 | 51±17 | 84±31 | 89±30* |
| AKI, grade 3 | 125±18 | 25±10 | 68±30 | 80±30* |

†versus baseline

*p<0.05

**Conclusions:** CN remains a common complication resulting in irreversible loss of GFR. PEG tube placement may be beneficial in patients with head and neck cancer receiving Cisplatin to maintain their volume status.

## SA-PO011

**Is It Safe to Use IV Ketorolac in Hospitalized Patients?** Joseph E. Chan, Debra J. Hain, Rute C. Paixao, Dianne T. Sandy, Fei Wang, Mauro Braun. *Dept of Nephrology and Hypertension, Cleveland Clinic Florida, Weston, FL.*

**Background:** Ketorolac has been known to cause acute kidney injury (AKI) but current data suggests that it is safe for those who have low risk for renal dysfunction. In our facility, there have been cases of AKI in patients treated with Ketorolac but the incidence is not known. This study describes the prescribing habits of Ketorolac in our facility and determines the incidence of AKI while on this therapy.

**Methods:** Electronic medical records of hospitalized patients who received Ketorolac were reviewed during a 3 month period. AKI was defined as an increase of serum creatinine of 0.3 mg/dl or greater and a decrease in eGFR to less than 60 ml/min while on treatment with Ketorolac. Treatment duration is defined as the time of initial administration up to the third day from the last dose of Ketorolac.

**Results:** A total of 633 medical records were reviewed and 341 patients met the inclusion criteria. The mean age was 45.7 years and 65% were females. The most common diagnosis for prescribing Ketorolac was Osteoarthrosis. The conventional prescribed dose was 30 mg IV every 6 hours. AKI developed in 6.4% of patients. In patients with AKI, 68% were 65 years or older, 68% had hypertension, 41% were diabetic, 40% were concomitantly receiving either an ACE-i or an ARB and 40% were also being given diuretics. The mean pre dose creatinine was 1.09 mg/dl and mean post dose creatinine was 1.72 mg/dl. During the time of AKI, 72% still received Ketorolac.

**Conclusions:** AKI occurs more commonly than previously anticipated in Ketorolac treated patients even at average doses. Hypertension and diabetes are the two most common comorbidities in patients who developed AKI. Those who are older than 65 years old may be at higher risk. Concomitant use of drugs that affect renal function, such as ACE-i, ARBs and diuretics, may also increase the risk of AKI. Ketorolac prescribing in the acute care hospital should consider individual comorbdities, and use of other drugs that can increase AKI. Awareness of current renal function through diligent review of daily labs may help prevent administration of Ketorolac in those with already impaired renal function.

## SA-PO012

**Creatinine Production Is Reduced in Acute Kidney Injury Patients with Sepsis** Rolando Claure-Del Granado,[1] Josee Bouchard,[2] Glenn M. Chertow,[3] Jonathan Himmelfarb,[4] T. Alp Ikizler,[5] Ravindra L. Mehta.[6] *[1]School of Medicine, IIBISMED, Universidad Mayor de San Simon, Bolivia; [2]Univ of Montreal, Canada; [3]Stanford School of Medicine; [4]Kidney Research Institute, Univ of Washington; [5]Vanderbilt Univ Medical Center; [6]Univ of California San Diego.*

**Background:** Diagnosis and staging of acute kidney injury (AKI) uses the serum creatinine concentration (sCr). In a previous animal model of AKI, Doi et al. have shown that sepsis dramatically decreases sCr and creatinine production. This phenomenon would limit early detection of AKI. We evaluated the effect of sepsis on sCr, creatinine production (Pc'), and creatinine degradation (Dc') in patients with AKI. We hypothesized that sepsis will reduce creatinine production and sCr.

**Methods:** We analyzed data from 234 critically ill non-dialyzed patients with AKI from five centers included in the PICARD study. Creatinine production was calculated using Cockcroft-Gault formula and using the Moran et al. formula (Kidney Int 1985, 27:928-937), which adjusts sCr for fluid balance. Creatinine degradation was computed using the Mitch et al. equation (Clin Sci 58:327-335, 1980) and adjusted for fluid balance.

**Results:** Of the 234 patients, 139 were septic (59%). Non-adjusted and adjusted sCr levels were lower in patients with sepsis (non-adjusted sCr median 2.0 mg/dL IQR [1.5 – 2.8] vs. 2.5 mg/dL IQR [1.8 – 3.5] and adjusted sCr 2.0 mg/dL IQR [1.4 – 2.7] vs 2.4 mg/dL IQR [1.8 – 3.6]; p <0.001). Pc' was significantly lower in septic than in non-septic (1,211 mg/day IQR [934 – 1,472] vs. 1,278 mg/day IQR [1,017 – 1,538] mg/day; p < 0.001); and after adjusting Pc' for fluid balance (1,092 mg/day IQR [828 – 1,295] vs. 1,124 mg/day IQR [892 – 1,344]; p < 0.001). Dc' was also significantly lower in septic than in non-septic patients.

**Conclusions:** Sepsis reduces creatinine production and reduces serum creatinine in critically ill patients with AKI thereby blunting the expected increases of serum creatinine. These observations could confound the early diagnosis of AKI. Sepsis also affects creatinine degradation.

*Funding:* NIDDK Support

## SA-PO013

**Remote Ischaemic Preconditioning Does Not Prevent Kidney Injury after Cardiac Surgery in Patients with Chronic Kidney Disease** Sean Gallagher,[1] Dan A. Jones,[1] Steven Michael Harwood,[3] Rakesh Uppal,[1] Magdi Yaqoob.[2] *[1]Cardiology, Barts Health NHS Trust, London, United Kingdom; [2]Nephrology, Barts Health NHS Trust, London, United Kingdom; [3]William Harvey Research Institute, Queen Mary Univ, London, United Kingdom.*

**Background:** Perioperative complications of cardiac surgery including myocardial infarction and acute kidney injury (AKI) are associated with substantial morbidity and mortality. We assessed the impact of remote ischaemic preconditioning (RIPC) upon these complications in patients with chronic kidney disease (CKD).

**Methods:** 86 patients with CKD (defined as eGFR < 60mL/min) undergoing coronary surgery were randomized 1:1 to standard care with (n=43) or without (n=43) RIPC. RIPC consisted of three 5-minutes cycles of forearm ischaemia and reperfusion. Primary end point was development of AKI defined as a 0.3 mg/dL increase in serum creatinine within 48 hours of surgery. Secondary end points included a comparison of biomarkers of renal injury (serum cystatin C (CyC), serum and urinary neutrophil gelatinase associated lipocalin (NGAL)) and a comparison of myocardial enzyme release (defined by 72 hour troponin T area under the curve (72 hrs AUC cTnT)).

**Results:** Clinical and operative characteristics were similar between RIPC and control groups. 27.9% (24/86) of the study cohort developed AKI following surgery. The incidence of AKI was similar in both groups (RIPC 27.9% (12/43) vs control 27.9% (12/43); p=1.0). There was no difference in biomarkers of renal injury or myocardial enzyme release between the groups following surgery.

**Conclusions:** RIPC using forearm ischaemia confers no meaningful additional renal or myocardial protection in patients with CKD undergoing cardiac surgery.

**SA-PO014**

**Effect of Hospital Volume on Acute Kidney Injury in Percutaneous Nephrolithotomy Admissions** Ankit Sakhuja,[1] Manoj Monga,[2] Juan C. Calle.[1] *[1]Nephrology, Cleveland Clinic, Cleveland, OH; [2]Urology, Cleveland Clinic, Cleveland, OH.*

**Background:** Acute Kidney Injury (AKI) is an important cause of morbidity and mortality in hospitalized patients. In general, procedural volume has been shown to be associated with worse outcomes. However, not much is known about AKI in hospitalized patients undergoing Percutaneous Nephrolithotomy (PCNL). We, therefore, designed this study to look at the frequency and effect of hospital volume on AKI in hospitalized patients undergoing PCNL.

**Methods:** We designed a retrospective cohort study using Nationwide Inpatient Sample database, which is the largest all-payer inpatient care database in US and contains a 20% stratified sample of US community hospitals. Patients aged ≥18 years undergoing PCNL in year 2010 were identified using ICD-9-CM codes. AKI was identified using ICD-9-CM codes 584.X. End Stage Renal Disease Patients were excluded. Chi square test was used to compare categorical variables. Hospital procedure volume for the year studied was generated and divided into low volume (<50), medium volume (50-100), high volume (>100) hospitals. A multivariable logistic regression was used to assess the effect of hospital volume on AKI.

**Results:** Of estimated 46,899 (95% CI: 42,710-51,088) PCNL admissions in the year 2010, 34.1% (16,010) had AKI. Patients with AKI tended to be more often older males, African Americans and on Medicare. They also more often had chronic kidney disease (CKD), Urinary Tract Infection (UTI) and hydronephrosis with UTI. Patients with AKI also seemed to be admitted more often to lower volume hospitals (57.0.% vs 66.3% of those with no AKI; p<0.001). On multivariable regression, in comparison to low volume hospitals, admission to high volume hospitals was associated with decreased odds of AKI (OR 0.59; 95% CI 0.44-0.81). The protective effect of high volume hospitals was persistent even after excluding those with primary diagnosis of hydronephrosis (OR 0.58; 95% CI 0.42-0.80).

**Conclusions:** AKI seems to occur over a third of hospitalized patients undergoing PCNL. Hospital volume of PCNL is an predictor of AKI with a protective effect of high volume hospitals.

**SA-PO015**

**The Economic Impact of Acute Kidney Injury in England** Marion Kerr,[1] Michael Bedford,[2] Beverley Matthews,[3] Donal O'Donoghue.[4] *[1]Insight Health Economics; [2]East Kent Hospitals Univ NHS Foundation Trust; [3]NHS Kidney Care; [4]Salford Royal NHS Foundation Trust.*

**Background:** Acute kidney injury (AKI) is one of the most serious and common complications affecting hospital inpatients. We estimate the prevalence of AKI in hospital inpatients in England, and the impact on mortality, length of stay and National Health Service (NHS) costs.

**Methods:** National data (Hospital Episode Statistics, HES) were examined to identify all recorded cases of AKI among adult inpatients in English hospitals in 2010-11. The findings were compared with data from East Kent Hospitals, where AKI was identified from laboratory records and classified using AKIN criteria, with the lowest serum creatinine recorded in the 12 months before admission used to estimate baseline. Multivariate regressions were used to estimate the impact of AKI on mortality and length of hospital stay. Unit costs were derived from national tariffs and NHS Reference Costs.

**Results:** AKI was recorded in 2.43% of hospital admissions in HES, and in 13.22% of admissions at East Kent (laboratory-identified and age- and gender-adjusted to match the HES population). The odds ratio for in-hospital mortality associated with AKI was 10.52 (95% confidence interval 9.93-11.16) in HES data and 5.38 (4.42-6.54) in East Kent data. AKI diagnosis was associated with a length of stay 2.59 (2.56-2.62) times as high as that for admissions without AKI in HES data and 1.63 (1.58-1.69) times as high in East Kent data. The annual cost of AKI-related care recorded in HES is estimated at £337 million. Based on the higher prevalence estimated from East Kent data, the annual cost of AKI-related inpatient care is estimated at £942 million, 5% of inpatient spending in England.

**Conclusions:** AKI prevalence in inpatients may be considerably higher than previously thought, and up to four fifths of cases may not be captured in routine hospital data. AKI is associated with large numbers of in-hospital deaths and with high NHS costs. Comparison of HES and East Kent data suggests that most of the cases recorded in HES may be relatively severe AKI (AKIN 2-3). These estimates do not include AKI in the non-hospitalised population.

**SA-PO016**

**Minocycline to Prevent Kidney Injury after Cardiac Surgery: A Randomized Double-Blinded Placebo-Controlled Pilot Study** Ladan Golestaneh,[1] Ziyad Al-Aly,[2] Michael I. Rauchman,[2] Kevin J. Martin,[3] Allon N. Friedman,[4] Pierre C. Dagher,[4] Tarek M. El-Achkar.[4] *[1]Montefiore Medical Center, Bronx, NY; [2]St. Louis VA Medical Center, St. Louis, MO; [3]St. Louis Univ, St. Louis, MO; [4]Indiana Univ, Indianapolis, IN.*

**Background:** Acute Kidney Injury (AKI) after cardiac bypass surgery (CABG) is a grave complication associated with significant morbidity and mortality. Minocycline therapy attenuates kidney injury in animal models of AKI but effects in humans are unclear.

**Methods: Study Design:** Randomized, double-blinded, placebo-controlled, multi-center study. **Setting & Participants:** We screened high risk patients who were scheduled to undergo CABG in 2 medical centers between Jan 2008 and June 2011. 40 patients were randomized and 19 patients in each group completed the study. **Intervention:** Minocycline prophylaxis given twice daily, at least for four doses prior to CABG to prevent AKI. **Outcome**: Primary outcome defined as AKI (0.3 mg/dl increase in Creatinine (Cr)) within 5 days after surgery. **Measurement:** Daily serum Cr for 5 days, various hemodynamic measures and length of stay (LOS).

**Results:** The two groups had similar baseline and intra-operative characteristics. The primary outcome occurred in 52.6% of patients in the minocycline group compared to 36.8% of patients in the placebo group (p= 0.51). Peak Cr was 1.6±0.7 vs. 1.5±0.7mg/dl (p=0.45) in the minocycline and placebo groups, respectively. Death at 30 days occurred in 0% vs. 10.5% in the minocycline and placebo groups, respectively (p=0.48). There were no differences in post-operative LOS, and cardiovascular events between the two groups, though there was a trend towards lower diastolic pulmonary artery pressure (16.8±4.7vs. 20.7±6.6 mmHg (p=0.059) and central venous pressure (11.8±4.3 vs. 14.6±5.6mmHg (p=0.13) in the minocycline group compared to placebo on the first day after surgery. In univariate analysis, obesity, peripheral vascular disease, duration of surgery and intraoperative hypotension were all significantly associated with developing AKI.

**Conclusions:** Minocycline did not reduce the risk of AKI in patients undergoing CABG. The potential effects of minocycline on pulmonary and cardiac compliance warrant a larger trial.

*Funding:* Pharmaceutical Company Support - Triax Pharmaceutical, Private Foundation Support

**SA-PO017**

**The Mechanism Underlying Nesiritide's Renal Protective Effects in Cardiac Surgery** Thomas M. Beaver,[1] Jessica A. Cobb,[1] Debra Robertson,[1] Maria Cecilia Lopez,[3] Henry V. Baker,[3] Lyle L. Moldawer,[1] Tomas D. Martin,[1] Philip J. Hess,[1] A. Ahsan Ejaz.[2] *[1]Surgery, Univ of Florida, Gainesville, FL; [2]Medicine, Univ of Florida, Gainesville, FL; [3]Molecular Genetics & Microbiology, Univ of Florida, Gainesville, FL.*

**Background:** Acute Kidney Injury (AKI) following cardiac surgery continues to be a problem; however, studies have demonstrated natriuretic peptides have renal protective effects. This study examined the protective mechanism of nesiritide, a human recombinant brain natriuretic peptide.

**Methods:** 29 cardiac surgery patients at risk for AKI were randomized to 48 hrs of nesiritide (0.01 mcg/kg/min) or placebo starting prior to surgery. AKI was based on KDIGO urine output criteria (<0.5mL/kg/hr over 6 hrs) and the biomarkers urineNGAL and urine IL-18. Serum pro-inflammatory cytokines and endothelin-1 were evaluated up to 24 hrs post cardiopulmonary bypass (CPB). In addition, microarrays of RNA from circulating neutrophils were examined. Statistical analyses were done with Mann-Whitney U and Fisher's exact tests.

**Results:** There was a trend towards decreased AKI based on urine output (57% of nesiritide group had AKI vs. 80% of placebo) and urineNGAL and IL-18 levels. Proteomic analysis found nesiritide had a mild anti-inflammatory effect (IL-6 and IL-10 levels decreased in the first hr after CPB). There was also a trend of lower endothelin-1 levels. Comparison of microarray expression patterns in patients with AKI vs. no AKI showed no significant difference. Table1: () denotes time post CPB in hrs. Values expressed as mean±SEM; NGAL in ng/mL; all others in pg/mL.

| | Nesiritide | Placebo | p-value |
|---|---|---|---|
| IL-6 (1) | 39.84±5.81 | 84.54±15.88 | 0.010 |
| IL-10 (0) | 1548.71±388.34 | 2399.49±327.25 | 0.021 |
| Endothelin-1 (4) | 2.674±0.207 | 3.567±0.429 | 0.163 |
| NGAL (2) | 230.27±71.49 | 554.39±263.31 | 0.253 |
| IL-18 (6) | 29.93±4.77 | 254.49±118.36 | 0.091 |

**Conclusions:** Nesiritide patients showed trends towards less AKI by biomarkers and greater urine ouput. A short-term anti-inflammatory effect and a trend of lower endothelin levels may explain nesiritide's renal protective effect.

*Funding:* Government Support - Non-U.S.

**SA-PO018**

**Clinical Acquired Resistance to Cisplatin-Induced Acute Kidney Injury in Cancer Patients** Naoko Tsuji,[1] Hideo Yasuda,[1] Takayuki Tsuji,[1] Naro Ohashi,[1] Akihiko Kato,[1] Yoshihide Fujigaki.[2] *[1]1st Dept of Medicine, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan; [2]Dept of Medicine, Teikyo Univ, Tokyo, Japan.*

**Background:** Animals that are recovering from cisplatin-induced acute kidney injury (AKI) are known to be resistant to kidney injury by repeated administration of cisplatin. This phenomenon, which is so-called acquired resistance, has not been reported in a clinical setting. We pick upped cancer patients who had cisplatin-induced AKI and investigated subsequent chemotherapy and kidney injury.

**Methods:** 54 cancer patients to which cisplatin were given in otorhinolaryngology/head and neck surgery and gastroenterology units of Hamamatsu University School Hospital from January 2007 to December 2011 were consecutively enrolled. We retrospectively investigated their clinical background and outcome. AKI was diagnosed by the criteria of Kidney Diseases: Improving Global Outcomes.

**Results:** 26 of 54 patients developed AKI after administration of cisplatin. Among 26 patients affected by cisplatin-induced AKI, cisplatin was administrated to 13 patients repeatedly in the following cycles. In the following cycles, only 3 patients (23.1%) developed AKI and but 10 patients (76.9%) did not. In 10 patients who did not repeat AKI in following cispaltin administrations, blood pressure, dosage of cisplatin, baseline serum creatinine, urine specific gravity, hydration volume, use of diuretics and blockades of renin-angiotensin systems, and other nephrotoxic agents such as non-steroidal anti-inflammatory drugs were not significantly different between the chemotherapeutic cycles with AKI and subsequent cycles without AKI. These suggested the first increases in serum creatinine were

caused by intrinsic kidney damage but not pre-renal hypovolemia. In 3 patients repeating AKI induced by cisplatin, the intervals of chemotherapeutic cycles tended to be longer than in acquired resistant patients (89.3±54.4 days vs 34.3±4.9 days).

**Conclusions:** Less incidence of AKI caused by repeated administrations of cisplatin after recovering from cisplatin-induced AKI suggested that the resistance to AKI has been acquired in the cancer patients.

## SA-PO019

**High Performance Information Search Filters for Acute Kidney Injury Content in PubMed, Ovid Medline, and Embase** Ainslie M. Hildebrand,[1] Arthur Iansavichus,[2] Nancy L. Wilczynski,[3] Amit X. Garg.[1,2,3] *[1]Div of Nephrology, Western Univ, London, Canada; [2]Kidney Clinical Research Unit, London Health Sciences Centre, London, Canada; [3]Dept of Epidemiology and Biostatistics, McMaster Univ, Hamilton, Canada.*

**Background:** We frequently fail to identify relevant articles when we search the large bibliographic databases PubMed, Ovid Medline or Embase.

**Methods:** We used computer automation to create information search filters to better identify articles relevant to acute kidney injury in these databases. We first manually reviewed a sample of 22,992 full text articles and used reference criteria to determine if each article contained acute kidney injury content or not. In the development phase (two thirds of the sample) we developed and tested the performance of over 1.6 million unique filters. Filters with high sensitivity and high specificity for the identification of acute kidney injury articles were then retested in the validation phase (remaining third of the sample).

**Results:** We succeeded in developing and validating high-performance acute kidney injury search filters for each bibliographic database with sensitivities and specificities in excess of 90%. Filters optimized for sensitivity reached at least 97.2% sensitivity and filters optimized for specificity reached at least 99.4% specificity. The filters were complex; for example one PubMed filter included over 140 terms used in combination, including 'acute kidney injury', 'tubular necrosis', 'azotemia', and 'ischemic injury'. In proof of concept searches physicians found more articles relevant to topics in acute kidney injury with the use of the filters.

**Conclusions:** These high performance information filters are now available online and can be used to better identify acute kidney injury content in large bibliographic databases.

## SA-PO020

**Sepsis Six Bundle of Care May Reduce the Incidence of Severe Acute Kidney Injury in Hospital** Edward Stern, Kelly Wright, Ben Caplin, Dorothea Nitsch, Nick R. Murch, Steve Shaw, Chris Laing. *Royal Free Hospital, London, United Kingdom.*

**Background:** Systemic sepsis is estimated to be a contributory cause in 47% of episodes of in-hospital acute kidney injury (AKI). There is limited evidence for interventions to reduce the incidence of sepsis-associated AKI. The Sepsis Six bundle uses fixed physiological cutoffs (triggers) on the bedside observation chart to identify patients at risk of severe sepsis. The bundle specifies six mandatory interventions (oxygen, fluid balance chart, arterial blood gas, intravenous fluid, blood cultures, antibiotics) to be performed within one hour of trigger. We investigated whether compliance with this bundle of care was associated with improved acute renal outcome.

**Methods:** We collected data on 415 consecutive patients who triggered for Sepsis Six shortly after introduction of the bundle to the Royal Free Hospital in London. We compared baseline with peak creatinine up to 10 days post-trigger to classify renal outcome using KDIGO delta creatinine criteria. We compared AKI outcomes between patients with complete and incomplete implementation of the Sepsis Six bundle.

**Results:**

| (number in group) | No AKI | AKI 1 | AKI 2 | AKI 3 | Total |
|---|---|---|---|---|---|
| 6 interventions | 140 | 22 | 23 | 11 | 196 |
| 0-5 interventions | 141 | 25 | 14 | 39 | 219 |
| Total | 281 | 47 | 37 | 50 | 415 |

We calculated relative risk ratio (RRR) for developing each stage of AKI (versus no AKI) with all six interventions completed and corrected for the presence of hypotension when triggering: AKI 1 RRR=0.9 (95% CI 0.5-1.7, p=0.73); AKI 2 RRR=1.5 (95% CI 0.8-3.1, p=0.24); AKI 3 RRR=0.3 (95% CI 0.1-0.6, p<0.01).

**Conclusions:** Sepsis Six triggers (simple bedside observations) identified a cohort with a high incidence of AKI (32%). Full compliance with the Sepsis Six bundle was associated with a 70% reduction in the risk of developing AKI 3, compared with incomplete compliance. A non-significant rise in the incidence of AKI 2 suggests potentially severe renal injury may have been converted to moderate injury by good compliance with the bundle. These data support the hypothesis that a simple, protocolised intervention, performed early in the course of sepsis, could protect against severe AKI.

## SA-PO021

**Does a Care Bundle Improve Management of Acute Kidney Injury? Results from Completed Audit Cycle** Jennifer R. Joslin, Daniel Zubli, Nathan Gauge, Hannah R. Wilson, Anna Buckingham, Tracy Ellimah, Cornelia Junghans Minton, Charlotte Masterton-smith, Sabrina Rossi, Joanna Sutherby, Katherine E.S. Warren, Mark Andrew Yates, Taryn Pile, Maria Ostermann. *Guy's and St. Thomas NHS Foundation Trust, London, United Kingdom.*

**Background:** An audit in 2011 showed poor recognition and management of patients with acute kidney injury (AKI) in level 1 inpatients (poster presentations, ASN 2011). In response, an AKI 'care bundle' was developed, in conjunction with the London AKI network, and AKI education among doctors and nurses was increased. This project completes the audit cycle.

**Methods:** The same methods were used as in the audit in 2011. During a 7 day period in April 2013, electronic patient records of all level 1 medical and surgical patients in a London teaching hospital were screened. Medical notes of patients with AKI as defined by the KDIGO criteria were reviewed by doctors independent from the treating team to assess compliance with recommended management. Patients receiving palliative care were excluded. Data were compared with results from 2011.

**Results:** 99 patients had AKI in the 2011 audit period and 95 in 2013. Mean ages were 73 and 69 years, respectively. Data of 244 AKI inpatient hospital days was available in 2011, and 250 for 2013.

| | 2011 AKI patient-days (%) | 2013 AKI patient-days (%) | p-value |
|---|---|---|---|
| AKI recognised by managing team | 59 | 74 | <0.001 |
| Documented AKI management plan if AKI recognised | 82 | 85 | 0.47 |
| Clinical assessment of fluid status | 37 | 66 | <0.001 |
| Complete fluid balance chart | 32 | 40 | 0.04 |
| iv fluids given if vulnerable to hypovolaemia | 82 | 75 | 0.2 |
| Contrast prophylaxis given if indicated | 44 | 56 | 0.5 |
| Timely antibiotics given if septic | 82 | 69 | 0.22 |
| Nephrotoxic drugs discontinued if appropriate | 31 | 76 | <0.001 |

There was also better recognition of AKI by the managing team on the 1st day of AKI (50% in 2011 vs 70% in 2013, p=0.015).

**Conclusions:** Implementation of an AKI bundle, together with enhanced education improves recognition of AKI and some aspects of care. However, more work is needed to understand the factors which limit recognition and good medical care.

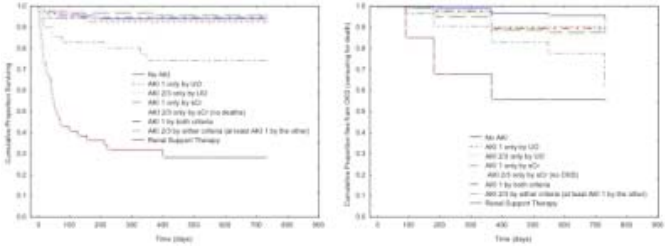
## SA-PO022

**Differential Prognosis after Cardiac Surgery-Associated Acute Kidney Injury According to Creatinine or Urine Output Criteria** Maribel Merino, Cesar Flores-Gama, Armando Vazquez-Rangel. *Nephrology, Instituto Nacional de Cardiologia, Ignacio Chavez, Mexico, Mexico.*

**Background:** Cardiac surgery-associated acute kidney injury (CS-AKI) results in increased morbidity and mortality. We analyzed short and midterm mortality and progression to chronic kidney disease (CKD) according to the criteria, either by serum creatinine (sCr) or by urine output (UO).

**Methods:** Retrospective analysis of adult patients who underwent cardiac surgery between March 2010 and June 2012 was made. CS-AKI was defined within 7 days according to KDIGO criteria. Progression to CKD was defined as having beyond 3 months an estimated glomerular filtration rate (eGFR) by CKD-EPI <60 ml/min/1.73 $m^2$ and a reduction from baseline eGFR of at least 15 ml/min/1.73 $m^2$. Kaplan-Meier curves were constructed for outcomes.

**Results:** A total of 794 patients were included, 56.9% were men, mean age was 53.5±15.8 years and baseline eGFR of 87.3±21.7ml/min/1.73 $m^2$ Any CS-AKI occurred in 494 (62.2%) patients, 103 (20.8%) had isolated UO criteria, 222 (44.9%) had isolated sCr criteria, and 113 (22.9%) had combined criteria, all of them without renal support therapy (RST). Just 56 patients required RST. With a median follow-up time of 365 days, 78 (9.8%) died and 52 (6.5%) progressed to CKD (censoring for death). In survival analysis, patients with AKI stage 1 by any or both criteria, patients with stage 2/3 but only by one of the criteria and not requiring RST, did not show significant difference in mortality and progression to CKD from patients without AKI. In contrast, AKI stage 2/3 with both AKI criteria or patients requiring RST, had a significant increase in mortality and progression to CKD.



**Conclusions:** CS-AKI contributes to a different short and midterm prognosis according to specific fulfilled criteria. Patients with stage 2/3 but only by one of the criteria showed a similar prognosis to those without AKI.

## SA-PO023

**Diverse Hemostasis Results in Cancer Patients with Acute Kidney Injury (AKI) Evaluated by Standard Coagulation Methods or Thromboelastography**

James Hung,[1] Tania Rubia Flores Rocha,[2] Elbio Antonio D'amico,[2] Luis Yu.[1]
*[1]ICESP, Universidade de Sao Paulo; [2]Universidade de Sao Paulo.*

**Background:** Routine tests, such as prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) are frequently ordered to assess clotting function but they might be inadequate. Thus, we compared the results of conventional tests with thromboelastography (TEG), which assesses clot formation in whole blood, including plasma and cellular components, in cancer patients with AKI.

**Methods:** We prospectively analysed patients admitted to the ICU of a Cancer University Hospital with sepsis. Renal dysfunction was classified according to the AKIN criteria. Besides standard coagulation tests (PT, aPTT and TT), TEG was used to evaluate the entire process of hemostasis.

**Results:** We included 103 patients: mean age 61±15y, 57% male, 85% solid tumors, 15% hematological tumors, 67% on vasopressors, 68% developed AKI (AKIN 1 - 19 patients, AKIN 2 - 20 patients, AKIN 3 - 31 patients).

| | Without AKI# | AKIN 1 | AKIN 2 | AKIN 3 | p |
|---|---|---|---|---|---|
| creatinine (mg/dl) | 0.8 | 1.29 | 1.4 | 2.59 | <0.05, # vs AKIN 2 and 3 |
| urea (mg/dl) | 44 | 66 | 87 | 142 | <0.05, # vs AKIN 2 and 3 |
| calcium (mg/dl) | 4.6 | 4.7 | 4.5 | 4.6 | 0.72 |
| hemoglobin | 8.7 | 9.8 | 8.5 | 9.7 | <0.05, AKIN 2 vs AKIN 3 |
| platelets ($10^3$/mm$^3$) | 184 | 214 | 236 | 160 | 0.56 |
| PT | 1.12 | 1.28 | 1.41 | 1.55 | <0.05, # vs AKIN 2 and 3 |
| aPTT | 0.99 | 1.02 | 1.14 | 1.15 | <0.05, # vs AKIN 2 and 3 |
| TT | 1.06 | 1.11 | 1.14 | 1.31 | <0.05, # vs AKIN 3 |
| **TEG** | | | | | |
| R | 8.5 | 8.9 | 8.4 | 10.6 | 0.08 |
| K | 2.4 | 2.6 | 2.8 | 3.2 | <0.05, # vs AKIN 3 |
| angle | 56.7 | 52.6 | 55.1 | 44.2 | <0.05, # vs AKIN 3 |
| MA | 64 | 60 | 64 | 58 | 0.53 |
| CI (coagulation index) | 1.6 | 1.4 | 1.8 | 0.7 | 0.52 |

**Conclusions:** Severe AKI caused impaired coagulation in cancer patients as evaluated by standard assays of hemostasis, suggesting a bleeding risk in this situation. In contrast, a normal result was shown in the same cancer patients using TEG. Thus, diverse hemostasis status was demonstrated in AKI cancer patients that may lead to distinct diagnostic and therapeutic maneuvers.

*Funding:* Government Support - Non-U.S.

## SA-PO024

**Acute Kidney Injury (AKI) Is Associated with Bronchopulmonary Dysplasia (BPD) in Very Low Birth Weight (VLBW) Infants**

Neha Patil, Rajesh Koralkar, Susan Keeling, Namasivayam Ambalavanan, David J. Askenazi. *Pediatrics, Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** Lung dysfunction has been linked with AKI in animal models and is thought to contribute to poor outcomes in critically ill children and adults with lung injury. Chronic lung injury is very common in neonates; yet the association between AKI and lung outcomes has not been explored.

**Objective:** To evaluate association between AKI and BPD in VLBW infants.

**Methods:** Between February 2012 to February 2013, 91 VLBW Infants (birth weight <=1200 gm. or gestational age <31 weeks) had serum creatinine (SCr) values prospectively collected on days 1, 2, 3, 4, and 14; and combined with clinically measured SCr to determine AKI status according to KDIGO; whereby AKI was defined if SCr ≥ 0.3 mg/dl or ≥ 150-200% from lowest previous value. Difference in the changes of SCr between day 4 and 14 were assessed between respiratory support categories using repeated measures ANOVA controlling for potential confounders.

**Results:** The incidence of AKI over first two weeks of life was (27/87) 31%. AKI infants were more likely to receive ventilator support at day 28 compared to those without AKI (p< 0.05). Changes in SCr from day 4 to 14 were different among respiratory support categories at day 7, 14, 21 and 28 (p value <0.01). When controlling for potential confounders individually, this association was significant for birth weight, surfactant, gender and race (p value <0.05), however; with combination of them and addition of gestational age this independent association was no longer present.

| Respiratory Support Day 28 | AKI Day 3 | | AKI Day 7 | | AKI Day 15 | |
|---|---|---|---|---|---|---|
| | No (66) | Yes (15) | No (60) | Yes (21) | No (57) | Yes (24) |
| **Room air /Oxygen Dome** | 48 (74%) | 7 (47%) | 46 (77%) | 9 (43%) | 45 (79%) | 10 (42%) |
| **NC / CPAP** | 9 (13%) | 2 (13%) | 8 (13%) | 3 (14%) | 8 (14%) | 3 (12%) |
| **Mechanical Ventilation** | 9 (13%) | 6 (40%) | 6 (10%) | 9 (43%) | 4 (7%) | 11 (46%) |
| **P value** | 0.05 | | 0.002 | | 0.0001 | |

**Conclusions:** In VLBW infants, AKI is strongly association with receipt of respiratory support. Whether AKI truly contributes to BPD in these infants is yet to be determined.

## SA-PO025

**Lysozyme-Induced Acute Tubular Injury: An Under-Recognized Cause of Acute Renal Failure**

Larry N. Cossey, Christopher Patrick Larsen. *Nephropath, Little Rock, AR.*

**Background:** Increased serum lysozyme has been reported in association with many diseases, however only rare case reports have shown lysozyme-induced tubular injury leading to acute renal failure. This report attempts to more fully describe the clinical and laboratory findings associated with this disease.

**Methods:** Two patients with lysozyme-induced acute renal failure were identified from our database in the past year. Routine light, immunofluorescence, and electron microscopy were performed. In addition, immunoperoxidase staining for lysozyme and laser microdissection of cortical tubules coupled with mass spectrometry were performed on both patients and two controls.

**Results:** The patients are 77 and 80 year-old males with acute-on-chronic renal failure and non-nephrotic range proteinuria. Both patients had bland urine sediment and no hematuria. Serum creatinine at time of biopsy was 2.0 and 2.1 mg/dl, respectively. Serum lysozyme level was available in one patient and was >12x reference range. Chronic myelomonocytic leukemia and myelodysplastic syndrome were present, respectively. By light microscopy diffuse acute tubular injury with epithelial thinning and loss of brush boarder was seen. Proximal tubules had brightly eosinophilic cytoplasm with round, slightly refractile (non-polarizable) protein droplets. Congo red stained positive within this material in the proximal tubules with no birefringence on polarization. Staining for lysozyme showed intense positivity within the proximal tubules and interstitium with mild tubular staining identified in proteinuric controls. Laser microdissection of cortical tubulointerstitium followed by mass spectrometry showed lysozyme as the most abundant protein in patients but not controls.

**Conclusions:** Lysozyme-induced acute renal injury is likely an under-reported cause of acute renal injury with this study confirming that the eosinophilic substance within the proximal tubules of these patients is lysozyme. By histology, we describe a unique constellation of findings including intensely eosinophilic protein droplets in proximal tubules with nonspecific staining for Congo red. Additionally, increased tubulointerstitial lysozyme staining can aid in diagnosis.

## SA-PO026

**Effects of *Schizolobium Parayba* Extract on Experimental Bothrops Venom-Induced Acute Kidney Injury**

Monique Silva Martinez,[1] Miriam Mendes,[2] Maria H.M. Shimizu,[1] Veridiana Avilla,[2] Isac De Castro,[1] Sebastiao R. Ferreira-Filho,[2] Denise M.A.C. Malheiros,[1] Luis Yu,[1] Emmanuel A. Burdmann.[1] *[1]Univ of Sao Paulo Medical School; [2]Uberlandia Federal Univ.*

**Background:** Venom-induced acute kidney injury (AKI) is a frequent complication of *Bothrops* snakebite, carrying relevant morbidity and mortality. The aim of this study was to assess the effects of *Schizolobium parayba* (SP) extract, a natural medicine with confirmed anti-phospholipase A2 effects in an experimental model of *Bothrops jararaca* venom (BV)-induced AKI.

**Methods:** Groups of 8-10 rats were assessed 20 min after injection of saline (VH), SP 2 mg/kg, BV 0.25 mg/kg and BV plus SP. The following parameters were analyzed: glomerular filtration rate (GFR, ml/min/100g, inulin clearance), renal blood flow (RBF, ml/min, Doppler), blood pressure (BP, mmHg, intra-arterial transducer), renal vascular resistance (RVR, mmHg/ml/min/, formula), urinary osmolality (UO, mOsm/kg, freezing point), urinary NGAL (NGAL, µG, ELISA), lactic dehydrogenase (LDH, IU/L, kinetic method), fibrinogen (F, mg/dl, Klauss modified) and blinded renal histology. Results (mean±SE) are shown below (*p<0.05 vs. control).

**Results:**

| | VH | SP | BV | BV+SP |
|---|---|---|---|---|
| GFR | 0.87±0.04 | 0.63±0.04* | 0.47±0.05* | 0.52±0.07* |
| RBF | 6.3±0.2 | 6.2±0.2 | 3.0±0.2* | 3.6±0.3* |
| BP | 134±4 | 132±4 | 127±7 | 120±4 |
| RVR | 20±1 | 21±1 | 39±7* | 34±3* |
| UO | 1305±113 | 1275±117 | 569±48* | 669±54* |
| NGAL | 11.8±2.3 | 11.3±1.5 | 23.8±4.5* | 18.4±2.3 |
| LDH | 107±38 | 267±156 | 2883±589* | 2946±912* |
| F | 164±12 | 144±19 | 60±5* | 60±1 |

BV caused acute tubular necrosis, which was not prevented by SP.

**Conclusions:** SP administered simultaneously with BV, in an approximate 10:1 concentration, was not able to prevent BV-induced AKI, hemolysis and fibrinogen consumption. SP alone caused GFR decrease.

## SA-PO027

**IgA Nephropathy with ANCA Seropositivity: A Case Series**

Angela K. Muriithi,[1] Lynn D. Cornell,[2] Fernando C. Fervenza,[1] Mary E. Fidler,[2] Sanjeev Sethi,[2] Samih H. Nasr.[2] *[1]Divs of Nephrology, Mayo Clinic; [2]Anatomic Pathology, Mayo Clinic, Rochester, MN.*

**Background:** Anti-neutrophil cytoplasmic antibodies (ANCA) are typically associated with pauci-immune necrotizing and crescentic glomerulonephritis (GN). There have been rare reports and small case series of ANCA positivity in patients with crescentic IgA nephropathy (IgAN). It is unclear if ANCA positivity in IgAN correlates with prognosis or the presence of crescents.

**Methods:** We report the clinical and pathologic findings of 18 patients with IgAN and concomitant ANCA positivity.

**Results:** We identified 13 men and 5 women from 1993 to 2012 among 425 patients with biopsy-proven IgAN who had ANCA positivity by ELISA (4%). Mean age was 50 years. 15 had myeloperoxidase (MPO), 1 proteinase-3 (PR3) and 2 both.

8 patients (47%) presented with proteinuria/microhematuria, 4 (24%) with rapidly progressive GN (RPGN), 4 (24%) with chronic kidney disease and 1 patient with acute kidney injury. All 18 had proteinuria (mean 3.4 g/24h), and 15/17 (88%) microhematuria. 2/17 (12%) had pulmonary involvement and 5/17 (29%) skin lesions. Mean serum creatinine was 2 mg/dl.

Histologically, 6 (35.3%) had crescents involving a mean of 35% glomeruli (range 10-59). 4 (24%) had glomerular necrosis; none had arteritis. All patients had IgA dominant mesangial staining on immunofluorescence. Mesangial deposits were seen in all 15 cases studied ultrastructurally.

Follow-up was available for 15 patients; 2 received ACE inhibition and/or fish oil only and 11 immunosuppressive therapy: cyclophosphamide/steroids then mycophenolate/azathioprine (n=6), mycophenolate/steroids (n=3), steroids only (n=2). Of the 6 with crescentic IgAN, 3 had RPGN and 4 received cyclophosphamide-based therapy. At a mean follow-up of 46 months, 2 died of unrelated causes, 4 progressed to ESRD (all 4 had RPGN, 3 with crescentic IgAN), and 9 had stabilized or improved their kidney function.

**Conclusions:** ANCA positivity is rare in patients with IgAN. Contrary to previous reports, we found that ANCA was associated with RPGN in only a quarter of IgAN patients and with crescentic disease in only a third. The outcome in crescentic IgAN patients with ANCA appears to reflect that of crescentic IgAN who present with RPGN.

## SA-PO028

**The Incidence, Etiology, and Outcome of Acute Kidney Injury in Patients with Liver Abscess** Seong Eun Yun, Dong Jun Park, Se-Ho Chang, Hyun Seop Cho, Yeojin Kang, Eunjin Bae. *Div of Nephrology, Dept of Internal Medicine, Gyeongsang National Univ Hospital, JinJu, Republic of Korea.*

**Background:** Acute kidney injury (AKI) can occur in various infectious conditions. Liver abscess is relatively rare disease leading to severe complications. Study about AKI in patients with liver abscess is rare.

**Methods:** We retrospectively reviewed the medical records of 404 patients who were diagnosed as liver abscess from January, 2000 to March, 2013.

**Results:** Male to female ratio was 61:39 and the mean age was 62.9 ± 13.1 years old. AKI occurred in 137 patients (34%). As per RIFLE classification, renal injury was in risk (35.8%), injury (47.4%), and failure (16.5%). One hundred fourteen patients (83.2%) initially had AKI on admission whereas others progressed to AKI during hospitalization. Fifty six (41%) patients had pre-renal azotemia whereas 19 patients (14%) were compatible with radio-contrast induced nephropathy. AKI occurred frequently in men and patients with liver cirrhosis (P<0.001 and P=0.005). Lower serum albumin and higher AST and ALT level were shown in patients with AKI, compared to patients with non-AKI. Isolation of organisms from blood culture and liver abscess aspirates were more frequent in AKI patients (P<0.001 and P=0.007). Hospitalization period was also longer in AKI patients (P<0.001). Existence of septic shock was positively correlated with occurrence of AKI (P=0.002). Twenty patients (5.0%) had chronic kidney disease (CKD). AKI was significantly more frequent in patients with CKD (P=0.04). Mortality had no difference between AKI and non-AKI. However, higher mortality was presented in CKD patients accompanied by AKI (P<0.001). Hemodialysis was done in three patients with AKI; two patients who had initially normal renal function completely recovered whereas one CKD patient progressed to ESRD requiring maintenance hemodialysis.

**Conclusions:** AKI is relatively common in patients with liver abscess although most are recovered completely. However, patients with previous renal impairment may have poorer prognosis if they are accompanied by AKI. Physician should make an effort to prevent, detect, and manage AKI early in patients with liver abscess.

## SA-PO029

**Clinical Characteristics of Acute Kidney Injury in Patients with Scrub Typhus – RIFLE Criteria Validation** In O. Sun, Kwang Young Lee. *Div Nephrology, Dept of Internal Medicine, Presbyterian Medical Center, Jeonju, Korea.*

**Background:** The aim of this study is to investigate the incidence and clinical characteristics of acute kidney injury (AKI) based on RIFLE criteria in scrub typhus.

**Methods:** From 2010 to 2012, 238 patients were diagnosed as scrub typhus. Of these, we included 223 patients who were followed up until renal recovery or for at least 3 months, and evaluated the incidence, clinical characteristics and severity of AKI based on RIFLE classification.

**Results:** Of 223 patients, 47 (21%) had scrub typhus-associated AKI. The incidence of AKI was 21.1%; of which, 10.7%, 9.4% and 1% were the Risk, Injury and Failure classes, respectively. In comparison with patients in non-AKI group, the patients in AKI group were older (70 ± 9 vs 61 ± 14 year, P=0.01) and had more comorbidities such as hypertension, diabetes and chronic kidney disease (77% vs 22%, P=0.01). The patients in AKI group had worse renal function than that of Non-AKI group at admission (37 ± 15 vs 82 ± 19ml/min/1.73m$^2$, P=0.01). Forty-five had AKI prior to admission, whereas 3 patients experienced AKI during their hospitalization.By multivariative logistic regression analysis, age and comorbidity were significant predictors of AKI. All patients recovered baseline renal function without renal replacement therapy following antibiotics such as doxycycline and supportive care.

**Conclusions:** The incidence of AKI in patient with scrub typhus is 21%. Age and co-morbidity are significant predictors of AKI in scrub typhus. In case of scrub typhus-associated AKI, anti-rickettsia agent and supportive care is very important.

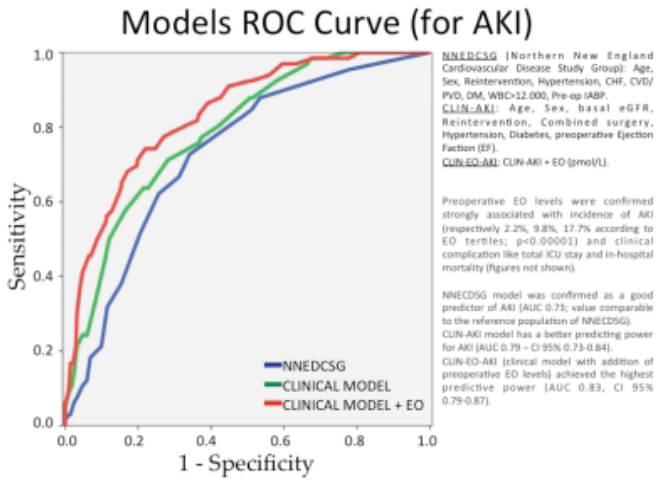
## SA-PO030

**A New Clinical Model for Postoperative AKI: Role of Endogenous Ouabain** Marco Simonini,[1] Nunzia Casamassima,[1] Chiara Lanzani,[1] Elena Bignami,[1] Elena Frati,[1] Roberta Meroni,[1] Elisabetta Messaggio,[1] John Hamlyn,[2] Paolo Manunta.[1] *[1]San Raffaele Scientific Institute, Milan, Italy; [2]Univ of Maryland, Baltimore.*

**Background:** AKI is a frequent complication of cardiosurgery. A lot of models predicting AKI have been proposed, but only few focused on milder AKI not requiring dialysis (AKI-ND), which is very common and contributes to several in-hospital outcomes. Endogenous Ouabain (EO) is an adrenal stress hormone with hemodynamic and renal effects. Recently it has been reported that higher pre-operative EO levels are associated with a worse renal outcome after surgery. Our aim is to develop a new risk model of AKI-ND using clinical aspects and EO as biomarker.

**Methods:** AKI according to AKIN was studied. We built predictive risk model (CLIN-AKI) considering clinical variables (see figure 1 for details). A further risk score (CLIN-EO-AKI) was developed adding preoperative EO values to the CLIN-AKI score. NNECDSG model, the only preoperative model for AKI-ND reviewed, was used for comparison.

**Results:** All models were tested on 802 patients admitted for elective cardiac surgery. 79 patients developed AKI (9.9%). NNECDSG model was confirmed as a good predictor of AKI (AUC 0.73, value comparable to the reference population). CLIN-AKI model has a better predicting power for AKI per se (AUC 0.79). Adding the preoperative EO level to the clinical model AUC increased to 0.83 (CI 95% 0.79-0.87). Inclusion of EO improved the risk prediction over the clinical models alone (ΔAUC respectively +0.06, p<0.03 and +0.1, p<0.01).




**Conclusions:** We developed a powerful and accurate risk model based on eight simple preoperative variables. This model was greatly improved by the addition of EO level as marker of kidney and vascular subclinical damage. Both these models are straightforward, useful and readily applicable at the bedside.

## SA-PO031

**Acute Kidney Injury from Calcium Channel Blocker-Clarithromycin Drug Interactions** Amit X. Garg,[1,2,3] Sonja Gandhi,[1,2] Jamie L. Fleet,[2] David G. Bailey,[2] Eric McArthur,[3] Ron Wald,[4] Faisal Rehman.[2] *[1]Western Univ; [2]London Health Sciences Centre; [3]Institute for Clinical Evaluative Sciences; [4]Univ of Toronto.*

**Background:** Calcium channel blockers are metabolized by the cytochrome P450 3A4 (CYP3A4) enzyme. Blood concentrations of calcium channel blockers can rise to harmful levels when this enzyme is inhibited. Clarithromycin is an inhibitor of CYP3A4 while azithromycin is not, making comparisons of the two co-prescriptions useful to understand the potential consequences of this drug-drug interaction.

**Methods:** We conducted a population-based retrospective cohort study of older adults (mean 76 years) who were newly co-prescribed clarithromycin (n=96,226) or azithromycin (n = 94,083) while taking a calcium channel blocker (amlodipine, felodipine, nifedipine, diltiazem, or verapamil) in Ontario, Canada from 2003 to 2012. The primary outcome was hospitalization with acute kidney injury and two secondary outcomes were hospitalization with hypotension and all-cause mortality. All outcomes were assessed within 30 days of a new co-prescription. Risks were expressed in both absolute and relative terms.

**Results:** Amlodipine was the most commonly prescribed calcium channel blocker (>50% of patients). Co-prescription with clarithromycin *vs.* azithromycin was associated with an increased risk of hospitalization with acute kidney injury [absolute risk increase 0.22% (95% confidence interval (CI) 0.16 to 0.27); relative risk (RR) 1.98 (95% CI 1.68 to 2.34)]. In subgroup analysis, this risk was highest with nifedipine [RR 5.33 (95% CI 3.39 to 8.38)]. Co-prescription with clarithromycin *vs.* azithromycin was also associated with an increased risk of hospitalization with hypotension [absolute risk increase 0.04% (95% CI 0.02 to 0.07); RR 1.60 (95% CI 1.18 to 2.16)] and all-cause mortality [absolute risk increase 0.43% (95% CI 0.35 to 0.51); RR 1.74 (95% CI 1.57 to 1.93)]. The primary associations proved robust in multiple additional analyses.

**Conclusions:** In older adults taking a calcium channel blocker, co-prescription of clarithromycin increases the risk of adverse events. Better prescribing to avoid CYP3A4 drug interactions with calcium channel blockers is warranted.

*Funding:* Government Support - Non-U.S.

## SA-PO032

**Bioelectrical Impedance as Prognostic Predictor in Acute Kidney Injury. Importance of Na/K Ratio, Total Water Corporal Volume and Its Distribution. Risk Stratification** Francisco Javier Lavilla, Jose Maria Mora Gutierrez, Nuria Garcia-Fernandez, Paloma L. Martin Moreno, Carmen Calderon Gonzalez, Diana Lopez Espinosa, Pedro Errasti. *Nephrology, Univ CLinic of Navarra, Pamplona, Navarra, Spain.*

**Background:** Evaluate prognostic implication of bioimpedance (BIA) in acute kidney injury (AKI). Importance of bioelectrical parameters, total body water and its distribution.

**Methods:** In 133 patients ( 64 years, EE: 1.57; 76 males) with AKI and bioimpedance analysis. We evaluated bioelectrical (phase angle -PA- and Na/K exchange ratio -Na/K-) and volemic parameters (liters -L-) (total body water -TBW-, extracellular water -ECW-, intracellular water -ICW- and ECW/ICW ratio), and relationship with prognostic index (Individual Severity Index -ISI– and Multi-Organ Failure Index –MOFI –), C reactive protein (CRP), prealbumin (PRALB) and natriuretic (BNP). Statistical analysis with SPSS 15.0.

**Results:** The PA, Na/K and ECW/ICW were associated with prognostic index, chronic health status, inflammatory status and protein metabolism.

| | ISI | MOFI | CRP | ALB | PRALB | KAR |
|---|---|---|---|---|---|---|
| PA<br>p | -0.295<br>0.039 | -0.235<br>0.018 | -0.300<br>0.003 | 0.324<br>0.007 | 0,440<br>0,005 | 0,443<br>0,000 |
| Na/K<br>p | 0.235<br>0.016 | 0.316<br>0.003 | 0.221<br>0.046 | -0.020<br>0.886 | -0,518<br>0,002 | -0,327<br>0,002 |
| ECW/ICW<br>p | 0.232<br>0.009 | 0.234<br>0.008 | 0.331<br>0.005 | | -0.433<br>0.002 | 0.281<br>0.015 |

PA: Phase angle. Na/K: Na/K exchange. ECW/ICW: extracelular/intracelular water ratio. ISI: Individual Severity Index. MOFI: Multi-Organ Failure Index. CRP: C-reactive protein PRALB: Prealbumin. BNP: Blood natriuretic peptide.

The NA/K was independently associated with death risk (p = 0.022 OR 5.05, 95% CI: 1.251-23.7), area under the curve of 0.714 p = 0.036 and cutoff of 1.1, sensitivity (0.889) and specificity (0.759). Mortality analysis showed differences in ECW/ICW, with greater values in deceased patients (1.5 EE EE 0.07 vs 1.97 0.20, p = 0.027).

**Conclusions:** The electrical bioimpedance analysis can be used as a prognostic parameter in patients with AKI. Na/K ratio may assess the risk of death, mainly with values greater than 1.1. The volume distribution is mostly related with prognosis, especially with extracellular-intracellular shift.

## SA-PO033

**Acute Kidney Injury in Pediatric Patients after Cardiac Surgery: Risk Factors and Prognosis** Maria Goretti M.G. Penido,[1] Nilzete Liberato Bresolin,[2] Vanessa Oliveira Duarte,[3] Andreia Elisa Baldissera,[3] Aline Patrícia Alves Pereira Bianchini.[4] *[1]Pediatric Nephrology Unit, Federal Univ of Minas Gerais, Belo Horizonte, MG, Brazil; [2]Joana de Gusmão Infant Hospital, Federal Univ of Santa Catarina, Florianopolis, SC, Brazil; [3]Dr J Amarante Faria Infant Hospital, Florianopolis, SC, Brazil; [4]Syrian Lebanese Social Responsibility Institute, Sao Paulo, SP, Brazil.*

**Background:** Studies on acute kidney injury (AKI) after cardiac surgery in children using the pediatric pRIFLE criteria are rare. The aims of this study were to analyze the prevalence of AKI, the association with cardiopulmonary bypass (CPB), with clinical variables and the prognosis after cardiac surgery in children admitted to a pediatric intensive care unit (ICU). The pRIFLE and the RACHS-1 score were analyzed as a prognostic tool.

**Methods:** This retrospective case-control study consisted of 160 children who underwent cardiac surgery. They were divided in G1 (without AKI, 75 patients) and G2 (with AKI, 85 patients) and classified according to the pRIFLE criteria. Data included: age, gender, weight, height, RACHS-1, prior renal function, use of mechanical ventilation, vasoactive drugs, need for dialysis, CPB time, length of stay (LOS) and outcome (discharge or death). Poisson multivariate analysis was used to estimate the associated factors.

**Results:** Eighty-five patients (53%) developed AKI. Association with the following parameters were observed: 69% had higher prevalence after the use of nephrotoxic drugs (p=0.008), 55% after surgeries with CPB (p=0.038). There was an inverse correlation between length/heigth and AKI (p<0.001). LOS in the ICU (p<0.001), CPB time (p<0.001) and mortality (p=0.006) were significantly higher in G2, as severity increased progressively among subgroups of G2 (p<0.001). Mortality was higher in patients with higher RACHS-1 scores (p<0.001). The cutoff time of CPB for risk of AKI was 70 minutes.

**Conclusions:** AKI was frequent and its incidence was higher in those with CPB-time ≥70 min. pRIFLE criteria were useful to define AKI and were also prognostic predictor. Mortality and LOS in the ICU were higher in G2. Use of nephrotoxic drugs, age, length/height, weight, and CPB were independent risk factors for AKI.

## SA-PO034

**Association between Low High-Density Lipoprotein Cholesterol Level and Increased Risk of Contrast Nephropathy in Non-Diabetic Patients with Myocardial Infarction** Hyung Wook Kim, Hoon Suk Park, Kyungyoon Chang. *Internal Medicine, St. Vincent's Hospital, The Catholic Univ of Korea, Suwon, Gyeonggi-do, Korea.*

**Background:** Hypercholesterolemia is a well-known risk factor for cardiovascular disease (CVD), and HDL cholesterol reportedly has a protective effect against CVD. However, whether hypercholesterolemia or hyperlipidemia is a risk factor for contrast induced acute kidney injury (CI-AKI) is not established. We performed this study to find the relationship between cholesterol levels and CI-AKI.

**Methods:** This study included 289 non-diabetic patients with myocardial infarction (MI) undergoing percutaneous coronary intervention.Demographic data and clinical findings were compared between patients with and without CI-AKI. Logistic regression analysis was performed to identify independent risk factors. We also compared clinical courses between the 2 groups.

**Results:** HDL cholesterol level (odds ratio [OR] 0.82, 95% confidence interval [CI]: 0.72–0.94; p = 0.003) was an independent risk factor for CI-AKI in multivariable logistic regression analysis, besides MDRD eGFR (OR 0.86, 95% CI: 0.79–0.95; p = 0.002) and troponin T level (OR 1.50, 95% CI: 1.13–1.99; p = 0.005). The group with CI-AKI had a longer hospital stay (10.2 ± 6.5 days vs. 6.6 ± 4.6 days, p = 0.047), increased in-hospital death (50% vs. 2.9%, p < 0.001), and poorer patient survival (p <0.001) than the group without CI-AKI.

**Conclusions:** Low HDL cholesterol level is associated with an increased risk of CI-AKI in non-diabetic MI patients. More intensive strategies should be implemented for preventing CI-AKI in patients with low HDL cholesterol levels, and HDL cholesterol can be a new target in CI-AKI prevention.

## SA-PO035

**Renal Blood Flow Characterization in Critically Ill Patients with and without Acute Kidney Injury** Htay Htay, Manish Kaushik, Han Khim Tan. *Renal Medicine, Singapore General Hospital, Singapore.*

**Background:** Although the clinical manifestations of acute kidney injury (AKI) are well-known, much less is known about the changes in renal perfusion in this setting. The study focussed on critically ill patients with severe AKI.

**Methods:** This was an observational cohort study of 16 critically ill patients from medical intensive care unit: 10 patients with AKI requiring continuous renal replacement therapy (CRRT) (AKI group) and 6 patients with normal renal function (non-AKI group). Bedside trans-abdominal Doppler ultrasound interrogation of the renal arterial and aortic circulation was performed by cardiovascular technicians upon successful informed consent taking. The indices measured were abdominal aortic blood flow, renal artery blood flow and resistive indices (RI). The machine algorithm converted blood flow velocities into blood flow rates (mL/min).

**Results:** The median age in years of patients in AKI and non-AKI group were 57 (interquartile range 49-74) and 72 (49-75); p=0.36, respectively. The male to female ratio was 8:2 in AKI group and 5:1 for non-AKI group. The median hospital admission creatinine (umol/L) were 236 (102-391) for the AKI group and for the non-AKI group, 69 (54-81); p=0.02.

Comparing AKI versus non-AKI groups respectively, the median abdominal aorta flows (mL/min) were 3117 (Interquartile range 2743-3506) versus 3119 (2348-5440, p=0.875, the median renal arterial blood flows (mL/min) 440 (307-570) versus 735 (529-940), p=0.02; and, the median resistivity indices 0.83 (0.82-0.86) versus 0.75 (0.72-0.8), p=0.02.

**Conclusions:** Reduction in renal arterial blood flow during severe AKI in critically ill patients was associated with a significant rise in resistivity index (RI) but comparable aortic blood flows in both AKI and non AKI patients. The pathophysiologic significance of these findings needs further elucidation.

*Funding:* Clinical Revenue Support

## SA-PO036

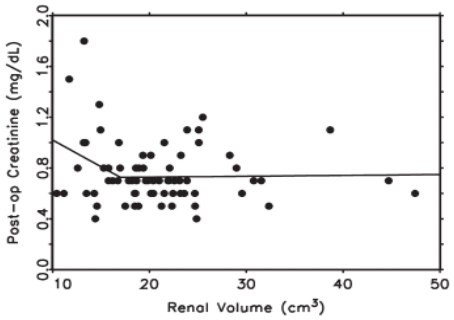
**Preoperative Renal Volume Predicts Peak Creatinine after Congenital Heart Surgery in Neonates** J. Bryan Carmody,[1] Michael D. Seckeler,[4] Cortney Ballengee,[5] Mark Conaway,[2] Kaimal A. Jayakumar,[3] Jennifer Richardson Charlton.[1] *[1]Div of Pediatric Nephrology, Univ of Virginia, Charlottesville, VA; [2]Dept of Biostatistics and Epidemiology, Univ of Virginia, Charlottesville, VA; [3]Div of Pediatric Cardiology, Levine Children's Hospital, Charlotte, NC; [4]Div of Pediatric Cardiology, Univ of Arizona, Tucson, AZ; [5]Dept of Pediatrics, Univ of Virginia, Charlottesville, VA.*

**Background:** Acute kidney injury (AKI) is common in neonates following surgery for congenital heart disease. We sought to determine whether neonates with smaller preoperative renal volume were more likely to develop postoperative AKI.

**Methods:** We reviewed 72 neonates who underwent congenital heart surgery at our center for any lesion other than patent ductus arteriosus from 2007-2011. Renal volume was calculated by ultrasound using the prolate ellipsoid formula. The presence and severity of postoperative AKI was determined both by measuring the peak serum creatinine in the first 7 days postoperatively and by using the Acute Kidney Injury Network (AKIN) scoring system.

**Results:** Using a linear change point model, a threshold renal volume of 17 $cm^3$ was identified. Below this threshold, there was an inverse linear relationship between renal volume and peak postoperative creatinine for all patients ($p = 0.036$) and the subgroup with

a single morphologic right ventricle ($p = 0.046$). There was a non-significant trend toward more AKI using the AKIN criteria in all neonates with renal volume ≤ 17 cm$^3$ ($p = 0.11$) and in the subgroup with a single morphologic right ventricle ($p = 0.17$).




**Conclusions:** Preoperative renal volume ≤17 cm$^3$ is associated with a higher peak postoperative creatinine and potentially greater risk for postoperative AKI for neonates undergoing congenital heart surgery. Infants with a single right ventricle may be at higher risk.

## SA-PO037

### Clinical Outcome of Patients on Combined ECMO and CRRT

Bijin Thajudeen, Mahmoud Kamel, Machaiah M. Madhrira, Amy Nicole Sussman. *Nephrology, Univ of Arizona, Tucson, AZ.*

**Background:** Extracorporeal membrane oxygenation (ECMO) is a supportive therapy, which provides cardiopulmonary and end-organ support in critically ill patients when other measures fail. Acute kidney injury in patients on ECMO is multifactorial. CRRT is a beneficial and efficient method of treatment in these patients. Since there is only limited outcome data on adult patients needing combined ECMO and CRRT, we looked at clinical characteristics of these patients and the factors determining mortality.

**Methods:** We did a retrospective observational chart review study of 40 consecutive patients above the age of 18, over a period of five years who received combined ECMO and CRRT. Outcomes measured included mortality and renal function at 30 days post initiation of combined ECMO and CRRT.

**Results:** Mean age of the cohort was 47 years. The most common indication for initiation of CRRT was maintenance of fluid balance in the presence or absence of acute kidney injury. 82.5 %( 33/40) of the patients died at 30 days post initiation of combined ECMO and CRRT. Mortality was found to be higher in patients above the age of 50 years compared to patients below the age of 50 years( P value=0.033). Renal function at the time of initiation, total fluid balance, serum albumin concentration and cardiac function at time of initiation of combined ECMO and CRRT did not predict mortality. Among the 7 (17.5 %) survivors, 5 patients required on going renal replacement therapy at 30 days( one required CRRT, four required intermittent hemodialysis). Two patients had complete recovery of their renal function.

**Conclusions:** Maintenance of fluid balance seems to be the most common indication for initiation of CRRT in adult patients on ECMO. Mortality is very high in adult patients receiving combined CRRT and ECMO. Age is an important predictor of mortality in these patients.

## SA-PO038

### Thrombotic Microangiopathy and Acute Renal Failure Associated with Intravenous Abuse of Opana ER: Report of Three Cases

Josephine M. Ambruzs,[1] Christopher Patrick Larsen.[1,2] *[1]Nephropath, Little Rock, AR; [2]Dept of Pathology, UAMS, Little Rock, AR.*

**Background:** There has been recent report and warning of a thrombotic thrombocytopenic purpura (TTP)-like illness associated with intravenous abuse of a prescription narcotic intended for oral use. Opana ER is an extended-release opioid agonist, oxymorphone hydrochloride, which has undergone a tamper-resistant reformulation. However, instances of melting and dissolving tablets with subsequent injection continue to occur.

**Methods:** We report the clinical and renal biopsy findings in three cases of acute renal failure associated with intravenous abuse of reformulated Opana ER.

**Results:** All cases involved young Caucasian adults including two males and one female. The clinical presentation was that of hemolytic anemia and acute renal failure. Two cases had concomitant accelerated hypertension. The hemoglobin on presentation ranged from 8.7-12.5 g/dL and there was an elevated lactate dehydrogenase and decreased haptoglobin in all cases. The serum creatinine ranged from 3.7-15.1 mg/dL. All cases reported a drug abuse history to include recent intravenous injection of reformulated Opana ER. On renal biopsy, the predominant finding was thrombotic microangiopathy consisting primarily of severe arterial mucoid intimal edema. Glomeruli showed ischemic corrugation of the basement membranes and segmental duplication as well as mesangiolysis. All cases required hemodialysis and two also underwent therapeutic plasma exchange. Renal outcomes included continued hemodialysis in one and persistent renal dysfunction in another after one month and three months follow-up respectively.

**Conclusions:** We report the first renal biopsy case series of thrombotic microangiopathy associated with the intravenous abuse of reformulated Opana ER. All of the cases were young adults presenting with hemolytic anemia and acute renal failure. Treatment involved hemodialysis as well as therapeutic plasma exchange. Early follow-up suggests renal outcome is poor. The specific component or components of reformulated Opana ER responsible for endothelial injury remains to be elucidated.

## SA-PO039

### Eculizimab Therapy for Gemcitabine Induced Hemolytic Uremic Syndrome

Ashok K. Ananthasayanan,[1] Pradeep Arora,[1,2] Rajiv Ranjan,[1,2] James W. Lohr.[1,2] *[1]Medicine, SUNY at Buffalo, Buffalo, NY; [2]Medicine, VAMC, Buffalo, NY.*

**Background:** Atypical hemolytic uremic syndrome (aHUS) is a rare but serious complication associated with gemcitabine therapy. We identified 4 cases of gemcitabine -induced aHUS over a 2 month period in patients being treated for lung cancer and biliary cancer.

**Results:** Three patients had kidney biopsies due to the atypical presentation of HUS (no schistocytes), confirming the diagnosis of aHUS. After stopping gemcitabine, all patients were initially or subsequently treated with eculizimab, a recombinant humanized monoclonal antibody that binds to terminal complement C5 protein. Treatment with eculizimab resulted in stopping hemolysis and improvement in renal function in all 4 patients. Details are shown in the table.

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| Age/Gender | 75/male | 70/male | 73/female | 69/female |
| Clinical Presentation | Hypertensive urgency,subacute renal failure | Subacute renal failure | Subacute renal failure | Acute renal failure |
| Schistocytes | No | No | No | Yes |
| Haptoglobin (mg/dl) | <10 | <10 | <10 | <10 |
| ADAMTS-13 | 74% | 79% | 83% | >95% |
| Kidney biopsy | Yes | Yes | Yes | No |
| Peak serum creatinine (mg/dl) | 2.6 | 2.8 | 4.3 | 2.8 |
| Time after stopping gemcitabine and start of eculizimab | 4 weeks | 3 weeks | 5 weeks | 3 weeks |
| Post-treatment haptoglobin (mg/dl) | 42 | 50 | 45 | 50 |
| Follow up creatinine | 2.0 | 1.4 | 3.4 | 1.2 |
| Outcome | improved | improved | died of cancer | improved |
| | | | | |

**Conclusions:** Gemcitabine induced aHUS is a rare but serious entity that has significant morbidity and mortality that requires early recognition and intervention. In view of the fact that we identified 4 cases over a 2 month period, we would recommend that patients being treated with gemcitabine be monitored for the development of HUS by serial serum haptoglobin levels on a regular basis. Eculizumab appears to be a well-tolerated, safe and effective treatment for gemcitabine-induced HUS.

## SA-PO040

### Women Are Prone to Develop a More Severe Course of Shiga Mediated HUS

Leyla Ramazan,[1] Jan Beneke,[1] Jan T. Kielstein,[1] Ulrich Kunzendorf,[3] Hermann G. Haller,[1] Rolf A. Stahl,[2] Jan Menne.[1] *[1]Medical School Hannover, Germany; [2]Univ Medical Center Hamburg-Eppendorf, Germany; [3]Univ Hospital Schleswig-Holstein, Kiel, Germany; [4]Klinikum Region Hannover, Germany.*

**Background:** In the 2011 German EHEC O104:H4 outbreak a high number of EHEC-HUS patients (68%) were women even so they represented only 58% of all EHEC cases. We analyzed in this study if gender did influence the clinical course of the disease.

**Methods:** 612 adult EHEC-HUS patients treated during German 2011 outbreak in 45 hospitals are included. Patients suffering from EHEC-associated enterocolitis without HUS were excluded. We prospectively and retrospectively collected demographic, clinical, and laboratory data on a standardized case-history form.

**Results:** 443 (72.4%) of patients were female and 169 (27.6%) male. The median age was comparable (45 vs. 43; n.s.). 50% of patients were admitted until day 3 of diarrhea. On admission the baseline laboratory values (LDH and platelets) were not different between m and f. Hemoglobin (Hb) was slightly lower and the eGFR was 51.2 vs. 68.5. ml/min. More women (62.5% versus 47.9%; p<0.001) required dialysis and time on dialysis was longer (median 10 vs. 8 days; n.s.). Furthermore, >100 days after the start of the disease 17.8% vs. 10.4% had still an eGFR <60 ml/min. Additionally, more women required intensive care treatment (60.3% vs. 45%, p<0.001). The ventilation rate was higher (25.3% vs. 17.8%, p=0.054) and the mortality was increased (3.6% vs. 0.6%; p=0.04). More women than men required intestinal surgery (2.9% vs. 1.2%, n.s.). The frequency of blood transfusions was higher in women (70.4% vs. 53.3%; p<0.001) and the median blood units given were 4 vs. 2 (p=0.002). Hospitalization was also longer in women compared to men (median 22 vs. 18 days, p=0.007). Surprisingly neurological symptoms (55.5% vs. 52.7%) and seizures (17.2% vs. 22.5%) were not significantly different. In both groups 85% of patients were treated with plasma exchange.

**Conclusions:** Women are more susceptible to develop severe clinical symptoms of HUS. The causes of these findings are unclear. Further investigations are required.

*Funding:* Clinical Revenue Support

**SA-PO041**

***E. coli* O104:H4 Induced HUS-Outbreak in Germany 2011: Symptoms and Clinical Course** Jan Menne,[1] Jan Beneke,[1] Leyla Ramazan,[1] Ulrich Kunzendorf,[3] Jan T. Kielstein,[1] Hermann G. Haller,[1] Rolf A. Stahl.[2] *[1]Medical School Hannover; [2]Univ Eppendaorf Hamburg; [3]UNiversity Schleswih-Holstein Kiel.*

**Background:** Consumption of sprouts contaminated with the Shiga-toxin 2 producing E.coli (STEC) O104:H4 resulted in the largest reported outbreak ofhemolytic-uremic syndrome (HUS).

**Methods:** For the present analysis, we collected demographic, clinical, and complete laboratory data of 658 EHEC-HUS patients (80% of total affected patients).

**Results:** The disease consists of four phases. The **incubation phase** from sprout consumption to the start of diarrhea lasted 7.4±3.3 days. The **gastrointestinal (GI) phase** started with the onset of GI symptoms (day 0 of the disease). Diarrhea was documented in 97.3%. During the early phase of the disease nausea and vomiting was common. 2.3% of the patients required intestinal surgery.

During the **HUS phase** between day 5-15, 50-60% of patients developed peripheral edema (often marked) and >50% also pleural effusion and/or ascites. During this phase of the disease 362 patients (55%) were treated on intensive care units and 22.6% of the patients required ventilation. Importantly, most patients (53.5%) developed neurological symptoms usually between day 10 and 14. 18.4% of patients had seizures and some patients had severe neurological problems without renal impairment.

The **recovery phase** was characterized by fatigue and weakness which started after day 5 and persisted in most patients for several months. Additionally some patients had symptoms comparable to a post-traumatic stress syndrome with night mares and anxiety for many weeks/months. Neurological symptoms disappeared; however, some mild residual symptoms were present. 6 (0.9%) remained permanently on dialysis (4 younger than 30 years) and in 15% an eGFR <60 ml/min persisted.18 (2.7%) of the patients died due to the following three main causes: 1. infection, 2. patient's refusal of advanced live support, or 3. interventional complications.

**Conclusions:** Shiga-toxin HUS is a severe and life-threatening disease. A high percentage of patients need dialysis and ventilation. Most patients were severely affected and require a long rehabilitation and eventually life long follow up.

*Funding:* Clinical Revenue Support

**SA-PO042**

**Sceleroderma Renal Crisis versus Atypical Hemolytic Uremic Syndrome** Ganesh Kambhampati,[1] A. Ahsan Ejaz.[2] *[1]Nephrology, Baypines VA Medical Center; [2]Nephrology, Univ of Florida.*

**Background:** We report a case of successful treatment of aHUS associated with scleroderma with the novel terminal complement inhibitor humanized monoclonal antibody eculizumab.

**Methods:** 62 year old WM with +SCl-70Ab/-ACA scleroderma presented with recent onset nausea, abd pain, SOB, weakness, uncontrolled BP (150-170mmHg) for the previous 2 months. PMH was significant for COPD, esophageal strictures, gastric antral vascular ectasia, Raynauds with digital ulcers and sclerodactyly, CVA with residual aphasia and absence of scleroderma crisis. PE revealed systolic murmur, bibasilar crackles and LE edema. Labs were as follows: BUN 29mg/dL, SCreat 1.55mg/dL (90 days earlier was 0.7mg/dL), Hgb 13g/dL, HCT 40.3%, Plt $61x10^3/mm^3$ (previously $190x10^3/mm^3$), LDH 414U/L, haptoglobin <15mg/dL, D-dimer 441mg/l, UA prot 100mg/dL, WBC 1/hpf, RBC 0/hpf, casts 0/hpf. An Echo performed demonstrated mod LVH, severe TR, severe RAE, LVEF 70-75%, mod RV dilatation, pulm HTN, and eRVSP 97.7 mmHg. Based on clinical and laboratory findings sceleroderma renal crisis (SRC) was one of the DDxs. Peripheral blood smear showed schistocytes suggestive of microangiopathic hemolytic anemia and thrombocytopenia. DDx now also included TMA (TTP/HUS) vs. SRC. ADAMTS13 Level was ordered and sent before planned initiation of plasmapheresis.

**Results:** Treatment initiated with ACE-inhibitor captopril (6.25-12.5mg mg PO q8hr) with no improvement in clinical condition. Patient received 5 sessions of plasmapheresis (TPE) and labs remained as follows: Plt $143x10^3/mm^3$, BUN 23mg/dL, SCreat 2.57mg/dL and elevated LDH 313U/L. ADAMTS13 activity was reported as 55%. Presumed diagnosis of atypical HUS was given. Due to persistent renal failure, thrombocytopenia and elevated LDH after treatment with plasma exchange, Eculizumab (Soliris) was empirically administered. TPE was discontinued, labs improved after three doses of Eculizumab to BUN 45 mg/dL, SCreat 1.90mg/dL, Hgb 12g/dL, Hct 38%, Plt $171x10^3/mm^3$ and LDH 212 U/L.Patient did not require renal replacement therapy.

**Conclusions:** Eculizumab successfully treated a patient with presumed diagnosis of aHUS associated with scleroderma and prevented need for renal replacement therapy, TPE and progression to severe renal failure.

**SA-PO043**

**Thrombotic Microangiopathy Associated with Hemolytic Uremic Syndrome: A Single Center 10 Year Experience** Prabesh Bajracharya,[1] Rossana Baracco,[2] Amrish Jain,[3] Tej K. Mattoo,[4] Gaurav Kapur.[5] *[1]Pediatric Nephrology and Hypertension, Children's Hospital of Michigan, Detroit, MI; [2]Pediatric Nephrology and Hypertension, Children's Hospital of Michigan, Detroit, MI; [3]Pediatric Nephrology and Hypertension, Children's Hospital of Michigan, Detroit, MI; [4]Pediatric Nephrology and Hypertension, Children's Hospital of Michigan, Detroit, MI; [5]Pediatric Nephrology and Hypertension, Children's Hospital of Michigan, Detroit, MI.*

**Background:** Microangiopathic hemolytic anemia with renal failure characterizes HUS. Atypical HUS (aHUS) linked to mutations in alternate complement pathway is less common. The aim of this retrospective study was to evaluate the clinical course of patients with aHUS seen in a tertiary center over a 10 year period.

**Methods:** A retrospective chart review (2003-2012) of patients aged 1 to 18yrs admitted with a diagnosis of HUS. Patients with no history of bloody diarrhea & absence of infection with Shigella, and Ecoli O157;H7 were grouped as aHUS.

**Results:** In aHUS group; 1 patient had C3 mutation & 1 had negative evaluation for known genetic mutations. In others (n=9) genetic evaluation was not done based on normal C3 & resolution of renal failure & hematological abnormalities. Clinical profile of patients with aHUS was similar to typical HUS group.

| | Typical HUS(N=32) | aHUS(N=11) |
|---|---|---|
| Median Age(Range) | 3(1.1-15) | 1.5(1-15) |
| Male/Female | 15/17 | 7/4 |
| At presentation<br>Mean Hb<br>Mean Platelet count<br>Mean GFR | <br>8.4±1.9<br>61±33<br>17±13 | <br>8.5±2.2<br>84±58<br>23±15 |
| % of patients with neurological symptoms(n) | 6.2(2) | 9.1(1) |
| % of patients requiring dialysis(n) | 62.5(20/32) | 36.6(4/11) |
| Median Duration of dialysis(range) | 11(4-120) | 9(9-11)one chronic PD |
| % of patients treated with plasma infusion(n) | 9.4(3) | 27.3(3) |
| Current GFR<br>CKD1-2 n(%)<br>CKD3-4 n(%)<br>CKD5 n(%) | <br>26 (81.2)<br>6(18.8)<br>0(0) | <br>9(81.8)<br>1(9.1)<br>1(9.1) |

One patient with stable renal function is receiving Eculizumab infusion.

**Conclusions:** aHUS is a heterogenous disorder associated. Genetic defects associated with complement pathway abnormalities are rare & Eculizumab is indicated in these patients.

**SA-PO044**

**Complement Activation and Plasmatic Membrane Attack Complex in Acute Kidney Injury of Different Etiologies** Eva Rodriguez, Marta Riera, Judit Rigol, Clara Barrios, Maria Jose Soler, Carlos Enrique Arias, Julio Pascual. *Nephrology, Parc de Salut Mar-IMIM, Barcelona, Spain.*

**Background:** Final component of complement pathway, membrane attack complex (MAC), has been demonstrated to be involved in models of ischemia-reperfusion. After injury, complement pathway is activated leading to the production of MAC and proinflammatory cytokines. The aim of this study is to establish if complement pathway is activated in different patterns of Acute Kidney Injury (AKI).

**Methods:** Prospective cohort study 133 patients with different conditions known to be at high risk of AKI. Overall, 53% had AKI and 47% normal kidney function. Plasma-EDTA sample was collected, four groups were classified according to the main risk factor for developing AKI: 1) septic model, patients admitted in intensive care unit with the diagnosis of sepsis (n=26, 50% AKI), 2) ischemia-reperfusion model, kidney transplant patients (n=46, 54% AKI), 3) nephrotoxic model, patients under colistin treatment (n=37, 48% AKI) and 4) multifactorial AKI (n=24, 62% AKI). Samples were tested for IL-6 and MAC using ELISA kit.

**Results:** AKI patients were classified according ADQI as Risk (n=14, 19,7%), Injury (n=12, 16,9%), Failure (n=25, 35,2%) and Loss (n=20, 28,2%). Among the 71 AKI patients, 23 (32,5%) needed renal replacement therapy. Eleven AKI patients died;31 survivors recovered full renal function, whereas 29 patients did not recover o recovered partially. Plasmatic MAC level was statistically different in patients with AKI as compared to normal renal function controls, regardless of the etiology of AKI (501±247 vs 388±150 mAU/mL, $p$=0.015). In AKI-patients, plasmatic MAC levels >460 mAU/mL were related with the probablility of not recovering renal function but no reached statistic significance whereas plasmatic MAC levels >460 mAU/mL were related with the need of renal replacement therapy (p=0,03). Plasmatic IL-6 levels were significant higher in a AKI patients compared to normal kidney function (10,47±2,8 vs 7,37±3,0 pg/mL, $p$=0,02).

**Conclusions:** Our data show that in AKI, regardless etiology, the complement system is activated, leading to an increase in MAC and pro-inflammatory cytokine stimulation (IL-6). High MAC levels are related to AKI severity.

**SA-PO045**

**Genetic Polymorphisms of Heme-Oxygenase 1 May Impact on Acute Kidney Injury, Bronchopulmonary Dysplasia and Mortality in Very Low Birth Weight (VLBW) Infants** David J. Askenazi, Brian Halloran, Neha Patil, Rajesh Koralkar, Namasivayam Ambalavanan. *Pediatrics, Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** The enzyme Heme Oxygenase 1 (HO1) catalyzes the rate-limiting step in heme degradation, producing biliverdin, iron and carbon monoxide. These products have anti-inflammatory and anti-oxidant properties which protect against AKI in several animal models. Genetic polymorphisms of HO-1 are associated with poor clinical outcomes in several critically ill cohorts.

**Methods:** Between Feb 2012 and Feb 2013, 91 VLBW Infants (birth weight ≤1200 gm. or gestational age ≤31 weeks) were prospectively followed. Serum creatinine (SCr) values on first two weeks of life were used to determine AKI status according to KDIGO; whereby AKI was defined if SCr ≥ 0.3 mg/dl or ≥ 150-200% from lowest previous value. Brochopulmonary dysplasia (BPD) was defined if an infant was receiving oxygen at 28 days of life. DNA was collected (Genotek Oragene) isolated (Qiagen Gentra Puregene) and the promoter region of HO-1 was sequenced (Qiagen Type-It Mutation Detect PCR Kit. The number of GT(n) repeats were counted for each infants' alleles and classified as short (≤ 27) or long (> 27). The –413 snp allele (A v T) was documented for each allele.

**Results:** AKI occurred in 27/87 (31%); mortality occurred in 9/87 (10.3%) and 36/78 (46.1%) survivors had BPD. The average number of GT repeats were not different by AKI or BPD category but those who died trended to have higher average GT repeats (27.7 +/- 1.12 vs. 25.7 +/- 0.4; p< 0.09). Categories of allele length (SS vs. SL vs. LL) did not differ by survival, AKI or BPD. A vs. T at –413 tended towards differences for AKI (p< 0.09), BPD (p< 0.08) and survival (p< 0.2), and was significant for the composite of BPD, AKI or mortality.

| -413 | No AKI, BPD or Death | Yes AKI, BPD or Death | P value |
|---|---|---|---|
| AA | 10 (29%) | 12 (24%) | < 0.05 |
| AT | 11 (31%) | 28 (56%) | |
| TT | 14 (40%) | 10 (20%) | |

**Conclusions:** In this small pilot study of VLBW infants, the number of tandem GT(n) repeats did not appear to predict AKI, BPD or mortality. However, a single polymorphism at position – 413 before the HO-1 promoter may predict clinical outcomes.

**SA-PO046**

**Levamisole-Associated Glomerulonephritis in a Habitual Cocaine User** Jonathan Scott Kurche,[1] Julie M. Simpson,[1] Shalini Tayal,[1,2] Jie Tang.[1,2] *[1]Univ of Colorado Denver, Aurora, CO; [2]Denver Health Medical Center, Denver.*

**Background:** Use of levamisole as a cutting agent for cocaine has resulted in increasing incidence of cutaneous vasculitis and agranulocytosis among abusers. Transient autoantibodies, including perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) are often present, and could lead to renal pathology. Levamisole has only been loosely associated with glomerular disease and the renal implications of levamisole-associated vasculitis are unknown.

**Methods:** We describe a 49-year-old female habitual cocaine user with a history of cocaine-associated cutaneous vasculitis characterized by a high p-ANCA titer, who developed acute renal failure with new nephrotic-range proteinuria. Urine sediment deonstrated dysmorphic erythrocytes, consistent with rapidly-progressing glomerulonephritis (RPGN) in the setting of recent cocaine use and laboratory-demonstrated levamisole exposure. Renal biopsy demonstrated crescentic glomerulonephritis (GN) involving more than 50% of glomeruli, as well as membranous nephropathy (MN) with immune complex deposition in the mesangium and subepithelium (figure). She developed granulocytopenia and signs of cutaneous vasculitis (figure). She was treated with IV steroids and cyclophosphamide and had significant improvement in her creatinine clearance and resolution of her skin rash.

**Conclusions:** We believe this to be the first description of biopsy-proven RPGN in the setting of levamisole exposure. This case demonstrates progression of disease in a patient with previously diagnosed cocaine-associated vasculitis, the presence of both GN and MN on biopsy, and the potential link of levamisole to MN. Levamisole is chemically similar to a class of characterized Toll-like receptor (TLR) agonists; we hypothesize that the variable presentation of this disease may be related to pleiotropic effects of TLR agonists on the immune system.

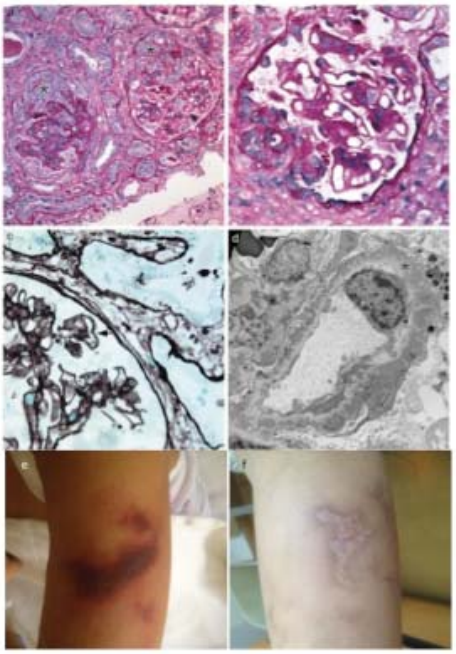


**Figure:** Spectrum of levamisole-associated injury in our patient. (a) Of 58 glomeruli, 10 (17%) were globally sclerosed, 27 (46.5%) demonstrated active crescents (21 complete cellular, 6 partially cellular), and 6 inactive crescents (10%, 2 fibrocellular, 4 fibrous), amounting to 43 of 58 (74%) of total glomeruli (PAS stain). Marked tubular injury was apparent, along with mild interstitial fibrosis (25%). Vessels were remarkable for thickened walls and tortuous appearance, but there was no definitive evidence of vasculitis. (b, c) Thickened basement membranes (b, PAS stain) with "spike & dome pattern" (c, arrow, silver stain). (d) Electron microscopy revealed sub-epithelial electron-dense immune complex deposits (single asterisk) and mesangial immune-complex deposits (double asterisks). Rare subendothelial deposits and extensive foot-process effacement were noted. No tuboreticular inclusions were present within endothelial cells. (e,f) Spectrum of both pristine (e) and mature (f) cutaneous upper extremity lesions in a patient with a history of cocaine-associated vasculitis. Lesion (e) developed in the absence of trauma on day 4 of hospitalization, lesion (f) was present at admission and, per patient report, previously appeared as lesion (e).

*Funding:* NIDDK Support

**SA-PO047**

**Synthetic Cannabinoids Triggered Thrombotic Microangiopathy Leading to Acute Kidney Injury** Jagadish B. Khanagavi,[1] Savneek S. Chugh,[2] Prajakta M. Phatak,[1] Randy A. Goldberg,[1] Praveen N. Chander.[3] *[1]Internal Medicine, New York Medical College(NYMC), Valhalla, NY; [2]Internal Medicine, Div of Nephrology, NYMC, Valhalla, NY; [3]Pathology, NYMC, Valhalla, NY.*

**Background:** Synthetic cannabinoids (SCs) like SPICE are psychoactive compounds marketed as bath salts, incense products and smoked as drugs of abuse. A few cases of acute kidney injury (AKI) attributed to it have been reported but the underlying pathophysiology is unknown. We report the first biopsy proven case of SCs-induced thrombotic microangiopathy (TMA) leading to AKI.

**Methods:** A 20-year-old man with no prior medical history presented with seizures, nausea, vomiting but no diarrhea. Blood Pressure was 176/88 mmHg and heart rate 104/minute. Physical exam was otherwise unremarkable. Lab data showed anemia, thrombocytopenia, elevated serum creatinine (SCr) (3.6 mg/dl) and LDH levels, low haptoglobin and schistocytes confirming thrombotic microangiopathic hemolysis. ADAMTS13 and Complement levels were normal. Urinalysis showed specific gravity of 1.018, 3+ protein, 5-10 WBCs and 5-10 RBCs per high-power field and occasional muddy brown and RBC casts. Urine toxicology revealed tetrahydrocannbinol. ANA, ANCA, anti-GBM antibodies, hepatitis screens, and HIV antibody screen were negative. Renal ultrasound was unremarkable. A diagnosis of atypical hemolytic uremic syndrome was made and plasmapharesis initiated. Patient showed clinical improvement but SCr remained elevated necessitating hemodialysis (HD). Patient later admitted to daily use of marijuana and SPICE over few months. Serum toxicology showed two SCs: SPICE (CP 47,497) and Hebrew University (HU)–320. Renal biopsy showed thrombotic vasculopathic lesions affecting glomeruli as well and acute tubular necrosis possibly due to vasculopathy. Three months later he remains on HD but is now non-oliguric.

**Conclusions:** We believe AKI associated with SCs in some cases is secondary to TMA possibly due to drug induced endothelial damage. Strong clinical suspicion and systematic analysis for such agents is required to promptly diagnose cases of unexplained AKI with TMA in young and otherwise healthy patients.

## SA-PO048

**Carbonyl Stress Induced Protein Modification – A New Link in Pathogenesis of Malarial Acute Kidney Injury** Pinaki Mukhopadhyay,[1] Debarati Mukherjee,[1] Raghwendra Mishra,[1] Monoj Kar,[1] Piyali Banerjee,[2] Gautam Mukherjee.[3] *[1]Nephrology, NRS Medical College, Kolkata, India; [2]Biochemistry, NRS Medical College, Kolkata, India; [3]Medicine, Bagnan Rural Hospital, Howrah, India; [4]Gynae & Obstetrics, North Bengal Medical College, Siliguri, India.*

**Background:** 1.To find out the incidence and degree of parasitism of falciparum malaria mediated acute kidney injury (FMAKI) and their outcome.

2. To measure the carbonyl and oxidative stress of FMAKI patients and correlation with outcome.

**Methods:** In all P.F malaria cases AKI was diagnosed by RIFLE criteria. Demographical, clinical and biochemical parameter were analyzed and they are followed from hospitalization to discharge/death . Oxidative and carbonyl stress markers [Advanced oxidation protein product (AOPP), Advanced Glycation End product (AGE), Pentosidine, Dityrosine, Thioberbituric acid reactive substance (TBARS) and Methyl glyoxal (MG)] were measured consecutively in 15 patient according to standard protocol. All data were analysed in appropriate statistical tool.

**Results:** Among 174 pts 50 (28.7%) had AKI (FMAKI).M: F was 2.6:1.About 45 had very low GFR (10.65 ±3.07ml/min).Among 50 cases of FMAKI 42% had parasite density <5%, 26% had 5-10% and 32% had >10%. Out of 29 cases of severe AKI 9 patients had parasite density<5% and 20 had >5%. Dialysis was required in 62%.Total 13 patients died of them majority belong oliguric severe AKI.There is positive correlation between parasite density, renal failure and surrogate outcome. The Oxidative stress indexes (OSI) of FMAKI patients were 1.89 times higher than normal control. The TBARS, MG level were 6.49 and 5.56 times higher indicating a significant carbonyl stress in these patients. AOPP level the marker of protein modification was also 2.33 times higher than normal control indicating that proteins are highly insulted in FMAKI. Areas under the curves for AOPP and MG were (0.735, p= 0.001),(0.691, p= 0.005) respectively. Mortality and need for dialysis was high in these patients.

**Conclusions:** 1. Parasitic index is independent surrogate predictor of FMAKI.

2. The carbonyl stress marker(MG) along with oxidative stress(AOPP) are significantly raised and possibly linked to the final kidney injury.

## SA-PO049

**ANCA-Associated Pauci-Immune Glomerulonephritis Presenting as Cryptogenic Organizing Pneumonia** Manish K. Saha,[1] Tarek Hamieh,[1] Vishal Sagar.[2] *[1]Medicine, Regions Hospital, St. Paul, MN; [2]Nephrology, Regions Hospital, St. Paul, MN.*

**Background:** Cryptogenic Organizing pneumonia (COP), formerly bronchiolitis obliterans-organizing pneumonia is usually idiopathic but has been associated with connective tissue disorder, infections and drug toxicity. Association of ANCA-related renal vasculitis with COP is rare.

**Methods:** A 68 year old male with history of type 2 diabetes mellitus presented with six weeks of fever, profuse night sweats and a nonproductive cough. His vital signs were normal. Physical exam revealed basilar crackles at bases. White blood cell count of 11.5k/ul, hemoglobin 6.8 g/dl, creatinine 1.53 mg/dl with eGFR 55.7 ml/min/1.73m². His baseline creatinine was 1.15mg/dl. Urinalysis showed 8 rbc/hpf with 30 mg/dl proteinuria and no casts/crystals. Total protein creatinine ratio was 0.5. CRP was 26.1 mg/dl .CT chest showed patchy infiltrate in left upper lobe and prominent bilateral interstitial infiltrate . Bronchoalveolar lavage was non-dagnostic. Cultures were negative .He underwent left lung biopsy which was suggestive of organizing pneumonia .There were no granulomas, or evidence of vasculitits. ANA was positive with MPO ANCA (myeloperoxidase–antineutrophilic cytoplasmic antibodies) >100 with normal proteinase-3. Viral hepatitis serology was negative. With active urine sediment and worsening renal function, kidney biopsy was performed. Pathology revealed pauci-immune type of focal segmental cresentric glomerulonephritis with no immunoreactivity and electron microscopy was normal. With the diagnosis of organizing pneumonia and ANCA positive pauci-immune glomerulonephritis, he was given a pulse dose methylprednisolone and started on Rituximab for four weeks. After completion of Rituximab and Prednisone taper, his creatinine was 1.26 mg/dl and ANCA was <6. Urinanalysis was bland. A repeat CT chest suggested improvement in patchy interstitial infiltrate .

**Conclusions:** In the appropriate clinical setting, even with minimal active sediment and mild renal insufficiency , the association of ANCA-related renal vasculitis and organizing pneumonia should be considered for timely evaluation and treatment to prevent progression of acute kidney injury.

## SA-PO050

**Midkine–A Novel and Early Biomarker of CI-AKI in Patients Undergoing Percutaneous Coronary Interventions-PCI** Jolanta Malyszko,[1] Jacek S. Malyszko,[1] Hanna Gajewska,[2] Ewa Koc-Zorawska,[1] Slawomir Dobrzycki.[2] *[1]Nephrology, Med. Univ, Bialystok, Poland; [2]Invasive Cardiology, Med Univ, Bialystok, Poland.*

**Background:** Midkine, a cytokine, expressed mainly in proximal tubular epithelial cells and is induced by oxidative stress. We tested the hypothesis whether midkine could represent an early biomarker of contrast-induced AKI in 89 patients with normal serum creatinine undergoing PCI.

**Methods:** Midkine, serum and urinary NGAL, cystatin C were evaluated before, and after 2, 4, 8, 24 and 48 hours after PCI using commercially available kits. Serum creatinine was assessed before, 24 and 48 hours after PCI.

**Results:** We found a significant rise in serum midkine as early as after 2 hours ($p<0.001$) when compared to the baseline values. It was also significantly higher 4 and 8 hours after PCI, then returned to the baseline values after 24 hours and started to decrease after 48 hours. Serum NGAL increased after 2, 4 and 8 hours, and in urinary NGAL after 4,8 and 24 hours after PCI. We found a significant rise in serum NGAL after 2, 4 and 8 hours, and in urinary NGAL after 4, 8&24 hours after PCI.Serum cystatin C increased significantly 8 hours, reaching peak 24 hours after PCI and then decreased after 48 hours. When contrast nephropathy was defined as an increase in serum creatinine by >25% of the baseline level 48 hours after PCI, the prevalence of CIN was 10%. Patients with CIN received significantly more contrast agent ($p<0.01$), but duration of PCI was similar.

Midkine were significantly higher 2, 4 and 8 hours after PCI in patients with CIN, while NGAL levels were significantly higher in patients with CIN starting 2 hours after PCI (serum NGAL) or 4 hours (urinary NGAL). Cystatin C were higher only 8&24 hours after PCI in patients with CIN.

**Conclusions:** Since the "window of opportunity" is narrow in CI-AKI and time is limited to introduce proper treatment after initiating insult, particularly when patients are discharged within 24 hours after the procedure, midkine needs to be investigated as a potential early marker for renal ischemia and/or nephrotoxicity. Inhibition of midkine can prevent the migration of inflammatory cells to the injured epithelial layer, reducing the severity of renal damage.

*Funding:* Government Support - Non-U.S.

## SA-PO051

**Distinct Populations of Label Retaining Cells (LRCs) Incorporate into Epithelia Post-Ischemia Reperfusion Injury (IRI) in Different Compartments of the Nephron** Sunil Rangarajan,[1] Paul W. Sanders,[1,2] Lisa M. Curtis.[1,2] *[1]Medicine, Univ of Alabama at Birmingham, Birmingham, AL; [2]Veterans Affairs Medical Center, Birmingham, AL.*

**Background:** Mechanisms underlying epithelial repair post-IRI are incompletely understood. One possible mechanism that has been explored is the contribution from LRCs residing in the kidney. LRCs represent a group of "stem-like" slow cycling cells that have been conventionally labeled using thymidine analogs. Labeling paradigms of LRCs were different in each of the previous studies that have described contradicting characteristics. Here, we have examined the dynamics of the differing populations of LRCs post-IRI.

**Methods:** Thymidine analogs, Chlorine-labeled deoxyuridine & Iodine-labeled deoxyuridine, distinguished by antibody staining, were administered at different postnatal times to the same mouse. This labeling allows for evaluation under identical conditions. At 12 weeks postnatal, these labeled mice were subjected to unilateral nephrectomy & contralateral IRI for 30 minutes. Nephrectomized kidneys obtained at surgery (internal control) & ischemic kidneys harvested 12, 24, 48, & 72 hours after IRI were analyzed for the dynamics of LRCs in specific compartments of the nephron.

**Results:** Majority of the neonatal LRCs resided within the epithelial structures in cortical & medullary regions. Nearly 1/3$^{rd}$ were outside the epithelia in the papilla. Adult LRCs were very few & were predominantly in the cortex. A large proportion of adult LRCs were outside the epithelia. Both LRCs increased 12 hours post-IRI & subsequently declined to levels at or below baseline. Most prominent dynamics of neonatal LRCs were seen in the collecting ducts while that of adult LRCs were seen in proximal tubules.

**Conclusions:** Different paradigms for labeling LRCs in the kidney result in identification of different populations of cells & each population exhibits compartment specific changes post-IRI. Consequently, they may contribute different functions during the post-IRI repair. Further characterization of these LRCs is required to understand their dynamics post-IRI along with the signaling processes involved, which might provide insight into possible therapeutic interventions.

*Funding:* Veterans Affairs Support

## SA-PO052

**Glucagon-Like Peptide-1 Attenuates Cisplatin-Induced Renal Injury: A Gut-Kidney Connection in Acute Kidney Injury** Daisuke Katagiri,[1] Yoshifumi Hamasaki,[1] Kent Doi,[1,2] Koji Okamoto,[1] Kousuke Negishi,[1] Masaomi Nangaku,[1] Eisei Noiri.[1] *[1]Dept of Nephrology and Endocrinology, Univ Hospital, Univ of Tokyo, Tokyo, Japan; [2]Dept of Emergency and Critical Care Medicine, Univ Hospital, Univ of Tokyo, Tokyo, Japan.*

**Background:** Accumulating evidence of the beyond-glucose lowering effect by glucagon-like peptide-1 (GLP-1) has been reported in the context of remote organ connections. This study investigated whether GLP-1 treatment using an inhibitor of dipeptidyl peptidase-4 (DPP-4), alogliptin (AG) and a GLP-1 receptor (GLP-1R) agonist, exendin-4 (Ex-4) attenuate cisplatin-induced AKI (CP-AKI).

**Methods:** C57Bl/6 mice were orally given 10 mg/kg of AG or vehicle once daily from 7 days before and to 96 hr after 15 mg/kg CP intraperitoneal injection. The apoptosis in tubular epithelial cells evaluated using immunoassay for ssDNA and cleaved caspase-3. The balance between pro-apoptotic (Bax and Bim) and anti-apoptotic (Bcl-2 and Bcl-xL) mechanism were evaluated by Quantitative real-time PCR. In another set of experiments Ex-4 was given once a day from 7 days before to 96 hr after CP injection. To demonstrate that GLP-1R–mediated pathway contributes to renal protection by AG, we also conducted an experiment using in vivo small interfering RNA (siRNA) against GLP-1R.

**Results:** Injection of CP to mice increased BUN and serum creatinine, and caused a remarkable renal pathological injury including tubular necrosis. AG treatment significantly reduced these renal injury induced by CP. The mRNA expression ratios of pro-apoptotic/

anti-apoptotic in the AG treated mice were significantly lower than those of the untreated mice. Blood GLP-1 showed significantly higher in the AG-treated mice, whereas other DPP-4 substrates (SDF-1, NPY) increased with the decline of renal function in the untreated mice and decreased according to AG treatment. Ex-4 also attenuated CP-AKI. Suppressing GLP-1R expression in the kidney by using siRNA injection reversed the protection of AG against CP-AKI.

**Conclusions:** These data suggest that a gut-released hormone GLP-1 would ameliorate CP-AKI via anti-apoptotic effects and this gut-kidney axis could be anticipated as a new drug target in AKI.

## SA-PO053

### Acute Kidney Injury in Mice Induces Specific Changes in Gut Microbiota

Daniel A. Peterson,[1] Yu Chen,[2,3] Sanjeev Noel,[2] Samatha Bandapalle,[2] Maria Noel Martina Lingua,[1] James Robert White,[4] Abdel Hamad,[1] Hamid Rabb.[2] *[1]Dept of Pathology, Johns Hopkins Univ, Baltimore, MD; [2]Dept of Medicine, Johns Hopkins Univ, Baltimore, MD; [3]Dept of Medicine, Nanjing Medical Univ, Nanjing, Jiangsu, China; [4]Independent.*

**Background:** Acute kidney injury (AKI) leads to distant organ effects. Crosstalk between organs may worsen or attenuate AKI. Prior work in germ-free mice demonstrated a direct effect of intestinal microbiota on experimental AKI (Jang & Rabb, *Am J Physiol* 2009). Thus current study examines how AKI modulates the bacterial composition of the gut microbiota by pyrosequencing. To identify changes specific to AKI we employed two different models of AKI, ischemia reperfusion injury (IRI) and nephrotoxic (cisplatin-CPN).

**Methods:** IRI was induced by bilateral clamping of the renal pedicles in C57Bl/6 mice for 30 minutes, or chemical AKI induced by CPN injection. Sham operated groups were also studied. To assess the microbiota, DNA was extracted from the fecal pellets (n=4-5 per group), before and day 3 intervention, and subjected to DNA extraction and PCR amplification of the 16S rRNA V3-V5 gene region using the 357F/926R primer set, amplicons were sequenced in multiplex using the Roche/454 platform. The sequences were clustered into 95% operational taxonomic units (OTUs) and taxonomic analysis performed by RDP classifier (rdp.cme.msu.edu).

**Results:** We observed a dramatic change in the gut microbiota that appears specific to AKI. There were key differences between IRI vs CPN-induced AKI effect on gut. The relative abundance of the taxonomic class Erysipelolotrichi, was expanded in AKI mice, IRI (21.3%, sem-2.6%, p =0.02) and in the CPN (3.5%, sem-1.2%, p=0.01). The level in the Sham group (0.4%) was similar to the average of the pretreated mice, (0.35%). This dramatic expansion (~11-65 folds) was compensated by a decrease in primarily the class Bacilli, but also other taxonomic classes.

**Conclusions:** The impact of both ischemic and nephrotoxic AKI on gut microbiota is profound, with key similarities and differences between IRI and CPN. Future studies are warranted to examine the microbiota as a target to improve our understanding and outcomes during AKI.

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## SA-PO054

### C5aR Deficiency and C5aR Blockade Protect Mice from Acute Pyelonephritis

Ke Li,[1,2] Naheed Chowdary,[2] Steven H. Sacks,[2] Wuding Zhou.[2] *[1]Core Research Laboratory, The Second Affiliated Hospital, Xi'an Jiaotong Univ; [2]MRC Centre for Transplantation, King's College London.*

**Background:** UTIs are mainly caused by uropathogenic *E. coli* (UPEC), and infections can progress from the bladder to the kidneys. Recent studies have suggested that complement (C) activation is actually harmful instead of beneficial for the host. However, the underlying mechanism remains to be established. It is unknown if the pro-inflammatory actions of C and specifically that mediated by C5aR signal represent an important pathogenic factor in UTIs.

**Methods:** We employed a well-established mouse model of ascending UTI leading to kidney infection and used C5aR-/- mice or C5aR antagonist to assess whether the severity of pyelonephritis is dependent on C5aR signaling. Using bone marrow chimeras between wild type (WT) and C5aR-/- mice, we evaluated the relative contribution of C5aR signaling on renal parenchymal cells and the infiltrating cells.

**Results:** We found that deficiency of C5aR or blocking C5aR significantly protected mice from kidney infection at 72h after bladder inoculation of E. coli. Compared with wild type (WT) mice, C5aR-/- mice exhibited a lower rate of kidney infection (31% vs 79%, n = 29), reduced bacterial load in the kidney and tissue damage, however, leucocytes accumulation was similar in both groups. C5aR antagonist treated mice also protected from the kidney infection with a similar protection level as that observed in C5aR-/- mice. Chimera studies showed that the absence of C5aR on renal or circulating cells attenuated kidney infection, suggesting that C5aR on both renal and circulating cells contribute to the infection. In vitro, C5a stimulation significantly increased TNF-α, IL-6 and KC production by macrophages and REC, while impaired neutrophil functions (bacteria uptake, reactive oxygen species activity), in the presence of LPS or uropathogenic E coli.

**Conclusions:** Our results demonstrate a pathogenic role for C5aR in pyelonephritis, implicating therapeutic potential of blocking C5aR in human UTI. it also suggest a mechanism by which activation of C5aR cross-talk with TLR4 exaggerates local inflammation, but impairs neutrophil bactericidal function, thus promoting kidney infection.

## SA-PO055

### The α-Intercalated Cell Detects Urinary Pathogenic *Escherichia coli* and Defends the Urinary Tract by Synchronized Secretion of NGAL and $H^+$

Neal A. Paragas,[1] Ritwij Kulkarni,[1] Catherine Forster,[1] Max Werth,[1] Kai M. Schmidt-Ott,[3] Thomas L. Nickolas,[1] Jonathan M. Barasch.[1] *[1]Medicine, Columbia Univ, New York; [2]Pediatrics, Columbia Univ, New York; [3]MDC, Berlin.*

**Background:** The intercalated (IC) cells of the collecting duct cells are responsible for acid base homeostasis with excretion of H+ by the alpha-IC and the excretion of bicarbonate by the beta-IC. We found a novel function of the A-IC, that it responds to Gram-negative urinary pathogenic *E. coli* (UPEC) in both cystitis and pyelonephritis urinary tract infections (UTI) with the graded expression of H+ and NGAL.

**Methods:** (1) Developed a conditional allele of NGAL. (2) Developed a bioluminescent pathogenic bacteria. (3) Knocked out intercalated cells lineage.

**Results:** A reanalysis of our multi-center ER study (n=1635) demonstrated that healthy patients with different severities of Gram-negative (but not Gram-positive) UTIs had significantly increased, dose-dependent expression of urinary NGAL which was reversible with antibiotics. Consistently, uNGAL levels and H+ secretion mirrored the intensity and timing of urinary CFUs (uCFUs) in mice. Sibling matched NGAL deleted mice took significantly longer to clear the infection and conversely, NGAL significantly inhibited UPEC growth in urine cultures by reversibly depleting bacterial iron. To visualize the UTI process, we developed a NGAL reporter mouse (NGAL-Luc2) and a reporter bacteria (UPEC-lux). Bladder inoculation of UPEC-lux induced NGAL-Luc2, Ngal RNA, and NGAL protein distantly in kidneys in a TLR4 dependent fashion. NGAL was predominately expressed by A-IC which directly bound the fluorescent UPECs. These data were reproduced in explanted medullary epithelia co-cultured with UPECs.

**Conclusions:** We show that A-IC are not only regulators of metabolic acids but immune defense cells with both sensing and effector functions. NGAL was necessary and sufficient for bacteriostasis; NGAL was co-regulated with the acidification of the urine. These findings provide an explanation for the intensive expression of kidney NGAL in septic and aseptic diseases: the kidney defends the urinary system via exocrine delivery of NGAL.

*Funding:* NIDDK Support

## SA-PO056

### Kidneys Recovering from Ischemia/Reperfusion Injury Modulate Specific Cytokine Responses to Subsequent Polymicrobial Sepsis in Mice

Takayuki Tsuji,[1] Ana C. Souza,[2] Xuzhen Hu,[2] Peter S.T. Yuen,[2] Robert A. Star.[2] *[1]1st Dept of Medicine, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan; [2]NIDDK, Bethesda, MD.*

**Background:** Sepsis frequently develops post-AKI and portends a poor prognosis. We previously showed that sepsis, induced 48 hr after partial recovery from AKI, had worse renal function and survival vs sepsis alone, despite less liver, muscle, spleen damage, and systemic cytokine response (HMGB1, IL-6, IL-10). Thus, partial recovery from AKI unexpectedly dissociated renal and systemic effects of sepsis. To test how recovering kidneys might affect cytokines, we removed the kidneys 0 hr post-CLP (48hrs post-I/R), then measured serum cytokines.

**Methods:** We performed sham I/R surgery or 40' bilateral ischemia/reperfusion (I/R) in male C57BL/6 mice. 48 hr later we induced polymicrobial sepsis by cecal ligation and puncture (CLP), then bilateral nephrectomy (Nx) or sham Nx. We measured outcomes pre-CLP (48 hr post-I/R) or 24 hr post-CLP.

**Results:** Bilateral nephrectomy at the time of CLP did not alter HMGB1 response after sham-I/R→CLP or I/R→CLP. However, IL-10 was significantly increased after Nx (vs sham Nx) after both sham-I/R→CLP and I/R→CLP. Strikingly, Nx increased IL-6 levels (vs sham Nx) in I/R→CLP but not sham I/R→CLP.

| | without bilateral nephrectomy | with bilateral nephrectomy |
|---|---|---|
| | sham→sham, I/R→sham, sham→CLP, I/R→CLP | sham→sham, I/R→sham, sham→CLP, I/R→CLP |
| HMGB-1 (ng/ml) | 2.7±0.9, 0.7±0.4, 70.2±15.1, 25.8±7.3 | 5.6±1.6, 4.9±2.6, 99.2±17.8, 39.1±4.6 |
| IL-6 (pg/ml) | 27±13, 67±8, 380,000±100,000, 64,700±35,000 | 84±7, 66.8±16.3, 443,000±89,500, 334,000±75,500 |
| IL-10 (pg/ml) | 33±18, 46±24, 20,600±1,020, 3,980±992 | 304±40, 258±41, 111,000±11,600, 58,900±18,500 |

**Conclusions:** After partial recovery from AKI, HMGB1, IL-6, and IL-10 had diminished responses to sepsis. Kidney removal did not affect the reduction of HMGB1, reversed the reduction in IL-6, and derepressed IL-10. Thus, the post-AKI kidneys selectively alter circulating cytokines, suggesting a complex interplay between systemic responses to infection and kidneys.

*Funding:* Government Support - Non-U.S.

## SA-PO057

### Krüppel-Like Factor 4 in Endothelial Cells Protects Mice from Renal Ischemia-Reperfusion Injury

Tadashi Yoshida, Maho Yamashita, Matsuhiko Hayashi. *Apheresis and Dialysis Center, School of Medicine, Keio Univ, Tokyo, Japan.*

**Background:** Krüppel-like factor 4 (Klf4) is a zinc-finger transcription factor involved in cellular differentiation and proliferation in multiple cell types. For example, results of our previous studies showed that deletion of the Klf4 gene in mice delayed down-regulation of smooth muscle differentiation markers, but accelerated neointimal formation following carotid injury. We demonstrated that enhanced neointimal formation in Klf4-deficient mice was caused by reduced induction of p21, a cell-cycle inhibitor, in smooth muscle cells following injury. Klf4 has also been shown to be expressed in aortic endothelial cells (ECs),

where it plays anti-inflammatory and anti-thrombotic roles. However, the role of Klf4 in the kidney remains unknown. The aims of the present studies were to determine the expression of Klf4 in the kidney, and examine if Klf4 plays a role in renal ischemia-reperfusion injury.

**Methods:** Klf4 expression in the kidney was determined by immunohistochemistry. Role of Klf4 in renal ischemia-reperfusion injury was investigated in EC-specific Klf4 knockout mice, which were generated by breeding Tie2-Cre transgenic mice and Klf4 floxed mice.

**Results:** Results showed that Klf4 was expressed in ECs in the kidney of control mice, but not of EC-specific Klf4 knockout mice. Following renal ischemia-reperfusion injury, EC-specific Klf4 knockout mice exhibited significantly enhanced acute tubular necrosis, enhanced expression of pro-inflammatory molecules such as Vcam-1, and highly elevated serum levels of urea nitrogen, as compared to control mice. Results in human umbilical vein endothelial cells showed that Klf4 inhibited cytokine-induced expression of Vcam-1 by binding to p65, an active component of NF-kB.

**Conclusions:** Results suggest that Klf4 in ECs plays a protective role in renal ischemia-reperfusion injury.

*Funding:* Government Support - Non-U.S.

## SA-PO058

**Low Energy Extracorporeal Shock Wave (SW) Ameliorates Renal Tubular Injury in a Rodent Acute Kidney Injury (AKI) Model** Mai Yoshida,[1] Takashi Nakamichi,[1] Takefumi Mori,[1] Kenta Ito,[2] Hiroaki Shimokawa,[2] Sadayoshi Ito.[1] *[1]Div of Nephrology, Endocrinology, and Vascular Medicine, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Dept of Cardiovascular Medicine, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan.*

**Background:** SW therapy recovers ventricular function in ischemic cardiomyopathy, which is associated with upregulation of angiogenic factors such as vascular endothelial growth factor (VEGF) and nitric oxide (NO) expression. Recently, SW has been clinically available to patients with angina pectoris. VEGF and NO are known to play important roles in AKI. The aim of our study is to investigate whether SW ameliorates AKI in a rodent model.

**Methods:** Renal ischemia reperfusion (I/R) surgery was performed for left kidney in male 8-week-old Sprague-Dawley rats. The operated rats were divided into the following 2 groups: SW-treated group (SW, n=11) and no SW-treated control group (CON, n=11). Both kidneys of only SW group were treated with SW (200 shocks/day for each kidney, 0.09 $mJ/mm^2$) just after I/R operation, day1 and day2. The rats of both groups were sacrificed immediately after the last SW treatment. Then, plasma, urine and both kidneys were collected to determine plasma creatinine concentration, urinary NO, renal VEGF mRNA expression, and histological changes of the kidneys.

**Results:** On day2, the left kidney weight was decreased in SW (SW 0.45±0.02 vs. CON 0.52±0.02 g/100 g body weight, $P<0.05$) and plasma creatinine of SW was significantly lower than CON (SW 0.32±0.03 vs. CON 0.43±0.03 mg/dl, $P<0.05$). A light microscopy revealed that tubular injury scores in the outer medulla of SW were significantly lower than CON (SW 1.6±0.2 vs. CON 2.0±0.1, $P<0.05$) on day2. Urinary $NO_2$ and $NO_3$, products of NO, of SW were significantly higher than CON (SW 0.06±0.014 vs. CON 0.051±0.013 mol/day, $P<0.05$). Realtime-PCR analysis demonstrated a significant upregulation of right renal VEGF mRNA expression of SW on day2 (SW 1.29±0.1 vs. CON 0.97±0.1 VEGF/GAPDH).

**Conclusions:** SW improved renal tubular injury and its function, in which angiogenic factors might play roles. SW may be an effective and non-invasive treatment for ischemic AKI.

## SA-PO059

**The Renoprotective Effect of Acute, but Not Delayed, Ischemic Preconditioning Depends on Tubular Cell Autophagy** Man Jiang Livingston,[1,2] Zheng Dong.[1,2] *[1]Dept of Cellular Biology and Anatomy, Georgia Regents Univ; [2]Charlie Norwood VA Medical Center.*

**Background:** Ischemic preconditioning (IPC) is an important mechanism of tissue protective in which application of one or more brief episodes of ischemia-reperfusion results in tolerance to subsequent ischemic injury. IPC is potentially useful for clinical applications in diseases including heart and kidney failure. The mechanism of IPC is very complex and remains poorly understood. Autophagy is induced in tubular cells during renal ischemia-reperfusion and protects against acute kidney injury (AKI). However, it is unknown whether autophagy contributes to the renoprotective effect of IPC.

**Methods:** This study examined the role of tubular cell autophagy in the effect of kidney IPC using both pharmacological and genetic approaches. IPC was induced in C57 mice by a non-lethal bilateral renal ischemia of 15 minutes. After either an hour (acute IPC) or 4 days (delayed IPC) of reperfusion, the mice were subjected to bilateral renal ischemia of 27 minutes followed by reperfusion. Renal function, histology and tubular cell death were monitored.

**Results:** Both acute and delayed IPC suppressed the renal function loss and tissue damage induced by subsequent severe ischemic AKI. While delayed IPC suppressed both tubular cell necrosis and apoptosis, acute IPC mainly inhibited tubular apoptosis. Autophagy was induced in kidneys to a comparable extent by both types of IPC, as indicated by LC3-II accumulation and P62 degradation. Pharmacological inhibition of autophagy by 3-methyladenine or chloroquine abrogated the renoprotective effect of acute IPC, but not that of delayed IPC. The renoprotective effect of acute IPC was abolished in proximal tubule-specific Atg7 knockout mice, whereas the effect of delayed IPC was preserved in this autophagy–defective model. Preconditioning treatment of C57 mice with Tat-beclin1 activated autophagy in kidneys and afforded significant renoprotection against ischemic AKI.

**Conclusions:** Together, these results suggest that tubular cell autophagy is indispensable for renoprotection by acute, but not delayed, IPC. Pharmacological up-regulation of autophagy may be an effective approach for prevention and treatment of AKI.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO060

**Mir-421 Regulates ATM after Acute Kidney Injury** Florian E. Toegel, Steven Leal-Ekman, Linshan Zhou, Joseph V. Bonventre. *Renal Div, Brigham and Women's Hospital, Boston, MA.*

**Background:** Fibrosis after AKI is an important contributor to CKD. Cell cycle arrest after AKI mediates maladaptive injury repair and induces a profibrotic phenotype. Ataxia Telangiectasia Mutated (ATM) is activated after AKI. ATM mediates cell cycle arrest causing up-regulation of profibrotic genes. MicroRNA-421 has been shown to down-regulate ATM expression.

**Methods:** Mir-421 levels were quantified by RTq-PCR. We knocked down Mir-421 expression with antimirs and enhanced expression by using mimics for *in vitro* studies.

**Results:** Mir-421 was down-regulated by ~80% in the injured kidney on day 5 after severe unilateral ischemia/reperfusion (28 min clamping) compared to the non-injured kidney. Bilateral ischemic injury (31 min clamping) caused mir-421 down-regulation on day 7, 12 and 14 post-injury by 44%, 74% and 74%, respectively. Mir-421 down-regulation correlated with increased ATM staining by immunofluorescence. Studies in vitro further defined the relationship between mir-421 and ATM. HK2 cells were transfected with antimirs or mimics and exposed to aristolochic acid (AA), which induces ATM. Mir-421 mimic significantly decreased the amount of p-ATM positive cells after AA exposure whereas antimir increased the p-ATM positive cells. Similar results were obtained when the mimic or antimir was applied after exposure to AA, suggesting a potential therapeutic use after the injury has occurred. Mir-421 mimic significantly decreased cell cycle arrest in HK2 cells after exposure to AA (G2/M 36.78 % control vs 16.44 % mimic). MTT assays showed no change in cell proliferation rate between cells treated with antimir or mimic compared to lipofectamine-treated controls. Antimir or mimic transfection did not change the time to closure of a wounded (scratch assay) monolayer indicating that mir-421 does not affect cell migration.

**Conclusions:** Down-regulation of mir-421 after ischemic AKI results in elevated ATM levels in vivo, and facilitates G2/M arrest after AA exposure in vitro. Mimics of mir-421, applied after injury, may represent a potential therapeutic approach to decrease maladaptive repair associated with AKI.

*Funding:* NIDDK Support

## SA-PO061

**Induction of Hypoxia-Inducible Factor Up-Regulates microRNA miR-21 in Renal Ischemia/Reperfusion** Xialian Xu,[1] Xiaoyan Jiao,[1] Yi Fang,[1] Mingyu Liang,[2] Xiaoqiang Ding.[1] *[1]Nephrology, Fudan Univ Zhongshan Hospital, Shanghai, China; [2]Physiology, Medical College of Wisconsin, Milwaukee, WI.*

**Background:** Hypoxia-inducible factor (HIF) is an important transcriptional regulator in cellular response to hypoxia. We have found that microRNA miR-21 protects mouse kidneys from ischemia/reperfusion injury and HIF mediates hypoxia-induced up-regulation of miR-21 in human renal epithelial cells. In present study, we examined the role of HIF induction in the regulation of miR-21 expression in C57BL/6N mice.

**Methods:** Mice received intraperitoneal injection of saline or cobalt chloride ($CoCl_2$), a classical inducer of HIF activation. 30min bilateral renal ischemia and 24h reperfusion was induced in mice after two injections. Renal injury and miR-21 abundance were analyzed.

**Results:** Compared with the sham group, elevation of Scr (2.49±0.88 vs 0.53±0.05 mg/dl, $P< 0.01$, n=6/group) and morphological injury were induced in the I/R group. Pretreatment with $CoCl_2$ afforded striking functional improvement (Scr: 0.71±0.11 vs 2.49±0.88 mg/dl, $P<0.01$, n=6/group) associated with amelioration of tubulointerstital damage. In the kidneys of mice treated with $CoCl_2$, protein levels of HIF-1α were up-regulated significantly. Ischemia/reperfusion induced up-regulation of miR-21 expression in the kidney. Renal abundance of miR-21 was increased in the $CoCl_2$ + I/R group by 236% ± 54% compared to the saline + I/R group ($p<0.05$, n=6/group).

**Conclusions:** These data, together with our previous findings, suggest HIF activation might mediate up-regulation of miR-21 in the kidney. miR-21 may be invovled in the mechanism of HIF-conferred renal protection against ischemic injury.

*Funding:* Government Support - Non-U.S.

## SA-PO062

**Induction of Mir-489 during Renal Ischemia-Reperfusion Protects against Acute Kidney Injury** Qingqing Wei,[1] Yong Liu,[2] Pengyuan Liu,[2] Mingyu Liang,[2] Zheng Dong.[1,3] *[1]Dept of Cellular Biology and Anatomy, Medical College of Georgia, Georgia Regents Univ, Augusta, GA; [2]Dept of Physiology, Medical College of Wisconsin, Milwaukee, WI; [3]Charlie Norwood VA Medical Center, Augusta, GA.*

**Background:** MicroRNAs have been implicated in acute kidney injury (AKI). While some microRNAs contribute to cell injury/death and tissue damage in AKI, others may protect kidney cells and tissues. By microarray analysis, we profiled microRNA expression during renal ischemia-reperfusion in mice and identified over a dozen of microRNAs showing significant changes. Among them, mir-489 was significantly induced both in vivo in ischemically injured kidney tissue and in vitro in hypoxia-treated renal proximal tubular cells (RPTCs).

**Methods:** Mir-489 induction during hypoxia was attenuated in HIF-1a knockdown cells, indicating that the induction is HIF-1 dependent. Consistently, proximal tubule-specific HIF-1a knockout mice showed lower mir-489 induction duringrenal ischemia-reperfusion than wild type. Functionally, transfection of anti-mir-489 in RPTCs increased apoptosis following ATP-depletion, suggesting a protective role of mir-489. Deep sequencing (RNA-Seq) was further conducted to identify the target genes of mir-489.

**Results:** Agonaute-2-bound RNAs in the presence or absence of mir-489 were immunoprecipitated, sequenced and compared. The analysis identified several classes of mir-489 targets, including the genes involved in cell stress response, wound-healing, cell communication, and cell differentiation.

**Conclusions:** Together, the results suggest that mir-489 is induced during renal ischemia-reperfusion to target some of the key pathways to protect kidney cells and tissues from AKI.

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support

## SA-PO063

**Urinary MicroRNAs as Biomarkers for Drug-Induced Nephrotoxicity** Oana M. Nicoara,[1] Krithika Ramachandran,[2] Daniel J. Antoine,[3] Vishal S. Vaidya.[2] *[1]Nephrology, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Medicine, Renal Div, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [3]Molecular and Clinical Pharmacology, MRC Centre for Drug Safety Science, Univ of Liverpool, Liverpool, United Kingdom.*

**Background:** Kidneys are vulnerable to injury due to their high filtration capacity and metabolic activity. Most drugs are eliminated by the kidney, thus causing drug-induced nephrotoxicity (DIN), that accounts for ~25% of the episodes of acute kidney injury (AKI). Traditional markers of AKI suffer from limitations of sensitivity, specificity, and timeliness of diagnosis. MicroRNAs (miRNAs) are a family of short, non-coding RNAs that stably exist in various body fluids and have emerged as potential biomarkers. Previously we performed an entire miRNome screen of the human urine and identified miR-21, miR-200c and miR-423 to be sensitive indicators of AKI in the hospital setting. The aim of our current study was to investigate if urinary miR-21, miR-200c and miR-423 could differentiate patients with acetaminophen (APAP)-overdose kidney injury from those without AKI.

**Methods:** Urine samples were obtained from APAP-overdose patients and healthy controls. The experiment was performed using a 384-well custom plate from Qiagen that contained specific probes for the 3 candidate miRNAs and miR-489 (previously identified as an invariant miRNA). A real time PCR analysis was performed using the ABI-7900HT instrument.

**Results:** The 3 candidate miRNAs were able to successfully distinguish the APAP overdose patients (n=70) from healthy controls (n=65). It was also found that these 3 miRNAs were significantly different between patients with APAP-induced kidney injury (n=43) and patients with APAP-overdose but no kidney injury (n=27): miR-21 (p=0.01), miR-200c (p=0.003) and miR-423 (p=0.003).

**Conclusions:** We report 3 urinary miRNAs as potential biomarkers of DIN. Further evaluation of these candidate miRNAs for sensitivity, specificity and early diagnostic abilities in an expanded longitudinal cohort of patients with APAP-overdose with or without AKI will help in establishing the value of these urinary miRNAs as non-invasive biomarkers for DIN.

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## SA-PO064

**A Critical Role of Class I Histone Deacetylasesin in Mediating Renal Tubular Cell Regeneration and the Recovery of Renal Function after Acute Kidney Injury** Shougang Zhuang. *Dept of Medicine, Rhode Island Hospital, Alpert Medical School of Brown Univ, Providence, RI.*

**Background:** Class I histone deacetylases (HDACs) are critically involved in the regulation of gene transcription, cell proliferation and renal development.

**Methods:** In this study, we tested the hypothesis that class I HDACs mediate renal regeneration and renal function recovery after acute kidney injury (AKI) in murine models of rhabdomyolysis or folic acid induced AKI.

**Results:** Intramuscular injection of glycerol to induce rhabdomyolysis or peritoneal injection of folic acid resulted in tubular damage and a rise in serum creatinine. Inhibition of class I HDACs with MS-275, a selective class I HDAC inhibitor, induced hyperacetylation of histone H3, and potentiated these responses. MS-275 administration also significantly enhanced rhabdomyolysis and folic acid-induced expression of NGAL and Kim-1, activation of caspase-3 and apoptosis of renal tubular cells. Conversely, this agent reduced renal expression of Pax2, vimentin and proliferating cell nuclear antigen in injured kidneys. In response to rhabdomyolysis and folic acid, multiple signaling molecules, including epidermal growth factor receptor (EGFR), AKT, signal transducer and activator of transcription-3(STAT3), and NF-kappa Bp65 were phosphorylated; two chemokines, MCP1 and RANTES, were also highly expressed in the kidney. Interestingly, MS-275 treatment suppressed phosphoryation of EGFR, AKT and STAT3, but enhanced NF-kappa Bp65 phosphorylation and MCP1 and RANTES expression.

**Conclusions:** Taken together, these data suggest that class I HDACs play a critical role in mediating renal tubular cell survival and regeneration after acute injury and EGFR and NF-kappB are downstream effectors of class I HDACs.

*Funding:* NIDDK Support, Government Support - Non-U.S.

## SA-PO065

**Isoform-Specific Increases in Histone Deacetylase (HDAC) Activity Contribute to the Progression of Renal Fibrosis** Scott R. Manson, Paul F. Austin. *Dept of Surgery, Washington Univ, Saint Louis, MO.*

**Background:** Histone deacetyalases (HDACs) are among the most widely expressed and important regulators of gene transciption. While the 10 HDAC isoforms function by similar biochemical mechanisms, they elicit disparate biological responses dependent upon their target genes. In this study, we examine the role of individual HDAC isoforms during the progression of renal injury along with their potential therapeutic importance.

**Methods:** Renal injury was modeled in mice by acute ischemia-reperfusion (IR) and chronic unilateral ureteral obstruction (UUO). The effects of HDAC inhibition were determined by treatment with the broad spectrum inhibitor Trichostatin A (TSA) and the isoform-specific inhibitors MS-275 (HDAC1-3), MC1568 (HDAC4, 5, 7, 9), CAY10603 (HDAC6, 10), PCI34051 (HDAC8).

**Results:** Each of the 10 HDAC isoforms demonstrate cell type-specific localization in the normal kidney. UUO leads to a 52.0% increase in total HDAC activity along with a 41.9% decrease in Histone H3 acetylation. This coincides with a dramatic increase in the expression of Class IIA HDAC proteins (HDAC4, 5, 7, 9), including an 11.3-fold increase in HDAC7 and a 6.7-fold increase in HDAC9 that localizes primarily to the proximal tubular epithelium. Additionally, infiltrating pro-fibrotic pericytes exhibit high levels of HDAC8 expression. Broad spectrum inhibition of HDAC activity with TSA results in a 31.1% decrease in renal fibrosis and a 43.4% decrease in the loss of renal architecture following UUO. While treatment with each of the isoform-specific HDAC inhibitors resulted in varying levels of renal protection, these compounds appeared to function by different mechanisms. [All results $p < 0.05$].

**Conclusions:** Increased HDAC activity contributes to the progression of renal fibrosis following both acute and chronic renal injury. This is primarily the result of an upregulation in the expression of Class IIA HDAC proteins. While broad-spectrum HDAC inhibition has significant renal protective effects, isoform-specific HDAC inhibition may provide a means of targeting specific cell populations and pathologic mechanisms during the progression of renal injury.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO066

**Vitamin D Deficiency-Induced Inhibition of p21 Upregulation in Ischemic Acute Kidney Injury: Negative Effects** Ana C. de Bragança, Rildo A. Volpini, Janaina Garcia Gonçalves, Daniele Canale, Maria H.M. Shimizu, Rosa M.A. Moyses, Antonio C. Seguro, Lucia Andrade. *Nephrology, Univ of Sao Paulo, Brazil.*

**Background:** Vitamin D deficiency (VDD) is a major predictor of mortality in critically ill patients. Coordinated cell cycle inhibition via increased expression of the cyclin-dependent kinase inhibitor p21 is necessary for optimum recovery from ischemia/reperfusion-induced acute kidney injury (IR-AKI). Because p21, a genomic target of 25-hydroxyvitamin D [25(OH)D], acts via vitamin D receptors (VDRs), VDD-induced inhibition of p21 upregulation could aggravate IR-AKI.

**Methods:** Wistar rats were fed 25(OH)D-depleted or normal diets for 30 days. We studied four groups: control (C), VDD, IR (both renal arteries clamped for 45 min on day 28) and VDD+IR. At 48 h after IR, we measured inulin clearance (Cin); serum 25(OH) D and PTH; and fractional interstitial area (FIA). Immunohistochemistry (for PCNA and ED1) and immunoblotting (for protein expression of p21 and VDRs) were performed in kidney tissue. Morphological kidney damage was scored from 0 to 4. Data are mean±SEM.

**Results:**

| | Cin (ml/min/100g) | p21 (%) | VDR (%) | PCNA+ cells | ED1+ cells | FIA (%) | Damage (score) |
|---|---|---|---|---|---|---|---|
| C (n=7) | 0.93±0.05 | 99±6.0 | 99.5±0.5 | 0.21±0.06 | 2.35±0.12 | 11.32±1.01 | 0.06±0.01 |
| VDD (n=8) | 0.76±0.03[a] | 100±27 | 57.5±8.5[a] | 1.2±0.14 | 5.8±0.73[a] | 14.04±1.02[a] | 0.1±0.01 |
| IR (n=7) | 0.43±0.04[a,b] | 290±4.8[a,b] | 198.5±0.7[a,b] | 29.41±5.12[a,b] | 9.98±0.68[a,b] | 16.70±0.7[a,b] | 1.44±0.12[a,b] |
| VDD+IR (n=8) | 0.29±0.03[a,b,c] | 182±29[a,b,c] | 172.7±10.9[a,b,c] | 33.17±5.88[a,b] | 10.38±0.75[a,b] | 19±0.49[a,b] | 1.72±0.09[a,b,c] |

[a]p<0.05 vs. C; [b]p<0.05 vs. VDD; [c]p<0.05 vs. IR

25(OH)D was decreased in VDD and VDD+IR; PTH was increased in VDD, IR and VDD+IR. Fractional excretions (Ca and P) were higher in VDD+IR than in VDD.

**Conclusions:** In this IR-AKI model, VDD-induced inhibition of p21 upregulation was associated with greater severity of morphological and functional kidney damage. VDD promotes renal interstitial expansion and inflammatory cell infiltration.

*Funding:* Government Support - Non-U.S.

## SA-PO067

**Chronic Nicotine Exposure Reverses Stimulatory Effect of ERK on Acute Renal Ischemia/Reperfusion-Dependent Induction of Heme Oxygenase-1** Istvan Arany, Dustin Reed, Robert Kampen, Luis A. Juncos. *Univ of Mississippi Medical Center.*

**Background:** Chronic nicotine (Ch-NIC) exposure exacerbates acute ischemia/reperfusion-mediated oxidative stress and renal injury (IR-AKI) in the mouse. Extracellular signaling regulated kinase (ERK)-dependent activation of heme oxygenase-1 (HO-1) is considered a major mediator of survival during IR-AKI. Hence, we determined whether Ch-NIC affects activation of those signaling pathways.

**Methods:** In vivo: Some mice were exposed to chronic NIC or vehicle (4 weeks) and subjected to 18-minutes of bilateral warm renal ischemia or sham operation followed by 1, 6 and 24 hours of reperfusion. In vitro: Cultured renal proximal tubule cells (LLC-

PK1) were treated with 200 μM NIC for 24 hours after which cell injury was induced by 400 μM $H_2O_2$. Extent of phosphorylation (U0126), duration of activation (via EGF or constitutively active MEK) or subcellular localization of activated ERK (cytoplasmic or nuclear constitutively active ERKs) was manipulated. Activation of the HO-1 promoter and the antioxidant response element (ARE) was determined in reporter luciferase assays.

**Results:** Ch-NIC attenuated IR-AKI-mediated induction of HO-1 while augmented phosphorylation, duration of phosphorylation and nuclear localization of the phosphorylated ERK (pERK) in the kidney. In vitro, $H_2O_2$-mediated activation of the HO-1 promoter was dependent on pERK-mediated activation of the ARE. Ch-NIC mitigated this induction of the HO-1 promoter through pERK-dependent attenuation of the ARE. Transient activation (by EGF) or cytoplasmic overexpression of an activated ERK construct augmented $H_2O_2$-dependent activation of the ARE while sustained activation (by constitutive MEK) or nuclear overexpression of an activated ERK construct mitigated it.

**Conclusions:** The activated ERK elicits opposing effects in induction of the HO-1 promoter: under oxidative stress ERK activation is transient and cytoplasmic that induces the ARE. In contrast, upon exposure to Ch-NIC the activated ERK is sustained and nuclear, which mitigates oxidative stress-dependent induction of the ARE. These results may offer therapeutic means to ameliorate adverse effects of Ch-NIC.

*Funding:* NIDDK Support

## SA-PO068

**The Role of Nicotinic Acetylcholine Receptor in Adverse Effects of Chronic Nicotine Exposure on Renal Acute Ischemic Injury** Istvan Arany, Dustin Reed, Robert Kampen, Luis A. Juncos. *Univ of Mississippi Medical Center.*

**Background:** Earlier we reported that chronic nicotine (Ch-NIC) exposure exacerbates acute renal ischemic injury (IR-AKI) in mice. NIC –a major component of cigarette smoke- is an agonist of the α7-nicotinic-acetylcholine receptor (α7nAChR). While acute activation of the α7nAChR is connected to anti-inflammatory signaling, its desensitization via Ch-NIC may activate signaling pathways that support inflammation and injury. In this work we tested the hypothesis that Ch-NIC exerts its adverse effects on IR-AKI through α7nAChR.

**Methods:** IR-AKI was established in wild type or α7nAChR knockout mice that were subjected or not to chronic NIC. 24 hours post-reperfusion, oxidative stress, renal dysfunction, injury and markers of survival/death signaling and inflammation were determined. In vitro, renal proximal tubule cells (LLC-PK1) were treated with 200 μM NIC for 24 hours followed by treatment with 400 μM $H_2O_2$. α7nAChR was inhibited by the antagonist α-bungarotoxin (αBTX); ROS production, mitochondrial depolarization, cell injury as well as activity of the MCP-1 gene were determined.

**Results:** IR-AKI-mediated renal dysfunction (plasma creatinine), injury (renal KIM-1), inflammation (MCP-1) and oxidative stress (MDA and nitrotyrosine expression) was attenuated in α7nAchR k.o. mice compared to their wild type counterparts. In vitro, αBTX attenuated NIC+$H_2O_2$-mediated production of ROS, mitochondrial depolarization and injury as well as activation of the MCP-1 promoter.

**Conclusions:** Our results suggest that the α7nAChR plays a pivotal role in transducing injury signals in the presence of Ch-NIC. Thus, manipulation of the receptor activity may offer therapeutic means to ameliorate adverse effects of chronic NIC/smoking.

*Funding:* NIDDK Support

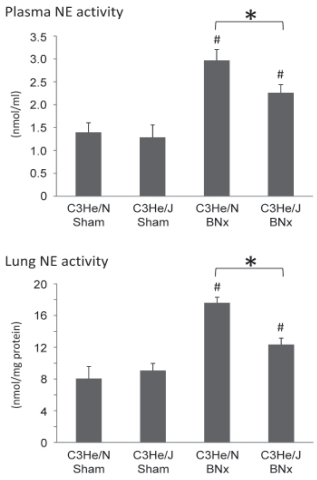
## SA-PO069

**High-Mobility Group Protein B-1–Toll-Like Receptor 4 Pathway in Acute Lung Injury Induced by Bilateral Nephrectomy** Kent Doi,[1] Tomoko Ishizu,[2] Yoshifumi Hamasaki,[2] Tetsushi Yamashita,[2] Masaomi Nangaku,[2] Naoki Yahagi,[1] Eisei Noiri.[2] *[1]Emergency and Critical Care Medicine, The Univ of Tokyo, Tokyo, Japan; [2]Nephrology and Endocrinology, The Univ of Tokyo, Tokyo, Japan.*

**Background:** Acute lung injury (ALI) and acute kidney injury (AKI) are severe complications in critically ill patients. These two organ dysfunctions synergistically increase mortality in intensive care units. Organ cross-talk between the kidney and the lung has been suggested recently as amplifying each organ injury. This study was conducted to identify a possible mechanism of AKI-induced ALI using a mouse bilateral nephrectomy (BNx) model.

**Methods:** Bilateral nephrectomy was conducted with TLR4-mutant C3H/HeJ and their TLR4 wild-type control strain C3H/HeN mice. Neutrophil infiltration, neutrophil elastase (NE) activity and cytokine expression (IL-6, KC, TNF-α) in the lung were examined. Plasma HMGB1 concentration was measured by ELISA. Anti-HMGB1 neutralizing antibody treatment and *s*plenocyte transfer from C3He/N to C3H/HeJ mice were conducted to demonstrate the contribution of HMGB1–TLR4 pathway to lung injury.

**Results:** TLR4-mutant C3He/J mice were resistant to lung injury including neutrophil infiltration and increased NE activity caused by BNx. Injection of splenocytes isolated from C3He/N mice to C3He/J mice reversed the suppression of NE activity in C3He/J mice. Blood concentrations of HMGB1 in C3He/J and C3He/N mice were increased significantly by BNx. Blockade of HMGB1 by neutralizing antibody reduced lung injury not in TLR4-mutant C3He/J but in TLR4-wild type C3He/N mice.




**Conclusions:** The obtained data suggest that enhanced HMGB-1–TLR4 pathway contributes to lung injury induced by BNx. Targeting the HMGB-1–TLR4 pathway will enable development of a new therapeutic strategy to improve the outcome of severely ill patients with ALI and AKI.

*Funding:* Government Support - Non-U.S.

## SA-PO070

**BMP and NF-κB Signaling Interaction in Acute Kidney Injury** Emilia Vigolo,[1] Lajos Marko,[2] Giulietta Roel,[1] Dominik Müller,[2] Ruth Schmidt-Ullrich,[1] Kai M. Schmidt-Ott.[3] *[1]Max Delbrueck Center for Molecular Medicine, Berlin, Germany; [2]Experimental and Clinical Research Center, Charité - Universitätsmedizin, Berlin, Germany; [3]Dept of Nephrology, Charité - Universitätsmedizin, Berlin, Germany.*

**Background:** Renal ischemia reperfusion injury (IRI) is a major cause of AKI. Both bone morphogenic protein (BMP) and nuclear factor-κB (NF-κB) signaling have been implicated in the pathogenesis of AKI. However, the precise timing of their activity and interconnection remain unknown. We hypothesized that BMP and NF-κB signaling interact to modulate the pathogenesis of AKI.

**Methods:** We induced IRI by clamping the renal pedicle for 25 minutes in NF-κB specific reporter (*κ-luc*) mice and Emx1-Cre;IκBαΔN$^{flox}$ (*Emx1-ΔN*) mice, in which NF-κB activity is specifically suppressed in renal tubules. We analyzed serum creatinine, renal neutrophil gelatinase-associated lipocalin (NGAL) mRNA expression, and histological damage scores. BMP signaling activation was detected by pSmad1/5/8 immunostaining.

**Results:** *κ-luc* mice displayed a gradual increase of NF-κB activity with a significant peak after 3 days following IRI, whereas tubular canonical BMP signaling was simultaneously down-regulated as verified by pSmad1/5/8 staining. To address the specific function of NF-κB in tubular cells, we generated the *Emx1-ΔN* mice. The mRNA expression of the NF-κB target genes IkBa, IL6, ICAM1 and VCAM1 was up-regulated in control, but not in *Emx1-ΔN* ischemic kidneys. 24 hours after IRI, *Emx1-ΔN* mice displayed reduced tubular injury, serum creatinine and NGAL expression compared to control littermates. Additionally, extracellular matrix expansion, inflammatory cell infiltration and CTGF expression were strongly reduced in the postischemic *Emx1-ΔN* mice compared to littermate controls. Conversely, tubular pSMAD1/5/8 was increased in postischemic *Emx1-ΔN* kidneys suggesting that NF-κB signaling suppresses the intrinsic tubular BMP activity.

**Conclusions:** These data provide initial evidence of a cross-talk between NF-κB and canonical BMP signaling in the postischemic kidney. Importantly, NF-κB-mediated repression of BMP signaling may participate in the maladaptive aggravation of tubular injury and perturbed regeneration following renal ischemia.

## SA-PO071

**The Effects of Aging on the Development of Renal Ischemia-Reperfusion Injury** Eun Hee Koo, Hye Ryoun Jang, Jung Eun Lee, Dae Joong Kim, Yoon-Goo Kim, Ha Young Oh, Wooseong Huh. *Dept of Medicine, Div of Nephrology, Samsung Medical Center, Seoul, Korea.*

**Background:** Ischemia-reperfusion injury (IRI) is the leading cause of ischemic acute kidney injury in both native and transplanted kidneys. IRI is known to increase acute rejection and have deleterious effect on long term outcome of allografts. Previous studies have reported that the elderly are more prone to ischemic acute kidney injury. In this study, we investigated how aging affects the development of renal IRI.

**Methods:** Both renal pedicles were clamped for 25 minutes on 9-week (9W, n=15), 6-month (6M, n=15), and 12-month-old (12M, n=11) male C57BL/6 mice. Renal function changes were evaluated with serum creatinine and cystatin C for 48 hours. Tubular injury, infiltration of CD 45 positive leukocytes, and cytokine expression on post-ischemic kidneys were analyzed.

**Results:** There was no difference in serum creatinine among groups, although serum cystatin C level was higher in 12M mice compared with 9W and 6M mice. Both 6M and 12M mice showed higher proportion of damaged tubules in the cortex than 9W mice, but medullary tubular damage was comparable. There was no difference in the infiltration of leukocytes into the post-ischemic kidney. Interleukin (IL)-6 expression was higher in 6M and 12M mice than 9W mice. The expression of interferon-γ was comparable. Both IL-4 and IL-10 were comparable among groups on baseline and day 2 after IRI. Vascular endothelial growth factor (VEGF) was lower in 6M and 12M mice at baseline. VEGF significantly decreased in all groups after IRI, and was much lower in 6M and 12M mice compared with 9W mice on day 2 following IRI.

**Conclusions:** Older mice showed more severe cortical tubular injury compared to young mice. Pronounced increase of IL-6 and decrease of VEGF on the post-ischemic kidneys in older mice seem to be the cause. However, pathologic changes did not correlate with age or changes in renal function.

## SA-PO072

**In Vivo Hydrogen Sulfide Measurements during Renal Ischemia Reperfusion** Bernardo Lopez, Cayetano Perez, Moises Hernandez, Francisco J. Fenoy, Miguel G. Salom, Francisca Rodriguez. *Physiology, School of Medicine at Universidad de Murcia, Campus de Espinardo, Murcia, Spain.*

**Background:** Hydrogen sulfide ($H_2S$) has recently been classified as a member of the gasotransmitter family. The enzymes involved in its production are mainly cystathionine β–synthase (CBS) and cystathionine γ–lyase (CSE) that generate $H_2S$ from L-Cysteine (L-Cys). Recent studies highlight $H_2S$ protective effects on renal ischemia-reperfusion injury (IRI), a major cause of acute renal failure and renal transplant dysfunction. The aim of this study was to determine the real-time cortical levels of the gaseous signaling molecule $H_2S$ during a renal IRI.

**Methods:** Anesthetized SD rats underwent 45/60 min of warm ischemia/reperfusion during intrarenal infusion of saline (IRI, n=8), 80 nmol/Kg/min NaHS (IRI+$H_2S$, n=6), 80 µmol/Kg/min L-Cys (IRI+L-Cys, n=4) or 2 µmol/Kg/min propargylglycine (PPG, a CSE inhibitor) + 0.5 µmol/Kg/min amino-oxyacetic acid [(AOAA, a CBS inhibitor), IRI+Inh, n=5]. Cortical $H_2S$ concentration was measured electrochemically; renal blood flow (RBF), and glomerular filtration rate (GFR) were also assessed. In other rats set, GFR was measured 24 h after ischemia in nonischemic, and in ischemic rats kidneys i.v. given (in mg/Kg) either saline (IRI, n=6), 1 NaHS (IRI+H2S, n=10), 50 L-Cys (IRI+L-Cys, n=8), or 40 PPG+3 AOAA (IRI+Inh, n=5).

**Results:** Renal ischemia increased cortical $H_2S$ levels, which dropped to preischemic values on reperfusion. Ischemic $H_2S$ increase was significantly blunted in the IRI+Inh rats group. NaHS, L-Cys, or PPG+AOAA infusion did not alter RBF and GFR basal values; RBF, but not GFR, came back to preischemic values on reperfusion. $H_2S$ donor, L-Cys, or PPG+AOAA had no effect on GFR (1154.6±177.1 µl/min/g) in nonischemized kidneys. 24 h after ischemia, GFR decreased in ischemized kidneys of IRI (226.7±75.8) and IRI+Inh (153.2±67.1) rats. The GFR fall was significantly improved in the ischemized kidneys of rats pretreated with NaHS (579.8±131.1) or L-Cys (698.2±155.3).

**Conclusions:** Renal ischemia produces a rise in endogenous renal cortical $H_2S$ levels, but only a pretreatment with an exogenous $H_2S$ source seems to contribute to the significant amelioration of the renal failure observed 24 h after reperfusion.

*Funding:* Government Support - Non-U.S.

## SA-PO073

**Urinary Excretion of Nidogen-1 by Meprin A during Acute Kidney Injury** Christian Herzog,[1] Randy S. Haun,[2,3] Gur P. Kaushal.[1,3] *[1]Medicine, UAMS, Little Rock, AR; [2]Pharmaceutical Sciences, UAMS, Little Rock, AR; [3]CAVHS, Little Rock, AR.*

**Background:** Nidogen-1 is an important component of the extracellular matrix (ECM) located at the basement membrane of tubular epithelial cells (RTEC) in the kidney. In a mouse model of acute kidney injury (AKI), nidogen-1 fragments appear in the urine long before the rise of the diagnostic markers BUN and serum creatinine, whereas these fragments are absent in normal urine. Actinonin, a potent inhibitor of meprin A (a metalloproteinase highly enriched in the brush border membrane (BBM) of RTEC) reduces the excretion of these fragments. Previous studies of our group have shown that meprin A (tetramer of α and β subunits) can degrade nidogen-1 in vitro. As several deleterious effects of AKI have been attributed to proteolysis by meprin A, its role in the excretion of nidogen-1 fragments was investigated.

**Methods:** Urinary excretion of nidogen-1 fragments was monitored by western blot (WB) in male C57Bl/6n mice as well as in knockout (KO) mouse models of meprin α and β after induction of AKI by cisplatin ± actinonin. Degradation of nidogen-1 was monitored in kidney homogenates by WB. Kidney distribution of meprins and nidogen-1 during AKI was visualized by immuno-fluorescence.

**Results:** Nidogen-1 fragments appear in the urine 12h after cisplatin-injection, long before the rise of BUN and serum creatinine levels and this excretion is markedly reduced by the meprin inhibitor actinonin. Meprin β KO-mice (characterized by the complete loss of membrane bound meprin A) show an even stronger reduction in urinary excretion of nidogen-1 fragments, whereas meprin α KO mice do not. Mice subjected to AKI show altered localization of meprin A from the BBM towards the cytosol and the basolateral membrane suggesting close contact with components of the ECM such as nidogen-1.

**Conclusions:** Meprin A, and particularly its β subunit, seem to be responsible for the proteolytic cleavage of nidogen-1 in the early stages of cisplatin-induced AKI long before the rise in BUN and serum creatinine levels. As meprin A changes its localization during AKI and targets molecules of the ECM, it becomes a valuable target for pharmaceutical intervention.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO074

**Therapeutic Manipulation of the Angiopoietin/Tie2 Pathway in Septic Acute Kidney Injury** Thomas Stiehl,[1] Kristina Thamm,[1] Hermann G. Haller,[1] Ansgar Santel,[2] Sascha David.[1] *[1]Nephrology, Medical School Hannover, Hannover, Germany; [2]Silence Therapeutics AG, Berlin, Germany.*

**Background:** Intensive basic research into sepsis and associated acute kidney injury (AKI) has yielded numerous putative molecular targets, but many have not translated into successful therapies. We have shown that Angiopoietin-2 (Angpt-2), a partial antagonist of the endothelium-stabilizing receptor Tie-2 secreted by endothelium, contributes to septic AKI and adverse outcomes in this disease. Here we report translational data modulating the Angpt/Tie2 pathway as a potential therapeutic strategy in murine experimental sepsis.

**Methods:** Endothelial Angpt-2 transcription was reduced via an Angpt-2 specific lipophilic in vivo siRNA approach in male C57Bl6 mice. Experimental polymicrobial sepsis was induced by cecal ligation and puncture (CLP). In this model we analysed the 1) renal expression of endothelial adhesion molecules (VCAM-1, ICAM-1) via qPCR and immunofluorescence (IF) staining, 2) infiltration of inflammatory cells (IF Gr-1), 3) organfunction, 4) pulmonary vascular leakage (Evans blue permeability assay) 5) severity of illness (activity score), and 6) mortality.

**Results:** Validation of the Angpt-2 siRNA showed a 45.5 ± 5.4 % reduction of Angpt-2 mRNA septic mice (p=0.01). We found a significant reduction in renal ICAM-1 and VCAM-1 expression in Verum treated septic animals, an observation that was associated with less organ infiltration of inflammatory cells (*Gr*-1 pos cells per high power field: Verum 19.3 ± 7.6 vs. Placebo: 36.5 ± 12.7 p<0.0001) and better organ function (*creatinin:* Verum 47.8 ± 8.4 vs. Placebo 66.2 ± 15.7 umol/l; *urea:* Verum 20.6 ± 13.4 vs. 41.2 ± 7.7 umol/l, p=0.008). Renal histological damage as visualized by brush border staining (lectin phythemaglutinin) was ameliorated (p=0.008). Moreover, Angpt-2 siRNA improved vascular severity of illness (p=0.01), reduced vascular leakage (p=0.008) as well as overall survival (after 72 hrs: Verum 50% vs. Placebo 0%, p=0.0002).

**Conclusions:** The Tie2 antagonist Angpt-2 might represent a novel promising target against sepsis and associated AKI. A specific lipophilic siRNA approach could be a clinically relevant way of application to reduce pathological Angpt-2 transcription.

*Funding:* Pharmaceutical Company Support - Silence Therapeutics

## SA-PO075

**Expression of DJ-1 in Renal Ischemia Reperfusion Injury** Guanhui Wu,[1] Kim-Chong Yong,[1] Yeong-Hau Howard Lien,[2,3] Li-Wen Lai.[1] *[1]Chemistry and Biochemistry, Univ of Arizona, Tucson, AZ; [2]Medicine, Univ of Arizona, Tucson, AZ; [3]AKDHC, Tucson, AZ.*

**Background:** DJ-1 (PARK7) is a ubiquitous cytoprotector against oxidative stress and cell death, acting as ROS scavenger, molecular chaperon, and regulator of other antioxidant genes. DJ-1 mutation causes autosomal recessive Parkinson disease and its role as a neural protector has been under intensive investigation. However, the protective role of DJ-1 in the kidney is largely unknown. Since oxidative stress is the major pathogenesis of renal ischemia/reperfusion injury (IRI), we measured the DJ-1 protein and mRNA level in the kidney.

**Methods:** Male NIH Swiss mice were subjected to 30 min of bilateral clamping of renal pedicles, followed by reperfusion for 1, 4, 8, 16, or 24 h. The total protein and mRNA were isolated and analyzed using Western Blotting and quantitative RT-PCR, respectively.

**Results:** The immunoblotting results showed a 50% reduction of renal DJ-1 protein level at 1 h after reperfusion in comparison to the control but the DJ-1 protein was restored back to the baseline level at 8, 16, and 24 h after reperfusion. In contrast, the qRT-PCR showed that renal DJ-1 mRNA abundance was slightly increased (130% of baseline level) at 1 h and 4 h, while it was dramatically increased to 260% at 8 h and remained elevated (150% of baseline level) after 16 and 24 h.

**Conclusions:** Upon oxidative stress, DJ-1 has been shown to undergo irreversible oxidative modification, leading to its inactivation and rapid degradation. Collectively, the transient decrease and reverse of DJ-1 protein level in combination of upregulation of DJ-1 mRNA abundance suggest that 1) DJ-1 is highly regulated at both protein and mRNA levels during renal IRI; 2) IRI causes oxidative modification and degradation of DJ-1 protein which is restored by the feedback upregulation of DJ-1 mRNA synthesis; 3) DJ-1 may serve as a potential drug target in the prevention/treatment of AKI.

*Funding:* NIDDK Support

## SA-PO076

**Sildenafil (SIL) Protects against Acute Kidney Injury (AKI)-Induced Cardiac Dysfunction via Direct Effects on Myocardial Cells** Andrea P. Soljancic,[1] Damian G. Romero,[2] Istvan Arany,[3] Rodrigo Maranon,[4] Kiran B. Chandrashekar,[1] Ruisheng Liu,[4] Luis A. Juncos.[1] *[1]Medicine-Nephrology, Univ of Miss Med Center; [2]Biochemistry, Univ of Miss Med Center; [3]Pediatrics, Univ of Miss Med Center; [4]Physiology, Univ of Miss Med Center.*

**Background:** AKI causes cardiac dysfunction. We reported that Sil protects against AKI and its associated cardiac inflammation. However, we did not discern if this beneficial cardiac effect was due to the less severe AKI or its direct cytoprotective effect (via heme

oxygenase-1; HO-1) in myocardial tissue. Therefore, we tested if Sil not only augments AKI-induced cardiac HO-1 (despite the less severe renal injury) in vivo, but also whether this augmentation was due to a direct effect on myocardial cells in vitro.

**Methods:** *In Vivo Studies*: SD rats treated with vehicle or Sil were subjected to 40 mins of bilateral renal ischemia-reperfusion (I/R). Blood and tissue were collected at 72 h. In Vitro Studies: H9c2 cells (embryo cardiomyoblasts) treated with vehicle or Sil (10 uM) were exposed for 24 h to rat serum collected 48 h post either 40 min of I/R-AKI or a sham surgery.

**Results:**

| In vivo | BUN mg/dl | Urine NGAL UI/ml | TNFα pg/ug | Cytochrome C ng/ug | HO-1 ng/ug |
|---|---|---|---|---|---|
| Sham | 21±2 | 700 ± 182 | 0.3±0.1 | 2±0.1 | 0.1±0.01 |
| Sham-Sil | 17±2 | 694 ± 92 | 0.3±0.1 | 1±0.1* | 0.1±0.01* |
| I/R-AKI | 76±3* | 3401 ± 139* | 4±0.2* | 10±0.6* | 0.7±0.03* |
| I/R-AKI-Sil | 41±3# | 2381 ± 62 # | 2±0.1# | 7±0.4# | 0.9±0.04# |
| **In vitro** | **TNFα (pg/ml)** | **Cytochrome C (ng/ml)** | **HO-1 (ng/ml)** | | |
| FBS | 3±0.5 | 6±0.1 | 6±0.1 | | |
| FBS-Sil | 3±1 | 6±0.1 | 16±0.4 δ | | |
| Sham | 4±0.5 | 6±0.6 | 7±0.1 | | |
| Sham-Sil | 3±0.3 | 6±0.3 | 17±0.2 λ | | |
| I/R-AKI | 255±6 λ | 97±6 λ | 24±0.3 λ | | |
| I/R-AKI-Sil | 74±2 ξ | 22±1 ξ | 38±0.3 ξ | | |

Data: Mean ± SEM. p<0.05: * vs Sham, # vs I/R-AKI, δ vs FBS, λ vs Sham, ξ vs I/R-AKI. FBS: Fetal Bovine Serum.

**Conclusions:** Sil directly enhances I/R-induced increases in HO-1 in myocardial cells, and blunts I/R-AKI-induced cardiac inflammation and apoptosis, suggesting that Sil-induced HO-1 in the heart may contribute to its protective effect during AKI.

*Funding:* Other NIH Support - NIH DK073401.

## SA-PO077

**Common Genetic Variation and Risk for Acute Kidney Injury in Trauma-Related Critical Illness** Paramita Mukherjee, Grant O'Keefe, Amber Burt, Jonathan Himmelfarb, Mark M. Wurfel. *Kidney Research Institute, Univ of Washington, Seattle, WA.*

**Background:** Acute kidney injury (AKI) is seen in 5-20% of critically ill patients. Better molecular and genetic markers are needed to assess individual risk for AKI. Our objective was to identify common genetic variants in the form of single nucleotide polymorphisms (SNPs) that influence susceptibility to AKI after major trauma by using a genome-wide association study (GWAS).

**Methods:** We used the Illumina Human610 Quad Beadchip to genotype 975 subjects from a cohort of Caucasian patients (age > 18) enrolled from the trauma ICU at Harborview Medical Center between 2003-2005 and followed for development of AKI. AKI was defined using a modified Acute Kidney Injury Network (AKIN) based on difference between the highest and lowest creatinine during the first 4 days of ICU admission and were coded as a 3-level ordinal outcome variable: stages 0, 1, 2 and 3 combined. We used logistic regression, assuming additive genetic effects (adjusting for age and sex) to identify SNPs associated with AKIN stage.

**Results:** The 975 subjects genotyped had a mean age of 45 years, were 74.2% male, and included 7% with chronic kidney disease. Injury was quite severe for most subjects as evidenced by a mean trauma related injury severity score (TRISS) of 33.7. Stage 1 and combined stage 2+3 AKI occurred in 52.8% and 5.9% of subjects respectively and was not correlated with the TRISS. Although, no associations achieved genome-wide significance, the top two SNP identified were in *CACNA2D4* encoding a voltage-dependent calcium channel and *ATP2C2* encoding a secretory $Ca^{2+}$ ATPase responsible for calcium uptake, with $p=2.1 \times 10^{-6}$ and $p=2.8 \times 10^{-5}$ respectively. We also identified 50 SNPs with $p < 1 \times 10^{-4}$ that fell within genes related to polycystic kidney disease , cell cycle and T cell regulation.

**Conclusions:** Common genetic variation in *CACNA2D4* and *ATP2C2* demonstrate moderate associations with risk for AKI after major trauma. Given that studies in rodent models of acute kidney injury have shown that increases in cytosolic calcium levels are associated with renal epithelial cell injury, these results warrant validation.

*Funding:* NIDDK Support, Other NIH Support - T32 training grant # T32DK007467-29; 5U01DK084012-03

## SA-PO078

**Impact of P-Glycoprotein Induction on Colistin-Induced Nephrotoxicity in Cultured Human Proximal Tubular Cells** Hyo-Wook Gil,[1] Soon Hyo Kwon.[2] *[1]Dept of Internal Medicine, Soonchunhyang Univ Cheonan Hospital, Cheonan, Chungnam, Korea; [2]Dept of Internal Medicine, Soonchunhyang Univ Seoul Hospital, Seoul, Korea.*

**Background:** Intravenous colistin is used to treat resistant infections caused by gram-negative organisms and is associated with nephrotoxicity. The pathogenesis of colistin-associated nephropathy has not been clarified and no effective therapeutic or prophylactic agent is available. This study aimed to examine the mechanisms underlying colistin-induced nephrotoxicity and to determine if P-glycoprotein (P-gp) induction can prevent nephrotoxicity.

**Methods:** We examined the cytotoxicity of colistin in cultured human proximal tubular cells (HK-2) by MTT assay and LDH assay. To evaluate colistin-based induction of reactive oxygen species (ROS), ROS were measured using DCF-DA. To investigate colistin-induced apoptosis, a TUNEL assay was conducted, and caspase 3/7 activity was evaluated. Autophagy was assessed by measuring the levels of LC3. To evaluate whether P-gp was involved in the efflux of colistin in HK2 cell lines, P-gp expression and cell toxicity were determined in cells treated with colistin with and without dexamethasone (i.e., P-gp inducer) and verapamil (i.e., selective P-gp inhibitor) by real-time PCR and MTT assays.

**Results:** Colistin showed cell toxicity in dose- and time-dependent patterns in the HK2 cell lines. Colistin induced ROS in a dose-dependent manner in the DCF-DA assay. Caspase 3/7 activity increased in a dose-dependent pattern at 6 h (25, 50, and 100 μg/mL) and was inhibited by N-acetylcysteine. Apoptosis was confirmed by TUNEL assay at 6 h (50.00 μg/mL). The ratio of LC3II to LC3Iincreased at 2 h. Colistin itself suppressed P-gp expression. P-gp expression and activity in the colistin-treated HK2 cell line were enhanced by treatment with dexamethasone, whereas colistin-induced cytotoxicity decreased. In addition, the induced P-gp transporter was shown to improve the efflux effect in colistin-treated HK2 cell lines, as shown by the Calcein-AM fluorescence accumulation assay.

**Conclusions:** P-gp is involved with colistin efflux in HK2 cell lines. This study suggests a protective effect of P-gp induction against colistin-induced nephrotoxicity.

## SA-PO079

**Ablation of C/EBP Homologous Protein (CHOP) Protects Ischemia/Reperfusion-Induced Acute Kidney Injury** Chiung-ying Huang,[2] Bo-lin Chen,[1] Ching-chin Yang,[1] Cheng-tien Wu,[1] Shing-Hwa Liu,[1] Chih-Kang Chiang.[1,2,3] *[1]Institute of Toxicology, National Taiwan Univ, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ, Taipei, Taiwan; [3]Dept of Integrated Diagnostics & Therapeutics, National Taiwan Univ Hospital, Taipei, Taiwan.*

**Background:** Renal ischemia-reperfusion (IR) yields high rates of acute kidney injury (AKI), but its potential mechanism remains poorly understood. Endoplasmic reticulum (ER) stress has been shown involved in the pathogenesis of AKI. This study investigated whether C/EBP Homologous Protein (*chop*), an important ER stress-related transcriptional factor, mediated IR-induced AKI.

**Methods:** Wild type and *chop*-knockout mice received bilateral renal arteries clamped by non-traumatic clamps for 15 min on the heat plate, followed by reperfusion. Renal pathology, renal functional assay, and ER-related signals were evaluated after reperfusion at the series time points. We also arranged bone marrow transplantation to clarify the influences of *chop* ablation in native immunity in AKI.

**Results:** *chop* deficiency retarded renal injury after IR-induced AKI. Both renal proximal tubule damage and collagen deposition were attenuated in *chop* deficiency mice. Furthermore, *chop* deficiency could decrease IR-induced caspase-3 cleavage and activity. Lipid peroxidation was enhanced after 24 h of IR, but it reversed by CHOP deficiency mice kidney. In addition to in vivo study, we evaluated the effects of reactive oxygen species in the *chop* knockdown renal tubule cells. *chop*-silenced tubule cells were treated by anaerobic/reperfusion condition and $H_2O_2$, and shown more resistant to apoptosis. In spite of lesser leavage of caspase-3 in *chop*-silenced tubular cells, mitochondria-related apoptosis signals, including Bax and Bcl-2, were not influenced by $H_2O_2$ and *chop* siRNA treatment. Finally, *chop* deficiency blocked NFκB activation and COX-2 expression, which suggested *chop*-related signals contributed to the oxidative stress-mediated inflammation responses.

**Conclusions:** To the best of our knowledge, this is first report demonstrated that *chop* deficiency attenuated IR-related AKI by modulating apoptotic, inflammatory and oxidative responses.

*Funding:* Government Support - Non-U.S.

## SA-PO080

**NRF2 Activators as Potential Modulators of Injury in Human Kidney Cells** Melanie S. Joy,[1] Amanda Atilano,[1] Xia Wen,[2] Lauren Aleksunes.[2] *[1]Univ of Colorado; [2]Rutgers Univ.*

**Background:** Cisplatin is a chemotherapeutic drug that is known to cause proximal tubule injury. We have demonstrated that the transcription factor nuclear factor e2-like 2 (NRF2, NFE2L2) protects against cisplatin-induced nephrotoxicity in rodents. The purpose of the current investigation was to study the ability of three NRF2 activators, oleanolic acid, sulforaphane, and oltipraz, to protect against the cytotoxicity of cisplatin and to regulate known NRF2 transcriptional pathways in human kidney cells.

**Methods:** Human renal proximal tubule epithelial cells (RPTEC) and HEK293 cell lines were exposed to cisplatin at 0-100 μM and cell viability measured by the MTT assay.Cells were treated with NRF2 activators (5 μM) either pre- or post- exposure (2 hrs) to cisplatin. Cells were harvested, total RNA isolated, and RT-PCR performed to quantify changes in expression of NRF2 itself and prototypical target genes GCLC and NQO1 in RPTECs.

**Results:** Pre-treatment of human kidney cells with NRF2 activators conferred greater protection than post-treatment. Interestingly, pre-treatment of RPTECs with oleanolic acid increased cell viability at high concentrations of cisplatin (30-100 μM) by ~40%. By contrast, HEK293 cells pretreated with oltipraz or sulforaphane had enhanced cell viability (60% and 43%, respectively) at high concentrations of cisplatin (30-100 μM). RPTECs showed reduced NRF2, GCLC, and increased NQO1 mRNA expression after exposure to cisplatin. Significant up-regulation of NRF2, GCLC, and NQO1 mRNA expression with NRF2 activators was observed in RPTECs treated with cisplatin.

**Conclusions:** Treatment of RPTECs with NRF2 activators with exposure to clinically-relevant cisplatin concentrations enhances human renal cell viability and increases expression of detoxifying genes (NQO1 and GCLC) and NRF2 itself.NRF2 activators are potentially novel treatments to mitigate drug-induced kidney injury.

*Funding:* NIDDK Support

## SA-PO081

**Renalase Protects against Ischemic and Toxic Acute Kidney Injury via a Receptor-Mediated Mechanism Independently of Catecholamines Metabolism**

Gary V. Desir,[1] Ling Wang,[1,3] Heino Velazquez,[1] John J. Chang,[1] Ahrom Ham,[2] H. Thomas Lee,[2] Robert L. Safirstein.[1] *[1]Yale, VACHS; [2]Columbia School Med; [3]Renji Hosp.*

**Background:** Acute kidney injury (AKI) is an important clinical syndrome predominantly caused by ischemic and toxic renal insults, and for which effective therapies are currently unavailable. Renalase, a secreted flavoprotein, oxidizes catecholamines, and certain gene polymorphisms are associated with essential hypertension, stroke and diabetes. Gene deletion aggravates acute ischemic kidney (AKI) and cardiac injury. Recombinant renalase prevents ischemic injury in wild type mice. Although renalase's crystal structure has been solved, its mechanism of action remains uncertain.

**Methods:** The effect of renalase and renalase peptides in cell models of cisplatin and hydrogen peroxide toxicity, and in in vivo models of cisplatin and ischemic acute kidney injury were determined. Cell signaling pathways were examined be western blot. Renalase peptides were chosen by examining renalase's crystal structure and by testing the effect of peptide antibodies on renalase function.

**Results:** Renalase increased the survival of human proximal tubular (HK-2) cells exposed to either cisplatin or hydrogen peroxide. We identified a 20 amino acid renalase peptide (RP-220) conserved in all known isoforms, and with no detectable oxidase activity, that was as effective as renalase at protecting HK-2 cells against toxic injury, and wild type mice against ischemic AKI. RP-220 and recombinant renalase rapidly activated AKT, ERK, and p38, and down-regulated. Inhibition of ERK and AKT activation abrogated RP-220's protective effect against ischemic AKI.

**Conclusions:** These data indicate that renalase protects against ischemic AKI independently of its enzymatic properties, and interacts with a yet to be identified receptor to activate intracellular signaling in a manner that promotes cell survival. Renalase and related peptides show potential as therapeutic agents for the prevention and treatment of AKI. We predict that the eventual identification of the renalase receptor will catalyze the development of additional novel therapeutic agents.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO082

**Paricalcitol Attenuates Renal Ischemia-Reperfusion Injury via Prostaglandin E2 Receptor EP4**

Hyeon Seok Hwang,[1] Cheol Whee Park,[2] Yoon-Kyung Chang,[1] Chul Woo Yang,[2] Suk young Kim,[1] Sangju Lee.[1] *[1]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Daejeon, Republic of Korea; [2]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

**Background:** Paricalcitol has protective effects in several kidney injury models but the mechanism by which this occurs remains unclear. We investigated whether paricalcitol regulates the prostaglandin E2 (PGE2) receptor EP4, and whether paricalcitol prevents renal ischemia-reperfusion injury (IRI) through the EP4 in models.

**Methods:** Human proximal tubular cell line (HK-2) was exposed to lipopolysaccharide (LPS) and ischemia by mineral oil monolayer. Male C57BL/6 mice were subjected to 23 min of bilateral kidney ischemia and 24 h reperfusion. The effects paricalcitol pretreatment with or without AH-23848 (EP4 antagonist) was investigated in both *in vitro* and *in vivo models*.

**Results:** Paricalcitol pretreatment significantly increased the cyclooxygenase (COX)-2 expression, PGE2 production and EP4 expression in HK-2 cells. Paricalcitol increased the viability of HK-2 cell exposed to ischemia or LPS. The cotreatment of AH-23848 with paricalcitol offset these protective effects of paricalcitol. LPS-induced p65 NF-κB phosphorylation was abolished in paricalcitol-treated HK-2 cells, and AH-23848 cotreatment blocked its inhibition of p65 NF-κB phosphorylation. In vivo, paricalcitol pretreatment improved serum creatinine levels, tubular necrosis and apoptotic cell death in IRI-mice kidneys. The infiltration of inflammatory cells (T cells and macrophages), and the production of proinflammatory cytokines (RANTES, tumor necrosis factor-α, interleukin-1β and interferon-γ, MIP-1α) were reduced in paricalcitol-treated kidney with IRI. These protective effects of paricalcitol on functional, tubular necrosis, apoptosis and inflammatory infiltration were inhibited by AH-23848 cotreatment.

**Conclusions:** In conclusion, our study demonstrated that paricalcitol attenuates proximal tubular cell death and inflammatory infiltration after renal IRI through the PGE2 receptor EP4.

## SA-PO083

**Vitamin D Deficiency Is a Risk Factor for Iodinated and Gadolinium Contrast Media Nephrotoxicity**

Weverton M. Luchi, Maria H.M. Shimizu, Daniele Canale, Pedro H.F. Gois, Antonio C. Seguro. *Nephrology, Univ of São Paulo - School of Medicine, Brazil.*

**Background:** Vitamin D deficiency(VDD) is widespread in general population. Iodinated(IC) and Gadolinium(Gd) contrasts may decrease renal function in high-risk patients.The aim of this study was to evaluate the effect of VDD on IC and Gd nephrotoxicity.

**Methods:** Male Wistar rats of 3-week-old were fed standard or vitamin D-free diet for 30 days. IC(Diatrizoate,6mL/kgBW) or Gd(Gadoterate 1.5mmol/kgBW) were given i.v. Six groups(n=12/group) were studied: Sham,IC,Gd,$VDD_{30}$,$VDD_{30}$+IC,$VDD_{30}$+Gd. Urinary thiobarbituric acid reactive substances were assayed(TBARS, oxidative stress marker). Inulin clearance(GFR),blood pressure(BP), renal vascular resistance(RVR) were measured 48h after contrast infusion. Renal tissue was immunoblotted for angiotensin II(AII) and endothelial nitric oxide synthase(eNOS).Data are mean±SEM.

**Results:** Serum 25(OH)D(ng/mL) was 15.4±1.0 in Sham and 4.1±0.8 in $VDD_{30}$ GFR was normal in IC and Gd. $VDD_{30}$ increased BP, RVR, TBARS, AII and eNOS. $VDD_{30}$+IC and $VDD_{30}$+Gd showed higher TBARS and AII and reduced eNOS, decreasing GFR.

| | GFR(mL/min/100gBW) | BP(mmHg) | RVR(mmHg/mL/min) | TBARS(nmol/24h) | AII(%) | eNOS(%) |
|---|---|---|---|---|---|---|
| Sham | 0.94±0.04 | 114±2 | 20.5±0.3 | 110±11 | 97±3 | 99±3 |
| IC | 0.91±0.03 | 121±2 | 21.5±0.4 | 152±8 | 120±6 | 156±15[b] |
| Gd | 0.92±0.03 | 116±2 | 21.1±0.3 | 168±24 | 149±16 | 125±9[a] |
| $VDD_{30}$ | 0.84±0.01[a] | 132±2[b] | 23.8±0.3[b] | 184±26[a] | 124±9[a] | 141±5[b] |
| $VDD_{30}$+IC | 0.64±0.03[eh] | 131±2[d] | 23.8±0.3[c] | 303±52[di] | 186±20[dh] | 92±5[eh] |
| $VDD_{30}$+Gd | 0.60±0.03[eh] | 132±2[e] | 24.1±0.4[e] | 310±42[fi] | 178±21[i] | 95±11[gh] |

[a]p<0.05,[b]p<0.001 vs.Sham;[c]p<0.001,[d]p<0.01 vs.IC;[e]p<0.001,[f]p<0.01,[g]p<0.05 vs.Gd;[h]p<0.001,[i]p<0.05 vs.$VDD_{30}$ BW was similar among 6 groups.

In 2 additional groups(n=5) on VDD for 60 days, a greater fall of GFR occurred:$VDD_{60}$+IC=0.48±0.04;$VDD_{60}$+Gd=0.46±0.04.

**Conclusions:** Our findings suggest that VDD is a risk factor for contrast nephropathy by oxidative stress and endothelial dysfunction.(CNPq, CAPES).

## SA-PO084

**Critical Role of Vitamin D Receptor Downregulation on Triggering the Inflammatory Process and Subsequent Fibrosis Formation in Vitamin D-Depleted Rats Submitted to Renal Ischemia/Reperfusion**

Janaina Garcia Gonçalves, Ana C. de Bragança, Daniele Canale, Maria H.M. Shimizu, Antonio C. Seguro, Lucia Andrade, Rosa M.A. Moyses, Rildo A. Volpini. *Nephrology, Faculty of Medicine - Univ of Sao Paulo, Sao Paulo, Brazil.*

**Background:** Renal diseases are generally followed by Vitamin D deficiency (VDD) which increases with the progression of CKD. Studies suggest that Vitamin D Receptor (VDR) could be a potential mechanism connecting inflammation and fibrosis development. We investigated the VDR expression and its relationship with TGF-ß, macrophages infiltration and fibronectin/collagen expression in VDD rats submitted to renal ischemia/reperfusion (IR).

**Methods:** For 90 days, male Wistar rats were fed a standard diet (control [C] and IR groups) or a 25(OH)D-free diet (VDD and VDD+IR groups). On day 28, IR and VDD+IR rats were submitted to 45-min clamping of both arteries. On day 90, we measured serum levels of 25(OH)D and PTH, immunoblotted for VDR/TGF-ß and performed IHC for collagen IV, fibronectin and ED1. We estimated fibrosis by fractional interstitial area (FIA) and collagen IV/fibronectin expression by escore (ranging from 0 to 4). Data are expressed as mean±SEM.

**Results:**

| Variable | C | VDD | IR | VDD+IR |
|---|---|---|---|---|
| 25(OH)D (ng/mL) | 15.4±1.0 | <1.5* | 15.0±0.6 | <1.5* |
| PTH (pg/mL) | 318.8±59.28 | 1470±628.3 | 453.2±95.92 | 2187±336.3[a,c] |
| VDR (%) | 100.0±10.11 | 14.12±1.98[a,c] | 103.6±11.80[b] | 7.31±1.35[a,b,c] |
| TGF-ß (%) | 100.0±4.49 | 146.8±33.16[a] | 152.4±49.23[a] | 252.2±64.52[a,b,c] |
| Collagen IV (score) | 1.01±0.10 | 1.34±0.04[a] | 1.83±0.15[a,b] | 2.04±0.09[a,b] |
| Fibronectin (score) | 0.31±0.03 | 0.67±0.03[a] | 0.68±0.05[a] | 0.89±0.04[a,b,c] |
| ED1 +cells (+cells per field) | 1.14±0.19 | 2.18±0.14 | 10.92±1.89[a,b] | 16.93±2.49[a,b,c] |
| FIA (%) | 7.3±0.5 | 17.2±0.8[a] | 24.4±2.9[a,b] | 34.9±0.55[a,b,c] |

*undetectable; [a]p<0.05 vs. C; [b]p<0.05 vs. VDD; [c]p<0.05 vs. VDD+IR.

**Conclusions:** Taken together, these data indicate that VDD combined with VDR downregulation may contribute to progressive chronic kidney disease.

*Funding:* Government Support - Non-U.S.

## SA-PO085

**Klotho Deficiency Aggravates Sepsis-Related Multiple Organ Dysfunction**

Lectícia Jorge,[1] Fernanda O. Coelho,[1] Talita R. Sanches,[1] Maria Irigoyen,[1] Maria H.M. Shimizu,[1] Antonio C. Seguro,[1] Niels O.S. Camara,[1] Makoto Kuroo,[2] Lucia Andrade.[1] *[1]Univ of Sao Paulo, Brazil; [2]UT Southwestern Medical Center at Dallas.*

**Background:** Sepsis-induced organ failure is characterized by a massive inflammatory response and oxidative stress. Klotho (Kl) has been shown to protect against renal ischemia-reperfusion injury and to be an antioxidant. We used a cecal ligation and puncture (CLP) model to analyze the role of Kl in sepsis-related organ dysfunction.

**Methods:** 8-12 week old male $Kl^{+/-}$ and $Kl^{+/+}$ mice (n=32) underwent CLP or sham operation. At 24 h post-CLP, we evaluated mean arterial pressure(MAP), baroreflex sensitivity, serum biochemistry, oxidative stress, and cytokines, as well as immunoblotting for Kl in kidney tissue. We also evaluated 14-day survival.

**Results:** Data are mean±SEM.

| Variable | CLP $Kl^{+/-}$ | CLP $Kl^{+/+}$ | Sham $Kl^{+/-}$ | Sham $Kl^{+/+}$ |
|---|---|---|---|---|
| Urinary Volume(ml/day) | 0.4±0.3[a,b] | 1.1±0.6[c] | 2.6±0.3 | 2.7±0.5 |
| MAP (mmHg) | 114±28[b] | 111±27 | 141±10 | 135±18 |
| Urea(mg/dl) | 77±14[a,b] | 57±4[c] | 39±8 | 40±8 |
| Lactate(mg/dl) | 60±23[a,b] | 31±12[c] | 19±12 | 20±15 |
| ALT(IU/L) | 92±39[a] | 39±12 | 40±19 | 37±11 |
| TBARS(nmol/ml) | 49.8±12.3[a,b] | 24.6±6.8[c] | 5.6±0.9 | 3.6±1.7 |
| Glutathione (µmol/ml) | 1.34±0.1[a,b] | 1.72±0.3 | 2.4±0.3 | 1.9±0.6 |
| IL6(pg/ml) | 18241±18486[a,b] | 138.7±104[c] | 20.3±8.4 | 28±5.9 |
| IL10(pg/ml) | 2495±2411[a,b] | 170±276[c] | 2.5±4 | 1.4±2.6 |
| TNFα(pg/ml) | 83±13.5[a,b] | 11.4±8.4[c] | 3.6±1.8 | 3±0.5 |
| KI(%) | 37±10[a,b] | 65±13[c] | 68±9 | 100±6 |

[a]p<0.05 vs.CLP $Kl^{+/+}$;[b]p<0.05 vs.sham $Kl^{+/-}$;[c]p<0.05 vs.sham $Kl^{+/+}$

Post-CLP baroreflex sensitivity and survival were significantly lower in $Kl^{+/-}$ than $Kl^{+/+}$.

**Conclusions:** Our results suggest that sepsis is a state of acute Kl deficiency. Sepsis induces inflammatory responses and multiple organ dysfunction in a Kl-dependent pathway. A modest decrease in Kl expression in chronic kidney disease can be a risk for systemic inflammation.

*Funding:* Government Support - Non-U.S.

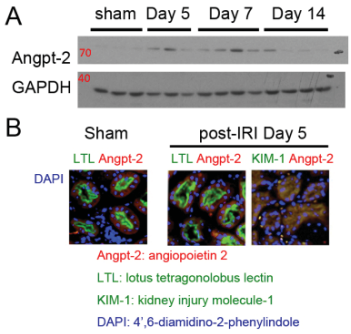
## SA-PO086

**Increased Expression of Angiopoietin-2 in Renal Tubular Epithelial Cells after Ischemia-Reperfusion Acute Kidney Injury** Chun-Fu Lai, Shuei-Liong Lin, Wen-Chih Chiang. *Dept of Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan.*

**Background:** Angiopoietin-2 (Angpt-2) has been identified as a regulator of vascular homeostasis, as well as a link between angiogenesis and inflammation. Clinical studies observed that circulating Angpt-2 levels were elevated in patients experiencing acute kidney injury (AKI). The present study aimed to examine the expression of Angpt-2 in the kidney after ischemic injury.

**Methods:** Using mouse unilateral ischemia-reperfusion-injury (IRI) and contralateral nephrectomy model, we analyzed gene and protein expression of Angpt-2. In addition, we used proximal tubular epithelial (NRK-52E) cells to investigate Angpt-2 expression under hypoxia.

**Results:** In response to the IRI, *Angpt-2* transcripts, measured by Q-PCR, increased in the kidneys significantly since day 5 after surgery and remained high thereafter. Western blot of kidney lysates showed that the expression levels of Angpt-2 protein were enhanced at day 5 and 7, and then decreased toward baseline at day 14. [Fig. A] Immunofluorescence staining indicated that Angpt-2 was expressed mainly in injured and non-injured proximal tubular epithelial cells after IRI. [Fig. B] This was further supported by *in vitro* experiments showing that low-oxygen condition (under 1% $O_2$) stimulated Angpt-2 expression in cultured NRK-52E cells.




**Conclusions:** Renal Angpt-2 expression by tubular epithelial cells is upregulated after IRI. Its role in kidney recovery after injury merits further study.

*Funding:* Government Support - Non-U.S.

## SA-PO087

**Mechanism of Protection by Renalase against Cisplatin-Mediated Cytotoxicity** Gary V. Desir,[1] Ling Wang,[1,2] Heino Velazquez,[1] Robert L. Safirstein.[1] *[1]Yale Univ School Med, VACHS; [2]Renji Hospital, Shanghai.*

**Background:** Acute kidney injury (AKI) is an important clinical syndrome most commonly caused by ischemic and toxic renal insults. Renalase is a secretory flavoprotein that oxidizes catecholamines, and protects against toxic and ischemic injury by its interaction with an as yet unidentified receptor leading to activation of intracellular signaling in a manner that promotes cell survival. A twenty amino acid renalase peptide (RP-220), which is conserved in all known isoforms but is devoid of any detectable oxidase activity is equally effective as intact renalase protein at protecting HK-2 cells and wild type (WT) mice against ischemic injury in a manner that is dependent on extracellular regulated kinase (ERK) and protein kinase B (AKT) activation.

**Methods:** To examine the mechanism of protection against cisplatin cytotoxicity, the effect of the renalase peptide RP-220 on HK-2 cells exposed to cisplatin was determined. Cell signaling pathways were examined by western blot, and chemical inhibition was used to determine the key pathways underlying renalase's protective effect.

**Results:** RP-220 increased the survival of cisplatin treated HK-2 cells by 45% at 24 hrs (n= 4, p<0.002). It rapidly increased the phosphorylation of AKT, p38 mitogen activated protein kinase (p38) and ERK. Chemical inhibition was used to determine the critical mediator of RP-220's protective effect. In control studies in the absence of cisplatin and RP-220, ERK and p38 inhibition had no effect on HK-2 cell survival rates, while AKT decreased cell survival by 37% (n=4, p<0.001) . In the presence of cisplatin alone, inhibition of ERK and p38, were protective and increased cell survival by 38 and 35 % respectively (n=4, p<0.01), while that of AKT was not. With the addition of cisplatin and RP-220, ERK inhibition was still protective, but p38 inhibition completed abrogated RP-220's protective effect.

**Conclusions:** These data indicate that p38 activation is the critical mediator of RP-220's protective action against cisplatin cytotoxicity, and suggest that renalase and related peptides show potential as therapeutic agents for the prevention and treatment of AKI.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO088

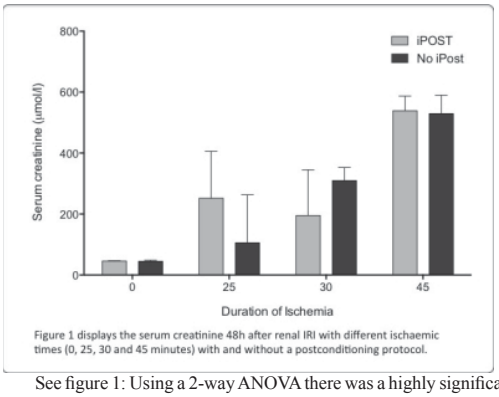
**Post-Conditioning the Kidney – A Cautionary Tale…** Kieran McCafferty, Conor J. Byrne, Magdi Yaqoob. *Dept of Translational Medicine, William Harvey Research Institute, Queen Mary Univ London, London, United Kingdom.*

**Background:** We have previously shown (Byrne/McCafferty, Circulation, 2012) that ischemic postconditioning (iPOST) is a potent cytoprotective strategy in the uremic heart. Others have shown that iPOST can confer cytoprotection in other organs including the kidney. Because iPOST can be applied at the point of reperfusion, it has led to increasing interest in the field of transplantation to reduce reperfusion injury and improve graft function. However, iPOST may be detrimental if the index injury is low, or may be ineffective at reducing reperfusion injury if the injury too great.

The role of the duration of ischemia on the effectiveness of iPOST in the setting of renal IRI is currently unclear.

**Methods:** 56 Male Wistar rats underwent a unilateral nephrectomy with contralateral renal ischemia (for 0, 25, 30 or 45 minutes) followed by either an iPOST procedure (5x10 sec ischemia/reperfusion) or a sham procedure. All animals were left to recover for 48h at which point they were sacrificed and assessed for renal injury.

**Results:**




See figure 1: Using a 2-way ANOVA there was a highly significant association between the duration of ischemia and the serum creatinine concentration (p<0.0001), however surprisingly, at all time points studied the addition of iPOST did not alter serum creatinine concentrations (p=0.63). In addition, other markers of renal injury showed similar trends, with a longer ischemic phase leading to higher urea, phosphate and potassium concentrations and oliguria, with no impact seen with iPOST.

**Conclusions:** Reassuringly iPOST does not appear to be deleterious even at low levels of injury. However in contrast to other studies, our experiments do not confirm cytoprotection over a spectrum of renal injury with iPOST. Further work is required before translational studies of iPOST in the context of renal transplantation are performed.

## SA-PO089

**Increased Transforming Growth Factor-β (TGFβ) Receptor Type2 (TGFBR2) Expression in Regenerating Proximal Tubule (PT) Cells Occurs by Phosphatidylinositol 3-Kinase (PI3K) Activity Mediated Transcription and Decreased Protein Degradation** Hui Geng,[1] Rongpei Lan,[1] Prajjal Kanti Singha,[1] Pothana Saikumar,[1] Joel M. Weinberg,[2] Manjeri A. Venkatachalam.[1] *[1]U. Texas; [2]U. Michigan.*

**Background:** TGFβ signaling increases after renal ischemia reperfusion injury (IRI), accompanied by enhanced expression of TGFβ and its receptors in regenerating PT. Failure of TGFβ signaling to regress during recovery induces tubule atrophy and fibrosis. PT regeneration in a tissue culture model is accompanied by increased TGFβ signaling and TGFBR2 expression, and to a lesser extent, of TGFBR1.

**Methods:** We studied TGFBR2 regulation in confluent differentiated PT cell cultures induced to regenerate by wounding or subconfluent passage, and localized signaling molecules related to TGFβ after IRI of rat kidneys.

**Results:** Wounding to remove ~90% cell mass or subconfluent passage of confluent PT cell cultures resulted in dedifferentiation and proliferation; and rapidly increased TGFBR2 mRNA, protein and reporter activity from a TGFBR2 promoter-luciferase construct. When concurrently increased PI3K signaling was decreased by adenoviral expression of dominant negative (DN) PI3K subunit p85, pan PI3K inhibitor LY294002 or specific PI3Kα or PI3Kβ inhibitors, TGFBR2 expression and reporter activity were suppressed. PDK1 inhibitor

OSU-03012, Akt inhibitor IV, or adenoviral expression of DN Akt similarly suppressed TGFBR2 expression. Conversely, Cre-Lox PTEN knockout increased Akt activity and TGFBR2 expression. On the other hand, normally high TGFBR2 protein degradation in confluent PT cells was diminished by wounding or subconfluent passage. Lysosomal inhibitors (chloroquine, NH4Cl, E64d/pepstatin A) but not proteasomal inhibitors (MG132, lactacystin) markedly increased TGFBR2 protein in confluent PT cells or PI3K inhibited subconfluent PT cells. *In vivo*, after IRI, PI3K activity assessed by phospho-Akt and phospho-S6 ribosomal protein increased rapidly and persistently in protein extracts and localized to regenerating PT, together with elevated TGFBR2 protein.

**Conclusions:** The data suggest that TGFBR2 becomes increased in regenerating PT due to enhanced PI3K activity dependent transcription as well as diminished protein degradation.

## SA-PO090

**Absence of CLIC4 Potentiates Acute Kidney Injury but Has Little Effect on Recovery** John C. Edwards, Jonathan M. Bruno, Phillip N. Key. *Internal Medicine, St. Louis Univ, St. Louis, MO.*

**Background:** CLIC4 is a member of the Chloride Intracellular Channel family expressed in kidney that has been implicated in angiogenesis and in TGFß signal transduction, both of which are important in the response to acute kidney injury (AKI). Specifically, CLIC4 in keratinocytes has been shown to redistribute to the nucleus and potentiate TGFß signalling. We investigated whether absence of CLIC4 alters the course of AKI.

**Methods:** CLIC4 null (KO) and matched wild type (WT) mice were subjected to folic acid-induced AKI. Functional injury was assessed by serum BUN levels at day 2, 7, and 21. Scarring/fibrosis was assessed by 21 day kidney weight. Activation of TGFß signalling was assessed by western blotting for phosphorlyated Smads 2/3 on day 2 following injury.

**Results:** Absence of CLIC4 increased susceptibility to acute kidney injury (median day 2 BUN 65 and 143 for WT and KO respectively, p=0.021), but did not alter functional recovery from AKI. There was no significant difference in day 21 BUN between WT and KO. Scarring was indirectly assessed by day 21 kidney weight. There was no difference in the relationship between severity of initial injury and final kidney size between the WT and KO mice. Phosphorylation of Smads 2/3 increased following injury and there was no difference in extent of phosphorylation between WT and KO. CLIC4 did not redistribute to the nucleus in tubular cells following acute injury. Kidneys were assessed for anatomical and functional differences that could predispose to AKI. CLIC4 KO mice were found to have increased proteinuria, smaller kidneys per body weight, have fewer glomeruli, and less dense peritubular capillary network than WT controls.

**Conclusions:** Mice lacking CLIC4 show increased susceptibility to folic acid-induced acute kidney injury, perhaps related to the underlying developmental angiogenic defect in these mice including less dense peritubular capillary density and fewer glomeruli. However, we did not find evidence that absence of CLIC4 alters the long term recovery or scarring following injury, indicating that CLIC4 is not a critical non-redundant regulator of the intensity or duration of TGFß signalling in kidney cells following folic acid injury.

*Funding:* Other NIH Support - NHLBI

## SA-PO091

**Combined Treatment of Glucocorticoid with Pioglitazone Enhances Protection against PAN-Induced Nephrosis** Shipra Agrawal,[1] Melinda A. Chanley,[1] Adam J. Guess,[1] Rainer Benndorf,[1,2] William E. Smoyer.[1,2] *[1]Clinical & Translational Research, The Research Institute at Nationwide Childrens Hospital; [2]Dept of Pediatrics, College of Medicine, The Ohio State Univ.*

**Background:** Oral glucocorticoids (GCs) are the primary therapy for nephrotic syndrome (NS), although GCs have serious side effects and are clinically ineffective in ~20% of patients. Notably, the FDA-approved diabetes II drugs, PPARγ agonists have been shown to slow progression of diabetic nephropathy and to partially protect against both puromycin aminonucleoside (PAN)-induced NS in rats and direct podocyte injury. We hypothesized that combination therapy of GCs with PPARγ agonists would improve clinical efficacy by reducing proteinuria in NS.

**Methods:** Proteinuria was induced in male Wistar rats by single PAN injection (50 mg/kg). Treatment groups received low-dose methylprednisolone (MP at 5 mg/kg), high-dose MP (15 mg/kg), pioglitazone (Pio at 10 mg/kg) and combinations of Pio with low- and high-dose MP. Urinary protein/creatinine ratios and serum BUN values were measured. PAN-injured (5 µg/ml) cultured podocytes were also treated with 0.1 and 1 µM Pio, dexamethasone (Dex) or a combination of Pio and Dex and cell viability measured after 3 and 5 days.

**Results:** PAN-induced NS resulted in peak proteinuria on day 11, which was ameliorated by the high-dose MP but not low-dose. Pio alone did not significantly reduce proteinuria. However, low-dose MP in combination with Pio ameliorated proteinuria significantly, similar to the high-dose MP alone. BUN values were also reduced with combination treatment, similar to the high-dose MP at day 9. Moreover, after PAN injury, combination of Pio with Dex tended to restore the viability of cultured podocytes better than either Pio or Dex alone.

**Conclusions:** Combination treatment of Pio with low-dose MP enhances the amelioration of PAN-induced proteinuria and provides similar protection to the high-dose MP. These data suggest that repurposing of Pio as a combination therapy with GCs could increase the clinical efficacy of GCs and/or enable reduced dosing and toxicity of GCs in patients with NS and potentially other glomerular diseases.

## SA-PO092

**A Model of Townes-Brock Syndrome Is Protected from Acute Renal Ischemia Reperfusion Injury** Sara Hirsch,[1] Tarek M. El-Achkar,[2] Michael I. Rauchman.[1,3,4] *[1]Biochemistry, Saint Louis Univ, St. Louis, MO; [2]Medicine, Indiana Univ, Indianapolis, IN; [3]Internal Medicine, Saint Louis Univ, St. Louis, MO; [4]Medicine, Saint Louis Veterans Affairs Medical Center, St. Louis, MO.*

**Background:** Acute kidney injury (AKI) affects 2-10% of hospital patients. Ischemia reperfusion injury (IRI) often contributes to the development of AKI. The cellular and molecular processes that govern injury and recovery from renal IRI are not well understood. Genes that are required for kidney development are upregulated following injury, but their function in IRI is unknown. *Sall1* is a transcription factor required for kidney development. Truncating mutations of *Sall1* cause Townes-Brock Syndrome (TBS). Renal manifestations of TBS include congenital hypoplasia and renal insufficiency.

**Methods:** We tested the response of 8 week old, male *Sall1* mutant mice to IRI. The renal pedicle was occluded for 22 or 30 min. Mice used for this study were: $Sall1^{TBS/+}$ - a model of human TBS expressing truncated Sall1 protein, $Sall1^{GFP/+}$ - a model of haploinsufficiency, $Sall1^{cKO}$ - postnatal homozygous deletion of *Sall1*, and $Sall1^{\Delta SRM/+}$ - where one *Sall1* allele is unable to bind the Nucleosome Remodeling and Deacetylase (NuRD) complex.

**Results:** The serum creatinine (sCr) of $Sall1^{TBS/+}$ mice was significantly lower than wild type (WT) mice 24 hrs after 22 or 30 min of ischemia. In contrast, sCr of other Sall1 mutant mice was not significantly different than WT. $Sall1^{TBS/+}$ mice had lower injury scores, decreased expression of biomarkers Kim-1 and NGAL, and 30% fewer proliferating cells as measured by BrdU incorporation.

**Conclusions:** $Sall1^{TBS/+}$ mice are protected from renal injury within 24 hours of IRI. Neither haploinsufficiency ($Sall1^{GFP/+}$) nor conditional knockout of *Sall1* replicate the protection observed in $Sall1^{TBS/+}$ mice, indicating that *Sall1* loss-of-function is not protective. $Sall1^{\Delta SRM/+}$ mice are also susceptible to IRI, suggesting that the protection observed in $Sall1^{TBS/+}$ mice is not due to mutant Sall1$^{TBS}$ protein interfering with WT Sall1-4 or NuRD function. We propose that $Sall1^{TBS/+}$ mice are protected from renal IRI through a novel, gain-of-function mechanism.

*Funding:* Veterans Affairs Support

## SA-PO093

**Early Intervention Prevents Sepsis-Induced Tubule-Interstitial Fibrosis, Endothelial Dysfunction and Tubular Apoptosis: Role of Coupled Plasma Filtration Adsorption** Giuseppe Castellano,[1] Alessandra Stasi,[1] Anna Maria Di Palma,[1] Margherita Gigante,[1] Angelica Intini,[1] C. Divella,[1] Giuseppe Stefano Netti,[2] Claudia Curci,[3] Clelia Prattichizzo,[2] Enrico Fiaccadori,[4] G. Grandaliano,[2] G. Pertosa,[1] Loreto Gesualdo.[1] *[1]DETO, Univ Bari, Italy; [2]Med and Surg Sciences Dept, Univ Foggia, Italy; [3]Consorzio Carso, Univ Bari, Italy; [4]Clin and Exp Med Dept, Univ Parma, Italy.*

**Background:** Sepsis-induced acute kidney injury(AKI) is characterized by tubular apoptosis and development of tissue fibrosis. Aim of our study was to investigate the effects of an early treatment by Coupled Plasma Filtration Adsorption(CPFA) in a swine model of sepsis-induced AKI.

**Methods:** After LPS infusion, 8 pigs developed AKI and underwent to renal biopsies at different time points. In the treatment group, 8 animals received CPFA for 6 h, after 3 h from LPS infusion. Endothelial cells (ECs) were cultured in presence of different swine sera for 12h and were analyzed by FACS.

**Results:** We found an acute induction of tubule-interstitial fibrosis in Sepsis-induced AKI, as indicated by extensive collagen deposition (Masson Trichrome staining); in addition, tubular apoptosis was an early event as indicated by intense caspase-3 staining. Finally, tubular damage was associated with dysfunction of ECs that expressed myofibroblast marker (α-SMA). Early treatment by CPFA was able to reduce collagen deposits (25.29 ± 8.9 vs. septic 51.54±8.28 p=.04), tubular apoptosis (Caspase-3$^+$ 20.96 ± 2.68 vs. septic 40.44±3.96 p=.004) and α-SMA$^+$ ECs (CD31$^+$/α-SMA$^+$ 4.05±2.19 vs. septic:16.5±3.53 fold change, p=.04). In vitro, EC cultured with septic sera showed reduced expression of specific EC markers (CD31 49±9.5 MFI) and VE-cadherin (11.1±2.3 MFI), with increased expression of markers of EC dysfunction such as N-cadherin and Vimentin. Surprisingly, sera of treated pigs preserved EC phenotype (CD31: 94.97±3.4 p=.04; VE-cadherin: 24.07±4.9 p=.03) without up-regulation of N-cadherin and Vimentin.

**Conclusions:** Our data indicated that an early treatment by CPFA might be pivotal to counteract the acute effects of LPS on renal tissue, by removing the cytokine responsible of EC dysfunction and tubule-interstitial damage.

## SA-PO094

**Protection against $H_2O_2$-Induced Cell Death by Angelica Sinensis and Astragalus Membranaceus in HK2 Human Kidney Cells** Muhammad Shahzad,[1,2] David M. Small,[1] Christudas Morais,[1] Glenda C. Gobe.[1] *[1]Centre Kidney Disease Res Sch Medicine, Univ Queensland, Brisbane, Australia; [2]Dept Pharmacol, Univ Health Sch, Lahore, Pakistan.*

**Background:** Oxidative stress is an important mechanism for renal epithelial cell destruction in several kidney diseases, including nephrotic syndrome. *Angelica* and *Astragalus spp* have a long history of medicinal use in traditional Chinese medicine, including for treating patients with nephrotic syndrome. The aim was to investigate the protection of *Astragalus membranaceus* and >*Angelica sinensis* against $H_2O_2$-induced cell death in HK2 proximal tubular cells.

**Methods:** Ethanol, methanol and aqueous extracts of roots of *Astragalus membranaceus* and *Angelica sinensis* were prepared by a three-solvent sequential process. HK2 cells were treated with 0.6mM of $H_2O_2$ alone and in combination with different concentrations of extracts for 24h. Cell viability was determined by MTT assay. Cell death was monitored microscopically. Expression of apoptosis-related proteins Bax, Bcl-xL, NF-κB (p65, p50), multi-functional TGFβ1 and pro-inflammatory TNFα were studied by Western blots.

**Results:** Ethanol, methanol and aqueous extracts were all protective. $H_2O_2$ reduced cell viability (approximately 50% control values), whereas significant protection against $H_2O_2$-induced cell death (in the forms of apoptosis and necrosis) was obtained with *Angelica sinensis* and *Astragalus membranaceus* extracts (viability restored to nearly 75% of control). $H_2O_2$ caused significant upregulation of pro-apoptotic Bax, NF-κB (p65, p50) and TNFα; and downregulation of anti-apoptotic Bcl-xL and TGFβ1. The extracts significantly decreased Bax, increased Bcl-xL, but had no significant effect on NF-κB isoforms and TNFα. TGFβ1 expression was upregulated. This may be anti-apoptotic, but a pro-fibrotic role for TGFβ1 should be investigated further.

**Conclusions:** The traditional use of these plant extracts in nephrotic syndrome was validated: oxidative stress-induced cell death in HK2 cells was prevented, and modulation of pro-apoptotic Bax and upregulation of anti-apoptotic Bcl-xL and pro-survival TGFβ1 may be the mechanisms of protection.

*Funding:* Government Support - Non-U.S.

## SA-PO095

**Effects of Leucine-Rich Repeat Kinase 2 (LRRK2) Deletion in Normal and Injured Rat Kidneys** Ravindra Boddu,[1] Joao Paulo Lima Daher,[4] Kyoko Kojima,[2] Lisa M. Curtis,[1,3] Anupam Agarwal,[1,3] Andrew B. West.[4] *[1]Medicine; [2]Surgery; [3]Veteran's Administration Medical Center, Birmingham, AL; [4]Neurology, UAB, Birmingham, AL.*

**Background:** Leucine-rich repeat kinase 2 (LRRK2) mutations account for 5-6% of familial Parkinson's diseases (PD) and 1-3% in sporadic PD. LRRK2 knockout (KO) mice do not develop neuropathological changes in the brain.Previous reports suggest that a loss of LRRK2 causes age-dependent accumulation of α-synuclein (60-fold) and ubiquitinated proteins in the kidney, in which LRRK2 is normally expressed at high levels (~6-fold compared to the brain).The role and biology of LRRK2 in the kidney is not well understood. Aged LRRK2 KO mice (~20 months) display impaired activation of autophagy with increased apoptotic cell death, inflammatory responses and oxidative damage. **Objective:**To understand the LRRK2 biology in kidney,we sought to characterize a rat model of LRRK2 deletion and evaluate the phenotype after acute kidney injury (AKI).

**Methods:** We utilized mass spectrometry (MS) and western blots to compare wild-type (WT) and knockout (KO) rat kidneys; and assessed renal function and structure following cisplatin-induced AKI in LRRK2 WT and KO rats. Localization of LRRK2 using immunofluorescence (IF) was also performed.

**Results:** LRRK2 KO kidneys are dark red in color and significantly heavier than WT kidneys, sinking in 10% sucrose solution. IF studies showed localization of LRRK2 in renal tubules. Protein expression for heme oxygenase-1, H and L ferritin and transferrin receptor are significantly decreased in 6 month KO rat kidneys. MS showed increased deposits of hemoglobin and fucosylated glycans (lipofuscin) in 12 month KO kidneys compared to WT kidneys. Following cisplatin-induced AKI, no differences in renal function were noted in young (~2.5 month) LRRK2 KO rats. However,macrophage infiltration was significantly increased in cisplatin-treated LRRK2 KO rats compared to WT rats.

**Conclusions:** The phenotype of the LRRK2 KO animal implicate LRRK2 to have biological significance in the kidney and further studies to dissect the possible role of LRRK2 in older animals will potentially identify a novel pathways for intervention in kidney injury settings.

*Funding:* NIDDK Support

## SA-PO096

**Involvement of Indoxyl Sulfate (IS) in Downregulation of Pulmonary Aquaporin (AQP)-5 in Rats Caused by Bilateral Nephrectomy (BNx)-Induced Acute Kidney Injury (AKI)** Masataka Sagata,[1] Chika Saigo,[1] Go Yoneda,[1] Yuko Yamamoto,[1] Yui Nomura,[1] Kazuhiko Nishi,[3] Hirofumi Jono,[2] Hideyuki Saito.[2] *[1]Dept of Clinical and Pharmaceutical Sciences, Kumamoto Univ, Kumamoto, Japan; [2]Dept of Pharmacy, Kumamoto Univ Hospital, Kumamoto, Japan; [3]Dept of Hemodialysis and Apheresis, Kumamoto Univ Hospital, Kumamoto, Japan.*

**Background:** Despite advances in understanding the pathophysiology, improvements in dialysis and supportive care, the mortality of AKI remains considerably high. The high mortality of AKI is associated with acute lung injury or acute respiratory distress syndrome, which are typical complications of AKI. It is suggested that dysregulation of lung salt and water channels following the AKI plays a pivotal role in ALI, however, the mechanism of dysregulation has not been elucidated. In this study, we examined involvement of a typical oxidative stress-inducing uremic toxin, IS, in dysregulation of pulmonary predominant water channel, AQP-5, in BNx-induced AKI model rats.

**Methods:** AST-120, clinically used oral spherical adsorptive carbon, was orally administered to rats (2.5 g/kg) at -24, -1 h before and 24 h after BNx surgery. Serum was collected at 4 and 48 h following surgery, and IS level was determined by HPLC. AQP-5 protein expression was examined by immunoblot analysis.

**Results:** At 48 h, BNx evoked AKI with the increases in serum creatinine (Scr) (29-fold vs sham-operated rats), BUN (12-fold) and serum IS levels (32-fold) in association with a marked downregulation of pulmonary AQP-5 expression (0.51-fold). Administration of AST-120 resulted in a significant decrease in serum IS level (0.64-fold), which was accompanied with the decreases in IS accumulation in liver and lung. AST-120 treatment had no effects on Scr, BUN and lung Na,K-ATPase expression, whereas the downregulation of AQP-5 was significantly restored (2.2-fold vs BNx rats). At 4 h after BNx surgery, serum interleukin-6 level was not affected by oral AST-120.

**Conclusions:** These results suggested that BNx-induced AKI causes downregulation and/or dysregulation of pulmonary AQP-5 expression, in which IS could play a toxico-physiological role as a mediator involved in renopulmonary crosstalk.

*Funding:* Government Support - Non-U.S.

## SA-PO097

**Pretreatment with Saline Loading Ameliorates Ischemic Acute Kidney Injury (AKI), and Reduces Intrarenal Upregulation of (Pro)renin Receptor in Rats** Masafumi Ono,[1] Yukitoshi Sakao,[2] Takayuki Tsuji,[1] Naro Ohashi,[1] Hideo Yasuda,[1] Yoshihide Fujigaki,[1,3] Akihiko Kato.[2] *[1]Internal Medicine 1, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan; [2]Blood Purification Unit, Hamamatsu Univ Hospital, Hamamatsu, Shizuoka, Japan; [3]Dept of Internal Medicine, Teikyo Univ School of Medicine, Tokyo, Japan.*

**Background:** (Pro)renin receptor (PRR), a trans-membrane receptor for renin and prorenin, is involved in the local activation of renin-angiotensin system (RAS) in the kidney. PRR also directly stimulates intracellular signaling pathway such as MAPK. However, it remains to be determined whether intrarenal PRR play a role in the development of AKI.

**Methods:** Male SD rats were subjected to left renal arterial clamping for 45 min just after right nephrectomy. We assessed the renal expression of PRR, renin/prorenin, angiotensinogen (AGT), and phospho-ERK1/2 protein by western blot and immunohistochemistry at 6, 24, 48 hr and 7 days after the injury. We measured intrarenal angiotensin II (Ang II) level by RIA. We also examined whether pretreatment with oral loading of saline solution (1.0% NaCl) for 48 hr just before AKI induction modifies intrarenal RAS components and MAPK system in the ischemic kidney.

**Results:** Serum creatinine (Scr) increased maximally at 48 hr after the reperfusion. Pretreatment with saline loading significantly decreased the peak of Scr (5.36±1.26 vs. 3.38±1.74 mg/dL, $P<0.05$). Renal ischemia increased the abundance of PRR protein at 48 hr, and then gradually decreased at day 7. PRR staining was mainly increased in the distal and cortical collecting ducts. There was a significant increase in renal AGT at 24 hr, while renal renin/prorenin remained at same levels. Phospho-ERK1/2 was upregulated at 6-24 hr after ischemia. Prior saline drinking suppressed the increment of PRR, AGT and phospho-ERK1/2 protein.

**Conclusions:** Renal ischemia increased the abundance of PRR concomitantly with AGT and phospho-ERK1/2. Saline loading mitigated ischemic injury, and prevented the increment of renal PRR abundance. These findings suggest that saline loading ameliorated ischemic AKI, mediating possibly through the suppression of RAS and MAPK systems via PRR downregulation.

## SA-PO098

**A Mouse Model of Type 1 Hepatorenal Syndrome (HRS)** Daniel E. Carl,[1] Siddhartha S. Ghosh,[1] Todd W. Gehr.[1] *[1]Internal Medicine/Nephrology, Virginia Commonwealth Univ, Richmond, VA; [2]Internal Medicine, Hepatology, Virginia Commonwealth Univ, Richmond, VA.*

**Background:** Type 1 HRS is a life threatening complication of cirrhosis with limited therapeutic options. The lack of a mouse model of HRS is a major gap in the field as it hinders molecular analysis of HRS and identification of therapeutic targets. The aim of this study was to develop a mouse model of type 1 HRS.

**Methods:** C57bl mice were administered CCl4 (1 ml/Kg) for 6-12 weeks to produce cirrhosis. Varying doses of lipopolysaccharide (LPS) were then administered intraperitoneally to identify a sublethal dose of LPS to induce AKI and a dose to cause 50% mortality. The rationale for LPS was the frequent development of HRS following an infection. These mice were compared to: (1) normal mice, (2) normal mice+LPS, and (3) mice treated with CCl4 alone. Animals were maintained in metabolic cages and urine obtained for analysis. Echocardiography was used to determine cardiac output.

**Results:** Mice developed bridging fibrosis with cirrhotic nodule formation after 6 weeks of CCl4. Hyperdynamic circulatory dysfunction (statistically significant increase in cardiac output and drop in systemic venous resistance) developed after 12 weeks of CCl4. Within 16 hours of a sublethal dose of LPS (2mg/Kg), 12 week CCl4-treated mice experienced a drop in urine volume and rise in creatinine. The urine Na decreased and there were no casts or proteinuria. Control mice treated with 2 mg/Kg LPS did not experience AKI.

| | Vehicle | LPS alone | CCl4 alone | **CCl4+LPS** |
|---|---|---|---|---|
| Urine volume(ml) | 1.33±0.6 | 1.28±0.4 | 1.7±0.7 | **0.84±0.4** |
| SCr(mg/dL) | 0.18±0.03 | 0.2±0.04 | 0.4±.1 | **0.8±0.2** |
| UNa(mmol/hr) | 140±57 | 115±34 | 137±37 | **46±12** |

Renal histology lacked glomerular or tubular pathology. A 6mg/Kg LPS dose led to 50% mortality rate in 12 week CCl4-treated mice; with no fatalities in controls+6mg/Kg LPS.

**Conclusions:** We describe a viable mouse model of type 1 HRS. Mice treated with 12 weeks of CCl4 develop histology and hemodynamics consistent with cirrhosis. Furthermore, cirrhotic mice develop AKI following LPS consistent with HRS.

**SA-PO099**

**Osmotic Nephrosis and Contrast-Induced Acute Kidney Injury Are Prevented by the RIPK1-Inhibitor Necrostatin-1 in Mice**
Andreas Linkermann,[1] Jan O. Heller,[1] Agnes Prokai,[2] Joel M. Weinberg,[3] Nina Himmerkus,[4] Attila Szabo,[2] Jan Hinrich Braesen,[5] Ulrich Kunzendorf,[1] Stefan Krautwald.[1] *[1]Clinic for Nephrology and Hypertension, Christian-Albrechts-Univ, Kiel, Schleswig-Holstein, Germany; [2]First Dept of Paediatrics, Semmelweis Univ, Budapest, Hungary; [3]Univ of Michigan, Div Univ of Michigan Medical Center, Ann Arbor, MI; [4]Institute for Physiology, Christian-Albrechts-Univ, Kiel, Schleswig-Holstein, Germany; [5]Institute for Diagnostic Histopathology and Cytopathology, Pathology Hamburg West, Hamburg, Germany.*

**Background:** The pathophysiology of contrast-induced acute kidney injury (CIAKI) is incompletely understood due to the lack of an appropriate *in vivo model that includes reduced kidney function before administration of radiocontrast media (RCM).*

**Methods:** We introduce a murine ischemia/reperfusion-injury (IRI)-based approach that allows induction of CIAKI by a single application of standard RCM. Whereas murine tubular cells and freshly isolated tubules rapidly absorbed RCM, plasma membrane integrity and cell viability remained preserved *in vitro* and *ex vivo* indicating that RCM do not induce apoptosis or regulated necrosis of tubular cells.

**Results:** In vivo, the IRI-based CIAKI model exhibited typical criteria of clinical CIAKI, like RCM-induced osmotic nephrosis and an increase in serum levels of urea and creatinine. Direct evaluation of renal morphology by intravital microscopy (IVM) following RCM-application revealed a brief increase in trans-tubular diameters in renal tubules and an initial dilation of peritubular capillaries within the first 20 minutes which was observed to a lesser extent also upon IRI-pre-treatment.

**Conclusions:** Mechanistically, necrostatin-1 (Nec-1), a highly specific inhibitor of the kinase domain of the receptor-interacting protein kinase 1 (RIP1) prevented osmotic nephrosis and CIAKI in vivo whereas an inactive Nec-1-derivate (Nec-1i) or the pan-caspase-inhibitor zVAD did not. Additionally, Nec-1 completely prevented the effects of RCM on peritubular capillaries, suggesting a previously unrecognized role of the RIP1-kinase domain in the pathophysiology of CIAKI and regulation of microvascular hemodynamics.

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**SA-PO100**

**Crosstalk between Interstitial Capillary and Tubular Injuries May Mediate Impaired Renal Microcirculation in Hepatic Acute Kidney Injury in Rats**
Akira Shimizu, Kayori Tsuruoka, Seiichiro Higo, Go Kanzaki, Yusuke Kajimoto, Megumi Fukui, Emiko Fujita, Shinya Nagasaka, Akiko Mii. *Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

**Background:** Hepatic acute kidney injury (hepatic AKI) is a common complication in the acute liver dysfunction. In AKI, impaired renal microcirculation is frequently evident with renal peritubular capillary (PTC) injuries. In the present study, we examined the crosstalk between PTC endothelial and tubular injuries in renal microcirculatory disturbance in hepatic AKI.

**Methods:** Acute hepatic failure was induced in rat by liver transplantation from DA (RT1a) to Lewis (RT1l) rats without immunosuppression. Acute rejection progressed, and rats were dead around day 11 with severe acute liver dysfunction. We examined the clinical and laboratory data and pathological characteristics of kidneys by day 11, focusing on the PTC and tubular injuries.

**Results:** During the development of liver graft rejection, acute liver dysfunction (T-Bil 8.9 ± 1.9; AST 844 ± 141 U/l, $p<0.01$) and acute kidney injury (BUN 130 ± 29.3 mg/dl; Cr 1.2 ± 0.2 mg/dl, $p<0.01$) were developed by day 11. During the progression of AKI, renal interstitial PTC injuries developed with decreased expression of eNOS on endothelial cells and reduction of PTC blood flow in vivo (320±122 μm/sec at day 11 vs 860±145 μm/sec in control, $p<0.001$). Renal interstitial microvasculature is controlled by the angiogenic factors secreted from tubular epithelial cells, such as vascular endothelial growth factor (VEGF), angiopoietin-1 (ang-1), and ang-2, and the expression of VEGF, ang-1, and ang-2 was gradually decreased in the kidney by day 11. Renal tubular degeneration with bile pigment accumulation, mitochondrial degeneration was also noted by day 11 with interstitial inflammation.

**Conclusions:** AKI developed in rats in acute liver dysfunction. The crosstalk between interstitial PTC endothelial and tubular injuries may mediate augmentation of impaired renal microcirculation in hepatic AKI.

*Funding:* Private Foundation Support

**SA-PO101**

**Radiocontrast Nephropathy: Role of Endothelial Injury, and Chronic Consequences**
Gunnar Schley,[1] Nada Cordasic,[1] Bernd Klanke,[1] Samuel N. Heyman,[2] Seymour Rosen,[3] Kai-Uwe Eckardt,[1] Carsten Willam.[1] *[1]Nephrology and Hypertension, Univ Hospital Erlangen, Erlangen, Germany; [2]Medicine, Hadassah Hebrew Univ Hospital, Jerusalem, Israel; [3]Pathology, Beth Israel Deaconess Medical Center, Boston, MA.*

**Background:** Contrast nephropathy (CIN) is one of the leading causes of in-hospital acute kidney injury. The classic rat model requires both inhibition of prostaglandin and nitric oxide synthesis followed by contrast medium (CM) administration. In this study, the pathophysiology of CIN is examined in a mice model, whose conditioning depends only on dehydration with subsequent CM administration.

**Methods:** CIN was induced in male mice by sodium iothalamate injection (3 g iodine/kg body weight) after 3 days of water deprivation producing an average weight loss of 20% without any obvious behavioral problems. Renal function and histology were determined 1 (N=28) and 14 days after CM (N=16).

**Results:** After 24 hours, 70% of mice went into renal failure and plasma creatinine rose from 0.09±0.02 to 0.95±0.22 mg/dl ($p<0.001$). The injury pattern at 24 hours was mainly located in the inner stripe of the outer medulla and consisted of capillary congestion, erythrocyte extravasation and necrosis, all of which correlated with the degree of renal failure ($R^2 = 0.85$, $p<0.001$). There was a clear gradient of injury, with the most severe damage in the inter-bundle zone. With progressive degrees of damage, there was increasing loss of endothelial immunohistochemical markers (MECA-32, CD34). Apoptosis of endothelial and tubular epithelial cells could be demonstrated using TUNEL stainings, and apoptotic changes were seen in the vasa recta. 14 days after the acute event, 25% of surviving mice still had renal failure. The injury in the inner stripe of the outer medulla now was chronic and the cortex then showed extensive chronic changes as well, reflecting injury of medullary thick ascending limbs with the loss of their associated convoluted tubules.

**Conclusions:** This novel mouse model underscores a role for medullary endothelial cell injury in the pathogenesis of CIN and shows that the long-term consequences of acute medullary injury are reflected by chronic changes in both the cortex and medulla.

*Funding:* Government Support - Non-U.S.

**SA-PO102**

**Iodinated Contrast Media Induce Tubular Necrosis and Dysfunction in Isolated Thick Ascending Limb of Rat**
Zhi Zhao Liu,[1] Kristin Schmerbach,[1] Yan Lu,[2] Erdmann Seeliger,[1] Kathleen Cantow,[1] Andreas Patzak,[1] Ruisheng Liu,[2] Mauricio Michalak Sendeski.[1] *[1]Institut für Vegetative Physiologie, Charite-Universitaetsmedizin Berlin, Berlin, Germany; [2]Dept of Physiology and Biophysics, Univ of Mississippi Medical Center, Jackson, MS.*

**Background:** The prevalence of contrast induced acute kidney injury (CI-AKI) is still increasing. In this study we test the hypothesis that contrast media induce tubular cell death accompanied with oxidative stress and reducing nitric oxide bioavailability.

**Methods:** Isolated thick ascending limbs (TALs) from male Sprague-Dawley rats were perfused with either vehicle solution or iodixanol. Propidium iodide was used to estimate the cell death rate. Superoxide concentration and nitric oxide (NO) bioavailability were quantified by the fluorescent methods. Further, the expression of oxidative stress related genes and the activity of superoxide dismutase (SOD) in tubuli were investigated.

**Results:** Propidium iodide intensity was significantly enhanced by iodixanol (n=8) compared to the control group (n=6) within 20min. Moreover, during total 4-hour perfusion, iodixanol increased fluorescence intensity to 17.7±3.1%, compared to control group (7.0±1.0%) in TALs. Iodixanol increased dehydroethidium fluorescence ratio, a measure for superoxide concentration (9.6±1.4%, n=7), compared to control group (1.7±1.0%, n=8) Tempol, a superoxide dismutase mimic, inhibited the increase. DAF-FM fluorescence, a measure for NO, decreased during iodixanol treatment (-0.6±0.6%, n=7) and by NO synthase inhibitor L-NAME (control: 1.6±0.3%, n=7) within 12min. Only two of 84 investigated genes related to oxidative stress were differentially expressed in the TALs. The SOD activity did not differ between treated and untreated animals.

**Conclusions:** The study suggests that tubular cell damage and accompanying oxidative stress in our model are consequences of contrast media cytotoxicity. Further, oxidative stress and cell damage may impair NO bioavailability.

**SA-PO103**

**Angiotensin III/Angiotensin Type 2 Receptor Axis Inhibits Hyperglycemia-Induced Fibronectin Synthesis through Inhibition of NADPH Oxidase Nox4 in the Kidney**
Robert T. Day, Rita de Cassia Cavaglieri, Khaled Khazim, Yves C. Gorin, Denis Feliers. *Medicine, Univ of Texas Health Science Center at San Antonio, San Antonio, TX.*

**Background:** Angiotensin II, a critical mediator of diabetic nephropathy, exerts its deleterious effects through AT1R. AT2R generally opposes the effects of AT1R, and has antifibrotic properties. Recent studies suggest that Ang III, and not Ang II, could be the physiologic ligand for AT2R.

**Methods:** Cultured rat mesangial cells (MCs) were exposed to high glucose (HG, 25 mM) ± Ang III and expression of fibronectin was measured. For in vivo studies, extracellular matrix and fibronectin deposition were compared in AT2R knockout mice (AT2R-KO) with type 1 diabetes and their wildtype counterparts .

**Results:** In MCs, HG increased fibronectin protein expression at 1 h and 24 h. This was prevented by co-incubation with a selective AT2R agonist or Ang III but potentiated by coincubation with Ang II. Inhibitory effect of Ang III was mediated by AT2R, but potentiating effect of Ang II was mediated by AT1R. Nox4-derived reactive oxygen species is required for HG-mediated oxidative stress and MC fibrotic injury. Ang III prevented rapid increase in Nox4 expression and NADPH-dependent superoxide generation induced by HG. Modelization of Nox4 mRNA 5' untranslated region suggested that Nox4 mRNA is readily translated. Polysome analysis showed that Ang III inhibited HG-induced translation of Nox4 mRNA. Ang III activated AMPK through AT2R in MCs and inhibition of AMPK prevented the inhibitory effect of Ang III on Nox4 expression and fibronectin synthesis.

In diabetic AT2R-wt mice as well as normoglycemic AT2R-KO mice, extracellular matrix expansion (including fibronectin) correlated with increased Nox4 expression and ROS generation, as well as with decreased Ang III and aminopeptidase-A expression.

**Conclusions:** Ang III activates AT2R to limit fibronectin accumulation in response to HG via AMPK-dependent inhibition of Nox4 mRNA translation. AT2R activators such as Ang III may become viable therapeutic interventions to complement traditional therapy to treat diabetic nephropathy.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO104

**Angiotensin 1-7 Prevents Systemic Hypertension and Ameliorates Kidney Injury via Inhibition of Oxidative Stress in Diabetic Akita Mouse Kidneys**

Yixuan Shi,[1] Chao-Sheng Lo,[1] Isabelle Chenier,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] Shao-Ling Zhang,[1] John S.D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hosp., Montreal, Canada; [2]Res. Ctr., Maisonneuve-Rosemont Hosp., Montreal, Canada; [3]Pediatr Nephrol Unit, Mass. Gen. Hosp. for Children, Boston, MA.*

**Background:** We investigated whether Ang 1-7 administration could affect systemic hypertension and ameliorate kidney injury via inhibition of oxidative stress in Akita mice with type 1 diabetes.

**Methods:** Ang 1-7 was administered daily (500 μg/kg body weight (BW)/day, subcutaneously) to male Akita mice starting at 14 weeks of age with or without co-administration of A779 (an antagonist of the Mas receptor) (10 mg/kg BW/day). The animals were euthanized at 18 weeks of age. Wild type (WT) mice of the same age served as controls. Blood glucose (BG), systolic blood pressure (SBP) and albumin/creatinine ratio (ACR) were monitored weekly.Kidneys were processed for histology including dihydroethidium (DHE) staining and immunostaining of heme oxygenase 1 (HO-1), WT-1 (podocytes marker), nephrin, transforming growth factor-beta 1 (TGF-β1), collagen IV and Ace2. Proximal tubule expression of TGF-β1, Collagen IV and Ace2 mRNA were evaluated by real-time qPCR; and expression of their proteins was evaluated by Western blotting. Glomerular tuft volume and tubular luminal area were assessed morphometrically.

**Results:** Ang 1-7 administration prevented the increase of SBP and normalized urinary ACR without affecting BG levels in Akita mice. Akita kidneys exhibited significant increase in DHE staining and immunostaining for HO-1, TGF-β1 and collagen IV, whereas WT-1, nephrin and Ace2 immunostaining were decreased. These changes were normalized with Ang 1-7 administration, and the effects of Ang 1-7 were reversed by A-779. Finally, Ang 1-7 administration decreased glomerular tuft volume and tubular luminal area in Akita mice, and these changes were also reversed by A779.

**Conclusions:** Our data suggest that intrarenal Ang 1-7 plays a protective role by attenuating SBP and RPTC injury in diabetes, predominantly through decreasing renal oxidative stress-mediated signaling.

*Funding:* Government Support - Non-U.S.

## SA-PO105

**Indoxyl Sulfate Induces Angiotensinogen Expression in Proximal Tubular Cells through Upregulation of CREB, NF-kB and NOX4**

Toshimitsu Niwa,[1] Hidehisa Shimizu,[1] Shinichi Saito,[1] Fuyuhiko Nishijima.[2] *[1]Nagoya Univ Graduate School of Medicine; [2]Biomedical Research Laboratories, Kureha Co.*

**Background:** In chronic kidney disease (CKD), indoxyl sulfate, a uremic toxin, accumulates in serum, and the expression of angiotensinogen (AGT) is upregulated in renal proximal tubular cells. The present study aimed to determine the relationship between indoxyl sulfate and the upregulation of AGT expression in proximal tubular cells.

**Methods:** For in vitro experiment, HK-2 cells derived from human proximal tubular cells were incubated with indoxyl sulfate (250 mM) . For in vivo experiment, the rat groups consisted of: (1) Dahl salt-resistant normotensive rats (DN, n=8), (2) Dahl salt-resistant normotensive indoxyl sulfate-administered rats (DN+IS, n=8). Indoxyl sulfate (200 mg/kg/day in drinking water) was administered to the rats for 32 weeks.

**Results:** Indoxyl sulfate induced expression of AGT in rat renal cortex and in cultured human proximal tubular cells (HK-2). In proximal tubular cells, indoxyl sulfate induced phosphorylation of cAMP response element-binding protein (CREB) on Ser-133, and small interfering RNA (siRNA) specific to CREB inhibited indoxyl sulfate-induced AGT expression. Our previous study demonstrated that indoxyl sulfate activated nuclear factor-kB (NF-kB) through reactive oxygen species (ROS) production. NF-kB inhibitors (pyrrolidine dithiocarbamate and isohelenin), NF-kB p65 siRNA, an antioxidant (N-acetylcysteine; NAC), and a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor (diphenyleneiodonum; DPI) suppressed indoxyl sulfate-induced AGT expression. Both NAC and DPI suppressed indoxyl sulfate-induced expression of NF-kB p65 and CREB. CREB siRNA suppressed indoxyl sulfate-induced NF-kB p65 expression, whereas both NF-kB inhibitors and NF-kB p65 siRNA prevented indoxyl sulfate-induced CREB expression. Indoxyl sulfate induced the expression of NADPH oxidase 4 (NOX4) in proximal tubular cells, which was suppressed by NAC, DPI, NF-kB inhibitors, NF-kB p65 siRNA, and CREB siRNA.

**Conclusions:** Indoxyl sulfate induces angiotensinogen expression in proximal tubular cells through upregulation of CREB, NF-kB and NOX4.

## SA-PO106

**Reactive Oxygen Species Contribute to Angiotensin II Activation of TRPC6 Channels in Rat Primary Podocytes in Isolated Glomeruli**

Stuart E. Dryer,[1,2] Marc Thomas Anderson.[1] *[1]Biology and Biochemistry, Univ of Houston, Houston, TX; [2]Div of Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** Angiotensin II (Ang II) evokes $Ca^{2+}$ influx into podocytes, but many aspects of its transduction pathway are unknown in this cell type. Some recent analyses have been carried out on immortalized podocytes heterologously over-expressing Ang II receptors, which runs the risk of promiscuous activation of non-physiological transduction cascades.

**Methods:** Whole-cell recordings from primary rat podocytes on the surface of glomeruli isolated by a sieving procedure. The podocytes were still attached to the glomerular capillary.

**Results:** Application of Ang II evoked inward currents with current-voltage characteristics of TRPC6. Robust responses were seen at [Ang II] = 1 nM, half-maximal activation occurred at [Ang II] =10 nM, and responses desensitized rapidly at [Ang II] = 1 μM. Ang II activation of cationic currents was blocked by losartan. Ang II-evoked currents were also inhibited completely by SKF-96365, by 50 μM $La^{3+}$, and by TRPC6 knockdown with siRNA, suggesting all of the Ang II-modulated cationic currents contain TRPC6 subunits. Responses to Ang II were completely blocked by including GDP-βS in recording pipette, and by the PLC inhibitor D-609, but not by the PKC inhibitor chelerythrine. Responses to hypoosmotic stretch persisted under those conditions. Effects of Ang II on TRPC6 were reduced by the NOX2 inhibitors apocynin and diphenylene iodonium and also by the reactive oxygen species (ROS) quencher MnTBAP.

**Conclusions:** Ang II acts through G-protein coupled AT1Rs through PLC to activate TRPC6. Generation of ROS through NADPH oxidase NOX2 contributes to TRPC6 activation by Ang II.

*Funding:* Pharmaceutical Company Support - Pfizer Inc.

## SA-PO107

**Endogenous NOX2 Interacts with Podocyte TRPC6 Channels and Contributes to Their Activation by Diacylglycerol but Not by Mechanical Stimuli:Essential Role of Podocin in Maintaining the NOX2-TRPC6 Complex**

Stuart E. Dryer,[1,2] Marc Thomas Anderson,[1] Eunyoung Kim.[1] *[1]Biology and Biochemistry, Univ of Houston, Houston, TX; [2]Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** The endogenously expressed TRPC6 channels of podocytes become active in response to reactive oxygen species (ROS) generated in several different signal transduction cascades. TRPC6 channels are also activated by diacylglycerol (DAG) normally produced during PLC-dependent cascades, for example in response to angiotensin II.

**Methods:** Whole-cell recording, immunoprecipitation, fluorometric analysis of ROS generation in immortalized mouse podocytes.

**Results:** Endogenous TRPC6 channels in mouse podocyte cell lines reciprocally co-immunoprecipitate with the NADPH oxidase NOX2 (gp91phox). However, this interaction is not detected in podocin knockdown cells[1]. In whole-cell recordings, we observed that TRPC6 activation by a DAG analog (OAG, 10 μM) was reduced in podocytes pretreated with the NOX2 inhibitor apocynin, by the pan-NOX inhibitor diphenylene iodonium and by the ROS quencher tempol. Tempol had no effect on TRPC6 activation by hypoosmotic stretch. Application of 10 μM OAG also increased generation of ROS, but not in podocin knockdown cells. OAG also increased surface expression of the NOX2 regulatory subunit p47(phox). Podocin is a cholesterol-binding membrane protein that may function to tether TRPC6 and other slit diaphragm proteins to cholesterol-rich membrane domains. We observed that cholesterol depletion using 10 mM methyl-beta-cyclodextrin reduced but did not eliminate activation of podocyte TRPC6 channels by OAG, and eliminated the NOX2-TRPC6 interaction as assessed by immunoprecipitation.

**Conclusions:** NOX2 assembles with TRPC6 at podocin-organized raft domains, and becomes catalytically active in response to DAG. The localized production of ROS contributes to TRPC6 activation by DAG. Importantly, podocin is required for formation of this signaling complex. 1. Anderson et al. (2013) Am J Physiol-Cell Physiol in press.

*Funding:* Pharmaceutical Company Support - Pfizer Inc.

## SA-PO108

**Overexpression of Heterogeneous Nuclear Ribonucleoprotein F Attenuates Systemic Hypertension and Normalizes Angiotensin-Converting Enzyme 2 Expression via Down-Regulation of Transforming Growth Factor-Beta 1/TGF-Beta Receptor II Signaling in Diabetic Akita Transgenic Mice**

Chao-Sheng Lo,[1] Shiao-Ying Chang,[1] Yixuan Shi,[1] Isabelle Chenier,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] Shao-Ling Zhang,[1] John S.D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hosp, Montreal, Canada; [2]Res. Ctr., Maisonneuve-Rosemont Hosp., Montreal, Canada; [3]Pediatr Nephrol Unit, Mass. Gen. Hosp. for Children, Boston, MA.*

**Background:** We investigated whether heterogeneous nuclear ribonucleoprotein F (hnRNP F) stimulates angiotensin-converting enzyme 2 (Ace2) expression in renal proximal tubular cells (RPTCs) via decreasing transforming growth factor-beta 1/TGF-β receptor II (TGF-β1/TGF-β RII) signaling in Akita mice.

**Methods:** Adult (20 weeks of age) male wild type (WT), Akita and Akita hnRNP F-transgenic (Tg) mice were studied. Kidneys were processed for histology. TGF-β1, TGF-β RII and Ace2 mRNA and their protein expression in renal proximal tubules (RPTs)

were evaluated by real time-qPCR and Western blotting, respectively. Freshly isolated mouse RPTs were studied ex vivo. Rat Ace2 gene promoter activity in pGL4.20 vector was studied in rat RPTCs in vitro.

**Results:** Akita mice exhibited increased systolic blood pressure and glomerular filtration rate as compared to WT mice. TGF-β1, TGF-β RII mRNA and their protein expression in RPTs were significantly increased whereas Ace2 mRNA and its protein were decreased in Akita mice compared to WT. These changes were normalized in Akita hnRNP F-Tg mice. Ex vivo, TGF-β1 inhibited Ace2 mRNA and protein expression in mouse RPTs and reversed by SB431542 (an inhibitor of TGF-β receptor I). In vitro, overexpression of hnRNP F inhibited TGF-β1 and TGF-β RII expression, whereas it increased Ace2 expression in rat RPTCs. TGF-β1 also attenuated Ace2 gene promoter activity and its effect was inhibited by SB431542 and small interference RNA of TGF-β RII. Finally, we identified a putative TGF-β1 responsive element in nucleotides N-1084 to N-499 upstream of transcriptional starting site of rat Ace2 gene promoter.

**Conclusions:** Our data suggest that intrarenal hnRNP F attenuates SBP and enhances Ace2 expression in RPTCs, predominantly through decreasing TGF-β1/TGF-β RII signaling.

*Funding:* Government Support - Non-U.S.

## SA-PO109

**Nuclear Factor Erythroid 2-Related Factor 2 Mediates High Glucose Stimulation of Renal Angiotensinogen Gene Expression and Induction of Hypertension in Diabetic Akita Mice**

Shaaban Abdo,[1] Yixuan Shi,[1] Isabelle Chenier,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] Shao-Ling Zhang,[1] John S.D. Chan.[1] *[1]Res Ctr, CHUM-Hotel Dieu Hosp, Montreal, Canada; [2]Res Ctr, Maisonneuve-Rosemont Hosp, Montreal, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp for Children, Boston, MA.*

**Background:** We investigated whether nuclear factor erythroid 2-related factor 2 (Nrf2) can mediate the effects of high glucose (HG) on stimulation of angiotensinogen (Agt) gene expression in renal proximal tubular cells (RPTCs), and induction of systemic hypertension (sHTN) and renal injury in type 1 diabetic Akita mice.

**Methods:** Adult male Akita mice specifically overexpressing catalase (Cat) in their RPTCs and Akita mice treated from 12 weeks ± insulin implants for 4 weeks were studied at 16 weeks. Non-Akita mice served as controls. Plasma glucose, systolic blood pressure (SBP) and urinary albumin/creatinine ratio (ACR) were monitored weekly. Kidneys were processed for histology. Renal proximal tubular (RPT) *Agt, Nrf2 and Keap1 mRNA* and their protein expression were evaluated by real time-qPCR and Western blotting, respectively. Freshly isolated mouse RPTs and rat RPTCs stably transfected with a plasmid, pGL4 containing rat *Agt* gene promoter ± Nrf2 activator (Oltipraz) or Nrf2 inhibitor (alkaloid trigonelline[C7H7NO2]) were also studied.

**Results:** Akita mice developed sHTN and exhibited renal hypertrophy.Overexpression of Cat or treatment with insulin normalized sHTN, attenuated renal hypertrophy and decreased urinary ACR in Akita Cat-Tg and Akita mice, respectively. RPT Agt and Nrf2, but not Keap1 expression, were significantly increased; these changes were normalized by Cat overexpression and insulin treatment. *Ex vivo,* Oltipraz stimulated Nrf2 and Agt expression in mouse RPTs and rat RPTCs and trigonelline abolished its effect. *In vitro,* HG and Oltipraz stimulated *Agt* gene promoter activity and their effect was prevented by inhibitors of oxidative stress and trigonelline as well as by transfecting RPTCs with small interfering RNA of *Nrf2*.

**Conclusions:** Our data indicate that HG induce RPTC injury and sHTN in diabetic mice through, at least in part, Nrf2-mediated stimulation of intrarenal *Agt* gene expression.

*Funding:* Government Support - Non-U.S.

## SA-PO110

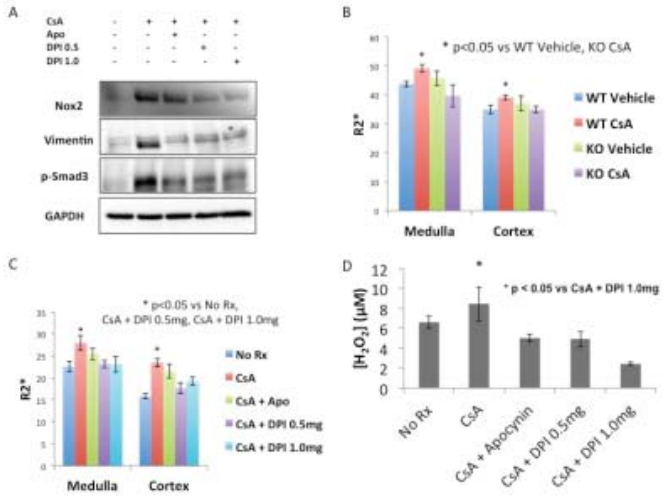
**Nox2 Mediates Cyclosporine A-Induced Hypoxia**

Omeed Hafez,[1] Shannon Reese,[1] Nancy A. Wilson Schlei,[1] Zaheer Akhtar,[2] Elizabeth Sadowski,[2] Arjang Djamali.[1] *[1]Medicine, Univ of Wisconsin; [2]Radiology, Univ of Wisconsin.*

**Background:** The use of Cyclosporine A (CsA), a calcineurin inhibitor, as maintenance immunosuppression in the management of solid organ transplantation is compromised by its chronic nephrotoxicity. This includes increased oxidative stress by an unknown mechanism. We hypothesized that the classical phagocytic Nox2 enzyme plays an important role in renal hypoxia mediated by CsA. We tested this hypothesis using the CsA-induced model of chronic nephrotoxicity in rats and mice.

**Methods:** Fisher344 rats received CsA 15mg/kg/24h, no treatment, or CsA with a non-specific inhibitor of Nox activity for 4 weeks (n=6-8/group). Inhibition was achieved using Diphenyleneiodonium (DPI; 0.5mg or 1.0mg/kg/24h) or Apocynin (Apo; 16mg/kg/24h). Wild-type (WT) and Nox2 knockout (KO) mice received CsA 30mg/kg/24h or vehicle for 8 weeks (n=4-5/group). To characterize the effects of CsA on oxygenation, animals underwent blood oxygen level dependent MRI (BOLD-MRI), a non-invasive imaging method that uses hemoglobin as an endogenous contrast agent. Oxidative stress was further evaluated by measuring $H_2O_2$ levels in rat serum. CsA-induced fibrosis was evaluated with Western blots.

**Results:**




Immunoblot analyses of kidney tissue lysates demonstrated that CsA therapy increased Nox2 and fibrogenesis (vimentin and p-smad3). Nox inhibition decreased fibrogenesis suggesting that Nox2 is a mediator of CsA-induced renal fibrosis (Panel A). BOLD-MRI demonstrated that CsA decreased renal oxygenation (increased R2* levels) while the lack of Nox2 prevented these changes in rats (B) and mice ©. These findings were supported by decreased $H_2O_2$ in serum from rats treated with Nox inhibitors (D).

**Conclusions:** These studies indicate that Nox2 modulates intrarenal oxygenation and is involved in the pathogenesis of CsA-induced hypoxia. Specific Nox2 inhibition may play a role in preventing CsA nephrotoxicity.

*Funding:* NIDDK Support

## SA-PO111

**Nox5 Exacerbates Filtration Barrier Damage and Increases Blood Pressure in a Mouse Model of Diabetes**

Chet E. Holterman,[3] Jean-Francois Thibodeau,[3] Mark E. Cooper,[1] Rhian Touyz,[2] Chris R. Kennedy.[3] *[1]Baker IDI Heart and Diabetes Institute, Melbourne, Australia; [2]Univ of Glasgow, Glasgow, United Kingdom; [3]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, Canada.*

**Background:** We have previously demonstrated that Nox5 is upregulated in human diabetic kidney and contributes to ROS–induced podocyte damage and filtration barrier dysfunction. While several studies implicate Noxes 1, 2 and 4 in diabetic nephropathy, nothing is known regarding the role of Nox5. Here we show that podocyte-specific Nox5 expression results in earlier and more severe albuminuria, increased systolic blood pressure, and interstitial fibrosis in streptozotocin (STZ) type 1 diabetes.

**Methods:** Transgenic mice expressing Nox5 specifically in podocytes ($Nox5^{Pod+}$) were subjected to low dose STZ (50mg/kg) injection daily for five days. Urinary albumin excretion rates from 24 hour urine collections were assessed by ELISA. Systolic blood pressure was measured weekly by tail-cuff plethysmography. Kidney pathology was further assessed by PAS and Mason-Trichrome staining and electron microscopy to determine morphological changes, glomerular sclerosis, and foot process effacement.

**Results:** Urinary albumin levels in $Nox5^{pod+}$ trended higher at 4 and 8 weeks post-STZ injection and were significantly higher 16 weeks post injection as compared to non-transgenic littermates. Systolic blood pressure was not increased in $Nox5^{pod}$ mice at 4 weeks post-STZ but showed increases over non-transgenic littermates at 8 and 16 weeks. PAS and Mason-Trichrome staining revealed increased glomerular sclerosis and interstitial fibrosis 16 weeks post-STZ in $Nox5^{pod+}$ animals.

| | **4wk post-STZ** | | **8wk post-STZ** | | **16wk post STZ** | |
|---|---|---|---|---|---|---|
| | non-tg | $Nox5^{pod+}$ | non-tg | $Nox5^{pod+}$ | non-tg | $Nox5^{pod+}$ |
| **Albumin**(μg/24hrs) | 1627±242 | 1980±200 | 1960±189 | 2396±396.4 | 1489±290 | 2527±494* |
| **Systolic BP**(mmHg) | 107±8 | 112±2 | 112±6 | 121±5 | 115±4 | 127±7* |
| Data are mean ±SEM, *P<0.05 vs non-tg 16wk post STZ | | | | | | |

**Conclusions:** These novel data identify Nox5 as an important NADPH oxidase isoform in the development of diabetic nephropathy. Nox5 may emerge as a novel therapeutic target for reducing the progression of this disease.

## SA-PO112

**TORC2 Signalling Regulates Stress Response and Longevity in C. elegans**

Elke Neumann-Haefelin,[1] Vanessa Ruf,[1] Gerd Walz.[1] *[1]Renal Div, Univ Hospital, Freiburg, Germany; [2]Dept of Genetics, Harvard Medical School, Boston.*

**Background:** The evolutionarily conserved *target-of-rapamycin* (TOR) kinase controls fundamental metabolic processes to support cell and tissue growth. TOR functions within the context of two distinct complexes, TORC1 and TORC2. The TORC2 with its specific component Rictor has been recently implicated in aging and regulation of growth and metabolism.

**Methods:** We established a C. elegans model system to study functional aspects of TOR signalling in vivo.

**Results:** Here, we identify *rict-1/Rictor* as a regulator of embryonic development and stress response in *C. elegans*. The transcription factor *skn-1* and its mammalian ortholog *Nrf* have conserved functions in stress detoxification and contribute to longevity. *skn-1* is also a critical regulator of embryonic development. Inhibition of TORC2 during adulthood confers stress resistance and longevity in a SKN-1-dependent manner. Moreover, genetic inactivation of *rict-1* partially suppressed the embryonic lethality associated with *skn-1* mutation.

**Conclusions:** These data indicate that TORC2 modulates the transcription factor SKN-1 during embryonic development and adult stress response in *C. elegans*.

*Funding:* Government Support - Non-U.S.

## SA-PO113

**The Regulation of the Nitric Oxide System Can Modulate the Klotho Expression in Kidney via TWIST-2 and E-cadherin** Jae Won Yang,[1] Jae Seok Kim,[1] Minseob Eom,[2] Seung-Ok Choi,[1] Byoung Geun Han.[1] *[1]Internal Medicine, Yonsei Univ Wonju College of Medicine, Wonju, Gangwon, Korea; [2]Pathology, Yonsei Univ Wonju College of Medicine, Wonju, Gangwon, Korea.*

**Background:** The klotho was originally identified as an anti-aging protein but was subsequently discovered to have a multitude of biologic actions. Animal experiments clearly showed a transient renal klotho deficiency in acute kidney injury from a variety of causes, including ischemia–reperfusion injury. We investigated whether relationship existed between the NO pathway and the klotho expression in kidney, and studied the possible pathway as basic helix-loop-helix transcription factors (TWIST)-1, 2, E-cadherin.

**Methods:** The 10th weeks Sprague-Dawley rats (N= 24, 200g, male) were divided four groups. We supplied low salt diet to the control group (N=6), L-NAME 1 mg/mL in drinking water to the L-NAME group (N=6), and udenafil 5 mgSQ to the Udenafil group (N=6), L-NAME and udenafil to the L-NAME and Udenafil group (N=6) for 4 weeks. After the collection of blood and urine on day 28, the both kidneys were ressected surgically.The serum creatinine,urine nitrate/nitrite,cGMP by ELISA, and tissues were investigated by immunohistochemical stain, and RT-PCR for klotho, TWIST 1, 2, E-cadherin.

**Results:** The serum creatinine and urine nitrate/nitrite level did not show the statistical difference between groups. The urine cGMP level showed 2.59±0.88, 1.79±0.99, 1.20±0.52, 0.69±0.59 pmol/well (p=0.0087). The klotho mRNA expression showed 0.98±0.01, 0.30±0.11, 0.68±0.15, 0.54±0.26 (p=0.0017). The TWIST-2 mRNA expression showed 1.90±1.65, 139.27±114.87, 10.33±8.42, 20.19±12.25 (p=0.0163). The E-cadherin mRNA expression showed 0.64±0.32, 1.57±0.97, 1.24±1.27, 13.82±3.04 (p=0.0029). The blocking of NO system decreased the klotho expression via the TWIST-2 increase. The induction of NO system increased the klotho expression via E-cadherin increase.

**Conclusions:** The regulation of the nitric oxide system can modulate the klotho expression in kidney via TWIST-2 and E-cadherin.

## SA-PO114

**Semicarbazide-Sensitive Amine Oxidase (SSAO) Inhibitor Inhibits Extracellular Matrix Deposition in Kidney Fibrosis** May Yw Wong, Jie Zhang, Sonia Saad, Carol A. Pollock, Muh Geot Wong. *Kolling Inst, Univ of Sydney, St. Leonards, NSW, Australia.*

**Background:** Novel anti-inflammatory agents targeting early phases of cellular response to injury are increasingly recognised to have a role in tubulointerstitial fibrosis. SSAO is a protein enzyme known for its role in inflammation by mediating the migration of leukocytes and producing reactive oxygen species. However, the role of SSAO inhibitors in kidney fibrosis is unclear. We sought to determine the role of a semicarbazide-sensitive amine oxidase (SSAO) inhibitor (PXS4728A) as an antifibrotic agent using *an in vivo* model of kidney fibrosis.

**Methods:** A 7 day unilateral ureteric obstruction (UUO) model of acute kidney fibrosis was examined in 6-8 week old male C57BL/6 mice (20–25g, n=5) with groups: (i) Sham operated; (ii) UUO-control; (iii) PSX4728A (2mg/kg;orally gavaged); (iv) Telmisartan, an angiotensin receptor blocker (3mg/kg; administered in drinking water), (v) PSX4728 and Telmisartan. Semiquantitative morphometric analyses of glomerulosclerosis and tubuloinsteritial fibrosis were performed. Kidney tissue was analysed for fibrotic and inflammatory mRNA and protein expression.

**Results:** The mRNA expression of Collagen-IV (C-IV) and Fibronectin (FN) in kidney tissue was lower in PXS4728A groups (2.25±1.59; $p<0.05$ and 3.20±2.73; $p<0.05$) as compared to UUO control (9.62±5.32 and 12.00±8.47). The mRNA expression of Transforming Growth Factor Beta-1 (TGFβ-1) and monocyte chemoattractant protein-1 (MCP-1) were effectively suppressed by PSX4278 (2.58±1.86; $p<0.05$ and 2.69±2.00; $p<0.05$) and Telmisartan (1.68±2.03; $p<0.05$ and 2.31±1.92; $p<0.05$) alone and also when combined (2.141±1.952; $p<0.05$ and 2.557±1.296; $p<0.05$) as compared to the UUO control. In addition, mice treated with PXS4782A had reduced tubular dilatation and glomerulosclerosis with lower FN and C-IV expression by immunohistochemistry.

**Conclusions:** Our data strongly suggest that PSX4728A is an effective antifibrotic agent in kidney fibrosis and comparable to Telmisartan.

## SA-PO115

**Advanced Glycation End-Products Induced Podocyte Migration via Downregulation α-Actinin-4 and Cytoskeleton Reorganization** Cailian Cheng, Zhenda Zheng, Chenggang Shi, Xun Liu, Zengchun Ye, Tan-qi Lou. *Dept of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China.*

**Background:** α-actinin-4 play an important role in podocyte function, and mutation of α-actinin-4 cause focal segmental glomerulosclerosis. In this study, we investigate the effects of advanced glycation end-products(AGEs) on the expression of α-actinin-4 and the changes of cytoskeleton, also we observed podocyte migration and the protection of losartan.

**Methods:** Podocytes were incubated with various concentrations of AGEs (0, 20, 40, 80 μg/mL)for 24 hours, α-actinin-4 protein levels were measured by western blot, cytoskeleton reorganization were observed by laser scanning confocal microscopy. Podocyte migration were assayed by wound healing test. Then podocytes were treated with losartan ($10^{-5}$M) and AGEs (80 μg/mL) for 24 hours, the changes of α-actinin-4, cytoskeleton, and podocyte migration were observed.

**Results:** Our results showed that incubation with AGEs resulted in a significant decrease in the expression of α-actinin-4 [(62±13)% vs. 100%, $P<0.05$] , AGEs also induced F-actin reorganization and increased podocyte migration[(250±32) vs. (75±20), $P<0.05$]. However, pretreatment with losartan ($10^{-5}$M) alleviated the downregulation of α-actinin-4 induced by AGEs [(80±14)% vs.(62±13)%, $P<0.05$]. Also, podocyte migration decreased significantly [(100±28) vs .(250±32), $P<0.05$].

**Conclusions:** AGEs increase podocyte migration via downregulation α-actinin-4 and cytoskeleton reorganization.

*Funding:* Government Support - Non-U.S.

## SA-PO116

**Advanced Glycation End Products Upregulated the Expression of Angiopoietin-Like Protein 4 via Activation the Renin-Angiotensin System in Endothelials** Cailian Cheng, Zhenda Zheng, Chenggang Shi, Xun Liu, Zengchun Ye, Tan-qi Lou. *Dept of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China.*

**Background:** To investigate the effects of advanced glycation end products (AGEs) on the expression of angiopoietin-like protein 4 and its mechanisms in endothelials.

**Methods:** Endothelial cells were incubated with various concentrations of AGEs for 24 hours, the expression of angiopoietin-like protein 4 were detected by real-time PCR and western blot analysis, the concentration of angiotensin IIin conditioned media and cell lysates were measured byenzyme-linked immunosorbent assay, FITC-labeled dextran filtration assay and transendothelial electrical resistance were performed to evaluate endothelial permeability. Then endothelials were pretreated with losartan for 60 minutes before AGEs added, and the changes of angiopoietin-like protein 4 expression and endothelial permeability were assayed.

**Results:** AGEs increased the expression of angiopoietin-like protein 4 mRNA and protein levels (2.60±0.18 vs.1.0, 250±32% vs.100%, respectively, $P<0.05$) parallel with an increase in the levels of angiotensin II[(2.8±0.21) vs. (1.3±0.19) pg/μl in media, (0.35±0.04) vs. (0.19±0.04) pg/μg protein in cell lysates ,$P<0.05$]. Incubation with AGEs also resulted in a significant increase in endothelial permeability [(0.46±0.09) vs.( 0.15±0.09) ]OD, [(43.39±2.82) vs. (73.28±2.64) ]$\Omega \cdot cm^2$ $P<0.05$). However, pretreatment with angiotensin Iireceptor blocker losartan($10^{-5}$M) blunted these effects induced by AGEs ($P<0.05$).

**Conclusions:** AGEs upregulated the expression of angiopoietin-like protein 4 via activation local renin-angiotensin system in endothelial cells, which may be a new mechanism for AGEs increasing endothelial permeability.

*Funding:* Government Support - Non-U.S.

## SA-PO117

**Effects of Advanced Glycation End-Products on Synaptopodin Expression and Cytoskeleton Reorganization in Podocytes** Cailian Cheng, Zhenda Zheng, Chenggang Shi, Xun Liu, Zengchun Ye, Tan-qi Lou. *Dept of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China.*

**Background:** To investigate the effects of advanced glycation end-products(AGEs) on the expression of synaptopodinandpodocyte migration, and to explore their mechanisms.

**Methods:** Podocytes were incubated with different concentrations of AGEs (0, 20, 40, 80 μg/mL)for 24 hours, the expression levels of synaptopodin were measured by western blot, cytoskeleton reorganization was observed by laser scanning confocal microscopy. Podocyte migration were assayed by transwell assay. Then podocytes were pretreated with losartan for 60 min, the changes of synaptopodin, cytoskeleton, and podocyte migration were observed.

**Results:** Our results showed that incubation with AGEs resulted in a significant decrease in the expression of synaptopodin[(50±11)% vs 100%, $P<0.05$], AGEs also induced F-actin reorganization and increased podocyte migration [(47±6) vs (13±3), $P<0.05$]. However, pretreatment with losartan ($10^{-5}$M) alleviated the effects induced by AGEs in podocytes.

**Conclusions:** AGEs downregulate the expression of synaptopodin, induce cytoskeleton reorganization, and increase podocyte migration via activation the local renin-angiotensin system in podocytes.

*Funding:* Government Support - Non-U.S.

## SA-PO118

**Mpv17 Protects Podocytes against Oxidative Stress and Apoptosis in Experimental Glomerulonephritis** Gabriella Casalena,[1] Stefanie Krick,[3] Ilse S. Daehn,[1] Liping Yu,[1] Shaolin Shi,[1] Vivette D. D'Agati,[2] Detlef O. Schlondorff,[4] Erwin P. Bottinger.[1] *[1]Dept of Medicine and The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Dept of Pathology, College of Physicians and Surgeons, Columbia Univ, New York, NY; [3]Dept of Medicine, Univ of Miami Miller School of Medicine, Miami, FL; [4]Dept of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY.*

**Background:** Mitochondrial dysfunction is increasingly recognized as contributing to renal glomerular diseases, including those secondary to mitochondrial DNA (mtDNA) mutations/deletions. Mitochondria maintain cellular redox and energy homeostasis and are a major source of intracellular reactive oxygen species (ROS).ROS accumulation may contribute to mitochondrial dysfunction/mutations and thereby to glomerulosclerosis. In mice, deletion of the Mpv17 gene predisposes to experimental glomerulosclerosis, but the underlying mechanism remains poorly defined.

**Methods:** Mpv17$^{+/+}$ and Mpv17$^{-/-}$ C57BL/6J mice were injected with nephrotoxic serum to induce a mild or a severe form of nephritis (NTSN). Mpv17$^{+/+}$ or Mpv17$^{-/-}$ podocytes were generated by crossing Mpv17$^{+/+}$ or Mpv17$^{-/-}$ with Immorto®transgenic mice (Charles River,MA).

**Results:** Mpv17 localizes to mitochondria of podocytes and its expression is reduced in several glomerular injury models and in human glomerular diseases. Using a mild or severe form of NTSN we found that deletion of Mpv17 resulted in increased proteinuria (mild NTSN), and increase in serum creatinine (severe NTSN). Renal defects were associated with mtDNA oxidation and depletion, and loss of protective mitochondrial enzymes (MnSOD, PGC1alpha). In vitro,Mpv17 protects mitochondria and hence podocytes from loss of mitochondrial membrane potential and mtDNA content, with Mpv17$^{-/-}$ podocytes showing increased apoptosis in response to oxidative stress-induced injury.

**Conclusions:** Mpv17 is a protective factor in podocyte mitochondria,with essential function for the maintenance of mitochondrial homeostasis and podocyte survival in response to oxidative stress-induced injury associated with experimental glomerulonephritis.

*Funding:* NIDDK Support

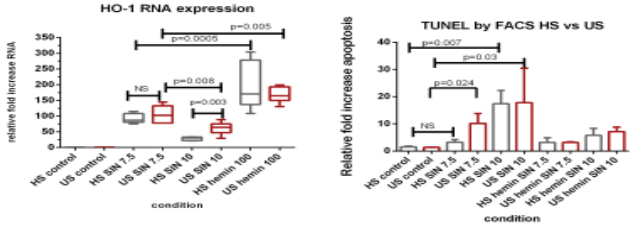
## SA-PO119

**Oxidative Stress Response and Heme Oxygenase 1 Expression in Human Umbilical Artery Endothelial Cells: Uremic versus Healthy Serum Conditions** Kristien El Daenen,[1] Marc Hoylaerts,[2] Bert Bammens.[1] *[1]Laboratory of Nephrology, KU Leuven, Belgium; [2]Molecular and Vascular Biology, KU Leuven, Belgium.*

**Background:** Heme oxygenase 1 (HO-1), an inducible heme-degrading enzyme, protects against atherosclerosis. Increased oxidative stress (OX) contributes to the accelerated atherosclerosis of CKD. We investigated the effect of oxidative stress on human umbilical artery endothelial cells (HUAECs) in uremic (US) compared to healthy serum (HS) conditions and studied HO-1 and its effects in control as well as short and persistent OX conditions.

**Methods:** HUAECs were conditioned in 30% human serum (pooled from 40 hemodialysis patients or 10 healthy volunteers) for 72h, followed by exposure to increasing concentrations of $ONOO^-$ (0.10-1.00 mM) for 5min or SIN-1 7.5 and 10mM for 8h (resp L.OX and H.OX). Additionally, HUAECs were pre-incubated with 100µM Hemin for 6h. HO-1 expression was evaluated by RT-PCR and western blot. Cell viability 30 minutes after $ONOO^-$ exposure was evaluated by MTT assay. Cell apoptosis after SIN-1 incubation ± Hemin pre-incubation (50 µM) was evaluated by western blot for caspase-3 and TUNEL staining by Flow Cytometry.

**Results:** HO-1 RNA expression and apoptosis: see figure.




Western Blot for HO-1 showed equal HO-1 expression in US and HS. $ONOO^-$ reduced US cell viability by MTT assay to a larger extent as compared to HS cells (43% vs 23%, P<0.05 between 0.1-0.5 mM).

**Conclusions:** Cell viability and apoptosis assays show higher OX-induced vulnerability in US, starting from lower concentrations and to a larger extent as compared to HS. In contrast to previously published animal data on kidney tubular cells, endothelial cell HO-1 inducibility is preserved in US. Whereas mild HO-1 induction is seen in response to oxidative stress, Hemin induces HO-1 significantly stronger. Pretreatment by Hemin reverses apoptosis, suggesting a beneficial role of hemin in Uremia.

## SA-PO120

**Activation of Nucleotide Binding Oligomerization Domain-Like Receptor Family, Pyrin Domain Containing-3 Inflammasome due to Production of Reactive Oxygen Species Induced by Calcium Oxalate Crystals in the Kidneys** Sunil Joshi,[1] Wei Wang,[1] Ammon B. Peck,[1] Saeed R. Khan.[1,2] *[1]Pathology, Univ of Florida, College of Medicine, Gainesville, FL; [2]Urology, Univ of Florida, College of Medicine, Gainesville, FL.*

**Background:** We have already shown that production of reactive oxygen species (ROS) is a crucial factor for renal injury and inflammation following exposure to oxalate (Ox) and calcium oxalate (CaOx) crystals. In this study we looked at the role of ROS in activation of Nucleotide binding oligomerization domain-like receptor family, pyrin domain containing -3 (NLRP3) inflammasome and associated genes involved in inflammation.

**Methods:** Eight week old male rats (n=6/group) were given hydroxy-l-proline (HLP) to induce hyperoxaluria and CaOx crystal deposition in the kidneys. Another set of rats getting HLP diet were also given Apocynin-supplemented water. Rats were euthanized on day 28 and kidneys were extracted. Microarray analysis was conducted using Illumina bead array reader™. Gene ontology (GO) analysis and the pathway analysis of the genes was done using DAVID (Database for Annotation, Visualization of Integrated Discovery) enrichment analysis tool.

**Results:** We analyzed a total of 22,226 genes and found that 20 and 24 pathways were highly significant in the cortex and medulla respectively. In the cortex, ECM-receptor interaction, complement and coagulation cascades, focal adhesion and hypertrophic cardiomyopathy were most significant pathways, whereas in medulla, complement and coagulation cascades, ECM-receptor interaction and dilated cardiomyopathy were the major pathways. Genes encoding for PYCARD (ASC: apoptosis –associated spec-like protein containing a CARD), other isoforms of CARD (Caspase activation and recruitment domains), TXNIP (Thioredoxin-interacting protein), caspase-1, IL-1β and IL-18 were significantly up regulated in HLP-fed rats, but the group receiving apocynin had these genes down regulated in the cortex and medulla respectively.

**Conclusions:** Our results show the role of ROS in the activation of NLRP3 inflammasome via TXNIP leading to a robust inflammatory response in the kidneys of rats with hyperoxaluria and CaOx nephrolithiasis.

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## SA-PO121

**Chitosan/siRNA Nanoparticle-Mediated COX-2 Knockdown Regulates Inflammatory and Oxidative Stress Response Induced by Unilateral Ureteral Obstruction in Mice** Line Nilsson,[1] Chuanxu Yang,[2] Jorgen Frokiaer,[1] Jorgen Kjems,[2] Rikke Norregaard.[1] *[1]Dept of Clinical Medicine, Aarhus Univ, Denmark; [2]Interdisciplinary Nanoscience Center, Dept of Molecular Biology and Genetics, Aarhus Univ, Denmark.*

**Background:** Cyclooxygenase type 2 (COX-2) is induced in response to unilateral ureteral obstruction (UUO). In this study the role of COX-2 in the progression of inflammation and oxidative stress in response to UUO was examined using a chitosan nanoparticle system containing anti-COX-2 siRNA that targets macrophages as a therapeutic strategy.

**Methods:** Mice were subjected to 3 days UUO and COX-2 knockdown were mediated by intraperitoneal injection of chitosan nanoparticles containing anti-COX-2 siRNA. Sham-operations were performed in parallel. Localization of Cy5-labeled chitosan was determined by in vivo optical imaging for nanoparticle tracking. COX-2 expression was evaluated by QPCR, immunoblot analysis and immunohistochemistry. Inflammation was examined by the expression of tumor necrosis factor-alpha (TNF-α) mRNA. Using immunoblot analysis, the regulation of the oxidative stress marker, heme oxygenase-1 (HO-1) and antioxidant enzymes, superoxide dismutase 1 and 2 (SOD1 and -2) were examined. HE staining was performed to investigate tubular morphology.

**Results:** Cy5-labeled chitosan nanoparticles predominantly accumulated in the macrophages in the obstructed kidney. COX-2 mRNA and protein increased in response to UUO and this was partly attenuated by treatment with COX-2 siRNA. TNF-α and HO-1 increased during UUO, which was prevented in response to COX-2 siRNA treatment. SOD1 and SOD2 protein levels were downregulated in response to UUO. Treatment with COX-2 siRNA attenuated the downregulation of SOD2. HE staining showed lesser tubular damage in COX-2 siRNA treated UUO mice. Plasma creatinine and urea were unchanged after COX-2 siRNA treatment in response to UUO.

**Conclusions:** This study demonstrates that nanoparticle-mediated COX-2 knockdown in mice may contribute to attenuate the development of inflammation and oxidative stress in response to 3 days UUO.

## SA-PO122

**RGD-Peptide Blocks Osteopontin Expression and Oxidative Stress and Prevents Early Diabetic Nephropathy in Type 1 Diabetic Mice** Taehoon Cho, Susanne B. Nicholas. *David Geffen School of Medicine, UCLA, Los Angeles, CA.*

**Background:** Osteopontin (OPN) may have both pro- and anti-inflammatory properties, and may also regulate the cellular response to oxidative stress. We have shown that OPN is highly up-regulated in renal injury and that global deletion of OPN can prevent diabetic nephropathy (DN), but the mechanisms have not been fully explored. OPN contains an

Arginine-Glycine-Aspartate (RGD) motif that binds to the integrin family of receptors, which activates critical intracellular signaling. This study examines the effect of RGD-blocking peptide on OPN expression and signaling in type 1 DN.

**Methods:** Diabetic $Ins2^{Akita/+}$ and non-diabetic $Ins2^{+/+}$ mice (n=4), aged 8 weeks were untreated or treated by intraperitoneal injection with an RGD-peptide against $\alpha_5\beta_1$-integrin and an RGE-control peptide (2,400μg/kg) for 12 weeks. Tail-cuff blood pressures, plasma glucose and 24hr urinary albumin-to-creatinine ratio (ACR) were measured monthly. At the end of the study, kidney cortex was harvested for protein isolation. Western blots quantified the expression of TGF-β1, OPN, and NADPH oxidase.

**Results:** Plasma glucose and urine volumes were 3–fold higher in diabetic compared to non-diabetic mice ($p<0.05$), but there were no changes in blood pressures. Treatment with RGD-peptide significantly attenuated ACR (3-fold, $p<0.001$) compared to either untreated or RGE-peptide treated $Ins2^{Akita/+}$ mice. Further, RGD treatment significantly down-regulated expression of TGF-β1 (2-fold, $p<0.01$), OPN (2.3-fold, $p<0.01$), and NADPH oxidase (p47; 2.5-fold, $p<0.01$).

**Conclusions:** RGD-peptide blockade of $\alpha_5\beta_1$-integrin function in type 1 diabetic mice significantly prevented early Type 1 DN possibly via attenuation of OPN and oxidative stress. The results suggest that RGD-peptide may have some therapeutic potential in human type 1 DN.

*Funding:* Other NIH Support - U54MD007598, Private Foundation Support

## SA-PO123

**Lipid Peroxidation in Metabolic Syndrome Affects Podocyte Physiology and Insulin Signaling** Krisztian Stadler, Christine Howard, Ellen Cleland. *Oxidative Stress and Disease, Pennington Biomedical Research Center, Baton Rouge, LA.*

**Background:** Podocytes are essential cells in the structure of the glomerular filtration barrier. Early podocyte loss is characteristic to chronic kidney disease in obesity, metabolic syndrome and diabetes. The role of redox imbalance has been implicated in chronic kidney diseases but it is not known how metabolic syndrome related accumulation of toxic end products affects podocyte physiology and insulin sensitivity. Lipid radicals are produced in a lipid peroxidation process where the accumulation of end products such as 4 – hydroxynonenal and isoprostanes is well known in human obesity.

**Methods:** To assess this relationship, we investigated the specific effects of lipid peroxyl radicals on podocyte physiology and function in vitro and in vivo. We used conditionally immortalized podocytes and two animal models of metabolic syndrome with renal impairments, our state of the art immuno-spin trapping approach and specific lipid radical scavenging.

**Results:** In vitro, in podocytes lipid peroxyl radicals reduced motility of the cells in a dose dependent fashion. Furthermore, these radicals compromised basal Akt phosphorylation affecting insulin sensitivity and cell survival of podocytes. In vivo, uninephrectomized DBA/2J mice on high fat diet and obese SHHF rats showed impaired Akt or p42/44 MAPK phosphorylation in their podocytes upon insulin stimuli. Increased secondary protein radical formation was detected by a novel immuno-spin trapping approach in the glomeruli. When animals were treated with a specific radical scavenger POBN for 14 days, podocyte insulin sensitivity has significantly improved.

**Conclusions:** These results suggest that redox imbalance and excess lipid peroxyl radical production, accompanying obesity and metabolic syndrome may affect podocyte physiology and insulin sensitivity and therefore may contribute to the loss of podocytes seen in the early phases of chronic kidney disease.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO124

**Albumin-Bound Fatty Acids Induce a Peroxide Mediated Redox Sensitive Apoptosis of Proximal Tubular Cells – The Role of Peroxiredoxin 2** Krisztian Stadler, Christine Howard, Ellen Cleland. *Oxidative Stress and Disease, Pennington Biomedical Research Center, Baton Rouge, LA.*

**Background:** Metabolic syndrome is an independent risk factor for albuminuria and chronic kidney disease (CKD). It is associated with an oversupply of the nutrient pool and the accumulation of related toxic end products from lipid peroxidation and irregular fatty acid metabolism. While podocytes are insulin sensitive, proximal tubular cells are almost completely lacking glycolysis and preferably oxidize fatty acids. We have hypothesized that a fatty acid overexposure therefore can induce tubular mitochondrial and cellular redox imbalance, ultimately contributing to the activation of redox sensitive apoptotic pathways.

**Methods:** To test this, we have exposed Nrk-52 proximal tubular epithelial cells to pure albumin(BSA), fatty acid contaminated albumin (BSA(FA)) and palmitate (Palm) in various doses and time points.

**Results:** Both BSA(FA) and Palm but not BSA compromised mitochondrial viability, potential and decreased basal respiration, ATP turnover and spare respiratory capacity. Apoptosis was evident in cells with longer exposure or higher doses, through the activation of a redox sensitive pJNK/caspase-3 pathway. This was associated with a decrease in peroxiredoxin 2 expression and an increase of the overoxidized dimer form $Prx\text{-}SO_3$ and catalase. This suggests a peroxide mediated redox sensitive signaling where FA exposure targets peroxiredoxin 2, ultimately leading to the apoptosis of tubular cells. Transfection with Prdx2 rescued cells from caspase 3 cleavage and death.

**Conclusions:** These results suggest that albumin overloaded with fatty acids but not pure albumin itself changes the redox environment in the tubuli, inducing a peroxide mediated redox sensitive tubular cell apoptosis. Thus mitigating FA levels may be an important factor in maintaining redox balance and for the prevention of tubular cell damage in proteinuric diseases.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO125

**Sphingomyelinase Activation Triggers in HIV-Induced Podocyte Injury through Altered Cellular Redox and NFkB Pathways** Kamesh R. Ayasolla, Partab Rai, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Medical School, New York, NY.*

**Background:** Sphingomyelinase- Ceramide (SM-Cer) cascade has been demonstrated to play an important role in several disease processes leading to inflammation, cell death, tissue and organ damage. However, the role of SM-Cer in the development and progression of HIV-associated nephropathy has not been investigated to date. In the present study, we investigated the effects of HIV-induced SM-Cer activation in altering cellular redox and NFkB pathway in a mouse model of HIVAN (Tg26) as we;as in human podocytes.

**Methods:** Renal tissue sphingomyelinase activity was measured in control and Tg26 (4 week old, n=4) mice. *In vitro* studies, human podocytes (HP) were transduced with either empty vector or HIV (NL4-3). Effect of Tempol (SOD mimetic agent,10 mM) and GW4869 (2 mM, a neutral sphigomyelinase [NSMase] inhibitor) was studied on HIV-induced podocyte reactive oxygen species (ROS) generation and NF-kB activation. In addition, the effect of GW4869 on podocyte activation of MAPK, PKCZ and IKKa, b, and p-p65 was studied in control and HIV milieus. To evaluate alterations in podocyte phenotype, protein blots of EV/HP and HIV/HP were probed for podocyte structural proteins.

**Results:** Renal tissues of Tg26 mice displayed 70-80 percent increases in neutral NSMase activity; similar increase in NSMase activity was observed in HIV/HPs. Both Tempol and GW4869 not only inhibited HIV induced podocyte ROS generation but also attenuated podocyte NF-kB activation. GW48 also inhibited HIV-induced MAPK signaling, PKCz activation, enhanced IKKa, b, p-p65 (Ser311) expressions in HPs. HIV also altered podocyte expression of fibronectin, nephrin, podocin and ZO-1, however, GW4869 and Tempol inhibited these effects of HIV.

**Conclusions:** HIV-induced sphingomyelinase activity contributes to an altered podocyte phenotype in HIVAN.

*Funding:* NIDDK Support

## SA-PO126

**The Uremic Solute Indole-3 Acetic Acid Induces Oxidative Stress via Endothelial Cyclooxygenase-2 Up-Regulation** Marion Sallée,[1,2] Laetitia Dou,[1] Claire Cerini,[1] Stéphane Poitevin,[1] Bertrand Gondouin,[1,2] Noemie Jourde-chiche,[1,2] Francoise Dignat-george,[1] Stephane Burtey.[1,2] *[1]UMR-S 1076, Aix Marseille Univ, Inserm, Marseille, France; [2]Centre de Néphrologie Dialyse Transplantation Rénale, APHM, CHU Conception, Marseille, France.*

**Background:** In chronic kidney disease, uremic solutes induce endothelial dysfunction, inflammation, and oxidative stress, leading to increased cardiovascular risk. Indole-3 acetic acid (IAA) is an indolic uremic solute of tryptophan metabolism. IAA is an agonist of the transcription factor aryl hydrocarbon receptor (AhR). The activation of cyclooxygenase-2 (COX-2) expression by AhR ligands contributes to inflammation and oxidative stress. We therefore hypothesized that IAA participates in inflammation and oxidative stress in chronic kidney disease patients by inducing COX-2 expression via AhR activation.

**Methods:** In cultured human umbilical vein endothelial cells, we studied the effect of IAA on COX-2 induction and activity and on COX-2-dependent reactive oxygen species (ROS) production. We then analyzed the signaling pathways related to AhR, involved in COX-2 up-regulation. We finally measured IAA, malondialdehyde, and C-reactive protein serum levels in 68 chronic kidney disease patients.

**Results:** In endothelial cells, IAA increased COX-2 mRNA and protein expression via an AhR/p38MAPK/NF-kB pathway. Furthermore, IAA increased COX-2 activity, reflected by prostaglandin E2 and ROS production. The participation of IAA in oxidative stress and inflammation was corroborated in chronic kidney disease patients by the association between IAA and the markers of oxidative stress and inflammation, respectively malondialdehyde and C-reactive protein.

**Conclusions:** In conclusion, the uremic solute IAA increases endothelial COX-2 expression via an AhR/p38MAPK/NF-kB pathway and enhances prostaglandinE2 and ROS production. IAA, via AhR activation and COX-2 up-regulation, could contribute to inflammation and oxidative stress in chronic kidney disease.

## SA-PO127

**Post-Transcriptional Regulation of UCP2 by Stanniocalcin-1 in Macrophages, Relevance to Inflammation** Roohi Khan, Luping Huang, Huiming Ju, Tatiana Belousova, Liping Zhang, David Sheikh-Hamad. *Nephrology/Medicine, Baylor College of Medicine, Houston, TX.*

**Background:** Uncoupling proteins (UCPs) reduce mitochondrial superoxide generation and are important for macrophage function. $UCP2^{-/-}$ macrophages generate more ROS compared to WT macrophages and display 5-fold greater toxoplasmacidal activity in vitro; the enhanced activity is abolished by ROS quencher. Mitochondrial ROS in $UCP2^{-/-}$ macrophages potentiate NF-kB, and amplify the inflammatory response. STC1 induces UCP2 in macrophages, reduces ROS – and is critical for macrophage activation; thus, understanding the regulation of UCP2 by STC1 is important. UCP2 mRNA levels are high, contrasting with low protein levels, and because STC1 induces UCP2 in macrophages within 1/2h (3-fold induction within 2-3h), we hypothesized that STC1 regulates UCP2 post-transcriptionally.

**Methods:** We determined: 1) UCP2 mRNA levels (RT-PCR) in STC1-treated macrophages in the presence or absence of actinomycin D, an inhibitor of transcription; 2) UCP2 protein levels (Western blot) in STC1-treated macrophages in the presence or

absence of cycloheximide, an inhibitor of protein synthesis; 3) $C^{14}$-PHE incorporation into UCP2, in macrophages treated with STC1 or vehicle; and 4) residual UCP2 $C^{14}$-PHE in STC1- or vehicle-treated macrophages in the presence of cycloheximide.

**Results:** Induction of UCP2 mRNA by STC1 occurs late (3-fold at 6h and 4-fold at 24h). Treatment with actinomycin D-abolishes UCP2 mRNA levels within 6h (3h half-life), and STC1 does not affect UCP2 mRNA stability. STC1 fails to induce UCP2 protein in the presence of cycloheximide, suggesting that UCP2 induction by STC1 is dependent on protein synthesis. Indeed, STC1 induces $C^{14}$-PHE incorporation into UCP2 10-fold within 3h; but, does not affect its degradation.

**Conclusions:** Early induction of UCP2 protein by STC1 occurs without significant change in mRNA level, consistent with translational control. STC1 induces UCP2 protein synthesis 10-fold; however, since protein degradation is high, STC1-mediated increase in UCP2 protein abundance is only 2-3-fold. This regulatory scheme allows immediate adjustment of UCP2/superoxide and the responses of macrophages to antigenic stimuli or cytokines.

*Funding:* NIDDK Support

## SA-PO128

**Gene Expression Profiling of Young and Aged Wild-Type Mice Reveals Pathways Involved in Kidney Aging** Christine E. Kurschat,[1] Valerie Bartels,[1] Fabian Braun,[1] Peter Frommolt,[2] Bianca H. Habermann,[3] Joachim Schultze,[4] Jan H.J. Hoeijmakers,[6] Peter Nuernberg,[2] Martijn E. Dollé,[5] Thomas Benzing,[1] Roman-Ulrich Mueller,[1] Bernhard Schermer.[1] *[1]Dept II of Internal Medicine, Cologne Univ, Germany; [2]Cologne Center for Genomics, Germany; [3]Max Planck Institute for Biology of Ageing, Cologne, Germany; [4]Life and Medical Sciences Institute, Bonn, Germany; [5]National Institute of Public Health and the Environment, Bilthoven, Netherlands; [6]Erasmus Univ Medical Center, Rotterdam, Netherlands.*

**Background:** Due to the worldwide demographic increase in the elderly population prevention and therapy of aging-related chronic kidney disease have become a major health concern. Currently, models to study renal aging are lacking. To elucidate molecular mechanisms involved in kidney aging we analyzed gene expression profiles of young and aged wild-type mouse kidney tissue to identify novel pathways involved in aging-related kidney disease.

**Methods:** Whole kidney RNA of wild-type mice was extracted at 4, 14, and 96 weeks of age. cDNA was hybridized to Affymetrix microarrays. Statistical analysis was conducted with R/ Bioconductor. GO enrichment and network analyses were performed using the DAVID server and NetBox software.

**Results:** GO term enrichment analysis revealed differentially expressed genes to be involved in lipid metabolism, immune and defense response, and blood vessel morphogenesis. These genes were part of growth receptor and chemokine receptor signaling, inflammation signaling, and complement response. Several genes were already known to be implicated in renal damage, such as Kim1, LFABP1 or N-gal. Histologically, aged mice showed a significant increase in glomerular tuft area and glomerular external diameter.

**Conclusions:** Aged mouse kidney tissue exhibited glomerular hypertrophy and aging-associated histologic alterations. Genes differentially expressed in aged kidney samples were preferentially involved in lipid metabolism, immune response, inflammation signaling and chemokine receptor signaling. Our results will contribute to identifying novel pathways and genes involved in regulating the process of kidney aging.

*Funding:* Government Support - Non-U.S.

## SA-PO129

**Hyperglycemia Enhances Kidney Cell Injury in HIV-Associated Nephropathy (HIVAN)** Partab Rai, Andrei Plagov, Rivka Lederman, Nirupama Chandel, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Medical School, Great Neck, NY.*

**Background:** Occurrence of insulin resistance and the development of diabetes has been reported to be increased with the use of protease inhibitors (PI) in HIV patients. In the present study, we evaluated the effect of short term hyperglycemia on renal lesions in a mouse model (Tg26) of HIVAN.

**Methods:** Control (FVBN) and Tg26 mice in groups (n=6) were administered either saline or streptozotocin. Blood sugar and urea nitrogen (BUN) levels were measured. After two weeks, Renal lesions were scored. Renal tissue lysates were probed for vitamin D receptor (VDR) renin, actin and assayed for Ang II. Renal cortical sections were assayed for ROS generation and DNA damage by immunolabeling (dihydroethidum and 8-OHdG).In *in vitro* studies, human podocytes (HPs) were transduced with either empty vector (EV) and NL4-3(HIV). To determine the role of Ang II blockade, oxidative stress, and VDR deficit status, EV/HP and HIV/HP were incubated in media containing either normal glucose (NG, 5mM) or high glucose (HG, 30mM)± losartan ($10^{-7}$M)/tempol (1μM)/EB1089(10pM, VDR agonist) for 24h followed by colabeling for γH2AX (DNA damage) and KU80(DNA repair).

**Results:** FVBN/STZ and Tg26/STZ mice displayed elevated BUN ($P<0.05$) and enhanced proteinuria ($P<0.01$) vs. respective FVBN and Tg26.Tg26/STZ displayed enhanced ($P<0.001$) number of sclerotic glomeruli and microcysts vs. Tg26. Renal tissues of Tg26 displayed down regulation of VDR and enhanced Ang II production vs. control. Hyperglycemia exacerbated down regulation of VDR and production of Ang II in FVBN and Tg26 mice. Hyperglycemia increased kidney cell ROS production and oxidative DNA damage in both FVBN and Tg26 mice. In i*n vitro* studies, HIV down regulated podocyte VDR expression and enhanced renin angiotensin (RAS) activation. Both glucose and HIV stimulated kidney cell ROS generation and DNA damage and compromised DNA repair; however, tempol, losartan (Ang II blocker) and EB1089 provided protection against DNA damaging effects of glucose and HIV.

**Conclusions:** Hyperglycemia activated the RAS and inflicted oxidative stress-mediated DNA damage via down regulation of kidney cell VDR expression in HIV milieu.

*Funding:* NIDDK Support

## SA-PO130

**The Role of Glycogen Synthase Kinase-3 in Podocyte Apoptosis under Diabetic Conditions** Ji Sun Paeng,[1] Hye-Young Kang,[1] Hyung Jung Oh,[2] Sung Jin Moon,[3] Seung Hyeok Han,[2] Tae-Hyun Yoo,[1,2] Shin-Wook Kang.[1,2] *[1]Severance Biomedical Science Institute, Brain Korea 21; [2]Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul; [3]College of Medicine, Kwandong Univ, Gyoeonggi-do, Korea.*

**Background:** Glycogen synthase kinase-3β (GSK-3β) is involved in the pathogenesis of various kidney diseases. In this study, we examined the changes in GSK-3β activity in podocytes under diabetic conditions and elucidated the functional role of GSK-3β in podocyte apoptosis, a characteristic finding in diabetic nephropathy.

**Methods:** In vivo, 32 rats were injected with either diluent (n=16, C) or with streptozotocin intraperitoneally (n=16, DM), and 8 rats from each group were treated with 6-bromoindirubin-3'-oxime (BIO) for 3 months. In vitro, immortalized mouse podocytes were exposed to 5.6 mM glucose (NG) or 30 mM glucose (HG) with or without BIO. Western blot using sieved glomeruli and cultured podocytes, and TUNEL or Hoechst 33342 staining were performed to identify apoptosis.

**Results:** Urinary albumin excretion was significantly higher in DM rats ($P<0.01$), and this increase was significantly abrogated in DM rats by BIO treatment ($P<0.05$). The protein expression of Tyr216-phospho-GSK-3β was significantly higher in DM glomeruli and in cultured podocytes exposed to HG, indicating that GSK-3β activity was significantly increased in podocytes under diabetic conditions. Western blot analysis revealed that the protein expression of Bax and active fragments of caspase-3 were significantly increased, whereas phospho-Akt, b-catenin, and Bcl-2 protein expression were significantly decreased in DM glomeruli and HG-stimulated podocytes. Apoptosis determined by TUNEL assay and Hoechst 33342 staining were also significantly increased in podocytes under diabetic conditions. The changes in the expression of apoptosis-related molecules and the increase in the number of apoptotic cells in DM glomeruli as well as in HG-stimulated podocytes were significantly ameliorated by BIO.

**Conclusions:** These findings suggest that enhanced GSK-3β activity within podocytes under diabetic conditions is associated with podocyte loss in diabetic nephropathy.

## SA-PO131

**Rosuvastatin Activates Transcription Factor Nrf2 through $p21^{cip1}$ Expression and Prevents Albuminuria through Preservation of Glomerular Endothelial Integrity in AKITA Diabetic Mice** Chieko Ihoriya, Minoru Satoh, Tamaki Sasaki, Naoki Kashihara. *Depto of Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Japan.*

**Background:** Transcription factor Nrf2 plays a crucial role in cellular defense against oxidative stress. Statins have been shown to reduce urinary albumin excretion and maintain the glomerular filtration rate in diabetic kidney disease; however, the mechanism is not fully elucidated. The renoprotective effects of statins may involve their pleiotropic effects, especially anti-oxidant activity. We hypothesized that statin decreases oxidative stress through the modulation of Nrf2 signaling pathway and inhibits urinary albuminuria excretion in diabetic nephropathy. The aims of this study were to assess the role of Nrf2 in rosuvastatin-mediated antioxidant effects in endothelial cells and to further elucidatethe molecular mechanisms of renoprotective effect of rosuvastatin (RSV) treatment.

**Methods:** Wild type (WT) and Akita diabetic mice (AKITA) were treated with RSV for 4 weeks. Urinary albumin excretion and renal histology were examined. Nrf2-antioxidant response element (ARE) activity was measured in human umbilical vein endothelial cell (HUVEC) with luciferase assay after transfection of reporter plasmids containing AREs. The expression of Nrf2-regulated genes was also examined.

**Results:** Increased urinary albumin excretion in AKITA mice was significantly reduced by RSV treatment. The amount of lectin-stained glomerular endothelial surface layer, important for permselectivity in the vascular wall, was significantly reduced in AKITA mice and preserved with RSV treatment. RSV significantly increased the transcriptional activity of the AREs and subsequent expression of Nrf2-regulated genes in HUVEC. Additional experiments with cycloheximide and actinomycin D indicated that RSV extended the half-life of Nrf2 protein. Furthermore, RSV increased $p21^{cip1}$ expression and thereby inhibited degradation of Nrf2 through direct binding of Nrf2 with $p21^{cip1}$.

**Conclusions:** These data indicate that rosuvastatin has anti-oxidative effects through activation of Nrf2, thereby restoring glomerular endothelial function and preventing development of albuminuria in diabetes.

## SA-PO132

**Beneficial Effects of Insulin in Subtotal Nephrectomy May Involve Atypical AMPK Activation** Joseph Satriano, Aihua Deng, Roland C. Blantz. *Nephrology, UCSD & VASDHS, San Diego, CA.*

**Background:** Chronic kidney disease (CKD) is a progressive disorder impacting kidney function and metabolic efficiency, eventually leading to renal lesions and fibrosis. We have previously shown that 5' AMP-activated protein kinase (AMPK) levels are low

in the subtotal nephrectomy (STN) model of CKD and that reestablishing AMPK activity in STN animals via agonist administration normalized kidney function (GFR), metabolic efficiency (oxygen consumption/sodium transport, QO2/TNa) and abrogated kidney lesions and fibrosis apparent at 30 days STN. Insulin administration has beneficial effects in CKD, yet would be expected to further decrease activity of the energy sensor AMPK. To investigate this conundrum we evaluated AMPK activity in STN animals administered insulin.

**Methods:** We examined the effects of acute insulin treatment (Aspart, 500mU/kg bolus, followed by iv 8mU/kg/min) in rats subject to STN for 7 days. Protein expressions were evaluated by Northern blotting. All values are relative densitometry units±SEM normalized against alpha-Tubulin.

**Results:** AMPK activity (p-AMPK T172/total AMPK) decreases from Con (1.26±0.21) to STN (0.094±0.02), yet surprisingly increases markedly with STN+Insulin (1.08±0.25). Evaluating mTOR components reveals mTORC1 activity (via p70S6K), which inhibits insulin sensitivity, expectedly increases from Con (38±1.8) to STN (6034±3017), but decreases to control levels with STN+Insulin (43±6.8). However, although p-Akt S473 does not increase from Con (3048±430) to STN (3430±116), it does increase with STN+Insulin (4692±177), suggesting a total mTOR increase via mTORC2 in STN+Insulin, which would promote insulin sensitivity. Further, PPAR-g is markedly increased in STN+Insulin (7978±881) animals beyond that of Con (1253±316) or STN (1347±274) animals.

**Conclusions:** Thus the unconventional induction of a AMPK activity by insulin rather than the prototypical mTORC1/insulin resistance response holds the potential of increasing the renoprotective AMPK axis as well as mTORC2/insulin sensitivity axis in the STN model of CKD. Following this line of preliminary data requires further investigation of the components involved.

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## SA-PO133

### Regulation of Macrophage Phenotype by M-CSF: GM-CSF Ratio and Its Implication in Diabetic Nephropathy

Satyesh K. Sinha,[1] Susanne B. Nicholas.[1,2] *[1]Charles R Drew Univ of Medicine and Science, Los Angeles, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA.*

**Background:** Diabetic nephropathy (DN) involves infiltration of pro-inflammatory macrophages (MΦ) into kidney tissues. In human, macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are known to independently promote anti- (M2) and pro- (M1) inflammatory MΦ, however, the effect of the ratio of these growth factors in regulating MΦ phenotype in DN is not known.

**Methods:** Using human peripheral blood monocyte, we characterized the phenotype of M-CSF- (M2) and GM-CSF-induced (M1) MΦ under varying concentrations (5, 15 and 25mM) of glucose and investigated the regulation of MΦ phenotype by IL-10 (M2 marker) or IL-6 (M1 marker) production in the presence of varying ratios of M-CSF:GM-CSF concentrations (2, 5 and 10ng/ml) by ELISA, RT-QPCR and microarray of isolated MΦ RNA.

**Results:** Under high glucose condition (25mM), M-CSF:GM-CSF 10:0 ratio stimulated MΦ secretion of IL-10, which was significantly inhibited when the ratio was changed to 10:2(12.94 vs 7.88pg/ml, $p<0.05$). On the other hand, 0:10 ratio of M-CSF:GM-CSF stimulated MΦ secretion of IL-6, which was counteracted by change in the ratio to 2:10(30.96 vs. 17.68pg/ml), $p<0.05$. Similarly, under high glucose, mRNA expression of IL-6 was up-regulated (9-fold) with M-CSF:GM-CSF 10:2 ratio compared to the 10:0 ratio, $p<0.05$. The expression of IL-6 was down-regulated with M-CSF:GM-CSF 2:10 ratio compared to the 0:10 ratio, $p<0.05$. Microarray analysis of MΦ RNA also identified significant (4-10 fold) up-regulation of several pro-inflammatory genes with M-CSF:GM-CSF 10:2 ratio compared to ratio 10:0 under high glucose, $p<0.001$.

**Conclusions:** The balance between M-CSF and GM-CSF is critical to define MΦ phenotype. Therefore, targeting the ratio of M-CSF:GM-CSF may be beneficial in DN.

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## SA-PO134

### Targeting a Novel Enhancer of TGF-β in Diabetic Nephropathy

Zhonglin Chai, Mark E. Cooper. *Diabetic Complications, Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia.*

**Background:** We have reported that Cell Division Autoantigen 1 (CDA1) synergistically enhances TGF-b signalling in diabetic nephropathy (DN) (Tu, et al, Kid Int 2011; 79:199). Using our recently generated CDA1 knockout (KO) and CDA1/ApoE double KO (dKO) mice, we have shown that genetic deletion of CDA1 retards diabetes associated renal injury (Chai, et al, J Am Soc Nephrol 2013, in press). These data suggest that CDA1 plays an important role in DN, which can be potentially retarded by pharmacologically inhibiting the activity of CDA1.

**Methods:** Yeast 2-hybrid system was used to indentify CDA1 binding protein 1 (CDA1BP1) which directly interacted with CDA1 and regulated the activity of CDA1. A 12mer short peptide of CDA1BP1 containing the binding site was used to interrupt the CDA1/CDA1BP1 interaction. CHA-061, a "retro-inverso peptide" of D amino acids was synthesized which contains a previously known "cell penetrating peptide" to allow it to enter cells with increased stability. ApoE KO mice were rendered diabetic by streptozotocin injections. At 5 weeks after diabetes diabetic mice were treated with CHA-061 or vehicle by ip injections twice a week for 5 weeks. Renal parameters as well as biochemical changes in kidney were analysed at 10 weeks after diabetes.

**Results:** CHA-061 reduced mRNA levels of target genes of CDA1 and TGF-b such as collagen I and III by >50% in HK-2 cells. Adenoviral-delivered CDA1 increased collagen I mRNA level by >2-fold, which was blocked by CHA-061 at 0.5 and 5 mM in HK-2 cells. A single ip injection of CHA-061 in ApoE KO mice at 1 and 5 mg/Kg bodyweight reduced renal mRNA levels of TGF-b target genes for >5 days. In the 10-week diabetes study in ApoE KO mice, renal gene expression of TGF-b1, TGF-b2, CTGF, MMP2, VCAM1, collagen III and fibronectin were increased by >2-6-fold in the diabetic group treated with vehicle and this effect of diabetes was blocked by CHA-061. CHA-061 also reduced gene expression of TGF-b type I receptor, TNF-α as well as attenuated diabetes associated increases in gene expression of collagens I, IV, MCP1 and ICAM1.

**Conclusions:** These results demonstrate that pharmacological targeting of CDA1 reduces TGF-b signalling and is potentially effective to retard DN.

*Funding:* Government Support - Non-U.S.

## SA-PO135

### tRNA Conformational Change Is the Early Marker of Ischemic Tissue Injury

Eikan Mishima, Daisuke Saigusa, Yoichi Takeuchi, Yasutoshi Akiyama, Takehiro Suzuki, Sadayoshi Ito, Yoshihisa Tomioka, Takaaki Abe. *Tohoku Univ, Sendai, Japan.*

**Background:** Tissue damage by ischemic and oxidative stress is a major factor in the progression of renal failure. Recently, it has been reported that transfer RNA (tRNA) metabolism is important response to protect cells. However, how tRNA senses such stress under these conditions is still unclear.

**Methods:** We examined the tRNA behavior using a newly generated antibody against tRNA-specific modified nucleoside, 1-methyladenosine (m1A). We also established measuring system for m1A by LC-MS/MS and for tRNA derivatives by ELISA.

**Results:** Oxidative stress directly generated the conformational change of tRNA structure detected by in vitro immunoprecipitation assay. The conformation change of tRNA was also confirmed in ischemia-reperfusion damaged tissues by IHC. Such conformation change was occurred at earlier phase than DNA damage detected by TUNEL or 8OHdG staining. By ELISA and LC-MS/MS, we next measured the tRNA derivatives in blood under tissue damage conditions. The circulating tRNA derivatives were increased in various animal tissue damage models (renal ischemia-reperfusion, cisplatin nephropathy, and radiation tissue injury). In addition, in human aortic arch replacement surgery which renal ischemia is inevitable, the circulating tRNA derivatives were increased after reperfusion procedure. The onset of the increase was earlier than that of urinary KIM-1. Furthermore, we examined serum m1A level in general population (n=1033) and their relationship with prognosis for 6.7year. As a result, the serum level of tRNA metabolites was correlated with mortality suggesting the continuous stress damage in the high level group. In CKD patients (n=29), high level of serum m1A was decreased by statin treatment.

**Conclusions:** These data suggested that i) under ischemia and other tissue damages, the conformation change of tRNA was induced, ii) following tRNA derivatives release to the blood occurred, iii) resultant circulating levels are correlate with cell damage and mortality. Thus, detection of intracellular tRNA condition and the measurement of circulating tRNA derivatives is important to detect early renal and tissue damages and for intervention.

*Funding:* Government Support - Non-U.S.

## SA-PO136

### TGF-β1 and TGF-β2 Synergistically Mediate the Glomerular Endothelial to Mesenchymal Transition Induced by High Glucose through ROCK Activation

Hui Peng, Yuanqing Li, Canming Li, Zengchun Ye, Meirong Zhong, Tan-qi Lou. *Nephrology, Dept of Medicine, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

**Background:** Our previous study found that EndMT occurs in glomeruli of DKD patients. However, the relevance of this change and the mechanism leading to this change remain unclear. In this study, we assessed the co-relationship between permeability of glomerular endothelial cells and EndMT. Additional, we explored the signaling pathway involved in high glucose induced glomerular EndMT in vitro.

**Methods:** Rat Glomerular Endothelial Cells (GEnCs), which pre-treated with or without TGF-β type 1 receptor inhibitor LY364947 or ROCK inhibitor Y27632, were incubated in medium containing normal glucose (5.5 mmol/L) or high glucose (30 mmol/L) for 48 h. In separated experiments, antibody against TGF-β1 or TGF-β2 was added into the medium in advance, respectively. GenC permeability was assessed by TEER. Real-time PCR was conducted to evaluate the expression of TGF-β1 and TGF-β2, while α-SMA, CD31 and phosphorylation of MYPT1 were determined by western blotting.

**Results:** High glucose caused EndMT which was associated with increased permeability in cultured GEnCs, which was evidenced by the down-regulation of CD31 and up-regulation of α-SMA. Simultaneously, high glucose stimulated the expression of both TGF-β1 and TGF-β2 that were associated with a significant increase in phosphorylation of MYPT1, reflecting an activation of ROCK. Pre-treatments of LY364947 or Y27632 improved GEnCs permeability and prevented the changes of CD31 and α-SMA stimulated by high glucose; moreover, LY364947 pretreatment blocked the phosphorylation of MYPT1 caused by high glucose. Notably, pre-incubating with antibodies against to TGF-β1 or TGF-β2 attenuated the influence of high glucose on α-SMA and CD31, as well as the phosphorylation of MYPT1.

**Conclusions:** Our study demonstrates that high glucose promotes glomerular EndMT which contribute to increased GEnCs permeability. TGF-β1 and TGF-β2 mediated this response synergistically via ROCK activation.

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*Funding:* Government Support - Non-U.S.

## SA-PO137

**Tempol Attenuates Renal Fibrosis in Mice with Unilateral Ureteral Obstruction: The Role of PI3K–Akt–FoxO3a Signaling** Hye Eun Yoon, Sung Jun Kim, Su Jin Choi, Sungjin Chung, Seok Joon Shin. *Internal Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

**Background:** Oxidative stress contributes to the pathogenesis of chronic kidney disease. Phosphatidylinositol 3-kinase (PI3K), Akt, and Forkhead box O (FoxO) transcription factors control oxidative stress. This study investigated whether tempol, an anti-oxidant, protects against renal injury by modulating PI3K–Akt–FoxO signaling.

**Methods:** Mice received unilateral ureteral obstruction (UUO) surgery with or without administration of tempol. We evaluated renal damage and expression of PI3K, Akt, FoxO3a and their target molecules, manganese superoxide dismutase (MnSOD), catalase, Bax, and Bcl-2 in the obstructed kidneys on days 3 and 7.

**Results:** Collagen deposition and F4/80 macrophage infiltration were significantly lower in tempol-treated mice compared with controls on day 7. The expression of PI3K, phosphorylated Akt, and phosphorylated FoxO3a decreased markedly in tempol-treated mice on days 3 and 7. Tempol increased the expressions of MnSODand catalase on days 3 and 7, and decreased the production of hydrogen peroxideand lipid peroxidation. Significantly less apoptosis, a lower ratio of Bax to Bcl-2 expression and fewer apoptotic cells in TUNEL staining, was observed in tempol-treated mice compared with controls.

**Conclusions:** In conclusion, tempol attenuates oxidative stress, inflammation, and fibrosis in the kidneys of mice with UUO, andthe modulation of PI3K–Akt–FoxO3a signaling may be involved.

*Funding:* Government Support - Non-U.S.

## SA-PO138

**Ethanol Causes Oxidative Stress in Podocytes by Inducing Cytochrome P450 2E1 (CYP2E1)** Ellen T. McCarthy,[1] Jianping Zhou,[2] Tarak Srivastava,[3] Ram Sharma,[2] Virginia J. Savin,[1,2] Mukut Sharma.[1,2] *[1]Kidney Institue, KUMC, Kansas City, KS; [2]Research Service, KC VA Medical Center, Kansas City, MO; [3]Nephrology, CMH, UMKC, Kansas City, MO.*

**Background:** Excessive ethanol (EtOH) consumption leads to hypertension while low amounts have beneficial cardiovascular effects. We have shown that low concentrations of EtOH (2µl/mL) increase podocyte expression of CYP isoforms, such as CYP4a12a, that synthesize 20-hydroxyeicosatetraenoic acid (20-HETE) and that 20-HETE protects the filtration barrier. High concentrations (10-20 µL/mL) derange the podocyte actin cytoskeleton. EtOH is metabolized by cell-specific cytosolic alcohol dehydrogenase (ADH) and microsomal CYP2E1. Metabolism of EtOH by CYP2E1 produces superoxide. The metabolic consequences of EtOH in podocytes are unknown.

**Methods:** We examined the effect of EtOH (2-20 µl/mL) on the expression of ADH and CYP2E1 in immortalized murine podocytes. These concentrations correspond to blood alcohol levels seen in humans. We measured expression of ADH and CYP2E1 using qRT-PCR and Western blotting and examined superoxide generation using the fluorescent probe hydroethidine (HE). We tested the protective effect of 20-HETE (100 nM) in some experiments.

**Results:** ADH gene and protein expression were detected in all conditions. ADH gene was upregulated by 2 ($P<0.001$) but not by 10 or 20 µl/mL EtOH. CYP2E1 expression was undetectable in untreated podocytes. EtOH (10 or 20 µl/mL) induced robust expression of CYP2E1 ($P<0.001$) and increased superoxide generation as indicated by HE fluorescence ($P<0.001$). Addition of 20-HETE prevented the increase in superoxide ($P<0.001$).

**Conclusions:** Low concentrations of EtOH upregulate both CYP4a12a and ADH expression. In contrast, higher concentrations cause oxidative stress through induction of CYP2E1 and also downregulate expression of CYP4a12a. This complex pattern of enzyme expression in podocytes is consistent with protection by low-level EtOH consumption via enhanced 20-HETE production. Protection from oxidative injury may be lost during excessive EtOH intake. Further studies are needed to determine the contribution of alcohol consumption to renal disease and progression in humans.

*Funding:* NIDDK Support, Veterans Affairs Support, Private Foundation Support

## SA-PO139

**Integrin Linked Kinase (ILK) Regulates AQP2 Independently of cAMP Axis. A New Nephrogenic Diabetes Insipidus Animal Model** Jose Luis Cano-peñalver,[1] Mercedes Griera,[1] Paloma Martín-sánchez,[1] Ines Mora,[1] DIEGO Rodriguez-Puyol,[2] Sergio De Frutos Garcia,[1] Manuel Rodriguez-Puyol.[1] *[1]Biología de Sistemas, Unidad Fisiología, Universidad de Alcala, Alcala de Henares, Madrid, Spain; [2]Fundacion de Investigacion Biomedica, Hospital Universitario Principe de Asturias, Alcala de Henares, Madrid, Spain.*

**Background:** AQP2 modifies the tubular water reabsorption through transcriptional and postraslational content as well as its quick cytoplasm-to-apical membrane trafficking. Phosphorilations by kinases are needed in both short and long-term regulations, including the canonical cAMP-dependent axis pathway: Vasopressin (AVP) binds to its tubular receptor V2R that activates Adenilate Cyclase (AC), which in turn produce cAMP that activates PKA. We are interested in the extracellular matrix messenger ILK (integrin linked kinase) implication that may lead renal functional changes.

**Methods:** We previously shown (ASN kidney week 2012) basal poliuria in transgenic conditional ILK-deleted adult mice (cKO-ILK) compared with controls (WT). Decreased urine osmolality was due to both AQP2 reduced expression and traffic.

**Results:** The present work focused in the in vivo ILK-dependent AQP2 regulation origin. Nephrogenic origin of the diabetes insipidus (NDI) observed in cKO-ILK was confirmed since plasma AVP levels and basal V2R renal expression were not different between groups. 24h treatment with the pharmacological V2R activator desmopressin did increase in both WT and cKO-ILK traffic and expression AQP2, as well as urine osmolality. However statistic differences remained between groups.

The canonical cAMP-dependent pathway was studied in ex-vivo kidney tissue that was short-time treated with desmopressin, AC activator FK or PKA activator 8-Br-cAMP. The pharmacological activation of all these enzymes increased the traffic, but did not completely restore the differences when ILK was depleted.

**Conclusions:** ILK regulates tubular water reabsorption, with unaltered changes in central production of AVP levels, V2R expression and activity. The ILK-dependent AQP2 expression and traffic regulation is independent of the canonical cAMP axis pathway.

*Funding:* Government Support - Non-U.S.

## SA-PO140

**Evaluation of Two Rat Chronic Kidney Disease (CKD) Models to Study Efficacy of Alternative Anemia Treatment** Anja Verhulst,[1] Felix W. Funk,[2] Patrick C. D'Haese.[1] *[1]Lab of Pathophysiology, UAntwerpen, Belgium; [2]Vifor(Int) Ltd, St. Gallen, Switzerland.*

**Background:** Next to erythropoietin (EPO), hepcidin (Hep), by limiting iron (Fe) efflux from enterocytes/macrophages, plays a central role in the development of CKD induced anemia. A lack of Hep clearance and the inflammatory status of CKD patients result in increased plasma Hep levels potentiating anemia upon the reduced renal EPO synthesis in these patients. The present study evaluates the potential use of rat CKD models to study efficacy of alternative anemia treatment.

**Methods:** Remnant kidney CKD (RK) was induced in 12 rats by 5/6 nephrectomy. Adenine induced CKD (AD) was generated in 12 rats by feeding 0.75% adenine for 4 weeks. RK and AD rats were followed-up for 11 and 8 weeks respectively. For each model a control (Ctr) group (n=5) was included. Blood/serum (S) parameters were followed-up: hematocrit (Hct), creatinine (creat), Hep and EPO. Liver/spleen Fe content was measured. $p<0.05$ was considered significant (sign).

**Results:** CKD was less severe in RK (Screat 1.02±0.15 vs Ctr 0.40±0.07mg/dl at 3 weeks) compared to AD rats (Screat 2.92mg/dl at 4 weeks). Hct was already sign lower in RK rats (40±3 vs Ctr 45±1%) after 1 week and remained constant thereafter. In AD rats Hct steadily decreased to much lower values: 32±2% at week 4 s and 25±3% at week 8. SHep levels were maximal in RK rats at week 3 (53.6±17.6 vs Ctr 32.3±12.4nM, $p<0.05$) and returned to normal at week 11 (20.5±6.2nM). In AD rats, SHep level was maximal at week 4 (66.3±17.4 vs Ctr 14.8±3.7nM, $p<0.05$) and returned to normal at week 8 (16.32±6.6nM). SEPO levels of AD rats were sign decreased (8.4±2.1 vs Ctr 14.5±6.9pg/ml) at week 8 while remained unchanged in RK rats. Liver Fe was sign increased in AD (86.7±17.5 vs Ctr 54.2±12.3 µg/g ww) and RK rats (107.9±30.1 vs Ctr 64.2±8.3 µg/g ww). In AD rats spleen Fe levels were sign increased also (1024.6±367.3 vs Ctr 334.71±135.6 µg/g ww).

**Conclusions:** In conclusion, AD is a suitable model to study efficacy of anemia treatment since the steep decrease in Hct values goes along with reduced SEPO levels, a substantial rise in SHep, and accumulation of Fe in liver and spleen, the latter pointing to deficient Fe incorporation in hemoglobin.

*Funding:* Pharmaceutical Company Support - Vifor Pharma

## SA-PO141

**Urinary Procollagen Type III Aminoterminal Propeptide Associates with Anemia Independent of eGFR in Male Pre-Dialysis CKD Patients** Akira Suzuki,[1] Kodo Tomida,[1] Tatsuya Shoji,[1] Noriyuki Okada,[1] Yoshiharu Tsubakihara,[2] Terumasa Hayashi.[1] *[1]Kidney Disease and Hypertention, Osaka General Medical Center, Osaka, Japan; [2]Conprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Japan.*

**Background:** Tublointerstitial fibrosis is the common final pathway to the end stage kidney disease. The severity of fibrosis assessed by renal biopsy is correlated with eGFR. Erythropoietin (EPO) is produced by the renal interstitial fibroblasts. In chronic kidney injury, sustained inflammation causes the proliferation and transformation of the fibroblasts to myofibroblasts, which leads to the renal fibrosis. Transformed fibroblasts were reported to be no longer able to produce EPO. Recent studies had demonstrated that the urinary procollagen type III aminoterminal propeptide (PIIINP) was correlated significantly with the fibrosis. In this study, the hypothesis was tested that the urinary PIIINP may be associated with the severity of renal anemia in pre-dialysis ESA naive CKD patients.

**Methods:** Forty one patients (CKD stage 3-5) were recruited at Osaka General Medical Center, who were not treated with ESAs. The following parameters were measured in each patient, such as urine and serum PIIINP, ferritin, Hb, serum and urine creatinine, serum albumin, urinary protein, and CRP. Correlation between Hb and each one of the parameters were cross-sectionally analysed. Patients with iron deficiency, all liver diseases and lung fibrosis were excluded.

**Results:** The population included 33 male and 8 female. Since female strongly correlated with low Hb level in this population, the analysis was stratified by sex. In female, any analysis couldn't be done because of the limited number of patients. In male, univariate analysis revealed that Hb level was correlated with urinary PIIINP/Cr ($R=-0.52956$, $P=0.0015$), but not with any other parameters. In multiple regression analysis including eGFR, urinary PIIINP/Cr was significantly and independently associated with Hb level.

**Conclusions:** These results suggested that renal fibrosis might be more important for anemia than eGFR in male pre-dialysis CKD patients.

*Funding:* Private Foundation Support

## SA-PO142

**Diagnostic Accuracy of Placental Growth Factor in Women with Chronic Kidney Disease/Chronic Hypertension and Superimposed Preeclampsia**
Kate Bramham,[1] Paul Seed,[1] Liz Lightstone,[2] Hayley Tarft,[1] Josephine Gill,[1] Lucilla Poston,[1] Lucy C. Chappell.[1] *[1]Div of Women's Health, King's College London, London, United Kingdom; [2]Imperial College Healthcare NHS Trust Renal and Transplant Centre, Imperial College London, London, United Kingdom.*

**Background:** Women with chronic kidney disease (CKD) and chronic hypertension (CHT) are at increased risk of superimposed pre-eclampsia (SPE). Diagnosis of SPE using blood pressure and proteinuria is challenging because both pre-exist or develop in women with CKD or CHT without SPE. Inaccurate diagnosis may result in unnecessary maternal admission and iatrogenic preterm delivery. Placental growth factor (PlGF) is a secondary marker of associated placental dysfunction in pre-eclampsia (PE), with known low plasma concentrations, but its role in diagnosing SPE in women with CKD or CHT has never been explored.

**Methods:** Women with CKD or CHT, SPE, PE and low risk controls, were recruited from two tertiary antenatal clinics after 20 weeks gestation. Diagnoses of PE or SPE were made according to International Society of the Study of Hypertension in Pregnancy guidelines. Plasma concentrations of PlGF were measured with Alere Triage® assay. Proportions of women with low PlGF (<100pg/ml) were compared.

**Results:** Samples from 129 women with CKD or CHT (90 CKD/39 CHT, including 18 with SPE), 24 women with PE and 71 healthy controls were analysed. Proportions of women with low PlGF are shown in Table 1.

| | PlGF ≥100pg/ml, N (%) | PlGF ≤100 pg/ml, N (%) |
|---|---|---|
| Low Risk | | |
| **PE: Yes** | 1 (4%) | 23 (96%) |
| **PE: No** | 40 (56%) | 31 (64%) |
| CKD or CHT | | |
| **SPE: Yes** | 3 (17%) | 15 (83%) |
| **SPE: No** | 78 (70%) | 33 (30%) |

Low PlGF for diagnosing PE in low risk women had high sensitivity(95.8%) and NPV(97.6%), but lower sensitivity (56.3%) and PPV(42.6%), and for diagnosing SPE had high sensitivity(83.3%), and NPV(96.3%), but lower specificity(70.3%) and PPV(42.6%).

**Conclusions:** Low PlGF is the first test to have such high sensitivity and negative predictive values for SPE in women with CKD or CHT. This finding has substantial implications for the future antenatal management of these women and their offspring.

*Funding:* Pharmaceutical Company Support - ALERE

## SA-PO143

**Adiponectin Receptor 1 Expression in End Stage Renal Disease**
Maria P. Martinez Cantarin, Bonita E. Falkner. *Medicine, Thomas Jefferson Univ Hospital, Philadelphia, PA.*

**Background:** Adiponectin is an anti-atherogenic, anti-inflammatory and anti-diabetic cytokine mainly produced in adipose tissue whose production is increased in End Stage Renal Disease (ESRD). Despite this increased level, ESRD is associated with increased insulin reistance, accelerated atherosclerosis and increased inflammation. The goal of the study was to examine Adiponectin Receptor 1 (AdipoR1) expression in skeletal muscle in ESRD.

**Methods:** A sample from the rectus abdominis muscle was obtained while ESRD participants were undergoing kidney transplantation. Another muscle sample was obtained from healthy kidney donors while they were undergoing donation surgery. AdipoR1 protein expression was quantified by Western Blot and AdipoR1 mRNA expression was determined by real time PCR. C2C12 myoblasts were grown and differentiated to myotubes under standard procedures. After differentiation, the myotubes were exposed to 20% uremic and normal serum for 8h and cells were lysed for protein blotting.

**Results:** AdipoR1 protein and mRNA expression was higher in muscle from ESRD participants than normal kidney function controls ($p<0.05$). C2C12 myoblasts exposed to uremic serum had higher AdipoR1 protein expression than myoblasts exposed to normal kidney function serum.

**Conclusions:** Our study suggests that higher adiponectin levels in ESRD patients are accompanied by higher expression of AdipoR1 in skeletal muscle. Our study also indicates that uremia is a direct stimulus for adipoR1 expression by skeletal muscle. Adiponectin resistance in chronic kidney disease is most likely downstream of the adiponectin 1 receptor.

*Funding:* Private Foundation Support

## SA-PO144

**Bone Histology in 25 ESRD Patients with High Turnover Renal Bone Disease in China**
Ying Qian,[1] Xiaonong Chen,[2] Nan Chen.[3] *[1]Nephrology, Ruijin Hospital, Shanghai, China; [2]Nephrology, Ruijin Hospital, Shanghai, China; [3]Nephrology, Ruijin Hospital, Shanghai, China.*

**Background:** To investigate the bone histology in CKD stage 5 patients on maintenance hemodialysis by bone biopsy, to observe the characteristic of all types of renal bone disease expecially the high turnover bone disease and the relationship between various traditional biomarkers (Scr ,Ca, P, ALP,25(OH)D,Alb, Hb,Hct,iPTH,PINP,OC,β-CTX) and bone tissure markers (BV/TV, % ,Tb.Wi, um ,O.Wi , μm ,OS/BS , % ,ObS/BS, % ,OcS/BS , % ,ES/BS, % ).

**Methods:** Bone biopsy were performed in 40 chronic kidney disease (CKD) stage 5 patients and 3 normal persons. Bone tissues were studied by histomorphometric measurement using an automatic image analysis system. Serum level of biomarkers and bone histomorphometric parameters were studied by correlation analysis.

**Results:** 25 uremic patients (62.5%) were found to have high turnover bone disease according to the bone histology . A significantly characteristic of high turnover bone disease was over activity of osteoclasts with or without bone mineral deposit impairment. There was a good correlation of serum calcium and osteocalcin concentrations with histomorphometric parameters (Ca vs Obs/BS r=0.041, P=0.047. Ca vs Ocs/BS r=0.474, P=0.017. Ca vs ES/BS r=0.485, p=0.014. OC vs Obs/BS r=0.678, P=0.031) . The rate of osteoporosis by BMD in highturnover renal bone disease was 40% and the abdominal aortic calcification rate was 60% by lateral abdominal X-rays. Compare with the BMD and bone X-ray, bone biopsy had the more positive findings and high value for diagnosing of high turnover renal bone disease.

**Conclusions:** High turnover bone disease is still the majority type of bone disease in CKD stage 5 patients in China. Serum level of calcium and osteocalcin may have predictive role in high turnover bone disease in clinical practice. Bone biopsy is still the golden standard for diagnose.

*Funding:* Government Support - Non-U.S.

## SA-PO145

**The Relationship between Insulin Resistance and Cardiovascular Disease in Non-Diabetic CKD Patients**
Hui Peng, Qianqian Wang, Jun Zhang, Cheng Wang, Xun Liu, Yuanqing Li, Meirong Zhong, Tan-qi Lou. *Div of Nephrology, Dept of Medicine, the Third Affiliated Hospital of Sun Yat-sen Univ.*

**Background:** Many studies have shown that insulin resistance is closely related to cardiovascular disease (CVD) in general population. However, the correlation has not been well established in non-diabetic chronic kidney disease (CKD). The present study wants to investigate the relationship of insulin resistance (IR) and CVD in non-diabetic CKD patients.

**Methods:** 25 non-diabetes non-dialysis CKD patients (stage 2-5) were enrolled in this cross-sectional observational study. IR index was assessed by the homeostasis model assessment. They were divided into two groups according to IR index: insulin-resistant group(HOME-IR≥2, N=7) and insulin-sensitive group(HOME-IR<2, N=18). SBP and DBP, left atrium diameter (LAD),interventricular septal thickness(IVST), left ventricular diameter(LVD), left ventricular posterior wall thickness (LVPWT),cardiac output(CO), ejection fraction(EF),E/A, cardiac index(CI), carotid artery intima-media thickness (CAIMT),HCO3-, serum creatinine, eGFR(calculated by CKD-EPI formula),blood-lipids and urine protein were collected. All data was analyzed by software SPSS 13.0.

**Results:** Serum creatinine, eGFR, blood-lipids and urine protein were not found significantly different between both groups. Correlation analysis showed that IR was not significantly related to SBP or DBP, LAD, IVST, LVD, LVPWT, CO, EF, E/A, CI, CAIMT or HCO3-. Nevertheless, SBP and DBP, LAD,IVST, LVD, LVPWT, CO, EF, E/A and CI were not significant different between both groups. However, insulin-resistant group had significantly thicker left carotid artery intima-media than insulin-sensitive group (1.050+0.129 vs 0.7455+0.242, P=0.035) as well as significantly less serum $HCO_3^-$ (18.329+2.888 vs 21.750+3.453, P=0.03).

**Conclusions:** This small-sample pilot study indicated IR may have no apparent correlation with cardiac function or cardiac morphological changes in non-diabetic CKD patients. However, those CKD patients with IR had thicker left carotid artery intima-media than insulin-sensitive patients, which suggested atherosclerosis was more severe in those non-diabetic CKD patients with IR.

## SA-PO146

**Important Factors Considered in Excluding Patients from Referral for Kidney Transplant – A Survey of Nephrologists' Views**
Ankita Tandon, Jeffrey P. Yourshaw, Kevin C. Roe, Nasrollah Ghahramani. *Dept of Medicine (Nephrology), Penn State College of Medicine - Hershey Medical Center, Hershey, PA.*

**Background:** Provider perceptions about appropriateness of certain patients for kidney transplant (KT) are potentially important contributors to disparities in KT. We examined nephrologists' perceptions about factors which they consider important in excluding patients from KT referral and analyzed the association between these perceptions and nephrologists' demographic and practice characteristics.

**Methods:** Invitations were sent to 3180 nephrologists in the eastern US. 822 expressed interest, and 250 were randomly invited to complete a questionnaire about demographics, practice characteristics, and their perceptions of the importance of various reasons a nephrologist might not refer patients for KT. A total of 216 surveys with complete responses were analyzed. Chi-square and stepwise logistic regression were performed.

**Results:** The three most common reasons regarded by nephrologists as important for excluding patients were "patient's inadequate social support" (70% of respondents), "limited understanding of the transplant process due to patient's inadequate education" (56%), and "patient's age above 65" (53%). In multivariate analysis, physicians with 2 or fewer transplant centers within a 50 mile radius were more likely to report inadequate social support (OR: 3.15; 95% CI:1.59-6.24; p=0.001) and age greater than 65 years (1.88; 95% CI: 1.01 to 3.49; p=0.04) as reasons to exclude patients from KT referral. Considering limited understanding due to patient's education, as an important reason to exclude patients for KT evaluation was more likely among nephrologists whose practice includes patients, majority of whom, have not completed high school (OR: 3.31; 95% CI: 1.60-6.86; p=0.001).

**Conclusions:** Patient's social support, understanding and age are the most common reasons regarded by nephrologists as important for excluding patients from KT referral. Practice location and overall characteristics of the patient population are important determinants of provider perceptions about the importance of these reasons.

*Funding:* NIDDK Support

## SA-PO147

**Abstract Withdrawn**

## SA-PO148

**Depression Is Associated with Missed Hemodialysis Treatment**

Veeda O. Landeras,[1] Michael S. Simonson,[1] Sharon D. Aaron,[3] Marcia R. Silver.[2] *[1]Medicine, Div of Nephrology and Hypertesnion, Univ Hospitals Case Medical Center, Cleveland, OH; [2]Medicine, Div of Nephrology and Hypertension, MetroHealth Medical Center, CLeveland, OH; [3]Social Services, Fresenius Medical Center, Cleveland, OH.*

**Background:** Depression is the most common psychological problem encountered in patients with end-stage renal disease (ESRD). Recent studies demonstrated that psychosocial factors are important predictors of patient outcome. Co-occurrence of depression has been shown to be one of the risk factors of poor prognosis in dialysis patients, partly because it is believed that depressed patients are less likely to adhere to their medication regimen and modify their lifestyle appropriately. Because clinical depression is prevalent among patients with ESRD on hemodialysis, we examined the relationship between clinical depression and missed hemodialysis treatments in an outpatient hemodialysis center.

**Methods:** We conducted a cross-sectional analysis of 71 outpatient adult hemodialysis patients. Inclusion criteria: age > 18 years and able to take written Beck Depression Inventory (BDI) in English. Exclusion criteria: clinical dementia. Patients scored positive for missed dialysis treatments if they had more than two unexcused absences over 12 months.

**Results:** Participantswere 38 % female and 45% African American, and average age was 55. The numerical BDI score correlated positively with missed dialysis treatments ($r$ = 0.248, $P$=0.037). The correlation between BDI score and missed dialysis remained significant after adjusting for age, race and sex in a logistic regression model.

**Conclusions:** Higher BDI score, suggesting the presence of depression, was associated with missed hemodialysis treatments in this pilot study. Future studies should include larger number of hemodilaysis patients and examine other covariates that might contribute to missed hemodilaysis treatments. Also, looking into whether effective intervention, Pharmacological and non-pharmacological, would be at value in this hemodialysis population subset to improve hemodialysis attendence.

*Funding:* Private Foundation Support

## SA-PO149

**Perceptions Regarding Genetic Testing in Populations at Risk for Nephropathy**

Barry I. Freedman,[1] Alison J. Fletcher,[1] Vivek R. Sanghani,[1] Angellina Graham,[1] Jessica N. Cooke Bailey,[1] Ana Iltis,[2] Nancy M.P. King.[2] *[1]Wake Forest Sch Med; [2]Wake Forest.*

**Background:** Genetic testing will become increasingly available over the next decade. It remains important to determine attitudes toward applying test results.

**Methods:** Relative to European Americans (EA), African Americans (AA) are at increased risk for ESKD and biologically mediated factors contribute. Concepts regarding acceptability of genetic testing for disease variants associated with common illnesses were explored in a standardized questionnaire administered to 130 individuals (64 AA; 66 EA) at risk for ESKD based on a 1st-degree relative with ESKD. In AA and EA, mean+SD age of surveyed relatives was 45.5+12.8 and 50.5+14.4 yrs, resp. (p=0.04); with similar relationships to ESKD relatives (child, sib, parent) (p=0.22).

**Results:** AA and EA, resp., wished to know their genetic test results if risk could be: (1) reduced by diet or exercise (100% and 98.5%, p=0.99); (2) reduced by physician treatment (100% and 98.5%, p=0.99); (3) no treatments were available (90.5% and 81.8%, p=0.21). If informed they did not have a disease susceptibility variant, 86.9% of AA and 87.9% of EA would be extremely- or pretty-likely to inform relatives of test results (p=0.84). If informed they had a disease risk variant, 91.8% of AA and 89.4% of EA would be extremely- or pretty-likely to inform relatives of test results (p=0.43). This exploratory assessment of attitudes toward accruing and applying genetic test data in family members at risk for ESKD revealed similar attitudes among AA and EA. Both populations generally appear equally receptive to gathering and applying their genetic test results for common disease. A substantial majority of people with a close relative having ESKD would want genetic susceptibility information regardless of the availability of prevention or treatment and would share this information with relatives.

**Conclusions:** These results suggest the importance of careful study design and disclosure regarding return of research results. They provide important information about how particular populations think about access to genetic information and highlight the desirability of extensive information about genetic susceptibility tests.

*Funding:* NIDDK Support

## SA-PO150

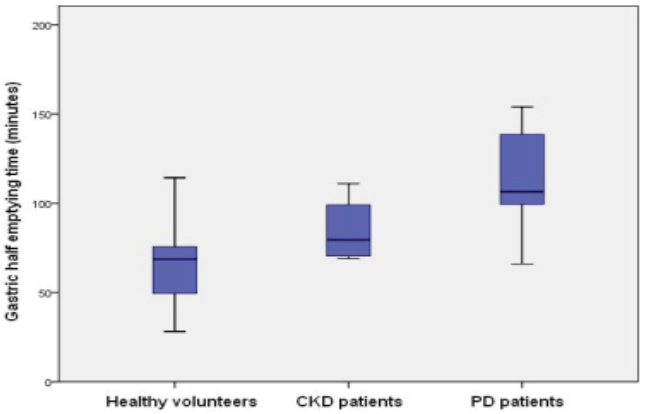
**Dynamic MRI Assessment of Gastrointestinal Motility in Chronic Kidney Disease Patients**

Laura E.A. Harrison,[1] Caroline Louise Hoad,[2] Luca Marciani,[2,3] Penny Anne Gowland,[2] Chris W. McIntyre.[1,4] *[1]Dept of Renal Medicine, Royal Derby Hospital, United Kingdom; [2]School of Physics and Astronomy, Univ of Nottingham, United Kingdom; [3]Nottingham Digestive Diseases Centre, Univ of Nottingham, United Kingdom; [4]Div of Medical Sciences and Graduate Entry Medicine, Univ of Nottingham, Derby, United Kingdom.*

**Background:** Gastrointestinal dysfunction is common in chronic kidney disease (CKD) and associated with malnutrition and inflammation. We assessed gastric emptying in Peritoneal Dialysis (PD) and CKD patients as well as Healthy volunteers (HV) utilising Magnetic Resonance Imaging (MRI) based techniques.

**Methods:** Participants underwent serial MRI fasted and after a mixed liquid/solid meal with measurement of gastric volumes, gastric emptying and motility. Gastric half emptying time (GE $T_{50\%}$) was calculated. 32 participants (8 PD, 8 CKD, 16 HV) were recruited. Data collection included demographics, bloods and GI symptom questionnaires.

**Results:** Gastric volumes immediately post meal ($T_0$) were around 340mls in each group but fell more slowly in PD patients compared to HV. Gastric half emptying time was progressively longer with worsening renal function and slowest in patients on PD. Median GE $T_{50\%}$ in HV was 69 mins [50-76], CKD 72 mins [70-80], PD 107mins [66-140], p=0.005. Three hours post meal, 25% of PD patients had over 30% of $T_0$ volume remaining, compared to 0% in CKD and HV groups. PD patients had significantly higher scores than HV on the Gastrointestinal Symptoms Rating Scale (p=0.01).




**Conclusions:** Gastric emptying is delayed with worsening renal failure and more prolonged in patients receiving peritoneal dialysis, suggesting that dialysis has an additional effect to uraemia related gastric dysmotility. Greater understanding of the factors influencing GI dysfunction is required to address the symptom burden and nutritional challenges these patients are subject to.

*Funding:* Pharmaceutical Company Support - Baxter Healthcare: Research Grant

## SA-PO151

**Structural, Physical and Functional Characterization of Soluble Ferric Pyrophosphate (SFP), a Novel Iron Compound for Pharmaceutical Applications**

Rameshwar Shukla,[1] Bhoopesh Mishra,[2] Raymond D. Pratt,[1] Ajay Gupta.[1] *[1]Rockwell Medical; [2]Illinois Institute of Technology.*

**Background:** Soluble ferric pyrophosphate (SFP) is the first complex iron salt that does not have a carbohydrate shell for parenteral use. Food grade SFP (FCC-SFP) is a poorly-defined preparation of uncertain composition and is not suitable for pharmaceutical applications. RM-SFP is produced under Good Manufacturing Practice (GMP). RM-SFP has an approximate molecular mass of about 1500 Da and is highly soluble in aqueous solutions. These properties allow its infusion via dialysate (Gupta, Kidney Int. 1999). SFP recently completed Phase III trials in CKD-HD patients.

**Methods:** We synthesized and characterized a RM-SFP, a water-soluble mixed chelate of iron with pyrophosphate (PPi) and citrate (RM-SFP), comprising 7-11% iron, 14-30% citrate and 10-20% PPi (Rockwell Medical Inc., Michigan, US Patent: US 7816404 B2). Because of the amorphous nature of the compound, structural characterization was carried out using Fe K edge X-ray Absorption Near Edge Structure (XANES) and Extended X-Ray Absorption Fine Structure (EXAFS) Spectroscopy to ascertain differences in the coordination environment of Fe in FCC-SFP vs RM-SFP.

**Results:** Linear combination fitting of Fe XANES data showed that iron is in the ferric ($Fe^{3+}$) state and does not complex with sulfate in FCC-SFP or RM-SFP. EXAFS analysis demonstrated that Fe complexation with O in the first coordination sphere (2.02 Å) is similar in both of these molecules. However, significant differences are observed in the coordination environment of Fe with P (3.22 Å) and Fe with and C (2.98 Å) binding in the second coordination sphere of Fe in FCC-SFP compared to RM-SFP. With RM-SFP, significantly faster iron transfer to apotransferrin was observed in-vitro as compared to FCC-SFP.

**Conclusions:** RM-SFP is significantly more soluble than FCC-SFP. In contrast to IV iron-carbohydrate iron complexes, SFP's fast binding kinetics allows $Fe^{3+}$ to bind to apotransferrin (in the blood) for direct transport to the erythron, thereby avoiding reticulo-

endothelial processing and storage. RM-SFP is a unique iron compound and a promising new means of providing iron to CKD-HD patients.

*Funding:* Pharmaceutical Company Support - Rockwell Medical Inc.

## SA-PO152

**Aortic and Mitral Annular Calcification in Patients with CKD and Diabetes**

Yoshitsugu Obi,[1] Takayuki Hamano,[2] Chikako Nakano,[1] Yasuo Kusunoki,[1] Akihiro Shimomura,[1] Kazunori Inoue,[1] Isao Matsui,[1] Hiromi Rakugi,[1] Yoshiharu Tsubakihara,[2] Yoshitaka Isaka.[1] *[1]Dept of Geriatric Medicine & Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Dept of Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

**Background:** Both CKD and diabetes are well-known risk factors for vascular calcification. We have reported that patients with CKD and diabetes have severe coronary artery calcification (CACS) and high serum calcium-phosphate product. Annular calcification may bring about valvular diseases with stenosis or regurgitation, potentially leading to heart failure. However, the prevalence and etiology of aortic (AAC) and mitral (MAC) annular calcification in CKD with diabetes still remain unclear.

**Methods:** In this cross-sectional study, we quantified AAC, MAC, coronary artery calcification (CAC), and descending aortic calcification (DAC) in patients with stage 3-4 CKD and diabetes using multi-slice computed tomography and the Agatston method. We also assessed the relationships among calcification scores using Spearman's correlation test. Then, the association of calcification scores with patient characteristics was evaluated using multivariate ordinal logistic regression.

**Results:** Among 44 participants, 19 (43%) and 6 (14%) patients had AAC and MAC, respectively. AAC showed a moderate correlation with DAC ($r_s = 0.37$), while only a weak correlation with CAC ($r_s = 0.13$). Older age and lower eGFR were significantly associated with higher AAC and DAC. Other identified risk factors were higher LDL cholesterol for AAC (OR, 3.1 [95%CI, 1.3 to 7.5] per SD, $P = 0.013$) and higher calcium-phosphate product for DAC (OR, 2.2 [95%CI, 1.2 to 4.1] per SD, $P = 0.017$).

**Conclusions:** In patients with stage 3-4 CKD and diabetes, AAC was more common than MAC, and was correlated with DAC, but not with CAC. LDL cholesterol, not calcium-phosphate product, was independently associated with AAC in this population. The different risk factors among AAC, MAC, CAC, and DAC suggest that the different mechanisms are involved in developing calcification.

## SA-PO153

**Reduced Kidney Function, Metabolic Disorders and Male Gender Are Correlates of Serum Uric Acid in Patients Hospitalized due to a Possible Acute Coronary Syndrome**

Zofia I. Niemir,[1] Malgorzata Stasiak-paczkowska.[2] *[1]Dept of Nephrology, Univ of Medical Sciences, Poznan, Poland; [2]Dept of Internal Diseases, HCP Medical Centre, Poznan, Poland.*

**Background:** Raised serum uric acid (Sua) is a known risk factor for the development and progression of both coronary artery and chronic kidney disease. Since acute coronary syndrome (ACS) is a leading cause of morbidity and mortality worldwide, we looked for Sua and its potential relates in patients with this complication.

**Methods:** The study involved 135 consecutive patients with an initial assumption of ACS made in the emergency unit during the period of 3 months and thus hospitalized. The group consisted of 62 females (F) and 73 males (M) with median ages 74 and 63, respectively ($p<0.001$). In addition to the routine cardiologic checkup, and before angiography, serum creatinine (Scr), Sua, lipid profile, albumin, glucose (G), and C-reactive protein (CRP) were measured. Congestive heart failure (CHF) was graded using the New York Heart Association classification. Body mass index (BMI) and estimated glomerular filtration rate (eGFR) were calculated.

**Results:** The median Sua was 5.3 mg/dl in F (2.3 to 15.2 mg/dl) and 6.2mg/dl in M (3.4 to 11.5mg/dl). Diabetes mellitus (DM) was diagnosed in 43.5% of F and 42.5% of M, while CHF in 69.3% of F and 64.4% of M. GFR below 60 ml/min/1.73m$^2$ was found in 54.8% of F and 41.1% of M. After all patient arrangement into Sua quartiles, 37.1% of F allocated to the first quartile vs. 19.5% of M ($p=0.03$). DM was stated in 29.7% of patients from the first compared to 60.6% in the fourth quartile ($p=0.0155$), while CHF in 48.6% and 87.8% ($p<0.001$), respectively. Significant differences between the first and fourth quartile values of Scr ($P<0.001$), eGFR ($p<0.001$), BMI ($p<0.01$), G ($p<0.01$), age ($p<0.05$), and triglycerides (TGL; $p<0.05$) were found. The results of multiple regression analysis showed that eGFR (b=-0.4504; $p<0.0001$), TGL (b=0.3219; $p=0.0002$), BMI (b=0.2598; $p=0.0029$), and male gender (b=0.1830; $p=0.0379$) were independent correlates of Sua in patients with ACS.

**Conclusions:** Our results point to Sua as a biomarker of metabolic and kidney disorders that are involved in the development of ACS.

*Funding:* Government Support - Non-U.S.

## SA-PO154

**Subclinical Cardiovascular Disease Is Associated with High Glomerular Filtration Rate in the Non-Diabetic General Population**

Bjorn Odvar Eriksen,[1,2] Kjell Arne Arntzen,[1,2] Marit Herder,[2] Ulla Dorte Mathisen,[2] Toralf Melsom,[2] Marit D. Solbu,[2] Maja-lisa Løchen.[1,2] *[1]Univ of Tromsø, Norway; [2]Univ Hospital of North Norway, Norway.*

**Background:** Reduced GFR in chronic kidney disease is a risk factor for cardiovascular disease. However, evidence indicates that high normal GFR in apparently healthy persons may also be a cardiovascular risk factor. At present, this issue remains unresolved due to a lack of longitudinal studies of manifest cardiovascular disease with precise GFR measurements. Previous longitudinal studies all used estimates of GFR based on creatinine or cystatin C, which are inaccurate at high levels. Therefore, we performed a cross-sectional study to assess the relationship between high GFR measured as iohexol-clearance and subclinical signs of cardiovascular disease.

**Methods:** GFR was measured in the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6), which consists of a representative sample of middle-aged persons from the general population without prevalent cardiovascular disease. A total of 1521 persons without chronic kidney disease, diabetes, or micro- or macroalbuminuria were investigated with carotid ultrasonography and electrocardiography.

**Results:** GFR in the highest quartile (> 101 mL/min/1.73 m$^2$) was associated with an increased odds ratio of having total carotid plaque area greater than the median of non-zero values (odds ratio 1.56, 95% confidence interval 1.02-2.39) or electrocardiographic signs of left ventricular hypertrophy (odds ratio 1.62, 95% confidence interval 1.10-2.38) compared to the lowest quartile. The analyses were adjusted for cardiovascular risk factors, urinary albumin excretion and fasting serum glucose.

**Conclusions:** High GFR is associated with carotid atherosclerosis and left ventricular hypertrophy in the general middle-aged population and should be investigated as a possible risk factor for manifest cardiovascular disease in longitudinal studies.

*Funding:* Government Support - Non-U.S.

## SA-PO155

**Effects of Low Sodium Intake on the Anti-Proteinuric Efficacy of Olmesartan in Hypertensive Patients with Albuminuria**

Chun Soo Lim,[1] Jin Ho Hwang,[1] Ho Jun Chin,[2] Sejoong Kim,[2] Bum Soon Choi.[3] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Seoul National Univ Bundang Hospital, Seongnam, Kyunggi-Do, Republic of Korea; [3]Dept of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Republic of Korea.*

**Background:** Blockade of the renin angiotensin aldosterone system (RAAS) reduces albumin excretion rate. The antiproteinuric effect of RAAS blockade can be magnified by dietary salt restriction. We tried to determine the effect of low salt diet in non-diabetic hypertensive patients with albuminuria on blood pressure and urine protein excretion.

**Methods:** This study is open label randomized controlled trial. During a run-in period of eight weeks, patients received Olmesartan. And then, patients were divided into two groups. One group was treated for another eight weeks with Olmesartan plus conventional low salt diet (LSD) education and the other group was treated for eight weeks with Olmesartan plus weekly intensive education on LSD. A total of 270 adult recipients were enrolled, and we performed interim analysis with 195 patients.

**Results:** During a run-in period, daily urinary albumin excretion was significantly decreased after taking Olmesartan for eight weeks (from 928.9 ± 980.8, to 508.6 ± 678.5 mg/day, $P<0.001$). Recipients with intensive LSD education showed more decreased urinary albumin excretion compared to control group (for Δproteinuria, 146.8 vs. -8.3 mg/day, $P=0.028$). The rate of more than 25% reduction in proteinuria was higher in the recipients with LSD education (61.9% vs. 36.7%, $P=0.003$). Urinary sodium excretion for 24-hr was decreased 26.0 mEq/day in the intensive education group. With more reduction in 24-hr urinary sodium excretion, there were tendency of more decrease in systolic BP and urinary albumin excretion (for ΔsBP, 10.1 vs. 8.5 mmHg, $P>0.05$; for Δproteinuria, 221.6 vs. -20.1 mg/day, $P=0.001$).

**Conclusions:** The reduction in salt intake reduced urine protein excretion and systolic BP in RAAS blockade treated hypertensives. Weekly intensive education on LSD would be a suitable method for clinical practice.

*Funding:* Pharmaceutical Company Support - Daiichi Sankyo Company

## SA-PO156

**Urinary Sodium and Kidney Failure in Chronic Kidney Disease**

Li Fan,[1] Hocine Tighiouart,[1] Andrew S. Levey,[1] Gerald J. Beck,[2] Mark J. Sarnak.[1] *[1]Tufts Medical Center; [2]Cleveland Clinic.*

**Background:** Current guidelines recommend less than 2 grams per day sodium intake in individuals with chronic kidney disease (CKD), but there are few data relating sodium intake to long-term outcomes in this population.

**Methods:** 840 patients with CKD enrolled in the Modification of Diet in Renal Disease (MDRD) Study were used to evaluate the association of mean baseline 24-h urinary sodium excretion (as a proxy for sodium intake) with kidney failure (defined as need for dialysis or transplantation) using multivariable Cox proportional hazards models.

**Results:** Mean age was 52 ± 12 years, 60% were men and 85% white. Median proteinuria was 0.32 (IQR, 0.07-1.51) g/day and mean 24-h urinary sodium excretion 3.46 ± 1.13 g/day. 617 participants developed kidney failure during a median follow-up of 6 (range, 0.25 -18.61) years. A two slope model with a cut-off urine sodium of 3 g/day best

fit the data. In those with urine sodium ≥ 3 g/day there was no association between urine sodium and kidney failure in multivariable models. In those with urine sodium < 3 g/day higher urine sodium was associated with increased risk of kidney failure in those with proteinuria <1 g/day [HR 1.72 (95% CI, 1.31-2.24) per 1 g/day urine sodium higher], and lower risk of kidney failure in those with proteinuria ≥1 g/day [HR 0.61 (95% CI, 0.42-0.89) per 1 g/day urine sodium higher]($p$ < 0.001 for interaction). Results were similar using time-dependant values for urine sodium.

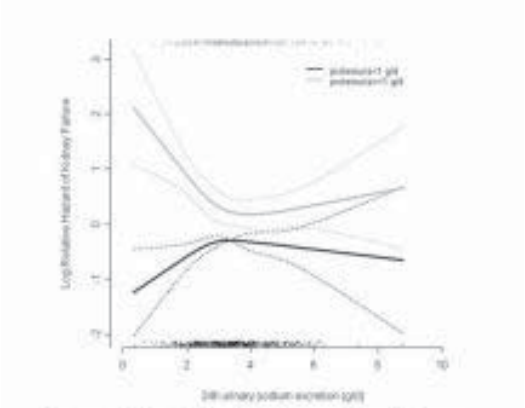


**Figure** Multivariable adjusted association between urine sodium and kidney failure stratified by baseline proteinuria. Splines were adjusted for age, sex, race, cause of kidney disease, measure GFR, log urine protein, BMI, SBP, LDL cholesterol, HDL cholesterol, smoking, diabetes, history of CVD, ACE inhibitor use, diuretic use, randomization to study A or B, and BP and dietary protein target.

**Conclusions:** The association of sodium intake with kidney failure depends on the absolute level consumed, as well as the level of proteinuria. These results need to be verified in additional studies and the mechanism explaining them explored.

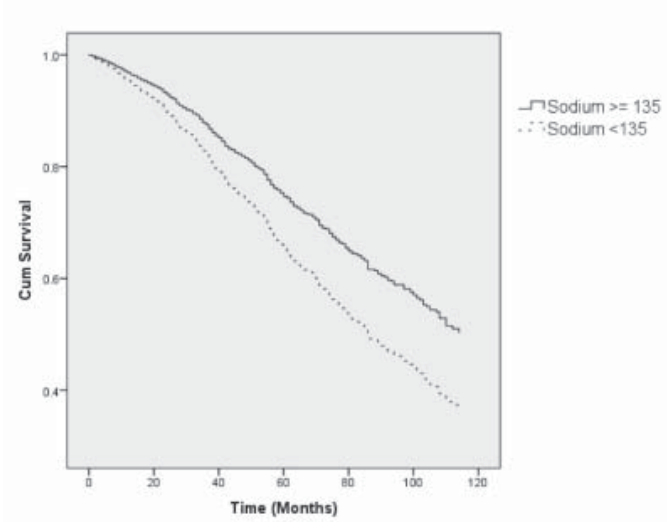
## SA-PO157

**Hyponatremia Is Associated with All-Cause Mortality in Chronic Kidney Disease** Diana Chiu, Darren Green, James Ritchie, Smeeta Sinha, Philip A. Kalra. *Salford Vascular Research Group, Univ of Manchester, MAHSC, Manchester, United Kingdom.*

**Background:** Hyponatremia is one of the commonest electrolyte abnormalities encountered in hospitalized patients. Few studies have investigated the association between hyponatremia and all-cause mortality in Chronic Kidney Disease (CKD).

**Methods:** A cross-sectional analysis of data from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS), a prospective observational study of outcome in patients with all-cause CKD stages 3-5, managed in secondary care, was carried out. Hyponatremia was defined as baseline serum sodium<135mmol/l. Predictors of all-cause mortality were selected from forward stepwise cox regression (including potential confounders: age, gender, smoking status, systolic and diastolic blood pressure, history of heart failure, previous myocardial infarction, diuretic use, renin angiotensin blocker use, eGFR, albumin, hemoglobin).

**Results:** From 2093 patients (mean eGFR 33±16 ml/min/1.73m$^2$), there were 1311 (63%) men, 660 (32%) diabetics, 346 (17%) had a history of heart failure, and 1002 (47.9%) patients were taking diuretics. Mean serum sodium was 140±3mmol/l and 96 (4.6%) patients had hyponatremia (mean 132±2mmol/l, range 125-134mmol/l). After a mean follow-up of 48±33 months, there were 684 (32.7%) deaths. In the final cox regression model, hyponatremia remained a significant independent predictor of all cause mortality (Hazard ratio 1.44, p=0.03, 95% CI 1.03-2.02).




**Conclusions:** There is an association between hyponatremia with all-cause mortality in CKD. It is uncertain whether this is a reflection of the adverse effects of hyponatremia or it is a surrogate marker of existing comorbidities. Further studies are needed to elucidate the cause.

## SA-PO158

**The Effects of Cigarette Smoking in Patients with Chronic Kidney Disease (CKD): Results from the Study of Heart and Renal Protection (SHARP)** Natalie Staplin. *On Behalf of the SHARP Collaborative Group, Clinical Trial Service Unit & Epidemiological Studies Unit, Univ of Oxford, United Kingdom.*

**Background:** Smoking tobacco is a major cause of vascular and non-vascular morbidity and mortality in the general population, but its absolute and relative importance in people with CKD, particularly its relevance to renal progression, is uncertain.

**Methods:** The SHARP study was a randomized trial of LDL cholesterol lowering with ezetimibe/simvastatin in 9270 patients with CKD followed for a median of 4.9 years. The relative risks of particular outcomes associated with baseline smoking status were estimated by Cox regression. Analyses were adjusted for age, sex, ethnicity, country, education and prior disease (vascular disease or diabetes mellitus).

**Results:** At screening, 1243 patients (13%) were current cigarette smokers (median consumption 10 per day), 3272 (35%) were former smokers and 4755 (51%) were never smokers. Compared with never smokers, vascular event risk was 36% higher among current smokers (adjusted hazard ratio 1.36, 95% CI 1.19-1.54) reflecting a 49% increase in atherosclerotic (1.49, 1.26-1.75) and a 25% increase in non-atherosclerotic (1.25, 1.05-1.49) events. All-cause mortality was 48% higher among current smokers than never smokers (HR 1.48, 95% CI 1.29-1.69), with significant increases seen for both vascular (1.35, 1.07-1.69) and non-vascular (1.59, 1.33-1.90) causes of death. Among 6247 patients not on dialysis at randomization, rates of end-stage renal disease (initiation of dialysis or renal transplantation) were similar among current and never smokers (HR 1.02, 95% CI 0.89-1.16) as were (the statistically more sensitive) estimates of annual rates of change in eGFR (mL/min/1.73m$^2$/year: current smokers -2.10 [SE 0.18], former smokers -2.04 [0.11], never smokers -1.97 [0.09]).

**Conclusions:** While smoking does not seem to accelerate renal progression in patients with CKD, it increases vascular morbidity and both vascular and non-vascular mortality to a similar proportional extent as is seen in other populations. The absolute excess risks attributable to smoking in this high risk population are therefore large, and the potential benefits of cessation substantial.

*Funding:* Pharmaceutical Company Support - The SHARP study was funded mainly by Merck/Schering-Plough Pharmaceuticals (North Wales, PA, USA), Government Support - Non-U.S.

## SA-PO159

**Association of Food Insecurity and End Stage Renal Disease (ESRD) among a National Cohort of Low-Income Adults with Chronic Kidney Disease (CKD)** Tanushree Banerjee,[1] Deidra C. Crews,[2] Donald E. Wesson,[3] Sai Hurrish Dharmarajan,[4] Rajiv Saran,[4] Sharon Saydah,[5] Nilka Rios Burrows,[5] Neil R. Powe.[1] *[1]UCSF; [2]JHU; [3]Texas A&M College of Medicine; [4]UM; [5]CDC.*

**Background:** Poor access to food among low income adults has been recognized as a risk factor for CKD, but there is little data on the impact of food insecurity on development of ESRD. We hypothesized that food insecurity would be independently associated with developing ESRD, that food insecurity would be associated with dietary acid intake and dietary acid intake would modify associations between food insecurity and development of ESRD.

**Methods:** We conducted a national,longitudinal cohort study of 1,451 adult (>20 years) with CKD enrolled in NHANES 2001-2004 with a household income ≤400% of the federal poverty level (FPL). Food insecurity was defined as ≥3 affirmative responses

on the 18-item questionnaire. Net Acid Excretion (NAE) was determined by the dietary recall questionnaire (24 hrs) using a model by Remer and Manz. Development of ESRD was ascertained over an average of 5.4 years of follow-up via linkage to Medicare ESRD Registry. We used a Fine-Gray competing risk model to estimate hazard ratio [HR] for ESRD associated with food insecurity after adjusting for demographics, socio-economic position, diabetes, hypertension, eGFR, urinary albumin:creatinine ratio,daily caloric intake, and NAE.

**Results:** 125 (8.6%) adults with CKD were food insecure. Median NAE in the food secure vs food insecure group was 48.4 mEq/day vs 52.8 mEq/day,(p=0.04). Food insecure individuals were more likely to be males (41.6%), blacks (30.4%), have diabetes (41.6%), or have albuminuria (74.4%) compared to their counterparts (p<0.05). Food insecure adults were far more likely to develop ESRD (HR [95% CI]: 5.5 [5.3-5.6]) compared to food secure adults after adjustment for potential confounders. NAE did not appear to be a mediator of the association between food insecurity and ESRD.

**Conclusions:** Among persons with CKD with a household income ≤400% of the FPL, food insecurity is strongly and independently associated with development of ESRD. Innovative approaches to address food insecurity among CKD patients may be warranted.

*Funding:* Other U.S. Government Support

## SA-PO160

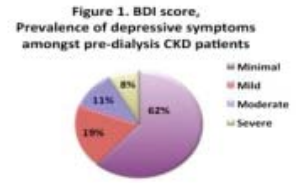
**The FRIENDS Study: Familiarization of Research Regarding Satisfaction with Life and Ways of Coping amongst Chronic Kidney Disease Patients at a Canadian Center** Sassan Ghazan-Shahi, Karen E. Yeates. *Medicine, Queen's Univ, Kingston, Canada.*

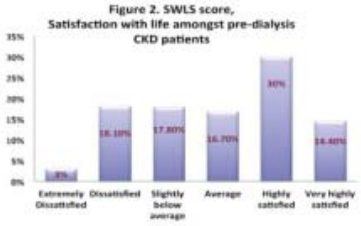
**Background:** The patients with moderate to advanced chronic kidney disease (CKD) are perceived to struggle with high co-morbidity burden related to living with chronic disease. It is the perception that patients may suffer from higher depressive symptoms and poor satisfaction with life and may lack coping mechanisms.The main objectives of this study were to determine the severity of reported depressive symptoms by the subjects and identify the self-perception of satisfaction with life and investigate the existence and frequency of coping strategies.

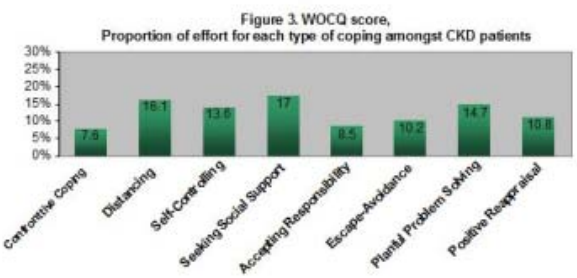
**Methods:** A cross-sectional observational study of Stage III-V CKD patients, enrolled in the multidisciplinary CKD clinic at Kingston General Hospital, Queen's University. The measures used in this study evaluate emotional health and coping through the use of following scales. **Emotional health** § Well-being:Satisfaction with Life Scale (SWLS); § Depression:Beck Depression Index (BDI); **Coping** § Strategy identification:Ways of Coping Questionnaire (WOCQ). Data collection was via self-administered questionnaires and interview.

**Results:** Total of 192 patients fully completed our questionnaires including demographic data and the study measures. 107 patients (55.7%) were male. Mean age ± Standard deviation (Min, Max):70.8±12.5(28.4, 94.9)

62% of patients described minimal depressive symptoms, and 8% described severe symptoms.30% were satisfied with their lives while 18% were dissatisfied. In the Ways of Coping,16% of subjects coped with stressors by distancing themselves and 17% sought social support.




**Conclusions:** Despite having chronic kidney disease, a large proportion of subjects described relatively good satisfaction with life and lower than expected depressive symptoms. Further adjustment with subject co-morbidity and degree of renal insufficiency may yield more specific correlation with these results.

## SA-PO161

**Neighborhood Poverty: A Surprisingly Poor Predictor of Lack of Pre-End-Stage Renal Disease Care at U.S. Dialysis Facilities** Laura Plantinga, Min Kim, Margarethe Goetz, Rachel E. Patzer. *Emory Univ, Atlanta, GA.*

**Background:** Receipt of nephrology care prior to end-stage renal disease (ESRD) is a strong predictor of improved ESRD outcomes, but 34% of U.S. ESRD patients begin dialysis having never seen a nephrologist. Because pre-ESRD care varies geographically, we examined whether neighborhood poverty was associated with lack of pre-ESRD care at facilities.

**Methods:** Geospatially linked data from the 2007-2010 Dialysis Facility Report and 2006-2010 American Community Survey were used in hierarchical mixed-effects models to assess the association of neighborhood poverty (≥20% of households in census tract living below poverty) with facility-level lack of pre-ESRD care (% of patients at a facility with no nephrology care prior to dialysis start), adjusting for facility and neighborhood confounders and allowing for neighborhood and network random effects.

**Results:** Among 5244 facilities, 1799 (34.3%) were located in a poverty area. With adjustment, facility location within a poverty area was not associated with lack of pre-ESRD care: the absolute increase in percentage of patients at a facility with no pre-ESRD care associated with facility location in a poverty area vs. other neighborhood was only 0.1% (95% CI: -13.2%, 14.7%; *P*=0.9). When poverty was examined continuously, potential effect modification by Gini index of income inequality was detected, such that the effect of poverty at a lower, but not higher, level of income inequality was modest but statistically significant.

| Tertile of Gini index | Tertile of % poverty | Absolute % increase in facility-level lack of pre-ESRD care (vs. 0% poverty) | *P* |
|---|---|---|---|
| High (45) | High (20%) | 0.66 | 0.258 |
| High (45) | Low (10%) | 0.32 | 0.258 |
| Low (35) | High (20%) | 1.59 | 0.005 |
| Low (35) | Low (10%) | 0.77 | 0.005 |

**Conclusions:** Despite previously reported detrimental effects of neighborhood poverty on health, it appears to have no substantial effect on facility-level lack of pre-ESRD care in this multi-level, ecological study. However, the observed statistical interaction with Gini index suggests that homogeneity of neighborhood poverty as a predictor of lack of pre-ESRD care may warrant further investigation.

*Funding:* Other NIH Support - NIMHD

## SA-PO162

**Perceived Racism and Discrimination in Health Care and Medical Mistrust Predict Poorer Health Related Quality of Life in End-Stage Kidney Disease Patients** Michael A. Freeman,[1] John R. Pleis,[2,3] Ron Shapiro,[1] Larissa Myaskovsky.[1,3] *[1]School of Medicine, Univ of Pittsburgh; [2]School of Public Health, Univ of Pittsburgh; [3]VA Pittsburgh Healthcare System.*

**Background:** Health-related quality of life (QOL) is a multi-dimensional concept which measures a patient's subjective experience of their physical, mental, emotional and social functioning. Although many non-medical factors (i.e. financial stress, crime) that influence QOL are beyond the scope of the medical community to address, factors such as experiences of discrimination, racism, and mistrust in health care occur within the medical setting and may be influenced during the course of medical care. Within medicine there has been a renewed focus on a holistic, patient-centered approach to patient care, rather than solely focusing on physiologic markers of health and disease. Given the potential for non-medical factors to affect a patient's QOL, it is important that practitioners be aware of these effects in order to optimize patient care.

**Methods:** Our group is prospectively following a large cohort of end-stage kidney disease (ESKD) patients as they proceed through the kidney transplant (KT) evaluation. In this subsample, 590 patients completed 2 structured interviews: 1 at the time of initial transplant evaluation (T1), and another when transplant evaluation is completed (T2). Perceived racism, experiences of race-based discrimination, medical mistrust and trust in physician were assessed as predictors at T1. QOL was assessed as an outcome at T2 using the KDQOL-SF.

**Results:** Preliminary results indicate that more experiences of discrimination in healthcare, greater medical mistrust and lower trust in one's physician significantly predict poorer QOL across all 9 subscales of the KDQOL-SF (p<0.05). Greater perceived racism in health care predicted poorer QOL on the symptom, effects on daily life, work status and cognitive function subscales of the KDQOL (p<0.05).

**Conclusions:** These data indicate the injurious effect that discrimination and mistrust in health care may have on our patients, and indicate areas for patient-, provider-, and system-level focused interventions.

*Funding:* NIDDK Support

## SA-PO163

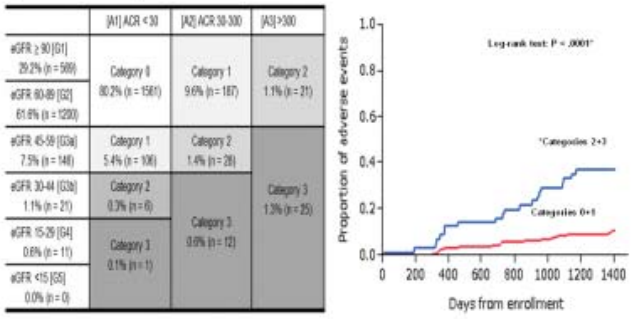
**Clinical Usefulness of KDIGO 2012 CKD Classification in an HIV Population: A Multicenter Study in Japan** Naoki Yanagisawa,[1,2] Minoru Ando,[1,2] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Dept IV of Internal Medicine, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Div of Infectious Diseases, Dept of Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan.*

**Background:** Chronic kidney disease (CKD) is epidemic among HIV-infected populations, and a determinant of their prognosis. The 2012 KDIGO CKD classification elaborated on how to identify patients at high risk for adverse outcomes.

**Methods:** Distribution of CKD in 1947 HIV-infected subjects (1829 men, 118 women, mean age: 44.9 ± 11.5 years) who regularly visited one of the 5 tertiary hospitals was studied, based on the 2012 KDIGO CKD classification. In addition, prevalence of HIV infection was studied in 176,839 chronic HD patients, according to the questionnaire survey in 1,951 dialysis facilities. Among 1947 pre-dialyzed HIV subjects, 661were prospectively followed up for 3.5 years to determine incidence of composite outcomes, including all-cause mortality, cardiovascular disease and a decline over 25% from baseline in eGFR. Four risk categories were constructed using the combination of 5 stages of eGFR and 3 grades of albuminuria. The cumulative incidence of the outcomes was analyzed with Kaplan-Meier method, and hazard risk (HR) for the outcome incidence was calculated using multivariable proportional hazards regression, adjusted for some known risk factors.

**Results:** The frequency of each category was shown in Table. The prevalence of HIV infection was 0.024% in the chronic HD patients. The Kaplan-Meier estimates were significantly increased over time in the risk categories 2 and 3, compared with the risk categories 0 and 1. The HR of risk categories 2 and 3 was 2-fold greater (HR = 2.00; its 95% confidence interval, 1.08 – 3.57; P = 0.0277), as compared to the other.

Figure. Distribution of CKD among HIV-infected individuals




**Conclusions:** The new KDIGO classification may facilitate targeting of high-risk CKD in the HIV-infected population as well as in the general population.

*Funding:* Government Support - Non-U.S.

## SA-PO164

**Early Start: Co-Morbidities Account for the Lack of Benefit Independently of the Vascular Access Type** Gustavo Laham, Gervasio Soler Pujol, Antonio R. Vilches, Carlos H. Diaz. *Nephrology Section, CEMIC, Ciudad de Buenos Aires, Argentina.*

**Background:** When and how to initiate Hemodialysis (HD) is still controversial. An early start (ES) on HD seems to show a lack of benefit. "Lead time bias" and comorbidities have been associated with different outcomes in ES groups. However, it is well accepted the impact that vascular access (VA) type has on patient survival (PS). Our aim was correlate PS with ES vs. late start (LS) on HD, taking into account the VA used.

**Methods:** Between 1995 and 2012, 322 incidental patients (Pts) initiated HD at CEMIC. eGFR was estimated by the MDRD-EPI equation. Co-morbid conditions were assessed using the Khan index (KI) and Pts were stratified accordingly as having low, median or high risk. Pts were divided in four groups: G1: ES (eGFR > 7 ml/min) with catheter (Cat) access (n=74), G2: ES with native or graft vascular (N/GV) access (n=87), G3: LS (eGFR< 7 /min) with Cat access (n=75), and G4: LS with N/GV access (n=86). The cut-off value to define ES or LS was based on median eGFR for these 322 patients.

**Results:** Mean age was 60.2 years, 60.6% were males, 23.3% were diabetics, median eGFR was 7ml/min/1.73m2 IQR (5-8.9), median follow up time was 30 months, by KI 39,8 %, 28,9 % and 31,4 % belonged to the low, median and high risk groups respectively. 53.4% had a N/GV access. Among the four groups the ES were significantly older (p<0.001), there were more diabetics (p<0.0001) and KI was higher (p<0.0001), while phosphatemia (p<0.0001), albumin (p<0.001), and Hemoglobin (p<0.005), were significantly higher in the LS groups. Survival analysis showed a higher mortality for ES P with a Cat access while LS P with N/GV access had the best survival: log rank p<0.0001. Multivariate analysis revealed that ES with either Cat access (p<0.005) OR 2.285, RIQ (1.29-4.04), N/GV access (p<0.031) OR 1.807, RIQ (1.05-3.08), median KI risk index (p<0.0001) OR 4.8 RIC (2.5-9.3) and high KI risk index (p< 0.0001) OR 12.7 IC (6.7-24.1) were predictors of mortality.

**Conclusions:** In this retrospective study, ES on HD was associated with a higher mortality rate than LS, this was independent of the VA used, but correlated directly with a high prevalence of co-morbidities.

## SA-PO165

**Carbamylated Albumin Is Better Correlated with Time Averaged Urea Levels in Chronic Kidney Disease Than in End-Stage Renal Disease** Tammy Hod, David J. Friedman, S. Ananth Karumanchi, Anders H. Berg. *BIDMC.*

**Background:** Urea carbamylates proteins and amino acids, and carbamylated proteins contribute to atherosclerosis. Deficiencies of free amino acid scavengers promote protein carbamylation. Amino acids stores are depleted in HD patients due to amino acid loss into the dialysate, in contrast to CKD patients in whom protein stores are preserved even when protein intake is decreased. We previously showed that increased urea and decreased amino acid concentrations were independently correlated with protein carbamylation in ESRD patients. Furthermore, the correlation between urea and carbamylated albumin (C-Alb) was significantly stronger in CKD patients as opposed to two ESRD cohorts. We hypothesized that serum levels of C-Alb better represent time-averaged urea concentrations in CKD patients compared to ESRD patients, and that carbamylation in CKD patients is less dependent upon serum amino acid levels.

**Methods:** Serum from 124 subjects with stage 3 or 4 chronic kidney disease were analyzed for carbamylated albumin (C-Alb) and free amino acids using LC-MS/MS. Blood urea nitrogen concentrations were measured using a clinical assay method. Associations between C-Alb, amino acids, and blood urea nitrogen were analyzed using Spearman correlations and partial correlations adjusted for amino acid levels.

**Results:** There was a strong correlation between carbamylated albumin and blood urea nitrogen concentrations amongst our CKD cohort which was not significantly altered when adjusted for differences in amino acid concentrations ($r_s$-0.75, P<0.001). Furthermore, the correlations between carbamylated albumin and serum amino acids were much weaker in these CKD subjects compared to our previously published analysis of ESRD patients.

**Conclusions:** Serum %C-Alb represents a clearer index of average urea concentrations in CKD patients compared to ESRD patients and may serve as an indicator of time-averaged urea levels similar to HbA1C in diabetes.

## SA-PO166

**Has the Prevalence of Hypertension, Diabetes, Obesity Changed among Adults with Chronic Kidney Disease in the U.S. from 1999 to 2010?** Sharon Saydah,[1] Edward Gregg,[1] Meda E. Pavkov,[1] Nilka Rios Burrows,[1] Neil R. Powe,[2] Rajiv Saran,[3] Yi Li,[3] Desmond Williams.[1] *[1]CDC; [2]UCSF; [3]UMich.*

**Background:** Diabetes, hypertension and obesity are risk factors for progression of chronic kidney disease. Whether the prevalence of hypertension, diabetes and obesity has changed in the past decade among adults with CKD in the U.S. is unknown.

**Methods:** Data from the 1999-2010 National Health and Nutrition Examination Surveys in 4 year periods were analyzed to examine trends among adults with CKD (n = 5708). CKD was defined as eGFR < 60 ml/min/1.73 m$^2$ or ACR ≥30 mg/g. Total diabetes (DM) was defined as self report or undiagnosed (undx) DM (A1c ≥ 6.5%). Hypertension (HYTN) was defined self reported HYTN treatment or undx HYTN (blood pressure ≥ 140/90 mmHg). Overweight was body mass index (BMI) 25 to < 30 kg/m$^2$) and obese was a BMI ≥30 kg/m$^2$. Estimates age, sex and race/ethnicity adjusted.

**Results:** Trends and 95% CI in the adjusted prevalence of DM, HYTN and obesity among persons with CKD are shown table.

| CKD population | 1999-2002 | 2003-2006 | 2007-2010 | Absolute % change (95% CI) |
|---|---|---|---|---|
| | % (95% CI) | % (95% CI) | % (95% CI) | |
| Total diabetes | 26.1 (22.8, 29.7) | 26.6 (23.2, 30.4) | 29.6 (27.0, 32.4) | 3.5 (-0.8, 7.9) |
| Undiagnosed diabetes | 4.9 (3.4, 6.8) | 4.0 (2.8, 5.5) | 4.6 (3.7, 5.7) | -0.4 (-2.3, 1.6) |
| Hypertension | 58.6 (55.3, 61.9) | 59.9 (56.9, 62.7) | 61.9 (59.3, 64.5) | 3.3 (-0.8, 7.4) |
| Undiagnosed hypertension | 19.4 (16.7, 22.3) | 14.4 (12.6, 16.4) | 11.5 (10.1, 13.1) | -7.8 (-10.9, -4.74) |
| Obese | 38.7 (33.8, 43.8) | 40.1 (36.8, 43.5) | 42.6 (39.7, 45.6) | 3.9 (-1.8, 9.6) |
| Overweight | 31.0 (27.6, 34.6) | 32.3 (29.6, 35.2) | 29.6 (27.2, 32.4) | -1.2 (-5.5, 3.1) |

The prevalence of undx HYTN decreased by 7.8 percentage points in the past decade.

**Conclusions:** The prevalence of risk factors for CKD progression is high among adults with CKD. While there has been no significant change in the prevalence of DM, HYTN or obesity among adults with CKD in the past decade, the prevalence of undx HYTN decreased.

## SA-PO167

**Natural Experiment with Bosnian Immigrants Who Settled Croatian Endemic Area – Final Epidemiological Evidence** Ivana Vukovic-Lela,[1] Sandra Karanovic,[1] Zivka Dika,[1] Jelena Kos,[1] Ante Cvitkovic,[3] Marica Miletic-medved,[3] Karen Edwards,[4] Arthur P. Grollman,[2] Bojan Jelakovic.[1] *[1]Dept of Nephrology, Arterial Hypertension, Dialysis and Transplantation, Univ Hospital Zagreb, Zagreb, Croatia, Croatia; [2]Dept of Pharmacological Science, State Univ of New York at Stony Brook, New York, NY; [3]Institute for Public Health Slavonski Brod, Slavonski Brod, Croatia; [4]Univ of Washington, Seattle, WA, Croatia.*

**Background:** Endemic nephropathy(EN) represents a form of aristolochic acid nephropathy(AAN) where AA was ingested *via* contaminated bread. Improvement in agriculture that occurred in EN area 25 years ago significantly decreased contamination of flour with AA. A group of immigrants from Bosnian non-EN area settled Croatian EN area 30 years ago(BoEN). This is considered as period long enough for intake of cumulative toxic AA dose. However, due to mentioned improvement it can be supposed that they were no longer exposed to AA. To test this hypothesis we analyzed proximal tubule damage(PTD),an early hallmark of EN, and renal function in BoEN.

**Methods:** 2347 farming villagers were enrolled and divided into 3 groups: autochthonous inhabitants of EN (CroEN;N=1687) and of nonEN villages (Cro-nonEN;N=372), and BoEN(N=102). Subjects were classified according to consensus criteria. Definitions: PTD=α1microglobuline/creatinine(α1CR)>31.5 mg/g;CKD=eGFR<60ml/min/1.73m$^2$.

**Results:** There was no difference in age,gender,BMI,BP,DM among groups(all p>0.05). α1CR values were significantly lower in BoEN than in CroEN diseased and suspect to have EN (7.04 vs.102 vs.48;p<0.001) but did not differ from Cro-nonEN(7.39;p>0.05). Prevalence of PTD and CKD was lowest in BoEN compared to CroEN and nonEN (1.3% vs.3.0 vs.8.3%; 5.9% vs.8.9% vs.15.8%,respectively;p<0.001). No EN cases were detected in BoEN. Twenty years ago BoEN observed significantly less *A.clematitis* in farming fields, less *Aristolochia* seeds among wheat seeds, and less frequently baked own bread ($\chi^2$=21.6;62.6;31.7, respectively; p<0.001).

**Conclusions:** This natural experiment confirmed our hypothesis that nowadays attenuated exposure to AA will lead to decrease in prevalence and probably to disappearance of EN.

*Funding:* Government Support - Non-U.S.

## SA-PO168

**Significance of Symptoms in Chronic Kidney Disease** Ajin John,[1] Anca Tilea,[1] Brenda W. Gillespie,[1] Michael Heung,[1] Fredric O. Finkelstein,[2] Margaret A. Kiser,[3] Peter Kotanko,[5] Rajiv Saran.[1] *[1]Univ of MI; [2]Metabolism Associates; [3]Univ of NC; [4]Med. Col. of Albany; [5]Renal Research Inst.*

**Background:** Little is known about the prevalence of symptoms in CKD. This study examined prevalence of symptoms in CKD and associations with patient outcomes.

**Methods:** We analyzed data from a prospective cohort (N=2,182; CKD stage 3-5). The Kidney Disease Quality of Life (KDQoL) was administered at enrollment and follow-up visits. A composite symptom score was calculated from KDQoL and symptoms were also abstracted by chart review. Seven KDQoL symptoms were matched with symptoms from chart review: *chest pain, itchy skin, shortness of breath, lack of appetite, washed out or drained, numbness in hands or feet, and nausea or upset stomach*. KDQoL-derived symptoms were dichotomized (no symptoms vs. any degree of symptoms) to allow for comparison with the chart review symptoms using McNemar test. Cox regression was used to test association of overall symptom score and individual KDQoL symptoms with mortality and ESRD, adjusted for age, gender, race, diabetes, BP and eGFR.

**Results:** 64% of patients completed at least one KDQoL. Mean age was 64yr; 54% were men and 70% white. Table below summarizes our findings. The most prevalent KDQoL symptom was 'washed out or drained' (n=949) of which only 31% (n=296) were identified as 'fatigue' by chart-review. The table also shows the results from the Cox survival analysis.

| Symptom (≤ 75) (Higher symptom score = better QoL) | $N_{KDQOL}$ (%) | $N_{Chart-Review}/N_{KDQOL}$ (%) | HR (95% CI) Death -- per 5 unit increase-- | HR (95% CI) ESRD -- per 5 unit increase-- |
|---|---|---|---|---|
| Overal Symptom Score | 533 (38%) | -- | 0.91 (0.86, 0.97) | 0.94 (0.92, 0.96) |
| Nausea or upset stomach* | 506 (36%) | 62/506 (12%) | -- | -- |
| Chest pain* | 317 (26%) | 34/371 (9%) | -- | -- |
| Shortness of breath* | 776 (55%) | 213/776 (27%) | 0.96 (0.92, 0.98) | -- |
| Lack of appetite* | 484 (34%) | 84/484 (17%) | 0.95 (0.91, 0.98) | -- |
| Faintness or dizzyness | 495 (35%) | -- | 0.98 (0.93, 0.99) | -- |
| Washed out or drained* | 949 (67%) | 296/949 (31%) | 0.95 (0.92, 0.99) | 0.97 (0.96, 0.99) |
| Numbness in hands or feet* | 729 (52%) | 117/729 (16%) | 0.96 (0.93, 0.99) | 0.96 (0.95, 0.98) |
| Dry skin | 717 (51%) | -- | 0.96 (0.93, 0.99) | 0.97 (0.96, 0.99) |
| Itchy skin* | 780 (55%) | 92/780 (12%) | -- | 0.97 (0.96, 0.99) |
| Cramps | 942 (67%) | -- | -- | 0.98 (0.96, 0.99) |

*Significantly different by McNemar's test

**Conclusions:** Symptom burden was high in this US cohort with CKD stage 3-5. Certain symptoms were associated with mortality; others with time to ESRD. The significantly lower frequency of symptom documentation in clinic charts points to a potential practice improvement opportunity to identify high risk CKD patients.

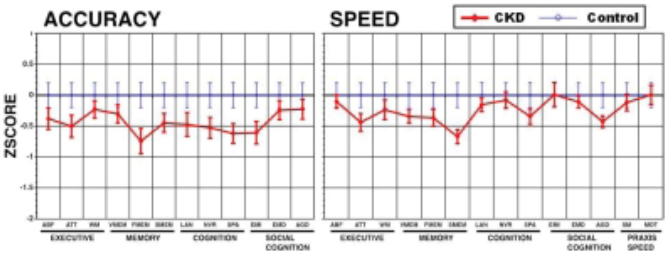
## SA-PO169

**Cognitive Performance on a Computerized Neurocognitive Battery in Children and Young Adults with CKD** Divya Ganeshmurthy Moodalbail,[2] Nina Laney,[2] Ruben C. Gur,[1] Allison M. Mott,[1] Stephen R. Hooper,[3] Jerilynn Radcliffe,[2] Abbas F. Jawad,[2] Kosha Ruparel,[1] Susan L. Furth.[2] *[1]Univ of Pennsylvania, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA; [3]Univ of North Carolina School of Medicine, Chapel Hill, NC.*

**Background:** Deficits in IQ, memory, attention and executive function have been reported in children with CKD, which may contribute to poor adherence to complex medical regimens and high rates of morbidity and mortality.

**Methods:** Cross sectional observational study of 42 children and young adults with CKD (eGFR ≤90 ml/min/1.73m2) and 25 healthy controls, ages 8-25 years. Cognitive performance was measured using a cognitive neuroscience-based computerized battery comprised of 14 tests scored on Accuracy and Speed: executive-control (abstraction/ mental flexibility, attention, working memory), episodic memory (verbal, facial, spatial), complex cognition (language and nonverbal reasoning, spatial processing), social cognition (emotion identification, emotion intensity differentiation, age differentiation), and sensorimotor and motor speed. Scores for CKD subjects were transformed to Z-scores using the mean and SDs of the control group as comparison.

**Results:**




Mean(SD) age and eGFR were 15(3) yrs and 42(23)ml/min/1.73m2 for CKD group and 15(3) yrs and 96(15)ml/min/1.73m2 for controls. CKD group was 64% male, 67% Caucasian, control group was 62% female, 58% African-American. Means+/-SEM of the NICK group compared to controls are shown in Figure 1. CKD subjects showed significant deficits in accuracy for face memory and spatial processing, and in speed for spatial memory and age differentiation.

**Conclusions:** Compared to controls, CKD subjects showed relative weaknesses in several areas, particularly for facial memory accuracy and spatial memory speed, with an effect size ≥ 0.5 SD.

*Funding:* Other U.S. Government Support

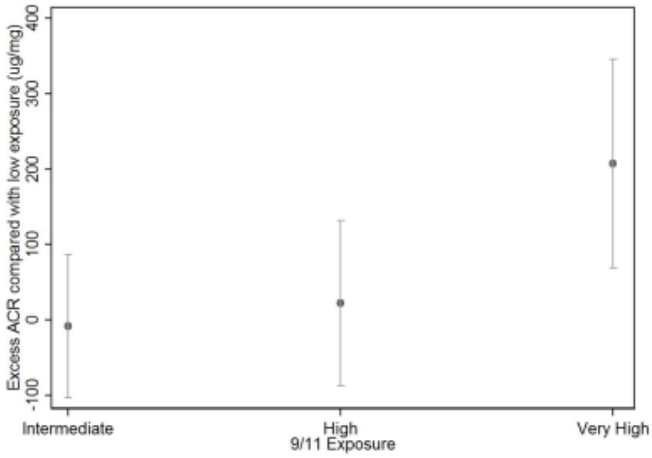
## SA-PO170

**New Evidence That Particulate Matter Exposure at Ground Zero Is Associated with Kidney Damage** Mary Ann McLaughlin,[1] Sarah F. Sanghavi,[1] Cynara Maceda,[1] Mark Woodward,[2] Laura E. Crowley,[1] Christina M. Wyatt.[1] *[1]Mount Sinai School of Medicine, New York, NY; [2]Univ of Sydney, Australia.*

**Background:** First Responders working at Ground Zero following the 9/11 tragedy were exposed to particulate matter including cement dust, smoke, glass fibers, and heavy metals. Particulate matter can deposit in microvascular walls, and inflammatory markers have been shown to be elevated post-exposure. We have previously demonstrated abnormalities in pulmonary and cardiac function in this population, but effects on the kidneys have not been explored. The purpose of this study is to assess renal endothelial damage in first responders exposed to particulate matter at Ground Zero using albuminuria as a marker.

**Methods:** We evaluated 183 consecutively enrolled first responders from the WTC –CHEST Program, a subset of the World Trade Center Clinical Center of Excellence. Participants provided information about particulate matter exposure on entry to the program, and exposure score was calculated based on proximity to Ground Zero, time of arrival, and duration of exposure. We measured urine albumin to creatinine ratio and performed a multivariable regression analysis adjusting for age, gender, race, smoking status, BMI, HbA1c, and hypertension.

**Results:** We demonstrated a significant linear trend (p=0.009) between level of exposure to particulate matter and albumin to creatinine ratio. Participants with the highest exposure to particulate matter had an albumin to creatinine ratio of 207ug/mg greater than the low exposure group (95% CI 69 to 345, p= 0.003) in the adjusted model.




**Conclusions:** We observed a statistically significant independent relationship of high exposure to particulate matter with albuminuria in this cohort after controlling for pertinent risk factors. This novel finding paves the way for future studies of environmental exposures and inflammation in the pathogenesis of albuminuria.

*Funding:* Other U.S. Government Support

## SA-PO171

**Skin Autofluorescence: A Non-Invasive Test to Improve Mortality Risk Prediction in Chronic Kidney Disease Stage 3** Maarten W. Taal,[1] Simon D.S. Fraser,[2] Paul J. Roderick,[2] Scott Harris,[2] Natasha J. McIntyre,[1] Richard J. Fluck,[1] Chris W. McIntyre.[1] *[1]Royal Derby Hospital; [2]Univ of Southampton.*

**Background:** Novel markers are needed to improve risk prediction in CKD. One potential candidate is tissue advanced glycation end product (AGE) accumulation, a marker of cumulative metabolic stress that can be assessed non-invasively by measurement of skin autofluorescence (SAF). SAF correlates with higher risk of cardiovascular (CV) events in people with diabetes and people requring renal replacement therapy. We aimed to investigate SAF as a predictor of all-cause mortality in a large cohort of people with CKD stage 3.

**Methods:** People with eGFR 59-30ml/min/1.73m$^2$ on 2 occasions prior to inclusion were recruited from Primary Care. Medical history was obtained and participants underwent clinical assessment as well as urine and serum biochemistry tests. SAF was measured using an AGE Reader® (DiagnOptics, Groningen, The Netherlands). The UK Health and Social Care Information Service provided date and cause of all deaths.

**Results:** Mean age was 72.9±9 years and baseline eGFR 52.2±10.4ml/min/1.73m$^2$. After a median of 3.6 years 179 of 1707 (10.5%) had died. The most common cause of death was CV disease (41%). Kaplan-Meier plots showed a progressive increase in all-cause mortality with higher SAF in tertiles. Cox proportional hazards models identified SAF as an independent predictor of all-cause mortality (Table).

| | | HR | 95% CI | p-value |
|---|---|---|---|---|
| SAF (units) | | 1.41 | 1.10-1.80 | 0.007 |
| Age (years) | | 1.07 | 1.05-1.10 | <0.001 |
| Previous CVD | | 2.13 | 1.54-2.94 | <0.001 |
| eGFR (ml/min/1.73m$^2$) | ≥60 | 0.39 | 0.21-0.75 | <0.001 |
| | 30-44 | 1.19 | 0.84-1.68 | |
| | <30 | 2.79 | 1.40-5.56 | |
| Albuminuria | | 1.79 | 1.25-2.56 | 0.001 |

**Conclusions:** We have identified SAF measurement as a simple, non-invasive test that predicts of all-cause mortality independent of several other well-established risk factors in a cohort of predominantly older people with CKD stage 3. Further research should focus on how best to incorporate SAF into risk prediction models and on interventions that may reduce AGE accumulation and the associated risk.

*Funding:* Pharmaceutical Company Support - Roche Products PLC; Sanofi, Private Foundation Support

## SA-PO172

**Prevalence and Determinants of Hyperoxaluria in Chronic Pancreatitis** Nathalie Demoulin,[1] Zaina Issa,[1] Ralph Crott,[2] Michel Y. Jadoul,[1] Pierre H. Deprez.[3] *[1]Nephrology, Cliniques Universitaires Saint-Luc, Belgium; [2]Public Health, Cliniques Universitaires Saint-Luc, Belgium; [3]Gastroenterology, Cliniques Universitaires Saint-Luc, Univ Catholique de Louvain, Belgium.*

**Background:** Chronic pancreatitis (CP) has been linked to enteric hyperoxaluria and acute oxalate nephropathy. Our aim was to evaluate the prevalence and determinants of hyperoxaluria in patients with CP.

**Methods:** Adults with CP (diagnosis based on CT or MRI imaging) seen at the gastroenterology day clinic in our hospital from 01/03 to 31/10/2012 were asked to participate. Pancreatic exocrine function was evaluated in all by fecal elastase (a pancreatic enzyme) and fecal acid steatocrit (a reliable measure of steatorrhea on a spot stool specimen). Oxaluria was measured twice.

**Results:** Forty eight patients accepted to participate. The cohort was 96% Caucasian, 50% male and 40% diabetic (63% on insulin). Etiology of CP was alcoholic in 29%, autoimmune, obstructive or hereditary in 19% and idiopathic in 52% of patients. CP was diagnosed a median of 4.5 years before inclusion. Estimated GFR was >60 ml/min/1.73m$^2$ in all patients, 2 had ACR between 30 and 300 mg/g. Clinical steatorrhea was present in 17% of patients and 48% were taking pancreatic enzyme supplementation. The 2 oxaluria measures were well correlated (ρ=0.68). Eleven patients (23%) had hyperoxaluria (>32mg/g of creatininuria, median 49 (35-75)). Oxaluria was moderately correlated with clinical steatorrhea (ρ=0.46), pancreatic enzyme treatment (ρ= 0.32) and fecal steatocrit (ρ=0.44) and inversely correlated with fecal elastase (ρ= -0.42). Age, diabetes, smoking and alcohol habits, BMI, HbA1c, vitamin A, D and E levels, INR and calciuria were not correlated with oxaluria.

**Conclusions:** To our knowledge, our study is the first to define the substantial (23%) prevalence of hyperoxaluria in a cohort of patients with CP. These patients are possibly at risk of developing acute oxalate nephropathy and nephrolithiasis, especially in case of intercurrent hypovolemia. Clinical and fecal indices of steatorrhea were moderately correlated with oxaluria. Intensifying pancreatic enzyme supplements and calcium supplementation could be proposed in patients with hyperoxaluria.

## SA-PO173

**Kidney Magnetic Resonance Imaging Reveals Structural Abnormalities That Are Associated with Kidney Function and Risk Factors for Adverse Outcomes in an Older Community-Based Cohort** Todd Woodard,[1] Sigurdur Sigurdsson,[2] John D. Gotal,[1] Alyssa A. Torjesen,[1] Lesley Inker,[3] Thor Aspelund,[2] Gudny Eiriksdottir,[2] Vilmundur Gudnason,[2] Tamara Harris,[4] Lenore J. Launer,[4] Andrew S. Levey,[3] Gary F. Mitchell.[1] *[1]Cardiovascular Engineering Inc; [2]Icelandic Heart Association; [3]Tufts Medical Center; [4]National Institute on Aging.*

**Background:** Because of the ability of the kidney to autoregulate perfusion and filtration, estimated glomerular filtration rate (eGFR) may underestimate early structural damage such as fibrosis, which contributes to disease progression and may be detectable by kidney imaging.

**Methods:** We performed dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) renography by using rapid 3D imaging of the kidneys for 5 minutes following injection of 1 mmol of Gd in 514 participants (79±4 years old, 57% women) in the AGES-Reykjavik Study. After tracing the kidney contour (excluding large cysts), custom software automatically segmented kidney tissue into 6 types: cortex, medulla, pelvis, fat, fibrosis or none of the above based on template matching of intensity-time data for each voxel. Fibrosis was defined empirically as voxels with persistently low signal intensity during DCE-MRI. A stepwise multiple regression model that entered age, sex, body surface area (BSA) and eGFR was used to examine relations between fibrosis and potential correlates of adverse outcomes.

**Results:** Mean total kidney volume was 335±82 ml, eGFR was 67±15 ml/min/1.73m$^2$ and fibrosis was 8.2±5.0% of kidney volume. Higher fibrosis % (model $R^2$=0.49, P<.001) was related to male sex (P=.002), higher BSA (P<.001), lower eGFR (P=.001), higher cardiac output (P<.001), higher heart rate (P=.006), lower hematocrit (P=.001), lower augmentation index (P=.023) and treated hypertension (P=.034).

**Conclusions:** In a community-based cohort of older people, modest kidney fibrosis detected by DCE-MRI was associated with reduced eGFR and other potential risk factors for adverse outcomes. The association of fibrosis with higher cardiac output and heart rate suggests that sympathetic nervous system activation may be a mechanism by which fibrosis detected by DCE-MRI contributes to chronic kidney disease.

*Funding:* NIDDK Support, Other NIH Support – NIA; NHLBI, Government Support - Non-U.S.

## SA-PO174

**The Prognostic Value of a Combined Renal Artery Patency Score in Atherosclerotic Renal Artery Stenosis** James Ritchie, Darren Green, Philip A. Kalra. *Salford Royal Hospital.*

**Background:** Atherosclerotic renovascular disease (ARVD) is often diagnosed by noninvasive biplane angiography. Most studies define significant disease by the minimum percentage stenosis (MPS) to the most affected kidney and consider bilateral disease in sub-group analyses. We hypothesized that considering both renal arteries (*Suresh et al, NDT 2000*) may better reflect the systemic impact of ARVD.

**Methods:** We compared MPS with a combined renal artery patency score (CPS). CPS is defined as *[200 – combined luminal loss to both kidneys]* such that a score of 200 represents bilateral 0% stenosis and a score of 0 represents bilateral 100% stenosis. 615 patients with ARVD diagnosed using CT or MRI angiography were assessed. Separate Cox models (adjusted for age, eGFR, blood pressure and proteinuria) were constructed to consider prognostic associations of MPS and CPS in relation to death, cardiovascular event (CVE) and progression to dialysis.

**Results:** Baseline age was 70±9 years; eGFR 32 [21-45] ml/min/1.73m$^2$; follow-up of 3.8 [1.8-6.0] years. As a continuous variable CPS significantly associated with risk for death and CVE, MPS did not. When considering bilateral disease, CPS associated more significantly with risk for death than MPS but MPS associated significantly with risk for CVE. Neither measure associated with risk for dialysis.

| | CPS | | | MPS | |
|---|---|---|---|---|---|
| | **Death** | **CVE** | | **Death** | **CVE** |
| Continuous variable | 1.03 [1.01-1.06]** | 1.03 [1.0-1.06]** | Continuous variable | 1.04 [0.9-1.1] | 1.03 [0.9-1.1] |
| <100 vs. >100 | 1.4 [1.1-1.8]*** | 1.3 [1.0-1.7]* | Bilateral >50% vs. not | 1.1 [0.8-1.4] | 1.4 [1.1-1.9]** |
| <80 vs. >80 | 1.3 [1.0-1.6]** | 1.3 [0.9-1.8]* | Bilateral >60% vs. not | 1.2 [0.9-1.6] | 1.5 [1.1-2.0]** |
| <60 vs. >60 | 1.3 [0.9-1.6]* | 1.2 [0.8-1.8] | Bilateral >70% vs. not | 1.5 [1.0-2.1]** | 1.6 [1.0-2.6] |

*Results presented as HR [95% CI]. Continuous variable assessed in 10-unit intervals. * p<0.1; ** p<0.05; ***p<0.01*

**Conclusions:** Defining patients by CPS and MPS appears to have differing prognostic associations in ARVD. Both methods are limited by their inability to consider cross sectional flow.

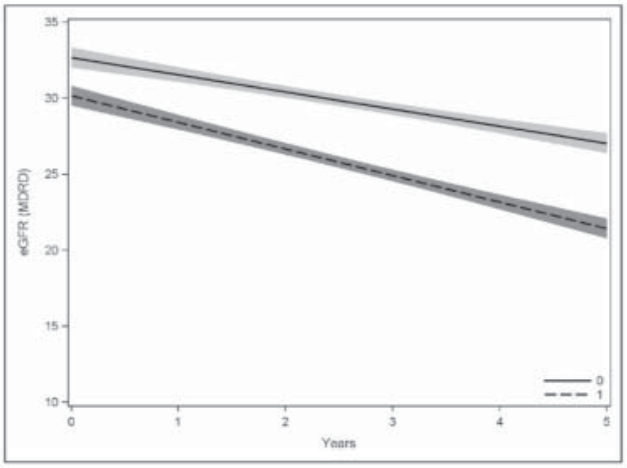
## SA-PO175

**Functional Status Associates with All-Cause Mortality in CKD** James Ritchie, Helen Alderson, Darren Green, Diana Chiu, Smeeta Sinha, Philip A. Kalra. *Salford Royal Hospital.*

**Background:** Global health assessments allow effects of multiple co-morbidities to be compared between patients. Measurements of functional status act as independent predictors for risk of death in the general and dialysis populations, but have not been assessed in a CKD cohort. One commonly used measure is the Karnofsky performance score (KPS).

**Methods:** Patients were selected from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS), a prospective observational study of outcome in patients with CKD 3-5 managed in secondary care. Associations between baseline KPS and risk for all-cause mortality were considered in a multivariate Cox model. Differences in rate of change in eGFR between groups of KPS were assessed in a mixed effects model.

**Results:** 1515 patients with a median follow-up 3.2 [1.5-5.0] years were included in this analysis (584 [39%] KPS 100; 648 [42%] KPS 90; 283 [19%] KPS ≤80). Patients with a higher KPS were younger, had higher eGFR and hemoglobin values and fewer previous macrovascular events. In multivariate analysis (adjusted for age, gender, eGFR, hemoglobin, albumin, diabetes, macrovascular events and diabetes), an association was observed between reductions in KPS at time of recruitment and risk for death; KPS 90 HR 1.2 [0.9-1.5] p=0.1; KPS 80 HR 1.8 [1.4-2.4] p<0.001. In a mixed effects model incorporating proteinuria, diabetes, hemoglobin and blood pressure, annual reduction in eGFR in patients with KPS >80 was 3%, and 5% in patients with KPS ≤80 p=0.008.




*eGFR trajectories predicted from mixed effects model. Solid line – KPS >80; dashed line KPS ≤80. Shaded areas 95% confidence intervals.*

**Conclusions:** This analysis suggests in a secondary care CKD cohort, reduced KPS predicts risk for death independently of renal function and vascular co-morbidities. This increased risk is associated with faster rates of eGFR loss in patients with lower KPS scores.

## SA-PO176

### The Marshfield Epidemiologic Study Area (MESA) Study on Urological Procedures in Urolithiasis and Their Impact on Chronic Kidney Disease

Radmila Mikan Savcic-kos,[1] Jingbo Huang,[1] Matthew R. D'costa,[1] Hong Liang,[1] Andrew D. Rule,[2] Narayana S. Murali.[1] *[1]Dept of Nephrology & Hypertension, Marshfield Clinic, Marshfield, WI; [2]Dept of Nephrology, Mayo Clinic, Marshfield, WI.*

**Background:** Epidemiological evidence exists that stone formers are at increased risk for ESRD. It is unclear if urological intervention impacts prevention or progression of CKD.

The MESA database, established in 1991, performs an automated daily review of all computerized databases of the Marshfield Clinic, finds and follows all residents, 90% of whom receive all care at the Marshfield Clinic or its affiliated centers and hospitals in a 24 zip code area that includes 89,291 patients.

**Methods:** We conducted a retrospective observational cohort study of the patients in MESA between Jan. 1991 and May 2007, where 1340 stone formers were matched by age, gender, and index date with 2712 control subjects. Cox proportional Hazards models adjusted for age, gender, and comorbidities using comorbidity propensity score were performed to evaluate the risk for incident Chronic Kidney Disease (CKD), elevated Serum Creatinine and any-cause mortality. Of the 1340 stone formers, 446 had urological procedures. Stone formers with procedures were compared to those without procedures.

**Results:** Stone formers with a mean of 9.1 yr. of follow-up were at increased risk for elevated SCr (HR (95% CI) = 1.30 (1.16-1.52)) and for CKD (HR (95% CI) = 1.30 (1.03-1.63)). Stone formers had lower rate of any cause mortality (16.2% vs. 25.6% in control group) (HR (95% CI) = 0.49 (0.40-0.59)).

Baseline comorbidities in stone formers with and without procedures were not significantly different except for obesity (p <0.0001). The results did not reveal any significant difference, in prevalence of CKD during the course of follow up after the index date.

**Conclusions:** Stone formers are at increased risk for elevated Creatinine and for CKD but urological procedures per se (e.g., lithotripsy) do not appear to impact prevalence of CKD.

*Funding:* Private Foundation Support

## SA-PO177

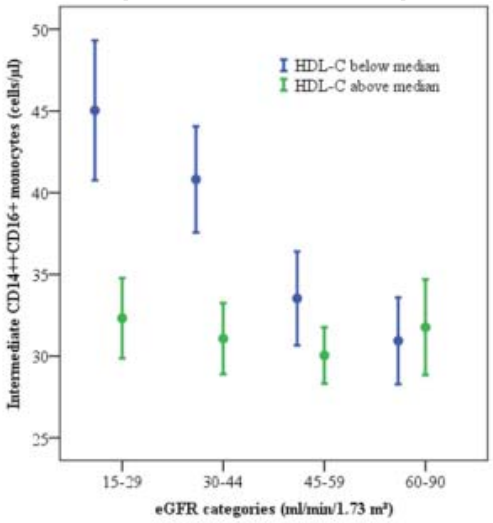
### Interrelationship between Low HDL Cholesterol and Monocyte Subset Distribution in Chronic Kidney Disease

Kyrill S. Rogacev, Adam M. Zawada, Sarah Seiler, Danilo Fliser, Gunnar H. Heine. *Internal Medicine VI, Saarland Uniersity Medical Centre, Homburg, Germany.*

**Background:** In patients with chronic kidney disease (CKD) low HDL and a skewed monocyte subset distribution towards intermediate $CD14^{++}CD16^{+}$ monocytes may contribute to the significantly higher risk of developing cardiovascular (CV) disease. Recent evidence suggests that HDL regulates monocytopoiesis in mice, but it is unknown whether HDL and monocyte subset distribution are linked in CKD patients.

**Methods:** Lipid profiles and monocyte subsets were measured in 438 CKD patients, who were followed-up for the occurrence of a composite CV endpoint.

**Results:** At baseline HDL was inversely correlated with intermediate $CD14^{++}CD16^{+}$ and nonclassical $CD14^{+}CD16^{++}$ monocytes (r = - 0.152; p = 0.001; r = -0.126, p = 0.008, respectively) but showed no significant association with total and classical $CD14^{++}CD16^{-}$ monocyte counts. A significant increase in intermediate monocyte counts with more advanced CKD was seen only in patients with HDL-C below the median [p (for trend) = 0.002], but not in patients with HDL-C above median [p (for trend) = 0.779].




In linear regression analyses, low HDL was an independent determinant of higher intermediate $CD14^{++}CD16^{+}$ monocyte counts (β = -0.138; p = 0.005), but not of total, classical $CD14^{++}CD16^{-}$ and nonclassical $CD14^{++}CD16^{+}$ monocyte counts (p = 0.244; p = 0.504; p = 0.059, respectively). In contrast to HDL, higher intermediate $CD14^{++}CD16^{+}$ monocyte counts predicted adverse cardiovascular outcome in univariate (p = 0.007) and multivariate models (Cox regression analysis: HR per increase of one cell / µl: 1.010, [1.001 – 1.019]; p = 0.034).

**Conclusions:** In CKD, low HDL is linked to higher intermediate $CD14^{++}CD16^{+}$ monocyte counts, which independently predicted adverse outcome. The relationship between HDL cholesterol and monocyte subset distribution in CKD deserves further evaluation.

*Funding:* Private Foundation Support

## SA-PO178

### Evaluation of Stakeholder Opinions on the Use of Surrogate Endpoints in Marketing Authorization of "Renal" Drugs

Bauke Schievink, Hiddo Jan Lambers Heerspink, Dick de Zeeuw, Jarno Hoekman. *Univ Medical Center Groningen.*

**Background:** There is a need for new drugs to delay or halt the progression to end-stage renal disease (ESRD). Drug development can be shortened by using surrogate instead of clinical endpoints. Yet, there is discussion regarding their validity. We designed an online survey to assess stakeholders' opinions on the use of surrogates in nephrology.

**Methods:** The population consisted of a pre-selected panel of experts with a professional background as regulator, industry official or academic researcher (N=145). Respondents were asked to rank 4 stakeholders (patients, industry, academia, regulators) based on the benefits they receive from the use of surrogates, and to value the validity of specific surrogates on a Likert scale from 1 (strongly disagree) to 5 (strongly agree). Analyses were conducted by computing mean scores and by comparing them between professional backgrounds and between nephrologists vs. non-nephrologists using Wilcoxon signed rank tests.

**Results:** Overall, 63 (41%) persons participated. Industry respondents considered use of surrogate endpoints the most beneficial for patients, while other stakeholders considered their use the most beneficial for industry (P<0.01). Respondents disagreed that drugs can only be marketed after hard outcome studies have been done (mean: 2.2, 95% CI: 1.9-2.5). Out of four proposed surrogates (blood pressure (BP), HbA1c, albuminuria, CRP) for two indications (ESRD, CV disease), only use of BP for CV disease was deemed accurate (3.8, 3.6-4.0). Other surrogates had mean scores smaller than 3 (P<0.01 compared to BP for CV disease). For ESRD, use of albuminuria was considered neutral (2.9, 2.6-3.2) and equally accurate as BP (2.8, 2.5-3.1, P=0.53), but more accurate than HbA1c (2.5, 2.2-2.8, P<0.05)

and CRP (1.9, 1.6-2.1, P<0.01). Opinions on the accuracy of these surrogates did not differ between nephrologists and non-nephrologists.

**Conclusions:** Stakeholders in drug development do not oppose to using surrogate endpoints in marketing authorization. However, most surrogates, including albuminuria and BP for ESRD, are not considered accurate.

*Funding:* Government Support - Non-U.S.

## SA-PO179

**Serum Cystatin C, Markers of Chronic Kidney Disease and Retinopathy in Indians without Diabetes** Charumathi Sabanayagam,[1,2,3] Boon Wee Teo,[3] Jiemin Liao,[3] Jie Jin Wang,[4] E. Shyong Tai,[3] Sunil Sethi,[3] Tien Yin Wong.[1,3] *[1]Singapore Eye Research Institute; [2]Duke-NUS Graduate Medical School, Singapore; [3]Yong Loo Lin School of Medicine, National Univ of Singapore; [4]Centre for Vision Research, Univ of Sydney, Australia.*

**Background:** Serum cystatin C, a novel marker for chronic kidney disease (CKD) has been shown to be superior to serum creatinine in predicting the onset of diabetes and its complications including nephropathy. Small clinical studies have shown correlations between cystatin C levels and diabetic retinopathy. However, it not clear if cystatin C is associated with retinopathy in subjects without diabetes.

**Methods:** We examined 1,757 Indian adults, aged 40-80 years who participated in the Singapore Indian Eye Study (2007-09) and were free of diabetes mellitus. CKD was defined as an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m$^2$ determined from serum cystatin C (eGFR$_{cys}$), and serum creatinine (eGFR$_{cr}$). Retinopathy was assessed from digital fundus photographs of both eyes. The associations of CKD-eGFR$_{cys}$ (n=200), CKD-eGFR$_{cr}$ (n=81) and confirmed CKD (eGFR$_{cys}$ <60 and eGFR$_{cr}$ <60, n= 58) with retinopathy were examined using logistic regression models adjusted for potential confounding factors including preexisting cardiovascular disease and albuminuria.

**Results:** The prevalence of retinopathy among those with CKD-eGFR$_{cys}$ and CKD-eGFR$_{cr}$ were 8.5% and 9.9%. In separate models, CKD-eGFR$_{cys}$ showed a significant association with odds ratio (OR, 95% Confidence interval [CI]) of 1.98 (1.06-3.72) while CKD-eGFR$_{cr}$ did not show a significant association with retinopathy 2.22 (0.96-5.13). In models including both markers, compared to optimal kidney function (eGFR$_{cys}$ ≥60,eGFR$_{cr}$ ≥60), confirmed-CKD was associated with a three-fold odds of having retinopathy (OR [95% CI] = 3.10 [1.20-8.00]).

**Conclusions:** In a population-based sample of Indian adults without diabetes, cystatin C in combination with creatinine was strongly associated retinopathy. If confirmed by future prospective studies, our findings may have clinical utility for predicting risk of retinopathy using an equation combining both cystatin C and creatinine.

*Funding:* Government Support - Non-U.S.

## SA-PO180

**The Relationship between the Reduced Number of Nephrons of the Congenital and Surgically Acquired Solitary Kidney with Arterial Hypertension Assessed by Means of the Glomerular Filtration Rate** Gheorghe Gluhovschi, Florica Gadalean, Silvia Velciov, Ligia Petrica, Cristina A. Gluhovschi. *Univ of Medicine and Pharmacy, Timisoara, Romania.*

**Background:** The concept between the number of nephrons and arterial hypertension (AHT) raises the question whether a reduced number of nephrons contributes to AHT. The solitary kidney (SK) represents a study model of this concept because of the presence of a reduced number of nephrons. The aim of our study was to assess the relationship between the reduced number of nephrons of the SK with AHT.

**Methods:** We conducted a study on 295 patients (pts) with a SK hospitalized in our department. Fifty-six pts (24M and 32F), mean age: 40.73±14.32 years had a congenital SK and 239 pts (69M and 170F), mean age: 56.81±13.42 years had a surgically acquired SK. All patients were assessed for the presence of AHT. GFR was measured by the CKD-Epi formula.

**Results:** Out of the 56 pts with a congenital SK 31 pts (55.35%) had AHT, the mean age at onset of AHT being 35.46±10.38 years. Out of the 239 pts with a surgically acquired SK 96 pts (40.41%) developed AHT after nephrectomy after a mean duration of 12.22±11.45 years. The distribution of pts according to stage of CKD and AHT is presented in Table 1.

| CKD STAGE | NUMBER OF PATIENTS | CONGEN-ITAL SK WITH AHT | CONGENITAL SK WITHOUT AHT | NUMBER OF PATIENTS | SURGICALLY-ACQUIRED SK WITH AHT DEVELOPED AFTER NEPHRECTOMY | SURGICALLY-ACQUIRED SK WITH AHT BEFORE NEPHRECTOMY | SURGICALLY-ACQUIRED SK WITHOUT AHT |
|---|---|---|---|---|---|---|---|
| 1 | 12 (21.42%) | 1 (8.33%) | 11 (91.66%) | 11 (4.6%) | 2 (18.18%) | 1 (9.09%) | 8 (72.72%) |
| 2 | 23 (41.07%) | 14 (60.86%) | 9 (39.13%) | 77 (32.21%) | 23 (29.87%) | 14 (18.18%) | 40 (51.94%) |
| 3 | 18 (32.14%) | 13 (72.22%) | 5 (27.77%) | 109 (45.6%) | 50 (45.87%) | 35 (32.11%) | 24 (22.01%) |
| 4 | 2 (3.57%) | 2 (100%) | 0 (0%) | 25 (10.46%) | 11 (44%) | 12 (48%) | 2 (8%) |
| 5 | 1 (1.78%) | 1 (100%) | 0 (0%) | 17 (7.11%) | 10 (58.82%) | 6 (35.29%) | 1 (5.88%) |

**Conclusions:** The prevalence of AHT increases with GFR decline, sustaining the concept of a relationship between a reduced nephron number and AHT. The prevalence of AHT is higher in patients with a congenital SK.

*Funding:* Clinical Revenue Support

## SA-PO181

**Targeted LC/MS Based Metabolomic Platform for Quantification of Nucleosides in Biofluids: Potential Biomarkers for Chronic Kidney Disease** Anna V. Mathew, Jaeman Byun, Subramaniam Pennathur. *Dept of Internal Medicine Nephrology, Univ of Michigan, Ann Arbor, MI.*

**Background:** Nucleoside metabolism plays an important role in several proliferative and catabolic conditions. Chronic kidney disease (CKD) is characterized by proliferation and increased catabolism, in addition to diminished clearance of the nucleosides. We hypothesized nucleosides such as uracil, pseudouridine, dimethyl guanosine, and allantoin would predict the rate of decline in renal function in CKD.

**Methods:** We developed a stable isotope-dilution liquid chromatography –mass spectrometry (ESI/LC/MS/MS) based quantitation technique to measure nucleosides in biological samples. Biological samples were purified with cation- exchange cartridges and the nucleosides were separated on a Luna $C_{18}$ column. The mobile phase was 0.1% formic acid and acetonitrile with 0.1% formic acid. MRM transitions monitored in positive mode were m/z 113 to 70 for uracil; m/z 116 to 71 for $^{13}C_1{}^{15}N_2$ uracil; m/z 245 to 209 for pseudouridine; m/z 312 to 180 for $N^2$,$N^2$-dimethyl guanosine, m/z 159 to 116 for allantoin and m/z 161 to 118 for $^{13}C$ allantoin respectively. Calibration curves were created with the concentration of the nucleosides spiked in the biological matrix.

**Results:** Initial validation of this method was done with pooled plasma samples from BKS db/db mice, a well-validated model of diabetic nephropathy. In order to determine if the nucleoside metabolism was able to discriminate rates of progression in CKD subjects, we utilized 15 baseline samples from subjects with Stage 3 and 4 CKD from the CPROBE, an ongoing biological and clinical data repository for CKD patients in the Michigan O'Brien Kidney Center. In this discovery cohort, we found three fold elevations in plasma levels of pseudouridine (3.56 vs. 1.04μM) and dimethyl guanosine (0.07 vs. 0.02 μM) in the progressors (defined as 20% decrease in eGFR or 1.5 fold increase in proteinuria in one year of follow-up) compared with non-progressors.

**Conclusions:** Further studies are warranted to validate the mechanistic and prognostic significance of these nucleoside markers by simultaneous assessment in plasma and urine from a larger cohort of CKD patients.

## SA-PO182

**Initial Characterization of Novel Loci of Kidney Function: The CKDGen Consortium** Adrienne Tin. *Johns Hopkins Bloomberg School of Public Health, On Behalf of the CKDGen Consortium.*

**Background:** Genome-wide association (GWA) studies of markers of kidney function and damage have yielded many loci, each with small effects, with much unexplained heritability. We conducted a meta-analysis of 50 population-based studies of individuals of European ancestry to identify additional loci and performed follow-up bioinformatics and functional experiments.

**Methods:** GFR estimated using the 4-variable MDRD study equation (eGFRcrea), CKD (eGFRcrea < 60 ml/min/1.73m^2), urinary albumin-to-creatinine ratio (UACR), and microalbuminuria (MA) were analyzed. The total sample sizes were 133,728 for eGFRcrea/CKD and 52,716 for UACR/MA. Study-specific results were pooled through inverse-variance weighted, fixed-effects meta-analyses. SNPs in significant loci were interrogated for their mapping to specific chromatin states in kidney tissues obtained from ChIP-Seq experiments. Based on pathway analyses and prior biological knowledge, eight genes were studied for their effects on kidney development using morpholino knock-down in zebra fish.

**Results:** The new analyses confirmed all previously identified loci for eGFRcrea and UACR. In addition, we identified 18 new independent loci for eGFRcrea (P-values from 6.0E-13 to 4.7E-8), and 16 of these showed direction-consistent association with cystatin C-based GFR, pointing towards a role in affecting kidney function. Chromatin state mapping analyses showed significant SNPs in these kidney function-associated loci mapped more often to regulatory regions in kidney tissues compared to those from other tissues studied in the ENCODE project. In zebra fish experiment, knock down of one of the eight genes analyzed led to visible tubular deformity in embryos.

**Conclusions:** We have identified additional novel loci that are associated with kidney function. Moreover, bioinformatics and functional experiments provide further evidence for their role in kidney development and function for many of these loci. Upon confirmation through independent replication, these new findings may advance our understanding of the biologic mechanisms underlying kidney disease.

## SA-PO183

**Association of Arsenic with Estimated GFR in U.S. Adolescents** Darcy K. Weidemann,[1] Ana Navas-acien,[2] Chin-chi Kuo,[2] Virginia Weaver,[2] Jeffrey J. Fadrowski.[1,2] *[1]Johns Hopkins Children's Center; [2]Johns Hopkins Bloomberg School of Public Health.*

**Background:** Emerging evidence suggests adverse health effects of long-term low-level exposure to arsenic. Few epidemiological studies have investigated the association of arsenic and kidney function, especially in children. Our objective is to examine the association of urinary arsenic and estimated glomerular filtration rate (eGFR) in a representative population of adolescents.

**Methods:** Cross-sectional study of 1,379 participants ages 12-17 years in the 2003-2010 National Health and Nutrition Examination Survey. Using linear regression adjusted for kidney disease risk factors, urinary dilution, and markers of seafood intake, we examined the association of eGFR and urinary total arsenic and DMA quartiles.

**Results:** The mean age was 14.5 years, 50% female, 63% white, 15% black, 13% Mexican-American, 78% active or involuntary tobacco smoke exposure, 35% overweight/obese, 2.4% hypertensive, and 0.4% diabetic. The median total urinary arsenic concentration and DMA (IQR, interquartile range) was 6.7 µg/L (IQR 3.8 – 12.1) and 3.6 µg/L (IQR 2.1 – 5.5), respectively. Higher quartiles of total arsenic and DMA were associated with higher eGFRs compared to the lowest quartile (Table).

| Difference in estimated GFR[a] associated with urinary total arsenic and DMA[b] | | | |
|---|---|---|---|
| **Total arsenic quartiles (µg/L)** | **eGFR (mL/min/1.73m²)** | **95% CI** | **p-value for trend** |
| 1 (<4.3) | - | | 0.05 |
| 2 (4.3 - 7.1) | 2.2 | (-1.4, 5.8) | |
| 3 (7.2 - 13) | 3.9 | (-1, 8.7) | |
| 4 (> 13) | 4.7 | (0.2, 9.3) | |
| **DMA quartile (µg/L)** | | | |
| 1 (<2.4) | - | | 0.03 |
| 2 (2.4 - 3.9) | 5.1 | (1.5, 8.6) | |
| 3 (4.0 - 6.0) | 3.3 | (-0.1, 7.2) | |
| 4 (> 6) | 7.4 | (1.6, 13.1) | |

[a] eGFR: bedside Schwartz equation
[b] Adjusted for urinary creatinine, arsenobetaine, and clinical/demographic characteristics in Results

**Conclusions:** Higher total urinary arsenic and DMA levels were associated with higher eGFR. This relationship could be explained by glomerular hyperfiltration induced by arsenic. Alternatively, among this population of adolescents with eGFR in the normal range, relatively higher eGFRs may be associated with greater urinary arsenic excretion, with implications for exposure assessment using urinary biomarkers.

*Funding:* NIDDK Support

## SA-PO184

**Genetic Polymorphism of Transporters and Protein in Renal Tubules and Urinary Excretion of Uric Acid in the Japanese General Population: The Takahata Study** Kazuko Suzuki, Keita Kamei, Hiroko Sato, Kosuke Kudo, Kazunobu Ichikawa, Tsuneo Konta, Isao Kubota. *Dept of Cardiology, Pulmonology, and Nephrology, Yamagata Univ School of Medicine, Yamagata, Japan.*

**Background:** A cluster of transporters and proteins expressed in renal tubules play a role in the metabolism of uric acid and the function of these molecules are genetically modified. To examine how the polymorphism of genes related with the metabolism of uric acid affect renal excretion of uric acid, we conducted a cross-sectional study in participants of community-based health checkup.

**Methods:** We genotyped the SNPs of 7 uric-acid-related genes, including 5 transporters (URAT1, GLUT9, ABCG2, SLC17A3 and SLC22A4) and 2 proteins (UMOD and ALDH2) in 1001 Japanese subjects. Urinary fractional excretion of uric acid (FEUA) was determined from morning spot sample and the association between FEUA and genotypes of these genes were examined.

**Results:** In total subjects, the analysis of variance (ANOVA) showed that FEUA was significantly different between the genotypes of rs505802 in URAT1, rs4293393 in UMOD and rs671 in ALDH2. Multiple regression analysis with adjustment for potential confounders showed that the genotypes of URAT1 and UMOD were independently associated with FEUA, in addition to male gender, systolic blood pressure, body mass index (BMI), salt intake and estimated GFR. The genotypes of ALDH2 was an independent factor of FEUA, not in total subjects, but in the subjects with renal insufficiency and BMI <25 kg/m$^2$.

**Conclusions:** This study revealed that the genotypes of URAT1, UMOD and ALDH2 were independently associated with FEUA. This indicates that genetic background of renal transporters and protein might affect renal excretion of uric acid in the Japanese general population.

## SA-PO185

**Factors Associated with Vasopressin Concentration in a General Population Cohort** Marieke Van Gastel,[1] Esther Meijer,[1] Lieneke E. Scheven,[1] Joachim Struck,[2] Stephan J.L. Bakker,[1] Ron T. Gansevoort.[1] *[1]Dept Nephrology, UMC, Groningen, Netherlands; [2]Sphingotec, Germany.*

**Background:** Vasopressin (AVP) plays an important role in maintaining volume homeostasis. Recent studies, however, suggest that AVP also plays a detrimental role in progression of chronic kidney disease. It is therefore of interest to identify factors that influence AVP concentration, particularly modifiable ones, because intervention on these factors may have beneficial renal effects.

**Methods:** This study was performed in a large, general population based cohort study (PREVEND Study, Groningen, the Netherlands). Copeptin (pro-AVP) was measured in 6,821 available plasma samples as surrogate for AVP. Associations of patient related factors with copeptin concentration were assessed in uni- and multivariable linear regression analyses.

**Results:** Median copeptin concentration was 4.70 pmol/L (IQR 2.94-7.62). We found a reverse association between copeptin concentration and eGFR (p<0.001) and a positive association with albuminuria (p<0.001) when analyzed univariable and when adjusted for age and gender. The final stepwise backward model (adjusted for age and gender) revealed associations with higher copeptin concentration for, in order of the strength of their association, lower fluid intake (p<0.001), higher sodium intake (p<0.001), higher blood pressure (SBP, p<0.001), current smoking (p<0.001), higher alcohol use (p<0.001), higher serum glucose (p<0.001), higher BMI (p<0.001), use of diuretics (p<0.01) and use of antidiabetics (p<0.05). No associations with copeptin concentration were found for characteristics as protein intake, C-reactive protein and use of non-diuretic antihypertensives.

**Conclusions:** A higher copeptin concentration is associated with a decreased kidney function and higher albuminuria, compatible with the alleged deleterious renal effects of AVP. Important modifiable subject related factors associated with copeptin concentration are current smoking, use of alcohol, and particularly intake of fluid and sodium. These data form a rationale to investigate whether intervening on these factors results in lower AVP concentration with concomitant beneficial renal effects.

*Funding:* Government Support - Non-U.S.

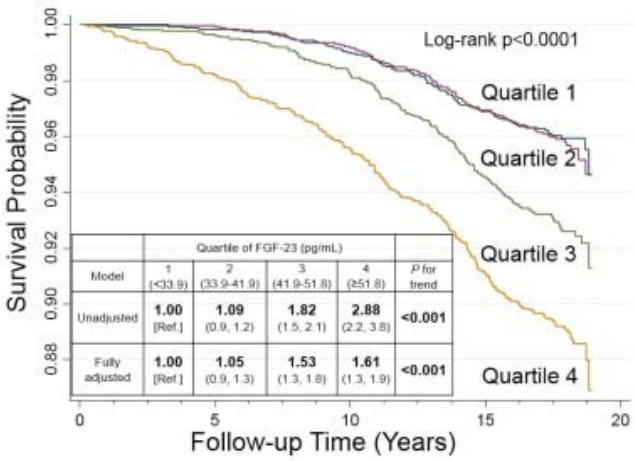
## SA-PO186

**Association of Serum Fibroblast Growth Factor-23 (FGF-23) Levels with Risk of Kidney Failure: 21-Year ARIC Follow-Up for the CKD Biomarkers Consortium** Casey M. Rebholz,[1] M. Grams,[1] Josef Coresh,[1] Elizabeth Selvin,[1] Lesley Inker,[2] Andrew S. Levey,[2] Paul L. Kimmel,[2] Vasan S. Ramachandran,[2] Harold I. Feldman,[2] Chi-yuan Hsu,[2] John H. Eckfeldt,[2] Pamela L. Lutsey.[2] *[1]Johns Hopkins Univ; [2]CKD Biomarkers Consortium.*

**Background:** FGF-23 is a bone-derived hormone with several endocrine functions in the kidney, including promoting urinary phosphorus excretion and inhibiting vitamin D activation. Elevated FGF-23 predicts kidney failure (KF) in moderate kidney disease patients, but has not been assessed in a community-based cohort.

**Methods:** FGF-23 levels were measured by Kainos ELISA (CV 14.5%) on 13,500 ARIC study participants in serum stored in 1990-92. KF was defined by ICD-9 and ICD-10 codes for hospitalizations and deaths from 1990 to 2010. Cox proportional hazards regression was used to relate baseline FGF-23 to KF during follow-up adjusted for baseline eGFR, covariates and clustering by study center.

**Results:** Mean age was 56.9 years, 56.0% were women, 5.8% had coronary heart disease, and mean baseline eGFR was 89.5 mL/min/1.73 m$^2$. During a median follow-up of 17.9 years, 599 (4.4%) participants developed KF. Time to KF was significantly different by quartile of baseline FGF-23 level (**Figure**; p<0.0001). There was an increased risk of KF among those with baseline FGF-23 levels in the higher quartiles after adjusting for eGFR, age, sex, race, diabetes, systolic blood pressure, antihypertensive medication, education, high-density lipoprotein, body mass index, C-reactive protein, $\beta_2$-microglobulin, hemoglobin A1c, and phosphate [**Table**; HR: 1.61; 95% CI: 1.34, 1.94; p<0.001]. Sensitivity analysis using USRDS-identified cases (n=256) showed similar results (HR: 1.94; p<0.001).




| | Quartile of FGF-23 (pg/mL) | | | | |
|---|---|---|---|---|---|
| Model | 1 (<33.9) | 2 (33.9-41.9) | 3 (41.9-51.8) | 4 (≥51.8) | P for trend |
| Unadjusted | 1.00 [Ref.] | 1.09 (0.9, 1.2) | 1.82 (1.5, 2.1) | 2.88 (2.2, 3.8) | <0.001 |
| Fully adjusted | 1.00 [Ref.] | 1.05 (0.9, 1.3) | 1.53 (1.3, 1.8) | 1.61 (1.3, 1.9) | <0.001 |

**Conclusions:** In a large, biracial, population-based study, higher baseline levels of FGF-23 were associated with an increased risk of KF independent of kidney function and other risk factors.

*Funding:* NIDDK Support, Other NIH Support - The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). Additional support was provided by R01HL103706 from the National Heart, Lung and Blood Institute, R01DK089174 from the National Institute of Diabetes and Digestive and Kidney Diseases, and 1U01DK085689 from the National Institute of Diabetes and Digestive and Kidney Diseases (CKD Biomarkers Consortium). Dr. Rebholz is supported in part by the National Heart, Lung and Blood Institute Cardiovascular Epidemiology training grant T32HL007024.

## SA-PO187

**Temoporal Trends in Area-Level Poverty and End-Stage Renal Disease (ESRD) Incidence** Bridget Garrity,[2] Holly J. Kramer,[1] Kavitha Vellanki,[1] David J. Leehey,[1] David A. Shoham.[2] *[1]Medicine, Loyola Medical Center; [2]Public Health Sciences, Loyola Medical Center.*

**Background:** Poverty is associated with end-stage renal disease (ESRD). The economic downturn in the U.S population which started during years 2007-2008 may have impacted ESRD incidence.

**Methods:** We examined ESRD incidence using data from the United States Renal Data System (USRDS). Analysis incuded adults aged ≥18 years who initiated dialysis during years 1995-2010 (n=1,253,541). Data were then linked with the 2000 and 2010 U.S. Census data. Negative-binomial regression was used to analyze temporal trends in the association between ESRD incidence and living in a poverty area defined by ZIP code with ≥20% of the population in that zip code living below the US federal poverty line. The numerator for each rate was the number of incident ESRD cases in a given race-ethnicity/sex/age group for a given period for each ZIP code. Analyses were stratified by time period (pre and post recession). Time period 1 was defined as January 1, 1995 - December 31, 2004 (pre-recession). Period 2 was defined as January 1, 2005 - December 31, 2010 (recession and post recession). Rates were calculated per million population per year.

**Results:** Between January 1, 1995 and December 31, 2010, there were 1,253,541 incident cases of ESRD in the USRDS network. Of these, 697,279 observations were in Period 1 and 556,262 observations were in Period 2. During period 1 (1995-2004), individuals living in a poverty area (17.4% of incident cases) had 24% higher ESRD incidence compared to those not living in a poverty area (95% CI 1.22, 1.25). During period 2 (2005-2010), a greater proportion of incident cases came from a poverty area (23.8%), and the association between poverty area and ESRD incidence was significantly stronger (p<0.001), with an incidence rate ratio of 1.28 (95% CI: 1.27, 1.30).

**Conclusions:** The influence of area-level poverty on ESRD incidence is increasing over time. Future surveillance and interventions for CKD prevention should target high poverty areas.

*Funding:* Clinical Revenue Support

## SA-PO188

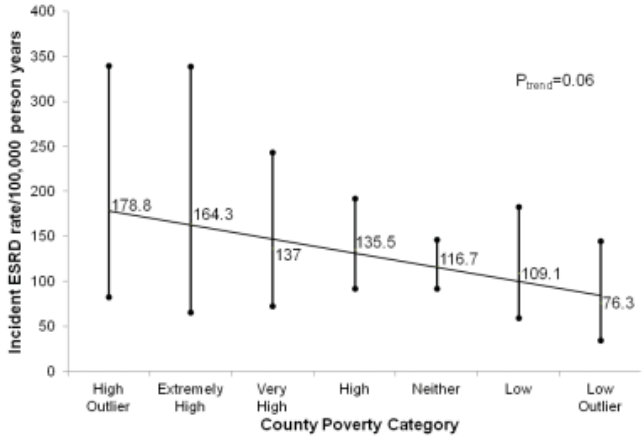
**Low Income, Community Poverty and Risk of End Stage Renal Disease**
Deidra C. Crews,[1] Orlando M. Gutierrez,[2] Suzanne E. Judd,[2] David G. Warnock,[2] William M. McClellan.[3] *[1]Johns Hopkins Univ; [2]Univ of Alabama, Birmingham; [3]Emory Univ.*

**Background:** The risk of ESRD is increased among poor individuals and in low income communities. We examined the associations of individual and community measures of socioeconomic status with incident ESRD.

**Methods:** In the population-based Reasons for Geographic and Racial Differences in Stroke (REGARDS) study of 23,314 U.S. adults aged 45 years and older, we used ANOVA and chi-square tests to assess participant differences across geospatially-linked categories of county poverty [outlier poverty, extremely high poverty, very high poverty, high poverty, neither (reference), high affluence and outlier affluence defined using data obtained from the 2000 US Census]. We used multivariable Cox proportional hazards models to examine the independent association of annual family income and county poverty measures with incident ESRD. Confounders included age, sex, race and region of residence (stroke belt, stroke buckle, other regions), and education.

**Results:** Participants residing in impoverished counties were more likely to be female and/or black, and were less likely to have completed high school than those in affluent counties (P<0.05 for all). There were 158 cases of ESRD per 100,000 person-years ($10^5$ py) of follow up. Incident ESRD rates declined as the affluence of counties increased.

**Figure. Incident ESRD rate/100,000 Person Years by County Poverty Category**




In adjusted models, only income was independently associated with risk of ESRD [hazard ratio (HR) 4.0, 95% confidence interval (CI) 1.7-9.2 comparing the <$20K group to the >=$75K group]; there were no statistically significant associations of geospatially-linked county measures of poverty and ESRD.

**Conclusions:** In contrast to annual family income, geospatially-linked measures of community poverty have little relation with risk of ESRD. Efforts to mitigate socioeconomic disparities in CKD may be best appropriated at the individual level.

*Funding:* Other NIH Support - National Institute of Neurological Disorders and Stroke, Pharmaceutical Company Support - Amgen Corporation, Private Foundation Support

## SA-PO189

**Testing the Validity of a New Quantitative Image Analysis Technique to Measure Severity of Abdominal Aortic Calcification on Lateral Lumbar Radiographs**
Mark D. Grant,[1] Rachel M. Holden,[1,2] Michael A. Adams.[1] *[1]Queen's Univ, Canada; [2]Kingston General Hospital, Canada.*

**Background:** Vascular calcification represents a serious complication of chronic kidney disease. Lateral lumbar radiograph images are inexpensive and prominently display abdominal aortic calcification (AAC); however, to date, there remains no fully quantitative method to report severity of calcification. The present study focuses on testing the reliability of a new quantitative image analysis technique compared to the previously validated semi-quantitative technique (AAC-24) when measuring severity of AAC on a lateral lumbar radiograph.

**Methods:** The quantitative image analysis technique was developed using Image Pro 6.0 and Microsoft Excel software. One hundred and ninety three subjects were selected from the Kingston cohort of the Canadian Multicenter Osteoporosis Study (CaMOS) based on availability of radiographic data. Subjects were sorted according to bone mineral density values and compared within standard deviation groups. Inter-observer reliability was assessed by having two readers analyze the same fifty images for severity of AAC using both the semi-quantitative technique, and the quantitative technique in proposal. Additionally, one reader analyzed the same set of fifty images twice, using both techniques previously mentioned, to assess their respective intra-observer reliabilities. Two scores were generated for each technique: L2-L4 and L3-L4 Sum.

**Results:** For the L2-L4 Sum, the intra- and inter-class correlation coefficients for the quantitative and semi-quantitative techniques were 0.975, 0.962 and 0.917, 0.892, respectively (n=35). For the L3-L4 Sum, the intra- and inter-class correlation coefficients for the quantitative and semi-quantitative techniques were 0.982, 0.964 and 0.899, 0.762, respectively (n=50).

**Conclusions:** The quantitative technique in proposal is a more reliable method to assess severity of AAC than the semi-quantitative technique. In addition to improved reliability, it is also a more informative assessment of AAC in the regions adjacent L2, L3, and L4 vertebrae. This quantitative method demonstrates promise for future application in clinical and epidemiological studies.

## SA-PO190

**The Characteristics of Advanced Aged IgA Nephropathy**
Yasuko Oshima, Takahito Moriyama, Chihiro Iwasaki, Kayu Tanaka, Kosaku Nitta. *Medicine, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** The susceptible age for IgA nephropathy (IgAN) is younger. However IgAN is sometimes developed in the advanced age, and its characteristics remains unknown.

**Methods:** We selected 600 IgAN patients, observed at least half a year and aged over 20 years old. We divided them into three groups as advanced aged group (AAG, n=31, aged over 50 years old), middle aged group (MAG, n=163, aged between 30 and 49 years old), and young aged group (YAG, n=407, aged between 20 and 39 years old). We analyzed clinical and histological background, renal outcome, and risk for progression.

**Results:** In AAG, mean arterial pressure (MAP) was significantly higher than YAG (97.0 vs. 85.7 mmHg, P=0.0004). In the clinical findings, total protein, serum albumin, and estimated glomerular filtration rate (eGFR) were significantly lower, and blood urea nitrogen, the amount of proteinuria (U-Prot) and N-acetyl-β-D-glucosaminidase (NAG) were significantly higher in AAG than in MAG and YAG [eGFR; 42.3 vs. 59.4 and 79.3 ml/min (P<0.0001, P<0.0001), U-Prot; 2.04 vs. 0.80 and 0.66 g/day (P=0.021, P=0.003), NAG; 10.6 vs. 5.8 and 4.5 U/l (P=0.003, P=0.004), in AAG vs. MAG and YAG, respectively]. In the histological findings analyzed by Oxford classification, interstitial fibrosis/tubular atrophy was severe in AAG than YAG (T0/T1/T2; 40.7/52.9/7.4 % in AAG vs. 76.9/29.0/4.1 % in YAG, P=0.001). Also arteriolar sclerosis was severe in AAG than MAG and YAG [grade as none/mild/middle/severe: 9.5/19.0/35.7/11.9 % in AAG vs. 20.6/47.5/24.5/7.5 % in MAG and 61.7/40.0/6.1/1.2 % in YAG (P=0.014, P<0.0001)]. Renal survival rate analyzed by Kaplan Meyer Method was significantly lower in AAG (27.2 % / 17.5 year in AAG vs. 69.2 and 84.9 % / 20 year in MAG and YAG, P<0.0001). The patients advanced to end stage renal disease (ESRD) in AAG were higher MAP and severe U-Prot than non-advanced patients [MAP: 103.5 vs. 92.5 mmHg (P=0.019), U-Prot: 3.02 vs. 1.57 g/day (P=0.036)].

**Conclusions:** The characteristics of IgAN in advanced age were higher blood pressure, lower renal function, severe proteinuria, severe interstitial change, and arteriolar sclerosis. The prognosis was very severe and over 70% was developed to ESRD within 20 years.

## SA-PO191

**Effect of Hematuria on the Outcome of IgA Nephropathy with Moderate Proteinuria**
Chihiro Iwasaki, Takahito Moriyama, Kayu Tanaka, Yasuko Oshima, Kosaku Nitta. *Medicine, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** Recently, hematuria has been recognized to be excreted from the active inflammatory lesion of glomerular capillaries in IgA nephropathy (IgAN). However, the relationship between hematuria and histological lesion, and the effect of hematuria on the reaction against steroid therapy and the outcome for IgAN have still unknown.

**Methods:** We examined 80 IgAN patients with proteinuria greater than 1g/day and were treated with predonisolone (0.8mg/kgBW). We divided them into two groups: low urinary red blood cells (U-RBC) group [(L group, n=30,U-RBC < 40 counts/high-power field(HPF)] and high U-RBC group (H group, n=50, U-RBC ≥ 40 counts/HPF). We analyzed the clinical and histological background, the relationship between hematuria and histological lesion, renal outcome, and the risk factors for progression.

**Results:** Median U-RBC in H group was significantly higherthan L group (L:16 vs.H:80counts/HPF, p<0.0001), and the other clinical findings were similar between both groups [Mean Arterial pressure (L group: 95.2 vs. H group: 93.4 mmHg), estimated glomerular filtration rate (eGFR) (67.9 vs. 74.1 ml/min) and proteinurea (2.58 vs. 2.16 g/day)]. Histological backgrounds were also similar between both groups. There was no correlation between U-RBC and active histological lesion. After starting steroid therapy, median proteinuria was soon decreased and remained less than 1g/g•creatinine until 5 years in both groups. Also, median U-RBC was soon decreased and remained less than 10 U-RBC/HPF in both groups. The 20-year renal survival rate was analyzed by Kaplan-Meier method was higher in H group (L:41.2 vs. H:49.5%), but it was not significant. By multivariate Cox regression analysis, lower eGFR(HR 3.22, 95%CI 1.74-6.23, p=0.0002) and severe histological lesion (HR 2.60, 1.11-6.59, p=0.0233) were independent risk factors for progression, but U-RBC was a factor for good prognosis (HR 0.55, 95%CI: 0.34-0.81, p=0.0016).

**Conclusions:** Our results indicate that the degree of hematuria at the biopsy in IgAN is not associated with severe clinical findings, active pathological findings, resistance to steroid treatmentand poor outcome.

## SA-PO192

**Obesity as a Risk Factor for Chronic Kidney Disease: Health Survey for England 2010** Helen L. MacLaughlin,[1,2] Wendy L. Hall,[2] Iain C. Macdougall.[1] *[1]King's College Hospital, London, United Kingdom; [2]King's College London, United Kingdom.*

**Background:** Observational studies in North America, Europe, & Asia indicate that obesity is a risk factor for CKD, yet there is no similar study of a nationally representative sample in the United Kingdom (UK). The aim of this study was to determine if overweight and obesity are independently associated with the prevalence of CKD in the Health Survey for England (HSE) 2010 cross-sectional cohort.

**Methods:** CKD was defined as estimated glomerular filtration rate (eGFR) <60ml/min/1.73m$^2$ using the CKD-EPI formula. Multivariable logistic regression models were developed to estimate CKD risk by BMI in 5 categories (reference category 18.5–24.9 kg/m$^2$), and were repeated with eGFR calculated using the 4-variable MDRD study equation for comparison with previous studies.

**Results:** The analytic cohort consisted of 3, 463 men & women (mean age 51y; SD 17y). The prevalence of CKD in the HSE was 5.9%. The prevalence of obesity was 43% in the CKD group vs 26% in the non-CKD group (p <0.001). Those with CKD were older, had more diabetes & hypertension, & a higher mean BMI than those without CKD. The risk of CKD was over 2.5 times higher in obese participants, but not under- or over-weight participants, compared to normal weight participants, after adjustment for age, gender, ethnicity, smoking, diabetes & hypertension: BMI <18.5 kg/m$^2$ adjusted OR 2.08 (95% CI 0.27 to 15.74 NS), BMI 25.0-29.9 kg/m$^2$ adjusted OR 1.47 (95% CI 0.94 to 2.31 NS), BMI 30.0-39.9 kg/m$^2$ adjusted OR 2.78 (95% CI 1.75 to 4.43, p<0.001), & BMI ≥40.0 kg/m$^2$ adjusted OR 2.68 (95% CI 1.05 to 6.85, p<0.05). Results were similar, although attenuated, using the MDRD equation to define CKD status: BMI <18.5 kg/m$^2$ adjusted OR 1.73 (95% CI 0.32 to 9.47 NS), BMI 25.0-29.9 kg/m$^2$ adjusted OR 1.37 (95% CI 0.95 to 1.99 NS), BMI 30.0-39.9 kg/m$^2$ adjusted OR 2.02 (95% CI 1.37 to 2.97, p<0.001) & BMI ≥ 40.0 kg/m$^2$ adjusted OR 2.01 (95% CI 0.89 to 4.54, NS).

**Conclusions:** The risk for CKD increases with obesity in the UK population, supporting findings from previous epidemiological studies. Future service delivery planning should account for increasing CKD risk in the years following an epidemic rise in population obesity rates.

*Funding:* Government Support - Non-U.S.

## SA-PO193

**The Impact of Initial and Subsequent Blood Pressure Control on Renal Outcme in Moderate to Severe Chronic Kidney Disease** Ping-min Chen, Ping Yu Chen, Tai-shuan Lai, Wen-Chih Chiang, Shuei-Liong Lin. *Dept of Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan.*

**Background:** Blood pressure (BP) is an important risk factor of end stage renal disease. The protective effect of BP control is well known in early stage chronic kidney disease (CKD). Limited data is available in late stage CKD. This study is to examine the impact of baseline and subsequent BP control on renal outcome in late stage CKD patients.

**Methods:** This is a retrospective single-center study, enrolling total 1210 stage 3B-5 CKD patients (age 18~80). The baseline BP when enrolling and in the first year were collected. The primary outcome was initiation of long-term renal replacement therapy (RRT). The outcome was first compared between different baseline BP groups. Good BP was defined as BP <130/80 mmHg, otherwise high BP. Patients were then stratified into four groups according to the baseline BP and subsequent BP control in the first year, if they were followed-up for more than one year. Survival analysis of the four groups was performed. Multivariate cox regression model was used to evaluate the effect of risk factors, baseline BP and subsequent BP on renal outcome. Subgroup analysis according to the baseline urine protein/creatinine ratio (UPCR) was performed.

**Results:** High baseline BP was a risk factor of RRT (HR 1.487, p = 0.001). Those with baseline and subsequent good BP (Group 1) showed the best renal survival, followed by those with baseline high BP and subsequent good BP control (Group 2). Group 3 (good baseline BP but high subsequent BP) and group 4 (high baseline and subsequent BP) patients had the worst outcome. Comparing group 2 and group 4, subsequent good BP control was associated with less risk of RRT (HR 0.515, p = 0.009). This renal protective effect of subsequent BP control was more prominent for patients with higher proteinuria ( UPCR ≥ 1 g/g, HR 0.485, p = 0.012).

**Conclusions:** Baseline high BP is a risk factor for CKD progression. Patients with subsequent good BP control had lower risk of RRT even baseline BP is high. This study indicats the beneficial role of good BP control in slowing CKD progression in late stage CKD patients.

## SA-PO194

**Gastrointestinal Bleeding in End-Stage Renal Disease** Juliana F. Yang, Aniko Szabo, Hariprasad S. Trivedi. *Medical College of Wisconsin.*

**Background:** We investigated the epidemiology of serious gastrointestinal bleeding (GIB) in end-stage renal disease (ESRD) subjects receiving dialysis.

**Methods:** Serious GIB was defined as GIB requiring hospitalization or during hospitalization for inter-current illness. The United States Renal Data System database was used to identify patients with non-HMO Medicare as payer and first service date between January 1, 1996 and December 31, 2005. The patients were followed 90 days after the first service date to the first occurrence of death, transplant, loss of Medicare, or December 31, 2006. Analysis of predictors of GIB incidence was performed using overdispersed Poisson regression. A Cox regression model with time-dependent predictors was used to evaluate the effect of GIB on survival. The process of recurrent GIB was modeled using a partially conditional Cox regression model.

**Results:** A total of 395,717 patients were followed for 841,192 person-years during which 66,679 GIB events (79/1000 person-years) were identified. The incidence of GIB was relatively stable from 1996 to about 2000, with an increasing trend thereafter. In 2006 the relative risk [RR] was 1.37 as compared to 1996 (95% CI 1.23-1.53). Females (vs. males), blacks (vs. whites), those with hypertension as cause of ESRD (vs. diabetes), and persons > 49 years of age (progressively in each age decile) had a higher RR of GIB. The first episode of GIB increased the hazard of death by about 2-fold (hazard ratio [HR] 1.91, 95% CI 1.88-1.95); each additional episode of GIB further increased the hazard by about 3% [HR 1.029, 95% CI 1.02-1.04). Having had a previous episode of GIB increased the hazard of recurrence substantially (HR 3.21; 95% CI 3.07-3.37), and each further episode kept increasing the hazard (HR 1.22; 95% CI 1.19-1.26).

**Conclusions:** In patients new to long-term dialysis the risk of serious gastrointestinal bleeding has increased by about 37% over a 10-year period and is associated with a higher risk of death. **Notice:** The data reported here have been supplied by the United States Renal Data System (USRDS). The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as an official policy or interpretation of the U.S. government.

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## SA-PO195

**Genetic Influence on the Variation in Serum Uric Acid Levels in Western Alaska Natives** V. Saroja Voruganti,[1] Shelley A. Cole,[1] Karin Haack,[1] Sandra L. Laston,[1] Sven O.E. Ebbesson,[2] Jean W. Maccluer,[1] Anthony Comuzzie,[1] Jason G. Umans.[3] *[1]Genetics, Texas Biomedical Research Institute, San Antonio, TX; [2]Norton Sound Health Corporation, Nome, AK; [3]Medstar Health Research Institute, Hyattsville, MD.*

**Background:** Serum uric acid (SUA) levels are heritable and are correlated not only with renal urate transporters but with adiposity and inflammation.

**Methods:** We assessed genetic contributions to the variation in SUA in both phases of the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) study, having reported previously that SUA was independently associated with prevalent CKD and hypertension in this population. Additionally, we investigated the relationship between SUA and cardiometabolic disease risk biomarkers in this population.

**Results:** Mean SUA levels were 5.27 ± 1.3 mg/dl (phase1 , 2000-04) and 5.26 ± 1.4 mg/dl (phase 2, 2006-10). SUA was significantly heritable in both phases; 0.50 (SE= 0.09, p = 3 x $10^{-9}$, phase1) and 0.37 (SE= 0.18, p 0.011), phase 2). Genome wide linkage analysis identified a novel locus on chromosome 15 (83cM) near marker D15S131 (15q21-q23) which may be regulating SUA. The LOD scores were 2.5 and 3.2 for the first and second phases, respectively. By contrast, we failed to detect evidence for linkage at sites on chromosomes 3 and 11, identified in our previous studies of Mexican Americans and American Indians, respectively. Our bivariate analyses showed significant genetic correlations between SUA and triglycerides (rhog = 0.41), percent body fat (rhog = 0.41), waist circumference (rhog = 0.51) at p < 0.005 and c-reactive protein (rhog = 0.48), diastolic BP (rhog = 0.31), and insulin (rhog = 0.42) at p < 0.05.

**Conclusions:** In summary, we detected a novel locus influencing SUA and significant pleiotropy between SUA and cardiometabolic disease risk factors in a unique Alaska Native population.

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## SA-PO196

**Association between Osteoprotegerin, Baseline and Change in Arterial Stiffness in CKD** Helen Alderson, James Ritchie, Philip A. Kalra. *Salford Royal Hospital.*

**Background:** Osteoprotegerin (OPG) is elevated in CKD and has been associated with vascular calcification and aortic stiffness. We sought to investigate the relationship between OPG and rate of change in arterial stiffness in a CKD population.

**Methods:** Patients were selected from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS), a prospective observational study of outcome in patients with CKD 3-5. OPG was measured in stored baseline sera from 140 patients who had a contemporaneous and follow-up measurement of arterial stiffness. Arterial stiffness was assessed using augmentation index corrected for heart rate (AIx) measured with a Sphygmocor device. Patients were grouped by quartiles of OPG.

**Results:** At baseline, mean age was 63±13 years, eGFR 31±12ml/min/1.73m$^2$, OPG 6.9 (5.5-9.2) pmol/L. 75% of follow up measure of AIx were performed at 12-months and 25% at 24-months. Baseline AIx was not significantly correlated with OPG level (r 0.09 p=0.3); no significant difference in AIx existed between quartiles of OPG. No significant correlation between OPG level and rate of change in Aix existed (r 0.12 p=0.2). When quartiles of OPG were compared, only patients in the highest quartile had a mean increase in vascular stiffness, with a significant difference observed between the highest and lowest quartiles (-1.5±4 vs. 0.7±5.7 p=0.03). Similarly when change in Aix /year was considered as a binary variable (increased / decreased), a greater proportion of patients in the highest OPG quartile had an increase in Aix.

| | Quartile 1 ≤5.5 | Quartile 2 5.5-6.9 | Quartile 3 6.9-9.2 | Quartile 4 >9.2 | p overall | p q1 vs q4 |
|---|---|---|---|---|---|---|
| N | 36 | 44 | 27 | 33 | | |
| Change in AIx /yr (Mean ±SD) | -1.5±4.3 | -0.4±3.1 | -0.9±3.9 | 0.7±5.7 | 0.17 | 0.03 |
| Increased AIx N (%) | 11 (31%) | 22 (50%) | 13 (48%) | 19 (58%) | 0.14 | 0.02 |

**Conclusions:** Any relationship between OPG level and rate of change in vascular stiffness appears to be non-linear, with patients who have the highest OPG levels at the greatest risk. Further work should include a larger sample size and consider possible confounding effects of renal function and other bone-mineral parameters.

## SA-PO197

**Association of Cystatin C and Urine Albumin to Creatinine Ratio with Change in Ankle Brachial Index: The Multi-Ethnic Study of Atherosclerosis** Pranav S. Garimella,[1] Joachim H. Ix,[2] Ronit Katz,[3] David Siscovick,[3] Holly J. Kramer,[4] Michael Shlipak,[5] Mark J. Sarnak.[1] *[1]Tufts Medical Center; [2]UC San Diego; [3]Univ of Washington; [4]Loyola Univ; [5]UC San Francisco.*

**Background:** Low ankle brachial index (ABI), a reflection of atherosclerotic disease, and high ABI, a reflection of calcified vessels, are both measures of subclinical cardiovascular disease (CVD). The association of serum cystatin C and albumin-creatinine ratio (ACR) with development of either high or low ABI is unknown.

**Methods:** We investigated the longitudinal associations of cystatin C and ACR with development of low (ABI 0.9-1.4 to ABI < 0.9), and high ABI (0.9-1.4 to > 1.4) over 10 years in 5,591 MESA (Multi-Ethnic Study of Atherosclerosis) participants with follow-up ABI values using multinomial regression.

**Results:** Mean (SD) age was 61 (10) years, 57% were women and 27% were blacks. Mean (SD) cystatin C was 0.88 (0.2) mg/L and median (IQR) ACR was 5.1 (3.2-10.0) mg/gm. Higher cystatin C was associated with progression to both low and high ABI in univariate, but not multivariable analyses (p>0.05). Elevated levels of ACR were significantly associated with progression to both low and high ABI in univariate and multivariable analyses.

**Conclusions:** Elevated levels of ACR are significantly associated with risk of progression to both low and high ABI.

| ACR | Progression to ABI<0.9 | | Progression to ABI>1.40 | |
|---|---|---|---|---|
| | Unadjusted | Multivariate Adjusted* | Unadjusted | Multivariate Adjusted* |
| Per Doubling | 1.26 (1.18, 1.35) | 1.10 (1.02, 1.20) | 1.14 (1.02, 1.29) | 1.14 (1.00, 1.30) |
| Quintiles | | | | |
| ≤3.0 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| 3.1–4.4 | 2.03 (1.19, 3.46) | 2.15 (1.24, 3.73) | 0.97 (0.47, 2.02) | 1.19 (0.56, 2.52) |
| 4.5–6.8 | 2.44 (1.44, 4.51) | 1.93 (1.12, 3.35) | 1.71 (0.89, 3.28) | 2.30 (1.20, 4.75) |
| 6.9–14.0 | 2.69 (1.60, 4.11) | 1.79 (1.04, 3.10) | 1.00 (0.47, 2.11) | 1.39 (0.63, 3.05) |
| >14.0 | 3.98 (2.42, 3.46) | 1.98 (1.15, 3.40) | 2.09 (1.09, 4.01) | 2.62 (1.26, 5.42) |

## SA-PO198

**Prognostic Value of Red Cell Distribution Width in Advanced Chronic Kidney Disease** Pablo Marcos Braillard Poccard, Cesar Garcia-canton, Noemí Esparza, Celia Lopez, Ana Ramirez Puga, Elvira Bosch, Rita Guerra, Eduardo Baamonde, Dolores Checa. *Nephrology, Hospital Universitario Insular, Las Palmas, Gran Canaria, Spain.*

**Background:** Increased red cell distribution width (RDW) is related to oxidative stress and pro-inflammatory states and is associated with higher mortality risk in the general population and in patients with cardiovascular diseases.

Our objective was to evaluate the association between RDW and mortality risk in a population with advanced Chronic Kidney Disease (CKD) and to describe the possible relationship with other prognostic factors.

**Methods:** Prospective observational cohort study of incident advanced CKD patients, who attended our clinic between January 2008 and December 2012 and were followed-up until March 30th 2013. The relationship between RDW and the rest of prognostic variables was studied. A survival analysis based on RDW terrtiles was conducted by using the Kaplan Meier method and the Cox proportional hazards model.

**Results:** The study included 533 patients aged 67.5 years, 55.9% male, 67.4% diabetic. Subjects were classified into RDW tertiles. Median follow-up times were: 16.7 months tertile 1 (T1), 17.6 months tertile 2 (T2), 17.9 months tertile 3 (T3). Mortality was 8.2%, 14.2% and 30.2% for T1, T2 and T3 respectively p<0.001. Tertile groups were diferents in age, %diabetes,%cardiovascular disease, hemoglobin, albumin, cholesterol T and CRP. and no differences were shown in gender, uric acid, phosphorus, GF MDRD, proteinuria or triglycerides. In a binary logistic regression model for mortality, RDW tertiles remained significant with OR of T3 against T1 2.929 (CI95% 1.498 -5.727) independently of the rest of variables. Kaplan-Meier survival curves for RDW tertiles showed survival means of 50.6m T1, 47.7m T2 and 37.8m T3; p<0.001. The Cox proportional hazards model showed a relative mortality risk of 2.325 for T3 against T1 (CI95% 1.266-4.271) adjusted for the rest of variables.

**Conclusions:** In this study, RDW appeared to be a marker for mortality risk independent of other known markers in CKD. The physiopathological meaning and prognostic value of this finding for advanved CKD patients should be verified in further studies.

## SA-PO199

**Prevalence and Control of High Blood Pressure in a German Cohort with Chronic Kidney Disease: Cross-Sectional Data from the German Chronic Kidney Disease (GCKD) Study** Karl F. Hilgers, Matthias Schmid, Silvia Huebner, Martin Busch, Hermann G. Haller, Anna Kottgen, Florian Kronenberg, Elke Schaeffner, Thomas Sitter, Georg Schlieper, Claudia Sommerer, Christoph Wanner, Stephanie Titze, Kai-Uwe Eckardt. *On Behalf of the GCKD Investigators, Germany.*

**Background:** We assessed blood pressure control in a large german cohort of chronic kidney disease (CKD) patients in nephrology specialist care.

**Methods:** The German Chronic Kidney Disease (GCKD) study is a prospective observational cohort study which enroled 5217 german patients with CKD of various aetiologies, who are under nephrology specialist care. Inclusion criteria were an estimated glomerular filtration rate (eGFR) of 30-60 mL/min×1.73 m2 or overt proteinuria in the presence of an eGFR>60 mL/min×1.73 m2. Office blood pressure measured according to WHO criteria by trained study nurses was available from 5181 participants. Hypertension was defined as systolic > 140 mm Hg or diastolic > 90 mm Hg or intake of antihypertensive medications. Blood pressure was considered as controlled if systolic <= 140 and diastolic <= 90 mm Hg.

**Results:** Office blood pressure (mean±SD) of 5181 participants was 139.5 ± 20.4 / 79.3 ± 11.7 mm Hg; no less than 4958 (95 %) of the patients were hypertensive. The prevalence of hypertension was only slightly lower in patients included because of proteinuria (94 %) than in patients included because of the eGFR criterion (95 %). Of all hypertensive patients, only 2410 (49 %) exhibited a sufficient control of blood pressure; the remaining 51 % exhibited a blood pressure of 156.3 ± 15.3 / 85.3 ± 11.7 mm Hg. Among patients with diabetes (35 % of the cohort), office blood pressure was 142.1 ± 21.4 / 76.3 ± 11.9 mm, 98 % were hypertensive, and 52 % of the latter were controlled. Blood pressure in uncontrolled diabetic patients was 158.2 ± 15.3 / 81.6 ± 11.6 mm Hg.

**Conclusions:** Blood pressure control of CKD patients in Germany is less than optimal, even in nephrology specialist care. Identifying potential reasons will require further analyses of e.g. medication patterns. Improving blood pressure control provides an opportunity to ameliorate progression of CKD and prevent some of its complications.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

**SA-PO200**

**Self-Reported Cardiovascular Disease and Long Term Renal Outcomes in Chronic Kidney Disease (CKD) – A Report from the Chronic Renal Insufficiency Cohort (CRIC) Study** Mahboob Rahman,[1] Dawei Xie,[2] Harold I. Feldman,[2] Alan S. Go,[3] Jiang He,[4] John W. Kusek,[5] James P. Lash,[6] Akinlolu O. Ojo,[8] Stephen L. Seliger,[9] Susan P. Steigerwalt,[10] Valerie L. Teal,[2] Raymond R. Townsend.[2] *[1]Case Western Reserve Univ; [2]Univ of Pennsylvania; [3]Kaiser Permanente; [4]Tulane Univ; [5]NIDDK; [6]Univ of Illinois; [7]Univ of Michigan; [8]Univ of Maryland; [9]St. Johns Health System.*

**Background:** Although CKD and cardiovascular disease (CVD) are commonly observed together, it is unclear whether CVD increases risk of progression of CKD. We evaluated whether CVD is an independent risk factor for progression of kidney disease among a cohort individuals with CKD.

**Methods:** A prospective study of 3939 participants with CKD enrolled in the CRIC Study between June 2003 and June 2008. Self-reported cardiovascular disease (myocardial infarction/revascularization, chronic heart failure (CHF), stroke or peripheral arterial disease) was ascertained by questionnaire at study entry. Our primary endpoint was a composite of end-stage renal disease or a decline in glomerular filtration rate (GFR) to one-half from baseline.

**Results:** Mean GFR at baseline was 42.8 ml/min/1.73m$^2$. One third (33.4%) of the study participants reported a history of cardiovascular disease, and 10.7 % reported a history of chronic heart failure. Over a median follow up of 3.3 years, 779 (19.8%) participants experienced a renal endpoint. In multivariable models, a composite of any prevalent CVD at baseline was not an independent predictor a renal endpoint (HR 1.08; 95% CI: 0.92 - 1.28). However, self-reported history of CHF was independently associated with a 32% higher relative rate of the endpoint (HR 1.32; 95% CI: 1.05 - 1.66). This relationship was consistent across subgroups defined by age, race, gender and diabetes status.

**Conclusions:** Although overall prevalent CVD was not an independent predictor of decline in kidney function, self-reported history of CHF was a strong predictor of CKD progression. Future work is needed to investigate mechanisms for this association.

*Funding:* NIDDK Support

**SA-PO201**

**Renal Function and Outcome in Thrombolysed Stroke Patients** Hardi Hassan,[1] Asim Majeed,[2] David Sandler,[2] Don Sims,[1] Shahid A. Kausar,[3] Indranil Dasgupta.[2] *[1]Geriatric Dept - Stroke Unit, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom; [2]Stroke Unit and Nephrology Dept, Birmingham Heartlands Hospital, Birmingham, United Kingdom; [3]Geriatric Dept - Stroke Unit, Sandwell and City Hospitals Birmingham, Birmingham, United Kingdom.*

**Background:** Stroke and renal impairment are common with ageing. Thrombolysis in stroke is offered to acute stroke patients aiming at significant reduction in disability after stroke. Little is known about the effects of eGFR on disability after stroke thrombolysis. This study tries to find any possible association between the baseline impaired renal function and disability in thrombolysed stroke patients.

**Methods:** A retrospective study analyzing the data from a group of thrombolysed stroke patients in three teaching hospitals in the West Midlands UK, between October 2008 and December 2012. Patients were categorized into two groups; group-1 with eGFR less than 60, and group-2 with eGFR more than 60. The outcomes studied were the admission National Institutes of Health Stroke Scale (NIHSS) as an indicator of severity of the stroke, discharge Modified Rankin Scale (mRS) as a general disability measure, and length of stay in hospital. We also looked at the discharge destinations for the two groups.

**Results:** Data from 263 patients were analysed. Among these, 68 patients belonged to group-1, and 195 to group-2. The mean age was significantly different (group-1 76 vs group-2 64, p =0.0001). The mean for admission NIHSS was 15.4 for group-1 vs 13 for group-2 (p = 0.0014). Group-1 were more disabled on discharge with mean mRS of 2.7 vs 1.9 for group-2 (p = 0.0001). The mean length of stay was 23 and 13 for group-1 and 2 respectively (p = 0.0061).

Mortality among group-1 patients was 16% compared to 6% in group-2, and more patients among group 2 went home compared to group-1 ( 75% vs 53% respectively) and less ended in institutional care ( 4% compared to 10% respectively).

**Conclusions:** This study shows that patients with impaired renal function are older, have more severe stroke and have poorer outcome following thrombolysis. These patients have longer length of stay in hospital, higher mortality,more disability, and increased risk of institutionalization.

**SA-PO202**

**Association of Vitamin D Status and Inflammation with Mortality in Hemodialysis Patients form the U.S. and Europe** Darius Mason,[1] Len A. Usvyat,[2,3] Daniele Marcelli,[4] Aileen Grassmann,[4] Inga Bayh,[4] Laura Scatizzi,[4] Peter Kotanko,[2] Mondo Consortium.[2,3] *[1]Albany College of Pharmacy/Health Sciences, Albany; [2]Renal Research Institute, New York, NY; [3]Fresenius Medical Care North America, Waltham, MA; [4]Fresenius Medical Care, Bad Homburg, Germany.*

**Background:** Neutrophil/lymphocyte ratio (NLR) and Vitamin D (vitD) deficiency are associated with inflammation and mortality in hemodialysis (HD) patients. Whereas NLR is an indicator of inflammatory status, vitD deficiency may promote a pro-inflammatory environment. The aim of this present study was to evaluate the relationship of NLR and vitD deficiency and mortality in HD patients.

**Methods:** The MONDO initiative encompasses global HD patients (Usvyat, Blood Purif, 2013). We enrolled incident HD patients from the US (n=6208) and Europe (EUR) (n=905) between 2000 and 2012. We included first vitD level, concurrent neutrophil and lymphocyte counts, and routine clinical and laboratory measurements. The NLR was calculated as the ratio of neutrophil and lymphocyte counts. We built Cox models to determine the association of NLR (≤4, >4) and vitD levels (≥20, <20 ng/mL) with all-cause death with adjustment for age, gender, diabetes, race, BMI, and albumin levels. Descriptive data are median (interquartile range).

**Results:** US and EUR, respectively, age at HD initiation was 62.0 (51.1-73.0) and 71.8 (58.6-80.8) yrs, 57% and 62% were male. In the US, 46% of patients were black. There were 830 (9.8 deaths per 100 pt yrs) and 164 (17.9 deaths per 100 pt yrs) deaths in the US and EUR, respectively. VitD and NLR were 19.5 (13.1-29.0) ng/mL and 3.5 (2.4-5.3) for the US, and 24.0 (13.0-36.5) ng/mL and 3.0 (2.1-4.4) for EUR, respectively. Concurrent vitD status modified the associations between NLR and death. While in EUR a NLR>4 was associated with mortality irrespective of vitD status (vitD ≥20: HR 1.57 (P=.06); vitD <20: HR 1.80 (P=.01)), this association was less strong in the US (vitD ≥20: HR 1.29 (P=.01); vitD <20: HR 1.17 (P=.14)).

**Conclusions:** NLR is useful for predicting mortality in US and EUR HD patients. Despite adequate VitD status, NLR maintains predictive ability for mortality in US patients with comparable trends in EUR patients.

*Funding:* Pharmaceutical Company Support - (MONDO) initiative

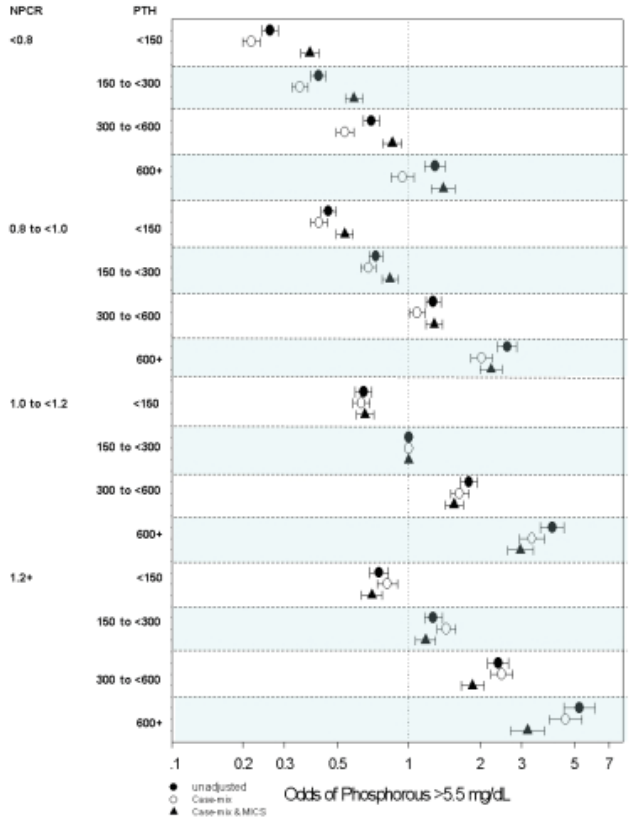
**SA-PO203**

**Concurrence of Higher Serum PTH and Higher Dietary Protein Intake Predict Hyperphosphatemia in Hemodialysis Patients** Elani Streja,[1] Wei Ling Lau,[1] Csaba P. Kovesdy,[2] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Memphis VA Medical Center, Memphis, TN.*

**Background:** High phosphorus (P) level is associated with higher risk of death in maintenance hemodialysis (MHD) patients. Previous studies have suggested that both higher serum intact parathyroid hormone (iPTH) level and higher dietary protein intake may contribute to higher levels of P. However, it is not well known how these two factors contribute to the risk of hyperphosphatemia when taken into account simultaneously. We hypothesized that the likelihood of hyperphosphatemia increases across higher serum iPTH and higher normalized protein catabolic rate (nPCR) levels, a surrogate of protein intake.

**Methods:** Over an 8-year period (7/2001-6/2009), we identified 69,355 MHD patients with iPTH, nPCR and P data. Logistic regression models were examined to assess the association between likelihood of hyperphosphatemia (P>5.5 mg/dL) and serum iPTH and nPCR increments.

**Results:** Patients were 61±15 years old and included 46% women, 33% blacks, and 57% diabetics. Both higher serum iPTH level and higher protein intake were associated with higher risk of hyperphosphatemia in dialysis patients (see Figure). Compared to patients with iPTH level 150-<300 pg/ml and nPCR level 1.0-<1.2 g/kg/day, patients with iPTH> 600 pg/ml and nPCR> 1.2 g/kg/day had a 3-fold increased risk of hyperphosphatemia (OR: 3.17, 95%CI: 2.69-3.75). Furthermore, increasing serum iPTH levels was associated with a linear incremental increase risk of hyperphosphatemia across levels of nPCR, and within each increasing level of nPCR the risk-trend was further incrementally magnified.




**Conclusions:** High serum P level is associated with both dietary protein intake and serum PTH level in MHD patients. Management of hyperphosphatemia not only requires attention to dietary interventions but also correction of hyperparathyroidism.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO204

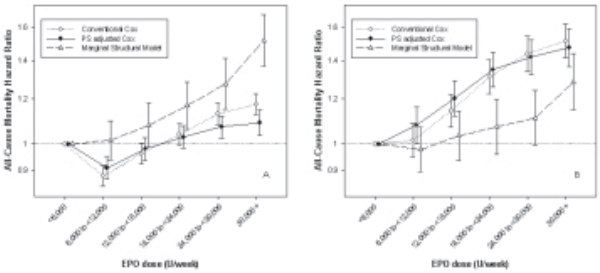
**Erythropoiesis-Stimulating Agent and Mortality in Hemodialysis: Multi-Level Model Comparison**

Elani Streja,[1] Jongha Park,[2] Ting-yan Chan,[1] Janet Lee,[1] Csaba P. Kovesdy,[3] Connie Rhee,[1] Daniel L. Gillen,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Univ of Ulsan College of Medicine, Ulsan, Korea; [3]Memphis VA Medical Center, Memphis, TN; [4]Dept of Statistics, Univ of California, Irvine, Irvine, CA.*

**Background:** It is unclear as to whether high erythropoiesis-stimulating agents (ESA) doses are harmful in hemodialysis (HD) patients. Dynamic ESA dosing can create time-dependent confounding because ESA dose is titrated in response to hemoglobin (Hb), a marker that is influenced by prior ESA dose and is also an independent prognostic factor for mortality.

**Methods:** Among a 2001-2006 cohort of incident and prevalent HD patients, ESA dose was averaged at successive *quarterly* intervals. ESA dose (U/week) was categorized into 6 levels. We compared the association between high-dose ESA and all-cause mortality across 3 models: a Cox model using baseline covariates, a propensity score adjusted Cox model stratifying on baseline propensity for ESA dosing, and a marginal structural model (MSM) weighting subjects by the inverse probability of ESA dosing in a time-dependent fashion. We adjusted for case-mix, inflammation, and nutrition covariates in all models.

**Results:** Compared to ESA<6000U/wk, mortality linearly increased with increasing ESA dose >1,200 U/week in overall (prevalent and incident; fig A) and incident HD patients (fig B) using MSM. These trends were comparable to those from the 2 comparison Cox regression models. ESA>24000U/week was associated with increased mortality in overall and incident patients in all models.




**Conclusions:** Higher ESA doses are associated with worse outcomes in MSM. Given randomized trials in which targeting higher Hb with ESA resulted in worse outcomes, our study suggests high ESA dose itself may be harmful. Focusing on ESA dose instead of Hb level should be considered in anemia management.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO205

**Change in Serum Phosphorus Level over Time and Mortality in Hemodialysis Patients**

Jongha Park,[1] Elani Streja,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[2] *[1]Ulsan Univ Hospital, Ulsan, Korea; [2]Harold Simmons Center, UCI, Orange, CA; [3]Memphis VA Medical Center, Memphis, TN.*

**Background:** Previous studies have found an association of hyperphosphatemia with mortality in maintenance hemodialysis (MHD) patients. However, an association between change in serum P over time and mortality has been insufficiently explored.

**Methods:** A total of 66,084 MHD patients treated from (6/2001 to 6/2006) in the US were analyzed. Patients were stratified by baseline serum P values (<3.5, 3.5 to <5.5 and ≥5.5 mg/dL), then were divided into 3 groups in within each stratum based on mean level during the 3rd calendar quarter. Cox proportional hazard models were used with adjustments for casemix and malnutrtion inflammation complex markers.

**Results:** Compared with patients whose serum P level was sustained greater than 5.5 mg/dL, survival benefit was revealed in patients with decrease in serum P level from ≥5.5 mg/dL to 3.5–<5.5 mg/dL. In the same vein, increase in serum P level from 3.5–<5.5 mg/dL to ≥5.5 mg/dL tended to be associated with higher mortality compared with no change in serum P level within 3.5–<5.5 mg/dL . In contrast, decrease to low level (<3.5 mg/dL) was paradoxically associated with increased mortality in comparison with no change in each baseline serum P stratum.

| Baseline P (mg/dL) | 3rd quarter P (mg/dL) | N | HR (95% CI) |
|---|---|---|---|
| ≥5.5 | <3.5 | 396 | 1.40 (1.24–1.58) |
| | 3.5–<5.5 | 9,525 | 0.93 (0.90–0.96) |
| | ≥5.5 | 22,720 | Ref |
| 3.5–<5.5 | <3.5 | 1,789 | 1.28 (1.21–1.36) |
| | 3.5–<5.5 | 19,723 | Ref |
| | ≥5.5 | 9,225 | 1.03 (0.99–1.06) |
| <3.5 | <3.5 | 669 | Ref |
| | 3.5–<5.5 | 1,689 | 0.78 (0.69–0.87) |
| | ≥5.5 | 348 | 0.86 (0.73–1.02) |

**Conclusions:** Decrease in serum P level may be associated with better survival. However, decrease in serum P level below normal range seems to be associated with increased mortality, in which low serum P may be a proxy of poor nutritional state.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO206

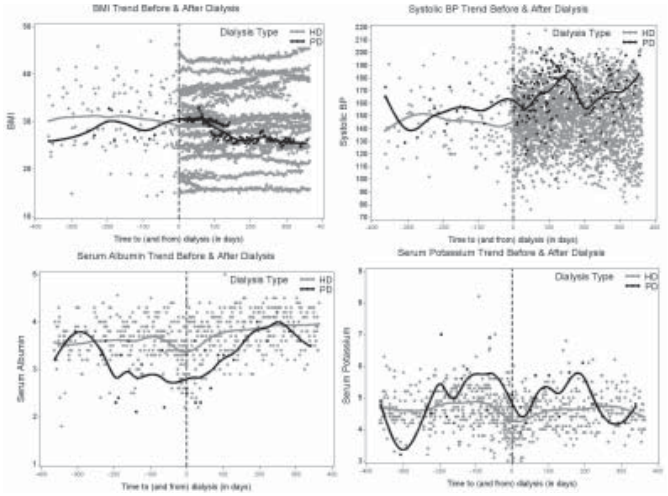
**Trends in Key Clinical Parameters before and after Initiation of Dialysis: The Renal Research Institute (RRI)-CKD Extension Study**

Aleksandar Milovanovic,[1] Anca Tilea,[1] Brenda W. Gillespie,[1] Michael Heung,[1] Peter Kotanko,[2] Rajiv Saran.[1] *[1]Univ of MI; [2]Renal Research Inst.*

**Background:** There is a paucity of studies examining the critical transition and continuum through advanced stages of CKD through initiation of dialysis and beyond.

**Methods:** We conducted a follow-up study of CKD patients at University of Michigan (UM), one of the sites in the RRI-CKD study (a 4-center prospective cohort), using electronic chart review among patients where follow-up had previously ended at or prior to ESRD. Electronic records were abstracted to track clinical events and outcomes 1year pre- and post-dialysis (hemodialysis-HD or peritoneal dialysis-PD).Spline regression examined trends in clinically relevant parameters in the period before and after initiation of dialysis, stratified by HD/PD.

**Results:** 74 of 280 UM patients were identified in the dialysis database.Mean age at dialysis initiation was 57 yrs.,83% were white,56% male;95% had hypertension and 40% were diabetic.52% were on HD and 39% on PD; 21 subjects had died. Comparative trajectories of key clinical parameters are shown in the Figure by dialysis type.Among HD patients,BMI,BP,and serum albumin were relatively stable pre- and post-dialysis. For PD patients, BMI steadily decreased and serum albumin showed a significant dip at 6 months pre- and post-dialysis,while systolic BP increased by an average of 20mmHg after initiation of dialysis.Serum potassium levels showed a dip for both dialysis types 3-months around the time of initiation of dialysis.However, PD patients on average had higher serum potassium levels than those on HD.




**Conclusions:** This pilot study points to significant differences between PD and HD patients in key clinical parameters during the transition from CKD to ESRD.Association between these trajectories and outcomes may provide guidance regarding patient management during this critical transition period.

## SA-PO207

**Healthcare Use before and after Initiation of Dialysis: Renal Research Institute (RRI) CKD Extension Study** Aleksandar Milovanovic,[1] Anca Tilea,[1] Brenda W. Gillespie,[1] Michael Heung,[1] Scott L. Hummel,[1] Peter Kotanko,[2] Rajiv Saran.[1] *[1]Univ of MI; [2]Renal Research Inst.*

**Background:** There is a paucity of studies examining the critical transition and continuum from late stage CKD through initiation of dialysis and beyond. We examined healthcare use 1 year pre- and post-dialysis in a CKD cohort.

**Methods:** We conducted a follow-up study of CKD patients at University of Michigan (UM), one of the sites in the RRI-CKD study (a 4-center prospective cohort), using electronic chart review to extend follow-up previously ending at or prior to ESRD. Clinical events and outcomes 1 year pre- and post-dialysis initiation (hemodialysis-HD or peritoneal dialysis-PD) were abstracted. Rates of hospitalization, emergency (ED), specialist and nephrologist visits were calculated and expressed per 100 person-days. Poisson regression was used to predict number of hospital-days within the year pre- and post-dialysis. Association between type of first vascular access and post-dialysis hospital days was examined adjusted for age, sex, race, diabetes and hypertension.

**Results:** 74 of 280 patients at UM were identified in the dialysis database. Mean age at dialysis initiation was 57yrs, 83% were white, 56% male; 95% had hypertension and 40% were diabetic. Initial modality was HD in 52% and PD in 39%; 21 subjects died (10 on HD, 11 on PD). PD patients had higher nephrology and specialist visit rates than HD patients (0.22 vs. 0.10 and 0.15 vs. 0.10, respectively), with the ED and hospitalization rates being similar between the two groups. Use of catheter as first vascular access type was associated with 2.3 more hospital days and AV Graft with 1.6 more days in hospital vs. arteriovenus (AV) Fistula. The 3 months pre-dialysis and 0-4 months post-dialysis were associated with greater hospital days compared with 5-12 months post dialysis. However, 4-12 months pre-dialysis was associated with the lowest number of hospital days when compared to 5-12 months post-dialysis.

**Conclusions:** Healthcare use was high among CKD patients both during the late CKD and early ESRD. AVF use as first vascular access was associated with lower number of days in hospital compared with AVG or catheter.

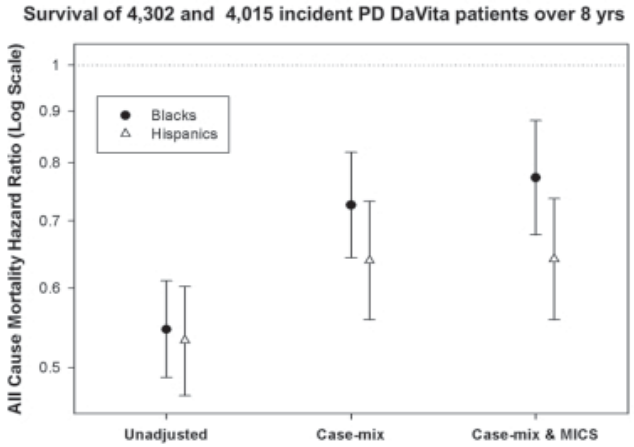
## SA-PO208

**Association of Race-Ethnicity with Mortality in Incident Peritoneal Dialysis Patients** Chunyang Li,[1] Elani Streja,[1] Connie Rhee,[1] Wei Ling Lau,[1] Miklos Zsolt Molnar,[1] Csaba P. Kovesdy,[3] Rajnish Mehrotra,[2] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Harborview Medical Center, Univ of Washington, Seattle, WA; [3]Memphis Veterans Affairs Medical Center, Memphis, TN.*

**Background:** It has been previously reported that African-American and Hispanic dialysis patients have greater survival than whites ("African-American Paradox"). Survival advantage has also been reported in Hispanics despitelower socioeconomic status and faster chronic kidney disease progression to dialysis dependency ("CKD Hispanic Paradox within Paradox"). However the association between race and mortality peritoneal dialysis (PD) patients is still unclear.

**Methods:** We examined 8-year (7/2001-6/2009) all-cause mortality for a cohort of 5,067 DaVita incident (< 90 days dialysis vintage days at the time of entry into cohort) PD patients including 1,052 African-Americans, 765 Hispanic, and 3,250 Whites using Cox proportional hazard models adjusted for case-mix and malnutrition-inflammation complex syndrome (MICS) covariates.

**Results:** Among African-Americans, Hispanics, and shites the mean age (mean + SD) was 52+14, 53+16, and 59+15 yrs old and included 55%, 48% and 42% women; and 46%, 57% and 48% diabetics, respectively. Compared to Whites, African-Americans and Hispanics have a lower risk of mortality; even after adjusting for case mix and MICS markers: HRs (95%CI) 0.77 (0.68-0.88) and 0.64 (0.56- 0.74), respectively. Compared to African-Americans, Hispanics had slightly lower risk of mortality.




**Conclusions:** Incident African-American and Hispanic PD patients have greater survival in comparison to white PD patients. Further studies are needed to examine these racial-ethnic differences.

*Funding:* NIDDK Support, Private Foundation Support

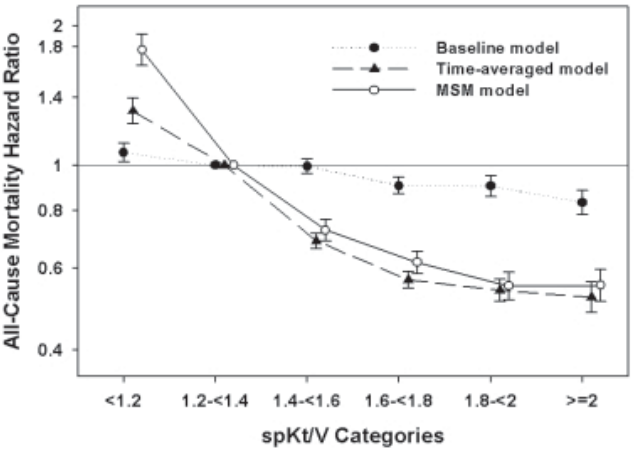
## SA-PO209

**Hemodialysis Dose and Survival: A Marginal Structural Model Analysis** Paungpaga Lertdumrongluk,[1] Elani Streja,[1] Onyebuchi A. Arah,[2] Jongha Park,[5] Csaba P. Kovesdy,[6] Connie Rhee,[1] Steven M. Brunelli,[3] Daniel L. Gillen,[4] Kamyar Kalantar-Zadeh,[1] Mark L. Unruh.[7] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Dept of Epidemiology, Fielding School of Public Health, UCLA, Los Angeles, CA; [3]DaVita, Needham, MA; [4]Dept of Statistics, Univ of California, Irvine, Irvine, CA; [5]Univ of Ulsan College of Medicine, Ulsan, Korea; [6]Memphis Veterans Affairs Medical Center, Memphis, VA; [7]Univ New Mexico, Albuquerque, NM.*

**Background:** Observational studies have consistently demonstrated the survival benefits of greater dialysis dose, whereas randomized controlled trials show conflicting results. The causal analysis of the impact of dialysis dose on mortality requires investigation with novel statistical methods such as marginal structural models (MSM) that account for time-varying confounding.

**Methods:** We quantified the effect of delivered dose of hemodialysis (spKt/V) on mortality in a 2001-2005 cohort of 68,060 patients undergoing thrice-weekly hemodialysis (HD). We compared conventional Cox regression and MSM analyses, accounting for time-varying confounding by applying longitudinally modeled inverse-probability-of-dialysis-dose weights.

**Results:** In Cox models, baseline spKt/V showed a weak negative association with mortality, while higher time-averaged spKt/V was strongly associated with lower mortality risk. In MSM analyses, a spKt/V <1.2 was associated with increased mortality (HR [95% CI] 1.77 [1.64-1.92], whereas lower mortality risk was observed with spKt/V values >1.4 (ref: spKT/V 1.2->1.4): HRs (95%CI) 0.72(0.69-0.76), 0.62(0.58-0.65), 0.55(0.51-0.59), and 0.55(0.51-0.60) for spKt/V ranges of 1.4-<1.6, 1.6-<1.8, 1.8-<2.0, and ≥2.0, respectively.



**Conclusions:** The data suggest higher dosing of hemodialysis is robustly associated with greater survival in MSM analyses that more completely account for time-varying confounding.

*Funding:* NIDDK Support, Private Foundation Support

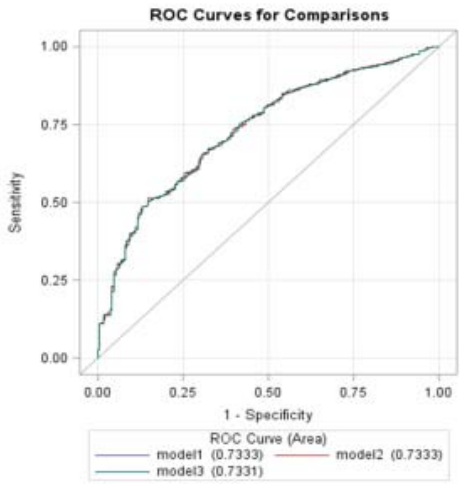
## SA-PO210

**Comparative Effectiveness of Laboratory Measures for One-Year Survival Prediction in Maintenance Hemodialysis Patients** Lu Bai,[1] Elani Streja,[2] Miklos Zsolt Molnar,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh,[2] Daniel L. Gillen.[1] *[1]Dept of Statistics, Univ of California, Irvine, Irvine, CA; [2]Harold Simmons Center, UCI, Orange, CA; [3]Memphis VA Medical Center, Memphis, TN.*

**Background:** It is not clear which potentially modifiable lab measures are associated with greatest survival in maintenance hemodialysis (MHD) patients. We aim to find the best subset of commonly measured markers for prediction of one year survival in patients initiating MHD.

**Methods:** In a 2001-2006 cohort of 63,758 incident MHD patients, we evaluated body mass index (BMI) and 16 lab measures with for ability to predict survival at one year. Logistic regression was used to model probability of survival at one year and the area under the receiver operating characteristic curve (ROC-AUC) was used to assess predictive performance of potential models.

**Results:** The final selected model (Model 1) included 14 variables (albumin, lymphocyte, serum creatinine, BMI, alkaline phosphatase, hemoglobin, phosphorus, ferritin, PTH, WBC, calcium, Kt/v, Kru and nPCR). The second highest rank model (Model 2) included the above covariates in addition to ISAT, while the third most predictive model (Model 3) included only 13 biomarkers, eliminating Kru from the top ranked model. Averaged ROC-AUCs resulting were for the three models 0.7352, 0.7350 and 0.7349, respectively. All three candidate models performed nearly equally (as seen by the heavy overlap in all estimated curves). Models 1 and 2 obtained the same estimated AUC when applied to the test data, however given the greater parsimony of Model 1 it is preferred.




**Conclusions:** We used rigorous prediction model building methods to identify 14 potentially modifiable biomarkers that influence survival at one year among new hemodialysis patients. Identification of these factors provides targets for treatment among new hemodialysis patients.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO211

**Abstract Withdrawn**

## SA-PO212

**Patients with Primary Glomerulonephritis on Renal Replacement Therapy May Be Cured: Data from the Veneto Dialysis and Transplantation Registry** Maurizio Nordio, Nicola Tessitore, Cataldo Abaterusso, Barbara Rossi, Giovambattista Virga, Francesco Antonucci. *Veneto Dialysis and Transplantation Registry, Padua, Italy.*

**Background:** Survival on RRT is affected by the primary renal disease (PRD). By using relative survival analysis (patients' mortality is compared with the general population) we observed that patients on RRT with primary glomerulonephritis (PGN) had the best survival. After ten years of RRT, patients with PGN experience the same mortality rate as the general population, suggesting the likelihood of cure. The study aims at estimating the fraction of cured patients with PGN and cure determinants.

**Methods:** We considered the cohort of incident patients with PGN on RRT in the period 01/01/1998 – 31/12/2010 in Veneto (Italy). Demographic features, RRT modality and the main comorbidities were considered. Cure models are a type of survival analysis assuming a proportion of subjects who will never experience the event. These models allow incorporation of the expected background mortality and thus estimate relative survival when cure is a possibility.

**Results:** In the study period, 813 of the 6962 RRT patients had a diagnosis of PGN. Median age was 56, 70% were male. The estimated cured fraction was 48%. Heart disease and cancer were associated with an increased risk of death (HR=2.7 [2.04 – 3.58] and 2.65 [1.85 – 3.80] , respectively), while anti-hypertensive therapy reduced the risk (HR=0.7 [0.49 – 0.84]). By postestimation forecasting, in presence of heart disease and cancer, the cured fraction fell down to 15% and 16%. If both were present, the cured fraction was virtually nil. Being on anti-hypertensive therapy increased the cured fraction up to 64%. Most patients alive after ten years were transplanted (77%).

**Conclusions:** Our study highlights the influence of PRD and comorbidities over the prognosis of RRT patients. Patients with PGN may be cured, since 48% of them reach the same mortality as the general population, indicating the effectiveness of RRT modalities in controlling uremia. Moreover, it underlines the relevance of comorbidities, because of their independent effect on survival rates. Our findings suggest that the tendency of considering RRT as a unique condition may be misleading.

*Funding:* Government Support - Non-U.S.

## SA-PO213

**Comparative Effectiveness of Iron Formulation and Dosing Practices on Health-Related Quality of Life in Patients on Hemodialysis** Janet K. Freburger,[1] Abhijit V. Kshirsagar,[3] Lily Wang,[1] Alan R. Ellis,[1] Wolfgang C. Winkelmayer,[4] M. Alan Brookhart.[1,2] *[1]Sheps Center for Health Services Research, Univ of North Carolina, Chapel Hill, NC; [2]Dept of Epidemiology, Univ of North Carolina, Chapel Hill, NC; [3]UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC; [4]Div of Nephrology, Stanford Univ, Stanford, CA.*

**Background:** While studies have examined the effects of intravenous (IV) iron on anemia parameters in the hemodialysis population, we are unaware of any that have examined how IV iron affects health-related quality of life (HRQOL). We examined the associations of different iron formulations and dosing strategies with HRQOL.

**Methods:** We conducted a retrospective cohort study using clinical data from a small dialysis provider (2006-2008) merged with data from the United States Renal Data System. We examined data on 1,253 patients who received center-based hemodialysis. Iron formulation use (ferric gluconate vs iron sucrose) and dosing (bolus: large doses over a short period vs maintenance: smaller, less frequent doses) were assessed during repeated 1-month exposure periods (n=1,782). Generalized linear mixed models, adjusting for dialysis facility, epoetin alfa (EPO) dose, and clinical/laboratory variables were used to estimate the effects of iron on HRQOL (measured by the SF-36) during a 3-month follow-up period. Effects were also estimated within clinically-relevant subgroups.

**Results:** Compared to maintenance dosing, patients receiving bolus dosing had higher HRQOL scores (mental health summary score 4.2 points higher, 95% CI: 0.9, 7.4; physical health summary score 2.4 points higher 95% CI: -1.3, 6.0). These differences were below the minimum clinically important difference of 5 points. The positive effects of bolus dosing were greater among individuals who were EPO hypo-responsive or who had lower baseline hemoglobin or iron indices. Overall, we did not observe clinically meaningful differences in HRQOL between patients receiving ferric gluconate versus iron sucrose.

**Conclusions:** Bolus iron dosing was associated with improvements in HRQOL, particularly for individuals who were EPO hypo-responsive or who had lower hemoglobin or iron indices at baseline.

*Funding:* Other U.S. Government Support

## SA-PO214

**Proteinuria with High Serum Level of Hepcidin-25 Indicates a Bad Prognosis in Non-Hodgkin's Lymphoma Patients** Masaki Hara,[1] Minoru Ando,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Renal Div, Dept of Medicine, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan; [2]Dept IV of Internal Medicine, Tokyo Women's Medical Univ, Shinjuku-ku, Tokyo, Japan.*

**Background:** Serum hepcidin-25 level increases in the state of chronic inflammation including kidney disease and cancers; however, its clinical impact on disease prognosis has not fully understood.

**Methods:** One-year prospective study was conducted to ascertain an impact of serum hepcidin-25 on mortality in non-Hodgkin's lymphoma (NHL) patients with CKD. The cohort comprised 50 NHL patients receiving chemotherapy (mean age, 66 years). Serum hepcidin-25 level was measured by liquid chromatography-mass spectrometry. Proteinuria, a simple sign of kidney disease, was defined as a dipstick test ≥1+, persistent in three consecutive examinations. Cumulative survival curves were drawn with Kaplan Meier method, which were stratified into 4 groups by both presence or absence of proteinuria and presence or absence of elevation in serum hepcidin-25 more than the mean value. Multivariable proportional hazards regression analysis was used to calculate hazard ratio (HR) with its 95% confidence interval (CI) for mortality.

**Results:** Prevalence of proteinuria was 14%. Mean serum hepcidin-25 level was 65.4 ± 60.9 ng/ml (the healthy reference: 22.2 ± 12.3 ng/ml). Distribution of the 4 groups was as follows: non-proteinuria with lower hepcidin-25 (58%), non-proteinuria with higher hepcidin-25 (28%), proteinuria with lower hepcidin-25 (0%), and proteinuria with higher hepcidin-25 (14%). The Kaplan-Meier estimate at 1 year was the highest (85.7%) in the group of proteinuria with higher hepcidin-25 among all. The HR (95% CI) of this group was 7.98 (2.20–29.5), as compared with the group of non-proteinuria with lower hepcidin-25 levels.

**Figure. Kaplan-Meier curves stratified by both proteinuria (presence or absence ) and elevation in serum hepcidin-25 (presence or absence )**

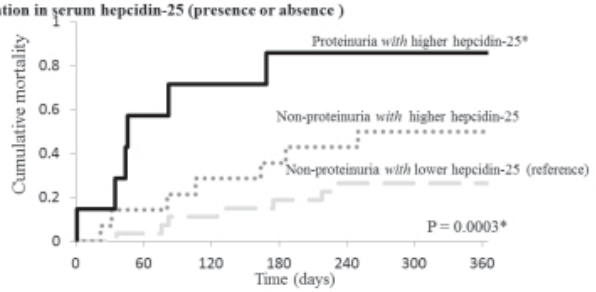



**Table. The joint risk of proteinuria and hepcidin-25 for one year mortality**

| | Multivariable analysis | |
|---|---|---|
| Variable | HR (95% CI) | P value |
| Age | 1.02 (0.99 - 1.06) | 0.2434 |
| Gender, men | 1.07 (0.32 – 3.10) | 0.9031 |
| Stages of NHL | 1.03 (0.57 – 2.15) | 0.9194 |
| Proteinuria *with* higher hepcidin-25 | 7.98 (2.20 – 29.5) | 0.0021 |

**Conclusions:** Proteinuria accompanied by serum hepcidin-25 elevation may predict poor outcome in NHL patients.

## SA-PO215

**Outcomes of Pediatric-Onset CKD Patients Who Underwent Health Care Transition Preparation** Maria E. Ferris, Nicole M. Fenton, Gerald A. Hladik, Abhijit V. Kshirsagar. *UNC Kidney Center, Univ of North Carolina at Chapel Hill.*

**Background:** The impact of health care transition preparation (HCTP) in pediatric-onset CKD patients transitioning from pediatric to adult-focused providers needs to be characterized.

**Methods:** We conducted an IRB approved cross-sectional study of adult nephrology patients who received at least one HCTP session at the UNC pediatric nephrology clinic between July '07 to May '13 (intervention). Disease self-management/readiness to transition was assessed by the provider-administered UNC TRxANSITION Scale and the self-administered STARx Survey. Adherence was ascertained based on health provider notes. Health outcomes were classified as no change or worse medical status, including transplant loss or death, based on chart review. Data was double entered and analyzed in SPSS.

**Results:** We enrolled 90 participants (66 intervention, 24 controls) with the following characteristics: 36 (40%) male; mean age of diagnosis 10.7 ± 5.67 years; 46% African American, 35% White, 8% Hispanic and 11% other race; 48% had private insurance and 50% came from a two-parent household. Currently, 25% of patients receive anti-depressants, 2% ADHD treatment and only 30% reported school attendance/employment. There was one death. Glomerular disease was reported in 59% of patients, transplant 34%, with a mean eGFR 66 ± 33 ml/min/1.73m$^2$(range 5-110); 50% were diagnosed at ≤ 12 years of age (30% prenatally or in the first year of life). Adherence was documented in 63% of patients and 17% were non-adherent. 49% of patients were overweight (BMI ≥ 25 kg/m$^2$), and 31% were obese (≥ 30 kg/m$^2$ ). The BMI of patients with glomerular disease was higher (28.71±7.45) than that of non-glomerular patients (26.93±5.85) (p=0.01). Intervention patients had superior outcomes than controls both in terms of clinical status (F= 5.64, p=0.02) and adherence($X^2$=3.76, p=.05).

**Conclusions:** In this single center cohort, survivors of pediatric-onset CKD have a lower employment rate and higher levels of depression compared to the general population. Health care transition preparation appears to play a significant role in improving health outcomes and adherence.

*Funding:* Private Foundation Support

## SA-PO216

**Renoprotective Effectiveness of Third Generation Calcium Channels Blockers** Ricardo M. Heguilen, Amelia Rita Bernasconi. *Nephrology, Hospital Juan A Fernandez, Buenos Aires, Argentina.*

**Background:** Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin 2 receptor blockers (ARBs) have widely recognized renoprotective properties. Third generation dihydropyridine calcium channel blockers (3dhpCCBs), acting on L as well as T-type calcium activated channels, would reduce intraglomerular pressure (P-GC) and urine protein excretion (UprotV) in several nephropathies. The main aim of this study was to assess whether the addition of the 3dhpCCB lecarnidipine (LER) has similar renal effects to the addition of an ARB in patients (Ps) with chronic kidney disease (CKD) receiving renoprotection with enalapril (ENL).

**Methods:** Normotensive, stage 2-3 CKD Ps with UprotV < 2 g/d, and receiving ENL for > 12 months were included. Renal function as well as UprotV were assessed at baseline; then Ps were assigned to fixed dose of losartan (LOS) or LER. Blood pressure, creatinine clearance and UprotV was assessed every 4 ± 0.5 months. All data are expressed as mean ± SD; repeated measures ANOVA was used and p values < 0.05 were considered statistically significant.

**Results:** Sixty four (44 men) 48 – 79 years old Ps, receiving ENL (10-25 mg/d) were included. LOS (50 mg/d) was added in 31 while LER (10 mg/d) was added in 33. The addition of LOS or LER produced no changes in BP. Serum creatinine –SCr- (LOS 1,48 ± 0,28 and LER 1,44 ± 0,29 mg/dL) and CrC (LOS 46.2 ± 5.8 and LER 45.1 ± 8.7 mL/min/1.73 m2) remained stable (p: NS) at 2 ½ years.

UprotV (g/day) decreased (p< 0,05) similarly in both groups (p: NS) from 1.36 ± 0,4 to 0,71 ± 0,3 –LOS- and from 1,32 ± 0,44 to 0,67 ± 0,31 –LER-).

**Conclusions:** 1. The addition of LER to the standard therapy with ENL has similar renoprotective effect to ENL plus LOS.

2. 3dhpCCBs could be an alternative for those Ps not responding to the dual blockade of the RAS or in whom such measure produced adverse consequences.

3. Additional adequately designed studies in a larger number of individuals are necessary to confirm the benefit of this group of agents.

## SA-PO217

**Effect of Steroid Pulse Therapy in Patients with IgA Nephropathy According to the Oxford Classification** Kyeong Woo Nho, Wonseok Yang, Su-Kil Park. *Div of Nephrology, Dept of Internal MedicineCollege of Medicine, Univ of Ulsan, Asan Medical Center.*

**Background:** Steroids have been shown to reduce proteinuria and preserve renal function. We investigated the effect of steroid pulse therapy in patients with IgA nephropathy according to the Oxford classification.

**Methods:** We assessed 33 IgA nephropathy patients. Pathologist reviewed biopsy slide was not over 6 years according to the Oxford classification. All patients had been maintained ARB or ACEi. 17 Patients were treated with 500 mg intravenous MP every 2 weeks for 6 months to improve renal function. The efficacy of MP pulse was analyzed by comparing the slopes of eGFR(ml/min/1.73m2) and log transformed urine albumin/creatinine ratios before,during,and after therapy using linear regression coefficients. We compare outcome between 17 steroid pulse groups(Group1) and 16 untreated groups(Group2).

**Results:** Median duration between biopsy and pulse therapy is 1month(range 0~73). Mean follow-up is 33.6months(12~46) after pulse.

| | ΔeGFR/month | P |
|---|---|---|
| Group 1(before&after steroid) | -0.68(0.52) vs -0.34(0.35) | 0.08 |
| Group1(before)&Group2 | -0.68(0.52) vs -0.29(0.55) | 0.05 |
| Group1(after)&Group2 | -0.34(0.35) vs -0.29(0.55) | 0.81 |

According to the Oxford classification 1/17 patient was M1, 2/17 were E1, 14/17 were S1, 6/17 were T0, 10/17 were T1, 1 patient was T2 in group1. Using $\chi 2$ test, we found no significant differences in Oxford classification of histological grade between group 1 and 2. Mean eGFR for group1 was 41.3±15.7. Linear regression analysis showed improvement of decline of eGFR after steroid pulse in 9 of 14 group1 patients with initial GFR>30. After steroid pulse, the rate of monthly decline of eGFR decreased in group1(-0.68±0.52 vs -0.34±0.35, p=0.08). There was no improvement of urine albumin/creatinine ratio in group1. As the grade of T increased, the rate of decline of eGFR increased in both group(p=0.02). Other parameters in Oxford classification were not a significant variable.

**Conclusions:** Steroid pulse therapy could improve the decline of eGFR in IgA nephropathy. Among the Oxford classification, only T grade affects the change of eGFR irrespective of steroid pulse therapy.

## SA-PO218

**Sedentary Activity Is Associated with Increased Mortality and Light Intensity Activity Is Associated with Decreased Mortality in CKD** R. Filipowicz,[1] G. Wei,[1] R. Marcus,[1] Michel Chonchol,[2] Tom Greene,[1] Srinivasan Beddhu.[1] *[1]U of Utah; [2]U of Colorado.*

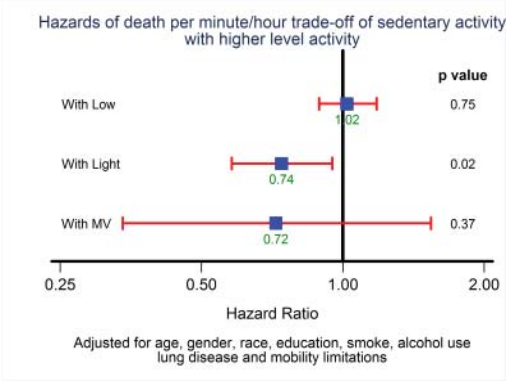
**Background:** Sedentary activity(SA) is emerging as a major risk factor for mortality in the general population. However, the associations of SA and physical activity (PA) intensity levels with mortality has not been studied in CKD. Therefore, we examined the associations of objectively measured SA and PA intensity levels with mortality in CKD using 2003-2004 National Health & Nutrition Examination Survey (NHANES) data.

**Methods:** PA levels were measured with Actigraph 7164 accelerometer in NHANES. Mortality data until Dec 31, 2006 were obtained by NHANES. For the current analyses, we included 405 NHANES participants aged >20 yrs with eGFR <60 ml/min/1.73m$^2$ & valid accelerometer data (wore the device ≥10 hr/day & ≥4 days). Cox regression models adjusted for age, gender, race, education, smoking, alcohol use, lung disease and mobility limitations were used to examine the associations of PA levels with mortality.

**Results:** The mean ± SD age was 69.3± 11.8 yrs. 39% were men and 6% were black. There were 154 deaths over 1190 years of follow-up. Table 1 summarizes the definitions and duration of PA.

| Definitions and duration of PA levels | | |
|---|---|---|
| | Accelerometer Counts (counts/min) | Average Duration Spent in PA Level (min/hr) |
| SA | <100 | 40± 6 |
| Low | 100 to 499 | 13 ± 4 |
| Light | 500 to 2019 | 6 ± 4 |
| MV | ≥ 2020 | 1 ± 1 |

Each min/hr ↑ in SA was associated with ↑ hazard of mortality (HR 1.10, 95% CI 1.04, 1.17, p=0.004). Figure 1 shows the association of trade-off of 1 min/ hr of SA with each of low, light or MV activity holding the other two activities constant.




These data suggest that trade off of SA with light PA is significantly associated with ↓ mortality while MV might also confer a lower mortality.

**Conclusions:** SA is associated with increased mortality in CKD and replacing SA with light or MV activities are likely to reduce mortality.

*Funding:* NIDDK Support

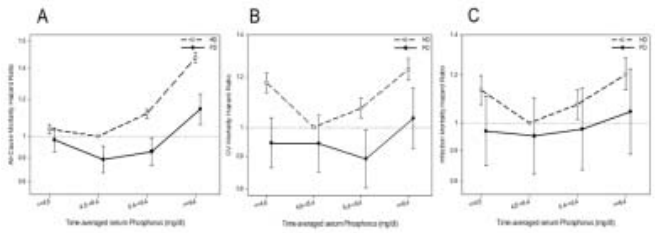
## SA-PO219

**Comparative Survival Predictability of Phosphorus and Albumin-Adjusted Calcium in HD and PD Patients** Vanessa A. Ravel,[1] Elani Streja,[1] Jongha Park,[3] Wei Ling Lau,[1] Connie Rhee,[1] Csaba P. Kovesdy,[4] Rajnish Mehrotra,[2] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Harborview Medical Center, Univ of Washington, Seattle, WA; [3]Univ of Ulsan College of Medicine, Ulsan, Korea; [4]Memphis VA Medical Center, Memphis, VA.*

**Background:** While the association of calcium (Ca) and phosphorous (P) levels with death risk is well established in patients undergoing hemodialysis (HD), there is a paucity of data for peritoneal dialysis (PD) patients. Furthermore, there are no data to determine whether the thresholdCa and P levels above which risk for death is increased is the same for those on PD vs HD.

**Methods:** Among a 2001-2006 cohort of 129,604 dialysis (10,066 on PD and 118,998 on HD at study entry) patients with Ca and P data. Time-averaged Cox models were examined to assess the comparative survival predictability of P and albumin-corrected Ca in HD vs PD patients.

**Results:** Risk for death was higher for HD patients with albumin-corrected Ca≤9.1mg/dl or >9.5mg/dl, but was lower in all sub-groups of PD patients with Ca<9.9 mg/dl (ref: HD patients with Ca 9.4-<9.5mg/dl). Risk for death was higher for individuals undergoing HD with P<4.5 mg/dl or >5.4 mg/dl (ref: HD patients with P 4.5-<5.4mg/dl), and PD patients with P>6.4 mg/dl. The risk for death for HD was higher than for PD patients at all levels of P and across all causes of death.




(A) All-cause mortality. (B) Cardiovascular mortality. (C) Infection related mortality. Reference group: hemodialysis patients with serum phosphorus levels from 4.5-5.4 mg/dl.

**Conclusions:** The associations between Ca and P with mortality are disparate between HD and PD patients. Low P level is associated with poor outcomes in HD, whereas high P levels predict mortality for patients with either modality, with the threshold above which risk is increased being higher in PD patients. Further studies exploring differences in the association of death risk of mineral metabolism parameters in HD vs. PD patients are warranted.

*Funding:* NIDDK Support, Private Foundation Support

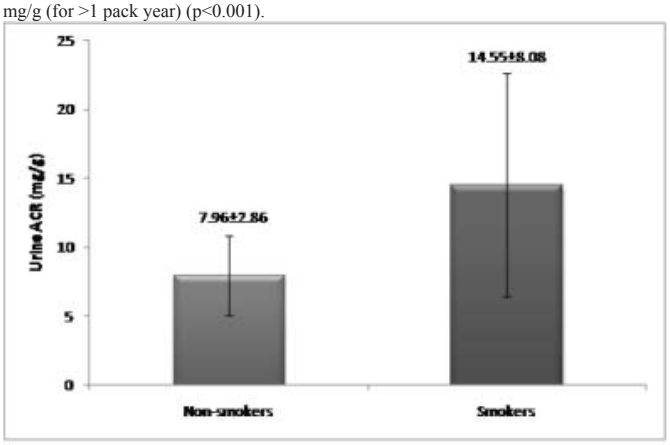
## SA-PO220

**Effect of Cigarette Smoking on Renal Function in Healthy Subjects** Om Parkash Kalra, Abhishek V. Patil, Sunil Agarwal, Amitesh Aggarwal, Ashok K. Tripathi, Anil Kumar Yadav. *Univ College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.*

**Background:** The prevalence of chronic kidney disease (CKD) is increasing, hence there is need to identify various risk factors associated with etiopathogenesis of renal disease. Smoking as a risk factor for pathogenesis of renal dysfunction has been less well studied. In our study, we attempted to study the effect of smoking on renal function in healthy adults.

**Methods:** This cross-sectional study was conducted on healthy male subjects in the age group of 31-40 years. The study consisted of 40 subjects in each of the two groups: non-smokers and smokers (cigarettes only). The claimed smoking status of study subjects was verified using smokerlyzer. Various laboratory investigations included assessment of renal function (eGFR) using CKD-EPI and MDRD equations, and urine albumin creatinine ratio (ACR).

**Results:** Using EPI equation, mean eGFR was 110.7±9.6 mL/min/1.73m$^2$ (non-smokers) and 110.7±9.4 mL/min/1.73m$^2$ (smokers) (p=0.999). Using MDRD formula, mean eGFR was 109.0±16.3 mL/min/1.73m$^2$ (non-smokers) and 110.1±15.7 mL/min/1.73m$^2$ (smokers) (p=0.750). Mean urine ACR was significantly (p<0.001) higher in smokers (14.55±8.08 mg/g) as compared to non-smokers (7.96±2.86 mg/g) as shown in Figure 1. Further within smokers, pack years of cigarette smoking showed a significant positive correlation with urine ACR levels (p<0.001) (r=0.858). In smokers urine ACR based on number of pack years of smoking was: 7.97±2.74 mg/g (for ≤1 pack year) 17.73±7.90 mg/g (for >1 pack year) (p<0.001).




**Conclusions:** In healthy young males, cigarette smoking was associated with significantly higher urine ACR even within normal limits of albumin excretion rates. Higher degrees of cigarette smoking was associated with increasing urine ACR levels; however, there was no significant effect of smoking on eGFR in our cross sectional study.

*Funding:* Government Support - Non-U.S.

## SA-PO221

**Risk of CKD Progression and Hypertension: U.S. Adults, NHANES 2007-2010** Lori D. Bash, Sheng-wei Wang, Panagiotis Mavros. *Global Health Outcomes, Merck & Co., Inc., Whitehouse Station, NJ.*

**Background:** We assessed 1839 US adults with chronic kidney disease (CKD), including 1336 with comorbid hypertension (HTN).

**Methods:** Among non-pregnant 2007-10 NHANES participants with blood pressure (BP), serum, and urinary measures, CKD patients were identified by KDIGO 2012 albuminuria and eGFR based definitions; HTN status was based on self-report and/or measured BP. Estimated risk of CKD progression was based on KDIGO 2012 guidelines for eGFR and albuminuria levels. All proportions are representative of the US population.

**Results:** Adults with CKD (13.5% of US), were on average 61 yrs old, 58% female, 72% white, 11% black, 7.5% Mexican American, 67% had HTN, 25% diabetes, 8.7% a history of CHD, and 54% dyslipidemia. Those with both HTN and CKD were older (66 vs 51 yrs), with more comorbidities compared to those without HTN. Among subjects with both, 76% were treated for HTN, 50% reaching target BP. Only those with CKD can be at risk of CKD progression. Those with both CKD and HTN were more often at higher risk than those without HTN; among them, those with controlled BP were less often at higher risk. Independent of patient characteristics, among adults with CKD, those with controlled HTN were 1.70 (95% CI 1.08-2.67) times more likely to be at high or very high risk compared to those without HTN. Those with HTN and uncontrolled BP were more likely to be at high or very high risk (OR: 1.88, 1.19-2.97).

| | US (n=10270) | All CKD (n=1839) | CKD, No HTN (n=503) | CKD and HTN (n=1336) | CKD&HTN, BP Controlled (n=653) | CKD&HTN, BP Not Controlled(n=683) |
|---|---|---|---|---|---|---|
| Low Risk (%) | 86.51 | 0 | 0 | 0 | 0 | 0 |
| Moderately Increased Risk (%) | 9.64 | 71.51 | 82.85 | 65.85 | 66.32 | 65.39 |
| High Risk (%) | 2.47 | 18.39 | 13.7 | 20.75 | 21.54 | 19.96 |
| Very High Risk (%) | 1.36 | 10.1 | 3.44 | 13.39 | 12.14 | 14.65 |

**Conclusions:** HTN, a risk factor for and of CKD, affects $^2/_3$ of those with CKD. HTN, especially uncontrolled, is independently associated with a higher risk of CKD progression. Observations suggest secondary prevention measures such as controlling BP may be critical in decreasing CKD progression among US adults. Findings should be further studied to inform treatment guidelines, therapeutic developments, and patient care.

*Funding:* Pharmaceutical Company Support - Merck & Co., Inc.

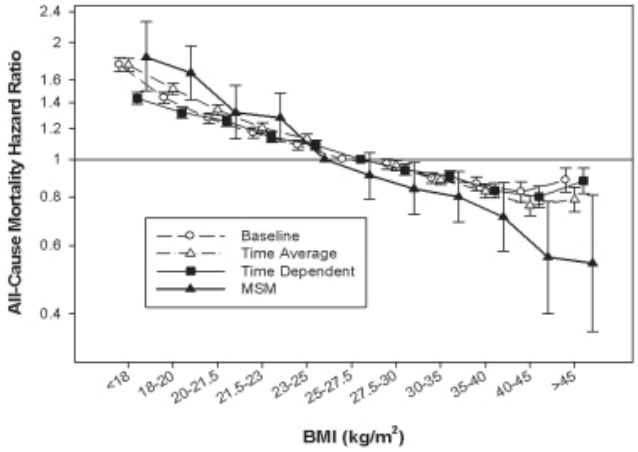
## SA-PO222

**Examining the Obesity Paradox in Hemodialysis: A Marginal Structural Model Analysis** Megha Mahendra Doshi,[1] Elani Streja,[1] Connie Rhee,[1] Wei Ling Lau,[1] Allen R. Nissenson,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Davita Inc., El Segundo, CA; [3]Memphis Veterans Affairs Medical Center, Memphis, VA.*

**Background:** Prior studies have found an inverse association between body mass index (BMI) and mortality for hemodialysis (HD) patients (obesity paradox). It has been suggested that these observations are a result of biases such as selection bias and that studies are needed to investigate the association between BMI and mortality accounting for time-dependent confounders using marginal structural modeling (MSM) and inverse probability of censoring weights. We hypothesize that even after applying these novel methods, the inverse association between BMI and mortality in HD patients is robust.

**Methods:** We examined the associations between BMI with all-cause mortality among a 2001-2006 cohort of 127,324 adult HD patients using 11 categories of BMI. We examined baseline, time-averaged, and time-varying BMI using multivariable adjusted Cox models, as well as an MSM considering time-varying BMI and time-dependent confounders.

**Results:** In all four models, BMI showed a linear incremental inverse association with all cause-mortality. In MSM analyses, compared to a BMI of 25-27.5, BMI<18 was associated with a 83% higher risk of mortality (HR 1.83, 95% CI 1.48-2.26), whereas the mortality risks were significantly lower with higher BMI: HRs (95%CI) 0.84(0.72-0.98), 0.80(0.69-0.93), 0.71(0.58-0.87), 0.56(0.40-0.78), and 0.55(0.36-0.81) for BMI ranges of 27.5-<30, 30-<35, 35-<40, 40-<45,and ≥45, respectively. The greatest survival advantage of higher BMI was observed with a BMI range of 40-<45 in MSM.




**Conclusions:** The linear inverse relationship between BMI and all-cause mortality is robust across models including MSM analyses that more completely account for time-dependent confounding and potential selection biases.

*Funding:* NIDDK Support, Private Foundation Support

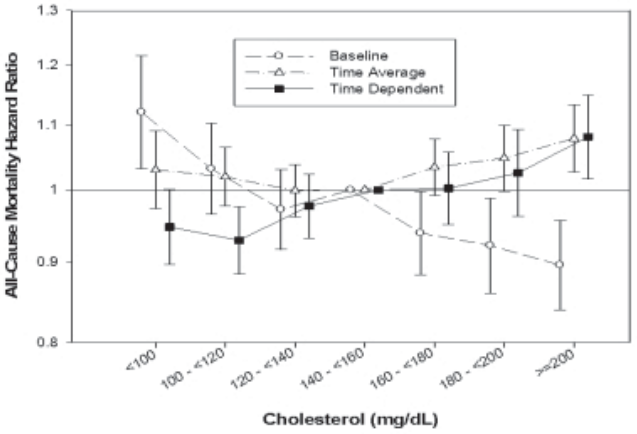
## SA-PO223

**Association of Cholesterol and Mortality in Hemodialysis Patients: A Joint Model Analysis** Megha Mahendra Doshi,[1] Elani Streja,[1] Hamid Moradi,[1] Connie Rhee,[1] Allen R. Nissenson,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, UCI, Orange, CA; [2]Davita Inc., El Segundo, CA; [3]Memphis VA Medical Center, Memphis, TN.*

**Background:** Earlier data has shown a paradoxical linear inverse association with cholesterol levels and mortality in hemodialysis (HD). However, these data did not account for cholesterol changes over time. We hypothesize that this inverse linear association is robust when using methods such as joint modeling that account for variations over time.

**Methods:** We examined associations between cholesterol with all-cause mortality in a 2001-2006 cohort of 22, 435 adult HD patients. We conducted Cox models for baseline, time-averaged, and time-dependent cholesterol, as well as a joint-model using longitudinal portion to model exposure over time combined with a Weibull proportional hazard survival submodel and accounts for the random effects variance covariance matrix. All models were adjusted for case-mix and malnutrition and inflammation (MICS) covariates.

**Results:** Baseline cholesterol showed a linear inverse association with mortality. When examining a time-dependent or time-averaged model, the pattern of association reverses direction. In joint model analyses, we observed a decreasing linear trajectory 0.98 for cholesterol over time, and an 18% mortality increase.




| Joint Models: Level of Adjustment | Estimate | 95% CI |
|---|---|---|
| Chol_50 longitudinal slope | 0.98 | 0.98-0.98 |
| Unadjusted HR | 0.89 | 0.86-0.93 |
| Casemix HR | 0.93 | 0.89-0.97 |
| Casemix + MICS HR | 1.18 | 1.13-1.22 |

**Conclusions:** The linear relationship between cholesterol and mortality is no longer inverse after accounting for cholesterol time variations. Further studies are needed to examine the relationship between change in cholesterol and mortality over time.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO224

**Age and Gender Variations in the Management of Renal Disease in South India: An Epidemiological Experience from State Sponsored Insurance Programme** Devaraju Sree Bhushan Raju. *Nephrology, Nizams Institute of Medical Sciences, Hyderabad, Andhra Pradesh, India.*

**Background:** There is no major epidemiological study done in India based on insurance data especially among those with various kidney diseases.The government of Andhra Pradesh has sponsoredfree, cash less health Insurance programme called Aarogyasri (AS) to cover all the people below the poverty line in the state.

We aim to study the Nephrology services provided under this programme especially the age and gender variations in them.

**Methods:** We analysed all the patients admitted under AS for Renal disease like Acute Kidney Injury ( AKI; n=9083), Chronic Kidney Disease stage V (CKD; n=14146), Nephrotic syndrome (NS; n=2951), Rapidly progressive Renal failure (RPRF;n=1785), maintenance hemodialysis (MHD; n=111158) and renal transplantation (Tx; n=737)was between April 2007 to July 2012.

**Results:** Younger patients (<40yrs) constitute nearly 40%. Patients of AKI, CKD and MHD were majorly between 40-60 yrs. RPRF was common between 20—40 yrs of age. NS was seen predominantly before 20 yrs (45%).Tx was not a preferable choice for many and majority continued on MHD. Nearly 70 % of the patients of Tx group were less than 40 yrs. Less than 10 % of patients on MHD were > 60 yrs. Only one third of patients in AKI, CKD and MHD groups were females. Tx was opted for males only and 80% of the Tx patients were males.

| Age groups | AKI | CKD | RPRF | NS | Tx | MHD |
|---|---|---|---|---|---|---|
| Total no of patients | 9083 | 14146 | 1785 | 2951 | 737 | 1111158 |
| <20 | 684 | 852 | 334 | **1322** | 102 | 4383 |
| 21-40 | 2862 | 4761 | **789** | 1149 | **501** | 42927 |
| 41-60 | **4136** | **6792** | 549 | 440 | 132 | **53515** |
| >61 | 1401 | 1738 | 110 | 37 | 1 | **10330** |
| Females | **3033** | 4129 | 638 | 1315 | 129 | 28964 |
| Males | 6050 | 10017 | 1145 | 1634 | **608** | **82194** |

**Conclusions:** 40% of patients with kidney disease were young (< 40 yrs) and is male preponderant. MHD was the preferred modality and Tx was not opted by many. Further analysis of this largest epidemiological study on renal diseases would help in planning preventive and treatment strategies at national level.

*Funding:* Government Support - Non-U.S.

## SA-PO225

**Clinical Outcomes in Australian Chronic Kidney Disease (CKD) Patients** Helen G. Healy,[1,2,3] Andrew John Mallett,[1,2,3] Anne Salisbury,[1,2,3] Zaimin Wang,[1,3] Wendy E. Hoy.[1,3] *[1]CKD.QLD; [2]Dept of Renal Medicine, Royal Brisbane and Women's Hospital (RBWH), Brisbane, Queensland, Australia; [3]Centre for Chronic Disease, School of Medicine, Univ of Queensland, Brisbane, Queensland, Australia.*

**Background:** CKD is the most common chronic disease in Australia, and renal replacement therapy (RRT) the most common cause of hospitalisation.

**Methods:** To describe longitudinal outcomes of patients with CKD managed in a metropolitan Australian Renal Unit through a registry of all consenting patients in public renal practices in Queensland (~10,800, CKD.QLD). Clinical outcomes and non-dialysis admissions were documented for all CKD patients followed in renal clinic at the RBWH over the first year after their recruitment to the CKD.QLD registry. At this analysis 46.7% of prevalent CKD patients at RBWH had consented and 612 had been enrolled for at least 12 months.

**Results:** After ≥12 months, of these 612 patients, 33 had started RRT (5.4%), 14 had died (3.2%), all prior to start of RRT (if planned), and 12 had been discharged or transferred. Cause of death was ESRD (4), cardiovascular (5), malignancy (3), other/unknown (2).

286 of these 612 patients (47%) had at least one RBWH hospitalisation, with a range of 1 to 25 episodes, and a median of 2. They had a total of 919 hospital episodes, with length of stay (LOS) ranging 1 to 95 days, mean LOS of 10.9 days and total LOS of 3110 days. Admitting units were renal (26.7%), general medical (22%), surgical (18.1%), specialist medical (12.1%), urology (7.9%), vascular (3.3%) and "others" (9.9%).

29% of hospitalised CKD patients, (13.6% of the total eligible cohort) generated 65% of admissions, LOS and costs. This group of 83 frequent and heavy users accounted for 602 admissions, 2035 bed days and AUD$1,523,130.51 of costs over the 1year period. Their mean age was 65.9 years, 50% were female and they most commonly CKD Stage 4 (33.7%). A higher proportion had CKD Stage 5 (18% vs 8.5%) and diabetic nephropathy (29% vs 18%).

**Conclusions:** This is the first report of longitudinal outcomes in CKD.QLD. The hospitalisations and costs are considerable with most generated by a subset of frequent heavy users. Further definition of this subset is required.

*Funding:* Government Support - Non-U.S.

## SA-PO226

### Heart Valvular Calcification: Epidemiology in Different Stages of Chronic Kidney Disease and Association with Cardiovascular Morbidity

Zhilian Li, Xinling Liang, Shuangxin Liu, Wenjian Wang, Ruizhao Li, Lixia Xu. *Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

**Background:** Heart valvular calcification (VC) in dialysis patients is associated strongly with incident all-cause and cardiovascular mortality. However, its epidemiologic data in earlier stages of chronic kidney disease (CKD) are scarce, and its association with cardiovascular (CV) morbidity remains unknown.

**Methods:** We retrospectively analyzed 2,452 Chinese CKD in-patients with complete clinical records and echocardiography data in 2008-2012. Patients were divided into 6 groups: CKD stage 1-4, Stage 5 for those still not on or initiated hemodialysis for <3 months, Stage 5D for patients undergoing hemodialysis for ≥3 months. Prevalence of PH and CV morbidity was investigated. Risk factors for CV morbidity were evaluated by logistic regression model. Cardiovascular morbidity were defined as myocardial infarction, angina, sudden death, heart failure, stroke, TIA, for hospitalization and peripheral vascular disease.

**Results:** VC was detected in 265(10.8%) patients. Prevalence of VC in CKD Stage 1-5D was 0.7%, 0.5%, 7.1%, 6.9%, 8.9% and 30.8%, respectively, whereas CV morbidity were found in 648(26.4%) patients, and in stage of CKD1-5D, 2.0%, 17.2%, 19.1%, 23.2%, 29.2%, 48.8%, respectively. CV morbidity was more prevalent in VC group than non-VC group. Patients with VC had higher risk for CV disease (OR: 1.56, 95%CI: 1.07-2.28) by logistic regression.

**Conclusions:** Our study provided the epidemiologic data of heart VC in different stages of CKD patients. Even in earlier stages of CKD, VC may be a risk factor for CV morbidity. However, evidence of VC in predicting CV morbidity from prospective studies in this population is needed.

*Funding:* Government Support - Non-U.S.

## SA-PO227

### Gender, Nephrosclerosis and Descent in Serum Uric Acid Are Prognostic Factorts for Hyperuricemic CKD Patients Treated with Febuxostat

Hidetoshi Kanai, Yasuhisa Tamura, Yasuhiro Kawai, Yoshi Koi Kaizu, Akihiko Nagase, Yasuyuki Somyo, Daisuke Oishi. *Nephrology, Kokura Memorial Hospital, Kitakyushu City, Fukuoka, Japan.*

**Background:** Hyperuricemia has been known as one of major risk factors for progress in CKD. Xanthine oxidase inhibitor (XOI) such as allopurinol was established as treatment, however, allopurinol might be limited to use for advanced CKD patients. Febuxostat, recently introduced as a novel XOI, potentiates to reduce serum uric acid (sUA) markedly and safely. We investigated the reno-protective effect of febuxostat on hyperuricemic CKD patients and the impact of prognostic factors.

**Methods:** 139 CKD patients (70yo, M/F=107/32, diabetes:30%, nephrosclerosis:33%) were administered 12.2 (10–40) mg/day of febuxostat for 10 (3-19) months. 63 patients had not been treatment with any OXI. 76 patients, administered 103 (50–200) mg/day of allopurinol previously, were converted to febuxostat (13.9mg/day). EGFR and sUA at 0, +/-1,3,6,12 months after starting febuxostat were applied. Multiple regression analysis was performed to select the independent variables, including gender,age, original kidney disease and sUA value, which have an influence on eGFR using stepwise variable selection method with p< 0.15.

**Results:** After administration of febuxostat, sUA was significantly reduced (p<0.01), and eGFR declining before febuxostat remained stable, independent with pretreatment by any doses of other XOI. There was no difference in eGFR with the dose of febuxostat (10 vs 20mg/day), age (over 65yo), presence of diabetes. Multiple regression analysis selected the change from baseline of sUA as the independent variable, which showed the estimated increase in eGFR per 1% decrease in sUA was 0.219 (p<0.05). Both female (p<0.01) and nephrosclerosis (p=0.03) also remained as beneficial factors for preserving eGFR. Alloprinol used was not selected as an independent variable in the model.

**Conclusions:** Treatment of hyperuricemia with XOI partially improved prognosis in CKD patients. Even in the patients already treated with allopurinol, converse from allopurinol to febuxostat may be benefit to maintain kidney function through enhanced lowering sUA, especially in nephrosclerotic female CKD subjects.

## SA-PO228

### N-Terminal Pro-Brain Natriuretic Peptide Is a Novel Valuable Biomarker for Progression of CKD Patients: A Longitudinal Follow-Up Study

Yoshiko Shimamura,[1] Kazu Hamada,[1] Koji Ogata,[1] Kosuke Inoue,[1] Yoshinori Taniguchi,[1] Masayuki Ishihara,[1] Taro Horino,[1] Kenji Yuasa,[2] Shigeo Yamanaka,[3] Tetsuro Sugiura,[3] Yoshio Terada.[1] *[1]Endcrinology, Matabolism and Nephrology, Kochi Medical School; [2]Kochi-Takasu-Hospital; [3]Laboratory Medicine, Kochi Medical School.*

**Background:** N-terminal pro-brain natriuretic peptide (NTpro-BNP) is known as diagnostic and prognostic biomarker of cardiac events. Recent report showed that cardio-renal interaction is an important problem in chronic kidney disease (CKD) patients. However , only few longitudinal follow-up studies have been reported to evaluate NTpro-BNP as a biomarker for renal prognosis. Accordingly, we elucidated the relation between NTpro-BNP and renal function in CKD patients.

**Methods:** Alongitudinal follow-up study for 24 months was performed in 318 consecutive CKD patients in Kochi prefecture. NTpro-BNP, serum creatinine, hemoglobin, albumin, calcium, phosphate and urine protein amount were measured. This study was approved by the Kochi Medical School review board. All patients provided written informed consent.

**Results:** The serum level of NTpro-BNP was positively correlated to creatinine (P<0.0001: r=0.437), age (P<0.05: r=0.136), urine protein amount (P<0.05: r =0.170) and phosphote (P<0.0001:r=0.379) and negatively to estimated glomerular filtration rate (eGFR) (P<0.0001: r=-0.300), hemoglobin (P<0.01: r=-0.308) and albumin (P<0.0001: r=-0.280). NTpro-BNP level in patients with heart failure was higher than in those without (P<0.0001). Furthermore, NTpro-BNP level elevated according to the progression of CKD stage. During the study period of 24 months observation, eGFR declined in all the patients and NTpro-BNPvalues had a positive correlation with the decline rate of eGFR (P<0.05: r =0.263). The eGFR decline rate had a correlation with urine protein amount (P<0.005: r =0.433), iPTH (P<0.005: r=0.407) and calcium (P<0.05: r =-0.235).

**Conclusions:** In CKD patients, serum level of NT-proBNP had a positive correlation with the decline rate of eGFR. Thus, NT-proBNP might be an novel valuable biomarker to predict the prognosis of CKD patients.

## SA-PO229

### Hyperuricemia Is a Risk Factor for the Progression to ESRD in Biopsy-Proven Benign Nephrosclerosis

Hiroyuki Suzuki,[1] Tomomi Endo,[1] Tatsuo Tsukamoto,[2] Eri Muso.[1] *[1]Dept of Nephrology and Dialysis, Tazuke Kofukai Medical Research Institute Kitano Hospital, Osaka, Japan; [2]Graduate School of Medicine, Kyoto Univ.*

**Background:** End stage renal disease due to benign nephrosclerosis is increasing in Japan. Diagnosis of benign nephrosclerosis is usually made based on existence of hypertension related organ damages (hypertensive retinopathy, cardiac hypertrophy etc) without renal biopsy. However, little is known about clinical characteristics and prognostic factors in biopsy-proven benign nephrosclerosis.

**Methods:** We selected 42 cases which were diagnosed by renal biopsy as having benign nephrosclerosis. Clinical and histological parameters and prognostic factors were investigated retrospectively.

**Results:** Clinical characteristics at renal biopsy were male to female ratio: 76.1%, age: 59.8±10.6 years, BMI: 24.9±5.0kg/m$^2$, hypertensives: 80.9%, hypercholesterolemia: 50%, diabetes: 14.2%, hyperuricemia: 73.8%, and smoker: 52.5%. Laboratory data were urinary protein excretion: 0.57±1.2g/day, occult blood: 26.1%, serum creatinine: 1.25±0.5mg/dl, and uric acid: 6.93± 1.6 mg/dl. Among these patients, 22 cases (male 68.1%) that could be followed more than 2 years (mean observation period, 3.2 years) were analyzed. Mean decline of eGFR in these patients was -2.95±4.4 ml/min/year. Four patients showed more than -5ml/min/year (rapid decline) and 1 patient reached end stage renal disease in 5 years. Existence of occult blood and hyperuricemia were related to rapid decline (RR 2.7, 95%CI:1.060, 6.880 and RR 2.0, 95%CI:1.260, 3.174, respectively). Blood pressure and urinary protein excretion tended to be higher in rapid decline but not significant (149.5±15/78.5±12 vs 121.5±34/71.7±22 mmHg p=0.069 and 2.5±3.5 vs 0.4±0.5 g/day p=0.323, respectively). Histological examination indicated that adhesion of glomerular tuft to Bowman's capsule was related to rapid decline (p=0.042), but rate of global sclerosis was not significant (p=0.095).

**Conclusions:** Hyperuricemia, occult blood, and adhesion were related to rapid decline of eGFR in biopsy-proven benign nephrosclerosis patients. There might be a possibility that hyperuricemia was related to formation of adhesion.

## SA-PO230

**Inter Study and Inter-Operator Variabilities of CMR-Derived LV Mass Measurements in CKD Patients** Ketan Kanji Vekaria,[1] Nihil Chitalia,[2] Debasish Banerjee,[3] David Goldsmith.[2] *[1]School of Medicine, King's College London, London, United Kingdom; [2]Nephrology, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom; [3]Nephrology, St. George's Healthcare NHS Trust, London, United Kingdom.*

**Background:** Patients with CKD have increased risk of mortality from Cardiovascular Disease (CVD). We are conducting the 5CVitaminD RCT with LVM cardiac remodelling as an endpoint. To validate the use of a small study population - ideal for a complex intervention such as this - we needed to use a cardiac imaging technique that was precise and accurate.

**Methods:** Subjects were imaged on a 1.5T Philips Intera MRI Scanner with 32 channel cardiac coil to obtain short axis stack (30 cardiac phases). Analysis was carried out on a Viewforum workstation (Philips Healthcare) by delineating the diastolic LV endocardial and epicardial borders. (a)5 patients were scanned twice using the same CMR protocol. Patients were scanned and then re-scanned again 10 mins later. Analysis was performed by the same observer twice. (b)16 patients on the 5CVitaminD study were analysed for LV mass using the same CMR analysis protocol by 2 independent observers.

**Results:**

| (a) Inter study | | | |
|---|---|---|---|
| Mean±SD LVM 1 | Mean±SD LVM 2 | Difference of mean (g) | Standard Deviation of mean difference |
| 108.52±30.52 | 108.43±31.74 | 0.09 | 1.53 |
| (b) Inter operator | | | |
| Mean±SD LVM 1 | Mean±SD LVM 2 | Difference of mean (g) | Standard Deviation of mean difference |
| 103.84±29.26 | 96.41±33.39 | 7.43 | 5.25 |

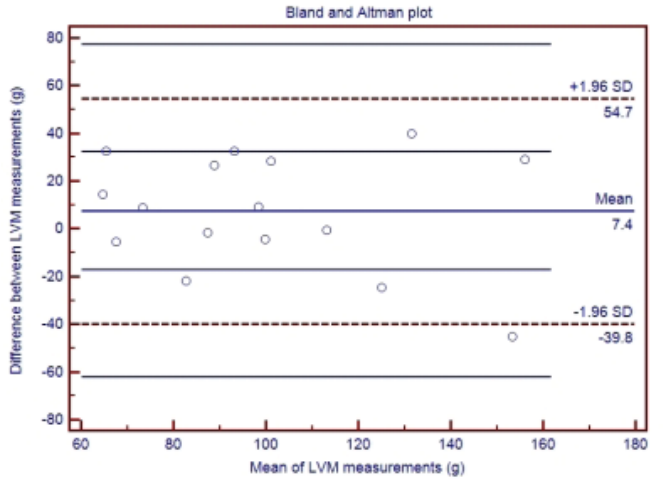



Fig. 1 Bland-Altman Plot of data from 16 paired samples analysed independently by two different observers.

**Conclusions:** The average discrepancy between the two LVM measurements was 7.43g, which is both small, and below the threshold of clinical significance for this study. These data validate our choice of CMR over echocardiography as the imaging technique for our study.

## SA-PO231

**Screening for Kidney Disease among Pre-Term and Low Birth Weight Children in Jalisco, Mexico** Jose Raul Reyna Raygoza,[1] Ricardo Martinez,[3] Laura Lopez,[3] Katia Valenzuela,[1] Alfonso Gutierrez Padilla,[2] Eusebio Angulo,[2] Susan M. Samuel,[2] Guillermo G. Garcia.[1] *[1]Div of Nephrology, Hospital Civil de Guadalajara, Mexico; [2]Univ of Calgary, Canada; [3]Dept of Pediatrics, Hospital Civil de Guadalajara, Mexico.*

**Background:** Pre-term infants (<37 weeks gestation) or low birth weight infants (<2500 grams) are at risk of developing hypertension and chronic kidney disease later in life. We sought to examine the prevalence of hypertension, albuminuria and either reduced estimated glomerular filtration rate (eGFR) or hyperfiltration among low birth weight or pre-term infants born in Jalisco, Mexico.

**Methods:** We evaluated records of all patients born at the Guadalajara Civil Hospital and those hospitalized in the Neonatal Intensive Care Unit during 1996-2011. Individuals born pre-term (<37 weeks gestation) and/or with low birth weight (<2500 gm) and/or intrauterine growth restriction were included. Pre-hypertension and hypertension were defined as BP between 90th to 95th percentile and BP ≥95$^{th}$ percentile respectively. Microalbuminuria was defined as urine microalbumin to creatinine ratio >30 mg/g. eGFR was determined from serum creatinine using Schwartz equation. In those with low eGFR or albumin to creatinine ratio >30 mg/g at baseline, the findings were confirmed 3 months later.

**Results:** Among 55 patients screened, mean gestational age was 34.2 (SD 2.5) weeks and mean chronological age at time of screening was 4 (SD 3.8) years. 56.4% were males. All patients were pre-term and 94.5% of patients had low birthweight. One patient had blood pressure >90$^{th}$ percentile. 32.7% (18/55) of patients had microalbuminuria and 33.3% (6/18) continued to have microalbuminuria upon re-test. One patient had hematuria. In total, 31(56.4%) patients had hyperfiltration. Of these, 22(40%) had eGFR 125-175 ml/min/1.73m$^2$, 8(14.5%) had GFR 176-225 ml/min/1.73m$^2$, 1(1.8%) had eGFR >225 ml/min/1.73m$^2$. Eight (14.5%) of patients had eGFR <90 mL/min/1.73m$^2$.

**Conclusions:** Glomerular hyperfiltration, reduced eGFR and microalbuminuria were common among children with a history of prematurity or low birth weight in Mexico. Continued surveillance for progression of chronic kidney disease is needed in this group of children.

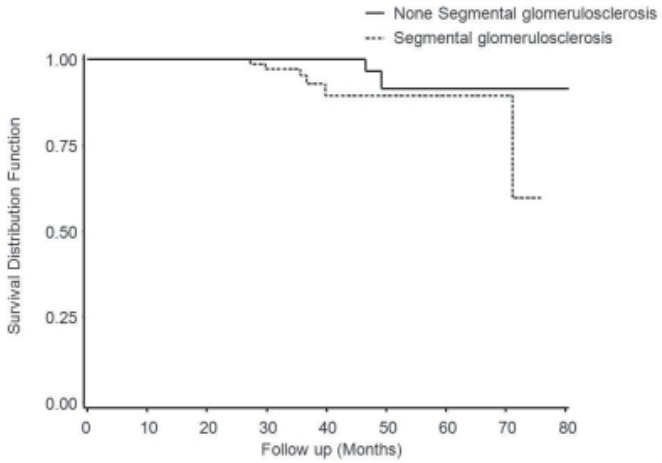
## SA-PO232

**Segmental Glomerulosclerosis in Oxford IgAN Classification Is Associated with the Poor Outcome of IgAN Patients: A Cohort Study of 240 Cases** Bin Zhu, Yongjun Wang, Yi Lin, Hongyu Chen. *Dept of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine (Guangxing Hospital), Zhejiang Chinese Medical Univ, Hangzhou, Zhejiang, China.*

**Background:** To investigate the parameters in Oxford IgAN classification associated with the outcome in a cohort of 240 Chinese patients.

**Methods:** A total of 240 patients with biopsy proven IgAN and CKD stage 1-4 were enrolled. Renal clinical and histopathologic parameters according to Oxford IgAN classification were assessed. A Kaplan-Meier survival analysis was performed to compare the survival rate. A Cox proportional hazard model was adopted to test the independent prognostic value of clincal paramters and pathological parameters. The primary outcome of chronic renal injury was defined as the serum creatinine increased to 1.5 times of baseline.

**Results:** Enrolled patients were followed up for a median of 38.35 (32.98, 44.76) monthes. A logrank test indicated that segmental glomeruloscerlosis is associated with the poor outcome of these patients(P=0.01).




A COX regression model indicated that segmental glomeruloscerlosis increased the risk of chronic renal injury by 6.39 times(95% CI, 1.28,31.95). The other parameters did not correlate to clincal outcomes.

**Conclusions:** This cohort study suggested that segmental glomerulosclerosis in Oxford IgAN classification is associated with the poor outcome of IgAN patients.

*Funding:* Government Support - Non-U.S.

## SA-PO233

**Outcome of Renal Biopsy Proven Membranous Nephropathy Receiving Immunosuppressive Therapy** Muhammad A. Imtiaz,[1] Rosa M. Montero,[1] Katie Ht Wong,[2] Mona Wahba.[1] *[1]Dept of Renal Medicine, St. Helier Hospital, Surrey, United Kingdom; [2]Dept of Medicine, St. Peter Hospital, Surrey, United Kingdom.*

**Background:** Membranous Nephropathy(MN) is the commonest cause of nephrotic syndrome in adults. Previous studies looked at progression of MN in patients having immunosuppressive therapy versus no immunosuppressive therapy.

**Methods:** Single centre retrospective study from 1995-2010 with biopsy proven MN. Data points of urine protein creatinine ratio(UPCR in mg/mmol), baseline eGFR at 2, 5 10 years, with onset renal replacement therapy(RRT) and patient survival. Immunosuppression regimens were; Prednisolone alone(P) and P+Cyclophosphamide(P+CYP). Those on Modified ponticelli were included in the P+CYP group. Those on maintenance therapy were on a combination of P+ Azathioprine(AZA) or P+ Mycophenolate mofetil(MMF).

**Results:** 48/125 were immunosuppressed. 18/48 were given P, 22/48 P+CYP, 4 P+MMF, 3 P+AZA, 1 MMF. All patients received angiotensin converting enzyme inhibitors(ACEi) or Angiotensin 2 receptor blockers(ARB) and a statin. 27 male, 22 female with median age of 60 years in P and 54.5 years in P+CYP group. UPCR was significantly lower at 5 years from baseline with P therapy($p<0.01$), contrary to those in the P+CYP who established significant reduction in UPCR at 2 and 5 years($p<0.05$, $p<0.01$, respectively). There is no difference in Cr or eGFR over time in either group. A trend in declining eGFR was seen over 10 years with P. The improvement in albumin from baseline continued over 2, 5 and 10 years($p<0.05$) with P. No difference between the two groups following the initial significant decline in cholesterol from baseline. Those treated with P show a significant improvement in Albumin at 2 and 5 years($p<0.01$) with a greater level of significance in

P+CYP($p<0.01$, $p<0.001$, respectively) compared with baseline levels. Those on P had 3 on RRT compared with P+CYP that had 5. In P treated group there were 3 deaths compared with 4 deaths in P+CYP.

**Conclusions:** Survival and dialysis dependency was similar amongst those on P or P+CYP treatment. Both groups had similar effects on creatinine and cholesterol levels. P+CYP treatment produced a significantly sustained reduction in UPCR and improving albumin over 10 years.

## SA-PO234

**Quo Vadis? Plasma Exchange and Intravenous or Oral Cyclophosphamide in Dialysis-Dependent ANCA-Associated Vasculitis** Wladimir M. Szpirt,[1] Elizabeth Krarup,[2] Martin Egfjord.[1] *[1]Nephrology, Rigshospitalet, Denmark; [2]Nephrology, Herlev Hospital, Univ of Copenhagen, Copenhagen, Denmark.*

**Background:** Salama et al. published in cJASN (2013,Nov.15) results on 41 ANCA Associated Vasculitis (AAV) patients (pts) requiring **dialysis** on admission, who were treated with Plasma Exchange (PLEX), standard prednisolone 1 mg/kg/day (STOCS), I.V. CYC (IVCYC) (6-10 pulses given over 13 weeks; 7.5 - 12.5 mg/kg depending on age). Their mortality and dialysis dependency at 3 and 12 months were superior to and compared with MEPEX oral CYC (ORCYC) arm (2.5 mg/kg daily). The total doses of IVCYC given were 2.96-7.38 g, whereas MEPEX pts. received up to 15-16 g in cumulative CYC dose.

**Methods:** We analysed this report in connection to a subgroup of 39 HD pts -out of-132 AAV pts referred to our centre between 2000-2010 and treated in 105 pts by 7 PLEX sessions, ***low*** ORCYC regimen (1.5 mg/kg/day < 65 years, 0.75 mg/kg/day > 65 years) and STOCS. Totally our pts received a estimated cumulative CYC dose of 4.75 g for older and 9.5 g for younger pts. Azathioprine or mycophenolate mofetil was given for maintenance of remission after 4 months.

**Results:** At 3 months 3 of IVCYC treated and 3 pts in our low ORCYC cohort died, 12 pts were dialysis dependent compared to our 8 low ORCYC pts.

At 1 year 13 IVCYC pts remained dialysis dependent, whereas 13 in of 39 our pts were on dialysis at 12 months. *Patient survival* in ORCYC cohort at 3 years was 72% (97% in no HD group) and 5 years 56% (87% in no HD group). *Kidney survival* in ORCYC cohort was 53% at 3 years (95% in no HD) and 40% (80% in no HD group) at 5 years. 11 of ICCYC pts had leukopenic episodes, 17 experienced infectious complications during 3 months of IVCYC therapy. This compared to 10 of our pts being leukopenic and 16 having infections during follow up. 5 relapses in IV CYC pts were observed compared to 12 relapses occurred during the 12 years of follow up in our pts. Furthermore no difference in observed malignacy can be reported (4 cases in ORCYC pts).

**Conclusions:** In our opinion low oral CYC induction regimen is not inferior to IV CYC in HD dependent AAV pts, when combined with PLEX and STOCS.

## SA-PO235

**Epidemiology of Cardiovascular Mortality in Patients with Chronic Kidney Disease and End-Stage Renal Disease** Navneet Kumar,[1] Peter A. McCullough.[2] *[1]Internal Medicine, Providence Hospital & Medical Centers, Southfield, MI; [2]Cardiology, St. John Providence Health System, Novi, MI.*

**Background:** Chronic kidney disease (CKD) patients, particularly end-stage renal disease (ESRD) have higher mortality than those without renal dysfunction. Estimated glomerular filtration rate (eGFR) and mortality have inverse relationship. ESRD patients die most commonly from cardiovascular events (acute myocardial infarction/heart failure/strokes/ventricular arrhythmias). But studies to individually subcategorize cardiac causes of death in CKD patients are lacking.

**Objective:** Prevalence of specific causes of in-hospital cardiac death in CKD and to find demographic and clinical factors associated with each cause.

**Methods:** Retrospective, non-randomized, multi-center, medical record review of patients with CKD (eGFR<60 ml/kg) who died at Providence Hospital and St. John Hospital and Medical Center during 1/1/2008-12/31/2011. Patients with circulatory system disease (ICD-9 codes:390-459) as cause of death were selected. 300 patients were randomly included. We collected demographic, clinical and co-morbid conditions data. History of coronary artery bypass graft, percutaneous angiography, pacemaker or implantable defibrillator and medications use related to circulatory system (statins, anti-platelet agents, anti-coagulant agents, beta-blockers, renin system inhibitors and diuretics) was obtained. Mortality was assigned into sub-classification as per Chronic Renal Insufficiency Cohort Study (CRIC). If patient cannot be assigned to these categories, then that patient was excluded.

**Results:** One-third CKD patients who died in-hospital were "Full Code". Most common terminal rhythm was sinus tachycardia. Most common cause of known death was congestive heart failure and primary witnessed arrhythmia.

**Conclusions:** It is the first attempt to find specific causes of in-hospital cardiac death in CKD patients. Study shows that the most common cause of known death was congestive heart failure and primary witnessed arrhythmia. This shows need for aggressive fluid management, telemetry, aggressive electrolytes management and to be more vigilant in these CKD patients who are not on dialysis.

## SA-PO236

**Serum Cystatin c (CyC) Has a Significant and Negative Correlation with Lymphocyte Count in Predialysis Chronic Kidney Disease (CKD) Patients** Hirotsugu Iwatani, Yusuke Sakaguchi, Hiroaki Kawabata, Ryohei Yamamoto, Hiromi Rakugi, Yoshitaka Isaka. *Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

**Background:** Serum creatinine (Cr) is a marker of renal function, but is affected by muscle volume or renal tubular excretion. Unlike Cr, CyC has been reported to be an early excellent marker of renal glomerular filtration rate (GFR). Although serum CyC has been regarded as a mere marker of GFR, CyC has a physiological activity of cysteine protease inhibitor and, therefore, potentially affects antigen presentation in antigen presenting cells such as dendritic cells. It is also well known that immune function is impaired in patients with decreased GFR. We assessed an association between CyC and lymphocyte count in patients with CKD in the predialysis stage.

**Methods:** The present cross-sectional study included 71 hospitalized CKD patients in the department of nephrology in Osaka University Hospital between April 2012 and May 2013. Because of its skewed distribution, lymphocyte count was normalized by logarithmic transformation. Associations of lymphocyte count ($\log_{10}[/\mu l]$) with Cr, CyC, or eGFR (based on Cr and CyC) were assessed in multivariate linear regression models adjusting for clinically relevant factors, including age, sex and total cholesterol.

**Results:** In univariate models lymphocyte count were significantly associated with Cr (per 1.0 mg/dL, $\beta$ -0.0222 ± SE 0.00575, $P<0.001$), CyC (per 1.0 mg/L, -0.0593 ± 0.0124, $P<0.001$) and eGFR (per 10 mL/min/1.73m$^2$, 0.0270 ± 0.00586, $P<0.001$). Even after the adjustment for clinically relevant factors, CyC had the significant association with lymphocyte count (-0.0409 ± 0.0178, $P=0.0257$), whereas not Cr (-0.0134 ± 0.00735, $P=0.0726$) and eGFR (0.0211 ± 0.0109, $P=0.0583$).

**Conclusions:** Cystatin c is significantly and negatively associated with lymphocyte count in CKD patients in the predialysis stage. Elevated serum cystatin c might be able to explain the decrease of lymphocyte count in renal failure.

*Funding:* Government Support - Non-U.S.

## SA-PO237

**The Use of a Previously Validated Renal Risk Score in an Irish Cohort of Stroke Patients** Caitriona M. McEvoy,[1] Sean Murphy.[2] *[1]Nephrology Dept, Mater Misericordiae Univ Hospital, Dublin, Ireland; [2]Stroke Dept, Mater Misericordiae Univ Hospital, Dublin, Ireland.*

**Background:** Patients presenting with a suspected cerebrovascular accident undergo emergency CT scanning to identify those suitable for thrombolysis. Imaging often involves administration of iodinated contrast, incurring the risk of contrast induced nephropathy (CIN) in those with underlying renal dysfunction. Scans are performed emergently, frequently without knowledge of the patient's renal function. A 5-point Renal Risk Score (RRS), aiming to identify patients with acute stroke at highest risk of CIN, in the absence of an available estimated glomerular filtration rate (eGFR) was recently described (Vergouwen et al). We examined the potential of the RRS to identify significant renal impairment in consecutive stroke patients at our institution.

**Methods:** We obtained data on 106 consecutive stroke cases from the hospital in-patient stroke portal. Demographic information, RRS components, presenting creatinine & eGFR were collected. RRS calculated as follows: Age (years) + (5 if female) + (5 if diabetic) + (15 if using insulin) + (10 if history of hypertension).The diagnostic value of RRS for predicting the presence of CKD was measured using sensitivity, specificity, and receiver operator characteristic (ROC) curves. Statistical analysis performed with SPSS Version20.

**Results:** Data from N=101 (45 male) patients were analysed. Average age: 70.02 years. 92/101(91%) patients had acute ischaemic events, 8/101 (7.9%) were haemorrhagic. Average RRS : 81.28 (30-113). The prevalence of moderate (CKD stage ≥3) and severe (CKD 4&5) renal impairment were 31.68% and 3.96% respectively. Using a ROC curve the diagnostic value of an RRS as a tool to predict CKD3 or greater was analysed: AUC 0.738 ($p<0.0001$). A cut-off value of 82.5 maximised sensitivity(80%) and specificity(63%). In a subgroup analysis of CKD4&5 patients, a RRS score >82.5 was associated with a sensitivity and specificity of 75% and 45.8% respectively.

**Conclusions:** Moderate to severe renal impairment is common in this population. The RRS is a useful tool in assessing for this at initial presentation. A cut-off score of >82.5 was most useful in our study population.

## SA-PO238

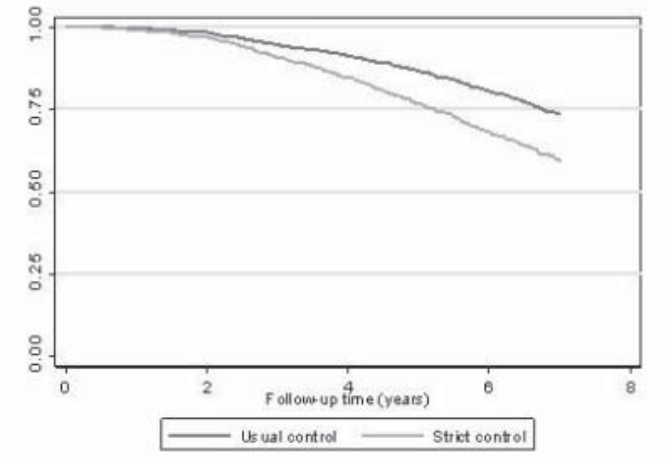
**The Use of a U.S. Veterans' Chronic Kidney Disease Database to Model the SPRINT (Systolic Blood Pressure Intervention) Trial** Anthony J. Bleyer,[1] Jun Ling Lu,[2] Miklos Zsolt Molnar,[3] Jennie Z. Ma,[4] Robert B. Canada,[2] Kamyar Kalantar-Zadeh,[5] Csaba P. Kovesdy.[6] *[1]Wake Forest Medical School; [2]Univ of Tennessee; [3]Univ of Toronto; [4]Univ of Virginia; [5]Univ of California, Irvine; [6]Memphis VA Medical Center, Univ of Tennessee Health Science Center.*

**Background:** Some retrospective studies accurately predict outcomes of prospective trials. In this study, we used a CKD database of U.S. Veterans to model mortality in the SPRINT (Systolic Blood Pressure Intervention) Trial, a prospective study comparing the effect of different SBP targets in CKD patients on cardiovascular (CV) outcomes.

**Methods:** Patients were selected if they met SPRINT criteria for BP, age (>50 years), and no diabetes. 2 groups were created modeling the SPRINT target SBP goups of <120 (strict control, SC) vs. 120 to <140 mm Hg (usual control, UC). As older patients with lower baseline SBP, CV disease, and higher Charlson index were more likely to have an SBP

<120 vs 120-<140 mm Hg, propensity scores were calculated from these characteristics plus gender, race, and baseline eGFR. 2500 patients were selected for each group based on the propensity scores using the 1:1 nearest neighbor matching method.

**Results:** The mean SBP in follow up was 119.4±5.5 mm Hg in the SC group and 132.8±5.7 mm Hg in the UC group. The death rate over a median of six years was 63.1/1000 patient-years (95%CI: 59.0-67.4) in the SC group and 37.8 (34.8-41.1) in the UC group (Hazard ratio, 95%CI: 1.74, 1.57-1.94, p < 0.001). Figure 1 shows the survival curves.




**Conclusions:** In our model of the SPRINT cohort to lower BP in patients with CKD stages 1 to 5, there was increased mortality in the strict control group vs. the usual control group. Significant limitations of this study included the small number of women and African-Americans in the study and its retrospective nature.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO239

**GFR Decline as an Alternative Endpoint for Clinical Trials in CKD – A Meta-Analysis of Individual Associations from Randomized Trials: Report of an NKF-FDA Workshop**

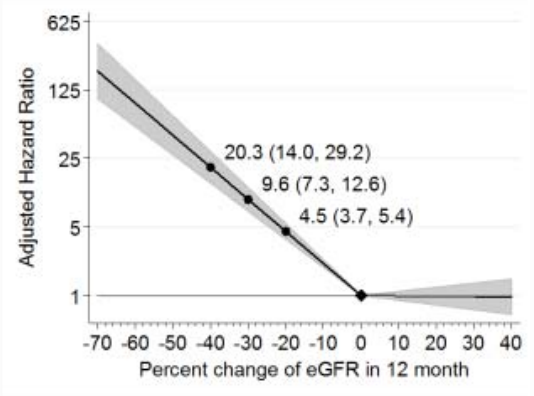
Hiddo Jan Lambers Heerspink,[1] Hocine Tighiouart,[2] Yingying Sang,[3] Shoshana Ballew,[3] Kunihiro Matsushita,[3] Josef Coresh,[3] Andrew S. Levey,[2] Lesley Inker.[2] *[1]UMC Groningen, Netherlands; [2]Tufts Medical Center, Boston; [3]Johns Hopkins, Baltimore.*

**Background:** ESRD and doubling of serum creatinine (2X Scr) are established clinical endpoints for studies of CKD progression. However, a Scr doubling (57% decline in eGFR) is generally a late event in CKD. Alternative endpoints defined by lesser eGFR declines may reduce trial duration and increase feasibility of trial conduct. As part of an evaluation of lesser declines in GFR as alternative endpoints, we describe the associations of a GFR decline with subsequent development of clinical endpoints.

**Methods:** Using a pooled dataset of 12821 patients from 43 clinical trials, we assessed the association between a 20%, 30%, and 40% decline in eGFR from baseline to month 12 with subsequent development of an established endpoint (EE) of dialysis, GFR <15 or 2X SCr. Cox analyses were performed within study, followed by random effects meta-analyses, and were adjusted for age, sex, race, baseline eGFR, proteinuria, and blood pressure, and diabetes. Meta-regression was used to explore baseline eGFR, proteinuria, disease aetiology, and intervention treatment as potential source of heterogeneity.

**Results:** Over a median follow-up of 2.0 [IQR 1.2–3.1] years, 2661 EE were observed after the 1-year baseline period. A strong linear association was observed between eGFR decline and subsequent EE (Figure 1). Hazard ratios of EE for 20%, 30%, or 40% decrease in eGFR were 4.5, 9.6, and 20.3, resp. The associations were consistent regardless of baseline eGFR, proteinuria, disease aetiologies, and treatments.

**Conclusions:** These analyses provide some support that changes in eGFR lesser than 57% can be used as alternative endpoints for trials of CKD progression.




## SA-PO240

**Association of Urinary Biomarkers of Kidney Injury with All-Cause Mortality and Cardiovascular Events: The Health Aging and Body Composition (Health ABC) Study**

Mark J. Sarnak,[1] Ronit Katz,[2] Anne B. Newman,[3] Tamara Harris,[4] Carmen A. Peralta,[5] Prasad Devarajan,[6] Michael R. Bennett,[6] Linda F. Fried,[3] Joachim H. Ix,[7] Suzanne Satterfield,[8] Eleanor Marie Simonsick,[4] Chirag R. Parikh,[9] Michael Shlipak.[5] *[1]Tufts Medical Center; [2]Univ of Washington; [3]Univ of Pittsburgh; [4]NIA; [5]UCSF; [6]Univ of Cincinnati; [7]UCSD; [8]Univ of Tennessee; [9]Yale Univ.*

**Background:** Kidney damage is a common sequel of several chronic pathologic conditions. Whether biomarkers of different forms of kidney damage are prognostic for cardiovascular disease (CVD) and all-cause mortality is unknown.

**Methods:** We measured three urinary biomarkers, kidney injury molecule (KIM) 1, urinary interleukin (IL) 18 and urinary albumin, in 3010 individuals enrolled in the Health ABC. KIM-1, IL-18 and albuminuria were all standardized to urine creatinine. Cox proportional hazards were used to investigate the associations of quartiles of urinary KIM-1/cr, IL-18/cr and albumin/cr (ACR) with all cause mortality and CVD. Multivariable models adjusted for demographics, traditional CVD risk factors, and estimated glomerular filtration rate.

**Results:** Mean age was 74 years, 49% were men, and 41% black. There were 1450 deaths and 797 CVD outcomes during a median follow up of 12.4 years. KIM-1/cr had a modest association with mortality, while the association of ACR with mortality was stronger and more linear. In similar analyses only ACR showed an association with CVD.

**Conclusions:** Urinary KIM-1 had a modest, independent association with all-cause mortality but not CVD, while urinary IL-18 showed no association. In contrast, albuminuria was strongly and independently associated with all cause mortality and CVD. Future studies should evaluate reasons for differences in the prognostic importance among kidney injury markers.

**Association of KIM-1, IL-18 and UACR with Mortality**

| | Demographic adjusted* | Adjusted for kidney function** |
|---|---|---|
| **KIM 1 standardized to UCr (ng/mg)** | | |
| < 497 | 1.00 (ref) | 1.00 (ref) |
| 497 – 814 | 1.27 (1.08, 1.48) | 1.21 (1.03, 1.41) |
| 815 – 1239 | 1.29 (1.09, 1.52) | 1.13 (0.96, 1.34) |
| ≥ 1240 | 1.77 (1.51, 2.09) | 1.28 (1.08, 1.52) |
| **IL-18 standardized to UCr pg/mg** | | |
| < 19 | 1.00 (ref) | 1.00 (ref) |
| 19 – 31 | 1.04 (0.90, 1.21) | 1.02 (0.88, 1.19) |
| 32 – 55 | 1.25 (1.07, 1.45) | 1.16 (0.99, 1.35) |
| ≥ 56 | 1.21 (1.03, 1.42) | 1.06 (0.90, 1.25) |
| **Urine ACR (mg/g)** | | |
| < 4.6 | 1.00 (ref) | 1.00 (ref) |
| 4.6 – 8.3 | 1.07 (0.91, 1.26) | 1.08 (0.91, 1.27) |
| 8.4 – 20.3 | 1.26 (1.08, 1.48) | 1.24 (1.06, 1.46) |
| >20.3 | 1.95 (1.67, 2.26) | 1.63 (1.39, 1.91) |

*Adjusted for age, sex, race, site and education
**Further adjusted for diabetes, hypertension, SBP, smoking, prevalent HF, prevalent CHD, albumin and CRP, further adjusted eGFR and UACR

*Funding:* Other NIH Support - NIA

## SA-PO241

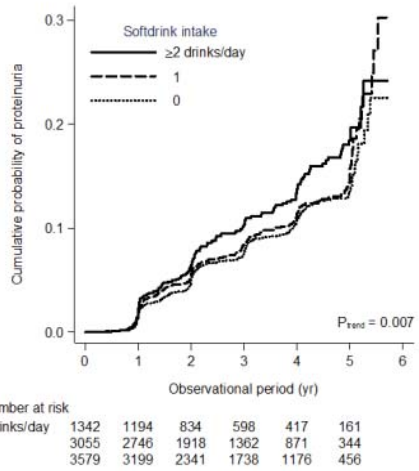
**Soft Drink Intake and Prediction of Proteinuria: A Retrospective Cohort Study**

Ryohei Yamamoto,[1] Maki Shinzawa,[1] Junya Teranishi,[1] Toshihiro Ishigami,[1] Noritaka Kawada,[2] Makoto Nishida,[2] Keiko Yamauchi-Takihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka,[1] Toshiki Moriyama.[2] *[1]Dept of Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Osaka Univ Health Care Center, Toyonaka, Osaka, Japan.*

**Background:** Association of soft drink intake and incidence of chronic kidney disease is controversial.

**Methods:** Study design: A retrospective cohort study in a single center. Participants: Among 12026 university employees who underwent their annual health checkup in Osaka University Health Care Center between Apr 2006 and Feb 2011, 7976 employees (66.3%) with ≥60mL/min/1.73m² of eGFR and negative or trace of urinary protein by dipstick test. Exposure: A questionnaire of daily soft drink intake (0, 1, or ≥2 drinks/day) at the first health checkup during the study period. Outcome: Incidence of proteinuria defined by ≥1+ of dipstick test. Statistics: Log-rank test for trend and multivariate Poisson regression model.

**Results:** Baseline characteristics of 7976 employees were as follows; age, median 33 yr (interquartile range 28 - 41), male 50.1%, BMI 21.4±3.2 kg/m² and blood pressure 113±14/71±12 mmHg. Among 3579, 3055 and 1342 employees with 0, 1 and ≥2 drinks/day of the baseline soft drink intake, 301 (8.4%), 272 (8.9%) and 144 (10.7%) employees developed proteinuria during median 2.9 years (interquartile range 1.5-4.2) of the observational period, respectively (P = 0.007 for trend).




Number at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| ≥2 drinks/day | 1342 | 1194 | 834 | 598 | 417 | 161 |
| 1 | 3055 | 2746 | 1918 | 1362 | 871 | 344 |
| 0 | 3579 | 3199 | 2341 | 1738 | 1176 | 456 |

Even after adjusting for clinically relevant factors, ≥2 drinks/day of soft drink was identified as a significant predictor of proteinuria (incident rate ratio [95%CI] of 0, 1 and ≥2 drinks/day: 1.00 [reference], 1.03 [0.88, 1.22] (P=0.698) and 1.28 [1.05, 1.56] (P=0.015), respectively).

**Conclusions:** At least 2 drinks/day of soft drink is associated with incidence of proteinuria.

## SA-PO242

**State Variation in Awareness of Chronic Kidney Disease in the United States** Sai Hurrish Dharmarajan,[1] Hal Morgenstern,[1] Neil R. Powe,[2] Delphine S. Tuot,[2] Rajiv Saran.[1] *[1]Univ of Michigan, Ann Arbor, MI; [2]Univ of California San Francisco, San Francisco, CA.*

**Background:** Although low awareness of chronic kidney disease (CKD) in the US has been reported, the extent of geospatial variation in CKD awareness is unknown. We estimated state-level awareness and examined variation across the continental US.

**Methods:** Using 2011 Behavioral Risk Factor Surveillance System (BRFSS) data, we identified adults who reported being told they have "kidney disease". Although the BRFSS provides state-level data on self-reported CKD, there is no clinical/lab information to serve as the "gold standard" for identifying who actually has CKD. To deal with that limitation, we estimated CKD awareness in each state indirectly by comparing--as a ratio--the observed prevalence of self-reported CKD in the BRFSS sample to the prevalence predicted from clinical data in the 2005-06 National Health and Nutrition Examination Survey (NHANES), using a logistic model adjusting for age, sex, race/ethnicity, hypertension status, and diabetes status. CKD in NHANES was defined as MDRD eGFR <60 ml/min/1.73m$^2$ or urinary albumin:creatinine ratio >30mg/g. We also compared estimated CKD awareness by category of age, race/ethnicity, sex, hypertension, and diabetes status.

**Results:** The state-specific prevalence of self-reported CKD ranged from 2% in Iowa to 4% in Arizona. The predicted prevalence of CKD ranged from 4% in Arkansas to 9% in Florida. Estimated CKD awareness in the US was 34%, and it ranged from 17% in Iowa to 61% in Arkansas. Overall CKD awareness in the US was higher in adults with self-reported diabetes (40%), non-Hispanic blacks (64%) and adults less than 65 years of age (96%).

**Conclusions:** The prevalence of self-reported CKD is low throughout the US, reflecting a low level of awareness that appears to vary appreciably across states and by category of major CKD risk factors. A method for indirectly estimating CKD awareness, perhaps improved by incorporating state variation, could be applicable in CKD surveillance programs to study CKD awareness.

*Funding:* Other U.S. Government Support

## SA-PO243

**Quality of Care of Chronic Kidney Disease in a Community Practice** Pradeep Arora,[1,2] Brian M. Murray,[2] Chester H. Fox,[2] Robert N. Anderson,[3] Rocco C. Venuto.[2] *[1]Medicine, VAMC, Buffalo, NY; [2]Medicine, Univ of Buffalo, Buffalo, NY; [3]Computer Task Group, Buffalo, NY.*

**Background:** Chronic kidney disease (CKD) is a major public health problem in the United States. There is a rising incidence and prevalence of kidney failure, with poor outcomes and high cost. Kidney disease is the ninth leading cause of death in United States. Data from United States Renal Data System (USRDS) indicates that there has been a 104% increase in the prevalence of chronic renal failure (CRF) between the years 1990-2002. There is an even higher prevalence of earlier stages of CKD. We aimed to study the prevalence and resource utilization of CKD in Western NY.

**Methods:** We analyzed data collected from 4.8 million claim records which included claims on >30,000 CKD patients from local payers and providers between 2006 and 2010. Patient demographics (age, gender), lab data and outcomes (hospitalization, ESRD, cardiovascular events and death) were captured from this data base. CKD was defined as per NKF-KDOQI guidelines.

**Results:** Mean age of the patients was 69.2+/-15.3 SD, 50.4% were male, 54% were above age of 70 years. 94% of patients with CKD had hypertension, and 55% had diabetes. Urine albumin/ creatinine ratio was available in only 25% of the patients with CKD. As per diagnsotic code for CKD,7028 had stage 3, 1592 stage 4, 244 stage 5 and 1428 were ESRD. The remainder were coded as CKD without stage.17% of patients with advanced CKD (stage 4 and 5) were not seen by a nephrologist. Only 5.7% of patients had AV fistula prior to initiation of dialysis and 22% required extensive hospital initiated dialysis.

**Conclusions:** More than 3/4 of patients with stage 4/5 CKD were seen by nephrologist. However AV fistula rate prior to start of dialysis was dismal. These data allows specific focus on regional deficiencies in CKD care that could be addressed and improve outcomes. The success of quality improvement programs can be evaluated by analysis of future claim based data.

## SA-PO244

**Enhancing ESRD Risk Prediction Using Age Based Risk Calculation in Stage 4 CKD** Shayan Shirazian, Candace D. Grant, Joseph Mattana. *Medicine, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Accurate ESRD risk prediction is essential in identifying patients who will require preparation for renal replacement therapy as well as low risk patients who are unlikely to reach ESRD in whom such preparation should be avoided. Stage based risk classification may overestimate risk especially in some older patients. In this study we applied a validated risk calculator, which incorporates age, to determine the frequency with which ESRD risk may be overestimated in a CKD-4 population.

**Methods:** In this cross-sectional study, the electronic health records of patients with CKD-4 seen in our nephrology practice were reviewed. Variables necessary for ESRD risk prediction were extracted, including age, sex, glomerular filtration rate (GFR), spot urine albumin to creatinine ratio, calcium, phosphorus, albumin, and bicarbonate. All CKD-4 patients are designated as very high risk according to KDIGO guidelines. Two year estimated risk of progression to ESRD was calculated using a risk calculator developed and validated by Tangri et al. (JAMA 2011;305(15):1553-9). Patients were considered to be low risk for progression to ESRD if their 2 year calculated risk was <10%.

**Results:** Two hundred and four records were reviewed and 85 patients met study criteria. The average age was 69.8 years, 61% were male, 74% were white and the average GFR was 22 mL/min/1.73m$^2$. The average 2 year calculated ESRD risk was 19%. Despite their all being deemed very high risk based on stage, 41% of patients were found to be low risk using the risk calculator. Further analysis of the low risk subgroup revealed a mean age of 76.5±13,which was significantly older compared to the remainder of the population (65.1±15.4, p< 0.01). Other variables including age, gender and race were not significantly different between the groups.

**Conclusions:** Using an age-based calculator, a substantial proportion of patients with CKD-4 were found to be at low risk for progression to ESRD over 2 years. These patients were significantly older than those at high risk of progression. Age-based calculators may better estimate risk of progression to ESRD in the elderly, potentially saving these patients from the risks of unnecessary dialysis preparation.

## SA-PO245

**Traditional Risk Factors Predict Renal Outcomes in Cancer Patients with Chronic Kidney Disease** Elerson Costalonga, Veronica T. Costa e Silva, James Hung, Luis Yu, Emmanuel A. Burdmann. *Nephrology, Cancer Institute of São Paulo.*

**Background:** Cancer patients have a high prevalence of chronic kidney disease (CKD). The aim of this study was to assess prognostic factors for kidney failure and CKD progression.

**Methods:** Among 350 outpatients with cancer referred to nephrology evaluation (2009-12) 173 had CKD according KDIGO definitions and at least 3 months of follow up. Clinical and biochemical data were retrieved from patient medical records. The primary endpoint was defined as kidney failure (eGFR < 15 ml/min/1.73m$^2$, CKD-EPI formula). Rapid CKD progression (sustained decline in eGFR of more than 5ml/min/1.73 m$^2$/yr) was defined as the secondary endpoint.

**Results:** Patients baseline characteristics and outcomes data are shown in Table 1. Results are expressed as mean±SD and percentage.

| | |
|---|---|
| Age (yr) | 66±12 |
| Male (%) | 69 |
| BMI (kg/m$^2$) | 26±5 |
| Solid tumors (%) | 87 |
| Metastasis (%) | 28 |
| Active cancer (%) | 68 |
| Hypertension (%) | 44 |
| Diabetes (%) | 25 |
| Cardiovascular disease (%) | 11 |
| ECA inhibitor use (%) | 31 |
| Baseline eGFR (ml/min/1.73 m$^2$) | 39±19 |
| **CKD etiology** (%) | |
| Diabetic nephropathy | 25 |
| Obstructive uropathy | 21 |
| **eGFR categories** (%) | |
| G1 | 1 |
| G2 | 9 |
| G3a | 18 |
| G3b | 40 |
| G4 | 26 |
| G5 | 6 |
| **Albuminuria categories** (%) | |
| <30 mg/g | 40 |
| 30-300 mg/g | 34 |
| >300 mg/g | 26 |
| **Risk categories** (%, KDIGO 2012) | |
| Low | 6 |
| Moderately | 16 |
| High | 25 |
| Very high | 53 |
| **Outcomes** (%) | |
| Rapid CKD progression | 26 |
| Kidney Failure | 14.5 |
| Death or Palliative care | 27 |
| Follow up (m) | 17±8 |

Multivariate logistic regression identified baseline eGFR (OR =8.9; 95% CI 2.4-33; p<0.01) and albuminuria categories (OR =7.9; 95% CI 2-31; p<0.01) as independent factors associated to the primary endpoint. Obstructive uropathy (OR =5.4; 95% CI 1.5-19.7; p=0.011) and ACR > 300mg/g (OR =3.8; 95% CI 1.4-10; p<0.01) were associated to rapid CKD progression.

**Conclusions:** Traditional risk factors as baseline eGFR and albuminuria can predicted renal outcomes in cancer patients with CKD.

## SA-PO246

### Prognostic Factors for Death or Palliative Care in Patients with Cancer and Chronic Kidney Disease

Elerson Costalonga, Veronica T. Costa e Silva, James Hung, Luis Yu, Emmanuel A. Burdmann. *Nephrology, Cancer Institute of Sao Paulo, Brazil.*

**Background:** Cancer patients have a high prevalence of chronic kidney disease (CKD). The aim of this study was to assess prognostic factors for death or palliative care in cancer patients with CKD.

**Methods:** Among 350 outpatients with cancer referred to nephrology evaluation (2009-12) 173 had CKD according KDIGO definitions and at least 3 months of follow up. Clinical and biochemical data were retrieved from patient medical records. The primary endpoints were defined as death or palliative care.

**Results:** After a follow-up of 17±8 m, 27% of the patients reached the endpoints. Their baseline features are shown in Table 1.

| | Surviving(n=126) | Death/Palliative Care(n=47) |
|---|---|---|
| Age (yr) | 66±11 | 66±14 |
| Male (%) | 71.4 | 61.7 |
| BMI (kg/m$^2$) | 26±5 | 24±4 |
| Diabetes (%) | 33 | 25 |
| Cardiovascular disease (%) | 10 | 6 |
| Solid tumors (%) | 85 | 91 |
| Metastasis (%) | 20 | 51* |
| Active cancer (%) | 59 | 94* |
| Karnofsky<80 (%) | 5 | 36* |
| Obstructive nephropathy (%) | 16 | 36* |
| eGFR (ml/min/1.73 m$^2$) | 40±20 | 35±18 |
| Serum albumin (g/dL) | 4.3±0.4 | 3.6±0.7* |
| Hemoglobin (g/dL) | 11.8±1.8 | 10.3±1.7* |
| PTH (pg/ml) | 108±94 | 157±135* |
| Vitamin D (ng/ml) | 19±8 | 18±8 |
| Protein C Reactive (mg/L) | 8±25 | 41±70* |

*p<0.01 vs Surviving

Multivariate logistic regression identified active cancer(OR = 25.8; 95% CI 3.2-206.9; p<0.01), serum albumin<3.5 g/dL(OR = 29.7; 95% CI 5.0-176.9.9; p<0.01), baseline eGFR<30 ml/min/1.73m$^2$(OR =4.3; 95% CI 1.4-12.8; p<0.01), and hemoglobin<10 g/dL(OR = 4.2; 95% CI 1.4-12.6; p<0.05) as independent factors associated to the endpoints.

**Conclusions:** Patients with cancer and CKD have a poor prognosis. Active cancer, lower baseline eGFR, albumin and hemoglobin were independent factors associated to poor prognosis.

## SA-PO247

### RAS Blockade in Diabetic Patients with Renal Dysfunction in China

Chuanming Hao,[1] Qionghong Xie,[1] Dayi Hu,[2] Danyi Zhang.[3] *[1]Div of Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China; [2]Peking Univ People's Hospital; [3]Vitalstrategic, Research Institute.*

**Background:** It is well documented that RAS blockade is associated with improved outcome in patients with diabetic kidney disease. This study examined the usage of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) in type 2 diabetes patients (2-DM) with or without chronic kidney disease (CKD) in China.

**Methods:** Data from the China Cardiometabolic Registries on blood pressure, blood lipid and blood glucose in 2-DM patients (CCMR-3B Study) were used to analyze ACEI/ARB use in this population. Outpatients who had been diagnosed of 2-DM for > 6 months were recruited to this non-interventional, observational, cross-sectional study. CKD was defined as having urine albumin creatinine ratio (ACR) > 30mg/g or eGFR < 60 ml/min. Serum creatinine-based eGFR was calculated using MDRD equation.

**Results:** A total of 25,454 outpatients from 6 regions in China (north, south, east, west, northeast and central China) were enrolled. The mean age was 63.0±11.9 yrs, 47.0% were male, 55.1% with HbA1C >7%, 63.8% with cholesterol >4.5mmol/L and 59.8% with hypertension (SBP > 130 mmHg, DBP> 80mmHg). Serum creatinine data was available in 22,628 patients, and 2258 (10.0%) had an eGFR<60ml/min. ACR was measured in 6,383 patients, and 3,231 (50.6%) of them had ACR > 30mg/g. Among hypertensive patients, 73.0% were on antihypertensive medications, and 39.7% were on ACEI/ARBs. A total of 2157 patients with hypertension had positive albuminuria, 1040 (48.3%) of them used ACEI/ARB, 22.9% used CCBs without ACEI/ARB, 7.7% used other antihypertensives and 21.1% not treated. In the non-hypertensive patients with or without albuminuria, ACEI/ARB utilizations were < 1%.

**Conclusions:** In diabetic patients with hypertension and albuminuria in CCMR-3B population, more than half of the patients were not treated with ACEI/ARB. This real world evidence suggest that the current treatment for diabetes with hypertension and albuminuria is sub-optimal.

## SA-PO248

### Classification Tree Method Analysis Related Factors of Early Renal Damage by Type 2 Diabetes Mellitus

Wenbo Zhao,[1] Lin Wei,[2] Hui Peng,[1] Xun Liu,[1] Jia-ling Rao,[1] Tan-qi Lou.[1] *[1]Nephropathy, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Neurology, The Guangdong Province Traditional Chinese Medical Hospital, Guangzhou, Guangdong, China.*

**Background:** To explore the factors influencing classification $T_2$DM early nephropathy by the tree model, in order to provide evidence for prevention and treatment.

**Methods:** The 1040 hospitial patients from January 2011 to January 2013, according to eGFR and the urine albumin, divided into $T_2$DM group (755 cases) and early diabeticrenal impairment group (285 cases ), to analyze the main factors to developed to microalbuminuria by Exhaustive CHAID classification tree.

**Results:** Classification tree model screened Fib, retinopathy, SBP, DPVD, gender from 34 candidate variables, related to the early renalimpairment. Elevated Fib was the main factor. The incidence of Fib>4.34 was 52.9%, higher than the Fib ≤ 3.30 (14.9%) . Fib between 3.30 and 3.85 group (26.0%) and between 3.85 and 4.34 group (41.5%), Fib≥4.34 group was high-risk group. Influencing factors screened with different fibrinogen were not same, SBP, DR, Gender and DR dividedly. Fib>4.34 group with DR were higher risk, 75.0%, comparing no DR, 43.9%. Fib>3.85 and≤4.34group, correlated with gender, the female was higher risk than male. Fib>3.30 and≤3.85 group with DR, had a higher risk; A history of DPVD without DR mean a higher risk. Female without DPVD was lower risk. Fib≤3.30 group screened influence factors was SBP; Respectively the three groups were 6.5%, 18.1% and 42.5% with SBP increasing. SBP 127 to 161mmHg group, the main influencing factor was retinopathy. Compared with no retinopathy team, the incidence was higher.


**Conclusions:** Classification tree model analysis of early renal impairment was feasible, multi-factors increased the risk of early renal impairment, that could improve the prevention and treatment as reference.

*Funding:* Government Support - Non-U.S.

## SA-PO249

**Renal Function as a Predictor of Mortality in Patients with Left Ventricular Assist Device Support** Jeffrey P. Yourshaw, Kevin C. Roe, David M. Anwar, Umar Farooq. *Internal Medicine, Penn State College of Medicine, Hershey, PA.*

**Background:** Impaired renal function is an independent predictor of mortality in patients with cardiac disease. We studied whether serum creatinine predicted mortality in patients receiving left ventricular assist device (LVAD) support.

**Methods:** We retrospectively collected data on all patients who underwent LVAD placement at Penn State Hershey Medical Center between June 2006 and February 2012. Indications of bridge-to-transplant and destination therapy were included. Creatinine before LVAD placement, 1, 3 and 6 months post-device placement were assessed as predictors of 1-year mortality using bivariate t-test analysis.

**Results:** 80 patients were included in the study. The mean age was 56.5 years (SD 11.67). 67 (84%) were males and 13(16%) females. Mean serum creatinine prior to device placement was 1.28 (SD 0.57) and mean creatinine at 1 year was 1.34 (SD 0.50). One year mortality was 26.25% (n=21). Baseline serum creatinine did not differ between living or deceased patients at one year (1.12 vs. 1.34, p =0.11). Similarly, serum creatinine at 1 month, 3 months, and 6 months also was not associated with mortality at 1-year (p=0.50, p=0.96, and p=0.52 respectively).

**Conclusions:** In patients at our institution, creatinine at baseline, 1 month, 3 months and 6 months did not predict mortality at 1-year post-LVAD placement.

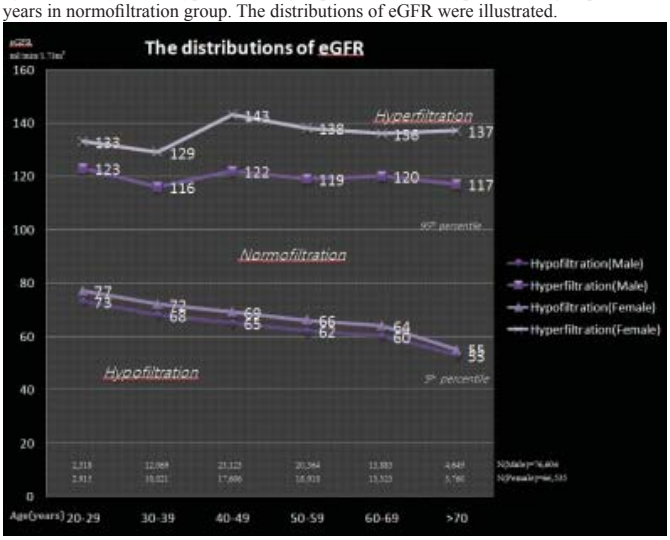
## SA-PO250

**Relationship between Glomerular Hyperfiltration and All-Cause Mortality in General Population** Kyung Don Yoo, Dong Ki Kim, Ho Jun Chin, Yon Su Kim, Hajeong Lee. *Dept of Internal Medicine, Seoul National Univ Hospital, Seoul, Jongno-gu, Korea.*

**Background:** Glomerular hyperfiltration has been known to be a marker of early damage of kidney disease. However, the association of glomerular hyperfiltration and individual's long term prognosis remains unknown.

**Methods:** Clinical and biochemical data had been collected in 143,271 routine health checkups from 1995 to 2009. We extracted the mortality data from Statistics Korea to our data using personal unique identifier. Hyperfiltration was defined as an estimated glomerular filtration rate (eGFR) over 95th percentile by age and sex-matched. Hypofiltration was defined as an eGFR below the 5th percentile by age and sex matched.

**Results:** A total of 143,271 individuals was included, and 53.5% of them were men. Among of them, 7.4% of participants had diabetes, and 24.8% had hypertension. The mean age was 50.5 years in hyperfiltration group, 50.2 years in hypofiltration group,and 52.6 years in normofiltration group. The distributions of eGFR were illustrated.




During the 58.9 months of follow up, 1,840 (1.3%) participants were dead. Interestingly,the mortality rate of hyperfiltration group was higher than normofiltration group in both men (3.8% vs 1.6%, P<0.001) and women (1.3% vs 0.7%, P<0.001). In the survival analysis, hyperfiltration compared with normofiltration was proved to be an independent risk factor for all-cause mortality after adjustment for by age,diabetes,hypertension,body mass index,lipid profile,C-reactive protein,and serum albumin in men (hazard ratio 1.119, 95% CI 1.002–1.437, P=0.047). In contrast, hyperfiltration failed to predict mortality in women.

**Conclusions:** In this study, we disclose that glomerular hyperfiltration might be an independent risk factor for all-cause mortality, especially in men. The mechanism and clinical implication of glomerular hyperfiltration should be more clarified.

## SA-PO251

**Clinical Significance of Subclinical Carotid Atherosclerosis and Its Relationship with Echocardiographic Parameters in Non-Diabetic Chronic Kidney Disease Patients** Jwa-kyung Kim, Sun Ryoung Choi, Jong-woo Yoon, Jung-woo Noh, Sung Gyun Kim. *Internal Medicine, Hallym Univ Sacred Heart Hospital, Kidney Research Institute, Korea.*

**Background:** Risk stratification of chronic kidney disease (CKD) patients is of particular interest and the presence of subclinical carotid atherosclerosis may be a good indicator of high-risk group. We investigated long-term outcomes of subclinical carotid atherosclerosis in non-diabetic CKD patients and tried to find echocardiographic parameters suggesting the presence of subclinical carotid atherosclerosis.

**Methods:** As a prospective design, a total of 182 patients underwent carotid ultrasonography and Doppler echocardiography at the time of CKD diagnosis. Carotid atherosclerosis was defined as carotid intima-media thickness >1.0 mm and/or the presence of plaque.

**Results:** Subclinical carotid atherosclerosis was found in 99 (54.4%) patients and they were significantly older and had a higher prevalence of hypertension, increased pulse pressure and serum high-sensitivity C-reactive protein (hs-CRP) levels than those without carotid atherosclerosis. Regarding echocardiographic results, left ventricular hypertrophy and markers of elevated LV filling pressure were closely related with the presence of carotid atherosclerosis. According to multivariate analysis, age, hs-CRP levels, and the ratio of early peak transmitral inflow velocity (E) to early diastolic mitral annular velocity (E') (E/E') were significant determinants of carotid atherosclerosis. During the study period of 28.8 ± 16.1 months, 23 cases of adverse CV events were occurred. Patients with carotid atherosclerosis showed significantly higher rates of annual cardiac event rates than those without (8.6% vs. 1.5%, p <0.001). Smoking (OR 3.13, 95% CI 1.10-9.09), increased E/E' ratio (OR 1.10, 95% CI 1.01-1.21), and the presence of carotid plaque (OR 7.80, 95% CI 1.45-45.97) were independent predictors for adverse CV events.

**Conclusions:** Subclinical carotid atherosclerosis was closely associated with poor prognosis in non-diabetic CKD patients. Increased age, hs-CRP, and E/E' ratio may be useful indicators suggesting the presence of subclinical carotid atherosclerosis.

## SA-PO252

**Characteristics of Uninephric Chronic Kidney Disease (CKD) Patients**
Andrew John Mallett,[1,2,3] Anne Salisbury,[1,2,3] Zaimin Wang,[1,2] Helen G. Healy,[1,2,3] George T. John,[1,3] Wendy E. Hoy.[1,2] *[1]CKD.QLD; [2]Centre for Chronic Disease, School of Medicine, Univ of Queensland, Brisbane, Queensland, Australia; [3]Dept of Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.*

**Background:** CKD due to uninephria is infrequently reported. 0.9% of Australian and New Zealand patients starting renal replacement therapy (RRT) in 2011 had either "Loss of Single Kidney" (n=6) or "Congenital Renal Hypoplasia and Dysplasia" (n=20). Its frequency in the general CKD population is undefined.

**Methods:** To define characteristics of uninephric patients with CKD. CKD.QLD is a registry and research platform involving all consenting patients in public renal practices in Queensland (~10,800). Uninephric patients among the first 2359 enrolled were sought through Primary Renal Disease coding for nephrectomy, hypo/dysplastic, atrophic, and congenital kidneys. They were compared to all patients in the CKD.QLD registry.

**Results:** 136 uninephric patients were identified, or 5.8% of the total. Of these 76 (56%) had a surgical nephrectomy, 17 (12.5%) had a congenital malformation, 16 (11.7%) had an atrophic/dysplastic kidney, 13 (9.5%) had congenital single kidney, 10 (7.3%) had a functional nephrectomy, and 4 (3%) had a partial nephrectomy.

Uninephric patients in total and by subgroups were most commonly CKD Stage 3b(30%) and 4(26%). Their mean age was 64.2 years, compared to 65.5 years for CKD.QLD. 47% of uninephric patients were female vs 45% of the CKD.QLD registry.

Among the 76 surgically nephrectomised patients, 6 were living kidney donors while 70 were for "other/non-living kidney donor" reasons. All living donors were female compared to 37% of the "other" group. CKD Stage 3a was most common in living donors (50%) and CKD Stage 3b most common in the "other" group (34%). Living donor mean age was 63yrs vs 67.5yrs in the "other" group.

**Conclusions:** There are small numbers of uninephric patients in this CKD group, though a greater proportion than represented in RRT data. They are younger that the broader CKD.QLD population and have greater prevalence in CKD stages 2 to 5. The identification of 6 living kidney donors is of concern.

*Funding:* Government Support - Non-U.S.

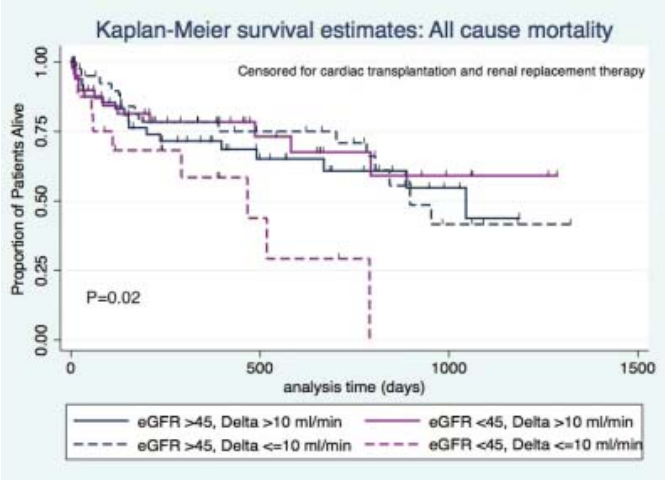
## SA-PO253

**Improvement in Renal Function Is Associated with Improved Outcomes after Ventricular Assist Device Implantation**
Jay L. Koyner, Abhijit S. Naik. *Sect. of Nephrology, Univ of Chicago.*

**Background:** Renal dysfunction is common in those with end stage heart failure including those requiring a ventricular assist device (VAD). Renal function improves following VADs in some patients although data on its impact on long-term mortality is limited.

**Methods:** We identified all patients who received a VAD from Jan. 2008 through Jan. 2012 at the University of Chicago and excluded subjects with ESRD and those who received pre-op renal replacement therapy (RRT). We defined Improved Renal Function (IRF) as a change in eGFR (MDRD) from baseline to day 7 of >10 ml/minute. The primary outcome was all cause mortality. The survival data was censored for cardiac transplantation and first RRT treatment. Kaplan Meier curves were then constructed and a Cox proportional hazards model was used to estimate the risk of mortality.

**Results:** 152 subjects were included in the cohort, 62 (41%) had baseline eGFR ≤45 ml/minute and 90(59%) had an eGFR > 45 ml/min There was no difference in the number of patients who had postop AKI (RIFLE- Risk) between the eGFR ≤ 45 and > 45 ml/min groups (p=0.57). Patients with eGFR≤45 and no IRF (n=18) had an increased risk of all-cause mortality [HR =2.87 (1.15,7.15) P=0.02] compared to those with an eGFR ≤45 ml/min and IRF (n=41). There was no mortality difference in patients with eGFR >45 ml/minute irrespective of their IRF status. Patients with eGFR ≤45 ml/min and IRF experienced outcomes similar to patients with eGFR > 45 ml/minute regardless of their IRF (p=0.85).




**Conclusions:** Patients with an eGFR ≤45 ml/min with IRF experienced outcomes on par with those who had a eGFR > 45 ml/min; these outcomes were significantly superior when compared to those with a eGFR ≤45 ml/min without IRF. The factors that impact IRF require further investigation which in turn may lead to improved VAD patient outcomes and may better identify patients who may benefit from a combined heart kidney transplant.

*Funding:* NIDDK Support

## SA-PO254

**Nephropathy in Illicit Drug Abusers – A Post-Mortem Analysis**
Oliver Jung,[1] Stefan Büttner,[1] Helmut Geiger,[1] Kerstin U. Amann,[2] Maike Julia Buettner.[2] *[1]Nephrology, Goethe Univ Hospital, Frankfurt/Main, Germany; [2]Pathology, Insitute of Nephropathology, Friedrich-Alexander-Univ, Erlangen, Germany.*

**Background:** Illicit drug abuse is an independent risk factor for chronic renal disease, but the pathogenic role of chronic exposure to illicit drugs and their contaminants under unsterile conditions remains unclear.

**Methods:** Retrospective investigation of all deceased that underwent forensic autopsy because of a suspected conjunction with illicit drug abuse between 01.01.2009 and 30.04.2011 in the Frankfurt/Main metropolitan area, Germany (3.8 million inhabitants).

**Results:** A total of 129 deceased were studied. Individuals were mostly Caucasian (99.2%), male (82.2%) and intravenous drug users (IVDU) (81.4%). Median age at time of death was 39 years and documented duration of drug abuse was 17 years, with the majority (87.2%) taking various drugs in parallel as assessed by toxicological analysis. Despite young age deceased had a high burden of co-morbidities, especially cardiovascular disease, liver cirrhosis and infections. Evaluation of kidneys demonstrated a broad spectrum of pathological alterations predominated by arteriosclerotic and ischemic damage, mostly mild interstitial inflammation, calcification of renal parenchyma and interstitial fibrosis/tubular atrophy, with hypertensive-ischemic nephropathy as the most common cause of nephropathy. Interstitial inflammation (OR: 16.59, 95%CI: 3.91-70.39) and renal calcification (OR: 2.43, 95%CI: 1.03-5.75) were associated with prolonged as well as severe IVDU, whereas hypertensive and ischemic damage were associated with toxicological confirmed cocaine abuse (OR: 6.00, 95%CI: 1.27-28.44) in multivariate analysis. Neither specific glomerular damage indicative for heroin- and hepatitis C related disease, e.g. FSGS or MPGN, nor signs of analgesic nephropathy were found.

**Conclusions:** In the majority of illicit drug abusers chronic progressive renal failure is attributable to repeated insults to the kidney caused by multiple antigenic and pharmacological challenges. In addition, our data demonstrate the deleterious role of cocaine abuse in this setting by inducing hypertensive and ischemic damage, promoting progression of renal disease.

## SA-PO255

**How to Estimate Glomerular Filtration Rate in HIV Patients?**
Corinne Isnard-Bagnis,[1] Laurence Pieroni,[2] Philippe Maksud,[3] Rachida Inaoui,[4] Stephanie Lallauret,[5] Nathalie I. Mbarga-Iobe,[5] Jerome Tourret,[1] Anne Bissery,[5] Alain Mallet,[5] Gilbert Deray,[1] Christine Katlama,[6] Sophie Tezenas du Montcel.[5] *[1]Nephrology, Groupe Hospitalier Pitie Salpetriere et Univ Pierre et Marie Curie, Paris, France; [2]Biology, Centre Hospitalier D'Avignon, Avignon, France; [3]Nuclear Medicine, Groupe Hospitalier Pitie Salpetriere et Univ Pierre et Marie Curie, Paris, France; [4]Rhumatology, Groupe Hospitalier Pitie Salpetriere et Univ Pierre et Marie Curie, Paris, France; [5]Clinical Research Unit, Groupe Hospitalier Pitie Salpetriere et Univ Pierre et Marie Curie, Paris, France; [6]Infectious Diseases, Groupe Hospitalier Pitie Salpetriere et Univ Pierre et Marie Curie, Paris, France.*

**Background:** Chronic kidney disease is a frequent complication in the HIV population. The optimal way to estimate glomerular filtration rate (GFR) in HIV patients is not known.

**Methods:** 44 $HIV_1$ patients were included in a transversal monocentric comparative study evaluating the accuracy of the different diagnostic tests available compared to the gold standard measurement of GFR. Adult, male, caucasian patients exhibiting an estimated (either Cockcroft or MDRD) GFR between 60 and 30 ml/min/1,73 $m_2$ were included. Plasma creatinine dosages (Jaffé and enzymatic), urea, albumin, proteinuria, Cystatin C were obtained. GFR was estimated using Cockcroft, MDRD, sMDRD, CKD Epi, CKD $Epi_{cyst}$, $CKDEpi_{cyst/creat}$ and measured using isotopic $Chrome^{51}$ EDTA clearance.

**Results:** Mean age was 62±10 with 82%>50 years, mean time from HIV diagnosis was 19±7 years. Mean BMI 23±4, with 9%>30. Prevalence of diabetes was 26%, HTA 47%. Viral load was <40 for 91% of patients and mean CD4 count was 438±195 cells/$mm^3$. Mean measured GFR was 63.39±13.47 ml/min/1,73$m^2$. All formulae under estimated GFR. Best precision was afforded by sMDRD and best accuracy by MDRD formulae. CKDEpi, $CKDEpi_{cyst}$ and $CKDEpi_{cyst/creat}$ performed worse than the MDRD formula.

**Conclusions:** In HIV patients in stable immunovirologic conditions with CKD stage 3 and high prevalence of metabolic associated conditions, MDRD formula performs best although all formulae under estimate GFR. Cystatin C formulae do not perform any better.

*Funding:* Private Foundation Support

## SA-PO256

**Hyperuricemia Predicts Progression of Chronic Kidney Disease in Patients with Reduced Kidney Mass** Isabel Galán Carrillo, Marian Goicoechea, Soledad Garcia de Vinuesa, Ursula Verdalles, Alba Santos, Santiago Andrés Cedeño Mora, Nicolas Macias, Abraham Rincon, Jose Luno. *Hospital General Universitario Gregorio Marañón, Madrid, Spain.*

**Background:** Hyperuricemia has been associated with an increase in the incidence of chronic kidney disease (CKD) in general population and with kidney disease progression in CKD patients. The aim of this study was to evaluate the role of hyperuricemia in kidney disease progression in patients with reduced kidney mass.

**Methods:** Three hundred thirty seven patients (182M, 155F), median age 59.8±17.1 years, being followed on an outpatient basis, were included in the study. The etiology of the reduced kidney mass was: nephrectomy in 188, kidney agenesis in 27 and kidney atrophy in 122. The median follow-up was 60.0 (36.0-97.5) months. The primary composite end-point was: doubling baseline serum creatinine, end stage renal disease or death.

**Results:** The mean reduction of GFR was 2.32 ml/min/1.73 m². Sixty-five (5.2%) progressor patients had a reduction of GFR higher than 2.32 ml/min/year/1,73m2. The composite end point was reached by 24 patients (14 deaths, 5 patients entering dialysis, 15 patients doubling serum creatinine) and 48 patients suffered from a cardiovascular event. The independent factors associated with the composite event adjusted to blood pressure were: age (HR 1,112 (1,055-1,173), p=0,001),hyperuricemia, defined as uric acid ≥7 mg/dl (HR 3,31 (1,24-8,85), p=0,017) and albuminuria (HR 3,56 (1,40-9,05), p=0,007). These factors also predicted CKD progression : age (HR 1,055 (1,027-1,083), p=0,001), hyperuricemia (HR 2,95 (1,64-5,28), p=0,001), and albuminuria (HR 2,44 (1,32-4,51), p=0,004). Age (HR 1,031 (1,006-1,056), p=0,014) and previous vascular disease (HR 5,14 (2,66-9,94), p=0,001),were independent predictors of cardiovascular events. Diabetes, dyslipidemia and renin angiotensin system blockers were not associated with CKD progression.

**Conclusions:** In patients with reduced renal mass due to lost of one kidney, progression of CKD is not frequent. Hyperuricemia is a risk factor for progression of renal disease and may be established as a new therapeutic target together with blood pressure and albuminuria.

## SA-PO257

**Plasmatic Renin Activity in Patients with Chronic Kidney Disease** Lidia Anguiano,[1] Marta Riera,[1] Julio Pascual,[1] Clara Barrios,[1] Angels Betriu,[2] Jose M. Valdivielso,[2] Elvira Fernandez,[2] Maria Jose Soler.[1] *[1]Nephrology, IMIM-Hospital del Mar, Barcelona, Spain; [2]Nephrology, Hospital Arnau de Vilanova, Lleida, Spain.*

**Background:** The renin-angiotensin system is activated in Chronic Kidney Disease (CKD), with an increase of Plasmatic Renin Activity (PRA). Our aim is to study PRA in patients in different stages of CKD patients without a history of cardiovascular (CV) disease.

**Methods:** A total population of 148 patients from the NEFRONA study in different stages of CKD and without a history of CV disease was analyzed. Patients were distributed into three groups: CKD stages 1-2 (CKD1-2, n=51), non-dialysis CKD stages 3-5 (CKD3-5, n=50) and dialysis (CKD5D, n=48). Variables analyzed were: gender, diabetes, dyslipidemia, hypertension, age, antihypertensive treatments (ACEi and ARA2) and treatment with vitamin D analogues (calcitriol, paricalcitol, colecalciferol and hidroferol). PRA was analyzed using a fluorimetric assay.

**Results:** Distribution of the population: mean age of 56.97±12.24, 60.1% men, 49.3% diabetics, 62.8% dyslipidemias, 69.6% hypertensive, 21.6% with ACEi treatment and 35.8% with ARA2 treatment.

There were significant differences in PRA levels between groups, with an increased PRA in CKD3-5 (199.1±12.1 RFU/µl/h) as compared to CKD5D (151.4±10.4) and CKD1-2 (116.5±6.1) ($p<0.05$).

In CKD3-5 patients, diabetic patients showed higher levels of PRA as compared to non-diabetic (229.9±20.9 vs 168.2±8.8; $p<0.05$). In this group, treatment with paricalcitol showed a decrease in PRA compared to those patients without treatment (137.2±11.9 vs 205.9±12.9; $p<0.05$). In CKD1-2, patients treated with ARA2 had higher levels of PRA compared to those not treated (153.8±12.5 vs 102.4±5.4 $p<0.05$).

**Conclusions:** In CKD patients without a history of CV disease, CKD3-5 showed an increase in PRA. Patients on dialysis have lower levels of PRA as compared to CKD predialysis. In the CKD3-5 group, diabetic patients have an increased PRA, while the treatment with vitamin D (paricalcitol) analogues is associated with lower levels of PRA.

## SA-PO258

**Impact of the Great East Japan Earthquake on Chronic Kidney Disease without Renal Replacement Therapy Patients in Severely Destroyed Coastal Area of Japan** Gen Yamada,[1] Mariko Miyazaki,[1] Tasuku Nagasawa,[2] Yasumichi Kinoshita,[2] Tae Yamamoto,[1] Kazuto Sato,[2] Masaaki Nakayama,[3] Hiroshi Sato,[1] Sadayoshi Ito,[1] Yaeko Murata.[1] *[1]Nephrology, Endocrinology, and Vascular Medicine, Tohoku Univ Hospital, Sendai, Japan; [2]Internal Medicine, Ishinomaki Redcross Hospital, Ishinomaki, Japan; [3]Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Medical Univ, Fukushima, Japan.*

**Background:** While there is serious impact by the great disaster for the dialysis patients, the disaster impact on chronic kidney disease(CKD) patients of pre-renal replacement therapy(RRT) has not been reported. The tsunami caused by Great East Japan Earthquake in March 2011 destroyed the coastal area of Miyagi prefecture. We conduct the prospective cohort study for CKD patients from 2006 in this area. We followed 156 patients treated in the severely devastated coastal zone, Ishinomaki. The applicable population is 220,000. The died or missed victims were 5,800 people, as well as 30,000 buildings totally collapsed in Ishinomaki medical zone.

**Methods:** Using data from Miyagi Gonryo study, we identified 156 patients who registered July and August/2008 in Ishinomaki Redcross Hopital.They were followed GFR, urinary findings, blood biochemistry findings, treatment and outcome annually. The Great East Japan Earthquake occurred after 3 years from registration. We described the outcome of 1 year after disaster compared with previous 3 years.

**Results:** One hundred and seventeen patients were valid in 2010. Annual check confirmed that 5 started dialysis and 3 died by disease. Four lossed after January 2011, 4 died by the tsunami. After 1 year from disaster, 1 lossed from June 2011, 7 moved, and 2 died by infectious disease in April 2011 and by heart failure in December 2011. Death and cardiovascular events didn't increase compared with 2010. While 29.9% of patients showed eGFR decline over 5%/year between 2008 and 2010 of 77 patients observed through study period to 2012, 58.4% of them decreased eGFR over 5%/year between 2011 and 2012.

**Conclusions:** We concluded that the great tsunami disaster was possible to affect pre-RRT CKD patients. We should treat them carefully for more long term about the cardiovascular event and renal outcome.

## SA-PO259

**Total Kidney Volume Is a Prognostic Biomarker for Worsening of Kidney Function in Patients with Autosomal Dominant Polycystic Kidney Disease** J.F. Marier,[1] Nathalie H. Gosselin,[1] Jason T. Chittenden,[1] Frank S. Czerwiec,[2] Daniel I. Levy,[3] Arlene B. Chapman,[4] Berenice Y. Gitomer,[5] Vicente E. Torres,[6] Eslie H. Dennis,[7] Klaus Romero,[7] D. Miskulin,[8] Ronald D. Perrone.[8] *[1]Pharsight, St. Louis, MO; [2]Otsuka, Rockville, MD; [3]Pfizer, Collegeville, PA; [4]Emory Univ, Atlanta, GA; [5]Univ of Colorado, Colorado, CO; [6]Mayo Clinic, Rochester, MN; [7]Critical Path Institute, Tucson, AZ; [8]Tufts Medical Center, Boston, MA.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. The growth of cysts increases total kidney volume (TKV) and result in interstitial fibrosis and renal failure. The goal of this project was to qualify TKV as prognostic biomarker for 30 and 57% decline of estimated glomerular filtration rate (eGFR) in ADPKD patients.

**Methods:** TKV data collected by the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) and longitudinal research registries were used (University of Colorado, Emory University, and Mayo Clinic). TKV was measured by ultrasonography, computer assisted tomography scan or magnetic resonance imaging. Longitudinal TKV and the probability of 30 or 57% eGFR decline was assessed using a joint model.

**Results:** Patients with 30 or 57% eGFR decline had higher baseline TKV (1.5 and 1.9 L, respectively) than patients without (1.0 L). Baseline age and predicted TKV at the time of 30 or 57% eGFR decline were statistically significant. Probabilities of not meeting the 30 or 57% eGFR decline are shown below.

| Endpoints | Baseline Age (Years) | Baseline TKV | | |
|---|---|---|---|---|
| | | 1 L | 2 L | 3 L |
| Probability of No 30%eGFR in 3 years | 20 | 88.5% | 81.8% | 76.0% |
| | 40 | 92.6% | 88.0% | 83.9% |
| Probability of No 57%eGFR in 10 years | 20 | 86.6% | 73.5% | 62.0% |
| | 40 | 80.5% | 63.4% | 51.2% |

**Conclusions:** Baseline TKV can be applied as a prognostic biomarker along with baseline age to predict eGFR decline. Patients with larger TKV have a greater risk of 30 or 57% eGFR decline. This information can be used to improve drug development programs and for trial enrichment.

*Funding:* Private Foundation Support

## SA-PO260

**Total Kidney Volume Is a Prognostic Biomarker for the Progression to End-Stage Renal Disease in Patients with Autosomal Dominant Polycystic Kidney Disease over 10 Years** J.F. Marier,[1] Mohamad-samer Mouksassi,[1] Fredrik Jonsson,[1] Frank S. Czerwiec,[2] Daniel I. Levy,[3] Arlene B. Chapman,[4] Berenice Y. Gitomer,[5] Vicente E. Torres,[6] Eslie H. Dennis,[7] Klaus Romero,[7] D. Miskulin,[8] Ronald D. Perrone.[8] *[1]Pharsight, St. Louis, MO; [2]Otsuka, Rockville, MD; [3]Pfizer, Collegeville, PA; [4]Emory Univ, Atlanta, GA; [5]Univ of Colorado, Colorado, CO; [6]Mayo Clinic, Rochester, MN; [7]Critical Path Institute, Tucson, AZ; [8]Tufts Medical Center, Boston, MA.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. The growth of cysts increases total kidney volume (TKV) and lead to renal failure. Radiological imaging can be used to measure TKV and monitor disease progression. The goal of this project was to qualify TKV as a prognostic biomarker for progression to end-stage renal disease (ESRD) in ADPKD patients.

**Methods:** TKV data collected by the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) and longitudinal research registries were used (University of Colorado, Emory University, and Mayo Clinic). Ultrasonography (US), computer assisted tomography (CT) scan and magnetic resonance imaging (MRI) were included. A joint model linking longitudinal TKV and the probability of ESRD was used.

**Results:** Mean baseline age of patients with and without ESRD were 42.8 and 33.1 years, respectively. Mean baseline TKV of patients with and without ESRD were approximately 2.2 and 1.0 L, respectively. Baseline age and the predicted TKV at the time of ESRD were statistically significant. Probabilities of not reaching ESRD are presented below.

| Baseline Age | Typical Baseline TKV (probability of no ESRD in 10 years) | | |
|---|---|---|---|
| | 1 L | 2 L | 3 L |
| 20 | 93.9% | 83.7% | 74.4% |
| 40 | 88.6% | 71.5% | 58.1% |

The above results were consistent with those derived with the US or CT-MRI datasets.

**Conclusions:** Baseline TKV can be applied as a prognostic biomarker along with baseline age to predict the risk of ESRD over 10 years. Younger subjects with large TKV are at greater risk of progression than older subjects with smaller TKV. This information can be applied to improve drug development programs and for trial enrichment.

*Funding:* Private Foundation Support

## SA-PO261

### Relationship between Renal Complications and Total Kidney Volume in Autosomal Dominant Polycystic Kidney Disease from the Consortium for Radiologic Imaging of Polycystic Kidney Disease Cohort

Frederic F. Rahbari-Oskoui,[1] Doug Landsittel,[2] Vicente E. Torres,[3] Kyongtae Ty Bae,[2] Michal Mrug,[4] Marie C. Hogan,[3] Lisa M. Guay-Woodford,[7] Jared J. Grantham,[6] Michael F. Flessner,[5] Arlene B. Chapman,[1] William M. Bennett.[8] *[1]Emory Univ; [2]U of Pittsburgh; [3]Mayo Clinic, MN; [4]U of Alabama-Birmingham; [5]NIDDK-NIH; [6]Kansas Univ; [7]Children's Nat Med Ctr, DC; [8]Legacy Good Samaritan Med Ctr, Portland, OR.*

**Background:** Inclusion of renal complications of autosomal dominant polycystic kidney disease (ADPKD) as new endpoints for interventional clinical trials has been suggested by the FDA. The relationship between these factors and height adjusted Total Kidney Volume (htTKV), is unknown.

**Methods:** We used the CRISP cohort to establish the frequency of kidney pain (P), hypertension (HT), urinary tract infection (UTI), gross hematuria (H) and nephrolithiasis (N) and their relationship to the last observed htTKV. Differences in htTKV by those with a baseline complication vs the No Complication group were calculated using the t-test; odds ratios (ORs) were calculated using logistic regression adjusting for age, BMI, gender, race, and baseline corrected iothalamate clearance.

**Results:** 11 year follow up data from 202 participants were analyzed. Baseline (& cumulative) percentage frequencies of P, HT, UTI, H and N were 60.8(84.7), 61 (71.4), 45.2 (52.7), 32.8 (48.1), and 13.3 (21.2) respectively. Mean (±SD) baseline htTKV, were significantly higher ($p < 0.05$) in: 1- HT patients vs non-HT (736.0 ± 394.5 vs 401.6 ± 224.9 cc), 2-Patients with H vs without H (728.6 ± 409.3 vs 537.3 ± 297.2 cc), 3- patients with P vs without P (690.1 ± 405.3 vs 478.5 ± 274.3 cc). The differences in htTKV based on UTI and N were not significant. The adjusted ORs (CI) for development of KP, HT, H, UTI, N based on (a 100 cc difference in) baseline htTKV were 1.17 (1.01, 1.36), 1.41 (1.19, 1.66), 1.17 (1.07, 1.28), 0.98 (0.89, 1.07) and 1.07 (0.98, 1.17).

**Conclusions:** In addition to established correlation with renal function, HtTKV is associated with specific renal complications of ADPKD. These findings reiterate the importance of htTKV as a marker of disease severity and support its use in the design of future studies.

*Funding:* NIDDK Support

## SA-PO262

### Kidney Length Measured by Ultrasound (US) Predicts Development of Chronic Kidney Disease (CKD) Stage 3 in Autosomal Dominant Polycystic Kidney Disease (ADPKD): Findings from the Consortium for the Radiographic Imaging Studies of Polycystic Kidney Disease (CRISP) Cohort

Arlene B. Chapman,[1] Vikram Smith,[1] Frederic F. Rahbari-Oskoui,[1] Jared J. Grantham,[2] Vicente E. Torres,[3] Kyongtae Ty Bae,[4] Doug Landsittel,[4] W. Charles O'Neill.[1] *[1]Emory Univ; [2]Kansas Univ; [3]Mayo Clinic; [4]Univ of Pittsburgh.*

**Background:** ADPKD causes kidney cysts, enlargement and ultimately renal failure. Total kidney volume (TKV) measured by magnetic resonance imaging (MR) is a measure of kidney cyst burden that strongly predicts future CKD Stage 3 within 8 years in ADPKD. Using simultaneous baseline US and MR imaging in the (CRISP), we established the predictive power of US length for future CKD Stage 3 in ADPKD.

**Methods:** Correlations between baseline US length and MR TKV and length and the future development of CKD Stage 3 by iothalamate clearance determinations were examined in 241 ADPKD subjects, 15-45 years, and baseline estimated creatinine clearance ≥ 70 ml/min. Correlations were assessed using Pearson's correlations. Area under the curve using receive operator characteristics were performed based on US measured renal length at baseline in CRISP.

**Results:** Correlations between Baseline US length and MR TKV and length were strong (r=0.98, r=0.97 respectively). Bland Altman plots demonstrated consistent agreement between US length and MR length and TKV until US length reached 17 cm. MR based lengths predicted future CKD Stage 3 (r=0.85, P<0.01) at an optimal length of 16 cm. Area under the curve ROC analyses demonstrated that US length predicted future CKD Stage 3 strongly (0.84. P<0.01) similar to MR baseline TKV.

**Conclusions:** Results support the strong predictive value of baseline US length for future kidney function decline (ie CKD Stage 3), consistent with MR based TKV measures in the CRISP II cohort. US length had a reduced agreement with MR based TKV and length when > 17 cm. US length can be used to predict future CKD Stage 3 in ADPKD.

*Funding:* NIDDK Support

## SA-PO263

### Correlation of Total Kidney Volume and eGFR in Patients with ADPKD: Results from the TEMPO 3:4 Trial

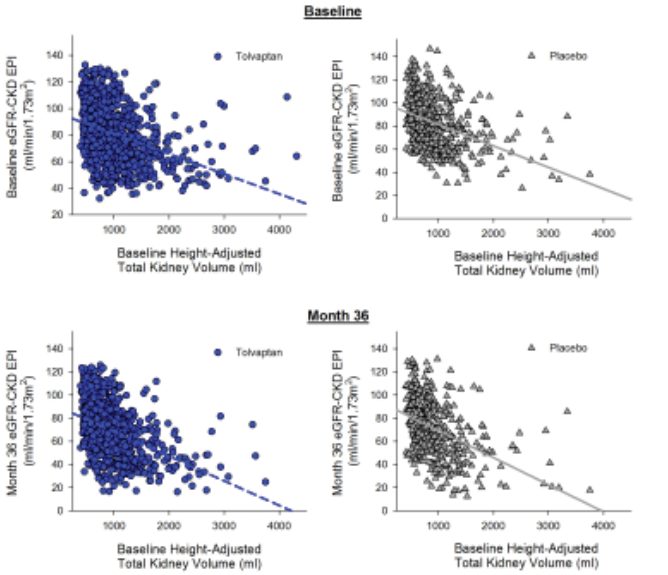
Ronald D. Perrone,[1] Arlene B. Chapman,[2] Frank S. Czerwiec,[3] Olivier Devuyst,[4] Ron T. Gansevoort,[5] Jared J. Grantham,[6] Eiji Higashihara,[7] Holly Krasa,[3] John Ouyang,[3] Vicente E. Torres.[8] *[1]Boston, USA; [2]Atlanta, USA; [3]Otsuka USA; [4]Zurich, Switzerland; [5]Groningen, Netherlands; [6]Kansas City, USA, Japan; [7]Mitaka, Japan; [8]Rochester, USA.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) causes kidney cysts often associated with pain, hypertension, and kidney failure. Total kidney volume (TKV) is a measure of kidney cyst development and enlargement that has been correlated with renal function decline in smaller studies.

**Methods:** Correlations between baseline TKV and height-adjusted TKV slope and changes in $eGFR_{CKD\text{-}EPI}$ were examined as post-hoc exploratory analyses. Correlations were assessed using Pearson's correlations at different time points and by treatment group.

**Results:** Correlations between htTKV at baseline and eGFR improved from Baseline to Year 3 with no difference between placebo and tolvaptan (Table). The correlation between baseline htTKV and eGFR slope on treatment was blunted in the tolvaptan compared to the placebo group.

| **Table** Pearson Correlation Coefficients with baseline height-adjusted TKV | | | |
|---|---|---|---|
| | **All Patients (N=1445)** | **Tolvaptan (N=961)** | **Placebo (N=483)** |
| Baseline eGFR | -0.378 | -0.370 | -0.395 |
| Month 36 eGFR | -0.435 | -0.434 | -0.439 |
| eGFR slope on treatment | -0.185 | -0.162 | -0.247 |
| *p<0.001 for all comparisons | | | |




**Conclusions:** Results support the strong predictive value of baseline htTKV for predicting GFR decline, consistent with data reported from the CRISP II cohort. Since there is approximately 30% less eGFR decline in the tolvaptan group regardless of CKD stage, greater variability likely reflects a differential response among the treated population which is not entirely accounted for by baseline htTKV or baseline eGFR.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical Development & Commercialization, Inc,

## SA-PO264

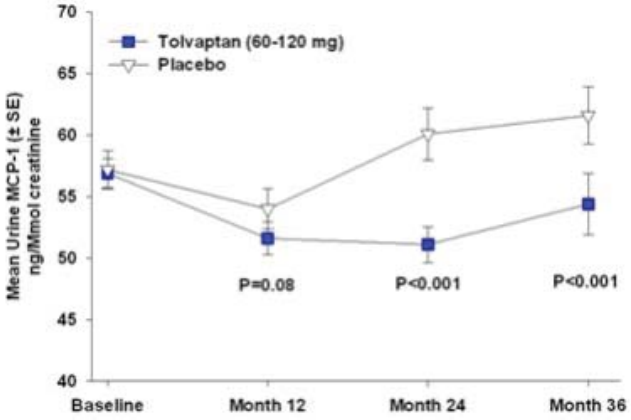
### Tolvaptan Administration to Patients with ADPKD Suppresses Urine MCP-1 Excretion: Results from the TEMPO 3:4 Trial

Jared J. Grantham,[1] Arlene B. Chapman,[2] Frank S. Czerwiec,[3] Olivier Devuyst,[4] Ron T. Gansevoort,[5] Eiji Higashihara,[6] Holly Krasa,[3] John Ouyang,[3] Ronald D. Perrone,[7] Vicente E. Torres.[8] *[1]Medicine, Kansas Univ, Kansas City, KS; [2]Medicine, Emory Univ, Atlanta, GA; [3]Development, Otsuka, Rockville, MD; [4]Medicine, Univ Zurich, Zurich, Switzerland; [5]Medicine, Univ Gronengin, Gronengin, Netherlands; [6]Urology, Kyorin Univ, Kyorin, Japan; [7]Medicine, Tufts Med Ctr, Boston, MA; [8]Medicine, Mayo, Rochester, MI.*

**Background:** Monocyte chemotactic protein-1 is produced by cysts and excreted in the urine in ADPKD. MCP-1 accumulates in cysts and interstitium and attracts macrophages. Urine MCP-1 derives from cysts that remain connected to downstream segments or from the cells of patent tubules. The effect of treatment that slows cyst development and enlargement on urine MCP-1 levels is unknown.

**Methods:** MCP-1 was prospectively measured (pre-treatment baseline, Month 12, 24, and 36) in 1445 patients treated with either tolvaptan or placebo for 36 months. Urine MCP-1 (ng/Mmol creatinine) was determined in 784 Tolvaptan and 429 Placebo subjects. Treatment group comparisons at each time point were determined using an observed case, intent to treat, MMRM analysis on log-transformed ratios.

**Results:** At baseline, the mean MCP-1 to creatinine ratio was balanced between treatment groups and associated positively with total kidney volume (R= 0.221, P<0.0001) and negatively with eGFR (R= -0.124, P<0.0001) and Uosm (R= -0.148, P<0.0001). Urine MCP-1 creatinine ratio was less with tolvaptan than placebo beginning at Month 12, with significant reductions at Months 24 and 36 (mean difference -12%).




**Conclusions:** Tolvaptan administered over a period of 3 years reduced the excretion of the biomarker MCP-1 into the urine relative to placebo, consistent with potential amelioration of renal interstitial inflammation.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical Development & Commercialization, Inc, Rockville, Maryland

## SA-PO265

**The Effect of Tolvaptan on Albuminuria in ADPKD. Results of the TEMPO 3:4 Trial** Ron T. Gansevoort,[1] Arlene B. Chapman,[2] Frank S. Czerwiec,[3] Olivier Devuyst,[4] Jared J. Grantham,[5] Eiji Higashihara,[6] Holly Krasa,[3] John Ouyang,[3] Ronald D. Perrone,[7] Vicente E. Torres.[8] *[1]For the TEMPO 3:4 Investigators, Groningen, Netherlands; [2]Atlanta, USA; [3]Otsuka Pharma, USA; [4]Zurich, Switzerland; [5]Kansas City, USA; [6]Mitaka, Japan; [7]Boston, USA; [8]Rochester, USA.*

**Background:** The TEMPO 3:4 Trial, including 1445 ADPKD patients, suggested that use of tolvaptan (T) had no effect compared to placebo (P) on albuminuria. However, the use of categorical "albuminuria events" to express albuminuria may have resulted in a loss of sensitivity to detect changes. In the present study we therefore specifically investigated the effects of T on albuminuria.

**Methods:** Post-hoc analysis of prospective, blinded RCT. Microalbuminuria or higher (MA) was a priori defined as spot albumin:creatinine ratio (ACR) ≥2.8 in males and ≥2.0 mg/mmol in females.

**Results:** Baseline median ACR was 3.1 (T) and 3.4 (P) mg/mmol. 58.6% of ADPKD patients had MA. Subjects with MA had higher TKV and lower eGFR, but were as likely to be male, hypertensive and to use RAASi than subjects without MA. During follow-up P treated patients with MA had a similar rate of growth in TKV when compared to patients without MA (5.51 versus 5.47 %/yr), but more rapid eGFR loss (median -4.13 vs. -2.63 mL/min/1.73m$^2$/yr). ACR rose in P treated patients (+0.23 mg/mmol), whereas it decreased in T treated patients (-0.40 mg/mmol). The difference in ACR in T versus P treated patients increased over time, reaching a maximum of 23% at month 36 (p<0.001), whereas no difference in blood pressure was observed. After T withdrawal the difference in ACR between the two treatment groups remained significant (16%, p=0.003). A beneficial effect of T on TKV growth and eGFR loss was observed in both MA subgroups, but stronger in patients with MA than without MA (effect on TKV growth: -53.7% vs -43.7%; on eGFR slope: -29.5% vs -20.2%%, resp.).

**Conclusions:** In ADPKD patients MA is associated with eGFR loss. Tolvaptan decreased albuminuria compared to placebo, independent of blood pressure, and this effect remained after withdrawal of study drug. Treatment efficacy of tolvaptan was more readily detected in patients with MA.

*Funding:* Pharmaceutical Company Support - Otsuka Pharma, USA

## SA-PO266

**Effects of Tolvaptan in ADPKD: Subanalysis of Japanese Patients from the TEMPO 3:4 Trial** Shigeo Horie,[1] Eiji Higashihara,[2] Satoru Muto,[3] Kikuo Nutahara,[4] Yasuhiko Iino,[5] Ichiei Narita,[6] John Ouyang,[7] Vicente E. Torres.[8] *[1]Urology, Juntendo Univ, Tokyo, Japan; [2]Polycystic Kidney Research, Kyorin Univ, Mitaka, Tokyo, Japan; [3]Urology, Teikyo Univ, Tokyo, Japan; [4]Urology, Kyorin Univ, Mitaka, Tokyo, Japan; [5]Nephrology, Nippon Medical School, Tokyo, Japan; [6]Clinical Nephrology and Rheumatology, Niigata Univ, Niigata, Japan; [7]Biostatistics, Otsuka PDC, Rockville, MD; [8]Internal Medicine, Mayo Clinic, Rochester, MN.*

**Background:** Tolvaptan, a vasopressin V2 receptor antagonist, slowed the increase of total kidney volume (TKV) and the decline of kidney function in ADPKD patients during a 3-years treatment period in the global TEMPO 3:4 study. This study included 177 Japanese patients in total 1445 patients. The purpose of this study is to evaluate the efficacy and safety of tolvaptan in Japanese patients.

**Methods:** 276 Japanese patients were screened and 177 patients were enrolled: assigned 118 patients to tolvaptan treatment and 59 patients to placebo treatment. We evaluated the same primary and secondary endpoint and safety profile in Japanese population as originally planned in the TEMPO 3:4 study (NEJM 368(13):1258-9.).

**Results:** 92 of 118 patients (78.0%) in the tolvaptan group and 55 of 59 patients (93.2%) in the placebo group completed the 3-year treatment. Baseline eGFR was lower and higher rates of proteinuria were noted in Japanese patients compared to global subjects. The increase of TKV was 1.3% per year in tolvaptan group, versus 5.0% per year in placebo (P<0.001). The relative treatment effect of 74.9% in Japanese patients was greater than global subjects (49.2%). Analysis of the key secondary endpoint showed fewer ADPKD-related events per 100 person-years of follow-up with tolvaptan than with placebo (41 vs. 52 events). Tolvaptam significantly inhibited the decline of renal function assessed by reciprocal of serum creatinine compared to placebo (-4.84 vs -6.28 (mg per milliliter)-1/year, P<0.05). Tolvaptan showed the similar safety profile in Japanese patients including hepatotoxicity.

**Conclusions:** Tolvaptan showed the similar efficacy and safety profile in Japanese ADPKD patients compared to the global subjects.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical Development & Commercialization Inc.

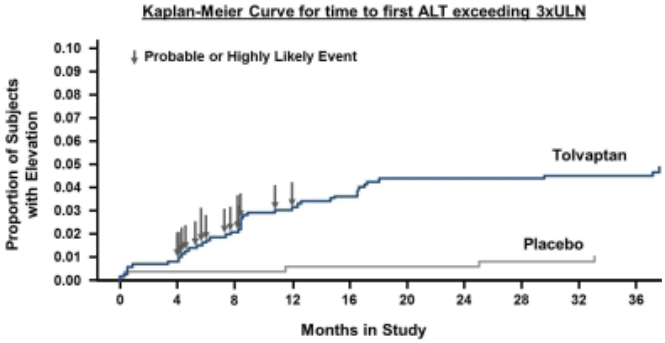
## SA-PO267

**Profile of Transaminase Elevations in Autosomal Dominant Polycystic Kidney Disease–Results from the TEMPO 3:4 Trial** Christopher Zimmer,[1] Arlene B. Chapman,[2] Frank S. Czerwiec,[1] Olivier Devuyst,[3] Ron T. Gansevoort,[4] Eiji Higashihara,[5] Holly Krasa,[1] John Ouyang,[1] Ronald D. Perrone,[6] Vicente E. Torres.[7] *[1]Otsuka, USA; [2]Atlanta, USA; [3]Zurich, Switzerland; [4]Groningen, Netherlands; [5]Mitaka, Japan; [6]Boston, USA; [7]Rochester, USA.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) subjects treated with tolvaptan(T) in the TEMPO 3:4 trial were more likely to have significant liver TA elevations than those treated with placebo(P). This imbalance was not previously observed with T, requiring further elucidation.

**Methods:** The potential of T to cause hepatocellular injury was explored with adverse event and laboratory data. Subjects with events meeting criteria indicative of liver injury were adjudicated. An expert panel assessed drug-causality considering co-morbid conditions, concomitant medication use, onset, offset, and dose-relationship.

**Results:** Forty-six of 1445 subjects (35 T and 11 P) met criteria for adjudication. 16 T and 1 P subject met drug-related causality criteria with onset of qualifying events after 3 months of treatment.




The incidence of new onset ALT elevations >3xULN in T subjects was low after 14 months. The TA elevations associated with T returned to 3xULN typically within 1 to 4 months and were not associated with fulminant liver failure, or permanent liver injury or dysfunction. No association with dose or exposure was found.

**Conclusions:** Hepatocellular injury was identified as a new risk of tolvaptan treatment in subjects with ADPKD enrolled in the TEMPO 3:4 trial. No subjects experienced hepatic failure, permanent liver damage or death from hepatocellular injury. Results from these analyses suggest that regular TA monitoring should be done during use of T. The mechanism of T-induced liver injury in ADPKD patients requires further study.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical Development & Commercialization, Inc

## SA-PO268

**Achievement and Maintenance of Blood Pressure Targets in HALT:PKD**
Vicente E. Torres,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Ronald D. Perrone,[4] D. Miskulin,[4] Theodore I. Steinman,[5] Franz Winklhofer,[6] William E. Braun,[7] Marie C. Hogan,[1] Frederic F. Rahbari-Oskoui,[3] Kaleab Z. Abebe,[8] Michael F. Flessner.[9] *[1]Mayo Clinic; [2]U CO; [3]Emory U; [4]Tufts U; [5]Beth Israel; [6]KUMC; [7]Cleveland Clinic; [8]U Pittsburgh; [9]NIH/NIDDK HALT Study Group.*

**Background:** HALT-PKD seeks to determine whether ACEI/ARB is superior to ACEI alone and low BP(<110/75) is superior to standard BP(120-130/70-80) in delaying cystic progression (eGFR>60mL/min/1.73m$^2$, Study A) and whether ACEI/ARB is superior to ACEI alone (BP target 110-130/70-80mmHg) in slowing eGFR decline (eGFR 25-60mL/min/1.73m$^2$, Study B).

**Methods:** Stepwise dosing of lisinopril(L) and telmisartan/placebo(T/P)(steps 1-4) followed by stepwise dosing of other agents (steps 5-10) is used to achieve BP targets. During 1/2006-4/2012, 519, 501, 490, 474, 433, 273, 154 A and 458, 447, 437, 411, 362, 191, and 80 B patients completed 4, 12, 24, 36, 48, 60, and 72-mth followup. BP control is assessed by home BP measurements.

**Results:**

| | Mths | Study A Std | Study A Low*[a] | Study B |
|---|---|---|---|---|
| MAP† | 4 | 91.5 (5.8) | 85.0 (6.5) | 91.0 (6.9) |
| | 12 | 91.7 (6.3) | 83.8 (6.7) | 91.5 (5.8) |
| | 24 | 92.1 (6.2) | 83.0 (6.5) | 91.6 (6.3) |
| | 36 | 92.7 (6.1) | 83.0 (6.7) | 91.6 (6.1) |
| | 48 | 93.3 (6.8) | 83.4 (6.7) | 91.7 (6.0) |
| | 60 | 93.0 (6.2) | 84.0 (9.0) | 92.0 (5.5) |
| | 72 | 93.6 (7.1) | 82.7 (6.3) | 91.6 (6.0) |
| Step; L, T/P mean dose | 4 | 2.2; 15.0, 50.7 | 3.4; 24.0, 64.3 | 2.9; 18.2, 55.9 |
| | 12 | 2.2; 15.6, 50.9 | 3.7; 25.0, 66.6 | 3.0; 18.7, 56.3 |
| | 24 | 2.3; 15.1, 50.9 | 3.8; 25.7, 65.7 | 3.1, 18.0, 56.0 |
| | 36 | 2.4; 15.3, 51.4 | 3.8; 25.4, 66.4 | 3.1; 17.4, 55.4 |
| | 48 | 2.4; 15.2, 51.5 | 3.8; 25.7, 65.8 | 3.0; 16.5, 54.2 |
| | 60 | 2.5; 15.6, 52.7 | 4.0; 25.8, 66.2 | 2.9; 15.9, 52.9 |
| | 72 | 2.7; 15.6, 54.4 | 4.2; 26.0, 67.4 | 2.7; 16.0, 51.8 |

†mean SD *Underlined: P<0.001 compared to Study A standard

[a] Step and dosage comparisons made using Wilcoxon test

Based on random regressions, negative MAP slopes in Study A low and significantly positive BP slopes in Study A standard result in increasing BP separation over time.

**Conclusions:** ACEI alone or ACEI/ARB achieves BP control and MAPs are within target in most subjects at 4-72 months of followup. Excellent separation in BP and doses of LIS and T/P between study A arms is achieved, without detectable differences in heart rate.

*Funding:* NIDDK Support

## SA-PO269

**Cardiac Magnetic Resonance Assessment of Left Ventricular Ejection Fraction in the ADPKD HALT A Cohort**
Kyongtae Ty Bae,[1] Diana Kaya,[1] Kaleab Z. Abebe,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Vicente E. Torres,[4] Theodore I. Steinman,[5] William E. Braun,[6] Franz Winklhofer,[7] Cheng Tao,[1] JinHong Wang,[1] Marie C. Hogan,[4] D. Miskulin,[8] Frederic F. Rahbari-Oskoui,[3] Michael F. Flessner,[9] Charity G. Moore,[1] Ronald D. Perrone.[8] *[1]U Pittsburgh; [2]U Colorado; [3]U Emory; [4]Mayo; [5]Beth Israel; [6]Cleveland Clinic; [7]U Kansas; [8]Tufts U; [9]NIH/NIDDK.*

**Background:** To measure left ventricular ejection fraction (LVEF) from magnetic resonance images (MRI) in HALT-PKD Study A subjects aged 15-49 with eGFR>60mL/min/1.73m$^2$ and to associate LVEF with clinical and imaging parameters including total kidney volume (TKV) and left ventricular mass index (LVMI).

**Methods:** ECG-gated cine cardiac MRI covering left ventricle (LV) were acquired in 458 subjects at baseline, prior to study intervention. From the end-diastolic (ED) and end-systolic (ES) MRI, ED and ES volumes of LV chamber were measured using a semi-automated program. LVEF was calculated as %(ED-ES)/ED LV volumes. LVMI was measured from end-diastolic MRI. TKV was measured from T1-weighted MRI of kidney. Univariate and multivariate analysis of the association of baseline parameters with LVEF was performed.

**Results:** Mean LVEF was 64.4±7.5% (range 40-87%) and did not differ between M and F. The baseline parameters that showed significant univariate associations with LVEF were LVMI, height-corrected TKV (htTKV), body mass index (BMI), age, and urine aldosterone. Only htTKV, BMI, and urine aldosterone remained significant in the multivariate regression. eGFR, RBF, BP, and urine albumin/sodium/potassium were not significant in the univariate analysis.

| | Coef | Standardized Coef | P |
|---|---|---|---|
| htTKV | -0.003 | -0.140 | 0.009 |
| BMI | 0.15 | 0.098 | 0.067 |
| Urine aldosterone | -0.096 | -0.116 | 0.030 |

**Conclusions:** In relatively young ADPKD subjects with preserved GFR, reduction in LVEF was associated with increased urine aldosterone and htTKV and decreased BMI. Subclinical renal hypoperfusion due to reduced LVEF or enlarged kidneys may result in increased urinary aldosterone excretion, contributing to the pathogenesis of HTN in ADPKD.

*Funding:* NIDDK Support

## SA-PO270

**Analysis of Octreotide LAR Therapy after Four Years in Polycystic Liver and Kidney Disease**
Marie C. Hogan, Eric J. Bergstralh, Maria V. Irazabal, James Glockner, Xujian Li, Vicente E. Torres. *Mayo Clinic, MN.*

**Background:** We completed follow up of 25 patients with ADPKD or ADPLD who received up to 4 yrs of the somatostatin analog OctLAR; 27/ 42 patients who participated in a prospective two-year clinical trial of OctLAR (40mg/mo) in PLD consisting of a 1 year RCT and a 2nd year of open label OctLAR re-enrolled into an open label extension study (OLE).

**Methods:** Liver/kidney MRIs at baseline, yr 1 & 2 were compared to re-enrollment scan for OLE (OLEi) and at OLE completion (OLEo). Primary end point was ΔTLV, annualized rate), 2º end points included ΔTKV & eGFR. TLV & TKV at OLEo were compared to baseline, yr 2 & OLEi.

**Results:** Twenty-five of 42 patients (60%) on OctLAR (16 of the original 28 (O→O) group)) or placebo (9 of original 14; (P→O) group) completed OLE. While off OctLAR, TLV increased at 3.4% per annum. After resuming OctLAR, TLV decreased again by 4.7% per annum. Despite regrowth off OctLAR, there was a progressive reduction in (mean ± SD) TLV (-11%; p=0.006; paired t test) on long-term OctLAR. Baseline TKV (777 ± 468ml) increased to 819 ± 508ml by yr2 & to 861 ± 525ml at OLEi & was again suppressed to (879 ± 593 ml; 0.7±13.6%) at OLEo. ΔGFR were similar in both groups.

| TLV (n=21)* | n | Baseline - Yr1(%) | Year 1 - 2(%) | Yr2 - OLEi(%) | OLEo(%) |
|---|---|---|---|---|---|
| OctLAR (n=13) | 13 | -6.5 (4.9) | -3.9 (5.9) | 4.0 (10.2)‡ | -5.7 (6.7) |
| Placebo (n=8) | 8 | 1.7(8.9)‡ | -8.3 (12.3) | 2.4 (4.7)‡ | -3.2 (5.1) |
| Total | 21 | n/a | -5.6 (9.0) | 3.4 (8.2)‡ | -4.7 (6.1) |
| p value (mean rate=0) | | n/a | 0.0113 | NS | 0.0020 |
| p value (On vs OFF drug) | | n/a | 0.0001 | -- | 0.0013 |
| **TKV (n=15)** | 15 | n/a | 4.0 (6.8) | 5.5 (7.7)† | 0.7 (13.6) |
| p value (mean rate=0) | | n/a | 0.0477 | 0.0249 | NS |
| p value (On vs OFF drug) | | n/a | NS | -- | NS |
| **eGFR%** | | | | | |
| OctLAR (n=16;11 w/o ADPLD, KTx) | | | | | -12 (21.8) |
| Placebo (n=9; 6 w/o ADPLD, KTx) | | | | | -0.3 (8.8) |
| Total (n=25; 17 w/o ADPLD, KTx) | | | | | -7.9(18.8) |
| p value (mean rate=0) | | | | | NS |

* Censored for liver cyst aspirations. ‡ Stat significant. † Off drug

**Conclusions:** Prolonged therapy with OctLAR over 4 yrs in a subgroup of symptomatic PLD patients shows the beneficial effects in arresting PLD progression. Discontinuation of therapy leads to further organ regrowth.

*Funding:* Pharmaceutical Company Support - Novartis

## SA-PO271

**Autosomal Dominant Polycystic Kidney Disease Cyst-Cell Implants Develop Cysts and Vasculature That Is Inhibited by Antiangiogenic Treatment**
Elsa Bello-Reuss. *Internal Medicine, Texas Tech Univ Health Siences Center, Lubbock, TX.*

**Background:** Previously we described angiogenesis in Autosomal Dominant Polycystic Kidney Disease (ADPKD) kidneys, as well as increases in Vascular Endothelial Growth Factor (VEGF) and its receptors in ADPKD kidney sections and cyst cells in culture. It has been reported that angiogenic growth factors expression correlate with disease severity in young patients with ADPKD and that interleukin-8 (IL-8) gene expression is also increased in ADPKD cells.

**Methods:** We studied the expression of IL-8 (at mRNA and protein level) and its receptors in ADPKD cyst cells and used ADPKD cyst cells -mixed with Matrigel™ - implanted in the immunoincompetent nude mouse and removed after 14 days, to study the development of cysts and angiogenesis in the implants and the inhibition of cyst growth by antiangiogenic drugs.

**Results:** We report that, in cyst cells in culture, there is constitutive expression of IL-8 (at the mRNA and protein levels) and that IL-8 secretion is inhibited by an anti-IL-8 neutralizing antibody (anti-IL-8) but no by dexamethasone (DEXA), a nonspecific IL-8 inhibitor. Cyst cells seeded in Matrigel and implanted in nude mice form cysts and the host provides the vasculature. Cysts grew to different sizes and the cells exhibit cuboidal or flat shapes. In this model of angiogenesis, cyst cells express IL-8, VEGF and their receptors. Cyst volume and vascularization is significantly decreased in the implants removed from mice treated with anti-IL-8, compared with untreated controls.

**Conclusions:** These studies underscore the importance of IL-8 secretion by ADPKD cyst cells in the recruitment of vasculature, creating a positive feedback for the continued growth of ADPKD cysts. The cysts in the nude-mouse implants reproduce the heterogeneous cyst size and morphology of the ADPKD kidney and could be used to study potential treatments for ADPKD using multi-target inhibitors.

*Funding:* Private Foundation Support, Clinical Revenue Support

## SA-PO272

**Analysis of Coronary Endothelial and Smooth Muscle Function Using $^{15}$O-Labeled Water PET in Early Stage Autosomal Dominant Polycystic Kidney Disease** Naoko Matsuoka,[1] Yasunobu Ishikawa,[1] Sekiya Shibazaki,[1] Osamu Manabe,[2] Keiichiro Yoshinaga,[2] Saori Nishio,[1] Tatsuya Atsumi.[1] *[1]Dept of Medicine II, Hokkaido Univ Graduate School of Medicine, Sapporo, Hokkaido, Japan; [2]Dept of Molecular Imaging, Hokkaido Univ Graduate School of Medicine, Sapporo, Hokkaido, Japan.*

**Background:** Cardiovascular problems are a major cause of morbidity and mortality in autosomal dominant polycystic kidney disease (ADPKD). Endothelial dysfunction (ED) has been used to predict future coronary artery disease before atherosclerotic changes. It has been reported that significant ED occurs in both normotensive and hypertensive patients with ADPKD. Polycystins are expressed is in endothelial and vascular smooth muscle cells. However, the association between ED and smooth muscle dysfunction has not been fully studied. Positron emission tomography (PET) can non-invasively myocardial blood flow (MBF). Using a cold pressor test (CPT) and adenosine triphosphate (ATP) infusion, PET can evaluate coronary endothelial function and coronary flow reserve (CFR). This study aimed to examine the coronary endothelium function in normotensive patients with ADPKD using $^{15}$O- labeled water PET.

**Methods:** Nine normotensive patients with ADPKD and 9 healthy controls were included in the study. Coronary endothelial function using was measured during CPT and vasodilator capacity, CFR was measured during ATP stress using $^{15}$O-labeled PET.

**Results:** There was no significant difference between groups regarding age, body mass index, blood pressure, and lipid levels. The resting MBF was significantly higher in patients than in control (0.93±0.07 vs 0.73±0.13; P<0.001). MBF during CPT was no significantly difference between the two groups. MBF during ATP infusion to that at rest, as an index of CFR, was significantly reduced in patients than in control (3.27±0.91 vs 5.06±1.28; P<0.01).

**Conclusions:** Normotensive patients with ADPKD with well-preserved renal function have reduced CFR indicating early atherosclerosis even in early stage of the disease. In contrast, there was no significant change in coronary endothelial function. Atherosclerotic changes might precede predominantly in vascular smooth muscle rather than endothelial dysfunction in ADPKD.

## SA-PO273

**Pravastatin and Angiogenesis in ADPKD** Wei Wang, Melissa A. Cadnapaphornchai, Michel Chonchol, Robert W. Schrier, Berenice Y. Gitomer. *Univ of Colorado Anschutz Medical Campus.*

**Background:** We have shown that serum levels of vascular endothelial growth factor (VEGF) correlate with total renal volume and renal function in young ADPKD patients suggesting that VEGF plays a key role in early cystogenesis. VEGF activity is regulated by its receptors including VEGF receptor -1 (Flt-1). A splice variant, soluble Flt-1 (sFlt-1) antagonizes VEGF signaling. Statins have diverse effects on angiogenesis and in human studies pravastatin has been shown to lower circulating VEGF levels. More recently pravastatin has been shown to slow the progression of renal structural disease in young patients with ADPKD. We hypothesized that statin may slow renal growth by mediating angiogenesis through reduction of VEGF levels in young ADPKD patients.

**Methods:** Subjects aged 8-22 years with ADPKD and normal renal function were randomized to either pravastatin (20mg or 40mg/day by age) or placebo. All subjects also received lisinopril. Serum and 24 hour urine were collected at each visit. VEGF and sFlt-1 levels were assayed by ELISA (R&D systems).

**Results:** After 18 months (m) of follow-up serum VEGF increased significantly compared to baseline in placebo (P) group (137.1±85.1pg/ml vs 109.2±71.0pg/ml, n=26, p<0.05) and was unchanged in the statin (S) group (157.1±115.9pg/ml vs 148.1±122.6pg/ml, n=25). Serum VEGF/sFlt-1, an index of VEGF bioavailability, increased significantly in group P at 18 m (1.5±1.2 vs 1.1±0.8, n=26, p<0.05) and 36m (1.2±0.6 vs 0.9±0.6, n=23, p<0.05) and was unchanged in group S at 18m (1.8±1.5 vs 1.7±1.4, n=24) and 36m (1.7±1.3 vs 1.4±1.2, n=23) relative to baseline. Urine VEGF levels increased significantly in group S at 36 m (7.0±4.0ng/mmol Cr vs 3.4±3.2ng/mmol Cr, n=15, p<0.05) and were unchanged in group P (7.6±4.7 vs 6.2±4.8ng/mmol Cr).

**Conclusions:** Based on the outcome of the pravastatin clinical trial in young ADPKD patients, statin therapy significantly slows renal growth. Our present results suggest that this is related to stabilization of serum VEGF and serum VEGF/sFlt-1 ratio as well as increased urine VEGF. The current study indicates a potential anti-angiogenic effect of pravastatin by regulation of VEGF level.

*Funding:* NIDDK Support

## SA-PO274

**Thombospondin and Autosomal Dominant Polycystic Kidney Disease** Wei Wang, Michel Chonchol, Godela M. Brosnahan, Robert W. Schrier, Berenice Y. Gitomer. *Dept of Medicine, Univ of Colorado Anschutz Medical Campus.*

**Background:** Thrombospondins(TSP) including TSP-1, TSP-2 are glycoproteins that inhibit endothelial cell migration, proliferation and survival as well as vascular endothelial growth factor (VEGF) activity. In contrast, VEGF and angiopoietins(Ang) 1,2 are established angiogenic factors that correlate with renal structure and function in autosomal dominant polycystic kidney disease (ADPKD). We hypothesized that dysregulated TSP expression results in an imbalance between pro- and anti-angiogenic factors in ADPKD that may favor angiogenesis early in disease.

**Methods:** Healthy adults were included and ADPKD patients stratified according to eGFR either ≥ 60 (I) or< 60 ml/min/1.73m$^2$(II). Serum levels of VEGF, Ang-1, Ang-2, TSP-1 and TSP-2 were assayed by ELISA.

**Results:** The key differences in pro and anti-angiogenic growth factor levels are summarized in table 1. Serum TSP-1 levels positively correlated with Ang-1 and Ang-2 levels in group I. Serum TSP-2 and Ang-2 levels were positively correlated in group II.

| | I | II | CON | p value |
|---|---|---|---|---|
| n | 26 | 25 | 10 | |
| Age(y) | 35±9 | 50±8*# | 36±14 | <0.001 |
| eGFR(ml/min/1.73m$^2$) | 100.2±13.2 | 36.6±5.4# | ND | <0.001 |
| TSP-1(µg/ml) | 10.5±5.5* | 10.7±5.9* | 0.45±0.2 | <0.001 |
| TSP-2(ng/ml) | 28.5±11.3 | 31.7±14.5 | 29.1±8.7 | NS |
| VEGF(pg/ml) | 229±165* | 204±190* | 15.3±15.5 | <0.001 |
| Ang-1(ng/ml) | 86.6±51.2 | 53.6±37.8# | ND | <0.05 |
| Ang-2(pg/ml) | 2100±1140 | 2878±2153* | 1539±690 | <0.001 |
| Ang-1/TSP-2 | 8.3±2.1 | 1.8±1.1# | ND | <0.001 |

*P vs Con; # P vs I; ND: not determined.

**Conclusions:** Serum levels of VEGF, Ang-2 and TSP-1 are elevated in ADPKD patients as compared to the normal controls. The relationships between pro and anti-angiogenic factors differ in ADPKD patients based on renal function. The increased Ang-1/TSP-2 ratio in patients with earlier stages of ADPKD favors a pro-angiogenic environment in earlier disease. TSP's are potent inhibitors of angiogenesis and tumor growth and as such may have potential therapeutic benefits in slowing cyst growth in ADPKD.

## SA-PO275

**Secreted Frizzeld-Related Protein 4 Is a Marker of Autosomal Dominant Polycystic Kidney Disease Progression** Stefan Zschiedrich,[1] Klemens Budde,[2] Christoph Wanner,[3] Claudia Sommerer,[4] Ulrich Kunzendorf,[5] Bernhard Banas,[6] Walter H. Hoerl,[7] Nicholas Obermueller,[8] Wolfgang Arns,[9] Hermann Pavenstaedt,[10] Jens Gaedeke,[2] Lothar Faerber,[11] Peter Wimmer,[11] Kai-Uwe Eckardt,[12] Gerd Walz.[1] *[1]Renal Div, Univ Hospital Freiburg, Freiburg, Germany; [2]Dept of Nephrology, Charité Universitätsmedizin Berlin - Campus Mitte; [3]Univ Hospital Würzburg; [4]Center for Nephrology, Heidelberg; [5]Univ Hospital Kiel; [6]Univ Hospital Regensburg; [7]Medical Univ of Vienna; [8]Univ Hospital Frankfurt; [9]Merheim Medical Center, Cologne; [10]Univ Hospital Münster; [11]Novartis Germany, Nuremberg; [12]Univ of Erlangen and Community Hospital of Nuremberg.*

**Background:** Secreted frizzeld-related protein 4 (sFRP4) is expressed at elevated levels in the tissue of autosomal dominant polycystic kidney disease (ADPKD) kidneys, and detectable in blood, urine and cyst fluid of APDKD patients. It is a molecule known to antagonize Wnt signaling, an intracellular pathway that controls cell migration, proliferation and cellular polarity. To monitor future therapeutic interventions, there is a need for reliable and inexpensive biomarkers of ADPKD disease progression.

**Methods:** We investigated sFRP4 levels from patient samples of the 'Everolimus in patients with Autosomal Dominant Polycystic Kidney Disease' trial to determine its capability as a progression marker. 3126 probes were analyzed and correlated to kidney function and total kidney volume of ADPKD patients followed over 24 months.

**Results:** sFRP4 levels were significantly increased after the two years follow-up of the study, correlating with an increase of serum creatine and deterioration of renal function. The increase was independent of the intervention and observed in everolimus- and placebo-treated patients. Total kidney volume (measured by MRI) did not correlate with sFRP4 levels.

**Conclusions:** Our results demonstrate that sFRP4 plasma levels increase in patients with ADPKD. Everolimus had no effect on sFRP4 levels. Thus, measurement of sFRP4 may be a reliable (and cheap) parameter of disease progression in ADPKD.

## SA-PO276

**Association of Plasma and Urinary GDF-15 Levels with Disease Severity in ADPKD Patients** Ivan Formentini,[1] Carolina Haefliger,[6] Paolo Piraino,[5] Maria Chiara Magnone,[1] Anders Fernstrom,[3] Peter F. Barany,[4] Guillemette Duchateau-Nguyen,[1] Matthias Meier,[1] Maria Bobadilla.[1] *[1]Cardiovascular & Metabolism DTA, F. Hoffmann - La Roche Ltd, Basel, Switzerland; [2]Dept of Medical Sciences, Univ Hospital, Uppsala, Uppsala, Sweden; [3]Dept of Medical and Health Sciences, Faculty of Health Sciences, Linköping Univ, Linköping, Sweden; [4]Div of Renal Medicine, CLINTEC, Karolinska Institutet, Stockholm, Sweden; [5]PP Statistical Consulting, Rende, Italy; [6]Novartis Pharma AG, Basel, Switzerland.*

**Background:** Growth differentiation factor 15 (GDF-15) is a distant TGF-β family member suggested to reflect inflammation and apoptosis processes under stressful conditions. Clinically, several investigations have demonstrated that circulating GDF-15 levels are increased and independently prognostic across a wide spectrum of cardiovascular diseases. The relationship between GDF-15 levels and renal functional loss /severity has been described but not yet fully established, making of this marker a potential integrative predictor of kidney damage and CV risk in all CKD patients.

**Methods:** In the current study, we aim to investigate if urine and plasma GDF15 levels are associated to renal functional parameters in a cross-sectional ADPKD patient (n=56) and controls (n=20) (TH-PO645, ASN 2012) cohort.

**Results:** Urine GDF-15 levels were not associated to disease stage ($R^2$=0.057, p=1.5e$^{-2}$), baseline $eGFR_{MDRD}$ ($R^2$=0.082, p=4.3e$^{-3}$) or UACR ($R^2$=0.056, p=7.2e$^{-3}$) in cross-sectional cohort of ADPKD patients. Matching serum samples instead demonstrated

a strong relationship to CKD staging ($R^2$=0.52, p<1.0e$^4$), $eGFR_{MDRD}$ at screening ($R^2$=0.58, p<1.0e$^4$) while lacking correlation to log urine albumin levels ($R^2$=0.0027, p=3.2e$^{-1}$). In a Bayesian additive regression trees classification model plasma GDF-15 contributed to the prediction of ADPKD vs controls ($ROC_{AUC}$=0.867) much better then urinary levels ($ROC_{AUC}$=0.687). Plasma levels were also capable of discriminating control subjects vs. renal impaired patients ($ROC_{AUC}$= 0.852).

**Conclusions:** In conclusion, the present results show that higher levels of plasma GDF-15 are associated to renal impairment state and CKD severity in ADPKD.

*Funding:* Pharmaceutical Company Support - F. Hoffmann - La Roche Ltd

## SA-PO277

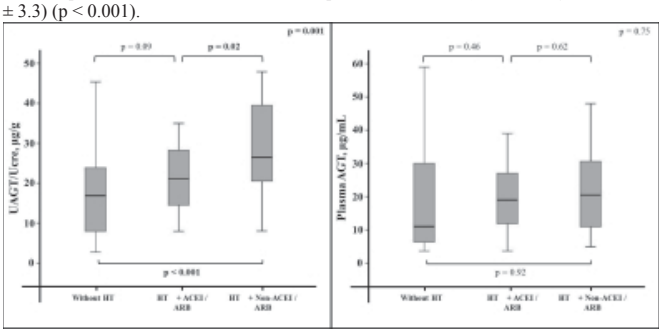
### Is the Intrarenal Renin Angiotensin System the Culprit for Hypertension in Patients with Autosomal Dominant Polycystic Kidney Disease?

Ismail Kocyigit,[1] Mahmut Ilker Yilmaz,[2] Aydin Unal,[1] Fahir Ozturk,[3] Eray Eroglu,[3] Cevat Yazici,[4] Ozcan Orscelik,[5] Murat H. Sipahioglu,[1] Bulent Tokgoz,[1] Oktay Oymak.[1] *[1]Dept of Nephrology, Erciyes Univ Medical School, Kayseri, Turkey; [2]Dept of Nephrology, Gülhane School of Medicine, Ankara, Turkey; [3]Dept of Internal Medicine, Erciyes Univ Medical School, Kayseri, Turkey; [4]Dept of Biochemistry, Erciyes Univ Medical School, Kayseri, Turkey; [5]Dept of Cardiology, Erciyes Univ Medical School, Kayseri.*

**Background:** Early onset of hypertension and its consequences account for the great majority of death in patients with autosomal dominant polycystic kidney disease (ADPKD). Renin angiotensinogen system(RAS) components have been shown in ADPKD kidneys. Thus we examined the urinary angiotensinogen (UAGT) levels as a biomarker of intrarenal RAS status in ADPKD patients with/without hypertension and healthy subjects.

**Methods:** Eighty-four ADPKD patients (43 with hypertension and 41 without hypertension) and 40 healthy controls were studied cross-sectionally. Hypertension diagnosed with ambulatory blood pressure monitoring. Urinary and plasma concentration of angiotensinogen, spot urine microprotein and creatinine levels recorded for each participants.

**Results:** UAGT/UCre levels were higher in hypertensive ADPKD patients (23.7 ± 8.4) compared with normotensive ADPKD patients (16.6 ± 5.2) and healthy controls (6.9 ± 3.3) (p < 0.001).




In univariate analysis, UAGT correlated with systolic blood pressure, diastolic blood pressure and proteinuria. The independence of these correlations were analyzed in a regression model, and shown UAGT to be significantly predicted by proteinuria and DBP.

**Conclusions:** Intrarenal RAS activation which is monitoring by UAGT levels clinically may be a harbinger for the development of hypertension and kidney disease in ADPKD patients.

## SA-PO278

### Prevalence of and Survival on Renal Replacement Therapy for ADPKD in Europe

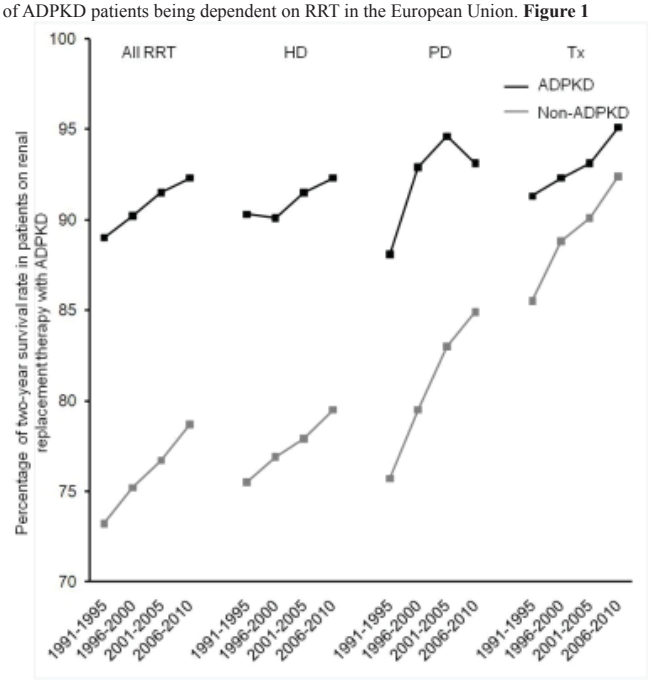
Edwin M. Spithoven,[1] Anneke Kramer,[2,3] Christoph Wanner,[3] Kitty J. Jager,[2,3] Ron T. Gansevoort.[1,4,5] *[1]Dept Nephrology, UMC Groningen, The Netherlands; [2]ERA-EDTA Registry, AMC Amsterdam, Netherlands; [3]For the ERA-EDTA Registry; [4]For the EuroCYST Consortium; [5]For the WGIKD.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the fourth most common diagnosis for starting renal replacement therapy (RRT). Still, there are few epidemiological data on the prevalence of and survival on RRT for ADPKD.

**Methods:** This study used data of the ERA-EDTA Registry on RRT prevalence and survival on RRT of 18 European countries with 385 million inhabitants. We studied four 5-year periods (1991-2010). Survival analysis was performed by the Kaplan-Meier method and by Cox proportional hazards regression.

**Results:** In 20 years time, the prevalence of RRT for ADPKD increased from 56.8 to 91.3 per million of the population (country range 71.1-104.0 pmp), whereas the percentage of ADPKD patients of the total number of patients on RRT remained stable at 9.8%. Two-year survival of ADPKD patients on RRT (adjusted for age, sex and country) has increased significantly from 89.0 to 92.3%, and was higher than for non-ADPKD subjects (Figure 1). Improved survival was noted for all RRT modalities (Figure 1): hemodialysis (adjusted Hazard Ratio for mortality last versus first time period 0.79; 95% CI 0.65-0.98), peritoneal dialysis (0.54; 0.37-0.80) and transplantation (0.53; 0.36-0.78). Especially cardiovascular mortality as cause of total mortality on RRT decreased in ADPKD patients i.e. from 53% to 29%, whereas it decreased from 44% to 35% in non-ADPKD patients. Of note, the incidence rate of RRT for ADPKD remained relatively stable at 7.6 vs. 8.1 pmp.

**Conclusions:** In ADPKD patients on RRT survival has improved markedly, especially due to lower cardiovascular mortality. This has led to a considerable increase in the number of ADPKD patients being dependent on RRT in the European Union. **Figure 1**




## SA-PO279

### Incidence of Renal Replacement Therapy for ADPKD in Europe

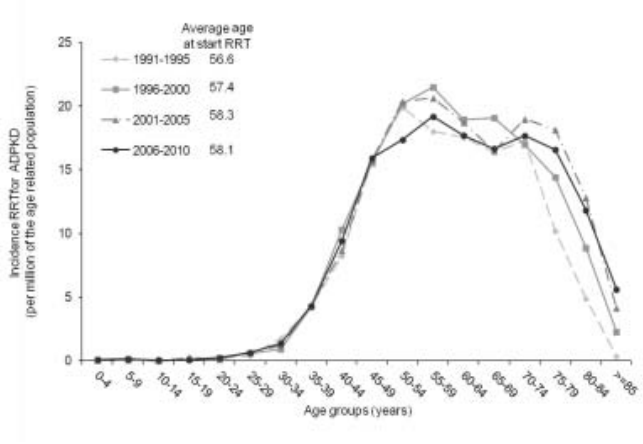
Edwin M. Spithoven,[1] Anneke Kramer,[23] Christoph Wanner,[3] Kitty J. Jager,[23] Ron T. Gansevoort.[145] *[1]Dept Nephrology, UMC Groningen, The Netherlands; [2]ERA-EDTA Registry, AMC Amsterdam, Netherlands; [3]For the ERA-EDTA Registry; [4]For the EuroCYST Consortium; [5]For the WGIKD.*

**Background:** Several treatment options, known to postpone the need for renal replacement therapy (RRT), have also been tested in RCTs including specifically ADPKD patients, with disappointing results. However, in general these RCTs were underpowered and selected patients with relatively stable eGFR, thus not allowing firm conclusions on renoprotective efficacy. We investigated whether epidemiological data on RRT incidence provide evidence for effective renoprotection in ADPKD.

**Methods:** This study used data of the ERA-EDTA Registry on RRT incidence rates in 18 European countries with 385 million inhabitants. We studied four 5-year periods (1991-2010).

**Results:** Over the four periods the average incidence rate of RRT for ADPKD, adjusted for general population age and gender, increased slightly from 7.6 to 8.1 per million of the population (pmp). Differences in the incidence of RRT for ADPKD were noted among countries (range 5.3-9.9 pmp). The mean age at start of RRT increased from 56.6 to 58.1 years (Figure 1) No change over time was observed in the incidence of RRT for ADPKD up to the age of 50 years (Figure 1) However, in recent time periods the incidence of RRT for ADPKD above the age of 70 years increased. Among countries there was a clear positive association between take-on rate of RRT for non-ADPKD renal disease and for ADPKD (r=0.64, p<0.001).

**Conclusions:** During the last two decades the incidence of RRT for ADPKD, as well as the age at which RRT was started, has increased slightly. Our data suggest that this is likely due to an increased access of elderly APDKD patients to RRT rather than providing evidence that effective renoprotective treatments have become available.




## SA-PO280

**Maternal and Fetal Outcomes in Women with Autosomal Dominant Polycystic Kidney Disease** Min Wu,[1] Diping Wang,[2] Peter C. Harris,[2] Ladan Zand,[2] Vesna D. Garovic,[2] Cindy A. Kermott.[3] *[1]Dept of Cardiovascular Diseases, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China; [2]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [3]Div of Preventive Medicine, Mayo Clinic, Rochester, MN.*

**Background:** Autosomal Dominant Polycystic Kidney Disease (ADPKD), a common genetic cause of chronic renal failure in children and adults, is characterized by the accumulation of fluid-filled cysts in the kidney and other organs. As the diagnostic techniques advance, more and more female patients are diagnosed with ADPKD before or during their birth age. However, it is still not clear how ADPKD affects the maternal and fetal outcomes in these patients. The aim of this study is to determine whether ADPKD is associated with adverse fetal and infant outcomes, maternal complications and long-term post-pregnancy prognosis.

**Methods:** We identified a cohort of 146 patients seen for pregnancy and cystic kidney disease at the Mayo Clinic from 1975 to 2010. From this cohort, 54 patients met the ultrasound diagnostic criteria for ADPKD (ADPKD group) while the other 92 patients had been diagnosed as 'Simple Cyst' (the control group). We compared the fetal and maternal outcomes of pregnancy between these two groups as well as report on long-term maternal prognosis.

**Results:** Overall, the fetal complication rates were similar between the ADPKD group and the control group. The spontaneous abortion rate (15.1% vs.14%, P=0.77) and premature rate (11.1% vs. 6.8%, P=0.44) were comparable in these two groups while the rates of fetal distress (3.4% vs. 0.7%, P<0.01) and birth defects (6% vs. 0%, P<0.001) were increased in ADPKD patients. Compared to the control group, pregnant ADPKD patients had higher risks for hypertension, proteinuria, edema, UTI, renal dysfunction and preeclampsia. Finally, follow-up studies after the last pregnancy clearly showed that rates of chronic hypertension and renal insufficiency were significantly increased in ADPKD patients.

**Conclusions:** ADPKD is associated with increased maternal complications during pregnancy but only has a slight potential of increased rates of fetal complications.

## SA-PO281

**Autosomal Dominant Polycystic Kidney Disease (ADPKD): A Risk Factor for New Onset Diabetes after Transplantation** Ankush Mittal, Ashtar Chami, Arlene B. Chapman. *Emory Univ Hospital, Atlanta, GA.*

**Background:** ADPKD is the fourth leading cause of ESRD and accounts for 8.3% of deceased donor and 11.7% of living donor yearly kidney transplants in the US. Our goal is to use an established cohort of PKD individuals evaluated for transplant to characterize their outcomes.

**Methods:** This is a retrospective cohort analysis of all ADPKD patients who presented for transplant evaluation at the Emory Transplant Center between 1/1/2002 and 1/1/2012, utilizing electronic medical records, prescription lists, and the organ transplant tracking record (OTTR). Individuals were evaluated with regard to their pre- and post-transplant course and conditions and complications that were related to ADPKD.

**Results:** 55 (32.5%) of transplanted patients developed new onset diabetes after transplantation (NODAT). 30.1% of Caucasians and 40.0% of African Americans developed NODAT (p>0.05). There was not a statistically significant difference in the BMI of patients who developed NODAT when compared to the rest of the population (28.7±0.9 vs. 27.3±0.4, p>0.05). Caucasians and African Americans with NODAT had BMIs prior to diagnosis of 28.9±1.0 and 28.3±1.9 (p>0.05), respectively, while those without NODAT had BMIs of 27.2± 0.5 and 27.2±1.3 (p>0.05), respectively. 32.1% of men and 32.9% of women developed NODAT (p>0.05). Patients with and without NODAT had BMIs of 28.0±1.3 and 28.2±0.05 (men, p>0.05) and 29.3±1.2 and 26.4±0.7 (women, p>0.05), respectively. 13.6% of transplanted patients were on belatacept. 21.7% of patients on belatacept and 34.7% of patients on tacrolimus developed NODAT (p>0.05).

**Conclusions:** Our cohort confirms previous reports of an increased incidence of NODAT in ADPKD patients despite similar BMIs, race, and gender profile and the use of belatacept in a proportion of the population.

## SA-PO282

**Nephrectomy and Renal Transplantation in Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Ankush Mittal, Ashtar Chami, Arlene B. Chapman. *Emory Univ Hospital, Atlanta, GA.*

**Background:** ADPKD is the fourth leading cause of ESRD and accounts for 8.3% of deceased donor and 11.7% of living donor yearly kidney transplants in the US. Our goal is to use an established cohort of PKD individuals evaluated for transplant to characterize their outcomes.

**Methods:** This is a retrospective cohort analysis of all ADPKD patients who presented for transplant evaluation at the Emory Transplant Center between 1/1/2002 and 1/1/2012, utilizing electronic medical records and the organ transplant tracking record (OTTR). Individuals were evaluated with regard to their graft and patient survival rates in those that did and did not have a nephrectomy.

**Results:** Of 286 ADPKD who presented for transplant evaluation, 169 (59.1%) were transplanted, 80 (28.0%) were evaluated but not yet transplanted, and 37 (12.9%) were ineligible for transplant. 5-year patient survival was 92.3% and 93.8% with and without a nephrectomy (p<0.001), respectively. 5-year patient survival for living and deceased donors was 98.0% and 92.9% (without a nephrectomy, p<0.001) and 100.0% and 89.7% (with a nephrectomy, p<0.001), respectively. 5-year graft survival was 82.9% and 89.8% with and without a nephrectomy (p<0.001), respectively. 5-year graft survival was 94.0% and 86.8% (without a nephrectomy, p<0.001) and 79.5% and 84.4% (with a nephrectomy, p<0.001) for living and deceased donor transplants, respectively. 5-year graft survival was 95.7% and 89.7% for African Americans and Caucasians, respectively, without a nephrectomy (p<0.001). 5-year graft survival was 75.0% and 84.1% for African Americans and Caucasians, respectively, with a nephrectomy (p<0.001). 5-year graft survival was 100.0% and 69.8% in men and women with a nephrectomy (p<0.001), respectively. 5-year graft survival was 77.7% and 88.9% for pre- and post-transplant nephrectomies (p<0.001).

**Conclusions:** Nephrectomy is a significant risk fact for graft survival in this ADPKD cohort. Men are shown to have significantly higher graft survival than women with a nephrectomy, and patients with a nephrectomy after transplant are shown to have significantly higher graft survival than those with a nephrectomy before transplant.

## SA-PO283

**Patient-Reported Pain in Autosomal Dominant Polycystic Kidney Disease: Initial Concepts Based on Patient Focus Group Discussions** Dorothee Oberdhan,[1] Arlene B. Chapman,[2] Sara N. Davison,[3] Frank S. Czerwiec,[1] Holly Krasa,[1] Jason C. Cole.[4] *[1]Otsuka Pharmaceutical Development & Commercialization, Inc., Rockville, MD; [2]Emory Univ, Atlanta, GA; [3]Univ of Alberta, Edmonton, Canada; [4]Covance Inc., San Diego, CA.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) involves significant kidney enlargement due to cyst development and expansion leading to kidney infections, bleeding, stones, chronic kidney disease and pain that is common, intermittent and debilitating, severely impacting a patient's health-related quality of life. Surgical intervention and medications may be needed. Previous research demonstrated consistency in disease burden across regions and suggested ADPKD patients report pain varyingly.

**Methods:** In 18 focus groups, conducted in 5 cities in Europe (n=72) and 4 cities in the US (n=53), patients were asked about their pain symptoms and impact on daily life. New conceptual domains discussed were summarized based on themes mentioned by ≥2 participants within a group.

**Results:** Patients report chronic and episodic dull kidney pain, chronic fullness/discomfort and acute severe kidney pain plus general pain. Severe kidney pain is usually associated with cyst rupture or infection. Kidney pain is easily distinguished from other pain, especially with progressing disease. Pain severity is distinct for each of the types of pain reported.

| General Pain | Chronic Dull Kidney Pain | Fullness/Discomfort | Severe Kidney Pain |
|---|---|---|---|
| Need warmth | Severity >7*<br>Comes and goes<br>On side/back<br>Easy to distinguish from other pain<br>Sleep disturbances<br>Impacts relationships<br>Irritable/on edge/impatient | Fullness/discomfort<br>Severity averages 2 or 3*<br>Impact on mild physical activity<br>Bending/stretching<br>Impact on moderate physical activity<br>Impacts relationships<br>Unable to eat/loss of appetite<br>Irritable/impatient | Pain<br>infrequent<br>memorable |

* Based on a scale of 1 to 10

**Conclusions:** Patients reported 3 distinct types of pain (chronic dull pain, fullness/discomfort and acute severe pain) beyond general pain. This categorization should allow easier pain identification using patient terms to help facilitate treatment.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical Development & Commercialization, Inc.

## SA-PO284

**Which Cyst to Drainage? — Mass Reduction Therapy for Autosomal Dominant Polycystic Kidney Disease Patients with Huge Liver Cysts**
Takashi Iijima, Tatsuya Suwabe, Koki Mise, Rikako Hiramatsu, Yoshifumi Ubara. *Nephrology Center, Toranomon Branch Hospital.*

**Background:** Percutaneous needle aspiration of cysts with subsequent sclerosis by injection of minocycline (drainage therapy) has been reported to be effective for patients with autosomal dominant polycystic kidney disease (ADPKD) who have one or a few very large and easily accessible cysts. However, a detailed investigation of the factors that influence the success of cyst drainage has not yet been performed. Accordingly, we evaluated the factors with an influence on this therapy.

**Methods:** At our institution, cyst drainage therapy was performed in 16 ADPKD patients with a hepatic cyst volume of over 500 ml between January 2010 and April 2012. The hepatic cyst area was calculated with area assessment software after precisely outlining the cyst(s) on CT scans, and the cyst volume was calculated by summing up each areas of 1-cm thickness CT slices. The mean CT value of each cyst was also calculated, as was the ratio of the cyst volume at one year after treatment to that before treatment. Drained cyst fluid was submitted for analysis of its specific gravity. Then CT values and specific gravity were compared between group A cysts showing over 80% volume reduction (mean of 92.4%) at one year after drainage and group B cysts with less than 80% volume reduction (mean of 31.1%). Statistical analysis was done by using the Mann-Whitney U test.

**Results:** Group A cysts had significantly lower CT values than group B cysts, with the values being 6 HU (4 to 10) and 19 HU(6 to 23) (p=0.004), respectively. Specific gravity was also significantly lower in group A than in group B, being 1.013 g/ml (1.007 to 1.017) versus 1.021g/ml (1.009 to 1.031) (p=0.017). When the cut-off value to predict responsiveness of drainage therapy is set at 13 HU of CT value, the sensitivity was 70% and specificity was 100%.

**Conclusions:** Drainage therapy was effective for hepatic cysts with lower CT values and lower specific gravity, while the response was poor when hepatic cysts had higher CT values and higher specific gravity, which probably indicate "complicated" cyst with prior infection and/or hemorrhage.

## SA-PO285

**Transcatheter Arterial Embolization with Embosphere: Evaluation of Technical Safety and Outcome**
Saori Nishio,[1] Yuske Sakuhara,[2] Naoko Matsuoka,[1] Junya Yamamoto,[1] Tasuku Nakagaki,[1] Daigo Nakazawa,[1] Daisuke Abo,[2] Sekiya Shibazaki,[1] Tatsuya Atsumi.[1] *[1]Dept of Medicine II, Hokkaido Univ School of Medicine, Sapporo, Hokkaido, Japan; [2]Dept of Radiology, Hokkaido Univ School of Medicine, Sapporo, Hokkaido, Japan.*

**Background:** Polycystic liver disease (PLD) is the most common extrarenal manifestation associated with autosomal dominant polycystic kidney disease (ADPKD). Patients with PLD often suffer from abdominal discomfort, dyspepsia, or dyspnea. Transcatheter arterial embolization (TAE) for hepatic artery branches using metallic coils has been reported as an effective and less invasive treatment for PLD. The disadvantages of coils are the need to use many of them before achieving complete obstruction and high cost. Furthermore, it is difficult to re-treat a patient in whom a previous TAE procedure with metallic coils had failed as a result of recanalization.

This study aimed to evaluate the technical safety and effectiveness of TAE using Embosphere for enlarged polycystic liver.

**Methods:** Five PLD patients with severe symptom (1 male, 4 females) underwent TAE for hepatic artery branches using Embosphere100-300μm and 300–500μm. One patient had undergone TAE with metallic coils had failed as a result of recanalization. We evaluated change of hepatic volume and intra-hepatic cyst volume by MRI, symptoms by visual analog scale and FACT-Hep health-related QOL scores before TAE and at 3, 6, and 12 months after treatment.

**Results:** TAE was considered technically successful when the target hepatic arteries were fully embolized, as demonstrated by hepatic arterial angiography performed at completion of the procedure. Technical success was achieved in all cases. No major complication related to TAE was found. Common adverse events were fever, epigastric pain, nausea, and vomiting. Two patients improved symptoms significantly one month after TAE. We found hepatic cyst volume reduction. No patient complained of worsening of the symptoms after the procedure.

**Conclusions:** We suggest that TAE using Embosiphere is effective and safe in treating symptomatic polycystic liver in ADPKD patients, even who had treated by TAE using metallic coils.

## SA-PO286

**Characterization and Management of Patients with Renal or Hepatic Cyst Infection**
Marten A. Lantinga,[1] Tom JG Gevers,[1] Wim J.G. Oyen,[2] Rudolphe De Sevaux,[3] Joost P.H. Drenth.[1] *[1]Hepatology, Radboud Univ Nijmegen Medical Centre, Netherlands; [2]Nuclear Medicine, Radboud Univ Nijmegen Medical Centre, Netherlands; [3]Nephrology, Radboud Univ Nijmegen Medical Centre, Netherlands.*

**Background:** Cyst infection is the most critical complication in autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (PCLD). Guidelines for diagnosis and treatment are absent. The aim was to assess the clinical characteristics and therapeutic approach of patients with cyst infection.

**Methods:** Patients with cyst infection were ascertained and identified from clinical and radiological registries in a tertiary referral center from 2001-2013. Patients were included when there was a positive cyst aspirate, positive $^{18}$FDG-PET/CT and/or clinical findings suggestive for cyst infection. Clinical, radiological, biochemical and/or microbiological data were derived from medical records.

**Results:** We included 49 patients (86% ADPKD, 45% renal transplant, mean age 56 years, male 41%) with renal (57%, RCI) or hepatic cyst infection (43%, HCI). Some 20% had diabetes mellitus and 47% used immunosuppressive drugs. Urine cultures were positive in 13/24 (RCI) and 2/15 (HCI) of patients, whereas blood cultures were positive in 6/19 (RCI) and 11/16 (HCI) of patients. *E. coli* was the most frequent pathogen and was cultured from urine (53%) and blood (59%). The antibiotic therapy of choice for RCI and HCI were fluoroquinolones in 52% and 52%, followed by cephalosporin in 26% and 14% and penicillin in 14% and 24% of patients. Some 21% of RCI were treated by nephrectomy and 10% of HCI by percutaneous cyst drainage or liver transplantation.

**Conclusions:** Diabetes mellitus and immunosuppressive drugs are associated with renal and hepatic cyst infection. *E. coli* is the most frequent isolated pathogen and fluoroquinolones the most often initiated antibiotic therapy. Persistent or recurrent cyst infection frequently demands invasive treatment.

## SA-PO287

**Cyst Infection in Autosomal Dominant Polycystic Kidney Disease: New Diagnostic Criteria and Prognostic Factors**
Tatsuya Suwabe, Yoshifumi Ubara, Takashi Iijima, Koki Mise, Satoshi Hamanoue, Keiichi Sumida, Junichi Hoshino, Rikako Hiramatsu, Kenmei Takaichi. *Dept of Nephrology, Toranomon Hospital, Tokyo, Japan.*

**Background:** Cyst infection is a common and serious problem in patients with autosomal dominant polycystic kidney disease and sometimes becomes refractory. However, diagnosis of cyst infection and the prognostic factors remain unclear. We have established new diagnostic criteria for cyst infection in ADPKD patients (Clin Exp Nephrol 2012; 16). This study was performed to clarify the characteristics and prognostic factors of patients with cyst infection and to evaluate the usefulness of our new diagnostic criteria.

**Methods:** All patients admitted to Toranomon Hospital with a diagnosis of cyst infection from 2004 to 2012 were reviewed. The symptoms, the laboratory and imaging findings at the onset, and the outcome of all patients were investigated. We classified the patients according to our diagnostic criteria and analyzed their prognostic factors.

**Results:** A total of 214 patients with 492 episodes were enrolled, among which 62.2% of episodes satisfied our diagnostic criteria for cyst infection and 27.8% satisfied the criteria for probable cyst infection. The sensitivity was increased in the episodes with positive cyst culture test (definite cyst infection). A total of 148 patients (306 episodes) had hepatic cyst infection and 85 patients (143 episodes) had renal cyst infection. Sixty-six patients died and 26 of them died from cyst infection. The age, ascites, serum cholinesterase (ChE) and hepatomegaly (>8,000 cm$^3$) were significant risk factors for all-cause mortality (HR [95%CI]: age, 1.048 [1.007-1.092]; ascites, 2.175 [1.093-4.325]; ChE, 0.991 [0.983-0.999]; hepatomegaly 3.550 [1.292-9.751]). Serum ChE and hepatomegaly (>8,000 cm$^3$) were significant risk factors for death from cyst infection (ChE, 0.830 [0.722-0.955]; hepatomegaly, 6.238 [1.522-25.561]). The prognosis of hepatic cyst infection was worse than that of renal cyst infection.

**Conclusions:** Our criteria were useful for diagnosis of cyst infection and for evaluating its severity. Hepatomegaly, liver dysfunction, ascites, and hepatic cyst infection were significant determinants of a poor prognosis.

## SA-PO288

**Causative Bacteria of Cyst Infection in Patients with Autosomal Dominant Polycystic Kidney Disease**
Tatsuya Suwabe, Yoshifumi Ubara, Takashi Iijima, Koki Mise, Satoshi Hamanoue, Keiichi Sumida, Junichi Hoshino, Rikako Hiramatsu, Kenmei Takaichi. *Dept of Nephrology, Toranomon Hospital.*

**Background:** Cyst infection is a common and serious problem in patients with autosomal dominant polycystic kidney disease (ADPKD) and these infections sometimes become refractory. Lipophilic antibiotics such as fluoroquinolones, which show good penetration into cysts, are recommended for the treatment of cyst infection. However, we sometimes encounter cyst infections that are resistant to lipophilic antibiotics.

**Methods:** All ADPKD patients with a positive blood culture or cyst fluid culture who were admitted to Toranomon Hospital with a diagnosis of cyst infection from 2004 to 2013 were enrolled. We investigated the causative bacteria detected by cyst fluid culture or blood culture and their sensitivity to lipophilic antibiotics.

**Results:** A total of 95 patients with 127 episodes of infection were enrolled from among 229 patients (453 episodes) who were hospitalized with a diagnosis of cyst infection. 80 patients were on dialysis. There were 91 episodes of hepatic cyst infection and 36 episodes of renal cyst infection. For hepatic cyst infection, 63 bacteria in blood culture and 98 bacteria in cyst fluid culture were detected. For renal cyst infection, 30 bacteria in blood culture and 24 bacteria in cyst fluid culture were detected. Escherichia coli (E. coli) was the most frequent bacterial isolate, while Enterococcus (which is not usually treated with fluoroquinolones) was the 2$^{nd}$ mostfrequent organism. Enterococcus infection showed a higher frequency in cyst fluid culture. Fluoroquinolone resistance was seen with 72.7 % of E. coli and 31.9% of other gram-negative organisms. Bacteria with extended spectrum beta lactamase, which require carbapenem treatment, were detected in 6 cyst fluid culture (4.9%) and in 2 blood culture (2.2%).

**Conclusions:** 20-50% of all causative bacteria were micro-organisms that are not usually treated with lipophilic antibiotics, and frequency of fluoroquinolone resistance was high in E. coli. This study suggested that lipophilic antibiotics are not always appropriate for treating cyst infection in ADPKD patients, although these are first-line agents.

**SA-PO289**

**The Compiling Data at the Time of Enrollment in J-PKD Registry**
Satoru Muto,[1] Toshio Mochizuki,[2] Ken Tsuchiya,[2] Saori Nishio,[3] Kazushige Hanaoka,[4] Kouichi Kamura,[5] Kazuhiko Tsuruya,[6] Eiji Ishimura,[7] Ichiei Narita,[8] Kouichi Kamura,[9] Yoshifumi Ubara,[10] Masahiko Ando,[11] Kikuo Nutahara,[12] Shigeo Horie.[13] *[1]Urology, Teikyo Univ, Tokyo, Japan; [2]Nephrology, Tokyo Woman's Medical Univ, Tokyo, Japan; [3]The 2nd Dept of Internal Medicine, Hokkaido Univ, Sapporo, Japan; [4]Nephrology, Jikei Univ, Tokyo, Japan; [5]Urology, Chiba East Hospital, Chiba, Japan; [6]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [7]Nephrology, Osaka City Univ, Osaka, Japan; [8]The 2nd Dept of Internal Medicine, Niigata Univ, Niigata, Japan; [9]Urology, Chiba East Hospital, Chiba, Japan; [10]Nephrology, Toranomon Hospital, Tokyo, Japan; [11]Center for Advanced Medicine and Clinical Research, Nagoya Univ, Nagoya, Japan; [12]Urology, Kyorin Univ, Mitaka, Japan; [13]Urology, Juntendo Univ, Tokyo, Japan.*

**Background:** The PKD Sectional Committee of a Grant-in-Aid for Progressive Renal Diseases Research, from the Ministry of Health, Labour and Welfare of Japan established the first nationwide, web-based, and prospective registry system, the Japan PKD Registry (J-PKD), to record clinical, and laboratory data in Japan. Although the follow-up periods of this study were 5 years, we will report the compiling data at the time of enrollment in J-PKD registry.

**Methods:** Patient data including age, gender, family history, complication, medical history, and laboratory data were electronically recorded at each institution and registered via the Internet Data and Information Center for Medical Research system, which is part of the University Hospital Medical Information Network.

**Results:** We included 271 ADPKD patients in this study (176 female and 95 male). Median age was 52 years. The mean estimated GFR was 49.3ml/min/1.73m$^2$. Several parameters including waist circumference ($r^2$=0.06, p=0.001), systolic blood pressure ($r^2$=0.05, p<0.001), total kidney volume ($r^2$=0.25, p<0.001) were significantly inversely correlated with eGFR. There were significant correlations between eGFR and Hemoglobin level ($r^2$=0.26, p<0.001), serum alubumin ($r^2$=0.08,p<0.001).

**Conclusions:** In this cohort study, we will clear the actual treatment course of PKD in Japan.

**SA-PO290**

**A Comprehensive Mutation Search within the *PKD1/2* for Japanese Subjects with Autosomal Dominant Polycystic Kidney Disease**
Mahiro Kurashige,[1,2] Kazushige Hanaoka,[1] Minako Imamura,[2] Yoshindo Kawaguchi,[1,3] Toshio Hasegawa,[1,3] Tatsuo Hosoya,[1] Shiro Maeda,[2] Takashi Yokoo.[1] *[1]Div of Kidney and Hypertension, Dept of Internal Medicine, The Jikei Univ, School of Medicine, Minato, Tokyo, Japan; [2]Laboratory for Endocrinology, Metabolism and Kidney Diseases, RIKEN Center for Integrated Medical Science, Yokohama, Kanagawa, Japan; [3]Dept of Medicine, Kanagawa Prefectural Shiomidai Hospital, Yokohama, Kanagawa, Japan.*

**Background:** Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common hereditary kidney disease, and most of its heritability could be explained by mutations in two genes, *PKD1* and *PKD2* in populations of European origin. However little is known about Japanese ADPKD. To elucidate genotypic and phenotypic characteristics of the Japanese ADPKD, we performed a comprehensive mutation search within *PKD1/2* by using 180 Japanese ADPKD patients from 161 unrelated families.

**Methods:** We screened the entire coding regions and their flanking regions of the *PKD1/2* by direct sequencing. We also examined large genomic rearrangements within *PKD2* by using quantitative PCR. We further investigated phenotype-genotype correlation using linear regression analyses.

**Results:** We identified 102 mutations within 128 families, through direct sequencing, including 81 *PKD1* mutations (43 truncated, 10 splicing, 23 missense, 5 in-frame) and 21 *PKD2*mutations (15 truncated, 3 splicing, 3 missense). We also found a novel 3.1-kb deletion within *PKD2* (intron13 to exon15). Decline of eGFR was significantly faster in patients with *PKD1* mutation than in patients with *PKD2* mutation (-3.11 and -2.01 ml·min$^{-1}$·year$^{-1}$ for *PKD1* and *PKD2*, respectively, p<0.01). Mutation type or nucleotide position was not associated with eGFR decline. Lower urinary osmolality was found to correlate with eGFR decline in a multiple linear regression analysis.

**Conclusions:** Mutations within *PKD1/2* contribute to the pathogenesis of most Japanese ADPKD. Our present finding suggests that *PKD1* mutations and lower urinary osmolality are risk factors for declining renal function in Japanese patients with ADPKD.

*Funding:* Government Support - Non-U.S.

**SA-PO291**

**In Vitro and Family Analysis to Assess the Significance of Indeterminate PKD1 Variants**
Binu M. Paul, Jamie L. Sundsbak, Christina M. Heyer, Katharina Hopp, Maria V. Irazabal, Marie C. Hogan, Vickie J. Kubly, Vicente E. Torres, Peter C. Harris. *Medicine, Mayo Clinic, Rochester, MN.*

**Background:** ADPKD molecular diagnostics is complex because of the high level of allelic heterogeneity at *PKD1 and PKD2*, and hence determining the pathogenic significance of some missense changes *in silico* is problematic. Quantitative *in vitro* assays of these alleles could help to determine their significance.

**Methods:** Segregation and detailed clinical analysis in conjunction with *in vitro* analysis of PC1 cleavage at the GPS domain was performed to test the significance of a group of unresolved variants.

**Results:** The proband in pedigree M599 presented with a few kidney cysts and multiple liver cysts at 45 years. Mutation screening revealed a novel variant, *PKD1*: p.S2612W, that was scored *in silico* as likely pathogenic. The *in vitro* assay indicated that p.S2612W significantly blocked cleavage proving a pathogenic role. The proband in M517 developed ESRD at 25 years and had the novel *PKD1* variant p.G2452C *in trans* with *PKD1*: c.9246_9252del7. A cousin with just p.G2452C had a few cysts in both kidneys. Although G2452C does not show strong evolutionary conservation, *in vitro* analysis showed a moderate impairment of GPS cleavage and so a likely modifying role. The proband in M190 presented with Potter's phenotype, PKD and perinatal death. The proband had the *PKD1*: p.W3298X mutation inherited from the mother and maternal grandmother with typical ADPKD and the novel *PKD1* variant, *PKD1*:p.R2213W (at a highly conserved site) from his unaffected father. *In vitro* analysis showed that this is a hypomorphic cleavage variant and *in trans* with p.W3298X results in just ~20% functional PC1. Collection and categorization of variants in the ADPKD Mutation Database can help with future diagnostics. However, in some cases limited clinical data is available to determine their significance. *PKD1*: p.R2516H is an example of such a variant that is at a highly conserved site but is a very conservative change. *In vitro* analysis showed that p.R2516H affects GPS cleavage and so is likely a pathogenic mutation.

**Conclusions:** Overall, our results indicate the value of family studies and *in vitro* assays to functionally test indeterminate variants.

*Funding:* NIDDK Support

**SA-PO292**

**Numbers and Activation State of Parenchymal Kidney Cells Change with Age and Disease State in db/db Mice**
Lisbeth N. Fink, Helena Cucak, Christopher Mayer, Carsten F. Gotfredsen, Maiken H. Pedersen, Bidda Rolin, Alexander Rosendahl. *Diabetes Research Unit, Novo Nordisk A/S, Maaloev, Denmark.*

**Background:** The db/db mouse model of diabetic nephropathy shows histological lesions reminiscent of early human diabetic nephropathy. We hypothesized that alterations in parenchymal cell numbers and activation state could serve as biomarkers for pre-late stage disease. Herein, we present a novel method for enumeration of kidney parenchymal cells and for cell-specific protein expression analysis during disease development in db/db mice.

**Methods:** db/db and db/+ mice were sacrificed at 8-28 weeks of age. Kidneys were enzymatically digested and the resulting cell suspensions were subjected to multiple parameter flow cytometric analysis. Staining of specific cell types was confirmed by immunohistochemistry. Kidney function was assessed by measuring plasma urea nitrogen.

**Results:** Proximal (PTEC) and distal (DTEC) tubular epithelial cells expanded dramatically with age (300% and 50% respectively from 8 to 28 weeks) in db/db mice, but not in db/+ mice. db/db DTECs progressively expressed higher levels of TLR4 and lower levels of PDGFR1 compared to db/+. In addition, PTECs acquired high TGFb1 expression at 28 weeks in db/db only. Arteriolar smooth muscle cells and endothelial cells also increased with age and db/db endothelial cells had elevated ICAM-1 compared to db/+. Although the frequency of podocytes was reduced, absolute podocyte numbers were not changed with advancing disease. Isolated db/db glomeruli became progressively more susceptible to in vitro cytokine-induced apoptosis. Histological analysis showed that db/db kidneys developed moderate mesangial expansion, but not glomerulosclerosis or interstitial fibrosis.

**Conclusions:** In db/db mice, progression of disease with increased age caused an increased expression of inflammatory markers and expansion of tubular epithelial cells, endothelial cells and arteriolar smooth muscle cells, and decreased podocyte frequency, but not numbers. The increase of TGFb in PTECs suggests a potential pre-stage 3 phenotype development in db/db mice even though neither sclerosis nor fibrosis was evident. These changes coincided with a decrease in plasma urea nitrogen.

*Funding:* Pharmaceutical Company Support - Novo Nordisk A/S

**SA-PO293**

**Effect of Enalapril on Albuminuria and Cellular Kidney Parameters in db/db Mice**
Lisbeth N. Fink, Helena Cucak, Maiken H. Pedersen, Carsten F. Gotfredsen, Alexander Rosendahl. *Diabetes Research Unit, Novo Nordisk A/S, Maaloev, Denmark.*

**Background:** Blockade of the renin-angiotensin system is the first-line therapy for microalbuminuric diabetes patients, whereas ACE inhibition has shown variable results in db/db mice. Herein, we characterized the kidney cellular dynamics in order to elucidate the albuminuria-reducing mechanism of action of Enalapril in db/db mice.

**Methods:** db/db mice were fed Enalapril for 12 weeks (5mg/kg/day) mixed with the chow. Albuminuria was measured before commencement of treatment and after 6 and 12 weeks of treatment. Kidneys were enzymatically digested and the kidney cell suspensions were analysed by flow cytometry for parenchymal and leukocyte cell parameters.

**Results:** Albuminuria increased in vehicle treated db/db mice during the first 6 weeks and was reduced to starting values after 12 weeks. Enalapril significantly reduced albuminuria after 6 weeks of treatment (P<0.001), whereas no significant reduction remained after 12 weeks of treatment compared to vehicle treated animals. Enalapril had no effect on mesangial expansion. After 12 weeks of treatment, Enalapril caused an expansion of podocytes, endothelial cells, arteriolar smooth muscle cells and distal tubular epithelial cells and consequently almost doubled cell numbers compared to vehicle control. Furthermore, Enalapril increased ICAM-1 protein expression on endothelial cells and restored epithelial

PDGFR1 expression, which was lost in db/db mice. Numbers of leukocytes (T cells and macrophages, including CD11c$^{+}$ macrophages) in Enalapril treated db/db mice were restored to comparable levels as in db/+ mice.

**Conclusions:** Enalapril treatment showed a temporary beneficial effect on albuminuria during the progression of diabetic nephropathy in db/db mice. However, despite lack of effect on albuminuria at 12 weeks of treatment, Enalapril increased numbers of distal tubular epithelial cells and other parenchymal cells and seemed to promote vascular inflammation, but also restored PDGFR1 and macrophage numbers to non-diabetic levels. The trophic effect and restoration of M1 macrophage numbers suggests beneficial effects even in absence of reduction of albuminuria.

*Funding:* Pharmaceutical Company Support - Novo Nordisk A/S

## SA-PO294

**Urinary Aminopeptidase A but Not ACE2 Is Upregulated by RAS Blockade in *db/db* Mice** Jan A. Wysocki, Philipp K. Haber, Christoph Maier, Minghao Ye, Daniel Batlle. *Div of Nephrology & Hypertension, Northwestern Univ, Chicago, IL.*

**Background:** Aminopeptidase A (APA) and ACE2 are Ang II-degrading enzymes abundantly expressed in the kidney. Abundance of these peptidases in the urine could indicate the capacity of the diabetic kidney to counteract the Ang II-overactivity characteristic of diabetic nephropathy.

**Methods:** Urinary APA and ACE2 activities were measured fluorometrically in the urines from db/db mice under physiological conditions and during pharmacological interventions with RAS blockers. Measurements from 4 consecutive urine collections 1-3 weeks apart were averaged for each experimental condition and expressed as enzyme activity/creatinine ratio.

**Results:** In the urines from db/db mice under baseline condition, APA (188±18 vs. 107±8, RFU/ug creat/hr, $p<0.001$) and ACE2 activity (46±12 vs. 10±2 RFU/ug creat/hr, $p<0.05$) were both increased as compared to db/m controls. When administered for several weeks, an ACE inhibitor, captopril (60 mg/kg/d) and a specific AT1 receptor blocker, telmisartan (2mg/kg/d), increased significantly the levels of urinary APA activity in db/db mice as compared to baseline (650±114 and 378±72 vs. 188±18, $p<0.001$ and $p<0.05$, respectively) whereas they had no effect on urinary ACE2 activity (39±6 and 51±7 vs. 46±12, respectively). Both RAS blockers reduced albumin creatinine ratio significantly ($p<0.05$).

**Conclusions:** Urinary APA and ACE2 activities are increased in db/db mice. Whereas RAS blockade increases urinary APA activity, ACE2 activity in the urine is unaffected by either captopril or telmisartan. Altogether these findings suggest that urinary ACE2 and APA appear to be differentially regulated by RAS blockers and, moreover, raise the possibility that the therapeutic renal action of RAS blockers involves up-regulation of kidney aminopeptidase A and thus enhanced Ang II degradation.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO295

**In Vivo Quantification of Nephrin Endocytosis Mediated by MAPK p38 in Diabetic Animals** Magdalena Woznowski, Sebastian Alexander Potthoff, Eva Koenigshausen, Johannes Stegbauer, Lars C. Rump, Lorenz Sellin, Ivo Quack. *Dept for Nephrology, Heinrich Heine Univ, Duesseldorf, Germany.*

**Background:** Microalbuminuria is an early sign of diabetic podocyte damage. We could recently demonstrate that even new-onset hyperglycemia causes nephrin endocytosis and albuminuria. So far it has been difficult to quantify the loss of surface nephrin in diseased animals. Therefore we developed a biotinylation assay which allows monitoring the amount of nephrin present at the slit diaphragm. Here we use this tool to dissect the role of p38 as mediator of hyperglycemia-induced nephrin endocytosis.

**Methods:** C57BL/6 mice were administered streptozotocin i.p. to induce hyperglycemia. For inhibition of p38, the mice were subsequently treated with SB202190 i.p. Albuminuria was quantified as albumin/creatinin ratio. To quantify the surface expression of nephrin the kidneys were perfused with biotin. Afterwards glomeruli were instantly harvested and analyzed with streptavidin via Western blot.

**Results:** Diabetic mice developed a significant albuminuria already four days after streptozotocin application. Inhibition of p38 nearly completely prevented albuminuria. For the first time we could show that new-onset hyperglycemia leads to a loss of surface expression of nephrin in glomeruli simultaneously to the occurrence of albuminuria. This cascade could be prevented by treatment with SB202190. Further we found evidence that endocytosis of nephrin is mediated by its phosphorylation by p38, facilitating binding of PKC$\alpha$ to nephrin.

**Conclusions:** Acute hyperglycemia rapidly induces albuminuria, but the underlying mechanisms have not been fully understood. Here we show for the first time in vivo that the occurrence of albuminuria in hyperglycemic animals is paralleled by nephrin endocytosis. Inhibition of p38 decreases nephrin phosphorylation and endocytosis, supporting the hypothesis that stabilization of the slit diaphragm prevents albuminuria. These findings suggest p38 as pivotal mediator of early diabetic damage and thus as a promising therapeutic target.

## SA-PO296

**Vitamin D Improves Podocyte Hyperpermeability Induced by High Glucose and Advanced Glycosylation Endproducts** Tae-Sun Ha,[1] JAE IL SHIN,[2] Se Jin Park.[3] *[1]Dept of Pediatrics, Chungbuk National Univ College of Medicine; [2]Dept of Pediatrics, Yonsei Univ College of Medicine; [3]Dept of Pediatrics, Ajou Univ School of Medicine.*

**Background:** The aim of this study was to investigate the effect of vitamin D on the pathologic changes of podocyte beta-catenin and P-cadherin and podocyte permeability induced by diabetic conditions.

**Methods:** We cultured mouse podocytes under: 1) normal glucose (5 mM, = control); 2) high glucose (HG, 30 mM); 3) AGE-added; or 4) HG plus AGE-added conditions and treated with vitamin D. The distribution of podocyte beta-catenin and P-cadherin was shown by confocal microscopy, and protein levels of them by western blotting. Podocytes were incubated with vitamin D at the concentrations of 10 and 50 nM for 6, 24 and 48 hours.

**Results:** HG and AGE increased the dextran filtration of monolayered podocytes at 18 hrs in permeability assay, which was improved by vitamin D. In confocal imaging, the distribution of beta-catenin and P-cadherin were internally concentrated by diabetic conditions, which was improved by vitamin D. In Western blotting, HG and AGE decreased beta-catenin protein levels at 6, 24 and 48 hours ($P<0.01$). Vitamin D improved the decreased beta-catenin protein levels at 6, 24 and 48 hours.

**Conclusions:** These findings imply that HG and AGE have an influence on the redistribution of beta-catenin and P-cadherin and amount of beta-catenin protein of podocytes thereby causing hyperpermeability, which can be reversed by vitamin D.

*Funding:* Private Foundation Support

## SA-PO297

**Therapeutic Potential of Suramin in Diabetic Nephropathy** Leah J. Siskind,[1,3] Midhun C. Korrapati,[1] Ryan Whitaker,[1] Judit Megyesi,[2] Rick G. Schnellmann.[1,3] *[1]Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC; [2]Dept of Internal Medicine, Univ of Arkansas for Medical Sciences, Little Rock, AR; [3]Ralph H. Johnson, Veterans Administration Medical Center, Charleston, SC.*

**Background:** Delayed administration of a single dose of suramin, a FDA-approved drug, decreased renal inflammation and fibrosis in an early model of type 1 diabetes-induced renal injury. These studies tested the hypothesis that delayed administration of a single dose of suramin decreases early and late stage diabetic nephropathy in a leptin receptor deficient C57BLKS/J *db/db* type 2 diabetic nephropathy (T2DN) mouse model.

**Methods:** Non-diabetic *db/m* and diabetic *db/db* mice of 8 and 17 weeks of age were treated with 10 mg suramin/kg, i.v. or saline and euthanized one week later. Measurements in 9 and 18 week mice before and following treatment included: body weight; blood glucose; urinary protein excretion; pathological lesions in glomeruli and proximal tubules; changes in protein of the pro-inflammatory transcription factor nuclear factor κB (NF-κB), endothelial intracellular adhesion molecule-1 (ICAM-1), profibrotic transforming growth factor-β1 (TGF-β1), phospho-SMAD-3 and alpha-smooth muscle actin (α-SMA); and IHC analysis of leukocyte infiltration and collagen 1A2 (COL1A2) deposition.

**Results:** Immunoblot analysis revealed increased NF-κB, ICAM-1, TGF-β1, phospho-SMAD-3, and α-SMA proteins in 9 and 18 week *db/db* mice as compared to *db/m* mice. IHC analysis revealed moderate leukocyte infiltration and COL1A2 deposition in 9 week *db/db* mice that increased in 18 week *db/db* mice. Importantly, suramin significantly decreased expression of all markers in 9 week *db/db* mice and partially decreased them in 18 week *db/db* mice. There was no difference in creatinine clearance between 9 week *db/m* and *db/db* mice ± suramin. Importantly, in the 18 week *db/db* mice suramin reversed impaired creatinine clearance and overt histological damage.

**Conclusions:** Delayed administration of a single dose of suramin in a model of T2DN attenuated inflammation and fibrosis as well as improved renal function, supporting the use of suramin to treat T2DN.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO298

**The Role of Glycosphingolipids in Diabetic Nephropathy** Leah J. Siskind,[1,2,3] Subathra Marimuthu,[1] Midhun C. Korrapati,[1] Lauren P. Wills,[1] Jennifer L. Blakely,[1] Rick G. Schnellmann.[1,2] *[1]Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC; [2]Ralph H. Johnson, Veterans Administration Medical Center, Charleston, SC.*

**Background:** Glycosphingolipids, a subtype of sphingolipids, are found in abundance in kidney diseases and an inhibitor of their synthesis prevented the progression of polycystic kidney disease in a rodent model. A role for glycosphingolipid species in diabetic nephropathy (DN) has not been determined. This study tested the hypothesis that glycosphingolipids accumulate in the kidney in response to hyperglycemia in type II diabetes and mediate DN.

**Methods:** Non-diabetic *db/m* and diabetic *db/db* mice were obtained from Jackson Laboratories. Glycosphingolipids were measured by HPLC-MS/MS in homogenates of kidney cortices and mesangial cells and proximal tubules cells purified from kidney cortices. Primary cultures of mesangial and proximal tubule cells (RPTC) were cultured under normal and hyperglycemic conditions (17 mM) and glycosphingolipids quantified. In addition, primary mesangial and proximal tubule cells were treated with exogenous glycosphingolipids and inflammatory cytokine expression and mitochondrial function, respectively, were determined.

**Results:** The glycosphingolipids glucosylceramide (GlcCer) and lactosylceramide (LacCer) were elevated in the urine of type II DN patients and the *db/db* mice model of type II DN. GlcCers and LacCers were also increased in kidney cortices of db/db mice, specifically within mesangial and RPTC. Hyperglycemia elevated GlcCer and LacCer in primary cultures of mesangial and RPTC and induced inflammatory cytokine production in mesangial cells and mitochondrial dysfunction and cell death in RPTC. Exogenous GlcCer and LacCer addition was sufficient to induce inflammatory cytokines in mesangial cells and mitochondrial dysfunction and cell death in RPTC.

**Conclusions:** Data support the hypothesis that hyperglycemia in type II diabetes leads to renal dysfunction at least in part by inducing GlcCer and LacCer accumulation in mesangial and RPTC, resulting in activation of inflammatory signaling pathways and mitochondrial dysfunction/cell death, respectively, and DN.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO299

**Cathepsin S Promotes Endothelial Dysfunction, Proteinuria, Podocyte Loss, and Glomerulosclerosis in Type 2 Diabetes** Murthy Narayana Darisipudi,[1] Onkar Kulkarni,[1] Shrikant R. Mulay,[1] Hartmann Guido,[2] Hans J. Anders.[1] *[1]Nephrologisches Zentrum, Klinische Biochemie, Medizinische Klinik und Poliklinik IV der LMU, Munich, Germany; [2]CV & Metabolism DTA, Hoffmann La Roche, Basel, Switzerland.*

**Background:** Cathepsin S is an elastolytic cysteine protease, known to drive atherogenesis and vascular wall degeneration in aging as well as in the accelerated atherogenesis of chronic kidney disease. To date a pathogenic role of cathepsin S in microvascular complications of type 2 diabetes is entirely speculative.

**Methods:** Male db/db uninephrectomized mice were fed either with food-drug mix contained cathepsin S inhibitor, RO5461111 and standard food from 16 weeks of age till 24 weeks. Kidneys were harvested for histopathological evaluation and spleens were used to estimate the Lip10 level. Blood and urine samples were obtained at monthly intervals.

**Results:** We found increased plasma cathepsin S activity and increased cathepsin S protein expression in kidney especially upon accelerating the onset of disease by early uninephrectomy. Immunostaining localized cathepsin S protein to tubular epithelial cells, endothelial cells and infiltrating leukocytes in advanced human diabetic nephropathy, while in db/db mice with less advanced disease the tubular signal was most obvious. However, tubular cathepsin S mRNA expression was excluded by in situ hybridization. Treatment with RO5461111 significantly reduced albuminuria, podocyte loss, and glomerulosclerosis in association with lower macrophage infiltrates as well as pro-inflammatory cytokines, adhesion molecules mRNA expression and restored eNOS expression. In vitro studies with glomerular endothelial cells documented a toxic effect of cathepsin S in terms of viability, detachment, and permeability. In vivo microscopy studies revealed that cathepsin S inhibition improved oxidative stress-induced microvascular permeability.

**Conclusions:** Cathepsin S is a circulating mediator of endothelial dysfunction driving albuminuria and progressive kidney disease in type 2 diabetes, a process that can be prevented by cathepsin S inhibition, e.g. with RO5461111.

## SA-PO300

**Complement C3a, C5a Regulate Endothelial-Myofibroblast Transition via Wnt/β-Catenin Signaling Pathway in Diabetic Nephropathy** Lijia Chen,[1] Ling Li,[1] Jing Zang,[1] Jie Zhang,[2] Qinghua Yin,[1] Lu Cheng,[1] Yanrong Lu,[2] Jingqiu Cheng,[2] Ping Fu,[1] Fang Liu.[1] *[1]Div of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China; [2]Key Laboratory of Transplant Engineering and Immunology, Ministry of Health, Regenerative Medicine Research Center, Chengdu, Sichuan, China.*

**Background:** Endothelial-myofibroblast transition (EndMT) is considered to be involved in the development and progression of renal fibrosis in diabetic nephropathy (DN). We aimed to investigate whether the C3a and C5a as the novel profibrotic factors could induce EndMT via Wnt/β-catenin signaling pathway in DN.

**Methods:** Male SD rats were randomized into four groups: normal control, DN, DN+C3aRA (C3a receptor antagonist) and DN+C5aRA (C5a receptor antagonist), which were sacrificed after eight weeks. In vitro Cultured human renal glomerular endothelial cells (HRGECs) were divided into ten groups: normal glucose, mannitol, TGFβ1, high glucose, HG+C3a, HG+C3a+C3aRA, HG+C3a+DKK1, HG+C5a, HG+C5a+C5aRA and HG+C5a+DKK1. DKK1 was used as Wnt/β-catenin pathway inhibitor. Double-labeled fluorescent staining was performed to detect the co-expression of CD31 with α-SMA or β-catenin. The expression of α-SMA, CD31, C3a, C5a, C3aR, C5aR, β-catenin, TGFβ and col-1 were detected by real time-PCR, western blot and immunohistochemistry.

**Results:** C3a, C5a, C3aR and C5aR were found up-regulated in glomeruli in DN group. Strong staining of α-SMA and β-catenin with decreasing staining of CD31 were observed in glomerular endothelial cells in DN group, which were retrieved by C3aRA or C5aRA treatment. And up-regulation of α-SMA, β-catenin, TGFβ1, col-1 and down-regulation of CD31 were also detected by real time -PCR, western blot and double-labeled fluorescent stainingin HRGECs stimulated by high glucose, C3a and C5a, which were retrieved by C3aRA or C5aRA and DKK1.

**Conclusions:** Complement C3a and C5a-induced EndMT is a novel mechanism in DN. Blockage of Wnt/β-catenin signaling pathway may alleviate EndMT and fibrosis in DN.

## SA-PO301

**Renoprotective Effects of Luseogliflozin (TS-071), a Novel SGLT2 Inhibitor, in Diabetic *db/db* Mice** Yumi Takiyama, Manami Kobayashi, Jun Honjo, Yukihiro Fujita, Tsuyoshi Yanagimachi, Hiroya Kitsunai, Yuichi Makino, Masakazu Haneda. *Div of Metabolism and Biosystemic Science, Dept of Medicine, Asahikawa Medical Univ, Asahikawa, Hokkaido, Japan.*

**Background:** Sodium glucose cotransporter 2 (SGLT2) represents a novel target for normalizing glycemia. To address *in vivo* the issue of the efficacy and tolerance of a novel SGLT2 inhibitor Luseogliflozin(LUSEO), we investigated whether LUSEO can prevent renal insufficiency and glomerulosclerosis in diabetic mice, and determined potential mechanisms of renoprotecitve effects.

**Methods:** *db/db* mice were treated with 15 mg/kg LUSEO for 8 weeks. The regulation of SGLT2 expression was evaluated using human renal proximal tubular epithelial cells (HRPTEC) by RT-qPCR.

**Results:** LUSEO significantly improved plasma glucose levels in *db/db* mice. Then, consistent with lowerd filtrated glucose load, urinary glucose excretion in *db/db* treated with LUSEO was decreased. Moreover, LUSEO showed renoprotective effects, which significantly attenuated urinary albumin excretion rates, glomerular mesangial matrix expansion, glomerular and interstitial fibronectin accumulation, and Armanni-Ebstein lesions in *db/db* mice. Of interest, LUSEO augmented pimonidazole staining of the S3 segment of the proximal tubules cells which expressed SGLT1, in the outer stripe of the kidney of *db/db* mice, suggesting hypoxia caused by the overload of glucose and sodium via blocking SGLT2 in the S1 segment. HRPTEC constitutively expresses SGLT2 mRNA. High glucose did not affect the expression of SGLT2 mRNA. Whereas, TGF-β1 markedly decreased SGLT2 mRNA expression. Intriguingly, Hepatocyte Nuclear Factor (HNF)-1α, a modulator of SGLT2 expression, was drastically decreased by TGF-β1 treatment, thereby providing a mechanism by which TGF-β1 represses SGLT2 mRNA expression through decreased HNF-1α. In addition, LUSEO alone failed to affect the expressions of SGLT2 and the inflammatory molecules, however, significantly inhibited TGF-β1-induced fibronectin expression.

**Conclusions:** These data demonstrate that LUSEO can protect against the progression of diabetic nephropathy, which is probably sufficient to overcome the adverse effects of TGF-β1 or compensatory hypoxia.

*Funding:* Pharmaceutical Company Support - Taisho Pharmaceutical Co., Ltd.

## SA-PO302

**Defective Autophagy Sensitizes Podocytes to Secondary Attack by Amplifying Inflammasome Activation in Diabetic Nephropathy** Li Fang, Weichun He, Chunsun Dai, Junwei Yang. *2nd Affiliated Hospital, Nanjing Medical Univ.*

**Background:** The mechanisms for the development of diabetic nephropathy (DN), the leading cause of end-stage renal disease (ESRD) in many industrialized countries, are not fully understood. Our previous data demonstrated that defective podocyte autophagy was associated with the progression of DN, here the underlying mechanism for defective podocyte autophagy leading to the progression of DN was investigated.

**Methods:** Both diabetic patients and animal experiment were included in this study. For the animal experiment, the CD-1 mice were divided into four groups: normal control, hyperuricemia group (250mg/kg/d uric acid intraperitoneal injection for 14 days), diabetic group (3 months after onset) and diabetic plus hyperuricemia group.

**Results:** In the diabetic patients, immunohistochemical staining for the kidney sections showed that thedefective autophagy in podocytes was always associated with the inflammasome activation in the glomeruli. In the animal experiment, more albuminuria, podocyte injury as well as inflammasome activation were detected in the diabetic plus hyperuricemia group compared to those in the other groups. In conditionally immortalized mouse podocyte cell line (MPC), 3-methylamphetamine (3-MA), an inhibitor of autophagy, could aggravate uric acid induced inflammasome activation in a dose- and time-dependent manner. Conversely, rapamycin, an inducer of autophagy, could alleviate uric acid induced inflammasome activation in a dose- and time-dependent manner.

**Conclusions:** These findings suggest that the defective autophagy sensitizes the podocytes to the secondary attack by amplifying inflammasome activation in DN. Thus, enhancement of autophagy will provide a novel avenue for preventing inflammasome activation and ameliorating podocyte injury in DN.

*Funding:* Government Support - Non-U.S.

## SA-PO303

**Hypovolemia, Not Hyperoxaluria, Causes Nephropathy following Gastric Bypass in Obese Rats** Benjamin Canales,[1] Marguerite Hatch,[2] Saeed R. Khan.[2] *[1]Urology, Univ of Florida, Gainesville, FL; [2]Pathology, Immunology and Laboratory Medicine, Univ of Florida, Gainesville, FL.*

**Background:** In the morbidly obese, Roux-en-Y gastric bypass (RYGB) can decrease renal hyperfiltration, improve glycemic control, and lower blood pressure. Its overall effect on renal function remains debatable, as RYGB can be also associated with recurrent oxalate lithiasis and post-procedural hypovolemia. We hypothesized that a diet-induced obese (DIO) model of RYGB with appropriate controls could address this question, providing end of study renal histology while controlling dietary oxalate content.

**Methods:** Male DIO Sprague-Dawley rats, aged 23 weeks, were randomly assigned to RYGB (n=74) or sham control (n=40). Once recovered, animals were placed ad lib normal calcium, high fat (40%) diet with 1.5% potassium oxalate (Ox) or without (No Ox). Weekly weights, food and water intake, complications, and clinical interventions were recorded.

Urine and serum were collected at baseline and every 5 weeks until euthanasia. At study endpoint, renal histology was assessed using a quantitative glomerular and tubular scoring system (0-4 scale) by two experienced veterinary pathologists, and animals were grouped into normal (NH) or abnormal (AH) histology. Animals that died prior to reaching endpoint were excluded from final analysis.

**Results:** Survival to endpoint was 63% (47/74) in RYGB and 90% (36/40) in controls. Over 36% (17/47) of RYGB animals had AH compared to 6% (2/36) of controls. Factors associated with developing RYGB AH included: age 20 weeks, 4 or more fluid boluses for hydration after surgical recovery, serum creatinine >0.6 mg/dL, clinical anemia, total body weight loss >28%, urine volume >30 ml/24 hours, and serum bicarbonate <25 mmol/L. There were significant increases in urinary oxalate in both RYGB No Ox and Ox groups as well as crystal deposits. However, neither dietary oxalate nor renal crystal deposition correlated to AH.

**Conclusions:** Results indicate that chronic hypovolemia in this RYGB model leads to AH, irrespective of dietary oxalate. The cause of volume loss appears to be of renal and not gastrointestinal origin. Studies to better define this polyuria mechanism are underway.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Ethicon Endo-Surgery, Private Foundation Support

## SA-PO304

**Effect of Angiotensin Receptor Blockade on Sodium-Glucose Co-Transporters and Renal Gluconeogenesis in Diabetic Rats** Akihiro Tojo, Satoshi Kinugasa. *Div of Nephrology & Endocrinology, The Univ of Tokyo, Tokyo, Japan.*

**Background:** The kidney has a crucial role in glucose reabsorption via sodium-glucose co-transporters (SGLTs), which amounts to approximately 144 g/day. Although the main organ of gluconeogenesis is the liver, about 10% of gluconeogenesis is undertaken by the kidney under normal conditions, and 40% in the fasting condition. It has been reported that angiotensin receptor blockade inhibits the onset of diabetes, although its mechanism is not clarified yet. We investigated the effect of angiotensin receptor blockers (ARBs) on the SGLT and renal gluconeogenesis in diabetic rats.

**Methods:** We prepared 3 groups of rats (each n=5); sham-injected controls, untreated STZ-induced diabetic rats at 4 weeks, and STZ-induced diabetic rats that were administered the ARB, telmisartan for 2 weeks. One kidney was used for immunohistochemistry and pre-embedding immune electron microscopy, and the other kidney was homogenized for western blot analysis.

**Results:** SGLT2 was expressed exclusively in the brush border membrane of proximal convoluted tubules in normal rats, and its expression was increased and also appeared in apical vesicles in the diabetic rat. Western blotting revealed that both SGLT2 and SGLT1 were increased in the membrane fraction of kidney homogenates in diabetic rats, and they were suppressed in rats treated with telmisartan. The restriction enzymes of gluconeogenesis, glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), were increased in the cytoplasm of proximal and distal convoluted tubules in diabetic rats. Both G6Pase and PEPCK were suppressed in kidneys of rats treated with telmisartan. Cytoplasmic glucose levels were significantly increased in the diabetic rats compared with controls (80.4±4.9 vs. 16.6±0.7 mg/mg protein, $p<0.005$), which were significantly reduced in rats treated with telmisartan (35. 7±5.5 mg/mg protein, $p<0.0005$).

**Conclusions:** The diabetic kidney showed increased cytoplasmic glucose levels mediated by enhanced SGLT expression in the proximal tubules and increased gluconeogenesis enzymes, which were suppressed by ARB treatment.

*Funding:* Government Support - Non-U.S.

## SA-PO305

**TNFα Causes Podocyte Cholesterol Accumulation and Apoptosis in Diabetic Kidney Disease** Christopher E. Pedigo,[1] Armando Mendez,[4] Matthias Kretzler,[2] Robert G. Nelson,[3] George William Burke,[4,5] Alessia Fornoni,[1,4] Sandra M. Merscher-Gomez.[1] *[1]Div of Nephrology and Hypertension, Univ of Miami, Miller School of Medicine, Miami, FL; [2]Univ of Michigan - Ann Arbor, MI; [3]NIDDK, Phoenix, AZ; [4]Diabetes Research Institute, Univ of Miami, Miller School of Medicine, Miami, FL; [5]Dept of Surgery, Univ of Miami, Miller School of Medicine, Miami, FL.*

**Background:** Diabetic Kidney Disease (DKD) is the most common cause of end-stage renal disease in the United States, and serum concentrations of Tumor Necrosis Factor alpha (TNFα) and TNFa Receptor 1 and 2 correlate with the development and progression of DKD. In DKD, decreased podocyte number and glomerular cholesterol accumulation are both associated with albuminuria. We tested the hypothesis that TNFα or TNFR1/R2 induce podocytes apoptosis via increased cholesterol accumulation.

**Methods:** Caspase 3 activity was used to quantify apoptosis in human podocytes treated with TNFα or TNFR1/R2. Lipid droplets were determined by Opera high Content Screening analysis of Bodipy 493/503 stained cells. $^3$H-cholesterol was used for efflux experiments and cyclodextrin to deplete cholesterol. Gene expression profiling was performed using Affymetrix GeneChip arrays.

**Results:** TNFα but not TNFR1/R2 induce apoptosis in podocytes ($p<0.05$). TNFα induced apoptosis was associated with a significant increase in the number of lipid droplets ($p<0.01$). ApoA1 mediated cholesterol efflux was decreased in TNFα-treated podocytes($p<0.05$) and was accompanied by decreased ATP-binding cassette transporter A1 (ABCA1) protein ($p<0.01$) and mRNA expression ($p<0.001$). Down-regulation of ABCA1 expression also occurred in glomeruli of patients with type 2 diabetes and DKD (N=70) when compared with healthy living donors (N=32). Cholesterol depletion with cyclodextrin partially prevented TNFα-induced podocyte apoptosis ($p<0.05$).

**Conclusions:** TNFα attenuates reverse cholesterol transport in podocytes leading to cholesterol accumulation and apoptosis. Our data suggest that strategies targeting the TNFα- cholesterol axis in podocytes may protect from the development of DKD.

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## SA-PO306

**Transcriptional Regulation of WNT Pathway Genes in Diabetic Nephropathy** Gareth J. McKay, David Kavanagh, Alexander P. Maxwell. *Renal Research Group, Queen's Univ Belfast, Belfast, N. Ireland, United Kingdom.*

**Background:** Diabetic nephropathy (DN) is a microvascular complication of diabetes. Members of the WNT/ β-catenin pathways have been implicated in interstitial fibrosis and glomerular sclerosis, characteristic hallmarks of DN. These processes are controlled, in part, by transcription factors (TFs), proteins which bind to gene promoter regions attenuating their regulation. We sought to identify known *cis*-acting transcription factor binding sites (TFBS) over-represented within the promoter regions of WNT pathway members compared to genes across the genome.

**Methods:** We used TFBS data from the JASPAR databases on 65 WNT pathway genes to identify known binding motifs. *P*-values were estimated on the hypergeometric distribution for each TF. Gene expression profiles of enriched motifs were examined from DN-related datasets to assess clinical significance.

**Results:** TFBS motifs Transcription factor AP-2 alpha (*TFAP2A*), Myeloid zinc finger 1 (*MZF1*), and Specificity protein 1 (*SP1*) were significantly enriched within WNT pathway genes (*P*-values$<6.83\times10^{-29}$, $1.34\times10^{-11}$ and $3.01\times10^{-6}$ respectively). *MZF1* gene expression was significantly increased in DN in a whole kidney dataset (fold change = 1.16; 16% increase; $P = 0.03$). *TFAP2A* gene expression was decreased in an independent dataset (fold change = -1.02; $P = 0.03$). *SP1* was not differentially expressed in any datasets examined.

**Conclusions:** We identified three TFBSs significantly enriched within the WNT pathway genes examined highlighting the use of *in silico* analyses for identifying key regulators of this pathway. Modification of TF binding to gene promoter regions affecting DN pathology may limit progression, making refinement of targeted therapeutic strategies possible through clearer delineation of their role.

## SA-PO307

**Involvement of Renal Cytochrome P450 and Arachidonic Acid Metabolites in Diabetic Nephropathy** Stephanie Atef Eid, Assaad Antoine Eid. *Anatomy, Cell Biology and Physiology, American Univ of Beirut.*

**Background:** Diabetic nephropathy (DN), a serious complication of diabetes leads to loss of renal function. Proximal tubular cells (PTs) contribute to the hypertrophic response of the kidney. Early stages of DN are also associated with alterations in renal sodium handling as well as hypertension; processes linked by involvement of the arachidonic acid (AA) metabolites 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs). This study aims to determine the specific AA-metabolizing CYP450 isoforms present in proximal tubules that are altered by high glucose (HG) or hyperglycemia and to investigate the effects of their alterations on DN.

**Methods:** Kidney injury assesment, ROS generation, 20-HETE and EETs formations, CYP4A and CYP2C11expression.

**Results:** We show that exposure of rats proximal tubular epithelial cells to high glucose (HG) resulted in increased extracellular matrix accumulation and hypertrophy. HG treatment increased ROS production and was associated with alteration in CYPs 4A and 2C11 expression and alteration in 20-HETE and EETs formation. HG-induced tubular injury were blocked by 20HETE inhibition. In contrast, inhibition of EETs promoted the effects of HG on cultured proximal tubular cells. We also show that alteration in CYPs 4A/2C expression and 20-HETE/EETs formation regulates the activation of the mTOR/p70S6Kinase pathway, having a major role in the development of DN. In parallel experiments we show that inhibition of the Cyp4A-mediated 20-HETE production or induction of EETs formation in animal model of diabetes prevent tubular cells injury and proteinuria. Our in vivo observations were correlated by increased urine levels of 20-HETE in diabetic rats and these levels were reduced by modulation of 20-HETE with its inhibitor HET0016.

**Conclusions:** Our results indicate that hyperglycemia in diabetes has a significant effect on the expression of AA-metabolizing CYPs, manifested by increased AA metabolism, and might thus alter kidney function through alteration of type and amount of AA metabolites. These observations provide a strong rationale to study cytochrome P4504A as a promising target for therapeutic drug development in DN.

*Funding:* Government Support - Non-U.S.

## SA-PO308

**Role of Bone Morphogenetic Protein-7 (BMP7) in Diabetic Tubulopathy** Ruixi Li, Wai Han Yiu, Hao-Jia Wu, Miao Lin, Dickson WL Wong, Loretta Y.Y. Chan, Joseph C.K. Leung, Kar Neng Lai, Sydney C.W. Tang. *Medicine, Queen Mary Hospital, Hong Kong, China.*

**Background:** The potential renoprotective role of BMP7 in diabetic nephropathy remains unknown.

**Methods:** Nine-week-old *db/db* mice and their *db/m* littermates underwent uninephrectomy (Unx) or sham operation, and received rh-BMP7 (300ug/kg body weight) or vehicle intraperitoneally every other day for 8 weeks before sacrifice. Primary human proximal tubular epithelial cells (PTECs) were growth-arrested and exposed to glycated human serum albumin (AGE-HSA) with or without rh-BMP7.

**Results:** Compared with vehicle control, Unx *db/db* mice treated with rh-BMP7 for 8 weeks had significantly lower urinary albumin-to-creatinine ratio (4,566±2,767 ug/mg *vs.* 7,338±5,748 ug/mg, $p<0.05$), serum BUN (33.3±3.46 mg/dL *vs.* 37.5±2.95 mg/dL, $p<0.05$), and renal cortical gene expression of IL-6, ICAM-1, CCL-2 and CCL-5. PAS staining of kidney tissue showed significantly less severe tubular damage and interstitial inflammatory cell infiltration in the BMP7-treated group. In cultured human PTECs, exposure to AGE-HSA induced overexpression of sICAM-1, CCL-2, IL-8 and IL-6, involving activation of p44/42 and p38 MAPK signaling. BMP7 dose-dependently attenuated AGE-induced upregulation of sICAM-1, CCL-2, IL-8 and IL-6 at both mRNA and protein levels. Moreover, BMP7 suppressed AGE-induced p38 and p44/42 MAPK phosphorylation and reactive oxygen species production in PTECs.

**Conclusions:** Our results demonstrated for the first time that BMP7 attenuates tubular pro-inflammatory responses in diabetic kidney disease by suppressing oxidative stress and multiple signaling pathways including p38 and p44/42 MAPK. Its potential application as a therapeutic molecule in diabetic nephropathy warrants further investigation.

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## SA-PO309

**Activated Local Renin-Angiotensin System within Glomerular Endothelial Cells Is Associated with Morphological and Functional Changes under Diabetic Conditions** Shin-Wook Kang,[1,2] Ji Sun Paeng,[1] Hye-Young Kang,[1] Hyung Jung Oh,[2] Seung Hyeok Han.[2] *[1]Brain Korea 21, Yonsei Univ, Seoul, Korea; [2]Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea.*

**Background:** The role of renin-angiotensin system (RAS) within glomerular endothelial cells (GECs) in the pathogenesis of diabetic nephropathy (DN) has not been fully explored. We aimed to investigate the existence and changes of RAS components in high glucose (HG)-stimulated GECs and the role of local RAS in morphological and functional changes of GECs under diabetic conditions.

**Methods:** In vitro, GECs were exposed to 5.6 mM glucose (NG) or 30 mM glucose (HG) with or without $10^{-7}$ M losartan for 24 hours. In vivo, 32 Sprague-Dawley rats were injected with diluents (n=16, C) or streptozotocin intraperitoneally (n=16, DM), and 8 from each group were treated with 10 mg/kg losartan for 6 weeks. The activation of local RAS was examined by Real-time PCR, Western blot analysis, and ELISA. Morphological changes were evaluated by scanning electron microscopy (SEM). FITC-tagged albumin permeability was determined by a transwell assay.

**Results:** Angiotensinogen mRNA and protein expression were significantly increased in HG-stimulated GECs compared to NG cells ($p<0.01$). AI and AII concentrations were also significantly higher in HG-conditioned media ($p<0.01$). However, there were no differences in the mRNA and protein expression of ACE and AII type 1 and type 2 receptors. On SEM examination, the number and diameter of fenestrae were significantly increased in HG-stimulated GECs and these changes were significantly abrogated by losartan ($p<0.05$). A transwell assay revealed that FITC uptakes in filtered media through HG-stimulated GECs were 4.3-fold higher compared to NG cells, and the increased uptakes were significantly ameliorated by losartan ($p<0.05$). In addition, diameter of GEC fenestrae and urinary albumin excretion were significantly greater in DM rats than those in C rats ($p<0.05$), which were significantly attenuated by losartan ($p<0.05$).

**Conclusions:** Local RAS within GECs was activated under HG conditions, and it seemed to be associated with alterations in GEC fenestration, resulting in the development of albuminuria in DN.

*Funding:* Government Support - Non-U.S.

## SA-PO310

**Oral Treatment with PBI-4050, a Novel First-in-Class Anti-Fibrotic Compound, Improves Kidney Function and Reduces Hepatic Steatosis in the Diabetic db/db Mouse Model** Lyne Gagnon, Lilianne Geerts, François Sarra-Bournet, Mikaël Tremblay, Kathy Hince, Shaun Abbott, Jean-Simon Duceppe, Boulos Zacharie, Christopher Penney, Pierre Laurin, Brigitte Grouix. *ProMetic BioSciences Inc., Laval, Canada.*

**Background:** Diabetic nephropathy is now the leading cause of end-stage renal disease. The aim of this study was to investigate the effect of PBI-4050, a novel first-in-class compound with anti-inflammatory, anti-oxidant and anti-fibrotic properties, on biological markers and kidney function in uninephrectomized (NX) diabetic (db/db) mice.

**Methods:** Total nephrectomy of the right kidney was performed on day 0 and animals were treated with vehicle or PBI-4050 (100 mg/kg, oral once a day) from day 1 through 130. Kidney function, kidney mesangial lesions, and hepatic steatosis were examined. Inflammatory/fibrotic and remodeling markers were assessed by qPCR in kidney and liver tissue.

**Results:** Urinary creatinine was significantly reduced in NX-db/db mice compared to sham and NX-C57BL/6 (negative control) mice, but returned to the normal level in PBI-4050-treated NX-db/db mice. Albuminuria was increased by 1.5 to 2 fold in NX-C57BL/6 and NX-db/db compared to sham C57BL/6 mice. Treatment of NX-db/db mice with PBI-4050 reduced albuminuria and proteinuria to the sham C57BL/6 mice level. NX-db/db mice demonstrated a significant increase in GFR (hyperfiltration), which was significantly reduced by treatment with PBI-4050. Db/db mice had larger glomeruli with increased mesangial matrix. Mesangial lesion scores were reduced in NX-db/db mice treated with PBI-4050. Lipid peroxidation (TBARS) in kidney was increased in NX-db/db mice and reverted to the control level following treatment with PBI-4050. Hepatic steatosis, observed in NX-db/db mice, was significantly reduced (50%) with PBI-4050-treatment. Furthermore, qPCR analysis of NX-db/db mice indicated that PBI-4050 treatment resulted in a significant reduction of the expression of IL-6, collagen I, TIMP-1 and MMP-2 in kidney tissue, and of TGF-β1, collagen I, TIMP-1 and MMP-2 in liver tissue.

**Conclusions:** Our results suggest that PBI-4050 offers the potential as a novel therapy for the reduction of diabetic nephropathy.

## SA-PO311

**Myo-Inositol Oxygenase (MIOX) Modulates Mitochondrial Dynamics and Autophagy in Diabetic Tubulopathy** Ming Zhan, Tatsuya Tominaga, Lin Sun, Yashpal S. Kanwar. *Pathology Dept, Northwestern Univ, Chicago, IL.*

**Background:** Diabetic nephropathy (DN) is often associated with mitochondrial injury and oxidative stress. MIOX, a tubular enzyme, modulates redox imbalance and apoptosis in tubular cells in diabetes, but the mechanisms remain unclear.

**Methods:** We investigated the role of MIOX in perturbation of mitochondrial dynamics and autophagy under high glucose (HG) ambience contributing to mitochondrial-mediated ROS generation and apoptosis in vitro and in vivo.

**Results:** Following HG treatment of HK-2/LLC-PK1 cells, an increased expression of MIOX and mitochondrial fission proteins, Drp1 & Fis1, and mitochondrial fragmentation was observed. Expression of fusion protein, Mfn2, and autophagy related proteins (LC3B, Atg5 and Beclin1) was decreased. MIOX-transfected tubular cells had an increased Drp1 expression and mitochondrial fission under LG & HG ambience, whereas LC3B, Atg5 and Beclin1 expression was decreased. At times, mitophagy was also suppressed. These changes were accompanied with Bax activation, mitochondrial cytochrome C release, ROS overproduction and apoptosis. MIOX siRNA & D-glucarate, an inhibitor of MIOX partially reversed these perturbations. An increased MIOX expression and mitochondrial fragmentation was also seen in proximal tubules during early stages of STZ-induced DN in mice, together with upregulation of Drp1, Bax and cytochrome C expression, but reduced expression of Mfn2 and autophagy related proteins. Dietary supplementation of D-glucarate to diabetic mice decreased MIOX expression and improved renal functions, as reflected by the reduction of serum creatinine, urinary albumin-to-creatinine ratio and tubular damage score. D-glucarate treated mice had partial attenuation of mitochondrial fragmentation and restoration of autophagy, accompanied with decreased oxidative stress and apoptosis in tubules. Conceivably, MIOX regulates mitochondrial dynamics, autophagy and apoptosis under HG ambience via modulation of Drp1 & Mfn2 activation.

**Conclusions:** Data suggest that MIOX modulates mitochondrial dynamics and autophagy in tubular cells in diabetic milieu, which may be a novel mechanism for ensuing redox imbalance and tubular cell injury and death.

*Funding:* NIDDK Support

## SA-PO312

**High Glucose Increases Formation and Pro-Oxidative Activity of Endothelial Microparticles** Dylan Burger, Kevin D. Burns. *Kidney Research Centre, Ottawa Hospital Research Institute, Univ of Ottawa, Ottawa, Canada.*

**Background:** Microparticles (MPs) are submicron membrane fragments released from the cell surface under conditions of stress or damage. In diabetic subjects, plasma endothelial MP levels are increased and predict risk of future cardiovascular events. Moreover, emerging evidence suggests that MPs may themselves exert deleterious biological effects. However, the stimuli responsible for increased MPs in diabetes, and the impact of diabetic conditions on the biological activity of endothelial MPs are not clear.

**Methods:** Human dermal microvascular endothelial cells (ECs) were cultured in media containing 5.6 mM glucose, 25 mM D-glucose, or 25 mM L-glucose (osmotic control). MP levels were assessed by Annexin V labeling and flow cytometry as well as nanoparticle tracking analysis (NTA). Finally, the effects of endothelial MPs, formed under normal and high glucose conditions, on formation of reactive oxygen species (ROS) was assessed by dichlorofluorescin fluorescence.

**Results:** Exposure of ECs to high glucose conditions was associated with a significant increase in MP formation over 24 hrs as assessed by flow cytometry (~2.5-fold vs. osmotic control, $P<0.05$, n=4) and NTA (~2.5 fold vs. osmotic control, $P<0.01$, n=6). Similarly, high glucose increased MP formation from human vascular smooth muscle or proximal tubule epithelial cells, although it had no effect on human mesangial cell MP formation. Interestingly, exposure to 25 mM glucose increased the mean size of endothelial MPs (5.6 mM: 203±7 nm vs. 25 mM: 260±14 nm, $P<0.05$ n=6). Moreover, MPs generated under high glucose conditions were more potent inducers of endothelial cell ROS production than MPs generated under normal glucose (25 mM MPs: ~6-fold vs untreated control, 5.56 mM MPs: ~2.5 fold vs. control, $P<0.05$, n=4).

**Conclusions:** Our results suggest that elevated glucose is a potent inducer of endothelial MP formation while also increasing the pro-oxidative effects of endothelial MPs. Such effects may contribute to progressive endothelial injury in diabetes.

*Funding:* Government Support - Non-U.S.

## SA-PO313

**The Paradoxical Effect of Lycopene on Diabetic Nephropathy in Type 2 Diabetic Mice** Young Sun Kang,[1] Mi Jin Lee,[1] Jin Joo Cha,[1] Young Youl Hyun,[2] Ji Eun Lee,[3] Hyunwook Kim,[3] Jung Eun Kim,[1] Mihwa Lee,[1] Hye Kyoung Song,[1] Jee-young Han,[4] Dae R. Cha.[1] *[1]Nephrology, Korea Univ Ansan Hospital, Ansan, Kyungggido, Republic of Korea; [2]Nephrology, Sungkyunkwan Univ Kangbuk Samsung Hospital, Seoul, Republic of Korea; [3]Nephrology, Wongwang Univ Sanbon Hospital, Gunpo, Republic of Korea; [4]Pathology, Inha Univ Hospital, Incheon, Republic of Korea.*

**Background:** Lycopene, a dietary carotenoid found in fruits, has been under investigation for its anti-oxidant benefits in cancer, CVD, and the metabolic syndrome. There is rare data of lycopene in kidney disease. The aim of this study is to investigate the effect of lycopene on diabetic nephropathy in type 2 diabetic animal models.

**Methods:** 8-week-old male diabetic *db/db* mice were treated with vehicle or lycopene of 1mg/kg/day intraperitoneally for 4 weeks. The biochemical parameters were measured and RT-PCR, western blot analysis, and IHC were performed.

**Results:** After lycopene treatment, there was a noticeable decrease in body weight, food and water intake, urine amount, and blood sugar level. But, lycopene had no significant effect on plasma Cr level, HbA1c level, BP, and HR. Interestingly, Lycopene improved ITT(insulin tolerance test), plasma lipid profile such as total cholesterol, triglyceride and LDL cholesterol in diabetic mice group. However, lycopene aggravated renal injury in diabetic animal model. This result was the opposite of the known anti-inflammatory effect. Lycopene increased proteinuria, the expression of renal inflammatory markers of PAI-1, TGFb1, and TNF-a, and 8-isoprostane as the oxidative stress marker. It increased the gene expression of lipid-regulating enzyme of FAS, and activated the insulin signaling pathway of insulin receptor, p85, and IRS. More interestingly, lycopene suppressed the nephrin expression and it increased the expressions of Akt, MAPK, IR, P85, and IRS.

**Conclusions:** Lycopene presented the paradoxical effect of the aggravation of proteinuria via the insulin signaling and nephrin pathway, although it is believed to activate AMPK in liver leading to improve the metabolic syndrome. Our results suggest the anti-diabetic or anti-lipid agents are able to have a harmful effect on the kidney.

## SA-PO314

**Collagen-Secreting Angiogenic Macrophages in Diabetic Nephropathy** Chhanda X. Bose,[1] Sudhir V. Shah,[1] Sundararaman Swaminathan.[3] *[1]Dept of Internal Medicine, Div of Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR; [2]Dept of Internal Medicine, Div of Nephrology, Univ of Virginia Health System, Charlottesville, VA.*

**Background:** Inflammatory mechanisms, including recruitment of macrophages into the glomerulus and tubulointerstitial region, play an important role in diabetic nephropathy. In diabetic kidneys, macrophage development and accumulation is angiotensin II-dependent.

**Methods:** We examined the effect of high glucose and/or angiotensin II on cellular responses of peripheral blood mononuclear cells (PBMC) in vitro, specifically on the development of collagen-secreting angiogenic macrophages. Total PBMC were incubated with 5mM (normal) and 25 mM (high) glucose for 2-8 days. Angiotensin II (0.1 or 1.0 μM) was added to the cultures for 24h to 4 days using immunofluorescence and Western blot analysis to check the expression of CD163, the fibrotic marker procollagen-1, and the angiogenic markers lectin and acetylated LDL by human PBMC in hyperglycemic conditions.

**Results:** Treatment of cells with 25 mM glucose induced CD163 and procollagen-1 expression. When 1 μM angiotensin was added to the glucose-treated cells, procollagen and CD163 expression were further enhanced. Further, we demonstrate that collagen-expressing macrophages are angiogenic in nature, as lectin and acetylated LDL (the angiogenic markers) were markedly up-regulated by high glucose and angiotensin II. Western blot analysis of glucose and angiotensin-treated cells confirmed our observations from immunofluorescence studies. We also demonstrate the infiltration of CD163+ macrophages and procollagen-1+ cells in the interstitium of human kidneys with diabetic nephropathy.

**Conclusions:** Overall, we present a novel observation that collagen-secreting fibrotic and angiogenic macrophages are induced in PBMC, which are further augmented with low doses of angiotensin II in hyperglycemic conditions. The presence of these cells in vivo may be an important pathophysiological mechanism for progression in diabetic nephropathy.

*Funding:* Private Foundation Support

## SA-PO315

**Association of Uromodulin Genetic Variant and Serum Levels with Renal Function in Diabetic Kidney Disease** Celine Klessens,[1] Verena Peters,[2] Claus P. Schmitt,[2] Ana Zutinic,[1] Emile De Heer,[1] Hans J. Baelde.[1] *[1]Pathology, Leiden Univ Medical Center, Leiden, Netherlands; [2]Pediatrics, Univ of Heidelberg, Heidelberg, Germany.*

**Background:** *CNDP1* codes for the enzyme carnosinase-1(CNDP1), in which the number of leucine repeats and resulting activity is associated with the susceptibility for diabetic nephropathy(DN) in woman with DM2. Considering the protective properties of carnosines, we hypothesize that local production and degradation in the kidney play a role in developing nephropathy in a subset DM2 patients.

**Methods:** Rabbit serum against CNDP1 and monoclonal antibody against carnosine synthase(CARNS) were used to investigate nephron segment-specific distribution of CARNS and CNDP1. Double label immune-stainings with megalin and Tamm Horsfall for the proximal and distal tubules respectively were performed. mRNA levels were measured in isolated glomeruli and whole kidney tissue from control and DN kidneys. Q-PCR was also performed on cultured podocytes, endothelial, mesangial and tubular cells.

**Results:** CNDP1 was predominantly detectable in the distal tubules and CARNS was found in proximal tubules(brush border pattern). In the glomerulus CNDP1 and CARNS were detectable to a lesser extent. mRNA measurements show expression of both CNDP1 and CARNS in the whole kidney, as well as in isolated glomeruli. We found high mRNA expression of *CARNS* and *CNDP1* in cultured podocytes, intermediate expression in mesangial and tubular cells, and very low expression in endothelial cells. No significant difference was found between normal and DN kidneys, but the mRNA expression levels of *CARNS* and *CNDP1* were significantly correlated(r=0.77).

**Conclusions:** We found evidence for local carnosin metabolism in the glomerulus (podocytes) as well as in the tubuli. mRNA level shows simultaneous regulation of both genes, which indicated common response elements in their promoters. The fact that their expression level is independent from diabetes provides the therapeutic opportunity to intervene in carnosine metabolism, since this can be manipulated without affecting diabetic disease. Future research will be focused on quantification and modulation of carnosine levels in DN kidneys.

## SA-PO316

**Oxidative Stress and Mitochondrial Disfunction in Genetic Model of Obese Rats** André Soares Trindade,[1] Thiago S. Rosa,[1] Priscila Afonso de Faria,[3] Anderson Sola de Haro,[1] Marcelo Macedo Rogero,[2] Tiago Rodrigues,[3] Elisa MS Higa.[1] *[1]Medicine, Universidade Federal de São Paulo, São Paulo, Brazil; [2]Shool of Public Health, Universidade de São Paulo, São Paulo, Brazil; [3]Natural and Human Science Center, Universidade Federal do ABC, Santo André, Sao Paulo, Brazil.*

**Background:** Obesity is characterized as chronic inflammatory disease associated with insulin resistance, increased activity of the renin-angiotensin-aldosterone system, and increased oxidative stress. In this study, the oxidative status and the mitochondrial integrity were screened in the cardiac, renal, and hepatic tissue by using the genetic model of obese rats (Zucker).

**Methods:** Isolation of rat mitochondria from liver, heart and kidney were isolated by standard differential centrifugation. Mitochondrial swelling was estimated from the decrease in the absorbance. The reduced glutathione (GSH) levels were estimated in a spectrofluorimeter by using OPT (o-phthaldialdehyde). The ROS generation was estimated by the changes in the DCF fluorescence, in a spectrofluorimeter. Statistical analysis were performed using the Microcal Origin 6.0 software.

**Results:** The homogenate and isolated mitochondria from liver of Zucker rats exhibited decreased GSH levels compared to Lean rats (33.3% and 46.5%, respectively). Similar result was found in the kidney homogenate but not in the isolated mitochondria. The production of ROS was significantly higher in the liver mitochondria from Zucker compared to Lean (50%), while no difference was observed in the heart. Mitochondria isolated from heart, liver and kidney were challenged with increasing Ca2+ concentrations and the swelling was higher in Zuckervs Lean, only in the liver.

**Conclusions:** Mitochondria from Zucker liver was more susceptible to permeabilization than that Lean. However, kidney and heart mitochondria showed no significant differences in the swelling. Our results show that obesity promotes oxidative stress with mitochondrial dysfunction primarily in the liver. Kidney and heart seems to be less affected. The increased susceptibility of liver mitochondria to permeabilization in obese rats may be associated to cell death and consequently to organ failure.

*Funding:* Government Support - Non-U.S.

## SA-PO317

**Association of Uromodulin Genetic Variant and Serum Levels with Renal Function in Diabetic Kidney Disease** Krishan L. Gupta,[1] Vinod Sharma,[2] Ashok Kumar Yadav,[2] Vinay Sakhuja,[1] Vivekanand Jha.[1] *[1]Nephrology, PGIMER, Chandigarh, India; [2]Translational and Regenerative Medicine, PGIMER, Chandigarh, India.*

**Background:** Uromodulin a UMOD gene encoded 95kDa, glycoprotein exclusively synthesized in kidney cells and released into urine. Mutation in UMOD lead to Uromodulin misfolding and retention in kidney cells, where it interact and stimulate cells of immune system to cause inflammation and progression of chronic kidney disease (CKD). Diabetic nephropathy is one of the most severe complications of type 2 diabetes leading to end-stage renal disease (ESRD). Recent genome wide association studies have identified the Uromodulin locus associated with hypertension and diabetic nephropathy. This study was designed to investigate variations in UMOD genes with type diabetes mellitus (T2DM) and susceptibility to nephropathy.

**Methods:** A total of 649 subjects (196 T2DM subjects without nephropathy (DM), 167 with diabetic nephropathy stage II-IV (DN); 186 healthy control (HC ) and 40 DN stage V C KD) were genotyped for UMOD variant rs4293393T>C by restriction fragment length polymorphism (RFLP). Serum uromodulin levels were quantified by using human uromodulin enzyme linked immunosorbent assay (ELISA).

**Results:** A significant difference was found in genotype and allelic frequency of UMOD gene (rs4293393 T>C ) among DM, DN II-IV, DN-V, C KD and HC . UMOD TC /C C genotype and C allele were found to be significantly more frequent in DN II-IV, DN-V compared to DM and HC ($p<0.01$). Significant higher serum uromodulin levels 12/ 10/ 12 WCN 2013 : Abst r act # 452065 Pr eview www. cal4abst r act s. com/ wcn/ f inalpr eview. php?absnum=452065 2/ 2 were found in DN-II-IV and DN-V compared to DM

(<0.003). Serum uromodulin levels showed a positive correlation with serum creatinine (R=0.42; p<0.0001), stages of disease (R=0.47; p<0.0001) and negative correlation with eGFR (R=-0.42; p<0.0001).

**Conclusions:** This study shows that variations in UMOD rs4293393 T>C , might have a bearing on susceptibility to nephropathy in type 2 diabetes. Serum uromodulin levels are increased in subjects with decreased eGFR.

## SA-PO318

**Angiotensin II Inhibition Corrects Increased Oxygen Consumption in Diabetic Rat Kidneys** Aihua Deng, Roland C. Blantz, Scott C. Thomson. *Nephrology/Hypertension, UCSD&VASDHS, San Diego, CA.*

**Background:** Studies have demonstrated that angiotensin (ANG) II blockade has beneficial effects on chronic kidney disease, especially for diabetic nephropathy, but the mechanism is unknown. The present study tests our hypothesis that ANG II blockade corrects increased oxygen consumption ($QO_2$) in diabetic kidneys, which may be part of the mechanism underlying the therapeutic effects of ANG II blockade.

**Methods:** Rats were made diabetic by a single intraperitoneal injection of streptozotocin (65mg/kg,). 4 groups of rats were used: 1) Normal; 2) Diabetic rats (DM) untreated; 3) DM + dual ANG II inhibition; and 4) DM + apocynin. Captopril (C, 20mg/kg/day), losartan (L, 20 mg/kg/day) and apocynin (40 mg/kg/day) were administrated in the drinking water for 4 weeks. Glomerular filtration rate (GFR) and renal blood flow (RBF), $QO_2$ and renal metabolic efficiency ($QO_2/T_{Na}$, where $T_{Na}$ = total sodium reabsorbed) were measured at week 4 after induction of DM.

**Results:** Compared with the normal, in 4wk untreated diabetic kidney GFR and RBF tended to increase but did not reach statistical significance. $QO_2$ was double increased and renal metabolic efficiency was decreased indicated by an elevation of $QO_2/T_{Na}$. The altered $QO_2$ and $QO_2/T_{Na}$ were corrected by ANG II inhibition but not by apocynin, a NADPH oxidase inhibitor.

| | Normal | 4wk DM | 4wk DM+ C/L | 4wk DM + apocynin |
|---|---|---|---|---|
| GFR (ml/min) | 1.63±0.05 | 2.34±0.35 | 1.85±0.19 | 2.04±0.2 |
| RBF (ml/min) | 9.72±0.54 | 13.5±1.87 | 12.57±1.16 | 13.8±0.88 |
| $QO_2$ (ml/min) | 0.22±0.01 | 0.59±0.08* | 0.29±0.02** | 0.52±0.05* |
| $QO_2/T_{Na}$ (ml/mmole) | 1.03±0.07 | 1.90±0.07* | 1.27±0.11** | 1.94±0.09* |
| BP (mmHg) | 113±2 | 112±8 | 79±3* | 117±3 |

**Conclusions:** The normal kidney is on the border of hypoxia. A huge increase in $QO_2$ occurs in diabetic kidney, which may intensify renal tissue hypoxia. The substantial elevation of $QO_2/T_{Na}$ suggests that diabetic kidney has a much higher demand for $O_2$ to support Na transportation and nontransport events. Chronic ANG II inhibition reduces the demand for $O_2$ without altering GFR.

*Funding:* NIDDK Support

## SA-PO319

**High Fat Feeding Accelerates Decline of Renal Function in db/db Mice** Li-Jun Ma,[1] Margaret S. Wu,[1] Yonghua Zhu,[2] Qing Shao,[2] Emamuel Zycband,[2] Effie Tozzo.[1] *[1]Diabetes & Endocrinology, Merck Sharp & Dohme Corp.; [2]PPDM Molecular Biomarkers, Merck Sharp & Dohme Corp., Kenilworth, NJ.*

**Background:** Most animal models represent early stage of human diabetic nephropathy (DN). Few mouse models showed decline in renal function. Accelerating the development of DN in mice would be desirable for experimental validation of potential targets that mediate the progression to late stage DN.

**Methods:** 12 week old male db/db mice were fed high fat diet (HFD, 60% fat) for 6-8 weeks (n=8 per time point) and the development of nephropathy was compared to age-matched db/db mice on regular chow (n=8 per time point). Urinary albumin was determined using a mouse Albuwell ELISA kit (Exocell). Serum and urine Creatinine levels were measured by GC/MS. Mesangial expansion was scored semiquantatively (score 0-5). mRNA expression of MCP-1 and nephrin in renal cortex was assessed by qPCR. Urine MCP-1 levels were analyzed by a quantikine ELISA Kit (R&D).

**Results:** db/db mice challenged with HFD for 8 weeks showed increased body weight (54.1±1.5 vs 44.4±1.3 g, p<0.01), increased ratio of kidney weight/body weight (1.1±0.1 vs 0.9±0.1 %, p<0.05), decreased urine volume (3.1±0.3 vs 10.3±2.2 ml/24hr, p<0.01), increased ACR (15-fold, p<0.01), increased total urine protein (5.7±1.0 vs 1.6±0.4 mg/24hr, P<0.01), decline of renal function as evidenced by increased serum creatinine (1.2±0.4 vs 0.7±0.1 mg/dl) and decreased creatinine clearance rate (99±20 vs 475±122 ul/min/mouse, p=0.07), and increased mesangial expansion (2.8±0.1 vs 0.4±0.1, p<0.05). We assessed following biomarkers after HFD feeding in db/db for 6 weeks, and our data showed an upregulation of renal cortex MCP-1 mRNA expression (1.5 fold increase), an elevation of urine MCP-1 levels (894.3±192.9 vs 6.6±2.2 pg/ml, p<0.01) and downregulated nephrin mRNA expression (p<0.05) when compared to db/db on regular chow. It is of interest to note that all these above changes occurred in the absence of an increase in glucose levels.

**Conclusions:** HFD feeding accelerates decline of renal function in db/db mice and increases kidney and urine MCP-1 levels. We are in the process of testing diets with more intermediate HF content closer to human diet such as 20-30 % fat.

*Funding:* Pharmaceutical Company Support - Merck

## SA-PO320

**Activation of $P2X_7$ Receptors and the Oxidative Stress in the Diabetic Nephropathy** Adelson M. Rodrigues,[1] Cassia T. Bergamaschi,[3] Maria José S. Fernandes,[5] Marcello Franco,[4] Giovana R. Punaro,[1] Fabiane R. Maciel,[2] Thamires Fernandes,[1] Guilherme B. Nogueira,[1] Elisa MS Higa.[1,2] *[1]Nephrology and Emergency Div, UNIFESP; [2]Translational Medicine, UNIFESP; [3]Cardiovascular Div, UNIFESP; [4]Investigative Pathology, UNIFESP; [5]Neurology, UNIFESP, Brazil.*

**Background:** Previous studies in our Laboratory showed the role of oxidative stress and the favorable effects of exercise training, on the progression of diabetic nephropathy in rats. $P2X_7$ receptors ($P2X_7$-R), in pathological conditions, are significantly up-regulated, increasing the levels of oxidative stress. The aim of this study was to evaluate the $P2X_7$-R and the oxidative stress in the kidneys of diabetic rats submitted to aerobic training.

**Methods:** Diabetes mellitus (DM) was induced in adult Wistar rats, with streptozotocin (60mg/kg, i.v.); control animals (CTL) received its vehicle. The animals were submitted to aerobic training on treadmill at a work rate of 16m/min for 60 min/day, 5 days a week/8weeks (DM+EX and CTL+EX); DM+SE and CTL+SE were kept resting on the treadmill at the same schedule (n=12 for each group). Half of the animals all groups were treated with N-acetylcysteine (NAC); the other, water as vehicle. One way ANOVA; significance at p<0.05.

**Results:** DM+SE vs CTL+SE increased the urinary excretion and renal tissue thiobarbituric acid reactive substances; there was also a reduced urinary excretion and renal tissue nitric oxide (NO). NAC and exercises reduced TBARS and increased NO in urine and the kidney. Confocal microscopy analysis of $P2X_7$-R in the kidneys showed with 100μM BZATP (preferential agonist to receptor) there was an increase in DM+SE vs CTL+SE (1.0 ±0.23 vs 0.14 ±0.06), which was reduced in groups received NAC (0.27 ±0.06), aerobic training (0.31 ±0.08) or both (0.17 ±0.04) , all p<0.05.

**Conclusions:** Our data show that the NAC administration, routine of exercise or both expressively decrease the activation of $P2X_7$-R in the kidneys of diabetic rats. The attenuation of oxidative stress through this mechanism, which also resulted in the increase of NO bioavailability, suggests that the control of these receptors' up-regulation could be useful to delay the progression of diabetic nephropathy.

## SA-PO321

**Diabetic Kidney Macrophages Display a Pro-Fibrotic Phenotype** Helena Cucak, Lisbeth N. Fink, Alexander Rosendahl. *Diabetes Research Unit, Novo Nordisk A/S, Maaloev, Denmark.*

**Background:** Diabetic nephropathy is one of the major complications of diabetes mellitus and is the leading cause of end-stage renal disease worldwide. Recently, it has been recognized that patients have low grade systemic inflammation, but how this is manifested on a cellular level in the diabetic kidney remains unclear. Experimental models suggest a central role for infiltrating macrophages in the pathogenesis, but their phenotype and the mechanism by which they contribute to tissue damage and fibrosis is unknown. Therefore, we evaluated the macrophage subsets present in the diabetic db/db and the healthy db/+ kidney during disease progression.

**Methods:** Kidneys from db/db and db/+ mice were enzymatically digested and kidney cell suspensions were analyzed by flow cytometry using an array of macrophage markers.

**Results:** 60% of the leukocytes in the kidney were macrophages in both healthy db/+ and diabetic db/db mice. The tissue derived macrophages could be divided into two major subsets based on expression of two cell surface markers; $CD68^+F4/80^+$ and $CD68^+F4/80^-$ macrophages. There were no differences in total macrophage numbers between diabetic and healthy kidneys. A similar fraction (40%) of both $CD68^+F4/80^+$ and the $CD68^+F4/80^-$ macrophages expressed CD206, a marker of alternatively activated 'M2' macrophages. In contrast, $CD68^+F4/80^-$ macrophages expressed 2.1-fold higher high levels of the 'M1' classical activation marker CD11c and displayed a 2.5-fold increase of the pro-fibrotic molecule galectin-3 compared to $CD68^+F4/80^+$ macrophages. Importantly, in diabetic kidney there was a clear shift towards a more pronounced pro-fibrotic macrophage phenotype. This was evident by 1.7-fold increased numbers of $CD68^+F4/80^-$ galectin-3-expressing cells, a 1.7-fold increase of TGFb-expressing $CD68^+F4/80^+$ cells, as well as a reduced CD11c expression.

**Conclusions:** We found that the db/db mouse kidneys are characterized by a qualitative phenotypic switch of resident macrophages towards more fibrotic phenotypes in late stage disease rather than a quantitative increase of macrophages. The emerging diabetic kidney macrophage subsets may possess a pathogenic function in the renal fibrosis central to the progression of diabetic nephropathy.

*Funding:* Pharmaceutical Company Support - Novo Nordisk A/S

## SA-PO322

**Megalin-Mediated Mechanism of High Fat Diet-Induced Kidney Disease** Michihiro Hosojima,[1] Shoji Kuwahara,[2] Hideyuki Kabasawa,[1] Hiroyuki Aoki,[2] Reika Kaneko,[2] Ichiei Narita,[1] Akihiko Saito.[2] *[1]Clinical Nephrology, Niigata Univ, Niigata, Japan; [2]Applied Molecular Medicine, Niigata Univ, Niigata, Japan.*

**Background:** Megalin, an endocytic receptor in proximal tubule cells (PTECs), is involved in uptake and metabolism of glomerular-filtered substances. This study aimed to investigate the role of megalin in high fat diet (HFD)-induced kidney disease.

**Methods:** Kidney-specific mosaic megalin KO mice (apoE cre+/-, megalin loxP+/+, ~60% megalin knocked out) and control mice (apoE cre-/-, megalin loxP+/+) were

uninephrectomized at age 10 weeks and fed a HFD (60% fat energy ratio) or normal fat diet (NFD) (10% fat energy ratio) for 12 weeks. Mice overexpressing LC3-GFP, an autophagosome marker, were also studied under the same protocol.

**Results:** Mice fed a HFD showed increases in body weights and blood glucose levels, compared with NFD-fed mice, with no differences in these parameters between HFD-fed megalin KO and control mice. In HFD-fed control mice, cytosolic vacuolar formation was observed in PTECs with increased nuclear expression of p27, a marker of cellular hypertrophy. The vacuoles were found to contain osmium-stained, onion-skin-like structures by electron microscopy. The vacuolar membranes were immunostained with LAMP1, LC3 and p62, indicating that the vacuoles were derived from dysfunctional autophagolysosomes. Autophagy in PTECs was actually impaired after 24-hr fasting in HFD-fed LC3-GFP transgenic mice. However, the vacuolar formation in PTECs was significantly suppressed in HFD-fed mosaic megalin KO mice with decreased nuclear expression of p27. Peritubular capillary rarefaction, demonstrated by decreased CD31 immunostaining, was induced in HFD-fed control mice, which was ameliorated in HFD-fed megalin KO mice. Glomerular hypertrophy with mesangial expansion, evident in HFD-fed control mice, was also ameliorated in HFD-fed megalin KO mice. Advanced glycation endproduct-modified BSA, an endocytic ligand of megalin, induced giant autophagolysosme formation in cultured PTECs.

**Conclusions:** In conclusion, megalin-mediated autophagolysosomal dysfunction in PTECs is primarily associated with the development of tubulo-glomerular alteration in HFD-induced kidney disease.

*Funding:* Pharmaceutical Company Support - Nippon Boehringer Ingelheim Co., Ltd. , Astellas Pharma Inc. , Sanofi K.K. , Dainippon Suitomo Pharma Co., Ltd. , Kissei Pharmaceutical Co., Ltd. , M.S.D K.K., Novartis Pharmaceuticals Japan , Eli Lilly Japan K.K. , Takeda Pharmaceutical Co., Ltd. , Mitsubishi Tanabe Pharma Corporation, Government Support - Non-U.S.

## SA-PO323

**Linagliptin Ameliorates Free Fatty Acid-Bound Albumin-Induced Tubulointerstitial Injury**

Yuki Tanaka,[1] Shinji Kume,[1] Shin-ichi Araki,[1] Daisuke Koya,[2] Masakazu Haneda,[3] Takashi Uzu,[1] Hiroshi Maegawa.[1] *[1]Dept of Medicine, Shiga Univ of Medical Science, Otsu, Shiga, Japan; [2]Dept of Diabetology and Endocrinology, Kanazawa Medical Univ, Kahoku, Ishikawa, Japan; [3]Div of Metabolism and Biosystemic Science, Asahikawa Medical Univ, Asahikawa, Hokkaido, Japan.*

**Background:** Severe proteinuria-induced tubulointerstitial injury indicates poor renal prognosis in patients with diabetic nephropathy. Establishment of new therapeutic strategy against proteinuria-induced tubulointerstitial injury in diabetic nephropathy is required. Dipeptidyl peptidase (DPP) 4 inhibitors, a new class of oral antidiabetic agents for type 2 diabetes, have recently been suggested exerting pleiotropic effects beyond glucose lowering.

**Methods:** We thus examined renoprotective effect of linagliptin, a DPP4 inhibitor, on free fatty acids (FFA)-bound albumin-induced tubulointerstitial injury in mice. Mice were fed either standard diet (SD) or SD with linagliptin, and intraperitoneally injected with FFA-bound albumin or PBS for 11 days. In addition, renoprotective effect of linagliptin was examined in cultured mouse proximal tubular (mProx) cells stimulated with FFA-bound albumin.

**Results:** We first confirmed that neither FFA-bound albumin injection nor linagliptin treatment altered systemic characteristics including body weight, fasting blood glucose and food intake. In SD group, FFA-bound albumin caused tubular cell apoptosis, interstitial inflammation characterized by macrophage infiltration and increased mRNA expression of inflammatory cytokines, such as MCP-1, IL-6 and TNF-α, and interstitial fibrosis with increased expression of fibronectin and PAI-1. These alterations were all prevented by the treatment with linagliptin. Furthermore, in cultured mProx cells, linagliptin inhibited FFA-bound albumin-induced increases in mRNA expression level of inflammatory cytokines and fibrotic markers.

**Conclusions:** These results indicate that DPP4 inhibitors exert anti-inflammatory and anti-fibrotic effect as an additional pleiotropic effect, and may serve as a therapeutic strategy to protect proximal tubular cells against proteinuria in patients with diabetic nephropathy.

## SA-PO324

**Linagliptin-Mediated DPP-4 Inhibition Ameliorates Kidney Fibrosis in Streptozotocin-Induced Diabetic Mice by Inhibiting Endothelial-to-Mesenchymal Transition**

Keizo Kanasaki, Shi Sen, Megumi Kanasaki, Swayam Prakash Srivastava, Daisuke Koya. *Diabetology and Endocrinology, Kanazawa Medical Univ, Kahoku, Ishikawa, Japan.*

**Background:** Kidney fibrosis is the final common pathway of all progressive chronic kidney diseases, of which diabetic nephropathy is the leading cause. Endothelial-to-mesenchymal transition (EndMT) has emerged as one of the most important origins of matrix-producing fibroblasts. Dipeptidyl peptidase-4 (DPP-4) inhibitors have been introduced into the market as anti-diabetic drugs.

**Methods:** Diabetes was introduced in CD1 mice by streptozotocin injection and some of the diabetic mice were treated with linagliptin (5mg/kgBW/day) 20 weeks after diabetes initiation. At 24 weeks after initiation of diabetes, all mice were sacrificed. In in vitro experiment, we used human dermal microvascular endothelial cells and analysed anti-EndMT effects of linagliptin.

**Results:** Streptozotocin-induced diabetic CD1 mice exhibited severe kidney fibrosis and strong immunoreactivity for DPP-4 24 weeks after the onset of diabetes. Linagliptin-treated diabetic mice exhibited an amelioration of kidney fibrosis and restored normal kidney structure. These anti-fibrotic effects of linagliptin were associated with the inhibition of EndMT. The therapeutic effects of linagliptin on diabetic kidneys were associated with the suppression of profibrotic programs, as assessed by mRNA microarray analysis. We found that the induction of DPP-4 observed in diabetic kidneys may be associated with suppressed levels of microRNA29s in diabetic mice. Using cultured endothelial cells, we found that linagliptin inhibited TGFβ2-induced EndMT and the motility of fibroblast-like EndMT cells.

**Conclusions:** These results indicate the possible novel pleiotropic action of linagliptin to restore normal kidney function in diabetic patients with renal impairment.

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## SA-PO325

**Autophagy Maturation Interruption in CKD and Diabetic Nephropathy**

Ying Wang,[1] Cynthia C. Nast,[2] Janine A. La Page,[1] Sharon G. Adler.[1] *[1]Nephrology, LABiomed Research Institute, Torrance, CA; [2]Pathology, Cedars-Sinai Medical Center, LA, CA.*

**Background:** Autophagy (Atg),induced by cell stress, aids in repair in modest injury, but facilitates death in major injury.DBA2J mice lack HGFIN (aka gpnmb) and are Atg insufficient. HGFIN recruits the Atg protein LC3B-II to autophagosomes (AP)(Li et al: FASEB J, 2010). Renal HGFIN mRNA is increased in rat SZ-DN & Type 1 DN. After 5/6Nx, HGFIN co-localized with LC3B in injured tubules (KI 79:1138-48). We tested if high glucose (HG) stimulates HGFIN and Atg in vitro, and if the DBA2J genome contributes to initiation/progression in CKD or DN in DBA2Js, Akitas (Ak, $Ins2^{+/-}$), & backcrossed Akitas (Ak/DBA2J).

**Methods:** 1. HGFIN was localized by immunohistochemistry (IH) in DN patients. 2. MDCT cells +/- HGFIN overexpression were cultured in normal (5.5 mM) or HG (10-25 mM) medium. LC3B activation (LC3B-II/I) was immunoblotted. 3. In mice, we measured urine ACR, plasma glucose (Glu), BUN, performed kidney lysate immunoblotting for LC3B-II/I and the autophagolysosome protein LAMP2, and assessed kidney light and electron microscopies (LM, EM) at 6 mos.

**Results:** 1. In DN patients, HGFIN was increased in glomerular & cortical tubular epithelial cells. 2. In vitro in the absence of Atg blockade, HG and HGFIN overexpression increased the LC3B-II/I ratio additively ($p<0.05$). 3. Glu was similarly elevated in Ak and Ak/DBA2Js (NS); normal in DBA2J. 4. ACR was highest in Ak/DBA2J; higher in non-diabetic DBA2Js than Ak at 3 mos ($p<10^{-4}$), then increased over the next 3 mos ($p<0.05$), and was similar to Ak as DN advanced in mos 4-6. 5. Mean BUNs were similar in DBA2J and Ak and higher in Ak/DBA2J (34, 35, 66 mg/dl; NS) 6. Renal lysate LC3BII/I was highest in mice with DBA2J and LAMP2 lowest in mice with DBA2J, consistent with arrested AP maturation contributed by DBA2J. 7. LM cytoplasmic tubule vacuoles (AP by EM) in DBA2J and Ak/DBA2J further substantiate AP maturation block.

**Conclusions:** In vitro, without Atg blockade, HG stimulated HGFIN and Atg. In vivo, high LC3BII/I and low LAMP2 implicate DBA2J contributing to initiation/exacerbation of CKD/DN, at least in part by interrupting Atg at the AP step. Modulating Atg and/or HGFIN may be renoprotective in CKD/DN.

*Funding:* Pharmaceutical Company Support - DaVita Clinical Research

## SA-PO326

**Tadalafil Integrates Nitric Oxide-Hydrogen Sulfide-AMPK Pathway to Inhibit High Glucose-Induced Matrix Protein Synthesis in Podocytes**

Hak Joo Lee, Denis Feliers, Meenalakshmi M. Mariappan, Kavithalakshmi Sataranatarajan, Yves C. Gorin, Goutam Ghosh-Choudhury, Balakuntalam S. Kasinath. *Univ of Texas Health Science Center, San Antonio, TX.*

**Background:** Hydrogen sulfide ($H_2S$) inhibits high glucose (HG)-induced matrix increment in renal cells. We hypothesized that tadalafil (Tada) inhibits HG stimulation of matrix proteins by generating $H_2S$ in podocytes.

**Methods:** Mouse podocyte culture, Immunoblotting, Polysome analysis.

**Results:** Tada abrogated 30 mM (HG)-induced increase in global protein synthesis, cellular hypertrophy, and, laminin$\gamma$1 and fibronectin expression. Tada abrogated critical events in mRNA translation induced by HG by inhibiting mTORC1. As AMPK inhibits HG-induced protein synthesis, its role was examined. Tada dose- and time-dependently stimulated AMPK phosphorylation. Compound C (AMPK inhibitor) abolished Tada effect on HG stimulation of mTORC1, mRNA translation and laminin$\gamma$1 synthesis. Tada rapidly increased the expression of cystathionine-γ-lyase (CSE), a $H_2S$ generating enzyme, by promoting translation of its mRNA (Polysome analysis) and not by transcription. DL-propargylglycine (PAG), a CSE inhibitor, inhibited Tada-induced AMPK phosphorylation and abrogated Tada effect on HG stimulation of laminin$\gamma$1. As Tada promotes cGMP accumulation by PDE5 inhibition, we examined the role of nitric oxide (NO) pathway. Tada rapidly augmented inducible nitric oxide synthase (iNOS) expression by increasing transcription. L-NAME and ODQ, inhibitors of NOS and guanylyl cyclase, abolished Tada induction of $H_2S$ and AMPK phosphorylation. DETA-NONOate and sodium nitroprusside, 2 structurally distinct NO donors, mimicked Tada effect on CSE expression and AMPK phosphorylation. Tada given intraperitoneally to normal mice augmented kidney expression of CBS and stimulated AMPK activity.

**Conclusions:** Tada ameliorates HG stimulation of synthesis of proteins including matrix proteins in mouse podocytes. This requires sequential stimulation of NO-$H_2S$-AMPK axis leading to inhibition of HG-induced mTORC1 activity and mRNA translation. Thus, Tada integrates NO-cGMP pathway with $H_2S$-AMPK axis to inhibit HG effect on podocytes, bringing together two gaseous signaling molecules.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO327

**Resveratrol Ameliorated High-Glucose Induced Oxidative Stress through Nrf2 Activation** Min Zhang, Chuanming Hao. *Nephrology Div, Huashan Hospital, Shanghai, China.*

**Background:** Hyperglycemia-mediated oxidative stress plays a crucial role in the progression of DN. Nrf2 controls the ARE-dependent gene regulation in response to oxidative stress. Resveratrol is a polyphenolic phytoalexin that exhibits benefits including antioxidant and anti-inflammatory. Resveratrol is also shown to confer renoprotection in animal models of type 2 diabetes. However, the mechanisms by which resveratrol exerts its antioxidative protective effects are not completely understood.The current study is aimed at examining the mechanism of resveratrol on defense against high glucose-induced oxidative damage in glomerular endothelial cells.

**Methods:** Endothelial cells were serum starved for 24h, and then exposed to media containing low glucose plus mannitol (2.5mM+22.5mM, LG+M), normal glucose plus mannitol(5mM+20mM, NG+M) or high glucose (25mM, HG) with resveratrol(1,5,10,25μM) for 24 hours. Then the cells were treated with Nrf2 activator sulforaphane (Sigma, USA) or transfected with Nrf2-siRNA. Cells were harvested for either protein or RNA assay. The expression levels of Nrf2, HO-1 and SIRT1 were measured by Western blot and qPCR. For ROS measurement, cells were incubated for 30 min with H2DCFDA (Sigma,USA), and the fluorescence intensity was measured by flow cytometry.

**Results:** High glucose increased ROS production in glomerular endothelial cells. The expression levels of SIRT1 and Nrf2 were down-regulated at high glucose concentration ($P<0.05$). Sulforaphane, an agonist of Nrf2, significantly increased the expression of HO-1 and decreased the production of ROS. Resveratrol, in a dose-dependent manner, significantly increased expression level of Nrf2. Accordingly, resveratrol significantly upregulated the expression of the Nrf2 target gene: HO-1. Resveratrol treatment also significantly attenuated high glucose-induced oxidative stress.The aforementioned effects were significantly attenuated by the small interfering RNA downregulation of Nrf2. The results of co-immunoprecipitation showed a relationship between Nrf2 and SIRT1.

**Conclusions:** Our results indicated that resveratrol ameliorated high-glucose induced oxidative stress through Nrf2 activation.

## SA-PO328

**Effect of an Oral Adsorbent AST-120 on Proteinuria, Albuminuria and Oxidative Stress in Metabolic Syndrome/Diabetes Rats** Rieko Aoki, Fujio Sekine, Yusuke Yamashita, Kaori Kikuchi, Kenji Bannai, Ryoko Tateoka, Yoshiharu Itoh. *Kureha Corporation, Tokyo, Japan.*

**Background:** Metabolic syndrome is known to be an important risk factor involved in the development of diabetic nephropathy. An oral adsorbent AST-120 has been used clinically as a drug for treatment of chronic kidney disease (CKD) patients to slow the progression of CKD. However, there is little evidence when AST-120 should be prescribed for subjects with early stage overt diabetic nephropathy. In this study, we aimed to assess the effect of AST-120 in the early stage of nephropathy using SHR/NDmc-cp, a rat model of metabolic syndrome/ type 2 diabetes.

**Methods:** Male SHR/NDmc-cp (SHR/ND) rats, aged 7 weeks, were administered 0% or 8% AST-120 for 12 weeks in their diets. WKY rats were used as a normal. At every 4 weeks, serum and 24-hour urine samples were collected for biomedical studies. We analyzed serum metabolites in normal and SHR/ND rats with or without AST-120 for 8 weeks by capillary electrophoresis mass spectrometry with time-of-flight (CE-TOFMS) and applied CE-TOFMS data to principal component analysis (PCA). We also examined the gene expression of oxidative stress markers by real-time PCR and the glomerular podocyte damage by immunohistochemistry in renal tissues treated with or without AST-120.

**Results:** AST-120-administered SHR/ND rats showed significantly lower level of urinary albumin excretion, urinary protein excretion and urinary 8-OHdG excretion as compared with SHR/ND rats. PCA score plot showed clear separation among three groups (Normal, SHR/ND and AST-120-administered SHR/ND). We could detect 40 metabolites, such as o-hydroxybenzoic acid, hippuric acid and indole-3-acetic acid, which accumulated in the serum of SHR/ND rats, and of which serum levels were reduced by administration of AST-120. The gene expression of oxidative stress markers in renal tissues was lower in AST-120-administered SHR/ND rats than in SHR/ND rats. And also the ratio of podocyte injured maker, desmin, positive glomeruli was lower in AST-120-administered SHR/ND rats than in SHR/ND rats.

**Conclusions:** It indicated that the administration of AST-120 at an early stage has a protective effect on the progression of diabetic nephropathy.

## SA-PO329

**Inflammation Stress Induces Lipid Redistribution in db/db Mice via the Disruption of LDL Receptor Pathway** Kun Ling Ma,[1] Yang Zhang,[1] Jing Liu,[1] Wu Yu,[1] Bi-Cheng Liu.[1] *[1]Institute of Nephrology, Southeast Univ School of Medicine, Nanjing, China; [2]Institute of Nephrology, Southeast Univ School of Medicine, Nanjing, China; [3]Institute of Nephrology, Southeast Univ School of Medicine, Nanjing, China.*

**Background:** Dyslipidemia and inflammation play crucial roles in the progression of diabetic nephrology (DN). Our previous studies *in vivo* and *in vitro* demonstrated that inflammation induced lipid accumulation in liver, aorta, and kidney in apolipoprotein E knockout mice through the disruption of low-density lipoprotein receptor (LDLr) feedback regulation. This study aimed to investigate the effects of inflammation on lipid redistribution in DN.

**Methods:** Twenty db/db mice were injected with casein to induce an inflamed DN model.Serum levels of inflammatory cytokines and lipid profile were respectively measured by enzyme-linked immunosorbent assay and clinical biochemistry assay. The effects of inflammatory stress on lipid accumulation in liver, aorta, and kidney were evaluated by hematoxylin-eosin staining, Oil Red O staining, filipin staining, and intracellular cholesterol quantitative assay. The protein expression of low density lipoprotein receptor (LDLr), sterol regulatory element binding protein-2 (SREBP-2) and SREBP-cleavage-activating protein (SCAP) in tissues was checked by immunohistochemical staining, immunofluorescent staining, or Western blotting.

**Results:** Significantly elevated serum levels of tumour necrotic factor-α and serum amyloid A in casein injected mice confirmed successful induction of inflamed diabetic model. Serum levels of lipid profile (total cholesterol and triglyceride, LDL and high density lipoprotein) in flamed mice were no obvious difference compared to the controls. Inflammatory stress increased lipid accumulation in liver, aorta, and kidney. Further analysis showed that inflammatory stress increased the protein expression of LDLr, SREBP-2, and SCAP in liver, aorta, and kidney of inflamed mice.

**Conclusions:** Inflammatory stress may induce lipid redistribution of db/db mice from circulation to target organs. The potential mechanism is through the disruption of LDLr feedback regulation.

## SA-PO330

**Role of HDAC in the Development of Type 2 Diabetes Related Nephropathy** Hang Yuan, Nian Liu, Ye Jia, Yingchun Cui, Ping Luo, Lining Miao. *Div of Nephrology, Second Hospital of Jilin Univ, Chang Chun, Ji Lin, China.*

**Background:** Histone deacetylase (HDAC) emerged as a new molecular target in the control of obesity and type 2 diabetes. Inhibition of HDACs has been demonstrated as players in ameliorated renal fibrosis, and the internal mechanism is associated with profibrotic genes regulation such as collagen and PAI-1. But the role of HDACs in the process of diabetic complications development is still unclear. In this study, we evaluated potential role of HDAC in type 2 diabetes related nephropathy.

**Methods:** Blood karyocyte and serum were separately collected from 20 health adults, new diagnosed type 2 diabetes (18 cases) as well as type 2 diabetic renal dysfunction patients (23 cases). We detected HDAC genes expression by QRT-PCR. In vivo relevance was tested using renal cortex come from high fat diet with streptozotocin injection induced type 2 diabetic rat, as well as diabetic rat fed with valproic acid, which was known as HDAC inhibitor.

**Results:** In compare with health adults, HDAC 1, 2, 3, 4, 10, 11 mRNA expression were increased in new diagnosed diabetes patients; while HDAC 2, 5, 9, 11 and SIRT 1 mRNA expression were significantly enhanced but HDAC 10, SIRT 4 expression decreased in diabetic renal dysfunction patients compared with new diagnosed diabetes patients. Interestingly, in vivo results showed that diabetic rats have more HDAC 1, 2, 3, 5, 11 mRNA expression in the renal cortex in compare with none diabetic control group; valproic acid administration inhibited HDAC 1, 2, 3, 11 expression; at same time increased proteinuria as well as profibrotic genes such as collagen 1a1, CTGF and α-smooth muscle actin (α-SMA) in diabetic kidney were attenuated by valproic acid.

**Conclusions:** These results demonstrate that overexpressed HDACs such as HDAC 1, 2, 3, 5, 9, 11 may involve in the pathogenesis of type 2 diabetes and related nephropathy; select inhibition of HDACs by valproic acid attenuated renal fibrotic genes expression and diabetes related proteinuria, therefore suggest HDACs might be a new target in the control of diabetic nephropathy.

*Funding:* Government Support - Non-U.S.

## SA-PO331

**Non-Esterified Fatty Acid Levels Modulate Adiponectin and Insulin Sensitivity in a Rat Model of Type 2 Diabetes Mellitus through a Nitric Oxide Dependent Mechanism** Sharma S. Prabhakar. *Internal Medicine, Texas Tech Univ Health Sciences Center.*

**Background:** Mechanisms leading to insulin resistance in type 2 diabetes mellitus (T2DM) remain incompletely understood. Adiponectin levels and signaling mechanisms are down regulated in obesity induced T2DM. Recent work from our laboratory showed that angiotensin blockade enhanced insulin sensitivity besides the renoprotection in diabetic rats (J Invest Med 2013). We hypothesized that adiponectin activity and insulin signaling is regulated by non-esterified fatty acid (NEFA) levels in obesity induced DM.

**Methods:** To test our hypothesis we used obese ZSF rat, a murine model of T2DM previously characterized in our laboratory (JASN 2007). Lean ZSF rats were used as controls as they do not develop DM. Obese rats were fed high calorie diet while control rats were given standard rat chow. Another group of obese ZSF rats received losartan (1mg/ml). All rats were obtained at 7th week and euthanized at 26 weeks and adipose tissue harvested. Plasma samples were collected at the start and end of the study to measure adiponectin (by ELISA), NEFA levels(by colorimetric assay) and Nitric Oxide metabolites or Nox (by chemiluminescence). Adipose tissue was homogenized and the protein was examined for the expression of adiponectin receptor 1, insulin receptor substrate 1 and glucose transporter GLUT 4 using immunoblotting.

| Group | Lean | Obese | Obese+Losartan |
|---|---|---|---|
| Adiponectin (μg/ml) | 4.15±0.56 | 2.93±0.44* | 3.89± 0.39 |
| NEFA (mM/L) | 0.194± 0.021 | 0.562± 0.042# | 0.275± 0.033 |
| NOx (μM) | 28±3.1 | 7.8±1.6# | 13.5± 2.4* |

N=6 in all groups, *p,0.01 #p<0.001 compared to lean rats.

**Results:** Our results showed that the plasma levels of adiponectin and NOx were decreased and NEFA levels increased in obese ZSF rats compared to lean ZSF rats. Furthermore the expression of AdipoR1 and IRS1 and GLUT4 were decreased in fat cells of obese rats compared to lean counterparts. Losartan treatment increased NOx and adiponectin levels and insulin sensitivity.

**Conclusions:** We conclude that NEFA may be involved in modulation of adiponectin activity and insulin signaling through mechanisms that involve NO generation in murine T2DM.

*Funding:* Private Foundation Support

## SA-PO332

**Glycemic Memory in Diabetes Nephropathy, Potential Role of SHP-1**
Farah Lizotte,[1] Nicolas Drapeau,[1] Andréanne Guay,[1] Chris R. Kennedy,[2] Marie-Odile Guimond,[1] Pedro Miguel Geraldes.[1] *[1]Medecine, Div of Endocrinology, Univ of Sherbrooke, Quebec, Canada; [2]Kidney Research Center, Canada.*

**Background:** Renal podocytes apoptosis induced by hyperglycemia and insulin resistance is an early event leading to diabetic nephropathy (DN).Intensive blood glucose control reduced the development of DN. However, if not started early in the disease, it is not sufficient to prevent DN progression, introducing the concept of **"**glycemic memory**"**. Recent studies demonstrated that SHP-1, a protein tyrosine phosphatase, is elevated in renal cortex of type 1 diabetic mice (Akita)causing insulin unresponsiveness and DN. We hypothesize that SHP-1 expression remains elevated in spite systemic blood glucose normalization after 4 months of diabetes that triggers insulin inhibition, podocytes apoptosis and glycemic memory in DN.

**Methods:** The in vivo contribution of SHP-1 in glycemic memory was evaluated using Akita mice treated with insulin implants after 4 months of diabetes. Also, mouse and human podocytes were cultured in normal (5.6mM; NG), high glucose concentrations (25mM; HG) or HG prior returning glucose levels to NG for an addition 24-48h (HG+NG).

**Results:** Renal function was assessed by measuring urinary albuminuria secretion and glomerular filtration rate. Both parameters were significantly increased in diabetic mice and remained elevated after normalization of blood glucose levels, compared to non-diabetic mice. Renal dysfunction was associated with a 98% increase of SHP-1 expression in renal cortex and insulin action inhibition that were not normalized following insulin implants to decrease glucose levels. In vitro, we observed that Akt and ERK phosphorylation induced by insulin (10 nM) was reduced in HG and maintained lower following glucose normalization (HG+NG). The increase of SHP-1 protein and mRNA expression in HG conditions remained elevated in spite returning glucose concentration from HG to NG.

**Conclusions:** In conclusion, hyperglycemia causes persistent and irreversible high expression of SHP-1 leading to podocyte unresponsiveness to insulin leading to glycemic memory in DN.

## SA-PO333

**TGF-β1-Mediated Signalling Is Increased by TZDs through Increased IHG-1 Expression**
Fionnuala B. Hickey,[1] James B. Corcoran,[2] Finian Martin,[2] Madeline Murphy,[2] Catherine Godson.[2] *[1]Trinity Health Kidney Centre, Trinity College Dublin, Dublin 2, Ireland; [2]UCD Diabetes Complications Research Centre, Univ College Dublin, Dublin 4, Ireland.*

**Background:** *Induced in high glucose-1* (IHG-1) is a highly conserved transcript upregulated in experimental models of renal fibrosis and in human diabetic nephropathy (DN). We have reported that IHG-1 enhances responses to TGF-β1 [Murphy *et. al.* JASN 2008]. IHG-1 is a mitochondrial protein and increases mitochondrial mass through stabilisation of PPARγ coactivator 1-alpha (PGC-1α) [Hickey *et. al.* JASN 2011]. Thiazolidinediones (TZDs) are PPARγ agonists that act as insulin sensitising agents and are effective therapeutic agents in diabetes. Here we have explored the effect of TZDs on TGF-β1-mediated signalling and fibrosis.

**Methods:** mRNA was measured by qRT-PCR. Mitochondrial biogenesis was assessed by comparison of mitochondrial to nuclear DNA and by analysis of a TFAM promoter reporter. Oxygen consumption was measured using a Seahorse XF analyser. TGF-β1-mediated signalling was analysed via western blotting. Expression of endogenous IHG-1 was inhibited using tetracyclin-inducible shRNAi.

**Results:** We demonstrate that increased IHG-1 expression in a human kidney epithelial cell line (HK2) results in enhanced mitochondrial biogenesis and oxygen consumption. We have confirmed that TZDs increase mitochondrial biogenesis. Critically we report that treatment of renal epithelial cells with TZDs results in increased IHG-1 expression. Renal tubular hypoxia has been proposed as a mechanism of disease in DN. In this context it is likely that increased oxygen consumption due to upregulated IHG-1 would exacerbate tubulointerstitial hypoxia. In addition, we demonstrate IHG-1-dependent enhancement of TGF-β1-mediated signalling downstream of TZDs.

**Conclusions:** Increased mitochondrial biogenesis has been postulated as a therapeutic benefit of TZDs. However, sustained increases in mitochondrial biogenesis would lead to elevated levels of IHG-1, which we have linked to increased oxygen consumption and enhanced TGF-β1-mediated signalling. Taken together, this leads us to hypothesise that TZD-mediated increases in IHG-1 may drive the hypoxia-fibrosis axis in DN.

*Funding:* Government Support - Non-U.S.

## SA-PO334

**Astragaloside IV Attenuates Glycated Albumin-Induced Proximal Tubular Epithelial-to-Mesenchymal Transition via Inhibiting ERK1/2 and JNK Phosphorylation**
Yong Gu,[1,2] Jianying Niu,[1] Weiwei Qi.[1] *[1]Div of Nephrology, The Fifth People's Hospital of Shanghai, Fudan Univ, Shanghai, China; [2]Div of Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China.*

**Background:** Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease (ESRD) in developed countries. Tubulointerstitial accumulation of extracellular matrixc leads to tubulointerstitial fibrosis which closely correlates with the development of ESRD. The epithelial-to-mesenchymal transition (EMT) is the main source of ECM in kidney. Our previous study showed that glycated albumin (GA) could cause EMT in tubular cells and Astragaloside IV (AS- IV), the main active ingredients of *Astragalus membranaceus,* alleviated such damage by inhibiting oxidative stress. In order to further study the mechanism of AS-IV on tubular cells in DKD, we observed the changes of redox balance and MAPK pathway by adding AS-IV under GA stimulation.

**Methods:** The NRK-52E cells's intercellular reactive oxygen species (ROS) level was detected by $H_2$ DCFDA. NADPH oxidase activity was assayed by adding exogenous NADPH oxidase. The activities of superoxide dismutase (SOD), glutathione peroxides (GSH-Px) and catalase (CAT) were tested by assay kit. Western blot was used to examine the protein expression of α-SMA, E-cadherin, phosphorylation of ERK1/2, JNK and p38 MAPKs.

**Results:** AS-IV significantly attenuated GA-induced generation of ROS, lowered the increased level of NADPH oxidase activity. GA reduced the contents of SOD, GSH-Px and CAT in NRK-52E cells but there was no obvious change by adding AS-IV. Moreover, GA-induced NRK-52E cells showed increased expression of α-SMA and decreased expression of E-cadherin which could be attenuated by AS-IV. GA stimulated the phosphorylation of ERK1/2, p38 and JNK and AS-IV reversed the changes in ERK1/2 and JNK but not p38 phosphorylation.

**Conclusions:** Our data demonstrate that GA could induce tubular cells EMT through oxidative stress. Supplementation of AS-IV attenuates GA-induced EMT. AS-IV weakens the oxidative stress by inhibited NADPH oxidase activity but not increasing the contents of antioxidants. ERK1/2 and JNK intracellular signaling pathways, not p38 pathway, mediate the effect of AS-IV on EMT.

*Funding:* Government Support - Non-U.S.

## SA-PO335

**PKC-Alpha Mediates Albuminuria by Heparanase-Dependent Loss of Endothelial Cell Glycocalyx in Diabetic Mice**
Joon-Keun Park, Torsten Kirsch, Nelli Shushakova, Anna Bertram, Putri Andina Agustian, Jan Menne, Hermann G. Haller. *Clinic of Hypertension and Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** We have recently demonstrated that hyperglycemia induces albuminuria in hyperglycemic mice via the activation of PKC isoform alpha. Since the polysaccharide-rich endothelial glycocalyx and surface layer (ESL) acts as a filtration barrier, and heparanase is the predominant enzyme that degrades heparan sulfate (HS), the main polysaccharide of the ESL we tested the hypothesis that PKC-alpha in hyperglycemia leads to loss of ESL on glomerular endothelium via the activation of heparanase.

**Methods:** PKC-α-/- and wild-type (WT) mice received STZ or buffer. After 2 or 8 weeks of hyperglycemia, the animals were analyzed. Albumin in urine was measured by ELISA. Immunohistochemistry was performed on cryostat /paraffin sections. EM analysis of ESL was carried out after perfusion with Alcian blue. Gene and protein expression was analyzed by real-time qPCR and western blot analysis.

**Results:** PKC-α was predominantly expressed in the glomeruli. Under diabetic conditions, an increased expression of PKC-α in glomeruli was observed. The increase in albumin excretion was prevented in hyperglycemic PKC-α-/- mice. Only a weak expression of heparanase was observed in nondiabetic control animals of both groups. Under hyperglycemic conditions, a significant increase of heparanase was observed in the WT but not in PKC-α-/- mice. Loss of HS in the glomeruli was significantly reduced in PKC-α-/- mice. We also observed that ESL-associated molecules such as thrombomodulin were regulated by PKC-α.

**Conclusions:** Hyperglycemia-mediated loss of endothelial glycocalyx is mediated by the PKC isoform alpha. PKC-alpha regulates heparanase expression under diabetic conditions. Inhibition of PKC-alpha in diabetic nephropathy may restore damaged glycocalyx and thereby influence inflammation and proteinuria.

## SA-PO336

**Low Dose of Vitamin D Analog, Paricalcitol, May Promote Carbonyl Stress and Kidney Disease in Diabetic Mice**
Xiaoyi Zheng, Wuxing Dong, Vivek Bhalla. *Medicine / Nephrology, Stanford Univ School of Medicine, Stanford, CA.*

**Background:** Diabetic nephropathy (DN) represents the primary cause of morbidity and mortality for patients with diabetes mellitus. Because Vitamin D analog therapy has been shown to attenuate diabetic nephropathy in combination with losartan, and genetic deletion of the Vitamin D receptor worsens diabetic nephropathy, we investigated the role of carbonyl stress in diabetic mice treated with paricalcitol, 19-nor-1,25-dihydroxyvitamin D2, an activated vitamin D analog.

**Methods:** Diabetic DBA/2J mice were induced by STZ injection (40mg/kg body weight) for 5 consecutive days, then administered paricalcitol (0.4 ug/kg body weight) or vehicle intraperitoneally thrice weekly for 24 weeks. Diabetes was validated by glucose tolerance tests at the end of the study. Urine albumin to creatinine ratio, glomerular volume

and podocyte number, and quantification of the carbonyl stress marker, 4-hydroxynonenal (4-HNE) were tested. Additionally, MES-13 mesangial cells were treated with 4-HNE and paricalcitol vs. vehicle prior to qPCR.

**Results:** Fewer diabetic mice survived until the study end-point with a trend toward decreased survival in the paricalcitol-treated vs. vehicle-treated diabetic mice. Surprisingly, paricalcitol-treated mice had significantly higher albuminuria (207.7 +/- 35.3 vs. 31.0 +/- 14.7 ug albumin / mg creatinine, $p<0.05$). Glomerular volume was not significantly different between these two groups, but there were significantly fewer podocytes per glomerulus in the paricalcitol-treated mice. Glomerular 4-HNE was lower in paricalcitol-treated mice (0.47 +/- 0.01 vs. 0.31 +/- 0.05 positive cells/mm2, $p<0.05$). Low-dose paricalcitol (0.5 nM) significantly increased 4-HNE-induced fibronectin1 expression while higher doses (5 nM or 50 nM) were not significantly different or lower vs. vehicle alone. Paricalcitol did not modulate 4-HNE-induced TGF-B1 expression vs. vehicle.

**Conclusions:** Taken together, in contrast to shorter duration of therapy, low dose, longer-term paricalcitol treatment may worsen diabetic nephropathy and survival, possibly by modulating the response to 4-HNE through increased fibronectin production, podocytopenia, and consequently increased albuminuria.

*Funding:* Pharmaceutical Company Support - Abbott-Renal Care

## SA-PO337

### Effects of Cannabinoid Receptor Type 2 (CB2) Agonist in a Mouse Model of Type 2 DN

Carlamaria Zoja,[1] Daniela Rottoli,[1] Daniela Corna,[1] Monica Locatelli,[1] Mauro Abbate,[1] Agnes Benardeau,[3] Karin Conde-knape,[3] Giuseppe Remuzzi,[1,2] Ariela Benigni.[1] *[1]IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; [2]Unit of Nephrology and Dialysis, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; [3]F. Hoffmann-La Roche AG, Basel, Switzerland.*

**Background:** Experimental evidence suggested that CB2 activation had a protective role in early STZ-induced diabetes in mice. Here we investigated the effects of the CB2 agonist RO6806207 given at a phase of overt disease in BTBR *ob/ob* mice, a recently characterized model of type 2 DN.

**Methods:** BTBR *ob/ob* mice received from 10 to 21 wks of age vehicle, CB2 agonist (10mg/kg i.p.) or lisinopril (30mg/kg in drinking water) as standard therapy for comparison (n=7-9 mice/group). BTBR wild-type mice (n=6) served as controls.

**Results:**

| Groups | Albuminuria (ug/24h) | Albuminuria (ug/24h) | Mesangial matrix (score) | Mesangial sclerosis % | Interstitial F4/80+cells/HPF |
|---|---|---|---|---|---|
| | 16wks | 21wks | | | |
| D+vehicle | 211±41 | 245±41 | 1.3±0.10 | 9.3±2.0 | 53±9 |
| D+CB2 | 86±16* | 158±30 | 0.9±0.05* | 3.5±0.9* | 34±12 |
| D+ACEi | 63±14** | 111±24* | 0.9±0.11** | 3.1±0.7* | 21±7* |
| Control | 30±4 | 36±8 | 0 | 0 | 18±6 |

Data are mean±SE; *p<0.05, **p<0.01 vs Diabetic mice (D)+vehicle. HPF: high power field

Both CB2 agonist and lisinopril reduced albuminuria. The antiproteinuric effect was associated with amelioration of defective nephrin expression of diabetic mice. CB2 agonist limited renal lesions to a similar extent as ACEi. Interstitial inflammation was lowered by 36% and 60% after CB2 agonist and lisinopril, respectively. Both compounds reduced osteopontin and TGFβ1 urinary excretion. Treatments did not affect hyperglycemia and dyslipidemia of diabetic mice.

**Conclusions:** CB2 agonist halted progressive renal damage when given to BTBR *ob/ob* mice during overt nephropathy. These findings may have therapeutic implications for type 2 DN.

*Funding:* Pharmaceutical Company Support - F. Hoffmann-La Roche AG, Basel, Switzerland

## SA-PO338

### L-carnitine Protects against Cyclosporine-Induced Pancreatic and Renal Injury in Rats

Can Li,[1] Chul Woo Yang.[2] *[1]Nephrology, YanBian Univ Hospital, YanJi, JiLin, China; [2]Nephrology, The Catholic Univ of Korea, Seoul, Korea.*

**Background:** L-carnitine has a protective effect against various types of injury. This study was designed to evaluate the beneficial effects of L-carnitine on pancreatic and renal injuries caused by cyclosporine A (CsA).

**Methods:** Rats maintained on a low sodium diet were given vehicle (olive oil, 1 mL/kg/day), CsA (15 mg/kg/day), L-carnitine (50 or 200 mg/kg/day), and a combination of CsA and L-carnitine for 4 weeks. The impact of L-carnitine on pancreatic injury was assessed by blood glucose level, plasma insulin concentration, and HbA1c. In addition, the protective effect of L-carnitine against CsA-induced renal injury was evaluated in terms of renal function, histopathology (inflammatory cell influx and tubulointerstitial fibrosis), oxidative stress (8-hydroxy 2'-deoxyguanosine, 8-OHdG), the transforming growth factor-beta1 (TGF-β1), apoptosis (caspase-3), and autophagy (LC3-II).

**Results:** CsA treatment caused diabetes, renal dysfunction, tubulointerstitial inflammation (ED-1-positive cells), and fibrosis, which were accompanied by an increase in 8-OHdG production and upregulation of TGF-β1, caspase-3, and LC3-II. Concomitant administration of L-carnitine increased plasma insulin concentration and decreased the levels of plasma glucose and HbA1c. In the kidney, L-carnitine induced a dose-dependent improvement of renal function, inflammation, and fibrosis in parallel with suppression of the expression of TGF-β1 and 8-OHdG. Furthermore, the administration of L-carnitine at a high dose inhibited the expression of caspase-3 and LC3-II.

**Conclusions:** These findings suggest that L-carnitine has a protective effect against CsA-induced pancreatic and renal injuries.

## SA-PO339

### Effects of Plant Steroid α-Spinasterol on Regulating Cell Proliferation and TGF-β Signaling Pathways through Thrombospondin-1 in Mesangial Cells

Jung Sub Song, Ju-Hung Song, Seon-Ho Ahn. *Internal Medicine, Wonkwang Univ College of Medicine & Hospital, Iksan, Jeonbuk, Republic of Korea.*

**Background:** TGF-β is the central cytokine responsible for the development of diabetic nephropathy, and usually secreted as a latent procytokine complex that has to be activated before it can bind to its receptors. It is reported that thrombospondin-1 (TSP-1) is the major activator of latent TGF-β in diabetic nephropathy in vivo. So this study is to examine the effect of α-spinasterol on TSP-1 production and downstream of TGF-β through TSP-1 induced by high glucose in mesangial cells.

**Methods:** α-spinasterol was isolated from roots of *Phytolacca americana*. Mouse mesangial cells were incubated for 24 hour with normal (5.6 mM) or high (30 mM) glucose-containing medium in the presence or absence of α-spinasterol (1 ng/mL). Mesangial proliferation was determined by cell number. Real time qRT-PCR was performed to observe the TSP-1, TβRI, TβRII, and fibronectin mRNA expression. Protein expressions of TSP-1, Smad2/3, p38, and fibronectin were determined by western blot.

**Results:** α-spinasterol inhibited the high glucose-stimulated proliferation of mouse mesangial cells, but did not affected to the growth of mouse mesangial cells cultured under normal glucose. TSP-1 mRNA expression and protein expression was increased in cultured mouse mesangial cells under the high glucose concentration. α-spinasterol attenuated TSP-1 mRNA expression and protein expression in cultured mouse mesangial cells under the high glucose, but not under the normal glucose concentration. TGFR1 mRNA expressions was increased in high glucose-stimulated mesangial cell, but decreased by α-spinasterol. Smad2/3 and p38 activities were increased in high glucose-stimulated mesangial cell, but decreased by α-spinasterol. Fibronectin mRNA and protein expression were decreased by α-spinasterol.

**Conclusions:** we demonstrated that α-spinasterol attenuated TSP-1 protein expression in mesangial cells under the high glucose, and resulted in regulating cell proliferation and TGF-β signaling pathways, Smad2/3 and p38 signaling. These results suggest that α-spinasterol may be considered as a promising future treatment option for diabetic nephropathy.

## SA-PO340

### Anti-Oxidative Effect of β-Blocker Carvedilol on Diabetic Nephropathy

Yuriko Yonekura,[1] Hideki Fujii,[1] Shunsuke Goto,[1] Kentaro Nakai,[1] Keiji Kono,[1] Riko Kitazawa,[2] Masami Shinohara,[3] Shinichi Nishi.[1] *[1]Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Japan; [2]Div of Molecular Pathology, Dept of Biomedical Informatics, Kobe Univ Graduate School of Medicine, Kobe, Japan; [3]CLEA Japan, Inc., Tokyo, Japan.*

**Background:** Oxidative stress has a major role in the pathogenesis of diabetic nephropathy. β-blocker carvedilol has been shown to have a protective effect from oxidative stress. In this study, we investigated the effect of carvedilol on the progression of diabetic nephropathy using non-obese type 2 diabetes mellitus model rats.

**Methods:** In the present study, we used Spontaneously Diabetic Torii (SDT) rats, which is a model of non-obese type 2 diabetes. Sprague-Dawley (SD) rats were used as control (SD, n = 5). At 20 week, SDT rats were divided into three groups: vehicle-treated SDT rats (DM, N=6), Insulin-treated SDT rats (INS, N=6), and carvedilol-treated SDT rats (CAR, N=6). At 30 week, blood and urine analysis, histomorphometrogical analysis, analysis of mRNA expression of NADPH oxidase and blood pressure measurement were performed among these four groups.

**Results:** At baseline, characteristics of these rats were comparable. At 30 week, despite comparable blood pressure, urinary excretion of albumin and 8-hydroxydeoxyguanosine (8-OHdG) were lower in the CAR group than in the DM group. In addition, relative kidney weight was lower in the CAR group compared to the DM group. The mRNA expressions of NADPH in the whole kidney were significantly decreased in the CAR group compared to the DM group. Further iummunohistological analysis revealed that the number of 8-OHdG positive cell in glomeruli and tubules was reduced by administration of carvedilol.

**Conclusions:** Our findings suggested that carvedilol prevent the progression of glomerular and tubular injury possibly through the reduction of oxidative stress in non-obese type 2 DM.

## SA-PO341

### COX-2, but Not mPGES-1 Mediates Renal PGE2 Synthesis and Nephropathy in STZ-Induced Type-1 Diabetes

Ying Sun,[1,2] Zhanjun Jia,[1,2] Kevin Yang,[1] Mi Liu,[1] Tianxin Yang.[1,2] *[1]Internal Medicine, Univ of Utah, Salt Lake City, UT; [2]VA Medical Center, Salt Lake City, UT.*

**Background:** It has been shown that local PGE2 in kidney is elevated in streptozotocin (STZ) induced diabetic mice and blockade of PGE2 action attenuates the diabetic nephropathy (DN). Present study is to elucidate whether mPGES-1, the best characterized PGE2 synthase, is involved in the development of diabetic nephropathy.

**Methods:** mPGES-1 WT and KO mice were employed to induce type I diabetes by STZ (120mg/kg IP).

**Results:** After STZ treatment for 6 weeks, mPGES-1 KO and WT mice presented the similar elevations of blood glucose (WTSTZ: 430±10.25 vs. KO STZ 436±47.8mg/dl, $p>0.01$). Meanwhile, both genotypes exhibited the paralleled increases of urinary and renal PGE2 productions (Urine: WTCont: 892.98±134.2 vs. WTSTZ: 4165.0±801.7 pg/24h, $p<0.01$; KOCont: 360.5±33.4 vs. KOSTZ 3145.1±465.4 pg/24h, $p<0.01$; kidney: WTCont: 810.9±48.4 vs. WTSTZ: 1153.3±147.8 pg/mg protein, $p<0.05$; KOCont: 634.7±76.6 vs.

KOSTZ: 1057.9±120.0 pg/mg protein, p<0.05). In parallel with these findings, kidney injury indexes including urinary ulbumin excretion (WTCont: 20.3±4.7 vs. WTSTZ 79.61±15.1 ug/24h, p<0.01, KOCont: 24.15±6.9 vs. KOSTZ: 84.12±15.1 ug/24h, p<0.01), kidney weight (WTSTZ: 0.825±0.032 vs. KOSTZ: 0.79±0.029%, p>0.05), and kidney histological changes (PAS staining) didn't show any difference between WT and KO mice. By western-blotting and quantitative real-time PCR, mPGES-1, mPGES-2, cPGES and 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) remain unaltered after six weeks of diabetes. However, administration of selective COX-2 inhibitor celecoxib (50mg/kg/day) significantly reduced urinary albumin excretion in KO mice (KOSTZ: 141.5±38.4 vs. KOSTZ+Celebrex: 48.7±20.8 ug/24h, p<0.05), as well as renal PGE2 content(KOSTZ: 588.7±89.2 vs. KOSTZ+Celebrex: 340.8±58.7 ug/24h, p<0.05), without affecting blood glucose(KOSTZ: 421.4±44.4 vs. KOSTZ+Celebrex: 375.8±28.8 mg/dl, p>0.05).

**Conclusions:** COX-2, but not mPGES-1, is responsible for renal PGE2 synthesis and the development of DN in STZ-induced diabetes. These results also suggest the existence of an unknown PGE2 synthase contributing to renal PGE2 production under diabetic status.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO342

**Angiotensin-Receptor Blockade Does Not Protect against Early Renal Tubular Injury in Streptozotocin-Induced Diabetes** Emily W. Nance, John Jason White. *Medicine, Georgia Regents Univ, Augusta, GA.*

**Background:** Diabetes is the leading cause of renal failure in the United States. Blocking the renin-angiotensin system (RAS) protects against glomerular injury but does not prevent the ultimate decline in renal function. Disease progression is strongly linked to the degree of tubulointerstitial injury, therefore, we examined the effects of RAS blockade on renal tubular injury in a rat model of diabetes.

**Methods:** Rats were made diabetic using streptozotocin (STZ) (I.V. 65mg/kg) and were supplemented with insulin allowing for modest hyperglycemia (n=10). One group (n = 5) received an angiotensin receptor blocker (ARB), candesartan (3 mg/kg/day) for 4 weeks. Blood glucose levels were monitored twice weekly and blood pressures were obtained weekly using the tail cuff method. After 4 weeks, rats were placed in metabolic cages to measure dietary intake and to collect 24 hour urine for protein and creatinine, and were then sacrificed. Tubular injury was assessed by measuring the urinary biomarkers, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and N-acetyl-β-D-glucosaminidase (NAG) using ELISA.

**Results:** As expected, all rats exhibited polydipsia, polyphagia, and hyperglycemia. ARB treatment resulted in lower blood pressure ($P < 0.05$) and a trend towards lower urinary protein excretion ($P = 0.08$). Our previous studies demonstrated after 4 weeks of diabetes, there was an increase in urinary KIM-1, NGAL, and NAG in diabetic rats compared to controls without a concomitant rise in proteinuria. In this study, ARB treatment had no effect on urinary KIM-1, NGAL, or NAG.

**Conclusions:** In conclusion, short-term treatment with ARB does not prevent early tubular damage in STZ diabetes. These findings justify additional studies of longer duration and also highlight the need for alternate therapies targeting the tubulointerstitium in diabetic kidney disease.

## SA-PO343

**Inhibition of Nephrin Signaling by SHP-1 in Diabetic Nephropathy** Benoit Denhez,[1] Chris R. Kennedy,[2] Marie-odile Guimond,[1] Pedro Miguel Geraldes.[1] *[1]Univ of Sherbrooke, Canada; [2]Kidney Research Centre, Ottawa, Canada.*

**Background:** Diabetic nephropathy is the leading cause of end-stage renal disease and is characterized by dedifferentiation and apoptosis of podocytes, which are highly specialized epithelial cells involved in the glomerular filtration process. Nephrin, a transmembrane protein found in the slit diaphragm, has been found to play a role in the integrity of the podocytes. Clinical observations indicated that nephrin expression was reduced in kidney biopsy of diabetes patients. However, recent studies have shown that phosphorylation of tyrosine residues of nephrin participate in intracellular pathways regulating actin dynamics and play a role in podocyte survival. Our laboratory has recently published that the expression of SHP-1, a protein tyrosine phosphatase which contains two SH2 domains, is elevated in cultured podocytes exposed to high glucose concentrations. Since nephrin can interact with proteins that contain SH2 domains, we hypothesize that SHP-1 binds with nephrin and deregulates nephrin-mediated pathways in diabetes.

**Methods:** Six-months of age male Akita mice, a type 1 diabetes model, were used for in vivo experimentations. Akita mice develop early renal dysfunction characteristic of diabetic nephropathy such as elevated albuminuria, glomerular filtration rate and glomerular hypertrophy.

**Results:** Immunoblots of proteins extracted from the kidney cortex show that nephrin expression and phosphorylation was reduced by 35% and 40% respectively, while SHP-1 expression was elevated by 100%. In vitro, coimmunoprecipitation assays in HEK cells expressing both nephrin and SHP-1 showed an interaction between the two proteins. Immunoblot analysis indicated that this interaction decreased tyrosine phosphorylation of nephrin by 50% (p=0.0071). Moreover, SHP-1 overexpression reduced phosphorylation of nephrin tyrosine 1176/1193 and 1217 by 65% (p=0.0004) and 55% (p=0.0077), respectively.

**Conclusions:** In conclusion, our results suggest that diabetes triggered SHP-1 expression in podocytes which reduced nephrin tyrosine residue phosphorylation that participate in actin remodeling and survival and contributed to the development of diabetic nephropathy.

## SA-PO344

**The Effects of COMP-Angiopoietin-1 on Lipolysis, Vascular Endothelial Cells and Macrophages in Streptozotocin-Induced Diabetic Mice** Won Kim, Aesin Lee, Dal Kim, Kyung Pyo Kang, Yujin Jung. *Internal Medicine, Chonbuk National Univ Medical School, Jeonju, Republic of Korea.*

**Background:** Streptozotocin(STZ)-induced diabetes mellitus increased inflammatory immunity and STZ-induced hyperglycemia leads to diabetic microvascular complications like diabetic nephropathy. Angiopoietin-1 (Ang1) is a potent angiogenic factor. We previously demonstrated that a variant of Ang1, COMP-Ang1 preserves diabetic nephropathy. However, there is controversy in mechanism of COMP-Ang1 in kidney protection. Especially metabolic effects of COMP-Ang1 in STZ-induced diabetic complications remain to be clarified. Therefore, in this study, we examined effects of COMP-Ang1 on fat droplet size, vascular endothelial cell (EC), and macrophage infiltrations in epididymal adipose tissue and metabolic parameters in STZ-induced diabetic mice.

**Methods:** To investigate the effects of COMP-Ang1, we administered COMP-Ang1 or LacZ adenovirus intravenously 2 weeks after STZ injection and harvested epididymal adipose tissue and blood 4 weeks after STZ injection.

**Results:** COMP-Ang1 ameliorated STZ-induced decrease of fat droplet size (lipolysis) and preserved PECAM-1-positive vascular EC in EAT. We also found that COMP-Ang1 decreased F4/80–positive macrophage infiltration. COMP-Ang1 diminished CD86 (M1 macrophage) and FIZZ1 expression (M2 macrophage) in EAT. COMP-Ang1 reduced STZ-induced hyperglycemia (fasting glucose). Serum levels of triglyceride and free fatty acid after COMP-Ang1 treatment were decreased than that after STZ injection. Phosphorylation of Akt in adipose tissue was significantly increased after administration of COMP-Ang1. We also injected COMP-Ang1 at same time with STZ to evaluate the preventive effect. Co-treatment COMP-Ang1 preserved PECAM-1-positive EC and reduced macrophage infiltrations in EAT. In addition, COMP-Ang1 decreased random blood glucose level in STZ-induced diabetic mice.

**Conclusions:** All of these results demonstrate that COMP-Ang1 treatment can decrease STZ-induced inflammatory molecule and hyperglycemia, and conserve vascular EC and lipid droplet size. Our data suggest that COMP-Ang1 may ameliorate STZ-induced diabetic nephropathy through metabolic regulation.

*Funding:* Government Support - Non-U.S.

## SA-PO345

**Interactions of PPAR Alpha and the Canonic Wnt Pathway in Renal Fibrosis** Rui Cheng, Lexi Ding, Jian-xing Ma. *Dept of Physiology, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK.*

**Background:** Peroxisome proliferator-activated receptor alpha (PPARα) has displayed reno-protective effects in animal models. However, its mechanism of action is unclear. The canonic Wnt pathway is known to regulate pathophysiological processes including inflammation and fibrosis. This study was to test the hypothesis that PPARα regulates renal fibrosis through interactions with the canonic Wnt pathway.

**Methods:** PPARα$^{-/-}$ mice were crossed with the BAT-gal Wnt-reporter mice to generate PPARα$^{-/-}$ or PPARα$^{+/-}$ mice carried the BAT-gal transgene. Streptozotocin (STZ)-induced diabetes and db/db mice were used as diabetic models. Diabetes and UUO were induced in age-matched wild-type (Wt) mice and PPARα$^{-/-}$ mice. Primary renal tubular cells were cultured from Wt mice or PPARα$^{-/-}$ mice.

**Results:** In STZ-induced diabetic rats and db/db mice, renal PPARα expression was down-regulated, compared to non-diabetic animals. PPARα agonist, fenofibrate, reduced urine albumin and renal fibrosis in STZ-induced diabetic rats. PPARα$^{-/-}$ mice with 3 months of diabetes showed more severe protein urea and significantly enhanced renal expression of fibronectin and CTGF, compared to diabetic Wt mice. Diabetic PPARα$^{-/-}$ mice showed more prominent increases of non-phosphorylated β-catenin levels in the kidney, compared to diabetic Wt mice. Wnt3a condition medium induced more prominent increases of phosphorylated LRP6 levels and transcriptional activity of β-catenin in PPARα$^{-/-}$ cells compared to Wt cells. TGFβ induced more fibronectin expression in PPARα$^{-/-}$ cells than in Wt cells, which can be rescued by over-expression of PPARα. The relationship of PPARα with the canonic Wnt pathway was further confirmed in PPARα$^{-/-}$/BAT-gal mice. X-gal staining at 7 days after UUO operation showed significantly higher β-catenin transcriptional activities in PPARα$^{-/-}$/BAT-gal mice vs their PPARα$^{+/-}$/BAT-gal littermates.

**Conclusions:** The beneficial effects of PPARα on renal fibrosis are at least in part through interactions with the canonical Wnt pathway. The interaction between PPARα and the canonic Wnt pathway renders a new therapeutic target for renal fibrosis.

## SA-PO346

**Effect of Ace2 Deletion and the Role of Gonadectomy in Male Mice with Diabetic Nephropathy** Sergi Clotet-Freixas, Maria Jose Soler, Julio Pascual, Marta Riera. *Nephrology, Hospital del Mar - IMIM, Barcelona, Spain.*

**Background:** Whereas ace2 deletion accentuates renal disease, its amplification ameliorates diabetic nephropathy.Gonadectomy(GDX) in ace2KO diabetic(DB) male mice has not been previously studied.

**Methods:** We study ace2 deletion in STZ–induced male mice. Groups: Wild-type(WT) cont, WTdb, KOcont, KOdb, GDX-KOdb. Variables: blood pressure(BP), urinary albumin excretion(UAE), kidney/body weight(KW/BW), glomerular size(GS), serum(s) and kidney(k) ACE activity and renal ACE and collagenIV gene expression.

**Results:** KW/BW and sACE were significantly higher in KOcont as compared to WTcont. Blood glucose(BG), KW/BW, GS, UAE and sACE were significantly increased in DB mice. GDX significantly decreased these parameteres. kACE activity and gene

expression significantly decreased in DB mice, and correlated(r=0.6, p<0.01). ACE gene expression decreased in KO groups. CollagenIV was higher in DB and KO groups.

| | WTcont(n=18) | WTdb(n=9) | KOcont(n=15) | KOdb(n=9) | GDX-KOdb(n=11) |
|---|---|---|---|---|---|
| BG(mg/dl) | 211.3±6.4 | 530.1±32.1* | 196.5±8.5 | 518.7±34.4* | 245.2±12.8*† |
| KW/BW(%) | 1.0±0.0 | 1.3±0.1* | 1.1±0.0$ | 1.4±0.0* | 0.8±0.0*† |
| UAE(μgAlb/mgCr) | 18.5±2.5 | 308.0±133.9* | 14.4±2.4 | 144.8±55.4* | 14.6±3.1† |
| GS(μm²) | 3147.9±121.7 | 3809.8±149.5* | 3002.9±161.9 | 3420.4±140.9 | 2956.1±69.6† |
| SBP(mmHg) | 96.4±1.6 | 97.6±1.9 | 96.2±1.9 | 104.2±1.7*$ | 95.4±0.9† |
| sACE(RFU/μl/min) | 2143.9±133.4 | 2984.1±198.1* | 2699.7±174.5$ | 3395.3±218.1* | 2266.6±190.5*† |
| kACE(RFU/μg/min) | 213.7±18.8 | 83.1±21.1* | 148.1±13.1$ | 73.7±11.4* | 70.8±13.8* |
| kACEgene(vs. actinβ) | 1.0±0.1 | 0.4±0.1* | 0.8±0.0$ | 0.2±0.0*$ | 0.2±0.0* |
| kCollVα1gene(vs. actinβ) | 1.0±0.1 | 1.5±0.1* | 1.3±0.1$ | 1.8±0.1*$ | 1.5±0.1 |
| *p<0.05vs.cont;$p<0.05vs.WT;†p<0.05vs.KOdb | | | | | |

**Conclusions:** Ace2KO mice show renal hipertrophy and fibrosis. These alterations are significantly accentuated by DB and reduced by GDX.

*Funding:* Government Support - Non-U.S.

## SA-PO347

**Endoplasmic Reticulum Was Stressed in Rat Kidney on Hyperinsulinemia Induced by High Palmitate Feeding** Jianling Tao, Yingjiu Liu, Yubing Wen, Hang Li, Xuewang Li. *Div of Nephrology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing, China.*

**Background:** Insulin resistance is the basic pathophysiological feature of metabolic syndrome, carrying higher risk to the renal diseases onset and progression. Endoplasmic reticulum stress(ERS) is believed to play a triggering role in many diseases development. We tried to explore if ERS occur in kidney challenged by hyperinsulinemia induced by high palmitate feeding.

**Methods:** Four-week-old Wistar male rats (n=36) were either fed by a regular diet (Control n=18) or a high palmitate diet(n=18). At three, six and nine weeks later, six mice in either group were sacrificed. Body weight, serum insulin, creatinine, BUN, lipid profile, and glucose were assayed. Renal histology were reviewed by PASM. WT-1 stain was applied to trace podocyte loss. Kidney synaptopodin, and ERS marker Glucose-regulated protein 78 (GRP78) was assayed by Western Blot.

**Results:** Serum insulin level was significantly increased after six weeks' feeding by palmitate. Even without statistical significance, serum glucose and body weight tended to rise at the same time point. Lipid profile, and renal functions remain similar between two groups at any observation time point. Renal histology including WT-1 positive podocyte number and kidney synaptopodin amount did not change much during nine weeks' palmitate feeding. While GRP 78 protein expression started to significantly increase at six weeks after palmitate feeding and continue to be higher at the nine weeks' point.

**Conclusions:** ERS is initiated by hyperinsulinemia in kidney before measurable biochemical or histological abnormalities occur.

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*Funding:* Government Support - Non-U.S.

## SA-PO348

**New Vertebrate Model for Human Obesity-Related Glomerulopathy** Tomoko Obara. *Cell Biology, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK.*

**Background:** Obesity, an ongoing significant public health problem, is a part of a complex disease characterized as metabolic syndrome. Despite the growing public health imperative, substantial hurdles exist in developing new animal models to study the mechanisms of obesity-related kidney diseases and identify newer modes of therapy to slow progressive chronic kidney disease (CKD) and halt end-stage renal disease (ESRD). We have recently generated a versatile vertebrate model that allows rapid *in vivo* dissection of the mechanisms of obesity-related-glomerulopathy (ORG). This model is the genetically tractable medaka that in response to a high fat diet (HFD) develops ORG, which mimics the human counterpart.

**Methods:** In the present study, to explore the possibility that the adult medaka fed with HFD (HFD-medaka) can be used as an animal model for human ORG, we analyzed, serum glucose, glomerular histopathology and alterations in protein expression in the kidney of HFD-medaka.

**Results:** In 4 weeks, the HFD-medaka exhibited hyperglycemia, developed enlarged glomerulular (glomerulomegaly) and mesangial proliferation. In addition, by proteomic analysis with mass spectroscopy, 18 human CKD-related proteins were found up-regulated while two proteins [Neprilysin (Nep) and Mitochondrial enoyl CoA hydratase]were down-regulated in kidneys from HFD-medaka compared to medaka fed a control diet. Recent studies involving humans and mice also suggest that NEP has a role in obesity. *NEP* knockout mice as well as mice treated with a NEP inhibitor (candoxatril) developed obesity due to dysregulation of hyperglycemia and lipid metabolism. The role of NEP in ORG has not been studied. Therefore, we knocked down the *nep* gene in medaka on a control diet and found that they developed glomerulomegaly, suggesting that mutations in *nep* or Nep protein deficiency could result in ORG.

**Conclusions:** This is the first study to systematically investigate using HFD-medaka as a metabolic syndrome model that will help us to explore the mechanism underling human ORG, to identify protein markers and candidate genes for ORG. Successful completion of this study will lead us for new therapeutic strategies for patients in combating ORG and halting progression of CKD into ESRD.

## SA-PO349

**The Hypoglycemic Events in the Patients with Diabetic Nephropathy on Insulin Therapy and Its Clinical Impacts on Cardiovascular Systems** Hitoshi Minakuchi, Shu Wakino, Kozi Hosoya, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio Univ, Tokyo, Japan.*

**Background:** Since the patients with CKD (chronic kidney disease) have insulin resistance, impaired glyconeogenesis in the kidney and/or reduced degradation of insulin, the circadian profile of blood glucose is supposed to be disturbed in CKD. However, this derangement has not been analyzed fully in the patients with diabetic nephropathy (DN) especially those on insulin therapy.

**Methods:** We recruited 20 DN patients on insulin therapy from March 2011 to April 2013 at the Ichikawa Hospital of Tokyo Dental College. They were all in the G3-G5 levels of CKD. The circadian blood glucose and blood pressure levels were monitored with CGMS (Continuous glucose measurement system) and ABPM (ambulatory blood pressure monitoring), respectively. To assess their vascular or neurological complication, head CT (Computed Tomography), IMT (intima-Media thickness) in carotid artery ultrasound, ABI (Ankle Brachial Index), PWV (Pulse Wave Velocity), and CVR-R (Coefficient of Variation of R-R intervals) were measured.

**Results:** Among all 20 participants, events of hypoglycemia (BS<70mg/dl) were detected in 9 participants (45.0%) by CGM analysis. Patients with hypoglycemic event (Hypo(+)) showed significantly lower eGFR, lower HbA1c, higher serum glucagon, and higher urinary excretion of free cortisol and metanephrine as compared with those in patients without hypoglycemic events (Hypo(-)). PWV levels were higher in patients in Hypo(+) as compared with those in Hypo(-), although CVR-R values were not different between the two groups. Non-dipper type alteration of sleeping blood pressure measured in ABPM were detected 8 out of 9 in Hypo(+) (88.9%) and 4 out of 11 in Hypo(-) (36.4%) with significant difference. Multiple logistic regression analysis revealed that the event of hypoglycemia was the independent risk factor for non-dipper type blood pressure change.

**Conclusions:** Among DN patients on insulin therapy, the events of hypoglycemia were not infrequently detected. This event was associated with the cardiovascular organ damages through non-dipper type blood pressure change or activation of sympathomimetic nerve system.

*Funding:* Government Support - Non-U.S.

## SA-PO350

**Can Urine Glucose Be Used to Adjust Diabetes Therapy** Anil K. Mandal,[1] Linda M. Hiebert,[2] Harry J. Khamis.[3] *[1]Dept of Medicine, Univ of Florida, Gainesville, FL; [2]Dept of Veterinary Biomedical Sciences, Univ of Saskatchewan, Saskatoon, Canada; [3]Statistical Consulting Center, Wright State Univ, Dayton, OH.*

**Background:** Assay of urine glucose is simple and cheaper than finger-stick glucose testing. Validity of urine glucose assay to adjust diabetes therapy has not been widely studied. In the kidney, glucose is filtered and reabsorbed by the proximal tubules until blood glucose exceeds 180 mg/dL then appears in urine, 180 mg/dL is tubular maximum. This number may be less with reduced kidney function. Our aim was to gauge if urine glucose levels relate to blood glucose in fasting or postprandial periods, determining if urine glucose testing could replace blood glucose testing to adjust therapy. Average monthly cost in the USA of urine glucose testing, $17.00, is much less than finger-stick glucose testing, $78.00.

**Methods:** 66 diabetic patients were treated with insulin Glargine twice daily, and regular insulin with meals. Urine samples were tested for glucose (UG) in the fasting state (FUG) and at 2-hour postprandial (2hPPUG), along with blood samples [fasting blood glucose (FBG), 2hPP blood glucose (2hPPG) and fasting hemoglobin (Hgb) g/dL]. Serum creatinine (Scr, mg/dL) and estimated glomerular filtration (eGFR, ml/min) were obtained at both time periods (FScr, 2hPPScr and FeGFR, 2hPPeGFR, respectively). UG was verified by Roche chemstrip IOUA as normal (negative), trace (50 mg/dL), 1+ (100 mg/dL), 2+ (250 mg/dL), 3+ (500 mg/dL), 4+ (≥1000 mg/dL). Correlation between parameters was determined using Spearman's nonparametric correlation. P<0.05 was deemed significant.

**Results:** Correlation was high between FUG and FBG (P<0.0001, r=0.4867) but not between FUG and FScr or FeGFR (P=0.8810 and 0.2005; r=-0.0193 and 0.1635 respectively). Likewise correlation was high between 2hPPUG and 2hPPG (P<0.0001, r=0.5228) but not between 2hPPUG and 2hPP Scr or 2hPPeGFR (P=0.5002 and 0.5084; r=--0.0911 and 0.0886, respectively). No correlation was seen between Hgb and FUG (p=3078, r=0.1388).

**Conclusions:** These data suggest that urine glucose predicts changes in FBG and 2hPPG but these changes are independent of renal function.

**SA-PO351**

**A Gene Variant in the CERS2 Gene Is Associated with Worsening Albuminuria in Diabetic Patients of ON TARGET and TRANSCEND**
Dov Shiffman,[1] Guillaume Pare,[2] Judy Z. Louie,[1] Charles M. Rowland,[1] James J. Devlin,[1] Matthew McQueen.[2] *[1]Celera, Alameda, CA; [2]Population Health Research Institute, Hamilton Health Sciences and McMaster Univ, Hamilton, Canada.*

**Background:** Microalbuminuria is a risk factor for chronic kidney disease (CKD) among diabetics. However, the rate of albuminuria worsening varies among patients. Genome-wide association studies identified genetic variants associated with estimated glomerular filtration rate (eGFR). We asked whether these genetic variants were also associated worsening albuminuria among diabetic patients of the ON TARGET and TRANSCEND studies—two randomized controlled trials of ramipril, telmisartan, both, or placebo in patients with vascular disease or high-risk diabetes.

**Methods:** We investigated 16 genetic variants that were reported to be associated with eGFR at a genome-wide level ($P<5\cdot10^{-8}$). We evaluated the association of these variants with annual rate of change in urine albumin: creatinine ratio (uACR).

**Results:** We found that only one of the 16 variants (rs267734) was associated with differential rate of uACR progression (P=0.0013, P<0.05 after Bonferonni correction for testing 16 variants). The annual rate of increase in uACR was 11.3% (95%CI 7.5% to 15.3%) for carriers of two risk alleles, 5.0% (95%CI 3.2% to 6.7%) for heterozygotes, and 1.6% (95%CI -1.8% to 5.2%) for non-risk homozygotes, after adjustment for age, sex, hypertension, treatment group, self-reported ethnicity, and the principal components of genetic variability. Consistent with previous reports, this variant was also associated with baseline eGFR in those with European ancestry (P=0.002), although not in other ethnic groups. This variant (rs267734) is in almost perfect linkage disequilibrium ($r^2$=0.96) with rs267738, a single nucleotide polymorphism encoding an alanine to glutamic acid change at position 115 of the ceramide synthase 2 protein encoded by the CERS2 gene. This enzyme catalyzes the synthesis of dihydro-ceramide from sphinganine and is expressed in the kidney. However, it is not known how CERS2 function might influence albuminuria.

**Conclusions:** We found that rs267734 in CERS2 is associated with uACR progression rate among diabetics in ON TARGET and TRANSCEND.

*Funding:* Pharmaceutical Company Support - Celera, Government Support - Non-U.S.

**SA-PO352**

**The Association of Glucose Variability with Diabetic Complication in Korean Diabetic Patients**
Mi Jin Lee,[1] Jin Joo Cha,[1] Young Youl Hyun,[2] Dae R. Cha,[1] Jung Eun Kim,[1] Mihwa Lee,[1] Hye Kyoung Song,[1] Young Sun Kang.[1] *[1]Nephrology, Korea Univ Ansan Hospital, Ansan, Kyunggido, Republic of Korea; [2]Nephrology, Sungkyunkwan Univ Kangbuk Samsung Hospital, Seoul, Republic of Korea.*

**Background:** Tight glycemic control lowers the risk of diabetic complications, but it is uncertain that glycemic variability influences diabetic complications.

**Methods:** We examined the associations of plasma glucose variability with diabetic complications in a prospective cohort of 161 Korean diabetic patients from 2002 to 2013. Intra-personal mean and standard deviation(SD) of fasting plasma glucose calculated. Also we calculated percent coefficient of variation ( %CV= 100X SD/mean).

**Results:** Over the duration of 11 years follow up, diabetic retinopathy and neuropathy, nephropathy developed 14%, 9%, 9.9% of patients compared to start of study. Patients who had retinopathy until the end of study, had higher percentage of coefficient of variation(23.25±10.31 vs 18.46±9.40, p[thinsp]=0.005) than patients without retinopathy. Patients who had neuropathy, nephropathy until the end of study, had higher percentage of coefficient of variation(25.56±10.73 vs 18.15±8.74, p <0.001, 24.08±10.77 vs 18.12±8.66, p<0.001) than patients without neuropathy, nephroapthy. Also patients who progressed to cerebrovascular disease had higher percentage of coefficient of variation(27.94±12.09 vs 20.34±9.34, p =0.014) than non-progressor.

**Conclusions:** Taken together, long-term glycemic variability was association with diabetic complication.

**SA-PO353**

**Differential Contribution of 25-Hydroxyvitamin D Status to Insulin Resistance According to Body Mass Index in Normoglycemic Korean Population**
Seong Woo Lee, Sejoong Kim, Kwon Wook Joo, Chun Soo Lim, Yon Su Kim, Dong Ki Kim. *Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea.*

**Background:** There has been a paucity of literature on the interaction between 25-hydroxyvitamin D (25OHD) status and insulin resistance (IR) according to the severity of obesity in Asian population.

**Methods:** Data from the Korea National Health and Nutrition Examination Survey in 2008-2010 were analyzed. The study subjects comprised 12,895 normo-glycemic participants aged ≥ 20 years. IR was estimated by Homeostatic model assessment (HOMA). Obesity and its severity were assessed by body mass index (BMI) and its quartile. The association between HOMA-IR and 25OHD was assessed by multiple linear regression analysis. In addition, the breakpoint between HOMA-IR and 25OHD was assessed by segmented linear regression.

**Results:** In scatter plot of HOMA-IR against 25OHD with segmented linear regression line, we found the break point within the range of 10-20 ng/ml of 25OHD. In multiple linear regression analysis, the beta value of 25OHD on HOMA-IR was -0.016 (p<0.001). In subgroup analysis according to the severity of obesity, the beta values of 25OHD on IR were -0.003 (p=0.68), -0.007 (p=0.39), -0.028 (p=0.002) and -0.033 (p=0.001) in Q1-4 of BMI, respectively.

**Conclusions:** The relationship between 25OHD and IR was inverse, but there might be threshold value in the range of 10-20 ng/ml of 25OHD in decreasing IR. Since the effect of 25OHD on IR increased as BMI increased, the benefit from 25OHD supplementation in reducing IR might be maximized by stratified approach according to the severity of obesity. Future studies need to be followed.

**SA-PO354**

**Independent Predictors of Development of Albuminuria and GFR Decline in Japanese Type 2 Diabetic Patients**
Michino Takagi, Tetsuya Babazono, Izumi Nyumura, Yasuko Uchigata. *Diabetes Center, Tokyo Women's Medical Univ School of Medicine, Tokyo, Japan.*

**Background:** Diabetic kidney disease has been diagnosed by albuminuria and decreased eGFR; however, whether these renal manifestations share common risk factors in type 2 diabetic patients remain to be elucidated. The aim of this study was to determine independent predictors of albuminuria and eGFR decline in Japanese type 2 diabetic patients.

**Methods:** This was a single-center observational cohort study involving 1,803 Japanese type 2 diabetic patients with normoalbuminuria and an eGFR ≥ 60 mL/min/1.73 $m^2$. There were 740 women and 1,062 men, and the mean age was 58 ± 12 years. Two independent endpoints were specified: development of albuminuria (≥ 30 mg/g creatinine, albuminuria cohort, N=1,777) and eGFR decline (< 60 mL/min/1.73 $m^2$, GFR cohort, N=1,655). Cox proportional hazard model was used to identify significant predictors of each endpoint.

**Results:** During the median follow up period of 6.9 (IQR: 3.5-8.1) years for albuminuria cohort and 8.0 (IQR: 3.8-8.5) years for GFR cohort, 181 and 316 patients reached each endpoint, respectively: the 5-year cumulative incidence of reaching the endpoint was 8.3% for albuminuria cohort and 10.4% for eGFR cohort. In the multivariate Cox model, increased albuminuria, higher HbA1c levels, and the presence of diabetic retinopathy at baseline were commonly associated with both endpoints. Additional independent risk factors for albuminuria were male gender and elevated uric acid levels; those for eGFR decline were higher age, lower eGFR, and lower HDL cholesterol levels.

**Conclusions:** Identification of some distinct predictive factors for albuminuria and GFR decline suggests that different mechanisms are implicated in the development of these two renal manifestations in diabetic patients.

**SA-PO355**

**Urinary Sodium Excretion in Regard to All-Cause Mortality and GFR Decline in Diabetic Nephropathy**
Gudbjörg Andrésdóttir,[1] Tine Hansen,[1] Peter Rossing.[1,2,3] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Århus Univ, Århus, Denmark; [3]Copenhagen Univ, Copenhagen, Denmark.*

**Background:** The association of urinary sodium excretion with all-cause mortality and decline in GFR in patients with type 1 diabetes and nephropathy, but without heart failure needs clarification.

**Methods:** In a retrospective observational setting we included all 281 patients with three or more 24h urinary samples during 2000-2010. Mean urinary sodium excretion was calculated from all (median 24) samples. Vital status was determined with a mean follow-up of 8.8 years. GFR($^{51}$Cr-EDTA) was measured annually. The excretion was analyzed as a continuous variable in regression analysis and as quintiles in mortality analysis due to nonlinearity (cutoff-points: 7.0, 8.6, 10.1 and 12.1 g/24h).

**Results:** At baseline 62% were men, mean age 47(11) years, GFR 79(28) ml/min/1.73$m^2$, $HbA_{1c}$ 9.2(1.4) %, >95% were treated with RAS blockade, and 10% had previous CVD. 42 (15%) died. Regardless of adjustments, patients in the 2.nd quintile consistently had the lowest mortality risk and the 1.st quintile the highest risk.

| Model | Variables | Hazard Ratio (95% CI) of mortality [1.st quintile with 2.nd as reference] | p |
|---|---|---|---|
| A | Sodium excretion | 4.49 (1.50- 13.42) | <0.01 |
| B | A + age, gender and previous CVD | 4.60 (1.53-13.78) | <0.01 |
| C | B + smoking and total cholesterol | 5.11 (1.67–15.58) | <0.01 |
| D | B + GFR, systolic blood pressure, $HbA_{1c}$ and albuminuria at baseline | 3.85 (1.04-14.32) | 0.04 |

The analyses were repeated after exclusion of 50 patients not prescribed diuretics - with same trend, albeit with higher hazard ratios. Mean (SE) GFR decline was 3.9(0.4), 3.0(0.4), 3.1(0.4), 2.5(0.3) and 3.2(0.5) ml/min/1.73$m^2$/year in ascending quintiles of sodium excretion. The rate declined 0.13(0.02-0.25) ml/min/1.73$m^2$ with each g/day rise in NaCl (p=0.02) in fully adjusted models.

**Conclusions:** In unselected patients with type 1 diabetes and nephropathy, but without known heart failure, the association of urinary sodium excretion and mortality appears j-shaped, and values below <7g/24h NaCl are associated with the highest mortality and the steepest rate of GFR decline independent of other risk factors.

## SA-PO356

**Diabetes Mellitus Is Not Associated with Increased 5-Year Mortality in Adult Patients with Vascular Access for End Stage Renal Disease** Paola De Rango, Basso Parente, Luca Farchioni, Beatrice Fiorucci, Lydia Romano, Giuseppe Giordano. *Vascular and Endovascular Surgery; Hospital S.M. Misericordia; Univ of Perugia, Perugia, Italy.*

**Background:** Patients with end stage renal disease (ESRD) are exposed to limited life expectancy. As to whether diabetes mellitus may further decrease survival in these patients is not universally demonstrated. This study aimed to investigate the impact of diabetes on survival of patients with vascular access performed at a single vascular surgery center.

**Methods:** Data from consecutive patients with ESRD admitted for vascular access surgery from 2006 to 2013 were reviewed. Data were collected prospectively. Five-year outcomes of diabetic and non-diabetic patients patients were compared. Primary end-point was 5-year survival assessed by Kaplan Meier analysis with log rank test.

**Results:** A consecutive series of 458 patients (293 males; mean age 69.9+14.3) received 686 vascular access procedures during the study period. One hundred nineteen were affected by Type II diabetes mellitus at the time of admission. Diabetic patients showed higher comorbidity profile due to the increased prevalence of hypertension (90.7% vs 77.6%;P=.002), coronary disease (58.3% vs. 42.8%;P=.06), hyperlipidemia (27.2% vs. 17.6%; P=.04) and were more common on statins therapy (51.0% vs 29.5%; P<.001) when compared to non-diabetic patients. Kaplan Meier survival rates at 3 and 5 years were 71.8%% and 61.3% for patients with diabetes mellitus and 70.9% and 58.5% for non-diabetics (P=.86). Cox regression analysis failed to show any significant association between diabetes mellitus and mortality. Primary patency rates of vascular accesses at 3 years were 44.8% in diabetic and 41.5% in non-diabetics (P=0.895).

**Conclusions:** Diabetes mellitus is not associated with worse major outcomes in patients with ESRD requiring vascular access.

## SA-PO357

**The Circulating microRNA Expression Profile of Diabetic Nephropathy Patients** Qiuling Fan. *Dept of Nephrology, The First Hospital, China Medical Univ, Shenyang, China.*

**Background:** To explore the pathogenesis and the biomarkers for early detection of diabetic nephropathy (DN), the circulating microRNA expression profile of DN patients was analyzed by AB Taqman human miRNA array.

**Methods:** We obtained serum samples from 5 diabetic nephropathy patients proven by renal biopsy as nodular diabetic glomerulosclerosis, 5 diabetic patients without microalbuminuria (DM) and 5 healthy controls (N). Serum miRNAs were analyzed with the TaqMan Low Density Array and then validated with a quantitative reverse-transcription PCR assay with 30 individual samples.

**Results:** The urinary microalbumin/creatinine ratio and serum creatinine in diabetic nephropathy patients were higher than that of diabetic patients and healthy control($p<0.05$). 20 miRNAs were upregulated and 22 miRNAs were downregulated in serum of diabetic patients compared with that of healthy controls. 42 miRNAs were upregulated and 19 miRNAs were downregulated in serum of diabetic nephropathy patients compared with that of diabetic patients. Among them, along with the progression of diabetes and diabetic nephropathy, miR-1179 was gradually increased (2.03 times in DM/N and 2.14 times in DN/DM) , miR-148b, miR-150 were gradually reduced (2.04 times in DM/N, 2.02 times in DN/DM and 2.03 times in DM/N, 2.02 times in DN/DM respectively).

**Conclusions:** The differentially expression of serum miR-1179, miR-148b and miR-150 may be responsible for the pathogenesis of diabetic nephropathy and are potential biomarkers for DN.

## SA-PO358

**The Usefulness of the New Classification for CKD by the KDIGO for Analyzing Progression of Diabetic Kidney Disease and Vascular Complications in Type 2 Diabetic Patients** Ming Li,[1] Huiqing Chen,[2] Xun Liu,[1] Wenbo Zhao,[1] Tan-qi Lou.[1] *[1]Dept of Nephrology, The Third Affiliated Hospital of SUN Yat-sen Univ, Guangzhou, Guangdong, China; [2]Dept of Paediatrics, The First Affiliated Hospital of SUN Yat-sen Univ, Guangzhou, Guangdong, China.*

**Background:** To investigate the clinical features of patients with type2 diabetic kidney disease and analyze the risk factors for the progression of Diabetic Kidney Disease, also to evaluate the risk for diabetic vascular complication.

**Methods:** 1645 Chinese adult patients with type 2 diabetic from June 2008 to September 2012 were included in this study. They were grouped according to Composite ranking for relative risks by GFR and albuminuria(KDIGO 2009).The clinical features and risk factors of DKD were compared and analyzed.

**Results:** 1) Rate of T2DM patients with CKD was 37.2%; According to Composite ranking for relative risks by GFR and albuminuria(KDIGO 2009), the low-risk group was 62.8%, and moderate risk group was 17.8%, the high-risk group was 7.9%, the very high risk group was 11.5%. (2) Multivariates regression analysis indicated that diabetic retinopathy (DR) (OR:1.770,95% CI 1.060-2.956), systolic pressure(OR:1.012, 95%CI 1.001-1.023), CysC (OR: 5.369, 95%CI 2.306 -12.499) were the independent risk factors for the progression of low risk group to moderate risk group. The risk factor for the progression of moderate risk group to high-risk group were DR(OR:3.318, 95%CI 1.399-7.039), systolic pressure(OR:1.033, 95%CI 1.013-1.053), CysC(OR:9.723, 95%CI 3.110 -30.394). Serum creatine(OR:1.105, 95%CI 1.069-1.142) was the risk factor for high-risk group to very high risk group. (3) Among the GFR(G1+G2,G3a,G3b and G4+G5), CVD% were 13.4%, 34.0%, 50.9%, 53.7% ($p<0.05$). Cerebrovascular disease% were 8.3%, 27.7%, 43.9%, 58.5% ($p<0.05$). DR% were 23.6%, 41.1%,67.9%, 54.3%($p<0.05$). The brachial-ankle index was significantly greater in the G3b group compared to the G3a group.

**Conclusions:** DR and CysC can predict the progression of DKD well. And good control of blood pressure can delay the progression of DKD.The subdivision of the G3 stage in the new classification proposed by the KDIGO is useful to evaluate vascular complications with T2DM.

*Funding:* Government Support - Non-U.S.

## SA-PO359

**The Correlation between Renal Tissue Disorder and Mast Cell Chymase on Patients with Diabetic Nephropathy** Sayuri Shirai,[1] Daisuke Ichikawa,[1] Kayori Tsuruoka,[1] Yugo Shibagaki,[1] Takashi Yasuda,[1] Kenjiro Kimura.[1] *[1]Nephrology and Hypertension, St. Marianna Univ School of Medecine, Kawasaki, Kanagawa, Japan; [2]Cardiovasucular Disease, Fukuoka Univ Chikushi Hospital, Chikushino, Fukuoka, Japan.*

**Background:** Human chymase is known to produce angiotensin II (AII) from angiotensin I (AI) but the pathophysiological role of chymase is not fully understood. We investigated relationships between chymase and renal pathological abnormality on patients with diabetic nephropathy.

**Methods:** We enrolled 34 type 2 diabetic patients with diabetic nephropathy who underwent renal biopsy from 2002 to 2012. Immunohistochemical staining for chymase (C) and tryptase (T) was performed on renal sections from patients. The number of C positive (C+) and T positive (T+) mast cell per unit area were counted. Correlation between those positive cells and patients' clinical and pathological indices was analyzed.

**Results:** Both C+ mast cells and T+ mast cells were found in the interstitium, but not in the glomeruli, of renal cortex. Both cell numbers had positive correlation with tubulointerstitial injury, progression of diabetic nephropathy, and advanced CKD stage, respectively. The ratio of C+ to T+ cell number had same correlation with above clinical parameters. The positive correlation between global sclerosis and interstitial injury were found on low intensity C+ group (<2 /mm$^2$), but was not found on high intensity C+ group (>2 /mm$^2$).

**Conclusions:** This study suggests that mast cells, especially C+ mast cells, are involved in development of diabetic nephropathy. Through release of bioactive substances like tryptase and chymase into the tubulointerstitial area, mast cells could promote renal inflammation and fibrosis, and thus contribute to diabetic nephropathy.

## SA-PO360

**Hyperglycemia Is Associated with Tubular Injury in Subjects with Type 2 Diabetes Mellitus** Ernesto Sabath,[1] Ma. Ludivina Robles-osorio.[2] *[1]Renal Dept, SESEQ; [2]Metabolism and Endocrinology, Universidad Autónoma de Querétaro.*

**Background:** Microalbuminuria indicates the presence of early glomerular disease in patients with diabetes and it has yet to be determined whether testing for markers of tubular injury can also identify persons who are at risk of progressive renal disease. The association of hyperglycemia with markers of tubular injury is currently unknown and the aim of the current study was to determine if hyperglycemia is associated with tubular injury.

**Methods:** Subjects aged >20 years old were included, and those with uncontrolled hypertension, current urinary tract infection and pregnant women were excluded. Blood samples were taken after a fasting ≥ 8hrs; measurements of the biochemical variables were done by standard technique. GFR was estimated using the CKD-EPI equation. Spot urine samples for albumin and α1-microglobulin (a1M) analysis were collected and the α1M determinations were done by ELISA. Descriptive statistics were calculated and comparisons between groups were done by t-student analysis and chi-square. For variables correlated with a1M excretion a multiple linear regression analysis was used.

**Results:** A total of 190 subjects with T2DM were included; the mean age was 52.6 ± 12.5 yrs, median time from diagnosis was 7.6 ± 7.2 years and mean BMI was 29.9 ± 5.4.

In 168 subjects we have determination for a1M, 76 (45.2%) had aIM higher than 10 µg/gCr; these subjects were older (56 ± 11 vs 48 ± 11, p= 0.002), and glucose serum concentration was significantly higher (227 ± 78 vs 166 ± 60 mg/dl, p= 0.00), but not differences in AUE was found; in multiple regression analysis the most important factor associated with a1M was serum glucose (p=0.001) and there was a significant correlation between fasting glucose levels with A1M excretion (r= 0.27, p=0.01). Subjects >50 yrs and abnormal a1M had a decrease in GFR (66.2 ± 16 vs 77.3 ± 18 ml/min, p=0.04) compared with those with normal values of a1M.

**Conclusions:** Hyperglycemia is associated with higher urinary excretion of a1M, reflecting a clinical significant effect of glucose concentration on tubule-interstitial injury; a1M excretion is associated with decrease in renal function in a subgroup of patients with T2DM.

*Funding:* Government Support - Non-U.S.

## SA-PO361

**The Clinical Evaluation of the Mechanisms of Natriuresis by DPP-4 Inhibitors** Koichi Kanozawa, Nobuyuki Onizawa, Hajime Hasegawa, Takatsugu Iwashita, Taisuke Shimizu, Juko Asakura, Tomonari Ogawa, Tetsuya Mitarai. *Div of Nephrology and Hypertension, Saitama Medical Center, Saitama Medical Univ, Kawagoe, Japan.*

**Background:** Glucagon-like peptide(GLP)-1 receptor has been expressed at renal proximal tubule, and acts on natriuresis through Na(+)/H (+) exchanger isoform 3 (NHE3). On the other hand, not only GLP-1 but also brain natriuretic peptide (BNP) is contained in the substrate of dipeptidyl peptidase-4 (DPP). The aim of study is to clarify the mechanisms of natriuresis of DPP-4 inhibitors, clinically.

**Methods:** We gave DPP-4 inhibitor (either of sitagliptin 50-100 mg, vildagliptin 50-100 mg, alogliptin 12.5-25 mg) for Japanese patients with Type 2 diabetes less than 2.0 mg/dl of serum creatinine level. The inpatients were collected first morning urine within one week around DPP-4 inhibitors administration, and the outpatients were collected first or second urine before and ten weeks and 20 weeks after DPP-4 inhibitors administration. The average value of twice urinary Na and K excretion was evaluated. Furthermore, the changes of BNP were also examined.

**Results:** The 46 inpatients and 60 outpatients ware enrolled, and the diuretics was used together at 43%, and 65% each, respectively. By administration of DPP-4 inhibitors, urinary Na excretion significantly increased from 112±16 to 179±19 mEq/gCr ($p< 0.01$) in the inpatients with diuretics combination, and also increased from 140±17 to 180±28 mEq/gCr ($p< 0.05$) in the diuretics combined use outpatients. In both the inpatient and outpatient who are without combination of diuretics, the increase of the urinary Na excretion was not statistically significant. The urinary K excretion also significantly increased from 44±5 to 53±6 mEq/gCr ($p< 0.05$) in the inpatients with diuretics combination, and increased from 34±3 to 39±4 mEq/gCr ($p< 0.05$) in the outpatients who combined diuretics other than K+sparing diuretics. On the other hand, BNP observed the upward tendency in 94±25 to 112±28 pg/ml in the outpatients by dosage of DPP-4 inhibitors.

**Conclusions:** It was considered that DPP-4 inhibitors regulate Na re-absorption in upper renal tubule since urinary Na and K excretion in diuretics combined use, and BNP might participate in natriuresis.

## SA-PO362

**25(OH)D3 Is an Effective Indicator of Mineral Metabolism for Patients with Diabetic Nephropathy** Li Wang. *Renal Dept, Sichuan Provincial People's Hospital.*

**Background:** Serum P, Ca and PTH concentration are traditional indicators to reveal the mineral metabolism. However they are usually affected by other factors, and hence not sensitive. VitD is also associated with mineral metabolism. We assess the impact of 25(OH) D3 on early screening of mineral metabolism for patients with type 2 diabetic nephropathy.

**Methods:** Cross-sectional study of 162 type 2 diabetic patients with diabetic nephropathy, where no statistical significance exists for age, gender, duration and season. They are sorted by different concentration of 25(OH)D3 according to KDOQI Clinical Practice Guidelines for Bone Metabolism. The distributions of three concentration groups are compared, for serum P, Ca and PTH respectively.

**Results:** This statistical analysis shows that, along with 25(OH)D3 concentration decreases, for insufficient and deficient groups, the proportions of high-P, low-Ca and high-PTH patients increases significantly, which are (3.6%-10.7%-85.7%, $p<0.05$), (9%-37.3%-53.7%, $p<0.05$) and (0%-12%-88%, $p=0.256$) respectively. The clinical data for PTH groups is limited (only 60 patients), which would be the reason for its non statistical significant difference. Meanwhile, a large proportion patients belonging to normal serum P, Ca and PTH groups have the phenomenon of 25(OH)D3 anomaly (deficiency or insufficient), which count to 82.9%, 83%, 100% respectively.

**Conclusions:** 25(OH)D3 is an effective indicator of mineral metabolism for patients suffering type 2 diabetes with diabetic nephropathy, and it is more sensitive comparing to serum P, Ca and PTH concentration. We suggest to detect 25(OH)D3 in mineral metabolism for early screening of the type 2 diabetic nephropathy.

## SA-PO363

**Role of Magnesium, Fibroblast Growth Factor-23 and Phosphorus in Cardiovascular Outcomes in Type 2 Diabetic Patients with Diabetic Nephropathy** Ana Paula Silva, André Fragoso, Pedro Neves. *Nephrology, Hospital de Faro E.P.E, Faro, Portugal.*

**Background:** To evaluate the role of magnesium (Mg), fibroblast growth factor-23 (FGF-23) and Phosphorus (Pi) levels in co-morbidity (hospitalization) and cardiovascular mortality. To evaluate the association between Mg and cardiovascular risk factors in type 2 diabetic patients with mild to moderate CKD.

**Methods:** An observational, prospective study involving 191 patients divided into groups according to baseline magnesium levels: 1< 1.2 mg/dL,2=1.2-2.3mg/dL and 3≥ 2.3 mg/dL. Baseline characteristics were analyzed and compared. Multivariate Cox regression was used to find out predictors of cardiovascular mortality and multivariate logistic regression was used to find out predictors of hospitalization. Simple linear regression and Pearson correlation were used to investigate correlations between magnesium and renal function, inflammation, oxidative stress, mineral metabolism parameters and left ventricular mass index.

**Results:** Patients' survival at 55 months on group 1, 2 and 3 was 21%, 30.1% and 91.2%, respectively ($P=0.004$). Mg (HR: 0.450, $p=0.007$), FGF-23 (HR: 1.005, $p=0.023$), Pi (HR: 1.105, $p=0.004$) and age (HR=1.954, $p=0.045$) were independent predictors of mortality. Mg (OR=0.889, $p=0.003$), FGF-23 (OR=1.027, $p=0.045$), Pi (OR=1.056, $p=0.032$) and creatinine (OR=1.016, $p=0.023$) were independent predictors of hospitalization. Mg levels were negatively correlated with left ventricular mass index ($R = -0.788$, $p = 0.0001$), creatinine ($R= -0.346$, $p = 0.0001$), PTH ($R= -0.7101$, $p = 0.0001$), FGF-23 ($R= -0.798$, $p = 0.0001$) and was positively correlated with vitamin D ($R= 0.840$, $p = 0.0001$).

**Conclusions:** Magnesium, FGF-23 and phosphorus levels might have a significant clinical use as markers/predictors of cardiovascular mortality and hospitalization in type 2 diabetic patients with mild to moderate CKD.

*Funding:* NIDDK Support

## SA-PO364

**What Is the Role of Apelin Regarding Cardiovascular Risk and Progression of Renal Disease in Type 2 Diabetic Patients with Diabetic Nephropathy?** Ana Paula Silva, André Fragoso, Nelson Almeida Tavares, Pedro Neves. *Nephrology, Hospital Faro E.P.E, Faro, Portugal.*

**Background:** To evaluate the association of different apelin levels with cardiovascular mortality, hospitalizations, renal function and cardiovascular risk factors in type 2 diabetic patients with mild to moderate CKD.

**Methods:** An observational, prospective study involving 150 patients divided into groups according to baseline apelin levels: 1≤ 98 pg/mL,2=98-328 pg/mL and 3≥ 329 pg/mL. Baseline characteristics were analyzed and compared. Multivariate Cox regression was used to find out predictors of cardiovascular mortality and multivariate logistic regression was used to find out predictors of hospitalization and disease progression. Simple linear regression and Pearson correlation were used to investigate correlations between apelin and renal disease, and between apelin and cardiovascular risk factors.

**Results:** Patients' survival at 83 months on group 1, 2 and 3 was 39%, 40% and 71.2%, respectively ($P=0.046$). Apelin, age and eGFR were independent predictors of mortality, and apelin, creatinine, eGFR, resistin and visfatin were independent predictors of hospitalization. Apelin levels were negatively correlated with cardiovascular risk factors IMVE ($R = -0.588$, $p = 0.0001$), creatinine ($R= -0.316$, $p = 0.0001$), IL-6 ($R= -0.708$, $p = 0.0001$), IMT ($R= -0.621$, $p = 0.0001$), systolic BP ($R= -0.441$, $p = 0.0001$), PP ($R= -0.588$, $p = 0.0001$) and OxLDL ($R= -0.669$, $p = 0.0001$), while it was positively correlated with eGFR ($R= 0.357$, $p = 0.0001$). Patients with lower apelin levels were more likely to start a depurative technique ($P=0.001$).

**Conclusions:** Apelin levels might have a significant clinical use as a marker/predictor of cardiovascular mortality and hospitalization or even as a therapeutic agent for CKD patients with cardiovascular disease.

*Funding:* NIDDK Support

## SA-PO365

**Endothelial Function in Type 2 Diabetic Patients with Renal Dysfunction** Lee Ying Yeoh, Tavintharan Subramaniam, Su-chi Lim. *Medicine, Khoo Teck Puat Hospital, Singapore.*

**Background:** Study the relationship of endothelial function, arterial stiffness in T2DM with reduced GFR.

**Methods:** Cross sectional study was done from August 2011 to May 2013 to explore novel risk factors for endothelial dysfunction and diabetic micro-angiopathies. Pulse wave velocity(PWV) was measured using applanation tonometry(SphymCorÒ).Iontophoresis followed by laser doppler flowmetry(Moor Instruments Ltd, England) were used to assess forearm cutaneous micro-vascular endothelial reactivity in response to acetylcholine chloride(Ach) and sodium nitroprusside(NT). Estimated glomerular filtration rate(eGFR) was calculated using CKD EPI equation and stratified into>90,60-90 or 30-59ml/min. ANOVA and multinomial logistic regression were used.

**Results:** There were 1515patients(48.5%Male and 51.2%Chinese). Mean duration of DM was 10.9±8.6years. GFR decline was associated with increasing age and albuminuria as opposed to HDL-cholesterol ($p<0.01$). Vascular function was affected when GFR<60ml/min as evidenced by impaired endothelium dependent vasodilatation(Ach) and increase PWV($p<0.01$).

| eGFR(ml/min) | 30-59 | 60-90 | >90 | Multivariate (compare to GFR>90) | |
|---|---|---|---|---|---|
| N | 211 | 448 | 856 | 30-59 | 60-90 |
| Age(years) | 64.2±9.2 | 61.7±9.1 | 53.5±10.0 | 1.15(1.13-1.18) | 1.11(1.09-1.13) |
| HDL-C(mmol/l) | 1.19±0.31 | 1.29±0.35 | 1.30±0.36 | 0.18(0.10-0.34) | 0.56(0.38-0.82) |
| ACR(ug/mg) | 412.8±815.5 | 165.8±557.3 | 91.1±257.4 | 1.001(1.001-1.002) | 1.001(1.000-1.001) |
| PWV(m/s) | 11.1±3.1 | 10.0±2.8 | 8.9±2.5 | 1.12(1.05-1.19) | 1.03(0.98-1.09) |
| Ach(%increment) | 86.5±67.3 | 118.7±99.1 | 129.0±96.1 | 0.997(0.994-0.999) | 1.000(0.999-1.002) |
| NT(%increment) | 58.8±46.9 | 72.1±54.3 | 81.4±57.1 | 0.997(0.993-1.001) | 0.998(0.996-1.001) |

**Conclusions:** Endothelial dysfunction and arterial stiffness are seen in T2DM patients when GFR<60ml/min. Changes in the micro-circulatory function and arterial stiffness might have taken place in early renal dysfunction.

## SA-PO366

**High Fibroblast Growth Factor-23 Levels Are Associated with Early Diastolic Dysfunction in Type 1 Diabetic Patients with No or Early Diabetic Nephropathy** Burcu Dogan,[1] Izzet Hakki Arikan,[2] Derya Guler,[2] Nursen Keles,[1] Banu Isbilen,[1] Ferruh Isman,[1] Aytekin Oguz.[1] *[1]Istanbul Medeniyet Univ Goztepe Hospital, Istanbul, Turkey; [2]Marmara Univ Pendik Hospital, Istanbul, Turkey.*

**Background:** Recent studies showed that higher fibroblast growth factor-23 (FGF-23) and lower soluble klotho levels are associated with cardiovascular disease. The aim of the study is to investigate the possible links between klotho and FGF-23 levels and cardiovascular risk factors in type I DM patients.

**Methods:** We included 87 type 1 diabetic patients with eGFR > 60 ml/min (DM) (50 female, mean age 34,2 ± 9,1 years) and 78 healthy controls (43 female, 34.5 ± 9.8 years) in the study. Demographic characteristics, albuminuria, lipid profiles, Ca, P, PTH and vitamin D levels were recorded. Echocardiographic examination was performed and eGFR was calculated. The presence of diabetic retinopathy and neuropathy were also noted. Serum klotho and FGF-23 levels were determined by ELISA method.

**Results:** FGF-23 levels were not different in diabetics (76.2±16.4 pg/ml) compared with controls (77.3±15.7 pg/ml). FGF-23 levels were only correlated with IVRT (isovolumetric relaxation time) (r=0,321, p=0,023) and E'med (early diastolic velocity at medial/septal annulus) (-0,293 p=0,041) in DM. There were no relationships between FGF-23 and albuminuria, eGFR, diabetic retinopathy, vitamin D levels and other parameters. Soluble klotho levels in DM (532,58±228,68 pg/ml) were also similar with controls (552,19±425,22 pg/ml). Soluble klotho levels were found not to be related with demographic, laboratory and echocardiographic parameters.

**Conclusions:** Elevated FGF-23 levels are associated with higher IVRT and E'med which indicate early diastolic cardiac dysfuction in type 1 DM patients with eGFR>60 ml/min. Soluble klotho levels are not changed in type 1 DM with early or no diabetic nephropaty in contrast with type 2 DM. High rate of hypertension, advanced age and other cardiovascular risk factors in type 2 DM might be responsible for this discrepancy between type 1 and 2 DM.

## SA-PO367

**Impacts of Dyslipidemia on Diabetic Nephropathy, Cardiovascular Mortality, and All-Cause Mortality in Patients with Diabetes: A Systematic Review and Meta-Analysis** Tadashi Toyama,[1] Miho Shimizu,[1] Kengo Furuichi,[1] Takashi Wada.[1] *[1]Div of Nephrology, Kanazawa Univ, Kanazawa, Ishikawa, Japan; [2]Dept of Disease Control and Homeostasis, Institute of Medical, 11 Pharmaceutical and Health Sciences, Kanazawa Univ, Kanazawa, Ishikawa, Japan.*

**Background:** Some meta-analysis reported the effects of statins for renal and cardiovascular outcome, and all-cause mortality. However, treatment of statins has two main effects. One is improvement of dyslipidemia and another one is other pleiotropic effects. When we treat patients with diabetes, we should understand precise impacts of dyslipidemia, because lifestyle changes are first-line treatment. In this study, we explored impacts of dyslipidemia on cardiovascular mortality, all-cause mortality, and renal failure in diabetes mellitus and examined inconsistency between studies.

**Methods:** Relevant studies were searched from MEDLINE and CINAHL by using key words of medical subject headings that included words related to "dyslipidemia", "diabetes mellitus," "renal failure," "cardiovascular diseases," and "mortality". Studies examining the risk of three endpoints (cardiovascular mortality, all-cause mortality, renal failure) associated with high LDL cholesterol (LDL-C) were included. The extracted estimates were combined using a random-effects model. To investigate the cause of heterogeneity, we performed a meta-regression analysis.

**Results:** The literature search yielded 8624 articles, of which 390 papers were reviewed in full. Seventeen studies, which fulfilled criteria, were included in the analysis. The presence of dyslipidemia (LDL-C +1 mmol/l) was 1.08-fold increase in risk of presence of albuminuria. The presence of dyslipidemia was also a risk factor for cardiovascular mortality (relative risk 1.37), and all-cause mortality (relative risk 1.21). In the meta-regression analysis, long-term follow-up period was associated with higher relative risks in the outcome of cardiovascular mortality and all-cause mortality.

**Conclusions:** High LDL-C is risk factor for cardiovascular mortality, all-cause mortality, and renal failure in diabetic patients. Long-term follow-up may have stronger impact of high levels of LDL-C on outcomes of diabetic patients.

## SA-PO368

**Impaired Physical Fitness in Obese Diabetic Patients with Chronic Kidney Disease** Vamsi K. Kanneganti, Eileen Collins, Jolene Butler, Susan O'Connell, Christine Jelinek, Conor McBurney, Anne Garabedian, Holly J. Kramer, David J. Leehey. *Medicine and Research, Hines VA Hospital, Hines, IL.*

**Background:** Patients with diabetes, obesity, and chronic kidney disease (CKD) are generally physically inactive which may be an important contributor to their high mortality rate.

**Methods:** We examined baseline data from a VA funded randomized controlled trial (NCT01036490) to assess physical fitness in this population. Inclusion criteria are type 2 diabetes, obesity (body mass index > 30 kg/m$^2$), chronic kidney disease (stage 2-4) and persistent proteinuria (> 200 mg/day for at least 3 months). Data from the baseline symptom-limited treadmill test were analyzed. We calculated a perceived exertion-workload index (PEWI) for each subject, defined as the ratio of peak perceived exertion (Borg scale) to the energy cost of peak physical activity (METS).

**Results:** Baseline data (mean ± SD) on 30 subjects were evaluated. Mean values (± SD) were as follows: age 66 ± 8.1 yrs, body mass index 36.5 ± 4.9 kg/m$^2$, percent body fat 41.3 ± 6.6%, hemoglobin 12.5 ± 1.7 g/dL, glycated hemoglobin 8.1 ± 1.8 %, serum creatinine 2.3 ± 1.0 mg/dL, creatinine clearance 46.5 ± 35.7 mL/min, urinary albumin excretion rate 1013 ± 1073 mg/24h. Average treadmill time was 6.8 ± 3.8 minutes and peak oxygen consumption ($VO_2$ peak) was 13.1 ± 3.6 mL/kg/min (peak METS 3.7 ± 1.0; range 2.0-5.8). Peak METS was positively correlated with baseline average weekly pedometer step count ($r = 0.62$, $p < 0.01$). The peak perceived exertion-workload index (PEWI) was 1.4 ± 0.86, with higher values indicating greater perceived exertion relative to workload. There was a negative correlation between peak PEWI and both treadmill time ($r = - 0.60$, $p < 0.001$) and average weekly pedometer step count ($r = - 0.63$, $p < 0.01$). There was no correlation between peak PEWI and hemoglobin, glycated hemoglobin, serum creatinine, creatinine clearance, body mass index, or percent body fat.

**Conclusions:** These data indicate that obese diabetic subjects with CKD have markedly impaired baseline physical fitness, which may in part be due to increased perceived exertion at a given workload with resultant decreased ability to exercise.

*Funding:* Veterans Affairs Support

## SA-PO369

**Phase 2 Clinical Trial of Orally Administered CCR2 Antagonist CCX140-B in Diabetic Nephropathy** Pirow Bekker, Antonia Potarca, Timothy Sullivan, Shichang Miao, Daniel Dairaghi, Lisa Lohr, Lisa C. Seitz, Zhenhua Miao, Bin N. Zhao, Jay P. Powers, Juan C. Jaen, Thomas J. Schall. *ChemoCentryx, Inc.*

**Background:** Monocyte chemoattractant protein-1 (MCP-1) and its C-C chemokine receptor CCR2 are implicated in diabetic nephropathy. The CCR2 antagonist CCX140-B has been tested in clinical trials in >550 patients. CCX140-B was effective in reducing glycemia, biochemical and histological markers of kidney disease in multiple mouse models. CCX140-B also reduced glycemia in a Ph2 clinical trial in type 2 diabetes and is currently in Ph2 testing in diabetic nephropathy (DN).

**Methods:** This is a randomized, double-blind, placebo-controlled Phase 2 clinical trial in 270 patients with DN. The primary efficacy measure is change from baseline in first morning urinary albumin:creatinine ratio (ACR). Eligible patients have been on stable ACE inhibitor or ARB, and anti-diabetic treatment for at least 8 wks, with ACR 100-3000 mg/g creatinine, HbA1c 6-10%, and eGFR ≥25 mL/min/1.73 m$^2$. Patients are stratified based on ACR and eGFR, and then randomized to receive placebo, 5 mg or 10 mg CCX140-B QD for 1 year.

**Results:** A total of 270 pts have been enrolled. Baseline characteristics, mean (SD): 64 (7) years old, 79% males, BMI 33 (5) kg/m$^2$, duration of diabetes 15 (8) years, duration of diabetic nephropathy 5 (4) years, geometric mean urinary ACR 475 (range 101-2708) mg/g creatinine, eGFR 60 (23) ml/min/1.73 m$^2$. 67% of patients had urinary ACR ≥300 mg/g and 33% between 100 and 299 mg/g inclusive. 52% had eGFR 25-59 ml/min/1.73 m$^2$ and 48% ≥60 ml/min/1.73 m$^2$. An external independent data monitoring committee has concluded after all reviews to date that there has been no evidence based on safety data to alter the protocol or study conduct. CCX140-B has been well tolerated in completed clinical trials, with no significant safety concerns. 12-week interim results from this trial are expected in Q3 2013.

**Conclusions:** Clinical development of the orally administered CCR2 antagonist CCX140-B is progressing well. CCX140-B has been well tolerated and safe in clinical trials, and has shown efficacy in a Phase 2 trial in type 2 diabetes. The first results from a clinical trial in DN are anticipated soon.

## SA-PO370

**Alogliptin Improves Steroid Diabetes by Decrease of Plasma Glucagon Levels in Chronic Kidney Disease Caused by Immunological Abnormalities** Naro Ohashi,[1] Takayuki Tsuji,[1] Hideo Yasuda,[1] Akihiko Kato,[2] Yoshihide Fujigaki.[3] *[1]First Dept of Medicine, Hamamatsu Univ School of Medicine, Hamamatsu, Japan; [2]Blood Purification Unit, Hamamatsu Univ School of Medicine, Hamamatsu, Japan; [3]Dept of Internal Medicine, Teikyo Univ School of Medicine, Tokyo, Japan.*

**Background:** Chronic kidney disease (CKD) is a risk factor for end-stage renal failure and cardiovascular disease, and a strategy to counteract CKD must be established. CKD caused by immunological abnormalities is treated by steroids, frequently resulting in steroid diabetes. Although insulin resistance is one of the mechanisms of steroid diabetes, and insulin is the most effective drug, it is not easy for patients to accept insulin therapy. Dipeptidyl peptidase-4 (DPP-4) inhibitors were developed for diabetes mellitus with a new mechanism of action. However, their efficacies and mechanisms of action for steroid diabetes are unclear.

**Methods:** We studied 11 CKD patients treated with steroids who were admitted to our hospital (3 men and 8 women; age, 66.0 ± 15.9 years). DPP-4 inhibitor alogliptin was administered for steroid diabetes. Levels of markers related to glucose metabolism were measured before alogliptin treatment and after alogliptin treatment, before the prednisolone dose was reduced.

**Results:** Alogliptin treatment significantly increased plasma glucagon-like peptide-1 (GLP-1) levels from 1.16 ± 1.71 pmol/L to 4.48 ± 1.53 pmol/L ($p = 0.0014$) and significantly reduced plasma glucose levels recorded 2 h after lunch and hemoglobin A1c (HbA1c). No significant differences were seen in either insulin secretory ability of homeostasis model assessment (HOMA) (HOMA-β) and insulin resistance index of HOMA (HOMA-R) before

and after alogliptin treatment. In contrast, alogliptin treatment significantly decreased plasma glucagon levels from 116.1 ± 38.7 pg/mL to 89.6 ± 17.3 pg/mL (p = 0.017). Moreover, there were significant correlations among HbA1c, GLP-1, and glucagon levels.

**Conclusions:** Alogliptin improves steroid diabetes by decrease of glucagon levels through an increase in plasma GLP-1 levels. Thus, alogliptin may be effective for the treatment of steroid diabetes in CKD caused by immunological abnormalities.

*Funding:* Private Foundation Support

### SA-PO371

**Insulin-Sitagliptin Combination Therapy Stabilizes Blood Glucose Level in Japanese Patients with Diabetic Nephropathy** Masao Toyoda, Masafumi Fukagawa. *Internal Medicine, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan.*

**Background:** The use of oral hypoglycemic agents in patients with diabetic nephropathy is often limited; therefore, intensive insulin therapy (IIT) is often prescribed. Similar to Western countries, the concomitant use of dipeptidyl peptidase-4 inhibitors and insulin has also been recently approved in Japan, and it is expected that the combination treatment could lead to stable euglycemia. In Western countries, adjusted doses of sitagliptin can be administered to patients with severe renal dysfunction or end-stage renal disease (ESRD); but this is contraindicated in Japan. Therefore, a retrospective study was conducted to evaluate the safety and efficacy of the combination of sitagliptin and IIT in Japanese type 2 diabetic patients with low estimated glomerular filtration rates (eGFR).

**Methods:** We selected 116 patients who were already receiving IIT combined with sitagliptin. From these patients, we selected 40 patients with eGFR of <60 mL/min/1.73 m$^2$. We analyzed hemoglobin A1c (HbA1c) levels, blood glucose (BG) levels, body weight, and changes in insulin dose. As an indicator of the stability of BG control, the M values were calculated through 4-times-daily self-monitoring of BG levels. Fluctuations in these levels were also analyzed using a continuous glucose monitor (CGM) in patients who could be monitored. The effect of treatment on BG level was assessed statistically.

**Results:** The mean eGFR in 40 patients was 47.8±9.1mL/min/1.73 m$^2$. The mean sitagliptin dose was 45.0±11.6mg, lower than the doses commonly used in Europe and North America. The mean HbA1c decreased significantly from 8.1±1.0 to 7.7±1.2%. The M value also improved significantly from 10.1±8.1 to 3.5±3.1. No significant changes were noted in body weight and insulin dose.

**Conclusions:** Our results showed that the combination of IIT and sitagliptin reduced fluctuations in BG level and reduced HbA1c level safely without weight gain even in patients with renal dysfunction. The lower dose of sitagliptin used in Japanese patients relative to their Western counterparts calls for further studies to examine inter-racial differences in the effects of incretin-related drugs.

### SA-PO372

**Effect of Angiotensin Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers on Cardiovascular Events in Patients with Diabetes Mellitus: A Meta-Analysis** Jianghua Chen. *Kidney Disease Center, First Affiliated Hospital, College of Medicine, Zhejiang Univ, Hangzhou, Zhejiang, China.*

**Background:** Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) maybe have different effects on cardiovascular (CV) events in diabetic patients. We conducted a meta-analysis to separately evaluate the effects of ACEIs and ARBs on major CV events in diabetic patients.

**Methods:** MEDLINE, Embase, Cochrane Library, and article reference lists were searched for RCTs that compared ACEIs and ARBs with controls in diabetic patients.

**Results:** Twenty-one RCTs compared ACEIs with controls (32,141 patients) and twelve compared ARBs with controls(23,617 patients). When compared with controls, ACEIs significantly reduced the risk of major CV events by 14%(0.86, 0.77-0.95), including myocardial infarction(MI) by 21% ( 0.79, 0.65-0.95) and heart failure by 19%(0.81, 0.71-0.93). ARBs treatment did not significantly affect major CV events(0.94, 0.87-1.01) except for heart failure(0.70,0.59-0.82). Both ACEIs and ARB agents did not significantly affect the risk for stroke.

**Conclusions:** In view of the high prevalence of CV disease in diabetic patients, ACEIs as the first choice of treatment may result in considerable benefit and reduction in MI and heart failure risk. More data are needed to clarify the role of ARB in survival benefits for diabetic patients.

### SA-PO373

**No Overall Increase in Volume Depletion Events with Empagliflozin (EMPA) in a Pooled Analysis of More Than 11,000 Patients with Type 2 Diabetes (T2DM)** Robert D. Toto,[1] Christoph Wanner,[2] John Gerich,[3] Afshin Salsali,[4] Thomas Hach,[4] Gabriel Kim,[4] Stefan Hantel,[5] Hans-Juergen Woerle,[4] Uli Christian Broedl.[4] *[1]Univ of Texas Southwestern Medical Center, Dallas; [2]Univ of Würzburg, Germany; [3]Univ of Rochester School of Medicine, Rochester, NY; [4]Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; [5]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.*

**Background:** The SGLT2 inhibitor EMPA is in development for the treatment of T2DM. SGLT2 inhibitors lead to osmotic diuresis, which could lead to volume depletion.

**Methods:** Using pooled data from Phase I, II and III trials, adverse events (AEs) consistent with volume depletion were evaluated through a search of investigator-reported AEs (8 prospectively defined preferred terms: BP decreased, BP ambulatory decreased, BP systolic decreased, dehydration, hypotension, orthostatic hypotension, hypovolemia, syncope) in patients with T2DM treated with placebo (PBO; n=3522), EMPA 10 mg (n=3630) or EMPA 25 mg (n=4602) in subgroups of age (<50, ≥50–<65, ≥65–<75, ≥75 yrs), eGFR (≥90, ≥60–<90, ≥30–<60, <30 mL/min/1.73m²) and diuretic use. Mean (SD) baseline age was 59.6 (10.0) yrs and eGFR 80.1 (22.1) mL/min/1.73m².

**Results:** The percentage of patients with volume depletion events was similar with PBO (49/3522 [1.4%]), EMPA 10 mg (52/3630 [1.4%]) and EMPA 25 mg (67/4602 [1.5%]). The percentage of patients with events increased from 0.9%, 0.4% and 0.7% with PBO, EMPA 10 mg and EMPA 25 mg, respectively, in the youngest group to 2.1%, 2.3% and 4.4%, respectively, in the oldest group. The percentage of patients with events increased from 0.8%, 0.9% and 0.6% with PBO, EMPA 10 mg and EMPA 25 mg, respectively, in the highest eGFR group to 9.6%, 14.3% and 7.1%, respectively, in the lowest eGFR group. Percentages were similar with PBO and EMPA in age and eGFR groups except as shown in the oldest group. More patients on diuretics (2.2–2.7%) than not (0.9–1.0%) reported these events; percentages were similar with PBO and EMPA.

**Conclusions:** EMPA was not associated with an increased frequency of volume depletion events versus PBO, except with EMPA 25 mg in patients ≥75 yrs of age.

*Funding:* Pharmaceutical Company Support - Boehringer Ingelheim

### SA-PO374

**Improvement in Insulin Resistance, Adipokines and Kidney Function following Bariatric Surgery** Sankar D. Navaneethan,[1] Steven K. Malin,[2] John P. Kirwan,[2] Philip R. Schauer.[3] *[1]Nephrology; [2]Pathobiology; [3]Bariatric Surgery, Cleveland Clinic.*

**Background:** Bariatric surgery improves metabolic function and is the most effective weight loss treatment for obesity. However, the efficacy of bariatric surgery on improving kidney function is unclear. Therefore, we determined the effects of bariatric surgery on kidney function, body composition, insulin resistance, and inflammation/adipokines in obese subjects with CKD.

**Methods:** Eleven subjects (8M/3F, age: 55 years, BMI 46 kg/m$^2$, iothalamate glomerular filtration rate [iGFR] 50 ml/min/1.73m$^2$) underwent either Roux-en-Y gastric bypass or gastric banding. Body composition (DEXA), inflammation (hs-CRP), insulin resistance (fasting insulin), adipokines (HMW-adiponectin and leptin) and kidney function (iGFR) were measured before and after 12 months of surgery. Pre-post data were compared with t-tests and Pearson's correlation was also performed.

**Results:** At 12 month follow-up, there was a significant decrease in BMI and fat mass. Bariatric surgery increased kidney function (Table). Fasting insulin, hs-CRP and leptin levels decreased, while HMW-adiponectin increased following surgery (Table).

| Variable | Median (Inter-quartile range) | p-value |
|---|---|---|
| Change in BMI (kg/m$^2$) | -9.3 (-15.2, -5.5) | 0.002 |
| Change in Fat mass (kg) | -25.4 (-32.6, -18.0) | 0.002 |
| Change in Log CRP | -0.54 (-0.69, -0.16) | 0.002 |
| Change in 1/insulin | -42.7 (-89.8, -0.21) | 0.03 |
| HMW Adiponectin change | 4666.9 (953.5, 6548.9) | 0.002 |
| Change in Leptin | -14.9 (-30.1, -5.9) | 0.004 |
| Change in Iothalamate GFR (ml/min/1.73 m$^2$) | 8 (5, 22) | 0.03 |

The improvement in kidney function correlated with the reduction in leptin (rho=-0.89, p<0.001) and insulin resistance (rho=-0.67, p=0.03). Decreased leptin was significantly correlated with lower fat mass (rho=0.63, p=-0.04) and insulin resistance (rho=0.73, p=0.01).

**Conclusions:** In CKD, bariatric surgery improves kidney function, inflammation, insulin resistance, and adipokines at one-year follow-up. Reductions in fat mass were linked to lower circulating leptin and insulin, suggesting that weight loss might contribute to the improvement in kidney function. Studies with longer follow-up data related to kidney function and the mechanisms that may contribute to the improvement in kidney function are warranted.

*Funding:* Other NIH Support - NCRR

### SA-PO375

**Glycemic Indices and Dialysis Modality in Diabetic (DM) and Non-Diabetic (NDM) Patients** Neal Mittman,[1] Lin Ma,[2] Mark E. Williams,[3] Julia I. Brennan,[4] Chinu M. Jani,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[2] Eduardo K. Lacson.[2] *[1]Long Island College Hospital, Brooklyn, NY; [2]Fresenius Medical Care, North America, Waltham, MA; [3]Joslin Diabetes Center, Boston, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

**Background:** Small studies have documented varying correlations between 2-3 glycemic indices. However, correlations among multiple types of novel glycemic indices have not been reported.

**Methods:** We explored simultaneous measurement of serum glucose (BG), glycosylated hemoglobin (A1c), serum fructosamine (SF), and glycated albumin (GA) in a national sample of 1659 DM and 1130 NDM hemodialysis (HD) and 318 DM and 324 NDM peritoneal dialysis (PD) patients from 26 FMCNA facilities distributed across the US. We

report mean values in these pts for the period January-March 2013, stratified by dialysis modality. In addition, SF was albumin-corrected (AlbF) and GA was expressed as a percentage glycation (%GA).

**Results:** We found that these glycemic markers correlated with each other and with blood glucose in both DM and NDM dialysis pts (all p≤0.001). However, there is a variability in the correlation coefficients (R-value) particularly in NDM relative to DM. Correlations were stronger in DM pts and less modality-related than in NDM pts. Interestingly, in the NDM pts, correlations were consistently stronger in PD vs. HD with BG and A1c. However, between the protein-based assays, the reverse was observed.

| r values HD/PD | A1c | SF | AlbF | GA | %GA |
|---|---|---|---|---|---|
| BG DM | 0.68/0.73 | 0.54/0.48 | 0.57/0.51 | 0.60/0.61 | 0.63/0.67 |
| BG NDM | 0.40/0.61 | 0.11/0.32 | 0.20/0.29 | 0.33/0.44 | 0.41/0.49 |
| A1c DM | | 0.69/0.64 | 0.66/0.63 | 0.77/0.78 | 0.78/0.83 |
| A1cNDM | | 0.19/0.34 | 0.13/0.25 | 0.35/0.50 | 0.36/0.54 |
| SF DM | | | 0.87/0.80 | 0.91/0.80 | 0.87/0.75 |
| SF NDM | | | 0.67/0.70 | 0.69/0.62 | 0.58/0.56 |
| AlbF DM | | | | 0.78/0.58 | 0.88/0.74 |
| AlbF NDM | | | | 0.38/0.18 | 0.58/0.35 |
| GA DM | | | | | 0.96/0.94 |
| GA NDM | | | | | 0.90/0.93 |

**Conclusions:** Differences between glycemic indices may reflect distinct exposure times to glycemia, i.e. A1c reflecting 2-3 months of exposure while SF and GA reflects only 2-3 weeks. Since GA is the predominant SF, stronger correlation between them is expected. In addition, modality comparisons may be influenced by differential protein losses and dialysate glucose exposure times.

## SA-PO376

**Effectiveness of the Diabetic StepAhead Program in Improving Patient Care among Diabetic ESRD Patients** Stephen D. McMurray,[1] Christine Ordway,[1] Carey Colson,[2] Pooja C. Oberai,[2] Anne N. Bubb.[1] *[1]DaVita VillageHealth, Vernon Hills, IL; [2]DaVita Clinical Research, Minneapolis, MN.*

**Background:** Diabetes mellitus (DM) is a leading cause of mortality and morbidity in end-stage renal disease (ESRD). StepAhead is an integrated DM care management program aimed at enhancing patient outcomes via physician-driven DM-management, regular eye exams and foot checks, and patient education on DM self-management. While the program is undergoing a year-long study to assess the reduction in lower limb amputations and mortality rates among this population, this interim analysis assessed its effectiveness after 6 months of implementation.

**Methods:** StepAhead was initiated in January 2012 and included 447 identified diabetic ESRD patients from 11 participating clinics. Information on clinical management of DM as well as DM-education was obtained from the electronic medical record at baseline and at 6 months.

**Results:** At 6-month follow-up, enrolled patients were 64 years old, 54.5% male, and 48.9% black. Cumulative outcome metrics (N = 356) show that 336 (94.4%) were provided physician management; 319 (89.6%) had an eye exam within the past year; 333 (93.5%) had received blood glucose education; and 330 (92.7%) had a glucometer by the end of six months. Of the 211 patients who were not physician managed for DM at baseline, 151 (72%) completed 6 months in the program, 136 (86.8%) became physician managed, and 36 (23.9%) were referred to a physician for DM-management at 6 months. Among the 218 patients who did not have an eye-exam within a year of enrollment at baseline, 120 (76.4%) received an eye-exam and 33 (21.0%) were referred for one at 6 months. Of the total 163 (73.0%) patients without a glucometer at baseline, 136 (84.0%) received one. Finally, 322 (89.2%) of the patients underwent at least one foot-check within 6 months of enrollment.

**Conclusions:** The data suggest that the StepAhead Program successfully enhances physician-driven DM-management and self monitoring. The preventive measures included in the program potentially allow for early detection of complications and delay progression, which will be evaluated in subsequent data cuts.

*Funding:* Pharmaceutical Company Support - DaVita HealthCare Partners, Inc.

## SA-PO377

**Effect on the Improvement of Prognosis in Simultaneous Pancreas-Kidney Transplantation and Kidney Transplantation Alone for Type 1 Diabetic Patients with End-Stage Renal Disease** Izumi Nyumura, Tetsuya Babazono, Michino Takagi, Yasuko Uchigata. *Diabetes Center, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** Kidney transplantation has been demonstrated to prolong survival in diabetic patients with ESRD; however, it is unclear whether simultaneous pancreas-kidney transplantation (SPK) yields additive benefit in type 1 diabetes (T1DM) patients with ESRD compared with kidney transplantation alone (KTA). We therefore conducted this study to compare prognosis in patients with T1DM undergoing chronic dialysis, KTA, and SPK. The results of transplantations were also compared between the two transplant groups.

**Methods:** 128 T1DM patients with EASD (84 women and 44 men) were studied, comprising of 54, 45 and 27 patients undergoing dialysis, KTA and SPK respectively. We compared survival among the three renal replacement therapy (RRT) groups after the start of dialysis. Patient and graft survival rates were also compared between the two transplant groups.

**Results:** Age of dialysis initiation, diabetes onset and duration of diabetes were significantly higher in SPK and KTA than chronic dialysis group. There were no significant differences among the three RRT groups in terms sex and dialysis treatment. Recipient's age was not different between SPK and KTA. In KTA 96% were from living donors. Ten-year patient survival rates after the start of dialysis was 63 % for the dialysis group, 94 % for the KTA group, and 92% for the SPK group, respectively (P<0.001). In multivariate analysis, SPK and KTA were associated with a lower mortality risk than dialysis patients, respectively (hazard ratio 0.10 and 0.28, p=0.003 and 0.029). Patient survival at 10 years was 90 % for the KTA group, 91 %for the SPK group, respectively (P=0.25). Graft survival at 10 years was 74 % for the KTA group, 59 % for the SPK group (P=0.52).

**Conclusions:** Prognosis for patients receiving SPK or KTA may be superior to that for patients undergoing dialysis. Patient and kidney graft survival were similar for SPK and KTA, because favorable results would be achieved with KTA.

## SA-PO378

**The Role of Circulating Fibroblast Growth Factor-21 in the Chronic Peritoneal Dialysis Patients** Jin Joo Cha,[1] Mi Jin Lee,[1] Young Youl Hyun,[2] Dae R. Cha,[1] Jung Eun Kim,[1] Mihwa Lee,[1] Hye Kyoung Song,[1] Young Sun Kang.[1] *[1]Nephrology, Korea Univ Ansan Hospital, Ansan, Kyunggido, Republic of Korea; [2]Nephrology, Sungkyunkwan Univ Kangbuk Samsung Hospital, Seoul, Republic of Korea.*

**Background:** In experimental studies, fibroblast growth factor-21(FGF-21) has shown to exert positive metabolic effects, inducing overweight reduction, lowering elevated glucose and triglyceride levels and increasing insulin sensitivity. A number of human studies also have reported elevated levels of FGF21 in patients with obesity, increased insulin resistance and fatty liver. However, the physiologic role in the humans is still controversial. We therefore investigated whether circulating FGF21 may act as a metabolic regulator in the setting of chronic renal failure.

**Methods:** Baseline plasma FGF21 levels were measured using an enzyme-liked immunosorbent assay in total 68 patients on peritoneal dialysis. Baseline metabolic parameters were assessed and prospective association of FGF21 with clinical morbidity was analyzed in a 2 year prospective study.

**Results:** All 68 patients (mean age= 49.1±10.6, PD vintage 44.5±29.9months) had residual GFR less than 2ml/min/1.73m2. Baseline FGF 21 showed wide variation ranging from 256.00 to 35896.00pg/ml (mean 4400.18±6100.35). Serum FGF21 level did not significantly differ in the presence of diabetes, hypertension. There were no associations between BMI, HOMA IR, HOMA beta levels, inflammatory markers(CRP, MCP-1, IL-6, TNF-α) and FGF21. Lipid profile and the fat component of the body (ATI) were also not correlated with FGF21. The use of CAPD decreased FGF21 level comparedto APD (p=0.01) and those with increased vascular calcifications (measured by calcification score) showed increased level of FGF21 (p=0.035). During median follow up of 30months, 9 (13.6%) patients had cardiovascular events and 35(53%) patients experienced infection which needed hospitalization. Serum FGF21 level did not increase the risk of cardiovascular complications nor infection.

**Conclusions:** These findings suggest that FGF21 might not have any metabolic role in the setting of chronic dialysis patients.

## SA-PO379

**Novel Coding Variants in NPHS1 Are Associated with Diabetic and All-Cause End-Stage Kidney Disease (ESKD) in African Americans** Jason A. Bonomo,[1] Maggie Ng,[1] Nicholette D. Palmer,[1] Barry I. Freedman,[2] Donald W. Bowden.[1] *[1]Ctr for Genomics & Personalized Med Res, Wake Forest School of Medicine, Winston Salem, NC; [2]Int Med/Nephrology, WFSOM.*

**Background:** Familial clustering and presumed genetic risk for diabetic- and non-diabetic ESKD appears strong in African ancestry populations. The genetic architecture of diabetes (DM)-associated ESKD has not been fully elucidated.

**Methods:** We reviewed exome sequencing data from 500 African American cases with DM-ESKD and 500 population-based controls in the T2D-GENES study focusing on previously implicated nephropathy genes (ie, NPHS1). Variants were genotyped in an independent replication cohort composed of 1,250 African American DM-ESKD cases and 850 population-based controls. Next, a trait discrimination study was performed in a cohort of 1,450 African American non-DM ESKD cases and 600 African American T2D non-ESKD cases. 1000 Genomes and the Exome Variant Server were used to identify additional rare- and low-frequency NPHS1 variants in African Americans. 25 additional NPHS1 missense variants were genotyped in DM-ESKD and non-DM ESKD cases and population-based controls. Finally Sequence Kernal Association Testing (SKAT) was performed.

**Results:** Initial analysis identified a low-frequency missense mutation (rs35238405, T233A) in the nephrin gene (NPSH1) associated with DM-ESKD (p=0.043, OR=3.01). Following adjustment for age, gender, admixture, and the apolipoprotein 1 (APOL1) G1/G2 alleles, association was confirmed in the replication cohort (p=0.038, OR=2.28). Rs35238405 was associated with non-DM ESKD (p=0.011, OR=2.5) but not T2D *per se* (p=0.14) after adjustment for covariates. In a combined, all-cause ESKD analysis, rs35238405 had an OR of 2.54 (p=0.0041) after covariate adjustment. Of the 25 genotyped NPHS1 variants, 9 were monomorphic and two were associated with all-cause ESKD in single-SNP association testing (rs146400394 p=0.0089, OR=0.04; rs143649022 $p=7.9\times10^{-6}$, OR=0.18) following covariate adjustment. P values of $4.2\times10^{-7}$ and $2.4\times10^{-4}$ were obtained in an unadjusted and adjusted SKAT analysis.

**Conclusions:** Coding variants in NPSH1 appear to alter the risk for common forms of diabetic and non-diabetic ESKD in African Americans.

*Funding:* NIDDK Support

## SA-PO380

**Does Iron Status Affect Platelet Counts in Hemodialysis Patients?** Kiyomi Koike,[1] Kei Fukami,[1] Kazumasa Shimamatsu,[2] Atsushi Kawaguchi,[3] Seiya Okuda.[1] *[1]Div of Nephrology, Dept of Medicine, Kurume Univ School of Medicine, Kurume, Fukuoka, Japan; [2]Shimamatsu Naika Iin, Chikushino, Fukuoka, Japan; [3]Biostatistics Center, Kurume Univ, Kurume, Fukuoka, Japan.*

**Background:** Normalizing hemoglobin(Hb) levels by ESAs has been reported to be associated with risk of cardiovascular death/events via thrombocytosis due probably to ESA-induced iron deficiency 'platelet link hypothesis' in CKD patients. However, whether or not disturbed iron status really affects platelet counts.

**Methods:** In our cohort, 117 HD outpatients followed for 4 years in a single center. Target Hb level was 11.0 g/dl. The outcome variable is increase in platelet counts. Potential predictors are iron indices such as transferring saturation (TSAT), serum iron and ferritin. We investigated the association between iron indices and increase in platelet counts by mixed effect model adjusted for time, age, gender, Hb, CRP, alb, a weekly ESAs dose and a monthly i.v. iron dose with non-linear analysis. Next, in the analysis for the first difference of a time series which is the series of changes from one period to the 3 months later (Δ), the correlation between Δiron indices and Δplatelet counts was examined. Further, we analyzed whether or not ESAs dose was associated with iron status.

**Results:** Mean age; 61.9 yrs and mean Hb; 10.8 g/dl. In multivariate regression analyses, lower TSAT levels (increase in platelet counts $0.3\times10^4/\mu L$ per 10% decrease in TSAT, 95%CI, -4.8 to -1.7; p<.0001) and higher ESAs dose (95%CI, 0.03 to 0.22; p=.0077) were significantly correlated with larger increase in platelet counts independently of Hb, Alb, CRP and i.v. iron dose. ΔTSAT was negatively and Δferritin was positively correlated with Δplatelet counts (95%CI, -4.9 to -1.7; p<.0001 and 95%CI, 0.0007 to 0.004; p=.003, respectively). Higher ESAs dose to keep target Hb levels was significantly associated with lower TSAT and ferritin (<330ng/ml) levels (p<.0001, respectively).

**Conclusions:** These observations suggest that iron status, especially decreased TSAT, may directly affect increased platelet counts and subsequently cause cardiovascular events in HD patients on ESAs treatment.

## SA-PO381

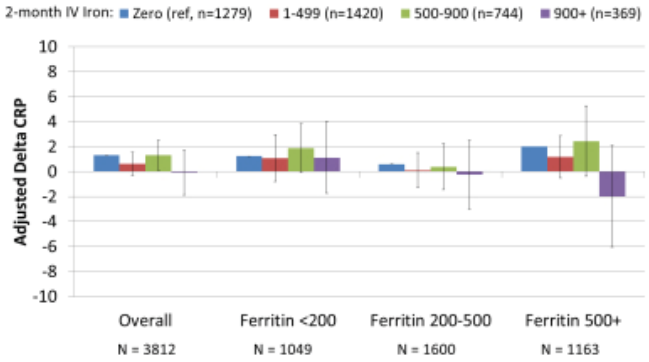
**IV Iron Is Not Associated with Change in CRP across Dose and Ferritin Levels: A DOPPS Study** Laura H. Mariani,[1] Keith McCullough,[2] Mark R. Marshall,[3] Patricia De Sequera,[4] Ananda Sen,[1] Masaaki Inaba,[5] Ronald L. Pisoni,[2] Bruce M. Robinson.[2] *[1]U. of Michigan; [2]Arbor Research Collaborative for Health; [3]Middlemore Hospital; [4]Hospital Infanta Leonor; [5]Osaka City U. Graduate School of Medicine.*

**Background:** Inflammation and CRP levels are positively associated with mortality in hemodialysis patients. The sustained effects of IV iron on CRP levels are unclear, and may depend on patient iron and inflammatory status. This study examined the association between iron dose and change in CRP.

**Methods:** Observational DOPPS phase 4 (2009-11) data from facilities that measured CRP at least quarterly on 75+% of patients, in seven European countries and Australia/New Zealand were used to determine IV iron dose over two consecutive months (iron dose measurement period, or IDMP). Baseline CRP was the most recent value prior to the IDMP. Final CRP was collected during the 2nd month of the IDMP or up to 1 month later. Linear models were used to determine the association between delta (final – baseline) CRP levels and total IV iron dose during the IDMP, controlling for patient demographics (age, race, sex, BMI); comorbidities (15 factors); and IV iron dose, TSAT, ferritin, Hgb, and ESA use in the month preceding the IDMP.

**Results:** Change in CRP did not differ meaningfully across iron dose categories, overall or by ferritin levels.

**Figure 1: Adjusted Change in CRP by Ferritin and IV Iron**




Additionally, no pattern for change in CRP was seen when excluding baseline ESA use, IV iron dose or lab values from the model, or in separate models based on the following at baseline: CRP < 10 v. 10+; TSAT < 20% v. TSAT 20%+; IV iron dose 0 v. >0 mg/month.

**Conclusions:** We found no association between two-month IV iron dose (including doses suggestive of replacement IV iron dosing) and change in CRP across a range of markers indicative of iron and inflammatory status. Associations with longer-term IV iron dosing also merit study.

## SA-PO382

**Achieved Iron Stores and Clinical Outcomes in a Trial of Ferric Citrate as a Phosphate Binder** Kausik Umanath,[2] Mohammed Sika,[1] Mark Koury,[1] Jamie P. Dwyer,[1] Julia Lewis,[1] The Collaborative Study Group.[3] *[1]Vanderbilt Univ; [2]Henry Ford Hosp; [3]CMM.*

**Background:** The safety of achieved iron stores in dialysis patients is controversial. Utilizing data from a multicenter, randomized trial in 441 dialysis subjects treated with ferric citrate (FC) or active control (AC) (calcium acetate, sevelamer carbonate, or a combination), we grouped subjects based on their achieved iron stores and examined their safety profiles.

**Methods:** Subjects were randomized 2:1 to FC or AC, and followed during a 52-week safety assessment period (SAP). IV iron use was at the discretion of the site as long as the serum ferritin was ≤ 1000ng/mL and the transferrin saturation (TSAT) was ≤ 30%. All oral iron and vitamin C supplements were prohibited. Adverse events, ferritin, and transferrin saturation (TSAT) were collected and analyzed.

**Results:** In the SAP, baseline mean serum ferritin was 594 + 297ng/mL. At week 52, mean ferritin was 776 + 444 ng/mL. Mean TSAT at baseline was 31 + 11%. At week 52, mean TSAT was 34 + 14%. Treatment emergent adverse events (TEAE), death, and serious adverse events (SAE) by tertiles of mean ferritin and TSAT averaged over 52 weeks are shown in Tables 1 and 2.

Table 1

| Ferritin, ng/mL | <500 | 500-1000 | >1000 |
|---|---|---|---|
| Subjects (*N*) | 117 | 232 | 92 |
| Subjects with Death (%) | 7.7 | 3.4 | 4.3 |
| Subjects with Any SAE (%) | 49.5 | 41 | 37 |
| Subjects with Any TEAE (%) | 95 | 99 | 97 |

Table 2

| TSAT, % | < 30 | 30-50 | >50 |
|---|---|---|---|
| Subjects (N) | 160 | 243 | 38 |
| Subjects with Death (%) | 6.9 | 3.7 | 2.6 |
| Subjects with Any SAE (%) | 52 | 37 | 39 |
| Subjects with Any TEAE (%) | 97 | 97 | 100 |

**Conclusions:** There was no evidence of an increase in adverse events associated with higher ferritin and TSAT levels.

*Funding:* Pharmaceutical Company Support - Keryx Biopharmacueticals

## SA-PO383

**Anemia Management: To HOLD or Not to HOLD** Adam E. Gaweda,[1] Michael A. Jacobs,[1] Yossi Chait,[2] Joseph Horowitz,[2] Michael J. Germain,[3] Christopher V. Hollot,[2] Michael E. Brier.[1] *[1]Univ of Louisville; [2]UMass Amherst; [3]Western New England Renal and Transplant Associates.*

**Background:** Safety issues related to the use of Erythropoiesis Stimulating Agents (ESA) in treating anemia of End-Stage Renal Disease (ESRD) affect practice patterns of anemia management. The FDA recommends ESA dose reduction or hold when hemoglobin (Hgb) exceeds 11 g/dL. We compared these two dose adjustment strategies through simulation of anemia management.

**Methods:** We performed a simulation of two clinical scenarios: 1) Hgb exceeding 11 g/dL following increase in ESA dose 2) Stable Hgb of 10.5 g/dL exceeding 11 g/dL due to random variability. The two dose adjustment strategies tested were (1) HOLD: hold ESA when Hgb reaches 11 g/dL and restart at 50% previous dose when Hgb falls below 10 g/dl, and (2) REDUCE: reduce ESA by 50% when Hgb reaches 11 g/dL. We calculated the following outcomes: a) percent time Hgb is less than 10 g/dL, b) percent time Hgb is in range 10-11 g/dL, c) mean weekly ESA dose. All outcomes were calculated over the period of 4 months after dose adjustment. We used an erythropoiesis model with two parameters: a) erythropoietic sensitivity and b) red cell lifespan, to generate a virtual population of 42 subjects. Statistical analysis was performed using mixed effect regression to account for multiple measurements within subjects.

**Results:** Scenario 1): HOLD resulted in the Hgb below 10 g/dL 50% of the time VS 0% of the time with REDUCE ($p < 0.001$). Mean weekly ESA dose was 21% lower with HOLD compared to REDUCE ($p < 0.001$)

Scenario 2): HOLD results in the Hgb below 10 g/dL 60% of the time VS 53% with REDUCE ($p = 0.017$). Mean weekly ESA dose was 10% lower with HOLD compared to REDUCE ($p = 0.138$).

**Conclusions:** When Hgb persistently rises above 11 g/dL, the choice of dose adjustment strategy should be based on the risk assessment for an individual patient (risk of transfusion due to low Hgb vs. risk of cardiovascular side effects due to ESA). When Hgb incidentally exceeds 11 g/dL, both strategies result in an unnecessary Hgb fall and should be avoided. To differentiate between the two scenarios in clinical practice, attention must be paid to proper monitoring and trending of Hgb.

*Funding:* NIDDK Support

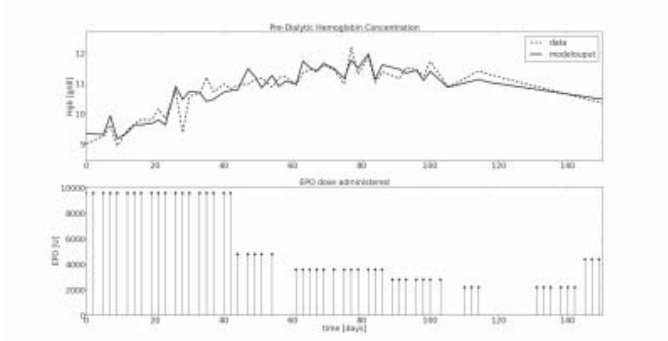
## SA-PO384

**A Mathematical Erythropoiesis Model Adjusted to Individual Hemodialysis Patients** Doris Helene Fuertinger,[1] Franz Kappel,[2] Stephan Thijssen,[1] Peter Kotanko.[1] *[1]Renal Research Institute, New York; [2]Univ of Graz, Austria.*

**Background:** Interindividual variability in red blood cell (RBC) lifespan, bone marrow response to erythropoietin (EPO), endogenous EPO production and half-life is high in hemodialysis (HD) patients. Routine measurement of these quantities is elusive, thus making it almost impossible to predict the individual response to EPO administration schemes. Here we describe the adaptation of a complex mathematical model of erythropoiesis to individual HD patients.

**Methods:** A mathematical model (Fuertinger et al, JMathBiol 2013) is adjusted to individual HD patients treated with EPO. The patient's blood volume and number of stem cells are estimated based on height and weight. Crit-Line™ devices were used for intradialytic hemoglobin (Hgb) measurements. Hgb data is used to identify the RBC lifespan, bone marrow response to EPO, endogenous EPO production, and EPO half-life in individual HD patients.

**Results:** We studied 1,866 HD sessions in 36 patients (mean (range) age 59.8 (25-85) years; 66.7% Blacks; 52.8% females; 61.1% diabetes; body mass index (BMI) 26.3 (17.9-50.4) kg/m$^2$). Independent of gender and BMI the mean (range) of the identified erythropoiesis parameters were biologically plausible: RBC lifespan 72.7 (50.5-103.4) days, and EPO half-life 6.98 (4.1-12.3) hours. The model accurately represented the Hgb dynamics in all patients. For an example for an adaption of the model to an individual HD patient, see Figure.




**Conclusions:** Our results indicate that this model can be adjusted to HD patients based on gender, height, weight, 3x weekly Hgb measurements, and EPO doses. The model quantitatively describes the specific dynamics of erythropoiesis and Hgb levels in EPO treated HD patients. The model can be used to investigate, compare, and improve EPO administration schemes based on metrics of accuracy, precision, and cost-effectiveness.

## SA-PO385

**Statin Therapy in Dialysis Patients and Its Effects on Erythropoietin Resistance Index** Jerome Pineault, Jean-Philippe Lafrance, Martine Leblanc, Georges Ouellet, Robert Zoël Bell, Michel Vallee, Vincent Pichette. *Medicine, Div of Nephrology, Maisonneuve-Rosemont Hospital, Montreal, Canada.*

**Background:** Erythropoietin (EPO) resistance and anemia is a burden among dialysis patients and efficient therapies are lacking. We studied the effect of statin therapy on EPO responsiveness in our dialysis population.

**Methods:** We reviewed the records of 662 patients receiving chronic dialysis from September 2008 to March 2011. We measured EPO resistance index (ERI) from the weekly dose of EPO/kg divided by Hb (g/dL) each month. Association between statin use and ERI was estimated using a multivariate linear mixed-effects, adjusting for sex, age, diabetes, dialysis modality and ACEi/ARB use.

**Results:** A total of 503 patients underwent analysis after exclusion. The multivariate regression analysis showed that ERI was not improved with statin therapy ($e^{\beta} = 0.97$, with $P > 0.05$). Patients getting started on statins during the study (N=42) had increased ERI after statin exposure and those getting off statins (N=47) had their ERI decreased after statin was stopped, the exact opposite of what was expected, even though results were non statistically significant. Male sex and peritoneal dialysis were associated with a lower EPO resistance. Also, ACEi/ARB and diabetes were associated (but not significantly) with higher EPO resistance.

**Conclusions:** Statin use was not associated with improved EPO responsiveness. Also, male sex and peritoneal dialysis appeared to be potential protectors from EPO resistance. However, a confirmation of these results is needed by larger randomized-controlled trials.

*Funding:* Clinical Revenue Support

## SA-PO386

**Hyporesponsiveness to Erythropoiesis-Stimulating Agent as a Prognostic Factor in Japanese Hemodialysis Patients: The Q-Cohort Study** Rieko Eriguchi,[1] Masatomo Taniguchi,[2] Kazuhiko Tsuruya,[2] Hideki N. Hirakata,[3] Satoru Fujimi,[1] Takanari Kitazono.[2] *[1]Fukuoka Renal Clinic, Fukuoka, Japan; [2]Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [3]Div of Nephrology and Dialysis Center, Japanese Red Cross Fukuoka Hospital, Fukuoka, Japan.*

**Background:** Several longitudinal studies have shown that responsiveness to erythropoiesis-stimulating agent (ESA) is related to the prognosis in hemodialysis (HD) patients. We investigated the effects of hyporesponsiveness to ESA on mortality in Japanese HD patients, taking factors modifying its effects into account.

**Methods:** A total of 2,905 Japanese HD patients aged ≥18 years, who received ESAs, were prospectively followed up for 4 years. The responsiveness to ESA was estimated using a erythropoietin resistance index (ERI), defined as erythropoietin dosage for a week divided by the post-HD weight and hemoglobin value. Patients were divided into three groups with tertiles of ERI values; low ERI: ≤5.10, intermediate ERI: 5.11-9.43, and high ERI: ≥9.44. The risk estimates were calculated by using a Cox proportional hazards model.

**Results:** During the follow-up period, 482 patients died from any causes. The mortality rate for 4 years increased linearly with higher ERI levels, being 12.5%, 17.1%, 28.0% for low, intermediate, and high ERI levels (p for trend < 0.001). Compared with those with lower ERI levels, the multivariate-adjusted hazard ratio (mHR) was significantly higher in patients with high ERI level (mHR, 1.59 [95%, confidence interval, 1.24-2.04]. Among high ERI group, patients with Kt/V≥1.57 had a significantly lower risk of death from any causes, than those with Kt/V<1.57 (mHR, 0.74 [0.55-1.00]).

**Conclusions:** Our findings suggest that ESA responsiveness can be considered a significant prognostic factor in Japanese HD patients, and higher dose of dialysis seems to be effective for improving prognosis of HD patients with hyporesponsiveness to ESA.

## SA-PO387

**Virdaglin, an Inhibitor of Dipeptidyl Peptidases 4 (DPPI-4), May Enhance the Effects of Continuous Erythropoietin Receptor Activator (CERA) in Treating Renal Anemia in Hemodialysis (HD) Patients** Jyunichiro Hashiguchi,[1] Satoshi Funakoshi,[1] Kenji Sawase,[1] Yoshiaki Lee,[1] Masatoshi Hayashida,[1] Takashi Harada,[1] Kenichi Miyazaki,[1] Tomoya Nishino,[2] Yoko Obata,[2] Shigeru Kohno,[2] Kazunori Utsunomiya.[3] *[1]Div of Blood Purification, Nagasaki Renal Center, Nagasaki, Japan; [2]Dept of Internal Medicine, Nagasaki Univ Graduate School of Medicine, Nagasaki, Japan; [3]Dept of Diabetology, Jikei Univ, Tokyo, Japan.*

**Background:** DPP-4 (=CD26) is expressed on activated lymphocytes and posess hematopoietic effects via pathways for regulating cytokines (Jones B, et al. Blood 2003). Continuous eythropoietin receptor activator (CERA) is a long-acting erythropoiesis-stimulating agent, and the effects may possibly be enhanced by hematopoietic effect of DPP-4 inhibitor.

We evaluated the erythropoietic effects of DPP-4 inhibitor in HD patients treated with CERA when converted from sulfonyl urea (SU) or phenylalanine analogues (PA).

**Methods:** From September to December 2010, 15 HD outpatients were enrolled in this study after appropriate IC, and were monitored by various parameters including fasting plasma glucose (FPG), HbA1c, Hb, albumin or body weight. CERA doses had stayed the same.

**Results:** All the subjects were administered CERA at 134 ± 27.5 mg. FPG and HbA1c level had been stabilized after dose adjustment of virdaglin. As shown in Table 1, there was significant increase in Hb level (10.8 ± 1.49 to 11.6 ± 1.08, p=0.018) though CERA doses had been unchanged.

| | SU / PA | Virdaglin | p value |
|---|---|---|---|
| HbA1c (%) | 6.87±2.21 | 6.55±2.89 | 0.25 |
| n-PCR (g/Kg/day) | 0.86±0.09 | 0.88±0.11 | 0.39 |
| T-cho (mg/dL) | 166.3±29.3 | 148.5±30.6 | 0.40 |
| i-PTH (μg/dl) | 117.6±86.9 | 85.87±0.4 | 0.15 |
| WBC (/mm$^3$) | 6510±3880 | 6920±4040 | 0.39 |
| **Hb (g/dL)** | **10.8±1.49** | **11.6±1.08** | **0.018** |
| Plt (/mm$^3$) | 20.5±8.5 | 19.4±9.9 | 0.27 |

Other nutritional parameters stayed the same.

**Conclusions:** Virdaglin can potentially enhance the hematopoietic effects of CERA in the treatment of anemia for diabetic HD patients.

*Funding:* Private Foundation Support

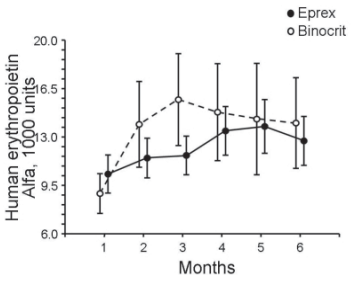
**SA-PO388**

**Human Erythropoietin Alfa Originator and Biosimiliar: Safety, and Therapeutic Equivalence in a Cohort of Emodialysis Patients. A Pilot Study** Paolo Lentini,[1] Luca Zanoli,[2] Massimo de Cal,[1] Stefania Rastelli,[2] Anna Basso,[1] Graziella Berlingò,[1] Valentina Pellanda,[1] Andrea Contestabile,[1] Roberto Dell'Aquila.[1] *[1]Nephrology, San Bassiano Hospital, Bassano del Grappa, Italy; [2]Internal Medicine, Catania.*

**Background:** Compared with erythropoietin originator, the use of erythropoietin biosimilar is suggested only for economic reasons.Therefore, we aimed to compare the therapeutic equivalence of human erythropoietin Alfa Originator (Eprex) and Biosimiliar (Binocrit) to maintain haemoglobin and haematocrit levels in the therapeutic range and the monthly cost of each therapy.

**Methods:** 63 chronic haemodialized patients(64±17yrs)on treatment with Eprex were assigned to Eprex or Binocrit harm and followed up for 6 months.42 subjects treated with Eprex and 11 subjects treated with Binocrit were included in this analysis.A general linear model for repeated measures,adjusted for haemoglobin and haematocrit levels was used.In this analysis a cost of £55.31 for 10,000 UI of Eprex and £50.91 for 10,000 UI of Binocrit was used.

**Results:** Monthly erythropoietin Alfa dose was 12,078±1,440 and 12,981±2,677 UI in Eprex and Binocrit group; mean haemoglobin and haematocrit levels were comparable between groups.In multivariate analysis,adjusted for monthly haemoglobin and haematocrit levels, erythropoietin Alfa dose was higher in Binocrit than Eprex group.




Even if Binocrit was 8% cheaper than Eprex, the monthly cost of erythropoietin Alfa to maintain haemoglobin and haematocrit levels in the therapeutic range was only 1% lower with Binocrit than with Eprex.

**Conclusions:** Our preliminary data showed that the switch from human erythropoietin Alfa originator to biosimiliar is associated with an increase of erythropoietin dose during a follow-up of 6 months and with a comparable monthly cost of Binocrit and Eprex therapy.

**SA-PO389**

**Elevated Cobalt Levels in Chronic Hemodialysis Patients Are Not Associated with Increased Morbidity or Mortality, but Are Associated with Higher Hemoglobin Levels and Decreased Erythropoiesis Stimulating Agent Requirements** Rebecca Backenroth,[1] Dvora Rubinger,[1] Shade Artoul.[2] *[1]Nephrology, Hadassah Univ Medical Center, Jerusalem, Israel; [2]Pediatrics, Kaplan Medical Center, Rehovot, Israel.*

**Background:** We have previously shown that chronic hemodialysis patients(CHD) have higher mean Cobalt levels (Co) than healthy controls. The higher Co were associated with higher hemoglobins(Hb), and lower erythropoiesis stimulating agent(ESA) doses. Furthermore, ESA free patients with good Hb had strikingly higher Co.

Since Co can be toxic, we evaluated possible detrimental effects of the spontaneously elevated Co.

**Methods:** Morbidity and mortality were evaluated retrospectively in all CHD who had Co determinations, with clinical and lab variables from charts and computerized lab and hospitalization records. Statistical analysis included T test, Mann Whitney, Chi Sq, Fisher's Exact, Pearson correlation, Regression analysis, Kaplan Meier survival and the Log Rank tests. Numbers are mean + SD.

**Results:** 150 CHD, 66% male, age 65±15, on dialysis for 6±4 yrs were studied.

There was no difference in underlying or new onset of illnesses, hospitalizations nor mortality between patients with Co lower or equal to the mean and those with higher levels, except the incidence of skin malignancy, which was less in CHD with lower initial Co. This relation disappeared when all Co levels were averaged. There were no melanomas or aggressive skin tumors. Kaplan Meier analysis was similar for low and high Co groups.

Mean Co was similar in groups with prevalent and incident diseases, with and without hospitalizations or survival.

Background illness and causes of mortality were similar to literature reports.

Targets of Co toxicity such as cardiac, thyroid and pulmonary disease were similar in low and high Co groups.

**Conclusions:** Spontaneously elevated Co in CHD are not associated with elevated morbidity or mortality. Minor skin malignancies may increase with higher Co. Since Co is associated with higher Hb and less ESA use, it may have therapeutic potential as an ESA. Randomized clinical trials are required to determine the possible role of low dose Cobalt in the treatment of anemia in dialysis patients.

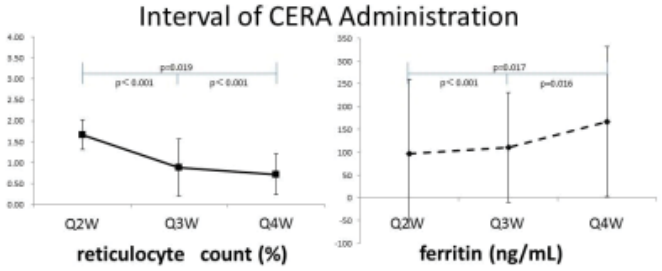
**SA-PO390**

**Biweekly Administration of a Continuous Erythropoietin Receptor Activator (CERA) Is Recommended in the Treatment of Anemia in Hemodialysis (HD) Patients: A Longitudinal, Single Institutional Study** Satoshi Funakoshi,[1] Jyunichiro Hashiguchi,[1] Yoshiaki Lee,[1] Kenichi Miyazaki,[1] Kenji Sawase,[1] Takashi Harada,[1] Tomoya Nishino,[2] Yoko Obata,[2] Shigeru Kohno,[2] Kazunori Utsunomiya.[3] *[1]Div of Blood Purification, Nagasaki Renal Center, Nagasaki, Japan; [2]Dept of Internal Medicine, Nagasaki Univ Graduate School of Medicine, Nagasaki, Japan; [3]Dept of Diabetology, Jikei Univ, Tokyo, Japan.*

**Background:** CERA is a long-acting erythropoiesis-stimulating agent that is approved for the treatment of renal anemia, but data on routine use of either once-monthly or biweekly in HD patients are scarce. In several phase 3 trials CERA once every two weeks were compared to results with other ESAs, and other phase 3 trials evaluated the effects of once-monthly CERA.

**Methods:** This study to evaluate the efficacious intervals of CERA administration was a prospective, single-center trial with duration of up to 18 months. Seventy-eight HD patients receiving CERA in our facility were included, and CERA administration was converted from once-monthly to twice-monthly (with half CERA dose). Since there were 5-week months, the parameters were compared in once biweekly (Q2W), every 3 weeks (Q3W) and every 4 weeks (Q4W). The CERA doses were adjusted in each patients to maintain Hb levels in the range 10-12 g/dL.

**Results:** At the end of the 18-month study, CERA doses per month were reduced in 24 out of 78 subjects. Mean Hb level was 10.5 ± 1.4 g/dL at baseline and 10.7 ± 1.7 g/dL at the end of the study. As shown in Figure 1, in biweekly administration of CERA, but not Q3W or Q4W, significantly higher reticulocytes counts and lowest ferritin levels were observed, indicating biweekly CERA was potentially the most efficacious in terms of red blood cell iron utilization or erythropoiesis.




**Conclusions:** Biweekly administration of CERA is recommended, and may potentially be cost-effective.

*Funding:* Private Foundation Support

**SA-PO391**

**The Relationship between Serum Angiopoietin-Like Protein 2 Levels and Atherosclerosis Factors in Hemodialysis (HD) Patients** Terumasa Nakagawa, Rika Yamazoe, Jun Morinaga, Yoshikazu Miyasato, Miki Ueda, Tomoaki Onoue, Teruhiko Mizumoto, Kohei Uchimura, Manabu Hayata, Yutaka Kakizoe, Kenichiro Kitamura. *Nephrology, Kumamoto Univ Graduate School of Medical Science, Kumamoto, Japan.*

**Background:** Angiopoietin-like protein 2 (Angptl2), a proinflammatory protein, was recently revealed to contribute to the pathogenesis of atherosclerosis in mice. However, its role in humans still remains poorly understood. The purpose of this study is to identify the factors associated with serum angptl2 levels and to elucidate the relationship between serum angplt2 levels and atherosclerosis in hemodialysis patients (HD).

**Methods:** Three hundred and eighty eight HD patients (men: 63%, mean age: 65±12 year, mean HD duration: 9.0±8.6 year) were enrolled. Serum Angptl2 levels and Hs-CRP levels were determined by the ELISA kit. Routine laboratory data, clinical history were collected. Cardio-ankle vascular index (CAVI) and carotid artery intima-media thickness were also measured.

**Results:** Mean serum Angptl2 level in all patients was 3.40μg/mL, which was higher than in healthy subjects in Japan. In diabetic HD patients (N=136, mean age: 65±10 year, mean HD duration: 5.1±3.9 year, mean serum Angptl2 levels: 3.53±1.09μg/mL), CAVI, the frequency of percutaneous transluminal AV shunt angioplasty, and serum triglyceride levels were significantly associated with serum Angptl2 levels (R=0.54, P<0.001) in multiple regression analysis. In non-diabetic patients (N=116, mean age: 61.7±11.5 year, mean HD duration: 13.7±9.95 year, mean serum Angptl2 levels: 3.09±1.17μg/mL), HD duration, BMI, and Hs-CRP levels were significantly associated with serum Angptl2 levels (R=0.73, P<0.001). Cerebral infarction in non-diabetic patients was related to serum Angptl2 levels (β=0.62, P=0.01) in multiple regression analysis (R=0.54, P<0.01). Furthermore, in a multiple logistic regression, serum Angptl2 levels ≥ 4μg/mL was a significant risk factor for cerebral infarction in male diabetic HD patients (P=0.04, odds ratio: 2.90, 95%confidence interval: 1.05 - 8.02).

**Conclusions:** Serum Angptl2 levels are associated with several atherosclerosis factors, and it could be a biomarker for cerebral infarction in HD Patients.

## SA-PO392

**Peripheral Monocytes May Be Predisposed to Uptake of Pro-Atherogenic Low-Density Lipoprotein through Genetically-Enhanced Expression of Scavenger Receptors in Hemodialysis Patients** Miki Nishida,[1] Minoru Ando,[2] Yusuke Iwamoto,[3] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Dept of Medicine, Kidney Center, Tokyo Women's Medical Univ, Sinjyuku-ku, Tokyo, Japan; [2]Dept of Nephrology, Tokyo Metropolitan Komagome Hospital, Bukyo-ku, Tokyo, Japan; [3]Dept of Nephrology, Saito Memorial Hospital, Warabi-shi, Saitama, Japan.*

**Background:** Atherosclerosis is accelerated in hemodialysis (HD) patients. Monocyte-macrophage scavenger receptor (SR) plays a pivotal role in promoting foam cell formation by binding and internalizing pro-atherogenic low-density lipoprotein (LDL).

**Methods:** Transcriptional levels of SR class A (SR-A) and class B (CD36) were simultaneously measured in peripheral monocytes by quantitative real-time RT-PCR, using the comparative threshold (Ct) method. Peripheral monocytes were magnetically labeled and isolated using a MACS Separator (Miltenyi Biotec Inc). Subjects included 39 chronic HD patients (mean age, 72 years) and 14 healthy controls. Multivariate logistic analysis was used to determine an association of SR expressions with prevalence of cardiovascular disease (CVD), including myocardial and cerebral infarctions and peripheral artery disease.

**Results:** The relative expressions of SR-A and CD36 mRNA ($2^{-\Delta\Delta Ct}$) were significantly greater in monocytes from HD patients than in those from controls (mean [95% CI of the mean]: 3.14 [2.58-3.69] versus 1.09 [0.79-1.39], P <.0001; and 4.45 [3.62-5.28] versus 1.15 [0.78-1.51], P=0.0004, respectively). Each SR receptor expression was significantly higher in HD patients who had CVD than in those who did not: SR-A, 3.56 [2.89-4.22] versus 2.19 [1.29-3.10], P=0.0137; and CD36, 2.43 [1.92-2.93] versus 1.65 [1.22-2.08], P=0.0479, respectively. Moreover, each SR expression was significantly associated with prevalence of CVD, after adjustment by age, gender, presence of diabetes, HD vintage and serum LDL-cholesterol level: odds ratio (95% CI), 1.95 (1.06-4.63) for SR-A and 4.18 (1.44-22.6) for CD36.

**Conclusions:** Transcriptional expressions of two SR types were enhanced in the HD patients, particularly in those with CVD. Prospective studies are warranted to ascertain the association between SR expressions and incidence of CVD.

## SA-PO393

**Plasma Calprotectin Levels in Chronic Kidney Disease** Denise Mafra,[1] Natalia Alvarenga Borges,[1] Amanda Barros,[1] Julie Lobo,[1] Denis Fouque.[2] *[1]Federal Univ Fluminense, Niteroi, Rio de Janeiro, Brazil; [2]Dept of Nephrology and Nutrition, CENS, Centre Hospitalier Lyon Sud, France.*

**Background:** Calprotectin (MRP8/14) is the main neutrophil cytosolic protein, which exerts potent proinflammatory effects through activation of Toll-like receptor-4 (TLR-4). Elevated plasma MRP-8/14 predicts the risk of future cardiovascular events. The aim of this study was to evaluate calprotectin plasma levels in CKD patients without acute infection under different treatments by comparison to healthy individuals.

**Methods:** Sixteen conservative treatment (CT) pts (44% men; 62.5±8.3 yrs; BMI 26.3±3.8 kg/m$^2$, CKD stage III - IV), nineteen hemodialysis (HD) pts (50.0% men; 55.1±13.1yrs; BMI 24.4±4.7; dialysis vintage, 49.5 [10-234]months), 8 peritoneal dialysis (PD) pts (12.5% men; 59.4±15.8yrs; BMI 23.1±2.4; dialysis vintage, 27.0 [20-52] months) and 16healthy subjects (43.7% men; 53.6±5.0yrs; BMI 24.6±2.7) were studied. Blood samples were obtained after a 12h fast and calprotectin was measured by enzyme immunometric assay (Bühlmann, France).

**Results:** Calprotectin levels were higher in all CKD groups (CT: 13.6±5.0, HD: 11.4± 3, PD: 12.0±3.9 ng/mL) compared with healthy individuals (9.5±0.8 ng/mL), but a significant difference was observed only between CT pts and healthy individuals (p<0.05). No correlation was found between plasma calprotectin levels and dialysis vintage.

**Conclusions:** We found increased levels of plasma calprotectin in CKD pts, particularly during conservative treatment. This may contribute to the chronic inflammation status and may allow a better identification of the cardiovascular risk in these patients.

*Funding:* Government Support - Non-U.S.

## SA-PO394

**Lipoxin A4 and Resolvin E1 Reduce Inflammatory Monocytes in Haemodialysis Patients** Eileen Nolan,[1,2] Debra F. Higgins,[1] Yvonne M. O'Meara,[2,3] Catherine Godson.[1,2] *[1]UCD Diabetes Complications Research Centre, UCD Conway Institute, Univ College Dublin, Dubin, Ireland; [2]UCD School of Medicine and Medical Science, Univ College Dublin, Dublin, Ireland; [3]Dept of Nephrology, Mater Misericordiae Univ Hospital, Dublin, Ireland.*

**Background:** An increase in CD16+ circulating monocytes occurs in haemodialysis (HD) and is associated with cardiovascular risk. CD16+ monocytes are pro-inflammatory and contribute to micro-inflammation. Lipoxin A4 (LXA4) and resolvin E1 (RvE1) are lipid mediators which promote the resolution phase of inflammation. Our hypothesis is that LXA4 and RvE1 may have a role in reducing inflammatory monocytes in HD.

**Methods:** Peripheral blood samples taken from 6 HD patients and 3 healthy controls were treated with LXA4 (0.01 nM) or RvE1 (0.01 pM), stained with mAb to CD14, CD16 and HLA-DR, and analysed by flow cytometry. ELISA was used to measure the cytokine content of plasma and of monocyte supernatant. THP-1 monocytes were stimulated with LPS (0.1 ng/ml) and treated with LXA4 or RvE1; protein was isolated and p-IkB-alpha levels assessed by western blotting.

**Results:** HD patients have higher levels of CD16+ monocytes (p<0.01) and higher plasma levels of TNF-a (p<0.001) and IL-6 (p<0.05) than healthy controls. Treatment of HD blood samples with LXA4 or RvE1 reduces CD16+ monocytes (p<0.05), while treatment of isolated monocytes with either agent reduces the production of TNF-a (p<0.01) and IL-6 (p<0.05). THP-1 monocytes cultured in uraemic plasma adopt an inflammatory phenotype with increased production of TNF-a compared to cells cultured in control plasma (p<0.001); this is attenuated by treatment with LXA4 and RvE1 (p<0.05). Treatment of stimulated THP-1 monocytes with LXA4 (p<0.001) or RvE1 (p<0.01) also leads to a reduction in p-IkB-alpha.

**Conclusions:** HD patients have evidence of inflammation and monocyte activation. Both LXA4 and RvE1 reduce inflammatory monocytes and cytokines in blood from HD patients. These agents may act through inhibition of the NF-kB signalling pathway. These results suggest that LXA4 and RvE1 have therapeutic potential in the management of inflammation in the haemodialysis population.

*Funding:* Government Support - Non-U.S.

## SA-PO395

**Upper Stream Reactions of Oxidative Stress in Hemodialysis Patients** Aki Hirayama,[1] Atsushi Ueda,[2] Sohji Nagase,[3] Hirofumi Matsui,[4] Kazumasa Aoyagi,[1] Shigeru Owada.[5] *[1]Center for Integrative Medicine, Tsukuba Univ of Technology, Tsukuba, Japan; [2]Tsukuba Univ Hospital Hitachi Medical Education and Research Center, Hitachi, Japan; [3]Nagase Naika Clinic, Moriya, Japan; [4]Faculty of Medicine, Univ of Tsukuba, Tsukuba, Japan; [5]Asao Clinic, Kawasaki, Japan.*

**Background:** Upper stream reactions against oxidative stress that evoke following cellular responses still remain unclear. This study aimed to reveal the dynamics of multiple reactive oxygen and nitrogen species (ROS/RNS) which act as stimulators of oxidative responses in hemodialysis (HD) patients.

**Methods:** Dynamics of multiple ROS, namely hydroxyl radical, superoxide radical, alkoxyl radical, alkylperoxyl radical, alkyl radical, and singlet oxygen, were investigated measuring the scavenging activities against these ROS by a newly developed electron paramagnetic resonance (EPR) method, MULTIple free-radical Scavenging (MULTIS). Nitric oxide (NO) metabolites and inflammatory cytokines were also measured. These methods were applied to a cohort of HD patients (n=23) and healthy control subjects (n=16).

**Results:** ROS scavenging activities of HD patients were significantly augmented against superoxide, alkoxyl radical and singlet oxygen, and attenuated against alkyl and alkylperoxyl radical, compared to the healthy group. Hydroxyl radical scavenging activity showed no difference between the two groups. In the healthy group, superoxide scavenging activity showed strong positive correlation with serum NOx concentration. This relation was not observed in HD patients, suggesting disintegration of superoxide-NO balance. Superoxide scavenging activity correlated with that of hydroxyl and alkoxyl radical in the healthy group, while this was related with alkylperoxyl radical in HD patients. ROS scavenging activities were not related with TNF-a, IL-6, and adiponectin in both groups.

**Conclusions:** These results indicate that alterations of cellular oxidative response stimulators in HD patients. Radical chain reaction pathway involving alkyl and alkylperoxyl radical are notably attenuated. Also, ROS-NO balance, which is a controlling factor of in vivo oxidation-antioxidation, is disintegrated in HD patients.

*Funding:* Government Support - Non-U.S.

## SA-PO396

**Decreased Insulin-Like Growth Factor-1 Levels Potentiate Association of Inflammation with All-Cause and Cardiovascular Mortality in Prevalent Hemodialysis Patients** Ilia Beberashvili,[1] Inna Sinuani,[2] Ada Azar,[3] Hadas Kadoshi,[3] Gregory Shapiro,[1] Leonid Feldman,[1] Zhan Averbukh.[1] *[1]Nephrology, Assaf Harofeh Medical Center, Zerifin, Israel; [2]Pathology, Assaf Harofeh Medical Center, Zerifin, Israel; [3]Nutrition, Assaf Harofeh Medical Center, Zerifin, Israel.*

**Background:** Insulin-like growth factor-1 (IGF-1) and inflammation have both been linked to high cardiovascular risk and mortality in the general population, as well as in hemodialysis (HD) patients. We hypothesized that the association of low IGF-1 with chronic inflammation may increase the mortality risk in HD patients.

**Methods:** We investigated the interactions between inflammatory biomarkers (IL-6 and TNF-α) and IGF-1 as predictors of death over a 4 years of follow-up (median - 47 months, interquartile range - 17.5-75 months) in 96 prevalent HD patients (35% women, mean age of 64.9±11.6 years).

**Results:** A significant interaction effect of low IGF-1 (defined as a level less than median) and high IL-6 (defined as a level higher than median) on all-cause and cardiovascular mortality was found: crude Cox hazard ratios (HR) for the product termed IGF-1 X IL-6 were 3.06, with a 95% confidence interval (CI): 1.59 to 5.88 (P=0.001) and 5.07, with a 95% CI: 1.72-14.94 (P=0.003), respectively. Across the four IGF-1-IL-6 categories, the group with low IGF-1 and high IL-6 exhibited the worse outcome in both all-cause and cardiovascular mortality (multivariable adjusted hazard ratios were 5.88, 95% CI 2.12 to 16.32, and 16.96, 95% CI 1.71 to 168.4, respectively). The main clinical characteristics of patients in the low-IGF-1-high IL-6 group didn't differ from other IGF-1-IL-6 categorized groups besides gender that consequently was inserted in all multivariable models together with the other potential confounders.

**Conclusions:** An increase in mortality risk was observed in HD patients with low IGF-1 and high IL-6 levels, especially cardiovascular causes.

## SA-PO397

**Fluid Retention in Hemodialysis (HD) Patients Is Associated with Endothelial Dysfunction through an Increased Pentraxin 3 (PTX3) and ROS Production by Neutrophils** Simona Simone,[1] Maria Pia dell'Oglio,[1] Marco Ciccone,[2] Roberto Corciulo,[1] Giuseppe Castellano,[1] Cosima Balestra,[1] G. Grandaliano,[3] Loreto Gesualdo,[1] G. Pertosa.[1] *[1]DETO, Nephrology, Dialysis and Transplantation Unit; [2]DETO, Cardiology Unit, Bari; [3]Dept of Medical and Surgical Sciences, Nephrology, Dialysis and Transplantation Unit, Foggia, Italy.*

**Background:** Fluid retention in HD patients is associated with increased cardiovascular morbidity and mortality. Long PTX3, a marker of inflammatory responses, may be involved in endothelial dysfunction and is a predictor of mortality in HD patients. The aim of the study was to evaluate in vivo the association between fluid retention, pulmonary artery systolic pressure (PASP), endothelial dysfunction (flow-mediated dilation, FMD), PTX3 and ROS production in HD patients.

**Methods:** Sixty uremic patients (mean age 62.5 yrs; mean dialytic age 44.3 months) on stable HD treatment with synthetic membranes and 20 healthy subjects were enrolled. Neutrophils activation ($CD62L^+$) and PTX3 protein expression were evaluated by flow cytometry and ELISA. Intracellular ROS generation in neutrophils was measured by 2',7'Diclorodiidrofluorescein. PASP and FMD of brachial artery were assessed non-invasively, using echocardiography and high-resolution ultrasound. The arterial stiffness was evaluated using cardio-ankle vascular index (CAVI).

**Results:** HD increased FMD from 4.23±1,8% (nv:7.5±2.1%; p<0.01) to 7.03±0.734%, ( p<0.001). These changes returned to baseline by 24h (5.59±0.4%). FMD, observed pre and post-HD, were significantly (p=0.002) and inversely correlated with PASP values(pre-HD:27,7±2,4mmHg; post-HD 18,1±1,6mmHg). The change in FMD and PASP were associated with a significant increase (p=0.0001) in neutrophil activation, intracellular and circulating PTX3 expression and ROS generation. Pre/post-HD (D) FMD was inversely correlated with CAVI (p<0.002) and P and PTH serum levels (p<0.02). PTX3, P serum levels and PASP were independent predictors of altered FMD.

**Conclusions:** In conclusion, fluid retention in HD patients may contribute to endothelial dysfunction and arterial stiffness by increasing PTX3 and ROS production.

## SA-PO398

**Increased Prevalence of Eosinophilia in a Hemodialysis Population: Longitudinal and Case Control Studies** Sarah Hildebrand, Neill D. Duncan, Damien Ashby. *West London Renal and Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

**Background:** Eosinophilia associated with dialysis reaction was frequently reported in the 1980s which decreased with the use of more biocompatible membranes. Recent observations at a local level suggest a possible recurrence of eosinophilia amongst hemodialysis patients without apparent relationship to dialysis sessions.

**Methods:** In this single-centre case-control study, hemodialysis patients with eosinophil count greater than 1 $x10^9$/litre during a 3 month period were identified and paired with controls matched for age, gender and dialysis satellite facility. Comorbidity, dialysis and medication data were collected. A sample of patients on hemodialysis 5 years ago was analysed to provide historical prevalence. Statistical analysis was performed using the Fisher exact test for categorical variables and t-test for continuous data.

**Results:** 24 patients (aged 31– 81, 46% male) from a population of 510 were identified as having eosinophilia with a mean level of 2.2 $x10^9$/l (range 1.1-7.5 $x10^9$/l). Between cases and controls there were no differences in comorbidities, dialysis vintage or most medications. There was a significantly higher frequency of angiotensin converting enzyme inhibitors (ACEi) use in patients with eosinophilia (10/24 vs 3/24, p=0.049). There were no patients with an allergic syndrome related to dialysis sessions, and generally, intradialytic symptoms were no more common in cases either. One patient did develop a dialysis-related febrile reaction 3 months after the onset of eosinophilia resolving with a change of dialysis membrane. A historical sample of 200 dialysis patients identified eosinophilia in only 3, demonstrating a significant increase in prevalence over time (1.5 vs 4.7%, p=0.049).

**Conclusions:** The prevalence of eosinophilia appears to have increased within this dialysis population over the last 5 years. No clear cause was identified in this study. More frequent use of ACEi may support a medication hypothesis for some patients. The development of intradialytic symptoms in one patient suggests components of the dialysis circuit as a possible cause so an empirical change of membrane is planned.

## SA-PO399

**Parathyroid Hormone (PTH) in Hemodialysis Patients Is Significantly, Positively Affected by Body Size: Both by Fat Mass and Lean Mass, Independently** Eiji Ishimura,[1] Senji Okuno,[1] Akihiro Tsuda,[1] Akinobu Ochi,[1] Shinya Nakatani,[1] Masaaki Inaba.[1] *[1]Osaka City Univ Graduate School of Medicine; [2]Shirasagi Hospital.*

**Background:** A significant positive relationship between PTH and body size has been reported in the general population (Eur J Endocrinol 2004) and in patients with primary hyperparathyroidism (J Clin Endcrinol Metab 2005). We hypothesize if PTH is affected by body size in hemodialysis patients, in whom PTH is abnormally regulated. We further examined whether fat mass or lean mass are associated with serum PTH.

**Methods:** 590 hemodialysis patients (age: 60.2±12.2 yr; median hemodialysis duration: 59.6 mo.; 343 males and 247 females; diabetics: 27.7%). were examined. Fat mass and lean mass were measured by dual X-ray absorptiometry.

**Results:** Intact PTH correlated significantly, positively with body weight and body mass index (p<0.001). Intact PTH correlated significantly and positively with fat mass and lean mass in males (p<0.01), and tended to correlate positively with fat mass and lean mass in females (p<0.1). In multiple regression analyses after adjustment for age, gender, hemodialysis duration, calcium, phosphate, vitamin D use and phosphate binder use, intact PTH was associated significantly with body weight, body mass index, fat mass and lean mass. Further, intact PTH was associated significantly and independently with both fat mass and lean mass after adjustment ($R^2$=0.206, p<0.0001).

| **Dependent variable: intact PTH** | | |
|---|---|---|
| **Independent variables** | β | p |
| Age (years) | 0.084 | 0.0551 |
| Gender (male vs. female) | - 0.159 | 0.0066 |
| Hemodialysis duration (years) | 0.229 | <0.0001 |
| Diabetes (yes vs. no) | - 0.153 | <0.0001 |
| Calcium (mg/dL) | - 0.233 | <0.0001 |
| Phosphate (mg/dL) | 0.188 | <0.0001 |
| Vitamin D use (yes vs. no) | 0.153 | <0.0001 |
| Phosphate binder use (yes vs. no) | 0.005 | 0.8934 |
| Fat mass (kg) | 0.134 | 0.0006 |
| Lean mass (kg) | 0.176 | 0.0047 |

**Conclusions:** We demonstrated, for the first time, that serum intact PTH correlated significantly, positively with body size in hemodialysis patients. Further, PTH was significantly, independently associated with fat mass and lean mass.

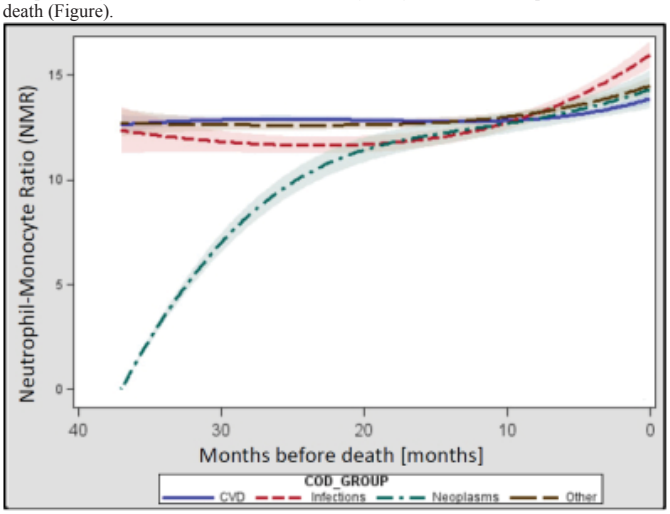
## SA-PO400

**Changes in Neutrophil-Monocyte Ratio Allow Prediction of Death from Infectious Causes in Incident Hemodialysis Patients: Results from a Retrospective Database Analysis** Laura Rosales,[1] Jochen G. Raimann,[1] Len A. Usvyat,[1] Stephan Thijssen,[1] Peter Kotanko,[1] Nathan W. Levin.[1] *[1]Renal Research Institute, New York, NY; [2]Fresenius Medical Care North America, Waltham, MA.*

**Background:** Chronic systemic inflammation determines survival and disease progression in hemodialysis (HD) patients. Neutrophil to lymphocyte ratio (NLR) was previously proposed as predictor of all-cause mortality comparing well to established inflammatory markers. Immune dysfunction may be associated with decreased functional toll-like receptors (TLR) with adverse outcomes. We investigated the predictive value of monocyte-lymphocyte ratio (MLR), neutrophil-monocyte ratio (NMR) and NLR for all-cause mortality and if death due to infectious causes is preceded by changes in MLR, NMR, and NLR.

**Methods:** Relevant laboratory markers and white blood cell count were collected monthly. Cox regression models (adjusted for age, gender, height, body mass index, diabetes, HD vintage, HIV and hepatitis C status) were developed to study the relationship between NLR, MLR and NMR to all-cause mortality and death from cardiovascular, infectious, neoplastic and other causes. Spline analysis of NLR, MLR and NMR prior to death was employed to depict their changes.

**Results:** 2269 incident HD patients (67.4±13.8years; 45% female; 55% White, 58% DM) were followed up to 3 years. Higher values of MLR, NLR and NMR were independently associated with all-cause mortality. Only NMR increased prior to infectious death (Figure).




NLR and MLR showed similar patterns and did not allow differentiation of infectious death from others causes.

**Conclusions:** Increase MLR, NLR and NMR predict all-cause death in incident HD patients. NMR shows a distinctive increase prior to infectious death and may be a potential tool to identify patients at risk of infectious death. The reasons are unclear but decreased in TLR expression is possibly involved.

## SA-PO401

**Importance of Food Labeling: Bromatological Study for Determination of Phosphorus and Potassium in Food Consumed by Hemodialysis Patients**
Camila Machado de Barros,[1] Isabela Santos Areias,[2] Bárbara Margareth Menardi Biavo,[1] Edeli Simioni Abreu,[2] Jacqueline Santos,[1] Elzo Ribeiro Júnior,[1] Carmen B. Tzanno-martins.[1] *[1]Grupo CHR; [2]Universidade Presbiteriana Mackenzie.*

**Background:** Adding nutritional information on food labels about these minerals becomes increasingly urgent, considering the growing population of patients on HD.

Conduct chemical analyzes of some processed foods in order to determine their amount of phosphorus and potassium, since this information does not appear on their label. Objective: Conduct chemical analyzes of some processed foods in order to determine their amount of phosphorus and potassium, since this information does not appear on their label.

**Methods:** The study was based on secondary data on food consumption by HD patients, from three hemodialysis clinics in São Paulo, Brazil. Laboratory tests were performed in the Bromatology Laboratory at the Mackenzie University. Each food was analyzed separately and twice. For the potassium measurement, it was used atomic absorption spectrophotometer; and for phosphorus, it was used phosphate by titration.

**Results:** We analyzed thirteen foods - Brazilian *human ration*; juice powder; milk pudding; hamburger, chicken nuggets; *bis* (a brazilian chocolate bar), instant soup, four cheese industrialized lasagna, industrialized bolognese lasagna; dark *cocada* (a brazilian coconut candy); white *cocada*; broth; *goiabada* (a brazilian guava candy). Regarding phosphorus concentration of per serving, the higher value foods were *ready lasagna* (39.2454 mg and 44.7886 mg), *chicken nuggets* (21.1259 mg) and milk pudding (19.2532 mg). When potassium concentration was analyzed, values shown were very low, even for patients undergoing hemodialysis, with recommended intake value of approximately 3000 mg daily.

**Conclusions:** Nutritional information with phosphorus content proved valuable and decisive in the choice of food for these patients. According to the foods surveyed, it was determined that the potassium content was not very high as was the level of phosphorus. This shows that one should pay greater attention to levels of inorganic phosphorus. However we believe there is need for more studies on the subject.

## SA-PO402

**Nutriton Education as a Tool for Knowledge about Potassium in Hemodialysis Patients**
Camila Machado de Barros,[1] Amanda Mafei,[2] Fabiana Valverde,[2] Luiza Sacanavini,[2] Edeli Simioni Abreu,[2] Rosana Farah,[2] Bárbara Margareth Menardi Biavo,[1] Jacqueline Santos,[1] Elzo Ribeiro Júnior,[1] Carmen B. Tzanno-martins.[1] *[1]Grupo CHR; [2]Universidade Presbiteriana Mackenzie.*

**Background:** Potassium levels are generally high (hyperkalemia) in patients with chronic kidney disease (CKD) on hemodialysis (HD). Therefore it is important to control food with high potassium content.

Objective: To evaluate the effects of a nutrition educational program regarding potassium in food on the knowledge of a patient with hyperkalemia in HD treatment.

**Methods:** This was a longitudinal study with 243 patients with CKD who perform dialysis in three nephrology clinics, located in São Paulo, being 36% of them with hyperkalemia. In a given dialysis session, we have applied the group dynamics, "Potassium Minefield". The game consisted of a board with 60 squares in which there were food illustrations distributed randomly. Some of these foods were "bombs", i.e. they contained high concentration of potassium and should be avoided. Other foods were low in potassium and could be consumed more often. Patients were asked to choose a food. Then we asked them if it was a "bomb" or not. After the answer, the square was turned. On the back of squares of foods with high potassium content, there was an illustration of a bomb. In order to assess the acquired knowledge, we applied a questionnaire with five multiple choice questions before and after the intervention.

**Results:** was observed that the knowledge on foods with high potassium content increased in the three units after completion of nutritional dynamics. The percentage of correct answers increased from 63.3% (± 13.9) before the intervention to 73.5% (± 16.3) after the intervention.

These values showed a good level of knowledge about the consequences of hyperkalemia and its treatment, as well as a better understanding of the nutritional recommendations regarding diet.

**Conclusions:** The nutritional intervention program applied resulted in increased knowledge about the low and high potassium content found in foods, demonstrating the effectiveness of nutrition education for the improvement and adjustment of treatment of patients in hemodialysis.

## SA-PO403

**Efect of Citrate on the Proteome of the Secondary Protein Layer of the Dialysis Membrane**
Jaromir Eiselt, Jan Mares, Jiri Moravec, Lukas Kielberger. *Internal Dept 1, Charles Univ.*

**Background:** A secondary protein layer formed on the dialysis membrane affects the efficacy of the purification procedure. Previous studies have shown higher Kt/V in dialysis using citrate instead of acetate as an organic acid in the acid concentrate. We tested the hypothesis that citrate in the dialysis fluid can change the amount and/or composition of the protein layer.

**Methods:** A total of ten patients were assessed during routine nadroparine high-flux hemodialysis using acetate (3.0 mEq/L) or citrate (2.4 mEq/L) in an acidic component of the dialysis solution. After the session dialyzers were flushed with 500 mL of saline and protein adsorbed to the membrane was eluted by 40% acetic acid. Amounts of protein and hemoglobin in the eluates were determined by spectrophotometric assays. Protein was resolved with 2D electrophoresis and spot patterns were compared between acetate and citrate dialysis. Spots showing difference ($p < 0.01$) were identified by tandem mass spectrometry.

**Results:** Total amounts of protein in the eluates in acetate and citrate dialysis were 5.11 mg (3.49-17.42) and 8.24 mg (3.39-10.26), respectively; $p = 0.72$. Total amounts of hemoglobin in the eluates of acetate and citrate dialysis were 3.57 mg (2.57- 8.21) and 2.75mg (2.,02-3.28), respectively; $p = 0.022$. In eluates, 208 protein fractions (spots) were considered (arbitrarily) characteristic, i.e. present in at least 75% of gels of either group. Of these, 88 protein fractions were differently expressed between both groups. Among these, 27 spots were identified as alpha fibrinogen fragments. Quantitatively, these fragments prevailed in citrate eluates by a factor of 3.04 (2.46-3.42), $p = 0.005$. Reuslts are expressed as median (interquartile range) and Wilcoxon test was used for statistical analysis.

**Conclusions:** After dialysis using citrate, dialyzer eluates contain significantly less hemoglobin and more alpha fibrinogen fragments. This finding could reflect an additional anticoagulation effect provided by citrate and, speculatively, achieved through inhibition of alpha fibrin crosslinking.

*Funding:* Government Support - Non-U.S.

## SA-PO404

**Efficient Removal of Beta2 Microglobulin and Leptin by Online Hemodiafiltration: Comparison of Three State of the Art Dialyzers**
Andreas H. Bock, Beatrice Paul. *Nephrology Div, Kantonsspital Aarau, Aarau, Switzerland.*

**Background:** Hemodiafiltration (HDF) removes high molecular weight solutes better than hemodialysis. This study compared three state-of-the-art highflux dialyzers in oHDF mode with respect to the elimination of leptin (18.6 kD), ß2- microglobulin (ß2M, 11.8 kD) and phosphate. Improved ß2M elimination is expected to delay ß2M amyloidosis, and lowering leptin levels should increase appetite, which may help to improve nutritional status.

**Methods:** During three consecutive weeks, each of 29 stable hemodialysis patients underwent a midweek study dialysis, using in randomized order a FX100 (FX100), Polyflux 210H (PF210) or FXCorDiax100 (CorDiax) dialyzer . The study dialysis was a 4 hour oHDF with 350 ml/' blood flow, 800 ml/' dialysate flow and 80 ml/' (19.2 L/4h) substitution rate. Arterial samples were taken at oHDF start, at 60/240 minutes (t60/t240) as well as 30 minutes after the end (t270). Bloodside clearances were computed using pre- and post-dialyzer blood samples at t60 and t240. Equilibrated removal ratios (eRR) were calculated from t0 and t270 samples. Dialysate obtained using a 1/25 split dialysate collector was assayed for ß2M, phosphate and albumin.

**Results:**

| | | PF210 | FX100 | CorDiax |
|---|---|---|---|---|
| **Leptin** | **eRR (%)** | 26.7 ± 23.0 | 39.2 ± 21.7 [p] | 40.6 ± 27.5 [p] |
| | **Clearance240 (ml/min)** | 89.7 ± 19.2 | 102.8 ± 20.4 [p] | 110.8 ± 13.4 [p] |
| **ß2M** | **eRR (%)** | 62.7 ± 5.3 | 67.9 ± 5.0 [pf] | 72.3 ± 5.3 [pf] |
| | **Clearance240 (ml/min)** | 115.5 ± 9.8 | 136.3 ± 12.8 | 150.4 ± 10.9 [pf] |
| | **Dialysate removal (mg/4h)** | 191.0 ± 67.8 | 204.9 ± 62.4 | 215.1 ± 63.3 [p] |
| **Albumin** | **Dialysate loss (g/4h)** | 1.31 ± 0.12 | 2.10 ± 1.00 [p] | 1.74 ± 1.01 |
| Data are mean ± SD [p] p<0.001 vs. PF210 [f] p<0.001 vs. FX100 | | | | |

The decrease of plasma leptin paralleled the height of baseline leptin ($r^2 = 0.62$). eRR, clearance and dialysate removal for phosphate was high and similar for all three dialyzers.

**Conclusions:** oHDF with modern highflux dialyzers removes significant amounts of ß2M and can substantially lower plasma leptin without relevant albumin losses, all of which should be beneficial at longterm. The CorDiax dialyzer in these real-life conditions performed better than the two other dialyzers.

*Funding:* Pharmaceutical Company Support - Fresenius

## SA-PO405

**A Study of the Immunoregulation of Double Filtration Plasmapheresis in Maintenance Hemodialysis Patients with Chronic Hepatitis C**
Cao Hongdi, Chunsun Dai, Junwei Yang. *2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

**Background:** To clarify the immunnoloregulation of double filtration plasmapheresis (DFPP) in maintenance hemodialysis (MHD) patients with chronic hepatitis C (CHC).

**Methods:** DFPP were performed in 20 MHD patients with CHC (HCV-antibody positive and serum HCV-RNA >500 IU/ml more than 6 months). The serum titer of HCV-RNA was monitored and peripheral blood mononuclear cell (PBMC) was analyzed by flow cytometry at the time of hour 0, hour 3, day 3, day 7 and day 28 during the DFPP.

**Results:** Compared to MHD patients without CHC, the frequencies of innate immune cells were similar in MHD patients with CHC, while the ratio of Th1 to Th2 was elevated and the frequencies of Th17 cells and Treg cells were higher in MHD patients with CHC. Serum HCV particles could be removed partially by the DFPP. The titer of serum HCV could remain in a lower level one month after the treatment. There were no significant changes of monocytes, NK cells, Th1, Th2 and Th1/Th2 in PBMC after DFPP in MHD patients with CHC. Single DFPP could reduce the frequency of Th17 cells in PBMC from 7 days after the therapy in MHD patients with CHC. Single DFPP could reduce the frequency of Treg cells in PBMC from 7 days after the therapy in MHD patients with CHC.

**Conclusions:** DFPP could partially remove the serum HCV particles mechanically. The titer of HCV-RNA could remain in a lower level at least for one month probablely due to the redistribution of the immunocytes in circulation.

## SA-PO406

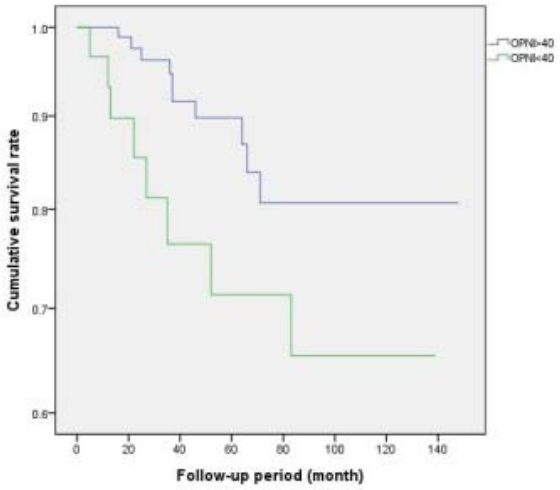
**Onodera's Prognostic Nutritional Index May Be a Significant Predictor of Mortality in Hemodialysis Patients**

Kiryong Park,[1] Sangeon Gwoo,[2] Ye Na Kim,[2] Ho Sik Shin,[2] Yeon Soon Jung,[2] Hark Rim.[2] *[1]Internal Medicine, Changwon Fatima Hospital, Changwon; [2]Internal Medicine, Kosin Univ College of Medicine.*

**Background:** No standard method for assessing the nutritional status in HD patients exists. Onodera's Prognostic Nutritional Index (OPNI) is a method that considers serum albumin level and total lymphocyte count. This simple method may involve common measures and can be applied rapidly in a large number of patients. Validation of OPNI has been performed for patients with end-stage liver disease, active tuberculosis, and gastrointestinal malignancies.

**Methods:** We examined the OPNI scores of 140 maintenance HD patients (59.8 ± 12.9 years; 64 men and 76 women) and followed these patients for 140 months. The OPNI is calculated based on the serum albumin level and total lymphocyte count and uses the following equation: OPNI = 10 × serum albumin (g/dL) + 0.005 × total lymphocyte count (/mL). Predictors of all-cause death were examined using a life table and Cox proportional analyses.

**Results:** The average OPNI value was 43.8 ± 6.5, and the values were negatively correlated with age. A total of 18 patients died during the 120-month follow-up period. OPNI values were normally distributed. Life table analysis revealed that patients with an OPNI < 40 (n = 31) had a significantly lower survival rate than those with an OPNI ≥ 40 (n = 109) (Wilcoxon test, P = 0.044).




Multivariate Cox proportional hazards analyses demonstrated that OPNI was a significant predictor of mortality (hazard ratio [HR], 6.491; 95% confidence interval [CI], 1.985–21.233; P = 0.002] even after the adjustment for age, gender, presence of diabetes, and body weight.

**Conclusions:** These results suggest that OPNI is a significant predictor of mortality in HD patients. The simple OPNI method is a clinically useful marker for the assessment of nutritional status in Korean HD patients.

## SA-PO407

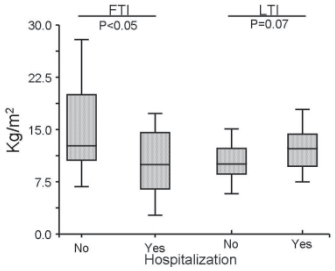
**Poor Nutritional Status Is Associated with Increased Calcifications and Higher Incidence of Hospitalization in Chronic Hemodialysis Patients**

Paolo Lentini,[1] Luca Zanoli,[2] Stefania Rastelli,[2] Massimo de Cal,[1] Graziella Berlingò,[1] Anna Basso,[1] Andrea Contestabile,[1] Valentina Pellanda,[1] Roberto Dell'Aquila.[1] *[1]Nephrology, San Bassiano Hospital, Bassano del Grappa, Italy; [2]Internal Medicine, Univ of Catania, Catania, Italy.*

**Background:** Calcifications are increased in chronic hemodialysis and associated with increased cardiovascular events.The nutritional status may influence, via the metabulism Ca/P, the outcome of the patients.We aimed to study the relations between the nutritional status, calcification score and hospitalization in chronic hemodialysed subjects during a follow-up of 1 year.

**Methods:** Body composition was evaluated with Bioimpedance spectroscopy technique (Fresenius BCM) and hospitalization was collected during follow-up; abdominal aortic calcifictions (Kauppila score) were evaluated by lateral lumbar radiographs at baseline and after 1 year.

**Results:** 36 hemodialysed subjects (age 64±15 yrs; 50% males; hemoglobin 11.3±0.7 mg/dl; Kt/Veq Daugirdas 1.50±0.29) were enroled.20 subjects (56%) were hospitalized during follow-up.Nutritional status (lean tessue index [LTI], positively; fat tissue index[FTI], negatively)was associated with the increase in Kauppila score during follow-up (Δ score: 1.2±1.0 points/year) and with hospitalizations, suggesting that subjects with poorest nutritional status are at risk of calcification and poor outcome.




In multivariate analysis adjusted for age, FTI was associated with Hospitalization (OR: 0.83; 95%CI 0.70-0.99;P<0.05). Adding the variation in Kauppila score during follow-up in a model with age and FTI produce the lost of significance of the association between FTI and hospitalization, suggesting that the effect of nutritional status on hospitalization is at least in part mediated by an increase in calcifications.

**Conclusions:** Nutritional status influences the development of calcifications and hospitalizations.

## SA-PO408

**Tryptophan Metabolism in Hemodialysis Patients**

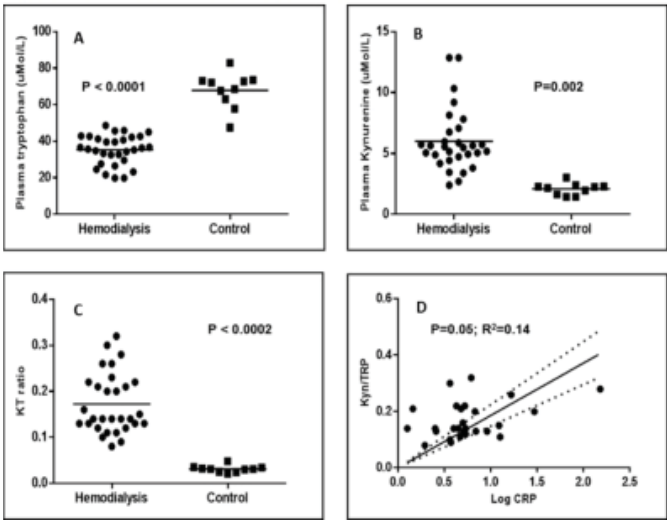
Rakesh Malhotra,[1] Vanja Persic,[2] Weifang Zhang,[3] Garry J. Handelman,[3] Laura Rosales,[4] Mary Carter,[4] Stephan Thijssen,[4] Len A. Usvyat,[4] Fredric O. Finkelstein,[5] Mark L. Unruh,[6] Nathan W. Levin,[4] Peter Kotanko.[4] *[1]UMDNJ-New Jersey Medical School; [2]Univ Medical Center Ljubljana; [3]Univ of Massachusetts Lowell; [4]Renal Research Institute; [5]Yale Univ School of Medicine; [6]Univ of New Mexico School of Medicine.*

**Background:** Tryptophan (TRP) levels and augmented concentrations of its metabolites have been shown to be significantly lower in animal models of renal insufficiency. We sought to compare the levels of TRP and its metabolites between hemodialysis (HD) patients and healthy subjects and to examine the extent to which TRP metabolites are associated with inflammation in HD patients.

**Methods:** Venous blood samples were drawn in healthy subjects and HD patients. TRP, para-cresol sulfate (PCS) and kynurenine (KYN) metabolites were measured by reverse-phase high-performance liquid chromatography (HPLC), with appropriate detection wavelength for each compound. C-reactive protein (CRP) was measured with an immunoturbidometric technique. We used Spearman rank test for correlational analysis.

**Results:** We studied 30 HD patients (70% male; mean age 58.8±13.3 yrs) and 10 healthy control subjects (60% male; mean age 43.9±12.4 yrs). KYN and KYN/TRP (6.0±2.5 uMol/L vs. 2.1±0.5 uMol/L; p=0.002 and 0.17±.06 vs. 0.03±0.01; P<0.0002, respectively) were significantly higher and TRP levels were significantly lower (35.3±8.1 uMol/L vs. 67.9±9.8 uMol/L; p<.0001) in the HD patients than those in the controls. KYN/TRP ratio and CRP levels were positively correlated in HD patients ($r^2$= 0.14; P=0.05).

**Figure 1: (a-c) Serum levels of TRP and its metabolites in HD patients and healthy subjects; (d) Correlation analysis between KYN/TRP and CRP in HD patients**




**Conclusions:** Low TRP and high metabolite levels were observed in HD patients, indicating increased TRP catabolism, possibly related to immune activation/inflammation. Further studies exploring the biological and functional consequences of increased TRP catabolism in HD patients are warranted.

**SA-PO409**

**Effect of Kibow Probiotic Renadyl™ on sCD30 Levels in Hemodialysis Patients** Subodh J. Saggi,[1] Eli A. Friedman,[1] Lorraine L.A. Thomas,[1] Natarajan Ranganathan,[2] Pari Ranganathan,[2] Gary R. Briefel,[1] Mary C. Mallappallil,[1] Usha N. Vyas,[2] Bohdan Pechenyak,[2] Peter Liang,[1] David Hochman,[1] Allen J. Norin.[1] *[1]SUNY Downstate Medical Center, Brooklyn, NY; [2]Kibow Biotech, Newton Square, PA.*

**Background:** Our prior studies in patients with CKD 3-4 (n=31) given Renadyl™, a safe proprietary dietary supplement, which metabolizes various nitrogenous wastes in the bowel, at a dose of 180-270 B CFU, over a 6 month period showed that BUN, creatinine and $K^+$ levels declined. How azotemia impacts QoL is unknown, though increased inflammation is a postulated mechanism. We now report on the effects of Kibow Probiotic Renadyl™ on 26 hemodialysis patients where we detected a reduction in their WBC counts, C-reactive protein (CRP) and total indoxyl glucuronide levels. In order to link the reduction to markers of inflammation, we looked at one biomarker of T cell activation, sCD30. This marker has previously been shown to be elevated in patients with CKD and lower levels of sCD30 have been associated with better prognosis in kidney transplant patients.

**Methods:** We conducted a prospective, double blind crossover trial with placebo and Renadyl™ for 8 weeks, followed by an 8-week washout period. Patient's serum was taken at 3 time points (baseline, after probiotics, and after placebo) and sCD30 levels were measured by ELISA (Bender MedSystems). Patient adherence was assessed by pill count and stool culture to verify probiotic growth during study and absence during placebo period. Data were analyzed with ANOVA for a crossover design with a mixed model methodology in SAS to detect differences in least square means in treatment, period and sequence effect.

**Results:** Mean sCD30 levels were 94.74ng/ml and decreased to 89.84ng/ml with probiotic administration. This difference is not statistically significant (p= 0.49).

**Conclusions:** Our results show that sCD30 levels are not affected with the administration of probiotics, which suggests that patients do not become immunocompromised by this treatment. Larger population studies or longer term studies might be needed to give a better insight into the role of T cell modulation by Probiotics in this population.

*Funding:* Pharmaceutical Company Support - Kibow Biotech, Inc

**SA-PO410**

**Effect of Kibow Probiotic Renadyl™ on NF-κB Levels in Hemodialysis Patients:** Subodh J. Saggi,[1] Eli A. Friedman,[1] Natarajan Ranganathan,[2] Pari Ranganathan,[2] Usha N. Vyas,[2] Bohdan Pechenyak,[2] Mary C. Mallappallil,[1] Gary R. Briefel,[1] Lorraine L.A. Thomas,[1] Peter Liang,[1] Allen J. Norin,[1] David Hochman.[1] *[1]SUNY Downstate Medical Center, Brooklyn, NY; [2]Kibow Biotech, Inc, Newton Square, PA.*

**Background:** End-stage renal disease (ESRD) is when the kidneys are unable to function effectively to sustain life and requires dialysis. As a consequence of this condition, uremic toxins build up and this has been associated with increased inflammation. NF-κB is an inflammatory marker that has been shown to increase in uremia. Previous studies showed that Renadyl™, a safe proprietary dietary supplement, decreased BUN, creatinine, and $K^+$. This study was done to see if Renadyl™ has an effect on NF-κB in ESRD patients on hemodialysis.

**Methods:** We conducted a prospective, double-blind crossover trial with placebo and Renadyl™ in 26 ESRD patients on hemodialysis. Each patient had 3 time points measured: Baseline, after taking probiotics for 8 weeks, and after taking placebo for 8 weeks. Peripheral blood mononuclear cells (PBMC) were extracted from the patient blood samples using ficoll hypaque and NF-κB levels were assayed using the TransAM p65 ELISA kit (Active Motif). Viability of cells was assessed using trypan blue exclusion. Patient adherence was assessed by pill count and stool culture to verify probiotic growth during study and absence during placebo period. Data were analyzed using ANOVA for a crossover study with a mixxed model methodology in SAS to account for treatment, period and sequence effect.

**Results:** NF-κB levels were 0.407U/uL when treatment with probiotic was first in sequence and 0.409U/uL when placebo was administered first. There were no differences in least square means between placebo and probiotic (p=0.9407).

**Conclusions:** These results indicate that NF-κB pathway is not activated and not modulated under the effect of Probiotic Renadyl™. This further suggest that the administration of probiotics to ESRD patients in itself is not harmful as the neither this pathway is activated nor suppressed as activation of this pathway is required in the settings of active infections.

*Funding:* Pharmaceutical Company Support - Kibow Biotech, Inc.

**SA-PO411**

**Daily Physical Activity and Physical Function in Relatively Healthy Maintenance Hemodialysis (MHD) Patients** Bryan B. Shapiro,[1] Jun Chul Kim,[1,2] Rachelle Bross,[1] Usama Feroze,[1] Rajeev Upreti,[1] Kamyar Kalantar-Zadeh,[1,3] Joel D. Kopple.[1,3,4] *[1]Harbor-UCLA Med Cntr; [2]CHA Gumi Med Cntr, CHA Uni, Korea; [3]UCLA Schl Pub Hlth, LA, CA; [4]UCLA Schl Med, LA, CA.*

**Background:** MHD patients reportedly display reduced daily physical activity (DPA). Such studies included debilitated patients, and it is unclear to what extent kidney failure and MHD per se contribute to decreased DPA. Physical performance capacity may influence DPA, and few data examined relationships between DPA and physical performance in MHD. We hypothesized that DPA is reduced even in healthier MHD patients and is correlated with physical performance.

**Methods:** DPA and 6-minute walk distance (6-MWD), sit-to-stand and stair climbing tests were measured in 72 relatively healthy MHD patients (52 years, 32% diabetics) and 39 normal adults(51 years) of similar age and gender mix. Patients very debilitated from comorbid illnesses were excluded from study. DPA was examined by a physical activity monitor usually worn for 10 days and by the Human Activity Profile(HAP).

**Results:** Evidence that the MHD patients were relatively healthy included serum albumin of 4.1g/dL, BMI 27.8 $kg/m^2$, nPNA 1.10 g/kg/day & Charlson Comorbidity Index 5.7. DPA with the activity monitor, time-averaged over 7 days, and all 3 physical performance tests were impaired in MHD patients, to about 60-70% of normal values ($p<0.0001$ for each measurement). HAP was also impaired ($p<0.0001$). MHD patients spent increased time sleeping or in marked physical inactivity($p<0.0001$) and less time in ≥moderate activity($p<0.0001$). These findings persisted when comparisons to normals were restricted to men or women alone or to non-diabetic or diabetic patients separately. In comparing non-diabetic to diabetic patients, only HAP scores and 6-MWD were more impaired in diabetics. DPA assessed by the activity monitor, after adjustment, correlated with 6-MWD but not the 2 other performance tests. HAP scores correlated more closely with all 3 physical performance tests than did DPA.

**Conclusions:** Even in relatively healthy MHD patients, DPA and physical performance are substantially impaired and correlated. Impairment in these measures was not much greater in diabetic vs nondiabetic patients.

**SA-PO412**

**Relationship between the Frequency of Monitoring Laboratory Parameters and the Outcome in Maintenance Hemodialysis Patients** Li Wang. *Renal Dept, Sichuan Provincial People's Hospital, Chengdu, China.*

**Background:** To compare different impact on clinical status by frequency of monitoring laboratory parameters in the maintenance hemodialysis patients(MHD).

**Methods:** 310 MHD patients in our hemodialysis center were enrolled with HD vintage from 13-216 month(58.78±39.68 months).We retrospective analysed the status of hemoglobin,serum calcium,serum phosphorus,serum albumin,iPTH and the frequency of monitoring the status above from July 2010 to June 2011.Acorrding to the KDOQI guidelines for CKD-anemia and the KDIGO guidelines for CKD-MBD,we calculated the qualified rate of the statuses above of each patient.The relationship between the frequency and the status was analyzed.

**Results:** Patients were divided into 3 groups according to the frequency of monitoring(A group,frequency≤3/year,B group,4-5/yesr,C group,frequency≥6/year). There was no significant difference among the 3 groups in age,gender,the course of CKD and HD.The Hgb was significantly lower in A group than in B and C group(A group vs B group,104.89±19.40g/L *vs* 111.06±15.37g/L,$p<0.05$.A group *vs* C group,104.89±19.40g/L *vs* 112.16±12.27g/L,$p<0.05$),and was not significantly different between B and C group(111.06±15.37g/L *vs* 112.16±12.27g/L,p=0.793).The same results were also observed in the qualified rate of Hgb(A group vs B group,18.06%±30.00% *vs* 24.39%±22.00%,$p<0.01$.A group vs C group,18.06%±30.00% vs 27.39%±22.23%, $p<0.01$),serum calcium (A group vs B group,40.20%±41.42% *vs* 43.86%±31.46%, $p<0.05$.A group *vs* C group,40.20%±41.42% *vs* 50.58%±32.37%,$p<0.01$)and serum phosphorus (A group vs B group,48.63%±45.48% vs 50.81%±38.64%, $p<0.05$.A group vs C group,48.63%±45.48% *vs* 52.15%±36.33%.$p<0.05$).

**Conclusions:** The frequency of monitoring laboratory parameters the KDOQI and KDIGO guidelines recommended was beneficial to MHD patients.Despite of the difficulties,the MHD patients should take clinical Lab test at least 4 times per year for better prognosis.

**SA-PO413**

**Single Purse-String Suture versus Double Purse-String Sutures: Complications in Shorter Break-In Periods CAPD Patients** Li Wang. *Renal Dept, Sichuan Provincial People's Hospital.*

**Background:** It recommends break-in periods at least 2 weeks to reduce catheter associated complications in CAPD patients. Double purse-string sutures were made on the posterior rectal sheath to fix the peritoneum when catheter implantation was done in Chinese CAPD patients. However, it prolongs hospitalization and complicates operative procedure. We investigated the complications and feasibility of different Tenckhoff catheter implantation methods in early initiate patients.

**Methods:** We reviewed surgical catheter placement in our unit from January 2012 to March 2013. 100 patients without history of abdominal operation were enrolled and f0llowed up at least two months. We defined break-in periods less than 14 days as shorter break-in periods patients. The single purse string suture group comprised 27 patients is using one purse string suture to fix peritoneum around the PD catheter, 44 patients with two string sutures were enrolled in double string sutures group, and 29 patients with two string sutures and break-in periods >2 weeks as control group.

**Results:** Time to initiation CAPD was shorter in single purse string suture group(9.77±2.37 days)and double string sutures group(8.60±3.06 days) than control group(25.00±9.86 days)($P<0.001$).The hospitalization after implantation was extended in control group than single purse string suture group and double string sutures group(28.00±10.43, 21.26±7.11, 18.00±6.99)($P<0.01$).2 single purse string suture group(7.41%), 3 double string sutures group(6.82%) and 3 control group(10.34%) observed catheter related complications including migration, peritonitis and tunnel infection. Catheter related complications were similar among groups($P=0.856$).

**Conclusions:** Shorter break-in periods of CAPD with single purse string suture technique in Tenckhoff catheter placement did not increase catheter related complications in our patients. The hospitalization was significantly shorten when PD was initiated <14 days. We suggest that single purse string suture and early initiation PD are applicable.

Future studies with long time follow-up and larger patient numbers are needed to validate the role of shorter break-in period and single string suture in PD practice.

## SA-PO414

**Non-Surgical Treatment to Reduce Periodontitis Associated Inflammation in ESRD** Wilner Samson,[1] George Fares,[3] Effie Ioannidou.[2] *[1]Medicine, Univ of Connecticut School of Medicine, Farmington, CT; [2]Medicine, Bay State Medical Center, Springfield, MA; [3]Univ of Connecticut School of Dental Medicine, Farmington, CT.*

**Background:** Periodontitis (PDT) is a polymicrobial infectious disease affecting the hard and soft tissues that leads to tooth loss. PDT is prevalent in ESRD, and may contribute to systemic inflammation associated mortality. In a randomized clinical trial, we assessed clinical and systemic response to non-surgical periodontal treatment in ESRD subjects with PDT.

**Methods:** 12 ESRD subjects were recruited using the following criteria: presence of chronic PDT, > 15 teeth, no periodontal treatment within the past 6 months, and no use of systemic antibiotics within 30 days. All subjects received a full-mouth periodontal exam including clinical attachment levels (CAL), pocket depths (PD), bleeding on probing (BOP) and plaque scores (PS). Chronic PDT was defined by the presence of minimum of one site with 5mm or greater PD and minimum of 2 sites with 6mm or greater CAL. ESRD patients with PDT were randomized to two groups by a computer generated randomization list. The test group received oral hygiene instructions and scaling and root planning, whereas the control group received oral hygiene instructions and full mouth supragingival debridement. Subjects were recalled at 2 months for clinical periodontal measures and systemic inflammatory assessment (serum CRP**).**

**Results:** Baseline periodontal parameters and systemic inflammatory markers were compared between the two groups, no difference was found. The serum CRP levels decreased for the test group after treatment, however increased for the control group showing a trend to significance (p=0.1). When comparing the difference between pre- and post-therapy measures, CAL in the test group decreased but increased for the control group (Δ=-0.1mm) confirming significance (p<0.05). PD was reduced in the test group, but in the control group PD showed increased trend (p=0.07). Reduction in serum CRP levels was significantly correlated with the improvement in PD, CAL, BOP and PS.

**Conclusions:** Non-surgical treatment for PDT in ESRD patients may improve gum disease as well as systemic inflammation, a marker of mortality.

*Funding:* Other NIH Support - NIH/ NIDCR K23 DE018689

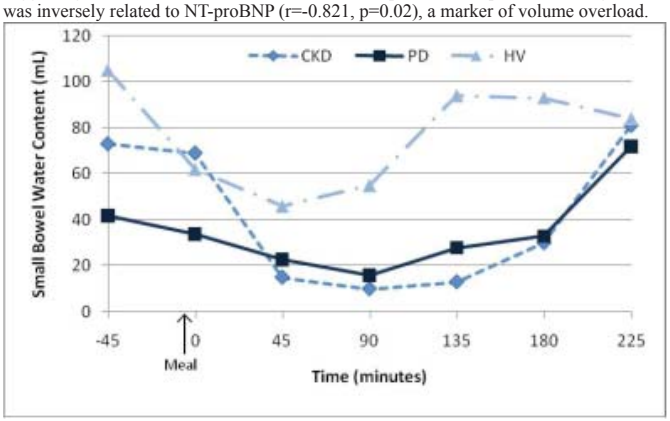
## SA-PO415

**Abnormal Gastrointestinal Function in Chronic Kidney Disease: Role of Uraemia, Peritoneal Dialysis and Volume Status** Laura E.A. Harrison,[1] Caroline Louise Hoad,[2] Luca Marciani,[2] Penny Anne Gowland,[2] Chris W. McIntyre.[1,3] *[1]Renal Medicine, Royal Derby Hospital, United Kingdom; [2]School of Physics and Astronomy, Univ of Nottingham, United Kingdom; [3]Div of Medical Sciences & Graduate Entry Medicine, Univ of Nottingham, Derby, United Kingdom.*

**Background:** Gastrointestinal (GI) dysfunction is common in chronic kidney disease (CKD), potentially driven by uraemia, volume status and dialysis modality. We assessed postprandial changes in the upper GI tract in a cross-sectional CKD study utilising Magnetic Resonance Imaging (MRI) techniques.

**Methods:** Participants underwent serial T2-weighted MRI fasted and after a 331kcal meal to assess small bowel water content (SBWC), a measure of free intraluminal water reflecting net intestinal secretion/absorption. 24 participants (9 Peritoneal Dialysis (PD), 6 CKD4, 9 non-CKD) were recruited.

**Results:** Fasting SBWC was lowest in PD patients, less than half that of non-CKD, (PD 34mL [IQR 10-63]; CKD 58 mL [IQR 22-138]; non-CKD 96 mL [IQR 50–157], p=0.04). Postprandially, SBWC fell to a nadir by $T_{90}$ in all groups (PD 12mL, CKD 7 mL, non-CKD 35 mL, p=0.01). PD and CKD patients had a delayed increase in SBWC, in contrast to non-CKD which returned to fasting levels by $T_{135}$. PD patients reported more postprandial abdominal pain and distension. AUC for total SBWC $T_0$-$T_{225}$ was significantly different between PD and non-CKD (PD 6772 mL/min vs non-CKD 16,430mL/min, p=0.001), suggesting reduced GI secretion or increased absorption. In PD patients, fasting SBWC was inversely related to NT-proBNP (r=-0.821, p=0.02), a marker of volume overload.




**Conclusions:** The altered GI physiological response to food in CKD patients is more marked in individuals receiving PD, and appears to be influenced by dialysis modality, in addition to uraemia. Volume overload and bowel congestion may be crucial factors in this process and potential future therapeutic targets.

*Funding:* Pharmaceutical Company Support - Renal Discoveries Extramural Grant awarded by Baxter Healthcare

## SA-PO416

**Development and Validation of Novel Equations for Estimating Lean Body Mass and Evaluation of Their Prognostic Values for Mortality in Peritoneal Dialysis Patients** Jie Dong,[1] Rong Xu.[1] *[1]Renal Div, Institute of Nephrology, Peking Univ First Hospital, Beijing, China; [2]Peking Univ First Hospital, China; [3]Peking Univ First Hospital, China.*

**Background:** Malnutrition is a strong predictor of mortality for dialysis patients. Early dignosis and intervention for malnutrition is based on a simple surrogate marker in clinical practice. Lean body mass is a good marker for somatic protein store but is not easily determined by the golden standare. Our study is to develop and validate estimating equations for lean body mass (LBM) in patients on peritoneal dialysis (PD), and then evaluate their prognostic value in a large prospective cohort.

**Methods:** Two equations for estimating LBM based on midarm muscle circumference (MAMC) and hand grip strength (HGS) respectively were developed in 106 and validated in 107 PD patients with dual-energy x-ray absorptiometry (DEXA) as referenced gold method, as compared to LBM estimated from creatinine kinetic (CK) and anthropometry (A) method. The prognostic values of LBM estimated from above four equations were further explored for all-cause mortality in 889 incident PD patients.

**Results:** Two new equations based on MAMC or HGS combined with gender, height and dialysis duration were developed. Bias of two new equations with LBM-DEXA were smaller than those of LBM-CK and LBM-A equation, with a median difference of 0.9 kg and -0.3kg between measured and estimated LBM by LBM-MAMC and LBM-HGS equation respectively, as compared to 3.0kg with LBM-CK equation and -5.0kg with LBM-A equation. Better precision and accuracy were achieved with LBM-MAMC and LBM-HGS equation reflected by smaller interquartile range of the difference and percentage of estimates that were 20% of measured LBM respectively. The prognostic value of LBM-MAMC and LBM-HGS was better than LBM-CK and LBM-A with the higher Chi-square by Omnibus tests for models of all-cause mortality.

**Conclusions:** LBM estimated from MAMC and HGS were verified to be more precise methods and reliable predictor of total mortality for PD population.

*Funding:* Government Support - Non-U.S.

## SA-PO417

**Clinical Assessment Is Not Sufficient to Evaluate Volume Status in Hemodialysis Patients** Branko Braam,[1,2] Ryan Reid,[1] Sylvia Kalainy,[1] Kailash K. Jindal.[1] *[1]Medicine/Nephrology, Univ of Alberta; [2]Physiology, Univ of Alberta, Edmonton, Canada.*

**Background:** Determining the dry weight of hemodialysis (HD) patients remains a challenge. Yet, hypervolemia is associated with hypertension, heart failure and mortality. Therefore, it remains important to search for clinical parameters that can predict hypervolemia in a HD patient. Using bio-impedance to assess fluid volume, we hypothesized that a combination of clinical parameters could be used to predict volume status in HD patients.

**Methods:** Multifrequency bioimpedance spectroscopy (BCM, Fresenius Canada) was performed before the start of a midweek dialysis session in 32 stable chronic HD patients to assess extracellular fluid volume (ECFV) and overhydration (OH). Physical signs (Blood pressure, BP; edema), plasma Na, K and albumin, urea reduction ratio (URR), and a recent CXR (cor-thorax ratio) were investigated as potential markers for fluid overload.

**Results:** Volume status of 32 HD patients (age 59 (23-83) years, 68% males, 53% diabetic) was divided into 2 groups: nomovolemia (OH<1.1L, n=17) and hypervolemia (OH≥1.1L, n=15). None of the parameters except for the OH and OH/ECFV were significantly different between groups. When the entire group was evaluated, no significant correlations between continuous variables and OH was detected. An attempt to combine variables into a 'fluid overload score' failed.

| | Normovolemic | Hypervolemic |
|---|---|---|
| Gender, M/F | 8/9 | 14/1 |
| Predialysis-BP, mmHg | 130±22/ 75±16 | 140±19/ 80±14 |
| Postdialysis-BP, mmHg | 137±24/ 77±19 | 138±16/ 75±11 |
| OH,L | -1.0±0.9 | 2.9±1.3 (P<0.05) |
| OH/ECV% | -0.8±4.5 | 14±5.4 (P<0.05) |
| HTN, % | 58 | 86 |
| Diabetes, % | 40 | 60 |
| Smoking, % | 11 | 53 |
| CTR | 0.47±0.06 | 0.45±0.05 |
| Edema, % | 11 | 40 |

**Conclusions:** Assessment of volume status using clinical parameters in HD patients failed to identify patients with volume overload. The absence of a relationship between OH and BP could be due to the use of antihypertensive medication. To identify and treat hypervolemia, more objective measures such as bio-impedance should be used rather than clinical parameters.

*Funding:* Pharmaceutical Company Support - The body composition monitor was provided by Fresenius Canada

## SA-PO418

**Life in the Fishtank: AVF Monitoring for Dummies** David H. King,[1] Asmaa Y.M. Al-Chidadi,[1] Michael Graeme Taylor,[2] Mo Al-qaisi,[1] Sumith C. Abeygunasekara,[1] Anthony Chan,[1] Eric S. Chemla,[3] William D. Paulson,[4] Abdelgalil Abdelrahman Ali.[1] *[1]Broomfield Hospital, Chelmsford, Essex, United Kingdom; [2]Div of Imaging Sciences & Medical Engineering, Kings College London, London, United Kingdom; [3]Div of Medicine & Cardiovascular Sciences, St. George's Hospital, London, United Kingdom; [4]Georgia Regents Univ, Augusta, GA.*

**Background:** Time and resources tend to limit attempts at effective global monitoring for AVF patients. Clinical monitoring appears to be a poor substitute. We have devised a non-invasive version of Static Pressure Ratio ***SPRn*** which features rapid 'point and click' data acquisition by ***BlueDop*** pocket Doppler (www.bluedop.co.uk), paired with a smart database, the 'Fishtank' which allows patients with failing AVF to 'float' to the top of the list for easy identification by the clinical team.

**Methods:** ***SPRn*** data was acquired during normal uninterrupted dialysis sessions using a unique algorithm to measure mean pressure at any desired point on the fistula, typically either distal brachial or radial artery. The ***SPRn*** algorithm is shown in Figure 1. Hospital number,Date,Time,BP and ***SPRn*** were automatically archived in the 'Fishtank' database. An ***SPRn*** value of greater than 0.64 was assumed to be associated with a > 60% (PSVR>6) significant stenosis in the venous outflow segment.

**Results:** 340 measurements were made over a 10 week period on 73 patients. A further 60 patients formed a control group. 23% of the monitored group rose to the top of the 'Fishtank' of which 82% were subsequently shown by Duplex Ultrasound to have a significant venous stenosis of 60% or greater.

**Conclusions:** Retrospective analysis over 30 months of clinically initiated Duplex Ultrasound requests for AVF assessment showed that only 38% were candidates for intervention, 48% had excellent blood flow and 14% had already thrombosed. This compares with 82% suitable for planned procedures shown by the 'Fishtank'. Figure 1. SPRn algorithm; SPRn = 1/(1+Pff/Vff); Pff = (S-D)/MAP derived from an automatic BP reading taken from the non fistula arm; Vff = (max-min)/mean derived automatically from the Doppler blood flow spectrum.

## SA-PO419

**Outpatient Dialysis Hospitalization Rates: A Comparison between Fellow (FL) and Non-Fellow (NF) Managed Units in NY State** George N. Coritsidis,[1] Adedoyin G. Akintide,[1] Carol Lyden,[2] Marie France R. DeLeon,[1] Jasjit Singh.[1] *[1]Internal Medicine/Nephrology, Elmhurst Hospital Center, Elmhurst, NY; [2]IPRO, Lake Success, NY.*

**Background:** Hospitalization rates of dialysis patients are an important measure of proper care in ESRD patients. Renal fellows may provide for greater physician presence or possibly lower the threshold for hospital admissions. We were interested to see how the presence of renal fellows impacts on this measure, and whether it provides for more efficient care.

**Methods:** We reviewed the de-identified records of Network 2 which includes all of NY State for 2010 regarding demographics, emergency department (ED)visits and hospitalization rates. The hospitalization rates were then compared to 2009 data. Fellow managed units (FL) were compared with non-fellow managed units (NF). Data was corrected for the number of patients per unit (pt).

**Results:** We identified a total of 24 fellow managed units (4432 patients) and 196 Non fellow units (29837 patients). Compared to the NF units, patients in the FL units were more likely to be African American (52.7% vs 38.5%, p<0.0001). Age, URR metrics, ethnicity and the incidence of diabetes and hypertension were similar.

| Year | Unit Type | ED Visit/pt | ED hospitalization/pt | Days hospitalized/pt | All hospitalizations/pt | Readmission within 30 days/pt |
|---|---|---|---|---|---|---|
| 2009 | FL | 1.21 | 0.63 | 8.59 | 0.97 | 0.34 |
| | NF | 1.20 | 0.60 | 8.25 | 0.98 | 0.32 |
| 2010 | FL | 1.24 | 0.65 | 6.50 | 0.95 | 0.33 |
| | NF | 1.26 | 0.64 | 7.40 | 1.00 | 0.33 |

**Conclusions:** There was a significantly higher percentage of African Americans in the fellow managed units. ED visits, hospitalization and readmission rates were similar regardless of the presence of a renal fellow.

## SA-PO420

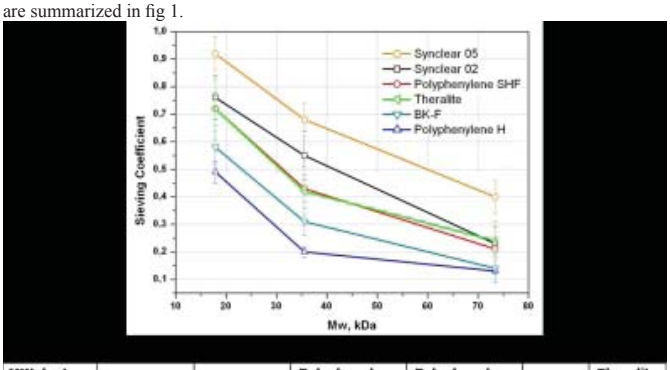
**In Vitro Characterization of High-Flux and High Cut-Off Membranes** Mauro Atti, Luca Corazza, Marialuisa Caiazzo, Luisa Sereni, Giuseppe Palladino. *Scientific Affairs, Bellco S.r.l., Mirandola, Modena, Italy.*

**Background:** The conventional dialysis membranes are classified as low-flux or high-flux membranes, depending on their permeability.Today a new class of High cut-off blood purification membranes are commercially available and this represent a challenge for blood purification in different pathologies treatable with extracorporeal treatments.

Dextran sieving experiments is an achievable and simple method, to classify High flux and High cut off membranes. Here we present preliminary results based on vitro method, obtained from six different membranes.

**Methods:** Dextrans (Sigma-Aldrich; Average MW 17.9, 35.6 and 73.4 kDa) solutions were prepared in distilled water at concentration of 1 g/L for each dextran. The dextran solution was recirculated at 300 ml/min flow rate and 50 ml/min as ultrafiltration flow. Feed (blood side entrance), retentate (blood side exit), and filtrate (dialysate exit) samples were taken at 15 and 60 min. Relative concentration of the samples were analyzed by spectrophotometric analyzer. The sieving coefficient SC was calculated according to Eq 1; SC= (2*Cf)/(Cp+Cr) , where Cf is the concentration of the solute in the filtrate, Cp its concentration in the permeate and Cr its concentration in the retentate.

**Results:** Six different commercially available dialyser were characterized and results are summarized in fig 1.




| MW dextran (kDa) | Syncear 0.5 | Synclear 0.2 | Polyphenylene SHF | Polyphenylene HF | BK-1.6F | Theralite 2100 |
|---|---|---|---|---|---|---|
| 17,9 | 0,92±0,06 | 0,76±0,08 | 0,72±0,06 | 0,49±0,04 | 0,58±0,06 | 0,72±0,11 |
| 35,6 | 0,68±0,06 | 0,55±0,09 | 0,43±0,05 | 0,20±0,02 | 0,31±0,05 | 0,42±0,09 |
| 73,4 | 0,4±0,06 | 0,23±0,06 | 0,21±0,03 | 0,13±0,01 | 0,14±0,05 | 0,24±0,07 |

As Aspected, we found an inverse proportionality between dextrans molecular weight and sieving coefficient .

**Conclusions:** The new membrane Synclear 0.5 showed encouraging behaviours in term of sieving coefficient, indicating a possible superior performance in the clearance of high molecular weight toxins. Innovative field for blood depuration could be explored due to its high depuration properties. Use of Synclear 0.5 membrane must be coupled with a specific sorbent system that allow reinfusion of albumin and retention of toxins substances.

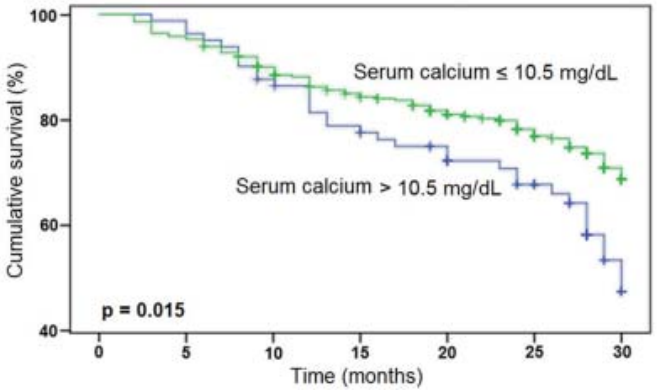
## SA-PO421

**Prevalence of Hypercalcemia and Hyperparathyroidism with Two Dialysate Calcium Concentrations** Patricia Coral Ruiz Palacios, Manolo Ramos Gordillo, Juan Francisco Fernández-pellón, Jose C. Pena. *Nephrology and Mineral Metabolism, Centro de Diagnostico Angeles, Mexico, Distrito Federal, Mexico.*

**Background:** The "calcium and alkaline" syndrome is a frequent complication in hemodialysis(HD) patients. The aims were to identify the presence of hypercalcemia in HD patients and to evaluate the effect of both a high dialysate calcium concentration compared to a low dialysate calcium concentration upon the serum calcium and PTHi concentrations.

**Methods:** From January 2010 to March 2011, 447 patients, age 52.6 ± 16 years old, 269 men(60%), were treated with high flux HD during 3 to 4 hours, 3 times per week, with a high dialysate calcium concentration(3.5 mEq/L). Then, during 14 months in the same cohort, the dialysate calcium concentration was lowered to 2.5 mEq/L. Oral calcium intake was held constant in all patients during both periods(1163 ± 757 mg/day). Hypercalcemia was defined as serum corrected calcium > 10.5 mg/dL. Serum calcium, PTHi and mortality were evaluated in all patients during both study periods. Results are expressed as mean ± SD. Mean values were compared with paired t test, hypercalcemia prevalence was compared with chi-squared test. Survival analysis was performed by Kaplan Meier curves and Log Rank(Mantel-Cox) test.

**Results:** Hypercalcemia prevalence was higher with high dialysate calcium(81 patients, 18.1 %) than with low dialysate calcium(15 patients, 3.3 % p < 0.01). With high calcium dialysate, mean serum corrected calcium was higher(9.61 ± 1.07 vs 9.24 ± 0.84 mg/dL p < 0.01) and mean PTHi was lower(898 ± 71.7 vs 992 ± 75.9 pg/mL p = 0.02) compared to low calcium dialysate. Survival rate was lower with high calcium dialysate compared to low calcium dialysate.




**Conclusions:** High calcium dialysate concentration was associated with hypercalcemia and increased mortality. Low calcium dialysate may stimulate PTH secretion, negative calcium balance and a reduction of the mortality rate.

## SA-PO422

**Comparing Accuracy of Assessment of Clotting of Dialyzer during Heparin Anticoagulation for Hemodialysis** Ki-won Kwon,[1] Young Hye Song,[1] Eun Hye Seo,[2] Jung-Hwan Park,[1,2] Jong-Ho Lee,[1,2] Young-Il Jo.[1,2] *[1]Dialysis Center, Konkuk Univ Medical Center, Seoul, Republic of Korea; [2]Div of Nephrology, Konkuk Univ Medical Center, Seoul, Republic of Korea.*

**Background:** Clinical assessment is useful for identifying coagulation problems during anticoagulation for hemodialysis (HD), the accuracy of visual assessment has yet not to be clarified. This study was designed to evaluate the accuracy of visual assessment of clotting within dialyzer during heparin anticoagulation for HD.

**Methods:** Adult ESRD patients who received maintenance HD were recruited. At the end of each dialysis session, the dialyzer was inspected for visible signs of thrombus formation and scored semiquantitatively (0=no clotting, 1=minimal, 2=moderate, 3=heavy, 4=complete clotting). The clotting volumes in dialyzer were calculated as follows: total blood compartment volumes (TBCV) of dialyzer before HD - TBCV after HD. The area of clotting in cross-section of dialyzer was measured by using squared paper. Each parameter was compared.

**Results:** A total of 244 dialyzer from 82 adult patients (male:female 41:41, age 62.6±11.6, DM 49%) were assessed and assorted into five groups (G-0:G-1:G-2:G-3:G-4=98:71:38:34:3) according to visual assessment of clotting within dialyzer. The grades of clotting within venous chamber estimated by visual inspection (G-0:G-1:G-2:G-3:G-4=239:4:1:0:0) were not correlated with the degrees of clotting within dialyzer. However, there were significant correlation ($p<0.001$) between the clotting within dialyzer and the coagulation volumes in dialyzer and the area of clotting in cross-section of dialyzer.

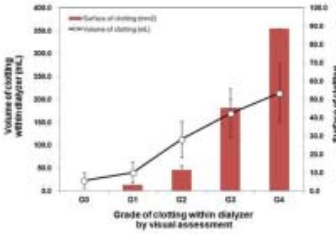



Figure 1. Correlation between the grade of clotting within dialyzer estimated by visual assessment and the clotting volumes within dialyzer and the surface areas of clotting of dialyzer.

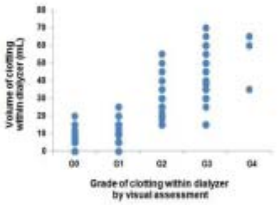



Figure 2. Distribution of volumes of clotting within dialyzer according to the grade estimated by visual assessment of clotting within dialyzer.

**Conclusions:** These results suggested the grade of clotting within dialyzer estimated by visual assessment may be useful indicator in decision of heparin dose for anticoagulation during HD.

## SA-PO423

**Ratios of Plasma Bicarbonate Increase to Dialysate-Plasma Bicarbonate Gradient at Early and Late Hemodialysis on 34 and 40 mEq/L Dialysate Bicarbonate Concentrations** David Tovbin,[1] Roberto Fudin,[1] Nayef Mohamed Habbashe,[1] Yakov Kuperman,[1] Saher Srur,[1] Alla Reitman,[1] Amir Abd Elkadir,[1] Shimon Storch.[2] *[1]Nephrology, Emek Medical Center, Afula; [2]Bnai-Zion Medical Center, Haifa, Israel.*

**Background:** Acidosis correction in hemodialysis (HD) requires bicarbonate (BIC) transfer to expanded extra-plasma compartments through narrow plasma compartment (P-EP transfer). Dialysate-plasma (D-P) transfer depends on D-P BIC concentration gradient (DBIC-PBIC). Intra-dialytic PBIC increase reach alkalotic range & counteracts gradient, D-P transfer & acidosis correction. We hypothesized that in pre-HD PBIC<22 mEq/L on standard BIC (SDBIC-34mEq/L), high DBIC (HDBIC-40 mEq/L) corrects inter-dialytic acidosis with limited intra-dialytic PBIC increase & alkalosis. Thus, we assessed intra-dialytic PBIC increases & their relations/ratios with DBIC-PBIC gradients on SDBIC & HDBIC.

**Methods:** In a prospective bi-center study, 15 patients were assessed for 3 weeks on SDBIC & HDBIC. Blood gases/electrolytes were assessed weekly at start, after 2 hours (mid-HD) & end HD. 3rd week data is presented as mean (SD) & evaluated using non-parametric tests.

**Results:** On SDBIC, pre-HD & mid-HD PBIC were 21.5 (2.7) & 27 (1.9) mEq/L, respectively. Early PBIC increase was 5.5 (1.9) mEq/L & correlated with DBIC- PBIC gradient ($r=0.71$, $p<0.005$). Mid-end PBIC increase was 1.3 (2) mEq/L. On HDBIC, pre-HD & mid-HD PBIC were 24.7 (2.3) & 33.2 (2) mEq/L, respectively. Early PBIC increase was 8.5 (1.7) mEq/L & correlated with DBIC- PBIC gradient ($r=-0.85$,$p<0.05$). Mid-end PBIC increase was 1.75 (2) mEq/L. In the 8 acidotic patients, on SDBIC pre-HD, mid-HD & end-HD PBIC were 19.3 (1.5), 26 (2) & 28 (2.9) mEq/L & on HDBIC 24 (2.6), 33.2 (2.4) & 34.9 (3.2) mEq/L, respectively. PBIC increase/DBIC-PBIC ratios decreased from early to late HD on both SDBIC & HDBIC {0.4357 (0.1124), 0.1634 (0.2834) & 0.5589 (0.1061), 0.2778 (0.3457), respectively, both $p<0.05$}.

**Conclusions:** Acidotic patients develop intra-dialytic alkalosis on SDBIC & more on HDBIC, which corrects inter-dialytic acidosis. Late HD decreased PBIC increase/DBIC-PBIC ratios suggest larger P-EP transfer for gradient. Thus, smaller gradient & DBIC are needed, which may limit alkalosis.

## SA-PO424

**Effects of Peritoneal Dialysis Using Icodextrin Dialysis Solution in Patients with Chronic Renal Failure Complicated by Refractory Congestive Heart Failure** Masahito Tamura, Tetsu Miyamoto, Ryota Serino, Yumi Furuno, Kenichiro Bando, Yoko Fujimoto, Akihiro Kuma, Emi Hasegawa, Yutaka Otsuji. *Dept of Nephrology, Univ of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

**Background:** Chronic heart failure is likely to cause renal impairment and often becomes resistant to conventional treatments. We evaluated the usefulness of peritoneal dialysis (PD) using icodextrin solution in patients with refractory congestive heart failure (CHF) complicated by chronic renal failure.

**Methods:** In patients at CKD stage 5 who presented with symptoms of NYHA III or above CHF resistant to conservative therapy and had a medical history of hospitalization for CHF, PD using icodextrin solution was initiated to prospectively evaluate its usefulness. One bag of icodextrin dialysis solution was administered overnight as the basal dose, and dextrose dialysis solution was added as renal function declined.

**Results:** Four patients classified as NYHA IV were enrolled to this study. The mean estimated GFR level was 7.12 ml/min/1.73m$^2$ in the PD initiation period. The mean amount of PD fluid removal was 817 ml/day. Urinary output increased from 812 ml/day before to 1,083 ml/day after PD initiation. The NYHA classification showed improvement to class II in all patients. Duration of hospitalization due to CHF decreased from 29 days/year before to 0 days/year after PD initiation. Improvements of the following parameters were observed after as compared to before PD initiation: atrial natriuretic peptide, from 294.1 to 12.7 pg/ml; left ventricular ejection fraction, from 37.5 to 48.3%; end-diastolic left ventricular diameter, from 56.5 to 46.6 mm; and end-systolic left ventricular diameter, from 42.8 to 31.9 mm. Body weight showed no significant change from 55.1 kg before to 57.2 kg after PD initiation. In 1 patient, residual renal function declined after PD initiation, and hemodialysis was used in combination due to poor body fluid control.

**Conclusions:** It was suggested that PD with icodextrin dialysis solution might be useful in patients with chronic renal failure complicated by refractory CHF. Improvement of the NYHA classification might be attributable to improved cardiac function.

## SA-PO425

**NeutroPhase Shows Improvement in the Infections Associated with Peritoneal Dialysis Catheters** Allan G. Kavalich,[1] Dmitri Debabov,[2] Ramin (ron) Najafi,[2] Russell Hoon,[2] Thomas Paulson.[2] *[1]Medical Director, San Bernardino Valley Home Dialysis Center, Inc., San Bernardino, CA; [2]NovaBay Pharmaceuticals, Emeryville, CA.*

**Background:** *S. aureus* accounts for most exit site and tunnel infections associated with peritoneal dialysis catheters. Gram negative bacteria and fungi are less common, but difficult to eradicate and frequently lead to peritonitis if catheter removal is delayed.

**Methods:** NeutroPhase spray (a stabilized 0.01% pure hypochlorous acid in a NS solution) was used to treat five patients with infections associated with peritoneal catheters.

**Results:** A 59 year old male with exduative tunnel infection due to heavy growth of multi-resistant E.coli was treated with Levequin. After 10 days he was re-tested and grew out resistant E.coli and started on NeutroPhase. Patient was brought into unit TID, NeutroPhase was induced into tunnel via 30cc syringe and flexible IV catheter to bathe area with 5cc for 10 minutes repeated X four. NeutroPhase was allowed to dry, catheter was anchored and dressing was applied. Process was repeated X 2 weeks, then at home three times per day for six weeks with complete resolution. 55 year old male developed a Candida Albicans PD exit site infection; was treated with IV Diflucan and Nystatin cream. Culture remained positive. Treated with NeutroPhase 4 sprays to the exit site BID X six weeks with complete resolution. Continues to use spray daily at exit site X six months with no further infections. A 64 year old male with MRSA exit site infection was treated solely with NeutroPhase spray TID for eight weeks with complete resolution. 55 year old female with chronic staph epi exit site infections in the past treated with Vanco and Rifampin. NeutroPhase spray started. Exit site clean after six weeks. Continued spray QD without infection X six months. 29 year old female with recurrent exit site infections treated with NeutroPhase spray QID with complete resolution X four weeks.

**Conclusions:** NeutroPhase was the only treatment showing improvement in these patients. Our data show that there is a clear utility for the use of NeutroPhase for treatment of infections associated with peritoneal dialysis catheters.

*Funding:* Pharmaceutical Company Support - NovaBay Pharmaceuticals

## SA-PO426

**Encapsulating Peritoneal Sclerosis in PD Patients or on Renal Transplantation after Peritoneal Dialysis** Valerio Vizzardi, Massimo Sandrini, Luigi Manili, Silvio Sandrini, Gisella Setti, Laura Econimo, Giovanni Cancarini. *UO Nefrologia, Spedali Civili and Univ of Brescia, Brescia, Italy.*

**Background:** Encapsulating Peritoneal Sclerosis (EPS) is one of the most severe complication of peritoneal dialisys (PD). Prevalence of EPS after PD discontinuation has been increasing in the last years, mainly after kidney transplantation (50% of cases).

**Methods:** Retrospective analysis of EPS diagnosed in our Center from Jan 2000 to Dec 2012.

**Results:** 13 cases of EPS(2.5%) occurred in 517 non-TX patients still on PD. In the same period, 81 PD patients received a TX and two of them (2.5%) developed EPS. **TX Pt1**: 53 yrs old man: hemodialysis (HD) from 1982 to 1987. 1st TX from 1987 to 2002 [cyclosporine (Cys) and steroids (ST)]. APD from 2002 to 2005. In 2005 he received a 2nd

TX (Mycophenolate (Myc) and ST). In 2009 renal function failed and the pt started PD. EPS was diagnosed on peritoneal biopsy obtained during the placement of the Tenckhoff catheter. Clinical history: no episodeof bowel obstruction. The patient was shifted to HD and continued Myc and ST (8mg/day). Abdomen CT scan (2011 after an episode of intestinal sub-obstruction) showed "distension ofbowel loops, some focal thickening along the parietal peritoneum (previously absent)." The pt started Tamoxifen 20 mg/day. In the following months the clinical condition deteriorated and in December 2012 parenteral nutrition was started. **TX Pt2**: 57 yrs old man, on APD for 79 months. On April 2007 he received a TX. Therapy: Myc, ST and Tacrolimus (after 36 months shift to Cys). The patient is still on Cys, Myc and ST. 2003, abdomen CT-scan: "calcifications of the parietal peritoneum"; peritoneal biopsy: "chronic peritonitis with fibrosis and sclerosis." 2005, abdomen CT-scan: no peritoneal calcification. In 2007 the pt underwent enterolysis surgery for bowel obstruction. He continue on 5 mg of ST and, at present, six years after enterolysis, is asymptomatic.

**Conclusions:** In literature EPS prevalence increases after PD discontinuation mainly after TX. In our experience the incidence of EPS do not differ between TX and non-TX PD patients. The evolution of the two cases was acceptable after 3 and 6 years since the time of diagnosis.

## SA-PO427

### Effect of Peritoneal Dialysis Modality and Peritoneal Membrane Characteristics on Phosphorus Level and Managment

Rafael Garcia, Sheru Kansal, Martin J. Schreiber. *Hypertension & Nephrology, Cleveland Clinic Foundation, Cleveland, OH.*

**Background:** Hyperphosphatemia is highly prevalent in peritoneal dialysis (PD). Prior studies have suggested that phosphate (P) removal in continuous ambulatory PD (CAPD) and automated PD (APD) was regulated by peritoneal membrane (PM) transport type. The current study examined the impact of PD modality and PM transport type on P values, P binder equivalent doses (PBED), calcium and PTH values.

**Methods:** In this retrospective and single cohort study, we included 40 patients (24 M), mean age 53 y/old all new to PD dialysis. Baseline PM transport type, PD modality, ultrafiltration, residual renal function (RRF), total creatinine clearance (TCrCl), PCrCl and Kt/V were identified. Serial P level and PBED were documented every 3 months for 1 year. Longitudinal data analysis on repeated measures of outcomes by mixed effects models for continuous binary outcomes were conducted.

**Results:** 65% of patients utilized APD and 35% CAPD. PM transport distribution: low-low average :17, high average- high:23. At baseline (mean): RRF 6.57 ml/min, PCrCl 39.39 L/wk, TCrCl 143.58 L/wk, Kt/V 2.55, P 4.7 mg/dl, PBED 1666 mg. At 12 months, P and PBED were higher at 5.1 mg/dl and 2853 mg. PM transport type did not affect P levels or PBED. The slope of P change was not affected by PD modality, however, PCrCl and TCrCl significantly correlated with P level. PD type was significant for PBED; CAPD demonstrated significantly higher PBED than APD. RRF and TCrCl but not PCrCl also significantly affected PBED.

| P levels | Estimate | P-value |
|---|---|---|
| PCrCl | -0.04 | 0.004 |
| TCrCl | -0.01 | 0.03 |
| **PBED** | | |
| CAPD | 942 | 0.03 |
| RRF | -329 | 0.0009 |
| TCrCl | 18 | 0.02 |

**Conclusions:** The change in P level or PBED was not significantly impacted by PM transport type. Patients on CAPD have higher PBED suggesting an advantage to APD for phosphate control. PCrCl significantly affected P levels but not PBED whereas TCrCl significantly affected both suggesting creatinine clearance may be a better measurement of adequacy with regard to phosphate control as compared to Kt/V and should be examine in all patients to optimize P control. Prescription design focused on achieving the highest TCrCl should be a priority in settings of hyperphosphatemia.

## SA-PO428

### Survival Predictors in Anuric Korean Peritoneal Dialysis Patients: Prospective Multicenter Propensity Score Matched Cohort Study

Jang-Hee Cho,[1,2] Hye Min Jang,[1,2] Yon Su Kim,[1,3] Shin-Wook Kang,[1,4] Chul Woo Yang,[1,5] Nam Ho Kim,[1,6] Ji-Young Choi,[1,2] Sun-Hee Park,[1,2] Chan-Duck Kim,[1,2] Yong-Lim Kim.[1,2] *[1]Clinical Research Center for End Stage Renal Disease in Korea; [2]Kyungpook National Univ School of Medicine; [3]Seoul National Univ College of Medicine; [4]Yonsei Univ College of Medicine; [5]Catholic Univ of Korea College of Medicine; [6]Chonnam National Univ Medical School.*

**Background:** This study aims to evaluate predictors of survival in the absence of residual renal function using anuric peritoneal dialysis (PD) patients.

**Methods:** Anuric PD patients (n=505, <100 ml of daily urine) enrolled in Korean nation-wide prospective cohort from September 1, 2008 to June 30, 2011 were analyzed. Survived and non-survived anuric PD patients were compared by propensity score matching analysis by a ratio of two to one. Propensity method was used to adjust patient age, dialysis duration and presence of diabetes.

**Results:** In total anuric PD patients, non-survived patients showed significantly older age, higher proportion of diabetes, coronary artery disease, arrhythmia, lower serum creatinine and albumin. On Kaplan-Meier analysis, patient survival was significantly lower in lower ultrafiltration volume (<1000ml/day), serum creatinine (<10.7mg/dL) and serum albumin (<3.7g/dL). After propensity score matching, only serum albumin and cholesterol were decreased in non-survived patients compared to survived patients (3.44±0.45g/dL vs. 3.63±0.52g/dL, p=0.0388 and 169.9±41.8mg/dL vs. 185.7±50.9mg/dL, p=0.0416, respectively). Multivariate analysis revealed serum albumin was independent predictor of mortality in matched cohort (hazard ratio=0.548, p=0.0085). Analysis using the receiver-operating-characteristic curve showed that mortality could be predicted with a sensitivity of 59.4% and a specificity of 63.2% using a cutoff value of 3.6mg/dL of serum albumin in unmatched total PD patients.

**Conclusions:** Mortality of anuric PD patients was associated with age, comorbidities, and nutritional factors such as creatinine and albumin. After adjustment by propensity score matching, hypoalbuminemia was independent risk factor for mortality in anuric PD patients.

*Funding:* Government Support - Non-U.S.

## SA-PO429

### Risk Factors for Peritoneal Dialysis Associated Peritonitis

Loke Meng Ong, N. Punitha, Z. Hadzlinda, B. Sunita, M. Lily, B.L .Goh, Ghazali T.V. Ahmad. *Ministry of Health (MOH), Malaysia.*

**Background:** Peritonitis remains the Achilles heel of peritoneal dialysis (PD) and adversely affect technique and patient survival. Many factors contribute to the risk of peritonitis.

**Methods:** We conducted a prospective observational study for 1 year to determine the patient and centre factors contributing to peritonitis. All episodes of peritonitis were independently verified.

**Results:** 2001 patients from 20 adult and paediatric PD centres in MOH were included. Their mean age was 48 years and 53% were females. 455 episodes of peritonitis occurred in 425 patients. The mean peritonitis rate was 41.8 patient-months per episode (PMEP). 29% of causative organisms were gram +ve, 32% gram -ve, 3% fungal and 32% had no organisms. The main risk factors for peritonitis were nasal Staph aureus carriage and manual PD (Table 1). There was a wide variation of peritonitis among PD centres (range 17.7-86.3 PMEP) but no centre factors could be identified. The number and experience of staff, staff-patient ratio, distance of residence to centre and number of home visits made did not affect the peritonitis risk.

**Table 1. Incidence Rate Ratio (IRR) for Peritonitis**

| | Univariate IRR (95% CI) | Multivariate IRR (95% CI) |
|---|---|---|
| Staph nasal carriage | 2.02(1.37-3.00) | 1.73(1.02-2.94) |
| Manual vs APD | 1.69(1.09-2.36) | 1.76(1.02-3.02) |
| Diabetes | 1.24(1.01-2.36) | 1.33(0.95-1.86) |
| Topical antibiotics | 0.74(0.58-0.94) | 1.08(0.65-1.77) |
| Need for assistance | 1.23(0.99-1.52) | 1.04(0.75-1.45) |
| Poor vision | 0.91(0.51-1.63) | 0.89(0.43-1.84) |
| Obesity | 1.56(0.99-2.47) | 1.23(0.62-2.48) |
| Female | 1.04(0.86-1.27) | 1.15(0.86-1.55) |
| Chinese | 0.69(0.54-0.88) | 0.85(0.49-1.48) |
| Elderly | 1.15(0.78-1.71) | 0.88(0.46-1.65) |
| High income | 0.80(0.56-1.15) | 0.77(0.44-1.36) |
| No education | 0.73(0.46-1.18) | 1.01(0.48-2.12) |

69% of peritonitis resolved with antibiotics and 2% relapsed then resolved. 23% required catheter removed and 6% of patients died.

**Conclusions:** There was a wide variation of peritonitis among PD centres. Staphylococcus aureus nasal carriage was the most important risk factor for peritonitis while automated PD reduced the risk.

## SA-PO430

### Effects of Walking Ability on Risk of Hospitalization for Cardio-Cerebrovascular Events in Hemodialysis Patients

Yoshifumi Abe,[1] Atsuhiko Matsunaga,[1] Ryota Matsuzawa,[1,2] Akira Ishii,[1,2] Kei Yoneki,[1] Manae Harada,[1] Toshiki Kutsuna,[1] Yutaka Takagi,[2] Atsushi Yoshida,[2] Yasuo Takeuchi,[1] Kouju Kamata.[1] *[1]Kitasato Univ, Sagamihara, Japan; [2]Sagami Junkanki Clinic, Sagamihara, Japan.*

**Background:** Hemodialysis (HD) patients are at high risk for cardio- and cerebrovascular events, yet very few studies have examined physical activity and function as a way to reduce the risk of cardio-cerebrovascular events among HD patients. We investigated the prognostic significance of walking ability on cardio-cerebrovascular events requiring hospitalization among HD patients.

**Methods:** A total of 148 Japanese outpatients undergoing maintenance HD three times per week at a HD center from October 2002 to August 2012 were followed up to 5 years. We measured maximum walking speed and characteristics (age, sex, body mass index, HD duration, comorbid conditions, serum albumin, and serum C-reactive protein) at baseline. HD patients were divided into two groups based on maximum walking speed: the fast speed group (women, ≥1.35 m/s; men, ≥1.50 m/s) and the slow speed group. A Cox proportional hazard regression was used to assess how walking speed affected hospitalization from all-cause, cardio-cerebrovascular events and non-cardio-cerebrovascular events.

**Results:** Median (25$^{th}$, 75$^{th}$ percentile) age of this study population was 64 (56, 72) at baseline, and 57.4% of the patients were women. Sixty percent of the patients were included in the fast speed group. There were 35 hospitalizations for cardio-cerebrovascular events and 21 hospitalizations for other causes. After adjusting for the effects of clinical characteristics, the hazard ratios for hospitalization from all-cause, cardio-cerebrovascular events and non-cardio-cerebrovascular events in the fast speed group were 0.48 (95% CI: 0.25-0.93; $P = 0.03$) , 0.33 (95% CI: 0.15-0.74; $P = 0.01$) and 0.89 (95% CI: 0.31-2.56; $P = 0.83$), respectively, compared with those in the slow speed group.

**Conclusions:** Walking speed is closely associated with physical activity of daily living.These results suggest that specific instruction on how to improve and maintain walking ability may be necessary to reduce the risk of cardio-cerebrovascular events among HD patients.

## SA-PO431

**Low Serum Magnesium Is Related with Vascular Stiffness in Patients on Maintenance Hemodialysis (MHD) but Not in Non-Dialysis Chronic Kidney Disease Patients (ND-CKD)** Takahiro Kuragano, Takeshi Nakanishi. *Internal Medicine, Div of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

**Background:** Recent clinical studies have shown that lower serum magnesium (Mg) levels are associated with vascular calcification and cardiovascular mortality in MHD. In this study, we evaluated the factors affecting Mg in MHD or ND-CKD. Furthermore we evaluated the relationship between Mg and the markers of arteriosclerosis in these patients.

**Methods:** We measured blood levels of Mg, hemoglobin (Hb), total cholesterol, creatinine (Cr), urea nitrogen (UN), β2microblobuin (MG), albumin (alb), Calcium (Ca), phosphate (P), intact parathyroid hormone (int-PTH), and tumor necrosis factor (TNF)-α in 69 patients with various stages of ND-CKD and 129 MHD. Furthermore, we measured brachial-ankle pulse wave velocity (ba-PWV) and ankle-brachial index (ABI).

**Results:** There was no significant difference in Mg between ND-CKD (2.19±0.26 mg/dL) and MHD (2.34±0.35 mg/dL). In MHD, Mg was significantly correlated with age (P=0.004, R=-0.25), Ca (P=0.02, R=0.21), TNF-α (P=0.005, R=-0.25), alb (P=0.001, R=0.32) and ba-PWV (P=0.001, R=-0.29). In multiple regression analysis, alb (P=0.0001, β=0.31) and Ca (P=0.029, β=0.18) were selected as the significant predictors of Mg in MHD. In the analysis for determining the factors affecting vascular stiffness of MHD, Mg (P=0.012, β=-0.22) was selected as the significant predictor of ba-PWV as well as systolic blood pressure (P=0.0001, β=0.32) and age (P=0.005, β=0.25). In ND-CKD, Mg did not related to Cr, UN, β2MG, alb, Ca, P, and int-PTH, ba-PWV and ABI.

**Conclusions:** Mg were associated with the index of vascular stiffness (ba-PWV) in MHD, and related with serum levels of Ca and alb. In ND-CKD, we could not show any parameters associated with Mg level.

## SA-PO432

**Neutrophil/Lymphocyte Ratio as a Predictor of Cardiovascular Events in Japanese Patients Starting Renal Replacement Therapy** Sawako Kato,[1] Bengt Lindholm,[2] Yukio Yuzawa,[3] Seiichi Matsuo,[1] Shoichi Maruyama.[1] *[1]Nephrology, Nagoya Univ Graduate School of Medicine, Aichi, Japan; [2]Baxter Novum & Renal Medicine, Karolinska Institute, Stockholm, Sweden; [3]Nephrology, Fujita Health Univ School of Medicine, Toyoake, Aichi, Japan.*

**Background:** Previous studies have suggested that a high neutrophil/lymphocyte ratio (NLR) is related to worse outcome in patients with cardiovascular diseases (CVD). Patients with chronic kidney disease (CKD) have an increasing risk for premature mortality, primarily as a result of CVD; however, the association between NLR and CVD risk is not known.

**Methods:** Eighty seven incident Japanese dialysis patients (58 males, age 58 ±11 years) without acute infections and severe liver dysfunction were enrolled and followed for a median of 38.7 months (range 1-68.9 months). Laboratory biomarkers including white blood cell count (WBC) and its differential count, were checked.

**Results:** NLR was 3.72 (1.19-24.4). NLR positively correlated to WBC ($R^2$=0.16, P<.0001), neutrophil count ($R^2$=0.55, P<.0001) and CRP levels ($R^2$=0.07, P=0.013), and negatively to lymphocyte count ($R^2$=0.51, P<0.0001). The duration from start of dialysis therapy to the first CVD events was significantly shorter in patients with higher NLR (Log rank 6.95, P=0.0084). The patients with high NLR had a significantly increased relative risk (Cox hazard model; 3.07 95% CI; 1.32-8.00) of CVD compared with those with lower NLR, even after adjustments for age, gender and presence of diabetes, while the relations between WBC, neutrophil and lymphocyte count and CVD events respectively were much weaker. The number of CVD events per year during the observation period was higher in patients with higher NLR (28.5 events per 100 person-years) than in patients with lower NLR (6.43 events per 100 person-years).

**Conclusions:** A higher NLR associates with increasing risk of cardiovascular events and is a stronger predictor of future CVD events compared with WBC, neutrophil, and lymphocyte counts.

*Funding:* Government Support - Non-U.S.

## SA-PO433

**Importance of Brain Natriuretic Peptide in Assessing Overhydration in Patients on Maintenance Hemodialysis: A Validation Study Using Three-Dimensional Echocardiography** Attila Kovács,[1] Mihaly B. Tapolyai,[2] Katalin Solymossy,[1] Maria Faludi,[2] Astrid Apor,[1] Andrea Nagy,[1] Virag Reti,[2] Klara Berta,[2] Bela Merkely.[1] *[1]Heart and Vascular Center, Budapest, Hungary; [2]Dialysis, Fresenius Medical Care, Semmelweis Univ, Budapest, Hungary.*

**Background:** Precise estimation of overhydration in end-stage renal disease (ESRD) patients remains a major clinical issue. We sought to investigate the significance of brain natriuretic peptide (BNP) levels in assessing overhydration and its correspondence with left ventricular geometry and function measured by three-dimensional (3D) echocardiography.

**Methods:** In our cohort study forty-four prevalent ESRD patients on maintenance hemodialysis (HD) were enrolled. Exclusion criteria were medical history of coronary artery disease, diabetes mellitus, any cardiomyopathy or significant valvular disease. Prior to regular HD session, overhydration percentage (OH%) was assessed with bioimpedance (Fresenius Body Composition Monitor) and blood sample was collected for BNP measurement. 3D echocardiography was performed both before and after HD (GE Vivid E9). End-diastolic (EDVi), end-systolic (ESVi) volumes indexed to body surface area, ejection fraction (EF) and sphericity were measured using dedicated software (4D AutoLVQ). Data are presented as median (interquartile range).

**Results:** EDVi and ESVi were reduced, while EF increased after ultrafiltration [pre- vs. post-HD; EDVi: 56.3(17.2) vs. 55.6(13.9), ESVi: 19.6(8.6) vs. 19.3(5.7) ml/m$^2$, EF: 63(9.5) vs. 66(10) %, all p<0.001]. 3D sphericity describing full shape of the left ventricle decreased with fluid removal [0.37(0.12) vs. 0.31(0.15), p<0.001]. Serum BNP levels significantly correlated with pre-dialysis values of EF (Spearman's rho=-0.478), sphericity (ρ=0.491), OH% (ρ=0.484, all p<0.01). In a multivariate linear regression model BNP (β=-0.455) and sphericity (β=-0.456) were found to be independent predictors of EF.

**Conclusions:** Brain natriuretic peptide implies overhydration through the alterations of cardiac geometry and function. 3D echocardiographic assessment of the left ventricle provides valuable and instantaneous information on intravascular fluid load.

*Funding:* Clinical Revenue Support

## SA-PO434

**Amputation-Free Survival in Dialysis Patients Undergoing Angioplasty of the Lower Limbs: Is Vascular Intervention Futile?** Ashraf Omar Oweis, Rory F. McQuillan, Joanne M. Bargman. *Nephrology, Univ Health Network, Toronto, Canada.*

**Background:** Previous studies have shown that dialysis patients are at increased risk for peripheral arterial disease. With improvement in interventional technology, more patients are undergoing angioplasty of the arterial tree to try to avoid amputation, and to maintain mobility and quality of life. However, there are few studies that have examined the outcome of lower limb angioplasty in these patients.

**Methods:** A retrospective analysis of all dialysis patients evaluated and treated by the interventional radiology department of a large referral hospital from 1 January 2005 to August 30 2012 with at least one-year follow up was conducted using electronic patient records. The Kaplan-Meier method was used to assess the main time-to-event outcomes (mortality and amputation) in comparison to non-dialysis patients during the same period of time. Cox proportional hazards models were fitted to examine the independent association among comorbidities, the use of antiplatelet and lipid-lowering agents, ACE/ARB and time-to-event outcomes.

**Results:** We evaluated 153 patients with lower limb angioplasty or stent of whom 51(33.3%) were on dialysis (2 PD and 49 HD) and 102 (66.7%) were not on dialysis. Mean time on dialysis was 76.9 months (SD 49.2). In the dialysis group there were 33(64.7%) deaths and 26(51%) amputations vs. 22 (21.6%) and 31(30.4%) in the non-dialysis group, with a P value of 0.0001 and 0.013 respectively. Almost half the deaths occurred within the first year after angioplasty: 16(31.4%) for the dialysis group vs. 10(9.8%) for the non-dialysis group, P=0.001. All patients in the dialysis group who had an amputation within a year of angioplasty died within that year.

**Conclusions:** This study shows a remarkably higher mortality and amputation rates in dialysis vs. non-dialysis patients who underwent lower limb angioplasty and/or stenting. This suggests that vascular intervention in dialysis patients may not be as effective as in those not on dialysis. A shift may be needed to focus on the benefits of early diagnosis, preventive measures, and the need for involving the palliative service early in the course along with nephrology and vascular teams.

*Funding:* Clinical Revenue Support

## SA-PO435

**Association of Physical Activity with HDL-C Level in Hemodialysis Patients** Ryota Matsuzawa,[1,2] Atsuhiko Matsunaga,[1] Akira Ishii,[1,2] Yoshifumi Abe,[1] Kei Yoneki,[1] Manae Harada,[1] Yutaka Takagi,[2] Atsushi Yoshida,[2] Yasuo Takeuchi,[1] Kouju Kamata,[1] Naonobu Takahira.[1] *[1]Kitasato Univ, Sagamihara, Japan; [2]Sagami Junkanki Clinic, Sagamihara, Japan.*

**Background:** The Japanese Society for Dialysis Therapy reports that decreased high-density lipoprotein cholesterol (HDL-C) levels are associated with increased mortality in dialysis populations. Although physical activity increases HDL-C levels in the general population, this remains unclear in hemodialysis patients. After confirming the relationship between HDL-C levels and survival, we investigated the effect of physical activity on HDL-C levels in hemodialysis patients.

**Methods:** First, 266 hemodialysis patients (age, 64±13 years; duration of hemodialysis, 9±8 years; HDL-C, 43.5±13.4 mg/dL) were monitored prospectively for 5 years. Serum lipid levels were obtained at baseline. Cox proportional-hazards regression was used to confirm the contribution of HDL-C level to mortality. Second, before investigating the association between physical activity and HDL-C level, we excluded patients who had severe comorbidities or who needed help in walking. As a result, 116 patients (age, 67±9 years; duration of hemodialysis, 9±9 years; HDL-C, 42.8±12.8 mg/dL) were recruited in the cross-sectional study. We evaluated their physical activity and characteristics including age, sex, body mass index, duration of hemodialysis, comorbid conditions, serum albumin and creatinine levels, smoking habit, and use of antilipemics. Physical activity was evaluated with an accelerometer as the number of steps per day for a consecutive 5-day period. We used multiple regression analysis to evaluate the association between physical activity and HDL-C level.

**Results:** During the follow-up period, 77 patients died. We confirmed that HDL-C level was a significant predictor of all-cause mortality (hazard ratio, 0.975; 95% CI, 0.954-0.997; $P = 0.03$). After adjustment for patient characteristics, physical activity was independently associated with HDL-C level (adjusted β coefficient, 0.244; adjusted $R^2$, 0.255; $P = 0.005$).

**Conclusions:** Physical inactivity was strongly associated with decreased HDL-C level in hemodialysis patients.

## SA-PO436

**Decline of Peripheral Resistance over Time in Patients with End Stage Renal Disease. Results from the CONvective TRAnsport STudy (CONTRAST)**
Irina Mostovaya,[1] Michiel Bots,[2] Muriel Grooteman,[3] Marinus A. Van Den Dorpel,[4] Pieter M. Ter Wee,[3] Peter J. Blankestijn.[1] *[1]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]2 Julius Center for Health Sciences and Primary Care, Univ Medical Center Utrecht, Utrecht, Netherlands; [3]Nephrology, VU Medical Center, Amsterdam, Netherlands; [4]Internal Medicine, Maasstad Hospital, Rotterdam, Netherlands.*

**Background:** Peripheral resistance is a well established marker of sympathetic activity in end stage renal disease (ESRD) patients. However, data on changes over time are scarce. The aim of this study was to examine changes over time of peripheral resistance (PR), cardiac output (CO), mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP).

**Methods:** A subpopulation of 84 patients from the CONvective TRAnsport STudy (CONTRAST), a randomized trial comparing online hemodiafiltration with low-flux hemodialysis, received echocardiography and simultaneous blood pressure measurements on a non-dialysis day at baseline, 6 months, 12 months and annually thereafter. PR was computed as MAP/CO. A total of 190 measurements were thus performed over a follow-up period of 3 years. The rate of change over time of PR, CO, MAP, SBP and DBP was estimated using linear mixed effects models.

**Results:** At baseline, patients had a mean age of 62 ± 14 years and 55% were male. Median PR was 17.7 (IQR 15.3 – 22.5) mmHg•min/L, mean CO was 5.5 ± 1.5 L/min, mean MAP was 97 ± 15 mmHg and mean SBP and DBP were 137 ± 29 and 76 ± 12 mmHg respectively. PR (Δ-1.6 95%CI:-3.0 to -0.2 mmHg•min/L per year), MAP (Δ-4.2 95%CI:-7.5 to -0.8 mmHg per year) and DBP (Δ-3.8 95%CI:-6.7 to -0.9 mmHg per year) decreased over time, while CO (Δ0.3 95%CI:-0.1 to 0.7 L/min per year) and SBP (Δ-4.3 95%CI:-10.3 to 0.9 mmHg per year) remained stable.

**Conclusions:** in ESRD patients MAP decreases over time, mainly due a decrease in DBP. As CO remained stable, this decline can be explained by a decrease of PR over time. Precise mechanisms were not investigated, but a decrease in renin-angiotensin-aldosteron system activation and renal sympathetic nerve activity could be a plausible explanation.

## SA-PO437

**Influence of Methodology on Left Ventricular Mass Measurement by Cardiac Magnetic Resonance in the Frequent Hemodialysis Network (FHN) Nocturnal Trial**
Gerald J. Beck, Glenn M. Chertow, Paul W. Eggers, Tom Greene, Brett Larive, Nathan W. Levin, Sanjay Rajagopalan, Michael V. Rocco, Javier Sanz, Tam Tran, Christopher Kramer, Alan S. Kliger, The FHN Trial Group. *NIH, NIDDK.*

**Background:** The FHN previously reported in the Daily Trial that left ventricular mass (LVM) by cardiac magnetic resonance (CMR) declined more over 12 months (unadjusted mean difference 13.9g) among patients randomized to 6x/week in-center hemodialysis (HD) compared to conventional 3x/week HD controls. In the smaller Nocturnal Trial (n=87 vs. 245), although LVM declined by 8.8g in the 6x/week nocturnal (NHD) vs. conventional HD (CHD) patients, these results were not significant. In the FHN trials, baseline and month 12 CMR studies were centrally read sequentially during the trial by 3 trained readers blinded to treatment group. Readers had rigorous training in standardized assessment of LVM and volumes.

**Methods:** Given the two trials' similar LVM effect sizes, we explored the impact of a single trained reader concurrently evaluating LVM for all Nocturnal Trial patients using matched baseline and 12 month MRIs (n=76) while blinded to treatment and time of visit.

**Results:** The standard deviation of the change in LVM was 28.2g using sequential measurements and 23.8g with concurrent measurements; a reduction of 16.6%. LV volumes showed closer agreement between sequential and concurrent readings. The original reported primary outcome (change in LVM or death) did not differ between the NHD vs. CHD arms: hazard ratio (HR) of 0.69 (95% CI 0.44 to 1.07, p=0.095). Use of concurrent readings yielded similar results: HR = 0.73 (95% CI 0.46 to 1.14, p=0.16).

**Conclusions:** Measurement of LVM using a single reader who read studies concurrently, modestly reduced the variability of LVM but did not change the primary outcome results from that reported previously for the FHN Nocturnal Trial. This was due to the magnitude of the treatment effect being reduced (along with the LVM variability) with the concurrent readings, which may reflect better reproducibility due to a single reader and use of matched (but blinded) pre and post-images.

*Funding:* NIDDK Support

## SA-PO438

**Hemodialysis Induced Cardiac Stunning: Impact on Segmental and Global Longitudinal Myocardial Strain**
Shih-Han S. Huang,[1,2] Lisa E. Crowley,[2] James O. Burton,[2] Aghogho Odudu,[2] Chris W. McIntyre.[3] *[1]Medicine, Nephrology, London Health Sciences Centre, Western Univ, London, Canada; [2]Dept of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [3]Div of Medical Sciences and Graduate School, Univ of Nottingham, Nottingham, United Kingdom.*

**Background:** Conventional hemodialysis (HD) results in recurrent cardiac injury. This is associated with left ventricular regional wall abnormality. Strain analysis derived from analysis of speckle tracked imaging (STI) echocardiography can be used to examine both global and segmental contractile function. In this study, we examined the relationship of HD induced circulatory stress with overall ventricular function (assessed as global longitudinal strain (GLS), segmental distribution of strain and their impact on patient survival).

**Methods:** Averaged values of segmental and GLS were determined from pre- and peak-dialysis (225 minutes) echocardiography of 104 conventional HD patients. These values were compared to the reference values of healthy individuals. Impact on survival was assessed over a follow up period of up to 7 years.

**Results:** The segmental and global strain values were -8.65 to -16.51 ± 4.91 to 8.13 and -11.48 ± 4.42, respectively. Significant HD induced changes to segmental strain were restricted to the mid- and apical-septal segments (r=-0.29 to -0.33 = p<0.05). Global and most of the segmental (10 of 12) contractile function was reduced in HD patients c.f. normal control values, with segmental and global strain values significantly higher than healthy individuals (2.70-9.83, p<0.001). Of the forty-six patients who died, there were statistically significant negative correlations between survival time and the GLS (r=-0.30, p=0.04) and the apical, inferior and septal segments (r=-0.31 to -0.32, and p<0.04).

**Conclusions:** Global and segmental strain as measured from STI provides additional information relating to the effects of HD induced cardiac injury. The clustering of dialysis induced segmental strain abnormalities to the apical, inferior and septal watershed area of the left ventricle suggests a higher degree of vulnerability to demand ischaemia in this part of the uremic heart to challenged perfusion.

## SA-PO439

**The Prevalence of Oral Lesions in People on HemoDialysis: Oral-D Study**
Giovanni F.M. Strippoli,[1,2,3,4] Suetonia Palmer,[5] Marinella Ruospo,[1,2] Patrizia Natale,[1] Valeria Maria Saglimbene,[1,2] Michela Sciancalepore,[1] Letizia Gargano,[1] Fabio Pellegrini,[2] David W. Johnson,[6] Pauline J. Ford,[6] Jonathan C. Craig,[4] Paul Stroumza,[1] Luc Frantzen,[1] Miguel Rodrigues Leal,[1] Marietta Torok,[1] Anna Bednarek,[1] Jan Dulawa,[1] Eduardo Jorge Celia,[1] Ruben Gelfman,[1] Jorgen B.A. Hegbrant,[1] Charlotta Wollheim,[1] Staffan Schön,[1] Michele De Benedittis,[3] Massimo Petruzzi.[3] *[1]Diaverum; [2]Mario Negri Sud Consortium; [3]Univ of Bari; [4]Univ of Sydney; [5]Univ of Otago; [6]Univ of Queensland.*

**Background:** It has been shown that oral diseases are common in the general population and are associated with socioeconomic status. the prevalence of oral diseases could be increased in people on hemodialysis, but this has not been formally established. We conducted a systematic prospective survey of oral lesions in hemodialysis and are also evaluating their association with death and adverse vascular outcomes.

**Methods: ORAL-D** is a multinational prospective cohort study. We consecutively enrolled adults receiving hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Europe and South America. A dental surgeon conducted a standardized oral examination. We analyzed prevalence of oral diseases using descriptive statistics and will analyze prospective association with death and adverse vascular outcomes with Cox regression.

**Results:** 4324 (mean age 61 years (SD 16) adults on hemodialysis in the participating clinics received a complete oral examination. Of these, 868 (20%) were edentulous, 996 (30%) had dental erosion. The mean decay/missing/filled teeth (DMFT) score was 22 (9), salivary pH was 7.3 (1.4). Salivary flow rate before dialysis was 0.5 ml/min (0.8), versus 0.4 ml/min (0.8) post dialysis. 1880 (43%) patients reported mouth dryness and 304 (7%) reported mouth pain. Periodontitis was present in 3036 (91%) of 3340 dentate patients. Analysis of association with death and adverse vascular outcomes is in progress.

**Conclusions:** Oral lesions are prevalent in people receiving hemodialysis and may indicate impaired healthcare practices, although further research on the predictors of oral disease in this population is needed.

## SA-PO440

**Oral Hygiene Habits in People on Hemodialysis and Association with Death and Adverse Vascular Outcomes: Oral-D Study**
Giovanni F.M. Strippoli,[1,2,3,4] Suetonia Palmer,[5] Marinella Ruospo,[1,2] Patrizia Natale,[1] Valeria Maria Saglimbene,[1,2] Michela Sciancalepore,[1] Letizia Gargano,[1] Fabio Pellegrini,[2] David W. Johnson,[6] Pauline J. Ford,[6] Jonathan C. Craig,[4] Paul Stroumza,[1] Luc Frantzen,[1] Miguel Rodrigues Leal,[1] Marietta Torok,[1] Anna Bednarek,[1] Jan Dulawa,[1] Eduardo Jorge Celia,[1] Ruben Gelfman,[1] Jorgen B.A. Hegbrant,[1] Charlotta Wollheim,[1] Staffan Schön,[1] Michele De Benedittis,[3] Massimo Petruzzi.[3] *[1]Diaverum; [2]Mario Negri Sud Consortium; [3]Univ of Bari; [4]Univ of Sydney; [5]Univ of Otago; [6]Univ of Queensland.*

**Background:** Oral hygiene habits in people with end end-stage kidney disease on hemodialysis are poorly described. We prospectively surveyed global oral hygiene habits in a large outpatient hemodialysis population, evaluating association with death and adverse vascular outcomes.

**Methods: ORAL-D** is a multinational prospective cohort study of oral diseases in people on hemodialysis. We enrolled adults on hemodialysis in 75 outpatient clinics selected randomly in Europe and South America. We assessed oral hygiene habits using standard self-administered patient questionnaires. We summarized data using descriptive statistics and association with death and adverse vascular outcomes will be analyzed using Cox regression.

**Results:** 4324 hemodialysis patients in the participating clinics from Italy, Hungary, Poland, Argentina, Portugal, France and Spain responded to the questionnaire. Of these, 2237 (52%) did not remember when they had last dental visit or reported they did not have a regular dental practitioner. 1171 (27%) reported their first dental visit at 30 years or older, 494 (11%) never brushed their teeth, 1570 (36%) used mouthwash and only 295 (7%) used dental floss. Only 1339 (31%) spent more than 2 minutes on daily oral hygiene cares. Analysis of association with death and adverse vascular outcomes is in progress.

**Conclusions:** Using validated instruments to evaluate oral hygiene, nearly half of adults on hemodialysis do not regularly visit a dental practitioner and many have poor oral hygiene habits. Additional studies of whether these variables on oral hygiene have any influence on clinical outcomes may be warranted.

## SA-PO441

**Association of Periodontal Disease and All Cause and Cardiovascular Mortality in Hemodialysis Patients: Oral-D Study**

Giovanni F.M. Strippoli,[1,2,3,4] Suetonia Palmer,[5] Marinella Ruospo,[1,2] Valeria Maria Saglimbene,[1,2] Patrizia Natale,[1] Michela Sciancalepore,[1] Letizia Gargano,[1] Fabio Pellegrini,[2] David W. Johnson,[6] Pauline J. Ford,[6] Jonathan C. Craig,[4] Paul Stroumza,[1] Luc Frantzen,[1] Miguel Rodrigues Leal,[1] Marietta Torok,[1] Anna Bednarek,[1] Jan Dulawa,[1] Eduardo Jorge Celia,[1] Ruben Gelfman,[1] Jorgen B.A. Hegbrant,[1] Charlotta Wollheim,[1] Staffan Schön,[1] Michele De Benedittis,[3] Massimo Petruzzi.[3] *[1]Diaverum; [2]Mario Negri Sud Consortium; [3]Univ of Bari; [4]Univ of Sydney; [5]Univ of Otago; [6]Univ of Queensland.*

**Background:** Periodontal disease is associated with increased cardiovascular mortality according to several studies done in the general population. We evaluated the association between periodontital disease and all-cause and cardiovascular mortality in hemodialysis.

**Methods: ORAL-D** is a multional prospective cohort study of consecutive adults receiving hemodialysis in 75 outpatient clinics selected randomly from a dialysis network in Europe and South America. A dental surgeon evaluated presence of periodontitis during a standardized oral examination. We are assessing survival at 12 months using centralized mortality data. We conducted analysis with Cox regression controlling for age, gender, previous cardiovascular event, income status, clinical performance measures, dialysis prescription and performance indicators.

**Results:** 3324 dentate hemodialysis patients in the participating clinics received a complete evaluation for periodontitis and completed follow up. Median follow up was 8.0 (6.5 to 8.7) months. 3036 patients (91%) had periodontitis and 344 (10%) died during follow up. While unadjusted analysis suggested an association between periodontitis and mortality, adjusted analysis had shown no associations with risks of all-cause (HR 1.11 [95% CI 0.68-1.82]) or cardiovascular (HR 2.20 [95% CI 0.94-5.11]) mortality.

**Conclusions:** Contrary to data in the general population, periodontitis at present follow-up of 8 months has uncertain associations with all-cause or cardiovascular mortality in patients on hemodialysis. ORALD will be completed at the end of 1 year follow up by September 2013.

## SA-PO442

**Left Ventricular Mass Is a Powerful Risk Factor for All-Cause and Cardiovascular Death in End Stage Kidney Disease (ESKD) Patients on Dialysis but Does Not Contribute to Prognosis: An Analysis in Two European Cohorts**

Giovanni Tripepi,[1] Bruno Pannier,[2] Francesca Mallamaci,[1] Gerard M. London,[2] Carmine Zoccali.[1] *[1]Nephrology Unit, CNR-IBIM, Reggio Calabria, Italy; [2]Service d'Hémodialyse, Hôpital F.H. Manhès, Fleury-Mérogis, INSERM, Nancy, France.*

**Background:** Left Ventricular Hypertrophy (LVH) is one of the strongest risk factors for death and CV events in end stage kidney (ESKD) patients. Causality apart, the concept that LVH is useful for risk stratification in ESKD has never been formally tested by state-of-art statistical analyses [risk discrimination(ROC curve area, AUC), calibration and re-classification].

**Methods:** We re-analysed the prognostic power of LVMI for all-cause and CV mortality in 2 independent ESKD cohorts in Italy and in France, the Cardiovascular Risk Extended Evaluation cohort (CREED,n=254) and the Hospital Manhes cohort (HM,n=270].

**Results:** In both cohorts, LVMI predicted all-cause [CREED, hazard ratio(HR):1.05;HM,HR:1.03] and CV death [HR:1.06 and 1.05] (P<0.001). In these cohorts, the AUCs of LVMI for all-cause death were 0.71 (CREED) and 0.67 (HM) and those for CV mortality were 0.64 and 0.69, figures lower than those by age alone both for all-cause (CREED:0.81;HM:0.88) and CV death (CREED:0.66;HM:0.78]. All predictive models were calibrated. In the CREED cohort a predictive model including Framingham factors, anti-hypertensive treatment, CV comorbidities, heart rate, Hb and albumin produced an AUC of 0.89 for all-cause and 0.76 for CV death, figures similar to those in the HM cohort (0.92 and 0.87). The incusion of LVMI did not improve the discrimination power of these models (all-cause death, CREED 0.89 vs.0.89; HM:0. 93 vs.0.92; CV mortality, CREED:0.76 vs.0.76; HM:0.88 vs.0.87). In an aggregate analysis of the 2 cohorts (n=524) the net reclassification index (NRI) by LVMI was not significant both for all-cause (NRI:4.5%,P=0.11) and CV death (NRI:3.4%,P=0.33).

**Conclusions:** LVMI is a strong risk factor in ESKD. However, the prognostic power of LVMI is by far lower than that by age alone or combined with standard risk factors. While LVH remains a fundamental treatment target in ESKD, measurement of LVMI solely for prognosis is unwarranted in these patients.

*Funding:* Government Support - Non-U.S.

## SA-PO443

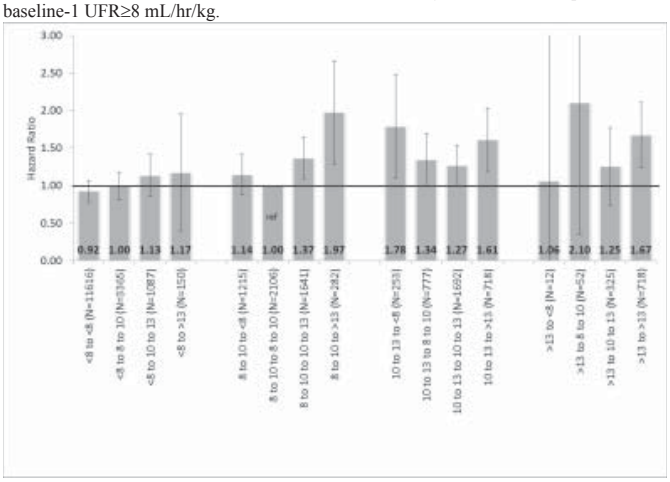
**Longitudinal Change in Ultrafiltration Rate in the First Year and Survival**

Len A. Usvyat,[1] Peter Kotanko,[2] Franklin W. Maddux,[1] Eduardo K. Lacson.[1] *[1]FMCNA, Waltham, MA; [2]RRI, NY, NY.*

**Background:** Higher ultrafiltration rate (UFR) during HD may be associated with poor outcomes. As UFR is measured in mL/hr/kg, interdialytic weight gain, HD time, and body size all contribute towards differences in mortality. We aim to determine association b/n changes of UFR at different levels of baseline UFR and survival.

**Methods:** All incident HD patients in FMCNA who initiated dialysis b/n Jan 1, 2010 and May 1, 2012, survived>365 days, with Kru<0.1 ml/min at the initiation were included. UFR (in mL/hr/kg) was calculated for 1st 6 months of dialysis [baseline-1] and in months 7 to 12 [baseline-2]. Patients were stratified into 4 groups at baseline 1 and 2 by UFR: <8, 8-10, 10-13, and >13 mL/hr/kg. Mortality was tracked until May 1, 2013. The Cox model adjusted for age, gender, DM status, race, ethnicity, and baseline BMI levels were constructed to assess survival after the first year on dialysis in 16 combinations of UFR categories at Baseline 1 and 2.

**Results:** Among 26009 incident patients, 16132 (62%) had stable UFR at both baseline 1 and 2. Mean age was 62.3±14.8; 52% male. The median survival follow-up time was 650 (range 1-851) days. We found significant negative correlation between UFR and BMI in baseline: r=-0.28 (p<0.001). Mortality risk tended to increase not only with an increment of UFR but also with decline of UFR in univariable and adjusted models, for patients with baseline-1 UFR≥8 mL/hr/kg.




**Conclusions:** This analysis demonstrates that patients with both increasing and decreasing UFR have a trend toward poorer survival than stable UFR (particularly >8 mL/hr/kg). Since larger patients have better outcomes, inverse correlation b/n UFR and BMI signals residual confounding. Further analyses are needed to determine contributions of the patients' body size, HD time, interdialytic weight gains, and/or changes in those parameters to the survival differences.

## SA-PO444

**Non-Traditional Blood Pressure Medications (BPM) Instructions for Hemodialysis (HD) Patients**

Sana Waheed,[1] Stephen M. Sozio,[1] Wendy L. St. Peter,[2] Patti Ephraim,[1] Jason Luly,[1] L. Ebony Boulware,[1] Tariq Shafi.[1] *[1]Dept of Medicine, Johns Hopkins Univ, Baltimore, MD; [2]Dept of Pharmacy, Univ of Minnesota, Minneapolis, MN.*

**Background:** Most studies of BPM use in HD patients assume that they take BPM similar to non-HD patients. However, with blood pressure (BP) fluctuations in HD patients, BPM are sometimes held before HD (HOLD) or administered as needed (PRN). Our study describes the frequency of the non-traditional instructions (HOLD and PRN) in HD patients.

**Methods:** We performed a retrospective cohort study of patients starting HD at Dialysis Clinic, Inc. facilities from 2003-2008 who were on a BPM at 6 months. Three independent abstractors reviewed medication instructions from free text comments entered in electronic medical records. We assessed distribution of HOLD and PRN instructions across patient demographics, dialysis related factors and BPM regimens.

**Results:** Of 11,291 incident HD patients who were on a BPM at 6 months, mean age was 62 years; 55% were men and 53% White. At 6 months, there were 30,212 BPM prescriptions of which 1,683 (5.6%) prescriptions had non-traditional instructions [HOLD: 1,191 (3.9%); PRN: 496 (1.6%)]. HOLD orders were commonest with β-blockers (5% of all β-blocker prescriptions) whereas PRN orders were commonest with clonidine (12.7% of all clonidine prescriptions). Patients with HOLD orders were likely to be older, white, had greater comorbidities and lower pre- and post- dialysis systolic BP whereas those with PRN orders where younger, less likely to be white and had higher pre and post dialysis systolic BP and greater BP variability (all p<0.05).

| | Overall (N=11,291) | Hold | | p-value | PRN | | p-value |
|---|---|---|---|---|---|---|---|
| | | No (N=10,317) | Yes (N=974) | | No (N=10,836) | Yes (N=455) | |
| Demographics | | | | | | | |
| Age – Mean (SD) | 61.9 (14.9) | 61.6 (14.9) | 65.8 (14.2) | <0.0001 | 62.2 (14.8) | 55.9 (16.2) | <0.0001 |
| Male – N (%) | 6189 (54.8) | 5645 (54.7) | 544 (55.9) | 0.4957 | 5972 (55.1) | 217 (47.7) | 0.0018 |
| White – N (%) | 6021 (53.3) | 5347 (51.8) | 674 (69.2) | | 5790 (53.4) | 231 (50.8) | 0.0005 |
| Clinical | | | | | | | |
| Diabetes Mellitus– N (%) | 7,324 (64.9) | 6993 (64.8) | 661 (67.9) | 0.0403 | 7,053 (65.1) | 271 (59.6) | 0.0155 |
| Cardiovascular Disease– N (%) | 6,318 (56) | 5,686 (55.1) | 632 (64.9) | <0.0001 | 6,069 (56.0) | 249 (54.7) | 0.5693 |
| Congestive Heart Failure– N (%) | 5,212 (46.2) | 4,676 (45.3) | 536 (55.0) | <0.0001 | 5,023 (46.4) | 189 (41.5) | 0.0436 |
| Comorbidity Index >=10, – N (%) | 1,448 (12.8) | 1,252 (12.1) | 196 (20.1) | | 1,410 (13.0) | 38 (8.4) | 0.0165 |
| BMI – Mean (SD) | 27.8 (7.6) | 27.8 (7) | 27.8 (7) | 0.7768 | 27.8 (7) | 27 (7) | 0.0255 |
| Pre-Dialysis Systolic BP | 150.0 (20.0) | 150.3 (20) | 147.2 (20.5) | <0.0001 | 149.7 (19.9) | 156.9 (21.3) | <0.0001 |
| Post-Dialysis Systolic BP | 141.8 (18.7) | 142 (18.7) | 139.8 (18.7) | 0.0004 | 141.4 (18.5) | 151.4 (20.9) | <0.0001 |
| Intradialytic Systolic BP Change | 13.8 (11.1) | 13.8 (11.1) | 13.5 (11.2) | 0.4392 | 13.8 (11.1) | 13.4 (11) | 0.5188 |
| Systolic BP Variability – Mean (SD) | 0.115 (0.030) | 0.115 (0.03) | 0.113 (0.028) | 0.0369 | 0.114 (0.03) | 0.122 (0.03) | <0.0001 |
| Relative Volume, L – Mean (SD) | 3.3 (1.4) | 3.4 (1.4) | 3.3 (1.2) | 0.0183 | 3.3 (1.3) | 3.4 (1.5) | 0.7079 |
| Laboratory Parameters | | | | | | | |
| Albumin – Mean (SD) | 3.66 (0.43) | 3.7 (0.4) | 3.6 (0.4) | 0.0043 | 3.7 (0.4) | 3.6 (0.5) | 0.1972 |
| Calcium phosphorous – Mean (SD) | 50.09 (15.05) | 50.1 (15.1) | 49.6 (14.4) | 0.3418 | 49.9 (15) | 53.5 (15.3) | <0.0001 |
| Hemoglobin – Mean (SD) | 12 (1.25) | 12 (1.3) | 12 (1.2) | 0.1707 | 12 (1.3) | 11.9 (1.3) | 0.1586 |
| Ferritin – Mean (SD) | 448.81 (335.3) | 450.8 (333.5) | 427.8 (353.5) | 0.0966 | 450.1 (336.6) | 417.4 (301.3) | 0.0269 |

**Conclusions:** Many HD patients have non-traditional BPM prescriptions. While underlying comorbidities may influence these instructions, studies are needed to understand the effect of these prescriptions on patient adherence and outcomes.

*Funding:* NIDDK Support, Other NIH Support - HRQ

## SA-PO445

**Association of Age and Blood Pressure Variability with Long-Term Mortality in Hemodialysis Patients** Ha Yeon Kim, Yong Un Kang, Chang Seong Kim, Joon Seok Choi, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Dept of Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea.*

**Background:** Blood pressure (BP) variability is known as a poor prognostic factor for cardiovascular disease and mortality. This study assessed the prognostic significance of BP variability in association with increasing age in hemodialysis patients.

**Methods:** We retrospectively analyzed 2,174 patients on hemodialysis at chonnam national university hospital between January 2000 and December 2012. BP data were collected three times in each hemodialysis session at start and finish times. Highest and lowest points of BP over the first month of dialysis initiation were collected as well. Intradialytic systolic BP variations over 20 mm Hg and diastolic BP variation over 10 mm Hg were defined as BP variability, respectively. Interdialytic systolic BP variations over 10 mm Hg and diastolic BP variation over 5 mm Hg were defined as BP variability, respectively.

**Results:** In patients aged <55 years, Kaplan-Meier survival curves for 5-year cumulative mortality showed significant higher mortality in patients with higher intradialytic systolic and diastolic BP variability as well as interdialytic systolic and diastolic BP variability (log-rank p=0.006, 0.001, 0.018 and 0.001). Cox proportional analysis revealed that 5-year mortality was associated with intradialytic diastolic BP variability (HR, 2.034 CI, 1.246-3.321). On the other hand, in older patient groups, 5-year mortality has no significant association between intradialytic systolic and diastolic BP variability as well as interdialytic systolic and diastolic BP variability by Cox proportional analysis (p=0.383, 0.828, 0.708 and 0.642 in 55 ≤ age < 75 group, n=1099, p=0.335, 0.246 0.272 and 0.059 in 75 ≤ age group, n=290).

**Conclusions:** For patients aged <55 years, the mortality was significantly higher in patients with BP variability. The association of BP variability with long-term mortality was weaker with increasing age, indicating that the prognostic significance of BP variability in hemodialysis patients is age dependent.

## SA-PO446

**Outcomes of Dialysis Patients after Coronary Revascularization: A Nationwide Cohort Study in Taiwan** Te-Chao Fang,[1,2,3] Chulin Chou,[2] Tsung-cheng Hsieh.[2] *[1]Dept of Internal Medicine, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; [2]Institute of Medical Sciences, Tzu Chi Univ, Hualien, Taiwan; [3]School of Medicine, Tzu Chi Univ, Hualien, Taiwan.*

**Background:** The outcomes of Chinese patients undergoing dialysis after coronary revascularization are unknown. We examined the outcomes of Taiwanese dialysis patients after coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), or coronary stenting.

**Methods:** Using data from the National Health Institutes Research Database, we determined the outcomes of 1,287 dialysis patients who underwent initial coronary revascularization between 1997 and 2008.

**Results:** The 7-year all-cause survival rates were 69% ± 4%, 68% ± 3%, and 57% ± 2% for the CABG, stent, and PTCA patients ($P$ = 0.001), respectively. After demographic and comorbidity adjustment, the hazard ratios (HRs) for all-cause death in the CABG (*versus* PTCA) and stent (*versus* PTCA) patients were 0.695 ($P$ = 0.015) and 0.721 ($P$ = 0.009). Additionally, no significant difference in all-cause death was found between the CABG and stent patients ($P$ = 0.906). Moreover, the ≥65-year-old CABG group patients and the <65-year-old coronary stent group patients showed better survival than the PTCA group patients. Compared with the PTCA and CABG groups, the coronary stent group was significantly associated with a higher risk for a recurrent AMI.Based on age stratification, the ≥65-year-old stent group had a higher risk for a recurrent AMI than PTCA group (HR, 1.486; $P$ = 0.031).

**Conclusions:** Chinese patients undergoing dialysis who underwent CABG or coronary stenting had better survival than those who underwent PTCA. Moreover, being ≥65 years old, CABG has better survival compared with PTCA; being <65 years old, coronary stenting has better survival compared with PTCA.

*Funding:* Private Foundation Support

## SA-PO447

**Hybrid Myocardial Imaging for Risk Stratification prior to Kidney Transplantation: Added Value of Coronary Calcium and Epicardial Adipose Tissue** Antonio Bellasi,[1] Cristina Karohl,[2] Luis D'Marco,[3] Paolo Raggi.[4] *[1]Dept of Nephrology, Ospedale Sant' Anna, Como, Italy; [2]Hospital de Clinicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; [3]Nephrology, Hospital Universitario Ruíz y Páez, Universidad de Oriente, Ciudad Bolívar, Venezuela; [4]Mazankowski Alberta Heart Institute, Univ of Alberta, Edmonton, Canada.*

**Background:** Patient selection and what clinical or imaging markers may help improve risk stratification prior to kidney transplantation remain uncertain. We looked for imaging markers that help predict an abnormal result in screening nuclear stress testing prior to surgery.

**Methods:** Cross-sectional study of 411 consecutive chronic kidney disease (CKD) stage 4-5D patients awaiting kidney transplantation referred for risk stratification. PET-computed tomography (PET-CT) or SPECT-CT were used to assess myocardial perfusion and quantify coronary artery calcium (CAC) and epicardial adipose tissue (EAT). Abnormal myocardial perfusion was defined as a perfusion defect involving >5% of the left ventricular myocardium.

**Results:** Fixed (necrotic myocardium) or reversible myocardial perfusion defects (ischemia) were present in 41 patients (10%). Male sex, smoking, and history of cardiovascular disease were more prevalent and age, CAC and EAT were greater in patients with myocardial perfusion defects than in those with normal myocardial perfusion (all p-values<0.05**).** On multivariate logistic regression, EAT and CAC were independent predictors abnormal myocardial perfusion while diabetes mellitus was of borderline significance ($P$=0.08). EAT added incremental diagnostic value to a model including age, CAC and diabetes mellitus [AUC 0.73 (95% CI: 0.64-0.81) to 0.76 (95%CI: 0.68 – 0.84); P=0.02] for the prediction of abnormal perfusion. Furthermore, the model containing EAT showed improved diagnostic discrimination (integrated discrimination improvement; IDI).

**Conclusions:** Abnormal myocardial perfusion on screening stress testing appears to be rare in patients awaiting kidney transplantation suggesting an excess of testing. EAT and CAC may help predict what patients are at higher risk of having an abnormal myocardial perfusion.

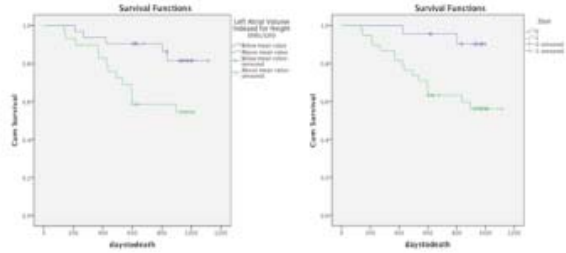
## SA-PO448

**Left Atrial Volume and Dialysis Induced Cardiac Injury** Lisa E. Crowley,[1] Adam Kirk,[1] Richard J. Fluck,[1] Maarten W. Taal,[1] Chris W. McIntyre.[2] *[1]Dept of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]Div of Medical Sciences and Graduate Entry Medicine, School of Medicine, Univ of Nottingham, Nottingham.*

**Background:** Left atrial volume (LAV) has been demonstrated to be a powerful determinant of cardiovascular mortality in HD patients. Haemodialysis (HD)-induced myocardial stunning driven by ischaemia is a recognised complication of HD and a pivotal part of the pathophysiology of the observed excess in CV mortality. We aimed to assess the hierarchy of importance of ventricular hypertrophy, increased LAV and presence/severity of dialysis induced cardiac injury.

**Methods:** 60 established HD patients underwent echocardiography at rest assessing cardiac structure and function. Dialysis induced cardiac stunning was detected and quantified from the measurement of left ventricular regional wall motion abnormalities (RWMA) under dialytic stress.

**Results:** 38/60 patients experienced dialysis induced cardiac stunning. Higher LAVI was significantly correlated with severity of stunning (as measured by number of RWMAs (r=0.37, p=0.004) and humoral evidence of volume overload (NT-proBNP r=0.56, p<0.001). Both increased LAVI and dialysis induced stunning were associated with reduced survival. There was a clear hierarchy of effect demonstrated in multi-variable Cox Proportional Hazard modelling. Stunning was most strongly associated with mortality (HR 4.92 [1.0-23.1] p=0.04), followed by LAVI (HR 1.065 [1.001-1.1000] p= 0.038) and LVMI (HR 0.99 [0.97-1.00] p=0.36).




**Conclusions:** This study demonstrates the hierarchical relationship (stunning>LAVI>LVMI) between the principal recognised morphological and functional echocardiographic determinants of increased CV mortality in HD patients and suggests that dynamic echocardiographic parameters may be more predictive of outcome than structural measures.

**SA-PO449**

**Association between Cardiac Valvular Calcification and Myocardial Ischemia in Asymptomatic High Risk Patients with End Stage Renal Disease**
Soo Jin Kim, Young Rim Song, Jwa-kyung Kim, Sung Gyun Kim, Jieun Oh, Jong-woo Yoon, Jung-woo Noh, Ja-Ryong Koo. *Internal Medicine, Hallym Univ, Hallym Kidney Research Institute, Seoul, Republic of Korea.*

**Background:** Valvular calcification is associated with significant morbidity and mortality in patients with end stage renal disease (ESRD). This study examined the hypothesis that valvular calcification is a marker of myocardial ischemia in asymptomatic high-risk patients with ESRD.

**Methods:** Echocardiography and myocardial perfusion single-photon emission computed tomography were performed in 285 asymptomatic high-risk patients with ESRD at initiation of dialysis. We evaluated the extent and severity of myocardial ischemia by the summed difference score (SDS) and defined the presence of myocardial ischemia as SDS ≥ 3 and moderate to severe ischemia as SDS ≥ 8. The presence of cardiac valvular calcification was assessed by echocardiography and defined as aortic valve calcification or mitral valve calcification.

**Results:** Eighty-five (29.9%) patients had echocardiographic evidence of cardiac valvular calcification. The presence of myocardial ischemia was significantly associated with aortic valve calcification (odds ratio [OR] = 3.19; 95% confidence interval [CI] = 1.76–5.78; p < 0.001), mitral valve calcification (OR = 3.31; 95% CI = 1.74–6.28; p < 0.001), and cardiac valvular calcification (OR = 3.18; 95% CI = 1.79–5.65; p < 0.001). The presence of moderate to severe myocardial ischemia (SDS ≥ 8) was independently associated with cardiac valvular calcification (OR = 2.86; 95% CI = 1.12–7.27; p = 0.028).

**Conclusions:** Valvular calcification was significantly associated with the presence of inducible myocardial ischemia in asymptomatic patients with ESRD, and may be a potential marker of patients at high-risk for the presence of silent myocardial ischemia.

**SA-PO450**

**Experience of Antegrade Trasseptal Percutaneous Transcatheter Aortic Valvuloplasty in Hemodialysis Patients**
Manabu Asano,[1] Kenichi Oguchi,[1] Masahiro Shimoyama,[1] Tokuya Nakahara,[1] Machiko Okamoto,[1] Hitoshi Iwabuchi,[1] Yoshihito Sakata.[2] *[1]Renal Unit, Ikegami Genaral Hospital, Tokyo, Japan; [2]Heart Center, Ikegami General Hospital, Tokyo, Japan.*

**Background:** Aortic stenosis (AS) due to accelerated sclerotic-calcific degeneration of valve is one of prevalent cardiovascular comorbidities in chronic dialysis patients. Percutaneous trans-catheter valvuloplasty (PTAV) is expected to function as an alternative AS treatment, providing immediate hemodynamic improvement with minimal invasion. The modification of procedure by a combination of trans-venous antegrade approach and utilization of Inoue balloon (Ante-PTAV) has been reappraised in terms of its improved therapeutic effect, procedural safety and possibly long-term symptomatic palliation. In this study, we evaluated the clinical efficacy of Ante-PTAV particularly on hemodialysis (HD) patients in order to define its therapeutic role.

**Methods:** Total 16 HD patients with severe AS (age 76.3±8.8 years old; 56.3 % males and 12.5 % diabetics) underwent Ante-PTAV with Inoue balloon (mean balloon size 20.5mm).Trans-Aortic valve gradients (ΔP) and valve area (AVA) were measured both by catheterization and echocardiography. Hemodynamic data were obtained in all treated patients, and symptomatic improvement over Ante-PTAV was evaluated and early clinical outcomes were followed up.

**Results:** From 1/2009 to 12/2012, 16 HD patients successfully underwent Ante-PTAV in our unit. The mean dialysis vintage of those patients was 119.9±110.4 months (range 11- 390 months; median 90 months). Critical aortic valve calcification was present in 94% of patients. The mean AVA increased from 0.65±0.17 $cm^2$ to 1.28±0.39 $cm^2$ ($p<0.05$) and mean ΔP decreased from 57.9±16.5 mmHg to 19.7±10.4 mmHg ($p<0.05$) over Ante-PTAV. In the short-term follow-up (within 3 month after Ante-PTAV), hemodynamic stability and tolerance during HD was remarkably increased in over 80% of treated patients. The median hospital stay was 4 days. No significant complications were noted.

**Conclusions:** Ante-PTAV is a useful and safe procedure to treat symptomatic critical AS in HD patients who are high risk for surgical valve replacement or ineligible for trans-catheter valve implantation.

**SA-PO451**

**The Impact of Non-High-Density Lipoprotein Cholesterol Levels on the Clinical Outcome in Incident Hemodialysis Patients**
Chan Ho Kim,[1] Mi Jung Lee,[1] Hye-Young Kang,[2] Hyung Jung Oh,[1] Tae-Hyun Yoo,[1,2] Shin-Wook Kang.[1,2,3] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul, Korea; [3]On Behalf of the Clinical Research Center for End-Stage Renal Disease Investigators.*

**Background:** Even though hyperlipidemia is a well-established risk factor for cardiovascular(CV) disease in the general population, the association between abnormal lipid levels and CV disease in ESRD patients remains unclear. Recently, non-high-density lipoprotein cholesterol(NHDL-C) has been demonstrated as a risk factor for CV events in patients with combined dyslipidemia. In this study, we investigated the impact of NHDL-C on the clinical outcome in incident hemodialysis(HD) patients.

**Methods:** A prospective cohort of 867 incident HD patients from 36 centers of the CRC for ESRD in Korea was selected for this study. The subjects were divided into 'high' and 'low' groups based on the baseline NHDL-C concentrations(≥ and <100mg/dL). Primary outcome was defined as a composite of all-cause mortality or CV events. Cox proportional hazard analysis was performed to determine the independent prognostic value of NHDL-C for the clinical outcome.

**Results:** The mean age was 57.6 years and 59.5% were male. During a median follow-up duration of 23 months, 53 patients(6.1%) died and CV events occurred in 78 patients(9.0%). The composite outcome was observed in 81 patients(15.1%) in the 'high' NHDL-C group compared to 35(10.6%) in the 'low' NHDL-C group, but this difference did not reach statistical significance(P=0.06). However, after adjustment for demographic and clinical characteristics, and parameters related to inflammation and malnutrition, the risk for reaching the composite outcome was significantly higher in the 'high' NHDL-C group compared to the 'low' NHDL-C group(HR,1.58;95%CI,1.03-2.44;P=0.038). In subgroup analyses, a significant association of NHDL-C with adverse clinical outcome was observed in patients with diabetes, patients without CV disease, and patients with increased high sensitivity C-reactive protein levels.

**Conclusions:** Serum NHDL-C at the time of HD commencement was a significant independent risk factor for adverse clinical outcomes in incident HD patients.

*Funding:* Government Support - Non-U.S.

**SA-PO452**

**Relationship between Pre HD Serum Sodium Concentration and Blood Pressure: Results from a Global Hemodialysis Cohort**
Jochen G. Raimann,[1] Michael Etter,[2] Jeroen Kooman,[3] Nathan W. Levin,[1] Daniele Marcelli,[4] Cristina Marelli,[5] Frank van der Sande,[3] Stephan Thijssen,[1] Len A. Usvyat,[1,6] Peter Kotanko,[1] Mondo Consortium.[7] *[1]Renal Research Institute; [2]FMC Asia/Pacific; [3]Maastricht Univ Medical Centre; [4]Fresenius Medical Care; [5]Fresenius Medical Care Argentina; [6]Fresenius Medical Care North America; [7]MONDO.*

**Background:** Recent evidence from a small study from the UKindicates a relationship between pre-hemodialysis (HD) serum sodium (SNa) and systolic (SBP) and diastolic (DBP) blood pressure (He 2013) in chronic HD patients. Here we extend this analysis to a global cohort of incident HD patients.

**Methods:** The Monitoring Dialysis Outcomes (MONDO) initiative encompasses HD patients from 26 countries who started HD between 2000 and 2010 (Usvyat 2013). Monthly pre-HD SNa levels were used as predictors of pre-HD SBP in a linear mixed model (LMM). LMMs were adjusted for age, gender, race (reference: white race), interdialytic weight gain (IDWG; in % of post-HD body weight), diabetes and serum albumin and calcium. A similar model was constructed with DBP as outcome. Analyses were carried out in the whole data set and after stratification by continent (North America; Latin America; Asia). LMMs were also constructed for the entire observation period, the first year, and the second year.

**Results:** We included 7675 incident patients. Including all patients, SNa was a significant predictor of pre-HD SBP and DBP (**Table 1**). The results were materially identical by by continent Separate analysis for first and second year gave comparable results. **Table 1:** Estimate of of Sna as a fixed effect in a Linear Mixed Model.

| Dependent Variable | Estimates SNa (per 1 mEq/L] (95% CI) | | | |
|---|---|---|---|---|
| | All patients | North America | South America | Asia |
| a) SBP | 0.23 (0.19 to 0.27) | 0.21 (0.16 to 0.26) | 0.24 (0.01 to 0.48) | 0.20 (0.12 to 0.28) |
| b) DBP | 0.12 (0.09 to 0.14) | 0.10 (0.07 to 0.12) | 0.18 (0.05 to 0.32) | 0.14 (0.10 to 0.18) |

**Conclusions:** Our analysis corroborates the findings of He et al. on a global level, indicating that the positive relationship between SNa and BP is a profound biological phenomenon in chronic HD patients.

**SA-PO453**

**Evaluating Time in Therapeutic Range for Hemodialysis Patients on Warfarin**
Laura Quinn,[1,2] Marisa Battistella,[1,2] Karen Cameron.[1,2] *[1]Pharmacy, Univ Health Network, Toronto, Canada; [2]Pharmacy, Univ of Toronto, Toronto, Canada.*

**Background:** Warfarin is frequently used in the hemodialysis (HD) population for patients with atrial fibrillation (AF) and venous thromboembolism (VTE); however, there is a lack of literature to support this practice. Given that HD patients have a 3-10 times higher risk for both stroke and bleeding than the general population, anticoagulation in these patients is controversial. Time in therapeutic range (TTR) has been accepted as a surrogate outcome of clinical effectiveness and safety of warfarin and is used extensively as an endpoint in clinical trials with a benchmark goal of 66%.

**Methods:** In order to evaluate TTR in an HD population, a six-year retrospective chart review was performed in 46 HD patients with AF or VTE on warfarin. One year of individual patient data was collected, which included weekly INR values, demographics, medication histories and clinical outcomes. TTR was calculated using the Rosendaal, *fraction of INRs in range* (FIR) and *cross-section-of-the-files* methods.

**Results:** The mean TTR using Rosendaal and FIR was, respectively, 49.2%±14.6 and 44.2%±13.5. The *cross-section-of-the-files* method captured INR data for 35 patients and TTR was 33.3%, 36.4%, 31.4% and 43.3% at four points during 2011. The percentage of time spent below and above target INR was 39.3%±16.2 and 10.0% (6-15.5) respectively for the Rosendaal method, suggesting that more often patients were at risk of inadequate efficacy rather than toxicity. There were 9 serious bleeding and 9 thrombotic events; these all occurred in patients with a TTR <60%. A survey of nephrologists in the unit revealed that they often target an INR range lower than 2-3 due to insufficient evidence and higher bleeding risk.

**Conclusions:** In conclusion, our data suggests this HD unit is not meeting the TTR goal previously established and that clinicians are conservative with INR management. Further studies to investigate ways to improve TTR are warranted. Ultimately, a prospective study evaluating the safety and efficacy of warfarin in HD patients is needed.

## SA-PO454

**Association of Sleep Apnea and Sleep Quality with Left Ventricular Mass in Hypertensive Hemodialysis Patients in Blood Pressure in Dialysis (BID) – SLEEP Study** Manisha Jhamb,[1] D. Miskulin,[2] Jennifer J. Gassman,[3] David W. Ploth,[4] Brigitte Schiller,[5] Raymond Y. Kwong,[9] Cynthia A. Kendrick,[3] Jose L. Vega,[6] John W. Kusek,[7] P. Zager,[6,8] Mark L. Unruh.[8] *[1]Pitt; [2]Tufts; [3]Cleveland Clin; [4]MUSC; [5]Satellite Hlth; [6]DCI; [7]NIH; [8]UNM; [9]Brigham Womens.*

**Background:** Sleep apnea (SA) and short and fragmented sleep may contribute to cardiovascular morbidity and mortality in hemodialysis patients. The association between SA severity and LV mass in these patients is not well described.

**Methods:** Maintenance HD patients joined this ancillary SLEEP study after enrolling in the Blood Pressure in Dialysis (BID) Pilot, a randomized trial of two levels of BP control. SA was monitored for 1 night with a portable home monitor (ApneaLink with oximetry). Sleep-wake behavior was assessed by actigraphy over a 5-day period. Subjective sleep quality was assessed with Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale. LV mass was measured centrally by MRI.

**Results:** Currently 27 patients (52.6±13.3 yrs, 63% male, 41% AA) have completed baseline sleep study. 23 had SA (85%) and of those 9 had mod-severe SA (AHI >15). Actigraphy showed 13 (57%) of patients slept <6 hrs/night. Most had poor sleep efficiency (87%) and poor sleep quality (86% with PSQI score>5). Body mass index, systolic BP, diastolic BP, heart rate, number of antihypertensive medications, inter-dialytic weight gain, hemoglobin level, history of myocardial ischemia, and MRI characteristics did not differ between those with no-mild SA (AHI≤15) as compared to those with mod-severe SA (AHI>15).

| | No-mild SA (n=18) | Mod-Severe SA (n=9) |
|---|---|---|
| | Mean±SD | Mean±SD |
| LV Mass(g) | 141 ± 38.4 | 140 ± 41.3 |
| LV Mass Index(g/m$^2$) | 74.3 ± 20.9 | 72.9 ± 21.7 |
| LV End Diastolic Volume(ml) | 189 ± 56.5 | 176 ± 78.5 |
| LV End Systolic Volume(ml) | 87.8 ± 34.7 | 85.3 ± 56.9 |
| RV End Diastolic Volume(ml) | 143 ± 41.9 | 159 ± 68.1 |
| RV End Systolic Volume(ml) | 69.7 ± 20.2 | 82.1 ± 47.3 |
| LV EF(%) | 54.1 ± 7.84 | 54.1 ± 10.0 |

**Conclusions:** Sleep apnea and poor sleep quality were common among hypertensive HD patients we studied. Based on preliminary data we did not observe a relationship between severity of sleep apnea and LV mass.

*Funding:* NIDDK Support, Pharmaceutical Company Support - Dialysis Clinic, Inc., Private Foundation Support

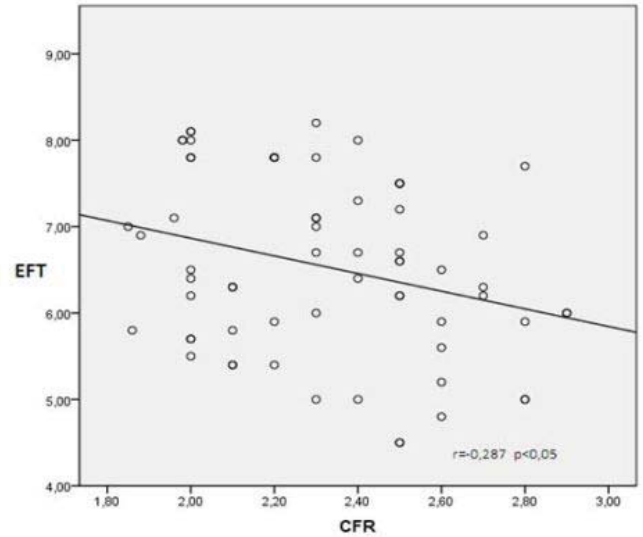
## SA-PO455

**Accelerated Atherosclerosis in Hemodialysis Patients; Correlation of Epicardial Fat Tissue Thickness with Coronary Artery Flow Reserve** Elif Ari Bakir,[1] Beyza Macunluoglu.[2] *[1]Nephrology, Kartal Research and Training Hospital, Istanbul, Turkey; [2]Nephrology, Uskudar State Hospital, Istanbul, Turkey.*

**Background:** The aim of this study was to evaluate the relation between epicardial fat tissue (EFT) thickness and coronary artery flow reserve (CFR) in HD patients as an indicator of accelerated atherosclerosis.

**Methods:** Seventy-one chronic HD patients and 65 age and sex-matched healthy controls were included in the study. EFT thickness and CFR were assessed by transthoracic Doppler echocardiography.

**Results:** EFT thickness was higher (6,53±1,01 mm vs 5,79 ±1,06 mm, p<0.001); CFR was lower (1,73[lrm]±0,11 vs 2,32[lrm]±0,28, p<0.001) in HD patients compared to controls. There was a significant negative correlation between EFT thickness and CFR (r = -0.287, p<0.05).




Multiple linear regression analysis showed that CFR, age, body mass index and total cholesterol levels were independently correlated with EFT thickness.

**Conclusions:** Our data have demonstrated that HD patients exhibit increased EFT thickness and decreased CFR. To our knowledge this is the first study to demonstrate the inverse relationship between EFT thickness and CFR in HD patients**.**

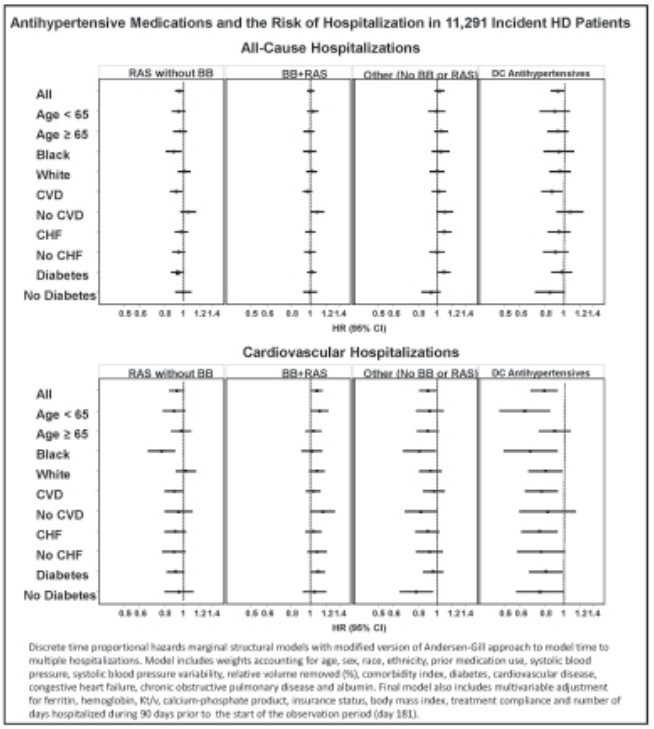
## SA-PO456

**Antihypertensive Medication Regimens and Hospitalization Risk in Incident Hemodialysis (HD) Patients** Tariq Shafi,[1] Stephen M. Sozio,[1] Wendy L. St. Peter,[2] Karen J. Bandeen-roche,[1] Patti Ephraim,[1] Jason Luly,[1] L. Ebony Boulware.[1] *[1]Johns Hopkins Univ; [2]Univ of Minnesota.*

**Background:** Optimal strategies to reduce hospitalizations, a frequent occurrence in HD patients, are not known. We compared the effectiveness of different blood pressure medication (BPM) regimens on reducing hospitalizations in HD patients.

**Methods:** We performed a retrospective cohort study of all adult patients initiating HD at Dialysis Clinic, Inc. facilities from 2003-2008 and prescribed a BPM at 6 months. We linked their dialysis electronic medical records to USRDS data, and the National Death Index. We categorized BPM use as 4 mutually exclusive regimens: β-blockers (BB), renin-angiotensin system agents (RAS), BB+RAS or OTHER regimens not including BB or RAS. Patients with BPM discontinued during follow-up constituted the DC group. We used discrete time proportional hazards marginal structural models accounting for repeated events, confounding and mediation by numerous clincial and demographic factors including blood pressure and HD session volume removal to quantify the association between BPM regimen and hospitalizations [all cause and cardiovascular (CV)] censoring for death.

**Results:** Of the 11,291 patients, mean age was 62 years, 55% were male and 53% were White. 22,384 hospitalizations (7,070 from CV causes) occurred during follow-up (median, 22 months). Compared to BB, patients treated with a RAS had lower risk of hospitalizations, particularly among those with CV disease at baseline [HR (95%CI) for all-cause: 0.92 (0.85-0.99); CV: 0.90 (0.80-1.01)]. DC group had lower risk of hospitalization but higher risk of death [1.5 (1.1-2.06)] compared with BB group.




**Conclusions:** HD patients treated with a RAS regimen (without BB) had a lower risk of hospitalization compared to BB containing regimens. RAS agents may be preferred antihypertensive medications for patients starting HD.

*Funding:* NIDDK Support, Other U.S. Government Support

## SA-PO457

**Prognosis of Dialysis Patients with Normal Stress Myocardial Perfusion Imaging at the Initiation of Dialysis: Long-Term Outcome and Predictors** Jwa-kyung Kim, Soo Jin Kim, Sun Ryoung Choi, Jong-woo Yoon, Jung-woo Noh, Sung Gyun Kim. *Dept of Internal Medicine, Hallym Univ Sacred Heart Hospital, Kidney Research Institute, Gyunggi-do, Korea.*

**Background:** Normal myocardial perfusion is closely associated with very low rates of cardiac events and better long-term outcomes in general population. However, little is known about its prognostic value in patients with end-stage renal disease (ESRD).

**Methods:** A total of asymptomatic 286 incident patients underwent baseline cardiac evaluation with echocardiography and stress/rest single-photon emission computed tomography. One hundred seventy seven (61.9%) patients displayed "normal" perfusion scan with a summed stress score (SSS) < 4.

**Results:** During the 4-year follow-up period, 79 cardiac events occurred. Patients with SSS<4 had significantly lower annual rates of cardiac events than those with SSS ≥ 4 (6.4% vs. 13.2%, hazard ratio, 0.54, 95% confidence interval, 0.31–0.94). Among 177 patients with SSS < 4, there were 40 cardiac events over 3.0 ± 1.9 years of follow up. In multivariable analysis, age, diabetes, a previous CAD history and C-reactive protein levels were significant clinical predictors of cardiac events in patients with normal myocardial perfusion imaging. In addition, baseline echocardiographic characteristics such as the presence of left ventricular (LV) systolic dysfunction, LV hypertrophy, and increased LV filling pressure strongly influenced to the occurrence of cardiac events.

**Conclusions:** Normal myocardial perfusion imaging does not always indicate a good clinical outcome in patients with ESRD. Echocardiographic parameters, diabetes, C-reactive protein level and a previous CAD history may improve the predictive value of normal myocardial perfusion imaging in patients with ESRD.

## SA-PO458

### National Study of Acute Stroke in Patients on Renal Replacement Therapies

Albert J. Power,[1] Retha D. Steenkamp,[2] James Fotheringham,[3] Damian G. Fogarty.[2] *[1]Imperial College London, United Kingdom; [2]UK Renal Registry, Bristol, United Kingdom; [3]Sheffield Kidney Institute, United Kingdom.*

**Background:** Stroke in patients on renal replacement therapy [RRT] remains poorly studied especially in peritoneal dialysis [PD] & transplant cohorts and particularly in Europe. We therefore conducted a national study to characterize the distribution and impact of stroke in all RRT patients.

**Methods:** We studied all patients incident to RRT in England [Jan 2002–Dec 2006] with follow up to Dec 2009 [n=21175, 62% male, 47% diabetic, 74% hemodialysis, 23% PD]. Demography & comorbidity at the start of RRT and hospitalization for first acute stroke were derived from ICD-10 codes in national Hospital Episode Statistic [HES] datasets linked to UK Renal Registry data.

**Results:** Overall 12% of the study cohort [n=2484] experienced first acute stroke [40% events prior to RRT start] compared to 2.3% in the general population. During RRT, 77% strokes occurred in hemodialysis [HD], 16% on PD & 4% in renal transplant patients.

Acute stroke patients were older [mean 65.9 vs 60.3yrs, p<0.001] with 62% strokes clustering in those ≥65yrs old. Stroke associated with cardiac & peripheral arterial disease [p<0.001], diabetes [p<0.001], and especially prior cerebrovascular disease [51% vs 7%, p<0.001]. There was no association between stroke and ethnicity [p=0.5], health network area [p=0.6] or social deprivation [Townsend index, p=0.1] with a trend to more events in females [p=0.08] in keeping with UK population data.

**Conclusions:** Stroke affects a significant proportion of patients on all RRT modalities and remains over-represented in dialysis cohorts particularly HD. Current ongoing analyses of national patient- & treatment-level stroke risk factors will allow for interventions to reduce stroke burden in all RRT populations.

## SA-PO459

### Ventricular Diastolic Function before, during and after Hemodiafiltration

Israel Campos González, Sujail Estrada, Patricia Zarate Rojas, Magdalena Madero, Armando Vazquez-Rangel, Hector Perez Grovas. *Div of Nephrology/ Echocardiography, National Heart Institute, Mexico City, Mexico.*

**Background:** Kidney disease is associated with cardiovascular(CV) mortality. There is limited data regarding the effect of hemodiafiltration(HDF) on cardiac function. The objective of the study was to evaluate the diastolic function(DF) at different HDF times.

**Methods:** Prospective study conducted between May-August 2011 in HDF patients. Tissue Doppler & 3D Echocardiography were captured pre, during & post-HDF. Dyssynchrony index and DF parameters were measured and the change in measurements was evaluated at different time points.

**Results:** 11 patients were included, the median age was 34±12y and 72% were female. The mean HDF duration was 232±18min. Blood flow(QB) was 442±37 mL/min, substitution volume was 17.4±1.2 L, weight gain was 2.63±0.58 Kg. Left ventricular ejection fraction was 52±0.3%, 51±0.2%, 51±0.5% for pre, during and post HDF(p=0.45). There were significant changes in diastolic function parameters at different time points.

| DIASTOLIC FUNCTION PARAMETERS | PRE-HDF | DURING-HDF | POST-HDF | p Value |
|---|---|---|---|---|
| E/A rate | 1.4±0.4 | 1.19±0.38 | 1.09±0.29 | 0.02 |
| Deceleration time of early mitral flow (DAT)/ms | 206±35 | 244±74 | 224±49 | 0.12 |
| Isovolumetric relaxation time in (IVRT)/ ms | 93±14 | 97±13 | 95.6±12 | 0.50 |
| Lateral mitral annular velocity in cm/s (Lateral E´) | 11.9±2.3 | 10.3±2.4 | 10.3±0.35 | 0.01 |
| E/E´rate | 8.3±2 | 6.6±2.1 | 6.7±2.1 | 0.01 |

Diastolic dysfunction occurred in 44% of the patients in the PreHDF phase and increased to 82% during and post HDF(p<0.05). Diastolic & systolic diameter, telesystolic, telediastolic and stroke volume(SV) had significant changes(p<0.001).There was significant correlation between SV & QB (r=0.63, p=0.03) and SV & transmembrane pressure (r=0.84, p=0.001).

**Conclusions:** Diastolic dysfunction is prevalent in HDF patients but increases dramatically during & after HDF. In contrast, systolic function remains intact throughout. These findings may contribute to understand CV disease in HDF. Larger studies are needed to confirm our findings.

## SA-PO460

### Mean Platelet Volume as a Predictor for Coronary Artery Disease in Chronic Hemodialysis Patients

Jong-woo Yoon, Myung Jin Choi, Youngki Lee, Won Jae Shin, Soo Jin Kim, Ja-Ryong Koo, Jung-woo Noh. *Hallym Univ, Kidney Research Institute, Chuncheon, Republic of Korea.*

**Background:** Mean platelet volume (MPV), as a marker of platelet activation, has been shown to be related to coronary artery disease and mortality after myocardial infarction. However, the effects of MPV on cardiovascular complication and vascular access failure in hemodialysis (HD) patients is not known.

**Methods:** 180 chronic HD patients (age 59.1 ± 12.1 years, male 47.8 %, diabetes 53.9 %, mean dialysis duration 48.3 ± 51.0 months) were enrolled. After baseline evaluation, all patients were monitored continuously for the development of coronary artery disease, cerebrovascular disease, vascular access failure and death.

**Results:** MPV was 9.14 ± 0.78 fl (range 7.7 to 12.0). Patients were grouped according to half-tile values of MPV (>9.0 fl, group 1 (n=98); ≤ 9.0 fl, group 2 (n=89)). Patients with higher MPV had a higher level of platelet count (p=<0.001) and a lower level of triglyceride (p=0.006), 25(OH) Vitamin D total (p=0.013) and parathyroid hormone (p=0.03). During a follow-up period of median 127 weeks, 133 composite events (31 deaths, 33 coronary artery disease, 9 cerebral artery disease, and 60 vascular access thrombosis or stenosis) occurred. The Kaplan-Meier curve showed significant difference in the cumulative events of coronary artery disease between two groups (23.5 % vs 12.3 %; log-rank test, p <0.05). In multivariate Cox analysis, MPV was an independent risk factor for coronary artert disease (hazard ratio, 0.459; 95% confidence interval, 0.211 to 0.998; p.049 CI 1.21-2.30 for 10mmHg increase; p<0.002).There were no significant differences between the two groups in death (14.3 % vs 20.7 %; log-rank test, p=0.301), cerebral artery disease (CVA) (6.1 % vs 6.7 %; log-rank test, p=0.452) and vascular access failure (33.7 % vs 32.9 %; log-rank test, p=0.932).

**Conclusions:** High MPV was significantly associated with increased risk of coronary artery disease in chronic HD patients, but not with CVA, access failure and death. Considering critical role of platelet in atherosclerosis, MPV may be a potential marker for prediction of coronary artery disease in HD patients.

## SA-PO461

### Prevalence of Abnormal Hemoglobin A1c in Non-Diabetic (NDM) Dialysis Patients

Neal Mittman,[1] Lin Ma,[2] Mark E. Williams,[3] Julia I. Brennan,[4] Chinu M. Jani,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[2] Eduardo K. Lacson.[2] *[1]Long Island College Hospital, Brooklyn, NY; [2]Fresenius Medical Care, North America, Waltham, MA; [3]Joslin Diabetes Center, Boston, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

**Background:** While a significant minority of ESRD pts begin dialysis without prior nephrologic care and CKD is known to be associated with insulin resistance, A1c measurement is not routinely performed in ESRD pts without a history of DM. In a single center experience, we (Desiraju et al, ASN 2005) identified NDM ESRD pts maintained on HD with abnormal A1c >5.6%.

**Methods:** We explored the distribution of A1c in a national sample of 1454 NDM pts from 26 FMCNA facilities distributed across the US. Residual blood from routine monthly draws was analyzed. We report on mean casual blood glucose and A1c in these pts for the period January-March 2013. Pts were classified as undiagnosed (UndxDM) if A1c≥6.5% and pre-diabetic (PreDM) if A1c was >5.6 but <6.5%.

**Results:** The A1c values were consistent with non-DM diagnosis in >98% of pts. However, ~16% met pre-diabetic A1c range and are potentially at risk for increased future morbidity (Table). Pre-diabetic status was associated with age, male gender, shorter dialysis vintage, and peritoneal dialysis (PD) modality (all p<0.05 vs. A1c≤5.6%), while race and ethnicity were similar. Undiagnosed DM was similarly associated with even shorter dialysis vintage and with use of PD.

| Table | N (%) | Mean A1c% (range) | Median | Mean glucose mg% (range) | Median |
|---|---|---|---|---|---|
| UndxDM | 25 (1.7) | 7.7% (6.5-11.5) | 7.4% | 187 (88-383) | 162 |
| PreDM | 230 (15.8) | 5.8% (5.6-6.5) | 5.8% | 116 (69-250) | 109 |
| NDM | 1199 (82.5) | 5.2% (3.8-5.6) | 5.2% | 102 (50-221) | 100 |

**Conclusions:** While the absence of a diagnosis of DM was consistent with A1c values for most patients, physicians should be vigilant in detecting rare cases of undiagnosed DM. One in every 6-7 dialysis patients may be pre-diabetic and it is not clear if this finding is associated with worse outcomes. We will prospectively follow these patients to determine the prognostic significance of the prediabetic range of A1c.

## SA-PO462

### Impact of Nephrological Care on Dialysis Initiation and Survival

Laura Sola,[1] Maria Carlota Gonzalez-Bedat,[2] Alejandro Ferreiro.[2] *[1]Renal Healthcare Program, Montevideo, Uruguay; [2]Uruguayan Registry of Dialysis, Montevideo, Uruguay.*

**Background:** Despite significant improvement in dialysis technology, mortality rate (MR) in chronic dialysis (CD) patients (Pts) still remains extremely high. Late referral to nephrologist has been linked to higher morbidity and short term mortality. The objective of the study is to analyze the impact of predialysis nephrological care (PNC) on selected clinical indicators and first year survival on CD.

**Methods:** Data from the Uruguayan Registry of Dialysis of Pts beginning CD between 11/1/2008-12/31/2011 were analyzed. PNC was assessed by stage 4 Pts included in the CKD Registry with follow-up ≥ 3 months. Quality of care indicators at CD initiation

included: hemoglobin (Hb) ≥10 g/dL, fistula ≥ 60 days (FAV60) and hepatitis B (HB) immunization. Initial CD modality was assessed. Hospitalization time at CD initiation and first year survival were compared regarding PNC, including PNC time and number of visits. Logistic regression analysis age, gender and diabetes adjusted was performed for hospitalization <5 days and Cox regression analysis for MR risk factors. Significant differences were considered as $p<0.05$.

**Results:** Of 1666 Pts that began CD, 194(11.6%) had PNC. Pts with PNC had older age (mean 67.1 vs 60.5 years) and were more commonly woman (49.5% vs 40.12%) than those without PNC. PNC was significantly associated to higher Hb (61.0 vs 41.5%), HB immunization (54.6 vs 23.1%), and FAV60 (34.6 vs 17.3 %), but not to PD choice (14.9 vs 9.8%).Hospitalization time was significantly lower in PNC Pts (8.5 ± 14.5 vs 15.6 ± 20.2 days) and didn't differ with PNC duration or number of visits. Less hospitalization was associated to PNC and Hb ≥ 10g/dL. First year survival was higher in Pts with PNC (Kaplan Meier). Adjusted to age, gender and diabetes, PNC reduced 34.8% mortality risk. Adjusted to FAV60, PNC didn't significantly improved survival.

**Conclusions:** Pts with PNC had better quality of care indicators, lower initial hospitalization time CD and of most importance greater first year survival allowing timely fistula confection. Our study emphasizes the importance predialysis care in order to reduce costs and improve outcomes.

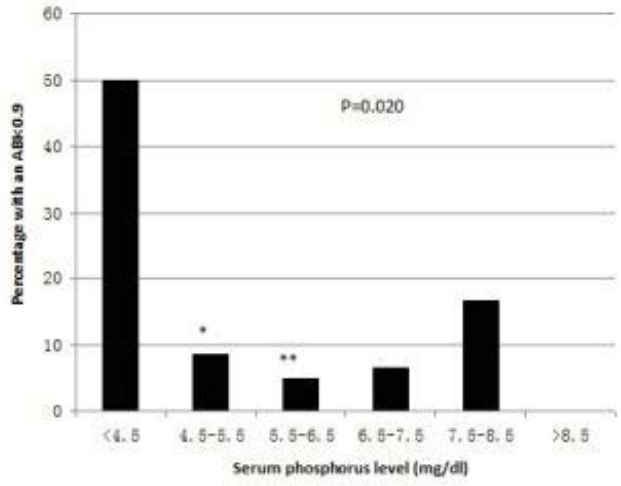
## SA-PO463

**Serum Phosphorus Is Associated with Ankle-Brachial Index in Maintenance Hemodialysis Patients: A Different Reference Range?** Ziyong Tang, Yue Wang, Ning Zhang, Huijing Ge, Xinhong Lu. *Renal Div, Peking Univ Third Hospital, Beijing, China.*

**Background:** Abnormally low ankle-brachial index (ABI) values (ABI<0.9) and high ABI (ABI>1.3) are associated with high cardiovascular morbidity and mortality in maintenance hemodialysis (MHD) patients. This study is aimed to assess the relationship of serum phosphorus level with ABI in MHD patients.

**Methods:** A total of 89 MHD patients were included in this retrospective study. ABIs were measured using an ABI-form device. The patients' serum phosphorus in the past 6 months were recorded. Other clinical and laboratory data were also collected.

**Results:** The mean of past six months' serum phosphorus levels in this group ranging from 2.45mg/dl to 12.34mg/dl, with the median 6.36 mg/dl. As normal homeostasis maintains serum phosphorus concentrations between 2.5–4.5mg/dl, serum phosphorus levels in our group were divided into following levels: level 1: <4.5, level 2: 4.5-5.5, level 3: 5.5-6.5, level 4: 6.5-7.5, level 5: 7.5-8.5, level 6: >8.5, respectively. The number of patients with low (<0.90) or high (>1.30) ABI were compared among the levels. Low ABI were present in 50%(3/6) of patients in level 1, 8.70%(2/23) in level 2, 5%(1/20) in level 3, 6.67%(1/15) in level 4, 16.67%(2/12) in level 5, and 0% in level 6. High ABI were present in 0% of patients in level 1, 4.35%(1/23) in level2, 15%(3/20) in level 3, 0% in level 4, 16.67%(2/12) in level 5, and 15.38%(2/13) in level 6. Chi-square tests show that there is a significant difference of low ABI among the levels (p=0.020), but the difference doesn't reach significant of high ABI among the levels (p=0.406).




**Conclusions:** In MHD patients, low ABI is prevalent in the phosphorus level lower than 4.5mg/dl, and high ABI is prevalent in the phosphorus level higher than 6.5mg/dl. The reference range of serum phosphorus level in MHD patients may be different from that of general population.

*Funding:* Government Support - Non-U.S.

## SA-PO464

**Reduced Number of Endothelial Progenitor Cells Predicts Future Cardiovascular Events in End Stage Renal Disease Patients on Maintenance Hemodialysis** Hong Joo Lee,[1] Dongyoung Lee,[2] Ju Young Moon,[3] Sang Ho Lee,[3] Chun-Gyoo Ihm,[3] Tae-won Lee,[3] Kyung Hwan Jeong.[3] *[1]Nephrology, Gachon Univ Dongincheon Gil Hospital; [2]Internal Medicine, Seoul Veterans Hospital; [3]Nephrology, School of Medicine, Kyung Hee Univ.*

**Background:** Endothelial progenitor cells (EPCs) have emerged as the key regulator of maintenance of endothelial integrity by repair of injured endothelium. The low number of circulating EPCs has been identified as an independent predictor of cardiovascular(CV) events, progression of atherosclerosis and CV death in general population. We ascertained whether the number of circulating EPCs may be the independent variables associated with incident CV events in ESRD patients on hemodialysis.

**Methods:** This study is the prospective observational descriptive study complemented by a retrospective chart review. We quantified EPCs in blood samples from 70 outpatients who underwent maintenance hemodialysis therapy, Circulating EPCs were quantified by flow cytometry as the number of $CD45^{low}CD34^{+}VEGFR2^{+}$ cells. The occurrence of CV events defined as coronary, cerebrovascular and peripheral vascular disease was observed.

**Results:** Mean age was 57.77 ± 12.68 years and 58.6% of all patients were male. The patients were divided into two groups according to a cutoff value for circulating EPCs determined by a ROC curve analysis. High ECP group had long CV event free period. In univariate analyses with Cox proportional hazard models, the incidence of CV events was significantly associated with a level of circulating EPCs lower than $9.5/2x10^4$ events, a history of CVD, the level of 25-(OH) vitamin D, serum creatinine, or hemoglobin $A1_C$. In multivariate analysis, a level of circulating EPCs lower than $9.5/2x10^4$ events was identified as independent predictors of CV events among ESRD patients on hemodialysis.

**Conclusions:** Reduced levels of circulating EPCs independently predict future cardiovascular events in ESRD patients on maintenance hemodialysis, thus supporting that endothelial injury in the absence of sufficient circulating EPC may unfavorably affect on occurrence of CV events.

## SA-PO465

**Impact of Genetic Polymorphisms of the Renin-Angiotensin-Aldosterone System on Coronary Artery Disease, Cardiovascular Complications and Mortality in Dialysis Patients** Katrin Ivens, Christos Bantis, Philip Reis, Nicoletta-Maria Kouri, Nicola Kuhr, Christina Schwandt, Peter J. Heering. *Dept of Nephrology, Heinrich-Heine Univ, Düsseldorf, Germany.*

**Background:** Renin angiotensin system plays an important role in the regulation of arterial pressure and the progression of atherosclerosis. We studied impact of the four major genetic polymorphisms of the renin angiotensin system [angiotensin converting enzyme gene I/D, angiotensinogen gene M235T, aldosterone synthasegene C-344T and A1166C polymorphism of angiotensin II-type 1 receptor ($AT_1R$)-gene ] on coronary artery disease (CAD) in chronic dialysis patients.

**Methods:** We studied n=462 patients on chronic dialysis with angiographically confirmed (n=217) or excluded (n=245) CAD followed up for 5.4±4.0 years. The genetic polymorphisms were determined by PCR amplification. Cardiovascular complications and the need for intervention (coronary angioplasty or coronary artery bypass grafting) were monitored.

**Results:** Patients with CAD were comparable to those without CAD regarding age at coronary angiography, time on dialysis, LDL cholesterol levels and prevalence of arterial hypertension (ns). Among patients with CAD there were significantly more men (73.9 vs. 61.2%, $p<0.01$), more diabetics (29.8 vs. 17.6%, $p<0.01$) and smokers (42.8 vs. 34.3%, $p<0.05$). Patients with CAD had also significantly higher CRP (1.15 ± 1.17 vs. 0.94 ± 1.15mg/dl, $p<0.05$) and fibrinogen levels (453 ± 134 vs. 417 ± 109 mg/dl, $p<0.05$). There was no difference in the genotype distribution of the four investigated polymorphisms in patients with or without CAD (ns). Furthermore, no impact of the investigated polymorphisms was detected in the Kaplan-Meier of intervention-free or myocardial- infarction-free survival as well in the general patient survival (ns).

**Conclusions:** Our results suggest that ACE gene I/D, angiotensinogen gene M235T, aldosterone synthase gene C-344T and $AT_1R$ geneA1166Cpolymorphisms are not risk factors for the development of coronary artery disease in chronic dialysis patients. Furthermore, they do not influence the incidence of myocardial infarction, need for intervention and mortality in this patient group.

## SA-PO466

**High Fibroblast Growth Factor (FGF) 23, CRP and IL-6 Levels Are Associated with Left Ventricular Hypertrophy (LVH) in Pediatric (ped) Patients (pts) Receiving Chronic Dialysis** Poyyapakkam Srivaths,[1] Rajesh Krishnamurthy,[2] Michael R. Bennett,[3] Qing Ma,[3] Rene' G. VanDeVoorde,[3] Stuart Goldstein.[3] *[1]Pediatric Nephrology, Baylor College of Medicine, Houston, TX; [2]Pediatric Radiology, Texas Childrens Hospital, Houston, TX; [3]Pediatric Nephrology and Hypertension, Cincinnati Children's Hospital, Cincinnati, OH.*

**Background:** LVH is a risk factor for mortality in adults recieving chronic dialysis (CD). LVH is widely prevalent in ped CD pts. Only 1 study has studied association of LVH and FGF23 in ped CD pts. No ped study has examined association of inflammation (inflam) and FGF23 and LVH in CD. Aim: Assess associations of inflam and mineral imbalance with LVH.

**Methods:** 2 centers Houston and Cincinnati (38 pts, 19 M, mean age 15.5 ± 4 yr) receiving CD (21 HD, 17 PD). Echo done within 3 months of enrollment. LVH defined left ventricular mass index (LVMI) >38.6 g/m^2.7 in pts <18 yr of age (yoa), >44 g/m^2.7 in F >18 yoa, and >48 g/m^2.7 in M >18 yoa. Cardiac calcifications (CC) assessed by ultrafast CT . Pts received thrice weekly HD for 3-3/12 hrs or daily CCPD. FGF 23, IL-6, IL-8 and CRP levels obtained on enrollment. Time averaged (6 months prior to enrollment) serum Ca, P, Alb and iPTH obtained. Patients on aspirin, with infection, underlying collagen vascular disease excluded.

**Results:** Mean LVMI 43.2 ± 13.1. LVH present in 63% (24/38) pts. FGF 23, CRP and IL6 levels significantly higher in patients with LVH (table).

| | FGF23 pg/ml[a] | P (mg/dl) | iPTH (pg/ml) | CRP(mg/dl)[b] | IL6(pg/ml)[c] | IL8(pg/ml) |
|---|---|---|---|---|---|---|
| No LVH | 1745(1367) | 6.4(1.6) | 396(251) | 0.33(0.47) | 3.44(2.04) | 39.3(65) |
| LVH | 2926(1482) | 6.8(1.6) | 425(335) | 1.8(2.02) | 5.29(2.8) | 28.1(16.8) |

values expressed mean(sd); a=0.02, b=0.018, c=0.04

No difference with age, gender, modality, vintage, serum P, Ca, albumin, PTH or IL8 levels and LVH. 11 pts had CC. Pt with CC had higher LVMI (54 ± 15.9 with CC vs. 41 ± 9.8 with noCC).

**Conclusions:** LVH is highly prevalent in ped pts receiving CD. Pts with CC have higher prevalence of LVH. High FGF 23 and CRP and IL6 levels are associated with LVH. We conclude that inflam and high FGF 23 contribute to cardiovascular morbidity in ped pts receiving CD.

*Funding:* Pharmaceutical Company Support - Baxter Clinical Grant, Private Foundation Support

## SA-PO467

### Improvement in Left Ventricular Hypertrophy and Diastolic Dysfunction in Patients on Nocturnal Home Hemodialysis as Assessed by Echocardiography and Cardiac Magnetic Resonance Imaging

Frederick Eng, Davinder S. Jassal, Tyler Friesen, Mike Zhu, Manish M. Sood, Navdeep Tangri, Claudio Rigatto, Paul Komenda. *Medicine, Univ of Manitoba, Winnipeg, Canada.*

**Background:** Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in patients with end-stage renal disease (ESRD). Left ventricular hypertrophy (LVH) is present in more than 70% of ESRD patients and is an independent risk factor for cardiac death in this patient population. Nocturnal home hemodialysis (NHD) is a form of renal replacement therapy whereby hemodialysis is performed for six hours overnight at least four days per week. NHD has several benefits over conventional hemodialysis (CHD) including improved blood pressure control, mineral metabolism, and degree of anemia. Limited studies are conflicting however, about the effects of NHD on cardiovascular remodeling as assessed by transthoracic echocardiography (TTE) and cardiac magnetic resonance imaging (CMR).

**Methods:** Patients enrolled in the NHD training program at a single tertiary care centre were invited to participate in the study between 2009-2011 inclusive. Eligible patients had a life expectancy greater than 12 months and had no reliable expectation of receiving a kidney transplant within 12 months. Participants underwent TTE and CMR at baseline and after one year of NHD. Data regarding blood pressure, mineral metabolism and degree of anemia were also collected and analyzed.

**Results:** A total of 11 patients were included in the study (6 male, mean age 48±16 years). Left ventricular mass index (LVMI) decreased significantly at one year by both TTE (152±7 to 129±8 g/m$^2$, p<0.05) and CMR (162±4 to 124±4 g/m$^2$, p<0.05). There were also significant reductions in left and right atrial volume as well as right ventricular mass index. Diastolic dysfunction, graded from 0 to 4, improved from a baseline of 3.4 to 1.2 at one year follow-up. There was no significant effect of NHD on blood pressure, mineral metabolism, or degree of anemia.

**Conclusions:** Long-term nocturnal hemodialysis leads to favorable cardiovascular remodeling with a reduction in cavity dimensions, regression of LVH, and improved diastolic function as assessed by both TTE and CMR.

## SA-PO468

### Relationship between Serum Cancer Antigen 125 (Ca125) and Left Ventricular (LV) Function in Patients on Maintance Hemodialysis

Hakki Yilmaz, Ozgul Malcok Gurel, Huseyin tugrul Celik, Ayse Mukadder Bilgic, Nuket Bavbek, Ali Akcay. *Turgut Ozal Univ, School of Medicine, Ankara, Turkey.*

**Background:** The aim of the study was to analyze associations between serum Ca125 levels and LV function in patients on maintance hemodialysis.

**Methods:** The study group included 110 patients {(54 women, 56 men) aged 62.83 ±13.66 years, treated with HD for 38.4±36.3 months} and 47 healthy age and sex matched volunteer controls were enrolled in the study. Ca125 and biochemical parameters were measured and echocardiography was performed. Hemodialysis patients were divided into different groups based on heart function.

**Results:** Mean Ca125 levels in patients were significantly higher compared with controls (38.78±35.48 vs 9.20 ±4.55 U/ml; P =0.003). Serum levels of Ca125 in the patient group1 were significantly higher than in the group2 and group3 (74.47±27.74; 13.14±4.84 ; 9.20±4.55 U/ml; p <0.001, respectively).

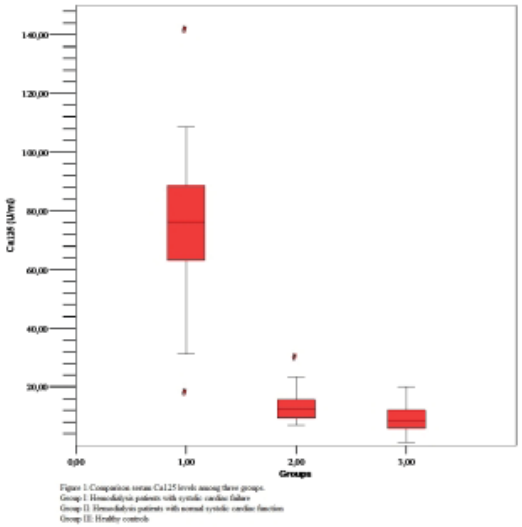



Figure 1 Comparison mean Ca125 levels among three groups.
Group I: Hemodialysis patients with systolic cardiac failure
Group II: Hemodialysis patients with normal systolic cardiac function
Group III: Healthy controls

Patients with elevated Ca125 (n=40) had significantly lower albumin, RWT, LVEF, FS while B-type natriuretic peptide levels, LVEDd, LVESd were significantly higher. Correlation analysis showed that CA 125 was positively related proBNP(r=0.596, P < 0.05), Crp ( r=0.439, P < 0.05), LVEDd (r=0.599, P < 0.001), LVESd( r=0.750, P < 0.001), LVMI( r=0.378, P < 0.05). But serum Ca 125 levels were negatively correlated with albumin (r=-0.513, P < 0.05), hemoglobin( r=-0.475, P < 0.05), HDL( r=-0.336, P < 0.05), EF( r=-0.878, P < 0.0001). Presence of depressed ejection fraction (ß=-1.121, p<0.0001) and increased CRP levels (ß= 0.247, p=0.035) were independent predictor of high Ca125 levels in whole group in multivariate-model.

**Conclusions:** Our study demonstrated an association between Ca125 and LV systolic dysfunction in patients with ESRD treated with HD. We conclude that Ca125 appears to be useful in establishing the presence of systolic heart dysfunction in HD patients.

## SA-PO469

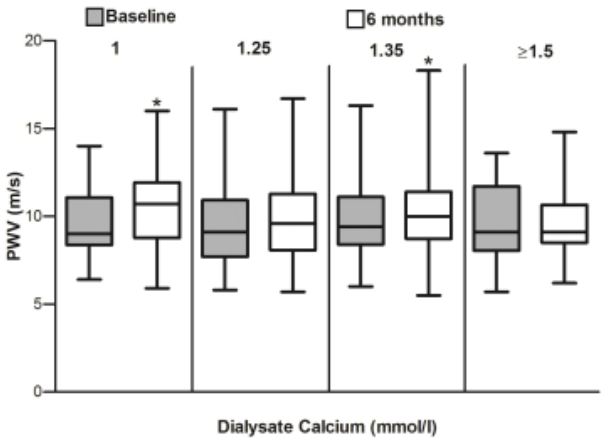
### The Effect of Dialysate Calcium Concentration on Vascular Stiffness in Haemodialysis Patients Measured by Aortic Pulse Wave Velocity

Evangelia E.M. Charitaki, Andrew Davenport. *UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom.*

**Background:** Higher dialysate calcium concentrations have been reported to acutely increase arterial stiffnessin haemodialysis patients. The aim of this study was to determine whether higher dialysate calcium concentrations are associated with chronic changes in arterial stiffness,raising the risk of sudden cardiac arrhythmic death, heart failure, and stroke in these patients.

**Methods:** This study included 289 maintenance dialysis patients [62.2% male, mean age 65.5±15.7 years,47.9% diabetic], dialysing with calcium concentrations of 1.0, 1.25, 1.35 and ≥1.5 mmol/l. The groups were matched for demographics and serum biochemistries. Patients dialysing with the lowest calcium dialysate concentration showed an increased incidence of hypertension and higher serum phosphate and parathyroid levels compared to those dialysing against the highest dialysate calcium concentrations. Pulse wave velocity(PWV) and aortic augmentation index(AoAXi) were measured before dialysisusing a brachial oscillometric device [Arteriograph]. Measurements were repeated after six months.

**Results:** Mean PWV increased over the six-months period (9.66±2.0 vs 10.13±2.16 m/s ,p<0.001), but no change was seen in AoAXi (38.7±16.3 vs 39.8±15.6%), or central aortic pressure (149.6±33.3 vs 150.4±31.9 mmHg).PWV did not differ between the four groups, either at the start or the end of the study, but increased significantly in the groups dialysing with a calcium concentration of 1.0 mmol/l (9.64±1.94 vs 10.45±1.98 m/s, p=0.0028) and 1.35 mmol/l (9.75±1.96 vs 10.21±2.18 m/s, p=0.02).




**Conclusions:** The increase in pulse wave velocity observed over time with the lowest calcium dialysate group suggests that factors other than the dialysis calcium balance determined by the dialysate calcium concentration, may be more important in promoting vascular stiffness in haemodialysis patients.

## SA-PO470

**Relation between Diastolic Dysfunction, Physical Function and Body Composition in Hemodialysis Patients** Jin Hee Jeong,[1] Pei-tzu Wu,[1] Peter J. Fitschen,[1] Brandon Kistler,[1] Hae Ryong Chung,[1] Annabel Biruete,[1] Ken Wilund,[1] Mohamed Ali,[2] Bo Fernhall,[2] Shane Phillips.[2] *[1]Kinesiology, Univ of illinois at Urbana-Champaign, Urbana, IL; [2]Univ of Illinois at Chicago, Chicago, IL.*

**Background:** Cardiovascular (CV) complications are the main cause of death in hemodialysis (HD) patients. Although metrics related to left ventricular systolic dysfunction (LVSD) such as ejection fraction (EF) are commonly used to predict adverse CV outcomes, LV diastolic dysfunction (LVDD) measures may provide better prognostic values in HD patients because they are less sensitive to blood volume changes. Additionally, muscle wasting and declines in physical function are common in HD patients. This can result from abnormalities in cardiac function, which can be further worsened by physical deconditioning. Little is known about the relationship between cardiac function and physical function in HD patients. **Aim:** To evaluate the prevalence of LVDD and to assess its relationship to physical function and body composition in HD patients.

**Methods:** Walking performance, leg strength, and whole body lean mass (WBLM) by DXA were measured in 83 HD patients (age=52.9± 11y). Echo was used to assess LVSD (EF) and LVDD (peak mitral inflow velocities (E and A), peak early diastolic mitral annular velocity(E') and deceleration time of E (DT)). LVDD classification: 1)mild DD (E/A< 0.8, E'<8cm/s, E/E'<8, and DT<200ms) and 2) advanced DD (E/A>0.8, E'<8, E/E'>9, and DT<200).

**Results:** The prevalence of LVDD was 48.2% (mild DD:14.5% and advanced DD: 33.7%) and of LVSD (EF < 40%) was 12.2%. 50% of patients with LVSD also had LVDD. BMI was significantly higher in patients with LVDD(p=0.017). After adjusting for age, gait speed, right leg peak strength, and WBLM% were significantly higher in the group without LVDD than with LVDD(p=0.006, 0.001 and 0.007;respectively). However, there was no significant difference in any measure of physical function or body composition between patients with and without LVSD.

**Conclusions:** This data indicates that LVDD is more closely related to physical function and body composition than LVSD in HD patients, and suggests that LVDD may be an important therapeutic target.

*Funding:* NIDDK Support

## SA-PO471

**Bradycardia as a Cause of Hypotensive Episodes during Routine Hemodialysis** D. Miskulin,[1] Klemens B. Meyer,[1] John E. Moran.[2,3] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Intelomed, Inc, Pittsburgh, PA; [3]Nephrology, Stanford Univ School of Medicine, Stanford, CA.*

**Background:** Patients on maintenance hemodialysis (HD) have a high cardiovascular (CV) mortality and often suffer CV events such as hypotension during HD. One manifestation of CV stress is the appearance of arrhythmias which may be associated with hypotension.

**Methods:** 25 patients with a history of frequent hypotensive episodes during routine HD were monitored during 4 HD treatments using the CVInsight device (Intelomed, Inc), which captures and processes the signal from a standard pulse oximeter (Nonin Medical, Inc) to provide real-time display of CV status based on rate of change of pulse rate and of pulse amplitude. Bradycardia was defined as a 15% decrease in pulse rate during treatment.

**Results:** 8 patients experienced 18 episodes of bradycardia during the study; 3 patients had episodes in all 4 HD treatments studied, 1 patient experienced 2 episodes, and 4 patients 1 episode. 15/18 episodes (83%) were associated with a defined CV event requiring intervention; in 2 of the other 3 episodes the systolic BP fell by more than 60 mm Hg. Four of the 8 patients were receiving beta blocker therapy. In 7 patients the fall in pulse rate was gradual over the course of treatment; in 1 patient there was an abrupt fall in all 4 treatments which was preceded by a fall in BP.

**Conclusions:** Some HD patients have an inappropriate fall in pulse rate during HD treatment, not necessarily related to beta blockade. Real-time monitoring will allow recognition of such events and facilitate intervention.

*Funding:* Pharmaceutical Company Support - Intelomed, Inc

## SA-PO472

**Mortality Rates among Prevalent Hemodialysis Patients in Turkey: A Comparison with USRDS Data** Gulay Asci,[1] Daniele Marcelli,[2] Aygul Celtik,[1] Aileen Grassmann,[2] Mustafa Yaprak,[1] Abdulkerim Furkan Tamer,[1] Mehmet Nuri Turan,[1] Mehmet S. Sever,[3] Ercan Ok.[1] *[1]Nephrology, Ege Univ, Turkey; [2]Fresenius Medical Care, Germany; [3]Nephrology, Istanbul Univ, Turkey.*

**Background:** There are significant differences between countries in mortality rates of hemodialysis patients(HD). We compared mortality rates in Turkey and the US and analyzed potentially related differences in patient-treatment characteristics.

**Methods:** All patients undergoing HD or hemodiafiltration in March 2009 for >3 months(n=4041) in the Turkish clinics of the NephroCare network were enrolled. Data were prospectively collected for 2 years through the database EuCliD. Mean age was 58.7±14.7 years, 45.9% female, 22.9% diabetic. Comparison with US data was performed by applying indirect standardization technique, using as reference specific mortality rates for patients on HD by age, gender, race and primary diagnosis as provided by the 2012 USRDS Annual Data Report.

**Results:** Crude mortality rate was 9.5 per 100 pt-yrs. Comparison of specific unadjusted mortality rates(per 1000 pt-yrs) of Turkish Patients with US whites is reported in table.

| | All | Female | Male | Diabetes | 20-44 | 45-64 | 65+ |
|---|---|---|---|---|---|---|---|
| Turkey | 95.1 | 90.4 | 99.0 | 154.8 | 21.6 | 72.4 | 164.2 |
| US white | 236.3 | 234.2 | 237.9 | 245.6 | 75.2 | 162.9 | 330.3 |
| Relative risk reduction | 0.59 | 0.61 | 0.58 | 0.37 | 0.71 | 0.55 | 0.50 |

The mortality advantage for Turkish patients was present in all subgroups. Standardized mortality rate of Turkish cohort was 0.50 (95%CI 0.46-0.54, p<0.001) compared to US. The differences in treatment characteristics between two populations are given in figure.

| | Turkish cohort | US cohort* |
|---|---|---|
| AV fistula (%) | 86.1 | 55.5 |
| Treatment time (min) | 240±13 | 214±17 |
| Systolic blood pressure >140 mmHg (%) | 24.1 | 89.0 |
| Hemodiafiltration (%) | 9.7 | NA |

**DOPPS-US data were used except AV fistula (USRDS)*

**Conclusions:** Annual mortality rate is significantly lower in Turkish cohort than US HD patients. Higher use of AV fistula, longer treatment time, better volume control and hemodiafiltration treatment are possible contributing factors.

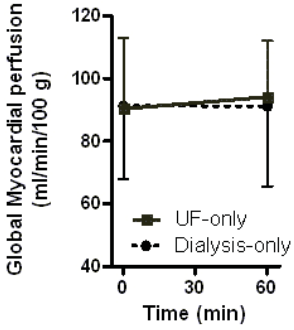
## SA-PO473

**Acute Effects of Isolated Ultrafitration and Isolated Dialysis on Myocardial Perfusion and Function Assessed by Intra-Treatment Positron Emission Tomography (PET)** Solmaz Assa,[1] Johanna J. Kuipers,[2] Esmee M. Ettema,[1] Judith J. Dasselaar,[1] Yoran M. Hummel,[3] Adriaan A. Voors,[3] Paul E. de Jong,[1] Ralf Westerhuis,[2] Rene A. Tio,[3] Riemer Hja Slart,[4] Casper F.M. Franssen.[1] *[1]Nephrology; [2]DCG; [3]Cardiology; [4]Nuclear Medicine, UMCG, Netherlands.*

**Background:** Previous studies showed that hemodialysis with combined dialysis and ultrafiltration is associated with significant reductions in myocardial perfusion and development of regional left ventricular (LV) systolic dysfunction. We studied the effect of isolated ultrafiltration (UF-only) and dialysis (dialysis-only) on myocardial perfusion and function.

**Methods:** 8 patients (7 male, 55±18 years) underwent 1h UF-only (UF-rate 1 l/h) and dialysis-only (zero fluid balance). Myocardial perfusion and LV ejection fraction (EF) were assessed by $^{13}$N-$NH_3$ PET before and during the last 15 min of treatment. Regional LV systolic function was assessed by echocardiography.

**Results:** Blood pressure, heart rate and body temperature were comparable during treatments. Global myocardial perfusion did not change during UF-only and dialysis-only, however, during dialysis-only a non-significantly greater number of LV segments showed reductions in perfusion compared with UF-only (7.9±5.3 vs 5.0±5.1). LVEF rose during UF-only (p=0.06) and did not change during dialysis-only. Two patients developed regional LV hypokinesia only during isolated dialysis (3 segments in both); 1 other patient developed regional LV hypokinesia during both UF-only and dialysis-only (in 4 and 2 segments, respectively).




**Conclusions:** Global myocardial perfusion was not compromised by isolated UF or isolated dialysis. However, regional decreases in myocardial perfusion and regional LV dysfunction were more pronounced during isolated dialysis indicating that, besides hypovolemia, acute dialysis-associated factors are involved in the pathogenesis of hemodialysis-induced LV dysfunction.

*Funding:* Pharmaceutical Company Support - Amgen

## SA-PO474

**Effect of Personalized Dialysate Sodium on Clinical Outcomes and Sodium Setpoint Stability, in Patients on Nocturnal and Frequent Hemodialysis Modalities** Benjamin Ka Thomson,[1] Christopher T. Chan,[2] Shih-Han S. Huang,[1] Robert M. Lindsay.[1] *[1]Western Univ; [2]Western Univ; [3]Univ Health Network, Univ of Toronto; [4]Western Univ.*

**Background:** Personalized dialysate sodium alters blood pressure, interdialytic weight gain, and perhaps mortality. We have previously shown that the Post- to Pre-dialysis sodium gradient (PPNa+) predicts clinical outcomes in home hemodialysis population. Moreover, modifiable factors affecting interdialytic weight gain in this population include dialysis duration and frequency, and dialysate sodium concentration, while patient age, sex and pre-dialysis serum albumin also have a role. Our objectives were to validate prospectively our equation for predicting interdialytic weight gain, and to confirm our retrosptive data that pre-dialysis sodium setpoint is modifiable.

**Methods:** All patients in the Southwestern Ontario regional home hemodialysis program were included in a randomized crossover design. Patients differed in dialysis frequency and duration. Each patient was treated with two consecutive three month periods with dialysate sodium concentration that was 3 mmol/L above then below (or vice versa) the most recently recorded pre-dialysis sodium setpoint. Clinical outcomes were compared between each three month interval. Clinical outcomes considered included pre-dialysis systolic and diastolic blood pressure (mm Hg), interdialytic weight gain (Liters). We also evaluated the stability of the pre-dialysis sodium setpoint by comparing the pre-dialysis sodium slope and difference across each 3 month interval.

**Results:** Data for this trial will be collected and analyzed completely by October 17, 2013. Results will be considered in two categories: 1. Clinical outcomes, which will be compared between each three month period and 2. Sodium setpoint, to determine if personalization of dialysate sodium alters the stability of the sodium setpoint.

**Conclusions:** The results of this trial will determine if and how dialysate sodium should be chosen in hemodialysis patients with increased dialysis frequency or duration. In addition, whether sodium setpoint can be altered prospectively will be determined.

*Funding:* Government Support - Non-U.S.

## SA-PO475

**Arterial Stiffness and Cardiovascular Outcomes in Minorities with End Stage Renal Disease** Kalyani Perumal,[1] Peter D. Hart,[1] James P. Lash.[2] *[1]Nephrology, John H Stroger Hospital, Chicago, IL; [2]Nephrology, Univ of Illinois at Chicago, Chicago, IL.*

**Background:** Cardiovascular disease is the leading cause of mortality in patients with end stage renal disease (ESRD). Although arterial stiffness is now recognized as an independent predictor of cardiovascular mortality in ESRD, there are no data on arterial stiffness in minorities with ESRD and its impact on cardiovascular outcomes.

**Methods:** We therefore conducted a prospective study to investigate the measures of arterial stiffness (pulse wave velocity, PWV) and reflected wave (augmentation index, Aix) in a predominately minority cohort of 77 patients on hemodialysis and its effect on composite outcome- cardiovascular event or all-cause mortality. PWV and AIx were determined from arterial waveforms recorded by SphygmoCor® device. 80% of measurements were taken prior to dialysis sessions. Cardiovascular events were defined as new onset myocardial infarction, congestive heart failure, arrhythmia, acute cerebrovascular event, peripheral arterial disease requiring medical or surgical intervention.

**Results:** In this group, 72% were African Americans , 24% Hispanics, and 4% were non-Hispanic whites. Mean age was 55 yrs; 61 % were male. Major causes of ESRD were diabetes (58 %) and hypertension was present in 91 %. Mean duration of dialysis was 41 mos. Mean PWV was 11 m/s and mean AIx was 24% in this cohort. All patients were followed for a mean of 36 mos. A total of 4 fatal and 24 non-fatal cardiovascular events occurred in 19 patients. In multivariate regression analysis, age and diabetes were identified as significant predictors of PWV ($p < 0.05$). In multivariate Cox analysis, after adjustment of confounding factors, the risk ratio for each 10% increase in PWV was 1.12 (CI 1.00- 1.23, $p< 0.05$) for composite outcomes. Survival analysis showed that the group in lowest tertile of PWV (PWV< 8m/s) had greater event free survival when compared to the group in upper tertile (PWV>12 m/s) ($p < 0.01$).

**Conclusions:** Thus, our study provides the first direct evidence that PWV is a significant predictor of cardiovascular morbidity and mortality in the minority population on hemodialysis.

## SA-PO476

**Pulmonary Hypertension in Hemodialysis Patients Is Associated with High Pulse Pressures** Brittany Jamille Thomas,[1] Muhammad Sarmini,[2] Rachel E. Patzer,[1] Janice P. Lea.[1] *[1]Emory Univ; [2]Univ of Missouri.*

**Background:** Pulmonary hypertension (PHT) is an independent predictor of mortality in ESRD patients. Etiology has been attributed to presence of arteriovenous fistulas, vascular calcifications, volume overload, and anemia. Aim of this study was to determine prevalence of PHT in our dialysis population and its relationship to blood pressure.

**Methods:** PHT defined as PAP > 35 mm Hg per echocardiogram( echo). Retrospective data analyses were conducted on a total number of 410 ESRD patients who had echos performed as usual standard of care with dialysis BP measurements averaged over a 6 month period prior to date of echo.

**Results:** We found that 61% had PHT and diabetics were more likely to have PHT , p=.021, OR- 1.7 (1.1-2.6). Mean values for pre and post systolic blood pressures (SBP), diastolic blood pressures (DBP), and pulse pressures are listed in table below. Patients with PHT had lower pre and post diastolic BP (p=.023 and .022), and higher pre and post pulse pressures, p=.003, and p=.015. Pre and post systolic BP was not statistically different between those with and without PHT. In addition, calcium-phosphorus product nor hemoglobin level were not statistically different between those with or without PHT.

| PAP | mean pre-SBP/post-SBP | mean pre-DBP/post-DBP | mean pre-pulse pressure | mean post-pulse pressure |
|---|---|---|---|---|
| > 35 | 154.3+ 2.2.8/140 + 19 | 83.5+14.4/76.1+11.7 | 70.7+15 | 63.9+13.3 |
| < 35 | 152.91+8.6/139.5+18 | 87.3+ 79.4+12.2 | 65.6+12.9 | 60.1+12.7 |

**Conclusions:** In summary, we show that ESRD patients with PHT have elevated pulse pressures attributed to lower DBP and not due to elevated SBP. Since pulse pressure is also an independent predictor of mortality, studies investigating mortality related to PHT need to be adjusted for pulse pressure. Furthermore, other potential mechanisms to explain these findings need to be explored.

## SA-PO477

**Progression of Pulmonary Hypertension in Dialysis Population Is Associated with Increased Mortality** Farhanah Yousaf, Nimra Sarfaraz, Haroon Rashid, Chaim Charytan, Bruce S. Spinowitz. *New York Hospital Queens, Flushing, NY.*

**Background:** There is increased prevalence of pulmonary hypertension in the dialysis population. Little is known about the progression and predictors of progression of pulmonary hypertension in patients undergoing dialysis. Therefore, a retrospective review was undertaken to explore the progression of pulmonary hypertension in dialysis patients, risk of all-cause mortality, and to investigate the routinely drawn biochemical parameters as predictors of progression of pulmonary hypertension.

**Methods:** IRB approval was obtained. Medical records of prevalent dialysis patients who had at least two right ventricular systolic pressures (RVSP) derived from echocardiography were reviewed. Patients were divided into normal (< 35 mmHg), mild (35 – 50 mmHg), moderate (51 – 70 mmHg), and severe (> 70 mmHg) pulmonary hypertension groups. Biochemical parameters from the interval between the two RVSPs were collected. Linear multi-logistic regression analysis was performed using SPSS.

**Results:** 38 patients, 18 males and 20 females, aged 72 ± 12 years, met inclusion criteria. The mean initial RVSP was 32 ± 16 mmHg and the follow up mean RVSP was 37 ±27 mmHg and the difference was statistically significant ($p<0.001$). Based on initial echocardiogram, 16 (42%) patients had pulmonary hypertension defined as a RVSP greater than 35 mmHg. On follow up, 20 (52%) patients remained stable, 12 (32%) worsened, and 6 (16%) improved. Among patients with worsening pulmonary hypertension, 6 (50%) of the 12 patients died during a mean follow up of 4.1 years compared to zero mortality among stable or improved RVSP group. Of the analyzed biochemical parameters, only ferritin was found to be an independent predictor of worsening pulmonary hypertension in our analysis ($r^2 = 0.354$, $p= 0.003$).

**Conclusions:** Our study confirms a high prevalence of pulmonary hypertension in end stage renal disease. There is a trend of worsening pulmonary hypertension in patients undergoing dialysis and it is associated with increased mortality. Additional studies in the dialysis population are warranted to explore the progression of pulmonary hypertension and its predictors.

**SA-PO478**

**Stroke and the "Stroke Belt" in Dialysis: Contribution of Patient Characteristics to Ischemic Stroke Rate and Its Geographic Variation**
James B. Wetmore,[2] Edward F. Ellerbeck,[3] Jonathan D. Mahnken,[2] Milind A. Phadnis,[2] Sally K. Rigler,[3] John Spertus,[4] Purna Mukhopadhyay,[2] Theresa I. Shireman.[3] *[1]Medicine, Div of Nephrology, Hennepin County Medical Center, Minneapolis, MN; [2]Biostatistics, Univ of Kansas Medical Center, Kansas City, KS; [3]Preventive Medicine and Public Health, Univ of Kansas Medical Center, Kansas City, KS; [4]Medicine, Div of Cardiology, Mid America Heart Institute of St. Luke's Hospital, Univ of Missouri - Kansas City, Kansas City, MO.*

**Background:** Geographic variation in stroke rates is well-established in the general population, with higher rates in the south than in other areas of the U.S. End-stage renal disease (ESRD) is a potent risk factor for stroke, but whether regional variations in stroke risk exist among dialysis patients is unknown.

**Methods:** Medicare claims from 2000-05 were used to ascertain ischemic stroke events in a large cohort of incident dialysis patients. A Poisson generalized linear mixed model was generated to determine factors associated with stroke and to ascertain state-by-state geographic variability in stroke rates by generating observed-to-expected (O/E) adjusted rate ratios for stroke.

**Results:** A total of 265,685 Medicare-eligible incident dialysis patients were studied. Older age, female sex, African-American race and Hispanic ethnicity, unemployed status, diabetes, hypertension, history of a previous stroke, and permanent atrial fibrillation were all significantly associated with ischemic stroke, while body mass index > 30kg/m$^2$ had an inverse association with stroke (P < 0.0001 for each). After full multivariable adjustment, the 3 states with O/E rate ratios > 1.0 were all in the south: North Carolina, Mississippi, and Oklahoma.

**Conclusions:** Regional efforts to increase primary prevention in the 'stroke belt,' or to better educate dialysis patients on the signs of stroke so that they may promptly seek care, may improve stroke care and outcomes in dialysis patients.

*Funding:* NIDDK Support, Private Foundation Support

**SA-PO479**

**Race and Ethnicity Largely Account for State-by-State Geographic Variation in Hemorrhagic Stroke in Dialysis Patients**
James B. Wetmore,[1] Milind A. Phadnis,[2] Jonathan D. Mahnken,[2] Edward F. Ellerbeck,[3] Sally K. Rigler,[3] Theresa I. Shireman.[3] *[1]Medicine, Div of Nephrology, Hennepin County Medical Center, Minneapolis, MN; [2]Biostatistics, Univ of Kansas Medical Center, Kansas City, KS; [3]Preventive Medicine and Public Health, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Geographic variation in stroke rates is well-established in the general population, with higher rates in the south than in other areas of the U.S. A similar pattern of geographic variation in ischemic strokes has also recently been reported in chronic dialysis patients, but whether this is also the case for hemorrhagic stroke is unknown.

**Methods:** Medicare claims from 2000-05 were used to ascertain hemorrhagic stroke events in a large cohort of incident dialysis patients. A Poisson generalized linear mixed model was generated to determine factors associated with stroke and to ascertain state-by-state geographic variability in stroke rates by generating observed-to-expected (O/E) adjusted rate ratios for stroke.

**Results:** A total of 265,685 Medicare-eligible incident dialysis patients were studied. Over a mean follow-up of approximately 2 years, 2397 (0.9%) of patients suffered a hemorrhagic stroke. African-Americans (adjusted rate ratio [ARR] 1.43, 95% confidence intervals [CI's] 1.30 – 1.57), Hispanics (ARR 1.78, 95% CI's 1.57 – 2.03) and individuals of other races (ARR 1.51, 1.26 – 1.80) had significantly higher risk for hemorrhagic stroke compared to Caucasians. In models adjusted for age & sex, four states had observed:expected ARR's for hemorrhagic stroke significantly > 1.0 (CA, 1.15; MD, 1.25; NC, 1.25; TX, 1.19), while only 1 had an ARR < 1.0 (WI, 0.79). However, after adjustment for race and ethnicity, no states had ARRs that varied significantly from 1.0.

**Conclusions:** Race and ethnicity, or some proxy for these, largely account for geographic variabilty in hemorrhagic stroke rates in the U.S.

*Funding:* NIDDK Support, Private Foundation Support

**SA-PO480**

**Stroke as a Watershed Event: Impact of Stroke on Mortality in Dialysis Patients**
James B. Wetmore,[1] Milind A. Phadnis,[2] Jonathan D. Mahnken,[2] Theresa I. Shireman,[3] Sally K. Rigler,[3] Edward F. Ellerbeck.[3] *[1]Medicine, Div of Nephrology, Hennepin County Medical Center, Minneapolis, MN; [2]Biostatistics, Univ of Kansas Medical Center, Kansas City, KS; [3]Preventive Medicine and Public Health, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Hemorrhagic stroke is a devastating clinical event, but the hazard ratio for mortality following a hemorrhagic stroke in chronic dialysis patients is unknown.

**Methods:** A large cohort of dually-eligible (Medicare-Medicaid) patients initiating chronic dialysis from 2000-05 was constructed. Medicare claims from this period were used to ascertain new hemorrhagic stroke events. A Cox regression model was generated to estimate the effects of time-dependent hemorrhagic stroke on mortality and to calculate survival time following hemorrhagic stroke.

**Results:** The cohort consisted of 69,371 incident dialysis patients with > 111,000 person-years of follow-up. There were 5.6 hemorrhagic strokes per 1000 patient-years. Of those with a hemorrhagic stroke, 81.8% died, compared to 52.0% of those without (P < 0.0001). One-quarter died by 0.35 months following a hemorrhagic stroke, 50% by 0.8 months, and 75% by 10.8 months. This survival pattern was similar whether the stroke occurred within the first, second, third, or fourth year following dialysis initiation. The hazard ratio (HR) for death was approximately 45 at 2 weeks, 24 at one month, and 7.0 at 2 months. The HR then fell rapidly thereafter to 2.8 at 4 months, 2.5 at 6 months, 1.7 at 12 months, and 1.1 at 18 months.

**Conclusions:** Hemorrhagic stroke confers substantial mortality, with a striking initial mortality and a dramatic decrease in remaining years of life. However, the HR for death after hemorrhagic stroke falls rapidly, and after only a year begins to approach 1.0. This pattern for mortality is generally similar whether the stroke occurs soon after dialysis initiation or after several years of dialysis.

*Funding:* NIDDK Support, Private Foundation Support

**SA-PO481**

**Fibroblast Growth Factor 23 and the Risk of All-Cause Mortality and Cardiac Hospitalizations and Death in Hemodialysis Patients: Results from the HEMO Study**
Kristen L. Jablonski,[1] Jessica B. Kendrick,[1] Alfred K. Cheung,[2,3] Tom Greene,[3] Michel Chonchol.[1] *[1]Univ of Colorado Denver, Aurora, CO; [2]VASLCHCS, Salt Lake City, UT; [3]Univ of Utah, Salt Lake City, UT.*

**Background:** Elevated serum levels of fibroblast growth factor 23 (FGF23), have been associated with adverse outcomes in patients requiring long-term dialysis therapy. Although the values of FGF23 often evolve over time, no study has examined the accumulated effects on important clinical end points.

**Methods:** The HEMO Study was a randomized multicenter study evaluating the effects of high-dose versus standard-dose and high-flux versus low-flux hemodialysis. Serum intact FGF23 levels were measured in stored serum samples obtained at baseline and annually in 1,340 patients in this cohort. Quartiles of FGF23 concentrations were chosen as the primary predictor variable, with the lowest quartile serving as the reference category. Time-dependent Cox proportional-hazards models were used to examine the association between FGF23 levels with all-cause mortality (ACM) and a composite of first cardiac hospitalization or death.

**Results:** Participants had a mean age of 57±14 years, 55% were females and 46% were white. During a median follow-up of 3.0 years, 582 (43%) died from any cause, and 514 (41%) had a cardiac event. Median (IQR) serum FGF23 level was 3118 [726, 12928] pg/mL. Overall, median levels of FGF23 rose during follow-up (p=0.01). After adjustment for potential confounders available in the database, including inflammatory markers and usage of calcitriol, the highest quartile of FGF23 was associated with an increase risk of ACM (HR;1.51, 95% CI 1.05-2.15; p=0.02) and cardiac events (HR; 1.51, 95% CI 1.07-2.13; p=0.02) when compared to the lowest quartile. Similarly, when FGF23 was evaluated as a continuous variable, higher levels of FGF23 were associated with an increased risk of ACM and cardiac outcomes (HR; 1.10, 95% CI 1.01-1.13 per doubling of FGF23; p=0.01).

**Conclusions:** In the HEMO study, higher serum FGF23 levels were independently associated with all-cause morality and cardiac hospitalizations and death in time-dependent Cox regression models.

*Funding:* NIDDK Support

**SA-PO482**

**Interdialytic Weight Gain Is an Independent Risk Factor for All-Cause Mortality and Cardiovascular Events in Incident Hemodialysis Patients**
Hye-Young Kang,[1] Shin-Wook Kang,[1,2,3] Mi Jung Lee,[2] Chan Ho Kim,[2] Hyung Jung Oh,[2] Seung Hyeok Han,[2] Tae-Hyun Yoo.[1,2] *[1]Brain Korea 21; [2]Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea; [3]On Behalf of Clinical Research Center for End-Stage Renal Disease Investigators.*

**Background:** Excessive interdialytic weight gain (IDWG) represents fluid overload and non-compliance in end-stage renal disease (ESRD) patients on hemodialysis (HD), and thus it is associated with unfavorable clinical outcome. Meanwhile, the amount of food intake also contributes to IDWG, which can reflect nutritional status. Such a paradoxical meaning of IDWG requires further investigation, particularly in terms of its prognostic value for adverse clinical outcomes.

**Methods:** A prospective cohort of 1,013 incident HD patients from 36 dialysis centers of the Clinical Research Center for ESRD in Korea was selected for this study. The percentage of IDWG (IDWG%) was expressed as a ratio of absolute IDWG to dry weight, and patients were divided into 5 groups according to IDWG%; <1.0, ≥4.0, and every 1.0 increment in between. Cox proportional hazard analysis was performed to determine the prognostic value of IDWG% for primary outcome, a composite of all-cause mortality or cardiovascular events.

**Results:** The mean IDWG% was 2.7±1.8%. During a mean follow-up duration of 18.7 months, 84 patients died and 99 cardiovascular events occurred. Compared to patients with IDWG% of 1.0-1.9, the adjusted hazard ratios (HRs) of IDWG% <1.0, 2.0-2.9, 3.0-3.9, and ≥4.0 groups were 1.30 [95% confidence interval (CI), 0.73-2.31; P=0.37], 1.37 (95% CI, 0.78-2.42; P=0.27), 1.91 (95% CI, 1.10-3.33; P=0.02), and 1.81 (95% CI, 1.04-3.16; P=0.03), respectively. In addition, sensitivity analysis revealed that a significant impact of high IDWG% (≥3.0) on the clinical outcome was consistent in male patients (HR, 1.57; 95% CI, 1.01-2.44; P=0.04), patients with older age (≥65 years) (HR, 1.69; 95% CI, 1.04-2.75; P=0.03), diabetes (HR, 1.75; 95% CI, 1.15-2.67; P=0.01), and BMI ≥22 kg/m2 (HR, 1.79; 95% CI, 1.08-2.99; P=0.02).

**Conclusions:** High IDWG% (≥3.0) was a significant independent predictor of all-cause mortality and cardiovascular events in incident HD patients.

*Funding:* Government Support - Non-U.S.

## SA-PO483

**Atrial Natriuretic Peptide Prophylaxis Does Not Improve Short-Term Survival or Contrast-Induced Nephropathy: A Meta-Analysis**
Sayyad F. Kyazimzade,[1] Daniel M. Pearlman,[1] Vinay Rao,[1] Alex L. Yerukhimov,[2] Jeremiah R. Brown.[1] *[1]The Dartmouth Institute for Health Policy and Clinical Practice at the Geisel School of Medicine, Lebanon, NH; [2]Dartmouth College, Hanover, NH.*

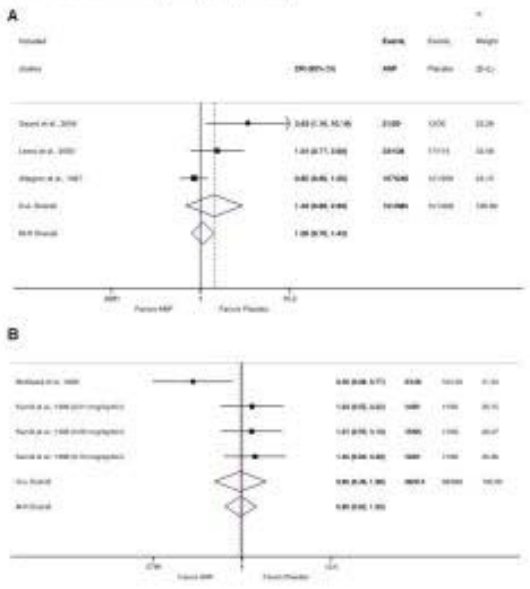
**Background:** Atrial natriuretic peptide (ANP) has been shown to improve renal function in individual randomized controlled trials (RCTs). Yet, other studies have not replicated this finding. To determine the true effect of ANP, we performed a systematic review of existing studies that assessed renal function and/or its relationship to patient survival. We hypothesized that prophylactic administration of ANP would improve the risk of contrast-induced nephropathy (CIN) and 21-day dialysis-free survival.

**Methods:** We conducted a meta-analysis of published RCTs involving prophylactic administration or treatment with ANP in participants who had renal failure. Eligible studies reported the number of participants experiencing 21-day dialysis-free survival or CIN among the total number of study participants. Our search identified 71 records, of which 5 RCTs, including 1288 patients, met our pre-specified eligibility criteria. We calculated random-effects summary estimate odds ratios (OR) and 95% confidence intervals (CI) for the 3 trials that provided data on 21-day dialysis-free survival and for the 2 trials that reported CIN occurrence.

**Results:** Pooled analyses revealed a nonsignificant difference in the odds of CIN occurrence among participants receiving ANP relative to those receiving placebo (15.3% *vs.* 13.8%; OR: 0.77; 95% CI, 0.44–1.35; $P$=.57; $I^2$=59.3%). Odds of 21-day dialysis-free survival in pooled ANP versus placebo study arms were also nonsignificant (39.2% *vs.* 37.8%; OR, 1.06; 95% CI, 0.79–1.43; $P$=.33; $I^2$=72.2%).

**Conclusions:** Existing trials show a nonsignificant improvement in the odds of both CIN and 21-day dialysis-free survival following prophylactic administration and treatment with ANP.

**Figure 1.** Atrial natriuretic peptide vs. placebo for 21-day dialysis-free survival (Panel A) and contrast-induced nephropathy (Panel B).




## SA-PO484

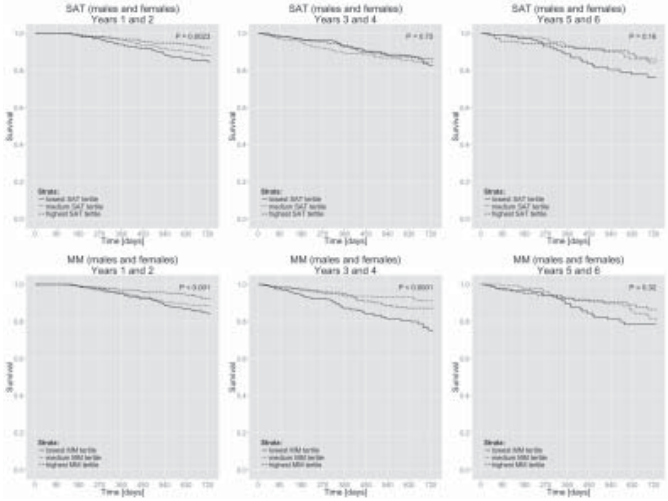
**Muscle and Fat Mass and Their Relationship to Survival in Hemodialysis Patients**
Stephan Thijssen, Nathan W. Levin, Peter Kotanko. *Renal Research Institute, New York, NY.*

**Background:** Mortality among dialysis patients is strongly associated with anthropometric measures, such as body mass index (BMI) and lean body mass, estimated as the volume of distribution of urea. In contrast to the general population, in dialysis patients a high body mass index is associated with improved survival. We have previously developed a set of regression equations for estimation of subcutaneous adipose tissue (SAT) mass and muscle mass (MM) in African-American hemodialysis (HD) patients (Thijssen, WCN 2007; $R^2$ ranging from 0.85 to 0.98). In this study, we applied these equations to elucidate the relationship between body composition and survival.

**Methods:** SAT and MM were estimated at baseline in 1,598 incident African-American HD patients who started HD between 1/1/2000 and 12/31/2010. For the purpose of run-in stability, only patients who survived for at least 120 days were analyzed. Crude time to death was compared between tertiles of fat and muscle mass by Kaplan-Meier analysis and log-rank test for consecutive 2-year segments up to 6 years after start of HD.

**Results:** There was a significant relationship between tertiles of SAT and mortality, with better survival in patients with greater SAT. The association was lost after the first 2 years on HD (Figure, top 3 plots). Similarly, greater MM was associated with significantly better survival, but this relationship was retained for the first 4 years on HD and only then disappeared (Figure, bottom 3 plots).

**Conclusions:** SAT and MM are significantly related to survival in HD patients, and it appears that the improved survival related to greater MM can be traced substantially longer than that related to SAT. Future studies dealing with the relationship between body composition and outcomes in HD patients should focus on individual components of body composition (like fat and muscle mass) rather than vague surrogates such as BMI.




*Funding:* Pharmaceutical Company Support - Renal Research Institute

## SA-PO485

**Opioid Analgesic Use among Dialysis Patients**
Eric D. Weinhandl,[1] Akeem Yusuf,[1] Yi Peng,[1] Wendy L. St. Peter.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Univ of Minnesota, Minneapolis, MN.*

**Background:** Pain is a common and potentially debilitating symptom among dialysis patients. Although opioid analgesics are frequently prescribed in the United States, there are relatively few data regarding use among dialysis patients. We used Medicare Part D data to describe utilization of opioid analgesics (OAs) among adult dialysis patients between 2007 and 2011.

**Methods:** For year *Y*, included patients initiated dialysis no later than March 31 of year *Y*-1; received dialysis and carried Medicare Parts A and B as primary payer from June 1 to December 31 of year *Y*-1; and carried Part D coverage from January 1 of year *Y* to the earliest of kidney transplant, death, or December 31 of year *Y*. Patients were categorized as having received the low-income subsidy (LIS) or not having received the subsidy (non-LIS).

**Results:** Between 2007 and 2011, the percentage of patients with ≥1 OA fill ranged from 60.8% to 62.2%, while the percentage of patients with ≥6 fills increased from 21.7% to 24.3%. Among users, cumulative OA supply increased from 108 days per patient-year in 2007 to 130 days in 2011. In 2011, most widely prescribed OAs were hydrocodone/acetaminophen (APAP) (42.1%), oxycodone/APAP (17.9%), tramadol (13.5%), codeine/APAP (7.1%), and oxycodone (6.1%); other agents with use in >1% of patients were fentanyl, hydromorphone, morphine, and tramadol/APAP. Use of propoxyphene was negligible, following withdrawal of branded formulations in November 2010. The prevalence of patients with ≥6 OA fills was relatively high with age <65 years, Native American race, and polycystic kidney disease as primary cause of end stage renal disease; and relatively low with Asian race and peritoneal dialysis. Furthermore, 27.1% of LIS versus 15.0% of non-LIS patients had ≥6 OA fills.

**Conclusions:** Opioid analgesic use is widespread among dialysis patients, exceeding estimates of 5% to 36% in a recent systematic review (*CJASN*, 6:326-333). Among users, the duration of use is increasing. Hydrocodone and oxycodone, each in combination with acetaminophen, are most commonly prescribed. Studies investigating the potential for opioid analgesic overuse and the relative safety of opioid analgesics in dialysis patients are needed.

*Funding:* NIDDK Support

## SA-PO486

**Widespread Opioid Use in the U.S. End-Stage Renal Disease Population**
Anne Mobley Butler,[1] Abhijit V. Kshirsagar,[2] M. Alan Brookhart.[1] *[1]Dept of Epidemiology, Univ of North Carolina at Chapel Hill; [2]UNC Kidney Center, Univ of North Carolina at Chapel Hill.*

**Background:** Almost half of patients with end-stage renal disease (ESRD) report chronic pain, and over three-quarters rate it moderate to severe. The etiology of the pain is multifactorial, often related to comorbidities, renal osteodystrophy, and complications of the dialysis procedure. Because non-steroidal anti-inflammatory drugs have adverse effects on renal function, opioids may be perceived as the preferred analgesic agents in this population. We sought to quantify the magnitude of opioid use among U.S. ESRD patients.

**Methods:** Using data from the United States Renal Data System (USRDS), a national registry of patients in the Medicare ESRD program, we identified patients ≥ 18 years receiving dialysis on January 1, 2008 with Medicare as a primary payer and parts A, B, and D coverage. Information on opioid use was ascertained from Medicare part D claims. We calculated the proportion of patients receiving opioid prescriptions. Patients were censored at: end of continuous enrollment in Medicare Parts A, B, and D; loss-to-follow-up; kidney transplantation; death; or end of study on June 30, 2008.

**Results:** During the 6-month study period, there were 335,888 opioid prescriptions. Of 156,476 eligible dialysis patients, 77,697 (50%) patients used an opioid. Hydrocodone, oxycodone, and propoxyphene were prescribed to 30%, 14%, and 10% of the study population, respectively. The study population was middle-aged (mean, 60 years), 52% male, 50% white, 43% black, and 8% low income subsidy status. Opioid use was higher among patients of younger age, female gender, black or white race, and without low income subsidy status. Opioid use varied by primary cause of ESRD, years on dialysis, and dialysis modality. In a separate analysis with similar eligibility criteria, quarterly opioid use increased slightly from quarter 3 of 2006 (38%) to quarter 4 of 2008 (39%).

**Conclusions:** We document widespread and increasing use of opioids in the U.S. ESRD population. Given increasing concerns about opioid safety in the elderly, research is needed to better understand the safety and effectiveness of opioids in ESRD patients.

## SA-PO487

**Depressive Affect in Incident Hemodialysis Patients Is Associated with Increased Hospital Days and Hospital Admissions** Kathryn A. McDougall, John W. Larkin, Len A. Usvyat, Rebecca L. Wingard, Eduardo K. Lacson, Franklin W. Maddux. *Fresenius Medical Care North America (FMCNA), Waltham, MA.*

**Background:** Depression is a key disorder that affects chronic dialysis patients. However, it is not consistently recognized and outcomes related to the disease have not been well-defined, particularly for the first 120 days in incident hemodialysis (iHD) patients. This study investigated the associations between depressive affect (DA) in iHD patients and hospital days/hospital admissions.

**Methods:** Among a random selection of 108 dialysis centers, 429 iHD patients in the first month of chronic outpatient dialysis at FMCNA clinics from Jan-Mar 2013 were identified and telephone contact was attempted for up to three times to administer the Patient Health Questionnaire 2 (PHQ2). The PHQ2 has two questions screening for DA in the previous two weeks. Scores range from 0-6 with a positive DA score defined as ≥3. Hospital admissions and clinical parameters were captured for 120 days after the patients initiated dialysis (up to 30 Apr 2013). Multivariate Poisson regression models were constructed to determine whether hospital days and hospital admissions differ for depressed patients.

**Results:** Among 172 screened iHD patients, DA was detected in 23.3%. Increased hospital days of 15.8 versus (vs) 7.4 days per patient year (ppy) occurred in DA positive vs non-DA patients. Hospital admissions were also greater with 2.9 vs 1.5 events ppy, respectively. Poisson regression identified DA as an independent predictor of hospital days ($p<0.001$) and a trending predictor of hospital admissions ($p=0.096$) even after adjustment for covariates typically associated with depression (figure 1).

Figure 1: Poisson regression outcomes

Poisson regression to predict of hospital days (offset = exp days)

| Parameter | Estimate | 95% LCL | 95% UCL | p-value |
|---|---|---|---|---|
| Intercept | -0.565 | -2.729 | 1.630 | 0.617 |
| Not DA [ref=DA Positive] | -0.587 | -0.833 | -0.345 | <.0001 |
| Age (years) | 0.012 | 0.021 | 0.041 | <.0001 |
| Male | -0.170 | -0.681 | -0.077 | 0.014 |
| Diabetic | 0.259 | 0.004 | 0.634 | 0.011 |
| Catheter | -0.143 | -0.407 | 0.121 | 0.289 |
| Hispanic | -0.983 | -1.420 | -0.546 | <.0001 |
| Race = White | 1.496 | 1.068 | 1.893 | <.0001 |
| BMI | 0.013 | 0.006 | 0.017 | <.0001 |
| Presence of Residual Renal Function | 0.425 | 0.080 | 0.770 | 0.016 |
| Number of Comorbidities | 0.059 | 0.009 | 0.090 | 0.000 |
| Albumin (g/dL) | -1.320 | -1.611 | -1.009 | <.0001 |
| Log of creatinine | 1.094 | 0.551 | 1.517 | <.0001 |
| Pre-dialysis SBP (mmHg) | -0.005 | -0.011 | 0.002 | 0.149 |
| IDWG (% of post weight) | 0.465 | 0.337 | 0.598 | <.0001 |
| URR (%) | 0.022 | 0.002 | 0.042 | 0.034 |

Poisson regression to predict of hospital admissions (offset = exp days)

| Parameter | Estimate | 95% LCL | 95% UCL | p-value |
|---|---|---|---|---|
| Intercept | 1.087 | -0.306 | 5.480 | 0.628 |
| Not DA [ref=DA Positive] | -0.451 | -0.883 | 0.080 | 0.096 |
| Age (years) | 0.023 | 0.001 | 0.046 | 0.044 |
| Male | -0.448 | -1.071 | 0.180 | 0.163 |
| Diabetic | 0.422 | -0.204 | 1.047 | 0.188 |
| Catheter | 0.289 | -0.313 | 0.892 | 0.351 |
| Hispanic | -0.543 | -1.354 | 0.269 | 0.190 |
| Race = White | 0.668 | -0.046 | 1.367 | 0.067 |
| BMI | 0.005 | -0.008 | 0.018 | 0.479 |
| Presence of Residual Renal Function | 0.304 | -0.299 | 1.008 | 0.397 |
| Number of Comorbidities | 0.215 | -0.007 | 0.038 | 0.178 |
| Albumin (g/dL) | -1.082 | -1.765 | -0.400 | 0.002 |
| Log of creatinine | 0.388 | -0.962 | 1.339 | 0.423 |
| Pre-dialysis SBP (mmHg) | -0.002 | -0.016 | 0.012 | 0.816 |
| IDWG (% of post weight) | 0.232 | 0.045 | 0.420 | 0.024 |
| URR (%) | -0.004 | -0.045 | 0.038 | 0.863 |

**Conclusions:** DA in iHD patients was significantly associated with higher hospital days after adjustment for covariates. Further studies are needed to elucidate if interventions that reduce DA improve outcomes.

*Funding:* Pharmaceutical Company Support - Fresenius Medical Care North America

## SA-PO488

**Increased Depression and Anxiety Are Associated with Decreased Adherence in ESRD Patients** Daniel Cukor,[1] Nisha Ver Halen,[1] Yvette Fruchter,[1] Subodh J. Saggi.[2] *[1]Psychiatry and Behavioral Science, SUNY Downstate Medical Center, Brooklyn, NY; [2]Medicine, SUNY Downstate Medical Center, Brooklyn, NY.*

**Background:** A significant relationship between depression and medical outcome has been demonstrated in ESRD patients on hemodialysis (HD). There may be a direct, neurobiological relationship between depression and medical outcome or the association may be mediated by adherence behaviors. The current study investigates depression, anxiety, and adherence at a Brooklyn dialysis center that serves a predominantly African American population.

**Methods:** Fifty-seven hemodialysis patients completed questionnaires during dialysis sessions. Depressive and anxious symptoms were assessed with the Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory (STAI), respectively. Higher scores on the BDI and STAI state and trait subscales represent increased severity. Adherence behaviors were measured by the End-Stage Renal Disease Questionnaire (ESRD-AQ) behavior subscale, a self-report questionnaire, concerning adherence with dialysis sessions, medications, fluid limitations, and dietary restrictions. Higher scores on the ESRD-AQ behavior scale represents better adherence.

**Results:** Depressive symptoms ($r= -.36$, $p = .01$), state anxiety ($r = -.38$, $p = .01$), and trait anxiety ($r= -.43$, $p=.003$) negatively correlated with adherence. Utilizing a regression, with a model correcting for age and gender, the Beck Depression Inventory explained 24.9% of the variance in adherence ($t=-2.84$, $p = .01$). Separate regressions were run with state and trait anxiety to examine their predictive abilities for adherence behaviors when correcting for age and gender. State anxiety explained 19.1% of the variance in adherence ($t= -2.07$, $p = .045$) and trait anxiety explained 25.7% of the variance in adherence ($t= -2.73$, $p = .01$).

**Conclusions:** Depression and anxiety play an important role in treatment adherence, a known predictor of medical outcome.

## SA-PO489

**NOTICE Initiative Post- versus Pre-Infection Control Evaluation (ICE) Results** Erik Roys, Natalie Scholz, Casey Parrotte, John Kalbfleisch, Rajiv Saran, Carol Chenoweth, Joseph M. Messana. *Univ of Michigan, Ann Arbor, MI.*

**Background:** The National Opportunity to Improve Infection Control in ESRD (NOTICE) project was designed to assess infection control practices at US dialysis facilities, to identify areas for quality improvement, and to reduce infections. Infection rates were measured before and after use of procedural and audit checklists, along with an on-site ICE.

**Methods:** Monthly NHSN vascular access-related infection (VAI), NHSN positive blood culture (PBC), and Medicare claims vascular access-related infection (ICD-9) data were collected from 34 facilities over 12 months with ICE visits occurring at 4-6 months. Generalized linear mixed models were used to assess changes in infection rates. Poisson models were used with log link and offset=log of the number of patients in the facility each month. Random effects for provider were included. The ICD-9 rates for NOTICE facilities were compared to controls matched on size, ownership, infection rates, and SES.

**Results:**

| Outcome | Pt mo. (Pre/Post) | Pre-ICE Visit Rate | Post-ICE Visit Rate | % Change | P-value |
|---|---|---|---|---|---|
| VAI | 134/185 | 1.42 | 0.98 | Post v Pre -28% | 0.01 |
| PBC | 134/185 | 0.88 | 0.69 | Post v Pre -17% | 0.23 |
| ICD-9 (NOTICE) | 162/240 | 2.53 | 2.12 | Post v Pre -18% | 0.03 |
| ICD-9 (NOTICE (N) & matched controls (C)) | 324/428 | N-2.53<br>C-2.31 | N-2.12<br>C-1.90 | Post vs. Pre -16%<br>N v C +9%<br>Interaction +1% | 0.04<br>0.65<br>0.92 |

The avg rates for all measures decreased from pre to post periods. VAI and ICD-9 rates were reduced by 28% and 18%, respectively. Differences were statistically significant ($p<0.03$). The reduction in PBC was not significant. When the change in ICD-9 rates were compared between NOTICE and control facilities, no difference was identified (RR=1.01, p=0.92).

**Conclusions:** Improvement in VAI and ICD-9 rates was statistically significant, and based on the pre post rates alone would suggest a marked improvement during the project. However, the ICD-9 rates showed similar declines in matched controls. The national decline in reported ICD-9 suggests that factors other than those included in NOTICE likely contributed to the pre-post changes reported here.

## SA-PO490

**Urgent-Start Peritoneal Dialysis: A Multicenter Clinical Trial** Arshia Ghaffari,[1,2] Tracey L. Milligan,[2] Mark H. Shapiro,[2,3] Michelle Cassin,[2] John E. Moran.[2,4] *[1]Nephrology, Univ of Southern California, Los Angeles, CA; [2]DaVita HealthCare Partners, Inc, Denver, CO; [3]Univ of California, San Diego, La Jolla, CA; [4]Nephrology, Stanford Univ School of Medicine, Stanford, CA.*

**Background:** Patients who present with advanced chronic kidney disease (CKD) in need of urgent dialysis are usually started on hemodialysis (HD), most commonly via a central venous catheter (CVC). Very few patients are given the option of peritoneal dialysis (PD) in this situation due to a number or issues, including nephrologists' unease and inexperience in urgently starting PD in unplanned, late-presenting patients. We initiated this study to demonstrate urgent-start PD can be safely applied in a protocol-driven, outpatient model.

**Methods:** Protocols were developed to standardize patient selection and management in the urgent-start period. Patients were provided with small-volume, in-center, supine PD thrice weekly less than two weeks after PD catheter placement. Catheters were placed by locally available methods.

**Results:** 181 patients (55% male, mean age 54 ± 16 years, 49.4% diabetic) started PD urgently at 36 facilities. 62.5% had received prior nephrology care. On average, PD was started in the outpatient facility 5.0 ± 3.2 days after PD catheter placement. 51 patients had a CVC placed and 50 had one or more HD treatments. 10 patients did not start PD within 14 days and were excluded from the analysis. With a mean follow-up of 6.8 months, 154 patients (90%) remain alive and on PD, 8 transferred to in-center HD (5 patient choice, 1 non-compliance, 1 bleeding during PD catheter insertion, 1 could not be trained for PD), 5 patients died (3 sudden cardiac deaths, 1 sepsis, and 1 liver cancer). 3 patients transferred to outside facilities while on PD, and 1 patient withdrew from dialysis.

**Conclusions:** This multi-center study demonstrates urgent-start PD can be broadly and successfully used in patients with advanced CKD in urgent need of dialysis in a structured, protocol-driven program. This management strategy allows patients the opportunity to choose PD as a modality. A major benefit is the avoidance of CVC use in many patients.

*Funding:* Pharmaceutical Company Support - DaVita HealthCare Partners, Inc

**SA-PO491**

**Peritoneal Dialysis First Policy of Thailand Brought about Much Higher Penetration Rate and Good Outcomes** Dhavee Sirivongs,[1] Piyatida Chuengsaman,[2] Siribha Changsirikulchai,[3] Adisorn Lumpaopong.[4] *[1]Renal Service Center, Srinagarind Hospital, Khon Kaen Univ, Khon Kaen, Thailand; [2]CAPD Service and Training Center, Banphaeo Hospital (Public Organization), Bangkok, Thailand; [3]Dept of Medicine, Srinakharinwirot Univ, Nakhon-Nayok, Thailand; [4]Phramongkutklao Hospital, Bangkok, Thailand.*

**Background:** Since October 2007, Thai government has provided PD first policy for non-health security population covering about 48 million people. This policy provides free of charge continuous ambulatory peritoneal dialysis(CAPD) for ESRD patients, so it has brought about rapidly increasing number of CAPD patients.

**Methods:** To analyze outcomes of CAPD treatment under the policy, we reviewed a first 60-month data from the 137 hospitals, 114 of them were new to CAPD, that recruited the patients during October 2007-September 2012.

**Results:** Total number of the registered patients was 19,464 cases, but only 15,211 cases actually received the treatment. Female to male ratio was 1:1.04, average age was 55.36 ± 14.3 years. Diabetes mellitus (DM) was found in 51.31%.Mean eGFR at the registered step was 8.01 + 5.0 ml/min. Drop-out rate was 3.47%: death in 30.10%, shift to HD 7.45 % and kidney transplantation 0.91%. Regarding causes of death, cardiovascular disease was the most common cause, only 10.96% of total death died from PD related peritonitis. The 1-year, 2-year, 3-year and 5-year patient survival rates were 82.1%, 66.3% 53.1% and 38.6% respectively. PD penetration was increased from 4% to 25% by 5 years of the policy. DM had significant negative impact to the survival ($p<0.05$).

**Conclusions:** PD first policy has markedly influence on patterns of treatment in Thailand, and saves a number of ESRD patients. Even most participating hospitals were new to CAPD, the outcomes of treatment are quite acceptable. The success of Thai PD-First policy is due to combination of strong policy, systematic manipulation, and good attitude of medical personnel to the treatment.

*Funding:* Government Support - Non-U.S.

**SA-PO492**

**Decreasing ESRD Patients' Hospital Length of Stay and Readmissions through Patient Centered Care Redesign Using Quality and Design Methods** Amy W. Williams, Kathryn Zavaleta, John J. Dillon, Mary Tibor, Susan Dornack, Mary Ann Ryan, Mark Nagele, Jerilyn Sue Wilson, Bradley D. Wick, Aaron Eckblad, Stephen F. Gudgell, Robert C. Albright. *Mayo Clinic, Rochester, MN.*

**Background:** In 2011 we began a quality and design methods approach to improve ESRD patient outcomes by creating high value patient-centered care across all settings. One aim was to decrease chronic dialysis patients' hospitalizations.

**Methods:** The multi-disciplinary team completed process mapping of current & future ideal states followed by small scale pilots and spread for in-center & home (PD & HD) dialysis patients (pts). Interventions included standardization of hospital care & hospital care team communication, roles and EMR tools, handoffs and transitions between care settings enhanced by consistent integrated flow of information, expansion of multidisciplinary care teams to include pharmacists and palliative care specialists, improved pt preference-focused education materials/decision aids for advanced CKD & ESRD, EMR alerts to all providers if GFR <25ml/min to consider CKD clinic referral and an evidence based practice CKD care algorithm electronically available for all providers. Hospitalization data was collected from the patients' medical records.

**Results:** The mean number of chronic dialysis pts per month cared for in the system from 2011 through YTD 2013 was 420.67 ± 15.50 pts/month. The number of in-hospital dialysis runs represented by quarter 1 data decreased each year: 2996 in 2011, 2032 in 2012, 1734 in 2013 (differences: 2011 vs. 2013, -1262 (-72.78%), 2012 vs. 2013, -298 (-14.67%)). Although end of year 2011 vs. 2012 data showed increased admission episodes in 2012 (508 vs. 543, +35 admissions (+6.89%)), length of stay decreased from 5.99 to 5.21 days (-0.78 days, -13.02%, $p=0.041$) and total pt hospital days decreased from 3043 to 2831 (-212 days, -6.97%). Total number of 30-day readmissions decreased 39.45% from 2011 to 2012 (difference in proportion of pts 23.6%, $p=0.000$).

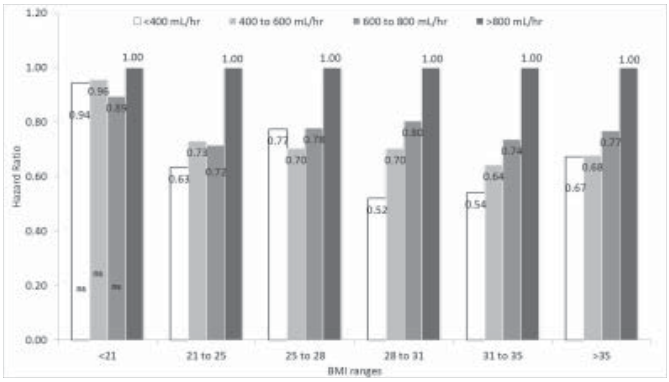
**Conclusions:** The use of quality and design methodology to redesign ESRD pt care to improve transitions of care and enhance pt focused care led to decreased total hospital days, length of stay and 30 day readmissions.

**SA-PO493**

**Higher Ultrafiltration Rates Are Associated with Greater Mortality at Higher BMI Levels** Len A. Usvyat,[1] Peter Kotanko,[2] Franklin W. Maddux,[1] Eduardo K. Lacson.[1] *[1]FMCNA, Waltham, MA; [2]RRI, NY, NY.*

**Background:** Higher ultrafiltration rate (UFR) during HD may be associated with poor survival. With the strong negative correlation b/n UFR and BMI observed by our group, we evaluated whether differences in survival persist if UFR is calculated in mL/hr within strata of BMI.

**Methods:** We studied incident HD patients in FMCNA who initiated dialysis b/n Jan 1, 2010 and Jan 31, 2013, tracking mortality until May 1, 2013. Only patients who survived first 90 days and with Kru<0.1 ml/min at initiation were included. The average UFR (in mL/hr) was computed in first 90 days. Patients were stratified into 4 groups of baseline UFR: <400, 400 to 600, 600 to 800, and >800 mL/hr and into 6 groups of BMI: <21, 21 to 25, 25 to 28, 28 to 31, 31 to 35, and >35 kg/m$^2$. Stratified Cox models adjusted for age, gender, DM status, race, ethnicity, and albumin levels were constructed to assess survival after the first 90 days in these 4 groups of UFR; in each analysis, UFR>800 was ref group.




**Results:** The study cohort consisted of 49,617 patients with mean age of 62.8±15.0, 54% male, 64% white, 63% DM and median survival follow-up of 304 (range 1-1126) days. All subgroups had >1000 patients except the group with BMI<21 and UFR of 800ml/hr (N=386). Except in patients with BMI<21, patients with UFR>800 mL/hr had consistently poorer outcomes than lower UFR ranges. Within each range of BMI>28, there was a gradient increase in the risk of death with increasing UFR (figure 1).

**Conclusions:** Higher UFR (in mL/hr) is associated with poorer outcomes at BMI>21 and there appears to be effect modification associated with BMI. Therefore, indexing UFR to body size requires further exploration, particularly with regards to gender/race. Although higher UFR is associated with worse mortality, the ultimate form and optimal threshold remains to be determined in future clinical trials.

**SA-PO494**

**Serum Phosphorus, Hemodialysis Social Networks, and Mortality Prediction** Avrum Gillespie,[1] Vladimir Ouzienko,[2] Heather Hammer,[3] Zoran Obradovic.[2] *[1]Nephrology, Hypertension, and Kidney Transplantation, Temple Univ School of Medicine, Philadelphia, PA; [2]Center for Data Analytics and Biomedical Informatics, Temple Univ, Philadelphia, PA; [3]SRBI, Silver Springs, MD.*

**Background:** Although the social diffusion and effects of healthy and unhealthy behaviors have been observed in studies of other chronic medical conditions, the characteristics of end stage renal disease patient social networks have yet to be well defined. Our study uses dietary and medication adherence measured by serum phosphorus correlations to demonstrate the existence of social links among hemodialysis patients and improve the accuracy of a mortality prediction model based on survey and medical record data.

**Methods:** A sample of 116 predominately African American patients receiving hemodialysis at two outpatient clinics in Philadelphia was divided into eight distinct groups based on the time and day of treatment. Patient interactions within groups were analyzed with social network methods, and a novel mortality prediction model was developed based on the discovered social links. Experiments validated the discovered social links and confirmed the contribution of the social network information to the accuracy of mortality prediction.

**Results:** Six of the eight social networks based on serum phosphorus were statistically significant with the highest network density (0.24) found in the Monday, Wednesday, Friday afternoon shift (t-value 5.97, Confidence 99.9%). Comparing the accuracy of our model to five alternative models showed that incorporating the social network data yielded the greatest predictive accuracy with an Area Under the Curve of 0.67 ± 0.02.

**Conclusions:** To the best of our knowledge ours is the only study to have modeled patient social networks within a hemodialysis clinic. We use phosphorus correlations to demonstrate the existence of social networks. Furthermore, we show that social link information improves mortality prediction.

**SA-PO495**

**Health-Related Quality of Life by Hemoglobin Status in Chronic Kidney Disease Patients Receiving Hemodialysis from 2010 to 2012** Carly J. Paoli,[1] Emmanuel A. Anum,[2] Akhtar Ashfaq,[1] Chris Evans,[3] Thomas Alfieri,[2] Matthew Gitlin.[1] *[1]Amgen, Thousand Oaks, CA; [2]DaVita Clinical Research, Minneapolis, MN; [3]Endpoint Outcomes, Boston, MA.*

**Background:** In 2011, reimbursement and regulatory changes in dialysis care altered treatment patterns and patient outcomes, possibly affecting health-related quality of life (HRQOL). This study examines HRQOL from 2010-2012.

**Methods:** This retrospective cohort study analyzed adult CKD patients on hemodialysis in a large US dialysis organization who completed the Kidney Disease Quality of Life (KDQOL)-36 survey in Quarter 1 of 2010, 2011, & 2012. Item scores were converted to a discrete scale from Likert-style responses and averaged over the cohort. Mean scores were stratified by Hemoglobin (Hb) status (Table) and assessed over the entire cohort (data not shown) and in patients whose Hb declined by ≥1g/dL (defined a priori) from 2010-2012.

**Results:** The cohort had 1017 patients; mean 2010 age was 60 yrs, vintage 3.70 yrs and 47% female. Mean(SD) Hb levels (g/dL) declined over time: 11.9(1.2) in 2010; 11.5(1.1) in 2011, 10.8(1.1) in 2012. Overall, patients had no changes in HRQOL, but for the 453 patients (45%) with Hb decline ≥1g/dL, mean scores by Hb category declined for many anemia-related items, items with most change shown in Table 1.

| Mean KDQOL-36 Item Scores: Patients With Hb Decline ≥1g/dL (n=453) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **KDQOL-36 Item #** | **2** | **3** | **6** | **19** | **22** | **30** | **32** |
| **Mean** | **52.4** | **43.7** | **71.1** | **73.7** | **82.6** | **70.8** | **60.2** |
| **<8(n=0)** | | | | | | | |
| **≥8<9(n=0)** | | | | | | | |
| **≥9<10(n=1)** | 100 | 100 | 100 | 75 | 100 | 100 | 25 |
| **≥10<11(n=26)** | 42.3 | 42.3 | 53.8 | 76.9 | 76 | 69.2 | 58.7 |
| **≥11<12(n=144)** | 53.1 | 41.7 | 72.9 | 71.7 | 83.2 | 71.2 | 62.5 |
| **>12(n=282)** | 52.8 | 44.7 | 71.6 | 74.5 | 82.8 | 70.6 | 59.3 |
| **2012** | | | | | | | |
| **Mean** | **47.9** | **40.3** | **66.2** | **77.3** | **78.8** | **74.5** | **64.2** |
| **<8(n=2)** | 25 | 25 | 50 | 87.5 | 62.5 | 75 | 62.5 |
| **≥8<9(n=34)** | 44.1 | 33.8 | 64.7 | 86.8 | 73.5 | 78.7 | 75 |
| **≥9<10(n=135)** | 44.1 | 37 | 67.4 | 78.3 | 74.1 | 75 | 67.2 |
| **≥10<11(n=190)** | 49.7 | 41.8 | 63.7 | 74.2 | 82.1 | 72.5 | 62.2 |
| **≥11<12(n=87)** | 51.1 | 43.7 | 69 | 77.9 | 80.7 | 77 | 59.8 |
| **>12(n=5)** | 60 | 60 | 100 | 90 | 85 | 65 | 65 |
| Higher KDQOL-36 scores indicate better HRQOL. | | | | | | | |

**Conclusions:** Overall, HRQOL did not change from 2010-2012 in this cohort, but in the sub-sample of patients (45%) who had Hb declines ≥1 g/dL, HRQOL decrements were observed in several anemia-related items.

*Funding:* Pharmaceutical Company Support - Amgen, Inc.

## SA-PO496

**Associations of Race, Obesity and Diabetes on Risk of Attrition from the Kidney Transplant Waiting List** John C. Sieverdes,[1] David J. Taber,[2] Titte Srinivas,[2] John McGillicuddy,[2] Frank Treiber,[1,2] Kenneth Chavin,[2] Prabhakar Baliga.[2] *[1]College of Nursing, Medical Univ of South Carolina, Charleston, SC; [2]College of Medicine, Medical Univ of South Carolina, Charleston, SC.*

**Background:** This study examined the associated variables related to why patients become inactive on the kidney transplantation waiting list.

**Methods:** Between January 2006 and December 2010, 4,494 referrals to the center for evaluation for transplantation wait-list suitability were cross-sectionally examined.

**Results:** Thirty-four percent (n=1,531) who met eligibility requirements were placed on the waiting list. Follow-up analyses revealed that 489 (32%) patients subsequently had their status changed from active to inactive. Multiple medical comorbidities was the predominant factor for removal from the wait list (26%), followed by socioeconomic status (18%), and cardiovascular events (16%)(Figure 1). Binary logistic regression demonstrated that age at referral (p=0.05), diabetes status (p=0.04), marital status (p=0.02), and education (p=0.05) were independently associated with wait list inactivity. African American race (odds ratio [OR]: 1.34 [CI: 1.08-1.66]) and obesity (BMI ≥ 30 kg/m$^2$) (OR 1.60 [CI: 1.27-2.03]) were risk factors for having diabetes. Patients with diabetes had a 41% increased risk of becoming inactive (OR: 1.41 [95% CI: 1.31-1.77]), twice as likely to be inactive due to cardiovascular events (OR: 2.08 [CI: 1.27-3.41]), and 73% more likely to die while waiting for a kidney (OR: 1.73 [CI: 1.13-2.66]).

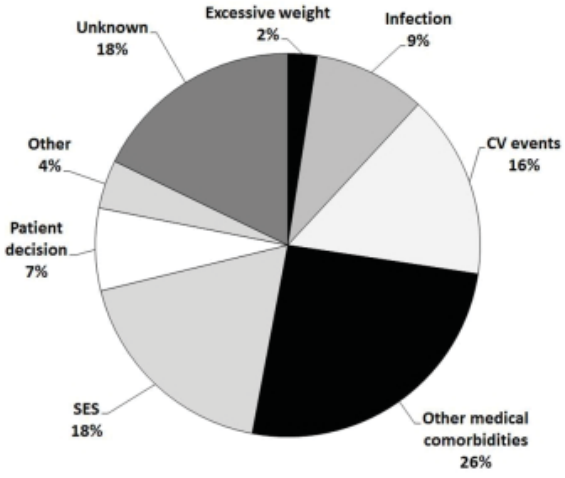



**Figure 1. Proportions for becoming inactive on the transplant list (n=489)**

**Conclusions:** These results demonstrate that obesity and race, which are well known risk factors for the development of diabetes, may indirectly increase the risk of being listed inactive or dying before receiving a kidney transplant. Future research should be focused on developing interventions to modify obesity rates in multiple socioeconomic and age-related areas.

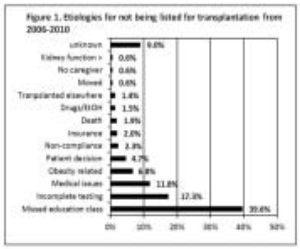
## SA-PO497

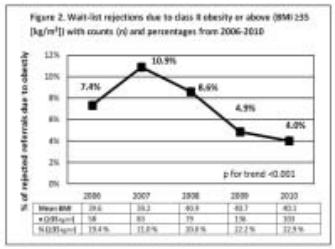
**Obesogenic Associations among End-Stage Renal Disease Patients Not Listed for Kidney Transplantation** John C. Sieverdes,[1] David J. Taber,[2] Kenneth Chavin,[2] John McGillicuddy,[2] Titte Srinivas,[2] Frank Treiber,[1,2] Prabhakar Baliga.[2] *[1]College of Nursing, Medical Univ of South Carolina, Charleston, SC; [2]College of Medicine, Medical Univ of South Carolina, Charleston, SC.*

**Background:** Rising obesity rates may be causing kidney transplant centers to shift their allowable BMI thresholds, which is a potential concern for successful outcomes. The purpose of this study is to examine the reported reasons end-stage renal disease (ESRD) referrals are not being listed for transplantation and to describe the rates of obesity at a large academic transplant program in the southeastern United States.

**Methods:** Between January 2006 and December 2010, there were 4,494 referrals who underwent evaluation for potential transplantation; 34% of which met eligibility and were placed on the waiting list, leaving 2,961 included in this cross-sectional analysis.

**Results:** The four predominant etiologies cited for not being initially listed over the 5-year period included missed education classes (39.5%), incomplete testing (17.3%), medical issues (11.8%), and obesity (6.8%) (Figure 1). Overall, the analysis demonstrated that even though a higher percentage of patients were presenting with class II obesity or above (body mass index (BMI) ≥ 35) in 2010 compared to 2006 ($X^2$,p=0.03), fewer patients were being rejected due to their BMI (p for trend <0.001) (Figure 2).




**Conclusions:** These results imply that the transplant community is extended the boundaries of weight-suitability in response to the obesity epidemic. Future studies are warranted to determine how this is impacting perioperative outcomes, quality of life, and graft and patient survival rates after transplant. Interventions promoting active lifestyle changes and weight management for ESRD patients should be used to manage these modifiable risk factors.

## SA-PO498

**Active Vitamin D and Nutrition Are Predictors of Infectious Diseases in Secondary Hyperparathyroidism of Uremia (SHPT) on Maintenance Hemodialysis Patients: Results from the MBD-5D Study** Yasuo Imanishi,[1] Ikue Kobayashi,[1] Masaaki Inaba,[1] Masafumi Fukagawa,[2] Tadao Akizawa,[4] Shunichi Fukuhara.[3] *[1]Osaka City Univ, Japan; [2]Tokai Univ, Japan; [3]Kyoto Univ, Japan; [4]Showa Univ, Japan.*

**Background:** Although nutrition is a significant predictor of mortality in dialysis patients, little is known about the role of nutrition in infectious diseases. This study examined the combination effects of nutritional status and intravenous (iv) vitamin D receptor activator (VDRA) administration on infectious diseases.

**Methods:** The Mineral and Bone Disorder Outcomes Study for Japanese CKD Stage 5D Patients (MBD-5D) is a multicenter, prospective observational study of hemodialysis patients with SHPT in Japan. This study is a subcohort of MBD-5D, and 2,022 prevalent patients, who were receiving hemodialysis for more than 3 months and met at least one of the following conditions: having intact PTH levels >180 pg/ml, or receiving iv vitamin D receptor activators (VDRAs) or oral VDRA analog, were employed. Nutritional status was assessed by Geriatric Nutritional Risk Index (GNRI) which was calculated using serum albumin, dry weight and ideal body weight. Patients were categorized into quartiles by GNRI and assessed the association between GNRI and infection related mortality or hospitalization.

**Results:** At follow-up time of 36 months, highest risks for both infection-related mortality and hospitalization were observed in the 1st GNRI quartile (poorest nutritional status). Time dependent Cox regression model revealed hazard ratio (HR) for the 1st GNRI quartile was 6.22 in mortality (95% confidence interval, 1.58- 24.45), and 1.85 in hospitalization (1.13- 3.02) in the iv VDRAs non-receiving group compared to the 4th GNRI quartile. In the iv VDRAs receiving group, these associations were not observed in mortality (HR 1.67, 0.83- 3.36) or in hospitalization (HR 1.07, 0.68- 1.67).

**Conclusions:** These results demonstrated that GNRI is a significant predictor for mortality in CKD-5D patients with SHPT. Administration of iv VDRA may have a preventive effect on the incidence of both infection related mortality and hospitalization in the patients with poor nutritional status.

*Funding:* Pharmaceutical Company Support - The MBD-5D study is supported by research grants from Kyowa Hakko Kirin co.,Ltd. without restrictions on publications.

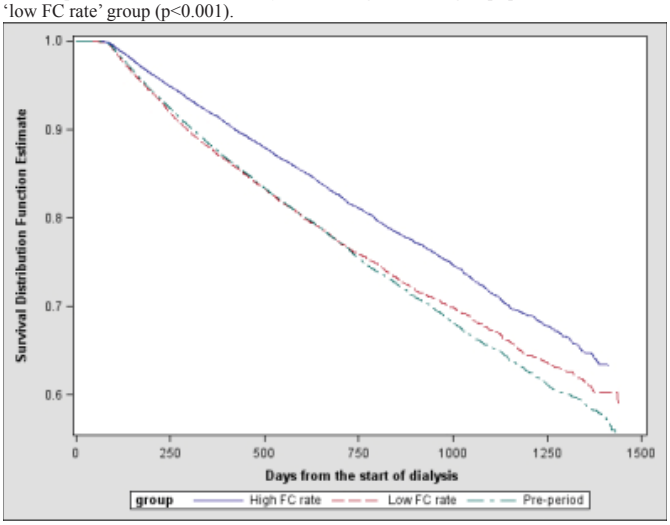
## SA-PO499

**Improvements in Survival and Major Amputations Observed with the Implementation of a Foot Check Program in Diabetic Hemodialysis Patients**
Andreja Marn-pernat,[1] Vanja Persic,[1] Len A. Usvyat,[2,3] John Rogus,[3] Franklin W. Maddux,[3] Eduardo K. Lacson,[3] Peter Kotanko.[2] *[1]Univ Medical Center, Ljubljana, Slovenia; [2]RRI, NY, NY; [3]FMCNA, Waltham, MA.*

**Background:** Diabetic HD patients (pts) are at high risk of lower limb amputations. In order to improve foot care in diabetic HD pts, monthly intra-dialytic foot checks [FC] were implemented as part of a standard clinic operating procedure in FMCNA clinics in January 2008 ['foot check program implementation date']. We aim to understand whether regular FCs are associated with reductions in major lower limb amputations and survival.

**Methods:** We compared incident diabetic HD pts before [pre-period;between 1/2004 and 12/2007] and after [post-period; between 1/2008 and 12/2011] FC program implementation. In post-period, we divided patients into those who are in clinics performing FC on >1/15 [high FC rate] or ≤1/15 [low FC rate] HD tx in diabetic pts. We employed Kaplan-Meier analysis to evaluate time to death. Poisson regression was constructed with # of major amputations.

**Results:** We studied 37624 pts in pre-period and 40651 pts in post-period. In latter cohort, 29335 were in 'high FC rate' group and 11316 were in 'low FC rate' group. Compared to pre-period, all-cause mortality decreased by 25% in 'high FC rate' group and 3% in 'low FC rate' group (overall log-rank test p<0.001, figure). The major lower limb amputation rate was reduced by 18% in 'high FC rate' group (p<0.001) and 16% in 'low FC rate' group (p<0.001).




**Conclusions:** Frequent FCs are associated with reduction of all-cause mortality and major amputations in diabetic HD pts (less frequent FC are associated with notably smaller reduction in mortality). Implementation of intra-dialytic FCs has a potential of saving lives and preventing major lower limb amputations. Further analyses should also check for overall reductions in mortality and amputation rates.

## SA-PO500

**Candidate Gene Analysis of Mortality in Dialysis Patients**
Gurbey Ocak,[1] Jeffrey J.W. Verschuren,[2] Carolien Rothuizen,[3,4] Friedo W. Dekker,[1] Ton J. Rabelink,[3,4] J.wouter Jukema,[2] Joris I. Rotmans.[3,4] *[1]Clinical Epidemiology, Leiden Univ Medical Center, Netherlands; [2]Cardiology, Leiden Univ Medical Center, Netherlands; [3]Nephrology, Leiden Univ Medical Center, Netherlands; [4]Einthoven Laboratory for Experimental Vascular Medicine, Leiden Univ Medical Center, Netherlands.*

**Background:** Dialysis patients have high mortality risks with cardiovascular mortality as an important cause of death. The aim of this study was to investigate the association between SNPs involved in vascular processes and mortality in a large population of incident dialysis patients.

**Methods:** We followed 1330 incident dialysis patients in which 42 SNPs in 25 genes involved in vascular processes (endothelial function and vascular remodeling, growth factors, inflammation, coagulation, and calcium/phosphate metabolism) were genotyped. Cox regression analysis was used to investigate the effect of these SNPs on five-years mortality.

**Results:** The mortality rate was 114 per 1000 person-years for the 1330 dialysis patients. We showed that vascular endothelial growth factor rs699947 (hazard ratio (HR) 1.48, 95% CI 1.14-1.92), vitamin D receptor rs2238135 (HR 0.58, 95% CI 0.35-0.94), interleukin 6 rs1800795 (HR 1.32, 95% CI 1.00-1.74), lymphotoxin alpha rs1799964 (HR 0.60, 95% CI 0.37-0.98) and CD180 rs5744478 (HR 2.27, 95% CI 1.07-4.79) were associated with an increased mortality risk.

**Conclusions:** In this large cohort of dialysis patient, we found that three SNPs related to inflammatory processes, one SNP involved in endothelial function, and one SNP related to vitamin D metabolism were associated with an increased mortality risk. This study provides further evidence for an important role of these processes in the comorbid conditions of dialysis patients. Future studies are warranted to unravel the underlying mechanisms responsible for the increased mortality in these patients.

## SA-PO501

**A Comorbidity Index for Outcome Analysis in Chinese Patients with End-Stage Renal Disease**
Jinn-Yang Chen. *Div of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan.*

**Background:** Chinese end-stage renal disease (ERSD) patients have different comorbidity patterns than Caucasian ESRD patients. We focused this study on the differential effects of these comorbidities on mortality in different populations and different dialysis modalities.

**Methods:** Records for patients who aged 20 years and older, started dialysis therapy between January 2006 and December 2009, and had received dialysis treatment for more than 90 days were reviewed for our study. Model was developed separately in peritoneal dialysis (PD) and hemodialysis (HD) patients. Integer weight of comorbid condition was derived from coefficient estimates of Cox regression for all-cause mortality, and the index was internally validated by data-splitting in 1000 simulations. The performance in discrimination, calibration and reclassification were assessed.

**Results:** 34371 patients were recruited for this study. Predictors for mortality and the weight for individual comorbid condition differed between PD and HD patients. Our index demonstrated the best model fit statistics and overall predictive ability in PD and HD patients. Although USRDS index did slightly better in discrimination (*c*-statistic = 0.644 and 0.766 in PD and HD), our index had the best calibration ability in PD patients (H-L test, $X^2 = 11.54$, $P = 0.17$) among 3 indices.

| | | AIC | $R^2$ | *c*-statistics | H-L Test |
|---|---|---|---|---|---|
| PD | Taiwan Index | 9093.74 | 0.457 | 0.630 | $X^2$=11.54, $P$=0.17 |
| | USRDS Index | 9148.4 | 0.431 | 0.644 | $X^2$= 59.07, $P$<0.0001 |
| | Charlson Index | 9108.83 | 0.451 | 0.636 | $X^2$= 33.61, $P$<0.0001 |
| HD | Taiwan Index | 163054.31 | 0.303 | 0.759 | $X^2$= 77.28, $P$<0.0001 |
| | USRDS Index | 163310.43 | 0.292 | 0.766 | $X^2$= 101.43, $P$<0.0001 |
| | Charlson Index | 163296.15 | 0.295 | 0.770 | $X^2$= 86.23, $P$<0.0001 |

Compared to USRDS index, our index significantly reclassified more patients to the right risk categories, with net reclassification improvements of 26.33 % ($P < 0.0001$) in PD and 14.57 % ($P < 0.0001$) in HD patients.

**Conclusions:** Our index improved the risk prediction for mortality in Chinese ESRD patients than other indices, especially in PD patients. External validation of this index is required.

*Funding:* Government Support - Non-U.S.

## SA-PO502

**A Propensity Score-Based Comparison of Mortality in Patients on Hemodialysis and Peritoneal Dialysis in Korea: A National Population-Based Study**
Dong-Ryeol Ryu,[1] Hyunwook Kim,[2] Jung-hwa Ryu,[1] Seung-Jung Kim,[1] Duk-Hee Kang,[1] Kyu Bok Choi.[1] *[1]Dept of Internal Medicine, School of Medicine, Ewha Womans Univ, Seoul, Korea; [2]Dept of Internal Medicine, Wonkwang Univ College of Medicine Sanbon Hospital, Gunpo, Korea.*

**Background:** Given a lack of prospective randomized controlled comparison, there have been many debates whether the choice of dialysis modality affects mortality rate of end-stage renal disease (ESRD) patients. In this study, we aimed to compare survival of incident dialysis patients between hemodialysis (HD) and peritoneal dialysis (PD) using the Health Insurance Review & Assessment Service (HIRA) database, with the intention for a complete survey.

**Methods:** We included 32,280 adults who initiated dialysis from January 1, 2005 to December 31, 2008 and followed up (median 26.5 months, range of 3-59 months). An intention-to-treat analysis was performed both in a propensity score-matched cohort and in propensity score-adjusted multivariate model.

**Results:** We matched 7,049 patient pairs with similar propensity scores from each modality and compared survival rate, and found that there was a significantly higher mortality rate in PD patients compared to HD patients (log-rank test, $P < 0.001$). In addition, propensity score-adjusted multivariate Cox regression analysis including the entire participants was performed and showed a similar pattern (PD vs. HD, hazard ratio, 1.23; 95% confidence interval, 1.17-1.29; $P < 0.001$). Subsequent subgroup analyses according to various comorbidities revealed that in older patients (≥ 55 years), except only for the subgroup of patients having no comorbidities, PD was consistently associated with a higher mortality rate, whereas, in younger patients (< 55 years), regardless of comorbidities, the survival rate of PD patients was comparable to that of HD patients.

**Conclusions:** While overall mortality rate was higher in PD patients, mortality rate of PD patients aged over 55 years with no comorbidities or those aged less than 55 years was comparable to that of HD patients.

## SA-PO503

**Asymmetric Dimethyl-Arginine (ADMA), Race, and Mortality in Hemodialysis Patients** David A. Drew,[1] Hocine Tighiouart,[1] Tammy Scott,[1] Kristina Lou,[1] Li Fan,[1] Saeed Kamran Shaffi,[1] Mario Plebani,[2] Daniel E. Weiner,[1] Mark J. Sarnak.[1] *[1]Tufts Medical Center; [2]Univ Hospital, Padova-Italy.*

**Background:** Asymmetric dimethylarginine (ADMA) levels are elevated in kidney disease and associated with mortality in studies evaluating predominantly Caucasian and European hemodialysis (HD) populations. No studies have explored whether this association extends to African American (AA) hemodialysis patients.

**Methods:** ADMA levels were measured at baseline in 258 maintenance HD patients. Multivariable Cox proportional hazards models were used to evaluate the association of ADMA to all-cause mortality. An interaction between race and ADMA level was evaluated and subgroup analyses were performed.

**Results:** The mean (SD) age was 63 (17) years, 54% were women and 22% were African American, with the remaining participants predominantly Caucasian. Mean (SD) ADMA level in non-AA patients was 0.79 µmol/L (0.16) vs 0.70 µmol/L (0.11) in A-As (p <0.001). 132 deaths occurred over median follow up of 2.3 years, with death rates of 23.4 and 7.0 per 100 person years among non-AA and AA, respectively. Higher ADMA levels were associated with an increased risk of all-cause mortality (adjusted HR [95%CI] =1.24 per SD increase [1.03, 1.49]). An interaction was found between AA race and ADMA level (p value = 0.01), such that, when analyzed by race, higher ADMA levels were associated with increased mortality in non-AAs (adjusted HR =1.34 [1.10, 1.63]) but trended towards protective in AAs (adjusted HR =0.51 [0.25, 1.04]).

Association between 1 SD increase (0.16 µmol/L) in ADMA level and risk for all-cause mortality

| | Unadjusted HR (95% CI) | Model 1* HR (95% CI) | Model 2** HR (95% CI) |
|---|---|---|---|
| Total Cohort | 1.25 (1.06, 1.48) | 1.28 (1.07, 1.53) | 1.24 (1.03, 1.49) |
| African American | 0.65 (0.30, 1.44) | 0.66 (0.31, 1.40) | 0.51 (0.25, 1.04) |
| non-African American | 1.20 (1.01,1.42) | 1.33 (1.11, 1.60) | 1.34 (1.10, 1.63) |

*Model 1: Adjusted for age and sex
**Model 2: Adjusted for age, sex, dialysis vintage, cause of ESRD, vascular access type, history of cardiovascular disease, Kt/V, and albumin

**Conclusions:** African Americans have lower ADMA levels in comparison with non African Americans. High ADMA was a risk factor for mortality only in non African Americans. Given historically better survival among AA dialysis patients, these results need to be explored in larger, racially diverse studies, which will be able to investigate potential etiologies for this relationship.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO504

**Health Related Quality of Life in the Saudi Arabia Dialysis Outcomes and Practice Patterns Study (DOPPS)** Mohammed A. Al-Ghonaim,[1] Lindsay Zepel,[2] Jamal S. Alwakeel,[1] Ayman Karkar,[4] Fayez F. Alhejaili,[5] Haroun Zakaria Ahmed,[3] Saeed Mohammed G Al-ghamdi,[6] Faissal A. Shaheen,[3] Ronald L. Pisoni,[2] E.L. Cope,[2] Brian Bieber,[2] Bruce M. Robinson.[2,7] *[1]King Saud U.; [2]Arbor Research; [3]SCOT; [4]Kanoo Kidney Center; [5]King Fahad Nat. Guard Hosp.; [6]King Faisal Specialist Hosp.; [7]UofM.*

**Background:** This study provides the first comparison of quality of life and depression in hemodialysis (HD) patients (pts) in Saudi Arabia (SA) with that in 12 other countries in DOPPS 4 (2009-2011).

**Methods:** SA dialysis facilities (N=20) were randomly chosen to represent different SA regions, facilities, and types of SA pts. Physical component summary (PCS), mental component (MCS) summary and kidney disease burden (KDB) scores were based on patient responses to the Kidney Disease Quality of Life survey. The Center for Epidemiologic Studies Depression (CES-D) survey assessed patient-reported symptoms of depression.

**Results:** Among SA pts (N=318), mean age was 54 years, 58% were male. Age-adjusted mean MCS (44.1) and PCS (36.7) scores were similar to pts in other DOPPS countries. In SA: (1) PCS scores were higher among pts of younger age and male gender; diabetes, coronary heart disease, and catheter use related to lower PCS (2) MCS did not differ by gender but was higher for married pts, and was lower for pts >65 yrs old. Age-adjusted KDB scores in SA were among the highest in all 12 DOPPS countries, indicating a lower perceived burden of kidney disease upon a pt's life. In SA, being married was strongly related to higher KDB scores. Physician diagnosed depression (18%) and patient-reported symptoms of depression (42%) were similar in SA to most other DOPPS countries.

**Table 1a:** Saudi Arabia DOPPS: patient characteristics by age group

| Characteristic (mean ± SD) or % | Age (years) 18-45 | 46-55 | 56-65 | > 65 | All |
|---|---|---|---|---|---|
| Patients, N (%) | 93 (29) | 75 (24) | 72 (23) | 78 (25) | 318 |
| QoL measure | | | | | |
| PCS | 42.4 ± 11.2 | 39.5 ± 10.3 | 35.6 ± 9.7 | 33.4 ± 11.3 | 37.9 ± 11.2 |
| MCS | 44.0 ± 11.5 | 46.0 ± 12.3 | 44.7 ± 13.6 | 41.7 ± 12.2 | 44.1 ± 12.4 |
| KDB score | 43.6 ± 26.9 | 49.5 ± 26.4 | 44.8 ± 25.6 | 40.2 ± 23.3 | 44.4 ± 25.7 |
| Depression categories (%) | | | | | |
| Patient-Reported (CES-D ≥ 10) | 38 | 43 | 42 | 46 | 42 |
| Physician-Diagnosed | 16 | 19 | 18 | 22 | 18 |
| *Patient-Reported Pruritus (%) | 42 | 41 | 46 | 44 | 43 |

*Moderately to extremely bothered by itchy skin

**Table 1b:** Association between patient characteristics and patient-reported outcomes

| Characteristic | MCS score[A] | PCS score[A] | KDB score[A] | OR CESD ≥ 10 (vs. CESD <10)[B] |
|---|---|---|---|---|
| Male (vs. female) | +2.4 | **+4.9**** | +4.2 | 0.53 |
| Married (vs. not married) | **+3.5*** | +2.0 | **6.0*** | 0.71 |
| Vintage, years on dialysis (vs. 1-3) | | | | |
| < 1 | -4.5 | -0.6 | -7.3 | **2.05*** |
| 3-6 | -3.0 | **-4.8**** | -3.0 | 1.30 |
| > 6 | **-4.8*** | -0.5 | **-7.3*** | 0.93 |
| Diabetes (yes vs. no) | -0.7 | **-4.8**** | -0.7 | 1.13 |
| Coronary heart disease (yes vs. no) | -1.6 | **-3.2*** | -6.1 | 0.86 |
| Congestive heart failure (yes vs. no) | -3.9 | -2.6 | -0.7 | 0.84 |
| Serum creatinine > 9.4 mg/dl (vs. ≤ 9.4 mg/dl) | +1.6 | **+2.8*** | +4.9 | 1.17 |
| Hemoglobin > 11 g/dl (vs. ≤ 11 g/dl) | +0.5 | +1.0 | +1.8 | 0.86 |
| Catheter (vs. AVF or graft) | +1.4 | **-3.7*** | -2.4 | 1.29 |
| Treatment time > 208 minutes (vs. ≤ 208 minutes) | -0.4 | -0.03 | -3.2 | 0.57 |

All models adjusted for age and account for facility clustering; mean vintage in SA was 5.3 yrs.
[A]Estimates were obtained from separate linear mixed models with each QoL measure as the outcome; estimate indicates difference in score from referent group
[B]Odds ratios were obtained from separate logistic models; odds ratios indicate likelihood of depression (score ≥10) compared with the referent group
*0.01 ≤ p < 0.05; **p <0.01

**Conclusions:** Health related quality of life was similar in SA pts versus pts in 12 other DOPPS countries, despite notable differences in pt characteristics and HD practices across countries.

*Funding:* Pharmaceutical Company Support - The DOPPS is supported by research grants from Amgen (founding sponsor, since 1996), Kyowa Hakko Kirin (since 1999, in Japan), AbbVie (since 2009), Sanofi Renal (since 2009), Baxter Healthcare (since 2011), and Vifor Fresenius Medical Care Renal Pharma (since 2012), with additional country-specific support provided in Canada by Amgen-Canada, Janssen, BHC Medical, Takeda and Kidney Foundation of Canada, and in Germany by Hexal and WiNe Institute. Support from the DOPPS sponsors is provided without restrictions on publications.

## SA-PO505

**Survival among Elderly versus Younger Maintenance HD Patients – Results from the International MONDO Consortium Database** Matan J. Cohen,[1] Daniele Marcelli,[2] Len A. Usvyat,[3] Cristina Marelli,[4] Michael Etter,[5] Peter Kotanko,[3] Yosef S. Haviv,[1] Mondo Consortium.[3] *[1]Hadassah-Hebrew Univ Medical Center, Jerusalem, Israel; [2]Fresenius Medical Care-Germany; [3]Renal Research Institute, NY; [4]Fresenius Medical Care-Argentina; [5]Fresenius Medical Care-Asia Pacific.*

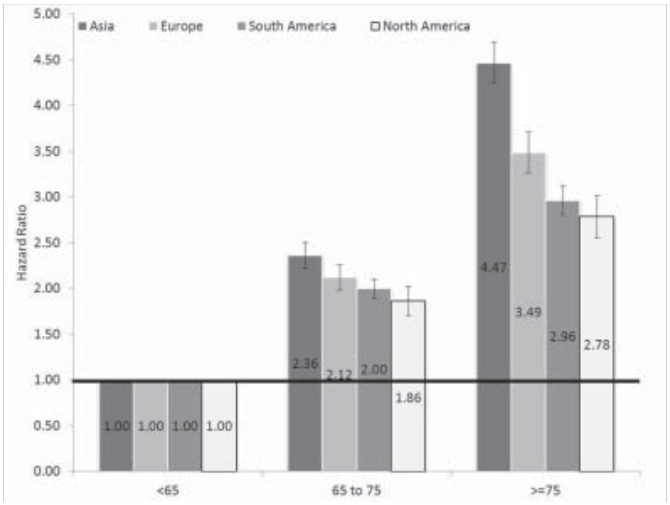
**Background:** The largest growing segment of hemodialysis (HD) patients is the elderly population. Previously the DOPPS III study described survival of elderly vs. younger HD patients (N=8,161) in 12 countries using age stratification of >45, 45-75 and >75-yr-old. In this study we evaluate the hazard ratio of elderly HD patients (≥75) vs. 65-75 vs. <65 in the MONDO international database (N=86,169).

**Methods:** The MONDO research initiative consists of HD databases from the US, Europe, Asia & Latin America [Usvyat, Blood Purif 2013]. We extracted data for all incident patients from RRI, FMC Europe, Latin America, and Asia Pacific between 2000 and 2012. Patients were stratified by age <65, 65-75, ≥75. Cox proportional hazards models were constructed for each database to assess the hazard ratio of death adjusted for gender, initial access type, race (for RRI only), and comorbidities (diabetes, cancer, cardiovascular and hepatitis).

**Results:** We studied 86,169 HD patients from 4 continents (Table):

| Continent | No. of Patients |
|---|---|
| South America | 20,880 |
| Asia | 12,521 |
| Europe | 41,698 |
| North America | 11,070 |
| Total | 86,169 |

In general, risk of death increased with age. In Asia, older patients had the highest hazard ratio of death vs. younger patients, compared to their counterparts in other continents. In the US, older patients had the lowest hazard ratio vs. younger patients (Figure):




**Conclusions:** The mortality hazard ratio of elderly vs. younger HD patients, within the same geographical region, varies across continents; It is highest in Asia and lowest in the US.

## SA-PO506

**Higher Mortality for Women Beginning Dialysis Treatment in the United States** Austin G. Stack,[1,2] Hoang Thanh Nguyen,[2] Ahad Abdalla,[1,2] Liam F. Casserly.[1,2] *[1]Nephrology and Internal Medicine, Univ Hospital Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Ireland.*

**Background:** Studies to date have yielded conflicting results on the survival of men and women who begin dialysis therapyin the US and may reflect differences in baseline comorbidity, differences in access to pre-dialysis care or differences in treatments following dialysis initiation. The overall goal of this study was to explore mortality differences between men and women who initiated dialysis from 1995-2008.

**Methods:** We identified 662, 985 men and 557, 015 women who commenced dialysis from 1995-2008 in the US Renal Data System. Baseline clinical health of patients was assessed at dialysis initiation and patients were followed from date of first dialysis to death and survival times were compared at the end of 2 years. Patients were censored at death, kidney transplantation, lost to follow-up or at the end of the study period. Mortality comparisons between men and women were stratified by race: White, Black, Asian and Native American. Adjustments were made for baseline clinical and laboratory health indicators, measures of pre-dialysis care and the timing of dialysis initiation. Ethical approval was received from the University Hospital Ethics Committee.

**Results:** Adjusted hazard ratios were significantly higher for White and Black women compared to White men and Black men respectively at follow-up. In contrast the hazard ratios of Asian and Native American women were similar to their male counterparts. White women experienced the highest overall risks.

| Race and Sex Group | Hazard Ratios for Death (HR) at 2 years | |
|---|---|---|
| | Unadjusted | Adjusted[2] |
| | **Unadjusted** | **Adjusted[2]** |
| White women (vs men) | 1.03 (1.02-1.03) | 1.07 (1.06-1.08) |
| Black women (vs men) | 1.15 (1.13-1.16) | 1.10 (1.08-1.12) |
| Asian Women (vs men) | 1.02 (0.98-1.05) | 1.02 (0.97-1.08) |
| Native Am. Women (vs men) | 1.05 (0.99-1.11) | 1.00 (0.92-1.10) |

| Race and Sex Group | Hazard Ratios for Death (HR) at 2 years | |
|---|---|---|
| | Unadjusted | Adjusted |
| White women (referent group) | 1.00 | 1.00 |
| Black women (vs white) | 0.69 (0.68-0.70) | 0.83 (0.82-0.84) |
| Asian Women (vs white) | 0.55 (0.54-0.57) | 0.65 (0.62-0.67) |
| Native Am. Women (vs white) | 0.62 (0.59-0.64) | 0.71 (0.67-0.76) |

**Conclusions:** Women experience higher mortality risks than men starting dialysis treatment in the US, with the greatest risks for white women. Differences in the severity of underlying disease or access to therapeutic interventions prior to or following dialysis onset may account for some of these differences and require further exploration.

## SA-PO507

**Factors Predicting Survival during the First 90 Days of Hemodialysis (HD): A Global Perspective** Adrian Marcos Guinsburg,[1] Cristina Marelli,[1] Daniele Marcelli,[2] Len A. Usvyat,[3,4] Michael Etter,[5] Peter Kotanko,[3] Mondo Consortium.[3] *[1]Fresenius Medical Care, Buenos Aires, Argentina; [2]Fresenius Medical Care, Bad Homburg, Germany; [3]Renal Research Institute, New York, NY; [4]Fresenius Medical Care North America, Waltham, MA; [5]Fresenius Medical Care, Hong Kong, Hong Kong.*

**Background:** Mortality during first 3 months on HD may indicate predialysis care (preDC) and patient status at HD initiation. We aimed to analyze factors predicting survival in the first 3 months on HD in a large international sample of incident HD patients.

**Methods:** The MONitoring Dialysis Outcomes (MONDO) consortium was described previously [Usvyat, Blood Purif, 2013]. Incident HD patients between 01/2000 and 12/2010 were followed until day 90 or death. Cox reg models were constructed to identify factors associated with survival in the first 90 days.

**Results:** We studied 14,829 patients (Asia 4,277; Europe 3,083; LA 2,500; USA 4,969). HR (p value) of factors are summarized in table 1.

| | Asia | Europe | Latinamerica | USA |
|---|---|---|---|---|
| Age (yrs) | *1.03 (0.03) | *1.05 (<.0001) | *1.03 (<.0001) | *1.03 (<.0001) |
| Male (yes) | 0.97 | 0.88 | *0.73 (0.04) | 1.01 |
| Diabetic (DBT, yes) | *3.07 (<.0001) | 1.32 | 1.03 | 1.01 |
| Albumin (g/dL) | *0.48 (0.01) | *0.4 (<.0001) | *0.4 (<.0001) | *0.33 (<.0001) |
| Calcium (Ca, mg/dL) | 1.16 | 0.97 | 1.12 | *1.28 (0.006) |
| Hgb (g/L) | *0.83 (0.03) | *1.17 (0.02) | 1.02 | 1.05 |
| PO4 (mg/dL) | 1.01 | 1.09 | 1.07 | 0.96 |
| Pre-SBP (mmHg) | 1.001 | *0.98 (<.0001) | *0.99 (0.0004) | *0.98 (<.0001) |

*p<0.05

Age predicts mortality in all regions while gender (male) only in LA and DBT only in Asia.

Albumin was positively associated to survival in all regions and pre-SBP in all regions except Asia. Ca and PO4 showed no effect (exception of Ca in USA). The association of Hgb with mortality was heterogeneous: no association in LA and USA, positive association in Asia and negative in Europe.

**Conclusions:** Age, albumin and preSBP showed a similar pattern across regions suggesting an underlying biology independent of practice patterns and health policies. Ca, PO4 and Hgb showed variable effects possibly indicating differences in preDC and policies.

## SA-PO508

**Uninsured Unemployed and Insured Employed ESRD Patients Have Similar Mortality Outcomes** Paul L. Kimmel,[1] Chyng-Wen Fwu,[2] Jonathan Ratner,[3] Kevin C. Abbott,[4] Paul W. Eggers.[1] *[1]Div of Kidney, Urologic and Hematologic Diseases, NIDDK, NIH, Bethesda, MD; [2]Social & Scientific Systems, Inc., Silver Spring, MD; [3]Westat, Inc., Rockville, MD; [4]Walter Reed National Military Medical Center, Bethesda, MD.*

**Background:** Uninsured US adults in the general population have less health-care access and utilization, but analogous evidence on mortality is sparse and contradictory. Nearly all US ESRD patients have Medicare available. Whether health insurance status before Medicare entitlement due to ESRD varies with probability of death is unknown.

**Methods:** We included ESRD adult patients less than 65 years old from 2000-2008 US Renal Data System. To account for competing risks (transplantation and transfer to peritoneal dialysis [PD]), we used Fine-Gray sub-distribution proportional-hazards regression models, estimating cumulative incidence of death, controlling for known risk factors including residential income and income inequality parameters. Separate models by employment status were analyzed, because of a significant interaction between insurance and employment status.

**Results:** 274,921 patients were included. 23% were employed and 86% were insured (including private insurance, Medicare, and Medicaid) at ESRD onset. After follow-up (median 26.8 months), 110,373 (40.2%) patients died, 37,741 (13.7%) patients received renal transplant, and 14,079 (5.1%) started PD. Uninsured patients were younger, poorer, and healthier than the insured. Among the employed, the uninsured had higher mortality than the insured (sub-hazard ratio: 1.12, 95% confidence interval: 1.06-1.19). Among unemployed patients, the uninsured survived longer than the insured (0.84, 0.82-0.86).

**Conclusions:** As expected, in ESRD patients, mortality increases with unemployment. In the employed at ESRD onset, mortality increases with lack of insurance. Counterintuitively, in unemployed ESRD HD patients, lack of insurance is associated with improved survival. Selection bias - people with better perceived health opting to not buy insurance - may explain these surprising findings. In addition, entry into the ESRD program may differentially benefit the previously uninsured.

*Funding:* NIDDK Support, Other U.S. Government Support

## SA-PO509

**Age, Race and Ethnicity, and Risk of Hospitalization among Patients Undergoing Hemodialysis** Guofen Yan,[1] Keith C. Norris,[2] Tom Greene,[3] Wei Yu,[1] Jennie Z. Ma,[1] Alfred K. Cheung.[3] *[1]Univ of Virginia; [2]Charles R. Drew Univ; [3]Univ of Utah.*

**Background:** Recent literature suggests overall hemodialysis (HD) mortality is lowest in Hispanics, intermediate in non-Hispanic blacks (blacks), and highest in non-Hispanic whites (whites), except for those under 30 years. Whether a similar pattern exists for hospitalization is unclear. We compared overall and age-stratified (18-30, 31-40, 41-50, 51-60, 61-70, 71-80, >80 yrs) hospitalization rates among the racial/ethnic groups.

**Methods:** We identified 563,281 (55% white, 33% black and 12% Hispanic) patients in the USRDS aged ≥18 yrs who initiated maintenance HD between 1995 and 2009, and survived the first 90 days, received in-center HD at day 91 for >60 days, with Medicare as primary payer. Hospital admissions and hospital days for all causes (AC), cardiovascular (CV), any infection, and dialysis-related infection (DRI) over one year were examined with Poisson regression.

**Results:** For both AC hospital days and admissions, unadjusted rates were highest in whites, intermediate in blacks, and lowest in Hispanics. After adjusting for various covariates, this pattern was evident only in the middle 2 age groups (51-60, 61-70 yrs), whereas among patients under 40 or above 70 years old, blacks had much higher rates than whites. Thus, the adjusted rate ratios (RRs) of blacks vs. whites exhibited a U-shaped relationship with age: RRs of 1.29 ($p<0.001$), 1.20 ($p<0.001$), 1.02 ($p=0.3$), 0.91 ($p<0.001$), 0.97 ($p=0.008$), 1.08 ($p<0.001$) and 1.22 ($p<0.001$) for the hospital days from younger to older groups. Hispanics consistently exhibited the lowest adjusted AC rates for all ages under 70 years (RRs ranging from 0.84 to 0.94 for the hospital days). However, hospitalization rates for DRI were consistently much higher in blacks than whites across all age groups, and higher in Hispanics than whites in age groups >60 yrs.

**Conclusions:** Our findings suggest that age modified the association of racial/ethnic factors with the overall and cause-specific hospitalizations. The delineation of the factors responsible for excess hospitalization rates among select subgroups may lead to reduced costs and improved quality of care for all dialysis patients.

*Funding:* NIDDK Support

## SA-PO510

**Association of Number of Comorbidities with Number of Readmissions** Eduardo K. Lacson, Weiling Wang, Franklin W. Maddux. *Fresenius Medical Care, North America, Waltham, MA.*

**Background:** Comorbidity influences hospital readmission rates in the general population. We evaluated the association between comorbid diagnoses and 30-day readmission rates in hemodialysis (HD) patients.

**Methods:** All adult HD patients, treated 3x/week on 1/1/11 in Fresenius Medical Care, North America facilities with ≥1 hospital discharge in 2011 were included and readmissions tracked until 12/31/11. Case-mix (age, gender, race, diabetes, vintage, access type) and lab variables as well as 23 comorbidity categories from the Medicare Dialysis Facility Reports were recorded as of 1/1/11. The frequency of comorbidities was counted per patient among 16 categories identified by univariate logistic models to associate with readmission rate and assigned into groups: 0, 1, 2, 3, >3 (Table). Poisson regression was used to assess the association between number of comorbidities & readmissions.

| Number of Comorbid Diagnoses | Patient N | Patient % | % Readmission Rate |
|---|---|---|---|
| 0 | 12194 | 19.0 | 32.4 |
| 1 | 18095 | 28.2 | 34.1 |
| 2 | 14565 | 22.7 | 37.7 |
| 3 | 10016 | 15.6 | 39.1 |
| >3 | 9258 | 14.4 | 43.8 |

**Results:** In 2011, 64,128 HD patients were hospitalized: mean age of 62.8±14.5 years, 52.5% male, 55.6% white, 39.3% black,and 28.8% HD catheters. Dialysis vintage was 3.8±3.7 years and the mean number of comorbidities was 1.9±1.5 (IQR: 1-3, median: 2). 50,380 readmissions occurred with mean of 0.79 ±8.1 (median: 0) per patient. Readmission rate increased by 8% for each additional comorbid diagnosis ($p<0.0001$). Compared to no comorbidity, the rate of readmission with 2 comorbidities increased by 23% and by 45% for >3 (all $p<0.0001$). Results were similar after case-mix & lab adjustment.

**Conclusions:** Comorbidity is a significant risk factor for 30-day readmission. Further study is needed to determine potentially actionable attributes of specific comorbid diagnoses that contribute to both specific and all causes of readmissions. Patients with multiple comorbidities will need to be considered in performance measure development to avoid unintended consequences such as selection bias that may limit subsequent access to care.

## SA-PO511

**Impact of Target Parathyroid Hormone (PTH) Range and Risk of Fractures in End-Stage Renal Disease (ESRD)** Chi-yuan Hsu,[1] David V. Glidden,[1] Jeffrey R. Curtis,[2] Barbara A. Grimes,[1] Linda McCann,[3] Brigitte Schiller,[3] Kirsten L. Johansen.[1] *[1]Univ of California, San Francisco; [2]Univ of Alabama, Birmingham; [3]Satellite Healthcare.*

**Background:** The optimal target range for PTH levels in hemodialysis (HD) patients is unclear. In the absence of randomized controlled trial data, "natural experiments" such as changes in protocol PTH target range implemented across a dialysis organization (in response to expert guidelines) regardless of individual patient characteristics may provide estimates of treatment effect that are relatively un-confounded.

**Methods:** We studied fracture risk among in-center maintenance HD patients treated at Satellite Healthcare, a non-for-profit dialysis organization, which has a uniform mineral metabolism protocol across all its dialysis facilities. The target PTH range was 150-300 pg/ml from April 2003-Jan 2005 and 150-400 after Feb 2005. We followed patients who had Medicare as their primary insurance starting Jan 2003. Using US Renal Data System data and time varying Cox regression analysis, we assessed the impact of changes in PTH target on rates of fracture. Fractures were identified using ICD-9 codes 820.x and 733.14 for hip, 813.x, 733.12, 812.x, and 733.11 for radius/ulna, 805.x, 806.x and 733.13 for spine and 814.x for wrist/carpal after 6-month lag. Outcomes were ascertained through Dec 2009 or 3 months after transfer out of a Satellite dialysis unit (for transplant or other reasons).

**Results:** We observed 36 factures over 1786 person-years of follow-up (2.0 per 100 person-yrs) when the target PTH was 150-300 pg/ml and 150 fractures over 6563 person-years of follow-up (2.3 per 100 person-yrs) when the target PTH was 150-400 pg/ml. The hazard ratio (HR) for 150-400 (vs. 150-300 pg/ml) was 1.04 (95% CI 0.71-1.53; $p=0.83$). Similar results were seen after controlling for demographics and comorbid conditions known to be fracture risk factors (adjusted HR 1.17; 95% 0.78-1.74; $p=0.45$).

**Conclusions:** Raising the upper limit of target PTH range from 300 to 400 pg/ml did not appear to be associated with significantly higher risk of fractures in maintenance HD patients, although confidence intervals were relatively wide.

*Funding:* NIDDK Support

## SA-PO512

**Relative Impact of Selected Indicators on Mortality: Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS)** Francesca Tentori,[1] Lindsay Zepel,[1] Angelo Karaboyas,[1] David C. Mendelssohn,[2] T. Alp Ikizler,[3] Brenda W. Gillespie,[4] Werner Kleophas,[5] Brian Bieber,[1] Ronald L. Pisoni,[1] Bruce M. Robinson.[1] *[1]Arbor Research; [2]Humber River Regional Hosp.; [3]Vanderbilt U.; [4]U. of Michigan; [5]Dialysezentrum Karlstrasse.*

**Background:** The proportion of facility patients meeting a clinical target is often used as an indicator of quality of care ("quality indicator"). Achievement of targets varies for different markers. The impact of quality indicators on outcomes is likely greater for indicators that are highly prevalent and have a strong association with mortality. We aim here to assess the proportion of deaths that could have been prevented by achievement of selected quality indicators.

**Methods:** We analyzed 15,118 hemodialysis patients from 539 facilities in DOPPS 3 (2005-08) and 4 (2009-11) in North America, Europe, Australia and New Zealand. Quality indicators were based on guidelines during the study period. In prior work, the 4 quality indicators included had the strongest association with mortality. The proposed goal was chosen based on observed distribution and clinical judgment about feasibility. Using an adjusted Cox model, we calculated the attributable fraction (AF) as the predicted reduction in number of deaths if facilities below the goal were set counterfactually to the target goal.

**Results:** Achievement of the quality indicators varied widely, with higher % of facilities not meeting the albumin and fistula guidelines. The AF ranged from 3.2% (for albumin) to 9.5% (for fistula use), with a cumulative AF of 23.4% (Table 1).

**Table 1**: Distribution of facility quality indicators and attributable fractions

| Quality indicator | Observed facility % of patients achieving indicator: (median [IQR]) | Proposed goal* | % of facilities below goal | AF** |
|---|---|---|---|---|
| Kt/V ≥ 1.2 | 90% (83%-95%) | 100% | 85% | 8.3% |
| Hemoglobin ≥ 10 g/dl | 92% (87%-96%) | 95% | 68% | 4.7% |
| Albumin ≥ 4 g/dl | 34% (17%-47%) | 40% | 60% | 3.2% |
| Fistula use | 67% (52%-79%) | 85% | 87% | 9.5% |
| **Cumulative AF** | | | | 23.4% |

*Goal = proposed % of facility patients to achieve the quality indicator
**The AF for mortality was adjusted for patients, demographics, comorbidities, all quality indicators shown, and the 4 additional quality indicators: IDWG < 5.7%, phosphorus 3.5-5.5 mg/dl, calcium 8.4-9.5 mg/dl, RAS inhibitor use; AF was averaged across 5 imputed datasets.

**Conclusions:** Though these results cannot establish causality, they indicate that ~23% of deaths could be prevented if all facilities below the Kt/V, hemoglobin, albumin, and fistula targets improved to the proposed goals. Increasing the facility % of patients with Kt/V ≥ 1.2 or fistula may yield the greatest improvement in mortality.

*Funding:* NIDDK Support, Pharmaceutical Company Support - The DOPPS is supported by research grants from Amgen (founding sponsor, since 1996), Kyowa Hakko Kirin (since 1999, in Japan), AbbVie (since 2009), Sanofi Renal (since 2009), Baxter Healthcare (since 2011), and Vifor Fresenius Medical Care Renal Pharma (since 2012), with additional country-specific support provided in Canada by Amgen-Canada, Janssen, BHC Medical, Takeda and Kidney Foundation of Canada, and in Germany by Hexal and WiNe Institute. Support from the DOPPS sponsors is provided without restrictions on publications.

## SA-PO513

**End Stage Renal Disease due to HIVAN in the United States 2000-2010** Donal J. Sexton,[1,2] Scott Reule,[1,2] Craig Solid,[1] Shu-cheng Chen,[1] Allan J. Collins,[1,2] Robert N. Foley.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, Univ of Minnesota, Minneapolis, MN.*

**Background:** The management of HIV has changed considerably in the last decade, and the effect of contemporary treatment on the standardized incidence of end stage renal disease (ESRD) due to HIV has not been characterized.

**Methods:** We combined US census data with data from the USRDS patients to determine whether incidence rates and outcomes of ESRD due to HIV associated nephropathy (HIVAN) have improved simultaneously with improvements in the management of HIV.

**Results:** 8839 of 1,141,026 (0.8%) patients initiated dialysis due to HIVAN between 2000 and 2010. Using incidence rates for 2000 to calculate expected rates for subsequent years (based on age, sex and race/ethnicity), standardized incidence ratios remained unchanged between 2000 and 2003 and then declined to 0.66 in 2010. Characteristics of HIVAN patients included residence in the South (59.5% vs. 41.5% in non-HIVAN), age < 45 yrs (55.7% vs. 13.1%), male sex (66.9% vs. 55.3%), African American race (87.6% vs. 28.3%), catheters for hemodialysis (90.9% vs. 82.5%), nephrology care < 1 year (90.9% vs. 76.4%), and body mass index < 18.5 kg/m$^2$ (12.2% vs. 4.4%). HIVAN was associated with a greater likelihood of failure to be listed for transplant (adjusted hazard ratio [AHR] 4.55), failure to receive a transplant (AHR 6.67) and death (AHR 2.53). Among patients with HIVAN, while mortality (AHR for 2005-2010 0.94 vs. 2000-2004) and non-listing declined (AHR 0.76), non-transplantation increased (AHR 1.50). Regional variation in outcomes was apparent, with HIVAN patients in the South having higher risks of non-listing (AHR 1.52 vs. the Northeast), non-transplantation (AHR 1.96) and death (AHR 1.17).

**Conclusions:** While ESRD due to HIV has declined in the United States in the last decade, associated outcomes remain poor and exhibit substantial regional heterogeneity.

*Funding:* NIDDK Support

## SA-PO514

**End Stage Renal Disease due to Lupus Nephritis in the United States 2000-2010** Donal J. Sexton,[1,2] Scott Reule,[1,2] Craig Solid,[1] Shu-cheng Chen,[1] Allan J. Collins,[1,2] Robert N. Foley.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, Univ of Minnesota, Minneapolis, MN.*

**Background:** Despite advances in the treatment of lupus nephritis (LN), the impact of these developments on the standardized incidence of ESRD due to LN in recent years has not been defined.

**Methods:** We combined US census data with data from the USRDS patients to determine whether incidence rates and survival of ESRD due to LN have improved in parallel with developments in treatment.

**Results:** 11,612 of 1,127,050 (1.0%) patients initiated dialysis due to LN between 2000 and 2010. Using incidence rates for 2000 to calculate expected rates for subsequent years (based on age, sex and race/ethnicity), standardized incidence ratios peaked in 2007 at 1.01, declining subsequently to 0.85 by 2010. Characteristics of ESRD due to LN included age < 40 yrs (53.7% vs. 8.4% in non-LN), female sex (81.5% vs. 44.3%), African American race (50.2% vs. 28.6%), catheters for hemodialysis (89.4% vs. 82.5%), and nephrology care > 1 year (30.5% vs. 23.4%). LN was associated with a lower likelihood of death (adjusted hazard ratio [AHR] 0.99) and a higher likelihood of listing for (AHR 1.58) and receiving a renal transplant (AHR 1.32). Among patients with LN, mortality did not change over time (AHR for 2005-2010 0.99 vs. 2000-2004), and although the likelihood of listing increased (AHR 1.36), transplantation actually decreased (AHR 0.9). Regional variation in outcomes was substantial, with LN patients in the South having lower survival (AHR 0.82 vs. the Northeast) coupled with a higher likelihood of non-listing (AHR 1.39) and non-transplantation (AHR 1.32).

**Conclusions:** ESRD due to LN in the United States has declined in the last decade, however significant regional variation exists in terms of LN patient outcomes.

*Funding:* NIDDK Support

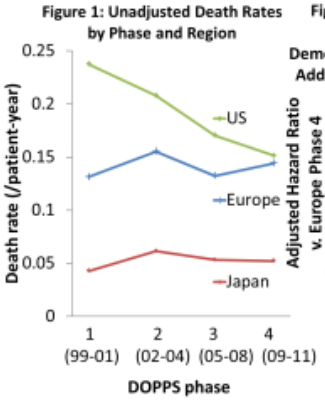
## SA-PO515

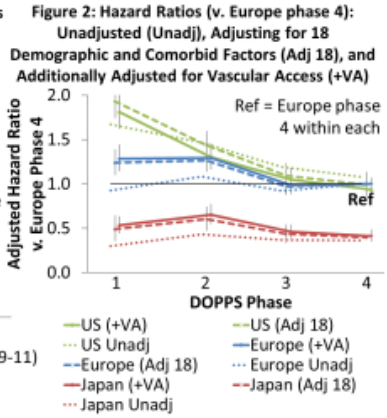
**International Mortality Trends among Hemodialysis Patients in the DOPPS** Keith McCullough,[1] Hugh C. Rayner,[2] David A. Goodkin,[1] Hal Morgenstern,[3] Friedrich K. Port,[1] Michel Y. Jadoul,[5] Akira Saito,[4] Ronald L. Pisoni,[1] Bruce M. Robinson.[1] *[1]Arbor Research Collaborative for Health; [2]Heart of England; [3]U. of Michigan; [4]Yokohama Dai-ichi Hospital; [5]U. of Louvain Medical School.*

**Background:** Case-mix-adjusted mortality rate in DOPPS has been higher in the US than in Europe or Japan. Patient population and clinical practices have changed over time. We are evaluating the effects of case mix and practice patterns on trends in mortality rates across three regions.

**Methods:** 1999-2011 DOPPS data from hemodialysis facilities in France, Germany, Italy, Japan, Spain, the UK, and the US were used to assess trends in mortality rates. Cox models with covariates for age, sex, vintage, 15 comorbid factors, and vascular access type among 29,660 patients selected from prevalent cross-sections within each phase were fitted to estimate adjusted hazard ratios (HR).

**Results:** The unadjusted death rate in the sample over phases 1-4 has fallen in the US (HR/phase=0.86; 95% CI 0.83-0.88), and has been stable in Europe (1.00; 0.97-1.04) and Japan (1.01; 0.95-1.08). [Figure 1] After phase 1, the HR/phase (unadjusted, case-mix adjusted (adj 18), and adjusted for vascular access (+VA)) were: in the US=(0.85*,0.82*, 0.83*), in Europe=(0.96^,0.88*, 0.86*) and in Japan=(0.93^,0.77*, 0.76*) (^p>0.1,*p<0.001). Mortality rates in Japan were lowest in all phases (p<0.0001). Adjustment for vascular access (+VA) explained some of the difference in the mortality rate between the US and Europe [Figure 2].




**Conclusions:** After phase 1, US and European trends in adjusted mortality have been similar. Differences in vascular access practices partly explain lower mortality rates in Europe v. US, but further investigation is needed to determine other reasons for patient-mix-adjusted differences in mortality rates over time and between regions.

*Funding:* Pharmaceutical Company Support - The DOPPS is supported by research grants from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, with additional country-specific support provided in Canada by Amgen-Canada, Janssen, BHC Medical, Takeda and Kidney Foundation of Canada, and in Germany by Hexal and WiNe Institute. Support from the DOPPS sponsors is provided without restrictions on publications.

## SA-PO516

**Estimated GFR at Dialysis Initiation: Associations with Clinical and Non-Clinical Factors** Yun Li,[1,2] Alissa Kapke,[2] Yan Jin,[2] Jeffrey Pearson,[2] Friedrich K. Port,[2] Bruce M. Robinson.[2] *[1]Univ of Michigan; [2]Arbor Research Collaborative for Health.*

**Background:** The average estimated glomerular filtration rate (eGFR) at dialysis start has continued to increase in the US. Our objectives are to examine the predictors of eGFR at dialysis start and its change over time.

**Methods:** The US Renal Data System ESRD database was used to identify 242,957 patients who initiated dialysis between 2006 and 2009, treated by 8,470 physicians and had complete information on variables of interest. Separate random effect models are used to model eGFR and its change over time (2006-2009).

**Results:** The eGFR at dialysis initiation averaged 10.9 (SD=4.6), rising by 0.5 from 2006 to 2009. On average, patients who were younger, female, uninsured, had fewer comorbidities, initiated dialysis in larger or free standing facilities, and/or in counties with higher poverty rates or lower education levels (Table 1) started dialysis at lower eGFR. The increase in eGFR at dialysis start was significantly steeper among patients who were non-Hispanic whites, received no prior nephrology care, and treated in hospital-based facilities or counties with less poverty (Table1). The mean eGFR and its change over time also varied significantly by geography (ESRD Network). Between-physician variation accounted for 10% of the total variation in eGFR at initiation. The total variation was reduced by 14% after adjusting for measured patient characteristics and by an additional 4% after further adjustment of facility/county contextual factors.

**Conclusions:** Analysis of national US data indicates that the majority of variation in eGFR at dialysis initiation occurrs at the patient level, though most is unexplained by measured variables in this dataset. Later initiation is correlated with some variables indicative of barriers in access to dialysis.

Table 1. Characteristics Associated with eGFR at Dialysis Start (2006-2009) and eGFR change over time

| | | | eGFR[a] Estimate | eGFR Change over Time[a] Slope |
|---|---|---|---|---|
| Patient-level | Age, per 10 years | | **0.17** | 0.037 |
| | Race/Ethnicity (vs. NH White) | Black | **0.14** | **-0.11** |
| | | Other | **-0.49** | **-0.27** |
| | Employment Status (vs. Unemployed) | Employed | **-0.76** | 0.014 |
| | | Other/Unknown | **-0.12** | 0.024 |
| | Any insurance (vs. No) | | **0.73** | 0.17 |
| | Prior nephrology care (vs. No) | | **-0.28** | **-0.14** |
| Facility-level | Free Standing Facility (vs. Hospital-based) | | **-0.12** | **-0.28** |
| | Facility-size | | **-0.034** | **0.004** |
| County-level | % Below Poverty, per 5% | | **-0.038** | **-0.088** |

a: Adjusted for listed variables and **year, sex, comorbid conditions**, cause of ESRD, **albumin, hemoglobin, modality, ESRD Network**, nurse to patient ratio, **facility size** and type (profit vs. non-profit and **urban vs. rural**), %high school graduates, and physician clustering. Bolded variables indicate p<0.05 in the adjusted model for eGFR. b: Estimate indicates average change in 4-year slope of eGFR for each listed variable after adjustments in a.

## SA-PO517

**Time Trends in the Association of Influenza Vaccination (IV) with Mortality and Hospitalization in U.S. Medicare Dialysis Patients (2006-2011)**

E.L.Cope,[1] K.A. Wisniewski,[2] M. Curran,[1] Bruce M. Robinson,[1] Ronald L. Pisoni.[1] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]KECC, Univ of Michigan, Ann Arbor, MI.*

**Background:** To assess the stability of the association between IV and improved health outcomes, we examined the association between facility-level IV and all-cause mortality and hospitalization over 5 seasons.

**Methods:** We examined 5 seasons (2006-2007 thru 2010-2011). Each season included Medicare ESRD patients on dialysis Oct 1-Dec 31. Vaccinations were counted using Medicare claims from any setting between Aug 1-Dec 31. Poisson regression was used to assess the relationship of facility IV rate with outcomes in the following year via each facility's standardized mortality (SMR) and hospitalization (SHR) ratios. Models were adjusted for facility and patient characteristics.

**Results:** Median facility IV rates fluctuated from 67.6%-71.1%. Facility IV rate was inversely associated with relative risk of mortality and hospitalization for all 5 seasons (Table 1). For mortality, the association was strongest during the 2006-2007 season (2.3% lower SMR per 10% higher facility IV rate, p<0.01), and weakest during the 2010-2011 season (0.8% lower SMR per 10% higher facility IV rate, p<0.01). The association between facility IV rate and hospitalization was relatively stable over time (1.6-2.2% lower SHR per 10% higher facility IV rate, all p<0.01).

Table 1. Mean facility SMR and SHR by percent of facility patients vaccinated against influenza.

| % Facility Patients Vaccinated (Quintiles) | 2006-2007 | 2007-2008 | 2008-2009 | 2009-2010 | 2010-2011 |
|---|---|---|---|---|---|
| Mean SMR | | | | | |
| 0-47.0 | 1.07* | 1.07* | 1.05* | 1.06* | 1.06* |
| 47.1-61.5 | 1.05* | 1.01 | 1.02 | 1.05* | 1.02 |
| 61.6-70.5 (Ref) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 70.6-78.0 | 0.96* | 0.97* | 0.99 | 1.00 | 1.01 |
| 78.1-100 | 0.93* | 0.92* | 0.97* | 0.97* | 0.99 |
| Mean SHR | | | | | |
| 0-47.0 | 1.08* | 1.05* | 1.07* | 1.05* | 1.06* |
| 47.1-61.5 | 1.04* | 1.04* | 1.07* | 1.06* | 1.05* |
| 61.6-70.5 (Ref) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 70.6-78.0 | 0.96* | 0.95* | 0.95* | 0.97* | 0.97* |
| 78.1-100 | 0.92* | 0.92* | 0.91* | 0.92* | 0.94* |

*p < 0.05

**Conclusions:** An IV performance gap remains with 29% of patients appearing not to be vaccinated in 2010. However, this may be an overestimate due to underreporting of IV in the claims. Associations between facility IV rate and lower hospitalization appeared robust across seasons, whereas those with mortality were less stable and suggest a more modest effect than previously published. Variation in vaccine efficacy and seasonal severity may contribute to fluctuations in the associations over time.

*Funding:* Other U.S. Government Support

## SA-PO518

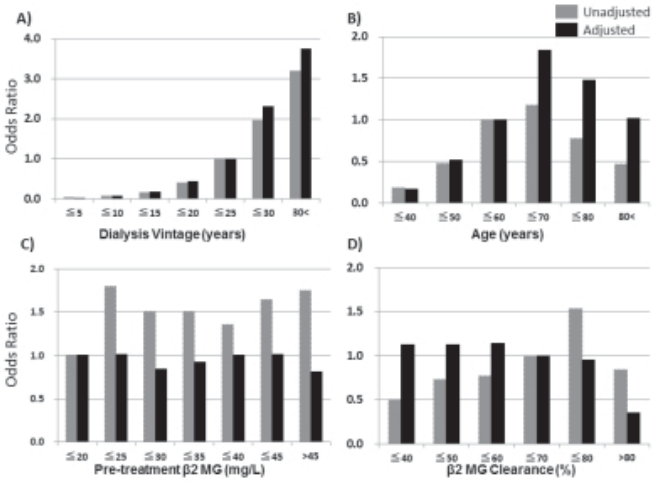
**Recent Risk Factors Associating with Development of Dialysis-Related Amyloidosis: A Nationwide Analysis from Japanese Dialysis Patient Registry**

Junichi Hoshino,[1] Kunihiro Yamagata,[2] Ikuto Masakane,[3] Shinichi Nishi,[4] Kunitoshi Iseki,[5] Yoshiharu Tsubakihara.[6] *[1]Nephrology Center, Toranomon Hospital, Tokyo, Japan; [2]Univ of Tsukuba, Ibaraki, Japan; [3]Yabuki Hospital, Yamagata, Japan; [4]Kobe Univ Graduate School of Medicine, Hyogo, Japan; [5]Univ Hospital of Ryukyus, Okinawa, Japan; [6]Osaka General Medical Center, Osaka, Japan.*

**Background:** The aim of this study was to clarify recent risk factors associated with development of dialysis-related amyloidosis, with risk of carpel tunnel syndrome operation (CTS) as a proxy.

**Methods:** The cohort consisted of 166,237 patients on dialysis (mean age, 66.1±12.4 years) who could be followed for a year between 2010 and 2011. Of these,2,275 (1.37%) needed a new CTS. Logistic regression was used to calculate adjusted odds ratios (OR) and 95 % confidence intervals (95% CI) for new CTS, after adjusting patient's backgrounds and dialysis factors.

**Results:** Adjusted OR (aOR) for dialysis vintage (vintage) 10-15, 15-20, 20-25, 25-30, and over 30 years were, respectively, 0.18 (0.12-0.26), 0.44 (0.31-0.62), 1.00 (referent), 2.32 (1.60-3.34), and 3.76 (2.43-5.82). The aOR for ages 40-50, 50-60, 60-70, 70-80, and over 80 were 0.52 (0.29-0.92), 1.00, 1.84 (1.38-2.46), 1.48 (1.05-2.08), and 1.01 (0.59-1.75), respectively. In addition, female , low serum albumin, and diabetic nephropathy were factors associated with new CTS. On the other hand, the pre-treatment β2-microglobulin (β2MG) and β2MG clearance less than 80 % were not significant, although β2MG clearance more than 80% was negatively associated with new CTS (OR 0.35 [0.13-0.92]).




**Conclusions:** The OR of new CTS almost doubled every 5 years vintage increased. The OR of CTS was highest for patients aged 60-70. The other factors associated with new CTS were gender, serum albumin, and diabetic nephropathy. β2MG clearance over 80% may decrease new CTS.

## SA-PO519

**End-Stage Renal Disease from Autosomal Dominant Polycystic Kidney Disease in the United States, 2000-2010**

Scott Reule,[1,2] Donal J. Sexton,[1,2] Shu-cheng Chen,[1] Craig Solid,[1] Allan J. Collins,[1,2] Robert N. Foley.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, Univ of MN, Minneapolis, MN.*

**Background:** As early detection and treatment are usually feasible and kidney disease is typically not a consequence of other organ dysfunction, autosomal dominant polycystic kidney disease (ADPKD) can provide a useful perspective on the overall quality of nephrology care. Hence, we determined whether incidence rates and survival of dialysis-requiring ESRD from autosomal dominant kidney disease (ADPKD, N=22,262, 2% of all ESRD in the US) changed between 2000 and 2010.

**Methods:** Using incidence rates for 2000 to calculate expected rates for subsequent years (based on age, sex and race/ethnicity), standardized incidence ratios, stable with until 2008, declined to 0.89 in 2010.

**Results:** Characteristics of ADPKD at dialysis initiation included age 40-65 (65.5% vs. 40.8%), white race (80.9% vs. 65.0%), fistulas (35.8% vs. 13.4%), and > 1 year nephrology care (45.3% Vs. 23.1%). On dialysis, ADPKD was associated with a lower likelihood of death (demography-adjusted hazards ratio [AHR] 0.41) and a higher likelihood of listing for (AHR 2.22) and receipt (AHR 2.83) of a renal transplant. Amongst patients with ADPKD, white race was associated with higher AHR for listing (AHR 1.34 Vs. African American) and transplantation (AHR 2.31).

**Conclusions:** While ESRD due to ADPKD may have declined and outcomes on dialysis are better than in many other conditions, disparities are present and seamless transition into renal replacement therapy appears to be the exception and not the rule.

*Funding:* NIDDK Support

## SA-PO520

**A Retrospective Study on Outcome of MPO-ANCA Associated Vasculitis in Renal Replacement Therapy**

Midori Hasegawa, Kyoko Hattori, Kazuo Takahashi, Hiroki Hayashi, Shigehisa Koide, Yukio Yuzawa. *Nephrology, Fujita Health Univ School of Medicine, Toyoake, Aichi.*

**Background:** There are few reports on the clinical course of myeloperoxidase - antineutrophil cytoplasm autoantibody (MPO-ANCA) associated vasculitis in renal replacement therapy (RRT). The aim of this study was to conduct a retrospective review of patients with MPO-ANCA associated vasculitis in RRT.

**Methods:** Birmingham Vasculitis Activity Score (BVAS), patient survival, relapse, and relationships with treatment were examined in our institution, 2 related hospitals, and 5 outpatient clinics in the past 21 years.

**Results:** Of 90 patients (67±13 years, M/F 51/39) recruited, 69 had microscopic polyangiitis, 20 had renal limited vasculitis, and 1 had granulomatosis with polyangiitis. BVAS at the start of RRT was 12.7±3.9. RRT was needed in 43.3% at the first medical examination. The initial dose of prednisolone (PSL) was 0.7±0.3mg/kg/day. The total prescribed duration of GC was 46.8 months. Cyclophosphamide (CY) was prescribed to 20%. During the period of 54±50 months after the onset of RRT, relapse occurred in 11.1%. Lung hemorrhage was noted in 8 patients, while 2 patients had generalized symptoms. The duration from the onset of RRT to relapse was 63±59 months. At the relapse, 5 patients were not receiving immunosuppressive therapy and PSL (4 ± 8 mg/day) was prescribed for the remaining 5 patients. Survival rates for 1, 3 and 5 years after RRT were 83.2%, 76.4% and 66.8%, respectively. The causes of death were infection (59.5%), cardiovascular event (24.3%), gastrointestinal bleeding (8.1%), malignancy (5.4%) and interstitial pneumonia

(2.7%). By Cox's multivariate analysis, patient year (HR1.09, P<0.0001) and pulmonary involvement (HR 3.39, P=0.043) were significant positive risks and the use of CY (HR 0.12, P=0.001) was a significant negative risk for mortality.

**Conclusions:** In patients with MPO-ANCA associated vasculitis, relapse could occur even after a long period from the onset of RRT . Infection was the most frequent cause of death and pulmonary involvement was related with mortality. It is important to clarify the optimal duration of maintenance therapy after RRT.

## SA-PO521

**Outcomes in Infants Initiating Chronic Dialysis Less Than 24 Months of Age** Kera E. Luckritz, Rachel Galimberti, Kathryn S. Plomaritas. *Pediatric Dailysis, Univ of Michigan, Ann Arbor, MI.*

**Background:** Infants < 2years of age make up less than 15% of the pediatric dialysis population. Survival is also the lowest of all the pediatric dialysis age groups. While peritoneal dialysis (PD) remains the preferred modality in this age group, hemodialysis (HD) is often necessary. Whether outcomes differ for either modality remains unclear.

**Methods:** Electronic health records were extracted for all patients who started chronic dialysis at <24months of age at our center between 1997 and 2012. Gender, cause of end stage renal disease (ESRD), age at dialysis start, dialysis modality, any modality changes, dialysis prescription, transplant status, age at transplant, and 6 month, 12 month, 18 month and 24 month survival were queried. Data was entered into a RedCap© database and analyzed with SAS© 9.2 (Cary, NC).

**Results:** Thirty three patients were identified with a mean age at dialysis initiation of 5.6 months (0.2-22). Most common diagnosis was dysplasia (48.4%). Seventy five percent of the patients started on PD. Twelve (36%) patients never required a change in modality. Six patients are still receiving chronic dialysis (2 HD, 4PD). Six patients (18%) died before reaching transplant. Only two of the patients who died were on HD at the time of death. Average age at time of death was 504 days. Causes of death included heart failure (1), liver failure (2), sepsis (2) and pneumonia/complications from ESRD (1). Average time from dialysis start to successful transplant was 446 days for those reaching transplant. One patient stopped dialysis and was successfully medically managed until transplant. Neither gender nor dialysis modality influenced mortality. There was no statistical significant in survival or transplant status by modality.

| | PD Alone N=11 | HD Alone N=2 | PD &HD N=20 | Total N=33 |
|---|---|---|---|---|
| Survival N(%) | 10(90%) | 2(100%) | 15 (75%) | 27 (82%) |
| Iransplant N(%) | 7(64%) | 1 (50%) | 13 (65%) | 22 (66%) |

**Conclusions:** The North American Pediatric Renal Transplant Collaborative Studies 2011 report cites a 75% survival in pediatric patients starting dialysis at less than one year of age. Our center demonstrates an overall survival rate >80% with no statistical difference between gender or dialysis modality.

## SA-PO522

**Changes in Incidence and Prevalence of Diabetic Kidney Disease (DKD) Patients on Renal Replacement Therapy (RRT) in Austria** Friedrich C. Prischl,[1] Martin Auinger,[2] Marcus Saemann,[3] Gert J. Mayer,[4] Alexander R. Rosenkranz,[5] Manfred Wallner,[1] Reinhard Kramar.[6] *[1]4th Dept Medicine, Klinikum Wels-Grieskirchen, Wels, Austria; [2]3rd Dept Medicine, Hospital Hietzing, Vienna, Austria; [3]Dept Nephrology, Medical Univ Clinic 3, Vienna, Austria; [4]Dept Medicine 4, Medical Univ, Innsbruck, Austria; [5]Clin. Div Nephrol., Medical Univ, Graz, Austria; [6]Austrian Dialysis and Transplant Registry, Rohr/Kremstal, Austria.*

**Background:** DKD is the leading cause of end stage renal disease (ESRD) in Austria.

**Methods:** We analyzed the Austrian Dialysis- and Transplant Registry for DKD-related ESRD for changes from 1965-2010.

**Results:** Over 45 years, 7557 and 20468 patients with DKD- and non-DKD-ESRD, respectively, started RRT. While DKD was not present until 1974, in 1975 7 type 1- (T1-DKD) and 1 type 2- (T2-DKD) diabetics started RRT (1.0 per million population - PMP). Around 1985, DKD increasingly led to RRT (1986: 14.7 PMP, 1996: 31.4 PMP) until 2006 (peak incidence n=436; 52.7 PMP). Then numbers decreased annually (2010: n=350 , 41.7 PMP) despite a growing diabetic population estimated to be 5.9% of population in 2006. Point prevalence of RRT-patients increased from 65.3 PMP in 1976 to 992.0 PMP in 2010 and also in DKD (1976: 0.9 PMP, 1986: 27.5 PMP, 1996: 96.4 PMP, 2006: 191.1 PMP ; 2010: 210.2 PMP). Mean age increased over time in all patients. In T2-DKD-patients (T1-DKD) mean age was 53.3 (33.0), 64.2 (47.3), 68.4 (46.3), 66.7 (45.1) and 69.0 (48.2) years in 1976, 1986, 1996, 2006 and 2010, respectively. T2-DKD females always were 3.4±3.3 years older than males. Numbers of females increased less than of males and reached peak incidence 2 years earlier. Finally, 5-year-survival probability in two DKD-cohorts (1997/98 versus 2007/08) was calculated. It was 28% in 1997/98 and 37.5% in 2007/08. Relative risk reduction unadjusted was 22% (HR 0.78, CI 95% 0.67-0.89; p=0.001).

**Conclusions:** Despite an increasing prevalence of diabetes, incidence of DKD-RRT-patients decreases since 2007. 5-year survival probability has improved significantly in recent years. It is speculated that RAAS-blockers and/or the recommended multifactorial therapeutic interventions may have resulted in this improvement.

## SA-PO523

**Dialyzing Ladies or Gentlemen: Does It Matter?** Mehmet S. Sever,[1] Serra Artan,[1] Fatih Kircelli,[2] Murvet Yilmaz,[3] Gulay Asci,[4] Cengiz Dogan,[5] Kutay Gunestepe,[2] Ali Basci,[4] Ercan Ok.[2,4] *[1]Istanbul Medical Fac., Turkey; [2]Fresenius Medical Care, Turkey; [3]Bakirkoy EAH, Turkey; [4]Ege Medical Fac., Izmir, Turkey; [5]CEND Consulting, Turkey.*

**Background:** Application and consequences of hemodialysis treatment may differ between genders. This observational study analyzes hemodialysis practice in a large cohort of male and female patients.

**Methods:** Data on 1,599,694 hemodialysis sessions performed in 7668 prevalent patients, who were treated in 55 centers between May 2009 - May 2012 were retrieved from the European Clinical Database (EuCliD)-Turkey, and submitted for analysis.

**Results:** Results on various demographic, clinical, laboratory and outcome features of the patients are provided in table-1.

*Table-1.* Various parameters of the male and female prevalent patients.

| | Female (n=3329) | Male (n=4339) | Overall (n=7668) | P |
|---|---|---|---|---|
| Age (yrs.) | 58,3±15,2 | 56,5±14,9 | 57,2±15,1 | <0,001 |
| Diabetic (%) | 28,5 | 27,2 | 27,7 | NS |
| A-v fistula usage (%) | 77,2±37,9 | 85,4±31,1 | 81,9±34,5 | <0,001 |
| Kt/V | 1,6±0,3 | 1,4±0,2 | 1,5±0,3 | <0,001 |
| Body mass index | 25,8±5,6 | 24,6±4,4 | 25,1±5,0 | <0,001 |
| IDWG/Dry weight (%) | 3,3±1,4 | 3,4±1,3 | 3,4±1,3 | <0,001 |
| Serum P (mg/dl) | 4,7±1,1 | 4,9±1,2 | 4,8±1,2 | <0,001 |
| Serum PTH (pg/ml) | 401,5±370,3 | 429,6±388,9 | 417,2±381,0 | 0,016 |
| Hb (g/dl) | 11,2±1,1 | 11,7±1,3 | 11,5±1,2 | <0,001 |
| ESA usage (%) | 75,2 | 65,0 | 69,4 | <0,001 |
| Hospitalization (%) | 31,4 | 27,7 | 29,3 | 0,001 |

*Abbreviations:* IDWG: interdialytic weight gain.

Survival at 3 years were similar both in female and male patients.

**Conclusions:** Many parameters, which may affect prognosis differ significantly between female and male dialysis patients. Measures to idealize unfavorable parameters in each group may improve ultimate outcome.

*Funding:* Pharmaceutical Company Support - Fresenius Medical Care - Turkey

## SA-PO524

**Associations of the Conicity Index, an Indication of Abdominal Fat Deposition, with Health-Related Quality of Life in Maintenance Hemodialysis Patients** Gildete Barreto Lopes,[1] Lucas Resende,[1] Elen S. Oliveira,[2] Barbara De Alencar Costa,[1] Pamella R. Conceição,[2] Gentil Luz Junior,[1] Luciana Ferreira Silva,[2] Antonio Alberto Lopes.[1] *[1]Universidade Federal da Bahia; [2]Universidade do Estado da Bahia, Brazil.*

**Background:** A high percentage of abdominal fat relative to peripheral fat represented by high **conicity index (CI)** is associated with higher death risk in maintenance hemodialysis (MHD) patients (Nephrol Dial Transplant 2010; 25: 562–68) but there is a lack of studies to investigate associations of **CI** with health-related quality of life (HRQOL). We investigated associations of the **CI** with HRQOL in MHD patients.

**Methods:** Cross-sectional analysis of data of 627 patients in the Prospective Study of the Prognosis of Chronic Hemodialysis Patients (PROHEMO) developed in 4 clinics in Brazil. SF-36 was used to generate HRQOL scores of 8 primary scales and two composite summaries, i.e., the physical (PCS) and mental components. **CI** was calculated by the following equation(J Clin Epidemiol 1991; 44:955-56): waist circumference (WC) in meters/ [0.109 x square root of (weight in kilograms/height in meters)]. **CI** represents how many times the WC is greater than the circumference of a cylinder formed by the person's height and weight. **CI** was categorized by quartiles: ≤1.23, 1.24 to 1.30, 1.31 to 1.36 and ≥1.37. Wilcoxon-type test was used to test the trend in HRQOL across **CI** quartiles.

**Results:** For all HRQOL scales there was a trend to reduction in scores toward higher **CI**. Steady reductions (P<0.005) in scores across **CI** quartiles were observed for PCS (scores of 44.4, 42.7, 41.5, 38.0), physical functioning (scores of 75.4, 70.4, 67.1, 54.7), role physical (scores of 49.4, 42.0, 41.5, 36.4) and pain (scores 74.1, 72.9, 68.8, 61.9). In linear models adjusted for numerous covariates each 0.2-point more in **CI** was associated (P<0.05) with decrease in HRQOL scores by 2.6 points for PCS, 9.1 points for physical functioning and 6.2 points for pain.

**Conclusions:** The results suggest that MHD patients with high abdominal fat relative to peripheral fat have, in general, poorer HRQOL, mainly for physical dimensions. **Conicity index** is a simple indicator of abdominal fat deposition that may help identify MHD patient with poorer HRQOL.

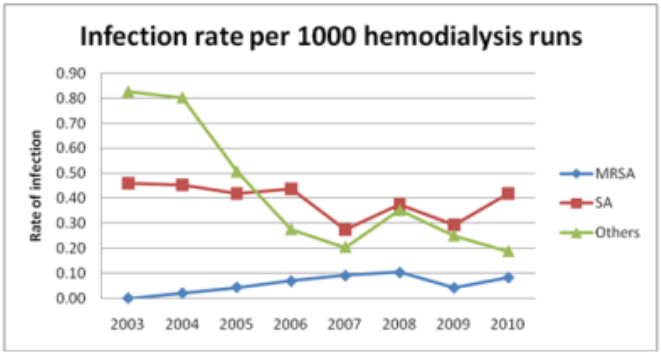
## SA-PO525

**Changes in Rate of *Staphylococcus aureus* Bacteremia in Hemodialysis Population** Emily A. Christie, Neesh I. Pannu. *Nephrology, Univ of Alberta, Edmonton, Canada.*

**Background:** Despite advances in antimicrobial therapies and preventative measures to decrease the rates of catheter-related bloodstream infection (CRBSI) in hemodialysis patients, these infections remain an overwhelming cause of morbidity and mortality.Serious complications from CRBSI occur in approximately 30% to 40% of hemodialysis patients. A landmark study published in 2003 demonstrated a significant mortality benefit of topical antibacterial ointment to central line sites. We evaluated the effects of Northern Alberta Renal Program (NARP) topical antibiotic protocol changes and to study any associated impact on the spectrum of pathogens.

**Methods:** The population studied were adult patients on hemodialysis within NARP, using temporary or tunneled catheters. All patients with documented bacteremia between January 1,2003-December 31,2009 within the Infection Control Database were included. Our analysis focused on the changes in the spectrum of pathogens over time.

**Results:** Our results revealed a decrease in the overall infection rate from 1.29 to an average of 0.69 bacteremias per 1000 HD runs, a 46.4% decrease. The analyis of the bacterial spectrum causing CRBSI showed a decrease in the proportion of infection caused by common pathogens and a corresponding rise in the rate of Staphylococcus aureus (SA). MRSA rates have shown a gradual increase over time.




**Conclusions:** The rate of hemodialysis catheter related infections in the NARP population has decreased over the past ten years. The spectrum of pathogens responsible for line-related bacteremias has shifted toward a greater proportion of SA infections. The rate of SA bacteremia in the NARP population is high compared to current published data. Further work is currently underway to investigate if there has been a corresponding impact on patient outcomes, as well as investigating the antibiotic regimens employed in this patient population.

## SA-PO526

**Overview of Regular Hemodialysis Treatment in China (as of 31 December 2012): Data from Chinese National Renal Data System** Xiang-Mei Chen, Dong Zhang, Qiang Qiu, Xuefeng Sun. *Dept of Nephrology, State Key Laboratory of Kidney Disease, General Hospital of Chinese People's Liberation Army, Beijing, China.*

**Background:** This report provides a summary of the 2012 Chinese Renal Replacement Therapy Registry Annual Report.

**Methods:** The data was recorded on the nationwide, web-based, and prospective registry system, the Chinese National Renal Data System (CNRDS). The patient data including age, gender, laboratory data and clinical and pathology diagnoses was recorded on the web page of the CNRDS. We analyzed the characteristics and trends of chronic hemodialysis in 2012.

**Results:** There were 3,540 hemodialysis centers in China having been registered in CNRDS. The total number of patients undergoing hemodialysis at the end of 2012 was 221,628. The mean age of all patients was 52.4±14.4 years and the proportion of male was58.7%. The mean duration of hemodialysis was 2.9±2.8 years. Primary diseases of all patients were respectively primary glomerular diseases (58.1%), diabetic nephropathy (16.5%), hypertensive nephropathy (10.1%), polycystic kidney disease (3.3%), renal calculus (2.0%) and others (10.0%). The standard-achieving rate of blood pressure in predialysis (≤140/90 mmHg) was 36.8%. The average dose of erythropoietin was 7498.33IU/week and the standard-achieving rate of hemoglobin in predialysis (≥110g/L) was 30.2%. The level of serum total calcium was 2.1-2.37mmol/L in 37.0% of patients. There were 32.8% of patients whose serum phosphorus was 1.13-1.78mmol/L. There were 24.9 percent of patients whose serum iPTH was 150 to 300pg/ml. 12,866 hemodialysis patients died in 2012. The causes of death were respectively cardiovascular events (45.6%), cerebrovascular events 20.8%), infection (11.1%) and others.

**Conclusions:** Compare to 2011, there was an increase of 7.2% with the number of hemodialysis centers in 2012. There was no obviously difference in primary diseases between 2011 and 2012. But the level of Hb and Alb were improved in 2012. There were no significant changes in standard-achieving rates of Ca, P and PTH. We should pay more attention to the management of disorders of calcium and phosphorus metabolism in Chinese dialysis patients.

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## SA-PO527

**Calciphylaxis in ESRD Patients from the USRDS** Lu Y. Huber,[1] Shalini Saith,[1] Mufaddal F. Kheda,[1] Stephanie L. Baer,[2] N. Stanley Nahman,[2] Rhonda E. Colombo,[1] Kristina W. Kintziger.[1] *[1]Nephrology, Georgia Regents Univ, Augusta, GA; [2]Nephrology, Charlie Norwood VAMC, Augusta, GA.*

**Background:** Calciphylaxis (CPX) is a complication of ESRD with a high mortality. It is a small vessel vasculopathy of unexplained etiology, but thought to be associated with calcium-phosphorus dysregulation. Because of its low prevalence, conducting large scale studies to assess the disease has proven difficult. We therefor queried the USRDS to investigate the natural history and relevant co-morbidities associated with CPX.

**Methods:** All prevalent ESRD patients in the USRDS were queried. CPX was defined using the ICD-9 diagnosis code 275.49 (other disorders of calcium metabolism) plus at least one of 7 additional relevant diagnosis codes identifying skin lesions common to the disease (i.e. nodules, ulcers, skin necrosis, etc) during the same inpatient visit. Basic descriptive statistics were used to assess frequencies and percentages for categorical variables, and mean ± standard deviation for continuous variables.

**Results:** A total of 2,133,440 individuals with ESRD diagnosed between 1963 and 2009 were identified, and 13,560,760 hospital records from 1977 through 2008 were available for analysis. There were 459 (0.02%) patients who met the criteria for CPX. For this group: age was 50.4±15.7 years with 20.3% > 65 years, 71.0% female, 59.0% white, and 37.3% African American. Treatment modality at the time of CPX diagnosis: center-based hemodialysis (HD) (361; 78.7%), peritoneal dialysis (PD) (68; 14.8%) and transplant (11; 2.4%). The median survival time for individuals with CPX was 1.1 years and 50% of the deaths were within 87 days of their CPX diagnosis. Co-morbidities associated with CPX included: obesity (79.1%), diabetes mellitus (65.8%), coronary artery disease (47.7%), bacteremia (43.1%), and history of parathyroidectomy (28.1%).

**Conclusions:** Although rare, CPX is associated with a very high mortality. It is more commonly seen in diabetics, obese individuals, females, and patients on in-center HD. Cardiovascular disease and severe mineral bone disorders may also be present. Bacteremia is a common complication and may contribute to the rapid demise associated with the diagnosis.

## SA-PO528

**Risks for Mortality in Hemodialysis Patients with Bacteremia** Stephanie L. Baer,[1,2] Rhonda E. Colombo,[2] Jay Desai,[1,2] Lu Y. Huber,[2] Puja Chebrolu,[2] Mufaddal F. Kheda,[2] N. Stanley Nahman,[1,2] Kristina W. Kintziger.[1,2] *[1]Augusta VAMC, Augusta, GA; [2]Georgia Regents Univ, Augusta, GA.*

**Background:** Bacteremia (BAC) occurs in over 20% of incident hemodialysis (HD) patients (Chebrolu, IDSA 2012). BAC is clinically associated with significant morbidity and mortality. We queried the USRDS to assess risk factors associated with 30-day mortality after a diagnosis of BAC.

**Methods:** All incident adult HD cases from the USRDS for 2005-2008 were queried for a diagnosis of BAC, mortality, and comorbidities, using ICD-9 codes and data from form 2728. Bivariable and multivariable analyses were performed.

**Results:** There were 359,882 adult incident HD patients available for analysis, of which 78,425 (21.8%) had BAC during the study period. Of these, 15,576 (19.9%) died within 30 days of BAC. Those with a significantly greater risk of 30-day mortality were 65+ years [relative risk (RR) 2.19, 95% confidence interval (CI) 2.12-2.27], and had: *Candida* colonization [RR 1.15, 95% CI 1.08-1.22], cirrhosis [RR 1.73, 95% CI 1.64-1.83], peripheral vascular disease [RR 1.20, 95% CI 1.16-1.24], meningitis [RR 1.62, 95% CI 1.28-2.0], pneumonia [RR 1.23, 95% CI 1.18-1.27], endocarditis [RR 1.30, 95% CI 1.21-1.40], pancytopenia [RR 1.17, 95% CI 1.02-1.35], decubitus ulcer [RR 1.53, 95% CI 1.48-1.59 ], *C. difficile* colitis [RR 1.18, 95% CI 1.12-1.24], TPN [RR 1.73, 95% CI 1.62-1.85], or critical care [RR 1.42, 95% CI 1.38-1.46]. The following factors significantly reduced the risk of 30-day mortality: being black [RR 0.79, 95% CI 0.77-0.82], Hispanic [RR 0.76, 95% CI 0.72-0.80], diabetes [RR 0.87, 95% CI 0.84-0.89], kidney transplant [RR 0.56, 95% CI 0.45-0.70], lupus [RR 0.79, 95% CI 0.68-0.93], glomerulonephritis [RR 0.81, 95% CI 0.72-0.92], osteomyelitis [RR 0.80, 95% CI 0.74-0.87], and MRSA infection [RR 0.72, 95% CI 0.67-0.77].

**Conclusions:** Of HD patients, over 20% will acquire BAC and 20% of those will die in 30 days. Risk factors for mortality include age, *Candida* colonization, cirrhosis, peripheral vascular disease, meningitis, pneumonia, endocarditis, pancytopenia, decubiti, *C. difficile*, TPN, or critical care.

## SA-PO529

**Restless Legs Syndrome and Drug Therapy among Dialysis Patients: USRDS Part D and Special Study Data** Nancy G. Kutner,[1] Rebecca H. Zhang,[1] Donald L. Bliwise.[1,2] *[1]USRDS Rehabilitation/QoI Special Studies Ctr, Emory Univ, Atlanta, GA; [2]Neurology, Emory Univ, Atlanta, GA.*

**Background:** Restless legs syndrome (RLS) is reported by 15%-30% of dialysis patients, but RLS contributors are not well specified. Use of medications with central nervous system (CNS) effects, shown to be associated with symptoms of RLS in the general population, merits study.

**Methods:** In USRDS Part D data for incident patients 10/1/06-12/31/10 we identified 4,879 RLS cases by ICD-9 RLS code (333.94) and 4 age-sex-race matched non-RLS controls (n=19,516), excluding patients with Parkinson Disease Dx. The odds of RLS Dx yes/no for antidepressants, anti-histamines, anti-psychotics, and metoclopramide were investigated in stratified logistic regression analyses, adjusting for vintage, drug supply days, and demographic variables, for drug use prior and following RLS Dx date.Use of drugs commonly prescribed for RLS was also examined. In addition, among 377 prevalent patients undergoing hemodialysis 2009-11 in the Atlanta USRDS ACTIVE-ADIPOSE study cohort, patient-reported RLS was identified by the International Restless Legs Syndrome Study Group criteria, with symptom frequency of 5 times/month or more and severity rated at least moderate or more severe over the last month, and patients' current home medication lists were reviewed.

**Results:** RLS Dx was clearly associated with use of Requip, Mirapex, and Sinemet, drugs commonly prescribed to treat RLS. However, the odds of patients with RLS Dx having CNS drugs prescribed were significantly greater for every comparison except anti-psychotics prior to RLS Dx.

| Drug Classes | RLS (n=4,879) Prior to Dx | After Dx | No RLS Dx (n=19,516) |
|---|---|---|---|
| Antidepressants, % | 25.7 | 37.8 | 20.5 |
| Anti-histamines, % | 2.8 | 5.3 | 2.3 |
| Anti-psychotics, % | 4.4 | 7.9 | 4.3 |
| Metoclopramide, % | 12.2 | 19.9 | 10.8 |

In the Atlanta prevalent cohort (median vintage = 3 years), RLS was reported by 17.8%, and 40% of these participants had one or more antidepressant, anti-histamine, anti-psychotic, and/or metoclopramide therapy prescribed.

**Conclusions:** These data suggest the value of carefully reviewing prescription of CNS drugs known to increase the risk for RLS symptoms.

*Funding:* NIDDK Support

## SA-PO530

**Maternal and Long Term Offspring´s Outcomes Derived from Pregnancies in Women Suffering from End Stage Renal Disease under Renal Replacement Therapy** Amelia Rita Bernasconi,[1] Ricardo M. Heguilen.[1] *[1]Medicine, Hospital J.A. Fernandez, Caba, Bs As, Argentina; [2]Medicine, Hospital J.A. Fernandez.*

**Background:** Despite fertility is compromised in women with end stage renal disease (ESRD), pregnancy occurs even in those receiving renal replacement therapy (RRT). In this retrospective study we report a 13-year experience of a nephro-obstetric group. The main aim was to analyze the outcome of a large cohort of pregnant women under RRT. Long-term maternal follow up with emphasis in perinatal and childhood outcomes are described.

**Methods:** Transversal study which including non-DBT RRT-requiring ESRD women and their offspring's.

**Results:** 40 consecutive P (3 twin, and 1 triple) in 38 non-DBT ESRD pts (30.6 ± 1.2 y/o), assisted from January 1, 2000 until June 1, 2013 were analyzed. 27/38 pts were already undergoing RRT (26 on HD and 1 on PD) at the time of gestation while 11 started HD because of P at 10.2 + 6.4 weeks of gestation. 3/4 of them were hypertensive and received amlodipine, alone or in combination with α methyldopa or labetalol. Hypertension worsened in 8 so dose had to be increased or an additional drug had to be added. Polyhydramnios developed in 17 P and preterm delivery was the rule (100%). Gestational age was 30.6 ± 0.9 wks and 28 P ended in caesarean sections. Leading causes of prematurity were polyhydramnios,premature membranes rupture, uncontrolled hypertension. Fetal weight at delivery was 1725 + 140 g while 23 weighed <1500 g. 14 of these 23 survived. Fetal demise was high, especially for the multiple pregnancies. Respiratory distress, congenital abnormalities and necrotizing enterocholitis were causes of fetal death. Low weight at birth neonates were followed up. Four developed retinopathy and underwent laser therapy, 2 developed hearing disorders, 4 developmental delay disorders with dyslexia, 1 had arrhythmia, 1 underwent ortopedic surgery because of congenital deformities. Children whose mothers receivded intensified HD had improved weight and no one reported adverse long term outcome.

**Conclusions:** Pts. In RRT seems to get benefits of intense dialysis, EPO and advances in dialysis, obstetrics and neonatal care improved the outcomes. It remains difficult to advise this women to conceive during HD.

## SA-PO531

**Procalcitonin (PCT) as Bacterial Infection Marker in Patiens with Renal Disease** Alba Santos, Nayara Panizo, David Arroyo, Javier Reque, Borja Quiroga, Isabel Galán Carrillo, Marian Goicoechea, Jose Luno. *Nephrology, Hospital General Universitario Gregorio Marañón, Madrid, Spain.*

**Background:** PCT is a helpful marker to identify bacterial infections in general population. However, the usefulness of this biological marker has not been proved in patients with renal disease. The aim of this study was to evaluate the accuracy of PCT as bacterial infection marker in patients with renal dysfunction.

**Methods:** 62 patients (mean age: 60.1 ±15.1 years, 33 M ,29 W) with renal disease (34 on dyalisis, 34 transplant recipients and 4 with AKI) who were admitted to the emergency department with fever ( > 38ºC) were included in the study. Baseline characteristics, analytical and microbiological data were collected.

**Results:** Positive microbiological cultures was found in 33 patients. PCT levels were higher in patients with positive cultures than in negative-cultured patients (17.7±33.4 vs 2.1±4.9 µg/l, p=0.012). No differences in CRP, leucocytes and fibrinogen levels were found between patients with positive or negative cultures. Positive cultures included 27 bacterial (17 gram–positive and 10 gram-negative ) , 2 fungi and 4 viral infections. PCT levels were higher in bacterial vs viral infection (20.06±35.02 µg/L vs 0.47±0.33 µg/L, p=0.018). PCT levels were correlated to CRP (r=0.689, p=0.001), fibrinogen (r=0.413,p=0.003) and neutrophil count (r=0.243,p=0.05). PCT concentration was negatively correlated with systolic (r=-0.412 p=0.01) and dyastolic blood pressure (r=-0.287,p=0.029). Positive microbiological cultures and CRP levels determined PCT concentration in mutivariate lineal regression analysis (β=0,258 y 0,484 , p=0,045 y p=0,001, respectively). The ROC curve for PCT was 0.640 with pooled specificity 97% and sensitivity 31%; LR positive: 10.33 and LR negative: 0.71.

**Conclusions:** PCT is a useful infection marker in patients with renal disease, as it helps to identify bacterial infection, with high specificity although low sensitivity. Other clinical and biological markers do not permit to identify bacterial infection in renal disease patients.

## SA-PO532

**Glycemic Control Is a Predictor of Infection in Hemodialysis Patients: Miyazaki Dialysis Cohort Study (MID Study)** Tatsunori Toida,[1] Yuji Sato,[1] Hiroyuki Komatsu,[1] Masao Kikuchi,[1] Shouichi Fujimoto.[2] *[1]Dept of 1st Internal Medicine, Faculty of Medicine, Univ of Miyazaki; [2]Dept of Hemovascular Medicine and Artificial Organs, Faculty of Medicine, Univ of Miyazaki, Japan.*

**Background:** There are limited data on the association between infection and glycemic control in diabetic patients on hemodialysis (HD). We investigated the impact of glycemic control on infection risk among diabetic patients on HD.

**Methods:** Patients with maintenance HD (n=1551) were enrolled in the MID Study in December 2009 and followed up for 3 years. The diagnosisof diabetes at baseline and information on clinical events of infection (1) hospitalization with infection, 2) hospitalization with pneumonia, 3) onset of pneumonia) during follow-up were extracted from the medical records.Cox regression analysis was used to examine the association between glycemic control and the events.

**Results:** At baseline, there were 493 patients with diabetes and 1058 patients without it. Compared with those in the bottom criterion of HbA1c level (HbA1c3.8-5.0%), the multivariable-adjusted hazard ratio for hospitalization with infection was not increased in the second and third criteria of HbA1c (HbA1c 5.1–6.0% to 6.1–7.0%), but was significantly increased to 8.43 (95% CI 2.62-27.11) in the group with poorest glycemic control (HbA1c≥7.1%). Similarly for hospitalization with pneumonia and onset of pneumonia, the multivariable-adjusted hazard ratios in the group with poorest control were 6.30 (95% CI 1.15-34.55) and 3.88 (95% CI 1.061-14.17), respectively.

**Conclusions:** Poor glycemic control (HbA1c≥7.1%) was associated with high infection risk among HD patients. The relationship between glycemic control and infection risk is not linear, so there might be a possibility of increasing the risk of infection depending on the glycemic control threshold.

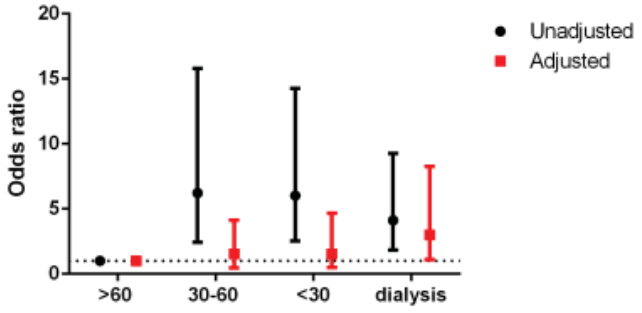
## SA-PO533

**p-Cresyl Sulfate Directly Associates with FGF23 and Aortic Calcification in Chronic Kidney Disease Patients, Independent of Kidney Function** Liesbeth Viaene,[1] Sam Heye,[2] Kathleen Claes,[1] Bjorn Meijers,[1] Pieter Evenepoel.[1] *[1]Nephrology, Univ Hospitals, Belgium; [2]Radiology, Univ Hospitals, Belgium.*

**Background:** Vascular calcification is common in patients with chronic kidney disease (CKD) and associates with increased cardiovascular morbidity and mortality. As opposed to coronary artery calcification, data on the prevalence and risk factors of aortic calcification (AC) in CKD are scarce. Recent studies suggested that uremia-related risk factors may be of great importance, as they may act synergistically with traditional risk factors. The present cross-sectional observational study aimed to clarify the role of *p*-cresyl sulfate (PCS), indoxyl sulfate (IndS) and mineral metabolism disturbances in the pathogenesis of AC in CKD.

**Methods:** Parameters of mineral metabolism, including FGF23, calcidiol, calcitriol and PTH, and PCS and IndS (HPLC) were assessed in 264 CKD patients (61% males; 58 ±14 years, CKD stage 1-5D). AC was assessed by lateral lumbar X-ray.

**Results:** AC was present in 60% of patients. Age and diabetes were identified as major determinants of AC. The adjustment for traditional risk factors attenuated but did not erase the association between CKD and AC.




PCS and FGF23 levels showed an inverse relationship with renal function and a direct relationship with AC. PCS was identified as determinant of FGF23, independent of classical determinants. In multivariate regression analysis, age, diabetes mellitus and PCS were identified as independent determinants of AC. When FGF23 was included, it replaced PCS in the final model.

**Conclusions:** High PCS and FGF23 levels are associated with an increased risk of aortic calcifications, independent of traditional risk factors and renal function. The link between PCS, FGF23 and AC requires further research. Experimental data suggest that Klotho deficiency might be the common pathway.

## SA-PO534

**Correlation of Fibroblast Growth Factor-23 with Carotid Atherosclerosis in Patients with Chronic Kidney Disease** Anil Kumar Yadav, Alok Goel, Sunil Agarwal, Shuchi Bhatt, Basu Dev Banerjee, Om Parkash Kalra. *Univ College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.*

**Background:** FGF-23 is increased early in CKD and is recognized as an important predictor of cardiovascular morbidity and mortality in these patients. Carotid Intima Media Thickness (CIMT) has been used as a surrogate marker of atherosclerosis and can be used to predict cardiovascular risk in these patients. Study was designed to estimate the levels of FGF-23 and intact parathyroid hormone(iPTH) and measure CIMT and then assess the correlation between these parameters in patients with various stages of CKD.

**Methods:** This cross-sectional study consisted of 75 subjects in the age group of 21 to 60 years, divided into 3 groups (25 in each). Group I: Healthy controls, Group II: CKD stage 2, 3 and 4 patients and Group III: CKD stage 5 patients on hemodialysis for ≥ 3 months. C-FGF-23 and iPTH estimation was done by ELISA method. CIMT was measured using Doppler ultrasound.

**Results:** All the 3 groups were age and sex matched. Mean C-FGF-23 was found to be significantly higher in Group III patients (622.17±42.45 rU/mL) when compared with group I (53.95±8.11 rU/mL), or Group II patients (213.59±107.21 rU/mL) ($p<0.001$ for all). Further, C-FGF-23 levels in stage 2 CKD patients were found to be significantly higher than healthy controls ($p<0.001$). Similarly mean iPTH was significantly higher in group III patients (543.90±59.24 pg/mL) as compared to group I (42.99±8.84pg/mL), and group II patients (150.39±126.48pg/mL) ($p<0.001$ for all). Further, no significant difference in iPTH was found between healthy controls and stage 2 CKD patients ($p=0.708$). Mean CIMT was significantly higher in Group III (0.71±0.1 mm) when compared with Group I (0.45±0.1 mm) or Group II patients (0.50±0.1 mm) ($p<0.001$ for all). A significant positive correlation was found between C-FGF-23 and CIMT in all groups ($r=0.716$).

**Conclusions:** FGF-23 is the earliest marker of mineral bone disorder and is increased in CKD even in stage 2 patients and shows a positive correlation with carotid atherosclerosis.

*Funding:* Government Support - Non-U.S.

## SA-PO535

**Urinary Soluble Klotho Significantly Predicts Renal Outcomes in Patients with Chronic Kidney Disease** Masashi Kitagawa, Hitoshi Sugiyama, Hiroshi Morinaga, Ayu Ogawa, Toshio Yamanari, Akifumi Onishi, Yoko Kikumoto, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Dept of Medicine and Clinical Science, Okayama Univ Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

**Background:** Low serum Klotho levels have been reported to be associated with adverse kidney disease outcomes, while the urinary Klotho levels have been suggested to be a more sensitive biomarker than the serum Klotho levels in patients with chronic kidney disease (CKD). The purpose of this study was to identify the relationship between the urinary Klotho levels and the renal function in patients with CKD and investigate whether the urinary Klotho levels can predict kidney disease outcomes in patients with CKD.

**Methods:** We analyzed the urinary soluble Klotho levels in a cohort of 161 patients with stage 1-5 CKD and assessed the relationships between the urinary Klotho-to-creatinine ratio (Klotho/Cr), proteinuria and the kidney function. The patients were prospectively followed for two years to monitor for doubling of the baseline serum creatinine concentration and the initiation of renal replacement therapy.

**Results:** The urinary Klotho/Cr level was positively correlated with eGFR and proteinuria and negatively correlated with changes in proteinuria during the follow-up period. The patients were categorized into two groups according to the baseline median urinary Klotho value. Renal survival was significantly lower in the patients with a urinary Klotho/Cr ratio of ≤ 0.321 μg/gCr than in those with a urinary Klotho/Cr ratio of > 0.321 μg/gCr ($p=0.0398$). A Cox regression analysis adjusted for age, gender, hypertension, diabetes, dyslipidemia, eGFR, proteinuria, hemoglobin, phosphate and fibroblast growth factor 23 showed that a urinary Klotho/Cr ratio of > 0.321 was significantly associated with a reduced risk for the renal end point. The adjusted odds ratio for a urinary Klotho/Cr ratio of > 0.321 was 0.62 (95% confidential interval: 0.37-0.99; $p = 0.0471$).

**Conclusions:** In this study, lower levels of urinary Klotho were significantly associated with renal outcomes, suggesting that a lower urinary Klotho level can serve as a novel biomarker for CKD progression.

## SA-PO536

**Klotho Deficiency Contributes to Uremic Cardiac Hypertrophy and Fibrosis in Mice** Jian Xie, Joonho Yoon, Makoto Kuroo, Chou-Long Huang. *UT Southwestern Medical Center, Dallas, TX.*

**Background:** Klotho (KL) is a membrane protein produced mostly in the kidney; its ectodomain is released as a soluble protein. We have reported that soluble Klotho (sKL) protects the heart against stress-induced cardiac hypertrophy and fibrosis, by inhibiting TRPC6 channel in the heart & independently of FGF23. We examined whether reduced serum sKL contributes to cardiomyopathy in CKD.

**Methods:** Wild type (WT) and heterozygous (Het) KL-deficient (*kl*) mice (129/SvJ) were rendered CKD by 5/6 nephrectomy (Nx) or sham surgery for 4 wk.

**Results:** Serum sKL levels (assayed by IP + western blot) were lower in Het vs WT mice, and both were further reduced by 5/6 Nx. CrCl was reduced ~60% by 5/6 Nx, but not different between WT and Het. Heart weight/body weight ratio (HW/BW, mg/g) of WT mice was increased by 5/6 Nx (sham & CKD: 4.1±0.1 & 5.4±0.2, $p<0.01$); CKD-induced increase was enhanced in Het (sham & CKD: 4.0±0.1 & 6.2±0.2, $p<0.01$; also $p<0.01$, WT/CKD vs Het/CKD). Supporting the HW/BW results, *BNP* expression was increased in hearts of WT CKD vs sham mice, and CKD-induced increase was enhanced in Het *kl* mice. BP was not different between WT and Het sham mice, but equally elevated in both CKD mice. Feeding CKD mice with antihypertensives and diuretics in drinking water normalized their BP to the level of sham mice. Under which, HW/BW ratio remained significantly increased in CKD vs sham mice. Hct levels were decreased in CKD vs sham mice, but not different between WT & Het mice. Serum Pi was not different between WT CKD & Het CKD. Ejection fraction (EF) measured by MRI showed no difference between WT sham and CKD mice (63±3 vs 61±3 %). LV end-diastole volume (LVEDV) was slightly lower & HW/LVEDV ratio increased in WT CKD vs sham, indicating concentric hypertrophy and diastolic dysfunction of WT CKD hearts. In contrast, relative to sham, EF was decreased in Het CKD (47±4 vs 62±4 %, $p<0.05$), LVEDV was markedly increased, and HW/LVEDV unchanged, indicating progression of Het CKD hearts to dilatation and systolic dysfunction.

**Conclusions:** Soluble Klotho is cardioprotective. Reduced serum sKlotho in CKD is an important cause of uremic cardiomyopathy, independent of serum Pi and FGF23.

## SA-PO537

**Role of Parathyroidectomy in Achieving Therapeutic Targets in Dialysis Patients with Mineral Bone Disorder in the Cinacalcet Era** Gurmukteshwar Singh, Abdul Qadir, Jane Garb, Dhivya Sundaramurthy, Barbara A. Greco. *Baystate Medical Center, Springfield, MA.*

**Background:** Parathyroidectomy (PTX) is recommended to manage mineral bone disorder (MBD) in dialysis patients who fail medical therapy. Over the past decade, surgical approach has shifted away from total PTX and cinacalcet was introduced. We evaluated MBD parameters, medication use, and mortality in a cohort of dialysis patients who underwent PTX during this time frame.

**Methods:** Adult patients from 8 Fresenius dialysis units who underwent their first PTX between 2000 and 2010 were included. Uniform, evidence-based protocols were used for management of MBD. Patients were referred for PTX if their MBD was uncontrolled on maximum tolerated treatment including phosphorus binders, vitamin D analogs, and cinacalcet. Medical records were reviewed to extract patient characteristics, laboratory data, and medication use pre- and post-PTX.

**Results:**

| **Clinical Characteristics of 101 patients included in the study** | |
|---|---|
| **Type of PTX** | |
| Total | 42.6% |
| Total with auto-implant | 43.6% |
| Sub-total | 13.8% |
| **Age at PTX (Mean)** | 50.1 |
| **Race** | |
| Caucasian | 37.2% |
| African American | 34.9% |
| Hispanic | 27.9% |
| **Years on dialysis** | 4.5 |
| **Cause of ESRD** | |
| HT | 37.2% |
| GN | 27.9% |
| DM | 18.6% |
| Oth | 16.3% |
| **Study end-point** | |
| Transplanted | 53.5% |
| Died | 27.9% |
| Alive | 4.7% |
| Moved | 14% |
| **Median follow-up (yrs)** | 7.3 |

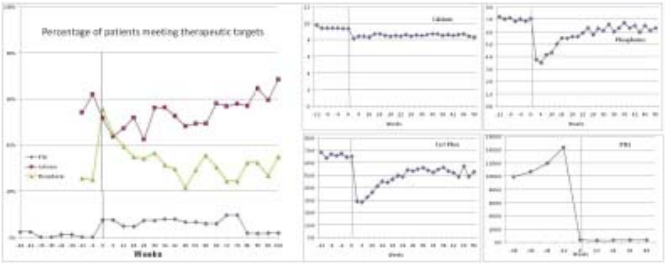



**Figure 1:** Percentage of Patients Meeting MBD Targets and Median Lab Values at Various Time Points

The percentage of patients meeting phosphorus goal increased following PTX but the benefit was transient. Most patients had sustained hypoparathyroidism after surgery despite attempts to preserve remnant tissue. Vitamin D and calcium requirements are being analyzed currently.

**Conclusions:** PTX does not uniformly lead to achievement of MBD therapeutic targets. PTX resulted in sustained hypoparathyroidism in a large percentage of patients. Further study is needed to determine risks and benefits of PTX in the cinacalcet era.

**SA-PO538**

**Comparison of Cinacalcet plus Paricalcitol to Cinacalcet plus Calcitriol Therapy in Hemodialysis Patients with Severe Hyperparathyroidism**
Siren Sezer,[1] Zeynep Bal,[1] Emre Tutal,[1] Öznur Kal,[1] Demet Yavuz,[2] Ibrahim Yildirim,[3] Burak Say?n,[1] Ruya Ozelsancak,[1] Sultan Ozturk,[4] Nurhan Özdemir Acar.[1] *[1]Dept of Nephrology, Baskent Univ Faculty of Medicine, Turkey; [2]Dept of Nephrology, Sakarya Univ Education and Research Hospital, Turkey; [3]Dept of Nephrology, Bulent Ecevit Univ Faculty of Medicine, Turkey; [4]Dept of Nephrology, Hitit Univ Education and Research Hospital, Turkey.*

**Background:** Clinical guidelines and literature reviews support the combination therapy with cinacalcet and VDRA treatment in patients with SHPT however, there is no consensus on the most effective type and dose of combination. The aim of this study is to evaluate and compare the effectiveness of cinacalcet and paricalcitol or calcitriol treatment of MHD patients with severe SHPT.

**Methods:** 146 patients with severe SHPT on chronic hemodialysis three times a week for at least 3 months were enrolled into the study. Patients with serum calcium concentration < 10.5mg/dL, serum Ca × P<75 and PTH level ≥ 1000 pg/ml were divided into two groups either who received cinacalcet plus intravenous paricalcitol (Group CP) or cinacalcet plus intravenous calcitriol (Group CC) for the treatment at least one year.

**Results:** 78 patients in group CP and 68 subjects in group CC were evaluated. In group CP, mean PTH values in 1st and 12th month were 1257.6 ± 668.4 pg/ml and 929.8 ± 497.3 pg/ml whereas in CC group, mean PTH values were 1226.9 ± 595.6 pg/ml and 1210.9 ± 574.8 pg/ml (p<0.003). At baseline both groups' alkaline phosphatase levels were similar however at the end of the study in group CP, ALP levels were significantly lower than the group CC (p<0.002). Despite the mean dose of vitamin D administration was significantly higher in paricalcitol group than the calcitriol group we observed less hyperphosphatemia and elevated CaxP in group CP (p<0.01, p<0.05 respectively).

**Conclusions:** This observational study showed that combination therapy with paricalcitol and cinacalcet is superior in terms of PTH response to treatment, less hyperphosphotemia and decrease in alkaline phosphatase levels to combination calcitriol and cinacalcet in dialysis patients with severe SHPT.

**SA-PO539**

**Vitamin D Analogs and Calcimimetics Use in CKD Can Influence Levels of Vitamin K Dependent Proteins (VIKI Study)**
Maria Fusaro,[1] Davide Miozzo,[2] Marianna Noale,[1] Giovanni Tripepi,[3] Antonio Piccoli,[2] Agostino Naso,[4] Rosalba Cristofaro,[2] Maurizio Gallieni.[5] *[1]CNR, Padua, Italy; [2]Univ of Padua; [3]CNR, Reggio Calabria; [4]Hospital of Padua; [5]Ospedale San Carlo Borromeo, Milano.*

**Background:** Vitamin K is involved in the production of Bone and Matrix Gla Proteins (BGP and MGP), regulating bone and vascular health. We carried out an observational study to evaluate associations between bone disease therapy and levels of BGP and MGP in patients on hemodialysis (HD).

**Methods:** Cross-sectional study of 387 HD patients. We determined BGP, MGP and routine biochemistry. We evaluated both vertebral fractures (VF) and aortic (AoVC) calcifications in the same spine radiograph. Three-year patients' survival was analyzed.

**Results:** VF and AoVC were associated with reduced BGP levels (for VF 213 *vs* 151 ng/ml, p<0.01; AoVC 262 vs 164 ng/ml). A total of 77 patients died during follow-up (19.9%). Mean follow-up was 2.7±0.5 years. Median MGP was significantly lower (15.0 versus 19.7 nmol/L, p=0.02) in nonsurvivors.

In a first multivariate stepwise regression BGP levels were 37.7% higher (p=0.0002) in patients treated with calcimimetics (19.4% of total). Patients treated with Vitamin D analogs (19.9% of total) also showed an increase of 20.1 % ( p=0.0228 ). Patients treated with calcium acetate (5.4%) showed increased MGP levels (+30.4%, p<=0.05), while in patients treated with calcimimetics MGP levels were increased by 21% (p=0.0142). A second multivariate stepwise regression considered alone or combined each of the previously identified predictors for BGP and MGP levels: calcimimetic alone increased BGP and MGP levels of 42% and 19%, respectively; combined treatment of calcimimetics, Vitamin D analogs and calcium acetate binding was associated with increased BGP levels (+59.8%) and MGP levels (+77%). Vitamin D analogs alone increased BGP levels (+24%).

**Conclusions:** We found lower BGP levels in patients with high prevalence of VF and AoVC. Lower MGP levels in non survivors. Both calcimimetics and vitamin D analogs are associated with increased BGP levels while increased MGP levels are found only in patients treated with calcimimetics. These drugs could have a relevant role in the protection of bone and vascular health.

*Funding:* Government Support - Non-U.S.

**SA-PO540**

**Ferric Citrate as a Phosphate Binder Has a Safety Profile Similar to Sevelamer Carbonate and Calcium Acetate**
Mohammed Sika,[2] Kausik Umanath,[1] Simin Goral,[4] Shahabul S. Arfeen,[5] Isai G. Bowline,[7] Gil Chernin,[8] Mark Koury,[2] Jamie P. Dwyer,[2] The Collaborative Study Group.[3] *[1]Henry Ford Hosp; [2]Vanderbilt Univ; [3]CMM; [4]Univ of Penn; [5]Neph Specialist; [6]Barzilai Med Center, Israel; [7]Wake Forrest Univ; [8]Ichilov Med Center, Israel.*

**Background:** Ferric citrate (FC), a phosphate binder, binds phosphorus safely and effectively in phase 2 trials. We report the safety profile of FC in a multicenter, randomized, open-label phase 3 trial in 441 dialysis subjects treated with FC or Active control(AC) [calcium acetate(CA), sevelamer carbonate(SC) or combo(CB)].

**Methods:** Entry criteria required subjects to be tolerant to AC. After a washout period in which all binders were stopped, subjects were randomized 2:1 to FC or AC, and followed during a 52-week safety assessment period (SAP). Adverse events were collected throughout the trial.

**Results:** 292 subjects were on FC. The 149 AC subjects included 78 on SC, 41 on CA and 30 (CB). During the SAP, 49% of subjects in the AC group experienced a serious adverse event (SAE) while only 39.4% of subjects did so in the FC group. There were 5.2% deaths in AC and 4.5% in FC. The percentages of AC subjects with SAE's in the infection, gastrointestinal (GI), cardiac and vascular categories were greater than those in the FC group.The number of treatment emergent adverse events (including serious and non-serious adverse events) per subject was 5.6 in FC and 6.5 in AC.14.2% of FC subjects and 9.4% of AC subjects discontinued from the trial or stopped study drug and continued in the trial. Despite a greater percentage of GI SAEs in the AC group, the single most common reason for discontinuation of study drug or withdrawal from the trial in the FC group was a GI adverse event. The difference in GI adverse events was expected because of the entry criterion requiring tolerance to AC drugs, while all subjects were naïve to FC.

**Conclusions:** FC, an efficacious phosphate binder, had an excellent safety profile when compared to currently available phosphate binders.

*Funding:* Pharmaceutical Company Support - Keryx Biopharmacueticals

**SA-PO541**

**A Prospective Study to Evaluate the Efficacy of Total PaRathyroidectOmy in Retarding Vascular/Valvular Calcification and Improving Bone MinEral Density in End-Stage Renal Disease Patients – PROCEED Study**
Angela Yee Moon Wang,[1] Brian Lang,[2] Yat Y. Yau,[3] Wai Kei Lo.[4] *[1]Medicine, Queen Mary Hospital, Univ of Hong Kong, Hong Kong, Hong Kong; [2]Surgery, Queen Mary Hospital, Univ of Hong Kong, Hong Kong, Hong Kong; [3]Radiology, Biomedical Imaging Center, Hong Kong, Hong Kong; [4]Medicine, Tung Wah Hospital, Hong Kong, Hong Kong.*

**Background:** Severe secondary hyperparathyroidism (SHPT) has been associated with increased risk of mortality & cardiovascular deaths in chronic kidney disease (CKD)-5D patients. We hypothesized that total parathyroidectomy (PTx) may retard vascular/valvular calcification & improve bone mineral density (BMD).

**Methods:** We conducted a prospective longitudinal study in 62 CKD-5D patients (mean age:55±11y;28% with previous fracture) with severe SHPT resistant to conventional medical therapy who underwent total PTx with forearm autograft. Primary endpoints were changes in vascular & valvular calcification assessed by plain coronary multislice computed tomography 1 year after PTx. Secondary endpoint was changes in BMD, Z-score & t-score by dual X-ray absorptiometry scan.

**Results:** Pre-operative median (interquartile range) total coronary artery calcium score (CACS) was 560 (190 - 1338) & increased significantly to 609 (228 - 1558)(P=0.014) at 1 year while mitral & aortic valve calcium score did not change significantly after 1 year. BMD at forearm [P=0.05], femur [P<0.001] & lumber spine [P<0.001] showed significant increase 1 year after PTx & was associated with sig. improvement in t-score & z-score at corresponding sites. CACS was stabilized or reduced in 30 patients while progression in CACS (as defined by ≥15% increase from pre-op CACS) was observed in 20 patients (40%). iPTH [P<0.001], serum calcium [P<0.001], phosphorus [P<0.001] & alkaline phosphatase [P<0.001] showed sustained significant reduction 1 year after total PTx.

**Conclusions:** Total PTx significantly improves biochemical parameters of CKD-mineral bone disease & BMD but did not necessarily retard vascular/valvular calcification in CKD-5D patients with severe SHPT, contrary to previous reports. The exact mechanisms whereby PTx improves clinical outcomes of 5D patients warrant further investigation.

*Funding:* Government Support - Non-U.S.

**SA-PO542**

**Ferric Citrate Binds Phosphorus, Delivers Iron, and Reduces IV Iron and Erythropoietic Stimulating Agent Use in End-Stage Renal Disease**
Julia Lewis,[1] Jamie P. Dwyer,[1] Mark Koury,[1] Mohammed Sika,[1] Gerald Schulman,[1] Mark T. Smith,[2] Frederick C. Whittier,[3] Douglas R. Linfert,[4] Claude Mabry Galphin,[5] Peale Chuang,[6] The Collaborative Study Group.[7] *[1]Vanderbilt Univ; [2]Kidney Care Assoc; [3]Clin Res; [4]Neph Assoc; [5]Renal Res Inst; [6]Metrolina Neph Assoc; [7]CMM.*

**Background:** We conducted a multi-center, randomized, open-label trial of an oral phosphate binder, ferric citrate (FC) in 441 dialysis subjects.

**Methods:** A 52-week Safety Assessment Period (SAP) in which subjects were randomized 2:1 to FC or active control (AC) (calcium acetate and/or sevelamer carbonate) was followed by a 4-week Efficacy Assessment Period (EAP) in which 192 subjects completing the SAP on FC were randomized 1:1 to continue FC or receive placebo.

**Results:** The EAP baseline mean serum phosphorus was 5.1 ± 1.2 mg/dL in FC subjects and 5.4 ± 1.5 mg/dL in placebo subjects. At the end of the EAP, serum phosphorus was lower in FC subjects (4.9 ± 1.3 mg/dL) vs placebo subjects (7.2 ± 1.8 mg/dL, p < 0.0001). During the 52-week SAP, serum phosphorus was similar in FC and AC groups (mean difference at week 52 = 0.04 mg/dL, p = 0.8). SAP baseline mean serum ferritins were 593 ± 293 ng/mL in FC subjects and 609 ± 308 ng/mL in AC subjects. At the end of the SAP, mean serum ferritin was 898 ± 489 ng/mL in FC subjects and 624 ± 361 ng/mL and AC subjects, p<0.0001. Transferrin iron saturation (TSAT) at baseline was 31% in both groups; at the end of the SAP, TSAT was higher, 39% FC group vs 29% AC group, p< 0.0001. IV iron use was prohibited, if serum ferritin was > 1000 ng/mL or TSAT was > 30%. FC subjects received less IV elemental iron (1.86mg/day) than AC subjects (3.84mg/day; p<0.001). FC subjects received less epoetin units (U) per day than AC subjects (756 U/day vs 993 U/

day, p<0.05). At week 52, hemoglobin was 11.4 and 11.1g/dL in the FC vs AC(treatment difference p=0.01).Serious adverse events and adverse events were similar in the EAP and the SAP in the FC, AC and placebo groups.

**Conclusions:** Ferric citrate is an efficacious and well-tolerated phosphate binder that also increases iron stores, and decreases IV iron and ESA use.

*Funding:* Pharmaceutical Company Support - Keryx Biopharmaceuticals

## SA-PO543

**Impact of Ferric Citrate, an Oral Phosphate Binder, on Mineral and Bone Metabolism Markers in Dialysis Patients** Daniel E. Weiner,[7] Mohammed Sika,[2] Jamie P. Dwyer,[2] Kausik Umanath,[3] Stephen Z. Fadem,[4] Marvin V. Sinsakul,[5] Peter N. Van Buren,[6] Jo Abraham,[1] The Collaborative Study Group.[2] *[1]Univ of Utah; [2]Vanderbilt; [3]Henry Ford; [4]Kidney Assoc; [5]Rush Univ; [6]UT Southwestern; [7]Tufts.*

**Background:** We conducted a multicenter, international, randomized, open-label clinical trial of a novel phosphate binder.

**Methods:** 441 dialysis subjects were enrolled in a trial consisting of a two week washout period during which all binders were stopped and a 52 week Safety Assessment Period (SAP) in which participants were randomized 2:1 to ferric citrate (FC, 1 gram caplets) or active control (AC), followed by a one month efficacy assessment period. There were 292 participants randomized to FC and 149 to AC. The AC group consisted of 78 participants treated with sevelamer carbonate, 41 with calcium acetate, and 30 with a combination of these agents.

**Results:** Results for mean serum phosphorus, calcium, iPTH and aluminum post-washout and at the completion of the SAP are in the table. Throughout the study, between 51% and 61% of participants had phosphorus in the 3.5 to 5.5 mg/dL range, without significant between group differences (p=0.41 to 0.98 at various time points). Four participants in the AC group had serum calcium greater than 10.5mg/dL, necessitating discontinuation of AC therapy versus none in the FC group; specifically, calcium was lower in FC subjects compared to calcium acetate only (9.1±0.9 vs 9.5±1.0, p=0.008).

**Conclusions:** Traditional markers of bone and mineral metabolism were similar in the AC and FC groups over a 52 week safety assessment period, with overall low aluminum levels in both groups despite the citrate component of FC and as expected given the use of calcium-containing binders, several episodes of hypercalcemia in the AC group. We conclude that FC can achieve control of MBD parameters comparable to current usual care.

| | Ferric Citrate | Active Control | p-value |
|---|---|---|---|
| Serum Phosphorus (mg/dL) | | | |
| Post-Washout | 7.4 ± 1.6 | 7.6 ± 1.7 | |
| Post-SAP | 5.3 ± 1.4 | 5.4 ± 1.6 | 0.96 |
| Serum Calcium (mg/dL) | | | |
| Post-Washout | 8.9 ± 0.8 | 9.0 ± 0.8 | |
| Post-SAP | 9.1 ± 0.9 | 9.3 ± 0.9 | 0.07 |
| Serum iPTH (pg/ml) | | | |
| Post-Washout | 624 ± 419 | 578 ± 444 | |
| Post-SAP | 456 ± 370 | 431 ± 331 | 0.9 |
| Serum Aluminum (mcg/L) | | | |
| Post-Washout | 7.1 ± 2.8 | 6.7 ± 2.1 | |
| Post-SAP | 7.4 ± 3.1 | 6.8 ± 2.0 | 0.11 |

*Funding:* Pharmaceutical Company Support - Keryx Biopharmaceuticals

## SA-PO544

**The Effect of JTT-751 (Ferric Citrate Hydrate) on Phosphorus and FGF-23 in Chronic Kidney Disease** Keitaro Yokoyama,[1] Hideki N. Hirakata,[2] Takashi Akiba,[3] Masafumi Fukagawa,[4] Masaaki Nakayama,[5] Kenichi Sawada,[6] Yuji Kumagai,[7] Geoffrey A. Block.[8] *[1]The Jikei Univ School of Medicine, Tokyo, Japan; [2]Japanese Red Cross, Fukuoka Hospital, Fukuoka, Japan; [3]Tokyo Women's Medical Univ, Japan; [4]Tokai Univ School of Medicine, Isehara, Japan; [5]Fukushima Medical Univ School of Medicine, Fukushima, Japan; [6]Akita Univ, Akita, Japan; [7]Kitasato Univ East Hospital, Sagamihara, Japan; [8]Denver Nephrology, Denver, CO.*

**Background:** JTT-751 is a novel iron-based phosphate (P) binder being developed for hyperphosphatemia in patients with chronic kidney disease (CKD).

**Methods:** A Phase 3, multicenter, randomized, double-blind, placebo-controlled study investigated the efficacy and safety of JTT-751 in non-dialysis dependent patients with CKD. A total of 90 patients with a serum P level of 5.0 mg/dl or higher were randomized 2:1 to JTT-751 (1.5–6.0 g/day, n=60) or placebo (n=30) for 12 weeks. The primary endpoint was change in serum P level from baseline to the end of treatment (EOT). Secondary endpoints included the percentage of patients achieving target serum P levels (2.5-4.5 mg/dl), and change from baseline in fibroblast growth factor-23 (intact-FGF-23) at EOT.

**Results:** The mean change in serum P level at EOT was –1.29 mg/dl in the JTT-751 group and 0.06 mg/dl in the placebo group (P<0.001). The percentage of patients achieving target serum P levels at EOT was 64.9% in the JTT-751 group and 6.9% in the placebo group (P<0.001). Treatment with JTT-751 significantly reduced intact-FGF-23 levels (P<0.001).

| | JTT-751:Baseline | JTT-751:EOT | Placebo:Baseline | Placebo:EOT |
|---|---|---|---|---|
| **P (mg/dl):Mean** | 5.66 | 4.37 | 5.57 | 5.62 |
| **FGF-23 (pg/ml):Median** | 453.0 | 209.0 | 358.0 | 478.0 |

JTT-751 significantly increased iron parameters (P<0.001) and hemoglobin (P=0.04). Adverse drug reactions were similar in patients receiving JTT-751 or placebo with gastrointestinal disorders occurring in 30.0% of JTT-751 patients and 26.7% of those receiving placebo.

**Conclusions:** In patients with non-dialysis dependent CKD, 12 week treatment with JTT-751 resulted in significant reductions in serum P and FGF-23.

*Funding:* Pharmaceutical Company Support - Pharmaceutical Company Support - Japan Tobacco Inc.

## SA-PO545

**Calcium Supplementation Affects Calcium Balance but Not Bone Mineral Density in Older Women with Moderate Chronic Kidney Disease** Anna Jeanette Jovanovich, Michel Chonchol, Gerard John Smits, Jessica B. Kendrick. *Div of Renal Diseases and Hypertension, Univ of Colorado Denver, Aurora, CO.*

**Background:** To examine whether calcium supplementation is associated with an improvement in bone mineral density (BMD) in women with moderate chronic kidney disease (CKD).

**Methods:** We performed a post-hoc analysis of the DECALYOS II, a 2-year randomized, double-blind, placebo-controlled study of 610 women randomized to calcium-vitamin $D_3$ fixed combination, calcium plus vitamin $D_3$ separate combination, or placebo. Both active treatment groups received the same daily amount of calcium (1,200 mg) and vitamin $D_3$ (800 IU). Dietary intake of calcium, 24-hour urine calcium excretion, and BMD of the distal radius measured by single X-ray absorptiometry were recorded at baseline (m 0), 12 (m 12) and 24 (m 24) months. Only participants (N=89) receiving calcium and vitamin D with CKD (eGFR < 60 ml/min/1.73m$^2$) after the baseline visit were included in this analysis. Linear regression models were used to assess the relationship between change in calcium balance and change in BMD.

**Results:** Mean age, eGFR, dietary calcium, urinary calcium excretion, and BMD were 78±5 years, 49±7 ml/min/1.73m$^2$, 527±191 mg/day, 103±63 mg/24h, and 0.32±0.1 g/cm$^2$. Serum calcium and intact parathyroid hormone (iPTH) were 9.3 ± 0.4 mg/dL and 66 ± 34 pg/mL, respectively. Fecal calcium excretion was assumed to be 1,000 mg/day. Participants were in negative calcium balance at baseline. During the course of the study the change in calcium balance was positive and significantly higher than baseline (m 0 to m 12: 1170.8 ± 67 mg/day; p<0.0001 and m 0 to m 24 1168.3±57 mg/day; p<0.0001). In linear regression models adjusted for age, height, weight, serum calcium, iPTH, 25-hydroxyviatmin D, eGFR, and intervention, a positive calcium balance was not associated with increased radial BMD at 12 (p=0.70) or 24 months (p=0.08). Serum calcium remained unchanged during the course of the study.

**Conclusions:** Calcium supplementation of 1,200 mg/day produced a positive calcium balance in older women with CKD, which was not related to improvements in radial BMD.

*Funding:* NIDDK Support

## SA-PO546

**Cinacalcet Improves Survival in Dialysis Patients – A European Cohort Study** Claudia Friedl,[1] Gilbert Reibnegger,[2] Reinhard Kramar,[3] Alexander R. Rosenkranz.[1] *[1]Dept of Internal Medicine, Clinical Div of Nephrology, Graz, Austria; [2]Institute of Physiological Chemistry, Graz, Austria; [3]Austrian Dialysis and Transplant Registry, Austria.*

**Background:** Dialysis patients are at excess risk of mortality. Observational studies have shown that high PTH, serum phosphorus and calcium are independent risk factors for cardiovascular and all-cause mortality in dialysis patients. Cinacalcet has proven to be efficient to improve these parameters. Aim of the present study was to test whether Cinacalcet treatment is associated with better survival in a cohort of Austrian dialysis patients.

**Methods:** Data from all prevalent dialysis patients from 01.01.2004 to 31.12.2009 (n=8225) were retrospectively evaluated. Data were obtained from the Austrian Dialysis and Transplant Registry. Patients without information about prescription of cinacalcet (n=164), age <18 years as well as a survival time of <90 days were excluded. Survival analysis was performed using the Kaplan Meier model and the Royston-Parmar model.

**Results:** The final study cohort included 7983 dialysis patients (n= 7251 hemodialysis; n=752 peritoneal dialysis), whereby 1572 (19.7%) had a prescription of cinacalcet. Compared to those without cinacalcet prescription patients receiving cinacalcet were younger (median [25th; 75th percentile] 57.9 [46.1; 67.7] vs. 66.0 [55.7; 74.8]), they were less likely to suffer from diabetes or cardiovascular diseases. During a median follow-up time of 2.6 years, 3574 (44.8%) patients died including 1342 (16.8%) deaths due to cardiovascular causes. Risk of all-cause and cardiovascular mortality was significantly decreased in the cinacalcet group. Odds ratio (OR) (with 95% CI) remained significant even after multivariable adjustment for age, sex, BMI, diabetes, cardiovascular comorbidity, registration status on kidney transplant waiting list, medication with phosphate binder and vitamin D compounds, hemoglobin, CRP, calcium, phosphorus and iPTH: all-cause mortality OR 0.50 (0.43-0.59), cardiovascular mortality OR 0.60 (0.48-0.75); p<0.0001.

**Conclusions:** In this observational retrospective study of a large homogenous European cohort, treatment with Cinacalcet significantly improves survival in dialysis patients.

**SA-PO547**

**Meal-Induced Change in Bone Mineral Variables in End-Stage Renal Disease** Hariprasad S. Trivedi, Aniko Szabo. *Medical College of WI.*

**Background:** Management of mineral and bone disorder is one of the important facets of care of end-stage renal disease (ESRD) subjects. As part of the same, bone mineral variables are regularly tested. However, these are randomly tested generally depending on when the patient presents for dialysis. Whether meals consumed by the patient affects these variables is not known.

**Methods:** As part of a study of circadian variation of bone mineral variables in ESRD we pre-planned evaluation of the effect of a mid-day meal on bone mineral variables in these subjects. Consenting subjects with ESRD and controls consumed a study diet containing 1200 mg calcium and 1000 mg of phosphorus per day for 5 days. In ESRD subjects phosphate binders, active vitamin products, and cinacalcet were discontinued prior to beginning the study diet. On the 6th day while the same diet was continued blood was drawn around 12 noon, followed by lunch and a second blood draw at 2:00 pm. In each sample phosphate, ionized calcium, intact parathyroid hormone (PTH), and 25-hydroxy vitamin D (25OHD) were tested. Within each group, mean difference of log-transformed values of each analyte, post-lunch versus pre-lunch, were analyzed using a paired t-test. Data are presented as ratios (post-lunch/pre-lunch). Two sample t-test was used to compare the effect of the meal between groups.

**Results:** 17 ESRD subjects (15 men, 2 women, mean age 45±12 years, 7 whites, 10 African-Americans) and 8 controls (6 men, 2 women, mean age 55±14 years, 4 whites, 4 African-Americans) participated in the study. In ESRD subjects post-lunch phosphate was significantly higher by about 12% (ratio 1.12, p= 0.0039) while ionized calcium was lower (ratio 0.98, p = 0.0002). PTH tended to be higher post-lunch but did not reach statistical significance (ratio 1.12, p=0.06). In control subjects only 25OHD was significantly different after the meal (ratio 0.94. p =0.0365). The between-group comparison showed that post-lunch the change in phosphate tended to be greater in ESRD subjects versus controls (p=0.05).

**Conclusions:** In ESRD phosphate is higher while calcium is lower after a meal. These variations should be considered during clinical testing.

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**SA-PO548**

**Circadian Variation of Bone Mineral Variables in End-Stage Renal Disease** Hariprasad S. Trivedi, Hershel Raff, Aniko Szabo. *Medical College of WI.*

**Background:** We investigated the circadian rhythm of bone mineral variables in end-stage renal disease (ESRD).

**Methods:** Subjects with ESRD on hemodialysis (n=17) and controls (n=8) were enrolled after written, informed consent. Diabetes mellitus was an exclusion criterion. After discontinuing all confounding medications (phosphate binders, active vitamin D, calcimimetics) for a period corresponding to 5 half lives, subjects ate a research diet controlled for the amount of daily calcium (1200 mg) and phosphorus (1000 mg), After overnight stay in the adult translational research unit, blood was drawn every 2 hours (8 am through following 6 am) for phosphate, ionized calcium, intact parathyroid hormone (PTH), and 25-hydroxy vitamin D (25OHD). A harmonic regression model with group-specific 24- and 12-hour periodic components was fitted to log-transformed values. Comparisons were made using autocorrelation adjusted repeated measures analysis of variance via generalized estimating equations.

**Results:** Characteristics of the subject population were as follows: ESRD subjects 15 men, 2 women, mean age 45±12 years, 7 whites, 10 African-Americans; Controls 6 men, 2 women, mean age 55±14 years, 4 whites, 4 African-Americans. The conditions of testing were optimal, based on typical circadian pattern of concurrent cortisol measurements in controls. Each molecule exhibited significant circadian variation in the ESRD subjects: phosphate (p<0.0001), ionized calcium (p=0.004), PTH (p=0.0004), and 25OHD (p=0.009). Peak and nadirs respectively were around the following times (hours): phosphate 3:30, 11:00; ionized calcium 12:15, 20:00; PTH 17:45, 10:15; 25OHD 9:45, 16:00. In controls only phosphate (p<0.0001; peak at 2:45, nadir 10:15) and PTH (P<0.0001; peak at 2:45, nadir 10:15) exhibited significant circadian variation. No statistical difference could be found between the circadian pattern of ESRD subjects and controls for any of the analytes.

**Conclusions:** Phosphate, calcium, PTH and 25-hydroxyvitamin D show a significant circadian rhythm in patients with ESRD. Knowledge of these phenomena is pertinent for clinical testing and may be valuable for understanding the pathophysiology of mineral bone disorder in ESRD.

*Funding:* Other NIH Support - Supported in part by grant 1UL1RR031973 from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources, National Institutes of Health, Pharmaceutical Company Support - Genzyme Corporation, Private Foundation Support, Clinical Revenue Support

**SA-PO549**

**Biochemical and Clinical Results (Phase I) of the Multicentric Study on the Prevalence of Vascular Calcifications and Vertebral Fractures in Parathyroidectomised (PTX) Dialysis Patients (Cave PTX Study)** Sandro Mazzaferro. *Sapienza Univ, Rome, Italy.*

**Background:** The CAVE PTX study aims to evaluate, in PTX dialysis pts, the control of divalent ions and medical therapies (phase I), and the prevalence of aortic calcifications and vertebral fractures (phase II).

**Methods:** Biochemistries (Ca, P, PTH) and therapies of PTX patients were collected from 149 Italian dialysis Units. A control group (C), comparable for age, sex and dialysis duration, was selected from the whole cohort. We report the data of biochemistries and therapies.

**Results:** Out of 12515 patients (HD = 87.7%; PD = 12.3%), 528 (4.22%) received PTX. PTX prevalence was higher in HD (4.5% vs 1,9% in PD). Cases and C (n=437) data are in tab 1.

| TAB 1 | Age, y | M/F, % | Dialysis, y | Ca, mg/dl | P, mg/dl | PTH, pg/ml |
|---|---|---|---|---|---|---|
| **PTX** | 58 ± 13 | 56/44 | 15 ± 8 | 8.8 ± 0.8 | 4.9 ± 1.3 | 182 ± 292 |
| **C** | 58 ± 17 | 54/46 | 12 ± 13 # | 9.0 ± 0.7 # | 5.1 ± 1.3 * | 334 ± 294 # |
| (*) p<.05, (#) p<.001 vs PTX | | | | | | |

| TAB 2 | PTH<150 | | PTH 150-300 | | PTH>300 | |
|---|---|---|---|---|---|---|
| | *PTX* | *C* | *PTX* | *C* | *PTX* | *C* |
| **Ca, mg/dl** | 8,6 ± 0.8 | 9.0 ± 0.9 # | 8.9 ± 0.7 | 9.0 ± 0.7 | 9.2 ± 0.8 | 9.0 ± 0.7 # |
| **P, mg/dl** | 4.8 ± 1.3 | 4.9 ± 1.5 | 4.9 ± 1.3 | 4.8 ± 1.2 | 5.1 ± 1.3 | 5.5 ± 1.3 # |
| **PTH, pg/ml** | 40 ± 30 | 93 ± 42 # | 216± 40 | 223 ± 41 | 630 ± 417 | 577± 331 * |
| (*)p<.05; (#)p<.001, PTX vs C | | | | | | |

Respectively in PTX and C, PTH was low (<150) in 64 vs 23%; optimal (150-300) in 17 vs 39%; and high (>300) in 19 vs 38%. Ca, P and PTH values in three groups are in tab 2. Prescribed drugs, respectively in PTX and C, were: Vitamin D (61 vs 64%); Pi binders (88 vs 75%) and Calcimimetic (13 and 35%). The most frequently prescribed drugs were Calcitriol and Ca based binders in PTX, and Paricalcitol and Sevelamer in C.

**Conclusions:** PTX has a low prevalence in Italy, and mainly involves relatively young, females and long-term haemodialysis patients. In PTX pts PTH values are mostly low and therapeutic choices are accordingly different. Different hard outcomes can be hypothesized.

*Funding:* Pharmaceutical Company Support - Amgen unrestricted grant

**SA-PO550**

**Success of Educational Interventions in the Management of CKD-MBD** Nimish Mehta, Anne Le. *Medscape, LLC, New York, NY.*

**Background:** Mineral and bone disorders in patients with chronic kidney disease (CKD-MBD) are frequently underrecognized and inadequately treated. A study was conducted to determine if on-line educational interventions could improve competence and performance of nephrologists and cardiologists with respect to CKD-MBD management.

**Methods:** A cohort of US-practicing nephrologists and cardiologists who participated in 1 of 3 educational interventions designed to address gaps in care of patients with CKD-MBD was assessed. The outcomes survey method, previously validated to measure performance, included knowledge- and case-based, multiple-choice questions focused on current evidence-based recommendations for the assessment and management of patients with CKD-MBD. Responses from the clinical cases and questions aligned with individual interventions were collected from the participant cohort and compared with a matched control group of nonparticipants.

**Results:** For each educational intervention, 200 nephrologists and cardiologists were assessed, divided equally between participants in the education and a matched control group of nonparticipants. Significant improvements were found as a result of participation in the educational interventions. For example, participants were more likely than nonparticipants to determine:

A greater calcium intake than output in a patient, resulting in a positive calcium balance (nephrologists 80% vs 60%, P =.001; cardiologists 74% vs 40%, P =.03)

· The role of the vitamin D-independent pathway for calcium absorption (nephrologists 56% vs 32%, P =.02; cardiologists 36% vs 18%, P =.04)

· Influence of diurnal fluctuation in serum phosphorus levels and dietary protein intake on a patient's serum phosphorus measurements

· Increased risk of cardiovascular calcification with calcium-containing phosphorus binders (cardiologists 52% vs 33%, P =.05).

**Conclusions:** This study demonstrated the success of targeted educational interventions on improving the practice patterns of nephrologists and cardiologists in the assessment and management of patients with CKD-MBD.

*Funding:* Pharmaceutical Company Support - Sanofi Renal

**SA-PO551**

**Examination of Tissue Phosphorus Deposition in Patients Undergoing Hemodialysis Using Current Standard Techniques** Toru Hyodo,[1,3] Noriko Mikami,[1] Yasuhisa Kurata,[1] Miho Hida,[1] Daisuke Ishii,[3] Kazunari Yoshida,[3] Masatsugu Iwamura,[3] Junko Kawakami.[2] *[1]Dialysis Center, Eijin Clinic and Kurata Hospital, Sagamihara, Kanagawa, Japan; [2]Clinical Nurtition, Sagami Women's Univ, Sagamihara, Kanagawa, Japan; [3]Urology, Kitasato Univ, School of Medicine, Sagamihara, Kanagawa, Japan.*

**Background:** With advances in dialytic techniques and the development of phosphorus (P) adsorbents, the tissue deposition of P may differ from that previously reported. In this study, we examined P loading in patients undergoing hemodialysis.

**Methods:** The subjects were 73 hemodialysis patients. They consisted of 53 males and 20 females. Thirty patients had diabetes, whereas it was absent in 43. The mean age was 65.4±14.6 years, dialysis period 8.1±13.3 years, dialysis time 4.23±0.47 hours, blood flow volume 209.7±25.7 mL/min, dialyzer membrane area 2.1±0.2 mm, and dialytic fluid flow volume 400 ml/min. The frequency of dialysis was 3 times a week. The tissue deposition of P was calculated using the following formula: Protein catabolic rate (PCR) x 15 x 0.65 – (Removal on dialysis) – (Removal with P adsorbent) – (Urinary excretion). P intake (calculated by multiplying the protein intake by a coefficient of 15) was adopted from the guidelines established by the Japanese Society of Nephrology in 2007. P removal on dialysis was measured by reserving dialytic fluid drainage. The urinary excretion of P was calculated using 24-hour urine. Lanthanum carbonate and sevelamer hydrochloride alone were administered to patients, and regarded as adsorbing 90 and 33.75 mg/g of P, respectively, for calculation.

**Results:** The dialytic fluid excretion of P was 298.6±164.9 mg/day. The urinary excretion of P was 79.1±53.3 mg/day (n=21). The volume of adsorption achieved by P adsorbents was 221.0±108.3 mg/day. The mean tissue deposition of P was -32.1±125.4 mg/day. The following correlation formula was obtained: [Dialytic fluid P removal per dialysis session] (mg) = 157.6 x [P](mg/dL) + 37, $r^2$=0.691.

**Conclusions:** Current dialytic therapy and advances in phosphorus adsorbents have reduced the tissue deposition of phosphorus. It may be important to prepare dialytic prescriptions and perform diet/drug therapies, considering P changes.

## SA-PO552

**PINP in Renal Impaired Patients: The Assay Matters** Etienne Cavalier, Pierre Delanaye. *Clinical Chemistry and Nephrology, Univ of Liege, CHU Sart-Tilman, Liege, Belgium.*

**Background:** The amino-terminal propeptide of type I procollagen (PINP) is produced by osteoblast in equimolar amounts with the collagen deposited in the tissue; it is considered as the reference bone formation marker by the IOF/IFCC. It circulates in different forms, a larger intact trimeric form and several fragment monomers. In healthy individual, circulating PINP is predominantly the trimeric intact with almost non-detectable monomers. In CKD patients, the monomeric form is elevated. Intact PINP (iPINP) assay measures the trimeric, Total PINP (TPINP) assay measures both trimeric and monomeric. We evaluated these two assays in CKD and hemodialyzed (HD) patients.

**Methods:** 157 stage 3-5 CKD patients were analyzed with the IDS-iSYS iPINP and Roche Elecsys TPINP assays; results were evaluated according to our laboratory established reference intervals and eGFR. 125 HD patient samples were also measured; results were compared with b-ALP values. The samples from 22 patients of before and after a single HD session were compared.

**Results:** In CKD subjects, observed ranges for T and iPNP were 8–822 and 8–146ng/mL. 96% (151/157) were within iPINP reference intervals (11–111ng/mL); 63%(99/157) were within TPINP range (15-90ng/mL). Relation between TPINP and eGFR tended to be exponential in <30mL/min whereas it remained linear with iPINP. In HD patients, values for TPINP and iPNP were 18-2192 and 16-641ng/mL. Using b-ALP cut-off of 20 µg/L to discriminate the high bone turnover patients, 56%(70/125) of HD samples were found to be below this threshold. Among these patients, 93%(65/70) presented normal iPINP values vs. 31% (22/70) in TPINP. A single hemodialysis session did not remove the monomers from the circulation.

**Conclusions:** PINP is a promising marker to evaluate bone formation in CKD patients. It might replace the b-ALP in patients where interferences with the liver form of the enzyme can be expected. We have compared two methods for PINP determination, one of which recognizes monomeric fragments that accumulate in CKD patients, TPINP. We have indirect data indicated that this method should not be used in those patients and the "Intact" method is more suitable.

## SA-PO553

**Analytical Performance of a New Bone Specific ALP Automated Immunoassay** Etienne Cavalier,[1] Pierre Delanaye.[2] *[1]Clincial Chemistry, Univ of Liege, CHU Sart-Tilman, Belgium; [2]Nephrology, Dialysis and Transplantation, Univ of Liege, CHU Sart-Tilman, Belgium.*

**Background:** Serum bone–specific alkaline phosphatase (bone ALP), a marker of bone turnover, has been proposed as an alternative biochemical target for clinical management of CKD-MBD patients. We assessed the analytical performance of the IDS-iSYS Ostase BAP (IDS, UK) automated assay and compared this method to the manual Ostase BAP Immunoenzymetric assay [EIA] (IDS, UK). The observed ranges for apparently healthy children and reference intervals for pre-menopausal, post-menopausal women and men were established.

**Methods:** The accuracy profile was determined with serum pool levels. The method linearity was verified with two sets of high/low serum samples. A total of 116 samples [6.1-75.7µg/L] was assayed by each method for method comparison. Serum specimens from overnight fasting, apparently healthy Caucasians subjects, normal Calcium, Phosphates, Intact PTH, and eGFR > 60, were selected to establish the reference intervals for males, pre-menopausal and post-menopausal. The observed ranges were established with apparent healthy young <20years males and females.

**Results:** The precision profile of the IDS-iSYS BAP assay demonstrates precision CV between 1.7% and 7.2%. The dilutions had good linear regression to the expected concentrations: Observed BAP = 1.047 x (Expected BAP) – 1.89; $R^2$ = 0.997. The method Passing Bablok regression was: IDS-iSYS = 1.05 x (EIA) + 0.25 with significant correlation r=0.994 (P<0.0001). We observed a peak between 7 and 8 years for girls, whereas boys showed the peak to be later, between 11 and 12 years.

**Conclusions:** The IDS-iSYS Ostase BAP assay performed well and showed suitable characteristics to achieve acceptable precision and accuracy. The patient comparison between the IDS-iSYS and the Immunoenzymetric showed an excellent relationship. The IDS-iSYS Ostase BAP is a suitable tool in clinical laboratories for the follow-up of osteoporosis therapy or for the differential diagnoses of other metabolic bone diseases such Paget's disease and renal osteodystrophy.

## SA-PO554

**Performance of a Novel Fully Automated Method for the Detection of Dephospho-Uncarboxylated Matrix Gla Protein (dp-ucMGP)** Etienne Cavalier,[1] Pierre Delanaye.[2] *[1]Clinical Chemistry, Univ of Liege, CHU Sart-Tilman, Belgium; [2]Nephrology Dialysis Hypertension, Univ of Lioege, CHU Sart-Tilman, Belgium.*

**Background:** Matrix Gla-protein (MGP) is one of the strongest inhibitor of vascular calcification, produced by vascular smooth muscle cells and chondrocytes. MGP can undergo gamma-glutamate carboxylation (vitamin K dependent step) and serine phosphorylation. Circulating dp-ucMGP reflects the amount of uncarboxylated MGP produced in the arterial vessel wall and is a direct marker for the vascular vitamin K status. Different studies, using cumbersome elisa methods have shown conflicting results regarding association of dp-ucMGP and abdominal aortic calcification score. We assessed the analytical performance of the fully automated IDS-iSYS InaKtif MGP assay (IDS, UK) and compared the results vs. an ELISA method in a cohort of haemodialysis patients.

**Methods:** Precision profile was determined with 4 plasma samples (264-2703pM) measured twice a day for 10 days. Linearity was assessed with 2 sets of high/low samples. dp-ucMGP concentrations were determined with iSYS and ELISA from 118 plasma sample of patients without Vitamin K supplementation and under thrice-weekly hemodialysis for at least 3 months, (mean age 68.7±14.4 years; median dialysis vintage: 21months, range: 3-396); 78(65.0%) had history of cardiovascular diseases.

**Results:** On iSYS, intra-assay coefficient of variation (CV) was 2.9-8.9%; Inter-assay CV was 4.1-13.4%. Linearity equation from 18 dilutions was Obtained = 0.95xExpected+209, $R^2$=0.99. The Passing-Bablock regression was iSYS=1.26xELISA+241. Bland Altman plot showed that iSYS gave higher results than ELISA, especially in the higher range.

**Conclusions:** The IDS-iSYS InaKtif MGP is the first fully automated dp-ucMGP kit which will be soon available commercially for the measuring of the dephosphor-uncarboxylated MGP levels. Different observations in the studies that used the ELISA method might be explained by the its poor linearity in the higher range. The dp-ucMGP blood test could be included as part of routine monitoring biomarkers for hemodialysis patients after further proven its clinical validity.

## SA-PO555

**Seasonal Vitamin D Levels in Untreated Incident Dialysis Patients** Gero D. von Gersdorff,[1] Philipp B. Marquardt,[1] Mathias Schaller,[1] Thomas Benzing,[1] Claudia Barth.[2] *[1]Nephrology - QiN Group, Univ Hospital Cologne, Cologne, Germany; [2]Curatorium for Dialysis and Kidney Transplantation, Neu-Isenburg, Germany.*

**Background:** Studies on the association between VitD and mortality are often not adjusted for season of sample collection. We report on seasonal VitD levels in a current large cohort of chronic ambulatory dialysis patients without VitD Supplementation.

**Methods:** The database of a large dialysis organization in Germany was queried for all patients >18 years in the period 2006–2012 who had a first VitD drawn within 1year after starting hemo- (HD) or peritoneal (PD) dialysis, respectively. VitD levels were grouped by month of draw and by patient's state of VitD supplementation. VitD sufficiency was defined as a level >30 ng/ml and severe deficiency as <12.5 ng/ml. Results are expressed as mean (SD) or %, and as odds ratio (OR) of mortality for VitD <12.5ng/ml.

**Results:** 19,474 patients (pt) were available for analysis. In 3568 VitD naïve pt (age 71.9(14) years, 61.6% male, 44.1% diabetic (DM)), VitD levels were 17.2 (10.9) ng/ml in winter and 20.4 (12.7) ng/ml in summer. Levels in PD were substantially lower than in HD (Fig. 1). VitD levels were also low in women and DM (Fig. 1). Of 3315 incident HD patients without supplementation, 17.5% were VitD sufficient and 36.3% were severely deficient. In corresponding PD patients (N=253), only 7.1% were sufficient and 58.5% were severely deficient. Mortality risk was 1.44 forHD and 1.35 for PD patients with a first VitD level <12.5 ng/ml.

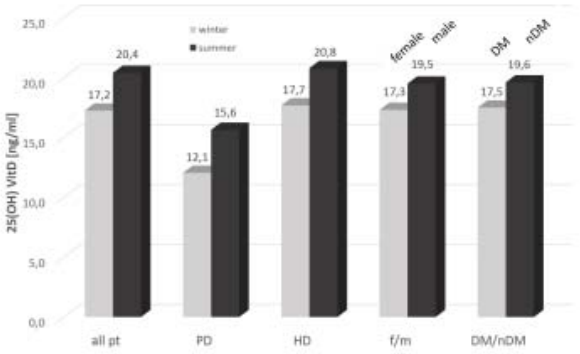



**Figure 1**: Left three columns: Levels of unsupplemented VitD in winter (November - April) and summer (July - October) in all incident, and incident PD and HD patients, respectively. Right two columns: Levels of VitD in women and men, and diabetics and non-diabetics, respectively.

**Conclusions:** In this analysis we could confirm and expand findings from an earlier 1997-2006 cohort (Krause et al, 2012) in ambulatory dialysis patients in routine practice. Severe deficiency was found in a large proportion of dialysis pt. Other factors that were associated with low VitD were female gender and DM as a comorbidity. Seasonal variation (sun exposure) contributed 2-4 ng/dl increase in the summer months.

## SA-PO556

**Seven Years of Experience with the German Calciphylaxis Registry** Vincent Brandenburg,[1] Thilo Krueger,[2] Jürgen Floege,[2] Markus Ketteler.[3] *[1]Cardiology, Univ Hospital RWTH Aachen; [2]Nephrology, Univ Hospital RWTH Aachen; [3]Nephrology, Klinikum Coburg.*

**Background:** Calciphylaxis is a rare disease (ORPHA280062) and devastating condition associated with high morbidity and mortality. Calciphylaxis is characterised by painful, ischemic, partly necrotic skin ulcerations. Pathomorphologically, media calcification of cutaneous arterioles and extracellular matrix remodelling are the hallmarks of the disease.

**Methods:** We established an international internet-based registry in 11/2006 to allow online notification for all cases of established or suspected CUA. A comprehensive data base including various parameters concerning patient characteristics, laboratory data, clinical background and presentation as well as therapeutic strategies was established. The diagnosis of CUA is made on clinical and/or histological grounds by the referring physician.

**Results:** Altogether 184 patients with CUA have been documented in 6.5 years: 61% females; 88% dialysis (HD and PD) patients, median age 67 (21-88) years. Stored serum samples were used for central laboratory analysis in core facility in n = 92 dialysis patients: PTH levels varied broadly between undetectably low and > 1100 pg/ml, mean 191 +/-176 pg/mL; fetuin-A: 0.21+/-0.01 g/L. Fetuin-A levels in control HD pts without CUA were significantly higher (n=65; 0.46+/-0.1 g/L, p < 0.01). Oral anticoagulation with Vit K antagonists was common in ESRD CUA pts (47%). Cutaneous lesions were localized in 79% at the lower extremities or gluteal region. Among the most frequently recorded therapeutic procedures were: surgical necrosectomy, intensifying dialysis modality, i.v. sodium-thiosulfat application. The median survival time was 516 days after online notification.

**Conclusions:** CUA is a rare diease among ESRD pts with increased mortality. PTH levels vary substantially but do not exceed current KDIGO target levels in most cases. Decisions on therapeutic strategies also vary significantly among centers. The present internet based ICCN registry is a valuable tool to collect data upon CUA cases and may become a basis for prospective systematic evaluations of treatment modalities in the near future.

*Funding:* Pharmaceutical Company Support - Amgen and Sanofi

## SA-PO557

**A 10-Year Retrospective Analysis of Parathyroidectomy (PTX) for Renal Hyperparathyroidism** Alasdair Duguid, Chern Beverly B. Lim, Samina Hussain, Zorica Vujovic, David Monro Smith. *Ninewell's Hospital, Dundee, United Kingdom.*

**Background:** Patients with advanced kidney disease are known to be at risk of developing renal bone disease (CKD-MBD) due to the development of secondary hyperparathyroidism. When this cannot be controlled with medical treatment alone surgical intervention may be indicated. Our aim was to assess the clinical and biochemical outcomes in patients who underwent PTX for renal hyperparathyroidism.

**Methods:** Retrospective review of all renal patients who had PTX from April 2003 to March 2013 at one institution. Relevant patients were identified from electronic medical records and theatre logs.

**Results:** 97 patients underwent PTX for renal hyperparathyroidism. There were 45 women and 49 men with a median age of 55 years (range 25-80 years) plus 3 who were excluded due to incomplete data. At the time of PTX, 20 patients had functioning renal transplants, 66 were on renal replacement therapy, 49 were on haemodialysis, 17 were on peritoneal dialysis and 8 patients were at a pre-dialysis stage. Of these patients, 30 had four parathyroid glands removed, 28 had three and a half removed, 11 had three removed, 9 had two removed and 16 had one removed. Histopathology showed 80 cases had hyperplastic changes, 12 had parathyroid adenomas and 2 were normal. 2 patients experienced recurrent laryngeal nerve injury and 1 patient died secondary to pneumonia. Recurrent hyperparathyroidism led to re-operation in 5 patients and 4 patients were on cinacalcet. Post PTX 66 patients were on alfacalcidol (median 0.5 micrograms, range 0-14 micrograms). When comparing pre and post-operative biochemistry, parathyroid hormone (PTH) levels were found to be significantly different using Wilcoxon matched-pairs test (W=-4183, P<0.001) with a median reduction of 75.2pmol/L (interquartile range 26.3-75.2). Corrected calcium levels were also significantly reduced (W=-3829, P<0.001) with a median reduction of 0.23mmol/L (interquartile range 0.09-0.39).

**Conclusions:** Most patients undergoing PTX were receiving haemodialysis. The majority of patients had a subtotal rather than total PTX with hyperplasia being the prevailing pathology. PTX has been shown in these patients to significantly reduce both PTH and corrected calcium.

## SA-PO558

**Analysis of CKD-MBD Markers in a Phase 3 Study of PA21 and Sevelamer in Patients with Hyperphosphatemia** Stuart M. Sprague,[1] Adrian Covic,[2] Markus Ketteler,[3] Anjay Rastogi,[4] Bruce S. Spinowitz,[5] Jürgen Floege.[6] *[1]NorthShore Univ Health System, Evanston, IL; [2]Gr.T. Popa Univ of Medicine and Pharmacy, Iasi, Romania; [3]Coburg Clinic and KfH-Dialysis Center, Coburg, Germany; [4]Univ of California; [5]New York Hospital Queens; [6]RWTH Univ Hospital Aachen, Aachen, Germany.*

**Background:** Effects of PA21 – a novel polynuclear iron(III)-oxyhydroxide phosphate binder (PB) – and sevelamer carbonate (SEV) on CKD-MBD markers were assessed.

**Methods:** Dialysis patients received PA21 1.0-3.0 g/day (n=710) or SEV 2.4-14.4 g/day (n=349) for 12 weeks' dose titration then a 12-week maintenance period. Eligible patients entered a 28-week extension study. Markers of CKD-MBD were measured.

**Results:** In both treatment groups (Table): serum intact parathyroid hormone (iPTH) decreased initially, then increased to Week 52; fibroblast growth factor 23 (FGF-23) decreased and serum $Ca^{2+}$ levels remained relatively stable over 52 weeks; tartrate-resistant acid phosphatase 5b (TRAP5b) decreased over 52 weeks; carboxyterminal cross-linking telopeptide (CTX) increased initially, then returned to near baseline values at Week 52; bone-specific alkaline phosphatase (BAP) increased initially, then returned to near baseline values with PA21 at Week 52; osteocalcin (OST) increased over 52 weeks. No significant differences between treatment groups were noted.

**Table:** Mean (SD) change from baseline in CKD-MBD markers.

| | Δ at 24 weeks | | Δ at 52 weeks | |
|---|---|---|---|---|
| **Marker** | **PA21 (n=707)** | **SEV (n=348)** | **PA21 (n=707)** | **SEV (n=348)** |
| iPTH (ng/mL) | –66.9 (268.86) | –35.4 (244.65) | 13.5 (355.58) | 34.1 (292.54) |
| FGF-23 (µg/L) | –30.76 (227.48)* | –44.08 (215.38)* | –54.60 (230.66)* | –78.55 (220.92)* |
| $Ca^{2+}$ (mg/dL) | 0.18 (0.84)* | 0.13 (0.82)* | 0.20 (0.79)* | 0.22 (0.91)* |
| TRAP5b (U/L) | –1.12 (2.53)* | –0.93 (2.59)* | –1.41 (2.78)* | –0.80 (2.99)* |
| CTX (ng/mL) | 2.92 (3.99)* | 3.55 (4.12)* | –0.44 (2.64)* | 0.15 (2.76) |
| BAP (ng/mL) | 2.36 (12.24)* | 4.97 (13.82)* | –0.11 (13.77) | 2.56 (13.19)* |
| OST (ng/mL) | 1.74 (19.18) | 2.05 (15.91)* | 8.69 (26.49)* | 12.33 (23.76)* |

*Significant change from baseline.

**Conclusions:** PA21 and SEV produced similar changes in CKD-MBD markers. Further analysis for clinical relevance is warranted, especially considering the FGF-23 lowering effect. As approximately 60% of patients had received prior $Ca^{2+}$-based PBs, treatment with non-$Ca^{2+}$-based PBs in this study may have led to an initial activation of bone metabolism.

*Funding:* Pharmaceutical Company Support - Vifor Pharma Ltd

## SA-PO559

**Administration of Activated Vitamin D for Patients with Predialysis Chronic Kidney Disease May Reduce Cardiovascular Disease Events after Dialysis Initiation** Daijo Inaguma. *Nephrology, Japan Red Cross Nagoya Daini Hospital, Nagoya, Aichi, Japan.*

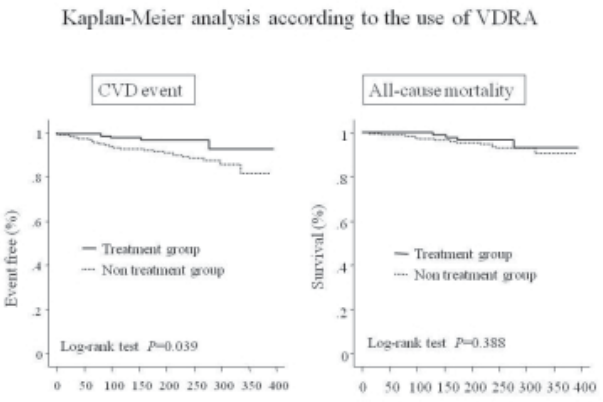
**Background:** Only a few studies have focused on whether or not administration of activated vitamin D (VD) during the predialysis stage can reduce CVD events in patients after dialysis initiation. We therefore examined whether activated VD therapy improves short-term survival after the initiation of dialysis.

**Methods:** We conducted a multicenter prospective cohort study of 487 patients with CKD who began dialysis during the period from October 2011 to January 2012. The last observation was conducted at the end of September 2012. Incidences of CVD events and all-cause mortality were compared between patients in the treatment group (130 patients) before initiation of dialysis to whom activated vitamin D was orally administered for at least 12 weeks, and patients in the non-treatment group (357 patients).

**Results:** The data at the time of dialysis initiation are presented.

| | treatment group | non-treatment group | p value |
|---|---|---|---|
| age | 66.7 ± 12.3 | 68.3 ± 12.7 | 0.216 |
| female gender (%) | 34.4 | 31.5 | 0.586 |
| diabetes (%) | 36.2 | 42.5 | 0.214 |
| eGFR (ml/min/1.73m2) | 5.2 ± 1.4 | 5.5 ± 2.0 | 0.080 |
| adjusted Ca (mg/dl) | 8.69 ± 1.01 | 8.59 ± 1.05 | 0.316 |
| phosphorus (mg/dl) | 6.03 ± 1.69 | 6.29 ± 1.80 | 0.148 |
| calcitriol (pg/ml) | 17.8 ± 7.1 | 11.4 ± 7.0 | <0.001 |

CVD events occurred in 111 patients during the follow-up period. Comparing the two groups revealed a significantly lower CVD-event occurrence in the treatment group.




Based on the results of multivariate analysis using the Cox proportional hazards model, with adjustment for age and gender, administration of activated VD has an inhibitory influence on CVD events.

**Conclusions:** We have shown that for predialysis CKD patients, the use of activated VD may reduce the CVD risk after initiation of dialysis.

## SA-PO560

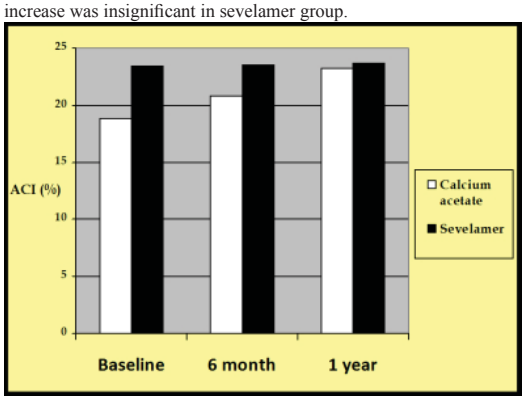
### Comparative Study between the Impact of Calcium Acetate versus Sevelamer Carbonate on Progression of Vascular Calcification in Pre-End Stage Renal Disease (CKD Stage 4 and 5) Patients

Sham Sunder, Himanshu Verma, Venkataramanan K, Satyanand Sathi. *Nephrology, PGIMER & Dr. Ram Manohar Lohia Hospital, Delhi, India.*

**Background:** Disorders of mineral metabolism,mainly hyperphosphatemia,may contribute to increased cardiovascular morbidity in CKD by promoting vascular calcification(VC).There is paucity of data on the extent of VC & the impact of sevelamer carbonate on progression of VC in pre-ESRD patients.

**Methods:** 150 pre-ESRD(CKD stage 4&5)patients were screened for abdominal aortic calcification(AC)using digital X-Ray lumbar spine and multi-slice abdominal CT scan.50 hyperphosphatemic CKD 4&5 patients having AC were selected & randomized to receive calcium acetate or sevelamer carbonate(25 in each group).The patients were serially studied for impact of phosphate binders on the progression of VC by measuring aortic calcification index(ACI)using CT scan at baseline,6 & 12 months.Clinical characteristics and laboratory variables(serum Ca,PO4,ALP,iPTH,Vit $D_3$,lipid profile,hs-CRP,IL-6) were also assessed.

**Results:** Among 150 patients,75% had AC with median ACI of 17.15%.ACI correlated positively with X-Ray AAC score(r=0.8106; *p<0.0001*).Sevelamer and calcium provided equivalent control of s.PO4(4.4+0.51 & 4.4+0.74,respectively,*p<0.01*).At baseline,the mean ACI in sevelamer group was significantly greater than the mean ACI in calcium group(23.45+1.8% vs 18.84+0.9%; *p=0.035*).In calcium treated patients, the mean ACI increased significantly at 6 months(20.8+1.06%) & 1 year(23.2+1.18%) *(p<0.01)*;but the increase was insignificant in sevelamer group.




hs-CRP & IL-6 decreased significantly at 6 month & 1 year with sevelamer, but increased with calcium at 1 year follow up *(p<0.01)*.

**Conclusions:** The prevalence of VC in pre-ESRD patients was 75%.Sevelamer carbonate was more effective in retarding the progression of VC than calcium acetate in pre-ESRD patients,due to it's pleiotropic properties.

*Funding:* Government Support - Non-U.S.

## SA-PO561

### Serum Sclerostin as an Independent Predictor of Mortality on Hemodialysis Patients: Results from a 10-Year Follow-Up Cohort

Flávia Letícia Carvalho Gonçalves, Rosilene M. Elias, Luciene dos Reis, Fabiana Graciolli, Claudio Luders, Benedito J. Pereira, Bruno C. Silva, Vanda Jorgetti, Hugo Abensur, Manuel C. Castro, Rosa M.A. Moyses. *Nephrology Div, Univ of Sao Paulo School of Medicine, Brazil.*

**Background:** Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a major syndrome that develops as a complication of CKD and usually predicts outcomes in observational studies. Recently, Wnt pathway disturbances have been identified as a common and early event in these patients, and probably play a role in CKD-MBD pathophysiolgy. In this study, we tested whether serum sclerostin (Sct), a Wnt pathway inhibitor, would be associated with mortality in a hemodialyisis (HD) patients' cohort.

**Methods:** On January 2003, all patients from a single HD center were evaluated (n=91). Clinical and biochemical data were collected and serum samples were stored for further analysis. Ten years later, serum Sct was measured and data was analysed.

**Results:** At baseline, their mean age was 42 ± 19 years, 60% male, 15% diabetic and had been on dialysis for 10 years. Their mean Hemoglobin (Hb), creatinine (Cr), albumin, PTH and Sct were respectively 11.6 ± 1.6 g/dl, 11 ± 3.3 mg/dl, 4.2 ± 0.5 g/dl, 169 (94-325) pg/ml, and 0.88 (0.54-1.6) ng/ml. After 10 years of follow-up, 28 deaths were observed. Variables associated with a higher mortality risk were older age, lower serum Cr, Hb and PTH, as well as higher serum Sct. When patients were divided into two groups according to median serum Sct , we found a higher prevalence of deaths in the higher Sct group (19 vs. 9, $p < 0.05$). Cox regression survival analysis showed that age (1.056), Hb (0.674) and Sct (1.874) were significant and independent predictors of death, even adjusted for diabetes, Cr and PTH.

**Conclusions:** Our data confirmed elevated Sct as an independent risk factor for mortality in HD patients. Prospective, interventional studies are needed to elucidate whether clinical interventions to modulate serum Sct would be able to improve these patients survival.

*Funding:* Government Support - Non-U.S.

## SA-PO562

### Serum Bicarbonate and Bone Mineral Density in United States Adults

Wei Chen, Michal L. Melamed, Matthew K. Abramowitz. *Dept of Internal Medicine/Nephrology Div, Albert Einstein College of Medicine, Bronx, NY.*

**Background:** Chronic acidosis leads to bone mineral loss by inducing calcium efflux from bone. In this study, we examined the association of serum bicarbonate levels with bone densitometry on a population-wide level in participants of the National Health and Nutrition Examination Survey 1999-2004.

**Methods:** Our analysis included 10,610 adults (≥20 years old) with estimated glomerular filtration rate (eGFR) ≥15 mL/min/1.73 $m^2$ who completed dual-energy X-ray absorptiometry (DEXA) testing. We excluded participants who were pregnant, taking bisphosphonates or had a diagnosis of chronic obstructive pulmonary disease. Bone mineral density (BMD) was measured by DEXA. Low total BMD was defined as 1.0 standard deviation below the sex-specific mean. Linear and logistic regression models were created to examine associations of serum bicarbonate levels with lumbar and total BMD.

**Results:** Participants with lower serum bicarbonate were younger, more likely to be female and smokers, had higher BMI, and were less likely to use diuretics. In the fully adjusted model, compared with participants with bicarbonate <24mEq/L, those with ≥27mEq/L had 0.012 g/$cm^2$ higher lumbar BMD (95% CI 0.00002, 0.025). In sex stratified analyses, compared with bicarbonate <24mEq/L, only bicarbonate ≥27mEq/L was associated with higher lumbar BMD among men; but among women, bicarbonate between 24-25, 25.1-26.9 and ≥27mEq/L were all associated with higher lumbar BMD. For total BMD, no statistically significant association was observed with serum bicarbonate among all participants. However, in sex-stratified analyses, bicarbonate level was independently associated with total BMD among women but not men. The odds of having low total BMD were 1.48 times higher in women with bicarbonate level <24 mEq/L compared to ≥27 mEq/L [OR 1.48 (95% CI 1.03, 2.14)].

**Conclusions:** Higher serum bicarbonate levels were significantly associated with higher lumbar BMD with a difference in threshold effect among men and women. For total BMD, an association with serum bicarbonate was observed only among women. This suggests a gender difference in the association of acidosis and bone density.

*Funding:* Other NIH Support - Clinical and Translational Science Award (CTSA) grants UL1RR025750, KL2RR025749 and TL1RR025748 from the National Center for Research Resources, a component of the National Institutes of Health (NIH), and by the National Center for Advancing Translational Sciences (NCATS), a component of the NIH, through CTSA grant numbers UL1TR000086, TL1RR000087, and KL2TR000088.

## SA-PO563

### Measures of Arterial Stiffness Independently Correlate with Bone Mineral Density and Osteoprotegerin in Patients with Long-Standing Type 1 Diabetes

Maria Lajer,[1] Simone Theilade,[1] Lise Tarnow,[1,2] Peter Rossing.[1,2,3] *[1]Steno Diabetes Center, Denmark; [2]Univ of Aarhus, Denmark; [3]Univ of Copenhagen, Denmark.*

**Background:** Abnormal bone metabolism and increased arterial stiffness are common in both diabetes and chronic kidney disease and are related to increased morbidity and mortality.

**Methods:** Prospectively followed cohort of 371 type 1 diabetic patients (55% men; age [mean±SD] 53±9 years, 42±8 years of diabetes, and 35% with long duration of diabetic nephropathy (DN)) Femoral neck bone mass density (BMD) ($g/cm^2$) was measured by dual energy x-ray. Measures of arterial stiffness were heart rate (HR) adjusted augmentation index (AI75), augmentation pressure (AP), and pulse wave velocity (PWV) (SphygmoCor, Atcor Medical). The calcification markers sclerostin, endostatin and osteoprotegerin (OPG) were measured in serum by immunoenzymetric assays (Biomedica Medizinprodukte).

**Results:** In total, 105 (28%), 197 (53%) and 69 (19%) had BMD (T-score) corresponding to normal, osteopenia and osteoporosis (>-1, -2.5 to -1.0 and <-2.5, respectively). Mean±SD AI75 18±10, 18±10, and 22±10; p=0.011 and PWV 10.2±3.3, 11.0±3.1 and 12.1±3.8 m/s; p=0.002 increased while AP tended to increase 10.4±7.7, 9.8±6.7, and 12.1±7.5; p=0.10 with deteriorating BMD group.

Following multivariate adjustment (ANCOVA: Sex, diabetes duration, $HbA_{1c}$, height, HR, mean arterial pressure, eGFR, urinary albumin excretion, cholesterol, smoking, and antihypertensive treatment), AI75 and AP were elevated with decreasing BMD status (p=0.002 and 0.006, respectively).

Similarly, sclerostin and endostatin correlated with PWV (p<0.018) while OPG correlated with AI75, AP and PWV (p<0.001). However, after the adjustment only OPG remained significantly related to all three measures of arterial stiffness (p<0.035).

**Conclusions:** These data suggest that in patients with longstanding type 1 diabetes, augmentation index and arterial pressure as measures of arterial stiffness correlates with bone mass and OPG independent of potential confounders.

## SA-PO564

**Relationship between Coronary Artery Calcification and Cardiac Valve Calcification at Hemodialysis Initiation in Patients with End Stage Renal Disease** Ken Kitamura, Hideki Fujii, Kentaro Nakai, Keiji Kono, Shinichi Nishi. *Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Japan.*

**Background:** Coronary artery calcification (CAC) and cardiac valvular calcification (VC) are common in patients with end-stage renal disease (ESRD) and may affect significant morbidity and mortality.The purpose of this study was to investigate CAC and VC at hemodialysis initiation in patients with ESRD.

**Methods:** This study included 63 patients with ESRD who were just planned to start hemodialysis therapy. Multi-detector computed tomography for quantification of CAC using the Agatston score and transthoracic echocardiography for assessing VC were performed for all the study patients. We semi-quantitated the severity of VC as VC score by counting the number of calcified valve cusp of aortic valve (AV), mitral valve (MV) and the presence of mitral annular calcification (MAC).

**Results:** Among 63 patients at hemodialysis initiation, 51 (81.0%) had CAC and 35 (55.6%) had VC. Twenty-five (49.0%) of 51 patients with CAC had severe CAC (CAC score ≥ 400). AV calcification, MV calcification and MAC were observed in 30 patients (47.3%), 17 patients (26.3%) and 8 patients (12.2%), respectively. CAC score is closely and significantly associated with VC score (r=0.39, p<0.005). Especially, significant associations of CAC score with AV calcification and MAC were detected. In addition, when the patients were divided into the following three groups on the basis of the degree of CAC and VC (group 1: severe CAC- and VC-; group 2: severe CAC+ or VC+; group 3: severe CAC+ and VC+), the number of patients with left ventricular hypertrophy was the highest in the group 3 and it was higher in the group 2 than in the group 1.

**Conclusions:** At hemodialysis initiation, most of the patients with ESRD had CAC and VC and these were well correlated with each other. These findings suggested that we need to follow hemodialysis patients with CAC and/or VC carefully as high risk patients for cardiovascular disease.

## SA-PO565

**Maxacarcitoriol Prevents the Progression of Cardiac Damage in Diabetes by Suppressing Oxidative Stress** Hideki Fujii,[1] Kentaro Nakai,[1] Keiji Kono,[1] Shunsuke Goto,[1] Riko Kitazawa,[2] Masami Shinohara,[3] Michinori Hirata,[4] Shinichi Nishi,[1] Masafumi Fukagawa.[1,5] *[1]Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Japan; [2]Div of Diagnostic Molecular Pathology, Kobe Univ Graduate School of Medicine, Kobe, Japan; [3]Planning and Development Section, CLEA Japan, Inc., Tokyo, Japan; [4]Fuji Gotemba Research Labs, Chugai Pharmaceutical Co., Ltd., Gotemba, Japan; [5]Div of Nephrology, Endocrinology and Metabolism, Tokai Univ School of Medicine, Isehara, Japan.*

**Background:** Diabetes mellitus (DM) is an important risk factor for chronic kidney disease (CKD) and cardiovascular disease (CVD). Recent reports have shown the significant association between vitamin D and CVD. Recently, we have reported that maxacarcitoriol (OCT), which is a vitamin D receptor activator, reduces oxidative stress. In this study, we investigated the effect of OCT on oxidative stress and cardiac damage in DM.

**Methods:** In this study, we used SDT rats, which are non-obese type 2 diabetes model rats. Twenty-week-old male SDT rats were divided into three groups: vehicle-treated SDT rats (DM, N=6), insulin-treated SDT rats (INS, N=6), and maxacalcitol-treated SDT rats (OCT, N=6). At 30 weeks of age, urinary and blood biochemical analysis and cardiac histological analysis were performed in these groups.

**Results:** At 30 weeks of age, despite comparable blood pressure and renal function, urinary excretion of 8-hydroxydeoxyguanosine (8-OHdG) and serum NT-proBNP levels were significantly lower in the OCT group than in the DM group. In addition, heart volume was significantly lower in the OCT group compared to the DM group. The mRNA expressions of NADPH p22 and BNP in the heart were significantly decreased in the OCT groups compared to the DM group. Further iummunohistological analysis revealed that the number of 8-OHdG positive cardiomyocyte was reduced and cardiac and perivascular fibrosis was ameliorated by OCT administration.

**Conclusions:** Oxidative stress may play a key role for the development of cardiac hypertrophy and cardiac fibrosis in DM. Furthermore, it is suggested that OCT suppresses oxidative stress and ameliorated the cardiac damage in DM.

*Funding:* Pharmaceutical Company Support - Chugai Pharmaceutical Co., Ltd.

## SA-PO566

**Higher Serum Alkaline Phosphatase Is Associated Not Only with Mortality but also the Incidence of Hip Fracture among Patients Receiving Hemodialysis in Japan** Yukio Maruyama,[1] Keitaro Yokoyama,[1] Takashi Shigematsu,[2] Masatomo Taniguchi,[3] Junichiro J. Kazama,[4] Tatsuo Hosoya,[1] Takashi Yokoo.[1] *[1]Div of Kidney and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan; [2]Div of Nephrology and Blood Purification Medicine, Wakayama Medical Univ, Wakayama, Japan; [3]Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [4]Div of Blood Purification Therapy, Niigata Univ Medical and Dental Hospital, Niigata, Japan.*

**Background:** The monitoring of serum alkaline phosphatase (ALP) is widely recommended in the management of chronic kidney disease-mineral and bone disorder (CKD-MBD). However, unlike calcium, phosphate, or parathyroid hormone, relationship between serum ALP and outcome of the patients receiving hemodialysis (HD) in Japan is unknown.

**Methods:** We collected the baseline data of 187,792 patients receiving HD thrice weekly (66 ± 12 years, males 61.9%, and median HD vintage of 7.9 years) extracted from a nationwide dialysis registry at the end of 2009 in Japan. Then we evaluated the patient survival and development of complication using the registry at the end of 2010.

**Results:** During one-year follow-up, 14,439 (7.9%) died of all causes including 6485 (3.6%) cardiovascular death. Additionally, 1,614 patients (1.1%) was newly-diagnosed as hip fracture. All-cause mortality, cardiovascular mortality, and the incidence of hip fracture were higher in line with the increase of baseline serum ALP. In a multivariable logistic regression analysis, patients of the highest quartile of ALP had higher all-cause and cardiovascular mortality and incidence of hip fracture compared with those of the lowest quartile (OR, 1.45; 95% CI, 1.34 to 1.57, and OR, 1.26; 95% CI, 1.13 to 1.40, and OR, 1.69; 95% CI, 1.37 to 2.09, respectively).

**Conclusions:** In this large observational cohort study, higher levels of serum ALP were independently associated not only with mortality but also the incidence of hip fracture among Japanese HD patients. Close monitoring of serum ALP is thought to be useful for the management of CKD-MBD.

## SA-PO567

**Aluminum Intoxication: The Problem Has Not Disappeared** Clarissa Jacob Barros Carvalho,[1] Camila Barbosa L. Oliveira,[1] Carla Queiroz Neves,[1] Alline S.A. Oliveira,[1] Vanda Jorgetti,[2] Jose Edevanilson Gueiros,[1] Ana Paula Gueiros.[1] *[1]Nephrology, UFPE, Recife, Pernambuco, Brazil; [2]Nephrology, USP, Sao Paulo, Brazil.*

**Background:** Aluminum intoxication (AI) is associated with low bone formation rates and incresead risk for fractures. The aim of this study was to evaluate the prevalence of AI in hemodialysis patients who underwent bone biopsy in a Brazilian center.

**Methods:** We performed a retrospective analysis of 158 patients underwent bone biopsy from March 2003 to March 2013. Patients were divided into 2 groups: AI group (patients with AI) and NAI group (patients free of AI). Clinical data were evaluated: age, sex, time on dialysis, occurrence of vascular calcification and fractures. Laboratory tests: total calcium, phosphorus, intact parathyroid hormone (iPTH pg/mL), alkaline phosphatase (ALP U/L). Bone biopsy was performed after double tetracycline labeling and specimens were classified as osteitis fibrosa (OF), mixed disease (MD), adynamic bone disease (ABD), osteoporosis (OS) or osteomalacia (OM). AI or iron intoxication were defined when more than 20% of the trabecular bone surface were covered by metal. We performed a comparative analysis between the groups to assess factors associated with AI. The frequency of IA was evaluated in two periods of 5 years (2003-2007 and 2008-2013).

**Results:** The prevalence of AI was 51.3% and did not decreased with the time. There were no diferences between the groups regarding clinical parameters. ALP (median 155 vs 430; p<0.001) and iPTH (median 814 vs 1732; p<0.001) levels were lower in the AI group. ABD (p=0.021) and iron intoxication (p<0.001) were closely associated with AI. Patients with MD had lower frequency of AI (p=0.003). A cox regression multivariate analysis showed that variables associated with AI were: time on dialysis (p=0.024), ALP (p=0.006) and iPTH (p=0.008).

**Conclusions:** Although the prevalence of aluminum-related bone diseases has declined in the world, AI still persists at a high prevalence in Brazil. Futhermore, IA had a close association with ABD and with iron intoxication.

## SA-PO568

**Post-Hoc Analysis of Pharmacodynamic Interaction of PA21 with Statins in a Phase 3 Study of PA21 in Dialysis Patients with Hyperphosphatemia**
Victoria Levesque,[1] Edward M.F. Chong,[1] Patrick Moneuse.[2] *[1]Vifor Pharma, Canada; [2]Vifor Pharma, Glattbrugg, Switzerland.*

**Background:** Dialysis patients often require phosphate binders (PBs) for hyperphosphatemia, and concomitant statins for hyperlipidemia. *In vitro* studies showed interactions between PA21 and atorvastatin, especially in an absence of phosphate. Clinical data showed sevelamer (SEV) lowered total cholesterol (Tot-C) and low-density lipoprotein cholesterol (LDL-C), but not triglycerides (TG). Therefore, an analysis of data from a Phase 3 study investigating PA21, a novel polynuclear iron(III)-oxyhydroxide PB, and SEV assessed whether these PBs altered the lipid-lowering effects of statins.

**Methods:** Dialysis patients received PA21 1.0-3.0 g/day (n=707) or SEV carbonate 2.4-14.4 g/day (n=348) for up to 52 weeks. A *post-hoc* analysis investigated lipid parameters to assess potential interactions between PA21 or SEV and atorvastatin, simvastatin, or other statins.

**Results:** The *post-hoc* analysis included 677 and 333 patients treated with PA21 or SEV, respectively. PA21 had minimal effect on lipid parameters in patients taking stable dose of atorvastatin, simvastatin, other statins or no statins (Table). Decreases in LDL-C and Tot-C, but minimal effects on TG, were observed in SEV subjects. Mixed model analyses confirmed that PA21 did not alter the lipid-lowering effects of statins.

**Table:** Mean (SD) change (mmol/L) in lipid parameters: baseline to Week 52 endpoint*.

| Baseline medication | PA21 (n=677) | | | SEV (n=333) | | |
|---|---|---|---|---|---|---|
| | LDL-C | Tot-C | TG | LDL-C | Tot-C | TG |
| Atorvastatin analysis | | | | | | |
| Atorvastatin (n=120) | -0.0 (0.82) | -0.1 (0.95) | -0.1 (2.27) | -0.7 (0.72) | -0.7 (0.84) | 0.2 (1.07) |
| Other statin (n=264) | -0.0 (0.58) | -0.0 (0.77) | -0.0 (1.20) | -0.6 (0.60) | -0.6 (0.73) | -0.0 (1.00) |
| Simvastatin analysis | | | | | | |
| Simvastatin (n=185) | -0.0 (0.58) | -0.0 (0.82) | -0.0 (0.96) | -0.5 (0.56) | -0.5 (0.72) | 0.0 (0.78) |
| Other statin (n=199) | -0.0 (0.74) | -0.0 (0.84) | -0.1 (2.04) | -0.7 (0.70) | -0.8 (0.80) | 0.1 (1.20) |
| Atorvastatin and simvastatin analyses | | | | | | |
| No statin (n=626) | -0.0 (0.72) | -0.1 (0.84) | 0.0 (0.88) | -0.6 (0.67) | -0.7 (0.84) | 0.0 (0.97) |

*Last available observation under stable dose of statin.

**Conclusions:** PA21 did not interfere with the lipid-lowering effects of statins, despite prior *in vitro* data showing an interaction. SEV lowered LDL-C and Tot-C, consistent with its known effects on these parameters.

*Funding:* Pharmaceutical Company Support - Vifor Pharma

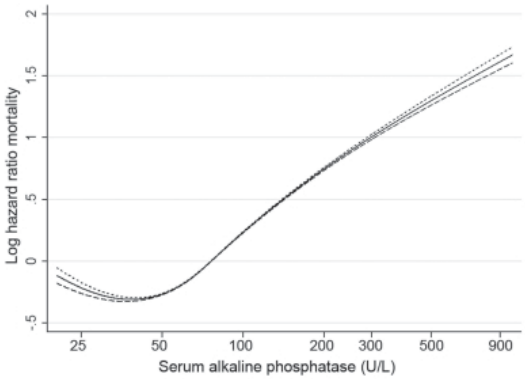
## SA-PO569

**Association of Serum Alkaline Phosphatase Levels with Kidney Function and with Mortality in a Nationally Representative Cohort of U.S. Veterans with Non-Dialysis Dependent CKD**
Csaba P. Kovesdy,[1,2] Miklos Zsolt Molnar,[3] Jennie Z. Ma,[4] Leigh Darryl Quarles,[2] Kamyar Kalantar-Zadeh.[5] *[1]Memphis VA Medical Center; [2]Univ of Tennessee Health Science Center; [3]Univ of Toronto; [4]Univ of Virginia; [5]Univ of California, Irvine.*

**Background:** Bone and mineral disorders are common and are associated with mortality in ESRD. The association of serum total alkaline phosphatase (ALP) with kidney function and with mortality in non-dialysis dependent (NDD) CKD has not been studied extensively.

**Methods:** We evaluated the association of ALP with estimated GFR (eGFR) and with all-cause mortality in a national cohort of 578,293 US veterans with stable NDD-CKD stages 1-5. Associations were examined in regression models, and in time-dependent Cox models, with adjustments for age, gender, race, comorbidities (including liver disease), eGFR, serum levels of liver enzymes and bilirubin, and medication use.

**Results:** The adjusted odds ratio (95%confidence interval [CI]) of ALP>120 U/L associated with a 10 ml/min/1.73m$^2$ lower eGFR was 1.25 (1.23-1.26, p<0.001). Over a median follow-up of 6.2 years 214,374 patients died (mortality rate: 70.0/1000 patient-years (95%CI: 69.7-70.3)). Compared to ALP levels of 50-119, ALP levels of <50, 120-199 and >=200 U/L were associated with adjusted mortality hazard ratios (95%CI) of 0.74 (0.73-0.75), 1.66 (1.64-1.68) and 2.56 (2.50-2.63), p<0.001 for all. The adjusted mortality hazard ratio (95%CI) was 2.02 (2.00-2.04, p<0.001, Figure 1) for one unit higher natural log-transformed ALP. Associations with mortality were similar in patients with all stages of NDD-CKD.




**Conclusions:** Hyperphosphatasemia is more common with decreasing kidney function, and is associated with increased mortality in patients with NDD-CKD. The effect of therapeutic interventions lowering ALP level will need to be examined in clinical trials.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO570

**Early Detection of Vascular Calcification in Retinal Vessels and Skin of Dialysis Patients**
Ramya Bhargava,[1] Faisal R. Ali,[1] Katie A. Law,[2] Jane Gray,[1] John Lear,[1] Nicholas D. Bryan,[2] David B. Henson,[2] Paul E. Brenchley,[1] Alastair J. Hutchison.[1] *[1]MRI, UK, United Kingdom; [2]Manchester Univ.*

**Background:** Vascular calcification is common in dialysis(HD) patients but early detection is difficult. We hypothesised that 1) retinal imaging by fundus photography & OCT scan may detect early calcification in retinal vessels and 2) that skin biopsies from calcified dialysis patients may have higher phosphorus(P31) & calcium(Ca43) content than normal.

**Methods:** HD patients with Xray evidence of vascular calcification in hands & pelvis with Adragao score ≥4 were invited to takepart in the study. 10 had fundus photography & OCT scans. 20 patients & 10 normal controls underwent skin biopsies. Biopsy samples were incinerated & acid digested; P31 & Ca43 were measured by spectroscopy. Skin content of P31 & Ca43 were compared in patients & controls. P31 was correlated with Serum PTH, Serum P & with Ca43.

**Results:** 1. No retinal vascular calcification was evident in any HD patients with vascular calcification in hands/pelvis; 2. No significant difference was seen in P31 & Ca43 between calcified patients & normal controls; 3. Positive correlation seen between P31 & Ca43 in patients & controls; 4. No correlation between P31 & serum PTH or serum P in patients & controls.

| | Calcified HD patients | Normal controls | P-value |
|---|---|---|---|
| N | 20 | 10 | |
| Age(mean±SEM) | 53.4±4 | 44.5±4.1 | NS |
| Gender(M:F) | 3:1 | 2:3 | |
| Serum Cr(mmol/L) | 875.2±56.4 | 70.2±4.5 | 0.0001 |
| Serum P(mmol/L) | 1.6±0.1 | 1.1±0.1 | 0.0017 |
| Sample Wt(mg) | 37.5±3 | 33.2±2.7 | NS |
| Skin P31(mcg/g) | 412.4±38.5 | 381.0±24.1 | NS |
| Skin Ca43(mcg/g) | 8.9±1 | 7.7±0.9 | NS |
| **Correlation of skin P31 with other parameters** r-Pearson's coefficient (95%CI), P-value | | | |
| vs Ca43 | 0.8 (0.5 to 0.9), 0.0001 | 0.9 (0.6 to 0.9), 0.0004 | |
| vs Serum PTH | 0.2 (-0.3 to 0.6), NS | | |
| vs Serum P | 0.1 (-0.3 to 0.5), NS | 0.2 (-0.5 to 0.7), NS | |

**Conclusions:** 1. Retinal vasculature is possibly protected from calcification that occurs elsewhere in HD patients; 2. Skin content of Phosphorus & Calcium is not a marker of vascular calcification.

## SA-PO571

**Clinical Significance of Breast Arterial Calcification in End-Stage Renal Disease**
Ekamol Tantisattamo, Na'Da Abouhassan, W. Charles O'Neill. *Renal Div, Emory Univ, Atlanta, GA.*

**Background:** The clinical significance of medial arterial calcification in end-stage renal disease (ESRD) is unclear because it often coexists with atherosclerotic calcification. As opposed to other sites, breast arterial calcification (BAC) is exclusively medial and is a convenient marker to address this question.

**Methods:** Mammograms were identified in 162 women with ESRD and those closest to the date of ESRD were reviewed. Coronary artery disease (CAD) was defined as a history of myocardial infarction or coronary stenosis of >50% by angiography. Peripheral artery disease (PAD) was defined as a history of bypass or amputation.

**Results:** Age at baseline mammography was 60.0 +/- 1.0 (SEM) y, ESRD duration was 4.7 +/- 0.4 y, 55% had diabetes, and 61% had BAC; follow-up was 2.9 +/- 1.7 y. Patients with BAC were older (62.6 +/- 1.2 vs. 53.2 +/- 1.6; p <0.0001) and more likely to be diabetic (65% vs. 40%; p=0.002). Serum calcium, phosphate, and parathyroid hormone did not differ significantly between patients with and without BAC and use of Ca-based and Ca-free phosphate binders, vitamin D compounds, and calcimimetics was similar. Patients with BAC had a higher prevalence of PAD (20% vs. 4.8%; p=0.006) but not CAD (27% vs. 22%) at follow-up. Follow-up duration was similar in the 2 groups. In a logistic regression

model, BAC (4.2; 1.1-16) and diabetes (10; 2.2-47) were independent determinants of PAD (odds ratios; 95% confidence intervals). After exclusion of patients with pre-existing disease at baseline, the incidence of PAD was greater in patients with BAC (10 % vs. 3.2%) but was not significant due to small numbers. The incidence of CAD was not increased.

**Conclusions:** Breast arterial calcification is independently associated with peripheral arterial disease in ESRD, suggesting that medial arterial calcification contributes to the pathophysiology of this disorder. Coronary artery disease is not associated with BAC in ESRD. In addition to being a marker, BAC may also predict PAD. Mammography may be a convenient and useful tool to identify patients at risk for peripheral arterial disease.

*Funding:* Pharmaceutical Company Support - Genzyme

## SA-PO572

### Prevalence and Determinants of Bone in Vascular Calcification

W. Charles O'Neill,[1] Randolph A. Hennigar.[2] *[1]Renal Div, Emory Univ, Atlanta, GA; [2]Dept of Pathology, Emory Univ, Atlanta, GA.*

**Background:** The occurrence of bone in calcified arteries has led to the hypothesis that vascular calcification is an osteogenic process. However, the prevalence and distribution of bone in calcified arteries and its relationship to the calcification and other factors are not known.

**Methods:** A total of 175 arteries in specimens of lower limb amputations from 60 patients were examined retrospectively by reviewing sections stained with hematoxylin and eosin. ESRD was present in 21 patients, CKD in 21, and no CKD (defined as a serum creatinine < 1.0) in 18. Mean age was 65.0 +/- 1.9 years and 58% had diabetes. Warfarin was prescribed in 15 patients.

**Results:** Calcification was present in 136 arteries from 52 patients, which was medial in 62, intimal in 11, and both in 63. Bone was observed in 16 arteries (medial in 14, intimal in 2) from 13 patients. There was no association with age, gender, diabetes, renal failure, or warfarin use, but bone was present in more than one artery in 3 patients, suggestive of patient-specific factors. Bone was present in 5% and 14% of arteries above and below the knee, with the highest prevalence in the posterior tibial artery (28%). The presence of bone did not correlate with the severity of calcification and was absent in over 80% of sections with severe calcification (>50% of arterial circumference). Medial cells staining for osteocalcin were noted in only 2 of 10 sections from heavily calcified arteries. Osteoclasts were noted in 9 sections from 7 patients and bone was present in all but one of these sections.

**Conclusions:** Bone formation is uncommon in arterial calcification, even when severe, indicating that not all calcification eventuates in bone formation. Although larger numbers are needed, the data suggest that patient-specific factors and arterial distribution, rather than severity of calcification are determinants of bone formation. Bone appears to be required for recruitment of osteoclasts to calcified arteries. These results question the notion that bone formation is an integral component of vascular calcification.

*Funding:* Clinical Revenue Support

## SA-PO573

### Statins and Vitamin D in U.S. Veterans with Chronic Kidney Disease (CKD)

Maryam Sharif- Hassanabadi, Andy Cheng, Seyed-ali Sadjadi, Navin Jaipaul. *Nephrology, Jerry L Pettis VA Medical Center, Loma Linda, CA.*

**Background:** Low(insufficient or deficient) Vitamin D and hyperlipidemia are common in CKD patients and associated with increased mortality. Prior studies have suggested variable effects of statins on 25-hydroxy-vitamin D [25(OH)D] levels. We conducted a single center retrospective cohort study of 632 patients with CKD stage 3 or 4 to determine whether vitamin D status is associated with statin use.

**Methods:** Computerized extraction was used to identify patients with at least one 25(OH)D level between January 2010 to October 2012. Data on patient demographics, laboratory variables, comorbidities, season of 25(OH)D level, and Statin use was collected by chart review. All statistical analyses were performed using SPSS version 20.

**Results:** Briefly, 592 (93%) patients were male with mean age of 71(+/-10.7). 477 (75%) had CKD stage 3. The prevalence of low 25(OH)D in our study population was 76.3%. The most commonly used Statin was Simvastatin (70.4%), followed by Rosuvastatin (15.3%), Pravastatin (7.5%), Atorvastatin (4.6%) and Lovastatin (2.2%). Unadjusted associations of additional study variables are summarized in table 1. Table 1.

| Characteristic | Statin Non-User (N=220) | Statin User (N=412) | p-value |
|---|---|---|---|
| Vitamin D, N(%) | | | <0.05 |
| *Normal≥30 ng/ml* | 62(28.2) | 89(21.6) | |
| *Insufficient 21-29 ng/ml* | 61(27.7) | 155(37.6) | |
| *Deficient≤20 ng/ml* | 97(44.1) | 169(41.0) | |
| Serum Calcium, mg/dL | 9.2+/-0.7 | 9.2+/-0.7 | <0.05 |
| Serum Phosphorus, mg/dL | 3.7+/-0.9 | 3.9+/-0.8 | <0.05 |
| Serum PTH, pg/mL | 141.0+/-146.8 | 140.4+/-134.7 | 0.45 |
| Body Mass Index (BMI), kg/m$^2$ | 28.2+/-6.0 | 31.2+/-6.9 | <0.05 |

In adjusted analysis, the odds of vitamin D insufficiency or deficiency increased by 6% or 7%, respectively, for each 1 kg/m$^2$ increase in BMI, and decreased by 33% or 27%, respectively for non-winter season. Statin usage was significantly associated with a higher prevalence of hypertension, coronary disease, higher BMI and phosphorus levels.

**Conclusions:** After adjusting for covariates, Statin use was not significantly associated with vitamin D status in our CKD study population.

## SA-PO574

### Results of a 12-Week Dose Titration Study Evaluating the Safety and Efficacy of Velcalcetide, a Novel Peptide Agonist of the Calcium-Sensing Receptor (CaSR), for the Treatment of Secondary Hyperparathyroidism (SHPT) in Hemodialysis (HD) Subjects

Geoffrey A. Block,[1] Kevin J. Martin,[2] David A. Bushinsky,[3] Gregory Bell,[4] Karen Pickthorn,[4] Saling Huang,[4] Yan Sun,[4] Reshma Kewalramani,[4] Christian Mix.[4] *[1]Denver Neph; [2]St. Louis Univ; [3]Univ of Rochester; [4]Amgen.*

**Background:** Velcalcetide (AMG 416/KAI-4169), a novel peptide agonist of the CaSR given intravenously thrice-weekly at the end of HD, is being evaluated as a treatment for SHPT.

**Methods:** A single arm, 12-week, dose titration study. Major inclusion criteria: serum iPTH ≥350 pg/mL (≥12 subjects with iPTH >700 pg/mL), corrected Ca (cCa) ≥8.5 mg/dL, and stable doses of vitamin D. Subjects receiving cinacalcet entered a ≥2-week washout period. Velcalcetide was initiated at 5 mg and titrated every 4 weeks (max dose 20 mg) to achieve iPTH ≤300 pg/mL. Serum iPTH, corrected Ca (cCa), P, and FGF23 were measured pre-HD weekly. The primary endpoint was % change from baseline in iPTH during the efficacy period (14 days before and 3 days after the last dose of velcalcetide).

**Results:** 37 subjects were enrolled and received at least one dose of velcalcetide. 32 subjects(87%) completed the 12-week treatment period; 5 subjects discontinued: adverse event(2), withdrawal of consent(1), "other" reasons(2); no subject discontinued for GI adverse event or symptomatic hypocalcemia. Mean(SD) baseline iPTH was 853(644) pg/mL. Mean reductions from baseline during the efficacy period included iPTH 53.6% (95% confidence interval:46.4%,60.8%), cCa 15%(13%,17%), P 10%(4%,17%) and FGF23 52%(34%,70%); 89% had ≥30% reduction in iPTH and 56% had iPTH ≤300 pg/mL.

The most common individual treatment-emergent adverse events (TEAEs) were blood calcium decreased (9 subjects,24%), diarrhea (5 subjects,14%), and symptomatic hypocalcemia (4 subjects,11%). Serious TEAEs were reported in 3(8%) subjects and none deemed related to drug. 2 subjects (5%) had cCa <7.5 mg/dL and 2(5%) experienced nausea; no vomiting events were recorded.

**Conclusions:** Velcalcetide was well-tolerated, resulted in substantial reductions in serum iPTH and FGF23 without discontinuation of therapy due to hypocalcemia or GI adverse events, and may address some limitations of oral daily medications.

*Funding:* Pharmaceutical Company Support - Amgen

## SA-PO575

### Long Term Safety and Efficacy of Velcalcetide (AMG 416), a Calcium-Sensing Receptor (CaSR) Agonist, for the Treatment of Secondary Hyperparathyroidism (SHPT) in Hemodialysis (HD) Patients

Geoffrey A. Block,[1] Kevin J. Martin,[2] David A. Bushinsky,[3] Yan Sun,[4] David M. Spiegel,[4] Reshma Kewalramani,[4] Christian Mix.[4] *[1]Denver Neph; [2]St. Louis Univ; [3]U of Rochester; [4]Amgen.*

**Background:** Velcalcetide (AMG 416/KAI-4169), a novel peptide agonist of the CaSR given intravenously (IV) thrice-weekly at the end of HD, is being evaluated as a treatment for SHPT.

**Methods:** Among subjects who completed a 12-week dose escalation study (parent study), data are reported from the subsequent 40 weeks of treatment in a single arm, open-label extension (OLE) study. The OLE study is currently ongoing. Major inclusion criteria for the parent study included iPTH ≥350 pg/mL, corrected Ca (cCa) ≥8.5 mg/dL and stable doses of active vitamin D. Following entry into the OLE study, doses of velcalcetide were adjusted to achieve and maintain iPTH ≤300 pg/mL. The primary objective for this observation period was assessment of long-term safety and tolerability. Change over time in iPTH, cCa and P from baseline in the parent study was also assessed.

**Results:** 30/37 subjects from the parent study enrolled in the OLE study. During the 40-week follow-up, 4(13%) subjects reported the adverse event of symptomatic hypocalcemia and 3 additional subjects(10%) developed at least 1 episode of asymptomatic serum cCa <7.5 mg/dL. No subjects experienced 2 consecutive cCa <7.5 mg/dL. The adverse events of nausea and vomiting were each reported by 2 subjects(7%). Mean(SD) baseline iPTH on entry to the parent study was 862(672) pg/mL. Velcalcetide decreased iPTH at week 13 (OLE study start) to 444(676) pg/mL, at week 26 to 305(550) pg/mL (n=25), and at week 52 to 252(131) pg/mL (n=20). At week 52, 17/20(85%) achieved at least a 30% reduction in iPTH, 16/20(80%) achieved a iPTH ≤300 pg/mL, and mean(SD) serum cCa and P were 8.9(0.5) mg/dL and 5.5(1.5) mg/dL, respectively (similar to week 13 values of 8.6(0.7) and 5.1(1.6) mg/dL, respectively).

**Conclusions:** This is the first report of long-term use of velcalcetide, an IV CaSR agonist given with thrice-weekly HD. Velcalcetide was well-tolerated with no subjects withdrawing from study drug due to hypocalcemia, nausea or vomiting; reductions in serum iPTH during this time were maintained.

*Funding:* Pharmaceutical Company Support - Amgen

## SA-PO576

**The Long Acting Calcimimetic R-641 Significantly Lowers Serum FGF23 in Experimental Chronic Kidney Disease** Thilo Krueger,[1] Chun Ouyang,[2] Peter Boor,[1] Nadine Kaesler,[1] Vincent Brandenburg,[3] Georg Schlieper,[1] Willi Jahnen-dechent,[4] Markus Ketteler,[5] Xiaodong Li,[6] William G. Richards,[6] Jürgen Floege.[1] *[1]Nephrology and Clinical Immunology, RWTH Univ Clinic Aachen, Aachen, Germany; [2]Nephrology, The First Affiliated Hospital of Nanjing Medical Univ, Nanjing, China; [3]Cardiology, RWTH Univ Clinic Aachen, Aachen, Germany; [4]Biomedical Engineering, RWTH Univ Aachen, Aachen, Germany; [5]Nephrology, Hospital Coburg, Coburg, Germany; [6]Amgen Inc., Thousand Oaks, CA.*

**Background:** Secondary hyperparathyroidism contributes to vascular calcification in chronic kidney disease (CKD). Calcimimetics lower parathyroid hormone (PTH) secretion by activating the calcium sensing receptor in the parathyroid gland. Here we investigated whether administration of a calcimimetic and PTH injection influences vascular calcification and serum levels of FGF23 in a rat CKD model.

**Methods:** CKD was induced in rats by adenine diet for 4 weeks. Four treatment protocols then continued for further 4 weeks: vehicle, PTH-(1-34), R-641+vehicle, R-641+PTH-(1-34). R-641 or vehicle were administered every third day. In the R-641+PTH group each R-641 dose was followed 48 hrs later by 80 µg/kg PTH s.c. Blood samples were collected; vascular calcification was assessed by measurement of calcium content in aortic walls. PTH-(1-84) and FGF23 serum levels were measured by ELISA.

**Results:** Compared to healthy controls, adenine significantly increased aortic calcium content. R-641 administration significantly reduced aortic calcium content (65 vs. 9 µg/g). Serum PTH was significantly lower in R-641 treatment groups compared to CKD rats (939 vs. 3212 pg/ml). Adenine diet induced a significant increase in FGF23 serum levels (1.4 vs. 198.4 ng/ml); R-641 treatment resulted in significantly lower values (71.4 ng/ml). PTH administration did neither change vascular calcium content in CKD nor FGF23 serum levels in CKD alone or with R-641 co-administration.

**Conclusions:** After 4 weeks treatment, administration of R-641 significantly reduced vascular calcification and lowered serum FGF23 levels in experimental CKD. PTH injection had no influence on vascular calcification or FGF23 serum levels.

*Funding:* Pharmaceutical Company Support - AMGEN Inc.

## SA-PO577

**The Long Acting Calcimimetic R-641 Does Not Induce Adynamic Bone Disease in Chronic Kidney Disease** Thilo Krueger,[1] Peter Boor,[1] Vincent Brandenburg,[2] Georg Schlieper,[1] Willi Jahnen-dechent,[3] Markus Ketteler,[4] Xiaodong Li,[5] William G. Richards,[5] Jürgen Floege.[1] *[1]Nephrology and Clinical Immunology, RWTH Univ Clinic Aachen, Aachen, Germany; [2]Cardiology, RWTH Univ Clinic Aachen, Aachen, Germany; [3]Biomedical Engineering, RWTH Univ Aachen, Aachen, Germany; [4]Nephrology, Hospital Coburg, Coburg, Germany; [5]Amgen Inc., Thousand Oaks, CA.*

**Background:** Secondary hyperparathyroidism is a frequent complication of CKD and contributes to renal osteodystrophy. Calcimimetics lower parathyroid hormone (PTH) secretion by activating the calcium sensing receptor. Constant suppression of PTH secretion, however, may lead to low bone turnover adynamic bone disease. Here we investigated whether repeated administration of a long acting calcimimetic leads to adynamic bone disease in a rat CKD model.

**Methods:** In a dose finding experiment we tested 5, 10 and 20 mg/kg of the long acting calcimimetic R-641 in rats. CKD was induced by adenine diet for 4 weeks. After 3 weeks 10 mg/kg R-641 was administered every third day for further 4 weeks. Bones were labelled with calcein (10 mg/kg) by injection at 10d and 3d prior to sacrifice. Femoral bones were subjected to histomorphometry and blood samples were collected weekly. Vascular calcification was assessed by calcium content determination from thoracic aortas. PTH-(1-84) was measured by ELISA.

**Results:** A single dose of R-641 lowered serum calcium for over 48 hours dose dependently while suppressing PTH levels for 12 hours. Compared to healthy controls, adenine treatment increased osteoid area, osteoblast and osteoclast numbers. Adenine reduced bone area compared to controls. Treatment with R-641 reduced osteoblast numbers and significantly decreased osteoid area without reaching criteria of adynamic bone disease. R-641 reduced vascular calcium content (9 vs. 65 µg/g). Serum PTH was lower in R-641 treatment groups compared to CKD rats (939 vs. 3212 pg/ml).

**Conclusions:** After 4 weeks treatment, a long acting calcimimetic reduced vascular calcification, improved secondary hyperparathyreoidism but did not lead to adynamic bone disease.

## SA-PO578

**Does Decrease of Plasma Fibroblast Growth Factor 23 Concentration during Cinacalcet Treatment Depends on the Decrease of Serum Phosphate Concentration?** Marcin Adamczak, Piotr Kuczera, Andrzej Wiecek. *Dept of Nephrology, Endocrinology and Metabolic Diseases, Medical Univ of Silesia, Katowice, Poland.*

**Background:** Fibroblast growth factor 23 (FGF23) is an important factor involved in the pathogenesis of calcium-phosphate abnormalities in patients with chronic kidney disease and increased plasma FGF23 concentration is a cardiovascular risk factor in these patients. The aim of this prospective, single-arm, open-label clinical study was to assess the influence of six-month cinacalcet treatment on plasma FGF23 concentration and its relationship to the serum phosphate changes in hemodialysed patients (HDP) with secondary hyperparathyroidism (sHPT).

**Methods:** In 58 HDP with sHPT (PTH>300pg/ml) serum PTH, FGF23, calcium and phosphate concentrations were assessed before the first dose of cinacalcet and after 3 and 6 months of treatment. The results are shown as means and 95% confidence index.

**Results:** Serum PTH concentration decreased significantly after 3 and 6 month of treatment. Plasma FGF23 concentration decreased after 3 and 6 months of treatment from 593 (457-730)pg/ml to 513 (380-645)pg/ml; p=0.099 and to 433 (304-561)pg/ml; p=0.015, respectively. FGF23 concentration decreased in 52% of patients. In patients with FGF23 decrease a significant ($p<0.05$) decrease in serum phosphate concentration after 3 and 6 months of treatment [1.92 (1.69-2.14); 1.74 (1.53-1.95) and 1.68 (1.47-1.88)mmol/l, respectively] but not in patients with stable plasma FGF23 concentration [2.13 (1.91-1.35); 2.21 (1.97-2.44); 2.13 (1.92-2.33)mmol/l were observed. There was a significant positive correlation between changes of plasma FGF23 and serum phosphate concentration (0-3 month of treatment) ($R=0.30$, $p=0.03$). In the multivariate analysis changes of plasma FGF23 concentration (0-6 month of treatment) was explained by the serum phosphate concentration changes ($\beta=0.29$; $p=0.04$), but not by the serum PTH and calcium concentration changes, nor by the dose of cinacalcet.

**Conclusions:** Decrease of plasma FGF23 concentration in HDP treated with cinacalcet seems to be related mostly to the decrease of serum phosphate concentration.

*Funding:* Government Support - Non-U.S.

## SA-PO579

**Use of Sevelamer Carbonate to Examine the Role of Intraluminal Phosphate in Secondary Hyperparathyroidism** Kenneth R. Phelps,[1] Darius Mason.[2] *[1]Stratton VAMC, Albany, NY; [2]Albany College of Pharmacy, Albany, NY.*

**Background:** In CKD, [PTH] rises with influx of phosphate (P), and filtrate [P] ($[P]_f$) rises in the cortical distal nephron (CDN). We surmised that increased $[P]_f$ promotes sequestration of Ca in complexes and thus creates a need for high [PTH] to maintain Ca reabsorption. Since $[P]_f$ in the CDN is roughly proportional to $E_P/GFR$ (P excreted/volume of filtrate), we hypothesized that [PTH] would correlate with $E_P/C_{cr}$ in CKD, and would fall with $E_P/C_{cr}$ as sevelamer (SC) reduced influx of P.

**Methods:** Patients with CKD (mean eGFR 28.7 ± 1.7) were randomized in double-blind fashion to SC (2.4 g with meals) or placebo (PL) for 4 wks. $E_P/C_{cr}$ was calculated as $[P]_u[cr]_s/[cr]_u$. [PTH]1-84 was measured by IRMA (Scantibodies), $[1,25(OH)_2D]$ by RIA (Labcorp), and intact [FGF23] by ELISA (Immutopics). Data are post-treatment means (SEM). Δ is change with treatment. Differences were analyzed by unpaired t-test, and regressions by least squares.

**Results:** In SC, $E_P/C_{cr}$ was lower and decrements in $E_P/C_{cr}$ were greater than in PL. [PTH] was not different between groups, but Δ[PTH] and % Δ[PTH] were different.

| Group | SC (n = 14) | PL (n = 15) | P |
|---|---|---|---|
| $E_P/C_{cr}$, mg/dL | 0.9 (0.1) | 1.4 (0.1) | 0.02 |
| $\Delta E_P/C_{cr}$, mg/dL | -0.5 (0.1) | 0.02 (0.11) | 0.01 |
| % $\Delta E_P/C_{cr}$ | -36.4 (7.2) | -0.06 (6.7) | 0.004 |
| [PTH], pg/mL | 66.4 (13.7) | 95.9 (13.4) | 0.14 |
| Δ[PTH], pg/mL | -12.3 (6.3) | 10.5 (8.6) | 0.04 |
| % Δ[PTH] | -11.7 (5.8) | 16.4 (10.0) | 0.02 |

[PTH] correlated with $E_P/C_{cr}$ in SC ($R^2 = 0.53$; $P = 0.003$) and PL ($R^2 = 0.35$; $P = 0.02$). Δ[PTH] correlated with $\Delta E_P/C_{cr}$ in SC ($R^2 = 0.56$; $P = 0.002$) and PL ($R^2 = 0.36$; $P = 0.02$). [PTH] was unrelated to $[P]_s$ and $[1,25(OH)_2D]$. In SC, [PTH] varied directly (not inversely) with [FGF23] ($R^2 = 0.37$; $P = 0.02$).

**Conclusions:** SC reduced $E_P/C_{cr}$ in CKD, and decrements in [PTH] were greater with SC. In both groups, [PTH] correlated with $E_P/C_{cr}$, a surrogate for $[P]_f$ in the CDN, and Δ[PTH] correlated with $\Delta E_P/C_{cr}$. Confounders did not explain the correlations. The results support the hypothesis that in CKD, high $[P]_f$ creates an impediment to Ca reabsorption in the CDN.

*Funding:* Veterans Affairs Support, Pharmaceutical Company Support - Genzyme Corporation

## SA-PO580

**Evidence for an Impediment to Tubular Calcium Reabsorption in Chronic Kidney Disease** Kenneth R. Phelps,[1] Kim Stote.[2] *[1]Stratton VAMC, Albany, NY; [2]SUNY Empire State College, Saratoga Springs, NY.*

**Background:** [PTH] is elevated in primary hyperparathyroidism (PHPT) and in hyperparathyroidism due to CKD. Plasma ionized Ca ($[Ca]_i$) and tubular Ca reabsorption/volume of filtrate ($TR_{Ca}/C_{cr}$) are increased in PHPT. Because $[Ca]_i$ is usually normal in CKD, we hypothesized that $TR_{Ca}/C_{cr}$ is also normal despite high [PTH].

**Methods:** We studied 29 patients with CKD (eGFR < 60), 7 patients with PHPT, and 28 control subjects (C). We obtained fasting serum/plasma creatinine ($[cr]_s$), ionized calcium ($[Ca]_i$), ultrafilterable calcium ($[Ca]_{uf}$), and [PTH] 1-84 (IRMA, Scantibodies), and urine cr and Ca ($[cr]_u$, $[Ca]_u$). $[Ca]_i$ was measured with an ion-selective electrode. $[Ca]_{uf}$ was measured by spectrophotometry after centrifugation in an Amicon Centrifree filter. Since the filtration rate of calcium ($C_{cr}[Ca]_{uf}$) is the sum of rates of excretion and reabsorption ($E_{Ca}$ and $TR_{Ca}$), $[Ca]_{uf}$ equals $E_{Ca}/C_{cr} + TR_{Ca}/C_{cr}$. $E_{Ca}/C_{cr}$ was calculated as $[Ca]_u[cr]_s/[cr]_u$, and $TR_{Ca}/C_{cr}$ as $[Ca]_{uf} - E_{Ca}/C_{cr}$. Means were compared with ANOVA.

**Results:** $E_{Ca}/C_{cr}$ was comparable in all groups. $[Ca]_i$, $[Ca]_{uf}$, and $TR_{Ca}/C_{cr}$ were similar in CKD and C even though [PTH] was higher in CKD. In PHPT, $[Ca]_i$, $[Ca]_{uf}$, and $TR_{Ca}/C_{cr}$ were higher than in CKD and C; [PTH] was intermediate between but not significantly different from concentrations in CKD and C.

| Group | CKD (n = 29) | PHPT (n = 7) | C (n = 28) |
|---|---|---|---|
| eGFR, mL/min/1.73m$^2$ | 28.7 (1.7)[a] | 83.3 (9.8) | 85.4 (2.0) |
| [PTH], pg/mL | 82.2 (9.8)[b] | 49.6 (8.0)[c] | 28.8 (2.3) |
| $E_{Ca}/C_{cr}$, mg/dL | 0.06 (0.01) | 0.07 (0.01) | 0.06 (0.01) |
| $[Ca]_i$, mg/dL | 5.0 (0.04) | 5.7 (0.1)[a] | 5.0 (0.02) |
| $[Ca]_{uf}$, mg/dL | 5.4 (0.05) | 6.1 (0.2)[a] | 5.4 (0.05) |
| $TR_{Ca}/C_{cr}$, mg/dL | 5.3 (0.04) | 6.0 (0.2)[a] | 5.3 (0.04) |

Values are mean (SEM). [a]P < 0.001 v other groups. [b]P < 0.001 v C. [c]P = 0.10 v CKD and 0.39 v C. All other differences NS.

**Conclusions:** $TR_{Ca}/C_{cr}$, $[Ca]_i$, and $[Ca]_{uf}$ were similar in CKD and C and higher in PHPT. The combination of normal $TR_{Ca}/C_{cr}$ and high [PTH] suggested the presence of an impediment to Ca reabsorption in CKD. It appears that [PTH] rose as necessary to compensate for the impediment and maintain normocalcemia.

*Funding:* Veterans Affairs Support, Pharmaceutical Company Support - Genzyme Corporation

## SA-PO581

**Effect of PA21, a New Iron-Based Phosphate Binder on FGF23 and Vascular Calcifications Compared with Lanthanum Carbonate and Sevelamer Carbonate in Uremic Rats** Olivier Phan,[1] Marc P. Maillard,[1] Felix W. Funk,[2] Olivier Bonny,[1] Michel Burnier.[1] *[1]CHUV; [2]Vifor (Internationnal) Inc.*

**Background:** Elevated serum phosphate and FGF23 levels are associated with cardiovascular disease in patients with chronic renal disease. Whether FGF23 can act on vascular calcification is still on debate. Few studies have analysed how to suppress FGF23 up-regulation using phosphate binders.

The aim of this study was to evaluate the effects of PA21 compared with lanthanum carbonate (La) and sevelamer carbonate (Se) on serum FGF23, phosphorus, calcium, iPTH concentrations and to investigate a potential effect on the development of vascular calcifications in an adenine-induced rat model of CRF.

**Methods:** After induction of CRF through a 4 week adenine-diet, renal function was significantly impaired in all groups. All uremic rats developed severe hyperphosphatemia and serum PTH increased significantly. Phosphate binders were then given for 4 weeks to all uremic rats, except for the uremic control rats. The concentration of each binder (% of binder added to the diet) was chosen to deliver approximately the same amount of active pharmaceutical moiety to each rat: PA21 5% (corresponding to 1% iron), La 2% (1% lanthanum), Se 1.5% (1% sevelamer). A computer-assisted automated quantitative measurement was used to assess the degree of calcification from von Kossa stained vessel sections.

**Results:** Hyperphosphatemia and increased serum PTH levels were controlled in the phosphate binder treated groups to the same extent. PA21 was the only phosphate binder that was associated with a decrease of FGF23. In uremic control rats, vascular calcifications were more prominently present in the thoracic aorta compared to the carotids and the abdominal aorta. Vascular calcifications of thoracic aorta were significantly decreased by the three phosphate binders to a similar extent. PA21 was more efficient than lanthanum carbonate to prevent calcifications in the upper part of the thoracic aorta.

**Conclusions:** PA21 was as effective in the control of hyperphosphatemia, secondary hyperparathyroidism and vascular calcifications as La and Se. The role of FGF23 as a potential factor of calcification needs to be confirmed.

*Funding:* Pharmaceutical Company Support - Vifor (Internationnal) Ltd

## SA-PO582

**Maxacalcitol Ameliorates Pathological Left Ventricular Hypertrophy by Inhibiting the Calcineurin-NFAT Pathway** Kazunori Inoue,[1] Isao Matsui,[1] Takayuki Hamano,[2] Akihiro Shimomura,[1] Yasuo Kusunoki,[1] Chikako Nakano,[1] Yoshitsugu Obi,[1] Yoshiharu Tsubakihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

**Background:** Cardiovascular disease, such as heart failure accompanied by pathological left ventricular hypertrophy (LVH), is the leading cause of death in CKD. Studies on vitamin D receptor knockout mice have revealed that active vitamin D (aVD) is one of the promising agents that ameliorate LVH. Both maxacalcitol (22-oxacalcitriol (OCT)) and paricalcitol are clinically available less calcemic analogue of aVD, however, their actions differ from each other in some points. Therefore, we examined (1) the potential of OCT as a therapeutic agent for LVH and (2) the underlying mechanisms especially focusing on the difference between OCT and paricalcitol.

**Methods:** Six-week-old male Wister rats were subjected to heminephrectomy and then divided into four groups; normal saline + vehicle (N+V), normal saline + OCT (N+O), angiotensin II (Ang II) + vehicle (A+V), or Ang II + OCT (A+O). Vehicle or OCT at a dose of 0.15 μg/kg BW was administered subcutaneously twice a day. Using neonatal rat ventricular myocytes (NRVM), we examined the difference between OCT and paricalcitol in view of the calcineurin-NFAT pathway, which plays a pivotal role in the pathogenesis of LVH.

**Results:** Compared with group A+V, heart weight, wall thickness, and mRNA expression levels of the hypertrophic markers were decreased in group A+O. We found that OCT inhibited Ang II-induced activation of calcineurin A, and that OCT recovered mRNA levels for atrogin-1 in AngII infused rats. In vitro analyses also demonstrated that OCT inhibited Ang II-induced hypertrophy by inhibiting the calcineurin-NFAT pathway. In comparison with the same concentrations of paricalcitol, OCT more effectively inhibited the hypertrophy by inducing atrogin-1-dependent ubiquitination of calcineurin A in calcinurin-overexpressed NRVM.

**Conclusions:** Our findings provide a novel approach to inhibit calcineurin-NFAT pathway in pathological cardiac hypertrophy.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## SA-PO583

**Response of Hyperplastic Parathyroid Gland to Withdrawal of a Calcimimetic Compound** Masahide Mizobuchi,[1] Hiroaki Ogata,[2] Ai Yamazaki,[1] Noriko Nunota Arai,[1] Naoto Kobayashi,[1] Fumihiko Koiwa,[3] Eriko Kinugasa,[2] Takanori Shibata,[1] Tadao Akizawa.[1] *[1]Dept of Medicine, Div of Nephrology, Showa Univ School of Medicine; [2]Dept of Internal Medicine, Showa Univ Northern Yokohama Hospital; [3]Dept of Medicine, Div of Nephrology, Showa Univ Fujigaoka Hospital.*

**Background:** Calcimimetics have been shown to have suppressive effects on PTH secretion and parathyroid cell proliferation. However, little is known about the mechanistic change in parathyroid cells when calcimimetics are withdrawn. We studied the response of parathyroid glands to the withdrawal of cinacalcet in 5/6$^{th}$ nephrectomized uremic rats.

**Methods:** Uremic rats (Nx) were fed a 1.2% high phosphate diet for 2 weeks to develop secondary hyperparathyroidism and then divided by 3 treatment groups: 1) with vehicle (Veh), 2) with cinacalcet (15 mg/kg/day, gavage) (Cina), and 3) with cinacalcet and a VDRA 22-oxacalcitriol (OCT: 0.15μg/kg, 3 times a week, intraperitoneally) (Cina+Maxa). After 2 weeks treatment, vehicle and cinacalcet were withdrawn while maxacalcitol treatment was continued. Immediately (day 0) and 7 days (day 7) after the withdrawal, blood and parathyroid tissues were obtained. Normal rats with vehicle at day 0 and 7 were utilized as normal controls (NC).

**Results:** Cina and Cina+OCT showed equally and significantly lower PTH than Veh at day 0 whereas PTH in the Cina and Cina+OCT was increased up to indistinguishable levels in the Veh at day 7. Serum P was comparable among Veh, Cina, and Cina+OCT in both day 0 and 7. Cinacalcet treatment significantly decreased ionized Ca compared with Veh at day 0, and this decrease was restored by day 7. At day 0, PCNAmRNA was significantly increased in Veh compared with that in NC and this increase was significantly suppressed in Cina and Cina+OCT. At day 7, however, Cina, but not Cina+OCT showed significant increase in PCNAmRNA compared with Veh.

**Conclusions:** These results suggest that, although PTH rebound is not present, simultaneous use of VDRA is preferred regarding parathyroid cell proliferation when cinacalcet is withdrawn.

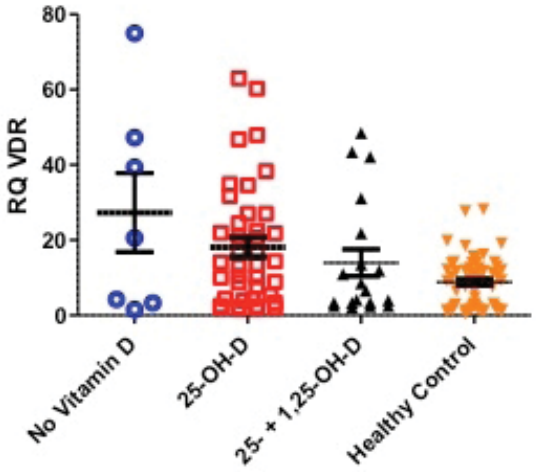
## SA-PO584

**Frequencies of Proinflammatory Monocyte Subsets Correlate with Vitamin D Receptor Expression** Eric Seibert, Christof Ulrich, Felix Kohler, Bogusz Trojanowicz, Roman Fiedler, Matthias Girndt. *Internal Medicine II, Martin-Luther-Univ Halle-Wittenberg, Halle (Saale), Germany.*

**Background:** Proinflammatory monocyte subsets are associated with cardiovascular mortality in HD patients. It is also known that mortality of dialysis patients is associated with vitamin D status and supplementation. We therefore examined the correlation between frequency of proinflammatory monocyte subsets and total leukocyte Vitamin D receptor (VDR) expression and their dependency on different vitamin D medications.

**Methods:** In 74 hemodialysis patients and 60 healthy controls, relative quantity (RQ) of total leukocyte VDR mRNA expression (qRT-PCR, normalized on beta-actin) and frequency of monocyte subsets (flow cytometry, MACS Quant, Miltenyi) were determined. Monocytes were subdivided into CD14++CD16-(Mo1), CD14++CD16+(Mo2) and CD14+CD16++(Mo3) cells. Vitamin D medication was classified by use of cholecalciferol, active vitamin D or none of them.

**Results:** RQ of VDR was significantly higher in HD patients than in healthy controls (19.0±18.9 vs. 8.9±6.6, $p<0.01$). Likewise, Mo2 were higher in HD patients than in controls (8.5±0.3 vs. 3.2±0.2%, $p<0.0001$). Mo2 frequencies and VDR RQ were dependent on vitamin D medication, being highest in patients without vitamin D and lowest in healthy controls. Use of active vitamin D compounds in addition to cholecalciferol was beneficial (fig.1). Frequencies of Mo2 were significantly correlated with VDR RQ ($R^2=0.11$, $p<0.001$).




**Conclusions:** Frequencies of proinflammatory monocyte subsets are correlated with VDR expression. Additionally, VDR expression and Mo2 frequencies vary between groups with different vitamin D compound medication. This may constitute a mechanistic link between beneficial effects of vitamin D supplementation and the prognostic value of proinflammatory monocyte subsets.

*Funding:* Clinical Revenue Support

## SA-PO585

**Local Synthesis of Calcitriol by 1αhydroxylase Is Involved in Vascular Calcification Induced by Uremia** Noelia Torremade,[1] M. Vittoria Arcidiacono,[1] Petya Valcheva,[1] Milica Bozic,[1] Sara Panizo,[2] Elvira Fernandez,[1] Jose M. Valdivielso.[1] *[1]IRBLleida; [2]Hospital Universitario Central de Asturias.*

**Background:** Vascular calcification is a complication of chronic kidney disease. We have previously demonstrated that uremia increases the expression of 1αhydroxylase in vascular smooth muscle cells (VSMC). The objective of this study is to determine the role of the local synthesis of calcitriol on uremia-induced vascular calcification.

**Methods:** Wild type and 1alpha hydroxylase KO mice (1αOHase KO) underwent 75% of renal mass reduction, and were treated with calcitriol (400 ng/kg/day) for two weeks. At the end of the experiment, serum samples were collected and Ca, P and BUN were quantified. The artery was used to evaluate vascular calcification by calcium quantification and alizarin red staining. In vitro WT and 1αOHase KO VSMC were treated with healthy and uremic rat serum to evaluate its effect on calcification. Normal rat VSMC overexpressing 1αOHasewere incubated under high P media.

**Results:** WT mice (n=7) show increased mortality when treated with calcitriol. However, 1αOHase KO mice (n=10) did not. Serum calcium levels (WT: 16.38 ± 0.48, KO: 15.18 ± 0.46 mg/dl), phosphorus (WT: 8.15 ± 0.42, KO: 8.43 ± 0.46 mg/dl), BUN (WT: 44.99±2.19, KO: 46.13±4.02 mg/dl), and 1,25D levels (WT: 150,21±0,89; KO: 123,28±14,171 pg/ml) increased and were similar in both calcitriol-treated groups. PTH levels decreased in both groups (WT: 29,25±0,84, KO: 30,48±0.70 pg/ml). Vascular calcium content significantly increased in the WT mice compared to 1αOHase KO mice (WT: 895,70±172,26; KO: 556.74±77.71 µgCa/mg protein p<0.05). Similar results were observed with alizarin red staining and immunohistochemical detection of Runx2, which increased only in WT mice. In vitro, WT VSMC treated with uremic serum showed a significant increase in calcification that was not observed in 1αOHase KO cells (WT: 3452.50 ± 498.07; KO: 510.72 ± 94.82 µgCa/mg protein. n=6 to 8, P <0.05). Normal VSMC overexpressing 1αOHaseshowed an increas of calcification when incubated in high P media.

**Conclusions:** These results suggest that local production of calcitriol in the artery may mediate vascular calcification observed in uremia.

*Funding:* Government Support - Non-U.S.

## SA-PO586

**Thyroid Hormones Decrease Plasma 1α,25-Dihydroxyvitamin D Levels through Transcriptional Repression of the Renal 25-Hydroxyvitamin $D_3$ 1α-Hydroxylase Gene (CYP27B1)** Hironori Yamamoto,[2] Mina Kozai,[1] Tomohiro Kagawa,[1] Otoki Nakahashi,[1] Shoko Ikeda,[1] Rina Onishi,[1] Yutaka Taketani,[1] Eiji Takeda.[1] *[1]Clinical Nutrition, Health Biosciences, Univ of Tokushima, Tokushima, Japan; [2]Health and Nutrition, Human Life, Jin-ai Univ, Echizen, Fukui, Japan.*

**Background:** Hyperthyroid patients have been reported to have low levels of plasma 1α,25-dihydroxyvitamin D ($1,25(OH)_2D$), however, its detailed mechanism is still poorly understood. The present study determined whether renal 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) gene expression was negatively regulated by thyroid hormones.

**Methods:** For hyperthyroid mice, C57BL/6 mice were given 3,3',5-tri-iodothyronine ($T_3$) at 20 or 200 µg/100 g of body weight by intraperitoneal injection for 24 hr. Real-time PCR were performed using kidney total RNA and gene specific primers. The transcriptional activity of CYP27B1 gene were measured by CYP27B1 luciferase reporter vector and renal proximal tubular opossum kidney (OK-P) cells.

**Results:** $T_3$-induced hyperthyroid mice showed marked decreases in plasma $1,25(OH)_32$[/sub]D levels and in renal expression of CYP27B1 mRNA. In addition, we observed that $T_33$[/sub] administration significantly decreased plasma $1,25(OH)_32$[/sub]D and renal CYP27B1 mRNA levels that were increased by low calcium diet, and induced hypocalcemia in mice fed a low calcium diet. $T_33$[/sub] decreases the basal transcriptional activity of the CYP27B1 gene through thyroid hormone receptors (TRα, TRβ1) and the retinoid X receptor α (RXRα) in OK-P cells. Interestingly, we identified an everted repeat negative thyroid hormone response element (1α-nTRE) overlapping the sterol response element (SRE) and the TATA-box in the human CYP27B1 gene. Finally, we established that CYP27B1 gene transcription is positively and negatively regulated by SRE-binding proteins (SREBPs) and $T_33$[/sub]-bound TRβ1/RXRα via the 1α-nTRE.

**Conclusions:** These results suggest that transcriptional repression of the CYP27B1 gene by $T_3$-bound TRs/RXRα, acting through the 1α-nTRE, results in decreased renal CYP27B1 expression and plasma $1,25(OH)_2D$) levels.

*Funding:* Government Support - Non-U.S.

## SA-PO587

**Vitamin D Is Cardioprotective but Not Essential for Fibroblast Growth Factor-23 Production** Carlos Cuervo, Gaston E. Zilleruelo, Michael Freundlich. *Pediatric Nephrology, Univ of Miami Miller School of Medicine, Miami, FL.*

**Background:** The mechanisms regulating FGF23 are unclear. $1,25(OH)_2D_3$ (D) and PTH both increase FGF23 levels. However, undetectable FGF23 levels in 1-α hydroxylase ablated mice and the regulatory transcription by a D response element in the FGF23 promoter, suggest D-indispensability for bone FGF23 secretion challenging the notion that PTH in the absence of D is sufficient for FGF23 production. Absent D also results in renin-angiotensin system (RAS) upregulation and may cause hypertension and cardiac hypertrophy (CH), as in CKD. These relationships were studied in vitamin D dependent rickets type I (VDDR-I), where abolished 1-α OH activity results in↓or undetected circulating D and ↑PTH levels.

**Methods:** After diagnosing VDDR-I at age 8 months, oral calcitriol and supplemental Ca were maintained for the subsequent 12 years, and measurements obtained while under Rx (on-Rx) or following periods of non-adherence (off-Rx).

**Results:** Hypocalcemia and rickets healed on-Rx but reappeared off-Rx. Final height and blood pressure were normal, the echocardiogram displayed normal ventricular dimensions and cardiac function. Comparing off-Rx vs. on-Rx, 1,25D levels were lower (15±4.5 vs.40±12.8 pg/ml, p=0.01), Alk-P higher (1,986±986 vs.1,532±184 U/L, p<0.05), PTH higher (931±228 vs. 43±24 pg/ml, p<0.005), FGF23 lower (70±3.5 vs. 270±0.3 RU/ml, p<0.0005), and renin higher (5.4±1.5 vs. 3.8±0.92 ng/ml/hour, p<0.02). 1,25D correlated with renin (r= -0.61, p<0.05) and PTH (r= -0.87, p<0.005). Plasma aldosterone and Ang II, and urine tetrahydroaldosterone were↑off-Rx and normalized on-Rx. During off-Rx at nadir 1,25D levels <8pg/ml, FGF23 levels were normal (70 RU/ml) while PTH was↑(751 pg/ml) and FEPO4↑(65%).

**Conclusions:** VDDR-I replicates data in animals deficient in D biosynthesis with RAS stimulation, and shows D acting as a negative regulator of renin production. Long-term D therapy in VDDR-I effectively prevented hypertension and CH strengthening the notion that D displays cardioprotective properties. Furthermore, maintenance of normal FGF23 concentrations with prevailing undetectable 1,25D levels, suggests D-independent PTH-mediated FGF23 secretion by the bone.

## SA-PO588

**$24,25(OH)_2$ D Concentration during Treatment of Vitamin D Deficiency** Hala M. Alshayeb,[3] Arif Showkat,[1] Leigh Darryl Quarles,[1] Geeta G. Gyamlani,[1,2] Valentin David,[1] Barry M. Wall.[1,2] *[1]Univ of Tennessee Health Science Center, Memphis, TN; [2]VA Medical Center, Memphis, TN; [3]Marshall Univ, Huntington, WV.*

**Background:** Although FGF23 upregulates Cyp24 in CKD, $24,25(OH)_2D$ levels have been shown to be directly related to eGFR in human subjects, possibly related to decreased total enzymatic activity in CKD (Kidney Int, 2012). Low 25(OH)D is a predictor of reduced $24,25(OH)_2D$, suggesting that production may be substrate dependent. To further assess these interactions, $24,25(OH)_2D$ levels were measured before and after treatment of vitamin D deficiency with cholecalciferol in patients with normal eGFR, CKD, and ESRD.

**Methods:** Patients with 25OHD level < 20 ng/ml received 10,000 IU/week of cholecalciferol for 8 weeks: CKD patients(n=14) matched with non-CKD (n=14) for age, sex, race, and diabetes and unmatched ESRD hemodialysis patients (n=14).

**Results:** There were no significant differences between CKD and non-CKD groups in basal levels of 25(OH)D and $24,25(OH)_2D$. Baseline iPTH and FGF-23 were higher in CKD patients. Cholecalciferol treatment resulted in increments in serum 25(OH)D (18.6 ± 8 ng/ml vs 12.2 ± 9, p=0.03) and in $24,25(OH)_2D$ (1.14 ± 0.89 ng/ml vs 1.02 ± 0.74, p=0.7) in non-CKD and CKD, respectively. $24,25(OH)_2D$ were not higher in CKD ( before or after cholecalciferol). Cholecalciferol resulted in an increase in FGF23 (44 ± 57 pg/ml, P=0.01) in non-CKD, but not in CKD or ESRD. In a multivariable analysis, low post-treatment serum $24,25(OH)_2D$ level was predicted by low post-treatment 25(OH)D (p<0.03), low eGFR( P=0.05) and high baseline PTH (p<0.01). The change in 25 (OH)D level in ESRD cohort was 16.5 ± 0.7 ng/ml, while 24,25OH2D were extremely low and did not change with cholecalciferol.

**Conclusions:** $24,25(OH)_2D$ increases with cholecalciferol therapy in CKD and non-CKD subjects, consistent with substrate dependent production. Despite decreased nephron mass in CKD, there was a similar increment in $24,25(OH)_2D$ following cholecalciferol suggesting Cyp24a1 up-regulation. Pesisistent markedly reduced $24,25(OH)_2D$ in ESRD is likely related to more severely reduced functioning nephron mass.

## SA-PO589

**Associations of Serum 25-Hydroxyvitamin D and 1,25-Dihydroxyviatmin D Levels with All-Cause Mortality and Cardiac Death in the Hemodialysis (HEMO) Study** Jessica B. Kendrick,[1] Alfred K. Cheung,[2,3] Tom Greene,[3] Michel Chonchol.[1] *[1]Univ of Colorado; [2]VASLCHCS; [3]Univ of Utah.*

**Background:** Determine whether serum vitamin D levels are related to all-cause mortality (ACM) and a composite of first cardiac hospitalization or cardiac death in patients requiring hemodialysis.

**Methods:** The HEMO Study was a randomized multicenter study evaluating the effects of high-dose versus standard-dose and high-flux versus low-flux hemodialysis. 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels were measured in stored serum samples obtained at baseline and annually in 1,340 patients in this cohort. Quartiles of serum 25(OH)D and $1,25(OH)_2D$ concentrations were chosen as the primary predictor variable, with the lowest quartile serving as the reference category. Time-dependent Cox proportional-hazards models were used to examine the association between vitamin D levels with ACM and a composite of first cardiac hospitalization or cardiac death.

**Results:** Participants had a mean age of 57±14 years, 55% were females and 46% were white. During a median follow-up of 3.0 years, 582 (43%) died from any cause, and 514 (41%) had a cardiac event. Median (IQR) serum 25(OH)D and $1,25(OH)_2D$ levels were 19.1 [14.2, 26.6] ng/mL and 6.3 [2.9, 14.5] pg/mL, respectively. After adjustment for potential confounders available in the database including inflammatory markers and usage of calcitriol, the highest quartile of 25(OH)D was associated with a decrease risk of ACM (HR;0.55, 95% CI 0.40-0.75) and cardiac events (HR; 0.70, 95% CI 0.52-0.0.94), when compared to the lowest quartile. Similarly, when 25(OH)D was evaluated as a continuous variable, higher levels of 25(OH)D were associated with a decreased risk of ACM and cardiac outcomes (HR; 0.77, 95% CI 0.66-0.90 and HR; 0.79; 95% CI 0.68-0.92, respectively per doubling of 25(OH)D level). There was no association between $1,25(OH)_2D$ levels with ACM or cardiac events.

**Conclusions:** Low serum 25(OH)D level, but not $1,25(OH)_2D$ level, is independently associated with ACM, cardiac hospitalizations and death in patients requiring hemodialysis.

*Funding:* NIDDK Support

## SA-PO590

**Human Cathelicidin and Vitamin D: Baseline Associates and Effects of Ergocalciferol** Ishir Bhan, Ravi I. Thadhani. *Nephrology Div, Massachusetts General Hospital, Boston, MA.*

**Background:** Cathelicidin is an antimicrobial peptide produced by the innate immune system that is regulated at a genetic level by vitamin D. Low plasma levels of cathelicidin have been to increased risk of infection in dialysis. We sought to determine baseline associates of plasma cathelicidin in a healthy population and to determine the effects of ergocalciferol ($D_2$) treatment.

**Methods:** We enrolled 100 healthy subjects free of chronic kidney disease or active infection at a university clinical research center. Baseline levels of 25-OH vitamin D were assessed. Subjects with levels ≤ 32 ng/ml were treated with 50,000 IU ergocalciferol x 5 doses (250,000 IU total) and returned for follow up laboratory studies (n=35). Levels of cathelicidin, 25-OH $D_2$, 25-OH $D_3$ and $1,25(OH)_2$ D, vitamin D binding protein, albumin, white blood count (WBC) with differential, and total and regulatory T-cells were assessed before and after treatment.

**Results:** Baseline plasma cathelicidin (total hCAP18) was associated with 25-OH D levels (R=0.25, p=0.015), which was largely driven by levels of 25-OH $D_3$ among individuals with total 25-OH D levels ≤ 32 ng/ml. This association remained significant and stable despite adjustment for demographic and laboratory factors. No association with 25-OH $D_2$ levels was observed. Additional predictors of cathelicidin included total WBC (R=0.32, p=0.002), absolute lymphocyte count (R=0.33, p=0.001), absolute neutrophil count (R=0.25, p=0.0.18), total T cell count (R=0.31, p=0.004), and regulatory T cell count (R=-0.25, p=0.23). Ergocalciferol treatment increased 25-OH $D_2$ (by 30 ng/ml, p<0.0001) and $1,25(OH)_2$ D (by 12 pg/ml, p<0.001) decreased 25-OH $D_3$ levels. No change in calcium, phosphorus, PTH, T-cells or plasma cathelcidin was observed with treatment.

**Conclusions:** Despite robust baseline associations with 25-OH $D_3$ levels, treatment with high-dose $D_2$ failed to augment plasma cathelicidin in a healthy population. The form of vitamin D ($D_2$ vs $D_3$) may be relevant to studies of its immunologic effects.

*Funding:* NIDDK Support

## SA-PO591

**Loop Diuretics Are Associated with Higher PTH in Patients with Normal GFR** Kristin M. Corapi,[1] Gearoid M. McMahon,[2] Julia Beth Wenger,[1] Julian L. Seifter,[2] Ishir Bhan.[1] *[1]Renal, Massachusetts General Hospital, Boston, MA; [2]Renal, Brigham and Women's Hospital, Boston, MA.*

**Background:** Loop diuretics are known to increase urinary calcium excretion and are associated with elevated PTH in patients with chronic kidney disease. We assessed whether loop diuretics are linked to higher PTH levels in patients with normal renal function.

**Methods:** We studied adult participants (≥18 years) from NHANES 2003-2004 and 2005-2006. Subjects with an eGFR<60ml/min were excluded. The primary outcome, PTH, was log-transformed due to non-normality. Univariate analysis was done using a chi square test or simple linear regression to identify significant predictors of PTH. The association between the use of loop diuretics and PTH was tested using a multi-variable linear regression model adjusted for known covariates after application of appropriate weights.

**Results:** 8,875 participants were eligible for inclusion, 157 of whom were using loop diuretics. Loop users and non-users did not differ in season of exam, serum calcium, or serum phosphorus. Loop users were more likely to be older, African American, female, and have a higher BMI. Loop users also had a lower mean 25-(OH) vitamin D and albumin, but higher alkaline phosphatase and uric acid. Median PTH was significantly higher in loop users than in non-users, 51.1 vs 37.3 pg/mL (p<0.001). In the multivariable-adjusted model, the use of loop diuretics was associated with significantly higher log PTH levels (p=0.002). After adjusting for other predictors, use of a loop diuretic was associated with a 12% higher median PTH value compared to non-loop users.

| Model | β | *p* value |
|---|---|---|
| **Univariate Model** | | |
| Loop diuretic | 0.036 | <0.001 |
| **Multivariable Model*** | | |
| Loop diuretic | 0.115 | 0.002 |
| * = adjusted for age, gender, race, BMI, uric acid, phosphorus, calcium, 25(OH) vitamin D, albumin, alkaline phosphatase | | |

**Conclusions:** In patients with normal renal function, loop diuretics are independently associated with higher PTH levels. Given the known adverse consequences of elevated PTH, it is important that providers be aware of this relationship.

## SA-PO592

**Bone Mineral Metabolism Parameters and Microalbuminuria in the National Health and Nutrition Examination Surveys 1999-2010** Timothy Ellam, Sheila E. Francis, Timothy Ja Chico. *Dept of Cardiovascular Science, Univ of Sheffield, United Kingdom.*

**Background:** Greater levels of serum phosphorus, parathyroid hormone (PTH) and alkaline phosphatase (ALP) are independent predictors of adverse cardiovascular outcomes in the presence or absence of kidney disease. Endothelial dysfunction has been implicated as a causal mechanism. We hypothesized that these factors are associated with higher-normal albuminuria and the likelihood of microalbuminuria, a cardiovascular risk marker considered to reflect endothelial dysfunction.

**Methods:** We examined associations of the above bone metabolic parameters with urine albumin:creatinine ratio (ACR), urine albumin concentration and likelihood of microalbuminuria (ACR>17mg/g in men and >25mg/g in women). Linear and logistic regression analyses were performed in the NHANES 1999-2010 cohorts (N=23,036 adults with eGFR≥60 and without macroalbuminuria) with adjustment for covariates including age, gender, BMI, race, blood pressure, diabetes, poverty:income ratio, eGFR, serum lipids, hemoglobin, and CRP. Bone-specific ALP and PTH measurements were available in NHANES 1999-2004 (N=10,341) and 2001-2006 (N=6,923) respectively.

**Results:** Greater serum phosphorus or estimated dietary phosphorus intake (whether absolute, indexed to body weight, calorie intake or calcium intake) did not predict higher log ACR or likelihood of microalbuminuria. Serum total ALP, bone-specific ALP and PTH were independent predictors of higher log ACR and log urine albumin (p<0.001 for all), though increased likelihoods of microalbuminuria with increasing levels of these parameters were non-significant following full covariate adjustment.

| | Fuly adjusted coefficients for prediction of LnACR | | |
|---|---|---|---|
| | β quintile 5 vs 1 | Corresponding fold-change in ACR | p |
| ALP | 0.07 | 1.08 | 0.002 |
| Bone-ALP | 0.18 | 1.20 | <0.001 |
| PTH | 0.16 | 1.18 | <0.001 |

**Conclusions:** Higher levels of ALP, Bone-ALP and PTH are independently associated with greater urinary albumin excretion in a representative sample of the general population. Whether targeting these parameters improves cardiovascular health remains to be determined.

## SA-PO593

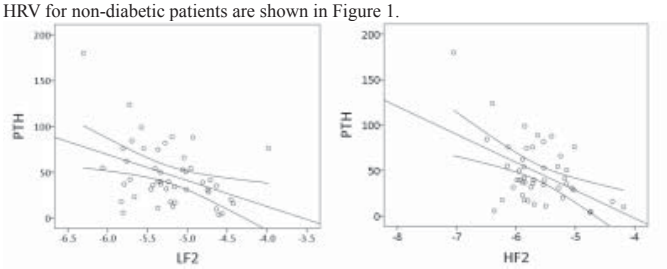
**Parathyroid Hormone and Heart Rate Variability in Hemodialysis Patients** Dimitrios J. Poulikakos,[1,2] Debasish Banerjee,[1,2] Marek Malik.[1] *[1]Cardiovascular Sciences Research Centre, St. George's Univ of London, London, United Kingdom; [2]Renal and Transplantation, St. George's Hospital NHS Trust, London, United Kingdom.*

**Background:** Depressed Heart Rate Variability (HRV) reflects abnormal cardiac autonomic regulation and has been linked with increased cardiovascular risk and sudden cardiac death. High parathyroid hormone levels have been associated with sudden cardiac death. We aimed to investigate the association between HRV indices and parathyroid hormone (PTH) in hemodialysis (HD) patients.

**Methods:** Continuous Holter electrocardiograms were obtained during HD and repeated 5 times at 2-weeks intervals in 80 stable HD patients. The high- (HF) and low-frequency (LF) components of HRV in absolute values were calculated every 5 minutes and averaged during the first and last hour of the recordings denoted with (1) and (2) respectively. Logarithmic transformation was applied to normalize the distribution of data. Pre-HD intact PTH, corrected calcium (Ca) and phosphate (P) levels were measured before the first recording.

**Results:** Data were available for 75 subjects (Age 60±15,32% females, 37% diabetics, intact PTH=44±32 pmol/L, Ca=2.3±0.2mmol/l, P=1.6±0.4mmol/l). All HRV indices showed

intrasubject stability. Diabetics had lower LF2 (-5.5±0.5 vs. -5.2±0.5 p=0.012). In non-diabetics PTH correlated negatively with all HRV indices (LF1 r= -0.348, p =0.016, LF2 r= -0.398 p = 0.006, HF1 r= -0.428 p 0.003, HF2 r= -0.491, p=0.000) and P correlated positively with LF1 (r=-0.415, p=0.005), HF1 (r=-0.437, p=0.003) and HF2 -0.319, p=0.033). There was no association between PTH and HRV indices in diabetics. Scatterplots for PTH and HRV for non-diabetic patients are shown in Figure 1.




**Conclusions:** High PTH and phosphate were associated with depressed HRV in non-diabetic HD patients. Prospective studies are necessary to further evaluate the role of mineral abnormalities in autonomic imbalance in HD patients.

## SA-PO594

**mTOR Is Central to Parathyroid Cell Proliferation in Experimental Secondary Hyperparathyroidism** Oded Volovelsky, Gili Cohen, Gilad Wasserman, Tomer Meir, Oded Meyuhas, Justin Silver, Tally Naveh-Many. *Hadassah Hebrew Univ, Jerusalem, Israel.*

**Background:** Parathyroid (PT) cell proliferation is central to secondary hyperparathyroidism (SHP). The signal transduction pathways for parathyroid cell proliferation are not defined. We demonstrate the role of mTOR in parathyroid cell proliferation of SHP.

**Methods:** We induced SHP by a low calcium diet or adenine high Pi CKD. PT cell proliferation was measured by BrdU and Ki67 staining of PT sections. Western blots of mTOR pathway activated PT proteins were performed. The mTOR inhibitor rapamycin was injected ip or added to PT glands in culture. Knock-in mice, whose ribosomal protein S6 (rpS6) is unphosphorylatable due to substitution of all five phosphorylatable serines to alanines ($rpS6^{P-/-}$) and wild type ($rpS6^{P+/+}$) mice were fed a calcium deficient diet.

**Results:** PT cell proliferation was increased in both models of SHP. Significantly, the mTOR pathway was activated in the PTs of the SHP rats, as measured by increased phosphorylation of components along this pathway. To examine the role of mTOR in SHP we injected the mTOR inhibitor rapamycin to the SHP rats. Rapamycin decreased PT mTOR activation and prevented PT cell proliferation in both models of SHP. Rapamycin decreased PT cell proliferation in CKD rats when injected at the start of the adenine diet (prophylactic effect) or later, to correct established PT cell proliferation. To verify whether the effect of mTOR inhibition is direct, PT glands from CKD rats were maintained in organ culture with and without rapamycin. Remarkably, proliferation continued in culture as measured by BrdU incorporation, and was inhibited by rapamycin, as observed in vivo, indicating a direct effect of the inhibitor on PT cell proliferation. rpS6 kinase is a downstream target of the mTOR pathway. Knock-in $rpS6^{P-/-}$ and wild type mice were fed a calcium deficient diet. Hypocalcemia led to the expected increase in serum PTH in $rpS6^{P+/+}$ wild type mice but less so in the $rpS6^{P-/-}$ mice, emphasizing the role of mTOR in SHP.

**Conclusions:** This is the first demonstration that mTOR is a significant regulator of PT cell proliferation and it exerts this effect, at least partially, through rpS6 phosphorylation.

## SA-PO595

**Elevated Sclerostin Levels Decrease Markedly after Successful Renal Transplantation and Correlate with Renal Function and Improvement of Persistent Hyperparathyroidism** Marco Bonani,[1] Daniel Rodríguez Gutiérrez,[1] Nilufar Mohebbi,[1] Thomas Fehr,[1] Jens G. Brockmann,[2] Rudolf P. Wuthrich.[1] *[1]Div of Nephrology, Univ Hospital Zurich, Zurich, Switzerland; [2]Div of Visceral and Transplantation Surgery, Univ Hospital Zurich, Zurich, Switzerland.*

**Background:** Sclerostin is a soluble inhibitor of Wnt signaling which inhibits bone formation. Secreted by osteocytes in response to mechanical unloading it acts as an important regulator of bone formation and localized bone remodeling. Sclerostin levels increase along CKD progression and are particularly elevated in patients on dialysis. The dynamic changes of sclerostin before and after renal transplantation have not been investigated.

**Methods:** We analyzed serum sclerostin levels in 42 patients (mean age 49±14years, 64%male, BMI 25.7±6.0kg/m$^2$) before, and 0.5, 3, 6 and 12 months after kidney transplantation. We also explored the correlation of sclerostin with relevant parameters of CKD-MBD.

**Results:** Sclerostin levels were significantly higher in patients with CKD stage 5 before transplantation (n=42) compared to control patients with normal renal function (n=96) (61.8±32.3 vs 28.4±10.9pmol/l;p<0.001). Within 14 days after transplantation and in parallel with the improvement of renal function, sclerostin values decreased markedly to levels slightly below the normal range (21.0±14.7). Sclerostin levels amounted to 21.0±12.5 and 23.8±14.9 after 3 and 6 months, and increased to 28.0±16.8pmol/l after 12 months. Sclerostin levels were similar in male and female, and in diabetic and non-diabetic patients at all timepoints. After transplantation, sclerostin levels correlated negatively with PTH (r=-0.170,p=0.033) and positively with age (r=0.311,p=0.045), but there was no significant correlation between sclerostin and BMI, eGFR, serum calcium, phosphate, magnesium, 25OH- and 1,25$(OH)_2$-vitamin D. Furthermore, sclerostin levels did not correlate with pre- and post-transplant bone mineral density at the hip and lumbar spine.

**Conclusions:** The marked reduction of elevated serum sclerostin levels shortly after kidney transplantation reflects the improvement of renal function and may contribute to the normalization of bone health after renal transplantation.

## SA-PO596

**Phosphaturic Hormones Predict the Progression of Anemia in Patients with CKD** Takayuki Hamano,[1] Chikako Nakano,[2] Naohiko Fujii,[3] Yoshitsugu Obi,[2] Isao Matsui,[2] Yoshitaka Isaka,[2] Yoshiharu Tsubakihara.[1] *[1]Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [3]Center for Clinical Epidemiology and Biostatistics, Univ of Pennsylvania Perelman School of Medicine, Philadelphia, PA.*

**Background:** It was reported that 25-hydroxyvtiamin D (25D) and 1,25-dihydroxyvitamin D (1,25D) levels are associated with severity of anemia in patients with CKD. However, it remains elucidated which MBD markers predict the progression of anemia in this population.

**Methods:** In the OVIDS-CKD study, we prospectively followed temporal change of hemoglobin (hgb) levels. At baseline, we measured 6 MBD markers including intact FGF23, 1-84 PTH, 25D, 1,25D, serum calcium, and phosphate. Outcome of interest is the slope of hgb. We followed the patients until the start of ESA therapy or the end of observation period. We employed a linear mixed effects model with hgb as a time-dependent outcome variable. The interaction term time*each MBD marker was entered into the model to examine if these markers modify the relationship between time and hgb (hgb slope). Since, we have already reported that FGF23 and 25D predict renal outcome (CJASN 2012), eGFR was adjusted as a time-dependent covariate in this model. Only for patients with hgb>10.5 and not receiving ESA at baseline, secondary outcome was defined as time to hgb<10.5 or the start of ESA therapy.

**Results:** We enrolled 738 Japanese predialysis outpatients. Cox models revealed that out of 6 makers only 25D and FGF23 levels predicted the time to the secondary outcome, in addition to prior CVD, proteinuria, baseline hgb, and eGFR. (adjusted HR per unit change of log FGF23 and 10 ng/mL of 25D: 1.65 [1.15-2.36] and 0.66 [0.46-0.96], respectively). However, this result was confounded by time-dependent eGFR. Mixed effects model adjusting for time-dependent eGFR revealed that FGF23 and PTH levels were associated with faster decline of hgb (-0.053 [-0.091~-0.016]g/dL/year and -0.044 [-0.079~-0.008] g/dL/year per SD increase, respectively).

**Conclusions:** Two phosphatonins predict the progression of anemia.

## SA-PO597

**The Association between Handgrip Strength and Vitamin D Status in Maintenance Hemodialysis Patient** Keita Kimura,[1] Akio Nakashima,[2] Yasuo Kimura,[1] Keitaro Yokoyama,[2] Takashi Yokoo,[2] Mitsuyoshi Urashima.[3] *[1]Shikashiwa Clinic, Chiba, Japan; [2]Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan; [3]Div of Molecular Epidemiology, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Sarcopenia is highly prevalent in patient undergoing hemodialysis. Assessment and prevention of this disorder are important, because it relates to impairment of activity of daily life, falling, and even to poor prognosis. Hand grip strength (HGS) is simple and reliable method for evaluating muscle function. In hemodialysis patient, HGS correlates with lean body mass and nutritional status. Vitamin D is thought to play a role in sarcopenia. In elderly people, 25-hydroxy vitamin D [25(OH)D] positively associates with muscle strength. However, in hemodialysis patient, the association between muscle function and vitamin D is not fully explored.

**Methods:** HGS was measured in 129 patients on maintenance hemodialysis. Laboratory data including serum 25(OH) D, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], calcium, phosphorus, alkaline phosphatase, intact-PTH, albumin, and hemoglobin were measured. Clinical characteristics of the patients were also obtained. The association of HGS with these factors was analyzed.

**Results:** Average serum 25(OH)D concentration was 19.4 ng/ml. Higher HGS was associated with younger age, male gender, higher hemoglobin, lower alkaline phosphatase and higher 25(OH) D. Neither 1,25$(OH)_2$D nor use of vitamin D were not associated. After adjusting by the potential confounding factors, the association between HGS and 25(OH) D was still evident.

| | Regression Coefficinet | Standard Error | P-value | 95% Confidence Interval |
|---|---|---|---|---|
| 25(OH)D | 0.30 | 0.09 | 0.001 | 0.13 to 0.44 |
| Age | -0.162 | 0.07 | 0.018 | -0.30 to -0.28 |
| alkaline phosphatase | -0.09 | 0.004 | 0.07 | -0.02 to 0.001 |
| Hemoglobin | 1.42 | 0.61 | 0.021 | 0.21 to 2.62 |

**Conclusions:** Our results clearly showed the association between 25(OH)D and HGS in hemodialysis patient. Considering high prevalence of Vitamin D deficiency and sarcopenia in this population, our results will provide the rationale for planning the controlled study using vitamin D for improving sarcopenia.

**SA-PO598**

**25-Hydroxy Vitamin D Deficiency Is Common in Patients with Chronic Kidney Disease in a Scottish Community Cohort: Results from the Triple A Kidney Project** Shona Methven,[1] Alan G. Jardine,[2] Mark S. MacGregor.[3] *[1]School of Clinical Sciences, Univ of Bristol, United Kingdom; [2]Institute of Cardiovascular and Medical Sciences, Univ of Glasgow, United Kingdom; [3]Renal Unit, Univ Hospital, Crosshouse, Kilmarnock, United Kingdom.*

**Background:** There is increasing awareness of the importance of chronic kidney disease-mineral bone disorders (CKD-MBD), but little is known about primary vitamin D deficiency in patients with CKD. We describe the prevalence of 25-hydroxy vitamin D (25-OHVitD) deficiency in patients with CKD in a geographical area with low levels of sunshine.

**Methods:** Participants with a diagnosis of CKD stage 3 were recruited from 7 primary care facilities across Ayrshire, Scotland in the Triple A Kidney Project. Detailed baseline clinical and laboratory assessment was undertaken, including measurement of 25-OHVitD and parathyroid hormone (PTH) using an immunoassay in all participants (Abbott Architect).

**Results:** Four hundred and eleven participants were recruited, mean age 70.6±9.6 years, 59% female, 99.5% white, 20% diabetic and median eGFR 54 (IQR 44–61) ml/min/1.73m$^2$. Serum adjusted calcium, phosphate and alkaline phosphatase levels were normal (2.2(±0.1) mmol/L; 0.9(±0.2)mmol/L and 78(±29)µg/L respectively). However, mean 25-OHVitD was 38 (±22)nmol/L, with 40.3% having insufficient 25-OHVitD levels (25-49nmol/L), 20.2 being deficient (14-24nmol/L) and 13.7% having undetectable 25-OHVitD (<14nmol/L). Mean PTH was 8.7 (±4.9)pmol/L (upper limit of normal [ULN] 7.5pmol/L) and 47.2% of the total cohort had elevated PTH, rising to 67.1% in those with CKD Stage 3B. Mild hyperparathyroidism was not related to severity of kidney disease (p=0.332), but PTH >3 times ULN was more common in Stage 3B (p<0.001).

**Conclusions:** Primary 25-OHVitD deficiency and mild secondary hyperparathyroidism are very common in those with CKD stage 3 in Scotland, presumably related to low levels of sunshine and diet. The degree of 25-OHVitD deficiency did not vary with renal function, whereas secondary hyperparathyroidism was more severe with poorer renal function. Vitamin D deficiency may predispose to more severe CKD-MBD in the future. Intervention studies are required to assess vitamin D replacement in this group.

*Funding:* Pharmaceutical Company Support - Unrestricted educational grant from Bristoll Myers Squibb

**SA-PO599**

**Dialysis Patients with Severe Secondary Hyperparathyroidism: A Homogeneous Population?** Emmanuelle Laurain,[1,2] Michele Kessler,[1] Luc Frimat.[1] *[1]Nephrology, Nancy Universitary Hospital, Vandoeuvre les Nancy, France; [2]Epidémiology, Nancy Universitary Hospital, Vandoeuvre les Nancy, France.*

**Background:** Diagnosis of Secondary hyperparathyroidism (SHPT) remains complex, and its management is not yet well standardized. One hypothesis that could explain these difficulties is that dialysis population with SHPT would not be homogeneous due to various pathophysiological types. The aim of this study was to identify subgroups in CKD patients with incident SHPT on the basis of clinical, biological and therapeutic data routinely available to the clinician.

**Methods:** EPHEYL is a prospective, multicenter, pharmacoepidemiological study including chronic dialysis patients (≥ 3 months) recently diagnosed with SHPT [serum parathyroid hormone (PTH) level ≥ 500 pg/mL or cinacalcet prescription or parathyroidectomy]. Multivariate analyses using multiple correspondence analysis and ascendant hierarchical clustering on clinico-biological (symptoms, PTH, phosphorus, calcemia and alkaline phosphatase levels) and therapeutic characteristics of SHPT (cinacalcet, vitamin D, calcium, or non-calcic phosphate binder) were performed to identify subgroups.

**Results:** 305 patients (261 patients with incident PTH ≥ 500 pg/mL, 44 patients with cinacalcet initiation) were included from December 1st, 2009 and May 30th, 2012. Their mean age was 67+15 years, and 60% were men. They had been on maintenance dialysis for a mean period of 28+35 months (92% on hemodialysis, 8% on peritoneal dialysis). Four subgroups of SHPT patients were identified: 1/ "intermediate patients" with hyperphosphatemia without hypocalcemia (n=113); 2/ younger patients with severe cardiovascular co-morbidities, hyperphosphatemia and hypocalcemia despite SHPT polymedication (n=73); 3/ elderly patients with few cardiovascular co-morbidities, controlled phospho-calcium balance, and few pharmacological treatments (n=75); 4/ patients who initiated cinacalcet despite a lower PTH (n=43). The quality criterion of the model had a threshold of 14 (> 2) suggesting a good classification.

**Conclusions:** Among dialysis patients, SHPT affects a very heterogeneous population at the time of SHPT diagnosis. The therapeutic management and issues seem to vary according to each subgroup.

*Funding:* Government Support - Non-U.S.

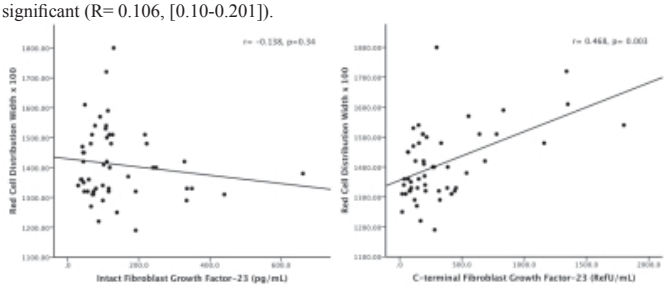
**SA-PO600**

**RDW Is Associated with cFGF23 and Not with iFGF23** Carlo A. Gaillard,[1] Fenna Breda, Van,[1] Mireille E. Emans,[2] Karien Van der Putten,[3] Branko Braam,[4] Frans J. van Ittersum,[1] Marc G. Vervloet.[1] *[1]Nephrology, UMCG/ VUMC, Netherlands; [2]Cardiology, UMCU, Netherlands; [3]Internal Medicine, TerGooiziekenhuis, Netherlands; [4]Medicine, Univ of Alberta, Canada.*

**Background:** Fibroblast growth factor-23 (FGF23) and red cell distribution width (RDW) are associated with poor clinical outcomes. RDW is associated with iron deficiency. Recent studies suggest a mechanistic link between iron deficiency and FGF23. In this post-hoc analysis we hypothesized that in CKD patients with heart and renal failure, iron metabolism links FGF23 and RDW.

**Methods:** Associations between levels of iFGF23 (intact), cFGF23 (c-terminal) and RDW were analyzed in 54 participants of the EPOCARES study (PMID 20383871). Two assays detecting FGF23 were used: one detected only iFGF23, the other detected free c-terminal FGF23 fragments and iFGF23.

**Results:** cFGF23 and RDW (correlation coefficient (r)= 0.468, p= 0.003) were associated, iFGF23 and RDW were not (r= -0.069, p= 0.627). Yet, iFGF23 and cFGF23 were both associated with cystatin C (r= 0.632, p < 0.001 and r= 0.414, p=0.02). Multivariable linear regression analysis, that included PTH, phosphate, BMI, smoking and cystatin C did not alter the strength of the association between cFGF23 and RDW (regression coefficient (R)= 0.163, [0.081-0.246]). After adjusting for markers of iron metabolism, this association was attenuated (R= 0.147, [0.061-0.232]). In the full adjusted model correcting for both markers of inflammation and iron metabolism the association remained statistically significant (R= 0.106, [0.10-0.201]).




**Conclusions:** cFGF23 and not iFGF23 levels were associated with RDW levels in CKD and CHF patients. This suggests a connection between c-terminal fragments and RDW, possibly independently of iron status and inflammation. Studies are needed that unravel what mechanisms are responsible for this association and whether this explains the link of RDW with outcome.

*Funding:* Other NIH Support - Netherlands Heart Foundation, Pharmaceutical Company Support - Roche

**SA-PO601**

**Klotho, Proteinuria and Acidosis in CKD Patients** Solenne Pelletier,[1] Laurence Dubourg,[2] Jocelyne Drai,[3] Sandrine Lemoine,[4] Aoumeur Hadj-aissa,[2] Denis Fouque.[1] *[1]Nephrology, Centre Hospitalier Lyon Sud, Pierre-Benite, France; [2]Explorations Fonctionnelles Renales, Hopital Edouard Herriot, Lyon, France; [3]Biology, Centre Hospitalier Lyon Sud, Pierre-Benite, France; [4]Nephrology, Hopital Edouard Herriot, Lyon, France.*

**Background:** a-Klotho(Kl) is a recently discovered FGF23 cofactor involved in phosphorus metabolism. Kl overexpression is associated with extended longevity, whereas Kl-KO animals die prematurely. We included 60 chronic kidney disease (CKD) stage 1-5 patients and studied relationships between renal function, proteinuria, bone biomarkers, acid-base status and serum a-Kl concentration.

**Methods:** Blood was drawn after overnight fast for PTH, FGF-23, calcium, phosphorus, 25OH vitamin D, bone specific alkaline phosphatase (bSAP). Serum a-Kl was measured by Elisa (IBL, Japan, normal values 239-1266 pg/mL); proteinuria was obtained from 24hr urine. Renal function was assessed by urinary inulin clearance.

**Results:** We found positive relationships between a-Kl and FGF23 (p<0.005, r=0.36), serum bicarbonate (p=0.05, r=0.26), and an inverse relationship with proteinuria (p=0.03, r=-0.32). No relationship was found between a-Kl and renal function, serum PTH, vitamin D, bSAP, calcium and phosphorus. Multivariate analysis including proteinuria, FGF23 and bicarbonate showed that FGF23 and bicarbonate were significantly associated with serum a-Kl (p<0.01).

**Conclusions:** The present findings show for the first time a potential link between 2 important amenable conditions occurring during CKD: proteinuria and acidosis. We suggest including these parameters during future Klotho studies in CKD patients. Whether correcting acidosis may improve serum a-Kl deserves further research.

**SA-PO602**

**Plasma Exchange Induces Vitamin D Deficiency** Thomas F. Hiemstra,[1] Alina L. Casian,[2] David R.W. Jayne.[2] *[1]Dept of Medicine, Univ of Cambridge; [2]Vasculitis and Lupus Clinic, Addenbrooke's Hospital.*

**Background:** Plasma exchange (PEX) is a widely used immune-modulating therapy for diseases mediated by antibodies or pathogenic proteins, or for transplant desensitisation. Vitamin D (25OHD) deficiency is associated with skeletal and extra-skeletal pathology, is

permissive to activity of a number of autoimmune diseases, and may be associated with poorer renal allograft survival. We asked whether PEX would induce vitamin D deficiency through removal of its carrier, vitamin D binding protein (DBP).

**Methods:** We performed a prospective cohort study of patients receiving plasma exchange at Addenbrooke's Hospital, Cambridge. Vitamin D metabolites, DBP and biochemical parameters were measured before the start and after every plasma exchange treatment, as well as 7 and 28 days after completion of PEX.

**Results:** 11 Caucasian patients (7 males) aged 59 ± 13 years received 5.5 ± 0.9 PEX treatments, for ANCA-associated vasculitis (n=5), myasthenia gravis (n=3), paraneoplastic neuropathy (n=2) and voltage-gated potassium channel antibody-mediated encephalopathy (n=1). Baseline estimated glomerular filtration rate was 56.9 ± 39.5 ml/min/1.73m$^2$, and 5 patients had chronic kidney disease stage 3 or worse.Baseline 25(OH)D levels were 50.6 ± 30.1 nmol/L. PEX significantly reduced 25OHD levels after 5 treatments to 22 ± 9.4nmol/L (p = 0.0017), and vitamin D remained low 7 days (26.4 ± 9.8nmol/L, p=0.02) and 28 days (30.8 ± 15.5, p=0.048) after cessation of PEX. $1_\alpha25(OH)_2D$ levels reduced from 103 ± 52pmol/L to 42 ± 4pmol/L (p = 0.003) with PEX, but had returned to baseline levels after 7 days. PEX also significantly reduced DBP levels from 206.5 ± 64.7µg/mL to 98.5 ± 34µg/mL (p = 0.0001), but levels had returned to baseline after 7 days. PEX significantly reduced corrected Calcium from 2.23 ± 0.12 mmol/L to 1.98 ± 0.08 mmol/L (p = 0.0007), but did not alter phosphate. Analyses of plasma effluent confirmed removal of DBP, vitamin D and PTH by PEX.

**Conclusions:** We identified sustained reduction in 25OHD and acute reversible reduction in $1_\alpha25(OH)_2D$ by a typical course of PEX, likely through its removal with DBP in plasma effluent. PEX-treated patients should receive vitamin D supplementation.

## SA-PO603

### Serum Phosphorus and Fibroblast Growth Factor 23 Do Not Modify the Association of Angiotensin II Inhibition with Outcomes in Subjects with Advanced Chronic Kidney Disease

,[1] Kristen L. Jablonski,[1] Jessica B. Kendrick,[1] Alfred K. Cheung,[2,3] Gerard John Smits,[1] Michel Chonchol.[1] *[1]Div of Renal Diseases and Hypertension, Univ of Colorado Denver, Aurora, CO; [2]VA Salt Lake City Healthcare System, Salt Lake City, UT; [3]Univ of Utah, Salt Lake City, UT.*

**Background:** In chronic kidney disease (CKD), there is a fibroblast growth factor 23 (FGF23) -mediated increase in fractional excretion of phosphorus to compensate for decreased kidney function. Higher levels of phosphorus and FGF23 are associated with kidney disease progression and mortality in advanced chronic kidney disease. However, it is unknown whether these associations differ by degree of hyperphosphatemia or FGF23 level.

**Methods:** We studied the effects of ACE/ARB use in 1753 subjects with advanced CKD (1099 CKD, eGFR=18±6 ml/min/1.73m$^2$; and 654 ESRD) who participated in the Homocysteine in Kidney and End Stage Renal Disease study. Outcome measures were dialysis initiation for those with CKD and death among the whole cohort. Cox regression models adjusted for important confounding variables and propensity score analysis was used to assess the association of ACE/ARB use with dialysis initiation and death. Analyses were stratified by serum levels of phosphorus and FGF23.

**Results:** Average age was 66±12 years, 36% were black, and 870 (50%) were taking ACE/ARB. Over a mean follow-up of 3 years, there were 714 (41%) deaths and 615 patients (56%) initiated chronic dialysis. In adjusted analyses, all subjects treated with ACE/ARB had a significantly lower risk of death [HR 0.81 (95% CI, 0.70-0.95; p=0.007)] and those with CKD had a lower risk of dialysis initiation [HR 0.86 (95% CI, 0.73-0.97; p=0.03)]. Neither serum phosphorus nor FGF23 levels significantly modified the association of ACE/ARB use with each outcome (p interaction 0.4 for death and p interaction 0.7 for dialysis initiation).

**Conclusions:** ACE/ARB use is associated with a reduced risk of kidney disease progression and death even when simultaneously accompanied by higher serum phosphorus and FGF23.

*Funding:* NIDDK Support

## SA-PO604

### Signaling of FGF-23-Klotho under Normal and Hyperglycemic Conditions

Sudha P. Chennasamudram, Ruchi Singh, Tetyana L. Vasylyeva. *Pediatrics, Texas Tech Univ Health Sciences Center, Amarillo, TX.*

**Background:** Fibroblast growth factor 23 (FGF23) is a protein produced by osteocytes and is involved in reabsorption of phosphorus in kidney. FGF23 binds to FGF receptors in the presence of a co-receptor called klotho to form a ternary complex in the distal tubule. This ternary complex acts on the proximal tubules to down-regulate tubular reabsorption of phosphate. In chronic kidney disease (CKD) patients, elevated levels of FGF23 have been observed leading to higher mortality rates. Diabetes is one of the leading causes of progression of CKD. The signaling pathways of FGF23-klotho under hyperglycemic conditions are poorly understood. Mechanisms involved in FGF23 pathways under hyperglycemic conditions could further provide information on the role played by FGF23 in the progression of CKD. The study objective is to investigate the effects of FGF-23-klotho signaling under hyperglycemic conditions.

**Methods:** Human proximal epithelial cells (HK2) and glomerular endothelial cells (GEC) were cultured and maintained at 37° C in a 5% CO2 incubator. Cells were treated with different concentrations (10, 25, 50 and 100 ng/mL) of FGF23 in the presence and absence of Klotho under normal (5mM) and hyperglycemic (25 mM) conditions. Formation of reactive oxygen species (ROS) in HK2 cells was investigated by flow cytometry. Effects of FGF23 on apoptosis in glomerular endothelial cells were also studied.

**Results:** Treatment of HK2 cells with 100 ng/mL of FGF23 under hyperglycemic conditions increased the percentage of ROS significantly compared to the cells under normal glucose. When klotho was added, there was a decrease in the percentage of ROS induced by FGF23 under hyperglycemic conditions. There was no change in the percentage of apoptotic cells before and after treatment of FGF-23 under hyperglycemic conditions in HK2 cells. But the percentage of apoptotic cells in GEC after 24 hours of FGF23 treatment under hyperglycemic conditions was higher compared to the cells under normal glucose.

**Conclusions:** High concentrations of FGF23 under hyperglycemic conditions are toxic to glomerular endothelial cells. The stress induced by FGF23 under hyperglycemic conditions is ameliorated by klotho in HK2 cells.

*Funding:* Pharmaceutical Company Support - Sanofi

## SA-PO605

### Klotho and Human Coronary Heart Disease

Juan F. Navarro-Gonzalez,[1,2] Javier Donate,[2] Mercedes Muros,[3] Horacio Perez Hernandez,[4] Violeta Cazaña,[2] Javier Garcia Perez,[1] Carmen Mora.[2] *[1]Nephrology Service, Univ Hospital Ntra. Sra. de Candelaria, Tenerife, Spain; [2]Research Unit, Univ Hospital Ntra. Sra. de Candelaria, Tenerife, Spain; [3]Clinical Analysis Service, Univ Hospital Ntra. Sra. de Candelaria, Tenerife, Spain; [4]Cardiology Service, Univ Hospital Ntra. Sra. de Candelaria, Tenerife, Spain.*

**Background:** Klotho is involved in maintenance of vascular health. We aimed to analyze the relationship between Klotho and human coronary artery disease (CAD).

**Methods:** Soluble klotho were measured in 371 patients underwent coronary angiography. Klotho gene expression was analyzed in thoracic aorta specimens from 70 patients underwent cardiac surgery.

**Results:** Soluble Klotho concentration was lower ($P < 0.001$) in patients with significant CAD (n = 233). The coronary stenosis severity index was significantly lower in patients with the higher soluble Klotho levels ($P < 0.001$). Multiple regression analysis showed that serum Klotho concentrations were inverse and significantly associated with CAD (adjusted $R^2 = 0.67$, $P < 0.001$). Multivariate logistic regression showed that risk factors for significant CAD included age, diabetes, smoking and inflammation, whereas serum Klotho levels were associated with a lower risk for CAD. Lower mRNA expression level of Klotho was observed in 46 patients with significant CAD, as compared with subjects without significant CAD ($P < 0.01$). Logistic regression analysis showed that *Klotho* expression was independently associated with lower risk for CAD.

**Conclusions:** Patients with significant CAD present lower concentrations of soluble Klotho, as well as reduced levels of *Klotho* gene expression in the vascular wall. Reduced serum Klotho concentrations and decreased vascular Klotho gene expression were significantly associated with the presence and severity of CAD, independently of established cardiovascular risk factors.

*Funding:* Government Support - Non-U.S.

## SA-PO606

### Bone FGF23 Expression Is Increased after Solid Organ Transplantation

R.C. Pereira,[1] Helena Liisa Valta,[2] Hannu J. Jalanko,[2] Isidro B. Salusky,[1] Navdeep Kaur Tumber,[1] Outi Makitie,[2] Katherine Wesseling-Perry.[1] *[1]Pediatrics, UCLA, Los Angeles, CA; [2]Univ of Helsinki, Helsinki, Finland.*

**Background:** Expression of FGF23 in bone cells is increased in patients with all stages of CKD and bone immunoreactive FGF23 correlates with skeletal mineralization in both pre-dialysis CKD and dialysis pts. Immunosuppressive agents, particularly glucocorticoids, have been associated with higher circulating FGF23 concentrations; however, the effect of organ transplantation of bone FGF23 values remains unknown.

**Methods:** Bone histomorphometry was performed in 12 pediatric pre-dialysis CKD pts and in 22 pediatric solid organ transplant (tx) pts. All tx pts were treated with a calcineurin inhibitor, prednisone, an antimetabolite and vitamin D3 (400 or 800 IU/d). No pts received calcitriol or other active vitamin D sterols. Five micron sections of non-decalcified bone were incubated with antihuman FGF-23 (225-244) followed by biotinylated 2o antibody, then developed with ABC complex/HRP and AEC kit (Vector). Number of FGF-23 expressing osteocytes was normalized by tissue area.

**Results:** GFR was lower in kidney transplant recipients than in recipients of other solid organs; however, bone FGF23 expression did not differ based on the type of organ received. Bone FGF23 expression was higher in patients with transplanted organs than in non-transplanted pre-dialysis CKD patients, despite higher GFRs in liver and heart allograft recipients. Considering all patients together, bone FGF23 immunoreactivity (FGF23/T.Ar) was inversely correlated with osteoid thickness ($r = -0.46$, $p<0.01$).

| | Liver or Heart Transplant (n=14) | Kidney transplant (n= 8) | Pre-dialysis CKD (n=12) | Normal range |
|---|---|---|---|---|
| Age (y) | 14.8 ± 0.8 | 16.9 ± 2.0 | 13 ± 1 | |
| Gender | 6M/8F | 4M/4F | 9M/3F | |
| Time since transplantation (y) | 4.8 ± 1.2 | 8.9 ± 2.2 | | |
| GFR (ml/min/1.73m$^2$) | 72.8 ± 4.4 * | 42.5 ± 3.8 | 54.7 ± 5.9 | |
| Calcium (mg/dl) | 10.0 ± 0.1 | 10.3 ± 0.1 ** | 9.4 ± 0.1 | 8.4-10.2 |
| Phosphorus (mg/dl) | 4.0 ± 0.1 | 3.5 ± 0.3 ** | 4.5 ± 0.3 | 2.2-4.7 |
| Alkaline Phosphatase (IU/l) | 138 (86, 216) | 108 (88, 146) ** | 229 (138, 343) | 31-103 |
| 25(OH)vitamin D (ng/ml) | 30 ± 3 | 31 ± 10 | 27 ± 2 | >30 |
| PTH (pg/ml) | 33 (29, 50) * | 73 (54, 79) | 68 (39, 155) | 8-73 |
| **Bone Histomorphometry** | | | | |
| Bone volume (BV/TV) (%) | 21.7 ± 2.2 | 24.8 ± 1.8 | 29.3 ± 2.6 | 8.9-34.4 |
| Osteoid volume (OV/BV) (%) | 2.0 ± 0.3 | 2.2 ± 0.5 | 7.2 ± 3.4 | 0.2-5.8 |
| Osteoid surface (OS/BS) (%) | 15.8 ± 2.7 | 16.4 ± 3.4 | 24.8 ± 5.4 | 4.3-31.7 |
| Osteoid thickness (O.Th) (um) | 7.0 ± 0.3 | 6.9 ± 0.6 | 11.4 ± 2.5 | 2.0-13.2 |
| Osteoid maturation time (OMT) (d) | 11 (10, 13) * | 15 (16, 18) | 9 (8, 19) | 1.2-11.5 |
| Mineralization lag time (MLT) (d) | 28 (22, 34) | 38 (33, 63) | 25 (13, 44) | 2.3-63.8 |
| Bone formation rate (BFR/BS) (um$^2$/um$^2$/y) | 10.1 (3.5, 26.4) | 9.8 (2.8, 18.4) | 14.7 (8.7, 28.5) | 8.0-73.4 |
| **Bone Immunoreactivity** | | | | |
| Bone FGF23/T.Ar (#/mm$^2$) | 2.0 ± 0.4<br>1.5 (1.1, 2.2) | 2.2 ± 0.7 **<br>1.5 (1.1, 2.8) | 0.8 ± 0.1<br>0.8 (0.4, 1.2) | 0.35 ± 0.1 |

* Indicates a difference between kidney transplant recipients and liver or heart transplant recipients (p<0.05)
**Indicates a difference between kidney transplant recipients and pre-dialysis CKD patients (p<0.05)

**Conclusions:** Therapy with immunosuppressive agents is associated with a marked increase in bone FGF23 immunoreactivity, regardless of the type of allograft received and despite, in the case of liver and heart allograft, higher GFR. The implications of these findings for bone biology and systemic complications remain to be evaluated.

*Funding:* NIDDK Support, Private Foundation Support

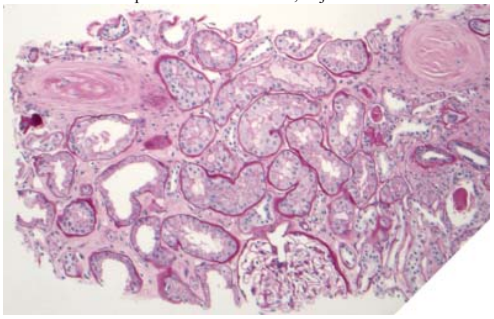
## SA-PO607

**Renal Sclerosing Peritubular Nodule – How Rare Is It?** Sajan Thomas. *Nephrology, Goldcoast Hospital, Goldcoast, Queensland, Australia.*

**Background:** Neurofibromatosis type 2 (NF2) is a rare autosomal dominant disorder. It affects about 1 in 25,000 people. Kidney involvement in NF2 has not been studied extensively. A relatively rare lesion, the renal sclerosing peritubular nodule (RSPN), was first described in a mother and 2 sons with NF2 who died and underwent autopsy in 1981.

**Methods:** A 53-year-old man with neurofibromatosis type II was referred for investigation and management of his deteriorating renal function. Physical examination revealed cutaneous manifestations of NF2. Serial blood tests showed declining renal function. Urine showed microscopic hematuria with no proteinuria. An ultrasound showed normal size kidneys and renal tract.A renal biopsy was performed.

**Results:** Light microscopy showed 3 out of 9 glomeruli globally sclerosed. Within the interstitium were paucicellular nodules, adjacent to tubules.



The nodules were lightly eosinophilic on H&E, PAS negative and argyrophilic on PASM.The nodules stained blue with Masson trichrome, resembling collagen. In the context of the clinical history, the morphology of the collagenous nodules was consistent with RSPN.

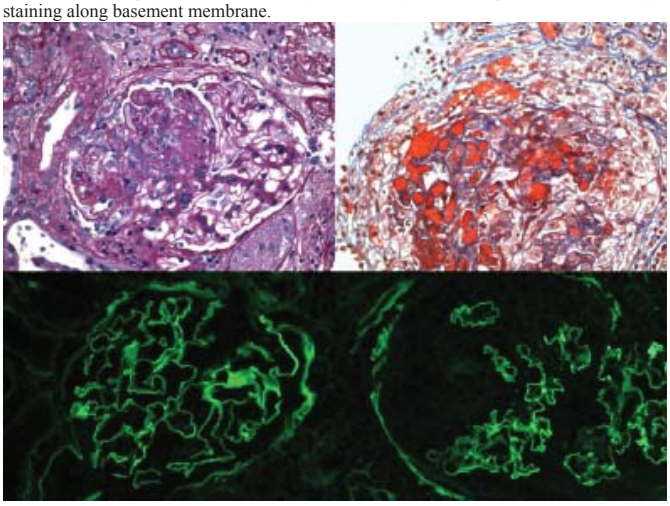
**Conclusions:** Our case represents a rare form of a hereditary renal abnormality in NF2. There are only two previous case reports. It has been thought that these nodules do not alter normal kidney function, even when they are extensive, however none of the previous patients survived into their fifth decade. With increasing frequency of NF2 patients surviving with advanced medical care, RSPN like lesions are more likely to be seen on their renal biopsy. Since these histologic lesions appear morphologically progressive early recognition , careful monitoring of the renal function and avoidance of potentially nephrotoxic agents is prudent.

## SA-PO608

**Necrotizing Crescentic Glomerulonephritis with Linear Anti-IgG Deposits in a Patient with History of Granulomatosis with Polyangiitis** Ninad D. Parekh, Elie El-Charabaty, Suzanne E. El Sayegh. *Dept of Medicine, Div of Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

**Background:** Granulometosis with polyangiitis (GPA) typically shows no or minimal immune deposits on immunofluorescence staining of renal biopsy and presence of large amount of immune deposits should prompt possibility of other etiologic process. It is essential to establish diagnosis to guide appropriate management therapy.

**Methods:** A 47-year-old Hispanic male presents with fatigue and lethargy. He has history of GPA, diabetes mellitus, hypertension, and hyperlipidemia. His medications include Zocor, lisinopril, azathioprine, and insulin. On exam, he is afebrile, vital signs stable. Remainder of physical exam is unremarkable. Laboratory data revealed creatinine 5.2 mg/dl (baseline 1.5 mg/dl) otherwise normal electrolytes. Urinalysis showed numerous dysmorphic red cells with red cell casts, and urine protien/creatinine ratio of 2.03 mg/mg. His serologies are all negative except anti-proteinase-3 (anti-pr3) antibody at very low titers. Kidney biopsy showed necrotizing crescentic glomerulonephritis with linear IgG staining along basement membrane.



The patient was treated with intravenous methyl prednisolone 1 gm/day for 3 days followed by oral prednisone at 1 mg/kg/day, and monthly intravenous cyclophosphamide 15mg/kg. Patient had with partial renal recovery at 2 months.

**Conclusions:** The presence of anti-pr3 antibody supports recurrence of GPA. However, linear IgG staining favours anti-GBM disease. Anti-GBM disease and GPA both are treated with prednisone and cyclophosphamide. However, anti-GBM disease is also treated with plamapheresis. In case of GPA, plasmapheresis is beneficial only in severe renal disease or pulmonary hemorrhage. Since our patient did not have detectable circulating anti-GBM antibody, decision was made not to proceed with plasmpheresis.

## SA-PO609

**ANCA Negative Renal Vasculitis in a Patient with Scleroderma: A Case Report** Sarah Khan, Bhupinder Sangha, Karina Sulaiman, Neville R. Dossabhoy, Zulqarnain Abro. *Nephrology, LSUHSC, Shreveport, LA.*

**Background:** There is no convincing evidence in the literature that a true vasculitis occurs in scleroderma. We describe a rare case of polyarteritis nodosa (PAN) vasculitis causing renal failure in a patient with localized scleroderma.

**Methods:** A 51 year old African American female with history of hypertension and syphilis presented with fatigue, severe dyspnea and significant swelling of hands and feet. She reported 6-8 months of polyarthralgia affecting the hands, feet and knees. She also reported a rash that developed on her trunk, back and legs 2 weeks prior. Physical exam was remarkable for lower extremity pitting edema and moderate sized, hypopigmented maculo-papular rash on her thorax and legs. Labs revealed Creatinine 8 mg/dL, BUN 68 mg/dL, Troponin 5.44, WBC 17,000, Hgb 8.7, CRP 3.6, ESR 32, Scl-70 Antibody +, ANA +, Hep B, C and HIV negative (neg), normal C3 & C4, ANCA neg, lupus anticoagulant neg. UA showed protein 500, RBC 50-75/hpf, WBC 20-30/hpf; urine drug screen neg. Echocardiogram showed large pericardial effusion with early tamponade, severe global hypokinesis and LVEF 20-25%. Patient was admitted to ICU and required emergent dialysis. A kidney biopsy showed inflammatory vasculitis associated with vascular thrombosis and focal renal parenchymal infarcts, suggestive of PAN. Renal angiogram showed moderate sized aneurysms consistent with PAN. A skin biopsy revealed increased hyalinized and thickened dermal collagen in superficial and deep dermis with diminished skin appendages, consistent with scleroderma. Patient was started on pulse steroids and IV Cytoxan. She was discharged on intermittent hemodialysis and prednisone 60mg PO daily, with no sign of renal recovery yet.

**Conclusions:** Systemic sclerosis patients can present with a vasculopathy called intimal fibro-proliferation. There is no convincing evidence in the literature that true vasculitis occurs in systemic sclerosis or localized scleroderma. Our patient, who did not meet the criteria for systemic sclerosis but had documented localized scleroderma on skin biopsy, with PAN seen on kidney biopsy and renal angiogram, could be the first reported case of ANCA negative vasculitis occurring in scleroderma.

## SA-PO610

**ANCA Vasculitis Associated with Influenza Vaccination** Megha Shah,[1] Tanu Duggal,[2] Paul E. Segal,[1] Naima Carter-Monroe,[3] Duvuru Geetha.[1] *[1]Dept of Medicine, Johns Hopkins Univ, Baltimore, MD; [2]Dept of Medicine, Sinai Hospital of Baltimore, MD; [3]Dept of Pathology, Johns Hopkins Univ, Baltimore, MD.*

**Background:** Administration of influenza vaccine has been associated with development of auto antibodies and autoimmune rheumatic disease.

**Methods:** Two patients were diagnosed with AAV at our institution 2 and 4 weeks after influenza immunization. Both patients had renal involvement, with one requiring

dialysis. Both patients were treated with cyclophosphamide(CYC) and prednisone(P) and in the patient with dialysis dependent renal failure plasmapheresis(PE) was added to the immunosuppressive regimen. Both patients achieved disease remission. The patient with initial dialysis dependent renal failure reached end stage renal disease. The characteristics and clinical outcomes of our 2 patients and 6 previously reported cases of AAV associated with influenza vaccine are reported in Table 1.

| Age | Sex | Time from immunization to disease onset | New diagnosis/ Relapse | Disease type | ANCA type | Treatment | Outcome |
|---|---|---|---|---|---|---|---|
| 83 | F | 2 wks | New | MPA | MPO | CYC/P | Remission |
| 28 | F | 2 wks | New | GPA | C/PR3 | CYC/P | Remission |
| 55 | F | 3 wks | New | MPA | P/MPO | CYC/P/PE | Remission, Dialysis Independent |
| 70 | M | 3 wks | New | GPA | P/MPO | CYC/P | ESRD, Death |
| 67 | F | 12 days | Relapse | GPA | C/PR3 | CYC/P | Remission |
| 20 | M | NK | Relapse | GPA | C | MMP/P/PE | Death |
| 50 | M | 2 wks | New | GPA | C/PR3 | CYC/P | Remission |
| 75 | F | 4 wks | New | GPA | P/MPO | CYC/P/PE | ESRD, Remission |

**Conclusions:** Renal involvement is common in AAV associated with influenza vaccine and treatment with conventional immunosuppression is successful in remission induction. While a causal role of vaccine in ANCA associated vasculitis cannot be confirmed with these case reports, the temporality suggests that influenza vaccine may be a triggering factor for induction of vasculitis in predisposed individuals.

## SA-PO611

**ANCA Associated Vasculitis Associated with Subacute Bacterial Endocarditis – A Match to Watch Out for** Asif A.K. Ansari, Diana L. Deitzer, Andres G. Chiesa-vottero. *Cleveland Clinic Foundation.*

**Background:** Infections such as sub-acute bacterial endocarditis (SBE) are seldom associated with elevated Antineutrophil cytoplasmic antibody (ANCA) titers and in some cases triggering features consistent with ANCA associated vasculitis (AAV). The presence of SBE and AAV in combination poses a clinical dilemma as therapeutic strategies are different and no complementary. We report a challenging case of AAV associated with SBE of mitral valve due to streptococcus mutans presenting with acute kidney injury (AKI).

**Methods:** 68 year old man presented with nonspecific symptoms of weight loss and weakness spanning 2 months. He was noted to have petechial rash on legs and a systolic murmur in mitral area on clinical examination. Laboratory studies showed hemoglobin of 7.6 mg/dl, platelets 70,000 cells/microliter, LDH 384 U/L, with undetected haptoglobins, low Complement C3 levels and c- ANCA positive. Serum creatinine was 3.6 mg/dl on admission and progressively worsened peaking at 6.88 mg/dl. Renal biopsy performed showed pauci-immune crescentric glomerulonephritis with fibrinoid necrosis. Blood cultures were positive for streptococcus mutans, with echocardiography revealing mobile mitral valve (MV) vegetation. Patient was treated surgically with MV repair and excision of vegetation, intravenous ceftriaxone and corticosteroids. Patient briefly required renal replacement therapy, and eventually had good clinical recovery.

**Conclusions:** Although rare, bacterial infections are being reported as triggers for AAV. This entity may be missed if appropriate investigations are not performed, resulting in inadequate therapy. Both SBE and AAV can result in AKI, and have an overlapping presentation and is often difficult to determine the underlying etiology. Optimal therapy in the presence of both conditions is not known, and immunosuppressive medications must be used judicially as can potentially worsen sepsis. Elevated ANCA with low complement levels should raise the suspicion of AAV associated with SBE. Our case supports the use of corticosteroids for life-threatening AAV complicating SBE.

## SA-PO612

**Anti-Glomerular Basement Membrane Disease after Nephrectomy for Xanthogranulomatous Pyelonephritis in a Child Expressing HLA DR15 Major Histocompatibility Antigens** Emma O'Hagan, Tamara Mallett, Karl McKeever, Mairead Convery. *Paediatric Nephrology, Royal Belfast Hospital for Sick Children, Belfast, Co. Antrim, United Kingdom.*

**Background:** The estimated incidence of Antiglomerular basement membrane (anti-GBM) antibody disease is 0.5 per million in adults but much rarer in children. We present the case of a 7 year old boy who developed anti-GBM antibody disease following nephrectomy for diffuse unilateral Xanthogranulomatous Pyelonephritis (XGP).

**Methods:** A previously well 7 year old boy was treated with anti-microbials and nephrectomy for diffuse, unilateral XGP, diagnosed on CT scan and confirmed on histopathology . Post-operative ultrasound scan of the contralateral kidney was normal as was estimated glomerular filtration rate (eGFR 128mls/min/1.73$^2$). Three months later the child represented with gross haematuria and acute kidney injury requiring haemodialysis (eGFR 9.9 mls/min/1.73$^2$ ). Renal biopsy showed severe crescentic glomerulonephritis with 95% of glomeruli demonstrating circumferential cellular crescents and strong linear IgG staining of the glomerular basement membranes. Anti-GBM antibodies were positive and anti-neutrophil cytoplasmic antibodies were negative. Treatment with plasma exchange, methylprednisolone and cyclophosphamide led to normalisation of anti-GBM titres. Frequency of haemodialysis has reduced as renal function gradually improves (eGFR 21.8 mls/min/1.73m$^2$).

**Conclusions:** Case studies in the adult literature have reported the development of a rapidly progressive anti-GBM induced glomerulonephritis following lithotripsy where patients expressed HLA DR2/HLA DRB15 major histocompatibility (MHC) antigens. Our patient expresses the HLA DRB15 MHC antigen which is positively associated with anti-GBM disease. We postulate that disruption of the GBM during nephrectomy exposed the target antigen to trigger anti-GBM disease in this patient. Anti-GBM disease is a rare but potentially treatable cause of acute kidney injury, which should be considered in patients who have a rapidly progressive form of glomerulonephritis and a history of recent renal surgery. We believe this to be the first reported case of a child with anti-GBM antibody disease following renal surgery.

## SA-PO613

**Goodpasture's Disease and Severe Thrombocytopenia: Thrombotic Thrombocytopenic Purpura or Not?** Howon Lee,[1] Jill Adamski,[2] Huma Fatima,[2] Eric L. Wallace.[1] *[1]Div of Nephrology, UAB; [2]Dept of Pathology, UAB.*

**Background:** Goodpasture's syndrome is a rare autoimmune disease caused by antibodies against cryptic epitopes in Type IV collagen and presents with rapidly progressive glomerulonephritis and pulmonary hemorrhage. The association of Goodpasture's syndrome with microangiopathic hemolytic anemia (MAHA) was made in 1984 prior to ADAMTS-13 testing. Subsequently, 2 case reports have associated thrombotic thrombocytopenic purpura (TTP) with Goodpasture's syndrome. Only one reported an ADAMTS-13 level, 17%, which is higher activity than usually reported in TTP. We report a case of Goodpasture's associated with MAHA and severe thrombocytopenia with a normal ADAMTS-13 level.

**Methods:** A 50 year old woman presented to the hospital with dyspnea, discolored urine, and lower extremity edema. Laboratory evaluation showed a creatinine 12 mg/dl, hemoglobin 6.2 g/L, and platelet count 459 x103 /cm$^2$ . Urinalysis showed a nephritic urine sediment. Serology test including ANCA was negative. Anti-GBM levels were 240 AI. She was given pulse dose steroids and initiated on dialysis. Renal biopsy, consistent with Goodpasture's, revealed linear IgG staining along the glomerular basement membrane, 100% crescents, but no evidence for thrombotic microangiopathy . Platelet count decreased to 131,000/μL. Plasmapheresis (TPE) was initiated, and IV cyclophosphamide given. Platelet count continued to decrease requiring 3 platelet transfusions. Laboratory evaluation revealed evidence of MAHA with schistocytes, decreased haptoglobin, increased LDH and indirect bilirubin. ADAMTS-13 activity was normal (55%). Heparin platelet factor 4 antibody was positive with a negative serotonin release assay, and a strong non-drug IgG antiplatelet antibody with unknown specificity was found. Despite 7 consecutive TPE, the platelet count decreased to 21,700/μL, and diffuse alveolar hemorrhage (DAH) developed. 3 weeks after initiation of therapy, platelet count improved and DAH resolved. The patient remained on dialysis.

**Conclusions:** This case illustrates that although there can be an association between Goodpasture's and MAHA, true TTP is unlikely on the basis of ADAMTS-13 levels in both our case and previous report.

## SA-PO614

**Plasmapheresis in Anti-Glomerular Basement Membrane Disease, How Much Is Enough?** Nitin Relia, Aleksandra Gmurczyk, Jennifer A. Tuazon, James J. Paparello. *Nephrology Div, Northwestern Univ, Chicago, IL.*

**Background:** Anti GBM disease is caused by pathogenic circulating antibodies and manifests as fulminant renal failure with crescent formation and pulmonary hemorrhage, although it can present as renal disease alone. The current standard of care for treating Anti-GBM disease is immunosuppression to reduce new antibody production and plasmapheresis to remove existing antibodies in circulation. Although plasmapheresis is central to the management of Anti-GBM disease, the duration of plasmapheresis that should be undertaken is unclear. KDIGO practice guidelines published June 2012 recommend Plasmapheresis for 14 days or until anti-GBM antibody titers are undetectable. These guidelines can be interpreted that a two week course of plasmapheresis is an adequate regimen for all anti – GBM disease.

**Methods:** The clinical course and data on 3 patients with anti-GBM disease without pulmonary involvement is presented. Two of the 3 patients required dialysis. All 3 patients were plasmapheresed until antibody titers became undetectable.

**Results:** The average number of exchanges required to achieve an undetectable anti-GBM antibody was 34. All 3 patients had improved renal outcome in follow up.

| Patient characteristics | Patient 1 | Patient 2 | Patient 3 |
|---|---|---|---|
| Age/Sex | 25 yr/Male | 19 yr/Female | 43 yr/Female |
| Creatinine at presentation (mg/dl) | 3.0 | 4.2 | 3.4 |
| Peak creatinine (mg/dl) | 9.5 | 7.6 | 13.0 |
| % of Crescents on Renal Biopsy | 60% | 75% | 100% |
| Dialysis (Y/N) | N | Y | Y |
| Number of Plasmapheresis sessions | 40 | 25 | 39 |
| Immunosuppression | Steroids, 3 monthly doses of Cytoxan, 2 doses of Rituximab | Steroids, 4 monthly doses of Cytoxan | Steroids, 6 monthly doses of Cytoxan |
| Follow up (months) | 36 | 36 | 12 |
| S.Cr (mg/dl) at most recent f/u | 1.8 | 1.6 | 2.5 |

The two dialysis dependent patients became dialysis independent.

**Conclusions:** The positive outcomes (in patients with poor prognosticators) of this small case series suggests that the course of plasmapheresis in patients with anti- GBM disease should be dictated by the anti-GBM antibody titer, and not simply a pre-specified number of treatments.

## SA-PO615

**IgA Vasculitis (Henoch-Schönlein Purpura Nephritis) Associated with Cystic Fibrosis** Sampath Kumar Thiruveedi, Robert L. Benz. *Dept of Nephrology, Lankenau Hospital, Wynnewood, PA.*

**Background:** IgA Vasculitis (HSP) is a systemic vasculitis characterized by tetrad of Palpable purpura, Arthralgia/arthritis, abdominal pain & renal disease. IgA Vasculitis is characterized by the tissue deposition of IgA-containing immune complexes. It is less

common in adults but represents a more severe clinical syndrome, with higher frequency of renal involvement. It is very rarely seen in association with cystic fibrosis with only 2 case reports on our literature search. Here we present one such rare association and discuss limitations in treatment.

**Methods:** 50 year old white male with history of cystic fibrosis, recurrent pulmonary infections, diabetes mellitus, Hypertension & CHF. He presented in October 2010 with abdominal pain & was found to have Jejunal inflammation on CT scan, ulceration on enteroscopy and Vasculitis on biopsy. Diagonisis of HSP was made but his kidney function was normal. In August 2011 he had microscopic hematuria. In April 2012 he had macroscopic hematuria, 1.3 gram proteinuria & increase in creatinine from 0.8 to 1.7. Serologic work up for proteinuria was negative and ultrasound showed normal size kidneys. In August 2012 He had nephrotic syndrome with 4.4 gm proteinuria & creatinine of 2.2. He was presumptively treated as IgA vasculitis with prednisone 1mg/kg & lisinopril. Despite 6 months of steroids his proteinuria increased to 6.6 grams & creatinine to 2.8. Kidney biopsy confirmed acute & chronic focal IgA glomerulonephritis. Cellcept was started cautiously as steroid sparing agent but discontinued a week later due to lobar pneumonia. He continues to be maintained on lower dose of prednisone.

**Conclusions:** Renal biopsy in Cystic fibrosis is technically difficult with inability to lie prone from poor respiratory reserve & coughing but can disclose a heterogeneous spectrum of nephropathies. Presented here is a case with typical demographics of Caucasian race and male sex for IgA vasculitis. Witnessed is the severity of illness with declining GFR and poor response to steroid therapy. Life threatening sepsis or infections limit the use of more aggressive immunosuppressive therapy in a group of patients particularly prone to resistant bacteria and recurrent infections.

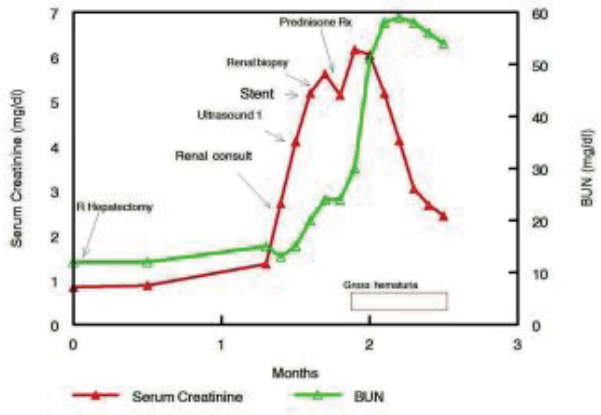
## SA-PO616

### IgA Nephropathy Precipitated after Hemi-Hepatectomy for Colorectal Metastasis

Siddiq Anwar, Derek Larson, Muhammad A. Ashraf, Helen Liapis, Aubrey R. Morrison. *Washington Univ School of Medicine, St. Louis, MO.*

**Background:** Mesangial IgA deposition in the renal glomerulus is a recognized complication of end stage liver disease (ESLD). We report a patient, who developed visible hematuria, acute kidney injury (AKI) and a lower legs rash suggestive of Henoch–Schönlein purpura(HSP) 8 weeks after a right hemi-hepectectomy. This was subsequently diagnosed as IgA nephropathy (IGAN).

**Methods:** A 50-year White male with a history of colorectal cancer developed AKI following his hemi-hepatectomy. This was initially attributed to a urinary tract infection and acute tubular necrosis, however he subsequently developed a 'rash' and proteinuria which raised the suspicion for a glomerular entity. Patient was readmitted with AKI 4 weeks later and an initial ultrasound revealed mild hydronephrosis. Urology was consulted for stenting, however, even after stenting, the patient continued to have an increase in his serum creatinine (SC) which eventually peaked at 6.2mg/dl. A renal biopsy showed mesangial hypercellularity with IgA dominant deposition consistent with IgA nephropathy. Three of ten glomeruli were globally sclerosed and one displayed crescent formation. The patient was subsequently placed on prednisone 80 mg daily with gradual improvement in SC. He did not require renal replacement therapy during his hospitalization.




**Conclusions:** In ESLD, it has been suggested that impaired clearance of immune complexes by hepatic kupper cells may allow them access to the systemic circulation leading to deposition of these complexes in kidney. This is the first reported case where IGAN and likely HSP has been associated acutely post liver resection. In this instance the clinical abnormalities resolved with a prolonged course of prednisone.

## SA-PO617

### Concurrent and Sequential Analysis of Glomerular Albumin Permeability, Soluble Urokinase Receptor, and Angiotensin 1 Receptor Antibody in a Renal Transplant Recipient with Recurrent Focal Segmental Glomerular Sclerosis

Siddiq Anwar, Nima Naimi, Muhammad A. Ashraf, George Jarad, Helen Liapis, Daniel C. Brennan. *Washington Univ School of Medicine, St. Louis, MO.*

**Background:** Recurrent focal segmental sclerosis (rFSGS) in kidney transplant (KT) is difficult to predict and causes graft loss. Early rFSGS is hypothesized to be secondary to one or more circulating factors: glomerular albumin permeability factor ($P_{alb}$ ), soluble urokinase receptor (suPAR), and angiotension-1 receptor antibody (AT1Rab).

**Methods:** We analyzed stored samples 1 yr pre-transplant, at transplant and on recurrence for $P_{alb}$, suPAR, and AT1Rab in a 23 yr. white male. He presented 8 months after 1 haplotype match KT with rFSGS and acute renal injury (AKI) requiring dialysis (HD). All 3 biomarkers were elevated 1 yr pre-transplant when he was not on HD. At transplant, Palb and AT1Rab were elevated, but suPAR level was not. 4 months later he developed CMV viremia and antimetabolite was stopped with resolution of viremia. Subsequently maintained on prednisone and tacrolimus. 8 months after KT, develops AKI and biopsy showed rFSGS with no rejection. C4d and anti-HLA antibodies were negative. At recurrence, all 3 biomarkers were elevated. 7 days after therapy with aborted plasma exchange (secondary to side effects) plus high dose steroids, Palb and suPAR remained significantly positive but AT1Rab was borderline positive. 12 weeks into treatment with high-dose steroids, rituximab and galactose, patient remains on HD.

| | 1 year pre-transplant | At transplant | 1 month Post-transplant | 3 months | 4 months | 8 months On admission | Post pulse steroids and failed PE and PP-1 week after admission |
|---|---|---|---|---|---|---|---|
| Creatinine mg/dl | 4.09 | HD | 1.3 | 1.2 | 1.2 | HD | HD |
| FK trough ng/ml | - | - | 6.4 | 6 | 5.8 | - | |
| CMV PCR copies/ml | - | - | - | 4533 | < 200 | < 200 | - |
| BK virus PCR | - | - | - | ND | - | ND | - |
| P/C ratio | - | - | 0.09 | - | - | 17.7 | - |
| DSA | - | ND | - | ND | - | - | - |
| $P_{alb}$ | 0.77 | 0.51 | - | - | - | 0.61 | 0.58 |
| SuPAR pg/ml | 3573 | 2898 | - | - | - | 4691 | 4428 |
| AT1R Antibody level U/ml | 20.4 | 23.2 | - | - | - | 16.8 | 13.5 |

HD: Hemodialysis, CMV: cytomegalovirus. Ig G: immunoglobulin G. PCR: polymerase chain reaction. ND: not detected. FK: tacrolimus. P/C: protein to creatinine. DSA: donor specific antibodies. suPAR: Soluble urokinase receptor-Suspicious: > 3000 pg/ml, >3500 pg/ml are suggestive of suPAR-mediated FSGS (Reiser lab, Rush University, USA). Glomerular albumin permeability: Palb. $P_{alb}$ -Normal: 0, Nonspecific: 0.2 to 0.5, Increased risk: Values greater than 0.5(Renal Research, KC VA Medical Center, Kansas City, USA). AT1Rab: Angiotension-1 receptor antibody: Neg :< 10, Borderline: 10 to 17, Positive: > 17 (Immunogenetics Laboratory, The Johns Hopkins University, Baltimore, USA)

**Conclusions:** Previous reports suggest elevated suPAR and high $P_{alb}$ at transplant confers risk of rFSGS. Although an N=1 study, this highlights uncertainties and difficulties in monitoring and interpretation of Palb, suPAR and AT1Rab pre and post transplantation. Further studies are needed to validate published reports and establish better surveillance protocols.

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## SA-PO618

### IgA Nephropathy with Renal Atypical Mycobacterium Infection

Zaheer Amin Virani, Anup Chaudhari, Hemant J. Mehta, Mohd. Majid Mohd. Ayyub Momin. *Nephrology, Lilavati Hospital, Mumbai, Maharashtra, India.*

**Background:** IgA Nephropathy due to mycobacterium tuberculosis elsewhere in the body has been described. However, presence of IgA nephropathy and mycobacterium infection in the kidney as a cause of IgA nephropathy is rare.

**Methods:** FK, F/18, was admitted with fever of 5 days duration associated with dry cough and coryza. There was no history of oedema, urinary complaints, rash or joint pains. On evaluation, BP 170/90 mm of Hg, bilateral pitting oedema feet. Her investigations showed microcytic hypochromic anaemia, Urine showed 4+ proteins, 80 to 100 rbc/hpf with hyaline and granular casts. Spot urine protein creatinine ratio was 6.15. Metabolic acidosis, hyperphosphatemia, Vit D deficiency and dyslipidemia were seen. Sr. Creatinine was 3.15mg%. (eGFR by MDRD 20.42 ml/min). She was negative for HBV, HCV, HIV, ANA, dsDNA, ASO-Titres, Anti-GBM and ANCA, C3 level was 120mg/dl. USG-KUB showed normal size kidneys with increased echogenicity. Histopathological diagnosis on kidney biopsy: MPGN with early cellular crescent formation with non caseating granuloma and chronic tubulointerstitial nephrites thought to represent mycobacterial infection associated IgA nephropathy. Search was made for evidence of mycobacterium infection. HRCT chest showed bilateral basal pleural thickening. Mantoux skin test and Gamma interferon detection for mycobacterium were positive. Bactec TB culture of urine showed occasional AFB on smear, and mycobacterium spps other than tuberculosis (MOTT) on culture. Serum IgA level was 378.3 mg/dl (upper limit of normal 348 mg/dl) She was started on Inj.

Methylprednisone 1gm daily for 3 days followed by oral prednisolone at 60 mg/day.Four drug anti tuberculosis treatment (H, R, E, Z) was started. Despite steroids and AKT patient continued to have worsening azotemia and was initiated on haemodialysis after 7 months.

**Conclusions:** There is evidence to suggest that tuberculosis, in addition to other conditions associated with mucosal exposure to antigens producing an IgA immune response can result in IgA nephropathy. This glomerulopathy is reported as a potential renal complication of concurrent mycobacterial infection. IgA nephropathy due to MOTT in kidney is rare.

## SA-PO619

**A Unique Case of IgA Heavy Chain Deposition Disease** Pradeep Dhakarwal, Anshul Kumar, Kathryn E. Ussai. *Nephrology Div, Lehigh Valley Hospital/ USF, Allentown, PA.*

**Background:** To our knowledge only 5 cases of IgA heavy chain deposition disease(HCDD) have been reported and 2 were female. We present a case of IgA HCDD and compare the clinicopathological features with earlier cases.

**Methods:** 65 year old white female was referred for edema, proteinuria and microscopic hematuria. She had history of hypertension and pulmonary embolism. Creatinine (Cr) at presentation was 0.81mg/dL, Hb, calcium were normal and urineP/Cr was 1.2 Urine microscopy had dysmorphic RBCs and RBC cast. ANA, anti dsDNA, ANCA, anti GBM, and complements were negative. SPEP, UPEP was negative but serum and urine kappa/ lambda was elevated. Renal biopsy light microscopy showed nodular scleroses with mesangial nodules. IF had 3+ staining of mesangial nodules and 2+ linear staining of GBM, TBM, vessel walls for IgA with negativity for IgG, IgM, kappa, lambda. On EM abundant powdery deposits within mesangial nodules were seen. Findings diagnostic of IgA HCDD. Bone marrow biopsy had hypercellular marrow involved by kappa light chain IgA plasma cells. She was started on cyc, bortezomib, and dexamethasone. Cr increased to 1.97 within one week of the first cycle and urineP/Cr increased to 9.3. She underwent 6 plasma-exchange (PLEX) and Cr decreased to 1.05. The decision was based on possible removal of heavy chains by PLEX, and for the possibility of developing light chain disease in addition HCDD. Second cycle of chemotherapy is ongoing, plan is for stem cell transplantation after induction.

**Conclusions:** All reported cases of IgA HCDD presented with hypertension, elevated Cr, microscopic hematuria, anemia and positive SPEP. 4 of 5 patients had nephrotic range proteinuria at presentation. In all cases kidney biopsy had crescentic features with nodular glomerulosclerosis; considered characteristic pattern in this subset of HCDD. Plasma cell dyscrasia was present in all patients. Our patient presented with normal Cr, 1.2g proteinuria, normal hemoglobin and negative SPEP. Renal biopsy had no crescents. This patient received PLEX with decrease in Cr. In summary, we report the sixth case of IgA HCDD, with multiple findings different from earlier cases, including an apparent response to PLEX.

## SA-PO620

**ACTH a Novel Treatment in IgA Nephropathy** Vinh Q. Nguyen,[1] Ashraf El-Meanawy.[2] *[1]Nephrology, Medical College of Wisconsin, Milwaukee, WI; [2]Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

**Background:** IgA nephropathy is the most non-infectious glomerulonephritis worldwide. Despite this, treatment options remains limited as current data on immunosuppressive therapy of MMF and cyclosporine have been inconclusive. This leaves supportive therapy and corticosteroids to be the cornerstone of current therapy for aggressive disease.

We report a case of IgA nephropathy treated with ACTH with improvement in proteinuria.

**Methods:** JB is a 38 y/o white male who was diagnosed with IgA nephropathy biopsy proven in his early 20's when he presented with hematuria. He now presented to the clinic with worsening creatinine with proteinuria. On PE his BP was 129/85, HR: 68 and well built. The remainder of his exam was unremarkable other than trace bilateral edema. His creatinine at the time was 2.04 ml/min with 0.9 g/g on spot P/C ratio. Patient was offered steroids and cellcept which he declined. He opted to have a second opinion at Mayo Clinic where a biopsy was performed showing positivity for IgA, IgM and moderate C3. He was treated with steroids. He returned to our clinic with worsening proteinuria of 3.3 g/g and a creatinine of 3.0 ml/min and was started on cellcept. Subsequently his renal function and proteinuria did not improve and ACTH was initiated. His serum creatinine stablize at 2.6-3.0 ml/min with improvement of proteinuria down to 1.7 grams/gram.

**Conclusions:** ACTH is a therapy that has been considered in primary membranous nephropathy. However its role in IgA nephropathy has yet of be described. Besides the glucocorticoid effects, it has been thought that ACTH has anti-inflammatory and immune modulating properties. We believe that in selective patients who failed corticosteroids and MMF, ACTH is a viable option.

## SA-PO621

**Successful Treatment of Rapidly Progressive Immunoglobulin A Nephropathy with Human Immunodeficiency Virus Infection by Steroid Pulse Therapy and Tonsillectomy** Daisuke Fujimoto,[1] Yoshikazu Miyasato,[2] Taku Miyoshi,[2] Masataka Adachi,[2] Kenichiro Kitamura.[2] *[1]Dept of Nephrology, Kumamoto General Hospital, Yatsushiro, Japan; [2]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan.*

**Background:** Several renal syndromes including Immunoglobulin A (IgA) nephropathy have been described in patients with human immunodeficiency virus (HIV) infection. Hotta et al. reported that steroid pulse therapy combined with tonsillectomy was effective for clinical remission in patients with IgA nephropathy (Am J Kidney Dis, 2001). Here, we report a case of rapidly progressive IgA nephropathy with HIV infection treated successfully with steroid pulse therapy and tonsillectomy.

**Methods:** A 43-year-old man was referred to our department for rapidly progressive renal impairment, proteinuria, and hematuria. He was under treatment of acquired immunodeficiency syndrome (AIDS) with Highly Active Anti-Retroviral therapy (CD4 count: >200/μL, HIV RNA: undetectable). Kidney biopsy revealed IgA nephropathy with cellular crescents. Firstly steroid pulse therapy (methylprednisolone 0.5-1g/day for 3 consecutive days, 3 courses) was initiated and prednisolone (30mg/day) and mizoribine (initially 50mg/day, loaded up to 150mg/day) were orally administered as aftertreatment. Two months later, he underwent tonsillectomy. One course of methylprednisolone pulse therapy was added after tonsillectomy. During steroid pulse therapy, CD4 count dropped below 200/μL, but no serious complication occurred. Serum creatinine (Cr) level increased up to 4.15mg/dL and gradually decreased to 1.1mg/dL. Although hematuria still exists, urinary protein declined from 3g/gCr to 0.3g/gCr during the 10 months treatment period.

**Conclusions:** Steroid pulse therapy combined with tonsillectomy dramatically ameliorated the rapid progression of IgA nephropathy in our current case and no serious complication occurred in such a highly immunocompromised host. This combined therapy could be considered in the treatment of rapidly progressive IgA nephropathy in patients with HIV infection.

## SA-PO622

**Resolution of C1q Deposition but Not of the Clinical Nephrotic Syndrome after Immuno-Modulating Therapy in Focal Sclerosis** Michelle L. Blake,[1] Eva Csongradi,[1,2] Tibor Fulop.[1] *[1]Internal Medicine, Univ of Mississippi Medical Center; [2]Medicine, Univ of Debrecen Medical and Health Science Center, Hungary.*

**Methods:** A 30 year-old Caucasian female referred for further management of biopsy-proven C1q nephropathy (C1qNP) and nephrosis. She was normotensive but had severe, bilateral pitting edema, serum albumin of 1.4 gm/dL and urine protein/creatinine (UPC) concentrations of 4800/133 mg/dL. Serologic work-up was negative, including complement C3 and C4 levels and antinuclear antibodies. A renal biopsy revealed minimal change nephropathy vs. focal sclerosis and C1qNP on immunopathology. She had failed a trial of high-dose oral prednisone. At follow-up she failed a regimen of mycophenolate mofetil 1,500 mg twice a day and prednisone 40 mg daily for 14 months and then monthly IV cyclophosphamide 750 mg x 9 cycles. She received the maximum tolerated ACE inhibitor and spironolactone therapy. Her random UPC ratio ranged 5-35 gm/day, serum creatinine (sCr) progressively rose from 1.0 mg/dL to 1.4 mg/dL. Two years after her initial visit we faced the dilemma of ongoing nephrotic-range proteinuria failing maximized therapy. The decision was made to repeat renal biopsy to reassess the underlying histology. The biopsy revealed focal sclerosis but no C1q deposition. Combined diuretic and ACE inhibitor therapy was continued for symptomatic control; restarting immune-modulating therapy was felt likely to be of little benefit. She was lost to follow-up up till 16 months post biopsy, when sCr rose to 2.8 mg/dL and she remained severely nephrotic with a UPC ratio of 23.

**Conclusions:** How an underlying glomerulonephritis changes with treatment remains a relatively little explored subject. While clinical nephrosis did not change with immunosuppressive therapy, C1q deposition ceased, making the latter entity likely an immunologically mediated process. Our case illustrates at least two points: first, an established pathologic diagnosis does not obviate the need for repeated renal biopsy later on, should diagnostic uncertainty persist. Second, histological diagnoses may evolve over time, especially in a patient receiving active and powerful immune-modulating treatment.

## SA-PO623

**Late Recurrence of Light Chain Deposition Disease after Kidney Transplantation Treated with Bortezomib: A Case Report** Tariq Javed, Abdul Moiz, Jorge C. Garces, Catherine G. Staffeld-Coit. *Ochsner Multi-Organ Transplant Institute, Ohsner Clinic Foundation, New Orleans, LA.*

**Background:** Herein, we present a case of a patient who developed recurrence of LCDD, several years after kidney transplantation, leading to allograft dysfunction requiring renal replacement therapy. The patient had a significant improvement in her renal function after receiving bortezomib and was able to come off dialysis.

**Methods:** A 55 YO Caucasian female with history of Type 2 DM and HTN, ESRD secondary to LCCD underwent living-unrelated kidney transplantation. Her induction consisted of IV solumedrol and rabbit thymoglobulin (4.5mg/kg in 3 divided dosages). She had immediate allograft function without the need of renal replacement therapy. Her creatinine stabilized between 0.8-1.0mg/dl. After 6 years of transplantation routine labs showed a Cr of 1.8mg/dl and nephrotic range proteinuria (9.5g/g) on spot protein to creatinine ratio. She subsequently underwent a kidney biopsy. A diagnosis of recurrent

light-chain deposition disease was made based on the biopsy findings. Oncology was consulted and a bone marrow biopsy was performed which was consistent with a plasma cell dyscrasia and she met criteria for multiple myeloma. Patient initially received five cycles of plasmapharesis without any improvement. She eventually required renal replacement therapy three times a week. She then received one cycle of Bortezomib (Velcade 1.3mg/m2 intravenously) along with IV dexamethasone. She received 4 doses of Bortezomib on day 1, 4, 8 and 11. There was no immediate clinical response to the treatment and she remained dialysis dependent. She was discharged to a rehabilitation center due to significant debility and deconditioning. Her renal function was closely monitored while she remained on dialysis. She gradually exhibited signs of renal recovery after staying on dialysis for 2 months. Dialysis was held when her urine output improved (> 1L/day), and her creatinine eventually settled between 1.5-1.7 mg/dl.

**Conclusions:** Bortezomib may provide an alternative to currently available treatment options in patient with LCDD recurrence after transplantation.

## SA-PO624

**Heavy Chain Deposition Disease: A Case Report** Sassan Ghazan-Shahi, Iain D. Young, Ralph M. Meyer, Christine A. White. *Internal Medicine - Nephrology, Queen's Univ, Kingston, Canada.*

**Background:** Heavy Chain Deposition Disease (HCDD) is a rare condition with few case reports in the literature. It is one of the three subtypes of monoclonal immunoglobulin deposition disease (MIDD) in the kidney. The most common clinical features are hypertension, microscopic hematuria, nephrotic range proteinuria, and variable degrees of renal insufficiency. The characteristic histopathological finding of HCDD is nodular glomerulosclerosis.

**Methods: Case Presentation**: An 82 yo female with proteinuria and microscopic hematuria. She reported increased bilateral leg edema, shortness of breath on exertion and generalized fatigue. There was no gross hematuria. She denied any other constitutional symptoms including fever, night sweats, weight loss or loss of appetite. There were no symptoms suggestive of a vasculitic or connective tissue disorder. **Past Medical History:** COPD and peripheral vascular disease. Her medications were perindopril/indapamide, metoprolol, ASA. **Physical examination:** BP=170/80 mmHg, clear lungs, broad and displaced PMI, S3 gallop and III/VI systolic ejection murmur over the aortic area, bilateral leg edema to knees. No ascites or organomegaly. **Table 1** shows categorized list of the investigations. **Image 1** and **Image 2** show light microscopy and immunofluorescence microscopy of the kidney biopsy.

**Table 1: Categorized list of investigational studies performed**

| | |
|---|---|
| **Hematologic/Chemistry** | |
| Hb (g/L) | 130 |
| Peripheral blood smear | Rouleaux |
| Cr umol/L (MDRD eGFR ml/min/1.73 m²) | 81 (59) |
| Electrolytes including Calcium | Normal |
| Total protein (g/L) / Albumin (g/L) | 68 / 33 |
| Liver enzymes | Normal |
| Total Cholesterol HDL ratio | 9.2 |
| Bone Marrow Aspiration & Biopsy | •normocellular marrow<br>•plasma cells= 5-10%<br>•Light chain staining: kappa restriction in the plasma cells |
| **Immunologic** | |
| Complement Levels (C3, C4) | Normal |
| ANA/Anti dsDNA, Anti-GBM, ANCA(P&C), RF | Negative |
| Hepatitis B | Previous infection |
| Hepatitis C | Negative |
| **Protein Electrophoresis** | |
| SPEP | •Positive<br>•M-Protein=10.1 g/L<br>•Immunofixation: IgG kappa |
| UPEP | •Positive<br>•Immunofixation: Whole IgG kappa |
| Serum kappa/lambda ratio(Nrl: 0.26-1.65)mg/l | 34.1/1.6 = 3.97 |
| **Urine** | |
| Analysis | •Proteinuria<br>•Microscopic hematuria |
| Microscopy | No cellular casts |
| 24 h collection for Protein | 4.4 gr/day |
| **Other investigations** | |
| Echocardiogram | LVEF=30%<br>Dilated cardiomyopathy |
| Skeletal survey | Osteoporosis |
| MRI | No lytic lesions |

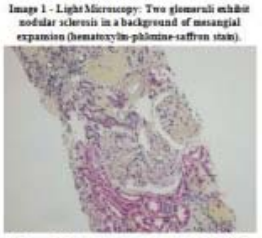


**Image 1 - Light Microscopy: Two glomeruli exhibit nodular sclerosis in a background of mesangial expansion (hematoxylin-phloxine-saffron stain).**

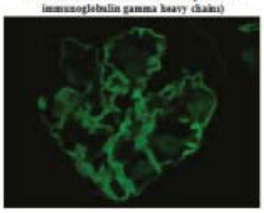


**Image 2 - Intense continuous staining of GBM. A similar pattern of linear staining of the basement membrane of one tubule is also apparent. (direct immunofluorescence with antisera specific for immunoglobulin gamma heavy chains)**

**Outcome:** She was started on Chlorambucil and Prednisone cycles The response to therapy over 9 months f/u period was favourable indicated by improvement of symptoms, lowered proteinuria and serum M-Protein.

**Conclusions:** Almost all reported cases of HCDD are in pateints with advanced renal insufficiency. Despite the severity of biopsy findings, the renal function in this case was reasonably well preserved on presentation and throughout the follow up period. Given the echocardiographic findings of possible cardiomyopathy, we are proposing possibility of cardiac deposition disease.

## SA-PO625

**A Case of Membranoproliferative Glomerulonephritis (MPGN) with Type I Cryoglobulinemia Associated with Monoclonal Gammopathy of Undetermined Significance (MGUS)** Shoko Hasegawa,[1] Toshiaki Nakano,[1] Akihiro Tsuchimoto,[1] Kazuhiko Tsuruya,[1,2] Takanari Kitazono.[1] *[1]Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

**Background:** The association of cryoglobulinemia and renal involvement is well known. Renal involvement usually is reported in mixed (type II or III) cryoglobulinemia, mostly associated with chronic hepatitis C infection. Renal involvement in monoclonal, type I cryoglobulinemia is unusual.

We report the a rare case of a patient exhibiting pathological characteristics of MPGN, which was induced by type I cryoglobulinemia associated with MGUS.

**Methods:** A 36-year-old Japanese woman was admitted because of proteinuria, hematuria and skin ulcers on the both legs. Urinary protein to creatinine ratio was 3.0 g/gCr and urinary sediment analysis revealed red blood cell of 60-70 /high power field and other various casts. The histopathology of skin biopsy showed an obstruction of superficial blood vessels with eosinophilic amorphous materials. Immunoelectrophoresis revealed a monoclonal immunoglobulin (Ig) G-K, type I cryoglobulin. Serum IgG was 1.3 g/dL (< 3 g/dl), and a bone marrow biopsy demonstrated 4% of plasma cells. The cryocrit was 35%. A renal biopsy showed mesangial hypercellularity, endocapillary proliferation, and double-contour formation of the glomerular basement membrane. Electron microscopic examination revealed a deposition of annular or curved cylinder tubular material in the glomerular basement membrane. The final diagnosis was MPGN caused by type I cryoglobulinemia associated MGUS. Within 2 months after initiation of Oral prednisolone therapy (50mg/day), proteinuria and hematuria had disappeared.

**Conclusions:** Renal histopathology of type I cryoglobulinemia shows generally noninflammatory glomerulopathies, including thrombotic and hypocellular lesions, without evidence of vasculitis (Karras A et al. Am J Kidney Dis 2002). We described a rare case of MPGN and skin ulcers with type I cryoglobulinemia associated with MGUS. The hematuria and proteinuria improved after therapeutic dose of prednisolone.

## SA-PO626

**Mixed Cryoglobulinemia and Membranoproliferative Glomerulonephritis Associated with Ehrlichiosis** Dawn J. Caster,[1] James T. Summersgill,[1] Paisit Paueksakon,[3] Rob Massung,[4] Wun-ju Shieh,[5] Kenneth R. McLeish.[1,2] *[1]Medicine, Univ of Louisville, Louisville, KY; [2]VAMC, Louisville, KY; [3]Pathology, Vanderbilt, Nashville, TN; [4]Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, GA; [5]Infectious Diseases Pathology Branch, Centers for Disease Control and Prevention.*

**Background:** Ehrlichiosis is a tick-borne disease with diverse clinical presentations, ranging in severity from a flu-like illness with fever and myalgias to a serious systemic disease with multisystem organ failure. Nephrotic syndrome has previously been reported in two cases of human ehrlichiosis. A kidney biopsy was performed in only one of those cases, and it demonstrated minimal change disease. This case describes a patient with ehrlichiosis who developed nephrotic syndrome, cryoglobulinemia, and membranoproliferative glomerulonephritis (MPGN).

**Methods:** A 40-year- old white male presented to the emergency department in late June complaining of shortness of breath, diffuse myalgias, headache, and lower extremity edema. Initial labs demonstrated pancytopenia, creatinine of 0.9 mg/dL, and albumin of 2.9 g/dL. The urinalysis showed protein concentration greater than 600 mg/dL and 15-29 red blood cells. A 24-hour urine collection contained 18 g of protein. He subsequently developed acute kidney injury and underwent kidney biopsy which showed MPGN and acute tubular injury. A tick-borne disease panel was positive for IgM and IgG to *Ehrlichia chaffeensis*. Serum testing revealed type 3 mixed cryoglobulinemia with no evidence of hepatitis C infection. Cryoprecipitate contained IgM and IgG to *E. chaffeensis*.

**Conclusions:** Mixed cryoglobulinemia is frequently associated with infections, particularly hepatitis C. Additional etiologies of mixed cryoglobulinemia include autoimmune disorders and hematologic malignancies. Our case is the first to describe ehrlichiosis associated with cryoglobulinemia and MPGN.

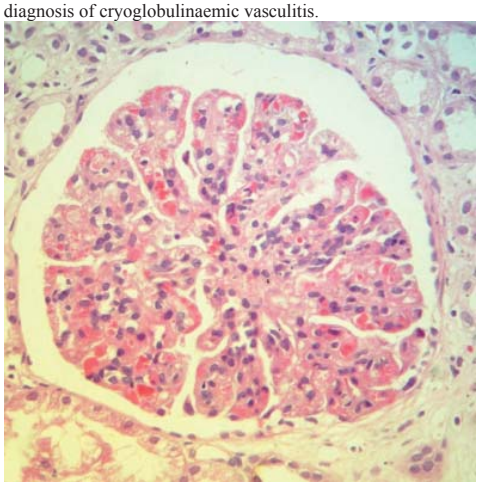
## SA-PO627

**Hepatitis C Negative Cryoglobulinaemic Vasculitis in a Young Woman** Talita Mourao Chaves Corrica, Tatiana Santos, Guilherme Fonseca Mendes, Talita Cardoso Proenca, Cláudia Fagundes, Luiz Fernando Christiani, Maria izabel Neves de Holanda Barbosa. *Nephrology, Hospital Federal de Bonsucesso, Rio de Janeiro, Brazil.*

**Background:** Cryoglobulinaemic vasculitis is caused by deposits of monoclonal or polyclonal imunoglobulins and complements compounds in small vessel walls in cold temperature, which promotes acute inflammation. This diseased is most often due to chronic HCV infection nevertheless, cryoglobulinemia is considered essential, in which no causal agent can be determined.

**Methods:** We report a case of a 39 year old woman presented with weakness, fever, arthralgia of small and large joints induced by cold temperatures. Upon examination the patient showed diffuse palpable purpura and nose and distal limbs ulcers. There was progressive worsening of skin lesions and anuria. Antibiotic therapy was started aimed to treat infectious vasculitis and hemodialysis was initiated. Sorology to HBV, HCV and

HIV, ANA, anti-DNA and ANCA were all negative, fraction of the complement C3 was normal and C4 reduced. Renal findings included renal failure with proteinuria and hematuria Ultrasound demonstrated normal kidney size and cortical-medullary relationship preserved. Cryoglobulinemia was suspected and subsequent serum cryoglobulin levels were positive. The peripheral blood smear showed characteristic findings and renal biopsy confirmed the diagnosis of cryoglobulinaemic vasculitis.



The patient was treated with steroids and monthly pulse of cyclophosphamide, with recovery of renal function after the second month of treatment and disappearance of the skin lesions.

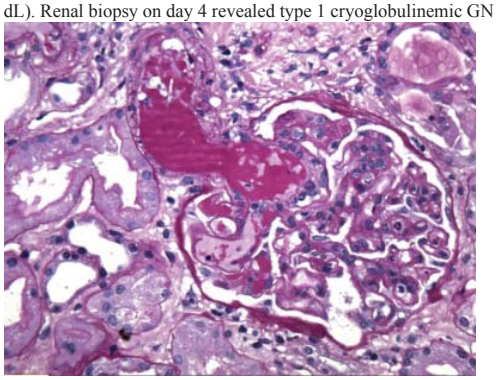
**Conclusions:** This case demonstrates that in patients with suspected vasculitis, even with negative sorology for hepatitis C, essential cryoglobulinemia should be considered, as early diagnosis and therapy promotes a higher chance of better outcome.

## SA-PO628

**The Cool Side of Acute Kidney Injury: Type 1 Cryoglobulinemic Glomerulonephritis in a Patient with Waldenstrom's Macroglobulinemia** Mamta Shah,[1] Rahul Mutneja,[1] Sparsha Kukunoor,[1] Dhwanil Vyas,[1] Andre A. Kaplan.[2] *[1]Internal Medicine, Univ of Conecticut, Farmington, CT; [2]Nephrology, Univ of Connecticut, Farmington, CT.*

**Background:** Renal manifestations of Waldenstrom's Macroglobulinemia (WM) include amyloidosis, glomerular/interstitial IgM deposition, cast nephropathy, intracapillary IgM deposition and cryoglobulinemic glomerulonephritis (GN). Type 1 cryoglobulinemic GN has been infrequently described.

**Methods:** 64 year old female with WM, not on treatment, presented with vomiting and blurry vision. She was found to be hypertensive with acute renal failure (Creatinine-5.2 mg/dl). Random urine protein was >600mg/dL. Labs 2 weeks prior showed a creatinine (Cr) of 0.6 mg/dL, cryoglobulins of 29% and IgM of 1330 mg/dL. She underwent immediate therapeutic plasma exchange (TPE) and hemodialysis (HD) for presumed hyperviscosity and renal failure. Serum viscosity drawn prior to TPE returned normal (1.5 U). Following HD and TPE Cr fell to 3.4mg/dL. 4 days later she received only TPE following which Cr was 2.5 mg/dL with concomitant decline of cryoglobulins (6%) and IgM (301 mg/dL). Renal biopsy on day 4 revealed type 1 cryoglobulinemic GN (courtesy Dr Herlitz).



She was started on bortezomib, rituximab and steroids and was sent home with follow up. Cr at discharge was 0.6 mg/dL.

**Conclusions:** Cryoglobulinemia (cryo) is present in 8-18% of WM patients. Cryo associated Raynaud's, palpable purpura or GN is seen in less than 5% of these patients. Symptoms related to hyperviscosity and cryo are indications for WM treatment. Bortezomib, rituximab and dexamethasone have been most studied. The rapid improvement in renal function post TPE, before immunosuppressive/steroid therapy is suggestive of rapid re-cannulation of cryoglobulin-clogged glomerular arterioles as is commonly seen with TPE treatment of cryo associated coalescing purpura of the skin.

## SA-PO629

**Sustained Remission of Refractory Membranoproliferative GN (MPGN) Associated with Monoclonal Gammopathy (MGUS) following Bortezomib/Dexamethasone Therapy** Marvin Aiko Schwarz,[1] Yonn-dschun Ko,[4] Thorsten Wiech,[3] Jürgen Floege,[1] Karl August Brensing.[2] *[1]Cardiology and Nephrology, RWTH Univ Hospital Aachen, Aachen, NRW, Germany; [2]Nierenzentrum Bonn, Bonn, NRW, Germany; [3]Dept of Pathology, Univ Hospital Hamburg Eppendorf, Hamburg, Germany; [4]Center of Oncology, Johanniter Krankenhaus Bonn, Aachen, NRW, Germany.*

**Background:** MPGN type 1 is known to be associated with monoclonal gammopathy of undetermined significance (MGUS). However, the causal relationship between the two entities is not well established.

**Results:** A 63-year-old man presented in 2005 with arterial hypertension, microscopic hematuria, proteinuria of 7.4 g/day and a GFR of 99 ml/min. Renal biopsy disclosed MPGN type I. An extensive screen for conditions associated with MPGN type I only revealed an MGUS IgG kappa in serum and Bence-Jones-proteinuria. From 2005 to 2012 the patient was treated with cyclosporine and low-dose steroids. While he initially responded with a partial remission of proteinuria, in 2011 and 2012 proteinuria relapse to full nephrotic syndrome (proteinuria 10-13 g/day, weight gain 10 kg). GFR decreased to about 55-60 ml/min. In view of the MGUS, no therapy with MMF was attempted. In 2012 his MGUS had remained stable with a maximal proportion of plasma cells of 10-12% in a bone marrow biopsy. Given this situation and the lack of alternative treatment recommendations in the 2012 KDIGO guidelines, in June 2012 we decided to switch the therapy to a plasma cell targeting therapy with bortezomib and dexamethasone (Bor/Dex). Proteinuria decreased from 10.5 g/day to 1.9 g/day within 5 months and to 1.1 g/day at 12 months after chemotherapy. GFR increased to 105 ml/min and the patient had full clinical remission. Chemotherapy was well tolerated. The maximal proportion of plasma cells decreased to 2-4% in 2013. A repeated kidney biopsy 3 months after 4 Bor/Dex courses therapy revealed almost complete regression of immunoactivity markers.

**Conclusions:** Our case suggests that the MGUS was indeed the cause of MPGN type 1 here. However, we can not fully exclude that Bor/Dex might have also acted directly on the kidney disease. Bor/Dex therapy warrant further investigation in similar situations.

## SA-PO630

**Hepatitis C Negative Cryoglobulinemic MPGN after Two Decades of Rheumatoid Factor Positivity** Ingrid Calliste, Liliana E. Rios Rojas, Joseph Mattana, Nobuyuki (Bill) Miyawaki. *Internal Medicine, Div Nephrology, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Membranoproliferative glomerulonephritis (MPGN) is is a lesion often associated with cryoglobulinemic (CG) glomerulonephritis in hepatitis C (HCV) infection yet rare cases of HCV negative CG MPGN have been documented. We present a case of an elderly female without clinical features of rheumatoid arthritis (RA) or HCV who had a positive rheumatoid factor (RF) titer as a surrogate for CG for 20 years and eventually developed CG MPGN with monoclonal IgM kappa deposits.

**Methods:** A 67 year old female with hypertension, intermittent microhematuria and gradually worsening proteinuria over eight years from 500mg/day to 3000mg/day despite angiotension receptor blockade and acceptable blood control was initially observed conservatively with serologies and biochemical testing given her preserved creatinine of less than 0.9mg/dL. She had a persistently positive RF since an arthritis assessment done nearly 20 years ago without any clinical features of RA. Her serologies were otherwise negative, including complement levels and HCV by PCR and antibody. Only one of numerous monoclonal assessments ordered through the years was transiently and faintly positive for IgM kappa. Quantitative immunoglobulin levels were unremarkable. Once her urine protein reached 3000mg/day a kidney biopsy was done and revealed MPGN with positive IgM and kappa deposits. Testing for cryoglobulins was positive.

**Conclusions:** Many cases of MPGN remain idiopathic but associated cases are often linked to HCV CG and the immune complex deposits, complement dysregulation, and thrombotic microangiopathies. Lymphoproliferative disorders, in the presence of paraproteinemias, have also been described in MPGN both with and without CG. Monoclonal IgM anti-IgG autoantibodies, commonly referred to as RF, are often considered a surrogate marker for CG. We speculate that this patient's slowly progressive course was likely due to years of low-grade production of IgM kappa. This case illustrates the possibility that isolated RF positivity in the setting of microscopic hematuria and proteinuria may signal the presence of an underlying paraproteinemic process.

## SA-PO631

**Membranoproliferative Glomerulonephritis (MPGN) and Central Venous Catheter (CVC) Infections** John Sy,[1] Cynthia C. Nast,[3] P.T.T. Pham,[2] P.C. Pham.[1] *[1]Nephrology, Olive View MC, Sylmar, CA; [2]Nephrology, UCLA MC, LA, CA; [3]Pathology, Cedars Sinai MC, LA, CA.*

**Background:** Chronic indwelling infected CVCs have been reported to be associated with MPGN *via* activation of the classical complement pathway.

**Methods:** We report a case of recurrent MPGN in association with recurrent CVC infections and review the literature for common clinical manifestations that may aid clinicians in early identification of both conditions. A 23 year-old male with prior small bowel resection due to trauma and chronic total parenteral nutritional support (TPN) *via* a CVC since the age of 17 presented with anasarca and low grade fevers. Evaluation: Urinalysis: 2+ blood, no casts, 2.0 g proteinuria, creatinine (Cr) 1.9 (baseline 1.4 mg/dL),

albumin 2 gm/dL. HIV, RPR, ANA, hepatitis B, C screen were negative. Complements: low C3 68, low-normal C4 19, low CH50 <28 mg/dL; Echocardiogram: no vegetations. Blood cultures: *Staphylococcus epidermidis*. Kidney biopsy: MPGN type I. Fourteen years later, he had a similar presentation with recurrent MPGN.

**Conclusions:** Literature review identified 3 similar cases; in all, the CVC was used for TPN for short bowel syndrome. All cases had multiple CVC infectious episodes prior to the diagnosis of kidney injury. Renal presentations ranged from incidental microscopic hematuria, non-nephrotic proteinuria, +/- cellular/granular casts, to relatively rapid to insidious rise in Cr, ranging from 0.5-2 months, in association with active urinary sediments. Extra-renal manifestations included edema/anasarca, fevers, and/or palpable purpura (leukocytoclastic vasculitis). Complement levels were depressed in all but one case. Blood cultures in 3 out of 4 cases revealed *Staph. Epidermidis*. Following CVC replacement and antibiotic therapy, Cr, proteinuria, and hematuria improved in all patients. Proteinuria improved within days and Cr within 2 weeks. Complete kidney function occurred within 3-10 months. Recurrent CVC infection in association with recurrent MPGN supports a causal relationship by the former. MPGN is associated with repeated episodes of CVC infections and may be predicted with surveillance urinalysis, Cr, +/- complements. Kidney injury may herald or coincide with CVC infections.

## SA-PO632

### Membranoproliferative Glomerulonephritis with Background Non-Collapsing Focal and Segmental Glomerulosclerosis in Untreated HIV Infection

Zaid Brifkani,[1] Raafat Farag Makary,[2] Leighton R. James.[3] *[1]Dept of Medicine/Nephrology and Hypertension, Univ of Florida, Jacksonville, Jacksonville, FL; [2]Dept of Pathology and Laboratory Medicine, Univ of Florida, Jacksonville, Jacksonville, FL; [3]Dept of Medicine/Nephrology and Hypertension, Univ of Florida, Jacksonville, Jacksonville, FL.*

**Background:** Membranoproliferative Glomerulonephritis (MPGN) is uncommon in patients with HIV-associated Nephropathy (HIVAN). The most common feature in HIVAN is a picture of collapsing Focal Segmental Glomerulosclerosis (FSGS).

**Methods:** We report the case of a young African American man with a history of cocaine use, who presented to the hospital with worsening 1-month lower extremity edema , two years after being diagnosed with HIV. Apparently, he never started recommended therapy and was not on any medications at the time of admission. He was found to have reduced $CD4^+$ cells (81 cells/mL) and elevated creatinine (4.4 mg/dL). Urinalysis revealed blood (small) and protein (600 mg/dL). Urine microscopy showed white blood cells (9 cells/high power field [hpf]), red blood cells (2/hpf) and hyaline casts (12/hpf). He had nephrotic range proteinuria (24 gm/24 hours), but negative workup for SLE and Hepatitis. Accordingly, a kidney biopsy was performed. Light microscopy demonstrated mesangial sclerosis, thickened glomerular capillary basement membrane (GBM), marked tubular atrophy and marked tubular dilatation. Immunohistochemistry showed no specific immunoglobulins or complement deposits. Electron microscopy revealed mesangial expansion, increased cellularity, thickened endothelial GBM, with subendothelial electron dense deposits along with tubuloreticular inclusions in the endothelium of interstitial blood vessels, all consistent with MPGN and non-collapsing FSGS. The patient was started on combined antiretroviral therapy (cART) and has maintained stable renal function without need for renal replacement therapy or immunosuppression.

**Conclusions:** This case demonstrates a rare presentation of MPGN in the setting of HIVAN with background non-collapsing FSGS, and highlights the importance of early initiation of cART therapy.

## SA-PO633

### Successful Antiviral Tripel Therapy in a Longstanding Refractory Hepatitis C Virus (HCV) Infection with an Acute Exacerbation (MPGN I with Acute Kidney Injury and Cryoglobulinemic Vasculitis)

David Callau, Joerg Latus, Niko Braun, Mark Dominik Alscher, Martin Kimmel. *Dept of Internal Medicine, Div of Nephrology, Robert Bosch Hospital, Stuttgart, Germany.*

**Background:** The HCV infection is a very common disease with about 170 million chronically infected patients worldwide. In almost 80% of cases the acute infection passes into a chronic form with possible hepatic and extrahepatic manifestations. Before 2011 the standard therapy of a chronic HCV infection consisted of interferon alfa as a single therapy or in combination with ribavirin. After approval of the two protease-inhibitors boceprevir and teloprevir in 2011 the standard therapy for patients with a genotype 1 changed. In patients with chronic kidney disease (CKD) these therapies are not approved due to side effects of ribavirin and have so far not been evaluated in studies.

**Methods:** In april 2012 a 58 y/o female was admitted to the hospital with severe complications of a known chronic HCV infection. In the past the HCV infection has been treated ineffectively (partial non-response) with pegylated interferon alfa and ribavirin. Currently she presents with a cryoglobulinemic vasculitis and a membranoproliferative glomerulonephritis with an acute kidney injury. We decided to treat our patient with a boceprevir based tripel therapy which was continued for a total of 48 weeks due to a beginning liver cirrhosis. After 10 weeks the viral load was negative for the first time and renal function improved significantly during the therapy.

**Conclusions:** Limited data suggests that a therapy with ribavirin in patients with CKD seems to be save under close monitoring. Our patient with severe renal and extrarenal manifestations was treated successfully with a protease-inhibitor based tripel therapy. Nevertheless, it is necessary to plan an interventional study to evaluate the exact risk-benefit-profile of a tripel therapy regimen in patients with CKD and hepatitis C.

*Funding:* Private Foundation Support

## SA-PO634

### Clinical and Histopathologic Maintenance of Disease Remission off Eculizumab in Two Patients with Recurrent C3 Glomerulopathy

Jonathan J. Hogan, Andrew S. Bomback, Leal C. Herlitz, Pietro A. Canetta, Jai Radhakrishnan, Michael B. Stokes, Glen S. Markowitz, Vivette D. D'Agati, Gerald B. Appel. *Columbia Univ Medical Center, NY, NY.*

**Background:** Eculizumab, a monoclonal Ab against C5, has emerged as a potential treatment for C3 glomerulopathies (C3G). Duration of therapy with this agent, however, remains an unresolved issue.

**Methods:** In 2012, we reported the 1st trial of eculizumab in C3G. Six subjects received the drug for 1 year:2 failed treatment,2 were re-started on eculizumab due to relapse, and 2 subjects remained off the drug with no evidence of relapse.We reviewed their clinical course and protocol biopsies performed before,upon completion, and one year after completion of eculizumab therapy.

**Results:** Both patients were white males, aged 20 and 42, with recurrent C3G in the allograft diagnosed within 2 months prior to initiating eculizumab therapy. Maintenance immunosuppression of MMF and FK506 was unchanged while on and off eculizumab. Genetic and autoAb testing revealed a mutation in MCP and a C3NeF in patient 1; patient 2 had no detectable mutation or autoAb. Patient 1's Cr fell from 2.0 to 1.4 mg/dl by completion of therapy. One year later, Cr remained 1.2-1.4 mg/dl with no proteinuria. Biopsies done at completion of therapy and 1 year later showed mild mesangial proliferation with no evidence of endocapillary proliferation or exudative features. Patient 2's proteinuria fell from 10.6 g/g by UPCR to 1.8 g/g by completion of therapy with stable creatinine. One year later, UPCR remained 1.4 g/g. Biopsy done at completion of therapy showed decreased mesangial proliferation and resorption of electron dense deposits on EM compared to pre-treatment. Biopsy performed 1 year later showed similarly mild mesangial proliferation and further resorption of deposits. Notably, in both patients, the de novo IF staining for kappa-restricted $IgG_2$ and $IgG_4$ (binding of eculizumab to C5 in renal tissue), present in biopsies performed at completion of therapy, was no longer seen one year off therapy.

**Conclusions:** In 2 patients with recurrent C3G,1 year of treatment with eculizumab was associated with clinical and histopathologic remission that persisted after discontinuation of therapy.

*Funding:* Pharmaceutical Company Support - Alexion

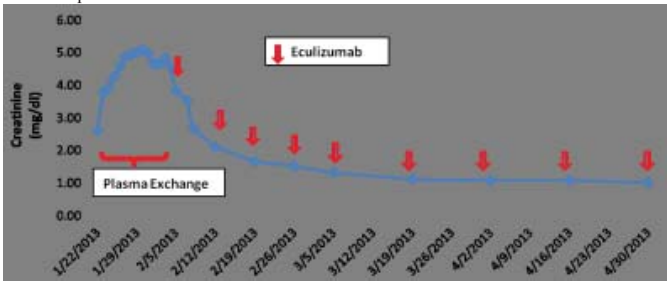
## SA-PO635

### Atypical Hemolytic Uremic Syndrome Treated with Eculizumab

Hilda E. Fernandez, Dan Negoianu. *Nephrology, Univ of Pennyslvania, Philadelphia, PA.*

**Background:** This case report of aHUS illustrates the efficacy of plasma exchange and Eculizumab in the treatment of thrombotic microangiopathy resulting from a mutation of MCP/CD46.

**Methods:** 23 yo male presented with non-bloody diarrhea and "wine-colored" urine with a rise in creatinine (Cr) to 1.9mg/dl. No prior history of renal dysfunction nor family history of renal failure. On exam, he was hypertensive with a blood pressure of 160/90, with 1+ non-pitting edema of legs. Laboratory findings included anemia (Hgb 8.6g/dL), thrombocytopenia (platelets 28/μL), and LDH 1800 U/L. C3 level was low (81mg/dL), and C4 was normal. Peripheral smear demonstrated schistocytes, shiga toxin was negative, and stool culture was negative for EColi O157:H7. ADAMST13 activity was normal. Genetic testing was performed as a send out lab. Patient was started on plasma exchange on hospital day #3 for concern for TTP/aHUS. Throughout hospitalization, the patient remained non-oliguric and did not require dialysis. Following daily plasma exchange for 2 weeks, LDH and platelet counts improved, though Cr remained elevated. He was started on Eculizumab weekly for 4 weeks, then every 2 weeks. Cr normalized to 1.01mg/dl, and LDH and platelet levels remained normal.




Genetic testing returned and demonstrated a CD46 (MCP) gene mutation of unknown significance at c.586G>A, p.Gly196Arg. The FH HUS Mutation Database lists this as a missense variant that indicates undetected C4b cofactor activity.

**Conclusions:** The use of Eculizumab was initiated as his creatinine had not improved to the same degree as his platelets and LDH following two weeks of plasma exchange. Prompt treatment with plasma exchange appeared to attenuate the disease, however initiation of Eculizumab allowed discontinuation of plasma exchange and was associated with further improvement of renal function and persistent remission of aHUS.

## SA-PO636

**Dermatomyositis-Induced Minimal Change Disease: A High Interferon State** Shailaja Chidella,[1] Hitesh H. Shah,[1] James M. Pullman,[2] Kenar D. Jhaveri.[1] *[1]Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY; [2]Pathology, Montefiore Medical Center/ Albert Einstein School of Medicine, Bronx, NY.*

**Background:** Dermatomyositis (DMS) is an autoimmune disease that characterized by involvement of proximal musculature and skin. Renal involvement is uncommon in dermatomyositis compared to other autoimmune disorders such as SLE. We report a patient who developed proteinuria in the setting of active DMS.

**Methods:** A 40-year-old Hispanic female was referred by rheumatology for evaluation of proteinuria. Three weeks prior to presentation, she had been diagnosed with DMS based on skin biopsy findings and systemic complaints. On presentation, she was normotensive and had trace lower extremity edema. She had normal lupus and other connective tissue serologies. A 24-hour urine collection revealed 2gm of proteinuria. Both serum albumin (3.7g/dl ) and serum creatinine were normal. Viral panels including hepatitis B and C, HIV, parvo virus B19 were negative. She has no history of diabetes mellitus or HTN. She denied any NSAID use. A kidney biopsy revealed normal light microscopy, normal IF but EM showed minimal change disease (MCD) with diffuse food process effacement. In addition, significant number of tubuloreticular inclusions (TRI) were noted. She had never received any interferon (IFN) therapy. Of all the connective tissue diseases, DMS has the highest IFN production. Given the podocyte effacement with presence of TRI, a secondary cause of MCD was considered. Given her recent diagnosis of DMS and new onset proteinuria, an association with DMS and MCD was made. At that point, she was started on oral steroids for treatment of DMS and MCD and her proteinuria is currently improving.

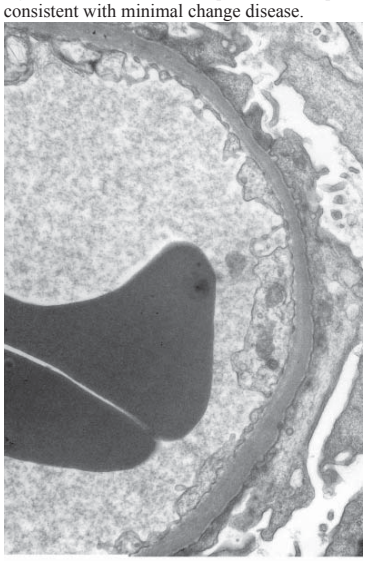
**Conclusions:** DMS is an autoimmune disease with varied systemic manifestations. Typically, it is not known to cause renal disease. We report a case of MCD secondary to DMS along with TRIs on kidney biopsy suggesting that active DMS is a high IFN signature state that can lead to the glomerular disease in these patients.

## SA-PO637

**Minimal Change Disease Associated with Waldenstrom's Macroglobulinemia** Olga Kuchmak[1] William L. Clapp,[2] A. Ahsan Ejaz.[3] *[1]Div of Nephrology, Hypertension and Transplantation, Univ of Florida, Gainesville, FL; [2]Dept of Pathology, Univ of Florida, Gainesville, FL; [3]Div of Nephrology, Hypertension and Transplantation, Univ of Florida, Gainesville, FL.*

**Background:** Waldenstrom's macroglobulinemia (WM) is a cancer of the B-lymphocytes, whereas minimal change disease (MCD) has been postulated to be associated with a Th2 immune response. MCD is common in children and WM is rare even in the adult predilection group. Occurrence of MCD associated with WM is rare, as described in this case report.

**Methods:** 73 year-old WM with history of diastolic dysfunction presented with generalized anasarca and recent 50 lbs weight gain. Labs were significant for the following: BUN 52mg/dL, Creat 2.58mg/dL, Total Prot 5.7g/dL, Alb 0.7g/dL, normal C3, C4, negative serology for hepatitis, HIV, ANCA and ANA. UA showed protein 3+, WBC 11/hpf, RBCs 2/hpf; random UPCr 12; UPEP and SPEP were positive for M (monoclonal) spike. Immunofixation electrophoresis revealed an IgM kappa monoclonal antibody and serum IgM level was 2,952 mg/dL. Subsequent bone marrow biopsy revealed mildly increased kappa predominant plasma cells present. Flow cytometry identified a small, kappa-predominant CD20(+) B-cell population. Overall findings were compatible with Waldenstrom's macroglobulinemia. A renal biopsy was performed. Light microscopy showed mild mesangial expansion. On immunofluorescence, kappa and lambda stains were approximately equal in intensity and distribution. Electron microscopy findings were significant for extensive foot process fusion presence with microvillous formation, features consistent with minimal change disease.



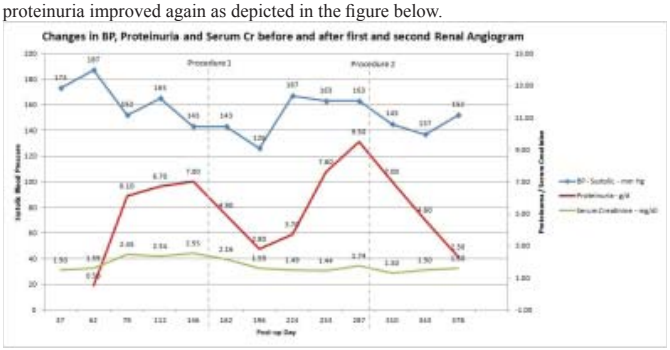
**Conclusions:** We report a rare case of MCD associated with WM. Although there was no evidence of complement activation by IgM or of Th2 response, the concurrence of MCD and WM points to the emerging role of immune response and immunoglobulins in renal diseases.

## SA-PO638

**Focal Segmental Glomerulosclerosis due to Transplant Renal Artery Stenosis** Salima Sadruddin, Anne L. King, Gaurav Gupta. *Nephrology, Virginia Commonwealth Univ, Richmond, VA.*

**Background:** Unilateral renal artery stenosis can result in Focal Segmental Glomerulosclerosis (FSGS) in the contra-lateral kidney due to hyperfiltration. Rarely FSGS has been diagnosed in an allograft with multiple arteries and a significant stenosis in a branch of the transplant renal artery.

**Methods:** A 45 year old white male with stage 5 chronic kidney disease due to diabetes underwent a pre-emptive deceased donor kidney transplant (KTx). Day 0 donor biopsy did not show any evidence of FSGS. The patient's serum creatinine plateaud at 1.5mg/dL within one month post-KTx and hypertension was controlled without medication. His blood pressure started rising after 6 weeks post-KTx and he developed nephrotic proteinuria of 7g/d. Allograft biopsy demonstrated FSGS with negative immunofluorescence and segmental podocyte effacement on electron microscopy. Due to absence of prior history of FSGS and presence of an accessory transplant renal artery a diagnosis of transplant renal artery stenosis (TRAS) was considered. A renal angiogram demonstrated upper pole TRAS which was successfully treated with angioplasty and drug-eluting stent placement. This was followed by a rapid improvement in proteinuria, renal function and blood pressure. Two months after the procedure, the patient's blood pressure and proteinuria again started to rise. A second renal angiogram showed in-stent restenosis as well as stenosis of another previously angioplastied lesion. It was once again successfully treated. The patient was switched to prasugrel from clopidogrel. He was continued on aspirin. Hypertension and proteinuria improved again as depicted in the figure below.




**Conclusions:** We report a case of FSGS due to the stenosis of a branch of the transplant renal artery. TRAS should be considered in the differential diagnosis of de-novo FSGS in patients with multiple transplant renal arteries.

## SA-PO639

**Focal Segmental Glomerulosclerosis Complicated by Hepatitis B Infection and Rheumatoid Arthritis** Kristin Mercado Luzentales, Beatrice P. Concepcion. *Section of Nephrology, Philippine General Hospital, Manila, Philippines.*

**Background:** Hepatitis B virus-associated nephropathy usually presents as membranous nephropathy (MN). Studies have reported a strong association between hepatitis B and MN. It is rarely associated with focal segmental glomerulosclerosis (FSGS). Rheumatoid arthritis (RA) has been associated with MN, amyloidosis, mesangioproliferative GN and diffuse proliferative GN but not with FSGS.

**Methods:** A 48-year old female consulted due to joint pains, tea-colored urine, edema and hypertension. Laboratory tests revealed anemia, azotemia, proteinuria and hematuria. Initial impression was systemic lupus erythematosus but antinuclear antibody test was negative. Other work-up revealed nephrotic-range proteinuria, hepatitis B surface antigenemia and high rheumatoid factor levels. HBV DNA was undetectable. Liver function tests and ultrasound were normal. She was diagnosed with RA and was given low-dose Prednisone. She was referred to a gastroenterologist for clearance prior to starting Methotrexate. She was given Lamivudine while on Methotrexate. Kidney biopsy revealed collapsing FSGS. Screening for HIV was negative. She was initially treated for idiopathic FSGS with high-dose steroids, with no response. Three months later, she had elevated levels of HBV DNA, hence Adefovir was added. On review of the biopsy, there was segmental foot process effacement, which is more consistent with secondary FSGS. Steroids were tapered off. Methotrexate was shifted to Hydroxycholoroquine. There was decreasing proteinuria, azotemia and HBV DNA levels six months later.

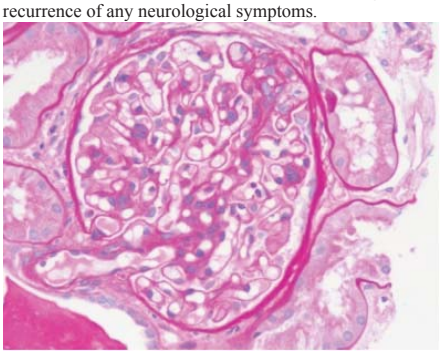
**Conclusions:** In the 8 cases reported before where FSGS was complicated by hepatitis B infection, HBV antigen was demonstrated in the glomeruli or tubules by immunostaining. In our case, this cannot be done due to its inavailablility in our country. It is well-known that drugs used in the treatment of RA can cause FSGS but there is only one reported case of RA associated with FSGS. This is the first reported case of FSGS complicated by hepatitis B infection and RA. Improvement was seen with treatment of both hepatitis B and RA. Treatment is challenging because the drugs used for RA could worsen hepatitis B infection.

**SA-PO640**

**Tip Variant Focal Segmental Glomerulosclerosis (FSGS) Associated with Interferon-β Treatment of Multiple Sclerosis (MS)**
Rhys David Russell Evans, Pandora Rudd, Andrew Rennie Hall, Mark Harber. *Whittington Hospital and UCL Centre for Nephrology, London, United Kingdom.*

**Background:** The nephrotic syndrome is a rare complication of treatment with Interferon. Minimal change disease and collapsing FSGS have been reported to occur in association with Interferon-b. We report a case of the nephrotic syndrome caused by tip variant FSGS in the setting of Interferon-b treatment of MS.

**Methods:** A 43 year old woman presented to the emergency department with swelling of her hands and feet. She had MS and had been treated with Interferon-b for the last 15 months without significant side effect. Other medical problems included migraine, oesophagitis, obesity and depression. She had been well until one week prior to presentation when she developed swelling of her hands and feet. She was hypertensive (199/110) with pitting oedema to the knee. Urine dipstick was positive for blood (3+) and protein (4+). Her creatinine was 64umol/L with an albumin of 27g/L, cholesterol 8.8mmol/L, and urinary protein:creatinine (PCR) 1284mg/mmol consistent with the nephrotic syndrome. ESR was 62mm/hour (CRP 8mg/L) with an otherwise unremarkable renal screen. A renal biopsy demonstrated tip variant FSGS. Her Interferon was held and she was treated with an ACE inhibitor, diuretics and tacrolimus. Her proteinuria and albumin have improved with latest urinary PCR 100mg/mmol and albumin 30g/L. Her creatinine has risen to 119umol/L in association with the above. Her oedema is resolving and she remains well in herself without recurrence of any neurological symptoms.



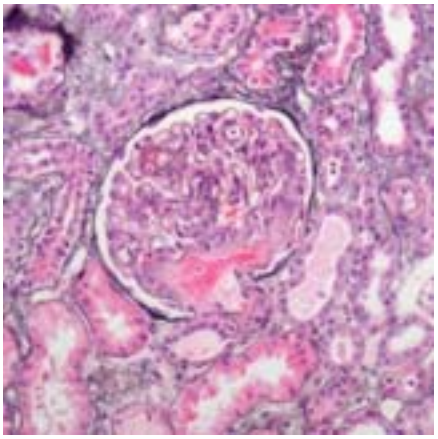
**Conclusions:** Minimal change disease and collapsing FSGS are rare complications of Interferon-b treatment of MS. This is the first report of the nephrotic syndrome caused by the tip variant of FSGS in this setting.

**SA-PO641**

**Thrombotic Microangiopathy in a Patient Treated with a Protease Inhibitor Based Regimen for Hepatitis C**
Rhys David Russell Evans, Mathena Pavan, Deepak Suri, Mark Harber. *Whittington Hospital, London, United Kingdom.*

**Background:** Current evidence advocates the use of triple therapy (pegylated interferon, ribavarin and a protease inhibitor) for the management of Hepatitis C genotype 1. We report a case of thrombotic microangiopathy in the setting of triple therapy with full recovery of renal function in response to cessation of antivirals and plasma exchange.

**Methods:** A 53 year old woman presented to the emergency department having collapsed in the hepatology outpatients. She had been started on peginterferon alpha, ribavirin and telaprevir for treatment of Hepatitis C 12 weeks prior to admission. The treatment had been complicated by a rash but had been otherwise well tolerated with undetectable Hepatitis C RNA at 4 weeks. She described a one-week history of fatigue, nausea and vomiting. She had a diffuse maculopapular rash extending from her trunk to her limbs but examination was otherwise unremarkable. Her creatinine had risen from a normal baseline to 444umol/L, with low haemoglobin (6.4g/dL) and platelets (88 x$10^9$/L). Her urinalysis was positive for blood (1+) and protein (3+). She had an elevated LDH (1147 iU/L), undetectable haptoglobins and red cell fragments on blood film. Renal biopsy demonstrated microthrombi and fragments in the glomeruli and small vessels and a diagnosis of thrombotic microangiopathy (TMA), thought to be a complication of her hepatitis C treatment, was made. She had a negative screen for other causes of TMA.



Her antivirals were discontinued and she was treated with 7 sessions of plasma exchange. This resulted in complete resolution of her acute kidney injury and improvement in her thrombocytopaenia.

**Conclusions:** Whilst TMA complicating interferon treatment of Hepatitis C has been documented, this is the first description of TMA complicating triple therapy. Protease inhibitors may exacerbate the side effects of interferon.

**SA-PO642**

**Diabetic Nephropathy without Diabetes: A Case Report**
Sara A. Combs, J. Pedro Teixeira, M. Scott Lucia, Richard J. Johnson. *Univ of Colorado, Aurora, CO.*

**Background:** Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in the US. We present a case of a 45 year-old Hispanic man with no known history of diabetes who presented with renal failure and nephrotic syndrome and had advanced DN on biopsy.

**Methods:** The patient presented to the ED with progressive dyspnea, chest pain, and lower extremity swelling over 3-4 weeks. Exam revealed a blood pressure of 220/100 mm Hg and generalized edema. His BMI was 29.9 kg/m2, though he reported a 50- to 100-pound weight loss in the past few years. Serum creatinine was 7.5 mg/dL (eGFR by MDRD of 8 mL/min), potassium 5.8 mEq/L, bicarbonate 15 mEq/L, serum albumin 2.0 g/dL, BUN 101 mg/dL, phosphorus 6.1 mg/dL, calcium 6.8 mg/dL, and hemoglobin 9.3 g/dL. Urine microscopy revealed 10 to 20 RBCs per hpf with few granular casts. Urinary protein was measured at 11 g by 24 hour collection. Fasting glucose was in the normal to impaired range (average 94, range 80-110 mg/dL) with normal random glucose levels. Hemoglobin A1c was 5.9%. SPEP and UPEP, serum lambda/kappa ratio, HIV and HCV antibodies, HBV surface antigen, and ANA were all negative. Renal ultrasound revealed normal kidney size and echogenicity. Hemodialysis was initiated with improvement in the patient's symptoms. Renal biopsy demonstrated marked nodular glomerulosclerosis, felt to be consistent with advanced DN. Dilated fundoscopic exam revealed severe bilateral diabetic retinopathy.

**Conclusions:** Advanced diabetic glomerulosclerosis in a subject without a known history of diabetes is rare. Some conditions such as light chain nephropathy may have a histologic lesion similar to diabetes. In this case, the presence of diabetic retinopathy makes true diabetic glomerulosclerosis likely. We suspect that he had diabetes but that it reversed with his weight loss, which may have been precipitated by his progressive renal failure. Decreased insulin clearance from the loss of GFR could have also contributed to the normalization of glucose levels. Clinicians should be aware that undiagnosed diabetes may remit leaving advanced retinal and diabetic disease that may be the first manifestations of the prior diabetic condition.

**SA-PO643**

**Membranous Nephropathy as a Manifestation of Chronic Graft versus Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation**
Yul Hee Cho, Ji Hee Lim, Min Young Kim, Myung Hyun Lee, Ji Hyun Yu, Keun Suk Yang, Seun Deuk Hwang, Chul Woo Yang, Yong-Soo Kim, Cheol Whee Park. *Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, College of Medicine, Seoul, Republic of Korea.*

**Background:** Membranous nephropathy (MN) is the most common glomerular disease after allogeneic hematopoietic stem cell transplantation (HSCT). The pathogenesis of MN after allogeneic HSCT is not well understood. We described the clinical and histopathological characteristics and outcomes of 12 patients with post HSCT MN.

**Methods:** We investigated the renal pathologic and clinical findings of 12 patients who developed MN after HSCT at Seoul St. Mary's Hospital. We also measured the anti-PLA2R (M-type phospholipase A2 receptor) antibodies using commercial antigen specific ELISA kit in the serum samples from the seven patients at the time of renal biopsy.

**Results:** Of the 12 patients, all had chronic Graft Versus Host Disease (cGVHD) and indication of biopsy was proteinuria (>1g/day) without microscopic hematuria, in 58% (7/12) of patients with nephrotic syndrome. Overall response rate, including complete remission (urinary protein level < 0.3g/day)and partial remission (urinary protein level

0.31-3.4g/day), was 83%. Two patients did not respond to treatment. The median follow-up period was 26 months and none of them developed end stage renal disease. All of the seven patients with MN after HSCT had negative for anti-PLA2R ELISA assay.

**Conclusions:** MN after HSCT is the most common of manifestation of cGVHD of renal disease. Eight-three percent of patients responded either partially or completely to immunosuppressive treatment. Anti PLA2R antibodies were all negative in these patients. These results suggest that MN after HSCT is one of characteristics of cGVHD and has a different pathogenesis from idiopathic MN.

## SA-PO644

**Membranous Nephropathy Post Allogeneic Haematopoietic Stem Cell Transplant – A Case Series** Karen Lok Yee Keung,[2] Subramanian K. Kumar.[1] *[1]Nephrology, Gosford Hospital, Gosford, NSW, Australia; [2]Nephrology, Westmead Hospital, Westmead, NSW, Australia.*

**Background:** Membranous nephropathy is a rare complication following allogeneic haematopoietic stem cell transplantation, and clinical observations to date suggest an association between chronic graft versus host disease (cGVHD) and membranous nephropathy in this setting. Whether this is a secondary process and perhaps a manifestation of graft versus host disease in the kidney is difficult to verify. Primary membranous nephropathy is a possibility, but there have been no published case reports of serum PLA2R antibody testing in these patients.

**Methods:** Here, we describe 5 cases of membranous nephropathy following allogeneic haematopoietic stem cell transplantation; serum anti-PLA2R antibody testing was available at the time of diagnosis for two of these patients.

**Results:** A history of cGVHD involving variable sites was identified in all five patients. All patients were treated with prednisolone with or without other agents after biopsy proven membranous nephropathy. At the time of last follow up, two patients had developed end stage renal failure and were dialysis dependent; two achieved complete remission with one remaining on low dose prednisolone alone, whilst the other was on a tapering immunosuppression regime. The fifth patient was in remission but required multiple agents to maintain this. In both patients who underwent testing for serum anti-PLA2R antibody, the result was negative.

**Conclusions:** The presence of a cGVHD history in all five of our patients with membranous nephropathy supports the findings in other cases series that there is likely an association between the two, but the exact nature of this relationship is difficult to establish. The absence of serum anti-PLA2R antibody in both patients in which the test was performed, suggests an alternative antigen to PLA2R is the target in patients with membranous nephropathy post allogeneic haematopoetic stem cell transplant. Furthermore, since the presence of the anti-PLA2R antibody supports the diagnosis of primary membranous nephropathy, the negative result in both cases would support the diagnosis of membranous nephropathy in this clinical context as a secondary process.

## SA-PO645

**Response to Oral Corticosteroids in Relapsing Membranous Nephropathy with Acute Kidney Injury following Influenza Vaccine** Chinmay P. Patel, Hitesh H. Shah. *Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY.*

**Background:** Membranous nephropathy (MN) is one of the most common forms of nephrotic syndrome in adults. Here, we describe a unique case of relapsing MN with acute kidney injury (AKI) following 2009 H1N1 influenza vaccine with management and long term follow-up.

**Methods:** A 60-year-old female presented with 1 week history of acute onset lower extremity edema 5 days after receiving 2009-10 influenza vaccine. Patient had refused influenza vaccination in the past as her father had died from Guillain-Barre syndrome after receiving influenza vaccine. She denied NSAID use. Physical exam was significant for hypertension and lower extremity edema. Laboratory data revealed AKI with serum creatinine (Scr) of 10.2. Her spot urine total protein to creatinine ratio (TP/CR) was 23. Serological workup and age appropriate cancer screening was negative. Kidney biopsy showed stage 1 MN with acute interstitial nephritis (AIN). She was started on daily oral prednisone (0.75mg/kg) with tapering doses over the next 2 months. 5 weeks after initiation of oral prednisone, her Scr had normalized (0.9) and spot urine TP/CR decreased to 1.3. However 1 week after completion of prednisone therapy, patient was found to have elevated Scr of 2.3. Her spot urine TP/CR increased to 28. At this point, patient underwent repeat kidney biopsy which showed MN with resolution of AIN. Daily oral prednisone was restarted at 0.75 mg/kg and tapered over the next 4 months. One month after initiation of steroids, her Scr decreased to 0.9 and spot urine TP/CR decreased to 0.2. Since then, she has refused further influenza vaccinations. At 3 year follow-up, our patient continues to remain in complete clinical remission with stable renal function and spot urine TP/CR of 0.1. Therefore, we strongly believe that abrupt onset MN in our patient was secondary to the activation of immune response triggered by the influenza vaccine.

**Conclusions:** To the best of our knowledge, this is the first described case of relapsing MN with AKI following influenza vaccination. Based on our experience, one can consider a course of oral corticosteroids in patients who develop MN and AKI after influenza vaccine.

## SA-PO646

**Syphilis-Associated Membranous Nephropathy Mimicking Class V Lupus Nephritis** Chinmay P. Patel,[1] Aditya Kadiyala,[1] James M. Pullman,[2] Hitesh H. Shah,[1] Kenar D. Jhaveri.[1] *[1]Hofstra North Shore-LIJ School of Medicine, Great Neck, NY; [2]Montefiore Medical Center, Bronx, NY.*

**Background:** Syphilis is a well known cause of secondary membranous nephropathy (MN), but is not commonly diagnosed in the US. We present a case of secondary syphilis presenting with acute onset nephrotic syndrome with unusual kidney biopsy findings.

**Methods:** A 21-year old Caucasian male was admitted with a 3 day history of worsening anasarca, facial edema & 10 lbs weight gain. He was recently diagnosed with a rectal fissure and was started on mesalamine for a presumed diagnosis of inflammatory bowel disease. He was homosexual. On physical exam, he was thin built, normotensive & had periorbital puffiness. Groin area showed bilateral erythematous macular rash. There was no adenopathy & the rest of the physical examination was unremarkable. Pertinent admission laboratory data showed normal CBC, liver enzymes & lipid panel. Serum creatinine was 1.1mg/dl & serum albumin was 2.1g/dl. A 24 hour urine revealed 15 gms of protein. Serological workup was negative for hepatitis B, C, HIV, ANA, & Anti-dsDNA antibodies. C3, C4 were in normal range. Patient had a positive RPR and FTA-Ab. In his kidney biopsy, LM showed thickening of glomerular basement membranes with no other changes. IF showed a "full-house " pattern with granular staining in the capillary walls & mesangium for IgG, IgA, IgM, C3 and C1q. EM showed extensive foot process effacement & many small subepithelial electron dense deposits diagnostic of MN. Clinical criteria for SLE were otherwise absent. The final diagnosis was syphilis-related MN with similarities to class V lupus nephritis. The patient's nephrotic syndrome went into remission within 2 weeks of treatment with 3 doses of benzathine penicillin, supporting syphilis as a primary cause. Six months after treatment, the patient remains in remission.

**Conclusions:** The presence of a "full-house" IF pattern on renal biopsy, usually characteristic of lupus nephritis, did not preclude the diagnosis of syphilis-associated MN. Despite its rare occurrence, syphilis associated renal diseases should be considered in the differential diagnosis of "lupus-like nephritis" especially in high-risk patient groups.

## SA-PO647

**Hypereosinophilic Syndrome Associated with Membranous Nephropathy– Third Case in the Literature** Shiba Khorsandi,[1] Salvatore Chillemi,[1] Arshia Abbasi,[1] Nitin Behl,[1] Deepthi Karanam,[1] Reza Amerinasab,[1] Tatyana Gavrilova,[1] Michael Yudd,[1] Joshua Kaplan.[1] *[1]Nephrology, UMDNJ; [2]Nephrology, UMDNJ; [3]Nephrology, UMDNJ; [4]Nephrology, UMDNJ; [5]Nephrology, UMDNJ; [6]Nephrology, UMDNJ; [7]Nephrology, UMDNJ; [8]Nephrology, UMDNJ; [9]Nephrology, UMDNJ.*

**Background:** The Hypereosinophilic Syndrome (HES) is a group of disorders characterized by persistent eosinophilia without an identifiable cause, and usually with eosinophil-mediated end organ damage. Skin, gastrointestinal, heart, and lung involvement are common, renal manifestations are rare. We report a case of HES with Membranous Glomerulopathy.

**Methods:** A 63 year old white man presented with pruiritc diffuse erythematous dermatitis and eosinophilia with no identifiable cause. He had mild eosinophilia during the previous 10 years. Skin biopsy suggested psoriasiform dermatitis. Four months later he had explosive new-onset nephrotic range proteinuria (9 g/day) with serum creatinine 1.3mg/dl. Absolute eosinophils were 1400/ mm3 and peaked at 4300/mm3 over one year. Serum IgE was > 50,000 IU/ml. Studies including, ANA, anti-DNA, ANCA, serum/urine protein electrophoresis, complement, hepatitis B/C serologies, and HIV were negative or normal. Studies for parasites and malignancy were negative, and he was on no medications. Bone marrow showed increased eosinophils (30%) without abnormal myeloid maturation, increased blast population or a lymphoproliferative disorder (absence of FIP1L1-PDGFRA, JAK2-V617F, BCR-ABL and 4q12 mutations). Renal biopsy showed classic Membranous Nephropathy (MN). Tubulointerstitial nephritis with eosinophils was not present. Cyclosporine and steroids induced a full remission of nephrotic syndrome and resolution of erythema and eosinophilia.

**Conclusions:** This is the third case in the medical literature describing the association of membranous nephropathy with HES, in this case HES lymphocytic variant. The close temporal onset of both HES and MN, and their resolution with treatment suggest a close relation, not merely a coincidental association. Both HES and the nephrotic syndrome responded to cyclosporine and steroids. The etiologic link of the 2 syndromes is unknown.

*Funding:* Private Foundation Support

## SA-PO648

**IgG4-Related Tubulointerstitial Nephritis with Minimal Change Disease** Shiba Khorsandi,[1] Joshua Kaplan,[1] Michael Yudd,[1] Nitin Behl,[1] Arshia Abbasi,[1] Deepthi Karanam,[1] Salvatore Chillemi,[1] Reza Amerinasab.[1] *[1]Nephrology, UMDNJ; [2]Nephrology, UMDNJ; [3]Nephrology, UMDNJ; [4]Nephrology, UMDNJ.*

**Background:** Immunoglobulin G4 (IgG4)-related tubulointerstitial nephritis (TIN) is a newly recognized clinicopathologic entity. We report a rare case of IgG4-related TIN with minimal change disease in a patient who has Sjogren's disease.

**Methods:** A 44 year-old Indian woman presented with bilateral leg swelling. Past medical history included autoimmune hepatitis, in remission on prednisone 5 mg daily. Her blood pressure was 140/77 mm Hg, and the physical examination was unremarkable other than 2+ pitting edema in her lower extremities. She was found to have proteinuria (17.9 g/24h). Serum Cr was 2.7 mg/dl with baseline Cr level 0.8 mg/dL. Antinuclear

antibodies titer was > 1:640. Anti-SSA and SSB antibody were elevated, confirming the diagnosis of Sjogren's disease. Complement studies showed normal C4 and decreased C3. Anti-dsDNA,HIV, hepatitis B/C were undetectable. Serum IgG level was high. Urinalysis revealed 3+protein with no red or white blood cells. Renal biopsy showed extensive foot process effacement and dense interstitial plasma cell infiltrates. No immune complex mediated glomerular lesions are identified. Staining for IgG4 was positive in plasma cells. She received pulse methylprednisolone 1 gram for 3 days, then prednisone 60 mg daily, which was slowly tapered down to 10 mg daily over one year. Cr decreased to 0.9 mg/dL and proteinuria to 1.3 g/24h after 3 months. Serum IgG level dropped and clinical symptoms improved.

**Conclusions:** A less commonly cause of TIN is within the IgG4 related systemic diseases that have been observed in many organ systems. More recently, it has been described in the kidney and typically presents with a gradual decrease in kidney and minimal or no proteinuria. We describe a rare case presenting with significant proteinuria and decreased GFR, with a kidney biopsy showing IgG4-related TIN superimposed on minimal change disease. One hypothesis is that the proliferating plasma cells produce IgG4 that is auto reactive against podocyte antigens. The mainstay of treatment is glucocorticoids, and nephrologists need to be aware of this entity that responds well to steroid therapy.

## SA-PO649

**Kaposi's Sarcoma in a 78-Year-Old Female with Membranous Glomerulonephritis** Hanna Bartosik,[1] Magdalena Krajewska,[1] Agnieszka Halon,[2] Krzysztof Letachowicz,[1] Katarzyna Jakuszko,[1] Katarzyna Madziarska,[1] Waclaw Weyde,[1,3] Marian Klinger.[1] *[1]Dept of Nephrology and Transplanatation Medicine, Wroclaw Medical Univ, Poland; [2]Dept of Pathomorphology and Oncological Cytology, Wroclaw Medical Univ, Poland; [3]Faculty of Dentistry, Wroclaw Medical Univ, Poland.*

**Background:** Nephrotic syndrome occurs in elderly patients, mainly as a secondary manifestation of vasculitis or neoplasm. In nephrology Kaposi's sarcoma is usually connected with immunosuppressive treatment in transplant recipients.

**Methods:** A 78-year-old Caucasian HIV-negative female with the history of diabetes mellitus and atheromatosis was admitted to Nephrology Department due to severe nephrotic syndrome (total protein level 41 g/l, albumin level 14 g/l) which had occurred 2 months earlier. The patient was treated with ACE inhibitors and diuretics. ANA, ANCA vaculities were excluded. Anti PLA2R antibodies were negative twice. All performed diagnostic tests did not reveal possible cause of nephrotic syndrome. Kidney biopsy was performed showing membranous nephritis of mild expression (the first stage in histological classification). Due to severe course of the disease and acute kidney injury (creatinine level 4.1 mg/dl) intravenous steroids, diuretics together with albumin were introduced. ACE inhibitors were stopped and cyclosporine treatment was started. Due to deterioration of kidney function and no remission of nephrotic syndrome cyclosporine was substituted with a low-dose of tacrolimus (2 mg per day with blood level 4 ng/ml). After 2 weeks of treatment improvement was noticed. After next 2 weeks skin changes were found, mainly on the left lower limb. Biopsy was taken. The diagnosis was Kaposi's sarcoma. A dose of tacrolimus was reduced to 1.5 mg per day and everolimus treatment was started 10 days later (0.75 mg per day). After 3 weeks of the regimen regression of sarcoma is observed. Remission of nephrotic syndrome and mild renal function impairment (creatinine level 1.7 mg/dl) is still maintained.

**Conclusions:** There is still a question whether Kaposi's sarcoma is connected with immunosuppressive treatment or membranous glomerulopathy is the first sign of the disease?

## SA-PO650

**Lupus Nephritis with a False Positive Antiglomerular Basement Membrane Antibody** Ekamol Tantisattamo,[1] Satyanarayana Chekuri.[2] *[1]Renal Div, Emory Univ School of Medicine, Atlanta, GA; [2]Dept of Medicine, Univ of Hawaii John A. Burns School of Medicine, Honolulu, HI.*

**Background:** Antiglomerular basement membrane (anti-GBM) disease is a rare cause of rapidly progressive glomerulonephritis (RPGN). It can be diagnosed by renal pathology or positive serum anti-GBM antibody (Ab). However, false positive anti-GBM Ab may occur and lead to delay in diagnosis and treatment of other medical conditions. We report a case with acute kidney injury (AKI) and positive serum anti-GBM Ab, but renal biopsy showing lupus nephritis (LN) class V.

**Methods: Case Description:** A 33 year-old man with history of nephrotic range proteinuria of 5.7 g/day lost to follow up for 5 years. He presented with progressive painless scrotal swelling for 3 weeks. Physical exam showed marked inflamed scrotum consistent with cellulitis. His BP was 123/75 mmHg. He was found to have AKI with a serum creatinine (SCr) of 6.8 mg/dL. Baseline SCr was 2.1 mg/dL 7 months earlier. He refused renal biopsy. Laboratory workup revealed positive ANA and anticardiolipin antibodies. C3 was low at 66 mg/dL and C4 was normal. Anti-dsDNA and ANCA were negative. RPR was reactive and FTA-ABS confirmed syphilis. He was treated for presumed late latent syphilis. Urine exam showed hematuria and 24-hour urine protein was 31 g. Anti-GBM Ab returned positive; as a result, plasmapheresis and pulse methylprednisone were started. Renal function had not improved after 1 week of treatment, and intermittent hemodialysis was initiated due to hyperkalemia. He finally underwent a renal biopsy which confirmed LN class V. Plasmapheresis was discontinued, and he was treated for lupus nephritis with intravenous cyclophosphamide and oral prednisone. SCr improved to 2.5 mg/dL.

**Conclusions:** Being a useful diagnostic tool for anti-GBM disease, serum anti-GBM Ab could be false positive. This leads to therapeutic management with highly potential complications, and delays definite diagnosis if renal biopsy is not performed. Renal biopsy in patients with a positive serum anti-GBM Ab especially whose clinical presentations are not typical for anti-GBM disease is warranted to confirm the definite diagnosis and prompt an appropriate treatment.

## SA-PO651

**How to Interpret Repeat Renal Biopsies in Lupus Nephritis? Lessons from the Transplantation of a Donor Kidney with Lupus Nephritis** Michael McRae, Mathieu Rousseau-Gagnon, Isabelle Houde, Julie Riopel, David Philibert, Sacha A. De Serres. *CHUQ - Hotel-Dieu de Quebec, Univ Laval.*

**Background:** The relevance of repeat biopsy in patients with lupus nephritis (LN) is controversial. Recent recommendations from EULAR/ERA-EDTA state that it provides useful information, suggesting a 6-month biopsy as an important adjunct to treatment. From a pathological standpoint, little is known about the time needed to clear immune deposits and proliferation within the kidney after successful treatment.

**Methods:** We describe the histological evolution of a kidney from a donor with proliferative mesangial LN at the pre-implantation biopsy, transplanted to a recipient with ESRD due to vesico-ureteral reflux. This case provides an opportunity to assess the clearance of immune lesions over time in a kidney explanted from its lupus environment.

**Results:** The donor was a 50-year old male, with past medical history limited to high blood pressure. Urine sediment showed mild proteinuria, but no hematuria. At pre-implant biopsy (Bx) there was 4% glomerulosclerosis, moderate mesangial enlargement, and mild hypercellularity, tubular atrophy and interstitial fibrosis. Immunofluorescence (IF) revealed moderate to strong mesangial IgG, IgA, IgM, C1q and C3. Electron microscopy (EM) showed numerous mesangial immune complex deposits and a single tubuloreticular inclusion. The recipient was a 26-year old man who received induction with daclizumab and maintenance therapy with prednisone, mycophenolate mofetil and tacrolimus. A for-cause Bx at 1 month posttransplant showed light microscopy findings similar to pre-implant Bx, and a slight attenuation of all IF stainings. A protocol Bx at 8 months showed stable mesangial expansion, but persistent IgM, C3 and C1q staining. A for-cause Bx at 33 months displayed normal mesangium and completely negative IF. Nine years post transplant, the patient maintains a stable renal function.

**Conclusions:** This case suggests that the resolution of histological LN lesions is a slow process. Persistent lesions after 6 months of treatment may be seen without ongoing systemic immune injury. Caution is advised in the interpretation of early biopsies following initial therapy for LN.

## SA-PO652

**Risks of Steroid Therapy in Lupus Nephritis – 3 Cases of Central Serous Retinopathy** Raquel Vaz,[1] Maria Francisca Barros,[1] Ana Teresa Nunes,[1] Inês Castro Ferreira,[1] Ricardo Neto,[1] Edite M. Pereira,[2] Eva Borka Mariz,[3] Isabel Tavares.[1] *[1]Nephrology, CHSJ, Porto, Portugal; [2]Internal Medicine, CHSJ, Porto, Portugal; [3]Rheumatology, CHSJ, Porto, Portugal.*

**Background:** Steroids are highly effective but associated with important morbidity. Central serous retinopathy (CSR), resulting from fluid accumulation behind neurosensory retina, is a rare but reversible condition described during treatment with steroids. We report 3 cases.

**Methods:** Case1:A 27-year-old female presented in May 2012 with lupus nephritis(LN) class IV and thrombotic microangiopathy(TMA), treated with 3 pulses of methylprednisolone(MP) followed by oral prednisolone(PDN) 1mg/Kg/day, intravenous(IV) cyclophosphamide(CYC) and plasma exchange(PE). As no improvement was observed she was given rituximab.TMA eventually remitted but she developed bilateral CSR. PDN was tapered to 7.5mg/day and mycophenolate mofetil(MMF) added. Eye lesions resolved and she mantains renal and haematological remission. Case2:A 32-year-old female developed LN class IV+V in 2010 treated with IV CYC and PDN 1mg/Kg/day. CYC was later switched to MMF, by then on PDN 20mg/day. 7 months later CSR was diagnosed. PDN was reduced to 2.5mg/day. Her eye symptoms improved but lupus disease exacerbated. Tacrolimus was added to MMF and PDN withdrawn. 1year later she is in renal and extrarenal remission, without new eye lesions. Case3: A 46-year-old female was admitted in 2001 with LN class IV. She was treated with 3 pulses of MP followed by oral PDN(1mg/Kg/day) and IV CYC. In 2005 PDN dose was increased and azathioprine(AZA) initiated due to renal flare. In 2007 she had a new flare PDN was increased and AZA switched to MMF. She was kept on low-dose PDN and stopped MMF in 2012. In January 2013 she presented with a new flare. MMF was restarted and PDN dose increased(0.5mg/Kg/day). She developed CSR and PDN was reduced to 10mg/day. Eye lesions improved and she maintains renal remission.

**Conclusions:** Discontinuation or reduction of steroid dose is essential in approach CSR and differential diagnosis with inflammatory disorders or toxicity from other drugs is mandatory. CSR highlights the need for new and less toxic therapies for lupus, including steroid sparing strategies.

## SA-PO653

**Catastrophic Antiphospholipid Syndrome in a Small Child** Marissa J. Defreitas, Alcia D. Edwards- Richards, Vimal Master Sankar Raj, Jayanthi Chandar, Wacharee Seeherunvong, Asumthia S. Jeyapalan, Gaston E. Zilleruelo, Carolyn L. Abitbol, Chryso P. Katsoufis. *Pediatric Nephrology, Univ of Miami/Holtz Children's Hospital, Miami, FL.*

**Background:** Catastrophic antiphospholipid syndrome (CAPS) is rare but associated with high mortality. We report a child with CAPS and multi-organ system failure with hepatic thrombotic microangiopathy (TMA) and markedly elevated antiphospholipid antibodies (aPL). Multimodal therapy including plasma exchange and Rituximab led to a full recovery.

**Methods:** A healthy 5 year old female presented with fever and gastrointestinal symptoms. Acute kidney injury, liver failure, and hemolytic anemia were revealed. Acute respiratory distress syndrome necessitated mechanical ventilation. Continuous renal replacement therapy was initiated for oliguric renal failure. Serratia marcescens bacteremia and metapneumovirus pneumonia were noted. Therapeutic plasma exchange was initiated for thrombocytopenia associated multi-organ failure. After 3 sessions, her clinical status improved. Liver biopsy noted microthrombi in the portal vein. aPL were markedly elevated. A diagnosis of CAPS prompted methylprednisolone pulse therapy. Proteinuria and an elevated Cystatin C led to a renal biopsy which showed acute tubular necrosis without TMA. She was started on enoxaparin prophylaxis and angiotensin receptor blockade for hypertension and renoprotection. A cumulative dose of Rituximab 1.5g/m2 was given and aPL subsequently decreased.

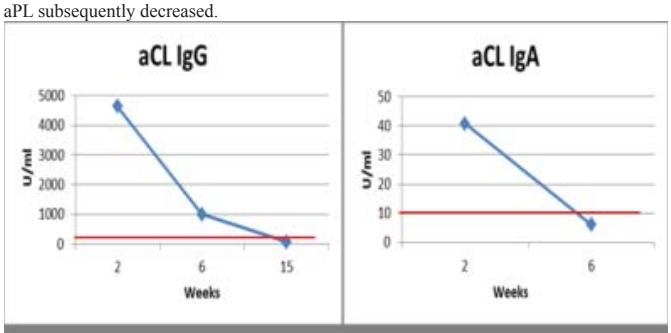



Figure1. Normal: Anticardiolipin (aCL) IgM , beta 2 glycoprotein (β2GP1) IgM, Lupus anticoagulant. Abnormal: β2GP1 IgG 150 to 127 SGU (normal <20). Red lines = normal values.

**Conclusions:** The patient showed complete recovery from CAPS with early initiation of multimodal therapy with plasma exchange, corticosteroids, Rituximab, and anticoagulation. The kidney biopsy did not show evidence of TMA despite the acute renal failure. This may reflect recovery of kidney injury after treatment or sampling error. Pediatric CAPS is uncommon, but a high index of suspicion allows for prompt diagnosis and treatment.

## SA-PO654

**A Case of a 62-Year-Old Male with IgG4-Related Disease Complicated by Systemic Lupus Erythematosus** Xiao-hu Shi, Xuemei Li, Mingxi Lee, Xuewang Lee. *Dept of Nephrology, Peking Union Medical College Hospital, Beijing, China.*

**Background:** IgG4 related Disease(IgG4-RD) was considered as an autoimmune disease which complicated by Autoimmune Pancreatitisa, Mikulicz's Disease. But it rarely involved SLE. We report a case of IgG4-RD complicated by SLE in a patient with chronic glomerulonephritis and progressive renal failure.

**Methods:** 62 y/o Asian male with a history of intermittent fever, hyperglobulinemia and increased Scr(0.78 to 4.24mg/dL) for 2yrs without any effect by kinds of antibiotics. On admission, Scr kept increasing(3.88 to 5.52mg/dL) in 2ws. PE revealed mild edema and Generalized Lymphadenopathy. Lab work revealed: globulin 70g/L while albumin 38g/L, and moderate proteinuria(1.9 to 2.98g/24h,UV 2 to 2.5L). Serumproteinelectrophoresis showedγ-globulin 43.4%. IgG subtype was fond extraordinary abnormal that: $IgG_1$ 20600mg/L(<11400),$IgG_2$ 5020mg/L(<6400),$IgG_3$ 2330mg/L(<1100), $IgG_4$ 39400mg/L(<1400). Immunologic test showed: $C_3$ 0.034g/L. ANA: 1:1280(homogeneous type), dsDNA: 1:40(ELISA),445IU/mL(IgG). Coombs' test was positive. But anti-GBM, ANCA, aSSA , aSSB, ACL, $\beta_2$GP1 and lupus anticoagulant were negative. USG & CT showed largish kidneys(12.8 and 13.1cm) and lymphadenectasis in mediastinum and peritoneal cavity without ureteralobstruction. BMB analysis showed no abnormal cell population. Renal biopsy showed: Glomerular proliferation in mesangial cells & matrix, severe plasma cell-rich interstitial inflammation(improved by immunohistochemistry test) and severe tubulointerstitial fibrosis which showed typically "storm form". IF was negative. Diagnosis of IgG4 related TIN and SLE(ACR 2009) were both confirmed. With a initial dose of 50mg/d prednisone and 100mg CTX. SCr was improved to 1.6mg/dL, IgG4 to 1224 mg/L, C3 to 1.2g/L, dsDNA to negative. In 7Ms follow-up, Prednisone gradually reduced to 15mg and CTX was stopped when accumulation reached to 12g.

**Conclusions:** IgG4-RD was more and more valued by clinician and was reported to associate with AIP, MD and WG, but rare case on IgG4-RD with SLE. Our patient developed IgG4-RD complicated by SLE suggesting that the pathogenesis and progress of this disease deserved further research.

## SA-PO655

**Diffuse Infiltrative Leukocytosis Syndrome, Immunotactoid Glomerulopathy, and HIV-Associated Nephropathy, in a Patient with Acute Kidney Injury: Case Report and Review of the Literature** Dinesh Kannabhiran, Saif A. Muhsin, Jeffrey I. Silberzweig, Surya V. Seshan. *NewYork-Presbyterian Hospital/Weill Cornell Medical Center, New York, NY.*

**Background:** Patients with HIV infection may suffer from a variety of renal diseases: functional disorders, HIV-associated nephropathy (HIVAN), lesions secondary to other infections, immune mediated diseases and drug-related disorders. We describe a case of acute kidney injury (AKI) with nephrotic range proteinuria in a patient with HIV infection, whose biopsy showed diffuse infiltrative lymphocytosis syndrome (DILS) and immunotactoid glomerulopathy (ITG) in addition to HIVAN.

**Methods:** A 51-year-old HIV infected woman presented with a 1 week history of lower limb swelling and Sicca symptoms. She stopped highly active anti-retroviral therapy (HAART) a year prior. Examination showed cervical lymphadenopathy, tonsillar enlargement, and lower limb pitting edema. Investigations revealed an elevated creatinine (2.0 mg/dL), and low albumin (1.1 mg/dL). Urinalysis showed 11 white and 41 red blood cells per high power field, a protein-creatinine ratio of 8.5 and no casts. The CD4 count was low ($287 \times 10^6$ cells/L) and the CD8 count was high ($1834 \times 10^6$ cells/L). C3 was markedly reduced. Testing was negative for Hepatitis C & B viruses, cryoglobulins, antinuclear antibodies, and rheumatoid factor. Protein electrophoresis was normal. Ultrasonography showed large kidneys. A kidney biopsy revealed findings suggestive of HIVAN in addition to ITG. There was diffuse CD8 lymphocyte interstitial inflammation suggestive of DILS. HAART was restarted. Serum creatinine was 1.38 before discharge. She received outpatient pulse steroids.

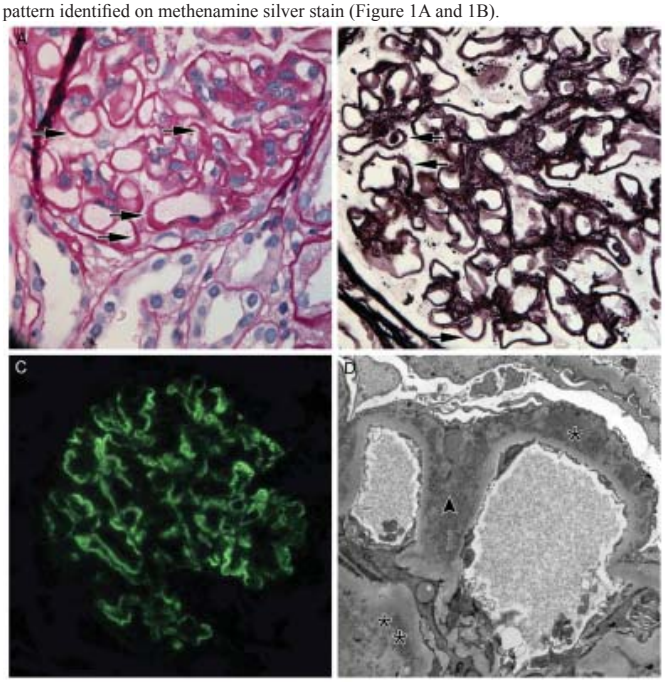
**Conclusions:** There have been few cases described in the literature of HIVAN in association with ITG, half of which were associated with viral hepatitis. We report the 4th case of HIVAN and ITG with negative viral serology leading us to question whether ITG could be triggered by factors related to HIV. To our knowledge, this is the first case describing the clinical presentation and kidney biopsy evidence of HIVAN, DILS, and ITG. Our case demonstrates the importance of renal biopsy for HIV infected individuals presenting with AKI.

## SA-PO656

**Atypical IgA Nephropathy with Membranous Features in a Patient with HIV** Sarah E. Panzer,[1] Shalini Tayal,[2] Jessica B. Kendrick.[1,2] *[1]Univ of Colorado Denver; [2]Denver Health Medical Center.*

**Background:** IgA nephropathy can exhibit a broad degree of plasticity in the clinical presentation, pathologic manifestations, and disease progression. The defining histologic lesion in IgA nephropathy is mesangial deposition of IgA; however, the findings on light microscopy can be quite variable. In this case report we describe an unusual histologic finding of a severe form of IgA nephropathy with membranous features in a patient with HIV.

**Methods:** A 63 year-old Caucasian male with HIV and new-onset hypertension was referred to the renal clinic for evaluation of nephrotic range proteinuria and intermittent gross hematuria. Renal biopsy demonstrated mesangial hypercellularity and expansion with diffuse global thickening of the glomerular basement membrane and a 'spike and dome' pattern identified on methenamine silver stain (Figure 1A and 1B).




Immunofluorescent staining showed strong IgA deposition in the glomerular basement membrane and mesangium (Figure 1C). Electron microscopy demonstrated numerous mesangial, intramembranous, and subepithelial electron dense immune-complex deposits with intervening membrane spikes between subepithelial deposits (Figure 1D). The patient was initially managed with angiotensin converting enzyme inhibitor therapy. However, renal insufficiency developed and the degree of proteinuria remained in the nephrotic range. Thus, the patient was treated with a six month course of alternating cyclophosphamide and prednisone and had a partial response to treatment.

**Conclusions:** Our case represents an atypical form of IgA nephropathy presenting with a membranous pattern histologically. Data regarding this form of IgA nephropathy is scarce and it is unclear whether the presence of histologic features of membranous incurs a worse prognosis. To our knowledge this is the first reported case of this constellation of histologic findings in a patient with HIV.

*Funding:* NIDDK Support

## SA-PO657

**Idiopathic Hypocomplementemic Immune Complex-Mediated Tubulointerstitial Nephritis: An Unattended Differential Diagnosis for Sub-Acute Renal Insufficiency in Elderly Patients** Fahima Nasreen,[1] Ana Paula Rossi,[1] Douglas M. Dressel,[2] James C. Wasserman.[1] *[1]Nephrology, Maine Medical Center, Portland, ME; [2]Pathology, Maine Medical Center, Portland, ME.*

**Background:** Immune complex (IC) deposits in renal tubular basement membrane (TBM) are commonly encountered with glomerular diseases. In Sjögren's syndrome, however, tubulointerstitial (TI) IC deposit occurs without significant glomerular involvement. We describe a case of idiopathic IC TI nephritis with distinct serologic, light microscopic (LM) and immunofluorescence (IF) findings, and response to steroid therapy.

**Methods:** A 74-year-old male with no prior history of kidney disease presented with fatigue, anorexia, weight loss of 40 lb over 9 months, and chronic sinus congestion for years. Physical examination was unremarkable and renal ultrasound was normal. Serum creatinine (SCr) level was 2.1 mg/dl, increase from a baseline of 1.0 mg/dl 6 months prior. The patient had mild proteinuria with no cellular or granular casts. Spot urine protein to creatinine ratio (UPCR) was 0.4 g/g. Immunological tests showed low complement (C3 77mg/dl, C4 < 3mg/dl), normal IgG, normal serum protein electrophoresis, negative ANA and ANCA. LM showed 49% globally sclerosed glomeruli with extensive tubular atrophy, diffuse interstitial infiltrate with reactive lymphoid cells and interstitial fibrosis. IF exposed granular deposits of IgG, C3, C1Q, and kappa and lambda light chains in the interstitium and TBM. The patient was started on prednisone 1mg/kg/d with excellent response. Clinical improvement was associated with improved renal function, which has been stable at 2 years post-diagnosis (SCr 1.1 mg/dl; spot UPCR 0.1g/g).

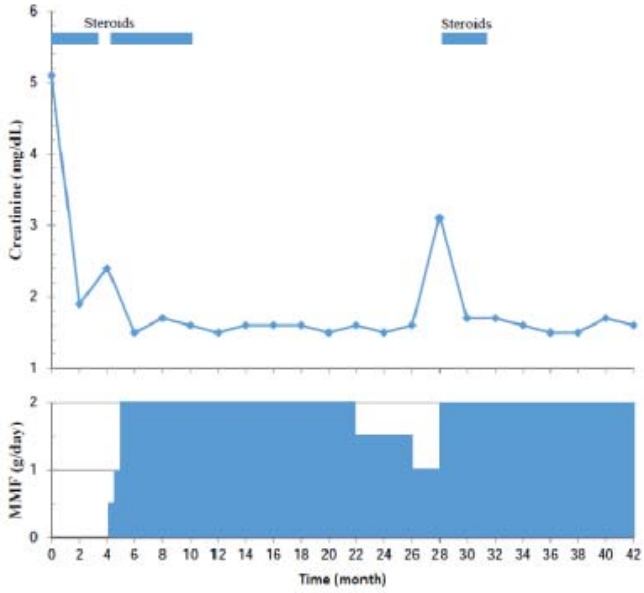
**Conclusions:** IC- mediated TI nephritis is a relatively new and rare entity with clinical features that may include sinusitis, anorexia, weight loss, and renal insufficiency. TI IC deposition and interstitial plasma cell infiltrates appear to be involved in local IC formation. Idiopathic IC mediated TI nephritis may occur in elderly patients presenting with acute or sub- acute kidney injury and, therefore, should be considered in the differential diagnosis of elderly patients presenting with renal insufficiency.

## SA-PO658

**Idiopathic Granulomatous Interstitial Nephritis Responding to Mycophenolate Mofetil Therapy** Napat Leeaphorn, Patompong Ungprasert, William W. LeCates. *Internal Medicine, Bassett Medical Center, Cooperstown, NY.*

**Background:** Granulomatous interstitial nephritis (GIN) is a rare histologic disease. Many etiologies have been reported such as sarcoidosis, drugs, and infections. Idiopathic GIN is diagnosed by exclusion. GIN generally responds well to steroids, but there is limited data with therapy other than corticosteroids.

**Methods:** A 68-year-old Caucasian man presented with fever. He was found to have acute renal failure (SCr of 3.7mg/dl, normal 4 months earlier) with bland urine sediment. Renal biopsy showed granulomas consistent with GIN. Oral methylprednisolone was initiated with good response, SCr~1.9 mg/dL. However, he developed hyperglycemia from steroids, and had a relapse after discontinuation of steroids. Therefore, MMF was chosen as a maintenance agent. We followed the patient for 3.5 years, and the patient has been in remission with MMF monotherapy with only one episode of relapse after an attempt to taper the dose of MMF.




**Conclusions:** The possible causes of GIN in this patient included drug-induced, sarcoidosis, tuberculosis and idiopathic. The patient was taking several medications that could potentially cause GIN. However, the need for long term MMF treatment with an inability to discontinue MMF after a long period of follow-up makes drug-induced GIN unlikely. The patient had never had any findings indicative of systemic sarcoidosis. Calcium, 1,25(OH)2 D, and ACE levels were normal. Tuberculosis was excluded by negative histologic stain and negative QuantiFERON-TB Gold. Since no obvious cause could be identified in our case, the diagnosis of idiopathic GIN was made. To the best of our knowledge, this is the first report of successful treatment with MMF of an adult patient with idiopathic GIN. This case supports the use of MMF as a potential alternative therapy for GIN.

## SA-PO659

**Leiomyosarcoma Associated with Nephrotic Syndrome** Ibrahim Qaqish,[1] Maxwell L. Smith,[2] Leslie F. Thomas.[3] *[1]Nephrology Fellowship, Mayo Clinic Arizona, Phoenix, AZ; [2]Div of Laboratory Medicine & Pathology, Mayo Clinic Arizona, Phoenix, AZ; [3]Div of Nephrology & Hypertension, Mayo Clinic Arizona, Phoenix, AZ.*

**Background:** The association of solid tumors with minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS) is uncommon. Renal cell carcinoma, colorectal carcinoma, lung and bronchus carcinoma, and thymoma account for the majority of reported cancers associated with MCD. Solid tumors associated with FSGS are rare with renal cell carcinoma and thymoma being the most commonly reported. Here we present the first reported case of leiomyosarcoma associated with MCD.

**Methods:** A previously healthy 46 year-old woman developed the nephrotic syndrome. The initial urine protein:creatinine (UPC) was 4.92. Renal biopsy demonstrated normal glomeruli by light microscopy and diffuse podocyte foot process effacement on electron microscopy. No secondary cause was identified. A retroperitonal ultrasound was normal, and additional imaging was not performed. After two months of prednisone therapy, the patient's proteinuria was significantly reduced (UPC 0.62). However, after three months of prednisone therapy, the patient's proteinuria began to worsen (UPC 1.64). Cyclosporine and mycophenolate mofetil were added, and triple immunosuppressive therapy was continued for an additional three months with improvement in proteinuria (UPC 0.15). Six months after her diagnosis of nephrotic syndrome, the patient developed lower back pain. Computed tomography revealed large masses located in her liver, skeleton, and lung. Biopsy showed a high-grade pleomorphic sarcoma consistent with metastatic leiomyosarcoma. Immunosuppressives were held, and chemotherapy with gemcitabine and docetaxel was initiated. A decrease and stabilization of tumor burden was observed. Nine months after the initiation of chemotherapy, the patient continues to demonstrate stable tumor burden and a complete remission of the nephrotic syndrome.

**Conclusions:** Leiomyosarcoma may lead to MCD and the nephrotic syndrome. Reduction in tumor burden achieved via targeted chemotherapeutic agents may result in a complete and sustained remission of MCD associated with leiomyosarcoma.

## SA-PO660

**Granulomatous Interstitial Nephritis: A Potential Precursor to Pauci-Immune Small Vessel Vasculitis** Liliana E. Rios Rojas,[2] Vladimir Liberman,[1] Ingrid Calliste,[2] James Drakakis,[2] Joseph Mattana,[2] Nobuyuki (Bill) Miyawaki.[2] *[1]Internal Medicine, Winthrop Univ Hospital, Mineola, NY; [2]Internal Medicine, Div of Nephrology, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Granulomatous interstitial nephritis (GIN) is a rare entity with a poor outcome. Little is known about the etiology, nor whether it may be a precursor to other renal pathologies. We present a case of GIN in which a repeat kidney biopsy revealed a substantially different diagnosis with important therapeutic implications.

**Methods:** A 57 year old woman with malaise and fever, without infection, was admitted with acute kidney failure with a serum creatinine (SCr) of 4.4mg/dL, hematuria and proteinuria. Pulse steroid and plasmapheresis were initiated for suspected rapidly progressive glomerulonephritis. Kidney biopsy revealed GIN. Mycobacterium, fungi, paraproteinemias, and ANCA tested negative. No evidence of tenosynovitis-interstitial nephritis-uveitis syndrome or sarcoidosis existed. Her GIN was attributed to ciprofloxacin or esomeprazole and maintained on prednisone. SCr improved to 3.2mg/dL at the time of hospital discharge and reached a nadir of 2.3mg/dL three months later. Prednisone was tapered off but in the next two months her SCr rose to 3.7mg/dL. Prednisone was resumed but the SCr increased further to 4.8mg/dL in a month. A repeat kidney biopsy was performed and instead of GIN, this biopsy revealed a pauci-immune small vessel vasculitis (ANCA testing was again negative). She received methylprednisolone and rituximab, then was maintained on prednisone and azathioprine. Her renal function steadily improved with a decline in creatinine to 3.0mg/dL within three months.

**Conclusions:** GIN is a poorly understood, rare entity associated with a poor outcome despite steroid treatment. This case suggests the possibility that some instances GIN may be a precursor to the development of pauci-immune small vessel vasculitis; hence treatment failure or a rising SCr after an initial favorable response to steroids might be an indication for re-biopsy. The possibility of missed or developing pauci-immune small vessel vasculitis may explain, in part, the poor overall outcome of GIN with steroid treatment.

## SA-PO661

**Minimization** Claire T. Kassakian, George P. Bayliss. *Dept of Medicine, Rhode Island Hospital, Providence, RI.*

**Background:** We present the case of a 48 yr old man with HIV and Kaposi's sarcoma who developed steroid responsive Minimal Change Disease, but who suffered a relapse of Kaposi's prompting an accelerated steroid taper regimen.

**Methods:** The patient is a 48 yr old man with a history of HIV for 10 yrs compliant with anti-retroviral therapy on Atripla and Kaposi's Sarcoma initially referred to renal clinic for a rising creatinine, proteinuria, and weight gain. In clinic, he was found to be hypertensive and 40 pounds above his baseline weight with periorbital edema and anasarca. Lab data was notable for a Creatinine of 1.97mg/dl from a normal baseline, 10.2g of protein on 24 hour urine collection, Albumin of 2.0, CD4 count 321 and viral load of 55. Urine sediment showed lipid laden renal tubular epithelial cells and occasional lipid droplets in casts. He was admitted to the hospital for emergent renal biopsy illustrating ischemic ATN in addition to a podocytopathy most consistent with Minimal Change Disease with subclinical IgA deposition. Tenofovir was held. His Creatinine continued to rise, peaking at 7.2 at which time he received IV pulse dose steroids before continuing oral Prednisone 80mg daily. Over three weeks, his Creatinine and proteinuria fell to 1.8 and 918mg respectively. While his proteinuria continued to decline with the lowest microalbumin to creatinine ratio of 151 mg/mg, he noted cutaneous lesions consistent with prior relapses of Kaposi's in addition to hyperlipidemia and glucose intolerance. Therapy with liposomal Daunorubicin was initiated with good initial response. After 6 weeks, his Prednisone dose was tapered over four weeks to 10mg daily. Despite the rapid taper, weekly microalbumin to creatinine ratios remained relatively between 151 to 361.

**Conclusions:** Treatment of Minimal Change Disease is not infrequently complicated by adverse complications of high dose steroid therapy including hyperlipidemia and glucose intolerance. We present a case of a patient with a history of HIV on antiretroviral therapy and Kaposi's sarcoma in remission whose treatment was complicated by recurrence of Kaposi's sarcoma prompting a rapid taper of his oral Prednisone in addition to chemotherapy with liposomal Daunorubicin.

## SA-PO662

**Primary Sjogren Syndrome with Minimal Change Disease** Xiaoxiao Shi,[1] Zhou Zhang,[1,2] Yubing Wen,[1] Hang Li,[1] Xuemei Li,[1] Xuewang Lee,[1] Limeng Chen.[1] *[1]Nephrology, Chinese Academy of Medical Sciences & Peking Union Medical College Hospital, Beijing, China; [2]No.4 Dept of Internal Medicine, Guizhou Hospital, Foshan City, China.*

**Background:** Glomerular involvement in primary Sjogren Syndrome (pSS), especially minimal change disease (MCD), has rarely been reported. We report four cases of pSS with MCD.

**Methods:** Four cases of MCD confirmed by renal biopsy in pSS patients were enrolled as pSS-MCD group. Ten pSS patients with membranous nephropathy (MN) of the same period were enrolled as control group (pSS-MN group). Medical records were summarized and compared.

**Results:** The four pSS-MCD patients were mainly middle-aged females (37-61y; female: male= 3:1). They were diagnosed as pSS by dry mouth, dry eyes, positive anti-SSA, anti-SSB antibodies, and labial gland biopsy. They all clinically presented as nephrotic syndrome, with severe proteinuria (3.70-19.02g/d) and remarkable hypoalbuminemia (12-19g/L). They also showed mild microscopic hematuria and leukocyturia but normal blood pressure. Only one female, who had the most severe proteinuria (19.02g/24h), showed renal function impairment (Scr 200μmol/L). None of them showed renal tubular acidosis. Compared with pSS-MN group, pSS-MCD patients had more severe hypoalbuminemia (15.0g/L vs. 26.5g/L, P=0.009), but no significant differences were shown in other clinical characteristics. In renal biopsy, they all showed typical glomerular lesions of MCD and focal distribution of tubulo-interstitial injury, which presented as thickened TBM, tubular atrophy, mild interstitial fibrosis and interstitial infiltration of mononuclear inflammatory cells. Besides the injuries of kidney and exocrine glands, the two cases with hypokalemia (3.0 and 3.2mmol/L) also showed hematologically involvement with decreased white blood cell count of $3.32 \times 10^9$/L and $3.36 \times 10^9$/L. Steroid (1mg/kg/d) was given to all patients and great improvement of proteinuria was observed.

**Conclusions:** We report the 4 cases of pSS with MCD with typical nephrotic syndrome, severe hypoalbuminemia and good response to steroid.

*Funding:* Government Support - Non-U.S.

## SA-PO663

**Periodic Fever, Aphthous Stomatitis, Pharyngitis, and Adenitis (PFAPA) Syndrome and IgA Nephropathy** Keisuke Sugimoto, Hitomi Nishi, Tomoki Miyazawa, Mitsuru Okada, Tsukasa Takemura. *Pediatrics, Kinki Univ School of Medicine, Osakasayama, Osaka, Japan.*

**Background:** Periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome (PFAPA) is a auto-inflammatory disease. Periodic abnormalities involving the mechanism regulating cytokine secretion have been suggested, the levels of IL-6, IFN-γ, and IL-1β increase markedly during episodes of fever. A recent study has demonstrated an involvement of the CX3CR1-fractalkine axis in the exacerbation of gross hematuria in IgAN. Fractalkine is enhanced by exposure to IL-1 and IFN-γ in mesangial cells. Immune complexes deposition in the glomeruli could promote both systemic and local secretion of various inflammatory cytokines and cell-proliferative hormones. Here, we present a child case with PFAPA and concurrent IgAN, not reported previously. This patient is of interest in terms of the pathogenic mechanisms underlying the two diseases, since a disoriented immune response and fractalkine has been suspected in both diseases.

**Methods:** A 10-year-old boy presented with periodic fever, exudative tonsillitis, oral aphthous ulcer, and cervical lymph node inflammation. These conditions had occurred at intervals of about 2-6 weeks since the age of 3 years. Microscopic hematuria, first detected at age 8 years, worsened during episodes of PFAPA-related fever; since 10 years of age, the hematuria was accompanied by sustained proteinuria. Histological evaluation of a renal biopsy specimen was IgAN. In the kidney specimen, fractalkine immunoreactivity and heavy macrophage infiltration were prominent. Multi-drug cocktail therapy improved the urinalysis findings, and subsequent tonsillectomy succeeded in controlling recurrences of PFAPA and IgAN. In a post-treatment, mesangial proliferation was decreased, and fractalkine immunoreactivity was absent.

**Conclusions:** Immunologic reactions against certain antigens in local mucosa, including tonsils, may be impaired in PFAPA and IgAN, as evidenced by the suppression of both diseases in our patient by tonsillectomy. Accordingly, the concurrence of PFAPA and IgAN in our patient appeared to be a consequence of shared autoimmune mechanisms and systemic and local increases in cytokine concentrations.

## SA-PO664

**ANCA Vasculitis and Renal Failure Associated with Cocaine Abuse – A Case Report** Wonngarm Kittanamongkolchai,[1] Robert M. Perkins.[2] *[1]Internal Medicine, Bassett Medical Center, Cooperstown, NY; [2]Div of Nephrology, Bassett Medical Center, Cooperstown, NY.*

**Background:** ANCA vasculitis is usually idiopathic. We report a rare case of possible levamisole induced ANCA vasculitis presenting with pulmonary hemorrhage and renal failure.

**Methods:** A 46 year-old female presented with fever, a 40-pound weight loss, and hemoptysis. She required mechanical ventilation for diffuse alveolar hemorrhage. Physical exam revealed palpable purpura and digital ischemic lesions. Blood evaluation showed neutropenia, serum creatinine of 4.1 mg/dL, negative ANA, anti-dsDNA > 45 IU/mL, anti-MPO > 8 U/mL, anti-PR3 >8 U/mL, and hypocomplementemia. Urine microscopy demonstrated dysmorphic red blood cells. Urine PCR was 3.68 g/g. A drug screen was positive for cocaine. Skin biopsy demonstrated leukocytoclastic vasculitis. Kidney biopsy showed focal segmental necrotizing and crescentic glomerulonephritis, pauci-immune type, with background segmental membranous glomerulopathy. She was treated with plasmapheresis, pulse steroids, and IV cyclophosphamide. She achieved remission and was transitioned to maintenance immunosuppression. Her subsequent course has been marked by persistent, downtrending, low-level anti-MPO and PR3 titers and no clinical relapse over a two-year period.

**Conclusions:** This case illustrates many features of drug-induced ANCA-vasculitis, including multiple autoantibody positivity and the histologic combination of membranous and necrotizing crescentic glomerrulonephritis. Levamisole, a common cocaine adulterant, has been reported as a cause of ANCA-associated vasculitis. Pathognomonic manifestations are dermatologic infarction, neutropenia, and dual MPO- and PR3-positivity, as in this patient. Pulmonary hemorrhage and renal involvement is rare in levamisole-induced vasculitis and would be challenging to distinguish from idiopathic ANCA vasculitis. Nephrologists should be aware of the consequences of the growing use of cocaine adulterated with levamisole in order to recognize characteristics of this reversible cause of glomerulonephritis.

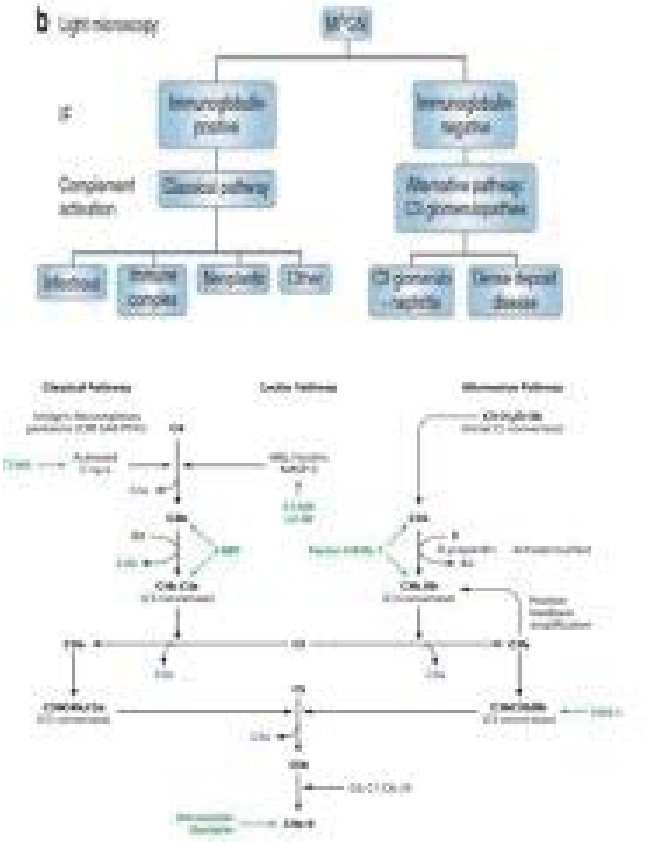
## SA-PO665

**C3 Glomerulopathy in a Young African American Adult** Julio C. Chirinos, Devasmita Choudhury. *Nephrology, UT Southwestern Medical Center, Dallas, TX.*

**Background:** Membranoprolipherative Glomerulonephritis (MPGN) is a pattern of glomerular injury recognized easily by light microscopy. Electron microscopy allows sub grouping in MPGN types I, II and III by localizing the area in which the electron dense deposits, and immunofluorescence detects immunoglobulin in MPGN type I and III, but not in type II. All 3 forms stain positive for C3. This sub grouping led to confusion, because of the presence of immunoglobulin negative MPGN I and III. This entities along with Type II MPGN are now under the subgroup of C3 glomerulopathy (C3G).

**Methods:** 35 year old man with PMHx of HTN diagnosed 3 years ago, on amlodipine only, referred referred by PCP for hyperkalemia and worsening renal function. Patient had no complaints at all, just that the last 1-2 months has been feeling more tiered than normal but was because has been exercising more for intentional weight loss. Reports he makes normal amounts of urine, but foamy for the last few months. **PE:** BP: 180/91 HR: 81; No distress. S1S2 normal. Lungs Clear, No LE edema. Labs: BMP: K 6 ; CO2: 19 scr: 59/4.42 (last Scr 1.4 in 2011); Hb 10. UA: No RBCs, no WBCs, P/C: 5 grams. Renal US: Bilateral echogenic kidneys (11 and 12 cm). Serology: Negative. SPEP, UPEP: negative. **Renal Biopsy:** Mesangiocapillary proliferation, consistent with MP type I. Diffuse interstitial fibrosis. **IF: C3 ++ no Ig. EM:** Effacement of visceral epithelial cell foot processes. Multiple mesangial proliferation.

**Conclusions:** This case give us the example of why a new classification for MPGN was needed.



This classification allows differences in therapy, since pathophysiology is also different. In types I and III therapy is focused in underlying disease triggering and driving the chronic antigenemia. The focus in C3G in contrast should focus on the dysrregulation of the alternative pathway.

**SA-PO666**

**Membranoproliferative Glomerulonephritis Type II with C3b-Autoantibodies – Potential Value of Immunoadsorption** Christina Taylan,[1] Peter F. Zipfel,[2] Rasmus J.C. Ehren,[1] Gesa Schalk,[1] Lutz Thorsten Weber.[1] *[1]Pediatric Nephrology, Univ Hospital of Cologne, Cologne, Germany; [2]Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany.*

**Background:** In Membranoproliferative Glomerulonephritis Type II (MPGN II) 50% - 80% of patients have autoantibodies (AAB) as complement activating agent.

**Methods:** We report a 7-year-old caucasian girl who was treated in January 2013 for the first episode of acute renal failure after viral upper airway infection. Renal biopsy revealed MPGN II. Additional findings like ANA, ANCA, GBM antibodies and ds-DNA-antibodies remained normal. Genetic analysis showed no mutation. Factor H (FH) Protein and the CFHR1 Protein with its two isoforms were unsuspectable. Functional complement showed low levels of C3 (0,08g/l min-0,8gl max) and an increase of SC5b-9 of 936 (<320) ng/ml. No autoantibodies to FH or C3Nef were found. Plasmapheresis was started after biopsy. Due to recurrent plasma intolerance and a high SC5b-9 therapy with eculizumab was indicated. Consistent with dysregulation of C3 convertase an autoantibody against this protein was found. Eculizumab was stopped after 10 doses. 3 months after diagnosis the child was set on Immunoabsorption (IA). To prevent new AAB development we started a medication with Mycophenolat mofetil 1200 mg/qm BSA/d. Under plasmapheresis renal function remained impaired though plasma C3 levels increased. With the beginning of Eculizumab plasma creatinine levels decreased and diuresis recured, but the child remained dialysis dependent. With the beginning of IA once a week proteinuria (from 6,4g/l to 0,35g/l) and plasma creatinine levels (from 7,26mg/dl to 1,80mg/dl) markedly decreased and diuresis normalized. Dialysis was stopped.

**Conclusions:** In AAB mediated cases of MPGN II IA may be the therapy of choice. However, it might be challenging to identify the individual dose and frequency of IA to preserve renal function and to control proteinuria. So far it remains open, if monitoring of antibody levels could be a helpful tool. In our patient antibody levels dropped from 870 AU to 303 AU after 4 weeks of treatment.

**SA-PO667**

**Retinal Cotton Wool Spots and Renal Impairment Associated with Interferon Beta 1 Alpha Therapy for Multiple Sclerosis** Eleni Chelioti,[1] Evangelia Gkalitsiou,[1] Evdokia Efthimiou,[1] Maria Sotiraki,[1] Spyridon Fradelos,[2] Alexia Papalexandrou,[1] Maria Tsilivigou,[1] Gabriel Papadakis.[1] *[1]Dept of Nephrologr, General Hospital of Piraeus, Greece; [2]Dept of Ophthalmology, General Hospital of Piraeous, Greece.*

**Background:** Interferon is now the mainstay of maintenance therapy for multiple sclerosis (MS). Retinopathy is a well-known adverse effect of interferon-alpha in patients treated for hepatitis C. On the other hand, retinal lesions are rarely related with subcutaneous interferon beta 1 alpha (IFNβ1α) in MS.IFNβ1α nephrotoxicity is also an extremely rare side effect. We report a case of IFN-associated retinopathy and nephropathy in a patient with MS receiving subcutaneous INFβ1α.

**Methods:** A 42-years old female with a history of MS was on treatment with IFNβ1α(44μg/3times a week)for 10 years. She presented with anemia, thrombocytopenia, albuminouria, mild elevation of liver enzymes and renal impairment. She displayed sudden hypertension without visual disorders. Ocular fundus exam showed several retinal cotton wool spots indicating IFN-retinopathy and interferon was discontinued. Subsequent imaging exploration revealed normal sized kidneys. Further biochemical and immunological analyses were negative for autoimmune renal diseases. The retinopathy disappeared without specific therapy 2 months after discontinuing IFNβ1α and kidney function reached normal levels. IFNβ1α resumed 16 weeks after stopping treatment due to MS relapse. On an iterative fundus exam 3 months after resumption of IFNβ1α, no further cotton wool spots have recurred.

**Conclusions:** Our case supports that both complications resolved after drug cessation and the diagnosis of IFNβ1α retinopathy and nephropathy was retained due to the lack of any other etiology. There are only eight reports on MS related interferon-β retinopathy. Likewise there is only one case of IFNβ1α associated retinopathy and nephropathy. To our knowledge this represents the second case of IFNβ1α associated cotton wool spots and renal impairment and the first in the English-language ophthalmic literature. Given this limited number of cases no safe conclusions regarding causality, screening, dose related issues, severityand treatment options can be made.

**SA-PO668**

**The Transplant Triumphs – Rapid Reversal of Nephrotic Range Proteinuria of Native Kidney Origin after Live Donor Kidney Transplant** Dhananjay P. Kulkarni, Ismail M. Qattash. *Internal Medicine/ Nephrology, The Univ of Illinois at Urbana- Champaign, Urbana, IL.*

**Background:** Proteinuria after renal transplantation increases both risk for graft failure and risk for death. In this case report we describe a finding of reversal of nephrotic range proteinuria after renal transplantation.

**Methods:** A 62 y/o Caucasian female with uncontrolled diabetes mellitus type 2, severe hypertension, coronary artery disease status post bypass grafting was referred to our clinic for abnormal renal function and proteinuria. Chronic kidney disease stage IV (GFR 16 ML/MIN/1.73 M^2) with nephrotic range proteinuria and high urinary protein-creatinine ratio was noted. Patient had findings consistent with diabetic process including ophthalmopathy and neuropathy. She continued to have heavy proteinuria and had a hospital visit for severe nausea and vomiting. Creatinine peaked at 3.4 and urea was 66. She was started on hemodialysis and her symptoms resolved. After 3 months patient underwent a living donor kidney donated by her son, with native kidneys in situ. Patient was on immunosuppressant therapy with mycophenolic acid and tacrolimus. TMP/SMX and valganciclovir were also continued for prophylaxis as per transplant nephrology service. Subsequent labs showed her creatinine return to normal and a complete resolution of proteinuria in 3.5 weeks from transplant. On follow-up at 2 years, patient remained free of any proteinuria and had normal renal function parameters.

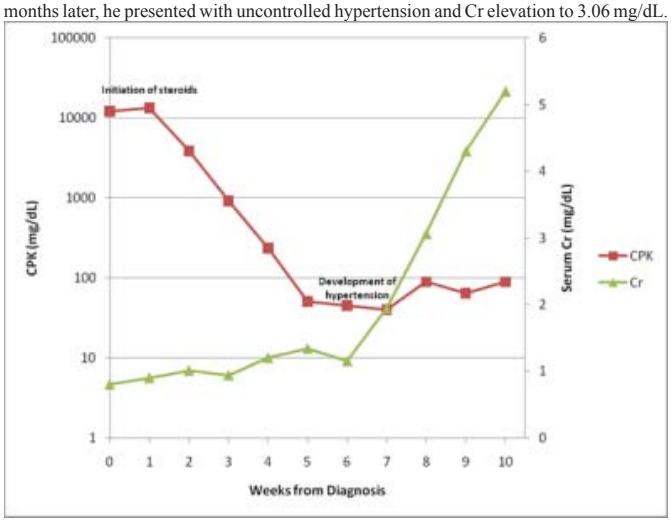
**Conclusions:** Proteinuria after transplantation carries poor prognosis, thus complete resolution of proteinuria post-transplant although rare is striking. A small proportion of live donor transplant recipients with immediate graft function have been known to have a complete resolution of proteinuria after average 4.5 weeks (range 1-10 wks). As our patient had findings suggestive of diabetic process including ophthalmopathy and neuropathy, a kidney biopsy was not performed. Thus a possibility of an alternate renal pathology responding to immunosuppression cannot be completely excluded. Although mechanisms explaining resolution of proteinuria of native kidney origin post-transplant remain unclear, they need to be studied in greater detail.

**SA-PO669**

**Scleroderma Renal Crisis as an Initial Presentation of Systemic Sclerosis in a Patient with Underlying Polymyositis** Yusra R. Cheema, Cybele Ghossein. *Nephrology, Northwestern Univ, Feinberg School of Medicine, Chicago, IL.*

**Background:** Systemic sclerosis (SSc) is a relatively rare connective tissue disease affecting 200 per 1 million adults. About 20% of patients with systemic sclerosis also have clinical or serologic evidence of another connective tissue disease. Of those with an overlap syndrome, 40% have evidence of both SSc and inflammatory myositis. Typically, patients with SSc/polymyositis overlap syndrome present first with signs of SSc and are diagnosed with polymyositis later in the course. Here we present the case of a patient initially diagnosed with polymyositis, started on treatment with corticosteroids and subsequently presenting with scleroderma renal crisis as the only manifestation of systemic sclerosis.

**Methods:** A 54 year-old man initially presented with complaints of fatigue and myalgias. On admission, a CPK level was elevated at 13,127 mg/dL. A rheumatologic work-up was unremarkable including negative anti-Jo1, anti-Scl 70, and anti-RNP antibodies. Serum creatinine (Cr) was 0.8 mg/dL. Patient underwent a muscle biopsy consistent with polymyositis and was started on high dose steroids with improvement in CPK levels. Two months later, he presented with uncontrolled hypertension and Cr elevation to 3.06 mg/dL.




Renal biopsy demonstrated vascular changes and focal thrombotic microangiopathy consistent with scleroderma renal crisis. Patient was started on escalating doses of captopril, amlodipine and aliskiren for BP control. Cr initially stabilized at 4.5mg/dL but renal function continued to worsen over ensuing 4 months, ultimately requiring initiation of hemodialysis.

**Conclusions:** In patients with an underlying connective tissue disease, the development of renal dysfunction particularly following initiation of corticosteroid therapy should raise suspicion for possible overlap syndrome and scleroderma renal crisis.

## SA-PO670

**Unusual Systemic Manifestations of Levamisole Induced Cocaine Vasculitis: Rapidly Progressive Glomerulonephritis** Rona S. Smith,[1] Ryan Jessee,[2] Aneel Kumar,[1] Laura D. Carbone,[1] Lekha K. George.[1] *[1]Univ of Tennessee Health Science Center, Memphis, TN; [2]Duke Univ, Durham, NC.*

**Background:** Levamisole exposure with tainted cocaine causes cutaneous vasculitis that has distinct lab and clinical features distinguishing it from classical ANCA associated vasculitis. We report an unusual sequelae of this syndrome –Rapidly Progressive Glomerulonephritis (RPGN) and discuss the potential role of Rituximab in its management in a patient with history of chronic cocaine abuse that developed vasculitis manifestations of midline facial lesions, neutropenia, skin lesions, and pulmonary and renal manifestations.

**Methods:** A 30 year old female, with long history of intranasal cocaine use, presented with worsening skin ulcerations. Physical examination showed violaceous, eschar-like skin lesions in various stages of healing on her hands, knees, and thighs along with upper airway midline lesions. Lab findings on admission showed an increase in serum creatinine from 0.8 (baseline) to 5.4 mg/dl, leukopenia (WBC count 2,200/mm$^3$), positive C-ANCA (13.4U/ml), and positive P-ANCA (15.9U/ml). Urinalysis showed gross hematuria, and proteinuria (150mg/dl). Antineutrophil elastase, specific for levamizole associated cocaine vasculitis, was also positive.

**Results:** CT scan showed paranasal sinus disease, septal defects, and a noncalcified nodule of the lung. Renal biopsy showed focal necrotizing and crescentic glomerulonephritis consistent with pauci-immune ANCA vasculitis. The patient was treated with total of 3gm (1mg/kg/day) of methylprednisolone. Due to fertility concerns, cytopenia, relapsing nature of disease with systemic manifestation, rituximab 375 mg/m$^2$ was also given. This resulted in disappearance of skin lesions, lung nodule, and improvement in serum creatinine to 1.6 mg/dl and WBC count to 4,200/mm$^3$.

**Conclusions:** This case of levamisole-induced vasculitis with palpable purpura, leucopenia, and positive ANCA and antineutrophil elastase along with RPGN, responded well with a regimen of high dose steroids and Rituximab. This response suggests a potential role of Rituximab in the management of such vasculitis with renal involvement.

## SA-PO671

**Renal Amyloidosis in Skin Poppers – Revisited** Vasil Peev,[1] Ruchir R. Chokshi,[1] Mario Carlos Ponce,[2] David B. Thomas,[3] Jair Munoz Mendoza.[1] *[1]Div of Nephrology and Hypertension, Univ of Miami; [2]Dept of Medicine, Univ of Miami; [3]Div of Pathology, Univ of Miami, Miami, FL.*

**Background:** Renal amyloidosis secondary to long-term subcutaneous (SC) drug use ("skin popping") originally described in African American population in New York in the 1980s, is nowadays rarely diagnosed despite the growing number of intravenous drug abusers.

**Methods:** A 47 year-old Caucasian male with history of hepatitis C, recurrent skin abscesses due to five year history of heroin and cocaine SC and intra venous drug use (IVDU), presented to our hospital with a right arm skin abscess. Physical exam revealed multiple skin needle marks, cachexia, hepatomegaly, 2+ pitting lower extremity edema and a diastolic 3/6 murmur in the aortic area. Initial laboratory data showed elevated serum creatinine (3.8 mg/dL), low albumin (2.1 g/dL), high LDL cholesterol (179 mg/dL) and nephrotic-range proteinuria (5.8 grams in 24 hours). Urine analysis showed no blood or pyuria. He tested positive for hepatitis C, and negative for hepatitis B and HIV. Culture from the skin abscess grew streptococcus hemolyticus and blood cultures were negative. A transesophageal echocardiogram showed vegetations in aortic and mitral valves. Further investigation revealed normal C3 (24 mg/dL) and C4 (47 mg/dL), negative rheumatoid factor and cryoglobulins, and absence of Bence Jones protein or monoclonal gammopathy. A kidney biopsy revealed AA amyloidosis involving multiple glomeruli, interstitium and vessels as well as foci of severe acute tubular necrosis with kappa and lambda immunofluorescence negativity for light chain monoclonality.

**Conclusions:** This case illustrates a rapidly progressive renal failure resulting from AA amyloid deposition secondary to sustained inflammatory response in a patient with SC and IVDU. Although this condition has mainly been reported in blacks, with more than 10-year history of IVDU, we now describe a case in a Caucasian man with a shorter period of IVDU. We also want to call attention to this nearly forgotten condition in an era of FSGS being the most commonly diagnosed cause of nephrotic syndrome in the African American population.

## SA-PO672

**Resolution of Syphilis Induced Rapidly Progressive Crescentic Glomerulonephritis (RPGN) with Penicillin Therapy** Deepak K. Nandikanti,[1] Rona S. Smith,[1] Aneel Kumar,[1] Barry M. Wall,[1,2] Lekha K. George.[1] *[1]Univ of Tennessee Health Science Center, Memphis, TN; [2]VAMC, Memphis, TN.*

**Background:** Renal manifestations of Syphilis are variable, with membranous nephropathy being the most commonly described lesion. We describe a patient with RPGN secondary to latent Syphilis, which resolved after Penicillin (PCN) treatment in the absence of immunosuppressive therapy.

**Methods:** A 28-year old black male with a history of HIV never on HAART therapy presented with one week of nausea, vomiting and abdominal pain. He was treated for STDs in past but never diagnosed or treated for Syphilis. He was afebrile and normotensive. Physical examination was normal. Serum Creatinine (SCr) was 7.2 mg/dl (1.1mg/dl one mg prior). Urinalysis: 3+ protein, WBC 15-20/HPF, many RBCs, and RBC casts. Spot urine protein to creatinine ratio (UPC) was 4.0. CD4 count was 412 with undetectable HIV viral load. Serologies for ANA, ANCA, anti-ASO, hepatitis B and C, anti GBM, C3, C4 were negative or normal. RPR was positive with titer of 1:32. Treponema pallidum particle agglutination assay was reactive, confirming Syphilis diagnosis. CSF was negative for neurosyphilis. Ultrasound showed normal sized kidneys. Kidney biopsy LM showed focal and segmental necrotizing lesions and crescents. IF showed mesangial granular staining for C3 (3+) and IgM (1+). EM showed effacement of foot process without deposits. He received weekly Benzathine PCN for 3 wk. RPR titers decreased to 1:4. SCr rapidly improved with the first dose of PCN. UPC improved to 2.0 after 3 weekly doses of PCN . His SCr was 1.1 mg/dl and RPR titres 1: 4 at three months follow up. These dramatic improvements in renal function occurred in the absence of any immunosuppressive therapy.

**Conclusions:** RPGN with crescents typically leads to ESRD in the absence of effective treatment, which usually includes immunosuppressive therapy. Our patient had dramatic renal recovery with PCN treatment, a finding which to our knowledge has not been previously reported. We strongly recommend that Syphilis be considered in the differential diagnosis of all patients with proteinuria or suspected glomerulonephritis, particularly in high risk populations.

## SA-PO673

**A Case of Fibronectin Glomerulopathy with Rapidly Progressive, Severe Nephrotic Syndrome Leading to End-Stage Renal Disease** Ikue Ishimoto,[1] Eisei Sohara,[1] Eisaku Ito,[2] Motoko Chiga,[1] Soichiro Iimori,[1] Tomokazu Okado,[1] Tatemitsu Rai,[1] Shinichi Uchida,[1] Sei Sasaki.[1] *[1]Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan; [2]Dept of Pathology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

**Background:** Fibronectin glomerulopathy occurs between the second and fifth decades of life in most patients, and it is known to be slowly progressive with mild proteinuria leading to kidney failure. The case of a 78-year-old woman with a rapid course of nephrotic syndrome due to fibronectin glomerulopathy is reported.

**Methods:** She had proteinuria that rapidly increased to 6.8 g/day in a month and microscopic hematuria. A renal biopsy specimen showed lobular glomerulopathy and membranoproliferative glomerulonephritis-like lesions on light microscopy. There was scanty staining for immunoglobulins and complement. Electron microscopy revealed granular deposits with fibril formation, which is 12-16 nm in diameter and typically arranged in an irregular fashion, indicating fibronectin glomerulopathy. Immunohistochemistry of the fibronectin showed intense staining in the mesangium and peripheral loop. Therefore, this case was diagnosed as fibronectin glomerulopathy. The kidney function was rapidly decreased, necessitating hemodialysis two months after renal biopsy.

**Conclusions:** To the best of our knowledge, such a rapid clinical course of nephrotic syndrome has not been previously reported in fibronectin glomerulopathy. In addition, this case is interesting because proteinuria occurred at 78 years of age, the oldest onset of fibronectin glomerulopathy reported to date. Taken together, it is important to consider fibronectin glomerulopathy in the differential diagnosis of nephrotic syndrome in older people.

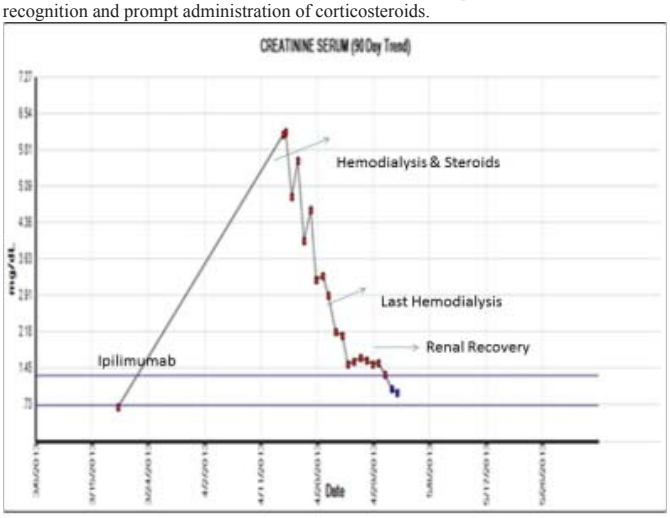
**SA-PO674**

**Ipilimumab-Induced Immune-Related Nephritis** Thuy-Trang T. Ngo,[1] Charles K. Minn,[1] Maen Abdelrahim,[2] Ala Abudayyeh.[3] *[1]Nephrology, The Univ of Texas Medical School-Houston; [2]Nephrology, The Univ of Texas Medical School-Houston; [3]Internal Medicine, Baylor College of Medicine; [4]General Internal Medicine, Section of Nephrology, Univ of Texas-MD Anderson Cancer Center, Houston, TX.*

**Background:** Ipilimumab, a fully human monoclonal IgG1 antibody against cytotoxic T-lymphocyte antigen-4(CTLA-4) expressed on the surface of activated T cells and monocytes, offers a new therapeutic option in the treatment of metastatic melanoma. By inhibiting CTLA-4, ipilimumab increases anti-tumor immune response. Since CTLA-4 plays a major role in regulating immune tolerance to self-antigens, its blockade can result in autoimmune destruction of tissues by enhancing T-cell response in peripheral organs. Common immune related side effects such as dermatitis, enterocolitis, hepatitis, and endocrinopathies are widely documented. However, renal involvement has rarely been described with only one case of ipilimumab-induced acute renal failure reported in the literature to date.

**Methods:** A 62 year old male presented with a history of uveal melanoma which progressed to metastatic disease was treated with ipilimumab (10mg/kg) which resulted in immune mediated nephritis in the presence of peripheral eosinophilia, enlarged kidneys, rash, colitis, autoimmune transverse myelitis, and uveitis.

**Conclusions:** Immune-related adverse effects from ipilimumab are generally dose dependent and typically occur between 3 to 12 weeks after drug administration. At the current approved ipilimumab regimen of 3 mg/kg every 3 weeks for four doses, immune-related adverse events occur in approximately 60% of patients. High-dose steroid therapy has been shown to effectively control side effects associated with ipilimumab immune stimulation. Greater awareness of this rare autoimmune nephritis may facilitate early recognition and prompt administration of corticosteroids.

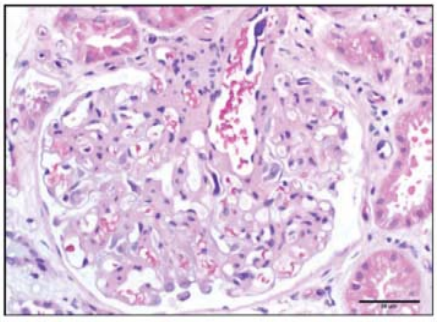



**SA-PO675**

**Primary Myelofibrosis Causes Glomerulopathy** Thuy-Trang T. Ngo,[1] Manjunath Kottalgi,[1] William F. Glass,[1] Ala Abudayyeh.[2] *[1]Nephrology, The Univ of Texas Medical School at Houston; [2]Nephrology, The Univ of Texas Medical School at Houston; [3]Pathology, The Univ of Texas Medical School at Houston; [4]Section of Nephrology, The Univ of Texas- MD Anderson Cancer Center.*

**Background:** Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders in which proliferation of one or more of the myeloid lineages occur resulting in fibrosis of the bone marrow. Renal involvement by MPN is infrequent. Glomerular abnormalities in primary myelofibrosis (PMF) patients have rarely been described.

**Methods:** A 66 year-old Caucasian male recently diagnosed with primary myelofibrosis (PMF) (on JAK-2 inhibitor) presented with increased leg swelling, poorly controlled hypertension,23 grams of proteinuria, and worsening serum creatinine. Renal pathology revealed diffuse mesangial matrix expansion and mild mesangial hypercellularity, effaced podocyte foot processes, and glomerular intracapillary megakaryocytes without immune deposits on immunofluorescence. These findings are consistent with myeloproliferative neoplasm-related glomerulopathy.



H&E: mesangial matrix expansion and intra-arteriolar and intracapillary megakaryocytes.

**Conclusions:** MPN-related glomerulopathy is thought to be a late complication of MPN. Previous study reported a mean time from diagnosis of MPN to renal biopsy was 7.2 years. Our patient underwent a renal biopsy in less than 3 months from diagnosis of PMF, suggesting glomerulopathy can develop sooner than previously thought. Renal biopsy is diagnostic, usually demonstrates variable degrees of mesangial hypercellularity and sclerosis, segmental sclerosis, features of chronic thrombotic microangiopathy (TMA), and intracapillary megakaryocytes cell infiltration on light microscopy. Steroids and renin-angiotensin system blockade appear to be ineffective in treating MPN-related glomerulopathy. Prompt recognition of disease entity is essential for diagnosis and proper management of this renal disorder.

**SA-PO676**

**Pauci-Immune Crescentic Glomerulonephritis Associated with Oropharyngeal Cancer** Amaka Edeani, Derek M. Fine. *Div of Nephrology, Johns Hopkins School of Medicine, Baltimore, MD.*

**Background:** Glomerulopathies of many forms have been described in association with solid and hematological neoplasms, most common is the association of membranous glomerulopathy with solid tumors. We report a case of ANCA-negative crescentic glomerulonephritis (GN) in a patient with oropharyngeal cancer.

**Methods:** A 52 year old male with tonsillar squamous cell carcinoma, diagnosed in 2011, treated to remission with tonsillectomy, radiation therapy and cisplatin. In June of 2012, he was diagnosed with a likely new squamous cell carcinoma of the right lateral tongue. He underwent resection and restarted a course of cisplatin and radiation therapy, completed in November 2012. He was admitted in December 2012 with gross hematuria and a serum creatinine of 1.7mg/dL, attributed at the time to cisplatin-induced nephrotoxicity. Hematuria spontaneously resolved and he was discharged with a creatinine of 1.9mg/dL. Three weeks later, he presented with recurrent hematuria, with creatinine now 4.5mg/dL and 24hr proteinuria of 5.24 gram. Serologic workup revealed negative anti-GBM, p-ANCA, c-ANCA, anti-streptolysin O, anti-myeloperoxidase antibodies and Hepatitis serologies. Urine and serum electrophoreses were negative. Renal biopsy revealed necrotizing crescentic glomerulonephritis with cellular crescents and acute tubular necrosis, with no immune complex deposits. Diagnosed with an ANCA-negative pauci-immune GN, he received pulsed steroids; however, renal failure worsened (creatinine up to 5mg/dL) and in addition to ongoing oral steroids, cyclophosphamide 75mg once daily was initiated with rapid improvement in renal function, with creatinine down to 3.3mg/dL by day of discharge 3 days later and 1.56mg/dL 4 weeks later.

**Conclusions:** Biava et al in 1984 described a case series of patients suggesting an increased incidence of solid tumors in those with pauci-immune crescentic glomerulonephritis. Subsequent observations have demonstrated these to be ANCA-associated. Associated tumors include prostate, lung, gastric and bladder cancer, and some hematologic malignancies. We describe here an unusual case of ANCA negative disease which has responded well to cyclophosphamide based therapy.

**SA-PO677**

**Single Center Experience with ACTH for the Treatment of Resistant Nephrotic Syndrome** Arafat Y. Melhem, Paul G. Schmitz. *St. Louis Univ, St. Louis, MO.*

**Background:** ACTH injection was approved by the FDA in 1952 for the treatment of the nephrotic syndrome (NS). Its use was largely abandoned with the development of oral corticosteroids. However, 2 recent case series in patients with NS revealed that ACTH induced a remission in >50%. In this report we examined our experience with ACTH for resistant NS.

**Methods:** We retrospectively examined 5 cases of resistant NS between 2011-2013 that received salvage therapy with ACTH. The age ranged between 23-59 years and the histology revealed: MCD (n=1), iMN (n=1), or FSGS (n=3). The subjects had been receiving multiple immunosuppressive agents (IS) at the time of initiation (mean = 3.3 IS). The duration of NS in our subjects was 4-16 years. The duration of ACTH administration was 7.4 ± 3 months. Complete remission (CR) was defined as stabilization of the serum Cr. coupled with a protein excretion of <0.5 g/24h. A partial remission (PR) was defined as a protein excretion between 0.5-3.5 g/24h. ACEi/ARBs and diuretics were employed in all patients.

**Results:** 2 of the 5 patients achieved CR and one exhibited a PR (but met the definition of a CR 2 months after stopping ACTH). The average follow-up was 11.8 months with a range of 7-16 months. Importantly, 2 patients with CR were disease free 6 months after

discontinuing ACTH treatment. Proteinuria decreased from an average of 6.2gm/24h to 3.4gm/24h. In addition, the eGFR increased from a mean of 63.8 mL/min to 71.4 mL/min. One patient experienced skin pigmentation, weight gain, worsening diabetes and cushinoid features. This patient remained on therapy, since she had failed 6 IS. While on therapy her Cr. decreased from 6.3 to 3.5 mg/dl and proteinuria declined from 6.7gm/24h to 3.5gm/24. Another patient experienced gastroesophageal reflux, which was controllable with conservative medical therapy. No other adverse events were reported.

**Conclusions:** In our center, we observed remarkable improvement in several patients that were highly resistant to IS. Our remission rate was comparable to previously published studies (60%). Moreover, the remissions persisted for up to 6 months after discontinuing the therapy. Subcutaneous ACTH is a unique and promising alternative to standard IS in patients with resistant NS.

## SA-PO678

### Proteinuria Relapse before Recovery of B Cells and Responsiveness to Rituximab in a Patient with Idiopathic Focal Segmental Glomerulosclerosis

Ilse M. Rood,[1] Job Huussen,[2] Jeroen Deegens,[1] Jack F. Wetzels.[1] *[1]Nephrology, Radboud Univ Nijmegen Medical Center, Nijmegen, Netherlands; [2]Nephrology, Slingeland Hospital, Doetinchem, Netherlands.*

**Background:** Focal segmental glomerulosclerosis (FSGS) is one of the most common causes of nephrotic syndrome (NS) in adults. FSGS is a heterogeneous disease with many underlying causes. A potential cause is the production of permeability factors by lymphocytes. This has been the rationale for targeted B-cell-therapy using rituximab (RTX). Several case reports showed remission of proteinuria in patients with idiopathic FSGS (iFSGS). We present a patient with a relapse before recovery of peripheral B cells, who achieved remission after renewed RTX therapy.

**Methods:** A 65-year old woman presented with proteinuria of 3.0 g/24h and a serum albumin of 24 g/l. Renal function was normal (serum creatinine 51 µmol/l). A renal biopsy showed lesions consistent with FSGS. After ruling out secondary causes, iFSGS was diagnosed. Treatment with prednisone was effective in inducing a partial remission. However, over the years there were multiple relapses necessitating repeated courses of prednisone. Treatment with cyclophoshamide or ciclosporin did not result in a lasting remission. Because of side effects of the therapies, RTX treatment was given at the time of new relapse (1000mg on day 1 and 15). A renewed partial remission occurred. Seven months later she had a new relapse (proteinuria 4.2 g/24h, serum albumin 24 g/L, serum creatinine 144 µmol/l). At that time B-cell CD19+ count was $0.01 \times 10^9$/L. Although B-cells were not recovered, a new dose of RTX was given, resulting in a new partial remission (proteinuria 1 g/24h, serum albumin 32 g/l).

**Conclusions:** This is the first reported case of a relapse of the NS after treatment with RTX when there was no evidence of B-cell recovery. Interestingly, a new dose of RTX resulted again in a partial remission. This case illustrates either that B-cells in secondary lymphoid tissues can still activate T-cells or that B-cell independent mechanisms of RTX are involved in the relapse (by directly affecting podocyte function).

## SA-PO679

### Successful Treatment with Dexamethasone and Bortezomib for Proliferative Glomerulonephritis with Monoclonal IgG Deposits in Multiple Myeloma

Rio Noto,[1] Nozomu Kamiura,[1] Yuichiro Ono,[2] Sumie Tabata,[2] Shigeo Hara,[3] Akihiro Yoshimoto.[1] *[1]Dept of Clinical Nephrology, Kobe City Medical Center General Hospital, Kobe, Hyogo, Japan; [2]Dept of Clinical Hematology, Kobe City Medical Center General Hospital, Kobe, Hyogo, Japan; [3]Dept of Diagnostic Pathology, Kobe Univ Hospital.*

**Background:** The pathophysiology of proliferative glomerulonephritis with monoclonal IgG deposits (PGN-MID) remains unknown. In 30% of PGN-MID cases, there was a monoclonal component detectable in the serum, but only two cases of myeloma were reported previously. Now we present the first case of PGN-MID leading to the diagnosis of multiple myeloma and subsequent successful treatment.

**Methods:** A 75 year old male with hypertension presented with progressive leg edema and fatigue. He was found to have nephrotic range proteinuria (13 grams on a spot protein-creatinine ratio) and hypoalbuminemia of 2.8 g/dL. His serum creatinine was 1.39 mg/dL. His urine showed 30–49 RBC/hpf, but no dysmorphic cells or cellular casts. On serum and urine protein electrophoresis, he had monoclonal spikes in both the serum and urine. Cryoglobulin test for serum resulted negative. Renal histopathology demonstrated lobular mesangial proliferation with moderate tubular atrophy and negative Congo red staining. Glomeruli showed granular capillary staining for IgG, C1q and C3 with light chain isotype restriction limited to kappa by immunofluorescence, although tubular deposits were absent. Analysis for IgG subclass confirmed restriction to IgG1 subtype. Granular electron dense deposits were present in subendothelial and mesangial locations. Bone marrow examination revealed 17% of IgG kappa positive monoclonal plasma cells. A diagnosis of PGN-MID in multiple myeloma was made and we started on bortezomib and dexamethasone. Patient has had significant positive response within 3 months with improvement of proteinuria to 0.66 grams/day and serum albumin to 3.4 mg/dL.

**Conclusions:** Redondo-Pachon *et al*. reported a case of PGN-MID and multiple myeloma refractory to treatment. However, we first demonstrated in this case that treatment for myeloma is effective for PGN-MID when plasma cell dyscrasia is involved in the pathogenesis.

## SA-PO680

### Seronegative Immune Complex Mediated Glomerulonephritis: Renal Limited Anti-Nuclear Antibody Negative Lupus?

Smitha Thomas-Mathew, Meenu Gaba, Mark Birkenbach, Iris J. Lee. *Dept of Nephrology and Pathology, Temple Univ School of Medicine, Philadelphia, PA.*

**Background:** Positive ANA is required for renal disease to be a stand alone clinical criterion sufficient for diagnosing systemic lupus erythematosus (SLE). Pathologic features of lupus nephritis are quite specific, yet cases of ANA negative lupus like renal disease exist. Whether such cases represent development of serologic SLE over time or a distinct disease entity that portend different prognoses remains unclear.

**Methods:** A 25-year old African-American female presented with lower extremity swelling. Her past medical and family histories were unremarkable. Her physical exam was notable only for trace to +1 pitting edema in the lower extremities. Complete blood count, basic metabolic panel, (BUN-15, creatinine-0.89 mg/dl), liver enzymes, were normal. Urinalysis was negative for blood, with 3+ protein and bland sediment. A 24 hour urine protein was 2926 mg. Serum C3, C4, ANA, dsDNA, anti Ro, anti La, antiphospholipid, hepatitis panel, RPR were negative. Our patient had no signs or symptoms of SLE. HIV1/2 antibody was initially positive, but the western blot was indeterminate and HIV viral load was not detected. Repeat HIV was negative. Renal biopsy demonstrated mild mesangial expansion and hypercellularity with immune deposits in a mesangial and capillary wall location observed by immunofluorescence and electron microscopy. Immune deposits demonstrated strong staining for IgG with weaker staining observed for IgM and IgA. Moderately intense staining for C3, C4, and C1q was observed. The patient was treated with prednisone and mycophenolate mofetil 1500mg twice daily.

**Conclusions:** The concept of ANA negative lupus as a distinct diagnostic entity remains problematic. Evidence from the literature, as well as utilization of Hep-2 cell lines for higher sensitivity ANA testing has decreased the incidence of ANA-negative lupus to < 2%. There is limited data available on treatment and prognosis of such cases. We report a rare entity; "seronegative lupus like nephritis" which needs further study to determine mechanisms and evolution of disease, and management to prevent rapid progression.

## SA-PO681

### A Case of Recurrent Uveitis and Tubulointerstitial Nephritis – An Elusive Oculorenal Syndrome

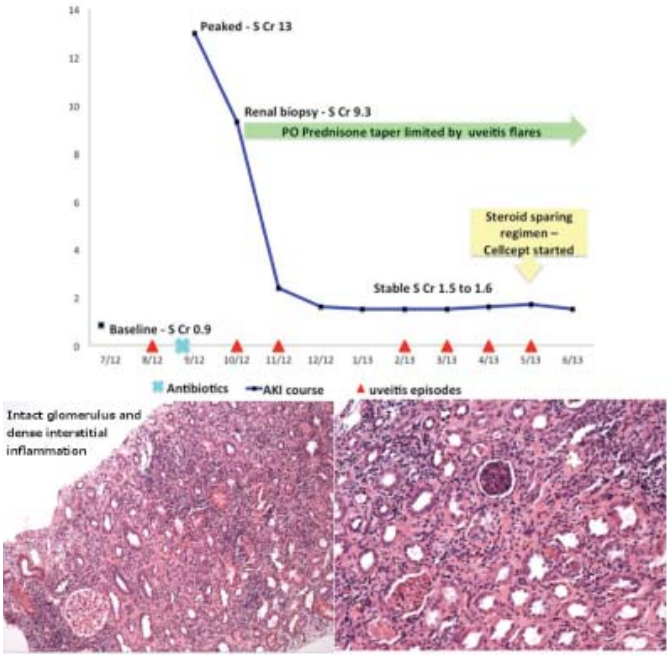
Nisha N. Patel,[1] Menaka Sarav.[2] *[1]Dept of Nephrology, Univ of Chicago, Chicago, IL; [2]Dept of Nephrology and Hypertension, NorthShore Univ HealthSystem, Evanston, IL.*

**Background:** We present a patient with acute tubulointerstitial nephritis and recurrent uveitis (TINU). This case demonstrates the complicated clinical course typical of oculorenal syndromes and the difficulty in distinguishing between these diseases.

**Methods:** A 49 year old African American female with history of pulmonary nodules (not biopsied), cerebral aneurysm, primary uveitis (2 months ago) and recent use of antibiotics for cough (2-3 weeks ago) presented with nausea, vomiting, malaise. Physical exam demonstrated volume depletion and labs showed AKI with a serum creatinine of 13. CXR showed no hilar lymphadenopathy. Working hypothesis was ATN from volume depletion, AIN from antibiotics and Oculorenal syndromes.

| Differential diagnosis for Oculorenal Syndrome |
|---|
| TINU |
| Sarcoidosis |
| Sjogren's syndrome |
| Infectious diseases such as tuberculosis, brucellosis, toxoplasmosis, histoplasmosis |
| SLE |
| Rheumatoid Arthritis |
| Behcet's disease |
| Drugs (NSAIDS, Antibiotics) |
| Wegener's Granulomatosis |

Renal biopsy demonstrated acute tubulointerstitial nephritis with absence of granulomas. ACE levels normal and Quantiferon gold test is negative to date. Prednisone was started with plans for taper over the next 2-3 months. Renal function improved, however steroid taper was limited by recurrent uveitis. She was recently started on mycophenolate mofetil as part of a steroid sparing regimen.




**Conclusions:** The diagnosis of TINU is suggested by the combination of uveitis and acute interstitial nephritis. The distinction of TINU from sarcoidosis and other granulomatous diseases is particularly difficult when there is an absence of a specific organ involvement. Case reports have shown the benefit of mycophenolate mofetil for recurrent uveitis and in a case of TINU.

## SA-PO682

**Urinary Levels and Renal Deposition of Bb Are Associated with the Severity of ANCA-Associated Glomerulonephritis** Shen-Ju Gou, Jun Yuan, Chen Wang, Min Chen, Ming-hui Zhao. *Renal Div, Peking Univ first Hospital, Beijing, China.*

**Background:** Our previous study revealed that complement activation products of the alternative pathway could be detected in renal specimens of human ANCA-associated vasculitis (AAV). The current study aimed to investigate the clinical and pathological significance of complement activation products in the urine and kidneys of patients with AAV.

**Methods:** Renal biopsy specimens from 29 patients with AAV were collected. Urine samples from 27 out of the 29 patients in active stage and 22 patients in remission stage were also collected. The renal deposition of Bb, C3d and C5b-9 were detected by immunohistochemistry. The urinary levels of Bb, C3a, C5a and soluble C5b-9 were determined by enzyme-linked immunosorbent assay.

**Results:** The deposition, measured by the mean optical density, of Bb in glomeruli correlated with the proportion of total crescents, the extent of interstitial infiltrate, interstitial fibrosis and tubular atrophy (r=0.50, P=0.006; r=0.59, P=0.001; r=0.45, P=0.01; r=0.55, P=0.002, respectively), while correlated inversely with the proportion of normal glomeruli (r=-0.49, P=0.008). The urinary levels of Bb, C3a, C5a and soluble C5b-9 were all significantly higher in active stage comparing with those in remission stage. The urinary levels of Bb in patients with active AAV correlated with the serum creatinine and correlated inversely with the proportion of normal glomeruli in renal specimens (r=0.56, P=0.002; r=-0.49, P=0.009, respectively).

**Conclusions:** The present study provided further evidence that complement activation via the alternative pathway occurred in the development of AAV. The renal deposition of Bb and urinary Bb levels were associated with the severity of renal injury.

*Funding:* Government Support - Non-U.S.

## SA-PO683

**Characteristics and Outcomes of ANCA-Associated Vasculitides** Vladimir Tesar, Zdenka Hruskova, Eva Jancova. *Dept of Nephrology, 1st Faculty of Medicine, Charles Univ, Prague, Czech Republic.*

**Background:** To present basic characteristics and long-term outcomes of ANCA-associated vasculitides (AAV) patients included in a nation-wide Registry.

**Methods:** Sixteen vasculitis centres (9x Nephrology, 4x Rheumatology, 2x Immunology, 1x Pediatrics) have participated in web-based data collection since 2009. Data consist of retrospective questionnaire completed once at entry, and of prospective follow-up with a visit recorded every 3-6 months. Statistical analysis included the Kaplan-Meier method and log-rank test for survival analysis.

**Results:** A total of 652 patients (M/F 311/341, median age at diagnosis 58, range 11-89 years) were included, 359 (55%) PR3(or c)-ANCA positive, 261 MPO(p) ANCA positive (40%) and 19 (3%) ANCA negative. Double positivity for ANCA and anti-GBM was found in 25 (4%). The mean time of follow-up was 66±57 months. Granulomatosis with polyangiitis was the most common diagnosis with 58%, and 34% suffered from microscopic polyangiitis (including renal-limited form). Cumulative organ involvement involved kidney in 89%, lungs in 61% and ENT in 38%. Mean S-creatinine at diagnosis was 211 ± 161 μmol/L, 161 patients required dialysis.Cyclophosphamide was used in 89% of the patients, plasma exchange in 232 (36%) and biological therapy in 34 (5%). The estimated 5-year survival was 81.5% (95% CI = 78.1% - 84.9%) and was higher in patients aged ≤65 years than in the older ones (85% vs. 67%, p<0.001) and in patients with better renal function at diagnosis compared to those with S-creatinine ≥ 200 and ≥ 500 μmol/L (91%, 80 % and 67%; p<0.05 and p<0.001, respectively). During follow-up, 41% of the patients relapsed. Last available VDI (Vasculitis Damage Index) ranged between 0 and 16 (median 4).

**Conclusions:** In the studied population, GPA was the most common AAV, reflecting the northern European type. Older age and severe renal involvement were associated with higher mortality. Long-term survival was similar to previous studies, often associated with a significant morbidity.

## SA-PO684

**Infection, Relapse and Mortality in Antineutrophil Cytoplasmic Antibody Associated Vasculitis** JulieAnne G. McGregor,[1] Roberto Negrete-Lopez,[2] Caroline J. Poulton,[1] Jason M. Kidd,[1] Suzanne L. Katsanos,[1] Lindsey R. Goetz,[1] Yichun Hu,[1] Patrick H. Nachman,[1] Ronald J. Falk,[1] Susan L. Hogan.[1] *[1]UNC Kidney Center, Chapel Hill, NC; [2]Hospital Universitario-UANL, Monterrey, Nuevo Leon, Mexico.*

**Background:** Study goal was to evaluate risk factors for infections, relapse and death within 2 years (yrs) of diagnosis in a cohort with biopsy-proven antineutrophil cytoplasmic antibody associated vasculitis (AAV) (1992-2011).

**Methods:** All recieved immunosuppression. Infection (inf), relapse and death within 12 & 24 months (mos) were assessed. Severe inf (intravenous antibiotics, intensive care unit, or cause of death) and prophylactic therapy was recorded. Inf number was grouped as no inf, 1-2 or >3 inf. Fisher exact or Kruska-Wallis tests were used with Cox regression to estimate hazards ratios (HR) and 95% confidence intervals.

**Results:** 490 patients (median age 59 yrs; 47% male, 55% MPO-ANCA) were followed for a median of 2.8 yrs. Within 12 mos, 55% had inf, 19% relapsed and 8% died. Within 24 mos, 66% had inf, 36% relapsed and 12% died.

More inf in 12 mos were associated with age (median age 57yrs with no inf, 60yrs with 1-2 inf and 66yrs with ≥3 inf; p= 0.02) and steroid-induced diabetes mellitus (DM) (19% in those with no inf, 27% in those with 1-2 inf, and 44% in those with ≥3 inf, p= 0.002). Female sex was the sole risk for more inf in 12-24 mos (39% female with no inf; 52% with1-2 inf, 57% with ≥3 inf, p=0.02), with urinary tract infections (UTI) most frequent (31% vs men 10%; p= <0.0001). Prophylactics did not reduce inf.

Relapse in 12 mos was more common in females (60% vs males 40%, p=0.014) and less likely with renal limited AAV (8% vs. GPA 33% and MPA 58%, p=0.019). Only PR3-ANCA was associated with relapse in 12-24 mos (p=0.005 vs. MPO).

Death was associated with more inf in 12 mos (0 3%; 1-2 11%; ≥3 13% p=0.002). A severe inf within 24 mos was a risk for death from any cause in this timeframe (HR 5.6, 95% CI 1.2, 27.2).

**Conclusions:** Elderly patients and those with steroid induced DM must receive less aggressive immunosuppression. UTI in females needs to prompt prophylaxis. This information on inf, relapse and death in the first 1-2 years following AAV diagnosis can guide immunosuppressive strategies.

## SA-PO685

**Use of Cyclophosphamide in ANCA Associated Nephritis: Experience of a Tertiary Care Center** Philippe Lachance, David Philibert. *Nephrology, CHUQ, Québec, Canada.*

**Background:** Cyclophosphamide (CYC) at 2mg/kg is the cornerstone of induction therapy in ANCA associated nephritis (AAN) with a 50 to 90 % remission rate but with infection and leucopenia in up to 50 % of cases. The aim of this study is (1) to describe retrospectively the safety and efficacy of reduced CYC regimen in our center and (2) to examine potential predictors of irreversible kidney injury (IKI).

**Methods:** Single arm retrospective study which included all incident AAN patients from January 2009 to July 2012 at Hotel-Dieu de Québec, a tertiary nephrology center. CYC dose, rate of remission, infection (from the medical file) and leucopenia (WBC<4) were reported and compared to those in the literature. Complete remission was defined as stable or normalizing creatinine with bland urinary sediment while it was considered partial if erythrocytes were presents. IKI was defined by need for chronic dialysis. All renal biopsies were reviewed. A damage score including tubular atrophy, glomerulosclerosis and interstitial fibrosis (0 to 3 pts each) was created. Patients with IKI were compared to the others to determine which features predict dialysis dependence.

**Results:** Twenty-six (26) patients (median age 59 (51, 70) years) were included with a median creatinine of 604 (263,880) μmol/L. Seven (27%) also had pulmonary manifestation. The median CYC induction and total doses were 1.13 (0.83, 1.38) mg/kg and 12 (7.7, 27) g respectively. Fourteen (54%) patients received plasmapheresis. After a median follow-up of 9 (4, 21) months, eighteen patients (70%) were in partial or complete remission. Infections and leucopenia occurred in six (23%) and nine (35%) patients respectively. IKI best correlated with peak serum creatinine (r=0.67). The histopathologic score correlated with IKI (r=0.52). AUC for this score was 0.85. A score > 5 yielded a 86% sensitivity and 74 % specificity.

**Conclusions:** Lower doses of CYC yielded remission rate similar to the literature with probably less infection and leucopenia. Peak serum creatinine seems to correlate well with IKI as well as histopathologic findings. However, combination of these two features is probably needed to accurately predict patients with IKI. More data are needed to draw firm conclusion.

*Funding:* Clinical Revenue Support

## SA-PO686

**Crescentic and Focal Necrotizing Glomerulonephritis in Patients with Normal Serum Creatinine** Stephen Paul McAdoo, Anisha Tanna, Olga Randone, Jeremy B. Levy, Megan Griffith, Frederick W.K. Tam, H. Terence Cook, Tom Cairns, Charles D. Pusey. *Imperial College Renal & Transplant Centre.*

**Background:** Focal necrotizing and crescentic glomerulonephritis (FN/CGN) usually present as RPGN and have a poor prognosis if untreated. These pathological findings, however, are not always accompanied by abnormal renal function at presentation. We aimed to establish the frequency and outcomes of patients presenting with FN/CGN and normal serum creatinine (sCr) at our centre.

**Methods:** We conducted a retrospective review (1995-2011) of all adult patients who presented with native renal biopsy proven FN/CGN and normal sCr (<120micromol/l).

**Results:** 38 patients were identified, median age 57 years (range 17-78), 29% male. Biopsies showed median 14 glomeruli (4-33), with 32% (4-100%) of glomeruli affected by necrosis/crescents. All patients received immunosuppression in accordance with local protocols. Median duration of follow-up was 45 months (2-184). Clinical features and outcomes are summarised in the Table below. The majority of patients had good outcomes at one year and at last follow-up. Only 18% of patients had pre-existing diagnoses of autoimmune disease associated with GN. 2 patients progressed to ESRF (both due to lupus nephritis, at 21 & 29 months) and 4 patients died during follow-up (2, 12, 96 & 122 months).

| **Diagnosis** | **Number of Cases (%)** | **% Crescents/Necrosis (range)** | |
|---|---|---|---|
| Pauci-immune GN | 28 (74%) | 32% (4-100%) | |
| Lupus Nephritis | 7 (18%) | 17% (4-50%) | |
| Anti-GBM disease | 2 (5%) | 36% (26-47%) | |
| IgA Nephropathy | 1 (3%) | 50% | |
| **Biochemistry** | **At Biopsy** | **At 1 Year*** | **At last Follow-Up*** |
| sCr (umol/l) | 84 (52-115) | 82 (58-145) | 77 (57-107) |
| Albumin (g/dl) | 29 (10-40) | 38 (30-46) | 37 (22-45) |
| uPCR (mg/mmol) | 71.2 (0-681) | 23 (0-272) | 15 (0-238) |

* censored for death/dialysis

**Conclusions:** This is the first reported series of severe renal pathology in patients presenting with normal creatinine. Low threshold of suspicion for FN/CGN, prompt renal biopsy and early initiation of treatment may prevent irreversible kidney damage and improve long-term outcomes in these patients.

## SA-PO687

**Analysis of 70 Patients with Severe ANCA Vasculitis Requiring Plasma Exchange at MGH: 2005-2013** William Franklin Pendergraft,[1-3] Charles T. Owens,[1-3] Andrew P. Murphy,[3] Colin M. Berry,[3] Karen A. Laliberte,[3] Robert Makar,[4] John Niles.[2,3] *[1]Joint Nephrology Fellowship Program, Massachusetts General Hospital (MGH) and Brigham and Women's Hospital, Boston, MA; [2]Div of Nephrology, MGH, Boston, MA; [3]Vasculitis and Glomerulonephritis Clinic, MGH, Boston, MA; [4]Blood Transfusion Service, Dept of Pathology, MGH, Boston, MA.*

**Background:** Anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis can cause necrotizing and crescentic glomerulonephritis and pulmonary capillaritis resulting in kidney and lung failure, respectively. When the disease process becomes severe, plasma exchange is often instituted to remove pathogenic autoantibodies.

**Methods:** We performed a retrospective analysis of clinical data related to all ANCA vasculitis patients in our center who required plasma exchange since 2004. Initially, those with acute organ-threatening disease had plasma exchange added to their treatment. For most, this happened within 24 hours of diagnosis or transfer to our institution. Standard exchange dose was typically 4 L/1.73 m2, and a total of seven exchanges were planned to occur over a 10-14 day period. On rare occasion, one to three extra exchanges were added for those with very active disease and exchanges were eliminated for several patients with rapid responses. Serologic and clinical parameters were measured and analyzed.

**Results:** 70 patients [41 women (59%)] with ANCA vasculitis required plasma exchange. There were 52 MPO-ANCA patients and 18 PR3-ANCA patients. In addition to plasma exchange, most patients also received cyclophosphamide, corticosteroids and rituximab induction therapy. Disease severity improved in the majority of patients. 41 patients developed acute kidney injury, but escaped the need for renal replacement therapy while 29 patients required dialysis during initial hospitalization of which 13 recovered renal function.

**Conclusions:** This study describes one of the largest single-center reports of its kind. Plasma exchange appears to contribute to the overall positive outcome of this group. Future work will revolve around identifying differences between these patients and others who did not require plasma exchange in order to determine its utility.

*Funding:* Pharmaceutical Company Support - Alexion Pharmaceuticals, Incorporated

## SA-PO688

**Late-Onset Neutropenia (LON) in ANCA Vasculitis: An Under Recognized Complication of Rituximab** William Franklin Pendergraft,[1-3] Andrew P. Murphy,[3] Karen A. Laliberte,[3] John Niles.[2,3] *[1]Joint Nephrology Fellowship Program, Massachusetts General Hospital (MGH) and Brigham and Women's Hospital, Boston, MA; [2]Div of Nephrology, MGH, Boston, MA; [3]Vasculitis and Glomerulonephritis Clinic, MGH, Boston, MA.*

**Background:** Rituximab (RTX) is an FDA-approved agent that depletes CD20-positive B cells for induction of remission in anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis patients. Our group manages the largest active cohort of ANCA vasculitis patients in the world, the majority of which are receiving RTX for durable maintenance of remission. LON has been reported to be a complication of chronic RTX exposure; however, these reports are limited in ANCA vasculitis patients.

**Methods:** We conducted a systematic retrospective analysis of ANCA vasculitis patients from 2006-2013 who were treated with RTX maintenance of remission therapy and developed LON. Continuous B cell depletion was performed in all patients by scheduled RTX infusion every four months. Complete blood counts with differentials (CBC) were checked every two months. Patients who developed neutropenia from cocaine use as well as patients with both MPO- and PR3-ANCA were excluded from this analysis.

**Results:** 215 patients treated by our group from April 2006 through June 2013 underwent continuous CD20-positive B cell depletion with RTX. 17 patients developed LON (defined as absolute neutrophil count (ANC) < 1000 cells/mm$^3$). Median ANC nadir was 350 cells/mm$^3$. Four episodes resolved without intervention by the time of repeat blood work; however, thirteen episodes were treated with granulocyte colony-stimulating factor (GCSF). Four patients had associated fever and were hospitalized for intravenous antibiotics. In all patients, neutropenia resolved in less than one week and in no patient was RTX discontinued due to this effect.

**Conclusions:** A serious and under recognized adverse feature associated with RTX use appears to be LON and concomitant risk of infection. Fortunately, LON resolves with GCSF use and typically does not recur with continued RTX use. A CBC with differential should be checked in every RTX-treated ANCA vasculitis patient every two months and immediately upon onset of fever.

*Funding:* Clinical Revenue Support

## SA-PO689

**Hypogammaglobulinemia in ANCA Vasculitis Patients Undergoing Continuous B Cell Depletion Using Rituximab** William Franklin Pendergraft,[1-3] Andrew P. Murphy,[3] Karen A. Laliberte,[3] John Niles.[2,3] *[1]Joint Nephrology Fellowship Program, Massachusetts General Hospital (MGH) and Brigham and Women's Hospital, Boston, MA; [2]Div of Nephrology, MGH, Boston, MA; [3]Vasculitis and Glomerulonephritis Clinic, MGH, Boston, MA.*

**Background:** Anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis is a systemic autoimmune disease characterized by small vessel inflammation caused by pathogenic autoantibodies primarily directed against proteinase 3 (PR3) and/or myeloperoxidase (MPO). Rituximab is an FDA-approved humanized monoclonal antibody that depletes CD20-positive B cells. There is increasing evidence that chronic B cell depletion can lead to hypogammaglobulinemia. Our group currently manages over 400 patients with ANCA vasculitis, the majority of which receive rituximab every four to six months to maintain durable remission; thus, we sought to investigate the effect of prolonged rituximab exposure on immunoglobulin concentrations.

**Methods:** We conducted a systematic retrospective analysis of ANCA vasculitis patients from 2006-2013 who were treated with rituximab maintenance of remission therapy and who developed hypogammaglobulinemia.

**Results:** 215 patients treated by our group from April 2006 through June 2013 underwent continuous CD20-positive B cell depletion with rituximab. Hypogammaglobulinemia, defined as an IgG level of < 400 mg/dL, developed in only 17 patients. Rituximab was discontinued in nine of these patients; however, none of these patients relapsed. As expected, the other eight patients, where rituximab was continued, also remained in remission. Six were hospitalized for infection or fever; however, three of these were associated with fever in the setting of late-onset neutropenia. Two patients received intravenous immunoglobulin (IVIg) for recurrent bronchitis.

**Conclusions:** Hypogammaglobulinemia was a rare, late and modest complication that improved with rituximab cessation and/or IVIg administration. There does not appear to be any apparent predictor or association for patients who develop hypogammaglobulinemia; however, as the size of this cohort grows, the power to identify an association may increase.

*Funding:* Clinical Revenue Support

## SA-PO690

**Clinical Characteristics and Renal Outcome in ANCA Associated Vasculitis with Renal Involvement: A 6-Year Retrospective Cohort Analysis** Ping Fu.[1] *[1]Dept of Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan, China; [2]Dept of Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan, China; [3]Dept of Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan, China.*

**Background:** Renal vasculitis is common manifestation in antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV), and is an important cause ESRD. This study aimed to analyze clinical characteristics and renal outcome of patients with renal involvement of AAV.

**Methods:** Patients who presented to the West China Hospital with renal involvement of AVV were retrospectively recruited. All patients were followed up from 3 to 60 months. Diagnostic subgroups were granulomatosis with polyangiitis (GPA); microscopic polyangiitis (MPA); eosinophilic granulomatosis with polyangitis (EGPA) or renal-limited vasculitis (RLD). We recorded patient demographics, laboratory tests, complications, treatments.The primary end-point outcome was the combination of doubling of base line of serum creatinine, end stage renal disease (ESRD), or death.

**Results:** 147 patients were analyzed. There were 69 men and 78 women with age of 56.02 ±15.33. The duration was 4.88 ±6.63 months. 8.8%patients were categorized as having GPA, 85.0% as MPA, 5.4% as PICGN. ANCA serology identified cANCA in 10.2% patients and pANCA in 87.1% patients. The mean serum creatinine at initial of disease was 363.97 umol/L. 15.6% patients required hemodialysis at the beginning of the disease. 11(7.4%) patients who presented with a doubling of creatinine level, ESRD occurred in 30 patients (20.4%), of whom 2 (1.4%) died. Relapse occurred in 29 patients (19.7 %). Renal survival at 1 and 5 years were 85.0% and 72.8% respectively. Old age more than 60 years, initial high serum creatinine level, high CRP level and infectious complication were predictors of poor renal outcome.

**Conclusions:** Age, serum creatinine level were the independent predictor of patients' survival of AAV. Old age and serious infection affect mortality and renal outcome of ANCA-associated systemic vasculitis. To improve renal outcome, earlier diagnosis, controlling infectiou complication should be established.

## SA-PO691

**Validation Study of the New Histopathologic Classification of ANCA-Associated Glomerulonephritis** Taewoo Lee, A. Gasim, JulieAnne G. McGregor, Caroline J. Poulton, Susan L. Hogan, Ronald J. Falk, Patrick H. Nachman, J. Charles Jennette. *UNC Kidney Center, The Univ of North Carolina at Chapel Hill.*

**Background:** A new histopathologic classification consisting of four classes (focal, crescentic, mixed and sclerotic) was recently introduced to predict prognosis of ANCA-associated glomerulonephritis (ANCA-GN) (Berden et al, J Am Soc Nephrol 2010).We aimed to ascertain the prognostic value of the classification in a large inception cohort.

**Methods:** ANCA-GN patients with >1 year of follow-up were included and categorized according to the new classification. Long-term patient and renal survival was assessed using competing risk regression analysis and adjusting for clinical characteristics (demographics, ANCA type, ANCA phenotype, estimated GFR (eGFR), and treatment) and additional histopathologic variables including chronicity score (0-16 points, sum of interstitial fibrosis, tubular atrophy, glomerular and crescent sclerosis) and arteriosclerosis (0-4 points).

**Results:** 207 patients were included: median age 65 years (IQR 49-73), 45% female, median eGFR 8.2 ml/min/1.73m2 (5.6-13.8), 42% MPO-, 58% PR3-ANCA. Patients and renal outcomes per histopathological classification are summarized in the table.

| | Focal (n=22) | Crescentic (n=77) | Mixed (n=71) | Sclerotic (n=39) |
|---|---|---|---|---|
| Renal death | 4% | 21% | 21% | 49% |
| Patient death | 30% | 33% | 25% | 15% |

The new classification was not associated with the composite outcome of patient or renal survival (P= 0.098 in Log-rank test and P=0.921 in trend test). In multivariate analysis, eGFR <15 ml/min [Subdivision hazard ratio (SHR) 1.85 (95% CI 1.43-1.96)] and severe chronicity [SHR per unit increase 1.10 (1.01-1.19)] were risk factors for renal death. Use of cyclophosphamide [SHR 0.3 (0.13-0.69)] and eGFR ≥ 15 ml/min [SHR 0.38 (0.15-0.97)] were significantly associated with patient survival after adjustment for age, ANCA type, and the new classification.

**Conclusions:** The new histopathologic classification alone is insufficient in assessing prognosis in ANCA-GN. A classification scheme that incorporates measures of tubulo-interstitial damage may be of improved prognostic value.

## SA-PO692

**MPO-Positive Cells and Extracellular MPO Are Associated with Capillary Injury in Glomeruli in Lupus Nephritis** Soko Kawashima, Shinya Kaname, Yoshinori Komagata, Yoshihiro Arimura, Akira Yamada. *First Dept of Internal Medicine, Kyorin Univ School of Medicine, Mitaka, Tokyo, Japan.*

**Background:** We have recently reported that in human MPO-ANCA-associated glomerulonephritis (GN), MPO exists along the glomerular capillary walls near infiltrated MPO-positive cells, suggesting that MPO release from neutrophils directly causes capillary injury (Clin Nephrol 2013). Here we investigated a possible role of MPO in the pathogenesis of glomerular capillary injury in patients with lupus nephritis, in which neutrophil infiltration and vascular necrosis are also frequently observed.

**Methods:** We analyzed 589 renal specimens obtained from 27 patients with lupus nephritis class IV. Glomerular infiltration of MPO-positive cells, deposition of extracellular MPO and endothelial cell injury were analyzed. Colocalization of MPO and CD34 deposition was also examined by immunofluorescence staining.

**Results:** The infiltration of MPO-positive cells and MPO deposition were observed in some glomeruli, especially in the glomeruli at an active injury phase with severe inflammatory cell infiltration, whereas MPO was rarely stained in sclerotic/fibrotic glomeruli with high chronicity. MPO deposition was mainly located in the glomerular capillary walls near the infiltrated MPO-positive cells. Interestingly, there were various degrees of MPO deposition along the capillary walls, and necrotic lesions were strongly associated with diffuse-type MPO deposition that covered more than 50% of the capillary loops, but was rarely associated with limited-type MPO deposition where only a small part of capillary walls was involved. Moreover, in some parts of necrotic lesions with MPO deposition phenomenon of neutrophil extracellular traps (NETs) was suggested to occur.

**Conclusions:** These results indicate that MPO deposition released from infiltrated MPO-positive cells, particularly diffusely distributed deposition along the glomerular capillary wall, may play important roles in the pathogenesis of lupus nephritis.

## SA-PO693

**Outcomes of Elderly Patients with ANCA-Associated Vasculitis Treated with Immunosuppressive Therapy** Parminder K. Judge, Richard Haynes, Edward J. Sharples. *Oxford Kidney Unit.*

**Background:** ANCA-associated vasculitis (AAV) is a common cause of acute kidney injury (AKI) in the elderly. Treatment requires immunosuppression (IS), which can have significant toxic effects. The aim of this study was to assess morbidity and mortality associated with immunosuppression in elderly patients with AAV.

**Methods:** A retrospective review of all patients presenting with AAV to a tertiary renal unit between 1990 and 2011 was conducted using the units' information database. 264 patients were reviewed and 32 who did not receive standard IS were excluded. 232 patients given induction therapy with prednisolone and cyclophosphamide (P&C) were studied.

**Results:** There were 146 (63%) males and 86 females (37%). 122 patients were PR3 positive and 110 were MPO positive. Mean creatinine at presentation was 528 µmol/L. Median duration of follow-up was 59 months. 76 patients had pulmonary involvement (18 severe). Older patients (aged >65) were significantly more likely to require dialysis at presentation (RR 1.66 [1.13-2.5]) and longer-term and were treated with lower total cyclophosphamide dose (mean 6.5g) than those <55 years (mean 10.5 g). Older patients were more likely to develop leukopaenia (RR 2.3 [1.7-3.3]) and infections in the first year (RR 2.4 [1.5-3.9]). After multivariable adjustment, age and dialysis at presentation were significant predictors of death (HR 1.07 [1.03-1.11, p=<0.001] and HR 2.2 [1.10-4.38, p=0.03] respectively).

**Conclusions:** Among patients treated with P&C, older age and dialysis-dependency were associated with worse survival. Older patients were also more likely to develop treatment-related complications despite lower cumulative doses. Morbidity and mortality associated with treatment must therefore be carefully balanced against that associated with the disease process itself.

## SA-PO694

**Laser Microdissection and Mass Spectrometry of ANCA-Negative Pauci-Immune Crescentic Glomerulonephritis Shows Accumulation of Components of the Alternative and Terminal Pathway of Complement** Sanjeev Sethi, Julie A. Vrana, Ladan Zand, Jason David Theis, Samih H. Nasr, Fernando C. Fervenza. *Mayo Clinic.*

**Background:** Pauci-immune crescentic glomerulonephritis (CRGN) is associated with positive-anti neutrophil cytoplasmic antibody (ANCA) titers in 80-90% of patients. The remaining patients are ANCA-negative CRGN. Recent studies have suggested a role for the alternative pathway of complement in the pathogenesis of ANCA-negative pauci-immune CRGN.

**Methods:** We identified 8 patients with ANCA-negative pauci-immune CRGN. Using laser microdissection, we dissected 2-4 non-scarred glomeruli/sample. Three samples were dissected for each case. For comparison, we identified 3 patients with ANCA-positive pauci-immune CRGN, one was proteinase 3 and two were myeloperoxidase positive. Peptides extracted from the microdissected tissue were subjected to liquid chromatography and mass spectrometry (MS). MS raw data files were queried using 3 different algorithms (Sequest, Mascot and X!Tandem), the results were combined and assigned peptide and protein probability scores in Scaffold. For each sample, a list of proteins with spectra numbers based on peptides identified by MS was generated.

**Results:** MS data showed high spectra numbers for C3 (14.75 ± 1.7) and moderate spectra numbers for C9 (6.3± 1.3) in glomeruli dissected from patients with ANCA-negative pauci-immune CRGN. On the other hand, MS showed low spectra numbers for C3 (4± 2) and no spectra numbers for C9 in ANCA positive pauci-immune CRGN. Importantly, C4B was absent in all cases of ANCA-negative pauci-immune CRGN. Spectra numbers of Ig- gamma-1 chain C region was detected in both ANCA positive (9 ± 5.2) and ANCA-negative (6.3 ± 1.3) pauci-immune CRGN. Both ANCA-negative and ANCA positive pauci-immune CRGN showed high spectra numbers for fibrinogen alpha chain, collagen alpha-2 and -1 (IV) chain.

**Conclusions:** Laser microdissection and MS studies indicate a role for the alternative and terminal pathway of complement in ANCA-negative pauci-immune CRGN. This is evidenced by the presence of large spectra numbers of C3 and moderate spectra numbers of C9 in glomeruli of ANCA-negative pauci-immune CRGN compared to ANCA-positive pauci-immune CRGN.

## SA-PO695

**Phase 2 Clinical Trial of CCX168, an Orally Administered C5aR Antagonist, in Patients with ANCA-Associated Renal Vasculitis (CLEAR)**
Annette Bruchfeld,[1] Matthias Schaier,[2] Lorraine Harper,[3] Michel Y. Jadoul,[4] Marten Segelmark,[5] Istvan Szombati,[6] Michael Venning,[7] Patrick Hamilton,[7] Christian Hugo,[8] Paul L.A. Van daele,[9] Ondrej Viklicky,[10] David R.W. Jayne,[11] Antonia Potarca,[12] Juan C. Jaen,[12] Thomas J. Schall,[12] Pirow Bekker.[12] *[1]Karolinska Institute, Sweden; [2]Univ Hosp Heidelberg, Germany; [3]Univ of Birmingham, United Kingdom; [4]Cliniques Saint-Luc, Belgium; [5]Linköping Univ, Sweden; [6]Budaclinic, Hungary; [7]Manchester Univ, United Kingdom; [8]Dresden Univ, Dresden, Germany; [9]Erasmus MC Center, Netherlands; [10]Instit of Clin and Exp Med, Czech Republic; [11]Univ of Cambridge, United Kingdom; [12]ChemoCentryx, Inc., Mountain View, CA.*

**Background:** CCX168 blocked C5a-induced neutrophil chemotaxis and CD11b expression in blood samples in Ph1. It markedly reduced glomerular crescent formation and necrosis, and proteinuria and hematuria in transgenic hC5aR mice with anti-MPO-induced GN.

**Methods:** This Ph2 trial tested whether CCX168 could substitute, at least partially, for CS. In Step 1, 12 pts were randomized 2:1 to 30 mg CCX168 BID + a reduced dose of prednisone (20mg/d) or placebo BID + a full dose of prednisone (60mg/d) for 12 wks. All patients received CYC 15 mg/kg IV up to 1.2g q2-3wk. If Step 1 were successful, in Step 2, 12 patients received the same treatments as Step 1, except the CCX168 group received no CS. Eligible patients had GPA, MPA, or renal limited vasculitis, were anti-PR3 or MPO+ and had active renal vasculitis. Primary objective: safety and tolerability. Secondary objectives: feasibility of reducing CS and effect of CCX168 on renal disease.

**Results:** Baseline characteristics are shown below.

| Baseline characteristic | Step 1 N=12 | Step 2 N=14 |
|---|---|---|
| Age | 59±14 | 54±15 |
| M/F | 6/6 | 9/5 |
| Newly diagnosed/Relapsed | 9/3 | 11/3 |
| Anti-MPO+/PR3+ | 7/6 | 7/7 |
| s-creat, µmol/L | 120±41 | 114±40 |

No SAEs related to CCX168 occurred in Steps 1 or 2. No rescue CS was required in Step 1 in the treatment period and two rescue events in Step 2 (treatment still blinded).

**Conclusions:** CCX168 shows promise in the treatment of ANCA-associated renal vasculitis.

## SA-PO696

**Endothelial Cell Injury Contributes to the Formation and Development of Extracapillary Lesions in Crescentic Glomerulonephritis**
Emiko Fujita,[1] Akira Shimizu,[2] Akiko Mii,[1] Megumi Fukui,[1] Shuichi Tsuruoka.[1] *[1]Dept of Nephrology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan; [2]Dept of Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

**Background:** Glomerulonephritis (GN) with extracapillary lesions is known to have a poor prognosis. However, the endothelial injury has not been elucidated. In the present study, we clarified the association of endothelial cell injury with the formation of extracapillary lesions and the development of crescentic GN.

**Methods:** Renal biopsy samples of crescentic GN including ANCA-related GN (n=30), lupus GN (n=30), and purpura GN (n=30) were diagnosed by pathologic studies. Endothelial cell injury was assessed electron microscopy and by immunostaining against CD34 that is expressed on all endothelial cells, and was characterized by the formation of extracapillary lesions and development of crescentic GN.

**Results:** In ANCA-related GN, lupus GN, and purpura GN, almost all active necrotizing glomerular lesions (n=36) began as focal and segmental leukocyte infiltration with loss of individual CD34+ endothelial cells. Subsequently, necrotizing lesions developed and were characterized by expansive loss of CD34+ cells together with exudation of fibrinoid materials (n=36), rupture of basement membrane (n=10), and crescent formation (n=83). By electron microscopy, capillary destruction was evident with fibrin exudation. In the chronic stage of crescentic GN (n=12), glomerular sclerosis developed without the appearance of glomerular capillaries. Furthermore, the remaining glomerular lobes without crescents had marked collapsing tufts with loss of endothelial cells and the development of glomerular sclerosis.

**Conclusions:** In crescentic GN, loss of glomerular capillaries with endothelial cell injury is strongly associated with the formation of necrotizing and extracapillary lesions. Furthermore, impaired capillary regeneration and collapse of remaining capillaries with loss of endothelial cells contribute to the development of glomerular sclerosis. The endothelial cell injury then induces the formation and development of extracapillary lesions in crescentic GN.

## SA-PO697

**Duration of Hematuria in ANCA Associated Glomerulonephritis (ANCA-GN): Does It Matter?**
Teresa K. Chen, Pradeep Manoharan, Duvuru Geetha. *Medicine, Johns Hopkins Univ.*

**Background:** Hematuria is considered a marker of active renal disease in ANCA-GN, both in activity tools for clinical trials and in routine clinical practice. Induction immunosuppression is often continued until hematuria has resolved. This study aims to describe predictors of hematuria resolution in ANCA-GN and whether hematuria duration is associated with estimated GFR (eGFR) at 1 year.

**Methods:** We conducted a retrospective study of 55 patients with biopsy-proven ANCA-GN. Proteinuria was defined as ≥1+ on dipstick and hematuria as >5 rbc/hpf. GFR was estimated using MDRD. The primary exposure was duration of hematuria, defined as <90 vs. ≥ 90 days post renal biopsy. The primary outcome was eGFR at 1 year. We used both linear and logistic regression, adjusting for age, gender, ANCA type, baseline eGFR, and proteinuria. Additional analyses were conducted to identify clinical and laboratory predictors of hematuria resolution.

**Results:** Mean age at diagnosis was 57 yrs (53% male, 80% Caucasian, 38% c-ANCA, 45% p-ANCA). At entry, all patients had hematuria, 94% had proteinuria, and mean (SD) serum creatinine was 3.1 (2.3) mg/dL. The majority (93%) were treated with cyclophosphamide or rituximab and steroids. Mean time to hematuria resolution was 91 (77) days, and 34 (62%) patients had hematuria resolution prior to 90 days. Older age and lower baseline eGFR were associated with lower eGFR at 1 year (p=0.03 and p<0.001, respectively). Hematuria resolution (<90 vs. ≥90 days) was not predictive of eGFR at 1 year (p=0.89). In sensitivity analyses, age, gender, baseline eGFR, proteinuria, and ANCA type were not associated with timing of hematuria resolution.

Table 1: Predictors of eGFR at 1 year.

| Baseline variables | Change in eGFR at 1 year | 95% CI | p-value |
|---|---|---|---|
| Hematuria ≥90 days versus <90 days | -0.87 | (-13.93, 12.19) | 0.89 |
| Age, per 10 years older | -4.78 | (-8.99, -0.58) | 0.03 |
| Female | -8.56 | (-21.44, 4.32) | 0.19 |
| ANCA type | | | |
| Negative | REF | REF | REF |
| p-ANCA | 2.08 | (-18.53, 22.68) | 0.84 |
| c-ANCA | -1.54 | (-19.00, 15.91) | 0.86 |
| Baseline eGFR, per 10 ml/min/1.73 $m^2$ lower | -7.55 | (-10.78, -4.31) | <0.001 |
| Proteinuria ≥1+ versus <1+ on dipstick | 10.08 | (-15.90, 36.05) | 0.44 |

**Conclusions:** Duration of hematuria does not predict eGFR at 1 year in ANCA-GN. We propose that among patients otherwise in clinical remission, the persistence of hematuria should not delay transition from induction to maintenance immunosuppression.

## SA-PO698

**Increased Risk for Antineutrophil Cytoplasmic Antibodies Associated Renal Vasculitis (AAV), Specifically Antimyeloperoxidase (P-ANCA) for Hispanic Patients (HISP)**
Salwa Rhazouani,[1] George N. Coritsidis,[1] Aleef M. Rahman.[2] *[1]Medicine/Nephrology, Elmhurst Hospital Center, Elmhurst, NY; [2]Stony Brook Univ Medical Center, Stony Brook, NY.*

**Background:** AAV is an uncommon diagnosis with most clinical observations arising from Europe, Japan and the United States. Recent data has suggested an increase in antiproteinase 3 associated (C-ANCA) vasculitis in Mexicans. Since data from Central & South America is limited, a retrospective analysis of biopsies from our institution that serves a large immigrant Hispanic population was conducted.

**Methods:** All renal biopsies (1999-2012) were analyzed for: age, gender, proteinuria(g/d), MDRD glomerular filtration rate (eGFR,ml/min/1.73 m2), renal outcomes and histology at presentation. Data presented as average ± standard error of mean. Multivariate logistic regression models were used to determine odds.

**Results:** Of 281 biopsies reviewed: AAV represented 12% (34/281) of the total; 21.9% of all HISP biopsies (25/114) and 5.4% of all non-Hispanic biopsies (9/167). AAV was the second most common glomerulonephritis after SLE (21.9 % vs. 23.7%) in HISP. HISP presented at 53.1± 3.4 years old, with proteinuria and eGFR of 3.4 ± 0.5 and 16.5 ± 3.2, respectively. 28% of HISP (7/25) had hemoptysis. Among 25 HISP with AAV, 56% (n=14/25) had P-ANCA and 40% (10/25) C-ANCA. Of all HISP AAV, 61% were South American and only 15% Mexican. 70% of South Americans had P-ANCA. 44% of HISP-AAV are dialysis dependent.

*P-Value<0.05, **P-Value<0.005

| Non-Adjusted Model | Characteristic | AAV OR, 95% CI | C-ANCA OR, 95%CI | P-ANCA OR, 95% CI |
|---|---|---|---|---|
| | Hispanic | 4.80 [2.14, 10.75]** | 5.12 [1.37, 19.07]* | 7.46 [2.093, 26.63]** |
| Adjusted Model | Characteristic | AAV OR, 95% CI | C-ANCA OR, 95% CI | P-ANCA OR, 95% CI |
| | Hispanic (ref: non-Hispanic) | 5.61 [2.37, 13.25]** | 5.38 [1.41, 20.49]* | 8.521 [2.266, 32.04]** |
| | Age | 1.06 [1.035, 1.098]** | 1.04 [0.999, 1.086] | 1.081 [1.038, 1.125]* |
| | Gender ( ref:females) | 2.017 [0.907, 4.48] | 3.36[ 0.975, 11.58] | 0.992 [0.334, 2.94] |

**Conclusions:** AAV was the second most common glomerulonephritis in HISP. The odds of having AAV is 5.6 times higher for HISP than non-Hispanics when adjusted for age and gender.The odds of having P-ANCA is 8.5 times higher for HISP than non-Hispanics.

## SA-PO699

**Rituximab for Remission Induction of Recurrent ANCA Associated Glomerulonephritis Post Kidney Transplant**
Duvuru Geetha, Christine Adefuin Murakami, Pradeep Manoharan, Naima Carter-Monroe. *Johns Hopkins Univ, Baltimore, MD.*

**Background:** Kidney transplantation (KTX) is the treatment of choice for patients with end stage renal disease (ESRD) due ANCA associated vasculitis (AAV). Relapses of AAV occur after KTX and may adversely affect allograft survival. Combined therapy with cyclophosphamide (CYC) and glucocorticoids has been the cornerstone of treatment for these relapses. B cells are implicated in the pathogenesis of AAV. Rituximab (RTX),

a B cell depleting monoclonal antibody is approved for remission induction in AAV. We report the clinical presentation and outcomes of 5 KTX recipients who were treated for recurrent glomerulonephritis (GN) with RTX.

**Methods:** We identified 37 patients who underwent KTX for ESRD due to AAV between 1999 and 2012. Seven patients experienced recurrent GN and 5 were treated with RTX. We collected demographics, details of immunosuppression, clinical features at relapse and outcomes following RTX use in these patients.

**Results:** The median age at the time of KTX was 26 years (4 Caucasians, 3 females). The disease phenotype was microscopic polyangiitis in 3 and granulomatosis with polyangiitis in 2 patients (p-ANCA=3, c-ANCA=2). All patients were in remission with 4 being ANCA positive at time of KTX, 3 received indcution therapy and all were maintained on steroids, mycophenolate mofetil and tacrolimus. Biopsy proven recurrent GN occurred at a median of 26 months. All patients had rise in serum creatinine and hematuria and were ANCA positive at relapse. RTX was used with steroids in all 5 patients. Four patients achieved disease remission after RTX, the fifth patient was refractory to RTX and CYC. All patients showed evidence of B cell depletion and 2 patients were ANCA negative post RTX. Follow up biopsies in 3 patients showed resolution of active GN in 2 patients and persistent active GN in 1 patient. At the time of last follow up, 2 patients had reached ESRD, one to refractory vasculitis and one to rejection and recurrent GN due to medication noncompliance.

**Conclusions:** RTX is an alternative to CYC for remission induction in recurrent AAV associated GN in KTX patients.

## SA-PO700

**ANCA-Associated Renal Vasculitis: Insights from an Observational Cohort Study** Mark N. Canney, Eoin O Sullivan, Philip Hugh Bredin, Michael Clarkson. *Dept of Renal Medicine, Cork Univ Hospital and Univ College Cork, Ireland.*

**Background:** ANCA-associated vasculitis (AAV) is a life threatening illness requiring robust immunosuppression that carries attendant risks of leucopenia and sepsis. Our unit is the sole tertiary referral centre for AAV in Southwest Ireland (population 650,000). Here we report the incidence and outcomes of renal AAV in an Irish population. Furthermore we explore the role of renal biopsy and IV vs oral cyclophosphamide (CYC) in the management of this condition.

**Methods:** The Cork Renal Vasculitis Registry captures all incident AAV patients in Southwest Ireland with clinical evidence of renal involvement. The period of interest was 2005-2012. Data was obtained from laboratory results and patient records, and analysed using SPSS v16.0.

**Results:** 73 patients met the inclusion criteria giving an incidence of 15.4 cases per million patient years. The majority were male (59%), anti-MPO ANCA positive (64%) and received CYC as induction therapy (68%). One year patient survival was 88%. Patients who received oral CYC tended to develop more severe leucopenia than those who received IV CYC (p=0.08). Patients who did not undergo renal biopsy had a higher creatinine at baseline than those biopsied (387 vs 279µmol/L, p=0.065). They received more plasma exchange (47% vs 10%, p<0.001) and pulse methylprednisolone (72% vs 40%, p=0.007) as induction therapy. Not having a renal biopsy associated with a higher risk of death and ESKD even after adjusting for confounding variables such as age, creatinine at presentation and initial BVAS (p=0.03). Intriguingly not having a biopsy associated with higher grade leucopenia (p<0.001) suggesting the potential of relative over-immunosuppression in more critically ill patients at presentation.

**Conclusions:** Induction with oral CYC may result in more severe leucopenia than IV CYC, in keeping with suggestions from clinical trial data. In severe renal AAV, not undergoing a renal biopsy associated strongly with more intense induction immunosuppression, higher grade leucopenia and higher risk of death/ESKD. Inability to perform a renal biopsy at presentation may be an important signal identifying patients at highest risk of adverse outcomes.

## SA-PO701

**In Sweden, ANCA Associated Nephritis Is a More Common and a More Severe Disease as Compared to Lupus Nephritis** Marten Segelmark,[1] Maria Weiner,[1] Christopher Sjöwall,[2] Ola Nived,[3] Per Eriksson,[2] Aladdin Mohammad.[3] *[1]Nephrology, Linköping Univ, Linköping, Sweden; [2]Rheumatology, Linköping Univ, Linköping, Sweden; [3]Rheumatology, Lund Univ, Lund, Sweden.*

**Background:** The aim of this study was to compare incidence rates and outcome between lupus nephritis (LN) and anti-neutrophil cytoplasm antibody (ANCA) associated nephritis (AAN) during a 12 year-period in two geographically defined populations in south Sweden.

**Methods:** In health care districts surrounding the University Hospital of Lund (population 237 000) and in districts surrounding the University Hospital of Linköping (population 417 000) all patients with biopsy proven LN and AAN during the period 1997 to 2008 were included in the study if they: (i) resided within the study areas at the time of onset of nephritis, (ii) had a clinical diagnosis of either SLE or AAV and (iii) experienced a first flare of biopsy-proven nephritis during the study period.

**Results:** During the study period 83 patients (Lund 44+ Linköping 39) with biopsy proven AAN were identified as compared to 27 patients with LN (13+14).

| Disease | n | Sex (M/F) | Age at diagnosis median (range) | p-creatinine (mg/dL) | Deaths | ESRD |
|---|---|---|---|---|---|---|
| AAN | 83 | 36/47 | 68 (15-88) | 2.82 | 36 | 19 |
| LN | 27 | 22/5 | 36 (18-72) | 0.88 | 2 | 1 |

The combined annual incidence rate per million inhabitants was estimated to be 10.5 (95% CI 8.2-12.7) for AAN and 3.4 (95% CI 2.1-4.7) for LN. Patients were followed until January, 2013. During the follow-up time 38 patients died (AAN 36, LN 2; p=0.033), and 20 patients developed end-stage renal disease (AAN 19, LN 1; p=0.021).

**Conclusions:** In Sweden AAN outnumber LN almost three to one, and outcome is considerably worse. In our country SLE is often diagnosed and treated early which in many cases prevent the development of severe nephritis, while AAN is often diagnosed at later stages leading to severe consequences.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## SA-PO702

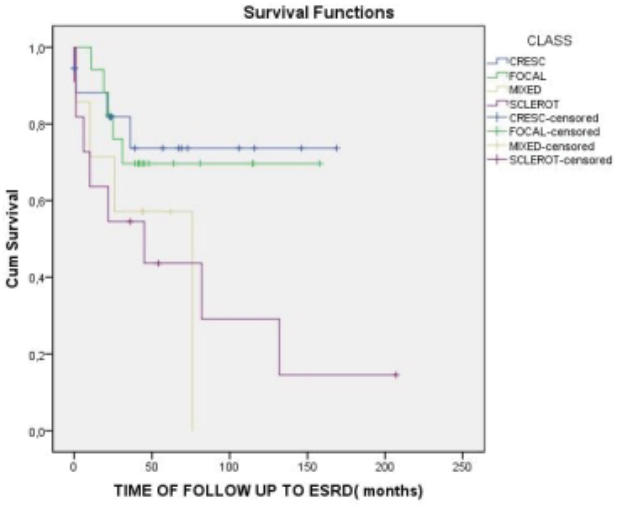
**Reclassification of ANCA-Associated Vasculitis (AAV) from a Nephrology Department According to a New Histopathologic Classification** Jesús P. Marin, Juan R. Gomez-Martino, Ines Castellano, Clarencio Javier Cebrian Andrada, Silvia Gonzalez S, Maria C. Jimenez, Sandra Gallego, Vanesa Garcia-Bernalt, Pedro J. Labrador. *Nephrology Div, Hospital San Pedro de Alcantara, Caceres, Spain.*

**Background:** AAV are the main cause of rapidly progressive glomerulonephritis. The aim of our study is to classify our patients with AAV diagnosed by renal biopsy according to histopathologic classification published by Berden et al in the JASN in 2010.

**Methods:** This is a retrospective descriptive study. From renal biopsies made during the last 20 years we analized AAV, describing: age, sex and histological category. Patients were classified in four classes:"focal" (≥ 50 % normal glomeruli),"crescentic"(≥ 50% glomeruli with cellular crescents)," mixed" (< 50 % normal, < 50 % crescentic, < 50 % globally sclerotic glomeruli), and "sclerotic" (≥ 50 % globally sclerotic glomeruli), following clinical evolution.

**Results:** From 629 biopsies, 67 (10,6%) corresponded to AAV. Patients had a mean age of 62,5 ± 12,6 years. Men/women 34:33. Histopathologic classification and evolution are represented in Table 1 and renal survival in Figure 1.

| CATEGORY | % | RRT (%) *p<0,05 | NO RRT (%) | EXITUS |
|---|---|---|---|---|
| **FOCAL(21)** | 31,3 % | 6(28,6%)* | 15(71,4 %) | 5(23,8%) |
| **CRESCENTIC(23)** | 34,4 % | 8(34,8%)* | 15(65,2%) | 5(21,74%) |
| **MIXED(8)** | 11,9 % | 5(62,5%)* | 3(37,5%) | 2(25%) |
| **SCLEROTIC(15)** | 22,4 % | 11(73,3%)* | 4(26,7%) | 1(6,67%) |




**Conclusions:** Following this classification, most of patients belong to crescentic class, followed by focal, sclerotic and mixed class. Patients of sclerotic class had the highest percentage of starting RRT, and focal class the least percentage. Highest mortality corresponded to mixed class. The severity of initial damage can predict evolution and renal survival.

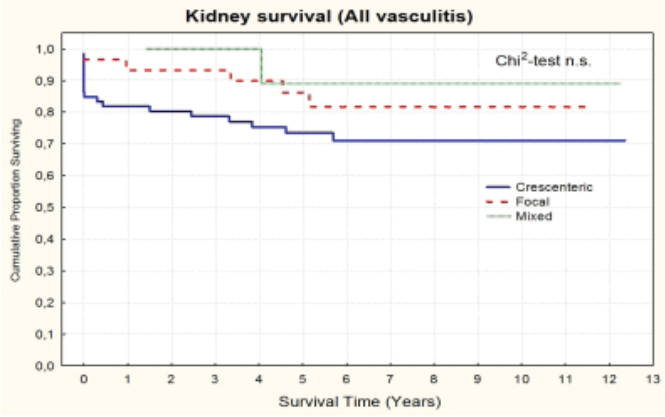
## SA-PO703

**Impact of Renal Histological Classification on Long Term Renal Outcome in ANCA Associated Vasculitis** Wladimir M. Szpirt,[1] Elizabeth Krarup,[2] Martin Egfjord.[1] *[1]Nephrology, Rigshospitalet; [2]Nephrology, Herlev, Copenhagen, Denmark.*

**Background:** The four histological subclasses in renal biopses of patients with ANCA associated vasculitis (AAV) have been proposed and shown to have an impact on renal outcome.

**Methods:** A prospective cohort study of all AAV pts. referred to our centre between 2000-2010 was performed. All pts. had AAV based on positive ANCA and a compatible clinical syndrome. 132 pts. were admitted and 118 followed for a mean of 5.7 years (range 0.2-12.3; 676 patient years) and 116 were kidney biopsed. 57% were male, 47% were MPO-ANC A positive. 36% had high creatinine >500. 40% were aged >65. 105 pts. were Plasma Exchange(PLEX)-d (mean 7 (5-11)). and the use was decided on severity of renal biopsy and ANCA titres. Immunsuppression consisted of prednisolone 1 mg/kg/day and a low dose of daily oral Cyclophosphamide (100 mg/day in pts. <65 years and 50 mg/day in pts. >65). AZA/MMF was given for maintenance of remission.

**Results:** The renal histological subclasses were classified into focal(≥50% normal glomeruli, n=30), mixed(<50% crescents and < 50% sclerotic, n=12), crescentic (>50% crescents, n=66) and sclerotic(>50% sclerosis, n=5 - not shown). 6 biopsies were inconclusive due to few glomeruli. Furthermore interstitial fibrosis was scored and 75 biopses were fibrosis free whether 41 had fibrotic changes. No significant differences were found between the classes, however at 5 years the best survival was found for mixed (89%), followed by focal (87%) and crescentic (73%) subclass, whether 3 out of 5 sclerotic kidneys were still functioning. Comparison of plus/minus fibrosis showed 5 years renal survival of 81% for non fibrotic kidneys compared to 72% with fibrosis (n.s.).




**Conclusions:** Only few renal biopses showed chronic sclerotic picture and the biopsy findings including interstital fibrosis were correlated to long term renal outcome, however the differences were not significant.

## SA-PO704

**Evaluating Lupus Nephritis with Serial Kidney Biopsies** Anthony Alvarado,[1] Ana Malvar,[2] Valeria Gabriela Alberton,[3] Bruno Jorge Lococo,[2] Maria Fernanda Toniolo,[3] Brad H. Rovin.[1] *[1]Ohio State Univ Wexner Medical Center, Columbus, OH; [2]Hospital Fernandez, Buenos Aires, Argentina; [3]C.D. Pathologico, Buenos Aires, Argentina.*

**Background:** The effectiveness of lupus nephritis (LN) therapy is determined mainly by improvement in proteinuria and renal function. The histologic response to therapy is generally not evaluated because serial kidney biopsies are not usually obtained in patients who have improved. Also, stopping therapy in responders is a clinical decision, and renal pathology is not taken into account. In this study serial renal biopsies were correlated with clinical response to determine the concordance of clinical and histologic findings.

**Methods:** Biopsies were done in 25 Hispanic LN patients at LN diagnosis (BX1), after 6 months of induction therapy with either MMF (2.4g/d) or cyclophosphamide (1g/mo X6) (BX2), and during maintenance therapy with either MMF or azathioprine, after at least 42 months of total treatment, and 24 months of clinical inactivity (BX3). Biopsies were read by a single renal pathologist (VA) and NIH activity (AI) and chronicity (CI) indices were calculated. Serum creatinine (SCr) and proteinuria (PR) were measured serially.

**Results:** Between BX1 and BX3, SCr and PR improved significantly (1.0±0.4 vs 0.8±0.3 mg/dl and 3.3±2.1 vs 0.3±0.2 g/d respectively, P<0.001). From BX1 to BX3 AI declined (9±4 vs 1.9±1.7, P<0.001) and CI increased (2.8±1.4 vs 4.3±1.6, P<0.01). However in individual patients who were complete responders (n=16, SCr normal; PR<0.5g/d) only 6 had an AI of 0-1 at BX3, while 8 had an AI≥3 at BX3 (range 3-5). Multivariate modeling showed that improvement in AI at BX3 correlated with cyclophosphamide induction plus the decline in SCr between BX1 and BX2, or cyclophosphamide induction plus the increase in complement component C4 between BX1 and BX3.

**Conclusions:** These data demonstrate discordance between clinical and histologic responses in LN patients on immunosuppressive therapy for more than 3 years, with 50% of complete responders still having active histologic lesions. The implications of this continued low-level activity for discontinuation of therapy remains to be determined.

*Funding:* Other NIH Support - National Center for Advancing Translational Sciences

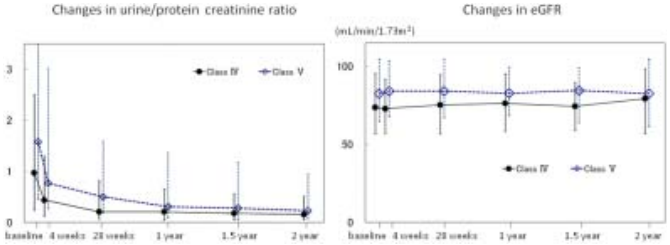
## SA-PO705

**Two-Year Maintenance Therapy with Tacrolimus for Class IV and V Lupus Nephritis Patients, Sub-Analysis of Post-Marketing Surveillance in Japan (TRUST Study)** Hirofumi Makino,[1] Naoko Wakasugi,[2] Tsutomu Takeuchi.[3] *[1]Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan; [2]Astellas Pharma Inc., Japan; [3]Keio Univ School of Madicine, Japan.*

**Background:** Tacrolimus (TAC) is an immunosuppressive macrolide that blocks T cell activation, and it is widely administered following organ transplantation. Recently some small randomized controlled trials have shown that TAC is an effective maintenance treatment for lupus nephritis (LN), and a small open-label trial showed that TAC is effective in class V LN. However, in any trials, the sample sizes were small and the follow-up period was not sufficiently long, and the safety and efficacy with class IV LN has not been clear enough.

**Methods:** Post-marketing Surveillance has been conducted in Japan. The design was an open-label, non-comparative, non-interventional observational study. Patients were registered centrally between 2007 and 2010, and we have conducted an interim analysis included all the registered 1375 patients. To clarify the safety and efficacy of TAC for class IV and V LN, the sub-analysis was performed.

**Results:** Of 1375 patients, we studied the 214 patients with biopsy-proven Class IV and 159 patients with Class V. There was no difference in the safety by class. The patients with Class V had higher urine protein/creatinine ratio(P/C ratio) with higher e-GFR at baseline, but the improvement of P/C ratio two years after TAC treatment were almost equivalent, and the eGFR was maintained in both classes.




**Conclusions:** Sub-analysis of data from TRUST showed that 2 years TAC treatment was a safe and effective treatment in both Class IV and V LN. It seemed to take a longer time to complete remission in Class V compared to Class IV, we found that 2 years TAC treatment achieved the maintenance of remission in both classes, and the renal functions were maintained similarly in both classes.

*Funding:* Pharmaceutical Company Support - Astellas Pharma Inc.

## SA-PO706

**The Achilles Heel of Lupus Nephritis Treatment – Identifying Non Adherence Using Hydroxychloroquine Blood Levels** Suceena Alexander, Gary Chusney, Vivienne D. Chusney, Janet Lee, Tom Cairns, Liz Lightstone. *Dept of Renal Medicine and Leslie Brent Laboratory, Imperial College Kidney and Transplant Institute, London, United Kingdom.*

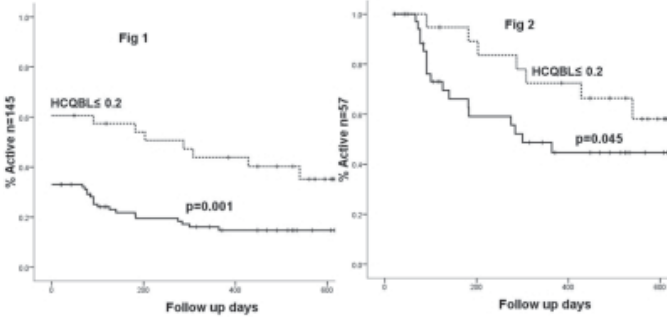
**Background:** Non adherence (NA) to lupus nephritis (LN) treatment (Rx) is the most important factor for non remission (NR) or renal relapses (RR). Hydroxychloroquine (HCQ) has very long half life, so low levels represent NA. In this first report in LN, we examined NA as defined by HCQ blood levels (HCQBL) ≤0.2ng/L and its correlation with clinical outcomes.

**Methods:** 145 patients (pts) with WHO class III, IV or V LN had HCQBLs measured during Rx. Complete remission (CR) was defined as protein creatinine ratio (PCR) <50mg/mmol and normal serum creatinine (SCr) or ±15% of baseline. RR was defined as >30% increase in PCR or SCr requiring renal biopsy or change in Rx.

**Results:** Comparison of NA and adherent pts at HCQBL testing (table).

| | HCQ≤0.2(N=33) | HCQ>0.2(N=112) |
|---|---|---|
| Gender M:F | 4:29 | 19:93 |
| Age(yrs) | 39.2±13.1 | 39.9±13.7 |
| Indian & SE Asians% | 54.5 | 41.1 |
| Blacks% | 24.2 | 28.6 |
| Class V% | 30.3 | 29.5 |
| Duration of LN(yrs) | 6.1±5.1 | 7.6±8.8 |
| Duration of HCQ Rx(yrs) | 3.1±3.1 | 4.1±4.4 |

All variables were comparable. Mean HCQ dose was 5.4±1.8 mg/kg/day. NA was present in 33 pts (22.8%). NA pts had significantly lower CR (39.4% vs. 66.1%, p=0.006) at testing and after median follow up of 16 mts (60.6% vs. 82.1%, p=0.012)(Fig1).




Time to CR was significantly longer in NA pts with disease activity at testing (p=0.045) (Fig2). Proportion of pts with RR was 11.1% at 12 mts and did not differ between groups.

**Conclusions:** These data demonstrate that >20% of pts are NA for HCQ and this is associated with poorer outcomes. Key questions are whether NA with HCQ reflects more generalized NA and whether HCQ levels can be used prospectively to improve adherence and outcomes.

## SA-PO707

**Soluble Forms of Urine Intercellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Molecule 1 (VCAM-1) Are High-Sensitivity Biomarkers for Lupus Nephritis Flare** Daniel J. Birmingham,[1] George Sigal,[2] Nicole Marie Reyes,[1] Pankaj Oberoi,[2] Brad H. Rovin,[1] Lee A. Hebert.[1] *[1]The Ohio State Univ Medical Center, Columbus, OH; [2]Meso Scale Discovery, Rockville, MD.*

**Background:** Identifying biomarkers of lupus nephritis (LN) flare would help elucidate LN flare pathogenesis, and could lead to forecasters of impending flare to allow earlier treatment. Cross-sectional studies of urine samples have identified a number of candidates. However, little has been done to rigorously test how these candidate biomarkers temporally change relative to LN flare onset.

**Methods:** The present study sought to fill this gap by testing 18 different candidate biomarkers of LN or acute kidney injury in serial bimonthly urine samples leading up to flare. The samples were from the Ohio SLE Study (OSS), a prospective longitudinal study of recurrently active lupus patients. Soluble biomarker levels, normalized to urine creatinine levels, were determined from 24-hour OSS urine samples collected at 10, 8, 6, 4, and 2 months prior to, and at, LN flare (n=16).

**Results:** Using a mixed-effects stepwise regression model, incorporating repeated measures ANOVA, and accounting for other covariates such as WHO classification, flare severity, age, race, C3, and use of medications, 9 of the 18 biomarkers were found to be significantly increased at the time of LN flare. The most sensitive of these were sICAM-1 and sVCAM-1 (both $P < 0.0001$), which increased by at least 25% at LN flare in 15/16 and 14/16 LN flares respectively. Both sICAM-1 and sVCAM-1 were also significantly increased at 2 months prior to LN flare. The next most sensitive biomarker was complement C5a ($P = 0.0002$), which increased by at least 25% at LN flare in 9/16 LN flares. Notable biomarker candidates that did not change relative to LN flare included neutrophil gelatinase-associated lipocalin, beta-2-microglobulin, vascular endothelial growth factor, osteopontin, and cystatin C.

**Conclusions:** Increases in urine levels of sICAM-1 and sVCAM-1 occur prior to LN flare and identify LN flare with high sensitivity, suggesting that enhanced leukocyte adhesion is a key factor in the pathogenesis of LN flare.

*Funding:* NIDDK Support, Other NIH Support - PO1 DK55546, UL1 RR025755

## SA-PO708

**Short Term Outcome of Induction Therapy in Pediatric Patients with Proliferative Lupus Nephritis** Murty Adabala,[1] Rudolph P. Valentini,[1] Rossana Baracco,[1] Tej K. Mattoo.[1] *[1]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [2]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [3]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [4]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI.*

**Background:** Lupus Nephritis (LN) & its treatment are associated with morbidity & mortality.The objective of this study was to evaluate the clinical outcome at 6 months following induction therapy in pediatric patients with proliferative LN.

**Methods:** Retrospective chart review (23 patints) of LN patients with WHO class IV & severe class III followed at vasculitis clinic (2004-2011). All patients received the induction treatment with pulse Methylprednisolone followed by daily prednisone & monthly cyclophosphamide for 6 moths. We reviewed the clinical presentation, laboratory parameters, renal histology & correlated these variables with the clinical outcome at six months.

**Results:** African Americans 10 (44%) & females 14 (61%). The mean age at diagnosis of LN was 13.8 y. At presentation 19 (82%) had WHO class IV LN, 15 (65%) had nephrotic syndrome, 19 (82%) had azotemia, 17 (74%) had hypertension. At six months 13 (57%) patients had complete remission, 3 (13%) progressed to end stage renal disease (ESRD) & 7 (30%) had partial remission. Rapidly progressive glomerulonephritis (RPGN) & severe activity (activity score of 15/24 and >) on renal biopsy at presentation were associated with progression to ESRD ($p<0.05$).

| Outcome<br>Variables at presentation | Complete remission ( n=13) | Partial remission (n=7) | End stage renal disease (n=3) |
|---|---|---|---|
| RPGN | 0 | 4 | 3 |
| Mean eGFR ( ml/min/1.73m$^2$) | 71 | 39 | 25 |
| Mean Serum albumin (gm/dl) | 2.5 | 1.9 | 2.1 |
| Activity Index (> 15/24) | 0 | 0 | 3 |

Logistics regression analysis identified serum albumin < 2 gm/dl and estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73m$^2$ at presentations as predictors of poorer outcome with partial response or progression to ESRD.

**Conclusions:** Patients with proliferative LN with hypoalbuminemia (serum albumin <2 gm/dl) and eGFR (< 30ml/min/1.73m$^2$)at presentation have a lower probability of attaining complete remission at 6 months with current induction treatment.

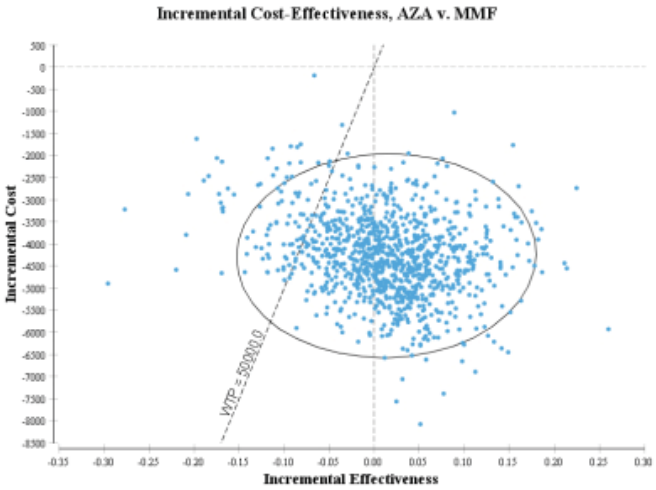
## SA-PO709

**The Cost-Effectiveness of Azathioprine versus Mycophenolate Mofetil as Maintenance Therapy for Proliferative Lupus Nephritis** Robert Nee, Ian Matthew Rivera, Christina M. Yuan, Dustin J. Little, Kevin C. Abbott. *Nephrology, Walter Reed National Military Medical Center, Bethesda, MD.*

**Background:** Clinical practice guidelines recommend that after induction therapy, patients with Class III and Class IV lupus nephritis (LN) receive maintenance therapy with azathioprine (AZA) or mycophenolate mofetil (MMF). However, the cost-effectiveness of these two treatment strategies has not been reported.

**Methods:** Using a third-party payer perspective, we constructed a Markov model with a 3-year time horizon to compare the cost-utility of generic formulations of AZA and MMF as maintenance therapy. We used individual-level simulations to account for patient age variability and probabilistic sensitivity analysis (PSA) to account for parameter uncertainty. We factored in drug adverse events and rescue therapy for LN relapse on either AZA or MMF. Probabilities, utility and disutility weights were based upon published studies, while costs of medications and health services were drawn from public sources. Model outcomes were costs, quality-adjusted life-years (QALY), incremental cost-effectiveness ratios (ICER) and net monetary benefit.

**Results:** Compared with AZA strategy, the ICER for MMF was $214,856/QALY gained. One-way sensitivity analysis showed that MMF is the favored strategy only if its yearly cost was less than $886, at a willingness-to-pay (WTP) $50,000/QALY. Results of PSA demonstrated that the probability that AZA was cost-effective compared to MMF was 90.9% at WTP $50,000/QALY.




**Conclusions:** Our state-transition model suggests that an AZA-based strategy is more cost-effective than MMF as maintenance therapy over a 3-year timeframe for proliferative LN. "*The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy of the Department of the Army, the Department of the Navy, the Department of Defense, or the US government.*"

## SA-PO710

**Podocyte Lesions Involved in Lupus Nephritis Based on the 2003 ISN/RPS System** Yan Wang, Di Song, Feng Yu, Suxia Wang, Minghui Zhao, Youkang Zhang. *Renal Div, Dept of Medicine, Peking Univ First Hospital; Institute of Nephrology, Peking Univ.*

**Background:** The podocyte lesions in lupus nephritis is still an intriguing controversy in ISN/RPS system. We assess the associations between podocyte lesions and clinico-pathological features in a large cohort of lupus nephritis patients.

**Methods:** Clinico-pathological data of 202 patients with renal biopsy proven lupus nephritis were reviewed. Morphometric analysis of podocyte foot process width(FPW) was performed to access the degree of podocyte injury, its correlations with clinical and pathological parameters were further analyzed.

**Results:** The podocyte foot processes in patients with lupus nephritis effaced variously, reflected by the FPW ranged from 626.16nm to 8253.78nm(median: 1397.39nm). 13 patients with lupus podocytopathy, including 9 with minimal change glomerulopathy and 4 with focal and segmental glomerulosclerosis pattern, met the histological criteria of lupus podocytopathy with a FPW of 2026nm±1793nm. The degree of foot process effacement was positively correlated with proteinuria($r=0.509$, $P<0.001$). The FPW of 1240nm can distinguish the nephrotic syndrome with sensitivity(81.5%) and specificity(62.7%). The degree of foot process effacement aggravated significantly from class I to class V, and patients with combined lupus nephritis presented with the severest podocyte lesions. The complete remission rate was significantly higher and the long-term renal outcome was better in group with calcineurin inhibitors than that with other regimens in patients with FPW higher than 1240 nm.

**Conclusions:** Podocyte damage was common in lupus nephritis. Lupus podocytopathy acted as an extreme form of lupus podocyte lesions. Podocyte injury mediated by immune complex deposits existed in different types of lupus nephritis, notably in class V and combined types. Hence, whether a subclass of lupus podocyte injury should be added to the ISN/RPS histopathological classification system for guiding therapy and judging prognosis, need further investigation.

## SA-PO711

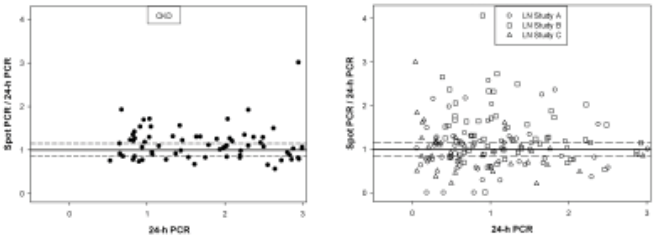
**Spot Urine Protein/Creatinine Ratio Is More Unreliable in Estimating 24-h Proteinuria in Lupus Nephritis Than in Other Forms of Chronic Glomerular Diseases**

Lee A. Hebert,[1] Daniel J. Birmingham,[1] Ganesh B. Shidham,[1] Derek M. Fine,[2] Roger A. Rodby,[3] Giuseppe Remuzzi,[4] Paul Louis Hebert,[5] Brad H. Rovin.[1] *[1]Internal Medicine, Ohio State Univ Wexner Med Ctr, Columbus, OH; [2]Johns Hopkins Univ, Baltimore, MD; [3]Rush Univ Medical Center, Chicago, IL; [4]IRCCS-Istituto di Richerche Farmacologiche Mario Negri, Bergamo, Italy; [5]Univ of Washington, Seattle, WA.*

**Background:** Spot urine protein/creatinine ratio (PCR) is often used clinically to estimate 24-h P. However, spot PCR shows considerable hour-to-hour variability. Spot collections (Cl) reveal this variability. Longer Cl conceal it. Spot PCR is useful in cohort studies where its variability is mitigated by averaging. However, clinical decisions often are based on a single spot PCR. Here, spot PCR variability is a liability. Here we compare spot PCR variability in lupus nephritis (LN) and chronic kidney disease (CKD).

**Methods:** For LN, we use the published works (N=3, 165 pts) that documented C content of the 24-h Cl (studies A, B, C); for CKD, the REIN cohort (98 pts). Almost all spot were morning Cl.

**Results:** Calibration plots show that most [spot PCR/24-hr PCR] values fall well outside the limits of agreement with 24-h PCR.




Coefficient of variation (CV) of [spot PCR/24-h PCR] is greater in LN than CKD (mean CV (95% CI) LN versus CKD is 0.538 (0.459, 0.621) versus 0.335 (0.185, 0.456), $p < 0.001$). KDIGO and ACR recommend spot PCR but its variability is confounding, especially in LN. For example, 0.6 PCR increase is a BILAG-A renal flare. Spot PCR would not be reliably detect this change (see Figure). Also, spot PCR is not reliable for screening. It over and under estimates about equally.

**Conclusions:** Spot PCR testing is substantially inaccurate, particularly in LN. Its impact on clinical decision making, compared to 24-h PCR, needs further study.

*Funding:* NIDDK Support

## SA-PO712

**Lower Renal miR-150 Expression Levels Are Associated with Better Response to Treatment in Lupus Nephritis Patients**

Hua Zhou,[1,4] Sarfaraz Hasni,[2] Howard A. Austin,[3] James E. Balow,[3] Ilias Alevizos,[1] Gabor Illei.[1] *[1]NIDCR; [2]NIAMS; [3]NIDDK/NIH; [4]Dept of Nephrology, The 1st Hospital, China Medical Univ, Shenyang, China.*

**Background:** We recently reported that renal miR-150 expression correlated significantly with chronicity index (CI) in lupus nephritis (LN) patients and showed a causative role of miR-150 in promoting renal fibrogenesis. In this study, we examined the association of pre-treatment renal miR-150 levels and response to treatment (cyclophosphamide and corticosteroids) in LN patients.

**Methods:** 28 renal biopsies (formalin fixed paraffin embedded tissue blocks) obtained from patients with active proliferative LN before starting treatment with cyclophosphamide and corticosteroids. Based on their response to treatment at 24 months patients were classified as treatment effective (n=16) and ineffective group (n=12). Total RNAs were extracted from the biopsies and renal miR-150 expression was measured by TaqMan RT-PCR. The renal miR-150 level was expressed in relation to the expression of U48 (ΔCt: U48Ct – miR-150Ct). Statistical analysis was done by Prism 5 software package.

**Results:** Consistent with our previous report in a different cohort of LN patients, renal miR-150 level positively correlated with CI ($r=0.63$, $p<0.05$) but not with activity index ($r=0.12$, $p=0.49$). Pre-treatment renal miR-150 level was significant lower in treatment effective group compared to ineffective group (ΔCt: $1.62 \pm 0.22$ vs. $2.72 \pm 0.38$, $p=0.015$, respectively). In a receiving operating characteristic analysis, renal miR-150 was an accurate predictor of treatment effectiveness in an area under the curve (AUC: 0.73, $p<0.05$).

**Conclusions:** Increased renal miR-150 levels are associated with an increased CI and poor response to immunosuppressive therapy in active proliferative LN patients. Pre-treatment levels of renal miR-150 have a good potential as biomarkers to predict the effectiveness of immunosuppressive treatment in LN, but its practical application needs further validation in larger cohort of patients. The potential use of miR-150 as a predictor of treatment response in proliferative lupus nephritis should be further assessed in noninvasive clinical samples such as urine and blood.

*Funding:* Other NIH Support - NIAMS

## SA-PO713

**An Open Label Randomized Controlled Trial to Compare the Efficacy and Complication between Low Dose and High Dose Intravenous Cyclophosphamide for Induction of Remission in Thai Patients with Proliferative Lupus Nephritis**

Ratana Chawanasuntorapoj,[1] Boonyarit Cheunsuchon,[2] Pisal Parichatikanond,[2] Kriengsak Vareesangthip,[1] Chairat Shayakul.[1] *[1]Medicine, Siriraj Hosp.; [2]Pathology, Siriraj Hosp., Mahidol U.*

**Background:** Proliferative lupus nephritis (pLN) is a major organ involvement affecting the worse prognosis in SLE patient. The pathogenesis consists of genetic, environment and immunology. The high incidence and poor prognosis are found in Asian, Afro-American ]and Hispanic. The cost and effective standard induction treatment, intravenous cyclophosphamide (IVCY) 0.75-1g/BSA (high dose, HDCYC) monthly for 6 doses provides the good response. The adverse effects are concerned: infection, gonadal toxicity and malignancy. The low dose intravenous cyclophosphamide (LDCYC) is effective in Caucasian with less complication. This study was to investigate the efficacy and complication of LDCYC comparison with HDCYC in Asian.

**Methods:** We randomly assigned 149 pLN patients to a HDCYC**,** 0.75-1 g/BSA IVCY monthly adjusted according to nadir WBC for 6 doses, and a LDCYC, 500 mg IVCY every 2 week for 6 doses. After induction therapy, both were followed by azathioprine.

**Results:** 76 and 73 patients were enrolled to HDCYC(G1) and LDCYC(G2) from Mar 2005 to Nov 2012. Median time to follow up was 40 months. Overall demographic data; age, blood pressure, onset, albumin, proteinuria, RPGN, activity and chronicity index were comparable. Median creatinine was 1.1 and 0.9 in G1 and G2, *p=0.04.* Induction treatment failure at 6 months was lower in G1, 24.7% compared to 34.3% in G2. The complete response at 12 months was significantly higher in G1, 76.5% compared to G2, 52.3. Renal flare was insignificantly low in G1, 35.5% and 45.2% in G2. 5 years renal and patient survival were comparable in G1 (90 and 83.2%) and G2 (89 and 86%). Regarding the complication including avascular necrosis, DM and infection were not different. There are 9 cases dead in each group and infection was a common cause. The gonadal toxicity was higher in G1 and malignancy was found 2 cases in G1.

**Conclusions:** The low dose CYC could be the effective to induce response in pLN. However, the complte response at 12 months was significantly high in high dose CYC.

*Funding:* Government Support - Non-U.S.

## SA-PO714

**Latin American Lupus Nephritis Cohort: Maintenance Therapy with Mycophenolate Sodium**

Vanina Vazquez,[1] Juan Jose Lopez,[2] Bruno Jorge Lococo,[3] Alicia Isabel Fayad,[4] Marcelo Alejandro De Rosa,[5] Vicente Altobelli.[6] *[1]Nefrologia, Hospital Simplemente Evita, Gonzalez Catan, Buenos Aires, Argentina; [2]Nefrologia, Hospital Gobernador Centeno, La Pampa, Argentina; [3]Nefrologia, Hospital Fernandez, Ciudad Autonoma de Buenos Aires, Buenos Aires, Argentina; [4]Nefrologia, Hospital de Niños A. Gutierrez, Ciudad Autonoma de Buenos Aires, Buenos Aires, Argentina; [5]Nefrologia, CyMSA, Ciudad Autonoma de Buenos Aires, Buenos Aires, Argentina; [6]Nefrologia, Centro Nefrologico Salta, Salta, Argentina.*

**Background:** Mycophenolate has emerged as promising alternative in Lupus Nephritis(LN) treatment but its effects on Latin American(LA) population to maintain response induced by cyclophosphamide (CYC) has been not well defined.

**Methods:** 46 pts,prospective study.Treatment:CYC iv induction followed by enteric-coated mycophenolate sodium (EC-MPS) 30 months maintenance.Analysis:EC-MPS efficiency to maintain complete response(CR)and to improve partial response(PR) achieved with CYC,persistent partial response(PPR), safety by adverse events(AE) listing.

**Results:** Induction:26% achieved CR,73.9% PR. Maintenance:all CR was sustained,50% PR switched to CR.End-study:63% achieved CR,36.9% showed PPR. PPR group showed > hypertension rate,sCr≥1.2, moderate interstitial fibrosis(IF) and tubular atrophy(TA) vs CR group.Risk predictor variables of association to RRP:elevated baseline sCr,moderate IF and TA.Most frequent AE:minor infections 36.6%,gastrointestinal intolerance 15.2%.

| | Total CTR | PPR | | Association and RR (95%IC) | |
|---|---|---|---|---|---|
| n 46 | n/% | n/% | p | | p |
| n pts | 29/63.04 | 17/36.9 | | | |
| WHO class | | | | | |
| IV % | 19/65.5 | 12/70.5 | 0.132 | | |
| Baseline sCr ≥ 1.2 mg/dl,% | 50 | 82.35 | 0.05 | 1.59(1.05-2.41) | 0.03 |
| Proteinuria >3.5 g/d,% | 53.33 | 76.47 | 0.19 | 1.48(0.95-2.29) | NS |
| HTN:BP≥140/90,% | 43.33 | 58.8 | 0.0001 | 1.59(0.98-2.58) | NS |
| Interstitial Fibrosis | | | | | |
| moderate,% | 0 | 23.52 | 0.0001 | 6.82(0.83-56.17) | 0.05 |
| Tubular Atrophy | | | | | |
| moderate,% | 3.33 | 29.41 | 0.015 | 8.53(1.09-67.05) | 0.04 |

**Conclusions:** Severe LN LA population can be safely and effectively treated with CYC and maintenance EC-MPS included those with risk factors for worse treatment response.

## SA-PO715

**Effects of an Intensified Treatment of Linphocyte Depletion without Immunosuppressive Maintenance Therapy in Severe SLE over a Four Year Follow-Up** Dario Roccatello, Daniela Rossi, Carla Naretto, Simone Baldovino, Mirella Alpa, Savino Sciascia, Ilaria Salussola, Vittorio Modena. *Giovanni Bosco Hospital and Univ of Turin, Italy.*

**Background:** B-lymphocytes (BL) play a critical role in Systemic Lupus Erythematosus (SLE). BL depletion therapy still remains an attractive option, despite the disappointing results of RCTs.

**Methods:** Twelve SLE patients [2 males, mean age 43.8 yrs (29-54)] with polyarthralgia and multiorgan involvement including class IV or V (ISN/RPS) glomerulonephritis (9 cases), skin lesions (9cases, with necrotizing ulcers in3), polyneuropathy (7cases, with CNS involvement in 2), lymphoadenopathy (6) e polysierositis (5) have been treated with an intensive BL depletion protocol for intolerance to conventional immunosuppressive therapy (6 cases) or as a front line therapy (6 cases). Protocol: Rituximab 375 $mg^2$ on days 1,8,15,22, and2more doses after one and two months, associated with 2 IV administrations of 10 mg/kg of cyclofosfamide and 3 infusions of methylprednisolone (15 mg/kg) followed by oral prednisone (0.8 mg/die, rapidly tapered to 5 mg/day in 10 weeks), without further immunosuppressive maintenance therapy.

**Results:** This protocol obtained a complete depletion of CD20+ BL for 12-18 months. Patients had been followed-up for 48.9 (25-93) months. ESR (baseline mean value: 54.2; 3 months: 33; end of follow-up: 14.9), anti-dsDNA antibodies (baseline: 192; 3 months:112; end of follow-up: 17) and proteinuria (baseline: 4.9 g/24 hours; 3 months:0.97; end of follow-up: 0.22) significantly declined ($p<0.05$), while C4 values (baseline 11 mg/dl) significantly increased ($p<0.05$) after 3 months (22 mg/dl) and at the end of the follow-up (20 mg/dl).3patients relapsed after 36,41 and72months respectively. They showed again a complete remission after retreatment over 13-48 months of observation.

**Conclusions:** These data confirm the opportunity to reconsider the regimens of BL depletion in the treatment of the most severe forms of SLE despite the disappointing results of RCTs. A promising role of Rituximab in protocols of "intensified induction therapy" in selected patients for whom avoiding immunosuppressive maintenance therapy could be particularly appealing.

## SA-PO716

**Oral Sirolimus Was More Effective Than Intravenous Cyclophosphamide in Patients with Proliferative Lupus Nephritis** Jian Hui Yang. *Renal Div, Zhejiang Provincial People's Hospital.*

**Background:** To evaluate the efficacy and safety of sirolimus on refractory lupus nephritis.

**Methods:** Forty-eight patients with proliferative lupus nephritis who failed to response refractory to regular dose of prednisone were randomized to sirolimus group (SRL) or intravenous pulse cyclophosphamide (CTX). The patients ages, gender, hematuria, proteinuria and other clinco-pathological features were of no marked differences between two groups. The former group were administrated with 0.3~0.5mg/d.kg-1 oral prednisone and 2 mg/d sirolimus (NCPC GENETECH BIOTECHNOLOGY CO. LTD) for 6 to 12 months. Sirolimus doses were adjusted so that the serum sirolimus concentration could be maintained between 4 to 10 ng/ml. The later group were given same dose of oral prednisone and intravenous CTX (0.6g)bimonthly. Patients who could not withstand the administration were excluded. No angiotensin-converting enzyme inhibitors or angiotensin receptor blockers were taken in these patients. The clinical manifestations, laboratory parameters and adverse reactions were observed.

**Results:** Three months after initiation of these therapy, the patients' SLEDAI, DNA antibody titer, urine protein decreased more markedly in SRL group compared with CTX group. Hypoalbuminemia and hypocomplementemia was alleviated more rapidly in the former group. Complete remission were induced in 19 patients treated with SRL while it was seen only 10 patients treated with CTX . Serum creatinine level was significantly reduced in SRL group than CTX group. Although hyperlipidemia and hypercholesterolemia were commoner in SRL group, no other serious adverse reactions such as leucocytopenia, bleeding cystitis and alopecia were found apart from acnes and upper tract infections.

**Conclusions:** Low dose of oral SRL was more effective and safer than CTX in patients with lupus nephritis. Hyperlipidemia or hypercholesterolemia in lupus nephritis was not the contradictions of sirolimus.

## SA-PO717

**Diffusion Weighted Imaging and Blood Oxygen Level-Dependent MR Imaging of Kidneys in Patients with Lupus Nephritis** Xiao Li, Nan Chen. *Dept of Nephrology, Ruijin Hospital, Shanghai Jiao Tong Univ School of Medicine, Shanghai, China.*

**Background:** To investigate the role of diffusion weighted imaging (DWI) and blood oxygen level-dependent (BOLD) magnetic resonance (MR) imaging in the assessment of renal involvement and pathological changes in patients with lupus nephritis (LN).

**Methods:** Thirty-eight patients with LN (34 biopsied) and 16 healthy controls underwent coronal echo-planar DWI and BOLD MR imaging of the kidneys with a single breath-hold time of 16s. The apparent diffusion coefficient (ADC) and R2* value of the kidneys was calculated with high b values (b=500 $s/mm^2$). Image analysis was performed on a GE workstation (Sun Microsystems, ADW4.2) with Functool 2 image analysis software. The relation between the renal injury variables and the ADCs or R2* values were evaluated.

**Results:** The mean ADC values of kidneys in patients with LN were $2.41\pm0.25 \times 10^{-3}$ $mm^2/s$, the mean R2* values of the renal cortex and medulla were 10.73/sec±1.74 and 13.48/sec±3.16 respectively, which were all significantly lower than those in volunteers ($p=0.048$, $p=0.048$ and $p=0.008$, respectively). In the patients with LN, the mean ADC values were correlated with eGFR ($r=0.558$, $p<0.05$). There was a negative correlation between the ADC values of the right kidneys and pathological chronic indexes ($r=-0.493$, $p<0.05$). Moreover, the R2* values of the renal medulla were negatively correlated with 24 hours proteinuria ($r=-0.344$, $P<0.05$), the degree of tubulointerstitial lesions($r=-0.342$, $P<0.05$). Among 10 patients who repeated MR scan after treatment for 9 to 12 months, 7 patients got complete remission (CR) and 3 had no response (NR). The ADC and R2* values of kidneys were significantly higher than before in CR patients (all $P<0.05$), while the ADC and R2* values being lower than before in NR patients with no statistical significance ($P>0.05$).

**Conclusions:** The functional MR imaging showed lower ADC values and R2* values of renal cortex and medulla in patients with LN than normal controls, which were associated with renal function, proteinuria and pathological changes. DWI and BOLD MR imaging may be used to non-invasively monitor disease activity, evaluate therapeutics efficacy in lupus nephritis.

*Funding:* Government Support - Non-U.S.

## SA-PO718

**Severely Low 25-Hydroxyvitamin D Levels Are Associated with Worse Renal Outcomes in Proliferative Lupus Nephritis** Alcino Pires Gama, Luciana Loureiro Nardotto, Lectícia Jorge, Cristiane Bitencourt Dias, Rui Toledo Barros, Viktoria Woronik. *Nephrology, Univ of Sao Paulo, Sao Paulo, Brazil.*

**Background:** The associations between 25-hydroxyvitamin D(25OHD) levels and lupus nephritis(LN) are not clearly known. The aim of this study was to evaluate if very low serum levels of 25OHD in proliferative LN patients are associated with worse renal outcome.

**Methods:** Medical records from 2009 to 2012 of 72 subjects with biopsy-proven proliferative LN were retrospectively analyzed. Severe 25OHD deficiency was defined as <10ng/ml. The study's endpoints were either progression of chronic kidney disease(CKD) - defined as a clearance reduction greater than 5ml/min/y - or progression to end-stage renal disease(ESRD).

**Results:** Median levels of 25OHD were low at baseline(16,5±7,9ng/ml). Clinical features, follow-up times, biopsy features and renal outcomes are shown on table 1.

| | 25OHD<10 n=17 | 25OHD>10 n=55 |
|---|---|---|
| CLINICAL | | |
| Age/y | 32,8±3,3 | 32±1,4 |
| Female(%) | 17(100%) | 10(82%) |
| Hb g/dl | 9±0,5 | 9,8±0,2 |
| C3 mg/dl | 61,5±7,5 | 65,4±4,5 |
| eGFR ml/min | 36±6,7 | 47±4,5 |
| Albumin g/dl* | 2,1±0,2 | 2,6±0,1 |
| Proteinuria g/24h | 4,9±0,8 | 4,4±0,5 |
| BIOPSY | | |
| Class IV(%) | 12(71%) | 47(85%) |
| Activity | 5,5±0,9 | 5,5±0,4 |
| Chronicity* | 2,5±0,6 | 3,8±0,3 |
| OUTCOMES | | |
| Follow-up/y | 1,7±0,2 | 2,3±0,1 |
| CKD progression(%)* | 9(53%) | 14(25%) |
| ESRD(%)* | 7(41%) | 8(14%) |

*p<0,05

Table 1. Clinical, biopsy features and outcomes. Results shown as median±SD or n(%)

After a median follow up 1,7±0,2 years, the 25OHD<10ng/ml group had worse renal outcomes: (1) progression of CKD 53% vs 14%, p<0,05; (2) ESRD 41% vs 14%, p<0,05. These outcomes were observed despite better biopsy features on the low-25OHD group at baseline (chronicity index 2,5±0,57 vs 3,8±0,32, p<0,05).

**Conclusions:** Severely low 25OHD levels at baseline were associated with worse renal outcomes (either progression of CKD or progression to ESRD) in biopsy-proven proliferative LN patients, even though this group presented better biopsy features at baseline.

## SA-PO719

**Persistent Proteinuria as a Major Predictor of Renal Outcome in Lupus Nephritis Class V (LNV)** Alcino Pires Gama, Luciana Loureiro Nardotto, Lectícia Jorge, Cristiane Bitencourt Dias, Rui Toledo Barros, Viktoria Woronik. *Nephrology, Univ of Sao Paulo, Brazil.*

**Background:** Natural history studies of LNV suggest a relatively low rate of progression to ESRD. The aim of this study was to review the prognosis and the predictors of renal outcome in LNV.

**Methods:** The medical records of patients (n=97) with biopsy-proven LN, WHO class V without any past history of proliferative lupus nephritis were reviewed retrospectively. The study endpoint was ESRD.

**Results:** Clinical and histological features are showed in table 1.

| Baseline and Histologic Features | ESRD (n=11) | No ESRD (n=86) |
|---|---|---|
| AGE(y) | 30±11 | 35±11 |
| Female | 10(90%) | 79(92%) |
| eGFR(ml/min)* | 40±29 | 93±41 |
| Proteinuria(g/day) | 6.4±4.7 | 4.2±4 |
| Serum Albumine(g/dl)* | 2±0.4 | 2.8±1 |
| C3 Level* | 58.7±32 | 93±38 |
| Hemoglobin(g/dl)* | 10.2±2.3 | 12±1.7 |
| Hematuria | 4(37%) | 34(40%) |
| Crescents* | 5(46%) | 8(10%) |
| Interstitial Fibrosis | 6(54%) | 29(34%) |
| At one year Features | | |
| eGFR(ml/min)* | 45±47 | 97±37 |
| Proteinuria(g/day)* | 5.3±5.5 | 1.3±1.1 |
| Serum Albumine(g/dl) | 3.8±0.7 | 3.8±0.6 |
| C3 Level | 94±36 | 109±34 |
| Hemoglobin(g/dl)* | 10.8±2.5 | 12.4±1.6 |
| Follow up(y) | 6.6±4.7 | 7.2±3.5 |

Results are shown as median±SD or n(%). *p<0,05

On a mean follow-up of 7.1 years, 11.3% of the patients reached ESRD. Logistic regression analysis showed that persistent proteinuria at one year and decreased serum C3 levels(hypoC3) on baseline was associated with the endpoint after adjustments for initial eGFR (OR 2.2 IC 1.1-4.2, p=0.020;OR 0.93 IC 0.87-0.99, p=0.024 respectively). The best cutoff of PP to identify patients who presented ESRD was a PP>6 with a positive predictive value of 100% and a negative predictive value of 91% defined by ROC curve (AUC 0,83 p=0.02).

**Conclusions:** Persistent proteinuria at one year and HypoC3 on baseline were a independent risk factors for ESRD in LNV in our study, these clinical features are helpful to identifying patients at high risk for poor outcome.

*Funding:* Government Support - Non-U.S.

## SA-PO720

**Could Proteinuria Less Than 500 mg/day Be an Actual Indicator of Lupus Nephritis Biopsy?** Marcelo Alejandro De Rosa, Jorge E. Toblli, Graciela Elena De Rosa, Alicia Marini. *Nephrology Div, Hospital de Clinicas. Univ of Buenos Aires, Buenos Aires, Argentina.*

**Background:** Renal biopsy is required to confirm the diagnosis of lupus nephritis (LN) according to the current ACR guidelines (Arthritis Care & Research 2012; 64: 797–808), in case of unexplained increasing serum creatinine level, proteinuria >1.0 g/day , or combinations of the following: a) proteinuria 0 .5 g/day plus hematuria, b) proteinuria >0.5 g/day plus cellular casts.

The goal of this pilot study was to determine the value of renal biopsy in lupus patients presenting with glomerular hematuria and proteinuria below 500 mg/day with normal renal function.

**Methods:** Renal biopsies from 38 lupus patients with glomerular hematuria and proteinuria below 500 mg/day were evaluated by LM and immunofluorescence microscopy and classified according to the ISN-RPS 2003. No patients with renal failure or previous renal biopsy were included. Glomerular hematuria was considered at the presence of 3 or more dysmorphic RBCs per high-power field plus acanthocytes more than 5% and RBC casts. The urinary sediment corresponding to every patient was observed by neprologists.

**Results:** The mean patient age was 35.5 years old (range 19 to 65) years. Male/female= 8/30. The 24hr proteinuria was negative in 2 pts. (5%), proteinuria trace in 16 pts. (42%), and between trace and 500 mg/day in 20 pts. (52%). From the total of 38 biopsies, 2 were class II (5%), 15 class III (39%), 18 class IV (47%) and 3 class V (8%).

**Conclusions:** The present results show that Class III, IV, or V are present in a substantial proportion of lupus patients with mild clinical manifestations of renal disease and it may suggest a new potential indication for renal biopsy. In addition, since these histological classes of LN are associated with poor prognosis and require an early diagnostic and appropriate treatment, we think the data presented in this pilot study acquire importance.

*Funding:* Government Support - Non-U.S.

## SA-PO721

**Treatment of Lupus Nephritis with Mycophenolate Mofetil (MMF). Long Term Outcomes** Beatriz Rodriguez Cubillo, Stelios A. Panagoutsos, Javier Juega Marino, Megan Griffith, Tom Cairns, Liz Lightstone. *[1]Renal, Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Hammersmith Hospital, London, United Kingdom.*

**Background:** Very little is known on long term outcomes of patients treated with lupus nephritis with MMF as compared to induction with CyP. We have used MMF for induction and maintenance treatment from 1999. We report the outcomes of patients treated for >5yrs.

**Methods:** Retrospective analysis of all adult patients with biopsy proven LN between January 1999 and March 2013 and on MMF either for induction and/or maintenance therapy for >5yrs. A 64/348 patients were identified from our lupus biopsy d/base. Renal remission – normal serum creatinine or no worse than 20% above baseline, and urine PCR <50mg/mmol.

**Results:** Median follow-up 80.87mths (72.01-102.90). 32(50.8%) patients received CyP induction. The rest received rituximab+ MMF(10) or MMF alone(11) as first induction therapy. Most frequent additional maintenance drugs were prednisolone & hydroxychloroquine. **Outcomes at latest f/up:**Maintenance prednisone treatment was stopped in 38 patients(67.9%); No patients died;5(7.9%) required RRT(0 of those whose urine PCR was<100 at 6mths), 4(6.5%) had sustained doubling of serum creatinine(SDSC); remission was achieved in 82% and 58% had a relapse. Serum creatinine improved from 90µmol/L [70.75-123.25] in yr 1 to 73.5/L [65.6-113.25]) by yr 5. There were no significant differences in long term outcomes whether induced with CyP or MMF; patients considered able to withdraw steroids had fewer relapses – 35% vs 67% in the steroid dependent group. Adverse events: Infections were documented in 33(52.2%); neutropenia in 11(17.2 %) & GI symptoms in 20.6%. There were 7 pregnancies in the 46 women(15.27%).

**Conclusions:** Our data, from a single centre retrospective study, suggest that treatment with MMF both for induction and maintenance is as efficacious in the long term as induction with CyP.

## SA-PO722

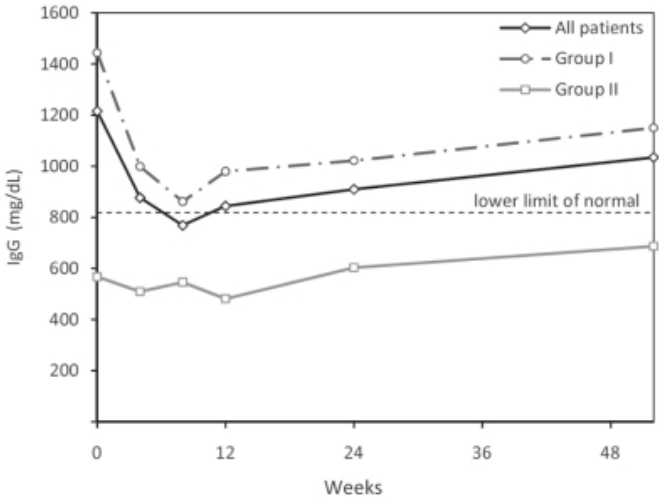
**The Effect of Corticosteroids and Mycophenolate Mofetil Treatment on Serum Immunoglobulin G Levels in Lupus Nephritis Patients** Desmond Y.H. Yap, Susan Yung, Gary Chan, Daniel Tak Mao Chan. *Medicine, The Univ of Hong Kong, Hong Kong, Hong Kong.*

**Background:** Depressed serum immunoglobulin G (IgG) is associated with heightened infective risk. The effect of corticosteroids and mycophenolate mofetil (MMF) on circulating IgG levels in lupus nephritis (LN) patients remains unclear.

**Methods:** Active class III/IV±V LN patients who received prednisolone (0.8mg/kg/d) and MMF (1g bd) as induction treatment were included and their longitudinal IgG profiles analyzed.

**Results:** 46 patients were included. Prior to treatment, 34 (73.9%) patients (Group I) had normal or elevated IgG (1444.0±600.5 mg/dL) while 12 (26.1%) (Group II) had IgG below the lower limit of normal (567.8±160.9 mg/dL). The mean IgG at baseline, 6- and 12-month after treatment were 1215.4±649.7 mg/dL, 914.5±362.4 mg/dL and 1034.6±452.5 mg/dL respectively. After initiation of prednisolone and MMF, IgG declined significantly after 2 weeks, reaching a nadir at 8-week, then followed by gradual normalization.

**Figure 1.**




Similar longitudinal IgG profile was observed in Group I patients while Group II patients showed insignificant IgG changes within the first 6 months. By 6-month, 18 (39.1%) patients had low IgG but only one had IgG <300mg/dL. IgG level was negatively correlated with proteinuria at 6-month (r=-0.657; p<0.001) but had no association with serum creatinine, serological markers, lymphocyte counts and MMF dose. 5 of 18 patients with low IgG had infections within the first year, but IgG level below the lower limit of normal was not associated with excessive infection (RR 1.863; 95% CI 0.466 to 6.818, p=0.280).

**Conclusions:** Proteinuria significantly affects serum IgG levels in LN patients. Treatment with prednisolone and MMF does not lead to clinically important IgG depression and excessive infection.

## SA-PO723

**Maintenance Therapy with Mycophenolate versus Azathioprine in Lupus Nephritis** Pavan Kumar Rao Navva.[1] *[1]Nephrology, Andhra Medical College, Vishakhapatnam, Andhra Pradesh, India; [2]Nephrology, Andhra Medical College, Vishakhapatnam, Andhra Pradesh, India.*

**Background:** Lupus nephritis is one of the commonest renal lesions affecting young women contributing to considerable morbidity and mortality. It requires long term treatment to preserve renal function. We tried to compare Mycophenolate mofetil, Azathioprine and Cylophophamide in Maintenance therapy of Lupus nephritis.

**Methods:** All biopsy proven Class 3 & 4 Lupus Nephritis patients in a Tertiary care centre in South East India were followed from May 2011 to May 2013. After initial induction with Cyclophosphamide for 6 months, maintenance therapy was started with either Mycophenolate(MMF) or Azathioprine(AZA) or quarterly Cyclophosphamide pulses(CYP). The decision to opt for any of the treatment modalities was given to the patient/attender with prior counseling regarding fertility and malignancy risk. We had 52 patients during the period, 4 patients were lost to follow up. Of the 48 remaining, 19 received MMF, 22 AZA and 7 CYP.

**Results:** Among 48 patients,the mean age in MMF group was 22.4±2.5yrs, AZA group 24.5±3.4 yrs and in CYP group 22.9±3.1 yrs. There was one male in each of the treatment

groups. At the start of the Maintenance therapy all the patients had attained either complete or partial remission. At the end of the study period 13 patients in the MMF group, 18 in AZA group and 5 in CYP group continued to be in remission. Four(21.1%) in the MMF group, 2(9.1%) in the AZA group and 1(14.3%) in the CYP group had died during the study period. 2 each in the MMF and AZA group and 1 in the CYP group had progressed to ESRD during the study period. The commonest cause of death was infection due to pneumonia in 4 and cellulitis leg in 2, leading in septicaemia. The incidence of flare either nephritic or nephrotic was 4 (21.1%) in the MMF group, 4(18.8%) in the AZA group and none in the CYP group, all of which responded to treatment.

**Conclusions:** We found that in our population the remission was equally maintained in all treatment groups. The mortality rates were higher in MMF group compared to AZA and CYP groups. The incidence of flares was similar in all the groups. Although all modalities were efficient we need larger studies to ascertain higher infection rates in MMF group in our area.

*Funding:* Government Support - Non-U.S.

## SA-PO724

### Lupus Podocytopathy: Clinical Characteristics, Pathology, and Therapy

Jason Cobb, Jose E. Navarrete. *Nephrology, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) in systemic lupus erythematosus (SLE) patients has been described as the lupus podocytopathy. Lupus podocytopathy pathology is characterized by diffuse foot process effacement with a lack of significant immune complexes.

**Methods:** We are presenting nine cases in our clinical practice with varying clinical characteristics at presentation, renal pathology, and response to therapy.

**Results:** At the time of kidney biopsy four patients were new diagnosis, and in five patients the average duration of known lupus was 7.1 years. At time of presentation the average creatinine was 3.26 mg/dL, 7.8 grams of urine protein per day, and serum albumin of 1.5 g/dL. All patients presented with hematuria. Renal pathology showed significant diffuse foot process effacement in all cases. Two patients with FSGS only, one patient with MCD only, one patient with minimal mesangial lupus nephritis with MCD, three patients with mild mesangioproliferative lupus nephritis with MCD, and two patients with mild mesangioproliferative lupus nephritis with FSGS. Seven of nine patients were treated primarily with corticosteroids and other therapies included calcineurin inhibitors, cyclophosphamide, and plasmapharesis. The patients were followed for an average of 3.4 years, with three patients requiring dialysis and only one required dialysis indefinitely.

**Conclusions:** We analyzed nine cases with varying presentations of the same disorder in our clinical practice. There were no correlation between severity of renal dysfunction and laboratory findings such as double stranded DNA elevation, decrease levels of C3/C4, and degree of proteinuria. No correlation for pathological changes such as presence of tubular reticular inclusion bodies or degree of interstitial fibrosis and atrophy, and amount of mesangial proliferation. The most severe renal dysfunction at time of presentation (creatinine > 5 mg/dL) was seen in the two patients with collapsing FSGS features. Our goal is to a complete analysis of all the cases of lupus podocytopathy in our large academic hospital system.

## SA-PO725

### Risk Factors to Not Achieve Complete Remission in Mexican Patients with Lupus Nephritis

Arreola Guerra Jose Manuel, Juan M. Mejia-Vilet, Rodrigo J. Rosado, Norma O. Uribe-uribe, Luis E. Morales-Buenrostro. *Nephrology & Pathology, National Institute of Medical Sciences and Nutrition SZ, Mexico City, DF, Mexico.*

**Background:** The induction therapy in Lupus Nephritis (LN) has been changed. The aim of this study is to compare the efficacy (complete remission) of different options of induction therapy in incipient patients with LN.

**Methods:** Retrospective cohort study of all patients with biopsy proven LN in the period between mar/08 and Feb/13. We included 185 patients with at least 3 months of follow-up. We evaluate the remission status in the month 3, 6 and 12. Time to complete remission (CR) was evaluated with the univariate and multivariate Cox regression analysis.

**Results:** Seventy-six patients received cyclophosphamide (CFM), Mycophenolate Mofetil (MMF) and 43 Azathioprine (AZA). Univariate and multivariate analysis is shown in table1. In the univariate analysis, the induction therapy with CFM was singnificantly less effective than MMF or AZA to achieve CR (p<0.029). In the multivariable analysis only SLEDAI index was statistically significant.

| Variable | CR (n=68) | w/o response or partial remission (n=117) | HRUnivariate (IC95%) | HR Multivariate (CI95%) | p value |
|---|---|---|---|---|---|
| Creatinine | 1.06(0.7-1.9) | 0.94(0.69-1.27) | 0.63(0.44-0.89) | 0.78 (0.55-1.11) | 0.179 |
| Pr/Cr index | 2.82(1.86-4.4) | 4.01(2.27-6.48) | 0.90(0.82-0.98) | 0.92(0.85-1.01) | 0.106 |
| dsDNA Ab | 95.6(34.1-394) | 132(24.9-489) | 0.99(0.99-1.00) | 0.99(0.99-1.0) | 0.860 |
| Chronicity grade | 3(2-5) | 3(2-6) | 0.67(0.51-0.88) | 0.80(0.58-1.10) | 0.175 |
| Class IV LN | 34(50%) | 78(66%) | 0.55(0.34-0.89) | 0.76(0.43-1.33) | 0.341 |
| **SLEDAI** | 12(8-12) | 12(8-12) | 1.07(0.96-1.19) | **1.12(1.003-1.25)** | **0.043** |
| CFM | 21(31%) | 55(47%) | 0.57(0.35-0.92) | 0.63(0.34-1.15) | 0.138 |
| AZA | 19(27%) | 24(20%) | 1.38(0.82-2.32) | 0.95(0.51-1.75) | 0.213 |
| MMF | 28(41%) | 38(32%) | 1.36(0.84-2.21) | 1.04(0.56-1.92) | 0.883 |

**Conclusions:** In Mexican patients with LN we don't find diferences in the outcomes of the diferents groups of induction. Only the SLEDAI index was significant related with CR. This represent the clinical practice of our center, were the most agresive cases were biased to receive CFM.

## SA-PO726

### Lupus Nephritis as the Main Histopathological Diagnosis in a Renal Biopsy Registry: Analysis of a Local Renal Biopsy Registry in Mexico

Juan M. Mejia-Vilet, Arreola Guerra Jose Manuel, Rodrigo J. Rosado, Norma O. Uribe-uribe, Luis E. Morales-Buenrostro. *Nephrology and Pathology Depts, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran, Mexico City, Mexico.*

**Background:** Renal biopsy (RBx) registries have been advocated worldwide to define the patterns of distribution of renal diseases.

**Methods:** Retrospective study of our institutional RBx registry, including 710 consecutive RBx analyzed by the same pathologist, from 04/2008-04/2013.

**Results:** 710 native RBx were performed in 689 adult patients. Secondary glomerulopathies (SGN) comprise 416 (58.6%), being lupus nephritis (LN) responsible for 319 out of 416 cases (76.6% of SGN), followed by diabetic nephropathy (9.8%), amyloidosis (3.8%), hereditary nephropathies (2.9%). Primary glomerulopathies (PGN) comprise 181 cases (25.5%), with focal and segmental glomerulosclerosis (FSGS) in 34.8%, followed by membranous nephropathy (27.1%), IgAN (16.6%), minimal change disease (11.0%), mesangioproliferative non-IgA (4.4%), membranoproliferative GN (3.9%). Vascular pathologies comprised 9.3% (pauciimmune GN 90.9%) and tubulointerstitial pathologies 3.9% of the total.

| | NEPHROTIC | NEPHRITIC | AUA | CKD | RPGMN |
|---|---|---|---|---|---|
| **FSGS (n=63)** | 57.1% | 1.6% | 36.5% | 4.8% | 7.9% |
| **MN (n=49)** | 91.8% | 0% | 8.2% | 0% | 0% |
| **IgAN (n=30)** | 23.3% | 0% | 70.0% | 3.3% | 3.3% |
| **MCD (n=20)** | 80.0% | 0% | 20.0% | 0% | 0% |
| **LN (n=319)** | 34.2% | 18.8% | 27.9% | 7.5% | 11.6% |
| **Vasculitis (n=41)** | 3.3% | 3.3% | 23.3% | 8.3% | 61.7% |

In LN, a 6.4:1 female predominance was observed, with 86.9% of cases diagnosed before age 40 (mean 30.4y) and 30.1% within 6 months of lupus diagnosis (mean 58.9). According to the ISN/RPS classification, the most common pattern was class IV+V (48.2%), followed by class IV (17.8%), class III+V (14.9%), class V (7.1%), class III (5.8%), class II (2.6%), class VI (2.6%) and lupus podocytopathy (1.0%).

**Conclusions:** In our center, LN surpasses all other glomerulopathies in frequency, being nephrotic syndrome it's most common presentation. Among PGN, FSGS is the most frequent, ressembling previously described changes in GN epidemiology. This highlights the importance of local RBx registries around the world.

## SA-PO727

### Clinicopathologic Features of Lupus Nephritis in African Americans and Hispanics in an Inner-City Safety Net Hospital

Albert M. Osei,[1,2] Richard A. Nunez Lopez,[1] Matilda Malm,[1,2] Mark A. Kraus,[1,2] Peter D. Hart.[1,2] *[1]John H. Stroger Jr. Hospital of Cook County; [2]Rush Univ Medical Center, Chicago.*

**Background:** Systemic Lupus erythematosus disproportionately afflicts women and ethnic minorities in the United States with renal involvement in nearly one half. To better understand lupus nephritis among African Americans and Hispanics, we reviewed the clinical and histopathologic features at the time of renal biopsy.

**Methods:** We retrospectively reviewed all renal biopsies from 2003 to 2012 at our institution to define patients with lupus nephritis. Cases were divided based on both the ISN/RPS classification and ethnicity. Tubulointerstitial involvement was classified as mild, moderate or severe if the corresponding percentages were less than 25, 25 to 50 or more than 50 respectively.

**Results:** Of 162 biopsies with lupus nephritis, 65% were performed in African Americans and 35% in Hispanics. Overall, and in each ethnicity the majority of patients were females (77% vs. males 23%). Mean age at the time of the biopsy was higher in African Americans compared to Hispanics (36±11 vs.33±10years, P≤0.05). Initial serum creatinine was also higher in African Americans than Hispanics (2.2±1.8 vs. 1.52±1.50mg/dL, P≤0.05). Degree of proteinuria, hypocomplementemia, and ANA panel did not differ. In African Americans, ISN/RSP class V was the most frequent lesion (26%), then class IV (20%), IV +V (14%) and III (11%). In Hispanics class V was also the most frequent (25%), followed by class IV (23%), III (18%) and III+V (18%). The serum creatinine did differ between class V and class IV (1.4± 1.3 vs.2.8±2.1mg/dl p≤ 0.05) and among tertials of tubulointerstitial fibrosis (severe 3.3±1.6; moderate 2.3±1.7; and mild 1.4±1.6mg/dl;p≤0.001). No such differences was noted for the degree of proteinuria. Also, lower levels of bicarbonate predicted severity of tubulointerstitial fibrosis (mild 24.6±2.9; severe 22.5±3.3mEq/L;p≤ 0.05) and the same was true for hemoglobin (mild 10.6±1.6; severe 9.8±1.5g/dl p≤0.05).

**Conclusions:** African Americans with lupus nephritis compared to Hispanics were older, had higher initial serum creatinine and more class IV +V disease. Further studies are needed to understand the impact of these differences.

## SA-PO728

### African Americans with Mixed Lupus Nephritis Have Better Prognosis Than Pure Proliferative Glomerulonephritis at a Safety Net Hospital

Jane Vernik,[1,2] Richard A. Nunez Lopez,[1] Mark A. Kraus,[1,2] Peter D. Hart.[1,2] *[1]Div of Nephrology, John Stroger Hospital of Cook County, Chicago, IL; [2]Internal Medicine, Rush Univ, Chicago, IL.*

**Background:** It has been described that adults with mixed Lupus Nephritis (combined membranous with focal or diffuse proliferative GN; MLN) have a worse clinical course than those with either segmental of diffuse proliferative lupus nephritis (PLN). Little information

is available regarding the outcome of African Americans with MLN. By reviewing our biopsy registry, we sought to evaluate if African Americans presenting with MLN had a different clinical outcome than patients with PLN.

**Methods:** We retrospectively examined medical records of the patients biopsied at our institution between January 01,2001 and May 30, 2012 and diagnosed with MLN or PLN based on either WHO or ISN/RPS classification. In patients who underwent multiple biopsies, only data related to the initial biopsy were included.

**Results:** Of the 769 biopsies performed, 141 had LN. 84 of these were identified as initial biopsies with MLN or PLN. 51( 60%) were of African American patients with 22 classified as MLN and 29 as PLN.

The incidence of crescents did not differ significantly between the MLN (41%; n=9) and PLN (45%; n=13) groups (p=0.12).There was no significant difference among serum creatinine, degree of interstitial fibrosis and tubular atrophy,C3, C4 and proteinuria at the time of biopsy.

All patients were induced with combination of high dose steroids and cytotoxic medications, with majority receiving cyclophosphamide and prednisone. Most of the patients were maintained on low dose prednisone and either Mycophenolate mofetil or Azathioprine.

A significant reduction in serum creatinine was noted in the MLN group at 1 year follow up (1.3+/- 0.6 mg/dL, N=13) compared to the time of biopsy (2.4+/-2.4 mg/dL, N=22) (p=0.05). Creatinine remained stable in the PLN group (2.8+/- 2.8 mg/dL to 2.8+/- 0.7 mg/dL; n=20; p=0.9). Serum creatinine was significantly lower in MLN vs PLN at 1 year follow up (p<0.05).

**Conclusions:** African American patients presenting with mixed lupus nephritis (MLN) have a better serum creatinine at one year follow up than those with proliferative lupus nephritis (PLN).

## SA-PO729

### Diagnostic Significance Determining the Level of Urinary Neutrophil Gelatinasa Associated Lipocalin in Patients with Lupus Nephritis

Violeta Rabrenovic,[1] Dragan Jovanovic,[1] Milica Petrovic,[1] Milan M. Radovic.[2] *[1]Dept of Nephrology, Military Medical Academy, Belgrade, Serbia; [2]Clinical Center of Serbia, Belgrade, Serbia.*

**Background:** The aim of the study was to monitor and compare the parameters of activity of lupus nephritis, between the examined and control groups of 20 pts each, at baseline, and 2 and 4 months after the start of treatment.

**Methods:** The prospective study included a group of 40 patients who were homogeneous in terms of demographic data (gender, age, body weight, type of lupus nephritis and therapeutic modality) in which 20 were associated with lupus nephritis in remission and 20 patients with active disease. We monitored and compared the parameters of the two groups in 3 visits over 2 months. : Proteinuria, the ratio of urinary protein and creatinine Upr/Cr, SLEDAI / r score, complement C3, C4, ANA, dsDNA antibodies, Urinary neutrophil gelatinasa associated lipocalin -uNGAL. All patients had creatinine clearance -Cockcroft-Gault ≥ 60 m / min. U NGAL was determined by CMIA immunochemical test (commercial kits of Abbott Diagnostic on ARCHITECT ® i2000 SR).

**Results:** A statistically significant difference (p <0.001) was observed for the comparison of anti dsDNA antibodies, proteinuria, U pr/ cre, SLEDAI index / r scores, uNGAL between these two groups of rounds. The uNGAL was significantly correlated with parameters of active disease p <0.001, the values of the area under the ROC curve (AUC) the maximum sensitivity of 95% sensitivity and 100% specificity expressed in u NGAL The coordinates of the ROC curve for the u NGAL, limit. "cut off" was 52.95 ng / ml. With that value, all patients with uNGAL more than 52.95 ng / ml, should be in the group with active disease, and none in the group with lupus nephritis in remission.

**Conclusions:** The results show the importance in the diagnosis of disease activity, and in confirmation of remission of lupus nephritis, which can result in application of more appropriate therapeutic modality and individual approach to treatment. This study suggests that the determination of uNGAL in patients with lupus nephritis in daily clinical practice can contribute to the optimal effect of treatment.

## SA-PO730

### Vitamin D Is Negatively Correlated to Urinary MCP1 in Lupus Nephritis Patients

Aline Lázara Resende, Cristiane Bitencourt Dias, Fabiana Graciolli, Luciene dos Reis, Vanda Jorgetti, Viktoria Woronik. *Sao Paulo Univ, Sao Paulo, Brazil.*

**Background:** The association between vitamin D levels and SLE disease activity remains controversial. Urinary levels of Monocyte chemoattractant protein-1 (uMCP-1) are recognized as a specific biomarker of Lupus Nephritis (LN) activity. The aim of this study was to characterize the vitamin D status of newly diagnosed LN patients and its relationship to uMCP1 levels.

**Methods:** We included pre-menopausal female patients with ≤ 2 months of diagnosed LN attended from 2010 to 2012. The levels of uMCP1 were determined by specific ELISA (R&D Systems) and standardized to urine creatinine (Cr). 25-hydroxyvitamin $D_3$ [25(OH) D] were measured by chemoimmunoassay. Age-matched healthy female control's samples were collected at the end of winter. Statistical analysis were performed by Mann–Whitney test, Spearman correlation coefficient and linear regression models.

**Results:** LN patients presented a mean age of 29.5±10 years and were on glucocorticoid use for 34±12 days. Proliferative LN was observed in 86.6% of cases, with a mean proteinuria of 4.7±2.9 g/day and an estimated GFR of 37 (31-87) ml/min/1.73m². All SLE patients presented vitamin D insufficiency (9.9±4.4 ng/ml, range 4-20). Clinical and biochemical features of LN patients and controls are summarized below.

| | LN patients (n=15) | Controls (n=15) | p |
|---|---|---|---|
| Age (years) | 29.5±10 | 31.7±6.4 | 0.25 |
| Body Mass Index (BMI) (Kg/m²) | 24±3 | 24±4 | 0.74 |
| Estimated GFR (ml/min/1.73m²) | 37 (31-87) | 90 (73-100) | 0.009 |
| 25(OH)D (ng/ml) | 9.9±4.4 | 24.3±6.2 | <0.001 |
| Urinary MCP1 (pg/mg Cr) | 1594 (595-2447) | 177 (113-267) | <0.001 |

The levels of 25(OH)D correlated negatively with uMCP1 (r= -0.63, p<0.001). The correlation between uMCP1 and 25(OH)D remained unchanged even after adjusted for age, BMI and estimated GFR (beta coeff. -0.56, 95% CI -0.008 to -0.002, p=0.002).

**Conclusions:** 25(OH)D levels were negatively correlated to uMCP1, independently of age, BMI and estimated GFR. The negative correlation between vitamin D and markers of disease activity in SLE support the presumed immunomodulatory effects of vitamin D and should be further evaluated.

*Funding:* Government Support - Non-U.S.

## SA-PO731

### Proliferative Lupus Nephritis-NIH Regimen versus Tacrolimus and Mycophenolate Combination

Krishna Patil, Mahesh Bennikal, Arpita Roychowdhury, Rajendra Pandey, Jayaprada Jadhav, Rajesh Waikhom, Dipankar Sirkar, Sanjay Dasgupta. *Nephrology, IPGMER, Kolkata, WB, India.*

**Background:** Is multitarget therapy superior to NIH regimen?Future lies in individualizing therapy.

**Methods:** Open label prospective randomized trial.Arm I:NIH(CYC based) regimen(n[thinsp]=[thinsp]14).Arm II:Tacrolimus plus Mycophenolate and Prednisolo ne(n[thinsp]=[thinsp]14).Primary endpoints:complete or partial remission(CR/PR),non response and mortality.Secondary outcomes:Change in SLEDAI,creatinine,eGFR,proteinu ria,hematuria,C3,anti dsDNA,LNDAI & histologic activity & chronicity indices.Episodes of infection & steroid toxicity over 6 months therapy.

**Results:** Primary outcomes:CR&PR(p=0.21 and 0.33 respectively),non response(p=0.33) and mortality(p=1.00)were similar.Secondary outcomes:Arm I faired better over arm II with respect to mean change in serum creatinine(p=0.017),eGFR(p=0.042),C3 (p=0.014), and histological activity index-HAI(p=0.036).The episodes of LRTI & UTI were higher in the arm I(p=0.019 & p=0.028 respectively).Steroid toxicity was higher in arm I(p= 0.001 ).GI intolerance was higher in arm II(p= 0.046). Subgroup analysis: In arm I: HAI of >/=15 predicted poor outcome(p=0.03) & In arm II a LNDAI of >/=9 predicted poor outcome(p= 0.055).12 out of 14 patients in the arm I had a LNDAI of >/=9.Dialysis dependence beyond 1 month of adequate immunosuppression predicted non responsiveness to therapy in both arms.3 of the 4 deaths in the arm II were amongst non dialysis requiring patients and 1 of them died after attaining PR,where as all 4 deaths in the arm I were among dialysis dependent patients.

**Conclusions:** Multitarget regimen should be reserved for patients with stable renal function, LNDAI <9 and having contraindication to CYC.CYC arm had greater improvement in the C3 levels and HAI. HAI of >/=15 predicts poor outcome with CYC. Dialysis dependence after 1 month of either regimen should prompt one to withdraw/switch immunosuppression.Infection episodes & steroid toxicity were more in NIH arm but early fatal infection episodes in arm II raises concern over its safety.Larger trials are needed to validate the utility of LNDAI or HAI in individualizing therapy,predicting outcomes & guiding when to choose alternatives at the outset.

## SA-PO732

### Managing Lupus Nephritis with Cyclophosphamide or Mycophenolate Mofetil in a Large Nephrology Centre

Hannah M.M. Burton, Tracey M.E. Salter, Rebecca Suckling, David Makanjuola, Fiona E. Harris. *St. Helier Hospital, United Kingdom.*

**Background:** Since the NIH trials of the 1980s cyclophosphamide has been the mainstay of induction therapy for lupus nephritis (LN). The Euro-Lupus Nephritis Trial (ELNT) then demonstrated the benefits of a low-dose cyclophosphamide regime. In 2012 a Cochrane review concluded that mycophenolate mofetil (MMF) was as effective as cyclophosphamide in inducing remission. We appraised our management of LN over two decades to ascertain if our protocol could be optimised.

**Methods:** We present our single centre observational data. We retrospectively analysed the records of patients presenting with LN from 1992-2012. Response to induction therapy was assessed at 1 year, with complete remission defined as serum creatinine (sCr) ≤124μmol/L and urine protein:creatinine ratio (UPCR) ≤33g/mol, and partial remission defined as ≤25% increase in sCr from baseline and 50% reduction in UPCR to ≤150g/mol.

**Results:** Sixty four patients (89% female, mean age 39) had biopsy-proven LN and were followed up for an average of 2351 days. The ethnic mix was Black (23%), Asian (32%), White (43%) and Other (2%). 23 patients received cyclophosphamide induction therapy; 25 received MMF. Of the remainder, 2 received rituximab, 5 received prednisolone/ azathioprine and 9 received prednisolone.

| | Cyclophosphamide | MMF | Other |
|---|---|---|---|
| No. of patients | 23 | 25 | 16 |
| WHO Class: | | | |
| I | 1 | 0 | 0 |
| II | 0 | 1 | 3 |
| III | 1 | 0 | 1 |
| IV | 15 | 9 | 5 |
| IV/V | 2 | 4 | 1 |
| V | 2 | 11 | 6 |
| Other | 2 | 0 | 0 |
| Deaths | 0 | 2 | 3 |
| No. of patients requiring renal replacement therapy | 1 (at 720 days) | 2 (at 1898 & 2388 days) | 2 (at 1819 & 3903 days) |
| Adverse events (mean no./ patient) | 2.8 | 2.3 | 1.9 |

**Conclusions:** In the cyclophosphamide group, 46% achieved remission at 1 year, compared with 71% and 54% at 6 months (low dose and high dose group respectively) in the ELNT. This discrepancy in results may be at least partially explained by the different demographics. The remission rate was 52% in our MMF group compared with 46% in our cyclophosphamide group. This suggests that MMF is not inferior to cyclophosphamide with respect to outcome, in keeping with the Cochrane findings. We acknowledge that indication bias may have influenced the results in our cohort.

## SA-PO733

**Renal Involvement in Sjögren Syndrome** Maryam Khosravi,[1] Chris Laing,[2] Stephen B. Walsh.[3] *[1]Royal Free & Univ College London Centre for Nephrology; [2]Royal Free & Univ College London Centre for Nephrology; [3]Royal Free & Univ College London Centre for Nephrology.*

**Background:** Sjögren syndrome (SS) is an autoimmune disorder that has been estimated to affect as much as 1-2% of the adult female population. Epithelial cells are targets for a cell-mediated autoimmune response, in the lacrimal and salivary glands, as well as the renal tubulointerstitial compartment.

The prevalence of renal involvement in SS has been estimated at approximately 30%. The renal manifestation of SS is classically of distal renal tubular acidosis (dRTA), decreased renal excretory function.

**Methods:** We characterised 13 patients with renal SS who presented to the UCL Centre for Nephrology.

**Results:** Of the 13 patients, all were female, the mean age was (55.6 ± 12.6 years). Immunologically, All were positive for extractable nuclear antigens: 12 were Ro (SSA) positive, 9 were La (SSB) positive, 5 had hypocomplementemia (low C4), 1 had ANA, 7 had hypergammaglobulinemia (mean IgG 20.4± 8). All had urinary acidification (UA) tests with both furosemide and fludrocortisone and then oral ammonium chloride. The mean nadir urinary pH was 5.58 ± 0.47; all individual UA tests confirmed the presence of dRTA. The mean serum creatinine was 112.6± 38.9, and isotopic GFR measurements confirmed impaired renal excretory function in all patients with a mean GFR of 42.5 ± 9.02 ml/min). No patients had evidence of Fanconi syndrome, no patients had glycosuria or evidence of tubular proteinuria, the mean protein creatinine ratio was 46.3± 62.5 mg/mmol. Renal biopsies were obtained in 9 patients; all showed a diffuse diffuse lymphoplasmocellular infiltrate of mononuclear cells with variable tubular atrophy. 8 patients were started on immunosuppressive treatment with reducing course corticosteroids and mycophenolate mofetil (mean dose 937.5 mg daily). 8 patients also required supportive treatment with sodium bicarbonate (mean dose 562.5 g daily) and potassium (mean dose 57.6 mmol/daily).

**Conclusions:** dRTA in renal SS is common, and this is usually due to a pronounced and diffuse interstitial nephritis which adversely affects the GFR, often significantly. This is often underappreciated and may respond to immunosuppression.

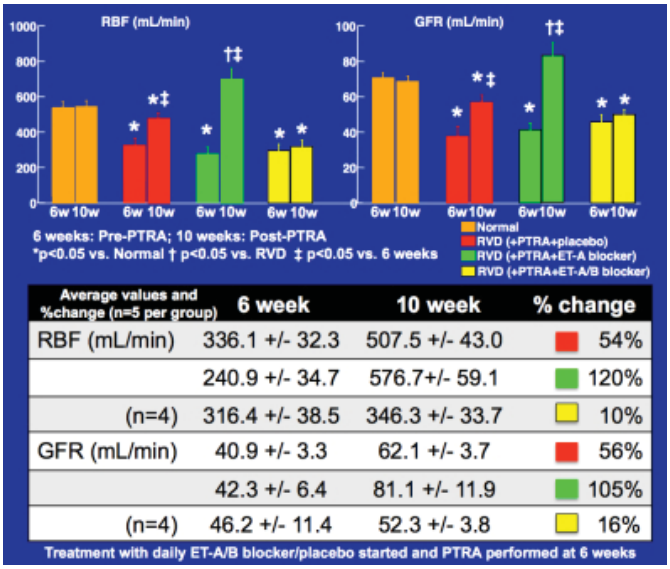
## SA-PO734

**Renal Angioplasty, Endothelin Receptor Blockers, and Recovery of Renal Function: A, Not B** Alejandro Chade, Nicholas Stewart. *[1]Physiology and Biophysics, [2]Medicine, [3]Radiology, Univ of Mississippi Medical Center.*

**Background:** Renal angioplasty and stenting (PTRAS) improves renal function in only 30% of patients with renovascular disease (RVD), underscoring the need for more effective therapeutic strategies. Because endothelin (ET)-A receptor blockade attenuates renal dysfunction and cardiovascular risk in chronic renal disease, we hypothesized that PTRAS followed by ET-A receptor blocker therapy would improve the renal outcomes in RVD. In addition, since little is known about the role of ET-B receptors in RVD, we determined whether PTRAS followed by dual ET-A and –B receptor blockade would synergize to provide greater renoprotection than ET-A antagonism alone.

**Methods:** Unilateral RVD was induced in 12 pigs by renal artery stenosis. After 6 weeks, single-kidney blood flow (RBF) and filtration (GFR) was quantified *in vivo* in the stenotic kidney using multi-detector computed tomography (MDCT). Then, all pigs underwent PTRAS and blindly randomized in: controls (RVD+PTRAS), and treated with ET-A blockers (Atrasentan, RVD+A) or with ET-A and ET-B blockers (RVD+PTRAS+AB, n=4 each). ET blockers/placebo were administered daily for 4 weeks and then in vivo MDCT studies repeated at 10 weeks.

**Results:** PTRAS resolved renal artery stenosis in all pigs and improved RBF and GFR. However, improvements were greater in RVD+PTRAS+A at 10 weeks, leading to almost a full recovery of renal function. In contrast, combined ET-A/B blocker therapy abolished the beneficial effects of both PTRAS and PTRAS+A.




| Average values and %change (n=5 per group) | 6 week | 10 week | % change |
|---|---|---|---|
| RBF (mL/min) | 336.1 +/- 32.3 | 507.5 +/- 43.0 | 54% |
| | 240.9 +/- 34.7 | 576.7+/- 59.1 | 120% |
| (n=4) | 316.4 +/- 38.5 | 346.3 +/- 33.7 | 10% |
| GFR (mL/min) | 40.9 +/- 3.3 | 62.1 +/- 3.7 | 56% |
| | 42.3 +/- 6.4 | 81.1 +/- 11.9 | 105% |
| (n=4) | 46.2 +/- 11.4 | 52.3 +/- 3.8 | 16% |

**Conclusions:** These results show that blockade of the ET-B receptors diminished the therapeutic effects of both PTRAS and ET-A blockade on RBF and GFR, implying an important role for a functional ET-1/ET-B pathway in the recovery of renal function in RVD. Furthermore, our study supports the potential therapeutic use of ET-A blockers to significantly improve the outcomes of PTRAS in RVD.

*Funding:* Other NIH Support - NIH-NHLBI, Pharmaceutical Company Support - Unrestricted grant from Abbvie

## SA-PO735

**Oxidative Stress: The Role in the Pathogenesis of Cardiorenal Syndrome Type 1** Grazia Maria Virzì, Alessandra Brocca, Massimo de Cal, Giacomo Mason, Sonya Day, Claudio Ronco. *Nephrology Dep-IRRIV, Vicenza, Italy.*

**Background:** Cardiorenal Syndrome Type 1 (CRS1) is characterized by acute cardiac events leading to acute kidney injury. Loss of redox homeostasis in reactive oxygen species (ROS) and reactive nitrogen species (RNS) results in a proinflammatory and profibrotic milieu via distinct mechanisms which promotes cardiovascular and renal structural and functional abnormalities. This pilot study examined the putative role of ROS/RNS-linked oxidative stress as a pathogenic mechanism of CRS1.

**Methods:** Subjects were selected from a prospective community-based cohort study with HF (58%male, mean age 80±8yrs, medianCrea 0.98mg/dl, IQR 0.87-1.15), CRS1 (54%male, mean age 76±10yrs, median Crea 1.34mg/dl, IQR 1.12-1.83). Quantitative determinations for IL6, Myeloperoxidase (MPO), Nitric Oxide (NO), Copper/Zinc Superoxide Dismutase (Cu/ZnSOD), and Endogenous Peroxidase Activity (EPA) were performed in HF (n=12), and CRS1 (n=11) patients. The Mann-Whitney U test was performed for cohort comparison (p<.05 was statistically significant) by STATA.

**Results:**

| | HF | CRS1 |
|---|---|---|
| MPO pg/ml | 505.6 (421.7-547.8 | 746.9 /665.2-940.0) |
| NO µM | 205.6 (95.0-277.5) | 507.3 (404.7-557.3) |
| Cu/ZnSOD pg/ml | 184.5 (160.5-192.0) | 274.5 (191.8-326.8) |
| EPA U/l | 274.5 (191.8-326.8) | 2978.4 (2071.8-4069.9) |
| IL6 pg/ml | 22.19 (16.6-24-6) | 90.68 (59.9-105.3) |

CRS1 patients displayed significant augmentation in circulating ROS and RNS, as well as expression of inflammatory cytokines, as IL6. Quantitative analysis of all oxidative stress markers showed significantly lower oxidative stress levels in HF compared to CRS1 patients (p<.05).

**Conclusions:** This pilot study demonstrates the significantly heightened presence of dual redox disequilibrium in CRS1 compared to HF patients: ROS/RNS production involving NADPH oxidase and MPO; superoxide production of hydrogen peroxide and NO; upregulation of proinflammatory mediators via peroxynitrite. Our findings indicate that oxidative stress is a potential therapeutic target, as it promotes inflammation by ROS/RNS-linked pathogenesis. For the first time we demonstrate evidence for the delineation of 2 distinct oxidative roles in CRS1 pathogenesis.

## SA-PO736

**In Vitro Study of Cell Responses at Radiocontrast Medium** Grazia Maria Virzì,[1] Alessandra Brocca,[1] Massimo de Cal,[1] Dinna N. Cruz,[2] Claudio Ronco.[1] *[1]Nephrology Dep-IRRIV San Bortolo Hosp, Vicenza; [2]Univ of California.*

**Background:** Radioontrast-induced nephropathy (RCIN) accounts for >10% of all causes of hospital-acquired renal failure, causes a prolonged inhospital stay and represents a powerful predictor of poor outcome. Mechanisms of RCIN are not completely understood. In this study, we investigated the in vitro effects of Contrast Media (CM) on renal tubular cells (RTCs) in terms of cell viability, and cell damage.Moreover, we evaluated the relationship between NGAL and RCIN in these patients.

**Methods:** In this study, we included 4 groups: 5RCIN patients with AKI(RCIN), 14 patients with AKI but no-exposition to CM(AKI_no CM), 5patients with exposition to CM but no AKI(CM_noAKI) and 16 healthy controls(CTR). Plasma from different cohorts were incubated with RTCs in standard condition for 24h and, subsequently, viability, apoptosis and necrosis was evaluated by cytofluorometric assay. Quantitative analysis of NGAL was performed in plasma. The Kruskal–Wallis test for multiple comparisons was applied to compare the groups. Pearson's Rank Order Correlation Coefficient ® was used to test the correlation between variables. A p-value of <.01 was considered statistically significant.

**Results:** RCIN groups is characterized by lower viability and higher apoptosis rates compared with other groups (p<.01).AKI_noCM and CM_noAKI groups have decreased viability and increased apoptosis compared with CTR. AKI_noCM group showed a stronger cell damage and apoptosis compared with CM_noAKI (p<.01). CM-noAKI group showed a significantly decreased viability rate and a significantly increased level of apoptosis compared with CTR (p<.01). There is a positive correlation between NGAL levels and Apoptosis (0.87) and Necrosis (0.75) and an inverse correlation between NGAL and cell viability (-0.84).

**Conclusions:** This study clearly demonstrates that CM-induced epithelial tubular renal cells apoptosis represents a key mechanisms of RCIN-AKI. In our results, the toxic effect of CM was evident and causes renal tubular cells death by apoptosis. These results suggest that AKI and CM may add their effects in RCIN patients and could induce in vivo a strong damage on kidney structures and functions.

## SA-PO737

**Ribonuclease 6: A Novel Antimicrobial Peptide in the Human and Murine Urinary Tract** Brian Becknell, Kirk M. McHugh, David S. Hains, John David Spencer. *Nationwide Children's Hospital.*

**Background:** Recent studies stress the importance of antimicrobial peptides (AMPs) in preventing urinary tract infections (UTIs). Members of the Ribonuclease A superfamily are potent AMPs that contribute to sterility in other organ systems. The goal of this study was to determine the expression and antimicrobial activity of Ribonuclease 6 (Rnase6) in the urinary tract.

**Methods:** Cystitis in mice was achieved via transurethral inoculation of $10^8$ colony forming units of uropathogenic E.coli (UPEC) into 8-12 week old C57BL/6 females. Organs were harvested for isolation of RNA/protein or fixed in 4% PFA. RNA/protein were also isolated from non-infected human bladder/kidney tissue and tissue with pyelonephritis. Human RNASE6 and mouse Rnase6 mRNA levels were measured by qRT-PCR. Immunohistochemistry localized Rnase6 expression. Immunoblot quantitated protein levels. Antimicrobial kill assays were performed using recombinant human RNase6 against $10^6$ CFU/mL UPEC.

**Results:** Human RNASE6 and mouse Rnase6 mRNAs are detectable at low levels throughout the uninfected urinary tract, but their encoded peptides are undetectable by immunoblotting. UPEC infection induces murine Rnase6 bladder mRNA 3-fold by 48 hours (p = 0.001). At this time point, murine Rnase6 protein localizes to myeloid cells infiltrating the bladder urothelium. Similarly, IHC demonstrates that leukocytes express Rnase 6 in kidney tissue with pyelonephritis. Human RNASE6 and mouse Rnase6 proteins are detectable by immunoblotting in kidneys with acute pyelonephritis as well as infected urinary sediment. Recombinant human RNASE6 protein binds to the bacterial cell wall and demonstrates dose-dependent bactericidal activity toward UPEC. Recombinant Rnase 6 demonstrates minimal cytotoxicity toward host tissues.

**Conclusions:** This study demonstrates that human RNASE6 and mouse Rnase6 proteins are expressed in the urinary tract and implicates human RNASE6 as a novel AMP toward UPEC. Our results suggest that RNase6 is a leukocyte-derived AMP that may play an important role in innate immunity of the urinary tract. RNase6 may represent a novel therapeutic target for children with complex UTI.

*Funding:* Other NIH Support - NIH K08DK094970-02 to JDS

## SA-PO738

**Single Photon Emission Computed Tomography Imaging of Kidney Aminopeptidase N Expression in a Transgenic Mouse Model of Urothelial Transitional Cell Carcinoma** Hariprasad Gali,[1] Gopal Pathuri,[1] Andria F. Hedrick,[1] Venkateshwar Madka,[2] Vibhudutta Awasthi,[1] Chinthalapally V. Rao,[2] Benjamin D. Cowley.[3] *[1]Dept of Pharmaceutical Sciences, College of Pharmacy; [2]Hematology/Oncology Section; [3]Nephrology Section, Dept of Internal Medicine, College of Medicine, The Univ of Oklahoma Health Sciences Center.*

**Background:** Aminopeptidase N (APN; CD13; EC 3.4.11.2) is a zinc-dependent membrane-bound exopeptidase that catalyzes the removal of N-terminal amino acids from peptides. APN is well known to be highly expressed on renal proximal tubules. APN expression has been demonstrated to be significantly decreased in renal cancer tissues compared to adjacent normal tissues. Thus, molecular imaging of APN expression noninvasively could provide useful information regarding tumor extent in the kidney.

**Methods:** Tc-99m labeling of a probestin conjugate containing a $PEG_2$ linker and a $N_3S$ tripeptide chelator sequence, Asp-DAP-Cys, was achieved using our reported procedure (Bioorg Med Chem Lett. 2012, 22:4567-70). Whole body SPECT imaging was conducted at 2 hr post-injection of $^{99m}$Tc-probestin in a transgenic UPII-SV40T mouse and a wild-type mouse. UPII-SV40T mice spontaneously develop urothelial carcinoma *in situ* and invasive transitional cell carcinoma that invade into renal tissues. Histopathology and immunohistochemistry analysis were used to evaluate APN expression in the kidney.

**Results:** Radioactivity uptake was not observed in the tumor region of the transgenic mouse kidney whereas the entire renal parenchyma was visible in the wild-type mouse. Histopathological studies confirmed that both the pelvis and parenchyma regions were normal in the wild type mouse kidney and the presence of transitional cell cancer in the renal pelvis of the transgenic mouse kidney. Proliferating cell nuclear antigen staining of the transgenic mouse kidney confirmed the presence of tumor. An immunohistochemical study confirmed that the APN expression in tumor tissue was much lower than that in the adjacent normal tissue.

**Conclusions:** Results reported here provide preliminary support for the use of $^{99m}$Tc-probestin SPECT for imaging kidney APN expression noninvasively in animal models of urothelial tumors.

*Funding:* Other NIH Support - NIH grant S10RR025652 funded the NanoSPECT system and NIH NCI-N01CN 53300

## SA-PO739

**Evaluation of Positron Emission Tomography Renography in a Rat Model of Autosomal Dominant Polycystic Kidney Disease** Hariprasad Gali,[1] Gopal Pathuri,[1] Andria F. Hedrick,[1] Vibhudutta Awasthi,[1] Benjamin D. Cowley.[2] *[1]Dept of Pharmaceutical Sciences, College of Pharmacy; [2]Nephrology Section, Dept of Internal Medicine, College of Medicine, The Univ of Oklahoma Health Sciences Center, Oklahoma City, OK.*

**Background:** We have identified *p*-$^{18}$F-fluorohippurate (F-18 PFH) as a potential radiopharmaceutical for PET renography. The objective of this study was to obtain an initial F-18 PFH PET renogram and kidney PET images of a male and a female Han:SPRD rat in order to assess the potential application of this novel technique to monitor progression of ADPKD.

**Methods:** F-18 PFH (~26 µCi) was IV injected into a 35 week-old male and a 38 week-old female Han:SPRD rat. Han:SPRD rats develop ADPKD, which is more severe in males. Dynamic PET imaging data in list-mode were acquired over a period of 30 min after injection followed by CT imaging data for 1 min. The PET scan time was divided into 30-sec time frames and the data was reconstructed using 2D filtered backprojection algorithm. Regions of interest (ROIs) were drawn over kidneys guided by CT images. Radioactivity within the ROIs of each frame was calculated, decay-corrected, and the renogram was plotted from the ROI activities.

**Results:** The renograms revealed a time-to-peak ($T_{max}$) of about 6 min and a time to half-maximal activity ($T_{1/2max}$) of >30 min for both kidneys for both rats. For comparison, our previously reported $T_{max}$ and $T_{1/2max}$ values for 10 week-old female healthy Sprague Dawley rats were 2.9 ± 0.4 min and 7.1 ± 1.3 min respectively (Nucl Med Biol. 2012, 39:1195-201). At the renogram peak, about 8 % injected dose (%ID) was observed in each kidney for male rat whereas about 14 and 12 %ID was observed in left and right kidney respectively for female rat. Kidney PET images of female rat at the renogram peak display higher activity compared to male rat indicating more functional tubular mass in female rat kidneys than male.

**Conclusions:** This preliminary study indicates that F-18 PFH PET renography may be a suitable technique to monitor progression of ADPKD in animal models. However, further research is required to validate the results and correlate the PET renogram parameters with the disease status.

## SA-PO740

**Enalapril Treatment Prevents and Reverses Renal Damage in the ZSF1 Obese Rat Model of Diabetic Nephropathy** Paul Harrison, Kathleen A. Lincoln, Hongxing Chen, Holly Clifford, Kristina Gueneva-Boucheva, Hu Sheng Qian, Glenn A. Reinhart, Carine Boustany. *Cardiometabolic Disease Research, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.*

**Background:** The prevalence of diabetic nephropathy is on a rise despite medical advancements. Novel therapies to slow the decline in renal function are urgently needed. Accordingly, preclinical models of diabetic nephropathy (DN) are warranted to support the advancement of novel compounds into the clinic. Recent reports suggest that the obese diabetic ZSF1 rat model closely approximates the pathophysiological conditions of human DN, including: hypertension, proteinuria and hyperglycemia. Angiotensinogen Converting Enzyme Inhibitors (ACEi) have been shown to slow the decline in renal function in patients with DN.

**Methods:** A 15 week time course study was conducted to assess the effect of enalapril initiated at different times on blood pressure, proteinuria and fibrosis. Male ZSF1 obese rats (n=9/group), telemetry instrumented for blood pressure recording, were randomly assigned to six treatment groups. Enalapril administration (3 mg/kg in the drinking water; achieving clinically relevant concentration) was initiated on study weeks 1, 3, 5, 8 and 10 for a treatment duration of 15, 12, 10, 8 and 5 weeks, respectively. Vehicle treated ZSF1 obese animals were used as comparators. Weekly urine collections, body weight, water and food consumption recordings, and clinical observations were performed. Blood pressure was continuously recorded. Blood collections for creatinine and pharmacokinetic determinations were performed on select weeks. At termination, renal tissue was assessed for incidence of glomerulosclerosis and interstitial fibrosis.

**Results:** Enalapril administered in a prophylactic (duration of 15 and 12 weeks) and therapeutic (duration of 10, 8 and 5 weeks) manner significantly decreased mean arterial pressure and urinary protein to creatinine ratio compared to vehicle. Glomerular and interstitial lesions were modestly reduced across all treatment groups.

**Conclusions:** In summary, the ZSF1 obese rat constitutes a preclinical model of diabetic nephropathy in which an ACEi both prevents and reverses renal damage.

*Funding:* Pharmaceutical Company Support - Boehringer Ingelheim Pharmaceuticals, Inc.

**SA-PO741**

**Cardiorenal Syndrome Type 5 Mechanism: A New Hypothesis for Organs Damage** Alessandra Brocca, Grazia Maria Virzì, Giacomo Mason, Massimo de Cal, Claudio Ronco. *Nephrology Dep-IRRIV, Vicenza, Italy.*

**Background:** Cardiorenal Syndrome Type 5 (CRS5) is characterized by the presence of combined cardiac and renal dysfunction due to systemic disorder. Severe sepsis represents the most common condition which can damage both organs. Activation and induction of cytokines, leukocytes and toll receptors are well established in organs damage in CRS5. CRS5 mechanism is not well-known, although specific cellular and molecular changes with time-specific pattern are reported. Our aim was to examine in vitro that CRS5 plasma was able to trigger a response in renal tubular cells (RTCs), resulting in apoptosis and in cytokine-release.

**Methods:** We enrolled 11 patients with CRS5 (68.4±10.8yrs), and 16 controls (CTR) (52.0±7.7yrs). Plasma from different groups were incubated with RTCs for 24h and, subsequently, cell apoptosis was evaluated by Annexin-V/Propidium Iodide assay and quantitative levels of IL-6 in plasma was performed by ELISA.

**Results:** In RTCs treated with CRS5 plasma, a quantitative analysis showed significantly higher apoptosis and necrosis rates compared to CTR (p<.001). A significantly lower viability was observed in RTCs incubated with CRS5 plasma compared to CTR (p<.001).

| | Viability (%) | Apoptosis (%) | Necrosis (%) |
|---|---|---|---|
| **CRS5** | 82.80 (81.20-86.20) | 15.10 (13.80-17.80) | 4.20 (1.60-12.20) |
| **CTR** | 96.60 (94.75-97.60) | 1.51 (1.20-2.00) | 0.98 (0.70-1.31) |

In CRS5 plasma, IL-6 levels (64.6pg/ml, IQR 59.1-96.3) were significantly higher compared with CTR plasma (6.17 pg/ml IQR 3.50-7.78) (p<.001). Moreover there were significant positive correlation between IL-6 levels and cellular apoptosis (rho .74, p<.001) and necrosis (rho .56, p<.01). A negative correlation (rho -.74, p<.001) was observed for viability.

**Conclusions:** Our study demonstrated the induction of cellular death, by apoptosis and necrosis pathways, in RTCs incubated with CRS5 plasma. Furthermore, we observed an increase of IL-6 plasma levels in CRS5 patients. In conclusion we could hypothesise a possible implication of inflammatory damage on the pathophysiology of CRS5. It is necessary to increase the patients sample size to validate our hypothesis.

**SA-PO742**

**Tubular Overexpression of Gremlin in Transgenic Mice Induces Renal Damage Susceptibility** Sergio A. Mezzano,[1] Alejandra M. Droguett,[1] Daniel Carpio Paniagua,[1] Bredford Kerr,[2] Raquel Rodrigues-Diez,[3] Jesus Egido,[3] Marta Ruiz-Ortega.[3] *[1]Nephrology, Universidad Austral de Chile, Valdivia, Los Rios, Chile; [2]Centro de Estudios Científicos, CECS, Valdivia, Los Rios, Chile; [3]Fundacion Jimenez Diaz, Universidad Autonoma, Madrid, Spain.*

**Background:** Gremlin is an embryogenic gene with a key role in nephrogenesis that is reexpressed in many human renal diseases, including diabetic nephropathy, pauci inmune glomerulonephritis and chronic allograft nephropathy. Some authors have proposed that gremlin could be involved in renal damage, acting as a downstream mediator of TGF-b.

**Methods:** To examine the *in vivo* role of Gremlin in the kidney we generated seven specific proximal tubular epithelial cells human gremlin (GREM1) transgenic mouse lines, presenting 1.2 to 200-fold increase of GREM-1 gene expression levels. These GREM1 transgenic mice presented a normal phenotype and non-proteinuria or renal function involvement.

**Results:** In response to acute renal damage cause by folic acid injection, tubular-specific GREM1 transgenic mice developed higher proteinuria after 7 and 14 days than wild type mice. Tubular GREM1 overexpression was associated to renal upregulation of profibrotic factors, such as TGF-b and a-SMA, and increased proliferating cells compared to wild type mice.

**Conclusions:** Our results suggest that GREM1-overexpressing mice might have an increased susceptibility to develop renal failure supporting the involvement of gremlin in renal damage progression.

*Funding:* Government Support - Non-U.S.

**SA-PO743**

**Xylene Induces DNA Methylation Changes in Podocytes and Tubular Epithelial Cells** Zhongxiu Xu, Wei-song Qin, Xianghua Cao, Cai-hong Zeng, Zhi-hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing Univ School of Medicine, Nanjing, China.*

**Background:** To determine if there is an epigenetic mechanism underlying organic solvent-induced renal injury, we investigated the change of DNA methylation in conditionally immortalized human podocytes and proximal tubular cell line HK2 treated with xylene.

**Methods:** DNA methylation changes in cultured human podocytes and proximal tubular cells were identified by comparing DNA methylation profiles of the cells treated with xylene and the untreated control. Illumina human 450k CpG DNA methylation microarray was used to analyze DNA methylation of the cells. Bisulfite sequencing PCR and pyrosequencing were used to validate the microarray results. Quantitative real-time PCR (qRT-PCR) was carried out to examine the correlation between the mRNA expression and methylation level of a gene.

**Results:** We identified 51 probes that displayed significant methylation level changes in xylene-treated podocytes compared with untreated control cells, among which 25 probes showed increased, while the remaining 26 decreased, methylation levels. mRNA qRT-PCR analyses of the corresponding genes revealed the correlations of IL-20 and p53 expressions with their methylation levels, respectively. For HK2 cells, there were 243 probes that displayed significant methylation change, of which 109 showed increased, while 134 decreased, methylation level. qRT-PCR analyses confirmed the correlation between the mRNA expression and the methylation level of EIF1AY or KDM5D. The results of pyrosequencing were well consistent with the microarray data.

**Conclusions:** Xylene can induce DNA methylation change in podocytes and tubular cells, which may be part of the mechanism by which xylene causes injury of these cells.

*Funding:* Government Support - Non-U.S.

**SA-PO744**

**Inhibition of Cyclooxygenase-2 Does Not Ameliorate Lithium-Induced Renal Microcystic Injury and Polyuria in Adolescent Rat** Kirsten Madsen,[1,2] Gitte Kjaersgaard,[1] Niels Marcussen,[2] Boye Jensen.[1] *[1]Dept of Cardiovascular and Renal Research, Univ of Southern Denmark, Odense, Denmark; [2]Dept of Pathology, Odense Univ Hospital, Odense, Denmark.*

**Background:** In human patients, chronic treatment with lithium leads to tissue injury with multiple microcysts that originate in cortical collecting duct and nephrogenic diabetes insipidus (NDI). In this study it was hypothesized that renal COX-2 activity promotes microcyst formation.

**Methods:** Microcystic kidney injury was induced in male adolescent rats by feeding dams with lithium [50 mmol Li/kg chow] from postnatal days 7 to 34.

**Results:** Lithium treatment induced cortical microcysts and dilatations in the distal nephron parts; it increased cortical cell proliferation and increased inactive pGSK-3β; it lowered AQP2 expression and induced polyuria with decreased ability to concentrate the urine and it increased COX-2 protein level in thick ascending limb. Concomitant treatment with lithium and a specific COX-2 inhibitor, parecoxib (5mg/kg/day, P10-P34) did not prevent lithium-induced microcystic injury and polyuria, but improved urine concentrating ability transiently after a dDAVP challenge. COX-2 inhibition did not reduce reno-cortical lithium-induced cell proliferation or inactivating phosphorylation of GSK-3β. COX-1 protein abundance increased in kidney cortex in response to lithium and was associated with microcysts and dilatations in collecting ducts in both rats and humans.

**Conclusions:** In summary, COX-2 is marginally stimulated in cortical loop of Henle cells in chronic lithium-treated adolescent rats; COX-2 is not colocalized with microcystic epithelium; mitotic activity and inactivated pGSK-3β in collecting duct; a blocker of COX-2 does not prevent cell proliferation, cyst formation or GSK-3β inactivation. It is concluded that COX-2 activity is not the primary cause for microcystic injury and polyuria in a NaCl-substituted rat model of Li-nephropathy while COX-1 is a likely candidate to affect the injured epithelium.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

**SA-PO745**

**The Effect of Pioglitzone on Mitochondrial Biogenesis in the Kidney of Dahl Salt Sensitive Hypertension Rat** Yoshimitsu Hayashi, Yuki Kusano, Kimio Watanabe, Makoto Kanno, Hiroshi Kimura, Kenichi Tanaka, Koichi Asahi, Hiroyuki Terawaki, Shigeatsu Hashimoto, Masaaki Nakayama, Tsuyoshi Watanabe. *Fukushima Medical Univ, Nephrology and Hypertension, Fukushima, Japan.*

**Background:** Thiazolidines (TZDs) improve insulin sensitivity but also appear to have antihypertensive effects in obesity. Increases oxidative stress in renal outer medulla by salt loading is thought to be one of the causes of hypertension and kidney damage. To closely examine the antihypertensive and renoprotective effects of TZDs, we used non-obese salt sensitive hypertension rat with pioglitazone (pio).

**Methods:** Aged 6week Dahl salt sensitive (DSS) rats were divided into the four groups: LS (0.3% Low salt fed n=10), LSP (LS fed containing pio n=10), HS (4.0% High salt fed n=20) and HSP (HS fed containing pio n=20). The concentration of pio in chow is kept at 0.005%. Rats were sacrificed at 12$^{th}$ week. Nitrotyrosine, SOD1 and SOD2 in kidney were observed by immunohistochemistry or western blot. We also determined two subunits (COX-I and SDH-A) of oxidative phosphorylation enzyme complex to elucidate mitochondrial biogenesis of kidney by western blot. COX-I is mitochondrial DNA-encoded, and SDH-A is nuclear DNA-encoded.

**Results:** Between HS and HSP groups, significant improvement were observed in systolic blood pressure (171 vs 156 mmHg), creatinine clearance (2.3 vs 3.1 ml/min) in HSP. No significant differences were observed in body weight, serum glucose and insulin levels, and urinary protein excretion. Serum triglyceride levels was decreased (96 vs 67 mg/dl, p<0.01), and adiponectin levels was increased (3.3 vs 5.2 mg/ml, p<0.01) in HSP. Nitrotyrosine was detected in fibrosis area in renal outer medulla and nitrotyrosine levels were significantly reduced (1.0±0.3 vs 0.71±0.3) in HSP. Expression of SOD2 levels (1.0±0.1 vs 1.3±0.2, p<0.05) and ratio COX-I / SDH-A (1.0±0.1 vs 1.3±0.4, p<0.05) were increased in HSP.

**Conclusions:** Pioglitazone suppressed salt sensitive hypertension, renal damage in DSS rat without apparent changes in serum glucose and insulin levels. Increased adiponectin to induce mitochondrial biogenesis might be involved in these renoprotective effects of pioglitazone.

## SA-PO746

**Alpha-1-Microglobulin Protects Renal Proximal Tubule Epithelial Cells from Apoptosis and Cell Damage following Hemoglobin-, Heme-, and Oxidative Overload** Magnus Gram, Sara Davidsson, Maria Johansson, Bo Akerstrom. *Infection Medicine, Clinical Sciences, Lund, Sweden.*

**Background:** Intravascular hemolysis, following e.g. cardiac surgery, with the release of large amounts of extracellular hemoglobin (Hb), is a highly pathogenic event causing substantial and sometimes non-reversible damage to the kidneys. Removal, scavenging, or neutralization of extracellular Hb and its down-stream metabolites may therefore represent a plausible strategy of protection of the kidney function and structure. The aim of this study was to investigate the structural and functional damage and characterize the inflammatory response in human primary renal proximal tubule epithelial cells following Hb, heme and oxidative insults and to evaluate the protective effects following treatment with the heme- and radical scavenger alpha-1-microglobulin (A1M).

**Methods:** Following exposure to Hb and Hb-metabolites we characterized the cellular and molecular response in human primary renal proximal tubule epithelial cells (HPRPTEC) with or without the addition of A1M.

**Results:** Exposure to oxyHb, metHb, heme, or ROS led to upregulation of interleukin-1β (IL-1β), heme oxygenase-1 (HO-1), ICAM-1 and p21 mRNA expression at 4 and 24 hours. Furthermore, results showed an increased necrosis (LDH), and apoptosis (caspase activation) in epithelial cells exposed to metHb and heme but not in cells exposed to oxyHb. Treatment with A1M reversed the metHb, and heme-induced effects on gene expression as well as necrosis and apoptosis responses.

**Conclusions:** Following systemic hemolysis, e.g. as a result of cardiac surgery or blood transfusion, there is a distinct increase of extracellular Hb-metabolites that have potential pathophysiological effects on the kidneys, sometimes rendering irreversible damage to the kidneys. We show that treatment of the Hb-, heme-, and ROS-induced inflammatory and stress response in the kidney cell line HPRPTEC could be inhibited by the heme- and radical scavenger A1M. Our studies therefore present a therapeutic opportunity and a means of decreasing the damage to the kidneys following intravascular hemolysis.

*Funding:* Pharmaceutical Company Support - A1M Pharma AB, Private Foundation Support

## SA-PO747

**Podocyte Injury with Hypercholestelemia Promotes Intraglomerular Lipid Deposition** Satoshi Hara,[1] Kazuo Sakamoto,[2] Yasutoshi Takashima,[1] Namiko Kobayashi,[1] Toshiharu Ueno,[3] Noriko Uesugi,[1] Taiji Matsusaka,[4] Michio Nagata.[1] *[1]Kidney and Vascular Pathology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Nephrology, Iizuka Hospital, Fukuoka, Japan; [3]Nephrology, Toranomon Hospital, Tokyo, Japan; [4]Internal Medicine, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan.*

**Background:** Focal segmental glomerulosclerosis (FSGS) cellular variant is characterized by endocapillaly proliferation mainly composed of foam cells which are derived from macrophage. The lesion is often associated with extracapillary proliferation. The present study aimed to investigate how foam cells infiltrate into the glomerulus in the setting of podocyte injury.

**Methods:** By mating low density lipoprotein receptor knockout (LDLRKO) mice with NEP25 mice which expressed human CD25 in podocyte, we generated NEP25;LDLRKO mice. In this model, immunotoxin injection provokes podocyte-specific injury leading to FSGS under hypercholesteremia. We compared NEP25;LDLRKO mice with LDLRKO mice and NEP25 mice as control. Mice have fed with Western-type diet or normal diet from 8 weeks old. At 12 weeks, immunotoxin for human CD25 or vehicle intravenous infusion was performed, and mice were perfused after 12 days. We performed Oil red O staining and immunohistochemically analyzed macrophage infiltration (CD68 staining), monocyte chemoattractant protein-1 (MCP-1).

**Results:** NEP25;LDLRKO and NEP25 mice revealed severe glomerular collapse with extracapillary proliferation. All groups showed macrophage and foam cells infiltration, although no significant differences were noted. Oil red O positive area in the glomeruli significantly increased in NEP25;LDLRKO mice ($p < 0.05$). Although MCP-1 was dominantly expressed at podocytes, MCP-1 expression were absent in the glomeruli with extracapillary proliferation in NEP25;LDLRKO and NEP25 mice.

**Conclusions:** Under hypercholesteremia, acute podocyte injury promotes excessive lipid deposition within the glomeruli probably due to injury of filtration barrier and mesangium. However, hypercholesteremia with podocyte loss did not cause macrophage attraction, suggesting mechanism of podocyte loss, but not podocyte loss per se may contribute to glomerular foam cell infiltration in FSGS.

## SA-PO748

**Vascular Eicosanoid Generation in Experimental Uremia** David Bishop-Bailey,[1] Julius Edward Kieswich,[2] Kieran McCafferty,[2] Scott Thomson,[1] Matthew L. Edin,[3] Darryl Zeldin,[3] Magdi Yaqoob.[2] *[1]Comparative Biomedical Sciences, Royal Veterinary College, London, United Kingdom; [2]William Harvery Research Institute, TMT, Queen Mary, Univ of London, London, United Kingdom; [3]Environmental Cardiopulmonary Disease Group, NIEHS, NIH, NC.*

**Background:** Cardiovascular disease is the leading cause of death for patients with moderate to severe chronic kidney disease. Eicosanoids and related lipids (EARLs), are products of cyclooxygenase, lipoxygenase and epoxygenase enzymatic pathways, and have established and emerging roles in renal and cardiovascular physiology. Here we examined the changes in vascular EARL production after experimental uremia in the rat.

**Methods:** Male Wistar rats (n=5 per group) underwent a 2-stage subtotal nephrectomy (SNx) or a sham procedure. The left kidney was two thirds resected with right total nephrectomy. After a further 4 weeks, blood was taken for serum analysis, and aorta's were removed and placed in serum free organ culture for 30min. The resulting conditioned media was analysed by LC-MS/MS for patterns of cyclooxygenase, lipoxygenase and epoxygenase: arachidonic acid and linoleic acid metabolites.

**Results:** Subtotal nephrectomy resulted in increases ($P<0.05$) in serum urea (sham 6.1±0.2; SNx 17.4±1.6 mmol/l), creatinine (sham 42.0±1.5; SNx 85.2±3.0 umol/l), $Ca^{2+}$ (sham 2.712±0.019; SNx 2.846±0.020 mmol/l) and mean arterial blood pressure (sham 137±26; SNx 157±19.6) but albumin, $Na^{+}$ and $K^{+}$ were unchanged. Sham and SNx aorta released an almost identical pattern of eicosanoids with prostacyclin the major EARL produced by both groups (sham 65±12; SNx 58±9 ng/ml). The one exception was a significant increase in the epoxygenase arachidonic acid metabolite 14,15-EET (sham 131±13; SNx 233±104 pg/ml, $P<0.02$).

**Conclusions:** 14,15-EET acts as a vasodilator and anti-inflammatory EARL which may represent a novel biomarker and mediator of possibly inadequate compensatory cardiovascular response to chronic kidney disease. The usefulness of drugs that elevate 14,15-EET levels, e.g. soluble epoxide hydrolase inhibitors, to limit the adverse cardiovascular outcomes associated with CKD remains to be determined.

## SA-PO749

**Sickle Nephropathy Is Mediated via ET-1 Upregulation of NOX2 and p47phox** Brett Heimlich, Joshua S. Speed, David M. Pollock. *Experimental Medicine, Georgia Regents Univ, Augusta, GA.*

**Background:** Sickle cell nephropathy (SCN) is a chronic manifestation of sickle cell disease (SCD) for which the mechanism is unknown. We previously found that reactive oxygen species (ROS) were elevated in the glomeruli of SCD mice. In vitro administration of the NADPH oxidase inhibitor apocynin prevented glomerular ROS production and importantly, in vivo treatment with ABT-627, a selective ET-A receptor antagonist, reduced glomerular ROS to levels observed in controls. Since ET-1 is elevated in patients with SCD, we hypothesized that ET-1 mRNA is elevated specifically in the glomeruli of SCD mice, which subsequently causes increased ROS creation via NOX2.

**Methods:** Renal tissues were carefully dissected and glomeruli were harvested from 14-week-old, knock-in HbS only sickle and C57BL/6J mice and were assessed for relative ET-1, NOX2, and p47phox mRNA.

**Results:** We found that sickle mice have 401±89% more glomerular ET-1 mRNA when compared to C57 control mice ($p<0.01$, n=6). Furthermore, we found that SCD mice have significant increases in both NOX2 and p47phox but not NOX4 mRNA in their glomeruli (186±21 and 301±64 % of control respectively, $p<0.01$, n=6). These results were recapitulated in the renal cortex of the same animals with the exception of NOX2. ET-1 mRNA was 269±36% of control while p47phox was 172±15% of control mice ($p<0.01$, n=6).

**Conclusions:** Combined with our previous experiment, these data support the hypothesis that chronically elevated ET-1 leads to the creation of ROS via NADPH oxidase in the glomeruli of sickle animals, contributing to renal damage in the progression of SCN.

*Funding:* Other NIH Support - NHLBI

## SA-PO750

**Clinical Assessment of Urine Magnesium Excretion Rate as a Predictor for Tubulo-Interstitial Disorders** Hajime Hasegawa, Chie Noiri, Taisuke Shimizu, Kunihiko Yasuda, Yoshimi Okada, Tatsuro Sano, Yuta Kogure, Tota Kiba, Tetsuya Mitarai. *Nephrol & Hypertens, Saitama Med Center, Saitama Med Univ, Kawagoe, Saitama, Japan.*

**Background:** Elevated urine magnesium excretion and its correlation with histological damage have been demonstrated in patients with tubulo-interstitial disorders. Present study aimed to assess the clinical significance of fractional excretion of magnesium (FEMg) for the prediction of interstitial disorders.

**Methods:** Adult patients of 94 cases with various renal diseases, including glomerulonephritis (41.5%), interstitial nephritis (23.4%), hypertensive nephrosclerosis (22.3%), diabetic nephropathy (6.4%), lupus nephritis (5.3%) and others, were enrolled and assessed cross-sectionally.

**Results:** Stratified analysis based on NAG-to-Cr ratio (NCR), which is a parameter for organic damage of proximal tubule cells, showed that high NCR group demonstrated significantly high FEMg although beta-2 microglobulin-to-Cr ratio (MCR) were not different. Univariate analysis using NCR as a predictor variate demonstrated that FEMg showed significant correlation with NCR ($R=0.596$, $p=0.0316$) but other parameters did not. Multivariate regression analysis confirmed the significance of FEMg as a effective predictor of NCR. FEMg also showed significant correlation with estimated GFR but NCR did not. Additionally, the correlation of FEMg with NCR was independently analyzed in patients with glomerulonephritis (G: glomerulus-predominant disorder, n=38), nephrosclerosis (N: disorders with both glomerulus and interstitium, n=21) and tubulo-interstitial nephritis (T: interstitium-predominant disorders, n=22), and FEMg demonstrated significant correlation with NCR in N ($R= 0.839$, $p<0.001$) and T ($R=0.743$, $p=0.002$) but not in G.

**Conclusions:** FEMg was only parameter showing significant correlation with both NCR and estimated GFR in all patients. In addition, clinical reliability of FEMg was more sensitive in patients with interstitium-predominant renal disorders. The present study might indicate that FEMg is a beneficial parameter to predict the severity of interstitial impairment.

**SA-PO751**

**Evaluation of Various Dendritic Cell Markers in Renal Biopsy Specimens of 48 Patients with Various Renal Diseases**

Hanako Takechi,[1] Takashi Oda,[1] Kojiro Yamamoto,[1] Naoki Oshima,[1] Yutaka Sakurai,[2] Hiroo Kumagai.[1] *[1]Dept of Nephrology and Endocrinology, National Defense Medical College, Tokorozawa, Saitama, Japan; [2]Dept of Preventive Medicine and Public Health, National Defense Medical College, Tokorozawa, Saitama, Japan.*

**Background:** Dendritic cells (DCs) are the most potent antigen-presenting cells, and their participation in the pathogenesis of glomerulonephritis has been suggested. We therefore investigated subtypes of DCs in kidneys of patients with various renal diseases.

**Methods:** We examined renal biopsy specimens from fifty-six patients with renal diseases or renal specimens of normal controls; 7 with ANCA-associated vasculitis (AAV), 9 with IgA nephropathy (IgAN), 5 with minimal change nephrotic syndrome (MCNS), 5 with focal segmental glomerulosclerosis (FSGS), 10 with membranous nephropathy (MN), 5 with membranoproliferative glomerulonephritis (MPGN), 8 with tubulointerstitial nephritis (TIN) and 8 normal control kidneys. Five distinct markers of DCs (CD1c, CD209, CD208, CD1a and CD303) and T cell subtypes (CD4, CD8) were analyzed immunohistochemically. The clinical data and histological features of renal biopsy were statistically evaluated for possible relation with immunostaining results.

**Results:** Among the five markers of DCs, three markers, i.e., CD1c (a marker of conventional DCs), CD209 (DC-specific ICAM3-grabbing nonintegrin; DC-SIGN, a marker of monocyte-derived DCs) and CD208 (DC-lysosome-associated membrane glycoprotein; DC-LAMP, a marker of interdigitating DCs), positive cells significantly increased in interstitium of the kidneys of AAV and TIN. The numbers of these interstitial DCs per area correlated with the number of interstitial CD4 positive cells and the grade of tubulointerstitial injury. Generally, DCs are rarely found in the glomeruli of most renal diseases, however, a few CD208+ DCs were observed in glomeruli in the patients with AAV.

**Conclusions:** These observations suggest that interstitial myeloid DCs may interact with CD4+ T cells and may induce tubulointerstitial injury in the patients of AAV and TIN. In addition, CD208+ DCs may induce inflammation not only in interstitium but also in glomeruli of patients with AAV.

**SA-PO752**

**The Protective Effect of Substance H in Chlorhexidine Gluconate-Induced Peritoneal Fibrosis Animal Model**

Kyung Don Ju,[3] Eun Kyoung Shin,[3] Seungmi Lee,[1] Miseon Park,[1] Hyo-jin Kim,[1] Tai Yeon Koo,[2] Curie Ahn,[1] Kook-Hwan Oh.[1] *[1]Div of Nephology, Dept of Internal Medicine, Seoul National Univ Hospital, Republic of Korea; [2]Transplantation Center, Seoul National Univ Hospital, Republic of Korea; [3]Biomedical Research Institute, Seoul National Univ Hospital, Republic of Korea.*

**Background:** Peritoneal fibrosis (PF) is a complication of long-term peritoneal dialysis. Inhibitory action of adenosine-monophosphate-activated protein kinase (AMPK) on the mTOR and smad-3 system has been suggested. Substance H is a novel and potent AMPK activator. The present study was implemented to investigate the protective effect of substance H against the development and progression of peritoneal fibrosis in an in-vivo and in-vitro models.

**Methods:** Peritoneal fibrosis rat were prepared by injecting 0.1 % chlorhexidine gluconate (CG) intraperitoneally. CG-treated rats without substance H treatment and vehicle-treated rats were used for control. Rat peritoneal mesothelial cells (RPMC) cultured primarily from wistar male rat using 0.25 % trypsin-EDTA and were treated in high glucose (HG, 30 mM) and normal glucose (NG, 5 mM) conditions.

**Results:** Substance H-treated peritoneal fibrosis rats exhibited marked decrease of peritoneal thickness, and cocoon formation compared with CG-treated control rat. The mRNA expression levels of Akt, mTOR and smad-3 in the peritoneal membrane were also ameliorated. From RPMC experiment, HG increased mRNA and protein expressions of Akt, mTOR and smad-3 in a time dependent manner. Substance H decreased the mRNA and protein levels of Akt, mTOR, smad-3 in a dose dependent manner.

**Conclusions:** Our findings suggest that substance H protects against peritoneal membrane fibrosis in the CG-induced peritoneal fibrosis model.

**SA-PO753**

**Renal Protective Effects of DPPIV Inhibition (LC15-0444) on Experimental UUO Model**

Hye Sook Min,[1] Mi Jin Lee,[1] Young Sun Kang,[1] Jin Joo Cha,[1] Young Youl Hyun,[2] Ji Eun Lee,[3] Hyunwook Kim,[3] Jung Eun Kim,[1] Hye Kyoung Song,[1] Mihwa Lee,[1] Jee-young Han,[4] Dae R. Cha.[1] *[1]Nephrology, Korea Univ Ansan Hospital, Ansan, Kyunggido, Republic of Korea; [2]Nephrology, Sungkyunkwan Univ Kangbuk Samsung Hospital, Seoul, Republic of Korea; [3]Nephrology, Wonkwang Univ Sanbon Hospital, Gunpo, Republic of Korea; [4]Pathology, Inha Univ Hospital, Incheon, Republic of Korea.*

**Background:** DPP IV is known to modulate substrates involved in metabolism, glucose regulation, inflammation, cell migration, and cell differentiation. However, the roles of DPP4 and its inhibitors are not fully understood in the renal setting. Therefore, we investigated the role of DPP4 inhibitor in experimental renal fibrosis model.

**Methods:** 8-week-old C57/BL6 mice were divided into 3 groups : I) sham II) vehicle + UUO, III) DPP4 inhibitor (LC15-0444) + UUO group. After inducing UUO C57Bl/6J mice were placed on a normal chow diet with DPP4 inhibitor (LC15-0444, 150mg/kg/day) as a food admix for 2 weeks.

**Results:** Interestingly, DPPIV activity was significantly increased in the obstructed kidney, which was ameliorated by DPPIV inhibition. Administration of LC15-0444 resulted in a significant decrease of microalbuminuria and proteinuria. However, there were no significantly changes of DPP4 activities in the heart and plasma. Gene expressions of MCP-1, PAI-1, Type IV collegen and TGF beta 1 in kidney were significantly decreased in DDP4 inhibitor (LC15-0444) group. In addition, Urinary excretion of 8-isoprostane was markedly increased in UUO, and DPPIV inhibition significantly decreased urinary excretion of 8-isoprostane. In cultured proximal tubule cells, DPPIV expression was upregulated by angiotensin II stimulation, and suppressed by DPPIV inhibition.

**Conclusions:** Altogether, these results suggest that activation of DPP4 in kidney has a role in the progression of renal disease and DPPIV inhibition may provide renoprotective effects independent of its hypoglycemic effects. In our study showed that DDP4 inhibitors have anti-inflammatory and anti-fibrotic processes in the kidney.

**SA-PO754**

**HIV-Induced Podocyte Ang II Production Sustains Downregulation of Vitamin D Receptor (VDR) through Upregulation of Snail**

Nirupama Chandel, Tejinder Singh, Noopur Goel, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

**Background:** Ang II plays an important role in the development and progression of HIV-associated nephropathy (HIVAN). Recently, we reported that HIV enhances the activation of renin angiotensin system (RAS) in podocytes through down regulation of VDR (Am J Physiol, 2013). We hypothesize that HIV may be sustaining podocyte Ang II production through up regulation of Snail.

**Methods:** Protein blots were prepared form renal tissues of age and sex matched control and HIV transgenic mice (Tg26; n=4) and probed for TGF-β, Snail, and VDR; the same blots were reprobed for actin. Renal tissue lysates of control and Tg26 mice were also assayed for their Ang II content by ELISA. Conditionally immortalized human podocytes (CIHP) were transduced with either empty vector (EV/CIHP) or NL4-3 construct (HIV/CIHP). Protein blots of cellular lysates EV/CIHPs and HIV/CIHPs were probed for VDR, renin, and TGF-β. Ang II content was measured in cellular lysates of EV/CIHP and HIV/CIHPs by ELISA. To determine the contribution of Ang II, EV/CIHPs and HIV/CIHPs were incubated in media containing either buffer or losartan ($10^{-7}$M, an Ang II blocker) for 24h (n=3). Subsequently, protein blots were probed for TGF-β, Snail and actin. To establish cause and effect relationship, podocytes transfected with Snail plasmid (Snail/CIHP) were evaluated for the expression of Snail, VDR and actin. To further confirm link between Snail and VDR, ChiP assay was carried out.

**Results:** Renal tissues of Tg26 mice displayed increased Ang II content and enhanced expression of TGF-β and Snail, but down regulation of VDR. HIV/CIHPs displayed down regulation of VDR but up regulation of renin; moreover, HIV/CIHPs showed enhanced generation of Ang II when compared to EV/CIHPs. HIV/CIHP also exhibited enhanced expression of TGF-β and Snail. Snail/CIHP also exhibited down regulation of VDR. Interestingly, losartan not only attenuated HIV-induced podocyte expression of TGF-β and Snail but also upregulated VDR.

**Conclusions:** HIV sustains generation of Ang II through down regulation of podocyte VDR and upregulation of Snail.

*Funding:* NIDDK Support

**SA-PO755**

**Hedgehog Pathway Plays a Vital Role in HIV-Induced Podocyte Epithelial-Mesenchymal Transition (EMT)**

Xiqian Lan, Kang Cheng, Partab Rai, Nirupama Chandel, Andrei Plagov, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Medical School, New York, NY.*

**Background:** HIV-associated nephropathy (HIVAN) is characterized by heavy proteinuria, progressive renal insufficiency, and distinct morphological changes in the form of collapsing glomerulopathy, microcystic dilatation of tubules, and tubulointersititial fibrosis. HIV-induced EMT and proliferation of renal cells are important involved mechanisms contributing to the development of HIVAN phenotype. We hypothesized the role of hedgehog pathway in the HIV-induced kidney cell EMT and associated fibrosis.

**Methods:** Protein blots were prepared and RNAs were extracted from renal tissues of 4 weeks old control and HIVAN (Tg26) mice (n=4) and probed for molecules involved in hedgehog pathway, including sonic hedgehog (SHH), PTCH, gli1, and gli2. For *in vitro* studies, human podocytes (HP) were transduced with either empty vector or HIV (NL4-3) and treated with either buffer or Grant58 (an inhibitor of gli1 transcription) for 48 hours. Subsequently, protein blots of EV/HP and HIV/HPs were probed for molecules involved in hedgehog, EMT (α-SMA, vimentin, FSP1,and Snail), and proliferative pathways. Additionally, effect of hedge pathway was studied in modulation of podocyte monolayer permeability by utilizing albumin flux assay.

**Results:** Western blot and real time PCR analysis showed enhanced expressions of SHH, PTCH, gli1, and gli2 in renal tissues of Tg26 mice. In vitro studies, HIV not only activated the hedgehog pathway but also enhanced the expression of EMT and proliferation markers in podocytes. On the other hand, the blockage of hedgehog pathway with Gant58, decreased expression of HIV-induced kidney cell EMT markers. Albumin flux assay showed that HIV infection increased podocyte monolyer permeability, which could be partially attenuated by Gant58.

**Conclusions:** These results indicate that hedgehog pathway plays a vital role in the development of HIVAN; additionally, it provides insight into a new target for HIVAN therapeutic strategy.

*Funding:* NIDDK Support

## SA-PO756

**The Role of MicroRNAs in Podocyte Differentiation and Injury**
Christopher P. Carrington,[1] Robert H. Jenkins,[1] Moin Saleem,[2] Timothy Bowen,[1] Donald Fraser.[1] *[1]Institute of Molecular and Experimental Medicine, Cardiff Univ, Cardiff, United Kingdom; [2]Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom.*

**Background:** Wholesale deletion of microRNAs (miRs) within murine podocytes is associated with proteinuria and progressive glomerulosclerosis. However little is known about the role of specific miRs in the maintenance of human podocyte phenotype.

**Methods:** Using a laser capture micro-dissection (LCM) based approach we have isolated and array profiled (Taqman Low Density Array (TLDA)) the miR expression patterns in glomeruli from 8 nephrotic patients with idiopathic membranous nephropathy (IMN). We then performed a miR array (Torey 3D Gene microarray) on conditionally immortalized podocytes stimulated with TGFβ. Using this approach we identified several miRs that were highly expressed in glomeruli of patients with IMN and altered with TGFβ stimulation.

**Results:** TGFβ treatment was associated with widespread and significant changes in podocyte phenotype including loss of podocyte specific markers, up-regulation of matrix associated genes and increased motility as assessed by scratch wound assay consistent with de-differentiation. MicroRNA array profiling on podocytes treated with 1.0ng/ml TGFβ for 24 hours revealed 20 microRNAs that were significantly down regulated with a de-differentiated phenotype, of these 2 miRs (miR 30a-5p and miR 26a) were also highly expressed in glomeruli from patients with IMN.

The expression of both miRs was confirmed by qPCR to be up regulated with the acquisition of a differentiated podocyte phenotype and down regulated in response to TGFβ induced de-differentiation. *In silico* analysis of these miRs revealed putative targets associated within the control of the actin cytoskeleton specifically the activation/ inhibition of the Rho GTPases, RhoA, Rac1 and cdc42.

**Conclusions:** MiRs 30a-5p and 26a are down regulated during podocyte de-differentiation and are also highly expressed in the glomeruli of patients with nephrotic syndrome caused by IMN. These microRNAs are predicted to target elements of the Rho GTPase signaling cascade controlling podocyte cytoskeleton rearrangement implicated in the development of podocyte foot process fusion and proteinuria.

## SA-PO757

**2-Photon Microscopy Reveals Stationary Podocytes in Living Zebrafish Larvae**
Nicole Endlich,[1] Ole Simon,[1] Ahmed Kotb,[1] Eric Schordan,[1] Henny Wegner,[1] Marcus J. Moeller,[2] Achim Goepferich,[3] Elisabeth Rumpel,[1] Karlhans Endlich.[1] *[1]Anatomy and Cell Biology, Univ Medicine Greifswald, Greifswald, Germany; [2]Internal Medicine, Div of Nephrology and Immunology, RWTH Aachen Univ Hospital, Aachen, Germany; [3]Pharmaceutical Technology, Univ of Regensburg, Regensburg, Germany.*

**Background:** Podocytes are an essential component of the glomerular filtration barrier and cover the outer aspect of glomerular capillaries. They form a complex actin-based cytoskeleton in vivo and show a prominent motility in vitro. Since a long time it has been speculated whether podocytes are stationary or mobile in vivo.

**Methods:** To address this question we performed two-photon microscopy (2PM) of the pronephros of translucent zebrafish larvae (casper) expressing eGFP specifically in podocytes (wt1a:eGFP larvae) over extended periods of time.

**Results:** By intravital 2PM, podocyte cell bodies as well as the interdigitating branching pattern of major processes could be resolved in zebrafish larvae at 5-7 dpf (days post fertilization) with a resolution of about 1 µm in the xy-plane. Time-lapse imaging demonstrated that podocytes neither migrated nor changed their branching pattern of major processes over a time period of up to 18 h. Podocyte motility was neither detected by recording at low rates (2-5 images per h) nor at high rates (up to 5 images per s). By contrast, weakly GFP-positive cells close to the pronephric glomerulus that were presumably of neural crest origin exhibited vigorous motility in 2PM time-lapse recordings.

**Conclusions:** In summary, we have generated a translucent zebrafish with fluorescently labeled podocytes for intravital 2PM revealing that podocytes are stationary cells in the intact glomerulus.

*Funding:* Government Support - Non-U.S.

## SA-PO758

**Increased Glomerular Water Permeability in Human and Streptozotocin-Induced Diabetes Mellitus**
Amy Russell, Yan Qiu, Kenton Arkill, Andy Salmon. *Microvascular Research Laboratories, Physiology & Pharmacology, Univ of Bristol, Bristol, United Kingdom.*

**Background:** Diabetic nephropathy patients show endothelial dysfunction, albuminuria and increased glomerular filtration rate. We hypothesised that glomeruli from humans and animals with diabetes would have increased water permeability.

**Methods:** Male Sprague Dawley rats were injected intravenously with streptozotocin (45 mg/kg) or citrate buffer alone. 7 days later, streptozotocin-injected rats were hyperglycaemic (before: 6.0±0.13 mmol/L; after: 27±1.7 mmol/L, p<0.0001) and proteinuric (sham: 11.6±0.9 mg/day; diabetic: 25.3±6.7 mg/day, p = 0.005). Kidneys were perfused via the abdominal aorta with 4% bovine serum albumin (BSA) under anaesthesia (sodium pentobarbital). Glomeruli were isolated via a sieving procedure. Human glomeruli were isolated from kidneys not suitable for transplantation, from donors with and without diabetes. Glomeruli were aspirated onto a micropipette and equilibriated in 1% BSA. The perifusate was exchanged to 8% BSA causing fluid efflux. The resultant glomerular volume decrease was used to calculate volume-corrected glomerular volume permeability ($L_pA/V$i: $min^{-1}$ $mmHg^{-1}$).

**Results:** $L_pA/V$i was significantly elevated in diabetic animals (sham: 1.00±0.09 (90/9); streptozotocin: 1.54±0.16(74/9)) {mean±sem (glomeruli/animals)} p<0.01). Human glomerular $L_pA/V$i was also increased in diabetes (healthy: 1.0±0.1 (25/3; diabetic donors {2.3±0.4 (16/3)} (p<0.001).

**Conclusions:** These are the first measurements of single glomerular permeability in diabetic humans. Glomerular $L_pA/V$i is elevated in early diabetes in humans and rats.

## SA-PO759

**Podocyte Hypertrophy Is Regulated by Ubiquitin C-Terminal Hydrolase-L1 Induced Cytoplasmic $p27^{Kip1}$ Accumulation in Rat Membranous Nephropathy**
Catherine Meyer-Schwesinger,[1] Tobias N. Meyer,[2] Maja Lindenmeyer,[3] Thorsten Wiech,[4] Clemens D. Cohen,[3] Rolf A. Stahl.[1] *[1]Nephrology, UKE, Germany; [2]Nephrology, AKB, Germany; [3]Nephrology, UZH, Switzerland; [4]Renal Pathology, UKE, Germany.*

**Background:** Podocytes are terminally differentiated and react to injury with hypertrophy and loss of barrier function. $p27^{Kip1}$ is a key regulator of cellular hypertrophy through induction of $G_1$ arrest. Subcellular localization of $p27^{Kip1}$ in tumours is influenced by ubiquitin C-terminal hydrolase L1 (UCH-L1), a key neuronal protease in the ubiquitin-proteasome pathway. We recently demonstrated *de novo* UCH-L1 expression and activation in podocytes of patients with membranous nephropathy (MGN) and in rodent models of MGN. Here, we investigated the role of UCH-L1 in podocyte $p27^{Kip1}$ homeostasis and hypertrophy.

**Methods:** UCH-L1 and $p27^{Kip1}$ co-localization studies were performed in human MGN and in the rat model of MGN, the passive Heymann nephritis (PHN). The effects of UCH-L1 overexpression, knockdown and inhibition of hydrolase function on podocyte hypertrophy and $p27^{Kip1}$ homeostasis were evaluated in cultured podocytes and in PHN.

**Results:** In human and rodent MGN, podocyte hypertrophy correlated with up-regulation of UCH-L1 and cytoplasmic $p27^{Kip1}$ protein in podocytes. In cultured podocytes, over-expression of UCH-L1 (1) increased levels of $p27^{Kip1}$ and $p27^{Kip1}$ phosphorylation at $thr^{198}$, which serves as a nuclear export signal, and (2) decreased poly-ubiquitination and proteasomal degradation of $p27^{Kip1}$. These altered post-translational modifications of $p27^{Kip1}$ resulted in cytoplasmic accumulation of $p27^{Kip1}$ protein in podocytes *in vitro* and *vivo*. UCH-L1 activity in podocytes decreased the percentage of cells in $G_1$ arrest, increased cellular turnover, hypertrophy, migration and cytoskeletal rearrangement, which are associated with known oncogenic functions of cytoplasmic $p27^{Kip1}$ in cancer. Inhibition of UCH-L1 hydrolase activity in rat MGN, and knockdown or inhibition of UCH-L1 *in vitro*, decreased cytoplasmic $p27^{Kip1}$ protein levels and attenuated podocyte hypertrophy, loss and proteinuria.

**Conclusions:** We demonstrate that podocyte hypertrophy and loss was induced by UCH-L1 through stabilization of $p27^{Kip1}$ in the cytoplasm of podocytes.

*Funding:* Government Support - Non-U.S.

## SA-PO760

**Regulation of Apolipoprotein-1L Splicing by Wilms Tumor-1 Protein**
Hidefumi Wakashiu, Jeffrey B. Kopp. *NIDDK, NIH, Bethesda, MD.*

**Background:** Apolipoprotein L1 (*APOL1)* genetic variants are associated with increased glomerular disease risk. ApoL1 has several RNA splice variants, producing distinct protein isoforms: APOL1-A is encoded by exons 1, 3-7; APOL1-B is encoded by exon 1-7; and APOL1-C is encoded by exons 1, 3, 5-7. Wilms tumor 1 (WT1) plays crucial roles in kidney development and podocyte differentiation.

**Methods:** We raised a rabbit antiserum against APOL1-B exon 2 peptide, which is specific for this isoform. We examined the expression of *APOL1* splicing variants, using by TA cloning,, RT-PCR and WT1 knockdown, and subcellular localization, using immunofluorescence (IF), Flag tagged vector transfection, in cultured human podocytes , WT1 inducible human osteosarcoma cells and human kidney.

**Results:** Seven APOL1 splicing variants (classed as V1-V4) were cloned from immortalized human podocytes. These include three splicing variants that contain exon2: APOL1-V2-1, V2-2 and V2-3, which encode ApoL1-B-1, B-2 and B-3 respectively. APOL1-V2-1 consists of exon 1-7. V2-2 is distinguished by having a longer exon 3. V2-3 lacks exon4, which contains a signal sequence. Human kidney expressed 5 APOL1 splicing variants. Using IF, we found that ApoL1-B was expressed by podocytes in normal human kidney and was decreased in FSGS kidney. Transfected C-terminal Flag-tagged ApoL1-B-3 was detected by both ApoL1-B ab and Flag ab, and co-localized with F-actin in immortalized human podocytes. ApoL1-B-1 and B-2 were not detected by ApoL1-B ab but were detected by Flag ab. In WT1-expressing UB27 human osteosarcoma cells, the APOL1-V2-3 RNA level was increased compared to control cells lacking WT1. WT1 expression dramatically increased ApoL1-B protein expression, assessed by WB and IF. Further, WT1 knockdown reduced the expression of APOL1-V2-3 in immortalized human podocytes while other APOL1 variant RNAs were reduced or unchanged.

**Conclusions:** ApoL1-B variants were expressed in the human podocytes. WT1 regulates ApoL1 splicing variants, and selectively increases expression of ApoL1B protein and APOL1-V2-3. A better understanding of the complexity of APOL1 RNA and protein isoforms will be required to define the role of APOL1 in podocyte function.

*Funding:* NIDDK Support

## SA-PO761

**Podocyte Slit-Diaphragm Protein Nephrin Associates with the Actin Cytoskeleton through Interacting with IQGAP1** Yipeng Liu,[1] Wei Liang,[1] Qian Yang,[1] Pravin C. Singhal,[2] Guohua Ding.[1] *[1]Div of Nephrology, Renmin Hospital of Wuhan Univ, Wuhan, Hubei, China; [2]Medicine, North Shore-Long Island Jewish Health System, Manhasset, NY.*

**Background:** Nephrin plays a crucial role in maintaining the structural and functional integrity of the glomerular filtration barrier. Nephrin is reported to contribute to dynamics of actin cytoskeleton in podocyte through several adaptor and effector proteins. IQGAP1 is a newfound scaffolding protein that is expressed in the slit-diaphragm (SD) and contains a F-actin biding domain. We evaluated the role of IQGAP1 in linking nephrin to the actin cytoskeleton.

**Methods:** Kidneys were harvested from male Sprague-Dawley rats (n=6) for isolation of glomeruli and immunofluorescence analysis. In vitro, mouse podocytes were cultured. The distribution and interaction of nephrin and IQGAP1 was detected by fluorescent microscopy and co-immunoprecipitation. Differential detergent extraction was used to evaluate the connection mode of nephrin and IQGAP1 to F-actin in the presence or absence of potassium iodide in vivo or cytochalasin D in vitro. HEK-293T cells were co-transfected with nephrin plasmid and IQGAP1plasmid containing full-length or domains (CHD, IQGAP repeat, WW, IQ, GRD and RasGAP-C) to assess the specific domain of IQGAP1 interacting with nephrin.

**Results:** Neprin and IQGAP1 are co-localized in the cellular membrane of cultured podocytes and along the capillary loops of glomeruli in a linear model. Analysis of differential detergent extraction indicated that nephrin co-precipitated with actin filaments and IQGAP1.Co-precipitation between nephrin and actin was prevented by potassium iodide or cytochalasin D which are able to depolymerize F-actin. However, the complexes of nephrin and IQGAP1 were not disrupted by the actin depolymerization. Co-transfection analysis in HEK-293T cell demonstrated that the IQ domain of IQGAP1 is the target module interacting with nephrin.

**Conclusions:** IQGAP1, as an adaptor protein, mediates the link of nephrin to actin cytoskeleton in podocyte.

*Funding:* Government Support - Non-U.S.

## SA-PO762

**PDZ and LIM Domain Protein 2 Forms a Protein Complex with Podocin and Nephrin at the Slit-Diaphragm** Liliana Garmendia,[1] Mariana Quiroz-munoz,[2] Stephanie Carol Acuña,[1] Eduardo A. Machuca.[1] *[1]Centro de Investigaciones Médicas, P.Universidad Católica de Chile, Chile; [2]Escuela de Medicina, P.Universidad Católica de Chile, Chile.*

**Background:** Nephrin and Podocin are main structural and functional components of the slit-diaphragm (SD). Inherited defects affecting Podocin and Nephrin encoding genes are the leading causes of congenital and early-onset nephrotic syndrome, respectively. Thoughtful knowledge of the SD structure and its signaling pathways is essential to understand glomerular filtration. Furthermore, understanding SD structure may lead to the discovery of targets to modulate its function in diseases known to be cause by disruption of the glomerular filtration barrier.

**Methods:** We used a proteomic approach to identify novel SD proteins. We isolated murine glomeruli, then, glomerular protein extracts were subjected to immunoprecipitation (IP) studies with anti-nephrin or anti-podocin antibodies coupled to Protein A-magnetic beads. Eluted proteins were analyzed by nano-LC/MS/MS. Mascot and Scaffold were used to validate MS/MS based peptide and protein identifications.

**Results:** We found novel podocyte actin-cytoskeleton components that interact with nephrin and podocin. One of them, PDZ and LIM domain protein 2 (Pdlim2), has been previously identified as a SD-related protein, although its interaction with nephrin or podocin has not been shown before. We validated Pdlim2 interaction with nephrin through *in vivo* and *in vitro* co-immunoprecipitation studies. Pdlim2 was shown to localize in glomerular capillaries by using double-staining immunofluorescence. In addition, we found that Pdlim2 partially co-localized with actin cytoskeleton components such as Synaptopodin, β -actin and Phalloidin. Using double-labeling electron microscopy we showed that Pdlim2 localizes in close proximity with Nephrin in foot-processes.

**Conclusions:** Our results suggest that Pdlim2 interacts with Nephrin at the SD. It has been shown that Pdlim2 is adapter protein located at the actin cytoskeleton that promotes cell attachment, and which may potentially interact with alpha-actinins, FLNA and MYH9. Further functional analyses are desirable to elucidate the role of Pdlim2 in the maintenance of the glomerular filtration barrier.

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## SA-PO763

**Formin-Binding Protein 1-Like Is a Novel Interacting Protein at the Glomerular Slit-Diaphragm** Mariana Quiroz-Munoz,[1] Liliana Garmendia,[2] Stephanie Carol Acuña,[2] Eduardo A. Machuca.[2] *[1]Escuela de Medicina, P. Universidad Católica de Chile, Chile; [2]Centro de Investigaciones Médicas, P. Universidad Católica de Chile, Chile.*

**Background:** The preservation of glomerular permselectivity relies critically on the podocyte and the architecture of its actin cytoskeleton. Podocytes intertwining to cover the capillary loops with interdigitating foot processes, which are connected through actin-based cell-cell adhesion proteins, forming a structure called slit diaphragm (SD). Nephrin is the major structural component of the SD, and is know to interact with podocin and CD2AP among others. Little is known about other interacting proteins within this complex which may connect the SD to the actin-cytoskeleton.

**Methods:** We isolated murine glomeruli by a modified sieving method. Then, protein lysates were immunoprecipitated with anti-podocin antibodies. Eluted proteins subjected to 1D-SDS PAGE, gels were stained with Coomassie blue and each lane was excised into ten segments. Gel bands were digested with trypsin and then they were analyzed by nano-LC/MS/MS.

**Results:** We identified a novel podocyte protein known as Formin-binding protein 1-like (Fnbp1l). We performed western blot analysis of protein lysates showing that Fnbp1l is ubiquitously expressed in mice. Using RT-PCR, we assessed whether a particular isoform of Fnbp1l isoform was express in kidney, finding that mRNA isoform 2 was predominantly expressed in kidney. We also performed immunofluorescence studies of frozen kidney tissue to locate Fnbp1l, resulting in Fnbp1l localization mainly in podocyte. Fnbp1l strongly colocalized with synaptopodin and partially with β-actin and phalloidin. Immuno-gold electron-microscopy showed partial Fnbp1l localization at the SD. Immunoprecipitation assays showed that Podocin co-immunoprecipitated with Fnbp1l.

**Conclusions:** We have shown that Fnbp1l interacts with Podocin at the SD. It has been previously shown that the function of Fnbp1l probably involves the coordination of actin assembly by promoting CDC42-induced actin polymerization. Thus, Fnbp1l-Podocin complex may link the SD to actin-cytoskeleton. Further work is necessary to elucidate the role of Fnbp1l in the maintenance of the glomerular filtration barrier.

## SA-PO764

**Mechanism of CLIC5A-Mediated Ezrin Phosphorylation** Abass Almomany, Laiji Li, Barbara J. Ballermann. *Medicine, Univ of Alberta, Edmonton, Canada.*

**Background:** CLIC5A deficiency in mice potentiates hypertension- and adriamyin-induced glomerular injury. CLIC5A is highly and uniquely expressed at the apical plasma membrane (PM) of podocyte foot processes as part of the Ezrin-Podocalyxin complex (*Am. J. Physiol. 298: F1492, 2010*). Phosphorylated ezrin ($^{P}$Ezrin) links podocalyxin to cortical actin. In podocytes of CLIC5A deficient mice, $^{P}$Ezrin abundance is reduced. This study explored the molecular mechanisms by which CLIC5A regulates ezrin function.

**Methods:** CLIC5A cDNA or vector (control) were introduced into Cos-7 cells, which express ezrin but not CLIC5A at baseline. Protein abundance was evaluated by immunoblot (IB) and protein localization by immunofluorescence (IF) confocal microscopy (CF). Since ezrin phosphorylation requires its binding to phosphatidylinositol 4,5-bisphosphate (PIP2), the distribution of the PIP2 reporter RFP-PH-PLC was evaluated with live-cell CF.

**Results:** CLIC5A expression induced surface ruffles (Scanning EM) and actin polymerization. The ratio of $^{P}$Ezrin : Total Ezrin was greater in Cos-7 cells expressing CLIC5A than in control cells (0.84±0.06 vs. 0.38±0.06, mean±SE, n=3, $p<0.01$), and $^{P}$Ezrin redistributed to the cytoskeleton. Ezrin phosphorylation was protein kinase C (PKC) dependent, but CLIC5A did not alter PKC activity, nor did CLIC5A block $^{P}$Ezrin de-phosporylation. The ratio of apical PM : cytosolic RFP-PH-PLC was much higher in CLIC5A expressing cells than in control cells (3.46±0.62, vs. 1.56±0.06; mean±SE, n=8, $p<0.01$), indicating increased apical PM PIP2 content. Activation of phospholipase C (with m-3M3FBS), which depletes PIP2, blocked CLIC5A-dependent ezrin phosphorylation. CLIC5A strongly co-localized with the PIP2 reporter and PIP5K1α (which generates PIP2) in discrete apical PM patches. GST-CLIC5A but not GST pulled down PIP5K1α from cell lysates.

**Conclusions:** Hence, CLIC5A may activate and/or recruit PIP5Kinase, resulting in localized PIP2 generation, ezrin phosphorylation and cell-surface re-organization. We postulate that in podocytes, where it is so highly expressed, CLIC5A maintains high, apical PIP2 and $^{P}$Ezrin concentrations, in turn contributing to the unique podocyte architecture and podocyte-dependent integrity of glomerular capillaries.

*Funding:* Government Support - Non-U.S.

## SA-PO765

**Caveolae May Enable Albumin to Enter Human Renal Glomerular Endothelial Cells** Takahito Moriyama, Yasuko Oshima, Kayu Tanaka, Chihiro Iwasaki, Ken Tsuchiya, Kosaku Nitta. *Medicine, Kidney Center, Tokyo, Shinjyuku-ku, Japan.*

**Background:** The pathophysiology of albuminuria remains unclear. We previously reported that caveolae on human renal glomerular endothelial cells (HRGECs) are increased in glomerular disease and correlate with the degree of albuminuria, suggesting that the proliferation of caveolae on HRGECs contributes to albuminuria(J Clin Pathol 2011; 64: 504-9). To assess the mechanism by which caveolae contribute to albuminuria, we investigated whether albumin enters into HRGECs through these caveolae.

**Methods:** HRGECs were incubated with Alexa Fluor 488 labeled bovine serum albumin (BSA), followed by immunofluorescence localization with antibody to caveolin-1 (Cav-1), the main structural protein of caveolae, to assess time course study of albumin internalization and whether BSA colocalizes with Cav-1. HRGECs were also incubated with albumin and caveolae disrupting agents, such as methyl beta cyclodextrin (MBCD) and nystatin, to determine whether disrupting the caveolae interfered with albumin endocytosis into HRGECs by western blot and immunofluorescence analysis. Albumin internalization into HRGECs transfected with Cav-1 small interfe-ring RNA (SiRNA) was also analyzed.

**Results:** Labeled BSA was expressed soon after the start of incubation, increasing gradually from 5.81±0.98 $\mu m^2$/cell at 5 minutes to 82.9±13.6 $\mu m^2$/cell at 6 hours after incubation. The ratio of BSA co-localized with Cav-1 was 17.3±3.06% at 5 minutes, and it was increased to about 50 at 15 minutes and remained 50 to 65 % during 6 hours after incubation. Incubation of HRGECs with MBCD or nystatin significantly decreased the intracellular amount of albumin and Cav-1 expression, relative to normal HRGECs, as

shown by western blotting and immunofluorescence. Also albumin and Cav-1 expression in HRGECs transfected with Cav-1 SiRNA were significantly decreased in comparison to normal HRGECs.

**Conclusions:** These findings indicate that albumin enters HRGECs through the caveolae. Caveolae on HRGEC play an important role in the pathogenesis of albuminuria by providing a new pathway through which albumin can enter glomerular endothelial cells.

*Funding:* Government Support - Non-U.S.

## SA-PO766

**Qualitative Changes in Vascular Endothelial Growth Factor A Expression in Kidney Disease** Rosanne Jane Turner, Ingeborg M. Bajema, Michael Eikmans, Jan A. Bruijn, Hans J. Baelde. *Pathology, LUMC, Leiden, Netherlands.*

**Background:** The cytokine vascular endothelial growth factor A (VEGF-A) is a key regulator in the maintenance of the glomerular filtration barrier by promoting survival of podocytes and endothelial cells. There are seven proangiogenic VEGF-A splicing variants. Longer isoforms containing heparin and neurophilin binding sites, such as VEGF-A 165 and 189, have a more prolonged and more effective mode of action than shorter isoforms, such as VEGF-A 121. In various kidney diseases quantitative changes in VEGF-A expression play an important role. In this study, we set out to investigate the renal splicing pattern of VEGF-A in various kidney disease entities in humans and mice.

**Methods:** Kidney biopsies from patients with acute rejection after kidney transplantation, patients with diabetic nephropathy, and from healthy controls were collected. Also kidney samples from mice with graft versus host disease and controls were collected. Lung, lymph node, spleen and thymus tissue samples from healthy mice were obtained. The relative expression of each individual splice variant was measured by RT-PCR followed by quantitative fragment analysis with capillary electrophoresis.

**Results:** In the healthy human kidney VEGF-A 121 was the dominant isoform with a relative expression of 75.08%. VEGF-A 165 and 189 showed relative expression levels of 21.66% and 3.26%. The splicing pattern in diabetic nephropathy and in transplanted kidneys with acute rejection and in graft-versus-host disease did not differ from that in control kidneys. In rodent kidneys VEGF-A 164 was the dominant isoform with a relative expression of 48.84%. VEGF-A 120 and 188 showed relative a expression of 43.24% and 7.74%, respectively. Different splicing patterns of VEGF-A were detected in samples from different organs.

**Conclusions:** VEGF-A splicing in the glomeruli is different between humans and rodents. Although it has been described that there are quantitative changes of VEGF-A in transplanted kidneys with acute rejection and in diabetic nephropathy, the splicing pattern does not change in these disorders. Apparently quantitative rather than qualitative changes of VEGF-A mRNA levels play an important role in renal diseases.

## SA-PO767

**Molecular Mechanism of Steroid-Induced Thrombus Formation in Kidney Disease** Noritaka Kawada,[1] Toshiki Moriyama,[1] Harumi Kitamura,[2] Hiromi Rakugi,[2] Yoshitaka Isaka.[2] *[1]Osaka Univ, Health Care Center, Toyonaka, Osaka, Japan; [2]Dept of Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

**Background:** The use of exogenous steroid positively associates with the risk of thromboembolism (Johannesdottir-SA et. al. *JAMA Intern Med*. 2013; 1, 1-10). Plasminogen activator inhibitor-1 (PAI-1) inhibits plasminogen activator (tPA), plasmin and matrix metalloproteinase (MMP) and is known as an established risk marker for cardiovascular disease and thrombus formation. We tested the effects of exogenous steroid on the PAI-1 expression in cultured cells originated from rat kidney.

**Methods:** Rat primary cultured mesangial cells (rMSC), renal fibroblast cells (NRK49F), and renal tubular cells (NRK52E), were exposed to dexamethasone (Dex) and/or TGFβ. The mRNAs were quantitated and the collected cultured mediums were processed to test the induction of PAI-1 protein and MMP activity.

**Results:** rMSCs exposed to Dex (10-6M) or TGFb (2ng/ml), 6 hours for mRNA and 16 hours for protein, induced both mRNA and protein for PAI-1 (mRNA: Dex +130%, $p<0.01$. TGFβ +190%, $p<0.01$; n=6 each). At the same experiment, the mRNA for tPA were reduced only in Dex (Dex -36%, $p<0.01$. TGFβ -20%, n.s.; n=6 each). The rMSCs exposed to both Dex and TGFβ further induced PAI-1 protein and mRNA (PAI-1 mRNA: +640%, $p<0.001$. tPA mRNA: -40%, $p<0.01$; n=6). This was accompanied by the inhibition of MMP activity ($p<0.01$, n=6). The induction in PAI-1 and the reduction in tPA were also observed in NRK49F and 52E cells exposed to Dex and/or TGFβ. The effects of Dex and TGFβ on PAI-1 and tPA expression in rMSCs were blunted by enhanced cAMP production with adenylate cyclase activator (Forscolin) or β2 stimulant (Salbutamol).

**Conclusions:** Steroid may promote thrombus formation in kidney disease through the induction of PAI-1 and the inhibition of tPA and MMP activity. Enhanced cAMP production has potential to moderate these unwilling side effects.

## SA-PO768

**PDGF Launches Mesangial Cell Proliferation via a Positive Feed-Back Loop Involving Erk5 and Akt Downstream of PI 3 Kinase** Falguni Das,[1] Amit Bera,[1] Nandini Ghosh-choudhury,[2] Balakuntalam S. Kasinath,[1] Hanna E. Abboud,[1] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA.*

**Background:** PDGF-forced proliferation of mesangial cells is an ominous feature of mesangioproliferative glomerulonephritis. The mechanistic underpinning of MC proliferation is not clear. We probed the role of most recently identified MAPK, Erk5.

**Methods:** Glomerular mesangial cells (MCs) were used.

**Results:** PDGF increased activating phosphorylation of Erk5 concomitant with enhanced tyrosine phosphorylation of several proteins including PDGF receptor in a time-dependent manner. XMD-8-92, an inhibitor of Erk5, attenuated PDGF-stimulated Erk5 phosphorylation, DNA synthesis and MC proliferation in the absence of any effect on Erk1/2 phosphorylation. Similarly, expression of dominant negative (DN) Erk5 or siRNA-mediated downregulation of Erk5 significantly inhibited PDGF-induced DNA synthesis and proliferation. Inhibition of Erk5 markedly blocked cyclin D1 mRNA and protein expression induced by PDGF, resulting in inhibition of its target cyclin-dependent kinase 4 (CDK4) phosphorylation and its substrate pRb inactivating phosphorylation. Interestingly, exogenous expression of wild type cyclin D1 or CDK4 reversed DN Erk5- and siErk5-mediated suppression of DNA synthesis and proliferation of mesangial cells induced by PDGF. As we have shown previously that PI 3 kinase/Akt axis regulates PDGF-induced MC proliferation, we interrogated its role. Inhibition of PI 3 kinase significantly blocked Erk5 phosphorylation. Since Akt is a downstream target of PI 3 kinase, we examined the contribution of Erk5 in its activation. XMD-8-92, DN Erk5 and siErk5 inhibited PDGF-stimulated Akt phosphorylation. Interestingly, MK2206, a specific inhibitor of Akt kinase and DN Akt abrogated Erk5 phosphorylation by PDGF.

**Conclusions:** Together our results provide the first evidence demonstrating the presence of a micro-circuitry mechanism involving Erk5 and Akt to instruct cyclin D1/CDK4 to drive cell cycle progression and proliferation of MC in response to PDGF.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO769

**Vitamin D Receptor Participate in the High Glucose-Induced Epithelial-Mesenchymal Transition of Mouse Podocytes through Wnt/GSK-3β/β-Catenin Signaling Pathway** Guo Jia.[1,2] *[1]Nephrology, The First Affiliated Hospital of Zhengzhou Univ, Zhengzhou, Henan, China; [2]Key-Disciplines Laboratory Clinical-Medicine Henan, Zhengzhou, Henan, China.*

**Background:** Proteinuria, closely related with the disease development, is the main clinical symptoms of diabetic nephropathy. Previous studies and our previous results were all confirmed that epithelial-mesenchymal transition (EMT) of mouse podocyte cells was the major pathophysiological mechanism of proteinuria in diabetic nephropathy. It has been reported that the Wnt/GSK-3β/β-catenin signaling pathway involved in the EMT process of renal tubular epithelial cell induced by high glucose. Vitamin D receptor was reported involving in the EMT of renal tubular epithelial cells of end-stage renal diseases. And decreased expression of VDR could lead the expression of β-catenin increased. The purpose of this study is to testing whether VDR participate in the EMT process of mouse podocytes caused by high glucose through Wnt/GSK-3β/β-catenin pathway.

**Methods:** The expressions of key molecules in Wnt/GSK-3β/β-catenin signaling pathway, as well as VDR expression, were detected in mouse podocytes with high glucose treatment and the normal control group by western blot and RT-PCR. Co-localization of the key molecules of Wnt/GSK-3β/β-catenin signaling pathway and VDR were observed by Immunofluorescence.

**Results:** High glucose cultured mouse podocytes had obvious EMT phenomenon. The activity and expression of GSK-3β were significantly increased compared with the normal control group ($P<0.05$). The expressions of β-catenin and its downstream snail were also up-regulated companied with glucose concentration increasing ($P<0.05$), while the VDR expression declined. By using the immunofluorescence assay, β-catenin in high glucose cultured mouse podocyte was found translocation to nuclear, showing less co-localization with GSK-3β and more co-localization with the VDR compared with the normal group.

**Conclusions:** Decreased expression of VDR may be involved in the EMT process caused by high glucose of mouse podocytes through Wnt/GSK-3β/β-catenin signaling pathway.

*Funding:* Government Support - Non-U.S.

## SA-PO770

**The Role of Dynamin in the Development of Proteinuria** Ramzi Khalil,[1] Reshma Lalai,[1] Rosalie Bor,[1] Reinhold Kreutz,[2] Emile De Heer,[1] Jan A. Bruijn,[1] Hans J. Baelde.[1] *[1]Pathology, Leiden Univ Medical Center, Leiden, Netherlands; [2]Institute of Clinical Pharmacology and Toxicology, Charité - Univ Medicine, Berlin, Germany.*

**Background:** Dynamin has been established as a protein essential in membrane scission, vesicular transport and interaction with actin in cytoskeleton regulation. Rats with a podocyte-selective knock out of Dynamin showed proteinuria, renal failure and foot process effacement. In a previous microarray experiment, we identified dynamin mRNA as differentially expressed in spontaneously proteinuric Dahl SS rats. This study aims to investigate the role of dynamin in the time course of the development of proteinuria and the direct relationship between dynamin deficiency and loss of glomerular permselectivity.

**Methods:** The involvement of dynamin in the development of proteinuria was studied in a time course experiment. Dynamin mRNA expression and urinary albumin excretion (UAE) were measured at 2, 4, 6, 8 and 10 weeks of age in Dahl SS rats. Additionally, dynamin mRNA expression was investigated in microdissected glomeruli of patients with various proteinuric kidney diseases. To clarify the direct relationship between dynamin and loss of glomerular permselectivity, a functional assay using dextran tracers was performed in zebrafish embryos, microinjected with a morpholino construct targeting the zebrafish homologue of dynamin.

**Results:** In the time course experiment, Dahl SS rats started showing significant and increasing albuminuria from week 6. Dynamin mRNA upregulation was seen at 4 and 6 weeks of age. Moreover, a statistically significant increase in dynamin mRNA expression was found in glomeruli of lupus nephritis and minimal change disease patients. The functional assay of the morpholino injected zebrafish demonstrated a loss of glomerular permselectivity.

**Conclusions:** Strong evidence is shown for the role of dynamin in the time course of proteinuria development, as dynamin mRNA expression was upregulated 2 weeks before onset of albuminuria. Also, the direct relationship between loss of dynamin and loss of glomerular permselectivity is shown in the functional assay using zebrafish embryos. The results from the human biopsies further confirm these results.

## SA-PO771

**EphB-Receptors Mediate Nephrin Tyrosine Phosphorylation – Mechanism for Adhesion and Repulsion** Eva Koenigshausen, Nils Tim Haep, Magdalena Woznowski, Ivo Quack, Lars C. Rump, Lorenz Sellin. *Nephrology, Heinrich Heine Univ, Duesseldorf, Germany.*

**Background:** Proteinuria is a hallmark for glomerular disease and results from disruption of the glomerular filter with the glomerular slit diaphragm. The slit diaphragm's major component is nephrin which is endocytosed upon the binding to beta-arrestin controlled by tyrosine phosphorylation. Eph-receptor family kinases are the largest group of tyrosine kinases and mediate cell adhesion and repulsion. Eph-receptor kinases bind to their cell-membrane bound ligands - ephrines. Ephrinb1 and EphB4 have been localized at the slit diaphragm. The molecular function of the EphB1 and B2 receptors in podocytes is unknown.

**Methods:** Murine and human podocytes were differentiated. After cell lysis, qPCR was performed. Cells expressing nephrin, beta-arrestin2 and Nck1/2 were lysed and co-immunoprecipitation was performed. For phosphorylation experiments cells were serum starved. Tyrosin phospho-specific antibodies were used. Preclustering was performed by incubation of IgG-Fc or ephrinb1-Fc with anti-human IgG with a final concentration of 1µg/ml.

**Results:** EphB1, B2, ephrinb1 and b2 are expressed in murine and human podocytes. EphB1 and EphB2 interact with the extracellular domain of nephrin. Expression of EphB1 and B2 results in a strong phosphorylation on nephrin at the Nck1/2 binding sites Y1176/1193 and Y1217. Stimulation with the ligand ephrinb1 leads to a decrease of nephrin-Y-phosphorylation. Expression of EphB1 and B2 increases the interaction of nephrin with nck2 and reduces the binding of nephrin with beta-arrestin2. Expression of an EphB2-kinase inactive mutant abolishes the enhancement of nephrin-Y-phosphorylation demonstrating that the kinase function is crucial for nephrin. PP2, a src family kinases inhibitor, decreases nephrin-Y-phosphorylation. EphB receptor induced nephrin-Yphosphorylation is reproduced in murine podocytes.

**Conclusions:** Like in other cell types EphB receptors may mediate adhesion and repulsion at the glomerular slit via modulation of nephrin-Y-phosphorylation. The understanding of adhesion and repulsion at the slit could help to understand pathophysiology in glomerular disease and identify new therapeutic targets.

## SA-PO772

**Proteomic Analysis of Formalin-Fixed Paraffin-Embedded (FFPE) Glomerular Tissue Suggests Depletion of Glomerular Filtration Barrier Proteins in Two-Kidney One-Clip (2K1C) Hypertensive Rats** Kenneth Finne,[1] Heidrun Vethe,[1] Trude Skogstrand,[1,2] Olav Tenstad,[2] Sabine Leh,[1,3] Rolf K. Reed,[2] Bjorn Egil Vikse.[1,4,5] *[1]Dept of Clinical Medicine, Univ of Bergen, Bergen, Norway; [2]Dept of Biomedicine, Univ of Bergen, Bergen, Norway; [3]Dept of Pathology, Haukeland Univ Hospital, Bergen, Norway; [4]Dept of Medicine, Haukeland Univ Hospital, Bergen, Norway; [5]Dept of Medicine, Haugesund Hospital, Haugesund, Norway.*

**Background:** It is well known that hypertension may cause glomerular damage, but the mechanisms are still incompletely understood. In the present study we investigated changes in the proteome of the glomerular filtration barrier in the non-clipped kidney of 2K1C hypertensive rats.

**Methods:** 2K1C hypertension was induced with a 0.2 mm silver clip in 6-weeks-old Wistar rats (n=6) that was sacrificed 23-weeks later and compared to age-matched sham operated controls (n=6). After sacrifice, the non-clipped kidney was fixed in formalin and embedded in paraffin. Using this tissue, we microdissected 100 glomeruli cross-sections that were trypsin digested and analyzed in a label-free manner using LC-MS/MS (Orbitrap system).

**Results:** While podocyte cytoskeletal proteins such as synaptopodin and α-actinin-4 remained unchanged in 2K1C compared to controls, we found reduced abundance of slit-diaphragm complex proteins in 2K1C. These included, but were not limited to, nephrin, podocin, neph1, dendrin and ZO-1. We also found lower abundance of important glomerular basement membrane (GBM) proteins including collagen IV, laminin and nidogen-1. Interesting glomerular markers of damage were desmin, heat shock 70kDa protein 1A/1B and transgelin.

**Conclusions:** In conclusion, we have demonstrated reduced abundance of proteins involved in the glomerular filtration barrier in FFPE tissue from the non-clipped kidney of 2K1C rats with proteinuria compared to non-proteinuric controls.

## SA-PO773

**Effect of Rho Kinase (ROK) Inhibition on Rho A Induced Podocyte Injury** Liming Wang,[1,2] William Eisner,[1,2] Robert F. Spurney.[1,2] *[1]Medicine, Duke Univ, Durham, NC; [2]Medicine, Durham VA Medical Center, Durham, NC.*

**Background:** We previously created transgenic (TG) mice that expressed a constitutively active Rho A construct (V14Rho) specifically in glomerular podocytes in a doxycycline inducible fashion. In these TG mice, induction of V14Rho caused albuminuria and foot process (FP) effacement (Kidney Int 81:1075, 2012). In this same study, V14Rho promoted apoptosis of cultured podocytes and this apoptotic effect was attenuated by treatment with the ROK inhibitor Y27632.

**Methods:** To determine if Y27632 attenuated V14Rho induced podocyte injury in vivo, we treated V14Rho TG mice with doxycycline for 2 wks followed by doxycycline and either Y27632 or saline for an additional 2 wks.

**Results:** Treatment with doxycycline alone caused albuminuria (43±3 vs 102±11 µg/24H; P<0.01). The addition of saline vehicle to doxycycline treatment had little effect on albuminuria (85±10 µg/24H; P=NS) but Y27632 increased the albuminuric response (142±26 µg/24H; P<0.05). Because abnormal podocyte motility may be associated with albuminuria and FP effacement, we determined the effect of Y27632 on podocyte motility using a wound-healing assay. We found that V14 Rho alone had little effect on wound closure. In contrast, Y27632 enhanced wound closure in either the presence or absence of V14Rho suggesting that Y27632 increased podocyte motility. We next determined the molecular signature of Y27632 induced motility by investigating the effects of V14Rho and Y27632 on the actin and microtubule cytoskeleton. In cultured podocytes, V14Rho promoted formation of detyrosinated and deacetylated tubulin as well as phosphorylation of both myosin light chain (MLC) and cofilin. Incubation with Y27632 inhibited V14Rho induced MLC phosphorylation, cofilin phosphorylation and tubulin deacetylation but not formation of detyrosinated tubulin. With the exception of cofilin, the effects of Y27632 were similar in V14Rho-expressing TG mice treated with Y27632.

**Conclusions:** The molecular signature of a motile podocyte in vitro was largely recapitulated in vivo. These data suggest that ROK inhibition promotes podocyte motility which, in turn, may diminish the beneficial effects of ROK inhibitors in glomerular diseases.

*Funding:* Veterans Affairs Support

## SA-PO774

**Urinary Microvesicles Differentiate Patients with Diabetic Nephropathy from Those with Active Glomerulonephritis** Ladan Zand, Muthuvel Jayachandran, Stephen T. Turner, M. Regina Castro, Joseph P. Grande, Vesna D. Garovic. *Mayo Clinic, Rochester, MN.*

**Background:** Microvesicles (MVs) are small membrane-enclosed sacs that are shed from activated or injured cells and have been identified in different body fluids including urine. They contribute to a variety of pathophysiological processes involving cell-cell interaction. We hypothesized that urinary MVs may serve as a diagnostic marker in differentiating between glomerulonephritis (GN) and diabetic nephropathy.

**Methods:** Digital flow cytometry was used to characterize urinary MVs expressing podocyte markers in patients with type 2 diabetes mellitus (DM) (n=31, male=19, female=12) and biopsy-proven GN (n=9, male=6, female=3) including focal segmental glomerulosclerosis (n=4) and membranous nephropathy (n=5). Of 31 patients with DM, 23 had urine albumin <300mg/L (Group I) and 8 had urine albumin >300 mg/L (Group II). For diabetic patients, other etiologies causing proteinuria including autoimmune diseases, hepatitis B, C, or monoclonal gammopathy were excluded. Data were analyzed by non-parametric Kruskal-Wallis test. Results are shown as median. Units for urinary MVs are expressed as MVs/µL of urine.

**Results:** Median age in GN group was 52 vs. 64.5 years in Group II (P=0.03) and protein to creatinine was 3.5 vs. 1.4 mg/mg (P=0.05). There was no difference in the GFR between the two groups (43 vs. 64 ml/min/1.73m$^2$). GN patients compared to Group II had significantly higher number of urinary MVs expressing podocyte markers such as nephrin (618 vs. 335 P=0.03), & podocalyxin (1376 vs. 587, P=0.04). There were also significantly greater number of parietal cell specific markers, claudin-1 plus CK-8 positive MVs (761 vs. 310, P=0.01). There were no differences in the number of urinary MVs expressing podocyte markers between GN group and Group I. There was no association between degree of proteinuria or GFR and presence of urinary MVs (data not shown).

**Conclusions:** The high number of urinary MVs found in GN patients is suggestive of active ongoing injury to the podocytes in contrast to patients with advanced diabetic nephropathy. Urinary MVs may be used as a novel biomarker in distinguishing GN from DM with macroalbuminuria.

*Funding:* Other NIH Support - NICHD

## SA-PO775

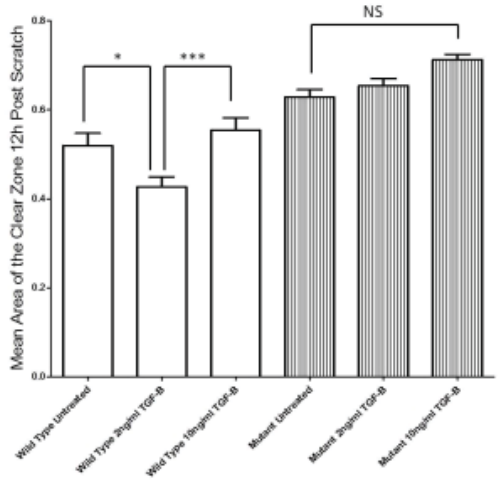
**Transforming Growth Factor Beta Signalling Impairment in *PLCE1* Mutant Podocytes** Carl James May, Gavin Iain Welsh, Moin Saleem. *Academic Renal Unit, Univ of Bristol, Bristol, Avon, United Kingdom.*

**Background:** *PLCE1* is a gene mutated in inherited nephrotic syndrome, causing diffuse mesangial sclerosis. It is unusual in not having a known role at the podocyte slit diaphragm or in actin regulation. We generated a conditionally immortalised cell line from a child with a PLCε1 mutation, and studied its biology.

**Methods:** Wild type and *PLCE1* mutant human podocytes were treated with 2ng/ml recombinant TGF-B1. Podocyte markers and TGF signalling activity were measured by western blot and IF. Podocyte motility and barrier function were assessed by scratch assays and Electric Cell-substrate Impedence Sensing (ECIS) respectively.

**Results:** The mutant podocytes expressed typical podocyte markers at similar levels to wild type cells, Interestingly, the mutant cell line appeared mesenchymal in culture, and significantly overexpressed mesenchymal markers such as fibronectin and α-SMA. Despite expressing TGFBR1 and TGFBR2 there was no phosphorylation of SMAD2 in the mutant cell line. Non-canonical TGF-B1 signalling pathways were similarly impaired. This lack of SMAD2 signalling in response to TGF-B1 treatment also had functional consequences. Wild type podocytes became significantly more motile in response to 2ng/ml TGF-B: the mutant podocytes were unresponsive. This has clear implications for TGF-B signalling *in vivo*.

TGF-B Treated Scratch Assay of Wild Type and *PLCE1* Mutant Human Podocytes




The wild type podocytes also demontrated a very tight dose response to TGF-B. Following 24 hour treatment with 2ng/ml TGF-B1, the wild type podocytes upregulated the EMT regulators *slug* and *snail*. There was no such upregulation in the mutant podocytes.

**Conclusions:** In the mutant cell line there are equivalent amounts of SMAD2 and SMAD3. Compared to the wild type podocytes which express significantly more SMAD2. the antagonism of SMAD2 and SMAD3 leads to impaired responses to TGF-B1 in the mutant cell line. In this way PLCε orchestrates the podocyte's response to TGF-B1.

## SA-PO776

**Sypl2 Ablation Causes Congenital Nephrotic Syndrome** Ling-Mei Chiang,[1] Pu Duann.[2] *[1]Paediatrics, Chang-Gung Memorial Medical Center, Keelung, Taiwan; [2]DHLRI, Ohio State Univ, Columbus, OH.*

**Background:** Synaptophysin-like 2 (Sypl2), originally cloned from transverse tubules in muscle. It also expressed subtle abundance in kidney. It was newly identified to podocytes. Although some deformity was found in Sypl2-null mices, i.e. disarrayed T- tubule and reduced mitochondria amount, there is no muscle dystrophy. In this abstract, we report ablation of Sypl2(-/-) on kidney function and pathophysiology.

**Methods:** renal function on Sypl2 null mice were studied in terms of protein filtration and Adriamycin-mediated glomerulonephropathy. The interaction between Sypl2 and other Slit diaphragm genes was explored.

**Results:** Sypl2 expression was further localized to podocytes. Immuno-colocalization with nephrin (Nphs1) or podocin (Nphs2) was not detected. Foot process syncytium and defects was found in both scanning and transmission electromycroscopy. Intracytoplaqsmic lipid droplets (1-5 μm), was found abundant in tubular epithelium cells suggests the pathognomic hyperlipidemia in nephrotic syndrome. Adult Sypl2-null mice displayed proteinuria (UpUc 0.52 ± 0.23 ) compared to their wild type littermates (0.22 ± 0.14). Genetic ablation of Sypl2 results in no abnormalities in fertility, litter sizes and Mendelian ratios. The pathohistology studies revealed distinct glomerular deformity in homozygous 21d-pups and 28d-heterozygotes in a esser extent. To test whether ablation of Sypl2 injured the integrity of the slit-diaphram thus interfere podocyte normal function, Adriamycin-mediated glomerulopathy was explored. Sypl2-null mice with C57BL/6 as parental strain, was anticipated to be adriamycin resistant. High dosage (17mg/KgBW) Adriamycin administration failed to give conclusive answer. Abnormal Glut-4 translocation and oral glucose intolerance were observed in Sypl2-null mice.

**Conclusions:** Sypl2 has no direct intermolecular contact with any known slit diaphragm molecules is not prone todevelop the Finnish-type nephrotic syndrome. Sypl2-null mice with intact slit diaphragm genes still develop pathognomic footprocess deformity and hyperlipidemia instead of the Finnish type nephrotic syndrome. It provide an animal model to study the metabolic-mediated nephrotic syndrome.

## SA-PO777

**Pathophysiology of Reductions in Renal Blood Flow (RBF) and Glomerular Filtrate Rate (GFR) in Sepsis** Ruben M. Sandoval,[1] George Rhodes,[1] Silvia B. Campos-bilderback,[1] Sarah E. Wean,[1] Bruce A. Molitoris.[1,2] *[1]Medicine/ Nephrology, IU School of Medicine, Indianapolis, IN; [2]Roudebush VAMC, Indianapolis, IN.*

**Background:** It is well known that the reduction in GFR in sepsis far exceeds the fall in RBF yet the mechanism(s) remain unknown.

**Methods:** We used the cecal ligation and puncture (CLP) sepsis model in Munich Wistar Frömter rats with surface glomeruli to evaluate glomerular vascular alterations during ongoing sepsis at 24 hours.

**Results:** Serum creatinine increased from 0.3 to 0.8 mg/dl at 24 hours and was associated with a marked reduction in glomerular capillary RBC flow rates from 1,771 ±467μm/sec in untreated rats to 576 ±327μm/sec (P≤0.01). At 24 hour post injury, WBC adherence increased from 0.42 ±0.33/25μm volume in untreated rats to 7.25 ±5.82/25μm 24Hrs post CLP (p≤0.05). Most noticeable was the heterogeneous nature of RBC flow within the glomerular capillaries. Areas of markedly reduced or stagnant flow were observed with channeling of flow into adjacent areas. We quantified capillary loop branching points, which we determined to occur at approximately 48 ±8.8 points per glomeruluar volume. Shunting of RBC flow was measured using changes in RBC density within the capillary loops showing a decrease in RBC density within occluded loops. Untreated rats had a visual RBC density of 80.8 ±3.5% while rats imaged 24HR post CLP had a decreased RBC density of 56.2 ±15.8% (p≤0.01). Glomerular permeability was measured using a 150kDa Rhodamine dextran which increased from 0.007 ±0.003 in untreated rats to 0.096 ±0.046 (p≤0.05) 24HR post CLP.

**Conclusions:** These data imply the marked dissociation between GFR and RBF during sepsis is due to a reduction in the glomerular surface area available for filtration and shunting of blood flow around the glomerulus via intraglomerular capillary branch points allowing for small decreases in RBF resulting from marked reductions in GFR.

*Funding:* Other NIH Support - NIH P-30 to Bruce A Molitoris

## SA-PO778

**Slit2-Robo2 Signaling Decreases Non-Muscle Myosin IIA Activity in the Podocyte** Xueping Fan, Anna Pisarek-Horowitz, Hila Milo Rasouly, Stefanie Chan, David J. Salant, Weining Lu. *Renal Section, Dept of Medicine, Boston Univ Medical Center, Boston, MA.*

**Background:** We have recently discovered that Robo2 is a novel podocyte protein localized to the basal cell surface of

the podocyte. Slit2-Robo2 signaling inhibits nephrin-induced actin polymerization and regulates podocyte foot process structure. However, the downstream signaling components of the Slit2-Robo2 pathway in podocytes are unknown.

**Methods:** Yeast two-hybrid screen and protein precipitation assays were performed to identify novel downstream signaling components of Slit2-Robo2 pathway in podocytes. Immunocytochemistry was used to verify protein expression of novel components in podocytes. We also performed genetic interaction studies in podocyte-specific knockout mice of Robo2 and the newly identified gene.

**Results:** Using yeast two-hybrid screen, we find that the myosin II regulatory light chain (MRLC), a subunit of non-muscle myosin IIA heavy chain (NM IIA, encoded by Myh9 gene), interacts directly with Slit-Robo Rho GTPase activating proteins (srGAPs). Protein precipitation assays show that NM IIA, MRLC, srGAPs, and Robo2 receptor form a protein complex that is enhanced by Slit2 ligand. Immunocytochemistry indicates that NM IIA, MRLC and srGAPs are expressed in podocytes. In vitro functional study demonstrates that Slit2-Robo2 signaling decreases NM IIA activity in podocytes. In vivo studies show that Myh9 and Robo2 podocyte-specific double knockout mice have fewer podocyte numbers compared with Robo2 single knockout mice.

**Conclusions:** We have identified srGAPs, MRLC and NM IIA (Myh9) as novel downstream protein components of the Slit2-Robo2 signaling in the podocyte. NM IIA activity is down-regulated by Slit2-Robo2 signaling, which may play a role in regulating podocyte cell adhesion and dynamics.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO779

**ERK1/2 Are Dispensable for Normal Podocyte Function and Initial Response to Injury In Vivo** Johannes S. Schlondorff, Eliyahu V. Khankin. *Div of Nephrology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

**Background:** The Erk pathway in one of the canonical mitogen activated protein kinase signaling pathways and plays a central role in critical cellular functions, including survival and differentiation. Erk activation is a common manifestation in multiple human glomerular diseases and mouse models. However, it remains unclear whether its role is as a mediator or mitigator of disease, with conflicting results from in vitro studies. Here we present data that deletion of Erk1/2 in podocytes is dispensable for normal glomerular function.

**Methods:** Mice with a germline deletion of ERK1 (ERK1 -/-), a flox allele of ERK2 (ERK2 flox/flox), and podocyte specific Cre expression were generated. These podocyte ERK1/2 deficient animals were compared to littermates without the Cre allele, or with at least one wild-type ERK1 allele. Urinary microaolbumin-to-creatinine ratios and renal histology were examined at various ages. Age and sex matched mice were injected with a single dose of nephrotoxic serum (NTS) to induce transient massive proteinuria with subsequent repair.

**Results:** Mice with ERK1/2 deficient podocytes demonstrate no gross or histologic renal abnormalities at 6 weeks of age and have no significant albuminuria at that time. At 8 months of age, male mice showed a trend toward mildly elevated microalbumin/Cr ratios. Podocyte ERK1/2 deficient mice did not differ from their littermates with regard to peak proteinuria after NTS injection, nor with initial recovery at 11 days post-NTS. However, one month after NTS, podocyte ERK1/2 deficient animals showed approximately two fold higher residual albuminuria.

**Conclusions:** Podocyte development and function is not dependent upon ERK1/2 in vivo. Similarly, podocyte ERK1/2 deficient animals show a similar initial response to acute, transient podocyte injury in the NTS model, though there is evidence that they do not recover as well from the injury. In summary, these findings suggest that ERK1/2 do not play an essential role in baseline podocyte function and likely has only a minor role in podocyte response to injury or stress.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO780

**Cyclosporine A Protects Podocyte via Upregulating the Expression of Cofilin-1** Xiaoyan Zhang,[1] Xiaoyan Li,[1] Youfei Guan,[2] Jie Ding.[1] *[1]Pediatric, Beijing Univ First Hospital; [2]Physiology and Pathophysiology, Beijing Univ Health Science Center.*

**Background:** Podocyte foot process is dysregulated in nephrotic syndrome. The effacement of podocyte foot processes typically arises owing to perturbations in the actin cytoskeleton. Calcineurin inhibitor Cyclosporien A (CsA) is currently used in the treatment of nephrotic syndrome. Recent data suggest that the effects of CsA on nephrotic syndrome are independent of its effects on the immune system. They identified that CsA can stabilize the actin cytoskeleton through stabilizing synaptopodin in podocytes and thereby reduce proteinuria directly. Other studies also showed that CsA induced cofilin phosphorylation and promoted stress fiber generation in proximal tubular cells. However, whether the antiproteinuric role of CsA is played by regulating cofilin-1 in podocyte has not been studied.

**Methods:** Acute podocyte injury and nephrotic syndrome were induced by puromycin aminonucleoside (PAN) injection in rats with or without CsA. Cultured podocytes were exposed to PAN with or without CsA treatment. Cofilin-1, nephrin, synaptopodin expression were determined by western blot or immunofluorescence. The cofilin-1 specific effect was determined using cofilin-1 siRNA.

**Results:** CsA reduced proteinuria, restored expression of cofilin-1, nephrin, syanptopodin and repaired foot process effacement of PAN induced nephropathy *in vivo*. *In vitro* studies showed that exposure of CsA restored the expression of cofilin-1 and nephrin which decreased by PAN. CsA also repaired actin cytoskeleton impaired by PAN. The protective effect of CsA was disappeared partially when cultured podocytes were exposed to cofilin-1 siRNA.

**Conclusions:** The antiproteinuric effect of CsA is derived from the stabilization of the podocyte actin cytoskeleton by upregulating expression of actin-binding protein cofilin-1.

*Funding:* Government Support - Non-U.S.

## SA-PO781

**Calbindin-1 (28kDa) Inactivation Leads to Proteinuria – Studies in Zebrafish (*Danio rerrio*), Mice and Mammalian Podocytes** Nils Hanke,[1,2] Laura L. Beverly-Staggs,[2] Patricia Ann Schroder,[2] Konstantin Deutsch,[1,2] Michelle P. Winn,[5] Gentzon Hall,[5] Ron Korstanje,[3] Christoph Kornauth,[4] Hermann G. Haller,[1,2] Mario Schiffer.[1,2] *[1]Div of Nephrology, Hanover Medical School, Hannover, Germany; [2]Mount Desert Island Biological Laboratory, Salisbury Cove, ME; [3]The Jackson Laboratory, Bar Harbor, ME; [4]Dept of Pathology, Medical Univ of Vienna, Vienna, Austria; [5]Div of Nephrology, Duke Univ Medical Center, Durham, NC.*

**Background:** Calbindin-1 (Calb-1) is an intracellular calcium-binding protein containing four active calcium binding domains. As calcium homeostasis is important for podocyte survival, we examined the effects of Calb-1 knockdown on the integrity of the glomerular filtration barrier and proteinuria in zebrafish.

**Methods:** Knockdown of Calb-1 was performed using morpholino injections in zebrafish *(Danio rerio)* eggs. We examined proteinuria development in the embryos by cardinal vein injection of 70 kDa FITC-labeled dextran and knockdown in a transgenic fishline (l-fabp:DBP-EGFP) expressing a 78kD GFP labelled protein. Pronephros structure was evaluated by TEM. Calb1 protein expression was analyzed in human biopsies as well as in wildtype and calreticulin deficient mice. Moreover proteinuria development was examined in aging Calb1-knockout mice.

**Results:** We found edema, proteinuria and foot process effacement in zebrafish embryos after knockdown of Calb-1.

Immunohistochemistry showed a strong expression of Calb1 protein in tubular cells and in addition we detected a cytoplasmatic vesicular expression pattern of Calb-1 in human podocytes. On immunofluorescence we detected a vesicular expression pattern in cultured human podocytes. Moreover we detected an upregulation of Calb1 in tissues with a calreticulin deficiency. Calb-1 knockout mice did not develop a spontaneous proteinuria indicating an indepence from Calb-1 in unchallenged mice.

**Conclusions:** In summary we found that Calb-1 knockdown results in a podocyte phenotype in zebrafish. In contrast in a rodent model the knockdown did not lead to proteinuria but to an upregulation of calreticulin indicating a delicate balance of calcium binding proteins in the podocyte.

*Funding:* Private Foundation Support

## SA-PO782

**Prostaglandin E2 Upregulates Olfactory Genes in Podocytes with Recovery from Nephrotoxic Serum Induced Injury** Nino Kvirkvelia, Maggie McMenamin, Afu Abdul, Michael P. Madaio. *Medicine, Georgia Regents Univ, Augusta, GA.*

**Background:** $PGE_2$ promotes resolution of Nephrotoxic Serum Nephritis in mice, and this was due, at least in part, to promoting glomerular cell recovery. In cell culture, $PGE_2$ reduced nephrotoxic serum (NTS) induced apoptosis of glomerular cells and promoted cell reproliferation after NTS-mediated injury.

**Methods:** To define the pathways involved, microarray analysis (Affymetrix oligonucleotide chips) was used to investigate $PGE_2$ regulated gene expression profiles in mouse glomerular epithelial cells after NTS treatment. Genes with a minimal 1.5-fold change and $P < 0.05$ were selected for network generation and pathway analyses by Ingenuity Systems. GenBank IDs of the selected genes were then mapped to the functional networks available in the Ingenuity Pathway Knowledge Base.

**Results:** A total of 237 genes with 3.5-1.5 changes ($p<0.05$) were identified, and the top 3 networks included functional categories of: a) Cell cycle, cell development, cell signaling; b) RNA post-translational modification, cell morphology, cellular assembly and organization; c) Cell-to-cell signaling and interaction, cellular compromise, cellular function and maintenance. The scores, reflecting the negative logarithms of the significant p values for these networks were 34, 29 and 27 respectively. The top upregulated $PGE_2$ targets included: NR4A2; NR4A1; OR5M11; TRDN; Olfr765/Olfr767; Olfr1085; Olfr582; Olfr948; OR10G2; Svs3a/Svs3b genes, with expression values of: 2.807; 2.681; 2.464; 2.229; 2.208; 2.110; 2.052; 1.951; 1.901; 1.893 respectively.

**Conclusions:** Among the highest $PGE_2$ regulated genes, a majority belong to an olfactory group of receptors, which are members of large family of G-coupled receptors, and share 7-transmembrane domain structure with many hormone and neurotransmitter receptors. Olfactory receptors have been shown to be expressed in the kidney at the RNA and protein levels. The hypothesis that $PGE_2$ acts as a specific ligand recognized by podocyte olfactory receptors to regulate podocyte survival and proliferation after injury are under investigation. The study will aid further understanding of signaling pathway regulated by $PGE_2$ in podocytes.

*Funding:* NIDDK Support

## SA-PO783

**Synaptopodin Limits Cell Surface Expression of TRPC6 in Podocytes** Hao Yu,[1] Andreas D. Kistler,[1] Stuart E. Dryer,[2] Jochen Reiser.[3] *[1]Nephrology, Univ of Miami, Miami, FL; [2]Biology and Biochemistry, Univ of Houston, Houston, TX; [3]Medicine, Rush Univ, Chicago, IL.*

**Background:** TRPC6 mutations have been shown to cause familial FSGS and increased TRPC6 in podocytes is found in acquired glomerular diseases. Synaptopodin (synpo) is a stabilizer of actin cytoskeleton in podocytes and it can be preserved by Cyclosporine A (CsA). We show that synpo interacts with TRPC6 and this interaction is functionally important and may have a role in regulating membrane localization of TRPC6.

**Methods:** Immunogold labeling and co-immunoprecipitation (co-IP) were performed to examine co-localization and interaction of TRPC6 and synpo, respectively. Cell surface biotinylation assay in synpo knock-down mouse podocytes was carried out to measure cell surface TRPC6 levels. Knockdown of synpo in podocytes and overexpression of wt TRPC6 and expression of FSGS-causing mutant $TRPC6^{M131T}$ in cultured and primary mouse podocytes were achieved by lentiviral system.

**Results:** Close co-localization of TRPC6 and synpo was observed in podocyte FPs by immunogold double labeling in mouse kidney sections. Interaction between TRPC6 and synpo was observed by co-IP in HEK293, cultured podocytes and mouse glomeruli. This interaction is significant because surface TRPC6 in synpo down-regulated podocytes showed a 2.4 fold increase in TRPC6 membrane expression suggesting synpo limits cell surface expression of TRPC6. Moreover, synpo was upregulated by ~2 fold in podocytes overexpressing wt TRPC6 or $TRPC6^{M131T}$, and decreased by ~70% in mouse $TRPC6^{-/-}$ glomeruli and $TRPC6^{-/-}$ primary podocytes. Reduction of synpo was rescued by expressing wt TRPC6 in $TRPC6^{-/-}$ primary podocytes. These data may reflect a feedback mechanism for podocytes to counteract the effect of malfunctioning or excessive TRPC6.

**Conclusions:** We demonstrate that synpo negatively regulates TRPC6 expression on cell membrane, presumably by direct binding. CsA, a drug that has been used to treat FSGS, was shown to stabilize synpo in podocytes. We propose a mechanism that CsA protects podocytes from excessive TRPC6 or FSGS-causing TRPC6 mutants by lowering cell surface expression of TRPC6 through stabilization of synpo.

*Funding:* NIDDK Support

## SA-PO784

**Dynamin1 Regulates Actin Cytoskeleton in Podocytes in a Phosphorylation Dependent Manner** Changkyu Gu, Sanja Sever. *Nephrology, Massachusetts General Hospital, Charlestown, MA.*

**Background:** Dynamin is an essential actin regulatory protein in podocytes, and loss of its function is closely connected to podocytes damage and proteinuria. Recently, our data has shown that dynamin directly regulates actin cytoskeleton via its oligomerization state. This dynamin oligomerization can be regulated through interaction with diverse cellular proteins, and it is reported that dynamin1 can differentially alter the affinity for its protein binding partners via phosphorylation by two different serine/threonine kinases, GSK3b and CDK5, in neurons. Based on these data, we hypothesize that phosphorylation-dependent dynamin1 oligomerization is an important molecular mechanism that regulates actin dynamics in podocytes.

**Methods:** Various phospho-dynamin1 mutants were generated by site-directed mutagenesis, and adenoviruses carrying these mutants were produced using Adenoviral Expression System (Invitrogen). Actin and paxillin in podocytes were stained to observe actin structures and focal adhesions. FLIM assay was done to detect oligomerization state of different phospho-dynamin1 mutants in podocytes.

**Results:** 1. Inhibition of GSK3b or CDK5 alters actin structures in podocytes.

2. Dynamin1 is phosphorylated by GSK3b and CDK5 in podocytes.

3. Overexpression of dynamin1 mutants lacking PRD domain inhibited the effects of GSK3b inhibitor on actin structures in podocytes.

4. Dynamin1 mutants which cannot be phosphorylated by GSK3b stimulate the formation of actin stress fiber and focal adhesions in podocytes.

5. FLIM assay showed that ablation of the GSK3b-specific phosphorylation site on dynamin1 induces dynamin1 oligomerization in podocytes.

**Conclusions:** The role of dynamin in actin cytoskeleton is essential to maintain the filtration barrier function of podocytes. Dynamin directly regulates actin structures via its oligomerization state. Our data show that dynamin1 oligomerization can be modulated by its phosphorylation state, and phosphorylation-dependent dynamin1 oligomerization contributes to regulation of actin dynamics in podocytes.

*Funding:* NIDDK Support

## SA-PO785

**Paricalcitol Ameliorates TGF Beta-Induced Glomerulopathy via Notch Pathway Activation** Taichi Murakami, Koji Okamoto, Shashi Shrivastav, Hidefumi Wakashiu, Jeffrey B. Kopp. *NIDDK, NIH, Bethesda, MD.*

**Background:** Vitamin D (VitD) is a promising approach to renoprotection. TGF-β induces kidney injury, and VitD antagonizes TGF-β signaling. TGF-β is engaged in cross-talk with the Notch pathway, with system-dependent agonistic and antagonistic effects. We tested whether VitD amplifies Notch signaling and thereby antagonizes TGF-β pro-fibrotic effect.

**Methods:** We investigated the effect of paricalcitol, a 1,25-OH VitD analog, on Notch activation in Alb/TGF-β transgenic (TG) mice, in which elevated plasma TGF-β, driven by the albumin promoter, induces progressive glomerulosclerosis. Paricalcitol was subcutaneously administered, beginning at 2 wk of age, when glomerular injury is mild, until 4 wk of age.

**Results:** Two-wk old TG mice exhibited the following: mild albuminuria, assessed as urine albumin/creatinine ratio (ACR), of 227 μg/mg; glomerular ECM accumulation; increased Smad3 phosphorylation; and down-regulation of VitD receptor protein. Notch pathway mRNA expression was significantly increased: Notch4 by 1.7 fold, Hey2 by 1.7 fold and HeyL by 4.0 fold. Immunostaining revealed a nuclear pattern of HeyL expression in glomeruli and tubules. Four-wk old TG mice had progressive kidney disease (table, mean±SD, * denotes significant compared to wild-type mice). Paricalcitol-treated mice exhibited less kidney damage, which reached statistical significance for urine ACR, glomerulosclerosis score, and HeyL/actin RNA ratio (#).

| | Urine ACR (μg/mg) | Glomerulo-sclerosis | FN/actin mRNA | COL1/actin mRNA | PAI-1/actin mRNA | HeyL/actin mRNA |
|---|---|---|---|---|---|---|
| Wild-type mice | 96±41 | 80±25 | 0.015± 0.0018 | 0.04±0.01 | 0.0011±0.00031 | 0.0014±0.00037 |
| TG mice | 4872±1733 * | 151±15 * | 0.038± 0.015 * | 0.22±0.077* | 0.013±0.0072* | 0.0047±0.0012* |
| TG mice + paricalcitol | 1187±1316 # | 125±12# | 0.027± 0.01 | 0.14±0.029 | 0.0077±0.0046 | 0.0074±0.0016# |

Paricalcitol did not alter Smad3 phosphorylation. The effect of paricalcitol plus TGF-β to increase HeyL mRNA expression was replicated in cultured mouse podocytes.

**Conclusions:** Paricalcitol may ameliorate glomerulopathy in part by amplifying Notch signaling and thereby antagonizing TGF-β signaling.

*Funding:* NIDDK Support

## SA-PO786

**Effect of Angiotensin II on Podocyte Autophagy** Imane Bensaada,[1,2] Carole Hénique-Gréciet,[1,2] Pierre-Louis F. Tharaux.[1,2] *[1]Paris Cardiovascular Research Centre - PARCC, INSERM, Paris, France; [2]Univ Paris Descartes, Sorbonne Paris Cité.*

**Background:** Injury and loss of podocytes are leading factors of glomerular diseases and renal failure. Angiotensin II (AngII) promotes albuminuria, aggravates non hypertensive glomerulosclerosis and severe hypertension nephropathy (HTN). Recently, autophagy has been identified as a major mechanism that delivers damaged proteins and organelles to lysosomes in order to maintain cellular homeostasis. Podocytes exhibit an unusually high level of constitutive autophagy.

**Methods:** The aim of this study was to evaluate the role of AngII on autophagy in podocytes. Expression of LC3 II, an autophagosomal membrane protein, was measured in kidney homogenates and in podocytes from HTN mice after chronic infusion of AngII or in vitro, in primary cultures of podocytes from mice with normal or constitutive activation of the AT1 receptor.

**Results:** Mice with chronic HTN presented decreased autophagy activity in podocytes. Furthermore, podocyte culture from mice with constitutively active AT1 mutant (CAM) displayed lower autophagy flux than wild type mice. Chronic activation of the AT1 receptor induced significant podocyte loss, as demonstrated by WT1 and podocin staining in aged mice. In primary podocyte culture, autophagy regulation is independent of the PI3-kinase-mTOR pathway, as shown after rapamycin treatment. To further investigate the importance of autophagy in podocyte survival, we used a mouse model with podocyte specific deletion of Atg5 alleles, that is required for autophagosome formation and maturation (Pod-Atg5 mice). Pod-Atg5 mice developed more severe glomerular injury than wild type counterparts upon chronic AngII infusion, with severe albuminuria and glomerulosclerosis.

**Conclusions:** In conclusion, these data indicate that chronic activation of the AT1 receptor promotes decrease in autophagy activity and unravel a protective function for podocyte autophagy in AngII-mediated glomerulosclerosis.

## SA-PO787

**TGF–β1 and VEGFa Differentially Regulate Notch Signaling in Podocytes** Mariya T. Sweetwyne, Katalin Susztak. *Renal, Electrolyte and Hypertension Div, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** The Notch family is comprised of receptors (N1-4) and the Jagged (Jag1-3) and Delta-Like (DLL1, 3, 4) ligands. The regulation of these receptors and ligands suggests that they are not functionally redundant in podocytes. We previously showed that loss of N1 from podocytes (via floxed Notch1/podocin Cre) protects glomeruli from diabetic injury in vivo. Conversely, expression of Notch2 is critical for podocyte development. To understand this specificity in podocytes, we investigated Notch signaling in response to growth factors TGF-β1 and VEGFa as they both regulate glomerulosclerosis.

**Methods:** Murine podocytes were cultured with either TGF-β1 or VEGFa (+/- gamma secretase Notch inhibitor - GSI) for 0, 1, 4, 8, 12, 24, 48hrs. Notch-related gene regulation was determined by qRT-PCR and protein expression confirmed by immunoblot (WB) and immunofluorescence (IF).

**Results:** Direct TGF-β stimulation of the ligand, Jag1, was detected (WB, mRNA) by 1-8h. We detected TGF-β/Jag1-induced Notch1, but not Notch2, cleavage by WB at 4-24h. Interestingly, we observed a second wave (24-48 hr) of Jag1 gene expression that was GSI sensitive/Notch cleavage dependant. TGF-β suppressed expression of the Notch ligand Dll1, while inducing expression of another ligand, Dll4, at 4-48h, which was also dependent on Notch activation. Examining individual cells, we found that the protein expression of Dll4 and Jag1 (IF) was increased heterogeneously, with cells expressing either Jagged1 or Dll4. Furthermore, TGF-β also induced Notch dependent Vegfa expression in podocytes. Direct stimulation of podocytes with VEGFa induced Notch dependent expression of Dll4 but not other ligands, suggesting that TGF-β-induced Dll4 expression is downstream of Jagged1 and Vegfa.

**Conclusions:** TGF-β directly induces Jagged1 expression and Notch1 activation in podocytes. At later time points Notch activation sustains the expression of different ligands including Jagged1 and Dll4, as well as growth factor Vegfa. Stimulation of podocytes with VEGFa is sufficient to induce Dll4. Our results reveal an important ligand-specific response to TGF-β1/VEGFa cross-talk converging on the Notch signaling pathway.

## SA-PO788

**Melanocortin 1 Receptor Protects against Puromycin Induced Actin Stress-Fiber Rearrangement** Johannes Elvin,[1] Annika Lindskog Jonsson,[1] Lisa Maria Buvall,[2] Anna Granqvist,[1] Jenny C. Nystrom,[3] Borje Haraldsson.[1] *[1]Dept of Molecular and Clinical Medicine, Institute of Medicine, Univ of Gothenburg, Sweden; [2]Dept of Nephrology, Massachusetts General Hospital & Harvard Medical School; [3]Dept of Physiology, Institute of Neuroscience and Physiology, Univ of Gothenburg, Sweden.*

**Background:** Drugs targeting the melanocortin 1 receptor (MC1R) have shown to possess beneficial properties in treatment of patients with nephrotic syndrome. We have earlier demonstrated reduction of proteinuria in combination with improvement in glomerular morphology following MC1R treatment in a nephrotic rat model. We are currently investigating the mechanisms behind the beneficial effects of MC1R stimulation, with focus on actin dynamic regulatory pathways in podocytes.

**Methods:** Conditionally immortalized mouse podocytes over-expressing human MC1R were subjected to the nephrotic agent puromycin aminonucleoside (PAN) and simultaneously treated with a selective synthetic MC1R agonist (SA) or a non-selective MC1R agonist (α-MSH). Following PAN and MC1R agonist treatment, F-actin was visualized with Rhodamine-Phallodin and stress fiber content in cells were calculated and compared between treatment groups.

**Results:** The MC1R agonists SA or α-MSH were both able to restore stress fibers in podocytes with disrupted actin cytoskeleton due to PAN treatment. Stress fiber analysis following MC1R agonist treatment, revealed restoration of up to 40% of the actin stress-fibers in cells compared to PAN treatment alone.

**Conclusions:** MC1R stimulation hereby demonstrates the ability to restore stress fibers in podocytes when exposed to nephrotoxic agents such as PAN. Adding to our previous work, we know that MC1R signals through a cyclic AMP-dependent (cAMP) pathway. These data could propose that MC1R protects glomerular podocytes by regulating the actin cytoskeleton through a cAMP dependent pathway.

*Funding:* Government Support - Non-U.S.

**SA-PO789**

**Tumor Necrosis Factor-α, Interleukin-6 and High Insulin Disrupt Podocyte Insulin Responses *In Vitro*** Abigail Charlotte Lay, Gavin Iain Welsh, Moin Saleem, Peter W. Mathieson, Richard Coward. *Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom.*

**Background:** We have previously demonstrated that podocytes are insulin-responsive cells *in vitro* and the podocyte-specific depletion of the insulin receptor (IR) *in vivo* results in glomerular dysfunction. It is recognised that insulin resistance is associated with albuminuria and type 1 and type 2 diabetic patients with insulin resistance are more at risk of developing nephropathy. Hyperinsulinaemia and increased circulating levels of the pro-inflammatory cytokines TNF-α and IL-6 are factors associated with insulin resistance. Here, the effects of Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6) and high insulin concentrations on podocyte insulin responses *in vitro* were examined.

**Methods:** Conditionally immortalised mouse podocytes were grown in the presence of insulin, TNF-α and IL-6, and the effects on podocyte insulin responses investigated. Insulin-stimulated glucose uptake was assessed via a 2-deoxyglucose uptake assay, western blotting was performed to determine changes in protein expression and insulin-stimulated phosphorylation cascades and focused insulin signalling PCR arrays were performed to determine any changes at the mRNA level.

**Results:** Prolonged exposure to TNF-α and IL-6 reduces insulin-stimulated glucose uptake in podocytes. Western blotting demonstrated no significant reduction in IR protein levels and insulin-stimulated phosphorylation of IGF-IRβ (Tyr1131)/IRβ (Tyr1146) and Akt (Ser473, Thr308). Other aspects of the insulin signalling cascade are currently being investigated in a focused insulin signalling PCR array. Preliminary data suggests podocyte exposure to high concentrations of insulin *in vitro* reduces IR protein expression and phosphorylation of IGF-IRβ (Tyr1131)/IRβ (Tyr1146). Furthermore, Akt phosphorylation (Ser473, Thr308) also appears reduced.

**Conclusions:** Prolonged exposure of mouse podocytes to insulin and the pro-inflammatory cytokines TNF-α and IL-6 *in vitro* disrupts podocyte insulin responses. Investigating factors contributing to podocyte insulin insensitivity will enhance our understanding of the causes of glomerular disease in settings of insulin resistance.

**SA-PO790**

**HIV Induces Podocyte Epithelial Mesenchymal Transition (EMT) through Modulation of Telomerase Reverse Transcriptase (Tert)** Tejinder Singh, Nirupama Chandel, Partab Rai, Gautam Kishore Valecha, Rivka Lederman, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

**Background:** HIV-associated nephropathy (HIVAN is charcterized by podocyte proliferative phenotype in the form of collapsing glomerulopathy. HIV induces podocyte proliferative phenotype through podocyte expression of TERT in a HIV transgenic mouse model. We earlier reported that proliferative phenotype of kidney cells in HIVAN was the consequence of HIV-induced epithelial mesenchymal transition (EMT). In the present study, we hypothesized that HIV induces EMT in podocytes through the modulation of kidney cell Tert expression.

**Methods:** Renal cortical sections of control and HIVAN mice (Tg26) were immunolabeled for Tert, and EMT markers. In in vitro studies, conditionally immortalized human podocytes (CIHP, these podocytes constitutively Tert) were transduced with either HIV(NL4-3, HIV/CIHP) or empty vector (EV/CIHP). CIHP proliferate at 33°C and differentiate at 37°C. To determine the effect of HIV on Tert expression and occurrence of EMT, EV/CIHPs and HIV/CIHPs were incubated in media at 33°C for 72h, followed by differentiation at 37°C for 72h (CIDHP) further followed by dedifferentiation at 33°C for 72h (CIDDHP). Cellular lysates of control and experimental cells were probed for Tert, vimentin, α-SMA, p-cadherin, and FSP-1. To probe relationship between Tert and podocyte EMT, HIV/CIDDHP were silenced for Tert and evaluated for vimentin, SMA and FSP-1.

**Results:** Renal cortical sections of Tg26 mice displayed enhanced Tert expression by podcytes. In in vitro studies, HIV/CIHP exhibited enhanced expression of Tert and robust expression of vimentin, α-SMA and FSP-1 when compared to EV/CIHPs. HIV/CIDHPs displayed moderate expression of Tert, vimentin, α-SMA, and FSP-1 but decreased expression of nephrin and synaptopodin. HIV/CIDDHP displayed robust expression of Tert, α-SMA, and FSP-1 and decreased expression of nephrin, synaptopodin and WT1. siRNATert/CIHP and siRNATert/CIDHP demonstrated attenuated expression of vimentin, α-SMA, and FSP-1.

**Conclusions:** These findings indicate that HIV-induced podocyte EMT is mediated through HIV-induced Tert expression.

*Funding:* NIDDK Support

**SA-PO791**

**ERK5 Involvement in the Regulation of Human Podocyte Phenotype** Irbaz Isaac Badshah,[1,2] Deborah L. Baines,[2] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, Surrey, United Kingdom; [2]St. George's Univ of London, London, United Kingdom.*

**Background:** Podocytes are specialised cells integral to the normal functioning kidney, however in diabetes injury occurs leading to podocyte dysfunction and a compromised phenotype. ERK5 is an atypical MAPK involved in pathways modulating cell survival, proliferation and phenotype. Previously we have demonstrated the expression and activation of ERK5 in human podocytes, the role of which remains largely unknown in these cells.

**Methods:** Conditionally immortalised human podocytes were stimulated with EGF (10ng/ml) and TGF-β1 (2.5ng/ml); inhibition of ERK5 activation was conducted with the chemical inhibitor BIX02188 (10μM) directed to the upstream MEK5. Intracellular signalling proteins were investigated by western blotting; phenotype was explored with markers of podocytes, epithelia and mesenchymal cells by immunofluorescence; proliferation was assessed with an MTS assay; motility was examined with a scratch assay; barrier function was studied using electric cell-substrate impedance sensing.

**Results:** TGF-β1 decreased P-cadherin staining and increased the myofibroblast marker α-SMA which was prevented by BIX02188; BIX02188 alone and co-incubation with EGF led to increased nuclear staining of P-cadherin. EGF and TGF-β1 increased podocyte proliferation which was prevented by BIX02188. Podocyte motility was reduced by BIX02188 alone and co-incubation with EGF and TGF-β1. Integrity of the podocyte barrier at 6h was impaired by TGF-β1, EGF and BIX02188; co-treatment of EGF and BIX02188 further reduced impedance, however BIX02188 co-incubation with TGF-β1 restored impedance to vehicle control levels.

**Conclusions:** We have previously demonstrated activation of ERK5 isoforms by TGF-β1 in human podocytes; here we provide strong evidence that TGF-β1-induced transition to a more mesenchymal phenotype, enhanced motility and reduced barrier function were all prevented by pan-inhibition of ERK5 activation. Furthermore, inhibition of ERK5 activity prevented EGF-induced proliferation. These results implicate ERK5 as a critical regulator of growth factor-induced pathological and physiological responses in human podocytes.

*Funding:* Private Foundation Support

**SA-PO792**

**ERK5 Involvement in the Regulation of Human Podocyte Survival** Irbaz Isaac Badshah,[1,2] Ekram Nabi,[1] Deborah L. Baines,[2] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, Surrey, United Kingdom; [2]St. George's Univ of London, London, United Kingdom.*

**Background:** Podocytes are highly focused cells central to kidney physiology, however in diabetic conditions injury occurs leading to an impaired function which is critically due to podocyte loss. ERK5 is an atypical MAPK involved in pathways modulating cell survival and apoptosis. Previously we have established the expression and activation of ERK5 in human podocytes, the role of which remains largely unknown in these cells under conditions associated with diabetic nephropathy.

**Methods:** Conditionally immortalised human podocytes were stimulated with the diabetic stimuli 30mM D-glucose and 200μg/ml advanced glycation end-product (AGE)-BSA. Inhibition of ERK5 activation was conducted with 10μM of the chemical inhibitor BIX02188 that targets the upstream MEK5. Intracellular signalling proteins were investigated by western blotting and protein modifications by immunoprecipitation; apoptosis was studied by western blot detection of cleaved caspase-3 and annexin V-FITC flow cytometry.

**Results:** High glucose treatment decreased p-ERK5 levels at 30min and over 48h increased apoptosis relative to the osmotic control of L-glucose as demonstrated through flow cytometry and raised cleaved caspase-3 protein levels. BIX02188 increased apoptosis, but co-incubation with D-glucose augmented the apoptosis observed with either treatment alone. AGE-BSA similarly decreased p-ERK5 at 30min, but did not cause apoptosis at 48h; contrariwise, AGE-BSA decreased the level of apoptosis relative to the non-glycated BSA control which was abolished following BIX02188 co-treatment. Immunoprecipitation revealed ERK5 to be SUMOylated in response to AGE-BSA treatment.

**Conclusions:** ERK5 is permissive for cell survival and inhibiting its activity leads to podocyte apoptosis under non-stimulated and diabetic conditions. ERK5 is SUMOylated under the diabetic stimulus of AGE-BSA treatment where there was no increase in apoptosis. This suggests that SUMOylation of ERK5 alters its localisation or activity in a manner not related to apoptosis. Manipulation of ERK5 signalling can be exploited to preserve the loss of podocytes that is observed in diabetic kidney injury.

*Funding:* Private Foundation Support

**SA-PO793**

**Effects of Serum Amyloid A on Glomerular Podocyte Inflammatory Responses** Robert J. Anderberg,[1] Rick L. Meek,[1] Sheryl K. Cooney,[1] Katherine R. Tuttle.[1,2] *[1]Providence Medical Research Center, Providence Sacred Heart Medical Center & Children's Hospital, Spokane, WA; [2]Div of Nephrology, Dept of Medicine, Univ of Washington School of Medicine, Seattle, WA.*

**Background:** Inflammatory mediators promote kidney damage that plays an important role in the pathogenesis of diabetic kidney disease (DKD). Acute phase serum amyloid A (SAA) is a family of apolipoprotein isoforms that likewise promote inflammation. We previously discovered that SAA isoform 3 (SAA3), a major acute phase reactant in mice, was increased in the glomeruli of mouse models of both type 1 diabetes/early DKD and

type 2 diabetes/advanced DKD. Notably, SAA3 was prominently expressed in glomerular podocytes. The aim of this study was to evaluate direct effects of SAA on podocyte inflammatory responses that may contribute to DKD.

**Methods:** Mouse podocytes were treated with SAA (human SAA isoform 1), advanced glycation end products (pro-inflammatory control), or normal control conditions for 6, 24 and 48 hours. Nuclear Factor kappa B (NF-kB) activity was measured in cell lysates. Podocytes were also treated with SAA for 1 day and mRNA measured (quantitative real-time PCR) using pathway arrays featuring NF-kB and inflammation-related genes. Mouse SAA3 protein was also measured from conditioned media (ELISA).

**Results:** Mouse podocytes exposed to SAA or AGE increased NF-κB activity. SAA exposure produced strong increases in expression of NF-κB gene targets comprising numerous inflammatory alpha and beta chemokines including ENA-78, RANTES, and MCP-1. Inflammatory cytokines were also induced, including IL-6, G-CSF, and SAA3 itself. SAA3 protein production was also augmented in podocytes exposed to SAA.

**Conclusions:** SAA induces a wide-ranging inflammatory response in podocytes, making it a likely candidate to instigate inflammatory mechanisms in diabetic glomeruli. Increased expression of an SAA gene, and consequent mRNA translation, in response to exposure to an SAA isoform suggests up-regulation via an autocrine positive feedback loop in podocytes.

*Funding:* Private Foundation Support, Clinical Revenue Support

## SA-PO794

**Blockade of AT1 Receptor Upregulates Vitamin D Receptors in Podocytes through Downregulation of CYP24A1** Tejinder Singh, Nirupama Chandel, Rivka Lederman, Partab Rai, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

**Background:** Blockade of AT1R leads to the slow down in the progression of renal lesions in several kidney disease models. We hypothesized that blockade of AT1R would provide protection against podocyte injury by *de nov*o up regulation of vitamin D receptor (VDR).

**Methods:** Control and HIV transgenic mice (Tg26) were evaluated under control and Telmisaratan treatment (2 wks, Tg/Tel) for renal tissues expression of VDR and CYP24A1. Human podocytes (HP) incubated in media containing either buffer, losartan ($10^{-7}$M) ± Ang II ($10^{-6}$M), or Ang II-stimulating miiieus (HIV, high glucose,30 mM) (n=3). To determine the role of AT1, CIHPs transfected with siRNA-AT1R or scrambled siRNA (SCR) in the presence/absence of losartan.To determine the role of tyrosine kinase, HPs were treated with either buffer or genistein ($10^{-7}$M) ± Ang II. To determine the role of Vit D3, CIHPs were incubated in serum free media (SFM) containing either buffer, vit D3 (10 pM) or losaratan for 24h (n=3). All Protein blots were probed for VDR and CYP24A1 expression.

**Results:** Renal tissues in Tg/Tel mice displayed attenuated VDR but enhanced CYP24A1 expression. In *in vitro* studies Ang II, glucose and HIV down regulated podocyte VDR expression; whereas, losartan enhanced podocyte VDR expression under basal as well as Ang II-, glucose-, and HIV-stimulated conditions. HP/siRNA-AT1 also displayed enhanced VDR but attenuated CYP24A1 expression. Ang II, HIV and glucose stimulated podocyte expression of CYP24A1. Interestingly, losartan enhanced VDR expression only in the presence of media containing either serum or vitamin D3. These findings suggested that AT1 blockade-induced down regulation of CYP24A1 prevented degradation of vitamin D3, which might have resulted in the up regulation of VDR. Since losartan treated HIV/podocytes silenced for VDR displayed greater apoptosis, it appears that losartan-induced de novo up regulation of VDR also contributed partially to the protection offered by AT1R blockade.

**Conclusions:** AT1 blockade upregulates VDR through down regulation of CYP24A1 and the former provides protection against HIV-induced podocyte injury.

*Funding:* NIDDK Support

## SA-PO795

**Rapid Multiplex Detection of *E. coli* and *K. pneumonae* by Loop Mediated Isothermal Amplification in Urine** Ashish Yeri,[2] Ishwad Chandra,[1] Abhay N. Vats.[1] *[1]Nephrology, Children's Hospital of Pittsburgh; [2]Chemical and Petroleum Engineering, Univ of Pittsburgh.*

**Background:** The need for rapid point-of-care diagnostics for infectious diseases has been steadily increasing globally, especially in low resource settings. *E. Coli* and *K. pneumonae* are responsible for a large proportion of urinary tract infections (UTI) as well as other infections. Detection of these organisms is generally done by bacterial culture which usually takes 48 hours or more. Recently, a loop mediated isothermal amplification (LAMP) technique has been developed which amplifies the DNA at around 63-65 °C with high specificity in 30-60 minutes or less. We utilized LAMP protocols and lateral flow display to detect these two common UTI associated organisms in a multiplex reaction.

**Methods:** The LAMP procedure utilizes 6 primers and can be performed on a regular water bath or a heating block maintained at a constant temperature of 60-65 C. The detection of the LAMP *E. Coli* and *K. pneumonae* amplification product was performed on lateral flow display (LFD) strips LFD primarily consists of a nitrocellulose membrane with antibodies immobilized at specified locations. Labeled primers, namely biotin, digoxigenin (*K. pneumonae)* and FITC labeled (*E. Coli* ) outer primers were and the detection is performed using a sandwich type immunoassay. We analyzed 98 de-identified residual urine samples from patients with suspected UTI with LAMP and LFD and compared results with culture and PCR.

**Results:** LAMP amplification took about 30 minutes or less which was followed by LFD detection in 5 minutes or less. LFDs showed a clear visible lines for *E. Coli* and *K. pneumonae* at the immobized anti-FITC or anti-digoxigenin locations and easy distinction between the positive and negative samples. LAMP had a higher sensitivity (98%) when compared to culture and was similar to PCR, making this assay a reliable, rapid and inexpensive diagnostic tool.

**Conclusions:** LAMP is an efficient and cost effective method for rapid detection of common UTI associated organisms. The technique reuires no sample preparation and can yield fast and precise results in appx 30 minutes. The technique is amenable for use in low resource settings as well as ER and out patient clinics.

## SA-PO796

**Pseudohyperphosphatemia during Exposure to Liposomal Amphotericin B Therapy** Nicole Bohm,[1] Katherine Hoover,[1] Amy E. Wahlquist,[3] Juan Carlos Q. Velez.[2] *[1]Dept of Pharmacy, Medical Univ of South Carolina; [2]Div of Nephrology, Medical Univ of South Carolina; [3]Dept of Public Health Sciences, Medical Univ of South Carolina.*

**Background:** Reports have emerged linking exposure to liposomal amphotericin B (L-AmB) therapy with artifactually elevated serum phosphorus (sPhos) levels (pseudohyperphosphatemia) caused by interference of the phospholipid component of L-AmB with the kinetic-based clinical assay. However, this phenomenon remains under-recognized.

**Methods:** We reviewed records of adult hospitalized patients treated with L-AmB or fluconazole (Fcz, control) at our institution in the last 5 years, excluding those with estimated glomerular filtration rate (eGFR) < 20 ml/min.

**Results:** Among those without chronic kidney disease (CKD) [eGFR > 59] and no concomitant acute kidney injury (AKI) [50% rise in serum creatinine], *de novo* high sPhos [>5.0 mg/dL] occurred more often during L-AmB therapy compared to Fcz therapy [36% (12/33) vs 13% (63/491), respectively, p<0.01]. Very high sPhos [>7.0 mg/dL] was also more common during L-AmB therapy [6% (2/33) vs <1% (2/491), respectively, p<0.01]. Among those with CKD [eGFR 20-59] and no concomitant AKI, high sPhos also occurred more often during L-AmB therapy [60% (12/20) vs 24% (45/184), p<0.01]. Similarly, very high sPhos was more common during L-AmB therapy [15% (3/20) vs 3% (6/184), p=0.02]. None of the L-AmB-treated subjects with high sPhos received phosphorus supplements. After adjustment for a reciprocal drop in serum calcium, use of phosphorus binders and baseline eGFR, L-AmB-treated patients remained more likely to have high sPhos than the Fcz group [4.0 (95% CI: 1.9 to 8.6) times in non-CKD and 4.3 (95% CI: 1.6 to 11.7) times in CKD, p<0.01]. In the presence of concomitant AKI, no differences were observed. In addition, we identified 6 cases where unexpectedly high sPhos levels during L-AmB therapy prompted a re-run of the sera utilizing a confirmatory endpoint-based assay. In all 6 cases, re-run values were normal [median spurious rise in sPhos: 1.7 (0.6 - 3.6) mg/dL].

**Conclusions:** We conclude that pseudohyperphosphatemia may not be uncommon during L-AmB therapy. Awareness of this phenomenon should be raised.

## SA-PO797

**Utility of the Urinalysis in the Diagnosis of Primary Glomerular Disease: Report of the NEPTUNE Collaborative Study** Howard Trachtman,[1] Richard A. Lafayette,[2] J. Troost,[3] Debbie S. Gipson,[3] Matthias Kretzler,[3] Peter J. Nelson,[4] Marie C. Hogan,[5] John C. Lieske,[5] Sharon G. Adler,[6] Heather N. Reich,[7] Kevin E.C. Meyers.[8] *[1]NYU Langone Medical Center, New York, NY; [2]Stanford Univ, Stanford, CA; [3]Univ of Michigan, Ann Arbor, MI; [4]Univ of Washington, Seattle, WA; [5]Mayo Clinic, Rochester, MN; [6]Harbor-UCLA Medical Center, Torrance, CA; [7]Toronto General Hospital, Toronto, Canada; [8]Children's Hospital of Phila., Philadelphia, PA.*

**Background:** Urinalysis (UA) is a key screening test in patients with primary glomerular disease (PGD) and proteinuria. Although differences in UA findings have been reported in patients with the various forms of PGD, there is little information about the utility of the UA in the diagnosis of PGD in a large unbiased disease population. Therefore, we assessed the value of the UA in making the diagnosis of minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS) and membranous nephropathy (MN) in patients enrolled in the NEPTUNE cohort study with a confirmed histopathological diagnosis (n=376).

**Methods:** Dipstick testing results were recorded for hematuria (n=189) and leukocyturia (n=167). All patients had proteinuria (> 500 mg/d) and required a diagnostic kidney biopsy. Hematuria or leukocyturia above trace was considered positive. UA results were compared between patients in the three categories of PGD and those with other diseases, predominantly IgA nephropathy, immune complex glomerular disease, and diabetic nephropathy.

**Results:** There were 56 cases of MCD, 47 of FSGS, 34 of MN, and 52 with other glomerular disease with UA testing. Hematuria was less frequent in those with MCD/FSGS (32%) vs MN (59%) or other glomerular diseases (67%) (P<0.01). Leukocyturia was generally absent in patients with MCD, FSGS, and MN but low-grade leukocyturia was more common (15%) in those with other glomerular diseases (P<0.05).

**Conclusions:** Patients with MCD and FSGS are less likely to have hematuria compared with MN. Although hematuria can be used to assess the probability of MN vs. MCD or FSGS, it is not a discriminating factor. The presence of leukocyturia was indicative of other PGD.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO798

**Short Synacthen Test: Outcome in Patients with Glomerulonephritis on Long Term Steroids** Roshni Rathore, Michael Venning. *Dept of Renal Medicine, Central Manchester Univ Hospitals NHS Trust, Manchester, United Kingdom.*

**Background:** Although potency, dose and duration of steroid use are important predictors of presence of hypothalamic-pituitary axis suppression, the preferred method to assess adrenocortical function is the response to synthetic ACTH. Short synacthen test is widely used for assessing adrenal function prior to weaning off long term steroids.

In our regional renal vasculitis clinic, we see a large population of renal patients with glomerulonephritides requiring long term immunosuppression. We specifically looked at our practice of performing short synacthen test before completely stopping steroids in this population.

**Methods:** We retrospectively analysed data obtained from biochemistry database at University Hospitals of South Manchester along with patients' case notes. We gathered data on 50 patients who had one or subsequent short synacthen test between 1993 and 2007. These patients were in remission and had been maintained at 5 mg daily or less of Prednisolone for some weeks or months. We divided them into those that had been successfully weaned, those that are still on steroids and those that were back on steroids after complete withdrawal.

**Results:** Time taken to completely come off steroids ranges from 3 months to 30 months. Three different regimes are being used for tapering the dose-1) Reduction by 1 mg every month 2) Missing dose a day and subsequently longer in a week 3) Alternate day dosing and reduction. Relapse or presence of active disease has been the main reason for prolonged continuation of steroids. There was no documented evidence of major complication in the form of addisonian crisis when steroids were weaned after adequate cortisol response to synacthen. Most patients have required 60 min test to confirm adequate response.

**Conclusions:** 1. A 60 minute cortisol rise of > 250 nmol/l can adequately predict safe steroid withdrawal if undertaken over several months; 2. An adequate cortisol rise of > 250 nmol/l at 30 min is not necessary for subsequent safe steroid withdrawal; 3. With this protocol, a peak rise in cortisol to >550 nmol/l is not necessary for subsequent safe steroid withdrawal as traditionally described.

## SA-PO799

**Exosomal mRNA for Biomarkers of Renal Injury Is Not Increased after Renal Transplantation** Zoltan H. Endre, Philip Peake, Timothy J. Pianta, Lena Succar. *Dept of Nephrology, Prince of Wales Hospital, Randwick, NSW, Australia.*

**Background:** mRNA extracted from urinary exosomes may provide a snapshot of altered renal cell metabolism after injury, preceding or paralleling changes in urinary biomarker protein concentrations.

**Methods:** Exosomes were isolated using nanomembrane concentrators from urine obtained immediately following renal transplantation, from patients with stable kidney transplant function, from healthy volunteers, and from patients with CKD complicated by proteinuria. The expression of exosomal mRNA for neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), cystatin C and kidney injury molecule-1 (KIM-1) was compared with levels of the corresponding urinary protein and18S RNA.

**Results:** Urinary protein concentrations of all biomarkers of injury increased after kidney transplantation, and exosomal18S RNA increased after transplantation and in CKD. Exosomal mRNA for NGAL, IL-18 and cystatin C was detected in all subjects with CKD and in some subjects in all groups, except in patients with stable transplant function. However, mRNA for KIM-1 was mostly undetectable. mRNA expression did not correlate with urinary protein concentrations. NGAL mRNA was lower following acute transplantation than in normal subjects and those with CKD, although urinary NGAL had risen by 4h post transplantation, and was high in CKD. IL-18 mRNA in CKD was higher than after transplantation, or in normal subjects, while urinary IL-18 levels in CKD were normal.

**Conclusions:** mRNA for NGAL, IL-18 and cystatin C is present in urinary exosomes, but levels do not reflect urinary protein concentration or acute injury. This may result from selective sorting of mRNA to exosomes by the parent cell, or by immunosuppression acting on these cells.

## SA-PO800

**Induction of Chitotriosidase in Human Macrophages by Incubation with Cystine Crystals: Clinical Utility in Nephropathic Cystinosis** Mohamed A. Elmonem,[1] Lambert Van den Heuvel,[2] Neveen Soliman,[3] Elena N. Levtchenko.[2] *[1]Clinical and Chemical Pathology, Inherited Metabolic Disorder Laboratory (IMDL), Faculty of Medicine, Cairo Univ, Cairo, Egypt; [2]Pediatric Nephrology, Growth and Retardation, Univ Hospital Leuven, KU Leuven, Leuven, Belgium; [3]Pediatrics, Center for Pediatric Nephrology and Transplantation (CPNT), Faculty of Medicine, Cairo Univ, Cairo, Egypt.*

**Background:** Nephropathic Cystinosis (NC) is an inherited autosomal recessive disorder. Mutations of the *CTNS* gene, coding for cystinosin, lead to accumulation and crystallization of cystine inside different cells. The aminothiol cysteamine is the only specific treatment for cystinosis, and so far, cystine content in white blood cells (WBC) is the main diagnostic tool and the only therapeutic monitor. Chitotriosidase enzyme is a human chitinase produced by activated macrophages. Its elevation was documented in many lysosomal disorders, and is used as a therapeutic monitor for enzyme replacement in Gaucher disease.

**Methods:** Different concentrations of cystine crystals were incubated with control human macrophages in-vitro to detect macrophage activation by assaying TNF-α in culture supernatant and chitotriosidase induction by measuring enzyme activity and mRNA expression in the cell lysate. Chitotriosidase activity was also measured in the plasma of 45 NC patients, and compared with matched 54 normal controls and 24 renal disease patients. Chitotriosidase levels were also correlated with cystine in WBC for 33 patients on cysteamine therapy.

**Results:** TNF-α, released in supernatant, increased up to ten folds after cystine crystal incubation and correlated with crystal concentration. Chitotriosidase activity and mRNA expression were also significantly increased. Enzyme activity (nmol/ml/h) in NC patients (0-3880, median 163) was significantly elevated compared to normal controls (0-72, median 14) and renal patients (2-144, median 39), $P<0.001$ for both groups. Chitotriosidase activity also correlated with cystine content in WBC ($r = 0.8$).

**Conclusions:** Chitotriosidase enzyme is a promising screening marker for NC patients among the clinically suspected and a probable therapeutic monitor for cysteamine therapy.

## SA-PO801

**Urinary microRNAs Are Stabilised by Association with Argonaute 2 Protein** Donald Fraser, Cristina Beltrami, Timothy Bowen. *Section of Nephrology, Cardiff Univ School of Medicine, Cardiff, United Kingdom.*

**Background:** microRNAs (miRs) are endogenous, short, non-coding single-stranded RNA transcripts that regulate gene expression at the post-transcriptional level. Recent work has shown that miRs found in serum and plasma are present in microvesicle-free and microvesicle-associated form, in each case showing a resistance to RNase activity that enhances their disease biomarker utility. Urinary miRs represent a potentially novel source of biomarkers for chronic kidney disease (CKD), but their localisation to microvesicles and sensitivity to degradation remain poorly characterized. The purpose of this study was to identify the location of miRs in urine, and to investigate their stability.

**Methods:** Microvesicle-free and microvesicular urinary fractions were prepared by sucrose gradient ultracentrifugation prior to analysis by flow cytometry, immunoblotting and RT-qPCR. Endogenous urinary miR stability was then compared with that of spiked-in, exogenous, C. elegans-specific cel-miR-39 using RNase or proteinase K digestion followed by RT-qPCR. Protein:miR associations were analysed by RNA-immunoprecipitation (RNA-IP).

**Results:** Over 95% of urinary miRs were present in microvesicle-free urine, with the majority of the remainder associated with exosomes. Endogenous miRs had significantly greater resistance to RNase degradation than cel-miR-39 in urine samples from both control subjects and proteinuric diabetic nephropaths. Proteinase K digestion significantly decreased endogenous miR stability, suggesting protection by protein binding partners. Investigation of putative partners using RNA-IP showed association between urinary miRs and Argonaute 2, a protein component of the RNA-induced silencing complex, but not albumin.

**Conclusions:** Our data demonstrate that the majority of urinary miRs are not associated with microvesicles, provide a mechanism by which their stability is enhanced, and suggest that miRs do not freely cross the glomerular filtration barrier. These findings have important implications for the use of urinary miRs as a novel class of CKD biomarkers.

*Funding:* Pharmaceutical Company Support - BBInternational

## SA-PO802

**Phospholipase A2 Receptor Staining in Biopsies of Stage I Primary Membranous Glomerulonephritis** Margaret Ryan, Gyongyi Nadasdy, Anjali A. Satoskar, Sergey V. Brodsky, Tibor Nadasdy. *Pathology, The Ohio State Univ Wexner Medical Center, Columbus, OH.*

**Background:** Most patients with primary membranous glomerulonephritis (MGN) have circulating IgG4 antibodies directed against the phospholipase A2 receptor (PLA2R) with positive glomerular capillary deposits for IgG4 and PLA2R by immunofluorescence. We recently described that the deposits in early (glomerular stage I) primary MGN tend to be IgG1 dominant (Huang et al., Mod Pathol 26: 799-805, 2013). To better characterize the role of PLA2R in primary early stage MGN, we evaluated the reactivity of glomerular stage I primary MGN biopsies with antibodies to the PLA2R.

**Methods:** From our biopsy archives, we identified fifteen cases of primary (by clinical history) glomerular stage I MGN. We stained the biopsies for the IgG subclasses by direct immunofluorescence on frozen sections and for PLA2R by indirect immunofluorescence on paraffin-embedded sections.

**Results:** The majority (n=11) of these cases were IgG1 dominant or codominant. Six cases were IgG1 dominant, four codominant for IgG1/IgG4, one codominant for IgG1/IgG2, and four IgG4 dominant. Two of the cases (one IgG1 dominant and one IgG1/IgG4 codominant) did not have glomeruli available for evaluation for PLA2R staining. All cases with IgG1 dominant glomerular deposits (n=5) were negative for PLA2R. One of these cases had serum PLA2R testing, which was also negative. Because of the retrospective nature of the study, we were unable to compare serum PLA2R antibodies with the histologic findings for the other cases. Three of the four cases with dominant glomerular IgG4 and the three cases with codominant IgG1/IgG4 staining were positive for PLA2R. The biopsy with IgG1/IgG2 codominant glomerular staining was negative for PLA2R.

**Conclusions:** The lack of PLA2R staining in all cases of IgG1-dominant early stage MGN suggests that in very early stage primary MGN antigens other than the PLA2R may be the target antigens. Alternately, it is possible the PLA2R initially evokes an IgG1 response which later may switch into an IgG4 response, but serial biopsies to prove this are unavailable.

## SA-PO803

**Biomarkers of Interstitial Kidney Pathology in Lupus Nephritis – The CKD Biomarker Consortium** Brad H. Rovin,[2] Huijuan Song,[2] Cassandra L. Hines,[2] Haifeng M. Wu,[2] Vasan S. Ramachandran,[4] Paul L. Kimmel,[3] John W. Kusek,[3] Harold I. Feldman,[5] Michael Merchant,[1] Jon B. Klein.[1] *[1]Univ of Louisville; [2]Ohio State Univ; [3]NIDDK; [4]Boston Univ; [5]Univ of Pennsylvania.*

**Background:** Interstitial inflammation (INF) correlates with long-term renal outcomes in lupus nephritis (LN). Monitoring the interstitium during therapy to determine if INF is resolving or interstitial fibrosis (FIB) is increasing could improve outcomes.

**Methods:** Discriminant analysis was used to design non-invasive biomarker panels that could distinguish INF and FIB in > 25% of interstitium. Urine biomarkers tested were chosen as plausible candidates from literature, or from differentially-expressed urine proteins identified by mass spectrometry followed by ELISA from LN patients with varying levels of INF and FIB on biopsy (n=81). Combinations of MCP-1, hemopexin (HPX), endothelial protein C receptor (EPCR), serum creatinine (SCr), and proteinuria were tested for the ability to discriminate between levels of INF and FIB ≤ and >25% involvement.

**Results:** The function that best discriminated ≤25% INF from >25% INF was Y=1.2*log(uMCP-1) + 3.7*log (SCr) + 1.2*log (uHPX) – 3.6, where Y≥0 identified >25% INF with 82% accuracy and an area under the ROC curve of 0.89. The discriminant function best distinguishing ≤25% FIB from >25% FIB was Y= -2.8*log (EPCR) + 1.8*log (uHPX) +10.2, where Y≥0 identified >25% FIB with 78% accuracy and an area under the ROC curve of 0.85. To determine discriminant functions' specificity for interstitial changes, they were applied to inflammatory or fibrotic glomerular lesions. The INF equation had only 63% accuracy identifying glomerular necrosis or cellular crescents in ≥ 25% of glomeruli. The FIB equation had 65% and 60% accuracy in identifying glomerulosclerosis or fibrous crescents in more than 25% of glomeruli, respectively.

**Conclusions:** These data demonstrate the potential value of combining biomarkers into diagnostic panels that discriminate between levels of interstitial pathology in LN with good accuracy and specificity. If validated, these panels can be of potential use to follow interstitial injury prospectively, and guide therapy.

*Funding:* NIDDK Support

## SA-PO804

**Urinary Podocyte Specific mRNA in Alport Syndrome** Larysa T. Wickman, Farsad Afshinnia, Su Qing Wang, Mahboob A. Chowdhury, Ryuzoh Nishizono, Jocelyn E. Wiggins, David B. Kershaw, Roger C. Wiggins. *Univ of Michigan.*

**Background:** There is increasing evidence that podocytes may play an important role in pathophysiologic mechanism leading to progression in Alport syndrome. It was shown that type IV collagen originates solely from podocytes. Studying urinary expression profiles of podocyte specific mRNA podocin may help to understand pathophysiologic mechanisms of Alport disease progression, guide treatment and lead to biomarker discovery that can aid assessment of novel therapies.

**Methods:** Urine pellet RNA is isolated and reverse-transcribed to cDNA using High-Capacity cDNA Reverse Transcription Kits. Quantitation of podocin mRNA was performed using the 7900 HT Fast Real-Time PCR System. All assays were performed against cDNA standards to allow data to be expressed in moles per gram of creatinine. Normal range was derived from single samples of urine from individuals without kidney disease and compared to average values for each clinic patient with Alport.

**Results:** We have collected and analyzed 20 urine samples from 10 patients with Alport syndrome and 291 urine samples from controls. This included 6 patients with biopsy proven Alport, 3 patients with hematuria/ proteinuria and family history of Alport and 1 patient with hematuria/proteinuria and thin GBM on biopsy. Majority of the patients (80%) were treated with Renin-Angiotensin System (RAS) inhibitors. Urine samples from Alport patients contained 23 fold elevated amounts of podocin mRNAs when compared to controls (P<0.001).

**Conclusions:** We have shown that podocin mRNA levels are significantly higher in representative samples of patients with Alport compared to normal controls. We need to extend the observation to examine whether this methodology can be used to identify Alport disease progression at an early stage before development of significant proteinuria and to determine whether initiation of RAS inhibition on early stages can slow the rate of podocyte detachment.

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## SA-PO805

**Urinary Podocalyxin Excretion Levels and Podocyte Damage** Masahiro Hagiwara,[1] Akira Hiwatashi,[1] Hirayasu Kai,[1] Joichi Usui,[1] Naoki Morito,[1] Chie Saito,[1] Keigyou Yoh,[1] Hiroyuki Kurosawa,[2] Kunihiro Yamagata.[1] *[1]Nephrology, Clinical Medicine, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Denka Seiken Co., Ltd., Niigata, Japan.*

**Background:** Podocalyxin (PCX), the major sialoprotein of glomerular epithelial cells (podocytes) is expressed on apical membrane, and helps maintain the architecture of the foot process and the patency of the filtration slits. PCX was excreted in urine as vesicle form in various glomerular diseases and this vesicle was originated from microvilli of podocyte membrane by shedding.

**Methods:** We examined urinary level of PCX and ultrastructural podocyte changes in rat animal models of membranous nephropathy (active Heymann nephritis (AHN)), minor change nephrotic syndrome (early phase of puromycin aminonucleoside nephritis (PAN)) and focal segmental glomerulosclerosis (late phase of PAN). AHN was induced by ip injection of Freund's complete adjuvant and renal cortex homogenate. PAN wad induced by 4 times (day 1, 28, 35, 42) ip injection of PA, and early phase (day 10) and late phase (day 52) were studied.

**Results:** Urinary PCX levels were higher in AHN and early phase PAN rats than normal control rats and late phase PAN rats.

| | n | proteinuria (mean ± SD, mg/day) | urinary PCX (mean ± SD,ug/day,) |
|---|---|---|---|
| active Heymann nephritis | 10 | 251 ± 100 | 20.1 ± 10.3 |
| early phase of PAN | 5 | 183 ± 69 | 6.3 ± 3.2 |
| late phase of PAN | 5 | 224 ± 74 | 1.3 ± 1.2 |
| normal control | 10 | 8 ± 6.5 | 2.3 ± 1.5 |

AHN rats showed a significantly higher level of urinary PCX than early and late phase PAN rats with same level proteinuria. Numerous microvilli formations of podocytes were observed in AHN than both phase PAN rats. Although, the podocyte numbers of late phase PAN rats showed only 10% decrease than early phase PAN, urinary PCX levels of late phase were markedly decreased to 20% of early phase PAN.

**Conclusions:** Among the proteinuric glomerular diseases, urinary PCX excretion was affected by podocyte microvilli formation, podocyte number and additional podocyte dysfunction.

## SA-PO806

**Light Microscopic Identification of Urinary Podocytes Using Latex Particles Bound with Anti-Podocalyxin Antibody** Masanori Hara,[1] Hiroyuki Kurosawa.[2] *[1]Pediatrics, Yoshida Hospital, Tsubame, Niigata, Japan; [2]Research and Development, Denka Seiken Co., Ltd., Gosen, Niigaata, Japan.*

**Background:** One of the causes for podocytopenia is detachment of podocytes from the GBM and leakage into the urine. While urinary podocytes are usually detected using immunofluorescence (IF), in the current study we developed a reliable light microscopic procedure using latex particles bound with an anti-podocalyxin (PCX) antibody.

**Methods:** An anti-PCX monoclonal antibody was coupled with latex particles (0.4-3.0 um) with various latex colors, and the particles checked for binding to PCX using an ELISA plate bound with purified PCX from isolated glomeruli. These particles were thenfurther examined for binding to PCX-transfected HEK cells, as well as urine samples from patients with glomerulonephritis. Optimal conditions for light microscopic identification were determined by comparing various factors, such as the types of anti-PCX antibodies, particle sizes, colors, pretreatment of the samples and incubation conditions. Quantitation of urinary podocytes counted by this new procedure was then directly compared with that by IF.

**Results:** The best conditions for the identification of urinary podocytes were as follows; 1) the antibody which reacts with sugar components of PCX, 2) 1 um of particle size, 3) blue color for particles, 4) 30 minutes for incubation period with two-way shaking, 5) pretreatment of urine samples by EDTA buffer. When 60 urine samples containing various densities of urinary podocytes were compared using these two detection methods, a significant positive correlation (rs=0.85 by Spearman; $P$<0.001) was found, which had both good specificity and sensitivity (0.90 and 0.80, respectively). The time needed for the entire new procedure, including microscopic examination, was ~ 60 min., compared to ~150 min. for the IF procedure.

**Conclusions:** We have developed a new method to detect urinary podocytes using antibody-bound latex beads that is faster, simpler, and equally reliable to the historical IF-based method. This new approach thus offers great advantages for the expansion of the use of semi quantitative podocyturia measurement as a practical biomarker for podocyte injury.

## SA-PO807

**Cytokines Profile in ICU Patients with Sepsis and AKI** Anna Clementi,[1] Grazia Maria Virzì,[2] Massimo de Cal,[2] Claudio Ronco,[2] Antonio Granata.[1] *[1]Nephrology Dep, Agrigento; [2]Nephrology Dep-IRRIV, Vicenza, Italy.*

**Background:** Sepsis, defined as systemic inflammatory response syndrome associated with an infectious disease, is a primary cause of morbidity and mortality in ICU patients (pts) with mortality rates 20-60% for septic shock. Sepsis is also a contributing factor for AKI, with cases requiring renal replacement therapy (RRT). Several mechanisms contribute to AKI development.

We examined the inflammatory profile in ICU pts and investigated the possible correlation between cytokines and the development of sepsis and AKI requiring RRT(AKI_ RRT), as well as the outcomes (out) in these pts.

**Methods:** 56pts (37Male, 63±15yrs) were recruited for the study at admission in ICU. 38/56pts had Endotoxin value higher than .40 units, used as cut-off value, 25/56pts had AKI_RRT. 40/56pts had negative out during ICU stay. We measured TNFα, IL6, IL18 in ICU pts sera. Mann-Whitney test was used for cohort comparisons. Statistical significance was set at p <.05.

**Results:**

| | No septic | Septic | NoAKI_RRT | AKI_RRT | Positive_out | Negativa_out |
|---|---|---|---|---|---|---|
| TNFα pg/ml | 28.7, 18.3-142.4 | 89.9, 34.2-135.7 | 131.7, 90.8-148.9 | 30.9, 19.5-70.1 | 44.1, 21.8-132.6 | 109.1, 81.8-186.2 |
| IL6 pg/ml | 407.7, 286-461 | 297.3, 177-449 | 332.6, 230.5-449 | 237.3, 183.9-445.1 | 305.6, 205.1-464.7 | 309.5, 195.6-439.7 |
| IL18 pg/ml | 398.4, 265-1189 | 1752, 1283-2597 | 933.5, 733.9, 1211.7 | 1886.9, 1470.7-2942.7 | 1586.3, 879-2156 | 2409, 1567-3125 |

There was no significant difference in TNFα and IL6 levels between septic and non septic pts, while IL18 resulted to be significantly higher (p<.01) in septic pts. TNFα and IL18 levels were significantly higher in AKI_RRT pts compared with NoAKI pts(p<.01),

while IL-6 did not differ significantly. TNFα and IL18 levels were significantly higher (p=.012 and p=.017, respectively) in pts with poor out, while there was no significant difference in IL6 levels.

**Conclusions:** Our study suggests that IL6 may be considered an unspecific inflammatory index, whose levels may increase in critically ill pts, independently on the development of sepsis or AKI. TNFα and IL18 levels seem to correlate sepsis and AKI in ICU pts and their increase is associated to poor out.

## SA-PO808

**Evaluation of Endogenous Insulin Secretory Capacity in Patients with Advanced Chronic Kidney Disease** Yukinari Yamaguchi,[1] Koji Harada,[1] Juri Tukahara,[1] Yuto Kasahara,[1] Kouichi Sumida,[1] Yasuhiro Akai.[2] *[1]Dept of Nephrology and Rheumatology, Rakuwakai Otowa Hospital, Yamashina, Kyoto, Japan; [2]Center for Diabetes Care and Research, Nara Medical Univ, Kashihara, Japan.*

**Background:** Diabetes is one of the major causes of ESRD, and adequate glycemic control is important to prevent the progression of diabetic nephropathy. Accurate evaluation of endogenous insulin secretory capacity (EISC) is important in choosing proper diabetic medications because the efficacy of these medications in lowering blood glucose could be dependent on the degree of EISC. There were few reports regarding EISC in advanced CKD patients. We conducted this study to investigate the methods to evaluate EISC in advanced CKD patients.

**Methods:** Thirty-four non-dialysis CKD patients were divided into 2 groups as follows: DM group (DM), patients with 2-h plasma glucose ≥ 200 mg/dl during the 75-g oral glucose tolerance test (OGTT); non-DM group (non-DM), 2-h plasma glucose < 200 mg/dl. To assess the EISC, we evaluated fasting serum insulin, plasma C-peptide, 24h-urinary C-peptide, and glucagon stimulation test (GST). Furthermore the insulinogenic index (II) or incremental area under the curve of insulin [ΔI(AUC)] divided by the incremental area under the curve of glucose [ΔG(AUC)] was calculated during the 0- to 30-min and 0- to 120-min time period of OGTT.

**Results:** Twenty-three patients were in DM and 11 in non-DM. There was no significant difference in the eGFR, HbA1c, and 24h-urinary C-peptide between DM and non-DM (22.0±9.7 vs 24.1±11.2 ml/min/1.73 $m^2$, $p$=0.64, 7.2±1.7 vs 6.2±0.9%, $p$=0.0525, 20.9±12.6 vs 25.9±24.6 μg/day, $p$=1.0). In 75-g OGTT, fasting serum insulin and C-peptide were not significantly different between 2 groups (7.1±6.1 vs 6.4±3.1 μU/ml, $p$=0.94, 3.4±1.7 vs 2.9±1.2 ng/ml, $p$=0.57). However, II, ΔI(AUC)/ΔG(AUC), and the difference of C-peptide after GST were significantly lower in DM than in non-DM (0.46±1.50 vs 0.70±0.68, $p$=0.0016, 0.12±0.11 vs 0.31±0.17, $p$=0.0010, 1.74±0.96 vs 3.41±1.99, $p$=0.0194).

**Conclusions:** Whereas the estimation of EISC using fasting blood sample is inappropriate, OGTT and/or GST should be performed to properly evaluate EISC in advanced CKD patients.

## SA-PO809

**A National Survey of the Availability and Use of Urine Microscopy in the UK** Shafi Malik.[1] *[1]Nephrology, Toronto General Hospital, Toronto, Canada; [2]Nephrology, Wrexham Maelor Hospital, Wrexham, North Wales, United Kingdom.*

**Background:** Urine microscopy has been used as a diagnostic tool since the 17th century,its use has been steadily declining.Studies have shown its use in differentiating between the causes of Acute Kidney Injury.Urine microscopy findings are found to correlate to renal histology.Urine microscopy is considered a basic and essential skill for a Nephrologist.We conducted a national survey of all the renal centers both adult & pediatric in the UK,aiming to study the availability and use of urine microscopy by Nephrologists. We also aimed to study whether its use helped in management decisions and the importance of it being included in the Nephrology training curriculum as an essential skill.Current Nephrology trainees(2011 - 2012)were also surveyed to get their view on urine microscopy being included in the training curriculum.

**Methods:** A web based survey was created.A link to the survey was sent by email to clinical directors of renal units and to Nephrology trainees in the country.All answers were anonymously collected.

**Results:** Of 70 Renal units,26 responded(37%).42% of respondents were from district general hospitals and 58 % from University teaching hospitals.In 5(20%)of the 26 hospitals,Nephrologists routinely performed urine microscopy.In 8(38%) of hospitals where Nephrologists did not perform urine microscopy,urine specimens were sent to Microbiology for microscopy.In centers where urine microscopy was done,63% said it helped in management decisions.In 80% of the hospitals that responded Nephrology trainees were not encouraged to learn and do urine microscopy.53% of Renal Unit clinical directors thought that urine microscopy should be part of the renal training curriculum.78 % of trainees who responded said that urine microscopy helped in management decisions and 57 % said it should be included in the training curriculum.

**Conclusions:** This is the first national survey studying the use of urine microscopy in the UK.Only 20 % of the hospitals that responded currently use urine microscopy.When performed, majority felt it helped in management decisions and more than 50% of trainees and Nephrology leads want it included in the Nephrology training curriculum.

## SA-PO810

**Renal Thrombotic Microangiopathy Associated with Chronic Graft versus Host Disease after Hematopoietic Stem Cell Transplantation** Akiko Mii,[1,2] Akira Shimizu,[2] Seiichiro Higo,[1,2] Shuichi Tsuruoka.[1] *[1]Dept of Nephrology, Nippon Medical School, Tokyo, Japan; [2]Dept of Pathology, Nippon Medical School, Tokyo, Japan.*

**Background:** Thrombotic microangiopathy (TMA) is one of the major complications after hematopoietic stem cell transplantation (HSCT). The pathogenesis of HSCT-associated TMA is controversial but considered to involve various factors such as irradiation, use of calcineurin inhibitors (CNI), infection, and GVHD. Previously, we reported the cases, which involve chronic GVHD after HSCT. The pathologic feature showed severe endothelial injury with glomerular's and peritubular capillary's C4d deposition, and we termed it chronic humoral GVHD.

**Methods:** We examined the clinical and pathologic findings of five renal biopsy cases with pathological TMA, in order to clarify the relation between GVHD and HSCT-associated TMA.

**Results:** All cases showed renal impairment. Acute and chronic GVHD occurred in four and three cases, respectively. Clinical evidence of TMA was detected in only one case. The predominant histologic findings were diffuse endothelial injury such as glomerular capillary collapse, GBM duplication with enlarged subendothelial space, and mesangiolysis. Hyalynosis was notable in small arteries. All cases showed glomerulitis, tubulitis and peritubular capillalitis (PTC-itis) with CD3+ cells infiltration. These suggest that GVHD is involved in the development of TMA. Furthermore, C4d deposition on glomerular capillaries and PTCs was evident, indicating that our cases may be mediated by antibody deposition and complement activation in chronic GVHD. In the clinical course, three cases were treated with prednisolone for renal GVHD, renal function ameliorated gradually. In one case, at that time, he was diagnosed as CNI toxicity. Renal impairment progressed by decreasing the dose of CNI, and finally he required dialysis.

**Conclusions:** Our cases were probably associated with the antibody-mediated renal endothelial injury in chronic GVHD. Three cases had an excellent course by treatment for renal GVHD. Kidney is one of the target organs of GVHD that may be associated with the development of TMA after HSCT. We need to take into consideration increase the dose of immunosuppressive drugs.

## SA-PO811

**Random Urine Protein to Creatinine Ratio (UPCR) Is a Relatively Poor Predictor of 24 hr Protein Excretion in Patients with Glomerular Disease** Marie C. Hogan, Heather N. Reich, Peter J. Nelson, Sharon G. Adler, Daniel C. Cattran, Gerald B. Appel, J. Troost, Debbie S. Gipson, Matthias Kretzler, John C. Lieske. *For the Nephrotic Syndrome Study Network.*

**Background:** A random UPCR is often used to estimate 24 hr urine protein excretion (24P). However, little data is available regarding sensitivity (SE) and specificity (SP), especially for specific glomerular diseases.

**Methods:** The multicenter Nephrotic Syndrome Study Network (NEPTUNE) applies standardized urine collections at defined study visits in the proteinuric diseases minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and membranous nephropathy (MN). Total protein (by pyrogallol red) and creatinine (by enzymatic creatinase) are measured in random and 24 hr urine collections at a centralized biobank at baseline (postbiopsy) and subsequent protocol visits within 2 yrs of diagnosis.

**Results:** Paired baseline random UPCR and 24P data were available from 177 participants (72% adults, 62% male). Mean (median, SD) UPCR = 3.8, (2.4, 4.6) g/g Cr and 24P = 1.8 (0.9, 2.8) g/ 24 hr. 24P was > 1 g in 45% and > 3.5 g in 12%. Biopsies revealed FSGS/MCD in 47%, MN in 12%, and other diseases in 40%. There was a significant relationship between UPCR and 24P (p<0.01) with correlations (r) of 0.51 and 0.84 before and after log transformation (P<0.001). The correlation was stronger in adults (r=0.65) than in children (r=0.36) and stronger in males (r=0.73) than in females (r=0.35). There was no difference by histopathological group. The correlation was particularly weak among those with 24P <1g (r=0.13) compared to those 1-3.5g (r=0.38) and >3g (r= 0.40). Nevertheless, UPCR was a reasonable predictor of a 24P > 1g (AUC by ROC analysis=0.86; SE 79% and SP 80% at UPCR of 2.2 g/g as well as >3.5g (AUC=0.85; SE 86% and SP 74% at UPCR of 1.4 g/g).

**Conclusions:** Among patients with MCD, FSGS, and MN, UPCR correlates moderately with 24P. The correlations are poorer in children, females, and those with 24P< 1g. Nevertheless, our data suggest UPCR can be used with caution to identify those patients with 24P above the clinically relevant cutpoints of 1g and 3.5g.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## SA-PO812

**Cross-Species Transcriptional Analysis of Human and Rat Experimental Membranous Glomerulonephritis Reveals Shared Mechanisms at the Gene and Coexpression Network Level** Viji Nair,[1] Sebastian Martini,[1] Clemens D. Cohen,[2] Matthias Kretzler,[1] J. B. Hodgin.[3] *[1]Internal Medicine, Univ of Michigan, Ann Arbor, MI; [2]Klinische Immunologie, Staedtisches Klinikum Muenchen Harlaching, Munich, Germany; [3]Pathology, Univ of Michigan, Ann Arbor, MI.*

**Background:** Passive Heymann nephritis (PHN) in the rat is the best characterized animal model of membranous glomerulonephritis (MGN). As seen in human MGN, the rat PHN model is distinguished by subepithelial immune deposits, diffuse podocyte foot

process effacement, and proteinuria. In order to elucidate the molecular similarities between human MGN and the rat PHN model, we compared differentially expressed genes (DEGs) from glomeruli of humans with MGN to DEGs from rat PHN glomeruli at the transcript and coexpression network level.

**Methods:** Microarray analysis on the human glomerular mRNA (AffymetrixHGU133A) from 21 participants of the European Renal cDNA Bank and 31 living donors revealed 2179 DEGs (FDR<0.05). Rat glomerular DEGs (2251, FDR<0.05) were obtained from publically available data [Hauser PV et al., Nephron Exp Nephrol 2009;112(2):e43-e58] (5 PHN rats versus 5 untreated controls, AffymetrixR230A).

**Results:** We found an overlap of 931 DEGs between human and rat glomeruli, of which 510 were concordantly upregulated and 223 concordantly downregulated. Pathway analysis revealed significant enrichment of upregulated genes in several KEGG canonical pathways including proteasome regulation, extracellular matrix-receptor interaction, cell cycle, and focal adhesion, whereas the downregulated genes were mainly involved in amino acid and fatty acid metabolism, glycolysis, and PPAR signaling pathways. Cytoskeleton organization, protein transport and localization were among the top enriched biological processes for the upregulated genes. Downregulated DEGs reflected similar metabolic processes. Coexpression network analysis revealed a highly significant conserved co-expression network enriched again for amino acid and fatty acid metabolic processes.

**Conclusions:** Our approach will lead to a better understanding of glomerular disease pathogenesis in human and animal models at the transcriptional and network level.

*Funding:* NIDDK Support

## SA-PO813

### Preservation of HIF-1 and ERK Phosphorylation Are Involved in Hypothermic Protection of Renal Ischemia-Reperfusion Injury

Dae Eun Choi,[1] Jin Young Jeong,[1] Sarah Chung,[1] Yoon-Kyung Chang,[2] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, Chungnam National Univ Hospital, Daejeon, Republic of Korea; [2]Internal Medicine, Daejeon Saint Mary Hospital, Daejeon, Republic of Korea.*

**Background:** Although hypothermia attenuates renal injury induced by ischemia-reperfusion (IR), precise molecular pathways have not been known yet. Our previous study showed ERK phosphrylation plays an important role in hypothermic protection in renal IR injury. Hypoxia-inducible factor-1(HIF-1) has been known as one of the potent protective proteins in IR injury. We evaluated the role of HIF-1 and interaction with ERK phosphorylation in hypothermic protection of renal IR injury.

**Methods:** C57Bl/6 mice were divided into four groups; sham operated mice, cold IR mice(30°C), warmIR mice(37°C) and PD98059(MAP kinase kinase inhibitor) treated cold IR mice(IR injury; reperfusion 27 minutes after clamping of both renal artery and vein). Kidneys were harvested at 10min and 27min after both renal artery ischemia and 24hr after IR injury. Renal HIF-1, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha(PGC 1-alpha), AMP-activated protein kinase(AMPK), and 8-hydroxydeoxyguanosine(8-OHdG) were evaluated by western blot and immuohistochemical stain. BUN and serum creatinine(s-Cr) were measured 24 hrs after IR injury. TUNEL staining of kidneys was performed.

**Results:** Serum creatinine(s-Cr), tissue injury score, and 8-OHdG and TUNEL positive cells in cold IR mice were significantly lower than those of warm IR mice (all, $p<0.01$).s-Cr, and tissue injury score,8-OHdG and TUNEL positive cells in kidneys of PD98059 treated cold IR mice were significantly higher than those ofuntreated cold IR mice(all, $p<0.05$). Renal HIF-1, PGC 1-alpha, and AMPK expression were significantly increased in the kidneys of cold ischemic mice at10min and 27min after both renal artery clamping compared to sham operated mice. PD98059 treatment in cold IR mice decreased renal HIF-1 significantly ($p<0.01$). However, PGC-1 alpha and AMPK were not changed.

**Conclusions:** In conclusion,HIF-1 preservation induced by ERK phosphorylation may be involved in hypothermic protection of renal ischemia-reperfusion injury.

## SA-PO814

### Multiorgan Involvement and Detection of Tissue Gadolinium in a Rodent Model of Nephrogenic Systemic Fibrosis

Tareq Issa Nassar,[1] Brent Wagner.[2] *[1]Internal Medicine Dept, Univ of Texas Health Science Center at San Antonio; [2]Nephrology Dept, Univ of Texas Health Science Center at San Antonio.*

**Background:** Nephrogenic systemic fibrosis (NSF) is an irreversible systemic disorder that is associated with exposure to gadolinium-based magnetic resonance imaging (MRI) contrast in patients with compromised renal function. Gadolinium (Gd) has been detected in many organs in patients with NSF, including the spleen (on autopsy). Although rodent models have been established, to date no laboratory has assayed for tissue gadolinium content in an experimental MRI contrast-induced fibrosis model.

**Methods:** Control-matched Hannover-Wistar rats with 5/6 nephrectomy were treated with 20 doses of gadodiamide/caldiamide (Omniscan, 2.5 mmol/kg IP) over 4 weeks. Sacrifice time points were week 1, week 3 and week 16 in order to follow the evolution and chronicity of the disease. Formalin-fixed and embedded tissues (e.g., skin, spleen, liver) were analyzed with scanning electron microscopy equipped with an energy-dispersive x-ray spectroscopy (EDS) silicone detector (EDAX Genesis). Spectra were obtained under low vacuum (30 Pa), 600 x, 10 mm working distance, 20 kV accelerating voltage. Estimated gadolinium content was compared.

**Results:** Skin and spleen demonstrated pathologic changes evident by light microscopy. In the contrast-treated animals, (normalized for controls), the estimated Gd in spleen (arbitrary, mean ± SD) was 0.01 ± 0.15, 0.51 ± 0.48 and 0.13 ± 0.53 for week 1, week 3 and week 16.

**Conclusions:** Spleens from MRI contrast-treated animals demonstrate pathology evident by light microscopy. This is the first prospective, experimental report to detect gadolinium in an NSF model. Together, these results suggest that gadolinium accumulation in specific tissues can elicit pro-fibrotic effects.

*Funding:* Veterans Affairs Support

## SA-PO815

### Hydrostatic Dialysis: A New Method to Enrich Urinary Vesicles for Clinical Analytics

Luca Musante,[1] Dorota Ewa Tataruch,[1] Alberto Benito Martin,[1,2] Dongfeng Gu,[1,3] Giulio Calzaferri,[1] Harry B. Holthofer.[1] *[1]Centre for Bioanalytical Sciences, Dublin City Univ, Dublin, Ireland; [2]Hematology/ Oncology Dept, TWeill Medical College of Cornell Univ, New York; [3]The Third Affiliated Hospital of Southern Medical Univ, Guangzhou, China.*

**Background:** Secreted vesicles in urine are released from all nephron segments and the epithelial lining the urinogenital tract and have been proposed as valuable source of disease biomarkers. Typically the vesicles contain specific signature proteins reflecting their cellular origin as well as DNA, and a variety of RNA species which suggest of important regulatory and communication functions. Despite their proposed importance, there are still significant challenges to overcome for successful clinical application, starting from simple method which allow to process large volumes of urine to enrich material for both proteomic and transcriptomic analysis. Here we show the full characterization of a novel easy method to simplify and enrich urinary vesicles and extract its transcript content.

**Methods:** In the enrichment method urinary vesicles are first concentrated and dialysed by a simple hydrostatic dialysis system which offers the following advantages: First, handling of large volumes of urine (~ 400mL) within a working day. Second, concentration of vesicle fraction up to 100 times. This easily meets the needs for the biobanking storage of urine sample material in smaller volumes Third, introduction of dialysis step necessary to equalize the physical-chemical features, like electrolyte concentration of the samples and thus to reduce the technical inter-individual variability.

**Results:** Application of this method mimicking a clinical pilot study has revealed that the minimum volume of urinary necessary to perform a proteomic analysis for a discovery phase is not less than 200ml and at least 100ml for a transcriptomic profiling. About 50ml of urine is needed for a validation analysis. Finally, 10ml of urine routinely collected may be utilised for specific targeted approaches including Western blots.

**Conclusions:** Our protocol for vesicle isolation paves the way to utilization of full diagnostic potential of urinary vesicles.

## SA-PO816

### Intraoperative Cell-Saver Blood Product: *In Vitro* Effect on Renal Tubular Cells

Grazia Maria Virzì,[1] Tommaso Hinna Danesi,[2] Alessandra Brocca,[1] Massimo de Cal,[1] Loris Salvador,[2] Claudio Ronco.[1] *[1]Nephrology Dep-IRRIV, Italy; [2]Cardiosurgery Dep, Italy.*

**Background:** The hazards of transfusions has led to an emphasis on blood conservation in cardiac surgery. Mediastinal shed blood has high inflammatory properties, related to the development of post-operatory complications as AKI and renal failure. Many modalities including intraoperative cell-saver (CS) have emerged as alternatives to avoid immunomodulatory effects. The aim of this study was to investigate the effect of CS product on renal tubular cells (RTCs).

**Methods:** 14 patients (64±14yrs) underwent on-pump cardiac surgery were enrolled. An intraoperative CS device was used in each patient. We collected 2 blood samples for each patient: one at the end of cardiopulmonary by-pass (CPB) from the venous reservoir and one from the CS treated blood pack. For each patient, CPB and CS product were incubated with RTCs for 24h and, subsequently, apoptosis and necrosis was evaluated by cytometer. Quantitative analysis of IL6, TNFα and oxidative stress (Endogenous Peroxidase Activity-EPA; Myeloperoxidase-MPO) were performed in CPB and CS samples.

**Results:**

| | %Vitality | %Apoptosis | %Necrosis | IL6 pg/ml | TNFα pg/ml | EPA U/l | MPO pg/ml |
|---|---|---|---|---|---|---|---|
| CPB | 93(89.6-94.6) | 2.5(1.8-6) | 2.9(2.2-7.0) | 136.3 (75.3-375.3) | 28.6 (26.2-31.3) | 49.5 (31.5-55.8) | 276.8(200.6-330.5) |
| CS | 74.9(62.1-79.7) | 21.3(13.9-37.4) | 7.1(4.4-9.2) | 24.8 (19.7-29.8) | 33.4 (33.1-35.2) | 65.2 (55.4-82.2) | 370.6(246.0-405.1) |

A quantitative analysis of cell viability showed significantly higher apoptosis and necrosis rates in RTCs incubated with CS group compared to CPB(both,$p<.01$) with a median Δapoptosis(CS-CPB) from the same patient of 19.5(IQR 11-28.5)and a median Δnecrosis(CS-CPB) of 3.3(IQR 0.7-7.1). IL6 and TNFα were lower in CS($p<.05$). An increase of oxidative stress was observed in CS($p<.05$).

**Conclusions:** Cell viability analysis indicated that CS product induces RTCs death by apoptosis and necrosis. CS product was able to induce a damage on RTCs and systemic disequilibrium caused by oxidative stress. In these patients, there could be injury to nephrons, which may lead to subclinical or recognized AKI with post-operatory complications and poor outcomes.

**SA-PO817**

**Metal-Ion Complexation to Serum Carnosinase Is Increased upon Hemodialysis** Sibylle Jenny Hauske, Shiqi Zhang, Sarah Kabtni, Emmanouil Ntasis, Eleni Stamellou, Bernhard K. Krämer, Benito Yard. *Vth Medical Dept, Medical Faculty Mannheim, Univ of Heidelberg, Mannheim, Germany.*

**Background:** We have previously shown that in serum the carnosinase (CN1) protein is only partly complexed with metal ions and that the relative proportion of not complexed (nc) CN1 negatively correlates with CN1 activity. ncCN1 can be detected by ELISA using RYSK173 (monoclonal IgG) antibody which we have raised against recombinant CN1.

**Methods:** In the present study we evaluated the relevance of Zinc binding to CN1 for detection by RYSK173. Since changes in divalent metal ion concentrations (conc.) have been reported to occur during hemodialysis (HD) we also assessed if HD affects the quality of CN1 by measuring total CN1 and ncCN1 conc. before and after HD.

**Results:** By making use of myc-tagged CN1 fragments we were able to narrow-down the RYSK173 epitope to a stretch of 40 amino acids in close vicinity to the metal binding region of CN1. While in serum ncCN1 constituted only 5% of total CN1, for recombinant CN1 expressed in Cos-7 cells approximately 50 % of total CN1 was recognized by RYSK173. When recombinant CN1 was spiked into human serum, it was poorly detected by RYSK173. Similarly, addition of $ZnCl_2$ to either serum or recombinant CN1 strongly diminished its detection by RYSK173. In HD patients (N=30) we measured both total CN1 and ncCN1 conc. before and after HD. While total CN1 conc. were significantly increased after HD, ncCN1 conc. were decreased. The increased total CN1 conc. positively correlated with the percentage of plasma fluid depletion during HD.

**Conclusions:** In conclusion our data further demonstrate the presence of different qualities of CN1 in human serum. These qualities are due to the extent of metal-ion complexation to CN1. Since the RYSK173 epitope is in close vicinity to the metal binding region of CN1 our data indicate that occupation of this region by metal-ion prevents RYSK173 binding. Interestingly, ncCN1 conc. as detected by RYSK173 significantly decrease after HD suggesting an increased concentration of divalent metal-ions that have complexed to CN1 after HD. The identity of the metal-ions as well as the consequence for CN1 activity remains to be assessed.

*Funding:* Government Support - Non-U.S.

**SA-PO818**

**Osteoprotegerin in Exosome-Like Vesicles from Human Cultured Tubular Cells and Urine** Alberto Benito Martin,[1] Alvaro C. Ucero,[1] Irene Zubiri,[1] Maria Posada Ayala,[1] Beatriz Fernández,[1] Maria Concepción Izquierdo,[1] Marta Ruiz-Ortega,[3] Jesus Egido,[1] Gloria Alvarez Llamas,[1] Alberto Ortiz.[1] *[1]IIS - Fundacion Jimenez Diaz; [2]IDIPAZ; [3]Universidad Autonoma de Madrid.*

**Background:** Urinary exosomes have been proposed as potential diagnostic tools. TNF superfamily cytokines and receptors may be present in exosomes and are expressed by proximal tubular cells. We have now studied the expression of selected TNF superfamily proteins in exosome-like vesicles from cultured human proximal tubular cells and human urine and have identified additional proteins in these vesicles byLC-MS/MS proteomics.

**Results:** Human proximal tubular cells constitutively released exosome-like vesicles that did not contain the TNF superfamily cytokines TRAIL or TWEAK. However, exosome-like vesicles contained osteoprotegerin (OPG), a TNF receptor superfamily protein, as assessed by Western blot, ELISA or selected reaction monitoring by nLC-(QQQ)MS/MS. Twenty-three additional proteins were identifiedin tubular cell exosome-like vesicles, including three (zinc finger protein 40, isopentenyl-diphosphate delta-isomerase and vitamin D binding protein) that had not been previously reported in exosome-like vesicles. Twelve were extracellular matrix proteins, including the basement membrane proteins type IV collagen, nidogen-1, agrin and fibulin-1. Urine from chronic kidney diseasepatients contained a higher amount of exosomal protein and exosomal OPG than urine from healthy volunteers. Specifically OPG was increased in autosomal dominant polycystic kidney disease urinary exosome-like vesicles and expressed by cystic epithelium in vivo.

**Conclusions:** OPG is present in exosome-like vesicles secreted by proximal tubular epithelial cells and isolated from Chronic Kidney Disease urine.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

**SA-PO819**

**Laser Capture Microdissection Enables mRNA and microRNA Quantification of Parietal Epithelial Cells (PECs) and Cellular Crescents from Paraffin Embedded Renal Biopsy Tissue** Clemens L. Bockmeyer, Karen Säuberlich, Stephanie Zell, Philip Zeuschner, Jan U. Becker. *Institute of Pathology, Hannover Medical School, Hannover, Lower Saxony, Germany.*

**Background:** Crescents develop from PECs and indicate a dismal prognosis. So far, studies on PECs were limited because PECs could not be analyzed by quantitative real time-PCR (qRT-PCR) for mRNAs and miRNAs.

This proof-of-principle study shows that our techniques can overcome this limitation and enable mRNA and miRNA expression analysis of PECs and the crescents derived thereof.

**Methods:** Patients with IgA glomerulonephritis (n=4), Schoenlein-Henoch purpura with cellular crescents (n=2) and mild tubulointerstitial nephritis with normal glomeruli (n=3) were analyzed. Four different compartments glomeruli (n=9), PECs (n=9), crescents (n=2) and periglomerular tubulointerstitium (n=9) were isolated from paraffin embedded renal biopsies. For quantitive mRNA and microRNA analysis we used real time quantitative PCR after preamplification (Applied Biosystems). We quantified the compartment markers WT1, PAX2, GLEPP1, CD44, CD45, CD68 against two reference genes (GAPDH, PGK1). microRNAs RNU48 and snRNU6 were also quantified. Results are given as mean value and standard deviation of the relative expression level.

**Results:** Relative expression of WT1 was 18.5±19.3 in glomeruli, 3.6±2.2 in PECs, 0.2±0.4 in periglomerular interstitium, 0.9±0.1 in crescents; of PAX2 0.2±0.3, 2.4±1.8, 3.8±6.2, 1.5±0.7; of GLEPP1 5.6±5.3, 0.8±0.6, 0.1±0.1, 0.2±0.1; of CD44 3.1±5.4, 1.3±0.9, 1.7±1.1, 1.5±0.6; of CD68 3.2±3.6, 3.4±1.9, 3.5±2.5, 4.7±2.8; of CD45 9.3±11.1, 5.3±2.7, 4.8±4.8, 3.6±1.3; of snRNU6 17.6±4.0, 20.1±3.6, not detectable, 17.2±5.6 and of RNU48 19.4±3,7, 21.6±3.0, not detectable, 18.7±1.8.

**Conclusions:** Our results show that it is possible to isolate PECs from other kidney compartments for mRNA and miRNA expression studies. WT1, PAX-2 and GLEPP-1 are the best compartment markers for the identification of PECs. mRNAs and microRNAs can be quantified in PECs and crescents with our method allowing advances in the research on PEC pathology and crescent formation not only in patients with Schoenlein-Henoch purpura.

*Funding:* Private Foundation Support

**SA-PO820**

**Optimal Level of Proteinuria Reduction for Renoprotection in Patients with IgA Nephropathy** Ki Heon Nam,[1] Fa Mee Doh,[1] Hyung Jung Oh,[1] Tae-Hyun Yoo,[1] Shin-Wook Kang,[1,2] Seung Hyeok Han.[1] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul, Korea.*

**Background:** Proteinuria is a target for renoprotection in various glomerular diseases. However, the optimal level of proteinuria reduction is not clearly defined in patients with IgA nephropathy (IgAN). In this study, therefore, we conducted a retrospective observational cohort study to investigate whether reducing proteinuria below the levels suggested by the current guideline may confer a more renoprotective advantage.

**Methods:** Among 644 patients, who were pathologically diagnosed with IgAN between 2000 and 2010, 500 subjects were eligible for the study. Time-averaged proteinuria (TA-P) was calculated as the mean of every 6-month measurements of spot urine protein-to-creatinine ratio. The study endpoints werea doubling of the baseline serum creatinine concentrations (D-sCr) and the onset of ESRD.

**Results:** There were 221 (44.2%), 135 (27.0%), 96 (19.2%), and 48 patients (9.6%) with TA-P of <0.5, 0.5-0.99, 1.0-1.99, and ≥2.0 g/g, respectively. During a median follow-up duration of 65 (12-154) months, D-sCr was observed in 1 (0.5%), 3 (2.2%), 18 (18.8%), and 30 patients (62.5%) of each group (P<0.001).There was no difference in the development of D-sCr between patients with TA-P <0.5 g/g and those with TA-P of 0.5-0.99 g/g. ESRD did not occur in these two groups, while 11 (11.5%) and 23 patients (47.9%) with TA-P of 1.0-1.99 and ≥2.0 g/g, respectively, progressed to ESRD. In a multivariable Cox model after adjustment for age, estimated glomerular filtration rate, blood pressure, pathologic findings, and treatment, the risk of D-sCr did not differ between patients with TA-P of <0.5 g/g and those with 0.5-0.99 g/g (HR, 3.67; 95% CI, 0.34-36.2; P=0.281), whereas itwas significantly higher in patients with TA-P of 1.0-1.99 g/g (HR, 27.6; 95% CI, 3.30-230.5; P=0.002) and those with TA-P >2.0 g/g (HR, 140.6; 95% CI, 16.4-1209.1; P<0.001).

**Conclusions:** The results of our study suggest that the optimal proteinuria levels are <1.0 g/g in patients with IgAN. Further studies are required to clarify whether reduction of proteinuria of <0.5 g/g may confer a more renoprotective advantage.

**SA-PO821**

**Development and Validation of a Prediction Rule Using the Oxford Classification in IgA Nephropathy** Shigeru Tanaka,[1] Toshiharu Ninomiya,[1] Ritsuko Katafuchi,[2] Kosuke Masutani,[1] Akihiro Tsuchimoto,[1] Hideki N. Hirakata,[3] Kazuhiko Tsuruya,[1] Takanari Kitazono.[1] *[1]Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Kidney Unit, National Fukuoka-Higashi Medical Center, Fukuoka, Japan; [3]Div of Nephrology and Dialysis Center, Japanese Red Cross Fukuoka Hospital, Fukuoka, Japan.*

**Background:** The risk assessment for developing end-stage kidney disease (ESKD) remains limited in patients with IgA nephropathy (IgAN). The aim of our study was to develop and validate a prediction rule for estimating the individual risk of ESKD in patients with IgAN.

**Methods:** We followed retrospectively 698 patients with IgAN diagnosed by renal biopsy at Kyushu University Hospital (derivation cohort). The Oxford classification was used to evaluate the pathologic lesions. The risk factors for developing ESKD were evaluated using a Cox proportional hazard model. Furthermore, we verified the prediction rule using the data of 702 patients diagnosed at Japanese Red Cross Fukuoka Hospital (validation cohort). The validation of the goodness-of-fit of the prediction model was assessed using a c-statistic and Hosmer-Lemeshow test (H-L test).

**Results:** In the derivation cohort, 73 patients developed ESKD during the median 4.7-year follow-up. As independent risk factors for developing ESKD, proteinuria, estimated glomerular filtration rate, mesangial proliferation, segmental sclerosis and interstitial fibrosis/tubular atrophy were selected. To create a prediction rule, the score for each variable were weighted by the regression coefficients calculated using the relevant Cox model. The incidence of ESKD was linearly increased with elevating the total risk scores (P for trend <0.001). Furthermore, the prediction rule demonstrated good discrimination (c-statistic: 0.89) and calibration (H-L test, P = 0.78) in the validation cohort.

**Conclusions:** We developed and validated a new prediction rule using clinical parameters and the Oxford classification for developing ESKD in patients with IgAN.

## SA-PO822

### Clinical Implication of Crescentic Lesion in Patients with IgA Nephropathy

Mi Jung Lee,[1] Tae ik Chang,[2] Hyung Jung Oh,[1] Seung Hyeok Han,[1] Dae-Suk Han,[1] Shin-Wook Kang.[1] *[1]Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea; [2]Internal Medicine, NHIC Ilsan Hospital, Gyoeonggi-do, Korea.*

**Background:** Early case series showed that the presence of crescents was associated with proteinuria and rapid deterioration of renal function in patients with IgA nephropathy (IgAN). To date, however, there has been much controversy about the role of crescentic lesions as a significant prognostic factor. In this study, therefore, we elucidated whether crescentic lesions predicted adverse renal outcomes in IgAN patients.

**Methods:** A total of 430 patients with biopsy-proven IgAN diagnosed between January 2001 and December 2010 were included. Histologic variables of the Oxford classification (Oxford-MEST) and the presence of crescents were assessed. The primary endpoint was a 50% decline in estimated glomerular filtration rate (eGFR).

**Results:** Among 430 patients, 81 (18.8%) had crescentic lesions. During a mean follow-up duration of 61 months, the primary outcome was significantly more observed in patients with crescents (19 patient, 23.5%) compared to patients without crescents (40 patients, 11.5%) ($P$ = 0.01). A Kaplan-Meier plot showed that the 10-year renal survival rates were significantly lower in patients with crescents than patients without crescents ($P$ = 0.01). However, in a multivariable Cox regression analysis including clinical factors and the Oxford-MEST score, crescents were not significantly associated with an increased risk of the primary outcome (HR, 0.78; 95% CI, 0.40-1.51; $P$ = 0.48). Moreover, adding crescents to the Oxford classification did not improve the discriminative ability for the prediction of renal outcomes [c-statistic: 0.86 (0.81-0.91) vs. 0.86 (0.80-0.91), $P$ = 0.35].

**Conclusions:** Crescentic lesions were not an independent prognostic factor in patients with IgAN, suggesting that crescents had limited value in predicting renal outcome in these patients. Further studies are required to clarify the clinical implication of crescentic lesions in patients with severe IgAN or IgAN with rapidly progressive nature.

*Funding:* Government Support - Non-U.S.

## SA-PO823

### Microbiota in Patients with Immunoglobulin A Nephropathy

Maria De Angelis,[1] Eustacchio Montemurno,[2] Maria Piccolo,[1] Gabriella Lauriero,[2] Lucia Vannini,[3] Valentina Maranzano,[2] Giuseppe Dalfino,[2] Sonya Siragusa,[1] Marco Gobbetti,[1] Loreto Gesualdo.[2] *[1]DiSSPA, Univ of Bari; [2]DETO, Nephr Unit, Univ of Bari; [3]Inter-departmental Centre for Industrial Agri Food Research, Univ of Bologna.*

**Background:** Immunoglobulin A nephropathy (IgAN) is one of the most common forms of primary glomerular disease. Along structural IgA abnormalities, hyperproduction of poorly galactosylated IgA1 is thought to play a role in the pathogenesis of primary IgAN. The downstream effector mechanisms triggered by mesangial IgA1 deposition and its etiology are poorly understood. Recently, it was showed a probable role of the enteric microbiota in educating the immune system and disease development. This study aimed at comparing the differences of the fecal microbiota between IgAN pts and Healthy Controls (HC).

**Methods:** Based on clinical data, IgAN pts were grouped into "non-progressors" (NP) and "progressors' (P). Each group was composed by 16 volunteers. The total and active faecal microbiota was characterized through an integrate approach of culture-dependent and -independent methods and metabolomic analyses. Bacterial tag-encoded FLX-titanium amplicon pyrosequencing (bTEFAP) and Biochrom 30 series Amino Acid Analyzer were carried out for genomic and phenotypic analyses.

**Results:** The main bacterial phyla (Firmicutes and Bacteroidetes) significantly (P<0.05) changed between the fecal microbiota of NP, P and HC. Overall, a decreased level of metabolically active and cultivable bacteria was found in NP and, especially, in P compared to HC. In addition, the composition of some genera mainly differed among NP, P and HC. Metabolites produced by intestinal microbes play a direct role in health and disease. According to the differences in the composition of the microbiota, the metabolome also differed in NP and P patients compared to HC. As shown by multivariate statistical analyses, the levels of free amino acids and volatile organic compounds of fecal and urine samples were markedly affected in NP and, especially, P pts.

**Conclusions:** The microbiota differences among NP , P and HC pointed out in this study, may have implications regarding specific diagnostic test, and/or for treatment and prevention.

## SA-PO824

### Glomerular IgG Staining on Immunofluorescence Microscopy Is Associated with Progression to ESRD in IgA Nephropathy

Andrew S. Bomback,[1] Pietro A. Canetta,[1] Blake M. Butler,[1] Ana Huerta,[2] Jonathan J. Hogan,[1] Jai Radhakrishnan,[1] Leal C. Herlitz,[1] Michael B. Stokes,[1] Vivette D. D'Agati,[1] Gerald B. Appel,[1] Glen S. Markowitz.[1] *[1]Columbia Univ College of Physicians and Surgeons, New York, NY; [2]Hospital Universitario 12 de Octubre, Universidad Complutense de Madrid, Madrid, Spain.*

**Background:** Autoantibodies against galactose-deficient IgA1 (Gd-IgA1) have been postulated as mediators in the progression of IgA nephropathy. Circulating IgG autoantibodies have been shown to predict this progression, but the predictive role of glomerular IgG staining on immunofluorescence (IF) microscopy remains unclear.

**Methods:** We reviewed clinical, histopathologic, and laboratory data in 95 patients with biopsy-diagnosed IgA nephropathy followed at the Center for Glomerular Diseases at Columbia University from 2005 through 2010. We used Cox proportional hazards models to evaluate whether IgG staining on IF was associated with progression to ESRD.

**Results:** The cohort was comprised of 61 men and 34 women with mean (SD) age of 35.7 (12.4) years. The majority of patients were either white (58%) or Asian (27%). At time of diagnosis, mean (SD) serum creatinine was 1.6 (1.0) mg/dl, mean estimated GFR was 66 (31) ml/min/1.73m$^2$, and mean proteinuria was 3666 (3393) mg/day. Staining for IgG on IF microscopy was positive in 37 (39%) patients: 1+ IgG in 32 cases and 2+ IgG (co-dominant with IgA) in 5 cases. Over a mean follow-up period of 58.8 months, 18 patients progressed to ESRD, including 8 of the 37 (22%) with IgG staining by IF and 10 of the 58 (17%) without IgG staining (p=0.6 for unadjusted comparison). In multivariate Cox proportional hazards models, adjusting for age, gender, race, baseline eGFR, and baseline proteinuria, the presence of IgG on IF microscopy was associated with significantly greater risk for progression to ESRD (HR 7.07, 95% CI 1.69-29.56, p=0.007).

**Conclusions:** The presence of IgG staining on IF microscopy, like serum levels of IgG autoantibodies, may predict outcomes in IgA nephropathy. These findings further support the pathophysiologic role of autoantibodies against Gd-IgA1 as mediators of disease progression and should be further examined in larger cohorts.

## SA-PO825

### Effect of Tonsillectomy Plus Steroid Pulse Therapy on Clinical Remission of IgA Nephropathy with Mild Proteinuria: A Multicenter Study

Hiroyuki Komatsu,[1,2] Yuji Sato,[1,2] Tetsu Miyamoto,[2] Takeshi Nakata,[2] Tomoya Nishino,[2] Masahito Tamura,[2] Tadashi Tomo,[2] Masanobu Miyazaki,[2] Shouichi Fujimoto.[1,2] *[1]First Dept of Internal Medicine, Univ of Miyazaki Hospital, Miyazaki, Japan; [2]Steering Committee for IgA Nephropathy from Four Univs (IgAN-4U), Japan.*

**Background:** Our previous multicenter cohort study of 323 patients (*JASN* 2012: 23; 58A) found that tonsillectomy plus steroid pulse therapy (TSP) can result in clinical remission (CR) for patients with IgA nephropathy and mild to moderate histological damage. Medical intervention for patients with IgA nephropathy and mild proteinuria (< 1.0 g/day) is controversial, and the effectiveness of TSP for such patients remains obscure.

**Methods:** Fifty-five patients who had mild proteinuria (0.4 to 1.0 g/day) at diagnosis and who were initially treated with steroid were eligible to participate in this study. We used univariate and multivariate analysis to evaluate the decline in renal function defined as a 100% increase in serum creatinine (sCr) and CR defined as the disappearance of hematuria and proteinuria (UP/Ucr < 0.3) between groups treated with TSP and steroid without tonsillectomy (ST).

**Results:** Background factors at diagnosis including age (mean, 31.9 vs. 34.0 y), ratio (%) of patients with hypertension (19.6% vs. 22.2%), sCr (mean, 0.74 vs. 0.86 mg/dL), proteinuria (mean, 0.62 vs 0.69 g/day), and histological severity did not statistically differ between the TSP and ST groups. None of the patients achieved a 100% increase in sCr during mean followed–up periods of 4.5 years. At the final observation, 69.6% and 44.4% of patients in the TSP and ST groups, respectively, achieved CR. Cox proportional hazards models revealed that CR was achieved about six-fold more effectively by TSP than SP (HR for CR; 5.85, p = 0.028).

**Conclusions:** TSP is a potential modality for inducing CR in patients with IgA nephropathy and mild proteinuria.

## SA-PO826

### Kidney Transplant following Crescentic IgA Nephropathy

Ziad S. Zaky, Jonathan J. Hogan, Gerald B. Appel, Andrew S. Bomback, Eric D. Braunstein, Demetra Tsapepas, Pietro A. Canetta. *Columbia Univ Med. Center, New York, NY.*

**Background:** Crescentic IgA Nephropathy (IgAN) represents an aggressive form of IgAN that often leads to ESRD. Little is known of the clinical course of patients (pts) with crescentic IgAN who undergo kidney transplant.

**Methods:** We reviewed the charts of 153 IgAN pts who underwent kidney transplant at CUMC between Jan-2000 and Jan-2013. Native kidney biopsy reports were available for n=70. Crescentic IgAN was defined as ≥30% glomeruli with cellular, fibrous, or fibrocellular crescents.

**Results:** Crescentic IgAN was found in 15 pts (21%), of whom 8 were male. Race/ethnicity included white (n=8), Af-Am (n=4), Asian (n=2), and Hispanic (n=1). Two pts had a second transplant. Of 17 transplants, 7 were living-related, 6 living-unrelated, and 4 deceased-donor. Mean ±SD age at first transplant was 36±11 yrs (range 22-55 yrs). Induction agents used were anti-thymocyte globulin (n=11), pulse steroids alone (n=5), and basiliximab (n=1). Mean follow-up after transplant was 55±44 mos (range 8.5-137 mos). All pts were alive at last follow-up (May-2013). 3/17 grafts (18%) eventually failed: 1 from interstitial fibrosis after 17 mos, 1 from BK virus at 8.5 yrs, and 1 from recurrent crescentic IgAN (see below). Mean ±SD serum creatinine in surviving grafts was 1.5±0.4 mg/dl. At last followup, 1 patient had significant (3+) proteinuria and 3 had (3+) hematuria. Routine surveillance biopsies were not done, but 9/17 (53%) grafts were biopsied for graft dysfunction or proteinuria. Recurrent IgAN occurred in 4/15 pts and 5/17 allografts, diagnosed at mean 9.2 mos post-transplant (range 0-23.4 mos), and characterized by IgA deposits without proliferation or crescents. One patient lost his first graft to recurrent crescentic IgAN. He had histological evidence of recurrent IgAN at 6 weeks and crescentic IgAN at 26.5 months. He was retransplanted at 48 mos and reperfusion biopsy of the 2nd transplant showed recurrent IgA deposition, but normal light microscopy.

**Conclusions:** 4/15 (27%) pts with native crescentic IgAN had recurrent IgAN in the allograft. Only 1/15 (7%) pts had histologic evidence of recurrent crescentic disease. Most pts had favorable outcomes following transplant.

## SA-PO827

**Circulating Tumor Necrotizing Factor α Receptors Predict the Outcome of Human IgA Neprhopathy** Yun Jung Oh,[1] Jung Pyo Lee,[2] Su Mi Lee,[3] Jin Ho Hwang,[3] Hyuk Huh,[3] Seung Hee Yang,[3,4] Dong Ki Kim,[3,4] Hajeong Lee,[3] Yun Kyu Oh,[2] Chun Soo Lim,[2] Yon Su Kim.[3,4] *[1]Internal Medicine, Cheju Halla General Hospital, Jeju, Korea; [2]Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea; [3]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [4]4 Kidney Research Institute, Seoul National Univ, Seoul, Korea.*

**Background:** The circulating TNF receptors (TNFRs) levels could predict the long-term renal outcome in diabetics, but the role of circulating TNFRs in other chronic kidney disease has not been investigated. Here, we investigated the correlation between circulating TNFRs and renal histologic findings in IgA nephropathy (IgAN) and assessed the notion that the circulating TNFRs could predict the clinical outcome.

**Methods:** 187 consecutive biopsy-proven IgAN patients between 2009 and 2012 were prospectively enrolled. Circulating TNFR levels were measured using serum samples stored at the time of biopsy. The primary clinical endpoint was the decline of estimated glomerular filtration rate (eGFR; ≥30% decline compared to baseline).

**Results:** Mean eGFR decreased and proteinuria worsened proportionally as circulating TNFR1 and TNFR2 increased (p<0.001). Interstitial inflammation and interstitial fibrosis were significantly more severe as TNFR1 levels increased regardless of eGFR levels. Also, the severity of interstitial fibrosis and tubular atrophy worsened with increasing TNFR2 levels. The risk of reaching the primary endpoint was significantly higher in the highest TNFR1 quartile compared with other quartiles by the Cox proportional hazards model (HR 12.924, p<0.001), but the risk was not evident in the highest TNFR2 quartile (HR 1.949, p=0.211).

**Conclusions:** In conclusion, the circulating TNFR1 and TNFR2 levels reflect the histologic and clinical severity of IgAN. Moreover, TNFR1 at the time of initial diagnosis may predict the long-term outcome of IgAN.

## SA-PO828

**Clinical and Histological Parameters Associated with the Recurrence of Proteinuria after Steroid Therapy in IgA Nephropathy Patients** Keita Hirano,[1] Tetsuya Kawamura,[2] Nobuo Tsuboi,[2] Hideo Okonogi,[2] Kazushige Hanaoka,[2] Takashi Yokoo.[2] *[1]Internal Medicine, Ashikaga Red Cross Hospital, Ashikaga, Tochigi, Japan; [2]Div of Nephrology, Jikei Univ School of Medicine, Minato, Tokyo, Japan.*

**Background:** We recently reported that the achievement of proteinuria <0.4 g/day at one year after six months of steroid therapy is associated with favorable renal survival in Japanese patients with IgA nephropathy (IgAN)(Clin Exp Nephrol 2012). However, the predictive factors of recurrence remain obscure.

**Methods:** Employing a historical cohort design, we enrolled 93 IgAN patients, who received six months of steroid therapy and achieved a reduction in the urinary protein excretion volume (UPE) to <0.4 g/day at one year. To evaluate the event of recurrence, the end point was defined as an increase in the UPE level of ≥1.0 g/day after the first one year following the initiation of steroid therapy. Concerning pathological predictors, we adopted the Oxford classification and the histological grades (HG) recently proposed in Japan, in which HG1, HG2 and HG3+4 correspond to <25%, 25-49% and ≥50% of glomeruli exhibiting cellular and/or fibrocellular crescents, global sclerosis, segmental sclerosis or fibrous crescents, respectively (J Nephrol 2012).

**Results:** During a median follow-up of 2.3 years after the first one year following the initiation of treatment, 18 patients (19.4%) reached the end point. Of note, mesangial hypercellularity and HG2 were significant pathological predictors of an increased risk of recurrence in the multivariate model (M1 vs. M0, HR: 2.98, 95%CI: 1.08-8.26; HG2 vs. HG1, HR: 8.58, 95%CI: 1.08-68.4). Moreover, females and tonsillectomy as concurrent treatment were associated with relatively reduced risk of recurrence (HR: 0.29, 95%CI: 0.10-0.85; HR: 0.20, 95%CI: 0.07-0.62).

**Conclusions:** Our data indicate that mesangial hypercellularity based on the Oxford classification and the histological grade in Japanese classification predict recurrence in Japanese IgAN patients that have received six months of steroid therapy. Tonsillectomy prior to the steroid therapy can protect them from the recurrence.

*Funding:* Clinical Revenue Support

## SA-PO829

**Comparison of the Predictability of Renal Outcome between the Oxford and the Haas Classifications in Patients with IgA Nephropathy** Kyoung Sook Park,[1] Chan Ho Kim,[1] Hyang Mo Koo,[1] Fa Mee Doh,[1] Hyung Jung Oh,[1] Tae-Hyun Yoo,[1,2] Shin-Wook Kang,[1,2] Seung Hyeok Han.[1] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul, Korea.*

**Background:** The Oxford classification, a new classification of IgA nephropathy (IgAN), has recently been proposed and gained worldwide acceptance. However, it is uncertain whether the Oxford classification can predict renal outcome better than the previous ones.

**Methods:** We conducted a retrospective cohort study to compare the predictability of renal outcome between the Oxford and the Haas classification in 500 Korean patients with biopsy-proven IgAN between January 2001 and December 2010. Primary outcome was a doubling of the baseline serum creatinine concentrations (D-SCr).

**Results:** During a mean follow-up duration of 68 months, D-SCr occurred in 52 patients (10.4%), and 35 patients (7.0%) progressed to ESRD. There was a significant stepwise increase in the development of D-SCr as the Haas classes increased (P < 0.001). In addition, the primary endpoint of D-SCr occurred significantly more in patients with the Oxford M and T lesions than those without such lesions (P < 0.001). In multivariable Cox regression analyses adjusted for age, blood pressure, proteinuria, and eGFR, the Haas class V (HR, 12.19; 95% CI, 2.61-63.8; P = 0.002) and the Oxford T1 (HR, 6.68; 95% CI, 2.86-15.6; P < 0.001) and T2 (HR, 12.16; 95% CI, 4.78-30.9; P < 0.001) lesions were independently associated with a higher risk for reaching D-SCr. Harrell's C indices of each multivariable model with the Haas and the Oxford classifications were 0.867 (P = 0.015) and 0.881 (P = 0.004), respectively, which was significantly higher compared to the model with clinical factors only (c-statistic = 0.819). However, there was no difference in c-statistics between the two models with the Haas and the Oxford classifications (P = 0.348).

**Conclusions:** Both pathologic classifications were comparable in predicting the progression of IgAN. Further studies are required to delineate relationship between pathologic features and treatment responsiveness in patients with IgAN.

## SA-PO830

**Urinary Angiotensinogen, a New Biomarker in Primary IgA Nephropathy Patients?** Danxia Zheng, Yimu Zheng. *The Renal Dept, Peking Univ 3rd Hospital, Beijing, China.*

**Background:** Urinary angiotensinogen (AGT) was supposed as a useful local marker of intrarenal Renin Angiotensin System (RAS) activity in renal injury. To investigate the inapporated increased RAS in IgA nephropathy, urinary AGT was measured, and its correlation with clinical and pathological injury index was investigated.

**Methods:** 73 IgA nephropathy patients (16-69 years, 44 male ) were recruited. All the patients were diagnosed by pathologist from renal biopsy, and any known secondary IgA nephropathy was excluded. The controls are 19 age-matched healthy volunteers. Urinary AGT (normalized by urine creatinine) levels on the biopsy day were measured by ELISA method.

**Results:** Urinary AGT was significantly higher in IgA nephropathy patients than in controls (162±234 vs 6.6±5.0μg/g, p<0.05). Urinary AGT was significantly lower in patients treated with RAS inhibitors compared with in patients not treated with RAS inhibitors (87±105 vs 217±284 μg/g, p=0.018). But even with the treatment of RAS inhibitors, urinary AGT level was still higher in IgA nephropathy than in the controls (87±105 vs 6.6±5.0μg/g,p<0.05). Urinary AGT was correlated positively with 24-h urinary protein(r=0.703, p=0.000) and renal crescent percent (r=0.428, p=0.000). This correlation was confirmed by multiple regression analysis. Urinary AGT was not correlated with gender, age, blood pressure, serum creatinine, serum albumin, or glomerulosclerosis percent. Taking hypertension and if RAS inhibitors being used before biopsy day into account, these IgA patients were divided into 4 subgroups.

| RAS inhibitors | + | + | - | - |
|---|---|---|---|---|
| hypertension | - | + | - | + |
| n | 14 | 17 | 25 | 17 |
| urinary AGT(μg/g) | 29±25 | 135±122 | 203±231 | 236±355 |

Urinary AGT in normotensive patients receiving RAS inhibitors treatment was lowest among 4 subgroups (p<0.05), but it is still higher than in controls (29±25 vs 6.6±5.0μg/g,p<0.05); which supported the necessary of earlier RAS inhibitors treatment in IgA nephropathy.

**Conclusions:** Our data support intrarenal RAS is elevated in IgA nephropathy, and correlated with renal injury activity. Earlier RAS inhibitors may never mean early in IgA nephropathy.

## SA-PO831

**Serum Under-*O*-Glycosylated IgA Level Does Not Correlate with Glomerular IgA Deposition Based upon Heterogeneity in the Composition of Immune Complexes in IgA Nephropathy** Yoshio Shimizu, Hitoshi Suzuki, Yusuke Suzuki, Yasuhiko Tomino. *Juntendo Univ Faculty of Medicine, Tokyo, Japan.*

**Background:** Although serum under-*O*-glycosylated IgA1 in IgA nephropathy (IgAN) patients may deposit more preferentially in glomeruli than normal IgA1, through self-aggregation and the formation of immune-complexes with autoantibodies, the relationship between glomerular IgA deposition level and *O*-glycan profiles of serum IgA1 remains obscure.

**Methods:** Serum total under-*O*-glycosylated IgA was quantified in 32 IgA nephropathy patients (11 males/22 females) by ELISA with *Helix aspersa* (HAA) lectin. Serum under-*O*-glycosylated polymeric IgA (pIgA) was selectively measured by a method that was developed by our group (pIgA trap). Immunofluorescence of biopsy specimens and image analyses were used to quantify the percentage area of IgA deposition in the whole glomeruli (Area-IgA). Correlations were assessed between the Area-IgA and data from HAA-ELISA or pIgA trap.

**Results:** While the under-*O*-glycosylated-IgA levels were significantly higher in IgAN patients than in healthy controls, when measured by HAA-ELISA (p<0.05), there was no significant difference in under-*O*-glycosylated-pIgA. There was no linear correlation between Area-IgA and data from HAA-ELISA or the pIgA trap.

Decision tree analysis was performed to determine the cause of the lack of a linear correlation between the Area-IgA and data from ELISA or the pIgA trap. The patients were initially classified into groups, A, B, C and D. based upon the gradual increases in the HAA-ELISA titers from A to D, and group C was divided into C1 and C2 groups, based on the pIgA trap. The area-IgA of each group (A, B, C1, C2 and D) (%) was 7.8±1.7, 12.5±1.7, 4.5±2.0, 5.9±3.4, 10.7±5.5 with significant differences. The backgrounds of the patients

in each group suggested that dominant immune complexes in the glomeruli consisted of 1. IgA-IgG and complement in groups A and B, 2. pIgA and complement in groups C1 and C2 and 3. monomeric IgA-IgA or aggregated monomeric IgA in group D.

**Conclusions:** Serum under-*O*-glycosylated IgA levels do not correlate with glomerular IgA deposition based upon heterogeneity in the composition of glomerular immune complexes in IgAN patients.

*Funding:* Pharmaceutical Company Support - Novartis, Tanabe-Mitsubishi, Kowa, Kyowa-Kirin

## SA-PO832

**Endothelial Nitric Oxide Synthase (eNOS) Gene Polymorphism and Risk for Progression in IgA Nephropathy** Dimitrios Xydakis,[1] Georgios Goulielmos,[2] Kostas Stylianou,[2] Antonia N. Papadaki,[3] Apostolos Papadogiannakis,[1] Eugenios Daphnis.[2] *[1]Nephrology, Venizeleio Hospital, Greece; [2]Univ Hospital of Crete, Greece; [3]Nephrology, Hospital of Chania, Greece.*

**Background:** Several studies have shown the influence of eNOS gene polymorphism on the progression of kidney disease. IgA nephropathy has a variable natural history. The aim of this study is to investigate any association between eNOS gene intron 4a/b polymorphism and clinical presentation and progression of IgA nephropathy patients in a geographical region.

**Methods:** We included in our study 103 patients with biopsy-proven IgA glomerulonephritis and a median follow up period of 5.1±2.8 years. All subjects where genotyped for the intron 4a/b polymorphism. The end-points were doubling in baseline serum creatinine and/or initiation of dialysis.

**Results:** At baseline, M/F 68/35, age was 41.5±13.5 years, creatinine 1.21±0.74 mg/dl, proteinuria 1094±1201 mg/24H, 78% of the patients had a baseline creatinine of ≤ 1.3 mg/dl, 57% had a proteinuria of <1000mg/24H. During follow up time, 12 patients (11%) have doubled serum creatinine and 7 of them (8%) started dialysis in a period of 5.1±4.2 years.

40(39%) patients presented eNOS intron 4 polymorphism a (6 aa,34 ab) and 63 (64%) polymorphism b. Patients reaching the outcome exhibited higher systolic blood pressure (144±20 vs 130±20 mmHg, p=0.02), diastolic blood pressure (82,1±13 vs 72,3±15mmHg, p=0.028), higher baseline creatinine (2.18±2,7 vs 1.34±0,8 mg/dl, p=0.009) and more extended tubulointertitial fibrosis (21% vs 13% , p=0.1)

In a multivariate Cox proportional hazard analysis, the presence of (a) allele of eNOS gene, showed an 8-fold hazard to reach the outcome (HR=8.09, p=0.04) after adjusting for age, sex, presence of hypertension, proteinuria and ACE inhibitors treatment.

**Conclusions:** A significant proportion of patients, present impaired renal function at baseline. The classical risk factors for progression of renal disease also apply to our population. Additionally, the genotype (a) of eNOS gene appears to be associated with a worse outcome. The early detection of (a) allele could indicate those patients at increased risk of renal impairment.

## SA-PO833

**Association between Low Plasma Renin Activity and Renal Interstitial Fibrosis in Patients with IgA Nephropathy** Hitomi Nishimoto, Hirofumi Ikeda, Yoriko Ura, Masaru Nakayama. *Div of Nephrology and Clinical Research Institute, Dept of Internal Medicine, National Kyushu Medical Center Hospital, Fukuoka City, Fukuoka Pref., Japan.*

**Background:** IgA nephropathy (IgAN) is the most common cause of glomerulonephritis. Renal interstitial fibrosis (RIF) is considered a poor prognostic factor in IgAN. An association between the renin-angiotensin system and RIF has been suggested, with low plasma renin activity (PRA) reflecting increased renal renin-angiotensin system activity. To date, no studies have evaluated the relationship between RIF and PRA in IgAN patients. Our aim was to determine whether PRA is associated with RIF in IgAN patients.

**Methods:** Forty-four patients (mean age, 42±15 years)with biopsy-proven IgAN were enrolled in this cross-sectional study. Subjects taking angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers or with estimated glomerular filtration rates (eGFRs) lower than 30 ml/min/1.73m$^2$ were excluded from the study. Mean eGFR and daily proteinuria of subjects were 76.2±20.5 ml/min/1.73m$^2$ and 1.1±1.4 g/day, respectively. Blood was drawn in a supine position early in the morning to measure PRA. PRA was log-transformed to achieve a normal distribution for analysis. We quantitated RIF by a point counting method and assessed the glomerular sclerosis index using semi-quantitative scoring.

**Results:** The mean percentage of RIF in subjects was 30±8 and the mean glomerular sclerosis index was 0.78±0.67. Univariate linear regression analysis showed that RIF was associated with age (β=0.38, p=0.01), systolic blood pressure (β=0.37, p=0.01), daily proteinuria (β=0.31, p=0.04), and log PRA (β=-0.54, p<0.01). In multivariate analysis, log PRA was the only independent predictor of RIF (β=-0.48, p=0.01). In univariate analysis, eGFR was associated with the glomerular sclerosis index (β=-0.42, p<0.01), but log PRA was not (β=-0.25, p=0.11).

**Conclusions:** In patients with IgAN, low PRA was independently associated with RIF. We speculate that low PRA might reflect enhanced tissue renin-angiotensin system activity, which activates RIF.

## SA-PO834

**The Role of Plasmin in the Development of Tubulointerstitial Change in IgA Nephropathy** Takahiro Uchida, Takashi Oda, Atsushi Watanabe, Hanako Takechi, Kojiro Yamamoto, Naoki Oshima, Hiroo Kumagai. *Dept of Nephrology and Endocrinology, National Defense Medical College, Tokorozawa, Saitama, Japan.*

**Background:** Plasmin activity has recently been reported to be associated with renal fibrosis in experimental models. However, the role of plasmin in human renal diseases is unclear. We therefore examined the renal plasmin activity in IgA nephropathy (IgAN).

**Methods:** Fifty seven patients with IgAN who were diagnosed by renal biopsy were retrospectively evaluated. Plasmin activity was assessed by in situ zymography with the use of plasmin sensitive synthetic peptide, and the percentage of positively stained area was calculated with image analysis software (LuminaVision Ver. 2.04). The relationships between the results of plasmin activity and the renal histological or clinical parameters were evaluated.

**Results:** Plasmin activity was observed only in tubulointerstitial (TI) space (and mainly in interstitium). Regarding the correlation with histological features, plasmin activity was significantly stronger in patients with TI change than those without TI change ($P$<0.05), and it was significantly correlated with the global glomerulosclerosis rate ($r$=0.45, $P$=0.0004). Regarding the correlation with clinical parameters, plasmin activity at the time of renal biopsy was significantly correlated not only with serum creatinine concentration (sCr) or estimated glomerular filtration rate (eGFR) at the time of renal biopsy ($r$=0.58, $P$<0.0001; $r$=-0.44, $P$=0.0005, respectively) but also with SCr and eGFR at the time of the end of the follow-up ($r$=0.58, $P$<0.0001; $r$=-0.46, $P$=0.0003, respectively). Double stainings for plasmin activity and CD68, and plasmin activity and neutrophil erastase in selected patients revealed the infiltration of CD68+ macrophages and neutrophils in TI space with upregulated plasmin activity.

**Conclusions:** These data suggest that TI plasmin activity may promote renal fibrosis by inducing inflammatory cells infiltration in human IgAN as well as in experimental models. Targeting to antagonize plasmin activity in situ may be a promising therapeutic approach to slow renal fibrogenesis and improve renal function.

## SA-PO835

**Crescentic IgA Nephropathy (C-IgAN) in Children** Yuko Shima,[1] Koichi Nakanishi,[1] Taketsugu Hama,[1] Hironobu Mukaiyama,[1] Hiroko Togawa,[1] Masashi Sato,[1] Kandai Nozu,[2] Ryojiro Tanaka,[3] Kazumoto Iijima,[2] Norishige Yoshikawa.[1] *[1]Pediatrics, Wakayama Medical Univ, Wakayama, Japan; [2]Pediatrics, Kobe Univ, Kobe, Hyogo, Jordan; [3]Pediatric Nephrology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Hyogo, Japan.*

**Background:** Crescentic glomerulonephritis (CN) is defined as nephritis with more than 50% of glomeruli presenting crescents. IgAN is one of causes of CN.

**Methods:** We analyzed retrospectively consecutive 515 children newly diagnosed as having biopsy-proven IgAN from June 1976 to May 2010. There were 25 children (4.9%) with C-IgAN. We compared clinical and pathological findings between cases with C-IgAN and the remained 490 cases.

**Results:** Among 25 C-IgAN, 16 children (64%) were referred to hospitals by annual school screening program for urinary abnormalities, 7 children for gross hematuria, a child for acute nephritic syndrome, and a child for nephrotic syndrome. There was no significant difference about gender and blood pressure. There were significant differences in ratio of gross hematuria (76 vs. 50%), excretion of proteinuria (1.9 vs. 0.5 g/day/m$^2$), eGFR (102 vs. 108 ml/min/m$^2$), and duration from onset to renal biopsy (4.0 vs. 8.0 M). As to the pathological findings, degree of tubular atrophy showed significant difference (IQR[5-10] vs. [0-5]%). Sixteen children of C-IgAN (64%) were treated with combination therapy including PSL and immunosuppressant. Mean observation period was 6.0±3.6 yrs. Four cases (16%) reached chronic renal failure (eGFR<60) at the latest observation. One case was treated with dipyridamole and warfarin, two cases were with combination therapy, and the last one's treatment was unknown. The Kaplan-Meier analysis showed that the cases with C-IgAN demonstrated significantly lower renal survival curve than the rest (p<0.001). But they showed significant better preservation of renal function compared with the previous reports and renal survival rates for 5 yr was 84.1%.

**Conclusions:** We confirmed the importance of school screening program to find even children with C-IgAN. In our study, most crescents were cellular/fibrocellular crescents, which are acute lesions. That may be the reason why the combination therapy is effective in children with C-IgAN.

## SA-PO836

**Tonsillectomy in a Pan-European Cohort of 1147 Patients with IgA Nephropathy** Rosanna Coppo, Roberta Camilla, Shubha Bellur, Ian Roberts, Daniel C. Cattran, John Feehally, Stephan Troyanov, Luca Vergano, Laura Morando. *On Behalf of VALIGA Study Group.*

**Background:** The beneficial effect of tonsillectomy in patients with IgA nephropathy (IgAN) is controversial.

While in Asia benefits have been claimed, in Europe small studies failed to show benefits.

**Methods:** The 1147 patients with IgAN enrolled in the European validation study of the Oxford Classification of IgAN (VALIGA), from 55 Centers of 13 Countries, offered a unique opportunity to investigate the effect of tonsillectomy in histologically and clinically well characterized IgAN patients followed in mean for 5 years.

**Results:** Tonsillectomy was performed in 62(5.9%), 10 children; e-GFR was calculated (MDRD and Schwartz formula). The e-GFR slope was calculated; end-points were ESRD and 50% loss of e-GFR. Values in 62 tonsillectomized and 987 non-tonsillectomized patients in Table 1.

| | NO tonsillectomy | YES tonsillectomy | P value |
|---|---|---|---|
| Age at renal biopsy (years) | 35.8 ± 16.5 | 37.4 ± 16.2 | ns |
| Follow-up (years) | 5.71 ± 4.48 | 5.62 ± 4.12 | ns |
| eGFR at renal biopsy (ml/min/1.73 m2) | 76.7 ± 34.6 | 73.4± 31.76 | ns |
| Proteinuria at renal biopsy (g/day/1.73 m2) | 1.20 (0.52-2.56) | 1.35 (0.59-2.83) | ns |
| Mean proteinuria over follow -up (g/day/1.73 m2) | 0.78 (0.40-1.62) | 0.73 (0.37-1.68) | ns |
| Loss of eGFR during follow-up (ml/min/1.73 m2/year) | -2.08 ±8.08 | -1.01± 7.75 | ns |
| ESRD | 112/987 (11.3%) | 9/62 (14.5 %) | ns |
| 50% loss of initial GFR | 137/987 (13.9%) | 9/62 (14.5%) | ns |
| combined end -point (ESRD or 50% loss of initial GFR) | 156/987 (15.8%) | 10/62 (16.1%) | ns |

**Conclusions:** A propensity score estimating the probability of receiving tonsillectomy paired 41 patients with tonsillectomy and 41 without tonsillectomy with similar risk of progression (gender, age, race, mean blood pressure, proteinuria, e-GFR at renal biopsy, previous treatments and Oxford MEST scores). No significant difference was found in the combined end point, nor in eGFR loss over follow-up.

## SA-PO837

**Proteomic Analysis of Whole Glomeruli in Patients with Immunoglobulin A Nephropathy Using Micro-Sieving** Shigeki Kojima, Kenichiro Koitabashi, Yugo Shibagaki, Takashi Yasuda, Kenjiro Kimura. *Div of Nephrology and Hypertension, Dept of Internal Medicine, St. Marianna Univ School of Medicine, Kawasaki, Kanagawa, Japan.*

**Background:** Immunoglobulin A (IgA) nephropathy (IgAN) is one of the most common primary glomerular disorders in the world. Pathogenesis of IgAN is still unclear. We recently established a method to isolate glomeruli from renal samples obtained by single needle biopsy, named as "micro-sieving". To identify glomerular proteins related to the pathogenesis of IgAN, we analyzed protein profiles of glomeruli obtained by micro-sieving in patients with IgAN.

**Methods:** Glomeruli were obtained by micro-sieving from renal biopsy samples of five patients with IgAN and five patients with minimal change nephrotic syndrome (MCNS). Then proteins, extracted from the isolated glomeruli, were separated by two dimensional fluorescence differential gel electrophoresis (2D-DIGE). By this method protein spots intensity of which was different between the IgAN and MCNS groups were detected and further identified by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF/MS). Some of the identified proteins were evaluated by immunohistochemistry.

**Results:** By 2D-DIGE, we detected in total 1170 protein spots matched among the samples. We found that intensity of 22 out of the 1170 spots was significantly higher in the patients with IgAN than in those with MCNS. Similarly, we found that intensity of 12 out of the 1170 spots was significantly lower in the patients with IgAN than in those with MCNS. Fifteen out of the 34 spots were successfully identified by MALDI-TOF/MS. Among them, we focused on two proteins, α-actinin-4 and glycine amidinotransferase, which intensity was significantly higher in IgAN. Also in immunohistochemistry, the expression of α-actinin-4 increased in the patients with IgAN than in those with MCNS.

**Conclusions:** Use of micro-sieving enabled us to obtain only glomerular proteins from renal biopsy samples and thus to analyze protein profiles of glomeruli. Future analysis of the identified proteins would help understanding of the pathogenesis of IgAN as well as MCNS.

## SA-PO838

**Adverse Events Associated with Immunosuppressive Therapy and Tonsillectomy in IgAN: The Nationwide Retrospective Cohort Study in IgAN in Japan** Yoshinari Yasuda,[1] Takashi Yasuda,[2] Sachiko Ohde,[3] Osamu Takahashi,[3] Tetsuya Kawamura,[4] Seiichi Matsuo.[1] *[1]Nephrol, Nagoya Univ, Nagoya, Japan; [2]Nephrol & Hypertens, St. Marianna Univ, Kawasaki, Japan; [3]Clin Epidemiol, St. Luke's Life Sci Inst, Tokyo, Japan; [4]Kid & Hypertens, Jikei Univ, Tokyo, Japan.*

**Background:** IgA nephropathy (IgAN) is one of progressive renal diseases requiring immunosuppressive therapy with/without tonsillectomy (Tx) when the prognosis is expected to be worse, however adverse events and complications associated with specific treatments have not been well documented. Thus, adverse events associated with immunosuppressive therapy and complications of Tx were analyzed in The Nationwide Retrospective Cohort Study in IgAN in Japan.

**Methods:** Study subjects were all IgAN patients diagnosed by the first renal biopsy in 49 collaborating hospitals during 2002 to 2004. Patients under 18 years old were excluded. Data at the time of renal biopsy and during the follow-up were collected, including death, complications of Tx and the following adverse events requiring specific treatment; infection, psychiatric disorder, aseptic necrosis, peptic ulcer, de novo diabetes, osteoporosis and others. Total 1,103 cases were registered till Dec. 1, 2012. Among them, we analyzed 931 cases which have sufficient data for the analysis.

**Results:** The median observation period was 5.4 years. Choice of therapy was as follows; conservative therapy (Cons) 475, oral steroids (Oral) 174, pulse methylprednisolone (mPSL) 121, and Tx with pulse methyprednisone (Tx+mPSL) 161. In this period, 11 patients died (6 malignancy, 2 CVD, 1 COPD, 1 druhg-induced lung injury and 1 unknown), and death cases were not obviously association with immunosuppressive therapy. Age (mean +- SD years old) and gender (female %) distributions were significantly different (Cons: 42.5 +- 16.8, 45.5%, Oral: 38.4 +- 15.0, 45.4%, mPSL: 39.6 +- 15.1, 43.0%, Tx+mPSL: 33.8 +- 15.8, 58.4%). Adverse event rates were significantly lower in Cons (2.3%) and in Tx+mPSL (3.7%) groups compared to Oral (12.6%) and mPSL (14.0%) groups. Complication of Tx was occurred in 3 out of 290 (1.1%) cases.

**Conclusions:** Adverse event rate was low in Cons and Tx+mPSL groups and complication of Tx was rare among Japanese IgAN patients.

*Funding:* Government Support - Non-U.S.

## SA-PO839

**Enhanced Urinary Angiotensinogen Is Correlated with the Impaired Reabsorption Capacity of Proximal Tubular Cells in Children with the Severe Henoch-Schonlein Purpura Nephritis** Yanjie Huang, Xiaoqing Yang. *Pediatrics, The First Affiliated Hospital of Henan Univ of Tranditional Chinese Medicine, Zhengzhou, Henan, China.*

**Background:** The mechanism of enhanced urinary angiotensinogen (UAGT) in hypertension and some kidney diseases remains to be clarified. The aim of this study was to investigate the levels of UAGT in children with Henoch-Schonlein purpura nephritis (HSPN), and the correlations of UAGT with urinary albumin (ALB), Cystatin C (CysC), and beta2-microglobulin (beta2-MG). Urinary CysC and beta2-MG have been recognized as the markers of the reabsorption capacity of proximal tubular cells.

**Methods:** Patients were classified into three groups, according to the initial clinical presentation. 1) HSPN 1, n=20, hematuria, 5 or more red blood cells in per high power field in a centrifuged specimen, urine protein/creatinine ratios (UPCR)<200mg/g; 2) HSPN 2, n=30, 200mg/g<UPCR<1000mg/g, with hematuria; 3) HSPN 3, n=30, UPCR>1000mg/g, with hematuria. We measured urinary creatinine (Cr), ALB, AGT, CysC, beta2-MG, and plasma CysC levels. The urinary levels of these parameters were expressed in ratios with Cr.

**Results:** The UAGT levels in HSPN 3 (11.74±3.14ug/gCr) were significantly increased, compared with HSPN 1 (versus 3.39±2.43ug/gCr, p<0.01 )and HSPN 2 (versus 5.76±1.84ug/gCr, p<0.01). The levels of urinary CysC and beta2-MG were also enhanced in HSPN 3, compared with other groups. However, the concentration of plasma CysC had no difference among groups. In HSPN 3, there were the significant positive correlations between UAGT and UCysC (r=0.794, p<0.01), also between UAGT and beta2-MG (r=0.757, p<0.01). But, the UAGT levels did not correlated with UALB (r=0.303, p>0.05) in HSPN 3.

**Conclusions:** These results suggest that the higher levels of UAGT in severe HSPN may mainly reason from the impaired reabsorption capacity of proximal tubular cells, when the molecular barrier function of the glomerular capillary wall is injuried, and massive AGT protein is leaked into the tubular lumen. Upregulation of UAGT may lead to the elevated generation of intrarenal angiotensin II along the nephron.

## SA-PO840

**Predicting End-Stage Renal Disease or Death in Henoch Schønleins Purpura Patients Using Predictive Model Absolute Renal Risk Developed for IgA Nephropathy** Ann-merethe Vaagane,[5] Thomas Knoop,[1] Bjorn Egil Vikse,[2] Einar Svarstad,[1] Sabine Leh,[3] Rune Bjoerneklett.[1] *[1]Dept of Medicine, Haukeland Univ Hospital, Bergen, Norway; [2]Dept of Medicine, Haugesund Hospital, Haugesund, Norway; [3]Dept of Pathology, Haukeland Univ Hospital, Bergen, Norway; [4]Dept of Transplant Medicine, Oslo Univ Hospital Rikshospitalet, Oslo, Norway; [5]Institute of Clinical Medicine, Univ of Bergen, Bergen, Norway.*

**Background:** The glomerulonephritis in Henoch Schønlein Purpura (HSP) is morphologically similar to primary IgA nephropathy (IgAN). It is however not well studied whether the clinical prognosis also is similar. Recently, a prognostic model to predict end-stage renal disease (ESRD) or pre-ESRD death in IgAN called absolute renal risk (ARR) was published (Berthoux et al, JASN 2011). With ARR, IgAN patients are scored from 0-3 based on the presence/absence of proteinuria ≥ 1gram/24 hours, hypertension and the severity of the histological findings.

**Methods:** We identified a cohort of HSP patients diagnosed with kidney biopsy in the Norwegian Kidney Biopsy Registry and cases with ESRD or pre-ESRD deaths in the cohort during follow-up were identified through record linkage with the Norwegian Nephrology Registry (ESRD) and the Norwegian Population Registry (deaths). We scored the patients according to ARR. We used Kaplan Meier statistics and log-rank test to compare risk of ESRD/death in the different prognostic groups. We also calculated 10-year cumulative risk of ESRD/death and compared our results with the findings of Berthoux et al.

**Results:** We identified 120 HSP patients with mean follow-up time 8.4 years (range up to 20 years). During follow-up, ESRD occurred in 13 and pre-ESRD death in 7 patients. Risk of ESRD/death significantly increased with increasing ARR score. Ten-year cumulative risk of ESRD/death were 0% if ARR was 0, 4% if ARR was 1, 11% if ARR was 2 and 66% if ARR was 3.

**Conclusions:** ARR is applicable also to risk stratify HSP patients. Ten-year risk of ESRD/death in each ARR group of HSP patients is fairly similar to what Berthoux et al observed in IgAN patients.

## SA-PO841

**Attenuated Megalin Expression in Proximal Renal Tubules Is Associated with Tubular Atrophy/Interstitial Fibrosis by Oxford Classification in IgA Nephropathy**

Ikue Kobayashi,[1] Yasuo Imanishi,[1] Masayo Yamagata,[2] Toshimi Michigami,[3] Eiji Ishimura,[1] Masaaki Inaba.[1] *[1]Metabolism, Endocrinology and Molecular Medicine, Nephrology, Osaka City Univ Graduate School of Medicine, Japan; [2]Pharmacology, Osaka Ohtani Univ, Tondabayashi, Japan; [3]Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan.*

**Background:** Although vitamin D receptor activator (VDRA) have been reported to have a beneficial effect on IgA nephropathy (IgAN) possibly via inhibiting renin-angiotensin system, vitamin D (VD) metabolism in IgAN is not well determined. Megalin, a multiligand endocytotic receptor involving in the reabsorption of 25-hydroxyvitamin D in renal proximal tubules, plays an important role in VD metabolism. To elucidate the role of megalin in IgAN, its interstitial expression was examined in IgAN kidneys and was compared to Oxford classification of IgAN.

**Methods:** Renal biopsy specimens were collected from histologically proven 43 IgAN patients (serum creatinine 1.13 ± 0.74 mg/dl, urinary protein 1.24 ± 1.19 g/day) with informed consent. Interstitial megalin expression was determined by immunohistochemistry using ScanScope Aperio Slide Scanner.

**Results:** In tubular atrophy and interstitial fibrosis (T) by Oxford classification, the megalin expression in T1/2 significantly decreased compared to T0(P=0.03 by Mann-Whitney U test). Other Oxford categories such as mesangial hypercellularity, endocapillary hypercellularity or segmental sclerosis did not make any difference in the interstitial megalin expression. No significant relationship was observed between interstitial megalin expression and biochemical parameters such as serum creatinine, eGFR, urinary protein, or urinary β2-microglobulin.

**Conclusions:** Advanced T classification, one of determinants for worse renal prognosis in IgAN, suppressed interstitial megalin expression in IgAN. The suppression of intrinsic VD activation may contribute to the progression of IgAN possibly via renin-angiotensin system.

## SA-PO842

**Different Impact of Proteinuria on Renal Outcomes in IgA Nephropathy and Membranous Nephrpathy**

Natalia I. Polanco Fernandez, Eduardo Gutierrez-martinez, Elena Gutierrez-solis, Paula Jara Caro Espada, Manuel Praga. *Grupo de Enfermedades Glomerulares de la SEN (GLOSEN), Spain.*

**Background:** Reduction in proteinuria in chronic glomerular diseases is considered to be a crucial therapeutic objective. However, a clear proteinuria target (mean proteinuria during follow-up below which no renal outcomes will develop) has not been established for all glomerular diseases. Some studies have shown a high number of negative renal outcomes in IgA nephropathy (IgAN) with proteinuria persistently > 1 g/24h, but if the same target could be applied to other diseases like idiopathic membranous nephropathy (IMN) is unknown.

**Methods:** Retrospective, multicenter study performed in 14 Spanish Hospitals that collected two cohorts of patients: biopsy-proven IgAN (n=77) and biopsy-proven IMN (n=129), with a long follow-up (129 and 96 months, respectively). All patients with IMN had achieved remission of nephrotic syndrome (proteinuria <3.5 g/24h) spontaneously. Time-average proteinuria (TAP) throughout follow-up was determined in every patient along with clinical, biochemical and histologic parameters. Main outcomes were progression of renal insufficiency (measured by a >50% increase of baseline serum creatinine and the annual rate of GFR loss) and ESRD.

**Results:** Both IgAN and IMN patients were divided in two groups, according to TAP > or < 1 g/24h during follow-up. Whereas a clear difference in renal outcomes between both groups was observed in IgAN, there were no differences in IMN.

| | | IgAN | | | IMN | |
|---|---|---|---|---|---|---|
| | <1g/day | >1 <3.5 g/day | p | <1g/day | >1 <3.5 g/day | p |
| Final serum Creatinine (mg/dl) | 1.5 ± 0.9 | 3.4 ± 2.2 | < 0.0001 | 1 ± 0.33 | 1 ± 0.27 | 0.9 |
| >50% serum creatinine increase (%) | 4 | 52 | < 0.0001 | 8 | 11 | 0.6 |
| GFR loss (ml/min/1.73m2/yr) | -0.07 ± 2.1 | -2.5 ± 3.2 | < 0.0001 | -0.06 ± 4.4 | -0.2 ± 8.1 | 0.09 |
| ESRD (%) | 0 | 33 | < 0.0001 | 0 | 0 | 0.9 |

**Conclusions:** TAP >1 g/day is associated with a poor renal prognosis in IgAN, but IMN patients exhibit a significantly better tolerance to similar values of proteinuria. Pathogenic mechanisms explaining this different proteinuria impact are unknown.

## SA-PO843

**Quantitative Evaluation of Renal Cortical Fibrosis and Renal Prognosis in IgA Nephropathy and Focal Segmental Glomerulosclerosis**

Hidekazu Iida,[1] Kensuke Asaba,[2] Satoshi Kinugasa,[2] Yoshitaka Ishibashi,[1] Masaomi Nangaku,[2] Akihiro Tojo.[2] *[1]Div of Nephrology, Japanese Red Cross Medical Center, Tokyo, Japan; [2]Div of Nephrology and Endocrinology, The Univ of Tokyo, Tokyo, Japan.*

**Background:** Renal prognosis is generally correlated with interstitial fibrosis in chronic kidney diseases. However, previous scoring systems to evaluate interstitial fibrosis are subjective and may show inter-observer variability. Thus, we used computer image analyzer software to evaluate the ratio of fibrosis area to the area of renal cortex in Azan staining sections in patients with focal segmental glomerulosclerosis (FSGS) and IgA nephropathy, and evaluated its association with renal prognosis during 20 years follow up.

**Methods:** The ratio of fibrosis area to the area of renal cortex was evaluated quantitatively by an image analyzer software (Image Pro Plus®) in Azan staining kidney sections in 52 FSGS patients and 159 IgA nephropathy patients diagnosed by renal biopsy at the University of Tokyo. Induction of hemodialysis or doubling of serum creatinine was used as a combined end point, renal prognosis was evaluated with the Kaplan-Meier method, and risk factors were examined by Cox's proportional hazards model.

**Results:** After correction with age, sex, and blood pressure at renal biopsy, the fibrosis area ratio was found to be an independent risk factor for renal survival in both IgA nephropathy and FSGS patients by Cox's proportional hazards analysis. The cut off values of the fibrosis area ratio, determined from the ROC curve using the least-square method, were 14.5% for IgA nephropathy and 15.8% for FSGS. The mean length of renal survival in patients with a cortical fibrosis area ratio lower than 14.5% was significantly longer than those with a ratio higher than 14.5% (23.1 vs. 12.5 years, p<0.00001) in IgA nephropathy. Likewise, it was longer in patients with a cortical fibrosis area ratio lower than 15.8% (20.4 vs. 8.2 years, p<0.00001) in FSGS.

**Conclusions:** The renal cortical fibrosis area ratio calculated by computer image software is an objective index that enables estimation of long-term renal survival in IgA nephropathy and FSGS patients.

*Funding:* Government Support - Non-U.S.

## SA-PO844

**Long-Term Prognosis of Clinically Early IgA Nephropathy Is Not Always Favorable**

Hajeong Lee, Dong Ki Kim, Ho Jun Chin, Yon Su Kim, Chun Soo Lim, Jung Pyo Lee. *Internal Medicine, Seoul National Univ Hospital.*

**Background:** Long-term prognosis of clinically early IgA nephropathy (IgAN) patients remains to be clarified. In this study, investigated long-term outcome of IgAN patients with apparently benign presentation and evaluated the prognostic factor for renal survival.

**Methods:** We included biopsy proven IgAN patients with estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73m$^2$, normal blood pressure, and proteinuria (PU) < 0.5 g/day at the time of biopsy. Patients who were younger than 15 years old or with other systemic disease were excluded. Primary outcome was defined as end-stage renal disease (ESRD) progression. Secondary outcome was defined as 50% increase of serum creatinine or increased amount PU >1 g/day. During the follow-up, cases with loss of microscopic hematuria, PU < 0.2 g/day and stable renal function were categorized to remission group.

**Results:** A total of 142 patients met these criteria and analyzed. Median systolic blood pressure was 119 (110-121)mmHg, initial eGFR was 85.1 (74.9 – 100.4) mL/min/1.73m$^2$ and proteinuria was 0.25 (0.13 – 0.38) g/day at diagnosis. More than 13% of patients showed moderate or severe tubular atrophy or interstitial fibrosis. Among them, 4 patients were died, 5 reached to ESRD. Twenty-year renal survival rate was 88.6% during 92 (46 – 204) months of follow up. Increase of serum creatinine levels was found in 3 patients, and development of PU in 11 patients. Remission was demonstrated in 35 patients. No patients were treated by immunosuppressive agents and 61 (43%) of patients were taken renin-angiotensin-aldosterone. Moderate or severe degree of interstitial fibrosis (adjusted odd ratio (OR) 6.828, 95% confidence interval (CI) 1.512 – 30.801, P = 0.013) and mild renal insufficiency (eGFR < 90mL/min/1.73m$^2$) (adjusted OR 3.866, 95% CI 1.004 – 14.933, P = 0.049) were independent predictors for elevation of serum creatinine or increased amount of PU.

**Conclusions:** This study demonstrated that the prognosis of early IgAN was not always favorable. Besides mild renal insufficiency, pathologic changes including interstitial fibrosis also should be considered as an important prognostic factor for clinically early IgAN patients.

## SA-PO845

**Effect of Hematuria on the Outcome of IgA Nephropathy with Mild Proteinuria**

Kayu Tanaka, Takahito Moriyama, Chihiro Iwasaki, Yasuko Oshima, Kosaku Nitta. *Medicine, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** Heavy proteinuria has been widely recognized as the risk factor for progression of IgA nephropathy (IgAN), and hematuria recently has been recently recognized to be excreted from the active inflammatory lesion of glomerular capillaries in IgAN. However the effect of hematuria on the outcome for IgAN remains unknown and the treatment of IgAN with severe hematuria is still controversial.

**Methods:** We retrospectively examined 88 IgAN patients with proteinuria less than 0.5g/day and were not treated with corticosteroid, immunosuppressive agents or tonsillectomy. We divided them into two groups: the low urinary red blood cells (U-RBC) group (L group, n=48); U-RBC was less than 20 counts/high-power field (HPF), and the high RBC group (H-group, n=40); U-RBC was greater than 20 counts/HPF. We analyzed the clinical and histological findings and renal survival rate until progression to end-stage renal disease.

**Results:** Median U-RBC in H group was significantly higher than L group (L group: 5 vs. H group: 30 counts/HPF, p<0.0001), and the other laboratory data were similar between both groups [estimated glomerular filtration rate (L: 77.1 vs. H: 81.9 ml/min) and proteinuria (0.26 vs. 0.27 g/day)], though some clinical findings were significantly different between both groups [male to female ratio (L: 25:23 vs. H: 9:31, p=0.004), and systolic and diastolic blood pressure (122.8/76.5 vs. 116.1/69.4 mmHg, p=0.0179/0.0073)]. Histological findings were not significantly different between both groups. During the 5 years after renal biopsy, median amount of proteinuria remained less than 0.5g/g•creatinine in both groups, and median amount of U-RBC was decreased to less than 5/HPF in the L group and to 10/HPF in the H group without any intensive therapy. 25 years renal survival rate by the Kaplan-Meier method was 100 % in H group, but it was decreased to 85.5% in L group, though it was not significant.

**Conclusions:** Severe hematuria at the time of biopsy naturally improved without any intensive therapy, and there were no effect of hematuria on the outcome for IgAN.

**SA-PO846**

**Clinical Feature and Long Term Renal Outcome of IgA Nephropathy in China: A Retrospective Cohort Study of 1835 Cases** Wei Qin, Xiaolei Chen, Junming Fan, Zi Li, Ling Ji. *Div of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

**Background:** This study was conducted to clarify clinical feature and long term renal outcome of IgA nephropathy (IgAN) in China.

**Methods:** 1835 patients with IgAN were included in this study. The clinicopathological features, treatments, responses and renal outcome were analyzed.

**Results:** Of the 1835 patients, 586 presented with nephrotic syndrome (NS); 114 presented with hematuria without proteinuria; 303 presented with renal dysfunction at biopsy; the rest 832 presented with nephritic syndrome with normal renal function. The median followup time was 40.4 months. Renal function in 94.1% patients remain stable. Only 109 patients developed ESRD or GFR reduced>50%. Multivariate analysis indicated that estimated glomerular filtration rate (eGFR) <90 mL/min, uncontrolled hypertension, NS, severe pathological lesions were the independent risk factors of decreasing of renal function. In patients with NS, 392 cases (66.9%) were IgAN with minimal change disease. Corticosteroid achieved high complete remission (CR) rate (86.2%) with a considerable relapse rate (28.1%). In the other 440 patients with NS and mesangial proliferation, corticosteroids and immunosuppressants achieved 57% CR, 31% partial remission (PR) and 12% no response (NR). In patient with proteinuria between 1-3g/d with or without renal dysfunction, immunosuppressive treatment achieved 28% CR, 37% PR, and 35% NR. RAS inhibitors (RASi) were prescribed in most patients. However, significant effect could only be observed in patients with hypertension. Renal function remained normal in every patient with normal renal function and proteinuria <1g/d during following up. Treatment of RASi or immunosuppressants did not show any benefit regarding as decreasing of proteinuria or protecting of renal function in these patients.

**Conclusions:** Renal function decline was observed in 6% of IgAN patients during following up. The prognostic factor of renal survival were eGFR<90 mL/min, uncontrolled hypertension, NS, severe pathological lesions. Corticosteroids and immunosuppressant was effective in most patients. RASi only had a limited effect in Chinese patients.

**SA-PO847**

**The Nationwide Retrospective Cohort Study in IgA Nephropathy in Japan** Takashi Yasuda,[1] Yoshinari Yasuda,[2] Sachiko Ohde,[3] Osamu Takahashi,[3] Tetsuya Kawamura,[4] Seiichi Matsuo.[2] *[1]Nephrology & Hypertension, St. Marianna Univ School of Medicine, Kawasaki, Kanagawa, Japan; [2]Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Aichi, Japan; [3]Center for Clinical Epidemiology, St. Luke's Life Science Institute, St. Luke's International Hospital, Tokyo, Japan; [4]Kidney & Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** We have started the Nationwide Retrospective Cohort Study in IgA nephropathy in Japan since Sep. 1, 2012. The main purpose is to clarify the choice of therapy, including tonsillectomy in combination with intravenous pulse methylprednisolone followed by oral prednisone (tonsillectomy with pulse), in patients with IgA nephropathy under various clinical presentations. In this interim analysis, we evaluated the therapeutic efficiency on renal outcome defined as 50 percent increase in the serum creatinine concentration from baseline between conservative therapy without steroids, oral steroids, pulse methyprednisone followed by oral prednisone (pulse methyprednisone), and tonsillectomy with pulse.

**Methods:** Adult patients with IgA nephropathy diagnosed by the first renal biopsy during the three years from 2002 to 2004 were eligible. Data at the time of renal biopsy and during the follow-up were collected, and total 1,103 cases from 49 facilities were registered till Dec. 1, 2012. Among them, we analyzed 931 cases which have sufficient data for the analysis.

**Results:** The median observation period was 5.4 years. Choice of therapy was as follow; conservative therapy 475, oral steroids 174, pulse methylprednisolone 121, and tonsillectomy with pulse 161. In this period, 103 patients reached the renal outcome. Cox regression analyses with adjustment for baseline covariates showed that, compared to the patients with tonsillectomy with pulse, the risk for those with other therapy was as follow; conservative therapy 3.74 (95%CI, 1.75-8.00), oral steroids 2.02 (0.87-4.68), pulse methyprednisone 2.76 (1.22-6.24).

**Conclusions:** This interim analysis seems to indicate the superiority of tonsillectomy with pulse in terms of improving renal prognosis. After registration of all cases, we will clarify proper choice of therapy by the propensity score analysis.

*Funding:* Government Support - Non-U.S.

**SA-PO848**

**Efficacy and Safety of Telmisartan, Clopidogrelin and Leflunomide in Patients with IgA Nephropathy — A Multicentre, Prospective, Randomized, Double-Blind, Double-Dummy Controlled Clinical Trial** Xiang-Mei Chen, Jie Wu, Shuwei Duan, Ying Zheng. *Dept of Nephrology, State Key Laboratory of Kidney Disease, Chinese PLA General Hospital, Beijing, China.*

**Background:** To evaluate the efficacy and safety of telmisartan combined with clopidogrelin and/or leflunomide for patients with lgA nephropathy and whether the combination therapy surpass telmisartan in decreasing proteinuria and protecting renal function.

**Methods:** We enrolled 400 patients aged 18–55 years from 13 centres in Beijing who had proteinuria 0.5~3.5g/d, baseline serum creatinine (SCr) <3mg/dl, All patients were eluted by taking telmisartan 80mg/d for 4 weeks and then randomly assigned to receive 24 weeks of treatment with telmisartan 80mg/d + clopidogrelin placebo + leflunomide placebo (group A), telmisartan 80mg/d + clopidogrelin 50mg/d + leflunomide placebo (group B), telmisartan 80mg/d + clopidogrelin placebo + leflunomide 20mg/d (group C), telmisartan 80mg/d + clopidogrelin 50mg/d + leflunomide 20mg/d (group D). Comparison of 24h urinary protein excretion, the serum creatinine, eGFR, albumin, cholesterol and uric acid after the therapy were assessed.

**Results:** After treatment for 24 weeks, proteinuria declined significantly in the four groups (P<0.05), while those in group C (1.20±0.76 vs 0.77±0.42 g/24h) and group D (1.16±0.63 vs 0.74±0.49 g/24h) were decreased more significantly than in group A (1.15±0.87 vs 0.92±0.58 g/24h) and group B (1.11±0.83 vs 0.89±0.42 g/24h) (P<0.05). Mixed effects model analysis showed that telmisartan, leflunomide and telmisartan combine leflunomide decreased proteinuria (P<0.01). The SCr declined in group C and group D more significantly than that in group A and group B (P<0.05). The level of eGFR in group C and group D were increased more than that in group A and group B. The serum uric acid in group C and group D decreased more significantly than group A and group B (P<0.05). There were no significant differences in the results of albumin and cholesterol among the four groups. No obvious adverse reactions were found in the four groups.

**Conclusions:** Telmisartan combined with leflunomide was safe and effective in decreasing proteinura and protecting short-term renal function.

*Funding:* Government Support - Non-U.S.

**SA-PO849**

**Eculizumab (ECU) Inhibits Thrombotic Microangiopathy (TMA) and Improves Renal Function in Pediatric Atypical Hemolytic Uremic Syndrome (aHUS) Patients (Pts)** Larry A. Greenbaum, Marc Fila, Michel Tsimaratos, Gianluigi Ardissino, Samhar I. Al-Akash, Jonathan Evans, Paul H. Henning, Kenneth V. Lieberman, Silvio Maringhini, Lars Pape, Lesley Rees, Nicole Van De Kar, Johan Van de Walle, Masayo Ogawa, Camille L. Bedrosian, Christoph Licht. *C10-003 Investigators.*

**Background:** aHUS is a rare, genetic, life-threatening disease of uncontrolled complement activation leading to systemic TMAand severe end-organ damage. ECU, a terminal complement inhibitor, is approved for the treatment of aHUS, and was shown to be safe and effective in a retrospective pediatric study. Here, we report safety and efficacy results at 26 weeks (wks) from the first prospective trial of pediatric aHUS pts.

**Methods:** This was an open-label, single-arm, Phase 2 trial of ECU in pediatric pts with aHUS. The primary endpoint was complete TMA response at 26 wks. Dosing was based on weight cohorts.

**Results:** 22 pts (age 1 month to 17 yrs) were enrolled; 19 completed 26 wks. 16 pts (73%) were newly diagnosed (median duration of 6 days prior to ECU). At wk 26, 14 pts (64%) achieved the primary endpoint.

| **Table: Wk 26 Results (N=22)** | |
|---|---|
| **Baseline Demographics and Disease Characteristics** | |
| Age (years) – mean (SD) | 6.6 (6.1) |
| Female sex – n (%) | 10 (45) |
| Identified complement regulatory protein mutation or auto-antibody – n (%) | 11 (50) |
| Time from aHUS diagnosis until screening (months) – median (range) | 0.56 (0.03–191.3) |
| Newly diagnosed pts – n (%) | 16 (73) |
| Duration of current clinical manifestation of aHUS (months) – median (range) | 0.2 (0.0–4.3) |
| PE/PI at baseline – n (%) | 10 (45) |
| Dialysis at baseline – n (%) | 11 (50.0) |
| Prior renal transplant – n (%) | 2 (9) |
| Platelet count <150x10$^9$/L – n (%) | 22 (100) |
| LDH >ULN – n (%) | 19 (86) |
| eGFR ≤60 mL/min/1.73m$^2$ – n (%) | 18 (82) |
| **Efficacy Outcomes** | |
| Complete TMA response – n (%) | 14 (64) |
| Complete hematologic response – n (%) | 18 (82) |
| Platelet count normalization – n (%) | 21 (95) |
| eGFR improvement from baseline ≥15 mL/min/1.73m$^2$ – n (%) | 19 (86) |
| eGFR increase from baseline (mL/min/1.73 m$^2$) – mean (95% CI) | 64 (50; 79)<br>*P*<0.0001 (wk 25) |
| Serum creatinine ≥25% decrease from baseline – n (%) | 16 (73) |

**Complete TMA response:** normalization of platelets and LDH, and ≥25% improvement in serum Cr from baseline on 2 consecutive measurements >4 wks apart. **Complete hematologic response:** platelet and LDH normalization at ≥2 consecutive measurements ≥4 wks apart. **Platelet Count Normalization:** platelet count ≥150x10$^9$/L ≥2 consecutive measurements ≥4 wks apart.

9/11 pts (82%) on dialysis at BL discontinued dialysis. Of the 11 pts not on dialysis at BL, 11 (100%) remained dialysis free through 26 wks. Of 10 pts on PE/PI at BL, all discontinued. QoL was significantly improved. ECU was safe and well tolerated. No pts withdrew due to Aes, no pts had meningococcal infections, and no pts died.

**Conclusions:** In this first prospective trial of pediatric pts with aHUS, early intervention with ECU improved hematologic and renal parameters, and decreased the burden of dialysis. These results confirm that ECU inhibits complement-mediated TMA, and is safe and effective at the approved dose regimen in pediatric pts with aHUS, including those receiving first-line ECU. Treatment in the clinical trial is ongoing.

*Funding:* Pharmaceutical Company Support - Alexion Pharmaceticals

## SA-PO850

**Eculizumab (ECU) in Atypical Hemolytic Uremic Syndrome (aHUS) Patients (Pts) with Long Disease Duration and Chronic Kidney Disease (CKD): Sustained Efficacy at 3 Years**

Yahsou Delmas,[1] Chantal Loirat,[2] Petra Muus,[3] Christophe M. Legendre,[4] Kenneth Douglas,[5] Maryvonne Hourmant,[6] Birgitta Maria Herthelius,[7] Antonella Trivelli,[8] Tim Goodship,[9] Giuseppe Remuzzi,[10] Camille L. Bedrosian,[11] Christoph Licht.[12] *[1]CHU Pellegrin-Bordeaux; [2]Hôpital Robert-Debré; [3]Radboud Univ Nijmegen Medical Centre; [4]Univ Paris Descartes and Hôpital Necker; [5]Beatson West Scotland Cancer Centre; [6]CHU de Nantes-Hôpital Hotel Dieu; [7]Karolinska Univ Hospital; [8]Gaslini Institute; [9]Newcastle Univ; [10]Istituto Mario Negri; [11]Alexion Pharmaceuticals, Inc.; [12]The Hospital for Sick Children.*

**Background:** aHUS is a rare, genetic, life-threatening disease of chronic complement activation leading to systemic TMA. ECU was shown to inhibit TMA, eliminate PE/PI, and improve renal function in pts with long aHUS duration and CKD in 1- and 2-yr reports of this prospective study. Here, we provide a 3-yr update.

**Methods:** aHUS pts ≥12 yrs with CKD receiving chronic PE/PI were enrolled. After 8 wks of observation, pts stopped PE/PI and started ECU.

**Results:** 20 pts were treated—16 pts for ≥30 mos. Median (med) time from diagnosis was 48 mos. Med duration of ECU was 156 (26—176) wks. Baseline mean eGFR was 31 mL/min/1.73m$^2$, and 90% of pts had eGFR≤60. Efficacy outcomes were consistent with a 2-yr analysis.

Table

| Key Outcomes* (N=20; ITT) | 114 wk Median ECU Duration | 156 wk Median ECU Duration |
|---|---|---|
| TMA event-free status[†,‡], % pts (95% CI) (Primary) | 95 (75–100) | 95 (75–100) |
| Hematologic normalization[†,§], % pts (95% CI) (Primary) | 90 (68–99) | 90 (68–99) |
| Mean change in eGFR from baseline, mL/min/1.73m$^2$, (95% CI) | +7.2 (0.76–13.6) (wk 104) | +4.1 (-12.6–20.9) (wk 152) |
| eGFR increase of ≥15 mL/min/1.73m$^2$, % pts (95% CI) | 40 (19–64) | 40 (19–64) |
| CKD improvement of ≥1 stage, % pts (95% CI) | 60 (36–81) | 60 (36–81) |
| ≥25% reduction in serum creatinine, % pts (95% CI) | 55 (32–77) | 55 (32–77) |
| Mean change in health-related quality of life (EQ-5D score)[‖] (SD) | 0.12 (0.15) (wk 104) | 0.16 (0.24) (wk 152) |

ECU dosage: 900 mg/week for 4 wks, 1200 mg at wk 5, then 1200 mg every 2 wks thereafter.
*All analyses are compared with baseline values.
†LOCF analysis.
‡Normalization of platelet and LDH counts for 2 measurements ≥4 wks apart.
§≥12 wks with no platelet decrease >25%, no PE/PI, and no new dialysis.
‖Clinically meaningful threshold ≥0.06.

3 pts had serious Aes possibly or probably related to ECU. 1 death (unrelated) occurred in the extension.

**Conclusions:** Improvements in hematologic parameters and kidney function were maintained at 3 yrs. These results confirm sustained efficacy with long-term ECU treatment and resulting terminal complement inhibition in aHUS pts with CKD previously managed with PE/PI.

*Funding:* Pharmaceutical Company Support - Alexion Pharmaceuticals

## SA-PO851

**Successful Treatment of DEAP-HUS with Eculizumab**

Damien Gerard Noone,[1] Fred G. Pluthero,[1] Peter F. Zipfel,[3] Christoph Licht.[1] *[1]Nephrology, The Hospital for Sick Children, Toronto, Canada; [2]Immunology, Univ of Heidelberg, Heidelberg, Germany; [3]Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany.*

**Background:** DEAP-HUS (deficiency of CFHR proteins and CFH autoantibody positive) represents a unique subgroup of complement-mediated aHUS. CFH autoantibodies block CFH surface recognition and mimic aHUS causing mutations. CFH autoantibodies are found in up to 15% of aHUS patients and typically occur in the background of CFHR3/CFHR1 deletions. The principal treatment concept for complement-mediated aHUS hinges on restoring alternative pathway regulation via plasma-based treatments or eculizumab, a monoclonal C5 antibody. Clinical practice guidelines for DEAP-HUS are lacking; strategies have been adopted from genetically-determined forms of aHUS and other autoimmune diseases. The literature supports PLEX initially, but with the addition of an antibody-suppressing or depleting agent with varied success reported.

**Methods:** We present a retrospective report of the successful use of eculizumab in 2 DEAP-HUS patients and their follow up.

**Results:** We show eculizumab to be safe and effective in the management of DEAP-HUS in 2 patients with confirmed CFHR3/CFHR1 deletion. One was dependent on bi-weekly plasma infusions. Successful induction of aHUS remission was achieved in the acute phase in the other. Immunosuppressive agents and midterm discontinuation of eculizumab are considered. We propose a biphasic treatment concept for DEAP-HUS with eculizumab used acutely to arrest the complement mediated damage and continued for at least 6 months (Phase 1), followed by the use of immunosuppressive agents targeting ongoing antibody production (Phase 2), with antibody titre measurement.

**Conclusions:** The complement C5 inhibitor eculizumab is safe and effective in maintaining a disease-free state in DEAP-HUS in the presence of ongoing anti-CFH autoantibodies. While immunosuppression alone may sufficiently address the autoimmune component of DEAP-HUS, the predisposing lack of CFHR1 – an identified C5 convertase inhibitor – and possible additional but unrecognized complement mutations would be left untreated.

## SA-PO852

**Eculizumab (ECU) Maintains Efficacy in Atypical Hemolytic Uremic Syndrome (aHUS) Patients (Pts) with Progressing Thrombotic Microangiopathy (TMA): 3-Year (Yr) Update**

A. Osama Gaber,[1] Chantal Loirat,[2] Larry A. Greenbaum,[3] Sunil Babu,[4] Richard R. Furman,[5] Neil S. Sheerin,[6] David J. Cohen,[7] Frank Eitner,[8] Yahsou Delmas,[9] Camille L. Bedrosian,[10] Christophe M. Legendre.[11] *[1]The Methodist Hospital; [2]Hopital Robert Debre; [3]Emory Univ; [4]Fort Wayne Medical Oncology and Hematology; [5]Weill Cornell Medical College; [6]Newcastle Univ; [7]Columbia Univ; [8]Bayer Pharma AG; [9]CHU Pellegrin-Bordeaux; [10]Alexion Pharmaceuticals, Inc; [11]Univ Paris Descartes & Hôpital Necker.*

**Background:** aHUS is a rare, genetic, life-threatening disease of chronic complement activation leading to systemic TMA. In 1 and 2 yr reports from this prospective study (C08-002), ECU was shown to inhibit TMA, prevent or reverse organ damage, and reduce the need for dialysis in aHUS pts. Here we provide a 3-yr update.

**Methods:** aHUS pts ≥12 yrs with progressing TMA (platelet decrease of ≥25% despite ≥4 PE/PI sessions 1 week [wk] before screening) entered an open-label, Phase 2 trial and long-term extension. Primary endpoints were platelet count change and hematologic normalization.

**Results:** 17 pts enrolled and 13 pts continued in the extension. Median duration of treatment was 100 wks, with a range of 2 to 186 wks. 5 pts remained enrolled for >130 wks. Continuous, long-term ECU treatment sustained or improved key hematologic and renal endpoints at 3 yrs.

Table

| Key Outcomes* (N=17; ITT) | 2-yr analysis | 3-yr analysis |
|---|---|---|
| Mean change in platelet count, x10$^9$/L (SD) (primary endpoint) | 94 (56) *P*=0.001 (wk 104) | 136 (41) *P*=0.007 (wk 156) |
| Hematologic normalization[†,‡], % pts (95% CI) | 88 (64–99) | 88 (64–99) |
| TMA-event-free status[†,§], % pts (95% CI) | 88 (64–99) | 88 (64–99) |
| Mean change in eGFR, mL/min/1.73m$^2$ (95% CI) | 35.2 (17.4–53.1) *P*=0.0005 (wk 104) | 54.0 (11.8–96.1) *P*=0.03 (wk 156) |
| eGFR increase of ≥15 mL/min/1.73m$^2$, % pts (95% CI) | 59 (33–82) | 59 (33–82) |
| CKD improvement of ≥1 stage, % pts (95% CI) | 71 (44–90) | 76 (50–93) |
| Serum creatinine decrease of ≥25%, % pts (95% CI) | 76 (50–93) | 76 (50–93) |
| Mean change in health-related quality of life[‖], EQ-5D score (95% CI) | 0.31 (0.28–0.34) *P*<0.0001 (wk 104) | 0.29 (0.28–0.30) *P*<0.0001 (wk 152) |

ECU dosage: 900 mg/wk for 4 wks, 1200 mg at wk 5, 1200 mg q2w thereafter.
*All analyses are compared with baseline values.
†LOCF analysis.
‡Normalization of platelet and LDH counts for 2 measurements ≥4 wks apart.
§≥12 wks with no platelet decrease >25%, no PE/PI, and no new dialysis.
‖Clinically meaningful threshold ≥0.06.

ECU was generally safe and well tolerated. 4 pts had serious Aes judged to be possibly drug related. No pts died.

**Conclusions:** The data demonstrate the safety and continued efficacy of ECU therapy in aHUS pts, highlighting the ongoing inhibition of TMA at this 3-yr update.

*Funding:* Pharmaceutical Company Support - Alexion Pharmaceuticals

## SA-PO853

**An Observational, Non-Interventional, Multicenter, Multinational Registry of Patients (Pts) with Atypical Hemolytic Uremic Syndrome (aHUS): Initial Pt Characteristics**

Christoph Licht,[1] Gianluigi Ardissino,[2] Gema Ariceta,[3] Jon Beauchamp,[4] David J. Cohen,[5] Larry A. Greenbaum,[6] Sally A. Johnson,[7] Masayo Ogawa,[8] Franz S. Schaefer,[9] Johan Van de Walle,[10] Veronique Fremeaux-bacchi.[11] *[1]The Hospital for Sick Children; [2]Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico; [3]Hospital Vall d' Hebron; [4]Alexion Pharmaceuticals International; [5]Columbia Univ; [6]Emory Univ; [7]Newcastle upon Tyne Hospital; [8]Alexion Pharmaceuticals; [9]Univ of Heidelberg; [10]UZ Gent Dienst Nefrologie; [11]Assistance Publique-Hopitaux de Paris.*

**Background:** aHUS is a rare, genetic, life-threatening disease of chronic complement activation leading to systemic thrombotic microangiopathy, with renal and other end-organ damage. The global aHUS Registry, initiated in April 2012, prospectively collects information on pts with aHUS. Here, we report baseline demographics from the initial pts in the aHUS Registry.

**Methods:** aHUS pts (regardless of treatment) are eligible for enrollment. Demographic, medical and disease history, treatments, efficacy and safety outcomes data are collected initially and every 6 months thereafter.

**Results:** By March 31, 2013, 53 pts from 8 countries were enrolled in the aHUS registry. 32 (60%) were treated with eculizumab [ECU], and 47 (89%) were ≥18 yrs.

Family history of aHUS, prior kidney graft, dialysis, and PE/PI were observed in ECU and non-ECU treated groups.

Table

| Demographics | | | |
|---|---|---|---|
| | Treated with Eculizumab (N=32) | Never Treated with Eculizumab (N=21) | Total (N=53) |
| Mean age at registry enrollment (SD), yrs | 38.1 (18.38) | 33.0 (15.62) | 36.1 (17.36) |
| Age at registry enrollment, n (%) | | | |
| ≥2 to <5 yrs | 2 (6.3) | 2 (9.5) | 4 (7.5) |
| ≥5 to <12 yrs | 1 (3.1) | — | 1 (1.9) |
| ≥12 to <18 yrs | — | 1 (4.8) | 1 (1.9) |
| ≥18 yrs | 29 (90.6) | 18 (85.7) | 47 (88.7) |
| Sex, n (%) | | | |
| Female | 22 (68.8) | 7 (33.3) | 29 (54.7) |
| Male | 10 (31.3) | 14 (66.7) | 24 (45.3) |
| Year of registry enrollment, n (%) | | | |
| 2012 | 17 (53.1) | 4 (19.0) | 21 (39.6) |
| 2013 | 15 (46.9) | 17 (81.0) | 32 (60.4) |
| **aHUS Disease Characteristics** | | | |
| | Treated with Eculizumab (N=32) | Never Treated with Eculizumab (N=21) | Total (N=53) |
| Mean age at initial symptoms (SD), yrs | 37.7 (19.84) | 24.2 (13.63) | 33.1 (18.94) |
| Mean age at diagnosis (SD), yrs | 39.2 (19.00) | 25.8 (15.97) | 34.5 (18.95) |
| Family history of aHUS, n (%) | 4 (12.5) | 6 (28.6) | 10 (18.9) |
| Prior kidney graft, n (%) | 4 (12.5) | 1 (4.8) | 5 (9.4) |
| Prior dialysis, n (%) | 15 (46.9) | 4 (19.0) | 19 (35.8) |
| Prior PE/PI, n (%) | 17 (53.1) | 4 (19.0) | 21 (39.6) |
| **Treatment Characteristics** | | | |
| | Treated with Eculizumab (N=32) | | |
| Mean age at eculizumab treatment initiation (SD), yrs | 38.1 (18.38) | | |
| Median dose at initiation of eculizumab (mg) | 900.0 | | |
| Mean time on eculizumab(SD), yrs | 0.9 (0.85) | | |
| Discontinuation of eculizumab, n (%) | 2 (6.3) | | |
| Restart of eculizumab (among those who discontinued), n (%) | 1 (50.0) | | |

Mean age at treatment initiation was 38 yrs, and mean time on ECU was 0.9 yr. Two pts discontinued ECU, with 1 of 2 restarting.

**Conclusions:** Analyses of data obtained through the aHUS Registry will increase our understanding of the history and progression of aHUS, and may help optimize pt care and quality of life. New clinical sites are encouraged to participate.

*Funding:* Pharmaceutical Company Support - Alexion Pharmaceuticals

## SA-PO854

**Spectrum of HIV-Associated Kidney Disease in the Era of Combination Antiretroviral Therapy**

John W. Booth,[1] Stephen Paul McAdoo,[2] Emil A. Kumar,[2] Tabitha Turner-stokes,[1] Partha Das,[3] Claire Melinda Naftalin,[3] Nadia Khatib,[5] Catherine Horsfield,[3] Patrick J. O'Donnell,[3] Deborah I. Williams,[4] Ed Kingdon,[4] Jyoti B. Baharani,[5] Bruce M. Hendry,[3] Rachel Hilton,[3] Jeremy B. Levy,[2] Rachael Jones,[6] John O. Connolly,[1] Frank A. Post.[3] *[1]UCL; [2]Imperial College; [3]King's Health Partners; [4]Brighton; [5]Birmingham; [6]Chelsea & Westminster NHS Trust, London, United Kingdom.*

**Background:** The spectrum of kidney disease in HIV positive patients has changed as a result of effective combination anti-retroviral therapy (cART) and ageing but remains poorly described.

**Methods:** We reviewed consecutive renal biopsies (2000-2012) of HIV+ patients attending eight clinics in the UK. We describe the clinical characteristics of 199 of 250 patients with HIVAN/FSGS (67), immune complex kidney disease (ICKD, 86) and tubulo-interstitial disease (TID, 46). Kruskall-wallis test was used to compare medians, chi squared test for proportions, and log-rank test to compare Kaplan-Meier estimates of end-stage kidney disease (ESKD).

**Results:** Patients with HIVAN and primary FSGS (NOS) were indistinguishable. ICKD encompassed membranous (16), undifferentiated immune complex disease (NOS) (34), IgA (26), MPGN (5) and SLE nephritis (5). TID included TIN (18), acute tubular injury (16) and interstitial fibrosis with tubular atrophy (12). Patients with HIVAN/FSGS, ICKD and TID differed by ethnicity (black: 97, 48, 36 %), median known duration of HIV (0.1, 6.3, 9.6 years), degree of immunodeficiency (median CD4 nadir 56, 128, 117; median CD4 at biopsy 122, 389, 289) and severity of CKD (median eGFR 23, 55, 33) ($p<0.001$ for all). At biopsy, 35, 68, 76 % of patients had initiated cART and 19, 53, 72 % had HIV RNA <200 c/mL; at 1 and 5 years post-biopsy, 28, 7, 7 % and 54, 21, 14 % of patients had progressed to ESKD ($p<0.001$ for all).

**Conclusions:** In this cohort, HIVAN/FSGS was typically present at HIV diagnosis. Patients with ICKD and TID were frequently stable on ART with suppressed HIV replication at biopsy. All forms of CKD were associated with a history of advanced immunodeficiency suggesting that early HIV diagnosis and appropriate cART initiation may constitute a renal risk reduction strategy.

## SA-PO855

**Tubulointerstitial Fibrosis Not Glomerular Sclerosis Predicts 1 Year Kidney Function after Donation**

Daisuke Ichikawa,[1] Sayuri Shirai,[1] Yugo Shibagaki,[1] Atsuko Ikemori,[2] Masahiko Yazawa,[1] Kayori Tsuruoka,[1] Naohiko Imai,[1] Takashi Yasuda,[1] Kenjiro Kimura.[1] *[1]Div of Nephrology and Hypertension, Dept of Internal Medicine, St. Marianna Univ School of Medicine, Kawasaki, Japan; [2]Dept of Anatomy, St. Marianna Univ School of Medicine, Kawasaki, Japan.*

**Background:** Studies have shown that the risk of end-stage renal disease in kidney donors appears to be similar to that in the general population; however, very little isknown about which histological findings correlate well with the prognosis of kidney function in healthy adults with a unilateral kidney. Here we investigated which parameters affected prognosis 1 year after donation.

**Methods:** This was a retrospective observational cohort study. The study included 41 consecutive adult living-related kidney donors who underwent kidney biopsy at the time of kidney grafting (0-hour donor kidney biopsy: 0hB) between 2009 and 2012. Data of kidney function by estimated glomerular filtration rate (eGFR) and risk factors at the time of donation were obtained from the medical records, and the kidney function of the donors was followed for 1 year. Biopsy tissues were evaluated for glomerular sclerosis (GS) rate (%),interstitial fibrosis (IF) rate (%), grade of arteriosclerosis (AS), and existence of arteriolosclerosis (ALS).

**Results:** A high IF rate was associated with a low eGFR at the time of 0hB ($r = 0.46$, $P < 0.01$), while a high GS rate was not with a low eGFR ($r = 0.08$). Grade of AS and existence of ALS did not affect eGFR. This association between the 4 histological findings and eGFR persisted at least for a year after donation. A decreasing eGFR a year after donation was only associated with a high IF rate. IF was evaluated, and the donors were divided into 4 quantile groups. The decreasing eGFR a year after donation was significantly higher in the highest IF group (> 10.5%) than in the lowest IF group (< 5.18%) ($P< 0.05$).

**Conclusions:** IF affected the progression of renal dysfunction 1 year after donation, while GS did not affect it. In healthy adults with a unilateral kidney, it is necessary to carefully check for severe IF.

## SA-PO856

**Syk Activation in Leucocytes in Acute Human Glomerulonephritis**

Jessica Ryan,[1,2] John Kanellis,[1,2] David J. Nikolic-Paterson.[1,2] *[1]Dept of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia; [2]Dept of Medicine, Monash Univ, Clayton, Victoria, Australia.*

**Background:** Spleen tyrosine kinase (Syk) plays an important role in Fc-γ receptor signaling. Syk inhibition is protective in rodent models of antibody-dependent glomerular disease; however, the potential role of Syk in human glomerulonephritis (GN) has not been investigated. Therefore, we examined activation of Syk in a range of human GN biopsies bydetecting phosphorylation of Tyr525/526 in the Syk activation loop (p-Syk).

**Methods:** Immunostaining for p-Syk, neutrophils and CD68+ macrophages was performed in renal biopsy sections in a cohort of 96 patients, which included: MCD (3), TBMD (8), post-infectious GN (5), Class IV SLE (19), ANCA vasculitis (13), IgAN (31), membranous nephropathy (7) and FGS (10).

**Results:** MCD and TBMD, which are diseases generally not associated with leucocytic infiltration or antibody deposition, had no infiltrating p-Syk+ cells. Likewise membranous GN and FGS had few glomerular p-Syk+ cells. In contrast, the proliferative GNs had numerous glomerular p-Syk+ cells (PIGN>ANCA vasculitis>Class IV SLE>IgA Nephropathy). p-Syk was seen in infiltrating glomerular leukocytes, mainly neutrophils and some macrophages, in 36/40 cases of crescentic GN. Glomerular p-Syk+ cells had a strong correlation with infiltrating neutrophils ($r = 0.85$, $P < 0.001$), and a weaker correlation with macrophages ($r = 0.22$, $P < 0.05$). Likewise interstitial p-Syk+ cells correlated with neutrophils and macrophages ($r = 0.63$ and $r = 0.35$ respectively, both $P < 0.01$). Glomerular and interstitial p-Syk+ cells correlated with renal function (GFR ml/min $r = -0.25$ and $-0.22$ respectively, both $P <0.05$) and systemic inflammation (C-reactive protein $r = 0.28$ and $r = 0.35$ respectively, both $P < 0.05$), but not proteinuria.

**Conclusions:** Syk is activated in infiltrating neutrophils and macrophages in acute forms of antibody-dependent glomerular disease. These findings support the therapeutic use of Syk inhibitors in rapidly progressive crescentic glomerulonephritis.

*Funding:* Government Support - Non-U.S.

## SA-PO857

**Baseline Immunosuppression Exposure in Neptune Study Cohort**

Keisha L. Gibson,[1] J. Troost,[2] Howard Trachtman,[2] Christine Sethna,[2] Alessia Fornoni,[2] Richard A. Lafayette,[2] Susan L. Hogan,[2] Michelle A. Hladunewich,[2] Matthias Kretzler,[2] Debbie S. Gipson.[2] *[1]UNC Kidney Center, Univ of NC at Chapel Hill, Chapel Hill, NC; [2]The Nephrotic Syndrome Network (NEPTUNE).*

**Background:** The Nephrotic Syndrome Study Network (NEPTUNE) includes a multicenter, prospective cohort of adults and children with proteinuria and a clinical indication for initial renal biopsy. We aimed to characterize the pre-biopsy immunosuppressive therapy (IST) and clinical features associated with therapy among the initial 313 enrollees.

**Methods:** IST was categorized as steroids only, calcineurin inhibitor, mycophenolate, cytoxan, multiple (MIST), and none. Indications for biopsy were classified as steroid dependent (SDNS), frequently relapsing (FRNS), steroid resistant (SRNS), or diagnosis. ANOVA and chi-squared tests were used for analysis. Data are presented as n(%) and median(interquartile range).

**Results:** 51(24%) of adults and 69(71%) of children were treated with IST ($p<0.01$). Median months cumulative IST was 2.0(14.0) adults and 6.0(10) children, $p <0.01$. Compared to adults without IST, adults with IST did not differ in terms of age of onset, obesity or race. Adults with IST compared to those without IST were more likely to have FRNS, SDNS or SRNS as the biopsy indication (8% v 0%, p=0.02), less likely to have FSGS (14% v 39%, $p <0.01$), and more likely to have MCD (27% v 7%, $p <0.01$). Children had greater exposure to second line or MIST compared to adults (37% v 7%, $p<0.01$). Children without IST were more likely to be black (64% v 36%, p =0.01), over age 12 years (68% v 20%, $p <0.01$) and obese (46% v 34%, p=0.04) and differed by histopathology (FSGS 44% v 25%; MCD 19% v 68%; other 38% v 5%, $p<0.01$) compared to children treated with IST.

**Conclusions:** In this contemporary prospective longitudinal cohort, a surprising 24% of adults with proteinuria are exposed to IST prior to initial clinically indicated kidney biopsy. Only a fraction of these have a history of childhood onset disease. Conversely, 29% of children did not have IST pre-biopsy. These children were more commonly of black race, older age, and obese. Pre-biopsy therapy in these pediatric settings is consistent with North American Clinical Practice Guidelines.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO858

**A Noninvasive Biomarker for Aristolochic Acid Exposure Detected in Exfoliated Urinary Cells** Tao Su,[1] Xiaomei Li,[1] Li Yang,[1] Byeong Hwa Yun,[2] Robert Turesky,[2] Arthur P. Grollman,[3] Kathleen G. Dickman.[3] *[1]Peking Univ First Hospital, Beijing, China; [2]NY State Dept of Health, Albany, NY; [3]Stony Brook Univ, Stony Brook, NY.*

**Background:** Aristolochic acid (AA), a nephrotoxin and upper tract urothelial carcinogen, is a component of all *Aristolochia*-based herbal drugs. In cases of suspected exposure, analysis of blood or urine for the presence of AA is not useful, as CKD and/or urothelial cancer can appear months to years following drug withdrawal. However, reactive AA metabolites form stable adducts with DNA that are resistant to repair and persist in cells with low turnover rates. Here we test the feasibility of detecting aristolactam (AL)-DNA adducts in urinary cells and evaluate their utility as a noninvasive biomarker of AA exposure in CKD patients with a documented history of *Aristolochia* use.

**Methods:** Cells were collected by centrifugation of 12-24 hour urine specimens from 40 patients; six cases were excluded due either to insufficient DNA for analysis or incomplete clinical data. AL-DNA adducts extracted from these cells were measured by $^{32}$P-postlabeling methodology or mass spectrometry.

**Results:** Based on eGFR, 32/34 patients had CKD, the majority at stages 4 or 5. All but two patients reported using *Aristolochia;* urinary AL-DNA adducts were absent in these two cases. Adducts, with levels ranging from 0.3-581 adducts/$10^8$ nucleotides, were detected in 28/32 of the remaining cases, representing a wide range of AA exposure times (2 mo-20 yrs), cumulative doses (0.14-19 g), and time following withdrawal (2 d-8 yrs). Adducts were found in 13/13 of cases with recent discontinuation of *Aristolochia* use (‹3 mo), and could still detected for as long as 8 yrs after the last exposure. Upper tract urothelial cancer was present in three patients at the time of sampling (adducts in 2/3) and developed in three additional adduct-positive patients during an 8-yr follow-up.

**Conclusions:** AL-DNA adducts can be detected in urinary cells several years after discontinuation of *Aristolochia* use. This noninvasive biomarker can be used to screen for persons at risk of developing AA nephropathy and upper tract urothelial cancer, providing an opportunity for early detection and intervention.

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## SA-PO859

**GQ-16, a New Peroxisomal Proliferator Activated, Receptor Gamma Represses Cell Proliferation and Tumor Necrosis Factor Alpha Promoter in Human Mesangial Cells** Alexandre Martini,[1] Michela Soares Coelho,[1] Francisco R. Neves.[1] *[1]Molecular Pharmacology Laboratory, Faculdade De CiêNcias Da Saúde, Universidade de Brasília, Brasilia, DF, Brazil; [2]National Institute of Science and Technology for Pharmaceutical Innovation, Universidade Federal de Pernambuco, Recife, PE, Brazil.*

**Background:** Tumor necrosis factor (TNF-α) induces mesangial proliferation and participates in kidney disease. Peroxisome proliferator activated receptor gamma (PPAR¡) agonists (pioglitazone –PIO and rosiglitazone - ROSI) improve insulin sensitivity and also ameliorate renal disease by their antiinflammatory action. Unfortunately, these ligands have been beset by side effects including weight gain, edema and cardiovascular toxicity. We developed a new compound (GQ-16) that exhibits partial PPARγ agonism, and a robust anti-diabetic activity in mice, yet did not elicit weight gain or edema. Here we investigate the role of GQ-16 on transcriptional activity of TNF-α promoter and mesangial cells (MC) proliferation upon TNF-α treatment.

**Methods:** Human MC were incubated at 37°C in DMEM supplemented with 10% fetal calf serum, treated with TNF-α (10 ng/mL) and GQ-16 (1μM to 50 μM) or PIO and ROSI at 1 μM. Thymidine incorporation assay was performed to evaluate mesangial cell proliferation. Gene reporter assays were carried out using luciferase reporter driven by human TNF-α promoter (fragment from -125 to -82), and the activity was measured in a luminometer. The data were analyzed by one-way ANOVA.

**Results:** In MC, GQ-16 significantly impaired TNF-α induced cell proliferation, in a dose-response curve (1μM -32%; 10μM -35% and 50 μM -52%). These results were similar to PIO (10μM -39%) and ROSI (10μM -35%). Also, GQ-16 significantly reduced transcriptional activity of human TNF-a promoter in a dose response manner. The IC50 of GQ-16 (91,98 nM) was 26 times higher than ROSI (3,46 nM), but at the highest concentration this response was comparable to ROSI.

**Conclusions:** These results suggest that in human MC, GQ-16 exerts an antiproliferative and represses the transcriptional TNF-α promoter activity through PPAR¡ suggesting an antiinflammatory effect similar to ROSI.

## SA-PO860

**Glomerulonephritis (GN) in a Chronic Kidney Disease (CKD) Population** Andrew John Mallett,[1,2,3] Anne Salisbury,[1,2,3] Zaimin Wang,[1,2] Helen G. Healy,[1,2,3] George T. John,[1,3] Wendy E. Hoy.[1,2] *[1]CKD.QLD; [2]Centre for Chronic Disease, School of Medicine, Univ of Queensland, Brisbane, Queensland, Australia; [3]Dept of Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.*

**Background:** GN is a heterogenous group of disorders accounting for 23% of Australian and New Zealand patients commencing renal replacement therapy in 2011. The characteristics of this group in Australian CKD populations requires require definition.

**Methods:** Aim: To define characteristics of CKD patients with glomerulonephritis. CKD.QLD is a registry and research platform involving all consenting patients in public renal practices in Queensland. Primary Renal Disease coding on the 2359 patients enrolled from 5 sites were searched for GN patients according to ANZDATA GN codes. These patients were compared to all patients in the CKD.QLD registry for age, gender and CKD stage.

**Results:** 286 GN patients were identified, representing 12.1% of the total. Of these 87 (30.5%) had Miscellaneous GN (MiscGN), 64 (22.5%) had GN with Systemic Involvement (GNSI), 63 (22.1%) had IgA Nephropathy (IgAN), 45 (15.8%) had Focal Segmental Glomerulosclerosis (FSGS), 21 (7.4%) had Membranous Nephropathy (MN) and 5 (1.8%) had Membranoproliferative GN (MPGN). There was similar prevalence among the 5 sites (9.6-13.3%).

CKD Stage 2 was most common for MN (38%) and FSGS (31%) whereas CKD Stage 4 was most common for MiscGN (27.6%), MPGN (60%) and GNSI (23.4%). CKD Stage 3b was most common for all GN combined (22.1%) and IgAN (27%).

48% of GN patients were female compared to 45% in the whole CKD.QLD registry. Females were most prevalent in GNSI (59.4%) and FSGS (51%), but males were more common in MN (76.2%).

Mean age was similar in all GN groups (51.6-58.8 years) with a mean of 54.2 years. Mean age for the whole CKD.QLD registry was 65.5 years. The most common age group for all GN was 55-64yrs (24.6%) compared to 65-74 years (29.2%) for all CKD.QLD.

**Conclusions:** There are significant numbers of patients with GN in these Australian CKD populations. They are younger than the aggregate CKD population. Stage and gender differ among several GN subgroups. Continued patient registration will allow enhanced GN subgroup analysis.

*Funding:* Government Support - Non-U.S.

## SA-PO861

**The Spectrum of Glomerular Diseases in Central Region of Saudi Arabia: A 5 Year Retrospective Study** Naveed Aslam, Ebadur Rahman, Dujanah Hassan Mousa, Seddeg Younis, Ahmed Soliman, Raees Farhan Mushtaq. *Nephrology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia.*

**Background:** Glomerular diseases continue to be the leading cause of end-stage renal disease globally. Hence it is important to recognize the glomerular disease pattern in any given geographical area to understand the pathobiology, incidence and progression of the disorder in that region.

**Methods:** A total of 348 native renal biopsies performed on patients with proteinurea > 1 gm, haematuria or renal impairment during a period of 5 years (between January 2005 and December 2009) in a tertiary care hospital of Kingdom of Saudi Arabia. All the biopsies were studied by histopathologist by (light microscopy, immunofluorescence and electron microscopy) and categorized. SAS version 9,2 was used for statistical analysis.

**Results:** Primary glomerular disease accounted for 55.1% of all renal biopsies. The most common histological lesion was focal segmental glomerulosclerosis (FSGS) (27.6%). Minimal change disease (MCD) was the second most common lesion (17.7%) followed by membrano-proliferative glomerulonephritis (MPGN)(13.0%) and IgA nephropathy (IgAGN) 11,5%. Secondary glomerular disease contributed to 37.9% of glomerular diseases with lupus nephritis (LN) as the commonest lesion (54.5%) followed by hypertension (HTN) (22%), post-infectious (7.5%), diabetic nephropathy (DN) (6.8%) and vasculitides (4.5%). We came across14 cases of crescentic glomerulonephritis (5 vasculitic, 2 post-infectious, 1 lupus nephritis, 1 IgA nephropathy, 1 mesangioproliferative, 1 diffuse proliferative and 3 not specified). ESRD accounted for 4 % of all renal biopsies whereas inadequate biopsies constituted 2.8 % of all the tissues.

**Conclusions:** This study demonstrates FSGS as the most common primary GN encountered in the studied cases, the second most frequent is MCD. Lupus nephritis is the most common secondary GN. The results of this study were compared with surveys from other studies in same region and rest of the world. Pattern of glomerulonephritis seems to vary in different regions of Saudi Arabia. We believe that Renal Biopsy Registry is mandatory to better understand the pattern of glomerular disease and to follow any trend change.

## SA-PO862

**Glomerular Descriptors Enhance Standard Renal Biopsy Diagnoses** Cynthia C. Nast,[1] L. Barisoni,[2] J. Troost,[3] A. Gasim,[4] Gerald B. Appel,[5] Daniel C. Cattran.[6] *[1]Cedars-Sinai; [2]U Miami; [3]U Mich; [4]UNC; [5]Columbia U; [6]U Toronto.*

**Background:** The Nephrotic Syndrome Study Network (NEPTUNE) has characterized a cohort with proteinuria, including FSGS and minimal change disease (MCD). In addition to standard morphologic diagnoses, biopsies are assessed using "descriptors" of renal lesions. Based on this evaluation, MCD was subclassified into MCD with only glomerular foot process effacement (FPE) (MCD-) and MCD with any other glomerular lesion (global sclerosis, ischemia, hypercellularity) (MCD+). These 3 groups (FSGS, MCD-, MCD+) were analyzed to discern whether presenting clinical features and FPE extent correlate with this descriptor-based subclassification.

**Methods:** 75 FSGS, 41 MCD- and 17 MCD+ patients had biopsies evaluated for FPE by two semiquantitative systems (3 or 5 grades), and analyzed relative to patient age, sex, baseline hypertension (HTN), urine Pr/Cr ratio, eGFR, and pre-biopsy steroid treatment.

**Results:** Sex ratio was similar, but ages differed in the groups (FSGS 36.3 yrs, MCD+ 27.9 yrs, MCD- 12.8 yrs, $p<.0001$). Urine Pr/Cr ratios were not different, but eGFR was lower in FSGS (73.6) vs. both MCD groups (MCD+ 111.6, MCD- 115.5; both $p<.0001$ vs FSGS). HTN was more common in FSGS and MCD- vs. MCD+ ($p<.02$). Overall, FPE extent and proteinuria correlated when groups were combined ($p<.0001$); however, there was no significant correlation when groups were separated regardless of the score system used.

Pre-biopsy steroid exposure was more frequent in MCD- (71%), less in MCD+ (56%) and least in FSGS (22%) ($p<.0001$); this difference occurred in children but more prominently in adults. Prior steroid treatment correlated with FPE extent only in MCD- ($p<.05$).

**Conclusions:** At baseline, FSGS patients were older with lower eGFR, more frequent HTN, and were less likely to receive pre-biopsy steroids. MCD+ had features between FSGS and MCD- including HTN, and intermediate age and pre-biopsy steroid exposure, with FPE similar to FSGS. This suggests morphologic glomerular descriptors may be used to identify a distinct clinical cohort in MCD. FPE overall correlated with degree of proteinuria, but did not discriminate among these diagnostic groups.

*Funding:* NIDDK Support

## SA-PO863

### Masked Monoclonal Membranous-Like Glomerulopathy

Christopher Patrick Larsen, Nidia Cordeiro Messias, Larry N. Cossey, Josephine M. Ambruzs, Patrick D. Walker. *Nephropath, Little Rock, AR.*

**Background:** Routine direct immunofluorescence on fresh tissue is the gold standard for the detection and characterization of immune deposits. We recently have observed an unusual form of glomerulonephritis in which the deposits are "masked" and require an antigen retrieval step to be visualized. Correlation with the patient information reveals this to be a unique clinicopathologic syndrome not previously described. We have termed this entity masked monoclonal membranous-like glomerulopathy (MMMG).

**Methods:** We identified 14 cases of MMMG in our case files over the past two years. LM, IF, and EM was performed in all cases. Retrospective analysis of clinical information was also gathered.

**Results:** The cases were characterized by subepithelial deposits and C3-predominant staining by routine immunofluorescence with weak to negative immunoglobulin staining. Repeat immunofluorescence after pronase digestion of the FFPE tissue demonstrated strong IgG with kappa restriction. Four patients had evidence of crescent formation without endocapillary proliferation. The remaining cases showed no proliferative changes. The patients were most commonly female (12/14) with a mean age of 24.8 years and 8/14 (62%) had evidence of vague autoimmune phenomenon. The clinical and demographic findings in MMMG patients do not correspond with those typically found in patients with monoclonal membranous glomerulopathy or proliferative glomerulonephritis with monoclonal IgG deposition as these patients were significantly older (mean age of 69.2 and 67.3, respectively) and rarely showed evidence of autoimmune phenomenon. Follow-up data in 9 patients showed one complete remission, 2 partial remissions and 6 with persistent disease (mean 14.9 months, range 2– 46 months).

**Conclusions:** We present the first description of a novel form of glomerulopathy that occurs predominantly in young females with vague autoimmune symptoms and proteinuria. The specific glomerulopathy is only revealed after addition of antigen retrieval. Previously, MMMG has likely been misdiagnosed as an atypical form of membranous glomerulopathy, infection-related glomerulonephritis, or C3 glomerulopathy.

## SA-PO864

### Quantitative T2 MRI to Measure Kidney and Liver Disease Progression in Autosomal Recessive Polycystic Kidney Disease (ARPKD)

Ying Gao,[1,2] Bernadette O. Erokwu,[1] Lan Lu,[1] Chris Flask,[1,2,3] Katherine MacRae Dell.[3,4] *[1]Depts of Radiology; [2]Biomedical Engineering; [3]Pediatrics; [4]CWRU Center for the Study of Kidney Disease and Biology, Case Western Reserve Univ, Cleveland, OH.*

**Background:** ARPKD is associated with significant morbidity and mortality in affected patients; however, there are no clinically-available methods to measure kidney and/or liver disease progression except in advanced stages, severely limiting the study of novel therapies in patients. We previously utilized quantitative T2 MRI to assess kidney and liver disease in the PCK rat. In the current study, we used T2 MRI to longitudinally examine disease progression.

**Methods:** Eight PCK rats were scanned at 1-, 2- and 3-mos of age in a 7T Bruker Biospec MRI scanner. Multislice, coronal kidney and sagittal liver images were obtained with a flow-saturated, respiration-gated, multiecho spin echo acquisition. For kidneys, T2 values were calculated using a linear least squares regression of a monoexponential decay model. Cysts (↑T2 values) and normal tissue (↓T2 values) were distinguished by manual thresholding. For liver, T2 values were calculated based on mean values of whole regions of interest via a nonlinear least squares fitting of a biexponential decay model. Means and standard deviations (SD) for disease and normal tissues were calculated at each time point and analyzed using 2-tailed Student's t-tests.

**Results:** For kidneys, the cystic volume increased over the 2 month study period. Mean±SD (%cystic) at 1,2,and 3 mos were 27.4±4.2%, 31.1±4.5% and 43.8±4.3%. Significant differences ($p=0.00006$) were seen in the 2-3 mos interval but not the 1-2 mos interval. For livers, mean T2 values (indicating increased biliary duct dilatation vs.normal parenchyma) also increased. Mean±SD values (msecs) at 1, 2, and 3 mos were 54.3±9, 64.4±7.8 and 74.6±9. Significant differences were seen at both the 1-2 mos ($p=0.032$) and 2-3 mos ($p=0.035$) intervals.

**Conclusions:** Quantitative T2 MRI assessments of PCK rats detected significant kidney and liver disease progression over the relatively short time intervals studied. These techniques, which are readily translatable to human imaging, may provide a useful assessment of disease progression in ARPKD patients as well.

*Funding:* NIDDK Support

## SA-PO865

### Maternal Anti-Human Laminin α5 IgG Causes Anti-GBM Disease in Perinatal Transgenic Mice Expressing Human Laminin α5

Brooke M. Steenhard, Larysa Stroganova, Adrian T. Zelenchuk, Patricia St. John, Dale R. Abrahamson. *Anatomy and Cell Biology, and the Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS.*

**Background:** Mammalian immune systems do not mature until well after birth, but transfer of maternal IgG to the fetus and newborn usually provides sufficient immunoprotection from infectious diseases. This IgG transfer occurs before birth in humans across the placenta and in many mammalian species, including rodents, continues after birth across the intestine. The transfer is selective and occurs by transcytosis across placental syncytiotrophoblasts and intestinal epithelium, which is mediated by the neonatal IgG Fc receptor (FcRn). Although maternal IgG is generally beneficial, maternal autoimmunity can be transferred to the fetus/infant, causing diseases such as myasthenia gravis, bullous pemphigoid, thrombocytopenic purpura, and systemic lupus erythematosus, including lupus nephritis.

**Methods:** Previously, we generated transgenic (Tg) mice that express human laminin α5 (huLama5) in basement membranes throughout the body (Steenhard et al., PLoS ONE 6(9): e23926, 2011). These Tg mice suppress murine Lama5 mRNA transcription and mouse laminin α5 protein deposition, but heterozygous animals have no renal phenotype.

**Results:** When we crossed huLama5 Tg males with wildtype (wt) females, we discovered development of a maternal anti-huLama5 immune response (presumably induced by embryonic Tg basement membranes) and maternal sera contained IgG that bound linearly to basement membranes of human kidney cryosections. Maternal alloantibody also crossed the placenta in vivo and bound in bright, linear patterns to glomerular basement membranes (GBMs) of Tg fetuses but not those of wt siblings. Foster nursing of Tg pups showed post-natal transfer of maternal anti-huLama5 IgG through suckling as well, which also bound to GBMs of Tg pups but not wt. By 18d, most Tg mice had glomerular C3 deposits. Electron microscopy showed neutrophil and monocyte infiltrates, thickened and split GBMs, and podocyte foot process effacement.

**Conclusions:** This novel model of perinatal anti-GBM disease may be useful for determining effects of autoantibodies on glomerular development in vivo.

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## SA-PO866

### Remodeling of Renal Urothelium Characterizes Renal Adaptation to Congenital Obstructive Nephropathy in the Megabladder Mouse

Ashley R. Carpenter,[1,2] Brian Becknell,[3] Jordan Allen,[4] Kirk M. McHugh.[1] *[1]Center for Molecular and Human Genetics, Nationwide Children's Hospital; [2]Biomedical Sciences Graduate Program, The Ohio State Univ; [3]Div of Nephrology, Nationwide Children's Hospital; [4]College of Medicine, The Ohio State Univ.*

**Background:** The *megabladder* (*mgb*) mouse model of congenital obstructive nephropathy (CON) presents the opportunity to describe renal adaptation to urinary tract obstruction. *Mgb*$^{-/-}$ mutants develop functional lower urinary tract obstruction due to a lack of proper detrusor muscle organization *in utero*, which leads to chronic kidney disease and death in early adulthood. To better understand the molecular mechanisms responsible for renal adaptation in CON, we performed transcriptome analysis of *mgb*$^{-/-}$ kidneys compared to controls.

**Methods:** Kidneys from age-matched male *mgb*$^{-/-}$ and wild type control mice were evaluated by ultrasound, Agilent cRNA microarray, qPCR and immunohistochemistry (IHC).

**Results:** Microarray analysis comparing severe *mgb*$^{-/-}$ kidneys with controls identified upregulation of urothelium-specific genes. These genes encompassed uroplakins (*Upk1a*, *1b*, *2*, *3a* and *3b*), cytokeratins (*Krt5*, *14*, and *19*), urothelial transcription factors (*Grhl3*, *Foxa1*) and additional genes with proposed functions in maintaining the urine permeability barrier (*Sprr1a*, *Gjb6*, and *Gsdmc4*). We confirmed increased expression of each of these transcripts by qPCR ($p<0.05$). Notably, *Upk1b* and *Krt14* mRNA were found to statistically differ in control versus mildly hydronephrotic *mgb*$^{-/-}$ kidneys. We characterized expansion of Upk3a and Krt14 protein expression and a significant increase in the number of Ki-67-positive proliferating cells along the renal urothelium as hydronephrosis worsened.

**Conclusions:** In the *mgb* mouse model of CON, proliferation and upregulation of urothelium-specific genes reflect an early adaptive response in the obstructed kidney. Further understanding of the molecular pathways responsible for urothelial proliferation and remodeling during renal adaptation will yield insights into the pathophysiology of chronic kidney disease as well as identify early diagnostic and prognostic markers of CON.

*Funding:* NIDDK Support

## SA-PO867

### Molecular Basis of Renal Adaptation in a Murine Model of Congenital Obstructive Nephropathy

Ashley R. Carpenter,[1,2] Brian Becknell,[1,3] Melissa Scott,[1] Michael Wilhide,[1] Susan E. Ingraham,[1,3] Kirk M. McHugh.[1] *[1]Research Institute, Nationwide Children's Hospital; [2]Biomedical Sciences Graduate Program, The Ohio State Univ; [3]Div of Nephrology, Nationwide Children's Hospital.*

**Background:** Congenital obstructive nephropathy (CON) is a common cause of pediatric chronic kidney disease (CKD) and a leading indication for renal transplant in children. The cellular and molecular responses of the kidney to CON are incompletely

characterized. In this study, we evaluated global transcription in kidneys with graded hydronephrosis in the *megabladder* (*mgb*$^{-/-}$) mouse.

**Methods:** Kidneys from age-matched male *mgb*$^{-/-}$ and control mice were graded by ultrasound, evaluated by Agilent cRNA microarray, and validated by qPCR and immunohistochemistry.

**Results:** This analysis indicates that CKD in the *mgb*$^{-/-}$ mouse model of CON involves a delicate balance between three canonical pathways including TGFβ directed pathogenesis, retinoic acid (RA) mediated remodeling/repair and steroid hormone modulation. Over half of the top twenty canonical pathways identified in this study involved renal response to injury, an observation that confirms our prior morphological and biochemical studies. The role of RA in kidney development has been well characterized and the results of this study suggest that these developmental functions are recapitulated during pathogenesis as a transient repair mechanism. Steroid hormones appear to play a dual modulatory role during renal adaptation that includes suppression of an acute inflammatory response as well as modulation of the genetic programs controlling cellular differentiation. Finally, this study identified the skewed expression of a group of sexually dimorphic genes in male *mgb*$^{-/-}$ kidneys as well as de-repression of Hdac target genes. These observations provide a potential mechanism for gender-based differences in renal pathogenesis.

**Conclusions:** The results of this study indicate that CKD in *mgb*$^{-/-}$ mice results in a highly orchestrated adaptive response that is designed to prevent permanent renal injury and permit rapid morphological and functional recovery. These observations will permit the development of novel biomarkers and therapeutic approaches to progressive renal injury in the context of CON.

*Funding:* NIDDK Support

## SA-PO868

**Global and Gene-Specific Hypomethylation by Maternal Undernutrition in Rat Embryonic Kidney** Midori Awazu, Mariko Hida. *Dept of Pediatrics, School of Medicine, Keio Univ.*

**Background:** Maternal undernutrition leads to low nephron number. We reported that ureteric bud branching is reduced and that genes involved in ureteric branching are methylated by maternal nutrient restriction in rat embryonic kidney. On the other hand, maternal undernutrition decreases global methylation in the baboon kidney. Since increased methylation traditionally decreases gene expression, we examined whether genes involved in negative regulation of kidney development are hypomethylated by maternal undernutrition.

**Methods:** The kidneys of embryonic day 18 fetuses from dams given food ad libitum (CON) and those subjected to 50% food restriction throughout pregnancy (NR) were examined. Global methylation was assessed by methylated DNA quantification kit. The methylation landscape around promoter CpG islands was analyzed using methylated DNA immunoprecipitation (MeDIP) coupled with microarray (NimbleGen Rat ChIP-chip 385K Promoter array) comparing methylated fractions of CON and NR. MeDIP probe significances were generated using the Kolmogorov–Smirnov test as implemented by NimbleScan. These values were transformed (-log10) to give peak scores, which reflect the probability of methylation at a p-value of less than 0.01.

**Results:** Global methylation was reduced in NR compared with CON by 30%. Of 15911 promoter regions included in the array, 7330 were hypomethylated in NR. Genes categorized into negative regulation of biological process were more frequently hypomethylated in NR vs CON (338 vs 262). The number of hypomethylated genes in the categories of pro-apoptosis was not different between CON and NR. Hypomethylated genes in NR known as negative regulators of kidney development were, in descending order of peak score, activin A receptor type IC, axin 1, STAT1, Osr1, TACSTD2, spry1, TGF-ß receptor II, activin A receptor type I, sema3a, noggin, and GSK3ß, most of which are implicated in branching morphogenesis. Pro-apoptotic genes that were hypomethylated in NR included Fas ligand, PKCδ, and TNFα.

**Conclusions:** Maternal nutrient restriction reduces global DNA methylation and the methylation of genes involved in negative regulation of branching morphogenesis, which may contribute to the reduced nephron number.

*Funding:* Government Support - Non-U.S.

## SA-PO869

**The Switch of Proteasome to Immunoproteasome in Peripheral Lymphomonocytes of Children with Henoch Schoenlein Purpura Differs from Primary IgA Nephropathy (IgAN)** Maria Elena Donadio, Elisa Loiacono, Licia Peruzzi, Alessandro Amore, Roberta Camilla, Luca Vergano, Giuliana Guido, Rosanna Coppo. *Nephrology Dialysis Transplanation, Città della Salute e della Scienza, Turin, Italy.*

**Background:** The proteasome (PS) plays a key role in activating transcriptional factors, cytokines and in presenting antigens; moreover, under the action of interferons, becomes immunoproteasome (iPS), by substituting beta1, beta2, beta5 catalytic uniots with low molecular weight proteins (LMP2 and LMP7) and endopeptidase-like complex (MECL-1). This modification confers optimal catalytic properties for professional presentation of peptides to MHC Class I. We previously demonstrated that adult patients with IgA-Nephropathy (IgAN) have an increased expression of iPS catalytic subunit, which correlated with severity of renal disease. Aim of this study was to investigate the switch from PS to iPS in children with primary IgAN and Henoch-Schoenlein purpura (HSP), sharing several immune system abnormalities.

**Methods:** Peripheral Lymphomonocytes (PBMC) from 18 children with IgAN, 57 with HSP and 33 healthy control subjects (HC), isolated by centrifugation gradient, were tested with real time PRC (Taqman) to assess quantitatively the mRNA levels of PS alpha subunit (constitutive), of the active subunit of PS (beta1, beta2, beta5) and of iPS (LMP2, LMP7 and MECL-1).

**Results:** The normalized values and the ratio between mRNA of corresponding subunit of PS and iPS are reported in the table. Differences between means were calculated with Student t test.

| | LMP2/beta1 | LMP7/beta5 | MECL-1/beta2 |
|---|---|---|---|
| Healthy control | **0.91 ± 0.42** | 1.08 ± 0.33 | 1.02 ± 0.29 |
| IgAN | **0.82 ± 0.39** | 1.44 ± 0.92 | 1.35 ± 0.70 |
| HSP | **1.23 ± 0.66** [a,b] | 1.45 ± 1.02 | 2.34 ± 3.92 |

**Conclusions:** A significantly higher switch from LMP2/beta1 subunits of PS/iPS was observed in children with HSP in comparison to IgAN and HC, which might indicate a more active antigen presentation and increased interaction between innate and adaptative immune system in this acute condition in comparison to chronic primary IgAN.

## SA-PO870

**Novel TNXB and ROBO2 Mutations Confirm Genetic and Phenotypic Heterogeneity of Hereditary VUR** Rasheed A. Gbadegesin,[1] Alison Homstad,[1] Gentzon Hall,[1] Andrew F. Malone,[1] Guanghong Wu,[1] Indra R. Gupta,[2] Patrick D. Brophy,[2] Michelle P. Winn.[1] *[1]Pediatrics, Medicine and Center for Human Genetics, Duke Univ, Durham, NC; [2]Pediatrics and Human Genetics, McGill Univ, Montreal, Canada; [3]Pediatrics, Univ of Iowa, Iowa, IA.*

**Background:** Vesicoureteral reflux (VUR) is the most common congenital anomaly of the kidney and the urinary tract and reflux nephropathy is a major cause of chronic kidney disease (CKD) in children. The gold standard for the diagnosis of VUR is VCUG, an invasive and unpleasant diagnostic procedure. VUR shows high heritability in family studies, however the genetic causes remain elusive and opportunities for non-invasive molecular and radiologic diagnosis of VUR have not been explored.

**Methods:** The objective of this study is to identify genetic causes for VUR as a first step towards identification of non-invasive molecular tools for diagnosis. We have ascertained and obtained clinical data and biologic samples from over 330 individuals with VUR, of these; we identified 27 kindreds with hereditary VUR. As a proof of principle that molecular diagnosis is feasible in VUR, we carried out genome-wide linkage analysis (GWLA) and/or whole-exome sequencing on two families with hereditary VUR.

**Results:** We identified a missense mutation T3257I in TNXB and a novel missense mutation A304D in ROBO2. The two mutations segregated with disease in the two families. Both mutations are conserved in evolution and are predicted to be deleterious with a polyphen score of 0.99 and 1 respectively. Some affected individuals with TNXB mutation demonstrated asymptomatic joint hypermobility while the phenotype in the family with ROBO2 is isolated VUR with no underlying renal dysplasia or extra renal manifestations.

**Conclusions:** Whole exome sequencing analysis identified novel mutations in TNXB and ROBO2 in two families with hereditary VUR showing that non-invasive molecular diagnosis may be feasible in a subset of patients with VUR. Our results highlight the genetic and phenotypic heterogeneity of VUR.

*Funding:* NIDDK Support, Private Foundation Support

## SA-PO871

**DNA Copy Number Variations in Human Alpha-Defensin 1-3 Gene Locus Are Associated with Urinary Tract Infections in Patients with Vesicoureteral Reflux** David S. Hains,[1,2] Huanyu Wang,[2] John David Spencer,[1,2] Brian Becknell,[1,2] Kirk M. McHugh,[2] Andrew L. Schwaderer.[1,2] *[1]Pediatrics, Nationwide Children's Hospital, Columbus, OH; [2]The Research Institute at Nationwide Children's Hospital, Columbus, OH.*

**Background:** DNA copy number variations (CNVs) can have profound effects on gene dosage. Specifically, CNVs in innate immunity genes, such as the alpha-defensin gene family, are associated with diseases involved with infection like HIV susceptibility. The *DEFA1A3* locus varies ranging from 2-12 copies per diploid genome. Despite the importance of alpha defensins in the immune response, CNVs in the alpha-defensin gene family have not been evaluated in patients with UTI. This study was designed to quantitate DNA copy number of alpha-defensin 1-3 (*DEFA1A3*) in patients with vesicoureteral reflux (VUR) and UTI enrolled in the RIVUR study.

**Methods:** Standard individual copy type: Southern blot and pulsed-field gel electrophoresis were performed to copy-type multiple individuals at the *DEFA1A3* locus. RIVUR study patients: 310 individuals from the RIVUR study (children with VUR and at least one UTI) were copy typed using multiplex real-time PCR and normalized to *ZNF80.* 310 age/ethnicity/gender-matched controls were also copy-typed. DNA copy calls: Exact *DEFA1A3* copy numbers were determined for each individual using known copy-typed individuals for standard curve. Tissue gene expression: Using validated exon-spanning primers for *DEFA1A3* mRNA, we copy-typed DNA and and quantitated *DEFA1A3* mRNA using real-time PCR on human kidney samples.

**Results:** On average, we identified that RIVUR patients had one less copy of *DEFA1A3* compared to controls ($p < 0.0001$). Furthermore, copy number distribution showed a significant overall skewing toward a lower copy number of *DEFA1A3* in RIVUR patients. Finally, DNA copy number correlated with mRNA message in the human kidney.

**Conclusions:** We present data in this study that suggests that lower DNA copy number in the gene for DEFA1A3 is associated with increased risk for UTI in children with VUR. Ultimately, decreased DNA copy number results in less transcribed mRNA. These findings serve as a potential risk factor when determining risk of UTIs in these patients.

*Funding:* NIDDK Support

**SA-PO872**

**APOL1 Variants Are Associated with LVH in the CKID Study**
Robert Woroniecki,[1] Cheryl Ann Winkler,[2] George W. Nelson,[2] Craig S. Wong,[3] Mark Mitsnefes,[4] Bradley A. Warady,[4] Susan L. Furth,[5] Frederick J. Kaskel,[6] Jeffrey B. Kopp.[2] *[1]Columbia U; [2]NIH; [3]U New Mexico; [4]Cincinnati Childrens' Hosp; [5]U Pennslyvania; [6]Albert Einstein U.*

**Background:** Left ventricular hypertrophy (LVH) is common among adults and children with chronic kidney disease, and is more common among African Americans (AA). We wished to determine whether APOL1 kidney risk alleles might contribute to this disparity.

**Methods:** We carried out a case/control study among AA subjects enrolled in the Chronic Kidney Disease in Children (CKiD) study to assess a role for *APOL1* variants in LVH. GFR was determined by plasma iohexol disappearance. Hypertension was defined according to Fourth Report for subjects ≤18 years and JNC7 for older subjects. LVH was defined by echocardiographic parameters. Proteinuria was assessed as first morning urine protein/creatinine ratio. *APOL*1 variants G1 (rs73885319, S342G) and G2 (rs71785313, NY deletion) were genotyped.

**Results:** DNA was available for 89 AA subjects, 43 (48%) had been diagnosed as ever having had hypertension and 62 underwent echocardiography. LVH was present in 9/33 (27%) of hypertensive children and 7/29 (24%) normotensive children (p=1). As expected, APOL1 risk alleles were common, with 23% of CKID subjects having 2 risk alleles compared to 12-15% in the general AA population. Two APOL1 risk alleles were present in 6/16 (38%) children with LVH and 5/45 (11%) without LVH, p=0.028. After adjustment for hypertension history, baseline GFR, and baseline proteinuria, the p value was 0.031. Among subjects with 2, compared to 0 or 1 risk alleles, the average GFR decline rates during the study were similar (10.4% vs. 8.8% respectively, p=0.82).

**Conclusions:** *APOL1* kidney risk alleles are associated with LVH in pediatric CKD. *APOL1* variants have been also associated with increased heart size at autopsy, in the absence of kidney disease (Hoy et al, Int Soc Hyperternsion 2012). It remains unknown whether the apparent effect of the *APOL1* variants on the heart arise from APOL1 gene expression in the heart or in the kidney. Further data on the duration and severity of hypertension will be required to exclude a role for hypertension as a mediator of cardiac damage.

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**SA-PO873**

**Mutations in the Insulin Receptor INSR Are Associated with Hypercalciuria and Nephrocalcinosis**
Arabella Simpkin,[1] Velibor Tasic,[4] Detlef Bockenhauer.[1] *[1]Great Ormond Street Hospital, London, United Kingdom; [2]Endocrinology, Addenbrookes Hospital, Cambridge, United Kingdom; [3]NIDDK, NIH; [4]Children's Hospital Skopje, Macedonia, The Former Yugoslav Republic of.*

**Background:** The insulin receptor INSR is expressed in kidney and studies in mice suggest a role in proximal sodium reabsorption as well as in podocyte function. Yet its physiological role in human kidney is unclear. Recessive mutations in INSR lead to Rabson-Mendenhall (RMS) or Donohue syndrome (DS). Observations in such patients can illuminate the role of INSR.

**Methods:** Retrospective review of patients with confirmed biallelic INSR mutations. Data for blood pressure, renal ultrasound, plasma creatinine and electrolytes, as well as urine protein, albumin and calcium excretion were sought from the referring clinicians.

**Results:** Clinical data were provided on 13 RMS and 5 DS patients, age 2-24 years (mean±SD: 10.5±6.5 years). All patients had low normal plasma creatinine for age (mean±SD: 0.24±0.11mg/dl) and plasma electrolytes were within the normal range in all. Blood pressure centile data (N=13) ranged between the 48th and 98th percentile for age, sex, height and weight (mean±SD: 65±23). 24-h urinary data (N= 13) revealed markedly elevated urinary calcium (mean±SD: 9±6 mg/kg/d; normal <4). 24-h urinary protein excretion (N=9) was borderline high (mean±SD: 5.5±4.7 mg/kg/h; normal <4) and urinary albumin excretion (N=9) ranged from 5.7-122.5 μg/min (normal <20). Renal ultrasound (N=10) showed nephrocalcinosis in all.

**Conclusions:** In contrast to mice with targeted Insr deletion, key renal abnormalities identified in humans are hypercalciuria and nephrocalcinosis, suggesting a role for INSR in tubular calcium handling. There was no obvious glomerular dysfunction. Whilst protein and albumin excretion rates were borderline elevated, it is unclear whether this reflects a primary role of INSR in podocyte function or just the long-standing hyperglycemia in these patients.

**SA-PO874**

**In Situ Evaluation of INF2 in Normal and Glomerular Diseases in Children**
Hiroshi Tamura. *Pediatrics, Graduate School of Medical Sciences, Kumamoto Univ, Kumamoto, Japan.*

**Background:** Mutations of the inverted formin 2 gene (INF2) are common causes of autosomal dominant focal and segmental glomerulosclerosis (FSGS). Its encodes a member of the formin family, which regulates actin and microtuble cytoskeletons. This study investigated the expression of INF2 in glomerular diseases.

**Methods:** We generated rabbit polyclonal antibodies against conjugated peptides from human INF2, and studied glomerular expression of INF2 and synaptopodin using kidney tissues of normal humans and those with glomerular diseases in children.

**Results:** Anti INF2 antibodies detected the original 135kD fragment by Western blot analysis using human isolated mature glomeruli. Immunohistochemically, INF2 was detected in a linear pattern along the glomerular capillary loop. Anti INF2 antibody stained the smooth muscles of renal arterioles and aorta. Among 42 patiennts, INF2 was normally expressed in glomeruli in purupura nephritis, IgA nephropathy, lupus nephritis and minimal change disease (MCD), while it was either decreased or absent in most subjects with focal and segmental glomerulosclerosis (FSGS). The expression of synaptopodin was similar to that of INF2, although some discrepancy existed.

**Conclusions:** Although indirect, our data suggest the existence of a vascular isoform of INF2 with a different molecular mass. We propose that examination of INF2 expression may help differentiate MCD from FSGS.

**SA-PO875**

**Epidermal Growth Factor Receptor Gene Polymorphisms in Childhood IgA Nephropathy**
Byoung-Soo Cho,[1] Jin-Soon Suh,[2] Yumi Choi.[3] *[1]Dept of Pediatrics, TheAll Medibio Research Institute, Seoul, Korea; [2]Dept of Pediatrics, Bucheon St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Bucheon, Korea; [3]Dept of Pediatrics, School of Medicine, Kyung Hee Univ, Seoul, Korea.*

**Background:** Recent studies indicated that epidermal growth factor receptor (EGFR) activation contributes to development and progression of renal diseases in animal models of obstructive nephropathy, diabetic nephropathy, and glomerulonephritis through mechanisms involved in activation of renal interstitial fibroblasts, induction of tubular injury, overproduction of inflammatory factors and/or promotion of glomerular and vascular injury. We investigated the association between single nucleotide polymorphisms (SNPs) of the *EGFR* gene and childhood IgA nephropathy (IgAN).

**Methods:** Three synonymous SNPs (rs1140475, rs2293347 and rs1050171) and one promoter SNP (rs6965469) were analyzed in 196 patients with childhood IgAN and in 288 healthy controls. The IgAN patients were divided and compared with respect to the presence of gross hematuria as a initial symptom, proteinuria and nephrotic-range proteinuria during disease course, and the presence or absence of podocyte foot effacement and pathologically advanced disease markers, such as interstitial fibrosis, tubular atrophy or global sclerosis at the renal biopsy.

**Results:** Significant differences in SNP frequencies between IgAN patients and healthy controls were observed for rs2293347 in the codominant model [p=0.027, OR (95% CI)=1.35 (1.03-1.77)] and the recessive model [p=0.028, OR (95% CI)=1.82 (1.07-3.12)]. Moreover, rs2293347 of *EGFR* gene was found to be associated with the presence of podocyte foot process effacement at the renal biopsy. In addition, rs1140475 was found to be associated with the presence of pathologically advanced disease markers and the presence of gross hematuria.

**Conclusions:** Our results suggest that the polymorphism of *EGFR* gene may associated with increased susceptibility to IgAN in children. They also suggest that the polymorphism of *EGFR* gene may be related to the development of proteinuria and the presence of foot process effacement or pathologically advanced states in IgAN patients.

**SA-PO876**

**Premature Immunologic Aging, Associated with Inflammation in Children with Chronic Renal Insufficiency and Dialysis**
Roshan P. George,[1,2] Aneesh K. Mehta,[1] Sebastian D. Perez,[1] Jean Kwun,[1] Brandi E. Johnson,[1] Stephanie A. Monday,[1] Jennifer Cheeseman,[1] Linda L. Stempora,[1] Barry L. Warshaw,[1,2] Allan D. Kirk.[1,2] *[1]Emory Transplant Center, Emory Univ; [2]Pediatric Nephrology, Children's Healthcare of Atlanta, Atlanta, GA.*

**Background:** Chronic Renal Insufficiency (CRI) and dialysis are known to be associated with chronic inflammation. Persistent antigen stimulation drives T cells toward exhaustion and senescence but the influence of non-specific inflammation such as that present in dialysis, has not been defined, particularly in children. We studied T cell maturation, senescence and inflammatory markers in children with CRI and those on dialysis.

**Methods:** Serum obtained from 100 children, [CRI or dialysis (n=80) and normal controls (NC, n=20)] was analyzed for proinflammatory cytokines using multiplex luminex assay. Multiparameter flow cytometry, specifically interrogating cells for indices of T cell maturation, exhaustion and senescence was done. Telomere length using PCR in DNA was studies to assess cell aging.

**Results:** Of the 80 children with CRI, [35 on dialysis, 19 with CRI and prior organ transplantation (pTx), and 26 with CRI, not on dialysis]; 20% had reversal of CD4/CD8 ratio. There was significant reduction in naïve CD4 T cell frequencies in dialysis vs. NC (p =0.04) and in the naïve CD8 T cell percentage in pTx vs. NC (p= 0.0004). Senescent $CD57^+$, CD8 T cells were also elevated in dialysis vs. NC (p= 0.05). $CD28^-$ late differentiated, CD8 T cells were significantly higher in patients with CRI and pTx (p= 0.002). $CD28^-$ T cells were associated with shorter telomere length (p=0.04). There was significant elevation of inflammatory cytokine IL-5 (p=0.04), IL-6 (p= 0.02) and VEGF (p=0.008) in children on dialysis compared to NC. Elevated ferritin correlated to higher percentage of $CD57^+$, CD8 T cells and $CD28^-$ T cells (p=0.008 and 0.03 respectively).

**Conclusions:** Children on dialysis have elevated markers of inflammation and a T cell repertoire skewed towards terminal differentiation, immune senescence and exhaustion. These data may form the basis for further studies, needed to assess the clinical impact of this immune phenotype with regards to risk for infections and cardiovascular morbidity.

*Funding:* Private Foundation Support

**SA-PO877**

**Management of Hypertension in Pediatric Pheochromocytoma/Paraganglioma** Mauricio Romero Olvera,[1] Gaurav Kapur,[2] Rossana Baracco,[3] Tej K. Mattoo,[4] Amrish Jain.[5] *[1]Pediatric Nephrology, Children's Hospital of Michigan (CHM), Detroit, MI; [2]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [3]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [4]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [5]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI.*

**Background:** Surgical manipulation of pheochromocytoma/paragangliomas(PH/PG) while being removed can cause hypertensive crisis and post resection hypotension. Guidelines on management of blood pressure (BP) in pediatric patients with PH/PG are lacking. We report our experience with preoperative adrenergic blockade.

**Methods:** This retrospective study included 7 patients, aged 1-18 yrs old with biopsy proven PH/PG at Children's Hospital of Michigan from 2003-13. Excluded were patients with elevated catecholamines due to other diagnosis(neuroblastoma). All patients had urinary/plasma catecholamines, imaging, genetic testing & multidisciplinary evaluation. BP was controlled with α blockade for 2-4 weeks and β blockade for 2-3 days before surgery. High salt diet was encouraged after 3 days of α blockade. Intraoperative esmolol/nitroprusside infusion ± fluid resuscitation was used for cardiovascular stability.

**Results:** Of 7 patients (10.4±5.8 yrs; 5 male), 6 had PH & 1(14.2%) PG. Bilateral disease was noted in 4 (57.1%) & 1 had 2 recurrences. Altogether 9 surgical removals were done in 7 patients. Plasma normetanephrine, urine normetanephrine and VMA were elevated in 6, 4, and 3 patients respectively. Adrenal mass was seen on CT scan (2/9)& MRI (7/9); confirmed by MIBG Scan(8/9). Nitroprusside used transiently(<2h) during surgery in 2; esmolol in 2. Five needed albumin/PRBC. All anti-hypertensives were discontinued immediately after surgery(72%) or within 4 days (28%).

| Intervention | Number | Mean dose (mg/kg/day) | Mean duration(days) |
|---|---|---|---|
| α blockade- Phenoxybenzamine | 7 | 0.64 | 33±16.5 |
| β blockade- | | | |
| Atenolol | 3 | 0.3 | 33 ±14.3 |
| Propranolol | 2 | 0.63 | 3 ±3 |
| Labetalol | 2 | 0.86 | 12 ±8.6 |
| Volume expansion | 7 | - | 14.8±11.4 |

**Conclusions:** A systematic and multidisciplinary approach of BP control with α blockade, volume expansion and β blockade ensures excellent outcome.

**SA-PO878**

**Podocyte Differentiation Arrest Is Associated with Aberrant Response of Notch to Down-Regulated Wnt Signaling** Haichun Yang,[2] Taiji Matsusaka,[3] Iekuni Ichikawa,[1] Agnes B. Fogo,[2] Ji Ma.[1] *[1]Pediatric Nephrology, Vanderbilt Univ, Nashville, TN; [2]Pathology, Microbiology and Immunology, Vanderbilt Univ, Nashville, TN; [3]Internal Medicine, Tokai Univ, Isehara, Kanagawa, Japan.*

**Background:** Forced re-entry of mature podocytes into the cell cycle by inducing Simian Virus 40 T antigen (SV40T) gene expression causes podocyte and glomerular damage, while induction of SV40T in podocytes of developing mice results in complete differentiation. Upregulated Wnt and Notch signaling are associated with podocyte damage in glomerulopathies. In contrast, during development, down-regulation of Wnt and activation of Notch suppresses proliferation and promotes differentiation. In the present study, we tested the possibility that coordinated interaction between Wnt and Notch pathways determines the fate of differentiated podocytes.

**Methods:** Primary podocytes from *Nphs2-rtTA:TRE-SV40T* (SV40T) and *Nphs2-rtTA* (Cont) mice were pre-treated with doxycycline to induce SV40T expression and incubated with Wnt inhibitor Dkk1.

**Results:** SV40T podocytes showed increased proliferation compared with Cont. Dkk1 increased p-β-catenin in both Cont and SV40T podocytes, indicating inhibition of Wnt/β-catenin pathway. Dkk1 did not change the proliferation profiles in these two genotypes of podocytes. Expression of PAX2, a transcriptional factor expressed in primitive podocytes, was significantly down-regulated by Dkk1 in Cont but not in SV40T cells. While there was no significant difference in the number of podocyte processes between Cont and SV40T at baseline, Dkk1 increased the process number in Cont (5.8±0.3 *vs.* 7.5±0.5 /cell, $P<0.05$) but not in SV40T (6.4±0.5 *vs.* 7.4±0.5). Dll4, a ligand for Notch signaling pathway, was significantly reduced by Dkk1 in Cont (1.03±0.07 *vs.* 0.39±0.05, $P<0.05$) while increased in SV40T (0.85±0.07 *vs.* 1.24±0.04, $P<0.05$).

**Conclusions:** Actively proliferating podocytes with induced SV40T have limited commitment for differentiation in response to inhibition of Wnt/β-catenin signaling. The concurrent proliferation-differentiation processes underlying complete podocyte differentiation requires coordinated interaction between the Wnt and Notch signaling pathways.

*Funding:* NIDDK Support, Other NIH Support - NICHD

**SA-PO879**

**A Polymorphism of Interleukin 10 and Its Receptor Polymorphism Is Associated with the Development of Childhood IgA Nephropathy** Jin-Soon Suh,[1] Yumi Choi,[2] Byoung-Soo Cho.[3] *[1]Dept of Pediatrics, Bucheon St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Bucheon, Korea; [2]Dept of Pediatrics, School of Medicine, Kyung Hee Univ, Seoul, Korea; [3]Dept of Pediatrics, TheAll Medibio Research Institute, Seoul, Korea.*

**Background:** Recent studies suggest that dysregulated innate immunity plays an important role in the pathogenesis of immunoglobulin A nephropathy (IgAN). The interleukin-10 and its receptor complexes elicit diverse hose defense mechanisms during many infectious and inflammatory diseases. In the current study, we investigated the association between the polymorphisms of IL-10 and its receptors, IL-10RA/IL-10RB genes and childhood IgAN.

**Methods:** 196 patients with IgAN and 288 normal controls were genotypes for two polymorphisms of the IL-10 gene (rs1518111 and rs1554286), two polymorphisms of the IL10RA gene (rs2256111 and rs4252243), and three polymorphisms of the IL10RB (rs2228054, rs999788 and rs2834167).

**Results:** Our case-control analysis showed that genotypes of rs4252243 in IL10RA gene were associated with IgAN. Individuals with genotypes containing the T allele (TC or TT) of rs4252243 had about 2-fold reduced risk of IgAN compared with those with the CC genotype in the codominant model (0=0.011) and in the recessive model (p=0.014). Furthermore, rs2228054 and rs999788 in the IL10RB gene were associated with the presence of proteinuria and nephrotic-range proteinuria during disease course, respectively.

**Conclusions:** Our data suggest that rs4252243 polymorphism in the IL10RA gene is associated with the reduced risk of IgAN and polymorphisms in the IL10RB are associated with the disease severity in IgAN patients.

**SA-PO880**

**Risk Factors for Progression in Children with IgA Nephropathy: Data from a European Cohort** Roberta Camilla, Rosanna Coppo, Shubha Bellur, Daniel C. Cattran, John Feehally, Francesco Emma, Kostantinos Giannakakis, Alessandro Amore, Stephan Troyanov, Ulla B. Berg, Magnus Soderberg, Gianna Mazzucco. *On Behalf of VALIGA Study Group.*

**Background:** The European validation study of Oxford classification of IgA nephropathy (IgAN) (VALIGA) has enrolled 1147 patients.

**Methods:** Children account for 174 cases, from 20 centers of 11 countries. Proteinuria was adjusted for body surface area and mean arterial pressure for gender and age. Schwartz formula estimated eGFR and the functional decline was defined as slope of eGFR.

**Results:** Mean age at renal biopsy was 12.7±3.7 years; median follow-up was 4.7 years (IQs 2.4-7.8 years); > 80% presented with normal renal function (eGFR > 90 ml/min/1.73m$^2$).

End-stage renal disesase (ESRD) was reached by 4%, 50% loss of eGFR by 5%; 7% reached the combined end point (ESRD or 50% loss of initial eGFR).

At renal biopsy 57% presented with mesangial proliferation (M1), 24% endocapillary proliferation (E1), 35% segmental glomerulosclerosis (S1) and 9% tubular atrophy/interstitial fibrosis (TA/IF; T1/2). Patients with segmental sclerosis and TA/IF showed a significantly worse eGFR slope (S0 vs S1 p=0.04; T0 vs T1/2 p=0.003).

At univariate linear regression, clinical data at renal biopsy (eGFR, proteinuria and MAP) were not associated with renal function decline, while data at 6-12 and 12-24 months and TA-proteinuria and MAP significantly predicted eGFR slope.

A multivariate linear regression model (including proteinuria and MAP at 12-24 months together with the difference of eGFR at renal biopsy and at 12-24 months as independent variables) performed well in predicting eGFR slope ($R^2$=0.39). This model was used to derive a formula able to estimate eGFR slope in ml/min/1.73m$^2$/year, which performs well in the VALIGA pediatric population (mean bias between estimated and really observed eGFR slope of 0.05 ± 6.6 ml/min/1.73m$^2$).

**Conclusions:** The Oxford classification of IgAN showed that in children segmental glomerulosclerosis and tubular-atrophy/interstitial fibrosis being more significantly associated with renal outcome. A formula was developed that predicts renal function decline based on clinical data after 1-2 years from renal biopsy.

*Funding:* Government Support - Non-U.S.

**SA-PO881**

**Enhanced Functional Complement Regulatory Proteins (CD46)-Induced Regulatory T Cells by High-Dose Pulse-Intravenous Meyhylprednisolone in Lupus Nephritis Patients** Ching-Yuang Lin. *Clinical Immunology Center, China Medical Univ Hospital, Taichung, Taiwan.*

**Background:** Defective suppressive regulatory T cell (Treg) function is crucial in lupus nephritis (LN) pathogenesis. Complement regulatory protein (CD46) is a newly defined co-stimulatory molecule for Treg activation; together with IL-10 production, it may help suppress kidney inflammation. We examine chemotaxis and adhesion molecule expression on CD3/CD46$^+$-activated CD4$^+$ T cells (Tregs) from Class III or IV LN patients to ascertain whether five-day pulse intravenous methyl-prednisolone (IVMP) therapy enhances CD3/CD46-activated Tregs suppressive function.

**Methods:** During 2009-2011, 40 active LN patients (Class III or IV) with heavy proteinuria, aged 12-18 years, were recruited. Peripheral blood mononuclear cells (PBMCs) were isolated, both from LN patients (before/after IVMP) and from healthy controls.

**Results:** Diminished IL-10 and CCR4 or CCR7 expression from CD3/CD46-activated Tregs appeared in LN patients. Therapy significantly increased CD3/CD46-activated Tregs with intracellular IL-10 and suppressed $CD4^+$ T cell proliferation. Percentage of CCR4 and CCR7 in CD3/CD46-activated Tregs significantly decreased in patients compared to healthy controls. IVMP therapy enhanced CCR4, CCR7, and CCR4 ligand-medicated migration and phosphorylation of protein kinase B expression of CD3/CD46-mediated Tregs.

**Conclusions:** Lower migratory capacity of suppressive CD3/CD46-activated Tregs might be involved in LN pathogenesis.

## SA-PO882

**Investigation of Principal Mechanism for Renal Sodium Retention in Children with Idiopathic Nephrotic Syndrome** Takeshi Ninchoji,[1] Hiroshi Kaito,[1] Kandai Nozu,[1] Taketsugu Hama,[2] Koichi Nakanishi,[2] Norishige Yoshikawa,[2] Kazumoto Iijima.[1] *[1]Pediatrics, Kobe Univ Graduate School of Medicine, Japan; [2]Pediatrics, Wakayama Medical Univ, Japan.*

**Background:** Renal retention of sodium is one of the principal mechanisms that leads to edema in children with idiopathic nephrotic syndrome (INS). Some animal experiments have documented that it is the epithelial sodium channel (ENaC) at collecting ducts or the sodium-hydrogen exchanger (NHE3) at proximal tubules that may contribute to sodium retention in INS. However, there are various theories of renal sodium retention and opinion is divided. In addition, another problem is that no structured studies have elucidated possible contributor to renal sodium retention in human with INS.

**Methods:** We retrospectively analyzed 10 patients with INS group and 10 with age-matched control (CON) group. All cases in INS underwent kidney biopsy during nephrosis, and those in CON underwent during complete remission. Paraffin-embedded serial sections were stained with immunostaining procedure, and the number of positive cells was counted by a single researcher. NHE3 was evaluated with CD10, a specific marker of proximal tubule, and both α- and β- subunit of ENaC with CK34βE12, a specific marker of collecting tubule. The ratios of NHE3 to CD10 and ENaC to CK34βE12 positive cells were compared each between two groups.

**Results:** The mean age at kidney biopsy was 5.3±1.9 years old in INS and 5.3±1.2 in CON. All cases showed no interstitial and tubular damage, and no cases deteriorated kidney function at biopsy. Serum albumin level in INS was significantly lower than that in CON (2.4±0.3 g/dl vs 3.5±0.3 g/dl; p=0.027). The ratio of NHE3 to CD10 positive cells in INS was significantly higher than that in CON (0.85±0.06 vs 0.77±0.06; p=0.013). On the other hand, there was no change in the proportion of α- and β-ENaC to CK34βE12 positive cells. Distribution of each channel had also no difference between two groups.

**Conclusions:** We could first elucidate the distribution and the trend of the sodium channel in children with INS. We demonstrated that it may not be ENaC, or collecting ducts, but NHE3, or proximal tubules, that contributes to sodium retention in INS.

## SA-PO883

**Patient Reported Outcomes in Pediatric Nephrotic Syndrome** David T. Selewski,[1] J. Troost,[2] Susan F. Massengill,[2] Rasheed A. Gbadegesin,[2] Larry A. Greenbaum,[2] Ibrahim Shatat,[2] Yi Cai,[2] Gaurav Kapur,[2] Diane Hebert,[2] Michael J. Somers,[2] Howard Trachtman,[2] Priya J. Pais,[2] Michael E. Seifert,[2] Jens W. Goebel,[2] John D. Mahan,[2] Heather E. Gross,[2] Emily G. Herreshoff,[2] Courtney L. Harkness,[2] Darren Dewalt,[2] Debbie S. Gipson.[2] *[1]Univ of Michigan; [2]PROMIS Pediatric Study Network.*

**Background:** PROMIS-II is a prospective longitudinal study evaluating patient reported outcomes (PRO) in pediatric chronic diseases. We report the initial PRO results for the nephrotic syndrome (NS) cohort.

**Methods:** 126 children (8-17 yo) with active NS were enrolled at 14 centers from 9/2010-3/2013 including incident and prevalent (mean 68 months from diagnosis) patients. Children completed PROMIS and PedsQL instruments. For PROMIS higher scores indicate more of a domain and population norms are not defined. For PedsQL, higher scores indicate better function (normative range 100±15).

**Results:** As shown in Table 1 (incident vs. prevalent unadjusted analysis, mean±sd), PedsQL scores were below the normative range (p<0.05). Multivariate analysis evaluating the unadjusted significant domains controlling for BMI, edema, and recent hospitalization showed that prevalent NS predicted worse PROMIS Pain (p=0.009), PROMIS Peer Relationships (p=0.048), PedsQL Social (p=0.004) and PedsQL School (p=0.008) scores.

**Conclusions:** PedsQL scores in children with active NS are well below population norms. Children with prevalent NS have increased burden in the domains of peer, pain, social and school functioning showing that disease duration negatively impacts PRO in these areas.

| **PROMIS** | | | |
|---|---|---|---|
| Domain | Incident (N=58) | Prevalent (N=68) | *p* |
| Anxiety | 49±11 | 50±10 | 0.57 |
| Peer Relationships | 51±10 | 47±11 | 0.02 |
| Fatigue | 49±13 | 50±12 | 0.6 |
| Mobility | 47±10 | 46±8 | 0.8 |
| Pain | 47±12 | 52±10 | 0.004 |
| Depression | 48±9 | 51±9 | 0.14 |
| **PedsQL** | | | |
| Physical | 69±26 | 70±20 | 0.85 |
| Emotional | 75±22 | 70±22 | 0.15 |
| Social | 86±14 | 76±19 | <0.001 |
| School | 68±20 | 58±22 | 0.016 |
| Overall | 74±18 | 69±16 | 0.09 |

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## SA-PO884

**Vitamin D in Pediatric Incident Idiopathic Nephrotic Syndrome** David T. Selewski,[1] Ibrahim Shatat,[2] Ashton Chen,[2] Priya J. Pais,[2] Larry A. Greenbaum,[2] Pavel Geier,[2] Raoul D. Nelson,[2] Stefan Kiessling,[2] Patrick D. Brophy,[2] Alejandro Quiroga,[2] Michael E. Seifert,[2] Caroline E. Straatmann,[2] John D. Mahan,[2] Maria E. Ferris,[2] Courtney L. Harkness,[2] J. Troost,[2] Debbie S. Gipson.[2] *[1]Univ of Michigan; [2]Midwest Pediatric Nephrology Consortium.*

**Background:** Idiopathic nephrotic syndrome (iNS) is a common kidney disease in children. While studies of children with prevalent NS have shown 25-Vitamin D (VitD) deficiency rates of 20-100%, there has not been a longitudinal study of 25-VitD levels in incident iNS. We assessed 25-VitD deficiency in children with iNS at diagnosis and over time (within 2 weeks of and 2-4 mos. after diagnosis).

**Methods:** Preliminary analysis of a longitudinal study of children (2-18yo) from 14 centers with incident iNS designed to investigate 25-VitD. 25-VitD deficiency defined as < 20 ng/mL. Exclusion criteria: evidence of secondary NS at diagnosis. Follow-up completes in 8/2013.

**Results:** 57 children are enrolled, 54 children have completed initial labs and 38 completed 2nd visit labs. Their characteristics are: males 70%; white 56%, black 35%, other race 9%, median age 5 yo. Table1 shows lab data by visit (mean±sd). All 54 (100%) had 25-VitD deficiency at diagnosis. 21 (55%) children had 25-VitD deficiency at follow-up. 81% of children were steroid responsive and 19% were steroid resistant. Children prescribed VitD supplements were less likely to have VitD deficiency at follow-up compared to those without (OR 0.2, 95%CI 0.1, 1.0). Steroid response, age, race, and season did not predict 25-VitD deficiency at follow-up.

**Conclusions:** In this incident iNS cohort, all children at diagnosis have 25-VitD deficiency and the majority continue to have a deficiency at 2-4 months. Furthermore, supplemental VitD decreases the odds of 25-VitD deficiency supporting a potential role for supplementation in incident NS.

| | Diagnosis (N=54) | 2-4 mos. Follow-up (N=38) |
|---|---|---|
| 25-VitD ng/mL | 9.0±3.7 | 22.4±19.5 |
| 1,25-VitD pg/mL | 23.3±14.4 | 54.5±27.9 |
| Int PTH pg/mL | 73.2±45.7 | 58.1±28.9 |
| Alb g/dL | 1.6±0.6 | 3.7±0.9 |
| Ca mg/dL | 7.9±0.8 | 9.1±1.2 |
| VitD Supplement (N) | 0 | 8 |

*Funding:* Private Foundation Support

## SA-PO885

**An Evaluation of a 6 Month versus 4 Month Steroid Protocol in Childhood Onset Nephrotic Syndrome** Andra Diana Popescu, Allison Dart. *Pediatrics and Child Health, Univ of Manitoba, Winnipeg, Canada.*

**Background:** Standard therapy for a first presentation of nephrotic syndrome is a course of corticosteroid, however there exists considerable practice variation in the duration of initial course. This study aimed to evaluate the effect of a 6-mo vs. 4-mo steroid protocol on clinical outcomes at 1 year.

**Methods:** This is a retrospective analysis of all children (1-18 yrs) with a first presentation of nephrotic syndrome treated at the Children's Hospital of Winnipeg with a 6-mo course of prednisone (July 2009 to Sept 2011) (Group B) compared to historical controls treated with a 4-mo course (Jan 2005 to June 2009) (Group A). Children with biopsy confirmed FSGS were excluded. Data was obtained from an electronic medical record. Differences in number of relapses, total steroid dose, time to first relapse and second line agent were evaluated between groups. Logistic regression models evaluating the association of clinical factors with 0 vs. >1 and 0-1 vs. >2 relapses were conducted. Follow-up time was 12 months.

**Results:** In Group A (n=20) 3 had 0, 4 had 1 and 13 patients had >1 relapse. In Group B (N=18) 5 had 0, 6 had 1 and 7 patients had >1 relapse. There were no differences in clinical characteristics between groups. The odds of any relapse (A vs. B) was not different between groups (odds ratio (OR)=2.8; 95% CI 0.5-14.9) and for 0-1 vs. >1 relapse the OR = 4.4 (CI 0.9-20.3). Sex, age, and initial albumin were not associated with outcome in either analysis. Survival analysis showed a mean of 4.8 mo to first relapse for Group A and 7.1 mo for Group B (NS), and 8.1 mo to initiation of second line therapy for Group A and 9.9 mo for Group B (NS). There was no difference in the total cumulative steroid dose at 12 months in mg/kg (p=0.3). The rates of steroid toxicity were low in both groups.

**Conclusions:** Our findings suggest that a 6-mo course of steroids for the first presentation of nephrotic syndrome may decrease the odds of frequent relapses, and delay the time to first relapse and time to initiation of a second line agent compared to a 4-mo course without increasing total steroid exposure. A randomized study with a larger sample size is required to confirm these identified trends.

## SA-PO886

**Vertebral Fractures in the 3 Year Period following Steroid Initiation among Children with Chronic Illnesses** Maury N. Pinsk, Monica Taljaard, Steven Arora, Lorraine E. Bell, Tom D. Blydt-Hansen, Guido Filler, Joanne Grimmer, Diane Hebert, Janusz Feber, Julian Paul Midgley, Veronique Phan, Leanne M. Ward, and The Canadian Stopp Consortium. *Canadian Pediatric Bone Health Working Group, Canada.*

**Background:** To describe the frequency of incident vertebral fractures (IVF) in steroid-treated children.

**Methods:** IVF were assessed prospectively each year following steroid initiation for 3 years, according to the Genant semi-quantitative method. To examine associations with baseline clinical factors, the 3-year total number of IVF was analyzed using multivariable Poisson regression.

**Results:** 404 children were enrolled at a median age of 6.2 years, range 1-17; 50% boys; 188 (46%) had leukemia, 136 (34%) rheumatic conditions, and 80 (20%) nephrotic syndrome. The baseline study visit occurred at a median of 18 days following steroid initiation (inter-quartile range 11-24 days). Overall, 17% of children (95% CI 13-22) had at least one incident VF over the 3 years. Among those with IVF, 24/55 children (44%) had 1 or more moderate or severe fracture. The proportions of children with IVF over 3 years were as follows: Leukemia: 24% (95% CI 16-32); rheumatic disorders: 13% (95% CI 6-19); nephrotic syndrome 9% (95% CI 1-17). The annual proportion of children with IVF peaked at 12 months and declined thereafter (p=0.04). In Poisson multivariable modeling assessing baseline clinical factors, the following were associated with higher, 3-year VF incident rates: the presence of VF at baseline (incidence Rate Ratio (RR) 6.3, 95% CI 3.2-12.4), female gender (RR 1.8; 95% CI 1.0-3.3), pre-pubertal status (RR 2.1; 95% CI 0.8-5.4), and lower BMD Z-scores (RR 1.4; 95% CI 1.1-1.7).

**Conclusions:** Within 3 years of steroid initiation, 17% of children had IVF. VF incidence peaked at 12 months, and almost half of the IVF were moderate or severe. Of the factors measured at baseline, prevalent VF were most strongly associated with IVF over the ensuing 3 years.

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*Funding:* Government Support - Non-U.S.

## SA-PO887

### Thrombosis in Childhood Nephrotic Syndrome: Contributory Risk Factors

Rezan Topaloglu,[1] Fehime Kara Eroglu,[1] Betul Tavil,[2] Fatih Ozaltin,[1] Mualla Cetin,[2] Fatma Gumruk.[2] *[1]Pediatric Nephrology, Hacettepe Univ Faculty of Medicine, Ankara, Turkey; [2]Pediatric Hematology, Hacettepe Univ Faculty of Medicine, Ankara, Turkey.*

**Background:** The aim of this study was to evaluate the prevalence and contributory risk factors for development of thrombosis in children with Nephrotic syndrome (NS).

**Methods:** Among 188 children with NS followed up at Pediatric Nephrology Unit in last seven years; 17 children (9%),identified as having thromboembolic complications and screened for thrombotic risk factors.

**Results:** The mean age of patients was 4.5±3-2 years at the diagnosis of NS and that was 7.1± 4.9 at the time of thrombosis. The mean time from NS diagnosis to the first thrombosis development was 2.6±2.3 years. More than half of the thrombosis (53%) occurred during the first year of NS. Among these children;14 (82%) had FSGS, 2 (14%) had congenital NS and 1 had minimal change disease. 88% of the thrombosis was venous and in regards to the localization; 6 deep venous thrombosis, 4 sino venous thrombosis, 2 portal venous thrombosis, 2 intracardiac thrombosis, 1 intracardiac thrombosis and cerebral infarct, and 2 had cerebral infarct. Among screened thrombotic risk factors high factor VIII levels (16.5%) was the leading risk factor followed by decreased antithrombin III level (29%) and high homocystein level (23.5%). The distribution of the hereditary risk factors were; factor V Leiden heterozygote mutation in 12.5%, MTHFR 677 heterozygote mutation in 37.5%, MTHFR 677 homozygote mutation in 6.2%, MTHFR 1298 heterozygote mutation in 23%, MTHFR 1298 homozygote mutation in 7.6%, PAI (4G/5G) polymorphism in 15.3% and PAI (4G/4G) polymorphism in 7.6%. Thrombotic risk factors were found in all children while 14 had multiple risk factors. Most of the children (70.5%) treated with only low molecular weight heparin ranging 3 to 12 months.

**Conclusions:** Unlike previously reported studies, in our cohort FSGS is associated with the highest incidence of venous thrombosis. All of our patients had predisposition to thrombosis that shows underlying genetic background influences the likelihood of thrombosis in nephrotic syndrome.

## SA-PO888

### Intermittent High-Dose Mizoribine Therapy Is Effective for Children with Steroid-Dependent Nephrotic Syndrome

Takayuki Okamoto, Takeshi Yamazaki, Asako Hayashi, Yasuyuki Sato. *Pediatrics, Hokkaido Univ Graduate School of Medicine, Sapporo, Hokkaido, Japan.*

**Background:** Daily high-dose mizoribine (MZB) for children with steroid-dependent nephrotic syndrome (SDNS) has been reported to be effective. Unlike preceding studies, we retrospectively investigated the efficacy of intermittent high-dose MZB therapy for children with SDNS within insurance support that means the maximum weekly dose of MZB was limited up to 1050mg.

**Methods:** Nine children (7 boys, 2 girls) who had SDNS with (n=5) or without (n=4) calcineurin-inhibitor (CNI)-dependency were enrolled in the present study to clarify the efficacy of limited-dose MZB therapy for sparing steroid and CNI. All patients were undergoing treatment at our hospital and associated hospitals between Jan 2009 and May 2013. The dosage of MZB was started at a single daily dose of 5mg/kg administered after breakfast and gradually increased to achieve the target concentration (3-h levels of 3 ug/ml) within 1050mg per week, such as daily (up to 150mg/day) and intermittent high-dose therapy; once in two days (300 mg alternate-day) and twice a week (550mg-500mg per week). The median dose of prednisolone (PSL) and CNI, the median number of relapses, the change in stature and body-weight were statistically analyzed before and after MZB therapy.

**Results:** 7 out of the 9 patients (78%) achieved the target concentration of MZB. In the 7 patients, all patients took intermittent high-dose MZB such as 6 patients (86%) took medication of MZB once in two days and 1 patient (14%) took twice a week. Intermittent high-dose MZB treatment (n=7) over a period of 12 months resulted in reduction of the mean PSL dose from 0.36 to 0.32 mg/kg/day and the median 12-month number of relapses from 2.1 to 1.3 episodes/12 months. In the 5 patients who had SDNS with CNI dependency, 3 patients (60%) could wean off CNI. No significant side effects such as hyperuricemia, liver dysfunction and leukopenia were revealed during the administration of MZB therapy.

**Conclusions:** Our retrospective study demonstrated that intermittent high-dose MZB therapy is effective for eliminating steroid or CNI to children with SDNS.

## SA-PO889

### Role of Early Volume Expansion in Mitigating Shigatoxin-Associated Hemolytic Uremic Sindrome. North Italian HUS Network

Gianluigi Ardissino, Francesca Tel, Sara Testa, Stefania Salardi, Rosaria Colombo, Ilaria Possenti, Silvana Tedeschi, Erminio Torresani. *Center for HUS Prevention, Control and Management, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico, Milan, Italy.*

**Background:** STEC-HUS is a severe acute illness for which there is no specific treatment. Among supportive therapy, the management of fluids has been traditionally concentrated on avoiding fluid overload because patients often present with oligo/anuric AKI. Hemoconcentration at disease onset is associated with more servere disease, however it is still unclear if early volume expansion can improve disease's outcome.

**Methods:** Since May 2010 a Network connecting pediatric hospitals in Northern Italy (10 millions gp) was developed aimed at early diagnosis and referral of STEC-HUS with the working hypothesis that prompt volume expansion with saline solution 0.9% may revert disease severity. All children with STEC-HUS referred to our Center from Jan. 2012 were addressed to intravenous hydration aimed at inducing an overhydration (+10-15% of the working weight within 48 hrs). The outcome was compared with an equal and sequential group of historical patients (group A) referred to our Center during 2007-2009 when patients were usually restricted in fluid intake.

**Results:**

| | Group A | Group B |
|---|---|---|
| N. | 22 | 22 |
| Gender (M/F) | 11/11 | 9/13 |
| Age (yrs) | 4.9 | 4.8 |
| sCreatinine at diagnosis (mg/dL) | 3.6 | 1.7 |
| Peak sCreatinine (mg/dL) | 5.6 | 2.9 |
| RRT (% of total) | 64 | 18 |
| Days of hospitalization | 14.1 | 9.4 |

**Conclusions:** Our data showed that early hydration of STEC-HUS can improve the outcome. It can be speculated that hypovolemia (due to diarrhea, reduced food intake, endothelial leakage and reduced onchotic pressure), if uncorrected, favours trombi formation and hipoxic/ischemic tissue damage.

Acknowledgement: thanks to the members of the North Italian HUS Network whose complete list is available at www.centroseu.org. The project has been supported by the " PROGETTO ALICE ONLUS – Associazione per la lotta alla SEU".

*Funding:* Private Foundation Support

## SA-PO890

### Discontinuation of Eculizumab Maintenance Treatment in Patients with Atypical Hemolitic Uremic Syndrome

Gianluigi Ardissino, Sara Testa, Francesca Tel, Ilaria Possenti, Fabio Paglialonga, Samantha Griffini, Massimo Cugno, Stefania Salardi, Silvana Tedeschi. *Center for HUS Prevention, Control and Management, Fondazione IRCCS Ca' Granda Osp. Maggiore Policlinico, Milan, Italy.*

**Background:** Atypical haemolytic uremic syndrome (aHUS) is a rare, systemic, life-threatening thrombotic microangiopathy (TMA). As many as 70% of aHUS patients have mutations in the genes encoding complement regulatory proteins. Since 2009, Eculizumab, a humanised recombinant monoclonal antibody targeting C5, that prevents the generation of membrane-attack complex C5b-9, has been successfully used in patients with aHUS. The standard maintenance treatment schedule requires administration of the drug every two weeks lifelong, but the possibility to and the risk of discontinuing the treatment has not yet been tested.

The present study analyzes the safety of treatment discontinuation with the rational of: 1. improving the quality of life; 2. minimizing the risk of adverse reactions; 3. reducing the risk of meningitis; 4. reducing the theoretical risk of development of neutralizing antibodies and 5. reducing the heavy costs of the treatment.

**Methods:** All patients with aHUS treated with Eculizumab at our Center, in stable remission were considered eligible to drug discontinuation. Strict routine home monitoring of indicators of disease activity was performed after treatment discontinuation: detection of blood in the urine with dipstick three times per week.

**Results:** Over a cumulative observation period of 59 months, three (2 CFH, 1 CFH+CFI) out of eleven patients (1 MCP, 2 Ab AntiFH, 3 CFI, 2 idiopathic) addressed to treatment discontinuation, relapsed. The recurrences occurred within 2 months from treatment discontinuation. Relapses were detected thanks to home urine dipstick that showed hematuria. Patients immedtely restarted the treatment with a complete recovery and return of serum creatinine to baseline level.

**Conclusions:** Our experience supports the possibility of discontinuing Eculizumab treatment in patients with aHUS in stable remission with a strict home monitoring of early signs of relapse.

*Funding:* Private Foundation Support

**SA-PO891**

**Interval Extension of Eculizumab Maintenance Treatment in Patients with Atypical Haemolitic Uremic Syndrome** Gianluigi Ardissino, Sara Testa, Donata Cresseri, Francesca Tel, Fabio Paglialonga, Ilaria Possenti, Samantha Griffini, Massimo Cugno, Stefania Salardi, Silvana Tedeschi. *Center for HUS Prevention, Control and Management, Pediatric and Adult Nephrology and Laboratory Unit, Fondazione IRCCS Ca' Granda Osp. Maggiore Policlinico, Milan, Italy.*

**Background:** Atypical haemolytic uremic syndrome (aHUS) is a rare, systemic, life-threatening thrombotic microangiopathy (TMA). As many as 70% of aHUS patients have mutations in the genes encoding complement regulatory proteins. Since 2009, eculizumab, a humanised recombinant monoclonal antibody targeting C5, that prevents the generation of membrane-attack complex C5b-9, has been successfully used in patients with aHUS. The standard maintenance treatment schedule requires administration of the drug every two weeks but the best treatment schedule is not yet defined. We tested alternative treatment schedule for prevention of relapses with the rational of improving the quality of life, reducing the risk of adverse reactions and reducing the heavy costs of the treatment.

**Methods:** All pts undergoing eculizumab treatment for aHUS in our Center (n: 22) were addressed to an extension of the interval between doses from the standard 2 weeks to 3 or 4 wks with a strict monitor of global complement activity. AP50 was routinely determined towards a target AP50 <25 before subsequent eculizumab dose. Strict home monitoring of disease reactivation with blood in the urine with dipstick, was performed at home.

**Results:** In the 12 pts (9 CFH, 1 CFH+CFI, 1 idiopathic, 1 C3) addressed to interval extension (five at 3 weeks and seven at 4 weeks), no aHUS relapses were observed over a cumulative observation period of 130 mos.

**Conclusions:** Our experience supports the possibility of tailoring eculizumab schedule for maintenance treatment in patients with aHUS based on global complement activity.

*Funding:* Private Foundation Support

**SA-PO892**

**Eculizumab Hepatotoxicity – When to Change Therapy?** Wesley N. Hayes,[1] Sibylle Tschumi,[1] Simon Ling,[2] Janusz Feber,[1] Christoph Licht.[1] *[1]Div of Nephrology, Hospital for Sick Children, Toronto, Canada; [2]Div of Gastroenterology, Hepatology and Nutrition, Hospital for Sick Children, Toronto, Canada.*

**Background:** Eculizumab is a first-in-class humanized anti-C5 antibody approved for treatment of paroxysmal nocturnal hemoglobinuria and atypical Hemolytic Uremic Syndrome (aHUS) in adults and children. Its use is increasing in children following reports of its safety and efficacy in inducing thrombotic microangiopathy response. No hepatic side effects have been reported to date.

**Methods:** We performed a retrospective review of case notes and investigations for patients treated with eculizumab for aHUS in a single pediatric center. A standard dosing regimen based on patient weight was used.

**Results:** Seven of 11 children aged 6 to 11 years experienced elevated aminotransferases following eculizumab treatment for aHUS. One patient with no pre-existing liver disease developed tender hepatomegaly 3 days following the first dose. Alanine transaminase (ALT), hepatic alkaline phosphatase (ALK), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) rose to over 20 times the upper limit of normal. Bilirubin, INR and albumin remained within normal limits. Liver biopsy taken following improvement in liver enzymes showed mild hepatocellular injury with no evidence of infectious or autoimmune hepatitis. Recurrent progressive elevation of ALT following re-dosing of eculizumab necessitated its discontinuation and transition to plasma therapy for aHUS. In 6 other children transient elevation of liver enzymes following eculizumab was observed. Infectious and other causes were excluded in each case.

**Conclusions:** Hepatotoxicity is a potentially important yet previously unreported and so far unexplained adverse effect of eculizumab. We recommend monitoring liver enzymes in all patients receiving eculizumab. In patients with excessive hepatic enzyme disturbance or clinical symptoms of hepatitis in association with eculizumab we would change to plasma therapy. Further research is required to clarify the magnitude of the issue, to characterize the mechanism of hepatotoxicity and to identify which patients are most at risk.

**SA-PO893**

**Frontline Treatment for Atypical Hemolytic Uremic Syndrome: Plasma-Exchange or Eculizumab?** Gianluigi Ardissino, Fabio Paglialonga, Sara Testa, Francesca Tel, Ilaria Possenti, Silvia Consolo, Silvana Tedeschi, Stefania Salardi. *Center for HUS Prevention, Control and Management, Fondazione IRCCS Ca' Granda Osp. Maggiore Policlinico, Milan, Italy.*

**Background:** The introduction of Eculizumab for atypical haemolytic uremic syndrome (aHUS) has quickly changed the management and the outcome of the disease. It is not clear what is the role left for "old" treatment, mainly plasma exchange (PEX) in the management of aHUS. The aim of the study is to retrospectively assess the outcome of treatment with PEX vs Eculizumab at our Center.

**Methods:** All patients with aHUS treated with PEX or Eculizumab since 2000 where considered for the present analysis. Patients who either remained dialysis dependent or who were switched to the other treatment modality because they did not reach remission, were considered non responders. PEX was performed with FFP in all cases, with an exchange volume of 50-150% (depending on the historical period) with a variable number of sessions (2-29).

**Results:** Twenty-eight patients underwent PEX for aHUS (16 CFH, 4 CFI, 2 MCP, 1 C3, 1 AbAntiFH, 4 Idiopathic) and since 2009, 26 patients were treated with Eculizumab (12 CFH, 4 CFI, 1 MCP, 1 C3, 2 AbAntiFH, 6 Idiopathic). Overall, the responders were 32 and 88% with PEX and Eculizumab, respectively. The response rate was not substantially different when analyzed separately for specific etiology: CFH Defect + AntiCFH Antibodies (41 Vs 100%), CFI Defect (25 Vs 75%), MCP (50 vs 100%), Idiopathic (0 Vs 66%).

**Conclusions:** In our experience, PEX was of limited efficacy for preventing ESRD in patient with aHUS compared to Eculizumab. Based on this finding, Eculizumab should be considered as the first-line treatment for aHUS.

*Funding:* Private Foundation Support

**SA-PO894**

**Presentation of Apolipoprotein L-1 (APOL-1) Nephropathy in Pediatric (Ped) and Young (Yg) Adult African American (AA) Patients (Pts)** Elizabeth I. Anyaegbu, Keith A. Hruska, Andrey S. Shaw, Sanjay Jain. *Washington Univ in St. Louis, St. Louis, MO.*

**Background:** 2 coding variants in the APOL1 gene have recently been found to associate with increased incidence of ESRD in the adult AA population (pop). Prevalence of 2 APOL1 risk variants (rvs) in the general AA pop is 9% compared to 0.04% in Caucasians. These rvs associate with hypertension (HTN) attributed ESRD, FSGS and HIVAN in adult AA pts. We hypothesized that as a genetic disease, *APOL1* nephropathy (APOL1N) has a ped phenotype, but it remains unclear if the *APOL1* rvs influence progression to ESRD in yg AA pts with HTN or FSGS and a family history (FHx) of ESRD.

**Methods:** We conducted a case-control study of 85 ped and yg AAs who presented with HTN or FSGS to determine the association with *APOL1* rvs, G1 and G2, using custom made TaqMan based alleic discrimination assays.

**Results:** Ped and yg AA pts presenting with either HTN (n= 39) or FSGS (n= 22) with a FHx of ESRD were cases. 24 healthy AA participants without a FHx of ESRD were controls. Mean ages (SD) for the FSGS and HTN pts were 24.3 (7.4) and 23.6 (6.9) years respectively; 27% of pts were < 18 years old. 66% of pts (40/61) had 2 APOL-1 rvs; significantly higher than the prev in the general AA pop ($p < 0.001$). 24/29 pts with HTN attributed ESRD had 2 APOL1 rvs while none of the HTN pts who presented without kidney disease had more than 1 APOL1 rvs. Of the ped pts, 35% (6/17) had 2 APOL-1 rvs; with majority of these rvs in the ped FSGS pts.

**Conclusions:** We demonstrated for the first time that 2 APOL1 rvs in HTN yg AA with a FHx of ESRD are strongly associated with ESRD ($p< 0.01$). We also found that 2 APOL1 rvs in yg AA with a FHx of ESRD were significantly associated with FSGS and not essential HTN in ped and yg AA pts. This study demonstrates that the ped presentation of APOL1N is not primary HTN. Prevalence of 2 APOL1 rvs in our controls (4/24) and ped HTN pts (1/11) were not significantly different from the general AA pop. Although a small cohort, our findings strongly suggest that APOL1N presents with kidney disease- FSGS or CKD in pts with a FHx of ESRD. Screening yg HTN AA with a FHx might aid in early identification of pts at risk for progression to ESRD.

*Funding:* Pharmaceutical Company Support - Genzyme Fellowship Grant

**SA-PO895**

**Cytoskeletal Changes in the Proteins of Podocytes by Interleukin-13** Se Jin Park,[1,4] Moin Saleem,[2] Tae-Sun Ha,[3] Jae Il Shin.[4] *[1]Dept of Pediatrics, Ajou Univ Hospital, Ajou Univ School of Medicine, Suwon, Republic of Korea; [2]Children's and Academic Renal Unit, Southmead Hospital, Univ of Bristol, Bristol, United Kingdom; [3]Dept of Pediatrics, Chungbuk National Univ College of Medicine, Cheongju, Republic of Korea; [4]Dept of Pediatrics, Severance Children's Hospital, Yonsei Univ College of Medicine, Seoul, Republic of Korea.*

**Background:** The aim of this study was to determine whether pathologic changes in the cytoskeletal proteins of podocytes are induced by interleukin-13 (IL-13) in the experimental minimal-change nephrotic syndrome (MCNS) model and to investigate whether montelukast, a leukotriene receptor antagonist, has an effect on the production of the cytoskeletal proteins in cultured human podocytes.

**Methods:** Human podocytes cultured on bovine serum albumin-coated plates were treated with different doses of IL-13 and montelukast and examined for permeability using monolayer semi-permeable membranes, for distribution using confocal microscopy, and for ZO-1 protein levels using Western blotting.

**Results:** As the concentration of IL-13 increased, the contents of ZO-1 and synaptopodin were decreased and their redistribution and rearrangement were observed in immunofluorescence studies. The redistribution of α-actinin & CD2AP by IL-13 were also found around the nucleus of the cell. α-actinin & CD2AP significantly decreased at 20 ng/mL IL-13 (both $P < 0.05$), whereas β-catenin and p130Cas significantly increased (both $P < 0.05$). At higher doses, IL-13 gradually increased the permeability of monolayered podocytes. ZO-1 was internalized and shown to accumulate in the cytoplasm of human podocytes in an IL-13 dose-dependent manner. High doses (50 and 100 ng/mL) of IL-13 decreased the levels of ZO-1 protein at 12 and 24 hr (both $P < 0.01$; n=3), which were significantly reversed by a high dose (0.5 μM) montelukast treatment ($P < 0.01$, n=3).

**Conclusions:** Our results indicate that IL-13 may increase podocyte permeability through the modulation of several cytoskeletal proteins in podocytes, and such alterations in the content and localization of the molecules may be relevant to the pathogenesis of proteinuria in the IL-13-induced MCNS model.

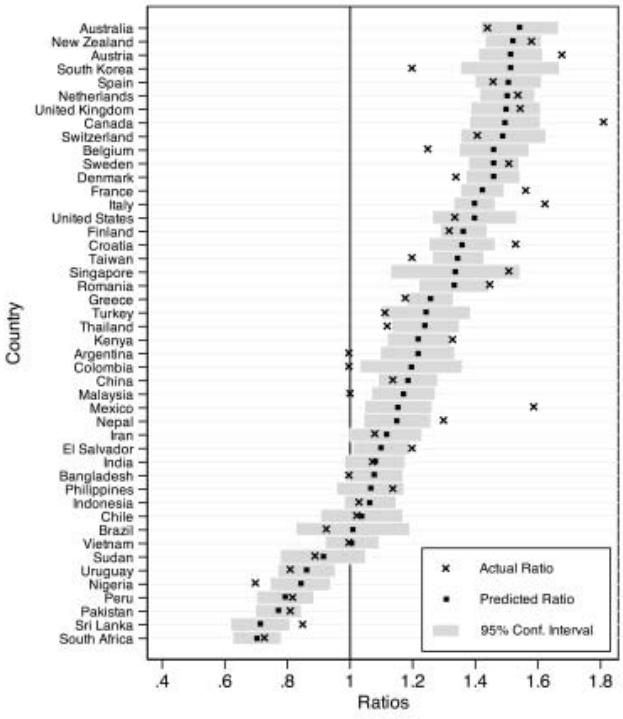
## SA-PO896

**Economies of Scale and Cost of Dialysis across the World: A Macroeconomic Perspective** Akash Nayak Karopadi,[2] Giacomo Mason,[1] Enrico Rettore,[3] Claudio Ronco.[1] *[1]Dept Nephrology - IRRIV, S Bortolo Hosp, Vicenza, Italy; [2]Dept Chem Eng and Dept Econ, BITS, Pilani, India; [3]Dept Statistical Sciences, Uni Padua, Padua, Italy.*

**Background:** ESRD treatment is a large expense in most health systems. The main dialysis methods, hemodialysis (HD) and peritoneal dialysis (PD), have significant differences in cost composition and production factors. In this paper we identify macroeconomic variables that influence the relative cost of such modalities across a sample of 51 countries, and finally draw policy-relevant conclusions.

**Methods:** We retrieved 75 articles from PUBMED and EMBASE estimating PD and HD costs in a 46 countries. We augmented this data with targeted surveys to prominent nephrologists in countries not covered by available literature. For each country, we estimated the ratio between cost of HD and PD. We finally conducted a multivariate regression analysis using the HD/PD cost ratio in each country as dependent, with several country-level indicators as regressors.

**Results:** We found a strong effect of the following variables on the HD/PD ratio: positive effect of a country's development (approximated by minimum wage or HDI); large positive effect of a country's economies of scale in PD equipment (presence of local manufacturing or absence of tariff restriction on PD equipment imports); negative effect of the percentage of private healthcare expenditure and APD usage. Actual and model-predicted ratios are in Figure 1.




**Conclusions:** Ceteris paribus, local manufacturing and the absence of import duties on PD equipment imply less costly PD: local manufacturing and no duties have very similar effects on the HD/PD cost ratio (0.42 vs. 0.33). The policy implication is that, in the absence of a market large enough to make local production of PD equipment feasible, a country can still reap the benefits of economies of scale by eliminating restrictions on import such as tariffs or quotas.

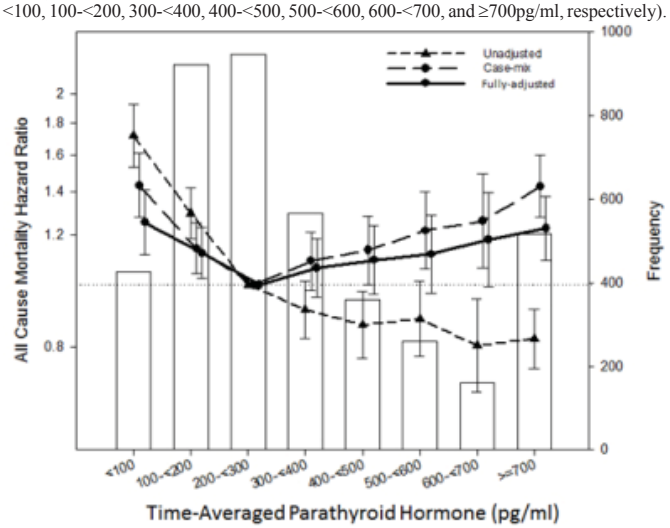
## SA-PO897

**Mortality-Predictability of Parathyroid Hormone in Peritoneal Dialysis Patients** Connie Rhee,[1] Wei Ling Lau,[1] Vanessa A. Ravel,[1] Elani Streja,[1] Csaba P. Kovesdy,[2] Rajnish Mehrotra,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmon Center, Orange, CA; [2]Memphis VA Medical Center, Memphis, TN; [3]Harborview Medical Center, Seattle, WA.*

**Background:** In hemodialysis (HD) patients, parathyroid hormone (PTH) derangements are associated with mortality. Prior studies examining the mortality-predictability of PTH in peritoneal dialysis (PD) patients have been limited and mixed, and the outcome-predictability of PTH in this population remains unclear.

**Methods:** In a cohort of 9,244 adult PD patients from a large national dialysis organization (entry period 2001-2006, with follow-up through 2009), we examined associations between time-averaged PTH with all-cause mortality using multivariable Cox models adjusted for case-mix and laboratory covariates. We then compared the mortality-predictability of time-averaged PTH among 9,244 PD vs. 99,323 HD patients.

**Results:** In PD patients, there was a U-shaped association between PTH and death risk, with PTH levels <200pg/ml and ≥700pg/ml associated with increased mortality (reference: PTH 200-<300pg/ml): HRs (95% CI) 1.25 (1.12-1.41), 1.12 (1.02-1.23), 1.06 (0.96-1.18), 1.09 (0.97-1.24), 1.12 (0.97-1.29), 1.18 (0.99-1.40), and 1.23 (1.09-1.38) for PTH levels <100, 100-<200, 300-<400, 400-<500, 500-<600, 600-<700, and ≥700pg/ml, respectively).




In analyses comparing the mortality-predictability of PTH among HD vs. PD patients, there was a U-shaped association among both HD and PD patients, with a greater magnitude of risk observed among HD patients particularly in lower and higher PTH categories (ref: PD patients PTH 200-<300pg/ml).

**Conclusions:** In PD patients, both lower and higher PTH levels are associated with increased mortality. Further studies are needed to confirm findings, elucidate underlying mechanisms, and to determine if correction of PTH to target ranges improves outcomes in PD patients.

*Funding:* NIDDK Support

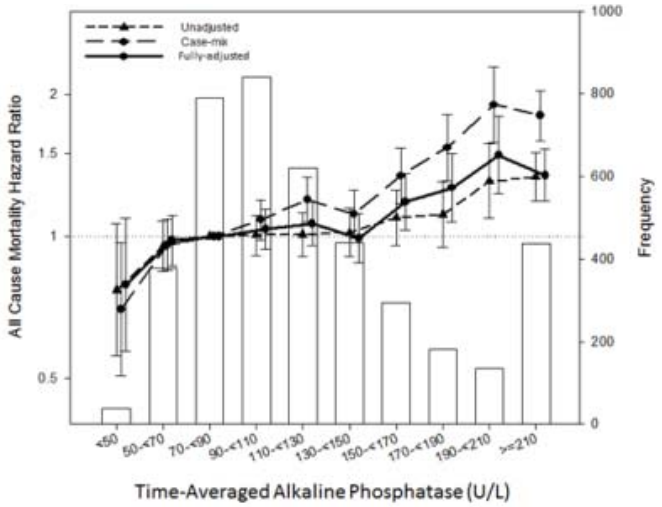
## SA-PO898

**Mortality-Predictability of Alkaline Phosphatase in Peritoneal Dialysis Patients** Connie Rhee,[1] Wei Ling Lau,[1] Vanessa A. Ravel,[1] Elani Streja,[1] Csaba P. Kovesdy,[2,3] Rajnish Mehrotra,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmon Center, Orange, CA; [2]Univ of Tennessee Health Science Center, Memphis, TN; [3]Memphis VA Medical Center, Memphis, TN; [4]Harborview Medical Center, Seattle, WA.*

**Background:** Kidney Disease Improving Global Outcomes guidelines recommend alkaline phosphatase (AP) as an adjunctive test in bone turnover assessment. Mounting evidence suggests AP derangements are associated with coronary artery calcification, cardiovascular events, sudden cardiac death, and increased mortality in chronic kidney disease and hemodialysis (HD) patients. However, the mortality-predictability of AP in peritoneal dialysis (PD) patients has not been well-studied.

**Methods:** In a cohort of 9,244 adult PD patients from a large national dialysis organization (entry period 2001-2006, follow-up through 2009), we examined associations between time-averaged AP with all-cause mortality using Cox models adjusted for case-mix and laboratory covariates. We then compared the mortality-predictability of time-averaged AP among 9,244 PD vs 99,323 HD patients.

**Results:** In analyses adjusted for case-mix and laboratory covariates ("fully-adjusted"), higher AP levels were associated with increased death risk in PD patients (ref: AP 70-<90U/L).




In analyses of time-averaged AP measurements, HD patients had increased mortality at all levels with the greatest risk in the highest and lowest AP categories, whereas PD patients had increased mortality with AP>150U/L only (ref: PD patients with AP 70-<90U/L). Sensitivity analyses adjusted for AST were qualitatively similar.

**Conclusions:** Higher AP levels are associated with increased mortality in PD patients. Further study of AP as a marker and/or mediator of vascular calcification and other underlying mechanisms, as well as the utility of AP in the management of chronic kidney disease-mineral bone disorders is needed.

*Funding:* NIDDK Support

## SA-PO899

**Prevalence of High Parathyroid Hormone and Alkaline Phosphatase Levels among Peritoneal Dialysis versus Hemodialysis Patients**

Connie Rhee,[1] Wei Ling Lau,[1] Vanessa A. Ravel,[1] Elani Streja,[1] Csaba P. Kovesdy,[2,3] Rajnish Mehrotra,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Orange, CA; [2]Univ of Tennessee Health Science Center, Memphis, TN; [3]Memphis VA Medical Center, Memphis, TN; [4]Harborview Medical Center, Seattle, WA.*

**Background:** Bone metabolism and morphology may be inherently different in peritoneal dialysis (PD) vs. hemodialysis (HD) patients, owing to heightened risk of vitamin D deficiency (ie, peritoneal effluent losses) and differences in mineral bone disorder management in PD (ie, greater oral vs. IV vitamin D use). Early studies suggested PD patients have a disproportionately higher prevalence of adynamic bone disease and low parathyroid (PTH) levels compared to HD patients, but were largely conducted prior to the adoption of lower calcium concentration peritoneal dialysate solutions. We thus compared the distribution of alkaline phosphatase (AP) and PTH levels according to modality in a contemporary cohort of dialysis patients.

**Methods:** Among a 2001-2006 cohort of 9,244 adult PD and 99,323 HD patients from a large national dialysis organization, we compared the distribution of AP and PTH levels. Using logistic regression adjusted for case-mix and malnutrition-inflammation-complex (MICS) markers, we then examined the association between dialysis modality and risk of higher PTH levels.

**Results:** Distributions of AP levels among PD vs. HD patients were similar, and >60% of patients receiving either modality manifested AP<110U/L. However, PD patients manifested a higher prevalence of PTH>300pg/dl (39.4% vs 48.1% of HD and PD patients, respectively) and PTH>600pg/dl (13.8% vs 20.1% of HD and PD patients, respectively). Compared to HD, PD was associated with higher risk of having a PTH>300pg/dl and a PTH>600pg/dl, after adjusting for case-mix and MICS covariates: ORs 1.68 (95% CI) 1.60-1.76 and 1.73 (95% CI) 1.62-1.84, respectively.

| PTH Level | Multivariable adjusted OR (95% CI) |
|---|---|
| PTH>300pg/dl | 1.68 (1.60-1.76) |
| PTH>600pg/dl | 1.73 (1.62-1.84) |

**Conclusions:** Contrary to earlier data, for a given level of AP and after adjustment for case-mix and MICS markers, PD patients have disproportionately higher PTH levels than HD patients.

*Funding:* NIDDK Support

## SA-PO900

**NT-proBNP Is a More Significant Prognostic Biomarker for Mortality among Three Cardiac Biomarkers in Incident Peritoneal Dialysis Patients**

Fa Mee Doh,[1] Hyung Jung Oh,[1] Chan Ho Kim,[1] Tae-Hyun Yoo,[1,2] Shin-Wook Kang.[1,2,3] *[1]Dept of Internal Medicine, College of Medicine; [2]Brain Korea 21, Yonsei Univ, Seoul, Korea; [3]On Behalf of the Clinical Research Center for ESRD Investigators.*

**Background:** Given multifactorial pathogenesis of cardiovascular (CV) disease in ESRD patients, integrated assessment using different biomarkers is required in risk stratification. In this study, we conducted a comprehensive analysis to identify the impact of three biomarkers (NT-proBNP, cTnT, and hsCRP) on mortality in incident continuous ambulatory peritoneal dialysis (CAPD) patients.

**Methods:** A prospective cohort of 335 incident CAPD patients from 36 centers of the CRC for ESRD in Korea was followed up to 33 mo. According to the baseline values of three biomarkers, patients were stratified into tertiles, and CV and all-cause mortality were compared among the three groups. Cox proportional analysis was performed to determine the independent prognostic value of each biomarker for mortality.

**Results:** During a mean follow-up duration of 21.5 mo, all-cause deaths were significantly more observed in the third tertile of NT-proBNP (29 patients, 25.7%) compared to the first (6 patients, 5.4%) and second tertiles (4 patients, 3.6%) (P<0.001). In addition, the third tertile of hsCRP had significantly higher all-cause mortality rates compared to the other tertiles (P<0.05). In contrast, there was a trend for higher mortality rates in the third tertile of cTnT, but this difference did not reach statistical significance. In multivariate Cox models, natural log values (Ln) of NT-proBNP and hsCRP, but not cTnT, were significantly associated with increased risks of all-cause mortality (Ln NT-proBNP, HR=12.81, P=0.001; and Ln hsCRP, HR=1.489, P=0.037), while NT-proBNP only significantly predicted CV mortality (HR=14.35, P=0.007). Moreover, among these biomarkers, NT-proBNP provided the highest positive predictive value for CV (AUC=0.826, P=0.004) and all-cause mortality (AUC=0.865, P<0.001).

**Conclusions:** NT-proBNP predicted CV and all-cause mortality better than hsCRP and cTnT in incident PD patients, suggesting that NT-proBNP could be a useful biomarker for adverse outcomes in these patients.

*Funding:* Government Support - Non-U.S.

## SA-PO901

**Serum Beta-2 Microglobulin Significantly Predicts Survival in Association with the Residual Renal Function in Japanese Patients on Peritoneal Dialysis – A Prospective Multicenter Cohort Study (PDR-CS)**

Hiroshi Morinaga,[1] Hitoshi Sugiyama,[1] Yasuhiko Ito,[2] Kazuhiko Tsuruya,[3] Hisako Yoshida,[3] Hiroki Maruyama,[4] Shin Goto,[4] Tomoya Nishino,[5] Hiroyuki Terawaki,[6] Masaaki Nakayama,[6] Hidetomo Nakamoto,[7] Seiichi Matsuo,[2] Hirofumi Makino.[1] *[1]Okayama Univ, Okayama, Japan; [2]Nagoya Univ, Nagoya, Japan; [3]Kyushu Univ, Fukuoka, Japan; [4]Niigata Univ, Niigata, Japan; [5]Nagasaki Univ, Nagasaki, Japan; [6]Fukushima Medical Univ, Fukushima, Japan; [7]Saitama Medical School, Saitama, Japan.*

**Background:** Beta-2 microglobulin (B2M) is an 11,800-molecular-weight polypeptide that is generated at a constant rate and eliminated by the kidneys. An elevated serum level of B2M is a potential risk factor predicting mortality in predialysis patients. However, it remains unknown whether B2M has an impact on the outcomes of patients with peritoneal dialysis (PD).

**Methods:** Five university hospitals participated in the PDR-CS and 188 PD patients were enrolled in the study, as of December 2011 (mean age, 58.0 years; male, 68.0%; diabetic nephropathy, 31.3%).

**Results:** The analysis showed that the group with a total Kt/V of 1.7 or higher to exhibit significantly lower levels of serum B2M than the group with a total Kt/V under 1.7 (23.1 vs. 27.3 mg/L; p<0.01). Dividing the patients based on whether they had a daily urinary volume of under 400 mL or 400 mL or higher revealed dramatic differences in the serum B2M levels (33.5 vs. 20.0 mg/L, p<0.0001). During the 24 months of follow-up, the group with serum B2M levels of less than 30 mg/L exhibited significantly better patient survival (p<0.01) and technique survival (p<0.05) than the group with serum B2M levels of at least 30 mg/L. The most significant determinant of the B2M level was the renal Kt/V (p<0.0005), as observed in a multivariate analysis after adjusting for the age and PD duration.

**Conclusions:** This study suggests that PD patients with a serum B2M level of less than 30 mg/L exhibit significantly better patient survival and technique survival in association with the residual renal function based on the renal Kt/V. The serum B2M level is thus considered to be a potential prognostic indicator in PD patients.

## SA-PO902

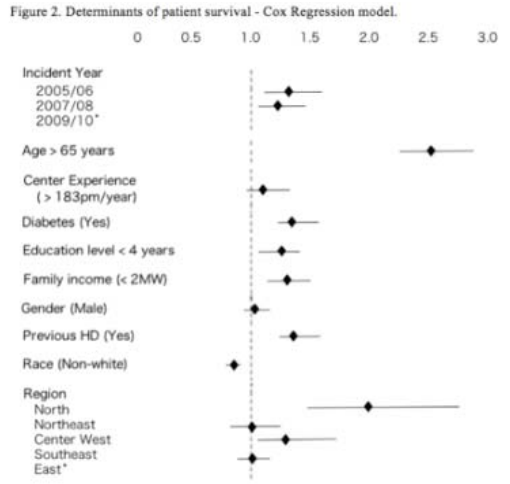
**Vintage-Related Improvement in Peritoneal Dialysis Patient Survival in the BRAZPD II Cohort**

Thyago Proença de Moraes,[1] Marcia Olandoski,[1] Ana Figueiredo,[2] Pasqual Barretti,[3] Roberto Pecoits-Filho.[1] *[1]Pontificia Universidade Catolica do Parana, Brazil; [2]Pontificia Universidade Catolica do Rio Grande do Sul, Brazil; [3]Universidade do Estado de Sao Paulo, Brazil.*

**Background:** Recent reports describe a trend to outcomes improving in peritoneal dialysis (PD) patients in the developed world. However, most of the recent growth in PD has been in the developing world, and studies looking at trends of outcomes in these regions are lacking. Our aim was to analyze temporal trends in patient and technique survival (TS) in BRAZPD II study.

**Methods:** This is a nationwide prospective study, in which incident adult patients on PD for at least 90 days from 122 centers were included between Dec 2004 - Jan 2011. Patients were divided based on the year of PD initiation: 2005/6, 2007/8, and 2009/10. Groups were compared using multivariate Cox regression.

**Results:** A total of 7007 patients (60±16 years old and 39% diabetics) were included. Main cause of dropout: death (51%); Main cause of death: cardiovascular disease(36%). Main cause of technique failure: peritonitis (63%). TS at 1, 2, 3, 4, and 5 year were 91%, 84%, 77%, 68%, and 58%. There was no change in TS over the years. Overall patient survival (PS) were 85%, 74%, 64%, and 54% at 1, 2, 3, and 4 years. PS improved with a striking 24% reduction of risk when 2005/6 was compared to 2009/10 (HR 0.76, CI95% 0.65-0.89).




Independent mortality predictors were: age ≥ 65 years (HR 2.55, CI95% 2.27-2.85), previous HD (HR1.41, CI95% 1.26-1.58), diabetes (HR 1.36, CI95% 1.22-1.52), White race (HR 1.25, CI95% 1.10-1.42), lower education level (HR 1.68, CI95% 1.11-1.44), low family income (HR 1.34, CI95% 1.19-1.51) and region (p<0.05).

**Conclusions:** A positive significant trend to patient survival improvement was observed throughout the vintages, even with no changes in technique survival and after adjusting for multiple covariates.

## SA-PO903

**Switching to Hemodialysis without a Functioning Arteriovenous Shunt Increased Risk of Mortality in Peritoneal Dialysis Patients** Shin Hung Tsai,[1] Jinn-Yang Chen.[2] *[1]Div of Nephrology, Cheng Hsin General Hospital, Taipei, Taiwan; [2]Div of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan.*

**Background:** Switching to hemodialysis was frequently encountered in peritoneal dialysis (PD) treatment. Whether switching to Hemodialysis without a functioning arteriovenous shunt will increase risk of mortality has not been fully addressed in the literatures.

**Methods:** Incident PD patients, aged ≥ 20 y/o, started dialysis therapy between January 2006 and December 2009, and had stayed on PD for ≥ 180 days were reviewed for our study. Switching to HD for ≥ 60 days was defined as a successful switching. Acute cardiovascular event (defined as admission due to acute heart failure, acute myocardial infarction or angina pectoris) and first peritonitis were analyzed as time-dependent covariates in the regression.

**Results:** Among 4333 incident PD patients, 163 (3.8 %) patients switched with a functioning arteriovenous shunt, 479 (11.1 %) switched with a central venous catheter (CVC) and 442 (10.2%) patients died within 60 days of hemodialysis with a CVC. Patients who switched with CVC were older (55.3±14.4 years), had higher prevalence of cardiovascular disease (41.3%) at baseline and had more episodes of acute cardiovascular events and peritonitis after initiating PD. After adjusting acute cardiovascular events, peritonitis and other baseline covariates in the Cox model, patients who switched with CVC had increased risk of mortality (HR:1.41, 95% CI: 1.07-1.86, *P*=0.014) than those who switched with functioning arteriovenous shunt (HR: 1.01, 95% CI: 0.59-1.74, *P*=0.97). Patients who switched with CVC also had higher non-dialysis out-patient and in-patient medical cost.

| USD/patient/month | Dialysis cost | OPD, non-dialysis | In-patient, non-dialysis |
|---|---|---|---|
| Switching a functioning AV shunt | 1629.6 | 127 | 293.6 |
| Switching with a central venous catheter | 1619.8 | 165.6 | 482.5 |

**Conclusions:** Switching to hemodialysis without a functioning arteriovenous shunt increased risk of mortality in PD patients. A planned arteriovenous shunt creation for patients not doing well in PD would avoid the increased mortality risk after initiating hemodialysis with a central venous catheter.

*Funding:* Government Support - Non-U.S.

## SA-PO904

**High Serum Cholesterol and Low Density Lipoprotein Predicted a Lower Early Death but Higher Late Death in Peritoneal Dialysis Patients** Qionghong Xie,[1] Xiaoling Ge,[1] Da Shang,[1] Rong Xu,[2] Yue Wang,[3] Yeping Ren,[4] Jianghua Chen,[5] Huiping Zhao,[6] Jie Dong,[2] Hai Yan Wang,[2] Chuanming Hao,[1] Tongying Zhu.[1] *[1]Huashan Hospital, Fudan Univ; [2]Peking Univ First Hospital; [3]Peking Univ Third Hospital; [4]Second Affiliated Hospital of Harbin Medical Univ; [5]The First Affiliated Hospital, Zhejiang Univ; [6]Peking Univ People's Hospital.*

**Background:** This study aimed to examine whether serum lipids were associated with all-cause mortality in PD patients.

**Methods:** Two retrospective cohorts were analyzed in this study. In the first cohort, 2,028 adult PD patients from seven PD centers in China were included and their lipids data at the beginning of PD were collected. The primary endpoint was all-cause mortality. In the second cohort, 171 PD patients who survived >24 months were included from one of seven centers. Their lipids were measured at the 1st, 3rd, 6th, 12th, 18th and 24th month and average levels were used to analyze their effect on mortality. Cox regression was used to analyze the association between lipids and all-cause mortality. Hazard Ratio was expressed as per 1 mmol/L increasing.

**Results:** The first cohort showed that higher cholesterol (p<0.001, HR=0.867), LDL (p<0.001, HR=0.823) and HDL (p<0.001, HR=0.606) but not triglyceride (p=0.073, HR=0.942) predicted a better prognosis in PD patients, consistent with a reverse epidemiology phenomena. When we analyzed the association between lipid and mortality using time-stratified Cox regression dividing follow-up time into 2 intervals: <1 year and > 1 year, we found that cholesterol (p<0.001, HR=0.719) and LDL (p<0.001, HR=0.052) were associated with the first-year death of the PD patients but not the late death (death after 1 year). To examine the association of lipid levels with later mortality, we analyzed the patients from second cohort who survived for more than 2 years and had two-year lipids available. The results: higher average cholesterol (p=0.008, HR=1.535) and LDL (p=0.002, HR=2.024) were associated with an increasing late mortality.

**Conclusions:** High lipid levels at beginning of PD predict a lower early death while high lipid levels are associated with late death in PD patients.

## SA-PO905

**Comparison of Three Comorbidity Indices as Predictors of Survival in Chinese Peritoneal Dialysis Patients** Terry Kw Ma, Cheuk-Chun Szeto. *Div of Nephrology, Dept of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese Univ of Hong Kong, Shatin, New Territories, Hong Kong, China.*

**Background:** In patients undergoing peritoneal dialysis (PD), various indices have been used to quantify the burden of comorbid conditions. The aim of this study is to compare the performance of three commonly used comorbidity indices in incident Chinese PD patients.

**Methods:** The cohort included 461 incident PD patients (245 males). Clinical information and presence of comorbid conditions were obtained by review of medical records. Comorbidity scores using the original Charlson Comorbidity Index, Brenda's modified Charlson Comorbidity Index, and Liu's Comorbidity Index were calculated. Patients were followed up for 45.5 ± 33.0 months for survival analysis.

**Results:** The mean age of our patients was 57.3 ± 13.5 years and 36.4% had diabetes. The median (inter-quartile range) of the Charlson, Brenda's, and Liu's scores were 6 (4-7), 1 (0-2), and 4 (2-5), respectively. There was a strong internal correlation between all three scores (p < 0.0001 for all comparisons). By univariate analysis, all three comorbidity scores significantly correlated with patient survival, with unadjusted hazard ratios (HR) 1.34, 1.31 and 1.21, respectively (p < 0.0001 for all). Multivariate analysis showed that only the original Charlson's score was independent predictor of patient survival (adjusted HR 1.34, 95% confidence interval 1.26-1.43, p < 0.0001).

**Conclusions:** When used alone, all three comorbidity scores have satisfactory performance in predicting patient survival of Chinese incident PD patients. However, the original Charlson's score appears to be the best predictor of patient survival amongst the three.

## SA-PO906

**Visit-to-Visit Variability in Systolic Blood Pressure Is Associated with Mortality in Incident Peritoneal Dialysis Patients** Han Ro,[1] Ae Jin Kim,[1] Hyung Soo Kim,[1] Chungsik Lee,[2] Sun Moon Kim,[3] Ji Yong Jung,[1] Jae Hyun Chang,[1] Hyun Hee Lee,[1] Wookyung Chung.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Gachon Univ Gil Medical Center, Gachon Univ of Medicine and Science, Incheon, Republic of Korea; [2]Div of Nephrology, Dept of Internal Medicine, Cheju Halla General Hospital, Jeju, Republic of Korea; [3]Div of Nephrology, Dept of Internal Medicine, Chungbuk National Univ Hospital, Cheongju, Republic of Korea.*

**Background:** Visit-to-visit blood pressure variability has been well known to be associated with mortality in general population. However, the relationships between the variability in blood pressure and mortality have not been studied in peritoneal dialysis (PD) patients.

**Methods:** In 98 patients who started PD in Gachon University Gil Medical Center between May 2007 and December 2011, 84 patients who continued PD until 36 weeks were retrospectively analyzed. The coefficient of variation (CV) of systolic blood pressure (SBP) was calculated from office blood pressures between 12 week and 36 week after PD. The patients were divided in to low and high BP variability groups according to the median value of CV of SBP.

**Results:** Higher BP variability predicted all cause mortality (log-rank test, P=0.008). Diabetes mellitus, congestive heart failure, history of previous cardiovascular event, serum sodium at 12 week, serum albumin at 12week, and use of β-blockers were the main correlates of CV of SBP. Higher BP variability was an independent risk factor of death after adjusting for confounding factors (hazard ratio 12.363, 95% confidence interval 1.017-150.293).

**Conclusions:** Peritoneal dialysis patients with high visit-to-visit variability in systolic blood pressure increased all cause mortality.

**SA-PO907**

**Ankle-Brachial Index Is Correlated with Mortality, Morbidity and Peritoneal Transport in Peritoneal Dialysis Patients**
Ana Rita Mateus Martins, Ricardo Vizinho, Patricia Quadros Branco, Maria Augusta Cabrita Silva Gaspar. *Nephrology, Santa Cruz Hospital, Carnaxide, Portugal.*

**Background:** Peripheral artery disease (PAD) is highly prevalent among patients with end stage renal disease. PAD is associated with high cardiovascular risk and is often unrecognized in peritoneal dialysis pts. Ankle-Brachial Index (ABI), a non-invasive method to diagnose PAD, show a U-shaped association with mortality. The ABI is highly correlated with subclinical PAD, but little is known about its association with morbidity and peritoneal transport in peritoneal dialysis pts.

**Methods:** We studied 72 pts (65,8% male, 27,4% diabetic, mean age 55±13,8 years) during 36±26,8months. Measurements of ABI were performed in supine position. Blood pressure was measured in the both arms (brachial artery) and ankles (posterior tibila arteries). Highest ankle SBP/Highest brachial SBP was used to calculate ABI. An ABI value greater than 0.9 was defined as normal. Subclinical PAD was defined as an ABI less than 0.9 in either extremity. ABI> 1.3 is a false negative caused by noncompressible arteries. ABI was correlated with functional parameteres of peritoneum, residual renal function, vascular calcification (Adragao Score) and cardiovascular events.

**Results:** A normal ABI were found in 84,9 %. Vascular calcification were found in 20 pts (27,4%).

During the follow up period, 7 pts died. In a multivariate analysis abnormal ABI (p=0,04) and high peritoneal transport (p=0,03) were independent predictors for amputation events, adjusted to diabetes and age. A survival cox regression showed that C reactive protein (p=0,02) and diabetes (p=0,05) were independent predictors for the survival, even when adjusted to BMI (body mass index), baseline cardiovascular disease and abnormal ABI.

**Conclusions:** ABI is correlated with peritoneal transport level, C reactive protein, diabetes, albumin and BMI in PD patients.

**SA-PO908**

**Metabolic Syndrome Investigation and the Risk Factors Analysis in Peritoneal Dialysis Patients: A Clinical Cohort Study in a Mono-Center**
Yiwen Li. *Kidney Disease Dept, ZheJiang Province Hospital.*

**Background:** To investigate metabolic syndrome status in peritoneal dialysis patients. To observe the effects of glucose-based peritoneal dialysate on the prevalence of metabolic syndrome in peritoneal dialysis patients. To identify the risk factors in peritoneal dialysis patients with metabolic syndrome.

**Methods:** A retrospective self-controlled study was performed with 77 peritoneal dialysis patients who had been long-term peritoneal dialysis treated in Division of Nephrology, the First Affiliated Hospital, Zhejiang University School of Medicine during January 1, 2009 to February 23, 2012. According to time after peritoneal dialysis (0 months, 6 months, 12 months, 18 months and 24 months) they were divided into 5 groups.

**Results:** 1. The prevalence of obesity was 14.86% in ESRD patients at the initiation of PD. There were no significant differences in the prevalence of obesity in PD patients with PD duration. 2. The FPG level of PD patients rose significantly with their prolonged dialysis duration, especially at 1 year after PD treatment (P<0.05), but there were no significantly high incidence rate of hyperglycemia.3.The prevalence rate of high TG was 98.68% in ESRD patients, and had increased significantly with PD duration (P<0.01). 4. The level of HDL-C increased significantly with PD duration (P = 0.005), but the prevalence rate of high HDL-C level decreased (P = 0.032). 5. The level of LDL-C increased significantly with PD duration (P <0.01). 6. The baseline prevalence rate of MS in ESRD patients was 59.21%. It increased up to 73.68% at 2 years after the initiation of PD therapy, though did not reach statistical significance. 7. FPG(B=4.549, P<0.01), BMI (B=9.338, P=0.002), FPG (B=6.983, P=0.008) , Systolic blood pressure (B=3.921, P=0.048), LDL-C(B=4.549, P=0.033), HDL-C(B=5.968, P=0.015), albumin (B=4.42, P=0.035) and dialysis additional energy (B=4.42, P=0.035)were the risk factors of MS.

**Conclusions:** The prevalence of obesity, dysglycaemia, dyslipidemia and MS were high in PD patients. ESRD and PD patients were high risk groups of MS. Long PD duration did not affect the incidence of MS. Dialysis additional energy and BMI along with FPG, Systolic blood pressure, LDL-C, HDL-C and albumin were the risk factors of MS.

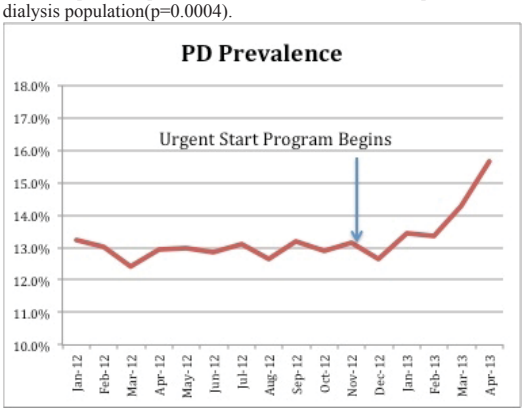
**SA-PO909**

**Urgent Start Peritoneal Dialysis Increases Peritoneal Dialysis Utilization without Compromising Outcomes**
Eric L. Wallace, Tarek Darwish, Russell Griffin, Gaurav Jain. *Univ of Alabama at Birmingham.*

**Background:** Peritoneal dialysis (PD) utilization remains low. A contributing factor is that until recently urgent dialysis required initiation using an intravascular catheter (IVC) on hemodialysis (HD) accounting for 60% of HD initiations. IVC usage is associated with increased morbidity and mortality, thus decreasing IVC use may lead to improved outcomes. Urgent start PD (UPD) allows for urgent initiation on PD by using the PD catheter prior to the standard 2 week healing time. We studied short term effects of UPD at a large academic center.

**Methods:** Clinical and outcomes data were collected on concurrent incident UPD and traditional patients using chart review. Incident PD patient was defined strictly as a patient having attended 1 day of PD training. Effect of UPD on PD prevalence (PD census/total dialysis census)was evaluated using Poisson regression. Outcomes were analyzed comparing UPD versus traditional cohorts. Pearson correlation was used to analyze IVC prevalence (total patients with IVC/total HD census) with relation to PD prevalence.

**Results:** UPD (N=11) and traditional (N=27) cohorts were similar in clinical characteristics. PD prevalence 6 months prior to UPD was stable at 13% (SD 0.002). After inception PD prevalence increased over a 6 month period to 15.7% of the total UAB dialysis population(p=0.0004).




Outcomes including need for a second PD related operation, major leaks, peritonitis, admissions, and patient retention were similar (p values 0.55, 0.52, 0.20,0.65, and 0.61 respectively). For every 1% increase in PD prevalence, there was a 0.6% decrease in IVC prevalence, though not statistically significant (p=0.25).

**Conclusions:** UPD increases PD utilization.Short-term clinical outcomes of urgent versus traditional start are similar. Increased PD prevalence may decrease IVC catheter prevalence although longer follow up will be needed to evaluate for significance.

*Funding:* Other NIH Support - 5UL1R025777 -Center for Clinical and Translational Science

**SA-PO910**

**Urgent Start PD: A Viable Mode of Dialysis Delivery to Increase PD Patients in the United States**
Heather L. Henderson, Quresh T. Khairullah, Heather M. Stamat, Robert Provenzano. *Nephrology, St. John Hospital and Medical Center, Detroit, MI.*

**Background:** There are over 500,000 people in the US with ESRD, of which only 7% use PD. There are many reasons for this low PD percentage, but the fact 60% of patients progressing to ESRD lack dialysis education or planning is a major factor. We developed an "Urgent-Start" PD program to address these issues.

**Methods:** Encouraged by Ghaffari and others showing urgent start PD (using the catheter earlier than 2 weeks) is a safe and viable option to initiate dialysis a protocol was devised involving a dedicated surgeon/nephrologist to place the Tenckhoff catheter, education of nurses, nephrologists, and one participating outpatient dialysis unit to deliver urgent start PD. All new start dialysis patients without prior dialysis education or plan were offered PD as their initial dialysis modality to avoid placement of a tunneled central venous catheter (CVC). After consent was obtained, a Tenckhoff catheter was placed and low volume exchanges were initiated immediately inpatient or at the outpatient unit as clinically appropriate. Once they were transitioned to the outpatient unit they received intermittent PD (IPD) thrice weekly until full fill volumes were tolerated and training was complete. After learning more about dialysis options those that chose to change to HD, an AVF was placed. While awaiting AVF maturation they continued in center IPD to avoid CVC placement.

**Results:** 19 new dialysis patients were enrolled in the program from May 2012 through May 2013. Two patients developed catheter complications requiring replacement or repositioning of their catheter and two patients developed a minor leak which resolved with holding PD temporarily. At six months of follow up, 6 patients (32%) transitioned or plan to transition to hemodialysis once their AVF has matured. No episodes of peritonitis and one exit site infection were observed.

**Conclusions:** Our urgent start PD program has resulted in increased PD growth (urgent and non-urgent starts) by 46% over last year with acceptably low incident complication rates. We believe our program should be considered to increase the use of PD by incident patients as well a method of CVC avoidance.

*Funding:* Private Foundation Support

**SA-PO911**

**Urgent Start Peritoneal Dialysis: A Centre's Experience**
Sunita K. Nair,[1] Rajinder S. Singh.[2] *[1]Dept of Nephrology, St. Paul's Hospital, Vancouver, Canada; [2]Dept of Nephrology, Vancouver General Hospital, Vancouver, Canada.*

**Background:** Traditionally, Hemodialysis ( HD)is the usual adopted initial modality for patients who need urgent unplanned dialysis. Peritoneal dialysis allows better quality of life than incentre hemodialysis and gives the option to dialyse at home. It offers an early survival advantage over HD (1). PD is cost effective (2). Recently it was shown by Gaffari that starting PD in unplanned patients needing urgent start is safe and feasible (3).

**Methods:** Retrospective study that looked at the urgent starts who were offered PD in the year Jan 2012- Feb 2013. These patients may or may not be followed up in the CKD clinics. PD catheter was inserted either at bedside by a Neprhologist or in the operating room by a Surgeon. Intermittent low volume PD (IPD) was commenced within three weeks of insertion. The study aimed to look at modality retention at 90 days since commencing on IPD and the complications encountered during their treatment.

**Results:** A total of 22 patients received PD as initial modality of dialysis in the period between Jan 2012-Feb 2103. IPD was commenced within three weeks of PD catheter insertion. 20 of these catheters were inserted bedside and 2 were surgical catheters. 11 patients remained on CCPD ( Continuous Cycler Peritoneal Dialysis), 5 patients stayed on CAPD ( Continuos Ambulatory Peritoneal Dialysis). So a total of 16 out of 22 ( 72%) patients retained PD modality beyond three months of commencing therapy. 2 patients died on PD and 1 patient moved territory. 3 patients were transitioned to HD. Of the patients, 2 developed Exit site leaks and 2 aptients had peritonitis. 1 patient needed brief HD for a hernia repair in between IPD and training for CAPD/CCPD. 2 patients had bloody effluent which subsequently cleared up.

**Conclusions:** PD can certainly be the initial modality for patients needing urgent start in a centre that has support in terms of bedside catheter insertion, space for allowing IPD within the hospital and rooms and staff for training at the earliest.

## SA-PO912

**Impact of a Dedicated Laparoscopic Peritoneal Dialysis Access Program on Clinical Outcomes** Tarek M. Alzahrani,[1] Mina Kashani,[2] Daniela Ghiculete,[1] Benjamin Shiff,[3] Philip McFarlane,[2] Jeffrey Perl,[2] Jason Y. Lee.[1] *[1]Urology, St. Michael's Hospital, Univ of Toronto; [2]Nephrology, St. Michael's Hospital, Univ of Toronto; [3]Faculty of Medicine, Univ of Toronto.*

**Background:** The success of peritoneal dialysis (PD) rests on the establishment of a functional access at time of PD initiation. Optimal methods of PD catheter insertion, which include laparoscopic (LAP), open surgical (OS), and fluoroscopic-guided (FG) techniques, continue to be debated. After the addition of a dedicated laparoscopic surgical access program, we compared the outcomes of PD catheters inserted at our institution using LAP, OS, and FG techniques.

**Methods:** After REB approval, a retrospective chart review was conducted on all PD catheters inserted at St. Michael's Hospital between January 2007 and March 2013. Patient demographic data, operative details, and clinical outcomes data were collected and analyzed for all 3 different insertion techniques.

**Results:** A total of 235 PD patients were included in the study (40 LAP, 36 OS, 159 FG) and each group had comparable patient populations; mean age, gender, BMI, prior history of abdominal surgery. The LAP group had a lower rate of early (<2 months post-insertion) mechanical complications (2.5% vs 39% OS vs 20% FG, p<0.001), a higher rate of successful PD initiation (94% vs 84% OS vs 79% FG, p<0.01), a higher rate of catheter technical survival (87% vs 39% OS vs 38% IR, p<0.01), and a lower rate of late (>2 months post-insertion) mechanical complications (5% vs 33% OS vs 18% FG, p<0.01The LAP group also had a lower rate of catheters requiring surgical/fluoroscopic revision (8% vs 40% OS vs 18% FG, p<0.01).

**Table 2 - PD Catheter Outcomes**

| | LAP | OS | FG | p-value |
|---|---|---|---|---|
| Mean Follow-up Duration (days) | **267.7** | 1331.7 | 987 | **<0.01** |
| Successfully Initiated PD | | | | |
| No<br>Yes<br>Still Buried | 2 (6%)<br>**31 (94%)**<br>7 | 5 (16%)<br>27 (84%)<br>3 | 33 (21%)<br>121 (79%)<br>0 | **<0.01** |
| Technical Survival of catheter * | | | | |
| No<br>Yes | 4 (13%)<br>**26 (87%)** | 22 (61%)<br>14 (39%) | 95 (62%)<br>59 (38%) | **<0.01** |
| Mechanical Complications ** <2 months from Insertion | **1 (2.5%)** | 14 (39%) | 32 (20%) | **<0.01** |
| Mechanical Complications ** >2 months after Insertion | **2 (5%)** | 12 (33%) | 28 (18%) | **<0.01** |
| PD catheter Manipulation *** Required | **3 (8%)** | 14 (40%) | 28 (18%) | **<0.01** |
| Manipulation Successful **** | 2 (67%) | 12 (43%) | 9 (69%) | 0.25 |

* Technical Survival defined as having started PD and still on PD at time of review
** Mechanical complication includes exit site infection, fluid leakage, mechanical obstruction, bleeding, tunnel infection
*** Manipulations performed by Interventional Radiologist or Laparoscopically
**** Successful manipulation defined as started on PD successfully

**Conclusions:** Our single-centre, retrospective review of 3 different PD insertion techniques demonstrated better outcomes using the laparoscopic technique, in comparable patient populations. Further follow-up is required to validate these promising early findings.

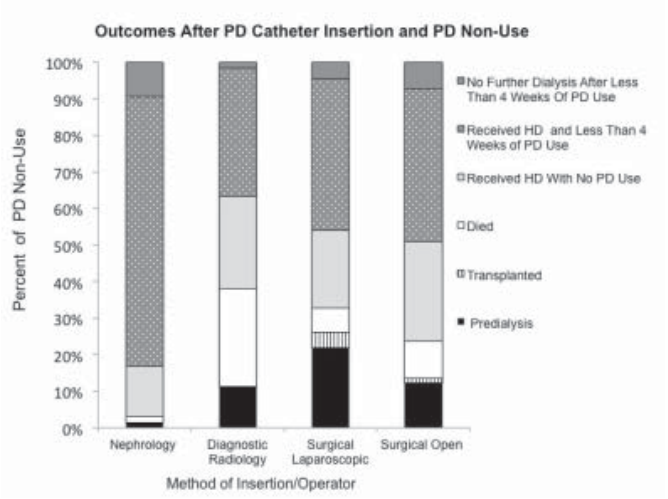
## SA-PO913

**Peritoneal Dialysis Use after Peritoneal Dialysis Catheter Implantation: A Population-Based Study** Jeffrey Perl,[1] Michael Paterson,[4] Arsh Jain,[2] Andreas Pierratos,[1] Brendan McCormick,[3] Gokulan Kandasamy,[5] Matthew J. Oliver.[1] *[1]Nephrology, Univ of Toronto, Tor, Canada; [2]Nephrology, Univ of Western Ontario, London, Canada; [3]Nephrology, Univ of Ottawa, Ottawa, Canada; [4]Chronic Disease Program, ICES, Tor, Canada; [5]Ont. Renal Network, CCO, Tor, Canada.*

**Background:** Llittle is known regarding outcomes of patients after peritoneal dialysis (PD) catheter implantation and predictors of the successful initiation of PD. We examined receipt of PD after catheter implantation exploring outcomes of PD non-use stratified by the method of catheter insertion.

**Methods:** Administrative data from Ontario identified 3886 predialysis adults with a first PD catheter implanted between 2002 and 2010. Patients were stratified by the method of PD catheter insertion: surgical-open (Su-O, n=1 884), surgical-laparoscopic (Su-L, n=1 154), nephrology-inserted (N-I, n=498), radiology-inserted (R-I, n=350). Patients were followed for at least 1 year to the successful receipt of PD (4 consecutive weeks of PD).

**Results:** The percent PD use among inserted catheters was: 85% of Su-O, 79% of Su-L, 87% of N-I and 79% of R-I. Diabetes, age, prior hemodialysis (HD) were associated with a lower risk of PD use. Relative to the Su-O group, the adjusted hazard ratio (AHR) of PD utilization was greater for N-I PD catheters AHR 1.4 (95% CI 1.2-1.5), similar for R-I inserted catheters AHR 1.0 (95% CI 0.9-1.2) but lower for the Su-L- group, AHR 0.8 (95% CI 0.7-0.8). Across groups, the majority of PD non-use resulted in the receipt of HD. More PD non-use in the Su-L, Su-O and in the R-I groups was driven by patients who remained predialysis and died prior to dialysis initiation.




**Conclusions:** Across all insertion methods, most PD catheter insertions result in successful PD use. N-I was associated with higher PD use. This may relate to the timing of insertion rather than the technical success of the technique.

*Funding:* Government Support - Non-U.S.

## SA-PO914

**Six Years Experience with Blind Percutaneous Insertion of Peritoneal Dialysis Catheters at a Brazilian Interventional Nephrology Center** Ricardo Franco,[1] Marcelo M. Nascimento,[1,2] Natasha Silva Constancio,[1] Joao otavio Ribas Zahdi,[1] Luciana Schmitt Cardon De Oliveira,[1] Leonardo Claudino Ribeiro,[1] Itamara P. Danucalov,[1] Tobias August Siemens,[1] Margarete Mara Da Silva,[1] Marcia Tokunaga De alcantara,[1] Miguel C. Riella,[1,3] Domingos Candiota Chula.[1,2] *[1]Interventional Nephrology Center, Pro-Renal Brazil Foundation, Curitiba, Parana, Brazil; [2]Internal Medicine, Federal Univ of Parana, Curitiba, Parana, Brazil; [3]Catholic Univ of Paraná, Curitba, Parana, Brazil.*

**Background:** Percutaneous peritoneal catheter insertion can be performed by trained nephrologists although the ideal method for inserting peritoneal dialysis catheters (PDC) remains controversial. This study was performed to evaluate the efficiency, the complication profile and the outcome of percutaneously placed PDC.

**Methods:** Our study population comprised 455 patients (55±17 years; 51%males) in whom 532 PDC were inserted blindly with a Tenckhoff trocar between January 2006 and December 2011 at our Interventional Nephrology Center (*Pro-Renal Brazil Foundation*).

**Results:** Early complications related to insertion included: four (0,7%) placement failures, three cases (0,5%) of bowel perforation , eight (1,5%) significant surgical wound bleeding and fifty five (10%) tip catheter migration (twenty-six out of them (47%) were successfully repositioned). Most common late complication and frequent cause for catheter removal was intractable and recurrent peritonitis which has been verified in eighty one patients (15%). In addition, exit site infection was present in twenty two patients (4%). Technical survival at 1 and 2 years was 80% and 60%, respectively. After 72-months follow-up, the survival rate by Kaplan–Meier analysis was significantly different according

to the presence of peritonitis ($\chi^2$=13.85; p<0,001). Finally, in the multivariate analysis only the presence of peritonitis was a significant risk factor for decreased catheter survival (HR=1.83; 1.35-2.47; IC (95%)).

**Conclusions:** PDC inserted percutaneously, by trained nephrologists, was associated with a very low complication rate and high primary success rate. This approach had reduced waiting time for PDC insertion and increased PD penetration in our dialysis population.

## SA-PO915

**Evaluating Outcomes of Patients with Bedside Peritoneal Dialysis Catheters Placed by Nephrologists Compared to Surgeons** Bhanu Prasad Tikkisetty,[1] Cathy Nadiger,[1] Jennifer St.onge.[2] *[1]Nephrology, Regina Qu Apelle Health Region, Regina, Canada; [2]Research and Health Information Service, Regina Qu Apelle Health Region, Regina, Canada.*

**Background:** Limitations in allocated OR time and staff resources in the RQHR have created long wait-times for patients to receive a catheter placement. Since 2007, nephrologists have also begun to perform PD catheter placements for non-complex patients. The purpose of this project was to evaluate whether nephrologists can also successfully insert PD catheters in non complex patients and therefore improve access.

**Methods:** We reviewed data from 78 charts of patients who received a PD catheter placement from 2007-2012 (Surgeon n=39; Nephrologist n=38). Our primary analyses examined the wait-time from referral to physician consult and the wait-time from physician consult to procedure between patients treated by a surgeon compared to a nephrologists. We also examined the percentage of patients with immediate (within one-month) and delayed complications following the procedure.

**Results:** The complication rate for nephrologists was 18% for immediate and 16% for delayed. Patients had to wait significantly less time to see a nephrologist (median 1.36 weeks) compared to seeing a surgeon (5.71 weeks; $p$<.05). Similarly, patients only waited a median 1.14 weeks from the time of consult to have the procedure by a nephrologist but 6.29 weeks for a surgeon placement ($p$<.05).

**Conclusions:** This research shows that nephrologists can successfully insert PD catheters with few patients experiencing immediate or delayed complications. The rates of complications were similar or lower than previous studies examining insertion of PD catheters. Given the current discrepancies in access to OR time and resources, this research suggests that nephrologists could continue to place PD catheters in non-complex cases, which would reduce wait-times without increasing the risk of complications for these patients. Surgeons should continue to handle complex cases. Decreasing the wait-time for PD catheters could also avoid some patients having to go on hemodialysis in the meantime, which is more costly to the region and disruptive to the patient.

## SA-PO916

**Long Term Results of Fluoroscopy-Guided Placement of Peritoneal Dialysis Catheter** Joo Hee Cho, Hong Joo Lee, Dongyoung Lee, Ju Young Moon, Sang Ho Lee, Chun-Gyoo Ihm, Kyung Hwan Jeong, Taewon Lee. *Depts of Nephrology, Kyung Hee Univ School of Medicine, Seoul, Korea.*

**Background:** Percutaneous fluoroscopy-guided insertion of peritoneal dialysis catheter provides accurate placement with little waiting time. Limited data suggest that this approach provides similar outcomes compared with more invasive techniques. Therefore, we report our experience with fluoroscopy assisted placement of PD catheters.

**Methods:** A Single- center, retrospective review of 401 consecutive PD patients from July 2001 to July 2012. We assessed post procedure pain scores using a 10-point visual analogue pain score scale, time to hospital discharge, complications related to PD catheters, causes of catheter removal and catheter survival. We also reviewed 89 PD patients undergoing fluoroscopic manipulation for mechanical failure of their PD catheter.

**Results:** We placed 537 PD catheters in 401 patients using fluoroscopy. There were 3 placement failures (0.56%). The average post procedure pain scores were 1.61 and 177 (44.13%) subjects had pain score of zero by 24 hours after procedure. Early complications defined as those occurring within 14 days of catheter insertion. Early complications related to PD catheter insertion occurred in 29 patients (7.23%) included 8 cases of leakage (27.58%) and 7 cases of peritonitis (24.13%). The most common cause of catheter removal was recurrent peritonitis (27.79%). 89 patients underwent fluoroscopic manipulation of PD catheters because of mechanical failure of PD catheters. The success rate of fluoroscopic manipulation was 86%. The 1- year catheter survival was 84%.

**Conclusions:** Percutaneous fluoroscopy assisted placement and manipulation of PD catheters are safe and effective. The survival rate of PD catheters placed using fluoroscopy was comparable to that of more invasive method. Radiological insertion of PD catheters to be available to a large proportion of patients who require PD.

## SA-PO917

**Outcomes of Fluoroscopic and Ultrasound Guided Placement versus Laparoscopic Placement of Peritoneal Dialysis Catheters** Dalia Dawoud,[1] Ahmed Elsayed,[2] Ahmed Kamel Abdel Aal.[3] *[1]UAB; [2]UAB; [3]UAB.*

**Background:** Peritoneal Dialysis (PD) is a renal replacement modality, with overall survival benefits that is higher or at least similar to hemodialysis. The timely and successful placement of PD catheters is crucial for the success of PD, especially in the urgent-start setting. Our aim is to compare the outcomes of PD catheter placement using fluoroscopy (FL) and ultrasound guidance (UG) with those placed using laparoscopic (LAP) technique.

**Methods:** We retrospectively compared the outcome of two groups of patients who initiated PD from January 2005 to December 2012: (1) Those who received (PD) catheter insertion using FL and UG under local anesthesia by Interventional Radiologists/ Nephrologists, and, (2) those who received PD catheter insertion using LAP under general anesthesia by surgeons. Patients with history of prior surgery were excluded from both groups. The primary endpoint was the occurrence of dialysis catheter complications at 1 year. Catheter complications including; technical, mechanical, and infectious complications were recorded. Differences between groups were analyzed using the Chi-square test.

**Results:** The overall complications at 1 year was significantly higher for the second group (p = 0.0019). The LAP approach is more likely to be complicated by catheter malfunction and peritonitis. The other complications occurred at a higher rate in the second group but the difference e was not statistically significant (Table).

| | ***FL and UG*** | ***LAP*** | ***p-value*** |
|---|---|---|---|
| ***N pts*** | 27 | 35 | |
| *Exit Site Infection* | 1 (4%) | 5 (14%) | 0.0923 |
| *Peritonitis* | 3 (11%) | 8 (23%) | 0.0570 |
| *Catheter malfunction* | 1 (4%) | 4 (29%) | 0.0326 |
| *Hernia* | 1 (4%) | 10 (11%) | 0.1559 |
| *Catheter removal* | 2 (7%) | 7 (20%) | 0.1585 |

**Conclusions:** The FL and UG technique is a minimally invasive technique that can be performed safely, and provides a significantly more favorable outcome compared to the LAP technique. The results may be due to lesser breach of the peritoneum with the former technique. This technique may allow for expeditious PD catheter placement in the late-referred patients with end-stage renal disease, thus facilitating urgent-start PD, and avoiding the need for temporary vascular access catheters.

## SA-PO918

**Vasopressin-2-Receptor Antagonist, Tolvaptan Preserved Residual Renal Function on Peritoneal Dialysis Patients with Diabetes Mellitus** Takeyuki Hiramatsu. *Dept of Nephrology, Konan Kosei Hospital, Konan-City, Aichi, Japan.*

**Background:** The contribution of residual renal function(RRF) as an independent predictor of survival or better cardiovascular health in dialysis patients has been confirmed by many observational studies. Vasopressin-2-receptor antagonist, tolvaptan was reported to augment water excression without changes in renal function. So we evaluated the short-term effect of tolvaptan on peritoneal dialysis (PD) patients with DM.

**Methods:** We studied 20 incident PD patients with DM. Patients were divided into group A and group B. Patients in group B received 15 mg/day of tolvaptan 2 weeks after PD initiation for 12 months, but not in group A. During study period, diuretics and PD prescription were not changed in both groups. Urine volume (UV), weekly Kt/V and weekly creatinine clearance (Ccr) were evaluated at baseline, 3 days, 6, and 12 months after PD initiation.

**Results:** RRF in group B were significantly increased from baseline to 3 days after PD initiation, (UV; P=0.005, renal Kt/V;P=0.0014, for at baseline and 3 days after PD initiation, respectively). After 6 and 12 months, RRF in group B were significantly greater than that in group A(*:P<0.005,**:P<0.001, ***P<0.05).

| category | Group | baseline | 6 months | 12 months |
|---|---|---|---|---|
| UV (ml/day) | A | 1406.8 ± 457.5 | 613.6 ± 282.0 | 218.2 ± 210.1 |
| | B | 1025.8 ± 225.2 | 1147.3 ± 462.3* | 816.0 ± 374.5** |
| renal KT/V | A | 0.706 ± 0.258 | 0.355 ± 0.280 | 0.320 ± 0.410 |
| | B | 0.659 ± 0.325 | 0.674 ± 0.401*** | 0.462 ± 0.302*** |
| renal Ccr (L/W) | A | 42.21 ± 17.05 | 20.39 ± 11.49 | 11.21 ± 14.14 |
| | B | 46.16 ± 23.36 | 45.83 ± 20.91* | 27.02 ± 13.68*** |

**Conclusions:** Short term treatment with tolvaptan preserved residual renal function, suggesting that tolvaptan might prevent cardiovascular events and improve quality of life in PD patients with diabetic end stage renal disease.

## SA-PO919

**Predictors of Residual Renal Function Decline in Patients Undergoing Continuous Ambulatory Peritoneal Dialysis** Chi-bon Leung, Bonnie Kwan, Cheuk-Chun Szeto. *Carol and Richard Yu Peritoneal Dialysis Research Centre, Dept of Medicine & Therapeutics, Prince of Wales Hospital, Shatin, Hong Kong.*

**Background:** Residual renal function (RRF) is an important prognostic indicator in continuous ambulatory peritoneal dialysis (CAPD) patients. We determine the predictors of RRF loss in a cohort of new CAPD patients.

**Methods:** We reviewed the record of 645 new CAPD patients. RRF loss is represented by the slope of decline of residual glomerular filtration rate (GFR) as well as the time to anuria.

**Results:** The average rate of residual GFR decline was -0.083 ± 0.094 ml/min/month. The rate of residual GFR decline correlated with proteinuria (r=-0.506, p<0.0001) and baseline residual GFR (r=-0.560, p<0.0001). Multivariate analysis showed that proteinuria, baseline residual GFR, and the use of diuretics had independent correlations with residual GFR decline. Cox proportional hazard model showed that proteinuria, baseline residual GFR, body weight, and the number of peritonitis episode were independent predictors of progression to anuria. Each 1 g/day of proteinuria is associated with 22.3% excess in the risk of progressing to anuria, each 1 ml/min higher baseline GFR is associated with 11.3% reduction in risk, while each peritonitis episode confers 7.9% excess in risk.

**Conclusions:** We conclude that proteinuria, baseline residual GFR, and the use of diuretics are independently related to the rate of RRF decline in CAPD patients, while proteinuria, baseline residual GFR, body weight, and the number of peritonitis episode are independent predictors for the development of anuria. The role of anti-proteinuric therapy and measures to prevent peritonitis episodes in the preservation of RRF should be tested in future studies.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## SA-PO920

**V2 Receptor Antagonist Tolvaptan Ameliorates Left Ventricular Mass Index (LVMI) and Urinary Output without Reduction in Residual Renal Function in Patients on Peritoneal Dialysis (PD)** Kenji Koizumi, Takefumi Mori, Ikuko Oba, Sadayoshi Ito. *Nephrology, Endocrinology and Vascular Medicine, Tohoku Univ Graduate School of Medicine.*

**Background:** Left ventricular (LV) hypertrophy is commonly observed in PD patients with poor control in volume overload. Loop diuretics and/or enhanced ultrafiltration are the common treatments for volume overload in PD patients, which may results in a decrease in residual renal function (RRF) and deterioration in peritoneal function, respectively. The V2 receptor antagonist, tolvaptan has been available for the treatment of heart failure to control volume overload in Japan. This study was conducted to examine whether tolvaptan could control volume status without reduction of RRF and improve left ventricular mass index (LVMI) in PD patients.

**Methods:** We studied 7 patients on peritoneal dialysis whose urinary volume (UV) increased more than 400mL/day after treatment with tolvaptan at Tohoku University hospital (Sendai, Japan). Body weight (BW), UV and weekly renal Kt/V (rKt/V) were compared before and after the first administration of Tolvaptan for volume control. Left ventricular mass index (LVMI), left ventricular ejection fraction (LVEF), E/E' and brain natriuretic peptide (BNP) were examined before and at least 18 weeks after the treatment. The difference of $p<0.05$ was considered as statistical significance.

**Results:** A significant increase in UV was observed in all patients. BW and BNP were not significantly reduced. An increase in rKt/V was observed and maintained over 4 to 6 months. On the other hand, significant reductions in LVMI and E/E' were observed ($p<0.05$) without changes in LVEF.

**Conclusions:** Tolvaptan increased urinary output and improved LVMI in patients on PD without deterioration in cardiac functions or residual renal function. This could be an effective novel strategy for volume control in PD patients.

*Funding:* Government Support - Non-U.S.

## SA-PO921

**Overhydration among Patient's Undergoing Peritoneal Dialysis in Relation to Age** Dorota Sikorska, Justin Nealis, Krzysztof Hoppe, Krzysztof Schwermer, Maria Wanic-kossowska, Krzysztof Pawlaczyk, Andrzej P. Oko. *Poznan Univ of Medical Sciences, Poland.*

**Background:** Overhydration is a prevalent problem in peritoneal dialysis (PD) patients and is associated with numerous complications. Multi-frequency bioimpedance analysis (BIA) offers the potential to accurately determine a patient's dry body weight (DBW). The main aim of this study was to assess the impact of age on fluid overload, and its' relationship with markers of cardiovascular risk in PD patients.

**Methods:** The study was performed on 70 PD patients. The cohort was divided into two subgroups according to gender (group A, 45 patients, <65 YO, mean age 47.98±12.76; group B, 25 patients, ≥65 YO, mean age 74.16±6.02). In both subgroups the degree of overhydration was assessed with BIA and clinical criteria. NT-proBNP and troponin T (TnT) concentration in the serum and the patient's nutritional status (SGA) was measured. Echocardiography and chest X-ray examinations were performed.

**Results:** There is a correlation between age and overhydration in BIA ($r=0.30$;$p=0.01$). The older group of patients had higher BIA overhydration (1.37±2.15 vs.2.08±2.21kg; $p=0.059$) and higher SGA (8.64±2.03 vs.9.20±1.29; $p=0.01$). The SBP and DBP (136.6±18.79 vs.140.36±22.92 mmHg; 82.66±12.06 vs.76.00±14.71mmHg) was comparable in both subgroups. The data revealed a tendency for higher NT-proBNP (3118.7±6872.7 vs.11377.2±13341.5 pg/ml; $p=0.03$) and TnT (0.04±0.03vs.0.17±0.17ng/ml;$p<0.01$) concentration in the older group. There was a correlation between BIA overhydration and NT-proBNP ($r=0.40$;$p=0.02$), TnT ($r=0.48$;$p=0.01$), SGA ($r=0.28$;$p=0.02$) and ejection fraction (EF,$r=-0.31$;$p=0.04$). The older group of patients were more overhydrated and presented with more aortic atherosclerotic changes (10vs.16;$p=0.02$) and had a reduced EF (58%vs.49%;$p=0.05$).

**Conclusions:** Older age may be a risk factor determining a higher tendency for overhydration. Overhydration seems to be a potential predictor for the development of cardiovascular complications. BIA appears to be a super technique for monitoring the hydration status, DBW and cardiovascular risk among PD patients as compared with clinical assessment alone.

## SA-PO922

**Is N-Terminal Pro-Brain Natriuretic Peptide a Useful Marker of Cardiovascular Disease or Volume Status in Peritoneal Dialysis Patients?** Ruth J. Pepper, Debbie Falconer, Andrew Davenport. *Centre for Nephrology, Royal Free Hospital, London, United Kingdom.*

**Background:** N-terminal pro-brain natriuretic peptide (NTproBNP) is used to diagnose heart failure in general medicine, and has been associated with both left ventricular hypertrophy (LVH) and extracellular water (ECW) expansion in haemodialysis (HD) patients. However whether NTproBNP has a role in peritoneal dialysis (PD), who typically have residual renal function remains to be determined.

**Methods:** Serum NTproBNP was measured in 287 PD patients and correlated with factors such as ejection fraction, echocardiogram findings and Kt/V. A linear regression model was used to delineate the relationship between the significant variables and log NTproBNP.

**Results:** The mean serum BNP was 776 pmol/l (SD 1883), and log NTproBNP 2.34 pmol/l (SD 0.64). Using the linear regression model, logNTproBNP was demonstrated to significantly correlate with logCRP [B co-efficient (B) = 0.184 ($p=0.002$)], low dialysis adequacy as measured by total Kt/V [B=-0.168] [$p=0.000$]. To assess for fluid overload the ratio of extracellular water to total body water (ECW/TBW) was calculated and was also significant [B=7.711] [$p=0.01$]. With respect to gender, logNT-proBNP was significantly associated with males [B=0.202] [$p=0.001$]. Although there was a significant correlation with ejection fraction [B=-0.017] [$p=0.000$]. However no relationship was observed with left ventricular mass index, urine output or residual renal function.

**Conclusions:** Serum NTproBNP was associated with reduced left ventricular ejection fraction, over hydration with ECW expansion, systemic inflammation as measured by CRP, reduced dialysis adequacy (total weekly Kt/V), and male sex. Therefore, serial NTproBNP measurements in stable PD outpatients may aid the clinical assessment of volume status, in the context of adequately dialysed patients with stable cardiac function and absence of systemic inflammation.

## SA-PO923

**Comparison of Cardiac Function via Echocardiography between Icodextrin and Glucose-Based Peritoneal Dialysis Patients: A Prospective One-Year Study** J. B. Chen. *Nephrology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan.*

**Background:** Icodextrin dialysis solution had been demonstrated effective ultrafiltration in a long dwell time in peritoneal dialysis (PD) patients. However, it is uncertain whether cardiac function preservation will be resulted from icodextrin ultrafiltration capacity. The aim of study was to assess the cardiac function in incident PD patients with icodextrin-based PD solution.

**Methods:** The study design was a prospective, open-label, parallel control groups. The incident PD patients were enrolled with 1:1 ratio by assigned dextrose and icodextrin-based PD solutions. Thirty patients were in icodextrin group, twenty-nine patients in dextrose group. The enrolled patients received echocardiography examinations at baseline and one-year apart. The variables of echocardiography including systolic and diastolic parameters were compared between two groups.

**Results:** At baseline cardiac examination, patients in icodextrin group showed inferiority in the variables of cardiac systolic function than those in dextrose group, i.e. LVESV (left ventricular end-systolic volume)($mm^3$), LVEF (LV ejection fraction)(%), ET(ejection time)(ms), decelceration time (ms). The trend approached to similar pattern in one-year apart. The similar phenomenon was observed in the variables of cardiac diastolic function, i.e. left atrium diameter (mm), aorta diameter, LVESD (LV end-systolic diameter)(mm). The comparison of variables at baseline and one-year apart in each groups demonstrated patients in dextrose group had significantly decrease LVEDD (47.7 vs 45.5 mm) and LVEDV (109.5 vs 98.1 $mm^3$) values at one-year period. In contrast, only one variable was found significantly increased in icodextrin group at one-year period, i.e. decelceration time (172.4 vs 201.3 ms).

**Conclusions:** Icodextrin-based PD solution could improve cardiac function both in systolic phase and in diastolic phase when those compared to dextrose-based PD solution in incident PD patients.

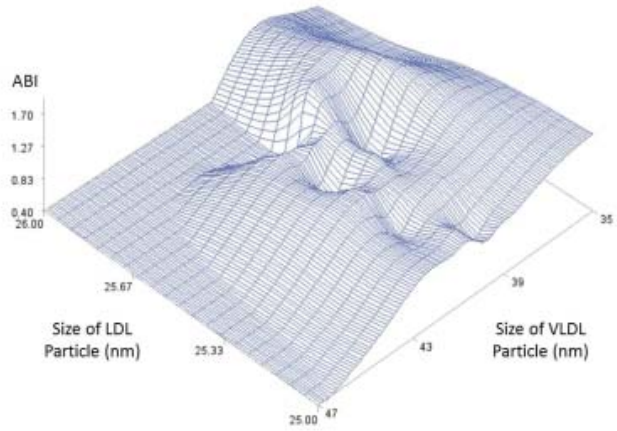
## SA-PO924

**Very-Low-Density Lipoprotein Affects Atherosclerosis of Peripheral Artery in Peritoneal Dialysis Patients** Eiichiro Kanda,[1] Masumi Ai,[3] Mitsuyo Okazaki,[3] Yoshitaka Maeda,[2] Sei Sasaki,[3] Masayuki Yoshida.[3] *[1]Nephrology, Tokyo Kyosai Hospital, Meguroku, Tokyo, Japan; [2]Nephrology, JA Toride Medical Center, Toride, Ibaraki, Japan; [3]Tokyo Medical and Dental Univ, Bunkyoku, Tokyo, Japan.*

**Background:** Peripheral artery disease (PAD) represents atherosclerotic disease and is a risk factor for death in peritoneal dialysis (PD) patients, who tend to show an atherogenic lipid profile. In this study, we investigated the relationship between lipid profile and ankle-brachial index (ABI) as an index of atherosclerosis.

**Methods:** Thirty-five PD patients were enrolled in this cross-sectional study in Japan. The proportions of cholesterol level to total cholesterol level (cholesterol proportion) in 20 lipoprotein fractions and the mean size of lipoprotein particles were measured using an improved method, high-performance gel permeation chromatography.

**Results:** The mean (standard deviation) age was 61.6 (10.5) years; PD vintage, 38.5 (28.1) months; ABI, 1.07 (0.22). A low ABI (0.9 or lower) was observed in 7 patients (low-ABI group). The low-ABI group showed significantly higher cholesterol proportions in the chylomicron fraction and large very-low-density lipoproteins (VLDLs) (Fractions 3-5) than the high-ABI group (ABI>0.9). Adjusted multivariate linear regression analysis showed that ABI was negatively associated with serum VLDL cholesterol level ($p=0.0074$) and the cholesterol proportions in large VLDLs (Fractions 4, $p=0.038$; 5, $p=0.0039$) and medium VLDL (Fraction 6, $p=0.014$). ABI was also negatively associated with the size of VLDL particles ($p=0.032$), which was associated with serum triglyceride level (Pearson's correlation coefficient $r=0.798$, $p=0.0001$).




**Conclusions:** This study showed that, among PD patients, the characteristics of VLDL particles were associated with atherosclerosis. Lowering serum VLDL level may be an effective therapy against atherosclerosis in PD patients.

## SA-PO925

**Overhydration in Peritoneal Dialysis: Clinical Perception versus Bioimpedance** Lourdes de la Vara Iniesta, Agustín Ortega-cerrato, Carmen Gómez Roldán, Juan Pérez-martínez. *Hospital of Albacete.*

**Background:** Overhydration is associated with increased morbimortality in peritoneal dialysis (PD) patients. Health professionals are capable of estimating our patients' hydration volume through clinical perception but, using other tools such as bioimpedance, it is possible to assess it more objectively. Objectives:Determine if there is a correlation between the hydration status of the PD patients treated in our unit when estimated using bioimpedance and when estimated by health professionals' clinical perception. Evaluate whether it is possible to improve the professional's clinical perception.

**Methods:** Descriptive, observational study. 37PD patients were included. 1resident,1nephrologist and 4nurses took part in the study. The estimation of the patients' hydration status was compared using the two methods. The data are given as mean±SD and percentage over/underhydration with respect to the Urea distribution volume(V). Pearson's correlation and the Bland-Altman plot were used for the statistical analysis.

**Results:** 111 determinations performed on PD patients were analysed. The mean overhydration estimated by bioimpedance was 2.77±2.05litres vs 2.04±1.67litres by the health professionals. The correlation between the two methods was significant (r:0.33;p<0.003). As can be seen in figure1A, there is no significant scatter between the estimations by the two methods, showing a tendency to underestimate our patients' hydration status. My clinical perception, as resident, became increasingly accurate as the 4-month study progressed [B].

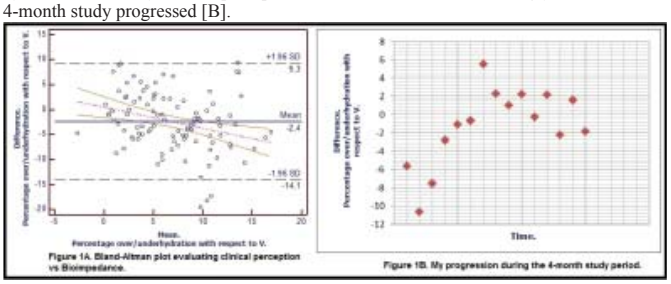



Figure 1A. Bland-Altman plot evaluating clinical perception vs Bioimpedance.

Figure 1B. My progression during the 4-month study period.

**Conclusions:** We found a high correlation between the two methods. Although bioimpedance may help the clinician to identify more objectively minor changes in volemia, it does not replace the professional's clinical perception. Furthermore, this perception can improve with input from all of the patient's clinical parameters, helping to give a more accurate estimation.

## SA-PO926

**The Effects of Paricalcitol on Cardiac Fibrosis in a Peritoneally Dialysed Uremic Rat Model** Zeynep Kubilay,[1] Arzu Velioglu,[1] Halil Tugtepe,[2] Deniz Filinte,[3] Izzet Hakki Arikan,[1] Mehmet Koc,[1] Serhan Tuglular,[1] Cetin Ozener.[1] *[1]Nephrology, ; [2]Pediatric Surgery, ; [3]Pathology, Marmara Univ.*

**Background:** VitaminD receptor activators are promising agents in mitigating cardiovascular complications in chronic renal disease. Herein, we evaluated effects of paricalcitol on cardiac fibrosis in a peritoneally dialysed uremic rat model.

**Methods:** 30 Wistar male rats were randomized to 4 groups. GroupN:Sham operation(n=6), GroupU:5/6 Nephrectomized(n=8), GroupPD:5/6 Nephrectomized+PD(n=8), Group PD-P:5/6 nephrectomized+PD+paricalcitol(0.24 µg/kg/day IP)(n=8).After 6 weeks, blood Cr, Ca,P,PTH levels, blood pressure, ultrafiltration capacity, cardiac fibrosis and media/lumen ratio of the intracardiac arteries, TGF-β and Collagen III staining were evaluated.

**Results:** When compared to Group U, Group PD and PD-P showed a decrease in cardiac fibrosis, media/lumen ratio and cardiac TGF–β expression, but significance was only found between Group PD-P and U for the studied variables.

| | N(n=6) | U(n=8) | PD(n=8) | PD-P(n=8) |
|---|---|---|---|---|
| Ca(mg/dL) | 10.8±0.13 | 10.9±0.14 | 9.6±0.13[c] | 9.7±0.52[b] |
| P(mg/dL) | 4.4±0.16 | 6.1±0.34 | 6.9±0.39 | 7.8±0.69[b] |
| PTH(pg/mL) | 372.5±54.1 | 244.9±41.8 | 478.7±50[c] | 605.8±76[b] |
| Ultrafiltration | 6.5±0.4 | 8.1±0.8 | -7.1 ±1.1[c] | -2.3 ±1.2[a,b] |
| Blood Pressure | 120±3.6 | 150±0.4 | 173±1.3 | 163±1.7[a,b] |
| Cardiac Fibrosis | 0.17±0.16 | 1.25±0.16 | 1.00±0.26 | 0.50±0.18[b] |
| Media/Lumen | 1.83±0.3 | 2.63±0.27 | 2.25±0.25 | 1.75±0.25[b] |
| Collagen III | 1.17±0.16 | 2.20±0.2 | 1.38±0.5[c] | 1.63±0.26 |
| TGF-β | 1.67±0.33 | 2.00±0.8 | 1.25±0.16 | 1.13±1.25[a] |
| a:p<0.05 group PD-Pvs.group PD,b:p<0.05 group PD-P vs.group U, c:p<0.05 group PDvs.group U | | | | |

**Conclusions:** Paricalcitol was found to decrease cardiac fibrosis. PD treatment alone did not improve this finding, whereas addition of paricalcitol significantly reduced cardiac fibrosis. Due to its beneficial effects on heart, paricalcitol could be preferred in peritoneal dialysis patients.

*Funding:* Pharmaceutical Company Support - Abbott, Private Foundation Support

## SA-PO927

**Relation between Volume Status and Health-Related Quality of Life in Peritoneal Dialysis Patients** Yun Jung Oh, Chungsik Lee. *Internal Medicine, Cheju Halla General Hospital, Jeju, Korea.*

**Background:** Volume status is an important predictor of outcomes in patients with end stage renal disease, and chronic fluid overload contributes to the high mortality in dialysis patients. However, it has not yet been identified whether to achieve euvolemia contributes to improve health-related quality of life(HRQOL) in peritoneal dialysis(PD). We investigated the relationship between volume status and HROQL in PD patients.

**Methods:** This study is a cross-sectional, noninterventional, and observational. We enrolled 41 prevalent PD patients, and accessed volume status using Body Composition Monitor(BCM). Korean version of Kidney Disease Quality of Life short form(KDQOL-SF), version 1.3 was used to evaluate HRQOL. We determined scores of three components summary of HRQOL; Kidney Disease Component Summary(KDCS), Physical Component Summary(PCS), and Mental Component Summary(MCS). The hydration status(HS) was normalized to extracellular water(ECW)(Relative hydration=HS/ECW) for the analysis, and overhydrated status was defined as OH/ECW value greater than 0.15.

**Results:** Mean values of HS, ECW, and relative hydration were 2.80±2.20L, 17.4±3.84L, and 0.151±0.101, respectively. In correlation analysis, relative hydration(HS/ECW) was negatively correlated with the scores of KDCS and MCS(r=-0.410; P=0.008 and r=-0.309; P=0.049 respectively). Among disease-specific KDCS domains, relative hydration was significantly associated with two subscales including burden of kidney diseases and cognitive function(ß=-82.813, P=0.018 and ß=-40.670, P=0.033, respectively). The result of multiple linear regression analysis showed that relative hydration had significant negative association with KDCS(ß=-37.565, P=0.033). Mean value of KDCS score was lower in overhydrated patients compared to normohydrated patients, but the difference was not significant(61.1±9.25 vs 66.7±17.17, P=0.075).

**Conclusions:** Overhydrated status showed a significant negative association with scores of KDCS and MCS, indicating that volume status is an important factor affecting HRQOL in PD patients. Close monitoring of volume status and a correct dialysis prescription to maintain euvolemia seem to be warranted to improve HRQOL in PD patients.

## SA-PO928

**Sympathetic Overactivity in Chronic Peritoneal Dialysis Patients** Dan Sapoznikov, Aharon Bloch, Haya Assayag, Dvora Rubinger. *Nephrology and Hypertension Services, Hadassah Univ Medical Center, Jerusalem, Israel.*

**Background:** Sympathetic overactivity is not well defined in chronic peritoneal dialysis (PD) patients.

**Methods:** Continuous beat-to-beat intervals (IBI) and systolic blood pressure (SBP) were monitored in 13 PD patients (pts.), 112 chronic hemodialysis (HD) pts. and in 32 control subjects (C).IBI and SBP oscillations in the low frequency range (LF, 0.04-0.15 Hz) were considered to be indicative of sympathetic activation. The prevalence of baroreflex and nonbaroreflex episodes (in which heart rate and SBP change in opposite or in the same direction) was interpreted as representative of vagal or central sympathetic activation, respectively.

**Results:** Mean SBP and IBI, sdIBI (a measure of overall autonomic activity), LF IBI (power spectrum of IBI in LF range), LF IBI/HF IBI (the ratio between amplitudes of IBI oscillations in LF and HF (high frequency) ranges, an index of sympatho-vagal balance) and LFα (baroreflex sensitivity in LF range) were (median and interquartile range):

| | C | HD | p vs.C | PD | p vs.C | p vs.HD |
|---|---|---|---|---|---|---|
| Age*(y) | 54±11 | 58±15 | NS | 55±14 | NS | NS |
| SBP (mm Hg) | 125 (25) | 137 (32) | 0.031 | 130 (33) | NS | NS |
| Mean IBI (msec) | 832 (172) | 838 (166) | NS | 712 (142) | 0.040 | 0.006 |
| sd IBI (msec) | 32 (15) | 20 (13) | 0.001 | 20 (14) | 0.001 | NS |
| LF IBI (msec$^2$/Hz) | 2327 (3048) | 739 (1569) | 0.001 | 520 (1409) | 0.001 | NS |
| LF IBI/HF IBI | 6.89 (3.60) | 3.15 (3.40) | 0.001 | 4.40 (7.20) | NS | NS |
| LFα (msec/mm Hg) | 5.06 (2.76) | 3.24 (2.57) | 0.001 | 3.13(1.37) | 0.002 | NS |

*mean±SD.

Baroreflex activity was predominant in HD, while in PD the prevalence of nonbaroreflex episodes was increased. The proportion of pts. With predominant (>90%) nonbaroreflex activity was higher in PD than in HD (p=0.013).

**Conclusions:** These results show: 1.Decreased IBI variability and baroreflex sensitivity are suggestive of sympathetic overactivity in both HD and PD. 2. The increased heart rate, the higher LF IBI /HF IBI and the increased prevalence of nonbaroreflex episodes point to a more pronounced sympathetic tonus in PD. The prognostic significance of these findings remains to be defined.

## SA-PO929

**An Analysis of Factors Predicting Peritoneal Catheter Tunnel and Exit-Site Infection in a Low Incidence Setting** Miguel Perez Fontan, Ana Rodriguez-carmona. *Nephrology, Univ Hospital A Coruña, A Coruña, Spain.*

**Background:** Catheter tunnel and exit-site infection (TESI) causes morbidity and Peritoneal Dialysis (PD) technique failure. Between 1990-2012 our Unit has kept homogeneous practices for prevention of TESI, including catheter design, insertion techniques, exit-site care, screening and management of Staphylococcus aureus (SAu) carriers, and diagnostic criteria of TESI.

**Methods:** We have explored the baseline profile for TESI in 665 patients initiatiating PD 1990-2010. Statistics: univariate tests, Kaplan-Meier and Cox models. We also applied time-dependent multivariate analysis to disclose the potential association between the risks of TESI and peritonitis.

**Results:** We observed 230 episodes of TESI (170 patients, 1631 patient-years). Univariate analysis disclosed PD intiated before 2000 ($p<0.0005$), kidney transplant before PD ($p=0.02$), SAu carriage ($p<0.0005$), short delay catheter insertion-PD initiation ($p=0.001$), lower GFR ($p=0.02$), fast peritoneal transport ($p=0.005$), lower hemoglobin ($p=0.008$) and higher C-reactive protein levels ($p=0.008$) as predictors of TESI. 68.3% of patients with TESI suffered peritonitis too, versus 49.8% of those without TESI ($p<0.0005$).

Multivariate analysis identified SAu carriage (HR 1.67, $p=0.03$), kidney transplant before PD (HR 2.58, $p=0.017$), fast peritoneal transport (HR 1.03, $p=0.001$) and delay <7 days between catheter insertion and PD (HR vs >30 days 3.45, $p=0.002$) as predictors of TESI. Time-dependent Cox analyses disclosed an independent association between TESI and peritonitis ($p=0.023$); there was a trend to a higher risk of peritonitis in patients with >1 TESI ($p=0.061$), while the time lapse to the first episode of TESI did not appear to modify this association ($p=0.30$).

**Conclusions:** Adequate catheter rest after insertion has a definite impact on the later incidence of TESI. Systematic screening and treatment of SAu carriers does not prevent an increased incidence of TESI in this subgroup. Our results confirm a strong time-dependent association between the risk of TESI and peritonitis.

## SA-PO930

**Rapid Progression of Brain Atrophy in Patients on Peritoneal Dialysis: A Longitudinal Study** Kazuhiko Tsuruya,[1] Hisako Yoshida,[1] Tohru Mizumasa,[2] Hideki N. Hirakata,[2] Takanari Kitazono.[3] *[1]Dept of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Div of Nephrology and Dialysis Center, Japanese Red Cross Fukuoka Hospital, Fukuoka, Japan; [3]Dept of Medicine and Clinical Sciences, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

**Background:** Brain atrophy has been reported in chronic kidney disease (CKD) patients, although its mechanism remains to be elucidated. We previously demonstrated that cerebral ischemia and dialysis-related hypotension play a role in brain atrophy in hemodialysis patients (Yoshimitsu T, et al., 2000, Mizumasa T, et al., 2004). On the other hand, to date, it has not been reported as to brain atrophy in patients with peritoneal dialysis (PD). Thus, we examined the progression rate of brain volume by a two-year longitudinal observation in the present study.

**Methods:** Thirty-two PD patients aged 37–79 (60 ± 11) years (men 19, diabetes 10) and 58 predialysis CKD patients aged 37–79 (61 ± 10) years (men 30, diabetes 15, eGFR 38.9 ± 11.6 mL/min/1.73m$^2$), who had no history of cerebrovascular disease, were recruited and underwent MRI scanning at baseline and after two years. T1-weighted MRI images were analyzed with statistical parametric mapping software. Total gray matter (GM), total white matter (WM), and cerebrospinal fluid were segmented and each volume was quantified using MRI voxel-based morphometry. GM volume (%GMV) and WM volume (%WMV) were normalized as percentages of intracranial volume to adjust for variations in head size. We compared the reduction rate of %GMV between PD patients and predialysis CKD patients.

**Results:** The percentage of GMV was lower in PD patients than predialysis CKD patients at baseline (39.09 ± 2.91% vs. 41.13 ± 2.47%). Annual reduction rate of %GMV was significantly higher in PD patients than predialysis patients (0.68 ± 0.42 vs. 0.26 ± 0.41 percentage-point/year). This significant difference remained even after stratification by age, gender, and diabetes.

**Conclusions:** Progression of brain atrophy is significantly higher in PD patients than predialysis CKD patients independent of age, gender, and diabetes.

## SA-PO931

**Does Peritoneal Dialysis Confer a Higher Risk for Calciphylaxis?** Sagar U. Nigwekar, Maria B. Luongo, Ravi I. Thadhani, Nina E. Tolkoff-Rubin. *Massachusetts General Hospital.*

**Background:** Calciphylaxis is a rare but highly fatal condition seen in dialysis patients. Prior literature speculates that peritoneal dialysis (PD) patients are at a higher risk for calciphylaxis compared to hemodialysis (HD) patients. However, no study has compared distribution of calciphylaxis risk factors in these populations to determine whether PD as a modality increases calciphylaxis risk.

**Methods:** Dialysis unit and pathology department records from two large academic centers were searched to identify calciphylaxis cases diagnosed between January 2002 and December 2012. Demographics, co-morbidities, dialysis adequacy, laboratory and medication data were compared between PD and HD calciphylaxis patients using a Fisher exact test (categorical variables) or a Wilcoxon test (continuous variables).

**Results:** We identified 69 cases of calciphylaxis; 7 were on PD (all on continuous ambulatory PD) and 62 were on HD. PD calciphylaxis patients were younger than HD calciphylaxis patients (median age 50 vs. 62 years, $p=0.03$) and were more likely to be non-Caucasian (62% vs. 36%, $p=0.04$). There were no significant differences between calciphylaxis patients on PD vs. HD for dialysis vintage (median 5.1 vs. 4.8 years), diabetes mellitus (28% vs. 35%, $p=0.10$), macrovascular disease (31 vs. 34%, $p=0.10$), calcium based phosphate binder use (29 vs. 33%, $p=0.13$), and vitamin D therapy (45% vs. 49%, $p=0.11$). Warfarin use (71% vs. 44%, $p=0.01$), obesity (57% vs. 37%, $p=0.01$), and hypoalbuminemia (100% vs. 65%, $p=0.02$) were more prevalent in PD calciphylaxis patients compared to HD calciphylaxis patients. Dialysis adequacy and serum calcium, phosphorous, and parathyroid hormone levels were similar.

**Conclusions:** Higher prevalence of calciphylaxis risk factors (such as warfarin, obesity and hypoalbuminemia) rather than dialysis modality likely explains calciphylaxis development in PD patients. Prospective studies are needed to confirm these findings. More judicious warfarin use and attention to malnutrition-inflammation complex may prevent calciphylaxis in PD patients.

*Funding:* Private Foundation Support

## SA-PO932

**Metabolic Syndrome and Body Fat Content of Peritoneal Dialysis Patients** Cheuk-Chun Szeto,[1] Terry Kw Ma.[2] *[1]Dept of Medicine & Therapeutics, The Chinese Univ of Hong Kong, Shatin, Hong Kong; [2]Dept of Medicine & Therapeutics, Prince of Wales Hospital, Shatin, Hong Kong.*

**Background:** In the general population, metabolic syndrome (MES) is strongly associated with cardiovascular risk. However, the definition of MES and its prognostic implication amongst patients undergoing peritoneal dialysis (PD) remains controversial.

**Methods:** We studied 329 prevalent PD patients and compared 4 sets of diagnostic criteria: the original WHO criteria, the International Diabetes Federation (IDF) criteria, the original National Cholesterol Education Program (NCEP) criteria, and the modified NCEP criteria. Nutritional status, body composition, and arterial pulse wave velocity were measured. Patients were followed for 31.7 ± 15.5 months for survival and hospitalization.

**Results:** Amongst the 329 patients, 175 (53.2%) fulfilled the WHO criteria, 177 (53.8%) by the IDF criteria, 199 (60.5%) by the original NCEP criteria, and 218 (66.3%) by the modified NCEP criteria. In general, the agreement between the 4 sets of criteria was fair to moderate (Cohen's kappa 0.352 to 0.580). Patients with MES defined by all 4 criteria had higher adipose tissue mass than the others, although the difference was most pronounced with the IDF criteria (18.2 ± 7.9 vs 10.7 ± 5.9 kg, $p<0.0001$), and the IDF criteria was least affected by overhydration. Furthermore, patients with MES, as defined by the IDF criteria, were hospitalized longer than those without MES (3.82 (inter-quartile range [IQR] 0.00 – 12.61) vs 1.07 (IQR 0.00 – 6.43) days per year of follow up, $p = 0.010$). There was no difference in overall survival, cardiovascular survival, or technique survival between patients with and without MES, irrespective to the diagnostic criteria after adjusting for diabetic status.

**Conclusions:** We conclude that the IDF criteria is probably the preferred choice for the definition of MES in PD patients as it best reflects the adipose tissue mass, least affected by overhydration, and best associated with the need of hospitalization during follow up.

*Funding:* Clinical Revenue Support

## SA-PO933

**Does Increased Peritoneal Dialysis Adequacy Correlate with Physical Activity and Energy Expenditure?** Sally Salah El-kateb,[1] Sivakumar Sridharan,[2] Kirsten L. Rennie,[3] Ken Farrington,[2] Andrew Davenport.[1] *[1]Royal Free Hospital; [2]Lister Hospital; [3]Univ Hertfordshire.*

**Background:** Whereas there is an established relationship between dialysis adequacy, nutrition and survival for haemodialysis patients, the same relationship has not been demonstrated for peritoneal dialysis (PD) patients. We therefore looked at the relationship between total energy expenditure (TEE), resting energy expenditure (REE), active physical energy expenditure (AEE), and dialysis adequacy and body composition.

**Methods:** All patients included are receiving PD treatment at the Royal Free hospital. Patient demographics, body composition measured using bioimpedance and PD adequacy (Total weekly urea KT/V and weekly litres of creatinine cleared (CrCl), along with residual urine output assessed by 24-hour urine collection) were recorded. Dietary protein intake (nPNA) and albumin serum levels were used as an indicator of the nutritional status. TEE, REE and PEE were calculated such that (TEE = REE * Mean daily MET) and REE = -2.497 * Age * (age) + 0.011 * height$^{2.023}$ + 83.573 * weight$^{0.6291}$ + 68.171. A physical activity assessment questionnaire was designed to determine daily MET.

**Results:** Seventy five patients (M=43,F=32) were included in this study, of which 25 were diabetic. Average age was 59.99±17.32(21-88) years. There was a significant association between AEE and age(β= -17.66, CI 95% (-34.8 --.47), $p=0.044$) and log vintage (β=-770.32, CI 95% (-1346.56 - -194.09), $p=0.01$). REE was significantly associated with skeletal muscle mass(SMM) (β= 88.51, CI 95% (72.23-104.79), $p<0.0001$), extracellular weight/total body weight(β= 66.78, CI 95% (24-109.5), $p=0.003$), BMI(β=-35.373, CI 95% (-59.39 - -11.35), $p=0.005$) and body fat mass(β=20.21, CI 95% (8.81 – 31.61), $p=0.001$). Finally, a significant association was depicted between TEE and SMM(β=129.84, CI 95% (25.56 – 234.11), $p=0.016$). No significance was detected between any EE and nPNA, CCr or total weekly urea KT/V.

**Conclusions:** There is no relationship between nPNA or dialysis adequacy measured by total weekly urea KT/V or litres of creatinine cleared per week or residual urine output and either total, resting or activity energy expenditure in PD patients.

### SA-PO934

**Leptin as a Key Factor in Obesity Pathophysiology: Clinical Relevant Associations in PD Patients** Ana Paula Bernardo,[1,2] Silvia Coelho,[1] Maria João Carvalho,[1] António Manuel Nunes Cabrita,[1] Anabela Rodrigues.[1,2] *[1]Nephrology, CHP; [2]UMIB/ICBAS/UP.*

**Background:** In peritoneal dialysis, obesity is assumed to result from intraperitoneal glucose absorption, and doubts still remain about its impact on patient survival, since most studies are simply based on body mass index (BMI) to characterize obesity.In general population, adipokines are known to play an important role in obesity pathogenesis and their associated metabolic complications such as dislipidemia and inflammation.Until now, no study explored body composition assessment with adipokines and inflammation in peritoneal dialysis.Our aim was to explore in a PD prevalent population the link between leptin and adiponectin, lipids, IL6 and fat mass evaluated with body composition monitor(BCM®).

**Methods:** We performed multifrequency bioimpedance assessment in 66 prevalent PD patients. We assessed lean tissue index (LTI), fat tissue index(FTI), relative lean tissue mass(Rel.LTM), relative fat mass(Rel.Fat), body cell mass(BCM), BMI, leptin, adiponectin, IL-6, triglycerides (Tg), HDL and LDL cholesterol, albumin and nPCR(protein catabolic rate).

**Results:** 48.5% female, 18.2% diabetics, 28.1% anurics, 20% fast transporters, mean age 50.3 years, PD vintage 18.7 months.Leptin correlated better with FTI and Rel.Fat than with BMI (r=0.67, r=0.63 and r=0,45, p<0.0001,respectively).Leptin also correlated with adiponectin(r=-0.24, p=.04), Tg(r=0.39, p=0.001) and nPCR(r=-0.41, p=0.004) but no correlation was found with IL-6, albumin, peritoneal membrane small solute transport or residual renal function in our population.Patients with leptin > median (17.25ng/mL) had lower adiponectin(15.7±7.3 vs 18.9±7.6µg/mL,p=0.034), lower LTI(12.4±2.7 vs 14.3±3.2Kg/m$^2$,p=0.025), lower BCM(18.4±6.1 vs 22.7±7.9Kg,p=0.018), higher FTI and Rel.FAT (14.1±4.9kg/m$^2$ vs 8.5±3.9Kg/m$^2$ and 37.5±8.4% vs 26.3±10.5%,respectively, both p<0.0001), higher Tg(190 vs 131mg/dl,p=0.008) lower HDL(38±9 vs 47±16mg/dl,p=0.013) and lower nPCR(1.0±0.3 vs 1.2±0.3g/kg/day,p=0.026).

**Conclusions:** Leptin shows better correlation with FTI and Rel.FAT assessed by bioimpedance than with BMI, and has important and clinically relevant associations with dislipidemia and nutrition parameters in PD patients.

### SA-PO935

**Body Composition Evaluation and Adipokines Profile in Risk PD Patients** Ana Paula Bernardo, Olivia Santos, Maria João Carvalho, António Manuel Nunes Cabrita, Anabela Rodrigues. *Nephrology, CHP, Porto.*

**Background:** Peritoneal dialysis has improved clinical results in the last decade. However aged patients, diabetics, anurics and fast transporters, leading to less favorable outcomes, still challenge therapy.Our aim was to document relevant corporal composition changes and adipokines profile alterations amenable to intervention in specific risk PD patient subgroups.

**Methods:** We performed multifrequency bioimpedance assessment in 66 prevalent PD patients, under updated PD therapy, treated with low-GDPs solutions, elective automated PD and free icodextrin use.We assessed relative fluid overload(RelOH), lean tissue index(LTI), fat tissue index(FTI), relative lean tissue mass(Rel.LTM), relative fat mass(Rel.Fat), body cell mass(BCM), leptin and adiponectin.

**Results:** 18.2% diabetics, 28.1% anurics, 20% fast transporters, mean age 50.3 years, 48.5% female, PD vintage 18.7 months. Diabetic patients had higher RelOH(15.3±8.1vs6.3±7.5%,p=0.001) but notably similar fat and lean tissue mass as well as adipokine profile. Anuric patients did not present significantly higher volume overload but had lower LTI (11.9±2.3vs 13.8±3.2Kg/m$^2$,p=0.04), and lower BCM (17.2±5.2 vs 21.7±7.5Kg,p=0.035), without other significant differences, namely adipokines.Fast transporters had no significant differences concerning adiponectin [19.3(11)µg/mL vs 15.9(10)µg/mL], leptin [13.8(42) ng/mL vs 19.5(56)ng/mL], FTI (10.5±5.3 vs11.4±5.2Kg/m2) and Rel.Fat(30.7±12.9 vs32.0±10.6%);additionally RelOH was similar(8.3±9.9 vs 7.8±8.0%).Aged patients (≥ 65 years) had significantly higher RelOH(12.5±6.4vs6.8±8.4%,p=0.018),without other differences concerning body composition or adipokines profile.

**Conclusions:** Updated PD schedules are able to avoid clinically relevant complications in risk patients: fast transport status and diabetes were not associated with increased fat mass or with a dismal adipokine profile; both anuric and fast transporters achieved good volume control but risk of protein malnutrition should be managed in anurics; older patients were prone to higher volume overload but preserved fat and lean tissue mass. Carefully body composition evaluation in risk PD patients is mandatory to adjust therapy and improve PD outcomes.

### SA-PO936

**Gabapentin Therapy for Pruritus in Automated Peritoneal Dialysis Patients: A Randomized Controlled Trial** Arturo R. Marin. *Nephrology, Hospital Regional ISSEMYM-Tlalnepantla., Mexico, Estado de Mexico, Mexico.*

**Background:** Uremic pruritus is a common and unpleasant symptom in patients with chronic kidney disease (CKD), the incidence is 42-52% and impact on quality of life in peritoneal dialysis and hemodialysis patients. Available treatments are not completely effective. Evidence with gabapentin and antihistamines indicate variable effects in dialysis patients.

Objective. To compare efficacy and side effects of gabapentin and loratadine in uremic pruritus in patients on automated peritoneal dialysis (APD).

**Methods:** We did a randomized, prospective, open, comparative clinical trial in APD patients with uremic pruritus. 60 patients were randomized to receive 12 weeks of treatment with gabapentin (n=30) 300 mg and loratadine (n=30) 10 mg every 24 hours, at night. To assess the severity of pruritus was used visual analogue scale (VAS), a reduction of pruritus of 50% was considered effective. To assess other features of pruritus was used 5D itch scale; a reduction of 2 points on the scale used was considered as effective.

**Results:** The gabapentin group were 22 male and 8 female patients, with 56.7+12.4 years old and the loratadine group were 21 male and 9 female patients, with 48.5+14.6 years old (p >0.05). All patients completed 12 weeks of treatment. Effectiveness was reported in 20 patients in the gabapentin group vs 10 patients in loratadine group (P = 0.025). With respect to the intensity of pruritus was found effective in 18 patients in the gabapentin group vs 9 in the loratadine group (P = 0.006), in terms of effectiveness duration was found in 15 patients in the group with gabapentin vs 7 in the loratadine group (P = 0.025). In the loratadine group was reported good tolerance to treatment and in the gabapentin group the more important adverse effect was somnolence (8 patients) but well tolerated in 6 patients, 2 decreased the dose. No statistically significant differences were found between ages, sex, underlying cause of CKD, PTH, and laboratory parameters between groups.

**Conclusions:** The use of gabapentin for treatment of uremic pruritus, in patients on APD is better than loratadine and is a good option for use in the treatment of uremic pruritus, in APD population of patients.

*Funding:* Government Support - Non-U.S.

### SA-PO937

**Use of the Mid-Day Exchange in a Large USA Peritoneal Dialysis Cohort** Bethany Greer Costello, Sue A. Tendering, Steven Guest. *Baxter Healthcare.*

**Background:** In peritoneal dialysis (PD), a mid-day exchange (MDE) is performed to increase total daily solute clearance or prevent ultrafiltration (UF) loss during the long dwell of automated PD (APD). The potential benefits of a MDE are offset by the additional burden of requiring patients to perform an additional daytime exchange. We sought to better understand the use of a MDE in US PD therapy.

**Methods:** The data used is maintained in a custom, purpose built, data warehouse containing PD supply shipment information for US patients using Baxter PD solutions, disposables, and cycler devices. The parameters were APD patients who ordered supplies from Nov 2012-Jan 2013, and ordered CAPD supplies (48,232 individual orders by 13,489 patients) implying a MDE. Three months of data were used to smooth fluctuation in ordering due to number of days in the month and patient ordering patterns. APD patients with a three month average of 15-36 CAPD solution bags per month were included.

**Results:** 971 clinics with a total APD population of 20,161 patients were analyzed. 16% were receiving CAPD solution shipments consistent with a MDE. 16% of APD patients translated to 3,226 patients in the database performing a MDE. Average number of CAPD solutions ordered per month was 26- suggesting the patients were consistent in the use of the MDE. Discussion: This analysis has revealed that 16% of APD patients in this USA cohort received CAPD solution shipments implying use for a MDE. This estimate may be conservative as it would not capture patients using the cycler for a MDE. If the MDE is used to improve long-dwell UF, use of icodextrin could favorably impact the therapy as it is indicated for a single daily exchange for the long (8-to-16-hour) dwell for APD or CAPD.

**Conclusions:** Analysis of a large USA PD cohort has suggested 16% of APD patients are prescribed a MDE and reasons for the MDE should be clarified. A significant number of these patients would be considered candidates for a prescription change to icodextrin.

*Funding:* Pharmaceutical Company Support - Disclosure of Financial Relationships: All authors are employees of Baxter Healthcare Corporation

### SA-PO938

**Peritoneal Dialysis Prescriptions during the Third Trimester of Pregnancy: A Meta-Analysis** Steven Guest,[1] Rodolfo Batarse,[2] Ralph M. Steiger.[3] *[1]Baxter Healthcare Corporation, Deerfield, IL; [2]Nephrology, Hypertension, Transplant Medicine, Rancho Mirage, CA; [3]Desert Regional Medical Center, Palm Springs, CA.*

**Background:** Management of the pregnant patient on PD is complicated by the uterine enlargement that may restrict dwell volumes and/or create catheter dysfunction or discomfort. A variety of publications describe successful outcomes of pregnancy in the PD literature. A meta-analysis of the 3rd trimester PD prescription was performed to describe prescription options.

**Methods:** A systematic review and meta-analysis was performed of the English literature to identify ESRD patients managed by PD in which sufficient information was provided to determine the PD prescription used during the 3rd trimester of pregnancy to the end of gestation. Pregnancy outcomes were also determined.

**Results:** Twenty-five publications describing 37 pregnancies were reviewed. Twenty-nine pregnancies were included in the analysis. These were selected as they were deemed to provide sufficient clinical and prescription information. Both APD and CAPD modalities were reported. --Five of 29 pregnancies were managed with dwell volumes of 2L or greater per exchange. The remaining 83 % of pregnancies were managed by lower volume dwells with increased number of exchanges, either manually or with a cycler device. Patients hospitalized in the last trimester often received extended APD shorter dwells in lieu of a long day dwell.

**Conclusions:** Pregnant PD patients have been managed thru full term gestation and delivery. Whether managed by CAPD or APD, most reports describe reduced dwell volumes with increased number of daily exchanges with dialysate dwelling throughout the 24-hour period. APD patients often had increased number of cycles prescribed with

an extended time connected to the cycler. Empiric increases in the total daily dialysate volume to enhance the delivered solute clearance but employing reduced dwell volumes is frequently reported in this literature.

### SA-PO939

**Peritoneal Dialysis for Stage 5 Chronic Kidney Disease Patients in China: Twenty-Seven Years of Experience in a Single Centre** Huiling Wang. *Div of Nephrology, Jimin Hospital, Shanghai, China.*

**Background:** We present the demographics, patient and technique survival as well as peritonitis rates of 841 stage 5 chronic kidney disease (CKD5) patients, who started continuous ambulatory peritoneal dialysis (CAPD) between 1 January 1985 and 31 December 2011 at renal division of Shanghai Jimin Hospital in China.

**Methods:** A retrospective cohort study of individuals 15 years of age and older, divided into three cohorts according to the year they started peritoneal dialysis (PD): the first cohort (1985-1994), the second cohort (1995-2004) and the third cohort (2005-2011). Demographic and biochemical variables, clinical outcomes, patient and technique survival, peritonitis rate, and factors affecting mortality were analyzed.

**Results:** Mean age at the start of PD was 58.5±16.7years. Chronic glomerulonephritis (CGN) was the most common cause of end stage renal disease (ESRD) (52.4%); ESRD incidence as a result of diabetes ($p<0.05$) and hypertension ($p<0.05$) were significantly more frequent in the second and third as compared with the first cohort. Cardiovascular disease was the main cause of death (41.9%); the common cause of transfer to haemodialysis (HD) was peritonitis (59.2%), psychosocial causes of technique failure were reported in only 3.9%. Patient and technique survival at 1, 3 and 5 years were estimated by Kaplan-Meier for each of three cohorts. The patients in the third cohort had significantly better 5-year survival rate ($p=0.000$) and longer technique survival ($p=0.000$). The peritonitis rate reduced to an average of 0.25 episodes per year at risk in the third cohort from 1.33 episodes per year at risk in the first cohort. In the Cox proportional hazards model analysis, age,diabetic nephropathy,transfer to PD from HD,serum albumin level, serum C-reaction protein (CRP) level,Total creatinine clearance (Ccr) and dialysate-to-plasma creatinine ratio (D/P creatinine) predicted mortality.

**Conclusions:** Patient and technique survival have significantly improved, and peritonitis rate has reduced in the most recent cohort compared with earlier periods. The causes of this improvement are likely to the appointment of dedicated PD nurses, a standardized follow-up system, and a "PD first" principle since 2005.

### SA-PO940

**Physical Activity: Peritoneal Dialysis versus Hemodialysis Patients** Daniel Teta,[1] Marie Paule Guillodo,[2] Anne Kolko-Labadens,[3] Catherine Deforges-lasseur,[4] Martial Levannier,[5] Marine Panaye,[6] Denis Fouque.[7] *[1]Nephrology, Univ Hospital, Lausanne, Switzerland; [2]AUB Santé, Brest, France; [3]Hémodialyse, AURA Nord, Saint Ouen, France; [4]AURAD Aquitaine, Gradignan, France; [5]Amgen, Neuilly/Seine, France; [6]Néphrologie, Hôpital H.E.Herriot, Lyon, France; [7]Néphrologie, U1060 CHLS, Pierre Bénite, France.*

**Background:** Physical inactivity, a risk factor for mortality in maintenance dialysis (MD) patients, has been essentially studied in hemodialysis patients (HD). We aimed to assess physical activity from peritoneal dialysis (PD) patients included in the Step by Step initiative, a large cohort of MD patients.

**Methods:** All French nephrologists were invited to participate in a national prospective multicenter observational trial. 149 investigators included 1163 adult prevalent (> 3 months) MD patients. Physical activity was assessed by the number of daily steps using a pedometer (NO PE317C, USA) for 7 consecutive days. All PD patients from the cohort (n=63, 5.4%) were analyzed and compared with randomly-matched computer-selected HD patients (n=123), 1:2 ratio, by age (10-year intervals) and dialysis vintage (3-months intervals), which mostly determined daily step number in the whole cohort.

**Results:** Median age (57 yr) and dialysis vintage (1 yr) were not different in PD vs HD patients, as expected. Sex ratio (males: 60% in PD vs 68% in HD), prevalence of diabetes (30% vs 25%), ischemic heart, peripheral arterial, neurological and joint diseases were comparable. The groups only differed by the prevalence of hypertension (89 vs 63%, p: 0.0002) and median body mass index (25.9 vs 24.2 kg/m$^2$, p: 0.036). Median daily step number was comparable in PD vs HD patients (3321: Q1 1478-Q3 5926 vs 3903: Q1 2156-Q3 7346; p: 0.193). 64% PD vs 61% HD patients were sedentary (< 5000 steps/d), and 22% PD vs 16% HD patients had a low physical activity (> 5000 and < 7500 steps/d).

**Conclusions:** This study is the first to compare physical activity of PD vs matched HD patients. Despite a less immobilizing treatment, 86% of PD patients had a sedentary/low activity profile, similar to HD patients. These data confirm the need to promote physical activity in all MD patients, independent of the dialysis technique.

*Funding:* Pharmaceutical Company Support - Amgen France

### SA-PO941

**The Relationship of Follow-Up Frequency and Peritoneal Dialysis Quality** Yan-ru Chen, Hui Peng, Jun Zhang, Tan-qi Lou. *Div of Nephrology, Dept of internal Medicine, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

**Background:** To investigate the relationship offollow-up frequency and quality of peritoneal dialysis.

**Methods:** 110 continuous ambulatory peritoneal dialysis(CAPD)patients were collected for retrospective analysis from October 2011 to December 2012. All patients were divided into two groups according to different follow-up frequency: group A(every 1~2 months) or group B(every 3~6 months).The dialysis quality of the two groups was compared. The dialysis quality including blood pressure, fluid balancing, nutritional status, serum calcium and serum phosphate, iPTH, peritonitis, exit infection were observed.

**Results:** Hemoglobin in group A (106.82±16.35)g/L was significantly higher than that in group B (94.32±20.77)g/L ($P=0.003$). Systolic pressure(141.73±27.12)mmHg, serum parathyroid hormone(335.85±187.36)pg/ml (iPTH), serum phosphate(1.37±0.36)mmol/L of group A were all lower than those of group B, which were respectively(156.87±22.36) mmHg,(463.95±354.65)pg/ml and(1.72±0.58)mmol/L. Thedifference of two groups was statistically significant (respectively $P=0.027$,0.033,0.001). The compliance of patients in group A is superior to those in group B ($P = 0.035$). In group A, patients with lower educational status, medical insurance covered less than 50% and patients whose home was far from the hospital (>50 km) were significantly less than in group B (respectively $P=0.025$,0.000,0.000).

**Conclusions:** According to our results, follow-up frequency affects quality of peritoneal dialysis. Short follow-up interval is better to ameliorate blood pressure, iPTH, serum phosphorus levels, anemia or patient compliance. Follow-up Frequency may be related to educational status, medical insurance coverage and the distance to the hospital.

### SA-PO942

**Prognostic Value of Blood Pressure at Initiation of Renal Replacement Therapy in Peritoneal Dialysis** Farsad Afshinnia, Jonathan H. Segal. *Internal Medicine, Univ of Michigan.*

**Background:** Reverse epidemiology manifested as higher mortality associated with lower blood pressure is described in patients with end stage renal disease (ESRD) on hemodialysis, but is not well described in peritoneal dialysis (PD). We evaluated the effect of baseline blood pressure at initiation of PD on all-cause mortality.

**Methods:** This is a retrospective observational study in a cohort of ESRD patients from the outpatient PD clinic of the University of Michigan from January 2007 to July 2012. Inclusion criteria were 18 years of age or older and having had at least one measured weekly KT/V. Baseline characteristics including demographics, comorbidities, medications, laboratory values as well as KT/V, PD prescription, and complications at follow up. The primary outcome is all-cause mortality. Multivariable Cox-regression survival models with increasing degree of adjustment by the background variables were applied for the analysis.

**Results:** Overall 88 patients were included and contributed to 2136 patient-months. There were 47 male- (53.4%), and 66 (75.0%) white patients. Median follow up time after initiation of PD was 35 months (interquartile range: 18 to 36 months). 22 patients reached the primary end point. There was a graded decrease in mortality from 38.9% to 13.2% for systolic blood pressure (SBP) <120 mmHg to SBP ≥160 mmHg. The first multivariable model revealed a 3.6 fold (95% CI: 1.1 to 12.7) higher risk of mortality with SBP <120 mmHg compared to SBP of 140-159 mmHg after adjusting for age, sex, and race. Subsequent models with stepwise addition of baseline albumin, KT/V, normalized protein catabolic rate, and etiology of ESRD further strengthened the association of SBP <120 mmHg with higher mortality as compared to SBP of 140-159 mmHg by stepwise increase in hazard ratio.

**Conclusions:** Reverse epidemiology manifested as higher mortality associated with lower blood pressure is observed in PD. This association may be representative of poor cardiac function in those patients with lower blood pressures. Randomized controlled clinical trials are needed to identify an optimal target of blood pressure control in patients performing PD.

### SA-PO943

**Tolvaptan Increases Urine and Ultrafiltration Volume for Patients with Oliguria Undergoing Peritoneal Dialysis** Toru Iwahori,[1,2] Shinya Nagasaka,[2] Akira Shimizu.[2] *[1]Immunology & Nephrology, Koyukai Memorial Hospital, Chiba, Japan; [2]Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

**Background:** Hypoalbuminemia caused by peritoneal dialysate protein loss, frequently occurs in patients on peritoneal dialysis (PD) and is associated with an increased risk of death. We investigate whether PD dialysis exchange volume (PD volume) could be reduced with Tolvaptan (TVP) through increased urine volume (UV).

**Methods:** In 23 stable patients with oliguria undergoing, the following parameters were examined diuretic response and the effect of TVP on peritoneal ultrafiltration (UF), body weight (BW), serum albumin, sodium, arm muscle area (AMA), PD volume, Kt/V, and urine and serum osmolarity (OSM).

**Results:** The average UV increased from 428 ± 178 mL to 906 ± 285 mL ($p = 0.018$ by paired t-test). Average weekly PD volume decreased from 28,836 ± 5,699 to 23,872 ± 3,569 mL ($p = 0.04$ by paired t-test). Average UF increased from 283 ± 147 to 575 ± 135 mL ($p = 0.019$ by paired t-test). On the other hand, there was no significant difference in the average dialysate Kt/V before and after TVP treatment. Serum sodium, AMA, and serum albumin levels were not statistically different before and after TVP treatment. The urine and serum OSM (us OSM) ratio of effective cases before TVP treatment was higher than that of ineffective cases ($p = 0.024$ by unpaired t-test).

**Conclusions:** Our results indicate that TVP is useful for patients on CAPD who have oliguria and high urine osmolarity. Furthermore, we can reduce PD volume to maintain their nutritional status.

**SA-PO944**

**The Effect of Folic Acid Therapy on Anemia in Patients with End Stage Renal Disease on Peritoneal Dialysis** Subir K. Paul, Shejuti Paul, Rajesh Boorgu, Jamie N. Cockrell, Narasimha Rao Boorgu, Cassandra (cassie) A. Miller. *Shoals Kidney and Hypertension Center, Florence, AL.*

**Background:** Despite maintenance of adequate iron saturation with intravenous iron and utilization of appropriate dose of Epogen therapy, anemia management remains sub-optimal in some patients with End Stage Renal Disease (ESRD) on peritoneal dialysis. We found macrocytosis and folic acid deficiency in some patients on peritoneal dialysis. This study tests the effect of folic acid treatment on plasma hemoglobin concentration in peritoneal dialysis patients with anemia.

**Methods:** 16 patients with ESRD on peritoneal dialysis with plasma hemoglobin concentration less than 11g/dl, folic acid level of 5 ng/ml or less, and iron saturation more than 20% were treated with 3 to 5 mg of oral folic acid daily. All patients had adequate KT/V and were free of infections or known inflammatory conditions. Vitamin B12 level was found to be normal in all patients. Mean age was 47.81 years. 7 were male and 9 were female. 9 were white, and 7 were black. Serum folic acid level and plasma hemoglobin concentration were evaluated at 4 weeks prior, initiation, and 4 weeks after initiation of therapy. Maintenance therapy with intravenous iron and Epogen were continued according to standard protocol. No subject received blood transfusion. Statistical analysis was done with student's t test.

**Results:** At four weeks prior to initiation, and at initiation mean folic acid level was 3.93 +/- 0.20 ng/ml and 3.96 +/- 0.22 ng/dl respectively (p=0.89). Following therapy with folic acid, mean folic acid level increased from 3.96 +/- 0.22 ng/dl to 19.26 +/-0.53 ng/dl (P<0.0001). During pre-treatment phase, mean plasma hemoglobin concentration remained unchanged from 10.42 +/-0.25 g/dl to 10.29+/- 0.25g/dl (P=0.97). Following treatment with folic acid, mean plasma hemoglobin concentration improved from 10.29 +/-0.25 g/dl to 11.30 +/-0.22 g/dl (P=0.005). Following treatment with folic acid, mean Epogen dose decreased from 14875+/-4968 units to 10437+/- 3407 units every 2 weeks (P=0.043).

**Conclusions:** We conclude that oral folic acid therapy may be a cost-effective addition to anemia management in patients with ESRD on peritoneal dialysis.

**SA-PO945**

**Change in Body Composition Measurements in Peritoneal Dialysis Patients According to Dialysate** Seokhui Kang,[1] Tae woo Kim,[2] Kyu-hyang Cho,[1] Jong-Won Park,[1] Kyung woo Yoon,[1] Jun-Young Do.[1] *[1]Internal Medicine, Yeungnam Univ Hospital, Daegu, Republic of Korea; [2]Internal Medicine, Soonchunhyang Univ Gumi Hospital, Gumi, Republic of Korea.*

**Background:** The accurate estimation of body composition is associated with prediction of morbidity or mortality in peritoneal dialysis patients. The aim of this study is to evaluate change in body composition measurements according to dialysate.

**Methods:** A total of 41 patients on maintenance peritoneal dialysis were enrolled in the study. Body composition analysis using bioimpedance spectroscopy (BIS) and multi-frequency bioimpedance analysis (MF-BIA) were performed in all patients, first with an empty abdomen (D-) and then with a full abdomen (D+, 2 liter). Differences in body compositions with D- and D+ were investigated. Biases were calculated as follows: measurement in D- – measurement in D+.

**Results:** In BIS, there were significant differences in fat mass and extracellular water between D- and D+. In MF-BIA, there were significant differences in free fat mass and extracellular/intracellular water between D- and D+. Bland-Altman analysis was performed. The mean biases in fat mass were 1.3 for BIS and -0.3 for MF-BIA. The mean bias in lean mass was 1.3 for BIS. That in free fat mass was -1.8 for MF-BIA. BIS underestimated the fat mass in D+. MF-BIA underestimated the lean mass in D+ and BIS overestimated the free fat mass in D+.

**Conclusions:** This study demonstrates that MF-BIA may be useful to evaluate fat mass, regardless of dialysate. Lean mass or free fat mass can be reliably done without dialysate.

**SA-PO946**

**Urinary Tract Infections in Kidney Transplant Recipients: Incidence and Risk Factors in a Contemporary Cohort** Joseph Kim, Stacey V. Konidis, Olusegun Famure, Yanhong Li, Shahid Husain. *Multi-Organ Transplant Program, Toronto General Hospital, Univ Health Network, Toronto, Canada.*

**Background:** Urinary tract infections (UTI) are a common cause of morbidity in kidney transplant recipients. This study aimed to evaluate the incidence, risk factors, causative organisms, and burden of urinary tract infections following kidney transplantation.

**Methods:** A retrospective cohort study of 907 kidney transplant recipients in Toronto, Canada from January 1, 2000 to December 31, 2010 was conducted. Time-to-first UTI was assessed using the Kaplan-Meier product limit method. Potential risk factors for the development of UTIs post-transplant were assessed using Cox proportional hazards regression models.

**Results:** Overall, there were 264 patients who had at least one UTI over a median of 3.0 years of follow-up, yielding an incidence rate of 7.5 (95% CI: 6.7 to 8.5) per 100 person-years. The cumulative incidence of UTI was 21.3% for male patients and 69.8% for female patients at 10-years post-transplant. The Cox proportional hazards model revealed that advanced age, female sex, and the presence of a structural urologic diagnosis were independently predictive of UTI post-transplant (hazard ratios 1.1 [95% CI: 1.0, 1.3], 4.8 [95% CI: 3.7, 6.2], and 1.5 [95% CI: 1.1, 2.0], respectively). Interestingly, ESRD due to polycystic kidney disease was associated with a reduction in the adjusted relative hazard of first UTI (hazard ratio 0.6 [95% CI: 0.4, 0.9]). Gram-negative bacteria were more common than gram-positive bacteria as the causative organisms for UTI events.

**Conclusions:** This observational cohort study found that kidney transplant recipients experience a significant burden of UTIs post-transplant. There are identifiable risk factors in this patient population that may help target preventive strategies to reduce the burden of this complication after kidney transplantation.

**SA-PO947**

**The Evolving Risk Factor Profile of New-Onset Diabetes Mellitus after Kidney Transplantation** Joseph Kim, Laura Rivera, Roman Zyla, Yanhong Li, Olusegun Famure. *Multi-Organ Transplant Program, Toronto General Hospital, Univ Health Network, Toronto, Canada.*

**Background:** New-onset diabetes after transplant (NODAT) is a common complication in kidney transplant recipients (KTR). The incidence of NODAT in KTR is high in the immediate post-transplant period and falls with time until it approaches that of diabetes in the general population, suggesting potential differences in the mechanism of diabetogenesis. An understanding of different risk factors for early- and late-onset NODAT may increase opportunities for prevention and/or early treatment.

**Methods:** The study population is composed of all individuals over the age of 18 who received a first kidney transplant between 1 Jan 2000 and 31 Dec 2011 at the Toronto General Hospital and were free of diabetes mellitus at the time of transplant. Patients were considered to have early-onset NODAT if they developed diabetes between day 14 and day 180 post-transplant inclusive, and late-onset NODAT if they developed diabetes after 180 days. A multivariable Cox proportional hazards model was used to identify risk factors for early- and late-onset NODAT.

**Results:** The study cohort was composed of 840 kidney transplant recipients, with a median follow-up of 3.53 years.Overall, 89 patients in the cohort developed early-onset diabetes, and 112 developed late-onset NODAT. Risk factors for early-onset NODAT were recipient age (HR 1.05 [95% CI: 1.03, 1.07]), deceased donor kidney (HR 2.04 [95% CI: 1.32, 3.13]) and expanded criteria donor kidney (HR 1.92 [95% CI: 1.15, 3.18]). Risk factors for late-onset NODAT were recipient age (HR 1.04 [95% CI: 1.03, 1.06]), non-white donor race (HR 2.04 [95% CI: 1.12, 3.85]), HLA mismatches (HR 1.21 [95% CI: 1.06, 1.39]), positive CMV serostatus (HR 1.69 [95% CI: 1.16, 2.50]), and deceased donor kidney (HR 1.69 [95% CI: 1.16, 2.50]).

**Conclusions:** The differences in risk factors for early- and late-onset NODAT suggests that they are likely the product of both shared and distinct mechanisms. Based on these results, the targets for diabetes risk reduction may partially depend on the timing of NODAT occurrence.

**SA-PO948**

**Transplant Renal Artery Stenosis in Kidney Transplant Recipients: Incidence, Risk Factors, and Outcomes** Joseph Kim, Ching Lucy Chau, Yanhong Li, Olusegun Famure. *Multi-Organ Transplant Program, Toronto General Hospital, Univ Health Network, Toronto, Canada.*

**Background:** Transplant renal artery stenosis (TRAS) has been implicated in poor allograft function and survival. As such, the prevention and early detection of TRAS may prove to benefit patient outcomes. This study aims to define the incidence, risk factors, and outcomes of kidney transplant recipients with TRAS in a large Canadian patient cohort.

**Methods:** This is a retrospective cohort study of 1224 kidney transplant recipients from 1 Jan 2000 and 31 Dec 2010 (followed until 31 Dec 2011). TRAS was confirmed through Doppler ultrasound indices (peak systolic velocity > 200 cm/second at mid-artery). Multivariable Cox proportional hazards models were fitted to examine the association between various risk factors at the time of transplant and the occurrence of TRAS over follow-up. In addition, the impact of TRAS as a time-varying exposure on the risk of total graft failure (i.e., return to dialysis or death with graft function) was also studied.

**Results:** A total of 106 incident cases of TRAS were observed over a median follow-up of 3.24 years. The incidence rate of TRAS was 0.46 cases per 100 person-years. The relative hazard for TRAS was significantly associated with living donor transplant [HR 2.69 (95% CI: 1.59, 4.56)] and male sex [HR 1.80 (95% CI: 1.16, 2.78)]. Our analysis showed an era effect with a lower relative hazard of TRAS in the most recent eras [HR 0.62 (95% CI: 0.41, 0.95) for 2005-2007 vs. 2000-2004 and HR 0.29 (95% CI: 0.15, 0.58) for 2008-2011 vs. 2000-2004]. TRAS was associated with an increased risk for total graft failure [HR 1.41 (95% CI: 0.58, 3.44)], although this failed to reach statistical significance.

**Conclusions:** Our results suggest that male sex and living donor recipients are at an increased risk for TRAS. A decrease in the risk of TRAS was also observed with the most recent transplant era. Further study is required to confirm and explore these associations in other kidney transplant populations.

**SA-PO949**

**Infection-Related Mortality Is Higher for Kidney Allograft Recipients with Diabetes Mellitus – A Population Based Cohort Study** Manvir Kaur Hayer, Daniela Farrugia, Adnan Sharif. *Renal Institute of Birmingham, Queen Elizabeth Hospital, Birmingham, United Kingdom.*

**Background:** Strong epidemiological evidence linking diabetes with risk for serious infections is lacking despite sufficient experimental evidence. The risk for infection-related mortality in kidney allograft recipients with diabetes, on immunosuppression, is unknown. This study investigates whether diabetes mellitus predicts mortality from infection post kidney transplantation.

**Methods:** We identified all kidney alone transplants done in England from April 2001 to March 2012 through a national database called Hospital Enquiry Statistics. Patient demographics were collected, and all deaths in the study group were identified via data linkage analysis with the Office for National Statistics. The primary outcome was death from infection post transplantation in diabetics versus non-diabetics. Cox proportional hazard models identified independent factors associated with infection-related mortality ($p<0.05$ was significant).

**Results:** 19,103 kidney allograft recipients were analysed, of whom 2,968 had diabetes at the time of transplantation. Diabetic allograft recipients were often older, male, non-white, of deprived socio-economic areas, received deceased-donor kidneys and had co-morbidities. 2,085 deaths occurred post transplantation; 433 were due to infection. Table 1 compares the risk of death in diabetics and non-diabetics.

| Risk for death (p<0.010) | % Diabetic | % Non-diabetic |
|---|---|---|
| Post kidney transplant | 16 | 10 |
| 1 year post kidney transplant | 4.9 | 2.6 |
| Due to infection post transplant | 3.3 | 2.1 |

Increasing age, deceased-donor kidneys, area socioeconomic deprivation, peripheral vascular disease and diabetes were independently associated with death from infection.

**Conclusions:** Infection-related mortality is common post transplantation especially in kidney allograft recipients with pre-existing diabetes mellitus. Diabetes is a risk factor for infection-related death post kidney transplantation independent of confounders. Further work is needed to decide whether personalised immunosuppression is beneficial for diabetic kidney allograft recipients.

*Funding:* Private Foundation Support

## SA-PO950

### Distinct Localization of HCMV-Encoded Chemokine Receptor US28 and Immediate Early Antigen after Renal Transplantation Suggests Microenvironment-Specific Expression

Wouter Lollinga,[1] Afsar Rahbar,[2] Martine J. Smit,[3] Annelies Riezebos-brilman,[4] Cecilia Söderberg-naucler,[2] Willem Van Son,[1] Johannes S. Sanders,[1] Jacob van den Born.[1] *[1]Nephrology, UMCG, Groningen, Netherlands; [2]Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Medicinal Chemistry, VU Univ, Amsterdam, Netherlands; [4]Medical Microbiology, UMCG, Groningen, Netherlands.*

**Background:** Human cytomegalovirus (HCMV) is associated with decreased renal graft function and survival, possibly through expression of chemokine receptor US28. US28 enables HCMV to benefit from the host cellular machinery and modify intracellular signaling. We hypothesized US28 to be expressed in vascular, inflammatory and tubular epithelial cells, linking HCMV infection to transplant dysfunction.

**Methods:** Recipient renal transplant biopsies (n=50) from HCMV-seropositive donors were obtained pre-transplantation, on indication or by protocol one year post-transplantation. Immunohistochemical staining was performed for US28 and immediate early antigen (IEA). Localization of both HCMV antigens was semi-quantitatively scored in renal compartments and analyzed by Spearman rank correlation.

**Results:** Both HCMV antigens were detected during recipient active and latent HCMV infections, with IEA exclusively localized in the nucleus and US28 mainly in the cytoplasm. Localization can overlap in renal compartments, but is regularly distinct. IEA displays widespread distribution but prevails in vascular endothelium and inflammatory cells. US28 distribution is more restricted and most prevalent in vascular smooth muscle and tubular epithelial cells ($r=0.557$, $p<0.001$). Strikingly, IEA and US28 expression within a single compartment didn't correlate.

**Conclusions:** We are the first to demonstrate US28 in renal transplant biopsies, and its specific localization. More pronounced than for IEA, the distinct localization of US28 in vascular smooth muscle and tubular epithelium suggests microenvironment-specific expression. Since these compartments frequently display histopathology, HCMV could instigate (chronic) transplant dysfunction through US28. The effect of US28 on transplant histopathology and recipient clinical outcome parameters are currently under investigation.

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## SA-PO951

### Long-Term Impact of CMV Infection on the Allograft and on Patient Survival in Renal Transplant Patients with Protocol Biopsies

Uta Erdbruegger,[1] Irina Scheffner,[2] Michael Mengel,[3] Anke Schwarz,[2] Hermann G. Haller,[2] Wilfried Gwinner.[2] *[1]Univ of Virginia; [2]Hannover Medical School, Germany; [3]Univ of Alberta, Canada.*

**Background:** The impact of CMV infection on allograft and patient survival is controversial.

**Methods:** We studied 594 patients with a protocol biopsy (pBx) at 6 weeks, 3 & 6 months post transplant (Tx). Chronic allograft changes were graded by the Banff classification (cGrade, cv, cg lesions). CMV infection was diagnosed by CMV antigenemia test. Follow up data was available up to 10 years.

**Results:** CMV infection was diagnosed in 153 of 594 patients (26%) in the first year after Tx, mostly within the first 3 months. In patients with CMV infection between the pBx at 6 weeks and 6 months, 11.5% of the biopsies had a cGrade>0 at 6 weeks compared to 3.5% in patients without CMV ($p=0.005$). At 6 months, the prevalence of a cGrade>0 rose to 40.6% in patients with CMV and to 34.2% in patients without CMV ($p=0.24$). In patients with CMV infection within the first 6 weeks post-Tx, 13% of the biopsies had a cGrade>0 at 6 weeks compared to 3.5% in patients without CMV ($p=0.06$). At 6 months, the prevalence of a cGrade>0 rose to 55% in patients with CMV and 34% in the group without CMV ($p=0.09$). Chronic vascular and glomerular changes were rarely seen and were not different. The annual GFR loss was greater in patients with CMV infection (median: 3.6 vs 2.4ml/min per year, $p=0.017$). Allograft survival was reduced in patients with CMV ($p=0.03$) as well as the combined allograft/patient survival ($p=0.008$). Clinical and laboratory factors that differed between patients with and without CMV were recipient and donor age, pre-Tx coronary heart disease, CMV donor/recipient IgG serostatus, and initial graft function. In multivariate analyses, higher donor age, lower initial graft function, and coronary heart disease were significant for graft loss whereas patient age and initial graft function were significant for death. In none of these analyses, CMV viremia or disease was a significant factor.

**Conclusions:** Patients with CMV post-Tx show more chronic allograft changes early-on, even before CMV infection. Thus, allograft loss and death is apparently not related to CMV.

## SA-PO952

### Effect of HIV and HIV/ HCV Coinfection in Kidney Transplant Recipients

Anjali Gupta, Graciela De Boccardo, Enver Akalin, Liise K. Kayler. *Montefiore Medical Center/Albert Einstein College of Medicine, NY.*

**Background:** Reports of kidney transplantation in human immunodeficiency virus seropositive (HIV+) recipients with or without hepatitis C (HCV+) are scarce and lack long-term outcomes.

**Methods:** We evaluated paired deceased-donor kidneys (derived from the same donor transplanted to different recipients) in which one kidney was transplanted into an HIV+ (n=207) or HIV+/HCV+ (n=43) patient and the other transplanted to a recipient without HIV or HCV (HIV-;n=250) using Scientific Registry of Transplant Recipients (SRTR) data between 2000 and 2010.

**Results:** While univariable analysis revealed death-censored graft survival (DCGS) was significantly worse in HIV+ patients compared to HIV- patients ($p=0.0331$), on multivariable analysis (adjusted for recipient risk factors), there was no significant difference (aHR 0.34, 95% CI 0.03, 3.91). There was no difference in patient survival by univariable ($p=0.7329$) or multivariable analysis (aHR 0.80, 95% CI 0.29, 2.18] between the two groups. HCV co-infection was a significant independent risk factor for DCGS (aHR 2.51; 95%CI 1.23, 5.14) and patient survival (aHR 3.03; 95%CI 1.31, 6.98). Acute rejection at one year occurred in 9.2%, 15.9%, and 16.3% of the patients in the HIV-/HCV-, HIV+/HCV-, and HIV+/HCV+ groups, respectively ($p=0.0724$). Acute rejection was significantly associated with African-American race (aOR 2.26, 95% CI 1.17-2.38) and HLA mismatch > 3 (aOR 2.18, 95%CI 1.01-4.66); however, neither HIV+(aOR 1.36, 95%CI 0.72-2.58) nor HCV coinfection (aOR 1.02, 95%CI 0.39-2.62) were risk factors for acute rejection.

**Conclusions:** Where as kidney transplantation in HIV+ recipients is associated with similar long term outcomes relative to non-infected recipients, HCV co-infection confers poor patient and graft survival. The higher rates of acute rejection amongst HIV+ kidney transplant recipients relative to HIV- recipients is at least partially due to the predominance of African-Americans in the HIV+ cohorts.

## SA-PO953

### The Impact of Hepatitis C Virus Infection on the Incidence of Post-Transplant Lymphoproliferative Disorder among Epstein-Barr Virus and Cytomegalovirus Seropositive Kidney Transplant Recipients: An Analysis of UNOS/OPTN Data

Hoang Anh Nguyen,[1] P.C. Pham,[2] P.T.T. Pham.[3] *[1]UCI Dept of Internal Medicine; [2]UCLA-Olive View Dept of Medicine, Nephrology Div; [3]UCLA Dept of Medicine, Nephrology Div & Kidney Transplant Program.*

**Background:** Post-transplant lymphoproliferative disorder (PTLD) is a serious complication after organ transplantation. Hepatitis C virus (HCV) infection is a well-established risk factor for the lymphoproliferative syndrome type II mixed cryoglobulinemia. In this study, we hypothesize that pre-transplant HCV infection increases the risk of PTLD among kidney transplant recipients who have Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) seropositivity.

**Methods:** Adult kidney transplant recipients (≥ 21 years old), of a deceased or living donor kidney transplant in the U.S., with PTLD diagnosis between 1999 and 2011 from the UNOS/OPTN data, were included the analysis using SAS. The difference of distributions of categorical variables between groups was investigated using chi-squared tests. Effect modifications of HCV antibody status on PTLD risk by selected demographic characteristics (age, sex, ethnicity) and clinical factors (CMV serology, EBV serology, pre-transplant malignancy, HLA mismatch, acute rejection episodes, use of medications for induction and maintenance of immunosuppression) were evaluated under the multiplicative model in the Cox regression models.

**Results:** There were a total of 923 PTLD cases (incidence, 1.78 patients per 1000 person-years) among 169,196 primary kidney transplant recipients between 1999 and 2011 (accrued 517,739.76 person-years at risk during the follow-up period). HCV prevalence at transplantation was 5.58% (N = 9,445). HCV infection did not increase PTLD risk in the total cohort even after adjusting for demographic and clinical risk factors. No significant effect modifications were found for HCV infection on the incidence of PTLD among EBV and CMV seropositive kidney transplant recipients.

**Conclusions:** Our findings suggest that HCV is not a major risk factor for PTLD among kidney transplant recipients, which is consistent with previous studies among other solid organ transplant recipients.

**SA-PO954**

**Prevalence and Risk Factor of Epstein-Barr Virus Viremia in Kidney Transplant Patients** Keun Suk Yang, Byung Ha Chung, Bum Soon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea.*

**Background:** Epstein-Barr Virus (EBV) infection is an important risk factor for post-transplant lymphoproliferative disease and increase in viral load of EBV also associated with the development of chronic allograft injury. In this study, we evaluated the prevalence and risk factors for the development of EBV viremia in renal transplant recipients.

**Methods:** One hundred ninety three patients were included in this study. The follow-up period from KT was 82.26±68.09 months and the mean age was 46.88±11.19 years. Plasma EBV loads were measured by real-time quantitative polymerase chain reaction and EBV Viremia was defined as having at least 500 copies EBV/ml.

**Results:** EBV viremia developed in 35.23% (68/193) of study population. Donors for the majority of patients in this study were living (74.1%). The patients with proteinuria showed significant difference in both groups ($p<0.01$). EBV viremia were more frequent in the patients treated with cyclosporine than with tacrolimus ($p<0.01$). Glomerular filatration rate was lower in EV group (53.74±21.39 ml/min/BSA) than NV group (60.54±17.58 ml/min/BSA) ($p<0.05$) and immunosuppression period was longer in EV group (124.92±83.63 months) than NV group (78.33±73.45 months) ($p<0.01$). No significant difference was shown between EV group and NV group on age at transplant, pre-transplant dialysis type, donor type, rejection history, ABO incompatible transplantation proportion.

**Conclusions:** The prevalence of [/bold]EBV viremia in stable renal transplant recipients was 35.23 % and follow up period and the type of immunosuppressant may be associated with the development of viremia.

**SA-PO955**

**BANFF Scores in Polyomavirus Nephropathy: How Do They Change over Time?** Harsharan Kaur Singh,[1] Rachel Cianciolo,[1] Carlos Jimenez,[1] A. Gasim,[1] Randal K. Detwiler,[2] Volker Nickeleit.[1] *[1]Div of Nephropathology, Univ of North Carolina, Chapel Hill, NC; [2]Nephrology and Hypertension, Univ of North Carolina, Chapel Hill, NC.*

**Background:** The morphology of Polyomavirus Nephropathy (PVN) resolution has not been systematically studied. It is unclear whether PVN contributes significantly to the development of chronic allograft injury over time.

**Methods:** Paired renal biopsies for cause from 14 patients (total biopsies n=28) were evaluated at time of initial PVN diagnosis and at time of PVN clearance following standard protocols (weeks between biopsies:mean=128; range=3.4-392). At time of index biopsy,9 cases were PVN stage A (miminal virally induced injury) and 5 were stage B (florid virally induced injury). Biopsies were scored using BANFF criteria. Renal function was assessed by serum creatinine titers (SCr). PVN clearance was defined as no staining for the SV40 T antigen. Statistical analysis: Wicoxon rank-sum test.

**Results:** No significant differences were seen in index and clearance biopsies for Banff acute lesion scores; all cases C4d negative. In clearance biopsies, overall inflammation decreased with new onset rejection induced changes (g,v,ptc); 15% C4d positive. Comparative analysis of chronic Banff lesion scoring showed increased chronic changes in clearance biopsies due to rejection (cg), and hypertension and/or calcineurin inhibitor toxicity (ci, ah, cv, ct) (figure 1B). PVN likely only contributed as a minor factor to chronic injury. Renal function-index biopsy: SCr(mg/dL) mean=2.79, range 0.43-11.3; clearance biopsy:SCr mean=1.98, range=0.59-4.4.

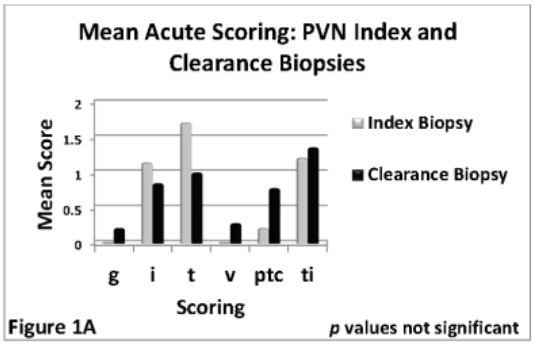



Figure 1A *p* values not significant

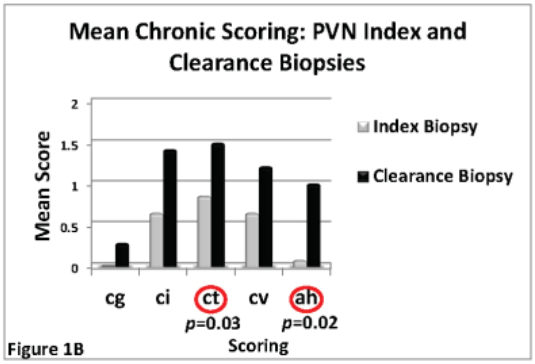



Figure 1B

**Conclusions:** In healed PVN stages A & B, renal function improved compared to time of PVN diagnosis. PVN does not necessarily contribute significantly to acute or chronic graft injury during disease resolution.

**SA-PO956**

**Bk-Virus and the Impact of Transient versus Persistent BK Viremia on Graft Function and Survival after Kidney and Kidney/Pancreas Transplantation – 5-Year Results** Nissreen S. Elfadawy,[1] Stuart M. Flechner,[1] Jesse D. Schold,[2] Titte Srinivas,[3] Emilio D. Poggio.[1] *[1]Glickman Urological and Kidney Institute, The Cleveland Clinic; [2]Dept of Quantitative Health Sciences, The Cleveland Clinic, Cleveland, OH; [3]Div of Nephrology, Medical Univ of South Carolina, Charleston, SC.*

**Background:** BK virus (BKV) reactivation is a serious complication after kidney transplantation. Currently there are limited data regarding long-term impact of transient BK viremia.

**Methods:** 609 patients who had kidney or kidney/pancreas transplant from 2007 to 2011 were screened for BKV infection. 130 patients (21.7%) developed positive BKV infection in blood during the first year post transplant. BKV positive patients were classified according to duration of infection from onset to clearance - if any (≥or < 3 months), and BK peak viral loads (≥ or < 10000 copies/mL) into: transient low viremia(n=42), transient high viremia (n=18), persistent low viremia (n=23), and persistent high viremia (n=47). Patients were followed up for a median of 36 months (3-66). BKV associated nephropathy (BKVAN), acute rejection, and the 1-year graft function were assessed and compared with the negative group.

**Results:** Patients and graft survival were comparable among the groups ($p>0.05$). Transient high viremia and persistent high viremia groups showed higher incidence of acute graft rejection (50% and 34% respectively vs 21.5%, p=0.01), and worse 1-year post transplant graft function (eGFR 42.1 and 44.9 vs 53.1, p= 0.01, 0.005 respectively). Tacrolimus and MMF blood levels were not significantly lower after diagnosis of transient viremia compared to the negative group (p= 0.3, 0.1).The 7 patients developed BKVAN were exclusively limited to the persistent high viremia group ($p<0.0001$). No graft loss occurred related to BKV.

**Conclusions:** Low BKV infection does not have a negative impact on graft outcomes. Although transient high viremia was not associated with BKVAN, poor graft function and high incidence of graft rejection in this patient group may suggest a new mechanism for the negative impact of BKV. In general, BK viremia and BKVAN are associated with excellent patient and graft survival in the era of BKV screening.

## SA-PO957

**The Genomics of BK Viremia and Nephropathy in Kidney Transplant Recipients Reveal a Heightened T Cell and Natural Killer Cell Response**

Michelle L. Lubetzky, Yi Bao, Maria Ajaimy, Graciela De Boccardo, Enver Akalin. *Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.*

**Background:** The genomics of polyomavirus BK viremia and nephropathy (BKVN) have not been well described. We aimed to study gene expression profiles of BK viremia and BKVN samples using microarrays.

**Methods:** 17 biopsy and 40 whole blood PAXgene samples from patients enrolled in our IRB approved "Immune Monitoring Study" were used for analysis. Biopsy samples were divided into 3 groups, Group 1: Normal biopsy,n=11, Group 2: BKVN, n=3, and Group 3: BK viremia with normal biopsy,n=3. Whole blood PAXGene samples were divided into 3 groups, Group 1: Stable renal function, n=14, Group 2: BK viremia, n=19, and Group 3: samples taken 1-2 months prior to BK viremia, n=7. All samples were analyzed using Affymetrix Human Gene 1.0 ST Arrays.

**Results:** When comparing BKVN biopsy samples to Group 1 and 3, there were 1442 and 1325 differentially expressed genes, respectively (FDR<0.05 and fold change >2). There was no significant difference between Group 1 and 3. Pathogenesis based transcript (PBT) analysis revealed a significant increased expression of interferon-gamma and rejection induced (GRIT), cytotoxic T-cell (CAT), constitutive and alternate macrophage, B cell and natural killer cell-associated transcripts (NKAT), indicating an active inflammatory immune response in BKVN biopsies. The top 50 differentially expressed genes were involved in immune activation such as toll-like receptor 10, CD40 ligand, B and T lymphocyte associated, CD4, and MHC-Class II DO Beta. Gene ontology analysis revealed pathways involved in immune activation, T cell activation and costimulation, B cell activation, and defense response to virus. While there were no significant differentially expressed genes in whole blood gene expression profiles of Group 1 and 3, the PBTs of BK viremia revealed significant increased expression of GRIT, QCAT and NKAT when compared to Groups 1 and 3.

**Conclusions:** Our results showed increased activity of cytotoxic T cells and natural killer cells in BKVN and viremia samples resembling acute rejection. Whether such response is to alloantigens or virus requires further studies.

## SA-PO958

**BK Nephropathy: A Paired Kidney Analysis from a Single Center**

Marilda Mazzali, André Ba Esteves, Luiz R.S. Ulisses, Leonardo F. Camargo, Gabriel G. Rivelli. *Renal Transplant Program–Div of Nephrology, School of Medical Sciences- State Univ of Campinas–UNICAMP, Campinas, Brazil.*

**Background:** Polyomavirus nephropathy (PVAN) has a negative impact on graft survival, and is related to donor and recipient risk factors. In order to identify recipient related factors, we analyzed a series of renal transplants from deceased donor, where one recipient developed PVAN and compared to their controls, recipients from a paired kidney, with persistent negative BKV viruria.

**Methods:** From the Transplant Unit database, 16 patients with biopsy proven PVAN, recipients from a deceased donor kidney were identified. For analysis, we considered 12 pairs of kidneys, one with biopsy proven PVAN (PVANs) and the paired kidney (Controls) with persistent negative BKV viruria. Two pairs, were both kidneys developed PVAN were excluded. All patients were screened for BK viruria every 3 months during the first 3 years post transplant.

**Results:** PVANs and Controls were comparable for age at transplant, primary renal disease, % PRA, induction therapy, and baseline immunosuppression, with a higher prevalence of male recipients in PVANs (91.6% *vs.* 66.6%, p<0.05). Despite a trend to a lower cold ischemia time (p=0.08) in PVANs, the incidence of delayed graft function and CMV infection were similar to Controls. However, incidence of acute rejection and severity of Banff grade were higher in PVANs (41% *vs.* 8%, p<0.05). Positive BKV viruria was observed 14±9 months post transplant, and biopsy diagnosis of PVAN was done 24±11 months post transplant. While serum creatinine in controls remained stable (1.2±0.3 mg/dL), renal function deteriorated over time in PVANs (1.8±0.8 mg/dL at viruria and 2.6±0.6mg/dL at biopsy, p<0.05 *vs.* controls). The mean follow up after diagnosis was 34.2 months for PVANs (Controls, 41 m, p=ns), with a significantly lower graft survival, starting one year after diagnosis (5 year graft survival 50% PVANs *vs.* 83% Controls, p<0.05).

**Conclusions:** In this series, PVAN was associated with male gender, number and severity of acute rejection. After a 5 year follow up, 50% of the grafts in PVAN group remained functioning, with a mean serum creatinine of 3.3 ± 1.6 mg%.

## SA-PO959

**Evolution of BK Viremia, Intragraft Inflammation and Tubulointerstitial Fibrosis following BKVN Diagnosis**

Jun B. Lee,[1] Surya V. Seshan,[3] John R. Lee,[2] Steven Salvatore,[3] Thangamani Muthukumar,[2] David Serur,[1] Manikkam Suthanthiran,[2] Darshana Dadhania.[2] *[1]Nephrology, Rogosin Institute, New York, NY; [2]Nephrology, Weill Cornell Medical College, New York, NY; [3]Pathology, Weill Cornell Medical College, New York, NY.*

**Background:** BKV Nephropathy (BKVN) is associated with greater than 50% risk of graft loss and earlier studies have mostly evaluated graft function and viral load following BKVN diagnosis, and the evolution of histological features following BKVN diagnosis has not been well-characterized.

**Methods:** We performed a systematic review of index and surveillance renal allograft biopsy findings in individuals with biopsy proven BKVN. We evaluated the change in the histology scores associated with inflammation and fibrosis following a decrease in BK viral load.

**Results:** We studied 22 patients with BKVN; 12 with BKVN stage A, 7 with stage B, and 3 with stage C. The surveillance biopsies were performed at mean of 9 months following BKVN diagnosis. Histological scores, renal function, and BK viral load at the time of BKVN diagnosis and at surveillance biopsy are summarized in the Table.

Table 1: Laboratory Data and Histology Scores

| Variable (mean+SD) | BKVN Biopsy | Follow-up Biopsy | P Value* |
|---|---|---|---|
| Creatinine (mg/dL) | 2.04+0.57 | 2.10+0.89 | 0.48 |
| Serum BKV PCR (copies/ml) | 1,299,158+2,535,182 | 1663+2650 | 0.03 |
| Post Transplant Months | 15.5+12.7 | 24.5+12.9 | |
| **Tubulitis Score** | | | |
| Stage A (N=12) | 0.58+0.90 | 1.00+0.95 | 0.34 |
| Stage B/C (N=10) | 1.50+1.18 | 0.40+0.52 | 0.03 |
| **Interstitial Inflammation** | | | |
| Stage A (N=12) | 1.17+0.72 | 1.42+0.90 | 0.52 |
| Stage B/C (N=10) | 2.00+0.82 | 0.90+0.57 | 0.003 |
| **Tubular Atrophy Score** | | | |
| Stage A (N=12) | 0.58+0.67 | 1.42+0.67 | 0.02 |
| Stage B/C (N=10) | 1.30+0.68 | 1.20+0.63 | 0.67 |
| **Interstitial Fibrosis Score** | | | |
| Stage A (N=12) | 0.50+0.52 | 1.08+0.90 | 0.09 |
| Stage B/C (N=10) | 1.20+0.63 | 1.20+0.63 | 1.0 |

* P Value calculated using paired T-test

**Conclusions:** The striking decrease in BK viral load was associated with a significant decrease in intragraft inflammation but tubulointerstitial fibrosis either increased (in BKVN stage A) or remained the same (in BKVN Stage B/C). Our new findings, in addition to providing a mechanistic basis for the poor long-term outcome in BKVN patients, suggest the hypothesis that anti-fibrosis therapy ( e.g., anti-TGF-beta antibody) may retard the progression of BKVN.

## SA-PO960

**Utility of ImmuKnow Assay in BK Infection after Kidney Transplantation**

Nashat Burhan Imran, Phuong M. Truong, Venkataraman Ramanathan. *Baylor College of Medicine.*

**Background:** By quantifying cellular ATP production after mitogenic stimulation, ImmuKnow assay values(IKAV) can provide an estimate of immune function. We studied the utility of this assay in patients with and without BK viremia (BKV) after kidney transplantation.

**Methods:** In this retrospective case-control study, IKAV and BK PCR was obtained in kidney transplant recipients at 1 month post-transplantation and then quarterly for a year. BKV was subdivided into groups based on PCR values: A<$10^3$, B:$10^3$-$10^4$, C:$10^4$-$10^5$ and group D>$10^5$. Delta IKAV was defined as IKAV on the date that BK was first ≥$10^3$ minus immediate previous IKAV. General linear mixed model was used to compare IKAV between different BK infection groups over time. Paired t-test was used to test the null hypothesis (i.e. no significant change in delta IKAV in incident BKV patients).

**Results:** We had 79 BKV cases and 99 of selected controls. Time-group interaction term was significant (P<0.0001) indicating that differences between groups depend on time. In BK cases, IKAV were significantly lower at months 3, 6, 9 and 12 months when compared to first month IKAV and BK PCR Groups appear have an inverse relationship. This appears to be consistent across time points; however, the association appears to dissipate by month 12. IKAV for group D were significantly lower than Group A (P=0.056) and Group B (P=0.015). Using paired t-test, we found that incident BKV was associated with a significant decrease in delta IKAV from month 1 to 3 (P<0.0001). On an average, there is a 367 point decrease in IKAV (SD=346, 95% CI: 269,486). Beyond 3 months, delta IKAV was not associated with incident BKV (P≥0.07).

| Effect | Time 1 (months) | Time 2 | Mean Difference (*Step-down Bonferroni P<0.0001)* |
|---|---|---|---|
| Time*Infection | 1 | 3 | -308 |
| | 1 | 6 | -315 |
| | 1 | 9 | -249 |
| | 1 | 12 | -273 |

**Conclusions:** Compared to controls, kidney transplant recipients who develop BKV have significantly lower IKAV after 3 months when compared to first month. Also, incident BKV is associated with a significant decrease in delta IKAV only in the first 3 months. Beyond that period, delta IKAV did not correlate with incident BKV.

## SA-PO961

**BK Viremia in Kidney Transplant Recipients**

Gopal Basu, Margabandhu Saravanan, Anjali Mohapatra, Anna T. Valson, Vinoi George David, Suceena Alexander, Shibu Jacob, Santosh Varughese, Chakko Korula Jacob, Veerasamy Tamilarasi. *Nephrology, Christian Medical College, Vellore, India.*

**Background:** BK virus associated nephropathy is a threat to kidney transplant (KT) graft survival.BK viremia precedes BKV nephropathy. We aimed to study the risk factors and outcome BK viremia in KT recipients with graft dysfunction.

**Methods:** KT Recipients who underwent transplantation at Christian Medical College, Vellore from January 2006 to December 2011 were included. Patients underwent BK viremia screening for evaluation of graft dysfunction [by Qualitative BK PCR (VP1 region Primers, Qiagen) with limit of detecting 100 genome copies/ml] and tissue histopathology. The risk factors, and outcomes were analysed using standard statistical methods.

**Results:** Of the 458 KT recipients (mean age 35.1±11.7 years; M:F=3.2:1) followed up for 23±14 months on predominantly prednisolone, tacrolimus and mycophenolate based regimen, BK PCR was tested in 196 individuals either for evaluation of graft dysfunction [Evaluation group] (n=165) or as routine screening [Screening group] (n=32). Among evaluation group, 33 patients had BK viremia at 18.0±13.7 months post transplant. Acute rejections [p=0.07] was a significant risk factor for BK viremia and use of basiliximab [p=0.032] had a protective effect. CMV disease [p=0.067] was associated with BK viremia. The decline in GFR with BK viremia was significantly higher than in non viremic patients (-1.48±1.46 ml/min/1.73m$^2$/month vs. -0.44±0.91 ml/min/1.73m$^2$/month [p=0.000]). All patients with BK viremia were treated with immunosuppression reduction and Leflunomide. With therapy, GFR stabilized and 15 patients became BK PCR negative after 6 months. Among patietns with graft dysfunction, BK viremia resulted in significantly poor graft survival and patient survival.

**Conclusions:** Among KTRs with graft dysfunction, BK viremia occurred in 20% of patients and was associated with acute rejections and CMV disease. Basiliximab use had a protective effect. Graft dysfunction patients with BK viremia had rapid decline in GFR, graft loss and mortality. Immunosuppression reduction and leflunomide cleared BK viremia or stabilized GFR in a considerable number of patients.

## SA-PO962

**HLA-A2, HLA-B44 and HLA-DR15 Associate with Lower Risk of BK Viremia while BK Viruria Is HLA Independent** Kosuke Masutani,[1,2] Parmjeet S. Randhawa.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Medicine and Clinical Science, Kyushu Univ, Graduate School of Medical Sciences, Fukuoka, Japan.*

**Background:** Human leucocyte antigens (HLA) modulate immunity to polyomavirus BK (BKV). Identification of HLA-antigens that alter the course of infection will facilitate risk stratification, and customization of pre-emptive intervention strategies.

**Methods:** We performed a retrospective cohort study with 998 kidney transplant patients with BKV infection status confirmed by polymerase chain reaction (PCR). Clinical parameters and donor-recipient matching for specific HLA-antigens were examined in relation to occurrence of viremia and viruria. Emphasis was placed on donor-recipient matching rather than the actual frequency of specific HLA-alleles, since a successful immune response requires sharing of HLA antigens between a virus infected target cell and the anti-viral effector cell.

**Results:** In multivariate statistics incorporating known risk factors of BKV, low risk of BK viremia was independently associated with matching of HLA-A2 (hazard ratio [HR] 0.51, 95% confidence interval [CI] 0.28-0.85), HLA-B44 (HR 0.31, 95%CI 0.076-0.85) and HLA-DR15 (HR 0.35, 95%CI 0.084-0.93) ($p<0.05$), whereas high risk of viremia was associated with male gender (HR 2.38, 95%CI 1.46-4.09, $p<0.001$). We did not find any difference in the distribution of HLA matching between BK viruria and BKV negative patients.

| | No BKV replication (n=515) | Viruria (n=381) | Viremia (n=102) | p-value |
|---|---|---|---|---|
| HLA-A2 match | 154 (29.9%) | 122 (32.0%) | 21 (20.6%) | 0.08 |
| HLA-B44 match | 56 (10.9%) | 42 (11.0%) | 3 (2.9%) | 0.04 |
| HLA-DR15 match | 55 (10.7%) | 30 (7.9%) | 3 (2.9%) | 0.03 |

**Conclusions:** HLA-A2, HLA-B44 and HLA-DR15 mismatches are associated with a lower risk of BK viremia. Control of low grade BK viruria not complicated by viremia may require HLA-independent mechanisms.

## SA-PO963

**Polyoma-BK-Virus Associated High Grade Carcinomas in Renal Allografts** Daniel J. Kenan, Harsharan Kaur Singh, Volker Nickeleit. *Div of Nephropathology, The Univ of North Carolina, Chapel Hill, NC.*

**Background:** Although polyoma-BK-virus (BKV) induced allograft nephropathy is seen in 2-4% of kidney transplant recipients, the role of BKV as a potential oncogenic pathogen is poorly understood.

**Methods:** Two transplant nephrectomies with high grade invasive transitional cell and collecting duct-like carcinomas were analyzed. Laser capture microdissection on formalin fixed normal and tumor tissue was performed and DNA was extracted. Tissue sections were analyzed by immunohistochemistry (SV-40 large T and VP antigens), in-situ hybridization and electron microscopy. Molecular studies were based on quantitative PCR assays, and targeted genomic DNA sequencing.

**Results:** Both study tumors strongly expressed SV40-T antigen (a proliferation marker) in tumor cell nuclei but not in adjacent normal tubules/parenchyma; no signals were seen for VP capsid antigens, by in-situ hybridization or electron microscopy in any tissue compartment (no evidence of productive viral replication). A TaqMan PCR assay showed BKV sequences (encoding T and VP ) in relatively low copy numbers in both the in situ and invasive tumor components but not in the adjacent normal tubular epithelial cells/ normal parenchyma. No PCR amplification products were detected with primers specific for polyoma-JC-virus. Conventional PCR further demonstrated the presence of the full length BKV genome. Sequencing of the PCR fragments proved the presence of BKV genotypes.

**Conclusions:** These new findings demonstrate that polyoma-BK-virus is closely associated with high grade carcinomas occurring in kidney transplants. The presence of BKV is restricted to tumor cells, and it is not associated with BKV replication or a productive infection. It is tempting to speculate that BKV is incorporated into the genome and promotes tumor growth. BKV: a potential tumorigenic pathogen?

## SA-PO964

**Efficacy of Ciprofloxacin for Prophylaxis of BK Virus Infection in Kidney Transplant Recipients** Tariq Shah,[1,2,3,4] Don Vu,[1,2,3] Elizabeth Cadag,[1] Yasir A. Qazi,[4] Robert Naraghi,[1,2] Caron Hutchinson,[2] David I. Min.[3] *[1]Saint Vincent Medical Center, Los Angeles, CA; [2]Transplant Research Institute, Los Angeles, CA; [3]Western Univ of Health Sciences, Pomona, CA; [4]Univ of Southern California, Los Angeles, CA.*

**Background:** BK virus nephropathy (BKVN) is a common viral infection that affects up to 10% of renal transplant recipients (RTRs), causing allograft dysfunction and graft loss. Fluoroquinolones have been shown in vitro to inhibit BK viral replication by direct inhibition of the BKV-encoded DNA gyrase. The aim of the study is to investigate the efficacy of prophylactic administration of ciprofloxacin for prevention of BK viremia after renal transplantation.

**Methods:** We retrospectively collected patient and transplant data for 104 patients undergoing kidney transplantation between April 2010 and January 2012 at St. Vincent Medical Center (SVMC). Forty-four patients received ciprofloxacin prophylaxis of 250 mg daily for 30 days (CP) and sixty patients received no ciprofloxacin prophylaxis (NP). Quantitative BK virus (BKV) DNA surveillance in plasma was performed at 1,3,6,9, and 12 months after transplantation.

**Results:** Baseline characteristics, transplant demographics, and 1-year incidence of acute rejection were similar between the two groups. The cumulative incidence of BK viremia at one year after transplantation was significantly reduced in CP (11.3% versus 28.3%, OR =3.07, p=0.036). Among recipients who developed BK viremia, the median time to BKV was 145 days in CP group, compared to 120 days in NP ($P>0.05$), however the CP group developed a significantly lower peak BK viral load, versus the NP group (median, 10.8 x $10^3$ copies/mL vs. 10.4 x $10^4$ copies/ml, p=0.044). The overall incidence of BKVN was 2.2 % in CP group and 6.7 % in NP group (P=0.39, OR=3.1). Additionally, patients in CP group were not found to experience a higher risk of fluoroquinolone-resistant infection.

**Conclusions:** Ciprofloxacin prophylaxis appears to be effective in preventing BK viremia within one year after transplantation. Future prospective, randomized studies are needed to determine the impact of ciprofloxacin prophylaxis for BKVN.

## SA-PO965

**Efficacy of Intravenous Immunoglobulinin the Treatment of BK Virus Nephropathy in Renal Transplant Recipients** Tariq Shah,[1,2,3,4] Robert Naraghi,[1,2] Don Vu,[1,2,3] Cameron Sheth,[5] Yasir A. Qazi,[4] Elizabeth Cadag,[1] Caron Hutchinson,[2] David I. Min.[3] *[1]Saint Vincent Medical Center; [2]Transplant Research Institute; [3]Western Univ of Health Sciences; [4]Univ of Southern California; [5]Univ of California, Santa Babara.*

**Background:** BK virus can cause clinically significant viral infection in the renal transplant recipients. The outcome may be BK virus associated nephropathy (BKVAN), leading to renal allograft dysfunction and the loss of the graft. The usual management of BKVAN involves reduction of immunosuppression and the addition of leflunomide, quinolones and cidofovir, but the rate of graft loss remains high. The aim of this study was to assess the impact of treatment with intravenous human immunoglobulin (IVIG) on the outcome of BKVAN in renal transplant recipients.

**Methods:** Upon diagnosis of BKVAN, patients remained on anti-polyomavirus strategy consisting of reduction of immunosuppression and the use of leflunomide therapy. Treatment with IVIG was given only to patients who did not respond to adjustment of immunosuppression and leflunomide. Response to IVIG therapy was monitored by measuring the viral load, serum creatinine level and repeated allograft biopsy if needed clinically. Clearance of viremia (<500 copies/mL) was considered a positive response to IVIG treatment.

**Results:** All 30 patients had persistent BKV viremia and BKVN with their mean BK viral loads higher than the baseline (range 15,000 – 2 million copies/mL). Mean peak BK load was 205,314 copies/mL compared to 697 copies/mL after one year follow up. Twenty seven patients (90%) had a positive response in clearing viremia. The actuarial patient and graft survival rates after 12-months were 100% and 96.7 %, respectively. The average total amount of IVIG was 102.4 ± 52.1 g per patient and the average cost per patient was $7,657.00.

**Conclusions:** IVIG administration appeared to be safe and effective in treating BK viremia, BKVAN and preventing graft loss in patients who had inadequate response to immunosuppression reduction and leflunomide therapy.

## SA-PO966

**The BK:KIDNI Trial – BK Viremia: Kinase Inhibition to Decrease Nephropathy Intervention Trial** Pietro Ravani, Brenda Hemmelgarn, Lee Anne Tibbles. *Medicine, Univ of Calgary, Calgary, Canada.*

**Background:** Although infection with human BK virus is common, this polyomavirus remains dormant in the kidney of affected individuals. However, following kidney transplant, BK viremia and nephritis are increasing problems as with immunosuppression BK virus reactivates and replicates, causing progressive interstitial nephritis in the renal allograft.

Observational studies suggest that reduction of immunosuppression can lead to clearance of BK viremia. However, reducing immunosuppression may also increase the risk of rejection. In addition, no consensus regarding how to reduce immunosuppression has been reached and studies on treatments other than reduction of immunosuppression are not available to inform clinical practice.

**Methods:** We are conducting a Phase III multi-centre randomized, parallel-group trial involving 300 patients (150 per arm), at 15 sites in Canada. De novo kidney transplant patients with positive PCR for plasma BK (viremia) are randomized to either reduced immunosuppression (control arm) or Sirolimus/Leflunomide (treatment arm). Biopsies are taken at first viremia and one year follow-up. Biopsy proven rejection is treated according to site-specific guidelines. The study period is at least one year in duration (last subject randomized will be followed for one year). Since recruitment will occur over approximately 3 years, the total duration of the trial and thus follow-up of first patients will be an estimated 4 years.

**Results:** To date we have screened 146 patients and recruited 27 across Canada.

**Conclusions:** The BK:KIDNI trial will test whether specific kinase inhibition is superior to standard therapy in reducing the risk of the multiple outcome of doubling of serum creatinine, need for renal replacement therapy for graft failure and death (primary objective). Secondary outcomes include rate of decline in renal function based on decline in eGFR, time to 50% increase in serum creatinine, time to clearance of viremia, biopsy proven acute rejection, grade of BK nephropathy on biopsy at 12 months, chronic allograft nephropathy at 12 months, change in Health Related Quality of Life measures, and differences in health care costs between the two strategies (secondary objectives).

*Funding:* Government Support - Non-U.S.

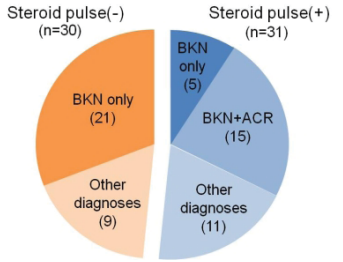
## SA-PO967

**The Outcome of Steroid Pulse Therapy in BK Viremia Patients Who Demonstrated Acute Rejection Like Lesion** Hyosang Kim,[1] Jong Cheol Jeong,[2] Jaeseok Yang,[2] Wonseok Yang,[1] Curie Ahn,[2] Duck Jong Han,[3] Su-Kil Park.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Asan Medical Center, Univ of Ulsan College of Medicine, Seoul, Republic of Korea; [2]Transplantation Center, Seoul National Univ Hospital, Seoul, Republic of Korea; [3]Dept of Surgery, Asan Medical Center, Univ of Ulsan College of Medicine, Seoul, Republic of Korea.*

**Background:** To investigate the change of renal function on renal transplant recipients with high BK viremia who received steroid pulse therapy for pathologic diagnosis of acute cellular rejection(ACR) along with BK nephropathy(BKN).

**Methods:** The study population consisted of 112 kidney transplant recipients with high BK viremia (defined as serum BKV load > $1x10^4$ copies/mL) detected more than once from January 1, 2004 to December 31, 2011 at Asan Medical Center and Seoul National University Hospital.

**Results:** Kidney biopsies were performed in 61 patients and 31 received steroid pulse therapy (>500mg) around the time of kidney biopsy.




Estimated GFR (eGFR) were 31.8±14.3 ml/min/1.73m² in steroid(+) group (n=31) at the time of steroid treatment and 47.9±26.9 in steroid(-) group (n=81) when serum BK load>$10^4$ ($p$<0.001). After 1 year, the difference of eGFRs has maintained (32.8±20.5 and 52.8±34.9, $p$<0.001). In Kaplan-Meier analysis for 50% decline of eGFR from baseline, renal function in steroid(+) group tended to deteriorate more rapidly.

Comparing between 15 BKN+ACR patients who received steroid pulse therapy and 21 BKN patients without steroid treatment, eGFRs were changed to 22.4±12.7 vs 31.7±13.2 after 1 year ($p$=0.05) from initial eGFRs 28.7±9.0 vs 31.3±9.4 ($p$=0.39) without any difference of BK viral loads. The renal function in BKN+ACR group tended to become aggravated earlier in Kaplan-Meier analysis.

**Conclusions:** In BK viremia patients who received steroid pulse therapy, the renal function tended to deteriorate more rapidly.

## SA-PO968

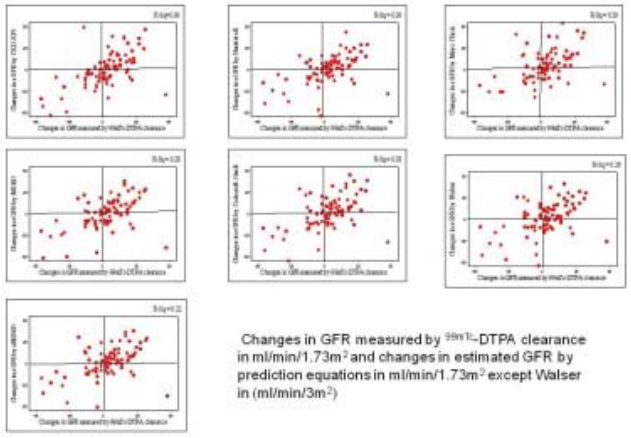
**Precision of Current Formulae to Detect Changes of GFR in Middle Eastern Kidney Transplant Recipients** Osama M. El-minshawy, Eman Elbassuoni. *Internal Medicine, School of Medicine, Univ of Tabuk, Tabuk, Saudi Arabia.*

**Background:** Assessment of graft function is crucial in kidney transplantion (Tx); Most formulae were developed for estimation of GFR in white and African Americans, Australian and Canadian people, Also their accuracy to detect changes of GFR in kidney Tx has not well studied. Aim of this work is to find out the precision of current formulae to detect GFR changes in kidney Tx recipients.

**Methods:** the study included 79 kidney Tx recipients; 46 (58%) were males, 45 (57%) were Egyptian, 34 from different Arab countries age 46±13 years, body weight 70±7 Kg, BSA 1.8±0.1m², BMI 25±4 Kg/m². serum creatinine 1.8±1 mg/dl, blood urea nitrogen 29±11 mg/dl. Serum albumin 3.7±0.6 g/dl. Their immunosuppression: prednisone, CNI, and MMF in 70 patients (89%), prednisone, and MMF in 9 patients (11%). GFR was measured by $^{99m}$Tc- DTPA clearance twice for each patient after follow up time 14±3 months and estimated twice at the same days of measurements by MDRD, abbreviated MDRD, Walser, Nankivell, Mayo clinic, Cockroft-Gault and CKD-EPI formula.

**Results:** measured GFR were: 8-90 ml/min/1.73m² (44±21) , absolute changes of measured GFR between 1st & 2nd measurement range from -45 to 46. changes of estimated GFR range from -52 to 48 by CKD-EPI, from -57 to 44 by Cockroft-Gault from -92 to 57 by Mayo Clinic formula, from -60 to 37 by MDRD, from -82 to 37 by aMDRD, from -53 to 43 by Nankivell, and from -53 to 40 by Walser.

| | No of Concordant | No of Discordant | % of concordance | $R^2$ |
|---|---|---|---|---|
| CKD-EPI | 58 | 21 | 73% | 0.39 |
| Nankivell | 57 | 22 | 72% | 0.29 |
| Mayo Clinic | 55 | 24 | 70% | 0.29 |
| MDRD | 55 | 24 | 70% | 0.28 |
| Cockcroft-Gault | 53 | 26 | 67% | 0.28 |
| Walser | 51 | 28 | 65% | 0.26 |
| aMDRD | 52 | 27 | 66% | 0.22 |




**Conclusions:** we conclude lack accuracy of current formulae to detect changes in GFR in kidney Tx.

## SA-PO969

**How to Estimate Kidney Function in Kidney Transplant Recipients with Mild to Moderate Kidney Impairment** Makoto Tsujita, Daijo Inaguma. *Kidney Disease Center, Nagoya Daini Red Cross Hospital, Nagoya, Japan.*

**Background:** With the recent increase in renal transplantations in Japan, accurate assessment of renal function is required.

**Methods:** This study included 73 patients who had undergone renal transplantationat Nagoya Daini Red Cross Hospital at least 6 months previously and had stable renal function for more than 3 months. Glomerular filtration rates (GFRs) were measured by inulin clearance (mGFR) and compared with estimated cystatin C-based GFRs (eGFRcys), estimated creatinine-based GFRs (eGFRcre) and their averaged values (eGFRave).

**Results:** mGFR was 43.3 ± 14.1 mL/min/1.73 m² and, eGFRcre was 39.6 ± 11.7 in eGFRcre, eGFRcys was 56.0 ± 17.1, and eGFRave was 47.8 ± 13.7 mL/min/1.73 m² , respectively.Serum cystatin C was 1.39±0.37 mg/L and serum creatinine was 1.58±0.51 mg/dL.The correlation coefficients between mGFR and eGFRcre, eGFRcys, and eGFRave was 0.768, 0.831, and 0.841, respectively (p <0.001, for all).The intraclass correlation coefficients were 0.754, 0.816, and 0.840, respectively (p <0.001,for all).The mean differences between measured and estimated GFR values were - 3.74 mL/min/1.73 m² with a root-mean square error (RMSE) of 9.06 for eGFRcre, + 12.64 with RMSE of 9.48 for eGFRcys, and + 4.45 with RMSE of 7.86 for eGFRave. Bland–Altman plots showed that eGFRcys overestimated GFR values compared with mGFR values in most cases and that eGFRave overestimated GFR values in 53 of 73 cases, whereas eGFRcre underestimatedthe values in 53 of 73 cases.

**Figure 1. Relation between measured GFR and estimated GFR**

| Estimated | Correlation Parameters | | | | | Mean | Accuracy |
|---|---|---|---|---|---|---|---|
| GFR | ICC | 95%CI | P values | Pearson's r | P values | differences | with 30% |
| eGFRcre | 0.754 | 0.635 – 0.838 | <0.001 | 0.768 | <0.001 | - 3.74 | 82.2 |
| eGFRcys | 0.816 | 0.722 – 0.881 | <0.001 | 0.831 | <0.001 | + 12.64 | 52.1 |
| eGFRave | 0.840 | 0.757 – 0.897 | <0.001 | 0.841 | <0.001 | + 4.45 | 86.3 |

GFR indicates glomerular filtration rate; ICC, intraclass correlation coefficient; eGFRcre, estimated creatinine-based GFR; eGFRcys, estimated cystatin C-based GFR; eGFRave, average of eGFRcre and eGFRcys

**Conclusions:** eGFRave may be the best marker to estimate kidney function in Japanese renal transplant recipients with mildly reduced or normal kidney function.

## SA-PO970

**Can Renal Function Equations Substitute for the Standard Method in the Measurement of the Glomerular Filtration Rate of Kidney Transplant Recipients?** Xiaoying Du, Jianghua Chen. *Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang Univ, Hangzhou, Zhejiang, China.*

**Background:** Accutate estimate of glomerular filraton rate (GFR) is crucial in the care of kidney transplant recipients. Since the reference methods to measure GFR cannot be easily implemented, several formulas for estimating GFR have therefore been developed. However, little is known about their diagnostic value in kidney transplant recipients. The aim of this study is to evaluate the performance of four creatinine-based formulas for estimating the GFR in this population.

**Methods:** Performances of the modified abbreviated Modification of Diet in Renal Disease (aMDRD) equation, aMDRD for Chinese chronic kidney disease patients (c-aMDRD), Cockcroft and Gault (CG) formula, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula were compared with the dual plasma sampling $^{99m}$Tc-DTPA plasma clearance method as the gold standard for measuring GFR in 155 stable patients after kidney transplantation. Correlation, bias, precision and accuracy within 15%, 30% and 50% of true GFR were determined.

**Results:** Though each GFR test displayed a significant correlation (P<0.0001) with $^{99m}$Tc-DTPA clearance, Pearson's correlation coefficients (r) were only 0.596 for aMDRD, 0.584 for c-aMDRD, 0.583 for CKD-EPI, and 0.556 for CG. Bias ranged from -46.07 to 34.39 ml/min/1.73m$^2$for aMDRD ,-45.13 to 49.33 ml/min/1.73m$^2$for c-aMDRD, -40.36 to 41.73 ml/min/1.73m$^2$for CKD-EPI, and -49.68 to 36.97 ml/min/1.73m$^2$for CG. The 15,30 and 50% accuracies were 44.5%, 83.9% and 98.1% for aMDRD respectively; 41.9%, 79.4% and 94.2% for c-aMDRD respectively; 47.7%, 79.4% and 94.8% for CKD-EPI respectively; 51%, 86.5% and 98.1% for CG respectively.

**Conclusions:** All of these equations can just roughly estimate the GFR of kidney transplant recipients. None of them can substitute for $^{99m}$Tc-DTPA plasma clearance. In clinical trials, renal graft function should be measured by a standard method.

## SA-PO971

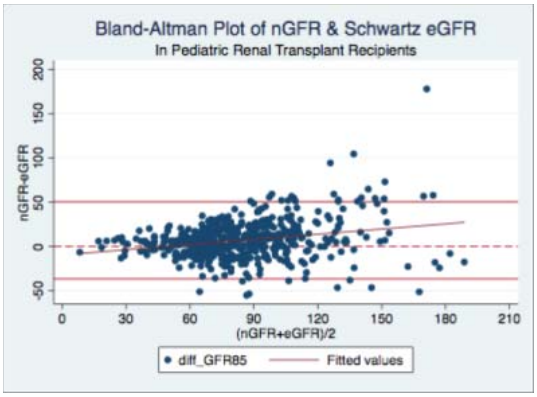
**Modified Schwartz Formula Accurately Reflects GFR in Pediatric Renal Transplant Recipients** Omar M.A.A. Alkandari,[1] Diane Hebert,[1] Valerie Langlois,[1] Lisa Robinson,[1] Rulan S. Parekh.[1] *[1]Hospital for Sick Children; [2]Hospital for Sick Children; [3]Hospital for Sick Children.*

**Background:** Kidney function is best assessed by measured GFR, however, this is impractical, complex, and costly especially in children. Our aim is to assess the validity of modified Schwartz formula in a pediatric renal transplant recipients.

**Methods:** We conducted a retrospective cross-sectional data analysis of pediatric kidney transplant patients less than age 18 (n=174) followed at our center between 2001-2012. We compared estimated GFR using modified Schwartz with enzymatic measured serum creatinine (SCr) to the two point $^{99m}$Tc-DTPA nuclear GFR (nGFR) and determined bias, precision, accuracy (within 30%), and agreement by Bland-Altman analyses in 505 paired measures. The eGFR was also determined using Counahan and Leger formulas.

**Results:** The study population included 62% males, 70% between ages 10 and 18 years , 46% with CAKUT as

cause of ESRD and 45 % of patients were living donor transplant recipients. Baseline median eGFR and nGFR were 77 and 86 ml/min/1.73m2, respectively. Out of the 505 nGFRs, 83% had a nGFR ≥ 60 ml/min/1.73m2. Overall, the modified Schwartz formula has a bias of 6.9 ml/min/1.73m$^2$ and accuracy within 30% of 83.6%. Bland-Altman analyses demonstrates good agreement across the range of GFR especially in the normal range of GFR.




Bias and accuracy within 30% vary for females (4.8; 86.7%), children less than 5 years (-3.0; 80%), older than 15 years (10.1; 77.7%), nGFR ≥ 90 ml/min/1.73m$^2$ (16.7;81.7%) and measurements done in first 3 months post-transplant (5.8; 78.3%). Cause of ESRD or CKD stage did not influence bias or accuracy. When compared to other SCr-based formulae, modified Schwartz and Counahan have better accuracy than Leger.

**Conclusions:** Modified-Schwartz is a useful bedside tool and a valid measure of GFR in pediatric kidney transplant recipients.

## SA-PO972

**Increased Risk of Late Graft Dysfunction from Rejection in New Onset Diabetes after Transplantation** Joseph B. Lockridge,[1] Kirsten L. Johansen,[2] F. Vincenti.[3] *[1]Div of Nephrology, Univ of California San Francisco, San Francisco, CA; [2]Nephrology Section, San Francisco VA Medical Center, San Francisco, CA; [3]Kidney Transplant Service, Univ of California San Francisco, San Francisco, CA.*

**Background:** New Onset Diabetes after Kidney Transplant (NODAT) is an independent predictor of graft loss and mortality. The mechanisms leading to graft loss in these patients remain unclear. Further, late allograft outcomes have not been specifically addressed in this population.

**Methods:** We screened 1949 patients transplanted at UCSF between July 16, 1997 and Dec 28, 2004. Of these, we identified 156 patients with stable graft function for one year and underwent biopsy for late onset graft dysfunction after one year. Participants were divided according to diabetes status into three groups: NODAT (n=45), pre-existing diabetes (n=38) and no diagnosis of diabetes (n=73). We used univariate and multivariable logistic regression analysis to determine whether NODAT was associated with rejection compared to those with pre-existing diabetes and those with no diabetes.

**Results:** Baseline characteristics were similar in all three groups with respect to donor status, gender, race, induction therapy, and panel reactive antibody. Patients with pre-existing diabetes had a higher age at transplant, while patients with no diabetes had a higher time post-transplant at the time of biopsy. We found that both patients with pre-existing DM and those without a diagnosis of DM had lower odds of rejection than patients with NODAT (OR 0.21, 95% CI 0.07-0.59 for pre-DM and OR 0.38, 95% CI 0.18-0.83 for no DM). Multivariable regression analysis confirmed a higher rejection rate in patients with NODAT compared to patients with no diabetes (OR 3.4 95% CI 1.01-11.50;p = 0.05) and to pre-existing DM when adjusted for allograft age, creatinine, and calcineurin inhibitor level.

**Conclusions:** Our study shows that in this cohort, NODAT was associated with a significantly higher risk of late allograft dysfunction from rejection. Larger studies are needed to determine the mechanism of disease and to guide immunosuppression management in this selected group.

*Funding:* NIDDK Support, Veterans Affairs Support

## SA-PO973

**Outcomes of Bisphosphonate Therapy in Kidney Transplant Recipients—A Meta-Analysis of Randomized Controlled Trials** Stephanie M. Toth, Jean M. Francis, Craig E. Gordon. *Renal Section, Boston Univ School of Medicine, Boston, MA.*

**Background:** Mineral and bone disorders that precede kidney transplantation are worsened in the post-transplant setting by immunosuppressive regimens and tertiary hyperparathyroidism. Bone mineral density (BMD) rapidly decreases following transplantation, leading to increased fracture risk. The benefits and risks of bisphosphonates in kidney transplant recipients are not well defined. The aim of this study is to evaluate changes in BMD and fracture rates in bisphosphonate-treated transplant recipients by meta-analysis.

**Methods:** We performed a systematic review of Cochrane, Embase, and Medline to identify studies that evaluated bisphosphonate therapy in kidney transplant recipients (last search 4/14/13). Randomized controlled trials comparing bisphosphonate therapy to standard of care in adults were included if follow-up duration was >6 months. We performed a random effects meta-analysis to determine the effect of bisphosphonates on BMD and fracture incidence.

**Results:** Of 793 retrieved studies, sixteen met inclusion criteria comprising a total of 748 patients. Mean age was 45.1 years, 69.8% were male, and mean baseline creatinine was 1.5 mg/dL. Treatment with bisphosphonates was associated with improvement in

lumbar and femoral neck BMD compared with controls (0.061 g/cm$^2$, 95% CI 0.039-0.082 and 0.056 g/cm$^2$, 0.020-0.093, respectively). This corresponded to an unweighted improvement in BMD of 7.8% in lumbar spine and 7% in femoral neck. Bisphosphonate therapy had no effect on serum creatinine or calcium. There was no difference in fracture incidence in the two groups.

**Conclusions:** Bisphosphonate therapy was associated with improved lumbar and femoral neck BMD in kidney transplant recipients without adversely affecting serum creatinine or calcium. Although there was no difference in fracture rates, this may have been due to short follow-up times or the small number of studies reporting clinical outcomes. Future studies should report fracture rates and plan for longer follow-up intervals to better evaluate whether bisphosphonates reduce fracture incidence in kidney transplant recipients.

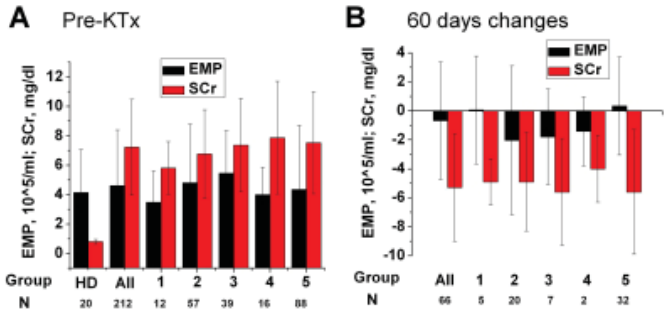
## SA-PO974

**Comparison of Endothelial Microparticles and Serum Creatinine after Kidney Transplantation** Zahida Qamri, Ronald Pelletier, Jon R. Von Visger, Kyle M. Ware, Anjali A. Satoskar, Tibor Nadasdy, Sergey V. Brodsky. *Dept of Pathology and Comprehensive Transplant Center, The Ohio State Univ, Columbus, OH.*

**Background:** Endothelial microparticles (EMP) are membrane vesicles shed from endothelial cell in response to injury, activation or apoptosis. Kidney transplantation (KTx) is the treatment of choice for patients with end-stage kidney disease (ESKD). The aim of this study was to compare changes in EMP and serum creatinine (SCr) in patients following KTx.

**Methods:** Blood was periodically collected from patients the day before (pre-KTx) and two months after KTx. EMP were identified as 1 µm CD31$^+$/CD42b$^-$ microparticles and quantified by fluorescence-activated cell scanning.

**Results:** This study included 212 KTx or kidney/pancreas (KPTx) recipients and 20 healthy donors (HD) (for comparison) prior to donation. The recipients were divided into 5 groups based on the cause of ESRD: 1: ESRD due to diabetes mellitus (DM) type I that received a KPTx; 2: ESRD due to DM (type I or type II) that received a KTx only; 3: congenital/obstructive causes of ESRD; 4: ESRD due to immune complex disease; 5: ESRD due to unknown causes. No differences in the quantity of circulating EMP were seen in the pre-KPTx or KTx recipient sera and HD sera (Figure 1, A). At day 60 after KTx, Groups 2, 3 and 4 had a reduction in both circulating EMP and SCr from pre-KTx values (Figure 1, B).




**Conclusions:** In our patient cohort the quantity of circulating EMP were similar in HD and ESRD patients before KPTx or KTx. Reduction in both circulating EMP and SCr was seen after KTx in selective groups. Mechanisms of these variations in post-KTx EMP in different patient groups are not clear yet, but may be related to an underlying systemic disease or immune suppression therapy.

*Funding:* NIDDK Support

## SA-PO975

**Oral Paricalcitol Ameliorates Post-Transplant Hyperparathyroidism and Proteinuria in Kidney Transplantation** Matias Trillini, Jorge Arturo Reyes Loaeza, Karen Courville, Claudia Ferrer Siles, Silvia Prandini, Flavio Gaspari, Antonio Cannata, Alessandro Villa, Annalisa Perna, Eliana Gotti, Norberto Perico, Piero Ruggenenti, Giuseppe Remuzzi. *IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Italy.*

**Background:** Secondary hyperparathyroidism (SHPT) is a major clinical problem in renal transplant recipients. In renal patients Paricalcitol (PAR) ameliorates SHPT and even reduces proteinuria, a major determinant of disease progression. Effects of PAR in SHPT renal transplant recipients are unknown.

**Methods:** In a randomized (1:1 computer-generated sequence), cross over trial (NCT01220050) we compared the effect of 6-month oral PAR added-on standard therapy vs 6-month standard therapy alone on serum intact parathyroid hormone (iPTH, primary outcome), markers of bone remodeling and proteinuria in 43 stable renal transplant recipients aged 54±9 yrs with iPTH persistently >80 pg/ml and no vitamin D therapy. PAR was started at 1 µg/d and, if tolerated, at 3 months was up-titrated to 2 µg/d. We compared treatment effects by repeated measures ANOVA, with participants as random and treatment and period as fixed factors.

**Results:** Serum creatinine averaged 1.39±0.33 mg/dl at baseline and was stable thereafter. Compared to standard therapy, PAR significantly reduced iPTH, ostase and osteocalcin levels, without affecting calcium and phosphate levels. Proteinuria significantly decreased on PAR, but not on standard treatment (Table). Reductions in iPTH, ostase and osteocalcin levels vs standard therapy were already significant at 3 months with PAR 1 µg/d. PAR was well tolerated.

Table. Outcome variables at baseline and at the end of each treatment period

| | Baseline | PAR 2 µg | Standard treatment |
|---|---|---|---|
| iPTH *(pg/ml)* | 115 (95 -152) | 63 (52 - 78)$^{\#\,\circ}$ | 128 (94 -166) |
| Ostase *(µg/l)* | 11 (8 - 14) | 9 (7 - 11)$^{\#\,\circ}$ | 13 (10 - 18) |
| Osteocalcin *(ng/ml)* | 25 (15 - 33) | 18 (10 - 28)$^{\#\,\circ}$ | 25 (16 - 38) |
| Calcium *(mg/dl)* | 9.6 (9.2 - 9.9) | 9.8 (9.5 - 10) | 9.6 (9.3 - 9.9) |
| Phosphate *(mg/dl)* | 3.2 (2.8 - 3.6) | 3.3 (2.8 - 3.9) | 3.2 (2.7 - 3.5) |
| Proteinuria *(g/24 h)* | 0.27 (0.13 - 0.60) | 0.14 (0.10 - 0.41)$^{\S}$ | 0.23 (0.10 - 0.54) |

Data are median (interquartile range)

$^{\#}p < 0.001$ vs standard therapy, $^{\circ}p<0.01$ vs. baseline, $^{\S}p < 0.05$ vs baseline

**Conclusions:** In renal transplant recipients oral paricalcitol safely ameliorates secondary hyperparathyroidism and may have beneficial effects on proteinuria that need confirmation in ad-hoc trials.

*Funding:* Pharmaceutical Company Support - AbbVie s.r.l., Italy.
*Funding:* Pharmaceutical Company Support - AbbVie s.r.l., Italy

## SA-PO976

**Functional Status Predicts Hospital Length of Stay and Rehospitalizations following Kidney Transplant (KTx)** Elizabeth C. Lorenz, Hatem Amer, Andrea L. Cheville, Fernando G. Cosio. *Mayo Clinic, Rochester, MN.*

**Background:** Decreased functional status has been associated with increased hospital length of stay (LOS) and rehospitalizations (RH) in patients with CKD. The goal of this study was to determine whether functional status predicts LOS and RH following KTx.

**Methods:** We performed a prospective cohort study involving all patients receiving KTx at our center between 12/2011 and 1/2013 (n=141). Providers assessed functional status with the following scoring system: A=regular exercise and no limitations, B=no regular exercise and no limitations, C=no regular exercise and slight limitations, D=no regular exercise and significant limitations. Providers assigned scores during the pre-KTx evaluation. The relationship of score to initial hospital LOS and number of RH during the first 4 months post-KTx was studied.

**Results:** Mean age was 51.0±13.6 yrs, 57.2% were male, 84.8% were Caucasian, 85.8% received LD KTx and 26.9% were diabetic (DM). Overall, 34.0% received a score of A, 46.1% a score of B, 16.3% a score of C and 3.5% a score of D. Scores of C or D were associated with DM (38.5% vs 12.8%, p=0.0006) but not with age, gender or dialysis status. Median initial hospital LOS was 4 days (range 3-13). A score of C or D independently predicted a hospital LOS longer than 4 days (OR 3.4, CI 1.4-8.1, p=0.003).

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| Variable associated with hospital LOS | OR (CI) | p | OR (CI) | p |
| Age (per 10 yr decrease) | 0.7 (0.6-0.9) | 0.03 | 0.7 (0.5-0.9) | 0.006 |
| Male | 1.6 (0.8-3.1) | 0.20 | | |
| Preemptive | 0.9 (0.5-1.9) | 0.90 | | |
| DM | 1.3 (0.6-2.7) | 0.53 | | |
| Functional status score of C or D | 3.4 (1.5-8.1) | 0.003 | 3.8 (1.6-9.9) | 0.003 |
| Thymoglobulin | 2.2 (1.1-4.5) | 0.03 | 3.6 (1.6-8.3) | 0.001 |
| BMI (per kg/m$^2$) | 0.9 (0.9-1.0) | 0.28 | | |

Overall 24.8% (n=35) underwent RH during the first 4 months post-KTx. Scores of C or D were independently associated with more RH (0.6 ± 0.1 vs 0.2 ± 0.1, p=0.02).

**Conclusions:** Functional status independently predicts morbidity following KTx. Limitations in functional status were associated with longer hospital LOS and more RH early post-KTx. Further study is needed to determine whether improving functional status prior to KTx could decrease hospital utilization post-KTx.

## SA-PO977

**A Pedometer-Based Exercise Prescription Is Associated with Improved Metabolic Parameters following Kidney Transplant (KTx)** Elizabeth C. Lorenz,[1] Hatem Amer,[1] Fernando G. Cosio,[1] Andrea L. Cheville.[2] *[1]Nephrology and Hypertension, Mayo Clinic; [2]Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN.*

**Background:** Exercise is associated with improved metabolic parameters in patients with chronic kidney disease. However, studies of exercise in KTx recipients are lacking. The aim of this study was to examine the effect of a pedometer-based exercise prescription on metabolic parameters following KTx.

**Methods:** We performed a prospective cohort study involving all patients who received KTx at our center between 12/2010 and 1/2013 (n=307). Patients transplanted between 12/2010 and 12/2011 received usual care (n=162) while patients transplanted between 1/2012 and 1/2013 received a 90-day pedometer-based exercise prescription beginning at KTx (n=145). Weight gain, glucose, lipids and automated office blood pressures (BP) were assessed 4-months post-KTx.

**Results:** Mean age was 51.5±13.3 yrs, 57% were male, 87% were Caucasian, 83% underwent LD KTx, 48% underwent preemptive KTx and 25% were diabetic. Mean BMI was 28.1±5.5 kg/m$^2$ and steroid-containing maintenance immunosuppression was used in 80%. Baseline demographics were not significantly different between cohorts. At 4 months post-KTx, patients in the pedometer cohort had lower BP and less impaired fasting glucose.

| Variable | Pedometer cohort (n=145) | Non-pedometer cohort (n=162) | p |
|---|---|---|---|
| Systolic BP (mmHg) | 122.5±18.1 | 125.8±15.6 | 0.049 |
| Diastolic BP (mmHg) | 73.1±10.4 | 76.8±9.2 | 0.004 |
| Number antihypertensive medications | 1.2±1.0 | 1.1±1.0 | 0.58 |
| Weight change (kg) | 1.5±5.3 | 1.0±5.7 | 0.44 |
| Triglycerides (mg/dL) | 152.1±81.8 | 154.4±85.9 | 0.77 |
| HDL (mg/dL) | 56.9±19.4 | 54.3±19.5 | 0.16 |
| Fasting glucose (mg/dL) | 112.0±31.6 | 113.6±33.8 | 0.43 |
| Impaired fasting glucose | 21.8% (n=31) | 33.3% (n=49) | 0.029 |
| NODAT | 4.2% (n=6) | 4.1% (n=6) | 0.95 |
| eGFR (ml/min/1.73m$^2$) | 53.9±15.4 | 54.6±15.1 | 0.56 |

Adherence to the prescription was 44.8%.

**Conclusions:** A pedometer-based exercise program was associated with lower BP and less impaired fasting glucose early post-KTx. Further study of personalized exercise prescriptions following KTx is needed.

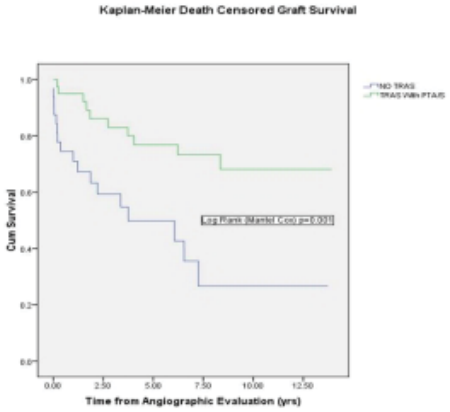
## SA-PO978

**Long-Term Outcomes of Angiographic Evaluation for Transplant Renal Artery Stenosis** Anum Ali, Muhammad Ahmad Mujtaba, Dennis P. Mishler, Tim E. Taber, Muhammad S. Yaqub, Asif A. Sharfuddin. *Nephrology/ Transplant, Indiana Univ.*

**Background:** The purpose of the present study was to evaluate graft survival, patient survival and long-term outcomes after angiographic evaluation and treatment for suspected transplant renal artery stenosis (TRAS).

**Methods:** From January 1999 to Aug 2012, 74 patients had an angiographic evaluation for suspected TRAS. Outcome measures were death-censored graft failure,all-cause mortality and long-term effects of TRAS treatment on efficacy,blood pressure (BP) control and graft function.

**Results:** TRAS was angiographically confirmed in 42 (56.7%) of the patients. The median time to angiographic evaluation in the TRAS group was 8.8 months, as compared to 48.3 in those who did not have TRAS (p<0.05). The median follow-up in the TRAS group was 67 months (range, 7-166) with a mean pre-procedure creatinine level of 2.2 ± 0.8 mg/dL. Systolic and diastolic BP fell from 166.2±19.7mm Hg to 137.9±5.8 mm Hg and 83.0 ± 14.5mm Hg to 68.6 ± 12.9 mm Hg (P<0.05).Post-procedure number of anti-hypertensive drugs was reduced from 3.5±0.9 to 2.7±1.0 (P<0.05). Primary Angioplasty (PTA) alone was performed in 57% of patients with TRAS, while the remaining had PTA with stent (PTAS). 8 cases of primary PTA treated had recurrent TRAS as compared to only 1 treated with primary PTAS. Death-censored graft survival after PTA/PTAS was significantly higher in TRAS group as compared to those without TRAS.




Overall transplant graft and patient survival was similar. Graft function at last follow up in TRAS treated group was 1.9±0.7mg/dl, as compared to 2.2±0.9 in those without TRAS treated with conventional medical therapy (p<0.05).

**Conclusions:** The long-term graft and patient survival were as good in TRAS treated patients as those without TRAS, but with particular benefit in superior graft survival post-intervention. Suspected TRAS should be intervened early with PTA/S as initial treatment.

## SA-PO979

**Urinary Clusterin Predicts Poor Recovery of Renal Function within Four Hours of Transplantation** Timothy J. Pianta,[1,2] Philip Peake,[2] Nicholas Buckley,[1] John W. Pickering,[3] Michaela Kelleher,[2] Zoltan H. Endre.[1,2,3] *[1]Prince of Wales Clinical School, Univ of New South Wales, Australia; [2]Dept of Nephrology, Prince of Wales Hospital, Sydney, Australia; [3]Dept of Medicine, Univ of Otago, New Zealand.*

**Background:** Impaired graft function is common after renal transplantation. We compared four urinary biomarkers of kidney injury, clusterin, neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1) with creatinine for early prediction of impaired recovery.

**Methods:** Serial urine and plasma biomarkers were measured before and for 7 days after transplantation in a single centre. Urinary biomarkers were normalised to creatinine excretion.

**Results:** Of 75 patients, 30 (40%) had immediate graft function, while 22 (29%) had delayed graft function requiring dialysis and 23 (30%) slow graft function. Urinary clusterin was increased at 4h in the 45 patients with non-immediate graft function (NIGF) (p=0.01). IL-18, KIM-1, and NGAL were increased (p< 0.05) by 8h, 1d and 2d respectively. At 4h only an elevated urinary clusterin [area under the curve (AUC): 0.68 (95% CI: 0.57 to 0.83)] and a low serum creatinine reduction ratio [AUC: 0.70 (0.59 to 0.82)] predicted NIGF. On multivariable analysis, only clusterin and the creatinine reduction ratio remained independently predictive of NIGF. Integrated discrimination improvement analysis demonstrated that elevated clusterin was independently additive to creatinine reduction ratio but a combined metric [clusterin or anuria] performed best. Addition of the combined metric of clusterin or anuria to a clinical model increased prediction of events (NIGF) by 0.05 (95% CI: 0.01 to 0.13); and of non-events (immediate function) by 0.08 (0.01 to 0.19). Clusterin performed best at 12h [AUC:0.72 (0.59 to 0.84)]. At this time [clusterin or anuria] also improved risk prediction independent of clinical factors and creatinine reduction ratio.

**Conclusions:** Urinary clusterin adds value to assessment based on creatinine, urine output and clinical factors in predicting poor recovery of renal graft function after transplantation.

*Funding:* Government Support - Non-U.S.

## SA-PO980

**Urine Liver-Type Fatty Acid-Binding Protein (L-FABP) Predicts Graft Outcome up to 2 Years after Kidney Transplantation** Myung-gyu Kim, Sang-Kyung Jo, Won-Yong Cho, Hyoung-kyu Kim. *Internal Medicine, Korea Univ Anam Hospital, Seoul, Republic of Korea.*

**Background:** In kidney transplant (KT) recipient, several new biomarkers have been investigated for predicting early tubular injury and our recent study identified day 2 urinary neutrophil gelatinase associated lipocalin (NGAL) to be useful in predicting slow graft function and adverse 1-year outcome. Here in this study, we further investigated the value of urinary NGAL and liver type fatty acid binding protein (L-FABP) in predicting long term graft outcome up to 2 years.

**Methods:** This was a single-center, prospective observational study. Serial urinary NGAL and L-FABP levels at 0 hrs, 2 days and 6 days after KT were measured and the clinical outcomes including acute allograft rejection, proteinuria and estimated glomerular filtration rate (eGFR) were collected during the 2-year period after KT.

**Results:** Of the 69 patients investigated, 14 and 7 experienced slow and delayed graft function (SGF and DGF), and urinary NGAL on day 2 after KT was significantly associated with SGF and DGF development, but L-FABP was not. During the 2-year follow up period, 13 (18.8%), 4 (5.8%) and 1 (1.4%) were diagnosed with acute T-cell mediated rejection, acute antibody mediated rejection (AMR) and chronic AMR, respectively. In addition, 10 (14.3%) developed calcineurin inhibitor toxicity and 6 (8.7%) developed BK viremia. The mean eGFRs at 1 and 2 years after KT were 65.1±19.1 and 58.5±22.6 ml/min/1.73m2. When poor long-term graft outcome was defined as eGFR at 2 year less than 50 mL/min/1.73 m2, elderly donor, AR and higher level of urinary L-FABP at 0hr were found to be significant risk factors. Furthermore, among the patients who did not develop AR, L-FABP showed more strong association with 2-year poor graft function (p=0.006). In the multivariate logistic regression analysis, higher L-FABP at 0 hr (p=0.015) as well as acute rejection (p=0.006) was also independent factor for predicting poor long-term graft function and ROC analysis showed that area under the curve (AUC) of urinary L-FABP was 0.692 (P=0.036).

**Conclusions:** Our results demonstrate the possibility that urinary L-FABP might be useful in predicting adverse longterm outcome in KT patients.

## SA-PO981

**Treatment of New Onset Diabetes after (Renal) Transplantation (NODAT) by Linagliptine and Insulin, a Comparative Analysis** Pratik Das,[1] Soumava Gupta.[2] *[1]Nephrology, Rtiics, Kolkata, West Bengal, India; [2]Nephrology, Rtiics, Kolkata, West Bengal, India.*

**Background:** NODAT has negative impact on renal allograft survival, cardiovascular risk and patient survival.Adequate glycemic control may alter the outcome.New generation dipeptidyl peptidase-4(DPP4) inhibitor linagliptine is now increasingly used to treat type2 DM. The present study has evaluated the safety & efficacy of linagliptine monotherapy in renal transplant recepients with NODAT,in comparison to insulin.

**Methods:** 200 renal allograft recepients with stable renal function & without past history of diabetes were evaluated for NODAT by routine 2 hrs oral glucose tolerance test.Those having a 2 hours glucose value more than 200mg/dl were randomly selected for either linagliptine monotherapy (5mg/d single dose) or insulin treatment.Fasting blood sugar was measured at 4 weekly interval &HbA1c at 12,24,36 &48 weeks. hypoglycemic &other treatment related events were noted.

**Results:** 67 patients fulfilled criteria for NODAT were randomly assigned to either linagliptine monotherapy(n=35) or insulin only(n=31).Fasting blood sugar level were comparable in 2 ggroups (124.56+_23.78 vs 118.69+_18.88 mg/dl,p>0.05), so also HbA1c %(7.6+_1.1 vs7.3+_0.88%,p>0.05).Weight gain was significantly lower in linagliptine group(0.88+_0.35 vs4.23+_1.79 kg,p<0.01). Hypoglycemic episodes were much lower in linagliptine group(1.01+_0.21 vs 8.76+_2.39 episodes,p<0.05).abnormal liver function test & sinnusitis were founnd in 2 linagliptine group.

**Conclusions:** Linagliptine monotherapy is as effective as insulin for glycemic control in NODAT with a much better safety profille.

## SA-PO982

### Febuxostat in Renal Transplant Recipients with Hyperuricemia

Youngjoo Jang, Su-Kil Park, Wonseok Yang. *Nephrology, Internal Medicine, Asan Medical Center, Seoul, Republic of Korea.*

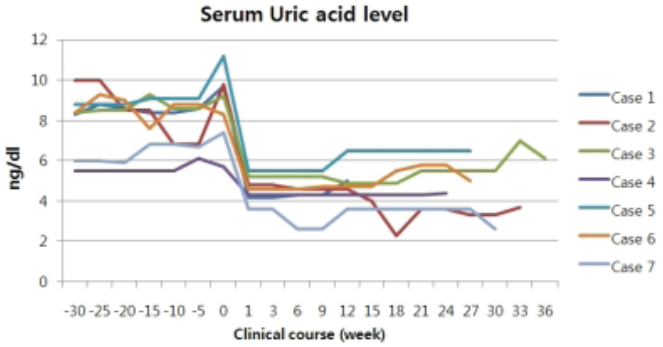
**Background:** Febusoxtat might be an alternative agent for the patient who cannot use allopurinol, because of more selective and less allergenic. However, information concerning the effectiveness and safety of this drug among renal allograft recipients is not well known.

**Methods:** Seven renal allograft recipients whose eGRF above 30 ml/min/1.73m$^2$ and need urate lowering therapy started febuxostat considering side effects or anxiety of using allopurinol. Febuxostat was administered 40mg daily in 6, 20mg daily and tapered on alternate days in 1 who is on Imuran. Uric acid, serum creatinine, hepatic enzyme, and serum cyclosporine or tacrolimus levels were monitored.

**Results:** The patients mean age was 60 ± 6.05 years. At the first visit after starting febuxostat ( Median duration 1.18 weeks, Range, 0.18 - 3 weeks ), UA levels ( mg/ml ) were lowered from 8.6 ± 1.74 to 4.6 ± 0.64 ( P = 0.018 ) and the other laboratory changes were not significant.

| | Before febuxostat | 1'st F/U | P value |
|---|---|---|---|
| Uric acid level ( mg/dl ) | 8.61 ± 1.75 | 4.60 ± 0.64 | 0.018 |
| Serum creatinine ( mg/dl ) | 1.42 ± 0.35 | 1.36 ± 0.30 | 0.141 |
| AST ( UI/L ) | 21.00 ± 4.08 | 21.14 ± 4.53 | 0.893 |
| ALT ( UI/L ) | 20.29 ± 7.50 | 19.86 ± 10.42 | 0.498 |
| Cyclosporine level ( ng/dl ) | 56.85 ± 26.68 | 52.68 ± 19.93 | 0.593 |
| Tacrolimus level ( ng/dl ) | 6.75 ± 2.62 | 8.50 ± 0.14 | 0.655 |

The UA level under 6.0 mg/dl was achieved in all subjects at first visit and maintained around 6.0 mg/dl ( 27.35 ± 7.57 weeks, Range 12.7- 36 weeks ).




Febuxostat was well tolerated and no adverse effects were noted.

**Conclusions:** Low dose febuxostat may effectively and promptly reduce serum uric acid level and maintained therapeutic serum uric acid level in renal transplant recipients without significant side effect.

## SA-PO983

### Cinacalcet Use at the Time of Transplantation Is Associated with a Significant Risk of Delayed Graft Function in Kidney Transplant Recipients

Laurent E. Weekers,[1] Stéphanie M.J.G. Grosch,[1] Catherine Bonvoisin,[1] Oliveir Detry,[2] Jean-marie H. Krzesinski,[1] Francois Jouret.[1] *[1]Nephrology, CHU ULg, Liege, Belgium; [2]Surgery, CHU ULg, Belgium.*

**Background:** The calcium-sensing receptor (CaSR) has been implicated in the ischemia/reperfusion cascade in cardiomyocytes and neurons. Renal ischemia/reperfusion occurs at the time of transplantation (Tx), with a deleterious impact on early graft function. Here, we retrospectively investigated whether the use of cinacalcet, a CaSR agonist, in kidney transplant recipients (KTR) influences early graft recovery.

**Methods:** All KTR from 2007 to 2012 were prospectively included in a database. Patients actively treated with cinacalcet on the day of Tx were retrospectively identified and matched with controls on (i) type of donor: living (LD), deceased after brain or circulatory death (DCD); (ii) cold ischemic time (CIT) ± 1 hour; (iii) residual diuresis ± 500 mL; and (iv) donor age ± 5 years. Delayed graft function (DGF) was defined as dialysis requirement after Tx. Baseline characteristics were compared between groups with t-test or Chi-2 as appropriate. The endpoint was the percentage of DGF in both groups.

**Results:** Among 337 KTR, 36 (10.7%) were treated with cinacalcet at Tx, they were matched with 61 controls. on the previously defined criteria. Characteristics of patients and donors are summarized in the table. DGF occurred in 42 % and 23 % of cinacalcet-treated and control groups, respectively (p=0.05).

| | | | Cinacalcet (n=36) | Controls (n=61) | p |
|---|---|---|---|---|---|
| Recipient | Age at Tx | years | 50.2±10.3 | 49±13.5 | 0.92 |
| | Sex ratio | % female | 47 | 41 | 0.55 |
| | Dialysis vintage | years | 3.7±2.1 | 3.3±3.8 | 0.57 |
| | Residual diuresis | ml | 430±655 | 444±541 | 0.91 |
| | Multi-organ Tx | % | 5.6 | 1.7 | 0.28 |
| Donor | Age | years | 46.8±11.4 | 47±11.4 | 0.93 |
| | Sex ratio | % female | 42 | 46 | 0.67 |
| | LD | % | 2.8 | 1.6 | 0.70 |
| | DCD | % | 30.6 | 21.3 | 0.31 |
| Transplantation | CIT | min | 779±297 | 825±255 | 0.43 |

**Conclusions:** These retrospective observations suggest that CaSR activation at the time of Tx impairs early graft recovery.

## SA-PO984

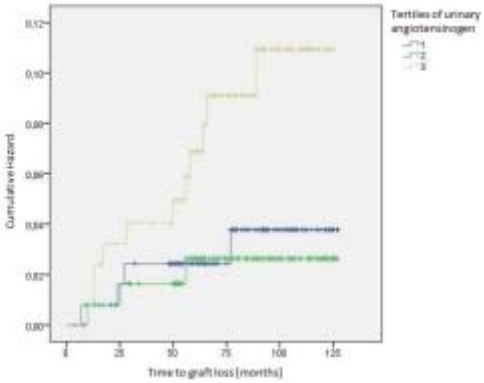
### Urinary Angiotensinogen Levels Are Associated with Graft Survival in Renal Transplant Recipients

Bernhard M.W. Schmidt, Wilfried Gwinner, Irina Scheffner, Mario Schiffer, Hermann G. Haller, Daniel Kayser. *Dept of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

**Background:** Intrarenal activity of the renin angiotensin aldosterone system (RAAS) is an important pathophysiological factor for the progression of renal disease. Urinary angiotensinogen (uAGT) levels have been shown to be a measure of intrarenal RAAS activity. We hypothesized that uAGT levels three months after renal transplantation (rTx) may predict long term graft survival.

**Methods:** We assessed uAGT in patients participating in our rTx protocol biopsy program using a commercially available assay (IBL, Japan). All samples had been stored at -80°C for at least five years to assure long term follow up of patients. Primary endpoint of our analysis was death censored graft survival. Tertiles of uAGT were built, Kaplan-Meier curves and log-rank test were used for analysis.

**Results:** The highest tertile of uAGT levels was associated with worse graft survival (figure), p=.048, log-rank test. Mean graft survival in the highest tertile was 117.0 (95% CI 112.4, 121.6) months vs. 124.5 (95% CI 121.7, 127.3) and 123.8 (95% CI 120.7, 126.9) in the middle and lowest tertile, respectively. uAGt was weakly correlated with donor age (r= .16 , p= .002) and best creatinine (r= .21, p< .001) but not modulated by treatment with ACE inhibitors or angiotensin receptor blockers.




**Conclusions:** In conclusion higher uAGT levels reflecting intrarenal RAAS activity are associated with worse graft survival. This reflects a potential pathophysiological role in graft failure e.g. due to profibrotic effects of angiotensin II and aldosterone. Therapeutic strategies to reduce intrarenal RAAS activity e.g. high dose RAAS blockade might be useful to improve long-term graft survival.

*Funding:* Private Foundation Support

## SA-PO985

### Adherence to Phosphate Binders and Low-Phosphorus Diet in Pediatric Kidney Transplant Candidates Is Associated with Post-Transplant Adherence to Immunosuppressant Medications

Oleh M. Akchurin,[1] Anita V. Tambay,[2] Frederick J. Kaskel,[1] Marcela Del Rio.[1] *[1]Albert Einstein College of Medicine / Children's Hospital at Montefiore; [2]Ponce School of Medicine and Health Sciences.*

**Background:** Non-adherence to treatment regimens is common in pediatric and adolescent transplant recipients and is the major cause of graft loss. During the transplant selection process, it is desirable to identify patients at high risk for non-adherence. Non-adherence to low-phosphorus diet and phosphate binders in patients with CKD and on dialysis is commonly used as a predictor of their post-transplant non-adherence. However, little evidence exists to support this assumption.

**Methods:** This is a retrospective IRB-approved study. Pre-transplant adherence to diet and phosphate binders was assessed by serum phosphorus levels during the last 2 years before transplant. Patients were categorized into non-adherent (NA) vs adherent (A) using the median serum phosphorus value as a cutoff. Post-transplant adherence was assessed by the number of sub-therapeutic and/or undetectable tacrolimus levels (UTL). Post-transplant adherence was analyzed for a 1-year period starting 1 year post transplant. Statistical methods included Chi-square and Mann-Whitney tests.

**Results:** Fifty three patients who received their first kidney transplants in our Center between 1990 and 2011 were included in the analysis. In the group that was NA to phosphate binders and low-phosphorus diet before the transplant, 69% of patients had at least one UTL during the time of post-transplant observation, which was statistically significantly higher than in the A group (40%, p=0.037). The median number of UTL episodes was also statistically significantly higher in the NA group compared with the A group: 2 [0.0-4.3] vs 0 [0.0-3.0] episodes respectively, p=0.04.

**Conclusions:** Pre-transplant adherence to low-phosphorus diet and phosphate binders was statistically significantly associated with post-transplant adherence to tacrolimus in our cohort. Analyses of the relationship between post-transplant adherence and other measures of pre-transplant adherence (e.g., interdialytic weight gain in hemodialysis patients), as well as multivariate analyses are ongoing.

## SA-PO986

**Adherence and Graft Survival in Pediatric Kidney Transplant Recipients Who Transition to Adult Services** Oleh M. Akchurin,[1] Anita V. Tambay,[2] Frederick J. Kaskel,[1] Rebecca Hashim,[1] Marcela Del Rio.[1] *[1]Albert Einstein College of Medicine / Montefiore Medical Center; [2]Ponce School of Medicine and Health Sciences.*

**Background:** Adolescent kidney transplant recipients have a high rate of graft loss. Transition of these patients from pediatric to adult care has been proposed as a risk factor for non-adherence and graft failure. However, data about adherence and graft survival after transition are limited and conflicting.

**Methods:** Retrospective IRB-approved study. Undetectable and/or sub-therapeutic serum tacrolimus levels and their variability were used to measure adherence and were compared between 2 years before and 2 after transition. Graft survival for pediatric patients during 4 years after transition was compared with 4 years before transition and also with post transplant of young adults starting 1-year post transplant.

**Results:** Three groups of patients were analyzed: (1) pediatric patients older than 18 years of age prior to their transition to the adult program (n=71), (2) those who were actually transitioned to the adult program (n=25), and (3) young adults 20-25 years of age who received their first transplant in the adult program (n=62). The median age of group 2 at transition was 22.5 [21.7–23.3] years. Overall, there were no significant differences in adherence within group 2 before and after transition, however patients with lowest tertile of baseline adherence showed improvement of their adherence after transition, which was significantly different from the patients with highest tertile of baseline adherence, p=0.02. The peak of graft loss in all pediatric transplant recipients occurred at 18.3±5.7 years. The rate of graft loss was 0.10 in group 1 (19 losses in 185.6 person-years), 0.06 in group 2 (4 losses in 67.9 patient-years), and 0.07 in group 3 (14 losses in 194.7 person-years). The differences in rate of graft loss between the groups were not statistically significant.

**Conclusions:** Transition of pediatric transplant recipients to the adult program was not statistically associated with worsening adherence and graft loss rate was not significantly different between the groups. Large-scale studies are needed to assess the nationwide impact of transition on adherence and graft loss.

## SA-PO987

**SHROOM3, a SNP and Renal Allograft Fibrosis** Madhav C. Menon,[1] Peter Y. Chuang,[1] Zhengzhe Li,[1] Weijia Zhang,[1] Yi Luan,[1] Philip O'Connell,[3] Robert B. Colvin,[2] Bernd Schroppel,[1] John C. He,[1] Barbara T. Murphy.[1] *[1]Icahn School of Medicine, Mount Sinai, NY; [2]Harvard Medical School, MA; [3]Westmead Clinical School, Sydney.*

**Background:** The Genomics of Chronic Allograft Rejection (GoCAR) is a multicenter study to understand the mechanisms of chronic allograft nephropathy (CAN).

**Methods:** Serial allograft biopsies were performed on 589 GoCAR participants. To identify molecular drivers of CAN, we examined genes whose expression level in the allograft 3 months after transplantation correlated with indices of allograft dysfunction(chronic allograft dysfunction index score(CADI), eGFR) at 12 months after transplantation in 160 participants.

**Results:** Higher allograft levels of *SHROOM3* transcript, antedated and correlated with CADI and, inversely with eGFR ($P<0.01$). Genome wide association studies have previously identified an intronic polymorphism (SNP) in *SHROOM3* (rs1731972) as associated with CKD. G and A are the major and minor alleles at this site respectively. We observed that allograft *SHROOM3* expression was higher with the A/G or A/A genotypes, compared to G/G **in the donor** ($P=0.02$). Recipients who received allografts from A/G or A/A donors were more likely to have a CADI≥2 at 12-mths than recipients of G/G allografts (OR-1.98; p=0.02). rs173192 is located within a consensus binding sequence for transcription factor 4 (TCF4/TCF7L2) downstream of Wnt-ßCatenin signaling, and overexpression of TCF4 increased *SHROOM3* expression in HK2 cells. The A-allele alone had enhancer activity compared to the G-allele with SHROOM3 promoter-enhancer reporter assays. TGF-β up-regulated *SHROOM3* expression through a ßCatenin-TCF4 mediated mechanism, while SHROOM3 facilitated canonical TGFβ-signaling (Smad3 phosphorylation) and Collagen-1 production.

**Conclusions:** In summary, we found that *SHROOM3* expression preceded the development of chronic allograft injury and demonstrated that SHROOM3 facilitates TGF-β signaling. The A-allele of rs173192 exhibited a TCF4-dependent enhancer function, and confers increased risk for CAN at 12-mths. This is the first description of any CKD-associated SNP that modulates gene expression. *SHROOM3* may serve as a novel therapeutic target common to both CAN and CKD.

*Funding:* NIDDK Support, Other NIH Support - NIAID, Private Foundation Support

## SA-PO988

**A Model to Identify Patients at Risk for Post-Transplant Kidney Disease after Liver Transplantation** Mario Schiffer,[1] Christian Lerch,[1] Eleni Evangelidou,[2] Hermann G. Haller,[1] Tobias J. Weismüller.[2] *[1]Nephrology, Hannover Medical School, Hannover, Germany; [2]Dept of Internal Medicine 1, Univ of Bonn, Bonn, Germany.*

**Background:** Following liver transplantation (LT) a significant subgroup of the recipients develops chronic kidney disease (CKD) ranging from mild impairment of kidney function to need for chronic hemodialysis treatment. Since CKD aftrer liver transplantation is multifactorial, so far no reliable models exist to predict the development of CKD in liver graft recipients. The goal of this study was the development of a predictive model to estimate the development of renal function after LT based on parameters known before LT.

**Methods:** We retrospectively analysed clinical and biochemical pre- and perioperative parameters of 328 liver recipients transplanted between 2004 - 2008 and developed ordinal logistic regression models which predict CKD stage 3 or CKD stage 4 or worse after LT. The five most important variables of this model were taken to build a more simplified model for clinical use. These variables were diagnosis of PSC, Hepatitis C, Age, GFR before LT and history of diabetes.

**Results:** The full model allowed with high accuracy the prediction of CKD stage 3 or CKD stage 4 or worse. The simplified model includes 5 parameters (diabetes mellitus, primary sclerosing cholangitis, hepatitis C, recipient-age, cystatin-c based glomerular filtration rate before LT) and showed a good accuracy in the prediction of CKD ≥ stage 3 (AUC =0.739) resp. CKD ≥ stage 4 (AUC = 0.774). To validate these data we performed an external validation and tested the model in a prospective cohort and confirmed an acceptable accuracy for the prediction of CKD ≥ stage 3 (AUC =0.716) resp. CKD ≥ stage 4 (AUC = 0.639).

**Conclusions:** In conclusion our model allows based on five parameters known before LT the identification of patients at risk to develop CKD Stage 3, 4 or worse after LT.

*Funding:* Government Support - Non-U.S.

## SA-PO989

**Serum Cystatin C in Renal Transplantation: Beyond GFR Estimation, a Prognosis Marker?** Ingrid Masson, Nicolas Maillard. *Nephrology Dialysis Renal Transplantation, Univ Hospital, Saint-Etienne, France.*

**Background:** In renal transplantation, death with a functioning graft remains one of the main causes of graft loss. In the general population, renal function impairment is strongly associated with cardiovascular and all cause mortality. Whether this association holds true for kidney transplant recipients (KTR) is unclear. This uncertainty is likely to be due, in part, to the fact that glomerular filtration rate (GFR) estimation based on serum creatinine (SCr) does not always provide an accurate evaluation of the graft function in KTR. As compared to SCr, we have recently shown in a large cohort of KTR that serum cystatin C (SCysC) is a much better marker of GFR.

Herein, we sought to study the ability of the 1-year-post-transplant renal function to predict all cause mortality according to the methods used to assess GFR.

**Methods:** Three hundred and forty two consecutive KTR for whom a measurement of GFR by inulin clearance was available at 1 year post-transplant were included. SCr and ScysC were measured with standardised methods. The association of the 1-year inulin clearance value the 1-year MDRD Study equation value and the 1-year CKD-EPI ScysC equation value with all cause mortality was studied by ROC analysis and Cox model.

**Results:** During a median follow-up of 145 months, 70 KTR died. Mean (±SD) inulin clearance at 1-year-post-transplant was 46 (±19) mL/min/1.73m$^2$. Aeras under the ROC curves were similar for inulin and CKD-EPI ScysC equation values (0.564 and 0.550, respectively, NS), and were both significantly superior to that of the MDRD equation (0.483, $p<0.01$). In Cox analysis, while all types of GFR evaluations were significantly associated to graft loss, only an inulin and a CKD-EPI ScysC equation values below 45 mL/min/1.73m$^2$ were associated with an excess risk of mortality (HR of 1.85 vs 1.01 for both inulin and CKD-EPI ScysC vs MDRD, respectively).

**Conclusions:** We conclude that ScysC-based GFR estimation might better predict KTR outcome as compared to a traditional SCr-based estimation. The one year-post transplant GFR value given by the CKD-EPI ScysC equation should be further evaluated as a potential surrogate marker for both graft and patient survival.

## SA-PO990

**The Performance of Cystatin C (CystC) and Creatinine Based CKD-EPI Equations to Estimate GFR in Kidney Transplant (KTx) Recipients** Mira T. Keddis, Hatem Amer, Nick Voskoboev, Andrew D. Rule, John C. Lieske. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** Performance of CystC- and creatinine-based CKD-EPI equations published in 2012 have not been evaluated in a large cohort of stable KTx recipients.

**Methods:** A prospective cross-sectional cohort of KTx patients presenting for routine annual visits and who are more than 1 year from transplant were actively recruited. GFR was measured by iothalamate clearance (mGFR). Plasma CystC was analyzed by particle-enhanced immunoturbidimetry assay, while serum creatinine (Cr) was measured by enzymatic assay, both traceable to international reference materials. eGFR was calculated using published 2012 CKD-EPI equations. Overall bias and accuracy (within 10% and 30%) for each eGFR equation were calculated.

**Results:** 1151 KTx patients were studied. Mean age was (mean+SD) 56.0 ± 14.2 years; 56.5% were male and 85.6% were Caucasian. mGFR was 55.7 ± 20.7 ml/min/1.73m$^2$.

| eGFR equations | GFR mean, median (range) | Percentage bias (95% CI) | Bias (log-logscale) (95% CI) | Accurate within 10% (P10) | Accurate within 30% (P30) |
|---|---|---|---|---|---|
| CKD-EPI Creatinine | 55.1, 52.8 (9.3, 168.1) | 4.1% (2.2, 6.1) | 0.1% (1.6, -1.5) | 34% | 80% |
| CKD-EPI CystatinC | 44.8, 43.2 (3.39, 113.9) | -17.5% (-18.8, -16.3) | -19.9 % (-18.8, -21.0) | 23% | 72% |
| CKD-EPI Creatinine-CystatinC | 48.6, 47.3 (9.93, 128.2) | -9.9% (-11.2, -8.6) | -12.1% (-13.2, -11.0) | 30% | 84% |

**Conclusions:** The creatinine-based CKD EPI equation outperformed CystC and CystC-Cr equations in a large cohort of stable KTx patients. CystC and CystC-Cr equations had significant negative bias.

*Funding:* Pharmaceutical Company Support - Gentian

## SA-PO991

**Endothelin-1 and Kidney Function in Renal Allograft Recipients** Rupesh Raina,[1] Michael S. Simonson,[2] Beth A. Vogt,[1] Joshua J. Augustine,[2] Donald E. Hricik.[2] *[1]Dept of Pediatrics, Nephrology, Univ Hospitals Rainbow Babies and Children's Hospital, Cleveland, OH; [2]Dept of Medicine, Nephrology, Univ Hospitals Case Medical Center and Case Western Reserve Univ, Cleveland, OH.*

**Background:** Endothelin-1 (ET-1), a vasoconstrictor and pro-inflammatory peptide, is elevated in chronic allograft nephropathy, and studies in experimental models suggest that ET-1 is a determinant of graft loss. However, whether elevated ET-1 is associated with allograft dysfunction in humans is unclear.

**Methods:** We examined the association between urinary ET-1, a non-invasive surrogate for ET-1 in kidney cortex, and renal function in an exploratory cross-sectional study of 28 renal allograft recipients 3 to 56 months post-transplant. ET-1was measured by ELISA in spot urine specimens and corrected for urinary creatinine. Glomerular filtration rate was estimated (eGFR) by the simplified MDRD equation.

**Results:** ET-1correlated inversely with eGFR ($r$=-0.599, $P$=0.01) independent of recipient age, sex, and donor age. ET-1 did not correlate with urinary albumin/creatinine ratio nor with IgG/creatinine, a marker of increased glomerular permselectivity. ET-1 was modestly elevated in patients with posttransplant hypertension (Systolic Blood Pressure > 140 mm Hg) compared to normotensive allograft recipients (5.4 ± 2.6 vs. 3.2 ± 1.5pg/mg creatinine, $P$=0.02). Consistent with findings in experimental models, ET-1correlated with a urine biomarker of allograft inflammation, macrophage chemoattractant protein-1 ($r$= 0.638, $P$<0.01). ET-1 was higher in participants with prior acute rejection (6.5 ± 1.6 vs. 3.9 ± 2.3 pg/mg creatinine, P=0.002) compared to those without rejection but not in those with prior delayed graft function (4.4 ± 2.5 vs. 4.3 ± 2.2, $P$=0.937).

**Conclusions:** These cross-sectional data are consistent with the hypothesis thatelevated ET-1is associated with low eGFR and inflammation in kidney transplant recipients, particularly in patients with prior episodes of acute rejection.

## SA-PO992

**Tubular Biomarkers in Serum and Urine of Stable Kidney Transplant Patients – Prospective Observational Study** Bertram Hartmann, Frieder Keller. *Internal Medicine 1, Nephrology, Univ Hospital, Ulm, Germany.*

**Background:** The surveillance and control of kidney function relay on serum creatinine and glomerular filtration rate (GFR) while interstitial fibrosis and tubular atrophy are leading pathological findings in kidney transplant biopsies.

**Methods:** This is a prospective observational study in a cohort of 39 stable kidney transplant patients. Lisa Schupp and the authors measured on routine appointments standard laboratory markers (e.g. serum creatinine, eGFR) as well as 8 different biomarkers in serum and urine: Kidney injury molecule-1 (TIM1/KIM1), monocyte chemotactic protein-1 (MCP-1), cysteine-rich protein 61 (Cyr 61), neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid-binding protein (L-FABP), fetuin A, interleukin-18 binding protein (IL-18 BP), retinol-binding protein 4 (RBP4). All specimens were measured with the ELISA method and the results were correlated with the eGFR (MDRD2 Formula) by linear regression analysis (SPSS).

**Results:** The eGFR was on average at 40 +/- 16 ml/min (range 6 – 76) but stable for the last 3 months. RBP4, Fetuin A, NGAL, Cyr61, MCP1 are higher concentrated in serum than in the urine. Urine and serum biomarkers were significancantly correlated to the eGFR ($p < 0.01$). Thus, urine and serum biomarker exhibit a perplexing pattern, the worse the kidney function the higher are the concentrations of urine as well as of serum biomarkers.

**Conclusions:** Since only patients with stable transplant function were investigated, the elevated biomarkers less likely can be correlated to cellular or humoral rejection. However, biomarkers that are high in serum and in urine could indicate interstitial fibrosis and tubular atrophy.

## SA-PO993

**Application of an Established CKD Biomarker Signature to Predict Chronic Renal Impairment after Solid Organ Transplantation** Jens Drube,[1] Eric Schiffer,[2] Harald Mischak,[2] Christian Lerch,[1] Hermann G. Haller,[3] Mario Schiffer.[3] *[1]Pediatrics, Hannover Medical School; [2]Mosaiques Diagnostics GmbH; [3]Nephrology, Hannover Medical School.*

**Background:** After solid organ transplantation (SOT) some patients develop CKD. This is a serious clinical problem as CKD negatively affects the survival of organs and patients. Momentarily, it is impossible to predict prior to transplantation patients at risk to develop CKD after SOT. Therefore, therapies cannot be adjusted to individual CKD risk profiles. The aim of this study was to test the ability of a CKD biomarker-pattern to detect previously published by *Good et al. 2010* to predict CKD after transplantation using prior transplantation urine samples.

**Methods:** From the prospective German RECAST register we generated two groups of transplanted subjects, a case group (lung n=5, heart n=5, liver n=1) and a control group (lung n= 7, heart n=3, liver n=2) who had a GFR below 55 or above 70 ml/min/1,73m² three months after transplantation, respectively. We conducted a capillary electrophoresis and mass spectrometry proteome analysis on urine obtained before transplantation. Each proteome profile was compared to the pattern published by *Good et al. 2010*. Additionally, we analyzed which individual biomarkers of the pattern correlated strongest with CKD outcome after transplantation.

**Results:** The biomarker-pattern published by >*Good et al.* was able to predict renal impairment three months after SOT with a sensitivity of 91% and specificity of 67%. The AUC of the ROC analysis was 0.71. Four of the 273 previously defined biomarkers showed a significant correlation with the GFR three months after SOT, another ten showed a significant trend, when P-values corrected for multiple testing.

**Conclusions:** This study shows that the biomarker-pattern published by *Good et al. 2010* can predict the development of CKD three months after SOT from urine obtained before transplantation. These results can be used to refine the biomarker pattern so it can be used in clinical practice.

*Funding:* Government Support - Non-U.S.

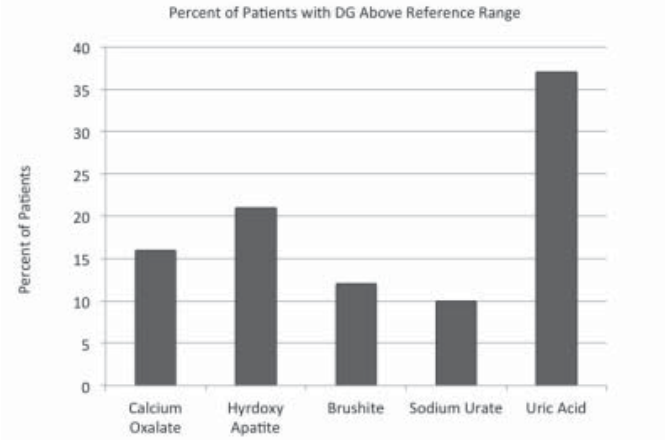
## SA-PO994

**Urinary Super Saturation in Newly Transplanted Kidney Transplant Recipients: Cause for Concern and Action** Hatem Amer,[1] Elizabeth C. Lorenz,[1] Dawn S. Milliner,[1] Andrew D. Rule,[1] Eric J. Bergstralh,[2] John C. Lieske.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Health Sciences Research, Mayo Clinic.*

**Background:** Renal allograft are vulnerable to calcification compared to native kidneys whichis a cause for decreased renal allograft function. Risk factors include pre transplant duration of dialysis and secondary hyperparathyroidism. Urinary composition plays a significant role. No systematic evaluation of post-transplant urine supersaturation has been performed.

**Methods:** Funded by the Rare Kidney Stone Consortium we prospectively enrolled a cohort of 100 consecutive incident kidney only transplant recipients. The study subjects completed a 24-hour urine collection for a super saturation profile 2-4 weeks from transplant.

**Results:** Age 52.4± 13.3 years, males 61%, Caucasian race 90%, pre-emptive transplants 51%, deceased donor organ source 14%, and first transplant 94%. Serum PTH 146 ± 168 [28-1269] pg/ml, serum calcium 9.5± 0.5 [7.9-11.2] mg/dL, serum phosphorus 3.0± 0.7 [1.1-5.3] mg/dL. 24-hour urine volume was mean 2540 ± 1028 ml, urine pH 5.9± 0.6 [4.9-7.8]. Daily urine excretion of sodium was 165± 73 [12-416], phosphorus 865± 373 [74-1868], oxalate 0.38± 0.14[0.05-0.84] mmol, calcium 112± 78[6-446], citrate 269±176 [24-881] mg.The Delta G (DG) as the measure of super saturation with positive values indicating super saturation was Brushite -1.9±1.6, Hyrdroxy apatite 1.8± 2.4, sodium urate 0.26±1.1, uric acid -0.5±2.9 and oxalate 0.9±0.9. More importantly figure 1 shows the proportion of patients with DG > reference limit for non-stone formers.




**Conclusions:** For the first time we present a series of urine super saturation profiles in incident kidney transplant recipients. A significant proportion of transplant recipients have urines super saturated above the reference for a variety of crystals putting them at risk for intra graft calcification and crystal deposition.

*Funding:* NIDDK Support, Other NIH Support - National Center For Advancing Translational Sciences (NCATS)

## SA-PO995

**Vitamin D Status and Long-Term Outcomes after Kidney Transplantation** Charlotte A. Keyzer,[1] Ineke J. Riphagen,[1] Michel M. Joosten,[1] Gerjan Navis,[1] Anna Muller Kobold,[2] Ido Peter Kema,[2] Stephan J.L. Bakker,[1] Martin H. De Borst.[1] *[1]Nephrology; [2]Laboratory Medicine, Univ Medical Center Groningen, On Behalf of NIGRAM, Netherlands.*

**Background:** Recent studies linked 25OH but not 1,25OH2 vitamin D deficiency to lower GFR one year after kidney transplantation (KTx), but whether this discrepancy also holds for long-term hard outcomes is unclear. We investigated the value of circulating 25OH and 1,25OH2 vitamin D levels as risk factors for graft failure and mortality in stable renal transplant recipients (RTR).

**Methods:** 25OH and 1,25OH2 vitamin D levels were measured by LC-MS/MS in a cohort of stable RTR transplanted in our center between 2001 and 2003, with a functioning graft for ≥1 year. Associations between vitamin D status and the risk of death-censored graft failure or all-cause mortality were investigated by multivariate Cox regression analyses adjusting for known risk factors.

**Results:** 448 RTR (51% men, age (mean±SD) 51.9±11.9 years, median 6.4 [IQR 3.1-11.8] years after KTx, eGFR (CKD-EPI) 47±16 mL/min) were followed for 7.1 [6.2-7.5] years. Median 25OH and 1,25OH2 vitamin D levels were respectively 50.3 [35.7-68.0] nmol/L and 104.4 [76.0-128.6] pmol/L. In multivariable Cox regression analyses, neither 25OH nor 1,25OH2 vitamin D was associated with graft failure (44 events). In univariate Cox regression, 25OH vitamin D was associated with all-cause mortality (HR 0.83 (95% CI 0.76 to 0.92) per 10 nmol/L increase; P<0.001; 99 events). Adjustment for potential confounders including renal function, seasonal variation and Framingham risk factors did not materially change this association. In univariate Cox-regression, 1,25OH vitamin D was associated with all-cause mortality (HR 0.92 (0.88 to 0.97) per 10 pmol/L increase; P=0.002). However, this association lost significance after adjustment for renal function.

**Conclusions:** Low circulating 25OH but not 1,25OH2 vitamin D is an independent risk factor for all-cause mortality after KTx. Given the abundant presence of 1α-hydroxylase in renal and extra-renal tissues, our findings supports a protective role for autocrine/paracrine vitamin D activation. Future studies should address whether vitamin D supplementation improves outcome after KTx.

*Funding:* Government Support - Non-U.S.

## SA-PO996

**Initial Bone Mineral Density Status Predicts the Development of New Vertebral Fractures during Kidney Transplantation** Carlo M. Alfieri,[1] Brigida Brezzi,[1] Fabio Massimo Ulivieri,[2] Maria Meneghini,[1] Riccardo Floreani,[1] Anna Regalia,[1] Francesco Barretta,[3] Manuela Curreri,[1] Maria Daniela Croci,[1] Maria Pia Rastaldi,[1] Piergiorgio Messa.[1] *[1]Nephrology, Dialysis and Transplantation, Fondazione IRCCS Ca'Granda Osp. Maggiore Policlinico, Milan, Mi, Italy; [2]Osteporosis Centre, Fondazione IRCCS Ca'Granda Osp. Maggiore Policlinico, Milan, Mi, Italy; [3]Clinical Sciences and Community Health,Epidemiology Unit, Dept of Preventive Medicine, Fondazione IRCCS Ca'Granda Osp. Maggiore Policlinico, Milan, Mi, Italy.*

**Background:** Bone disorders and related skeletal fractures are common events during kidney transplantation (KTx). In our study we evaluated: 1) the prevalence of asymptomatic vertebral fractures (VFx) at baseline and 1 year after KTx; 2) the factors associated with the occurrence of new VFx during the first year after KTx; 3) the role of bone mineral density (BMD) in the prediction of new VFx.

**Methods:** 109 consecutive KTx patients (57 M–age range 17-73 years) were evaluated at the 1st and 12th mth for VFx by vertebral X-ray (Genant et al, J Bone Miner Res. 1993 Sep;8(9):1137-48). In addition to the routine biochemical evaluation, FGF-23, Fetuin, Osteoprotegerin (OPG) and 25OH-VitD levels were also evaluated at 1, 6 and 12 mths after KTx. In 87 of these patients BMD, assessed by dual energy X-ray absorption (DEXA),was also performed.

**Results:** VFx were present in 22% and 37% of pts at 1st and 12th mth resp. VFx at 1 mth correlated only with serum phosphorus (P) (directly) and PTH (inversely), whereas at 12 mths only with the number of fractures at baseline. Of the studied patients, 25% had one or more new VFx during the first 12 mths of KTx (VFx-PROG+). VFx-PROG+ had lower BMD at baseline, which was a good predictor for new VFx (ROC:AUC 0,6962), with no difference in PTH, Vit-D status, cumulative steroid doses, FGF-23, Fetuin, and OPG levels compared with VFx- non progressors.

**Conclusions:** According to our results: 1) the prevalence of VFx is high in KTx patients and 25% of them have at least one new VFx during the first year after KTx; 2) baseline BMD might be predictive of the occurrence of new VFx; 3) mineral metabolism related parameters do not seem to play a major role in the occurrence of new VFx.

## SA-PO997

**Can Steroids Be Withdrawn Safely in Routine Practice? Prospective, Four-Year Data from the Mycophenolic Acid Observational REnal Transplant (MORE) Study** V. Ram Peddi,[1] Kimi Ueda Stevenson,[1] Anne Wiland,[2] Kevin M. McCague.[2] *[1]California Pacific Medical Center, San Francisco, CA; [2]Novartis Pharmaceuticals Corporation, East Hanover, NJ.*

**Background:** Corticosteroid withdrawal (CSW) after kidney transplantation is widespread, but prospective data are lacking on the long-term implications for acute rejection, graft survival and graft function vs corticosteroid continuation (CSC) in routine clinical practice.

**Methods:** Prospective data were analyzed from the observational, four-year MORE study of de novo adult kidney transplant patients receiving mycophenolic acid at 40 centers in the US, managed according to local practice. Cox regression analysis adjusted efficacy outcomes for recipient gender, African vs non-African American, deceased vs living donor, PRA status (<30% vs ≥30%), delayed vs immediate graft function and baseline mycophenolic acid dose (<2g/day vs ≥2g/day).

**Results:** 363 CSW and 509 CSC patients were analyzed. All patients received tacrolimus, with similar trough levels in the CSW and CSC cohorts. The observed four-year incidence of efficacy events in the CSW vs CSC groups was 10.1% vs 14.3% for biopsy-proven acute rejection (BPAR, p=0.12), 3.1% vs 6.3% for graft loss (p=0.030) and 4.4% vs 5.0% for death (p=0.65). Cox regression analysis showed hazard ratios (HR) for CSW vs CSC to be 0.75 (95% CI 0.46, 1.21; p=0.25) for BPAR, 0.35 (0.12, 0.86; p=0.032) for graft loss and 0.82 (0.31, 2.09; p=0.68) for death. Mean estimated GFR (CKD-EPI formula) for CSW vs CSC was 59.5 vs 57.5mL/min/1.73m$^2$ (p=0.84) at one year post-transplant, 59.7 vs 60.3mL/min/1.73m$^2$ (p=0.42) at two years, and 57.7 vs 58.1mL/min/1.73m$^2$ (p=0.44) at three years (numbers at four years were too low for meaningful analysis).

**Conclusions:** After adjustment for confounding variables, CSW by month 3 after kidney transplantation was associated with significantly higher four-year graft survival than CSC, with no increase in BPAR or reduction in graft function vs CSC in routine practice.

*Funding:* Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

## SA-PO998

**Impact of Maintenance Steroids versus Rapid Steroid Withdrawal in African-American Kidney Transplant Recipients: Comparison of Two Centers** Divya Jain Arwindekar,[2] Revathi C. Belur,[1] Woojin James Chon,[2] Amishi S. Desai,[1] Ignatius Yun-Sang Tang,[1] Michelle A. Josephson,[2] Sanjeev Akkina.[1] *[1]Medicine, Univ of Illinois at Chicago, Chicago, IL; [2]Medicine, Univ of Chicago, Chicago, IL.*

**Background:** Rapid steroid withdrawal (RSW) is used increasingly in kidney transplantation but long-term outcomes in African-American (AA) recipients are not well known. Our objective was to compare 5 year transplant outcomes in a large cohort of AA patients who underwent RSW to those who were maintained on steroids (CS).

**Methods:** Post-transplant courses of AA receiving a kidney allograft between 2003-2011 at two urban transplant centers were followed. Both centers use thymoglobulin induction with tacrolimus and mycophenolate mofetil as maintenance therapy. The patients at Center 1 (C1) underwent RSW with completion of therapy by day 5. At Center 2 (C2), patients were generally tapered to a maintenance dose of 5 mg of prednisone daily by 1 month post-transplant. The data were analyzed to compare death-centered graft survival, graft survival, patient survival, and MDRD GFR at 1 and 5 years post-transplant between the RSW and CS groups. Additionally, biopsy-proven acute rejections (BPAR) in the first year were compared between the two groups. An intention-to-treat analysis was used with C1.

**Results:** There were 381 AA recipients at C1 and 299 at C2. C1 recipients were younger (48.6 vs. 50.6, p = 0.048), had more living donors (64% vs 20%, p < 0.0001), more expanded criteria donors (9.5% vs. 5.0%, p=0.03), more hypertensive kidney disease (68% vs. 32%, p<0.0001), and less diabetic kidney disease (6.6% vs 22%, p<0.0001) compared to C2. Death-censored and patient survival was similar between the two centers. Other outcomes are shown in the table.

| Outcome | C1 | C2 | p-value |
|---|---|---|---|
| 1 Year Graft Survival | 97% | 96% | NS |
| 1 Year GFR(mL/min/1.73m2) | 53.4±17.8 | 58.9±25.0 | 0.002 |
| 1 Year Biopsy Proven Rejection | 19.4% | 11.4% | 0.004 |
| | | | |
| 5 Year Graft Survival | 75% | 72% | NS |
| 5 Year GFR (mL/min/1.73m2) | 52.5±20.4 | 56.4±23.2 | 0.18 |

**Conclusions:** Graft survival and GFR were similar between the two centers but BPAR was higher in the RSW group. Our findings suggest RSW regimens can be used in AA with similar outcomes to CS regimens.

*Funding:* NIDDK Support

## SA-PO999

**The Impact of the Transplant Pharmacists on Cardiovascular Risk Reduction in Kidney Transplant Recipients** Caitlin R. Musgrave, David J. Taber, Nicole A. Pilch, Beje S. Thomas, Titte Srinivas. *Medical Univ of South Carolina, Charleston, SC.*

**Background:** Reductions in acute rejection have not translated into comparable improvements in long-term graft survival in kidney transplant (KTX) pts. Cardiovascular (CV) disease is the leading cause of late graft loss following KTX, and control of HTN, DM, and hyperlipidemia is an essential strategy to prevent CV events. The aim of this study was to determine the impact of txp PharmD interventions on the management of CV risk factors in KTX.

**Methods:** This prospective controlled pilot study involved 2-3 mos of enrollment followed by 6 mos of intervention. Patients were 3 mo-3 yr post-KTX and enrolled into the control group (CG) or intervention group (IG). IG pts participated in a pharmacist-run clinic during which the PharmD evaluated and modified CV-related medications through collaborative practice agreements. The control group received standard of care post-KTX.

**Results:** There were no significant differences in baseline characteristics between the two groups aside from more pts in the IG being on baseline ASA (p=0.05, tables 1-2). At study end, significantly more pts in the IG were started on CV meds for compelling indications (p=0.042, table 3) and started on ASA (p<0.001, table 2). Pts in the IG were also more likely to be started on an ACE I/ARB, ASA, or statins during follow-up (OR 2.4, 4.7, 5.9, respectively). No statistically significant changes in lipids, A1C, or pulse were noted; however, paired repeated measures analysis demonstrated that patients in the IG had significant reductions in SBP, which was not demonstrated in CG pts (-8.4±18.0 vs 5.7±31.6, p<0.05).

| | Control Group n=30 | Intervention Group n=24 | p-value |
|---|---|---|---|
| **Table 1. Baseline Characteristics** | | | |
| **Age**, mean ± SD | 55.9 ± 11.7 | 60.8 ± 10.8 | 0.116 |
| **Male sex**, n (%) | 23 (76.7%) | 13 (54.2%) | 0.093 |
| **Weight**, mean ± SD | 90.5 ± 20.3 | 93.2 ± 21.1 | 0.634 |
| **Race** | | | 0.156 |
| Caucasian | 14 (46.7%) | 6 (25.0%) | |
| African American | 16 (53.3%) | 18 (75.0%) | |
| **SCr**, mean ± SD | 1.51 ± 0.35 | 1.56 ± 0.48 | 0.645 |
| **Immunosuppressant use**, n (%) | | | |
| Tacrolimus | 27 (90.0%) | 23 (95.8%) | 0.620 |
| MMF | 26 (86.7%) | 20 (83.3%) | 1.000 |
| MPA | 0 (0.0%) | 2 (8.3%) | 0.193 |
| Prednisone | 27 (90.0%) | 23 (95.8%) | 0.620 |
| Everolimus | 1 (3.3%) | 0 (0.0%) | 1.000 |
| Belatacept | 0 (0.0%) | 1 (4.2%) | 0.444 |
| **Cardiovascular risk factors**, n (%) | | | |
| Hypertension | 28 (93.3%) | 24 (100%) | 0.497 |
| Diabetes | 29 (96.7%) | 22 (91.7%) | 0.579 |
| Previous CV history | 10 (33.3%) | 11 (45.8%) | 0.407 |

| **Table 2. Cardiovascular Medication Use at Baseline and Follow-Up** | Baseline | Follow Up | Baseline | Follow Up | p-value (baseline) | p-value (follow-up) |
|---|---|---|---|---|---|---|
| **Antihypertensives** | | | | | | |
| Beta blocker | 21 (70.0%) | 19 (63.3%) | 22 (91.7%) | 21 (87.5%) | 0.087 | 0.062 |
| ACE inhibitor/ARB | 10 (33.3%) | 13 (43.3%) | 8 (33.3%) | 13 (54.2%) | 1.000 | 0.584 |
| CCB | 20 (66.7%) | 18 (60.0%) | 19 (79.2%) | 18 (75.0%) | 0.370 | 0.384 |
| Diuretic | 9 (30.0%) | 10 (33.3%) | 10 (41.7%) | 9 (37.5%) | 0.404 | 0.781 |
| Vasodilator | 7 (23.3%) | 8 (26.7%) | 6 (25.0%) | 6 (25.0%) | 1.000 | 1.000 |
| Other | 10 (33.3%) | 9 (30.0%) | 5 (20.8%) | 3 (12.5%) | 0.370 | 0.190 |
| **Antidiabetics** | | | | | | |
| Insulin | 23 (76.7%) | 22 (73.3%) | 20 (83.3%) | 10 (79.2%) | 0.736 | 0.753 |
| Oral antidiabetic | 7 (23.3%) | 6 (20.0%) | 3 (12.5%) | 2 (8.3%) | 0.483 | 0.277 |
| **Antihyperlipidemics** | | | | | | |
| Statin | 18 (60.0%) | 17 (56.7%) | 15 (62.5%) | 18 (75.0%) | 1.000 | 0.252 |
| Fibrate | 1 (3.3%) | 2 (6.7%) | 1 (4.2%) | 1 (4.2%) | 1.000 | 1.000 |
| Niacin | 1 (3.3%) | 1 (3.3%) | 1 (4.2%) | 1 (4.2%) | 1.000 | 1.000 |
| Fish oil | 3 (10.0%) | 4 (13.3%) | 3 (12.5%) | 3 (12.5%) | 1.000 | 1.000 |
| **Aspirin Therapy** | | | | | | |
| Aspirin 81 mg | 7 (23.3%) | 9 (30.0%) | 12 (50.0%) | 19 (79.2%) | *0.051* | **<0.001** |
| Aspirin 325 mg | 4 (13.3%) | 3 (10.0%) | 2 (8.3%) | 1 (4.2%) | 0.682 | 0.620 |

| **Table 3. Patients Started on Medications with Compelling Indications** | | | p-value | Odds Ratio |
|---|---|---|---|---|
| Any medication with a compelling indication | 5 (16.7%) | 10 (41.7%) | **0.042** | 3.571 |
| Beta blocker | 0 (0.0%) | 1 (4.2%) | 0.444 | 0.434 |
| ACE inhibitor/ARB | 3 (10.0%) | 5 (20.8%) | 0.443 | 2.368 |
| Aspirin | 2 (6.7%) | 6 (25.0%) | 0.067 | 4.667 |
| Statin | 1 (3.3%) | 4 (16.7%) | 0.114 | 5.880 |

| **Table 4. Cardiovascular Risk Factors** | Baseline | Follow Up | Baseline | Follow Up | p-value* |
|---|---|---|---|---|---|
| LDL | 83.7 ± 37.0 | 101.9 ± 40.0 | 80.6 ± 24.0 | 92.8 ± 28.8 | 0.528 |
| HDL | 51.2 ± 24.0 | 49.9 ± 13.3 | 51.0 ± 13.2 | 50.4 ± 18.9 | 0.418 |
| Triglycerides | 165.1 ± 141.5 | 136.6 ± 68.0 | 150.4 ± 86.1 | 168.9 ± 97.3 | 0.430 |
| A1C | 7.01 ± 2.36 | 6.90 ± 1.45 | 7.12 ± 1.37 | 6.69 ± 1.16 | 0.839 |
| Systolic blood pressure | 136.7 ± 22.3 | 141.8 ± 26.6 | 145.5 ± 20.2 | 136.8 ± 12.8 | *0.066* |
| Diastolic blood pressure | 73.5 ± 11.6 | 81.1 ± 14.1 | 71.5 ± 10.0 | 74.4 ± 10.9 | 0.143 |
| Pulse | 71.2 ± 12.7 | 74.0 ± 15.3 | 70.6 ± 11.4 | 78.5 ± 13.3 | 0.216 |

***p-value for change from baseline to follow up in the control group vs. change from baseline to follow up in the intervention group**

**Conclusions:** The involvement of txp PharmDs in CV risk factor management led to improved prescribing of medications with compelling indications and lowers SBPs. Further studies are warranted to determine the impact of txp PharmD involvement on long-term KTX outcomes.

## SA-PO1000

### Patient Survival in Simultaneous Pancreas Kidney Transplant Recipients and the Paired Kidneys

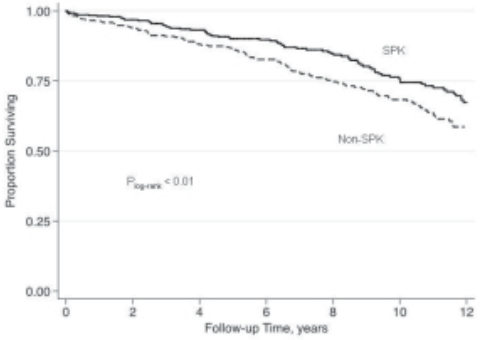
Brenda L. Muth,[1] Brad C. Astor,[1] Hans Sollinger,[2] Jon S. Odorico,[2] Arjang Djamali.[1] *[1]Nephrology, Univ of Wisconsin, Madison, WI; [2]Surgery, Univ of Wisconsin, Madison, WI.*

**Background:** Survival after simultaneous pancreas kidney (SPK) transplantation is better than deceased donor transplantation. However, it is unclear what roles donor and recipient factors play in this advantage.

**Methods:** We conducted a paired-organ analysis to examine patient and kidney graft survival in 453 pairs of recipients transplanted at the University of Wisconsin between 1994 and 2010.

**Results:** Non-SPK recipients were older, more likely to be sensitized and have DGF, less likely to be white and to recieve a pre-emptive transplant, and had longer cold ischemia time (CIT) than those with SPK.

93 (20.5%) SPK and 108 (23.8%) non-SPK recipients died over a median of 5.9 years.




SPK was associated with a lower risk of death (adjusted hazard ratio [HR]=0.50; 95% CI: 0.30, 0.82). Donor (0.49; 0.28,0.76) and recipient age <45 years (0.59; 0.43, 0.80) were also associated with improved patient survival. DGF was associated with a two-fold higher risk of death (2.04; 1.35,3.10).

| | SPK | Non-SPK | p |
|---|---|---|---|
| Age (Mean ± SD) | 40 ± 7 | 44 ± 12 | <0.001 |
| Female (%) | 39 | 44 | 0.08 |
| Diabetes | 100 | 13 | <0.001 |
| PRA > 10% (%) | 4 ± 21 | 27 ± 44 | <0.001 |
| Pre-emptive Transplant (%) | 33 | 12 | <0.001 |
| CIT (hr ± SD) | 14 ± 5 | 19 ± 7 | < 0.001 |
| DCD (%) | 12 | 12 | |
| Donor Age (Mean ± SD) | 30 ± 13 | 30 ± 13 | |
| DGF (%) | 10 | 20 | <0.001 |

Kidney grafts failed in 179 (39.5%) non-SPK vs 147 (32.5%). SPK (0.59; 0.39, 0.88) and white race (0.59; 0.42, 0.83) were associated with lower risk of failure, whereas donor age >45 years (1.74; 1.36, 2.23) and DGF (1.79; 1.32, 244 were associated with greater risk of failure. Adjustment for CIT did not change these associations.

**Conclusions:** Both donor (age) and recipient (SPK vs non-SPK, age, race) characteristics as well as DGF are related to patient and kidney graft survival.

## SA-PO1001

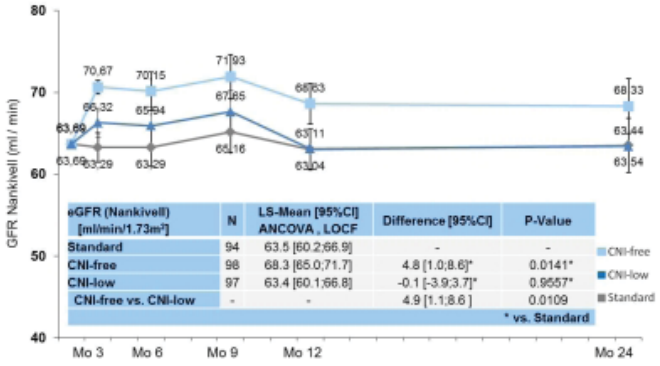
### HERAKLES at Month 24: Maintained Superior Renal Function in Patients on an Everolimus-Based Calcineurin Inhibitor Free Regimen Compared to Standard Cyclosporine/Mycophenolate and Low Cyclosporine/Everolimus

Martin G. Zeier,[1] Wolfgang Arns,[1] F. Lehner,[1] Markus Guba,[1] Claudia Sommerer,[1] Hans-Hellmut Neumayer,[1] Johannes Jacobi,[1] Peter Weithofer,[1] Daniel Baeumer,[2] Christoph May,[2] Eva-Maria Paulus,[2] Oliver Witzke,[1] Klemens Budde.[1] *[1]HERAKLES Study Group, Germany; [2]Novartis Pharma Nuremberg, Germany.*

**Background:** To follow up(FU) on the evolution of renal function(RF) of 3 different immunosuppressive regimen with different calcineurin inhibitor(CNI) exposure at month(Mo)24 post renal transplantation(Tx).

**Methods:** 802 patients (pts) were included in an 1year, prospective, open-label, randomized (RDZ), controlled multi-center study. After induction with basiliximab pts received cyclosporine A(CsA), enteric-coated mycophenolate sodium(EC-MPS) + steroids. 3Mo post Tx 499 pts were RDZ 1:1:1 to either continue standard (STD) CsA(100-180ng/ml) + EC-MPS(n=166), or to convert to a CNI-free regimen+ everolimus(EVR;5-10ng/ml) + EC-MPS(n=171) or to convert to CNI-low regimen+ EVR(3-8ng/ml) and reduced CsA(50-75ng/ml)(n=162). Mo24 FU visit was performed by 131(96%)STD, 132(96%) CNI-free and 125(93%) CNI-low pts. RF (primary endpoint) was assessed as Glomerular Filtration Rate (cGFR;Nankivell).

**Results:** Trough levels: CsA 99±32ng/ml in STD, 83±34ng/ml in CNI-low pts; EVR 6.7±3.1ng/ml in CNI-free, 6.2±2.3ng/ml in CNI-low pts. RF was similar at RDZ and had significantly improved at Mo12 by +5.6mL/min/1.73m2(95%CI:[+2.9;+8.3];p<0.001) in favor of CNI-free regimen and remained significantly improved by +4.8mL/min/1.73m2 at Mo24 (ITT;p=0.014). Rejections since RDZ were similar in all 3 groups (12%STD,14%CNI-free,12%CNI-low), safety profile was overall comparable.




| eGFR (Nankivell) [ml/min/1.73m²] | N | LS-Mean [95%CI] ANCOVA , LOCF | Difference [95%CI] | P-Value |
|---|---|---|---|---|
| Standard | 94 | 63.5 [60.2;66.9] | - | - |
| CNI-free | 98 | 68.3 [65.0;71.7] | 4.8 [1.0;8.6]* | 0.0141* |
| CNI-low | 97 | 63.4 [60.1;66.8] | -0.1 [-3.9;3.7]* | 0.9557* |
| CNI-free vs. CNI-low | - | - | 4.9 [1.1;8.6 ] | 0.0109 |
| | | | | * vs. Standard |

**Conclusions:** Reduced CsA in combination with EVR did not result in better RF compared to STD regimen with EC-MPS. However, CNI-free regimen lead to better RF maintained for 2years, confirming previous reports.

*Funding:* Pharmaceutical Company Support - Novartis Pharma Germany

## SA-PO1002

**HERAKLES at Month 24: Follow-Up Results on Efficacy and Safety of Three Different Treatment Regimens in De Novo Renal Transplant Patients Demonstrate Options for Individualized Immunsosuppression**

Martin G. Zeier,[1] Volker Kliem,[1] Wolfgang Arns,[1] Oliver Witzke,[1] Claudia Sommerer,[1] Hans-Hellmut Neumayer,[1] F. Lehner,[1] Johannes Jacobi,[1] Daniel Baeumer,[2] Christoph May,[2] Eva-Maria Paulus,[2] Markus Guba,[1] Klemens Budde.[1]
*[1]HERAKLES Study Group, Germany; [2]Novartis Pharma Nuremberg, Germany.*

**Background:** To follow-up (FU) on safety and efficacy of 3 different immunosuppressive (IS) regimen at 24 months (Mo) after renal transplantation (Tx).

**Methods:** 802 patients(pts) were included in this 1year, prospective, open-label, randomized (RDZ), controlled, multi-center study. After induction therapy with basiliximab all pts received cyclosporine A(CsA), enteric-coated mycophenolate sodium(EC-MPS)+steroids. 3Mo post Tx 499pts were RDZ 1:1:1 to either continue standard(STD) treatment CsA(100-180ng/ml)+EC-MPS(n=166) or convert to a calcineurin inhibitor (CNI)-free regimen with everolimus(EVR;5-10ng/ml) +EC-MPS(n=171) or to a CNI-low regimen with EVR(3-8ng/ml) +reduced CsA(50-75ng/ml) (n=162). All pts continued on steroids. Mo24FU visit was performed by 131(96%)STD, 132(96%)CNI-free and 125(93%) CNI-low regimen pts of the FU-population.

**Results:** From RDZ to Mo24 BPAR was reported in 17/144(12%) STD, 20/146(14%) CNI-free and 17/141(12%) CNI-low pts (ITT). Two (1%) deaths occurred in the CNI-low, none in the other groups. 1(1%) graft loss was observed in the STD and 4(3%) in the CNI-free group. Premature discontinuation due to AEs occurred in 1/147(1%) STD, 3/148(2%) CNI-free and 1/141(1%) of CNI-low pts (safety-pop) from Mo12 to Mo24. Renal function expressed as cGFR (Nankivell) was significantly improved by +4.8mL/min/1.73m2(95%CI:[+1.0;+8.6]) in favor of CNI-free regimen at Mo24(ITT;p=0.014).

| N (%) * Safety Pop. | Standard | CNI-free | CNI-low |
|---|---|---|---|
| Infections | 32 (21.8) | 50 (33.8) | 35 (24.8) |
| Severe infections | 6 (4.1) | 7 (4.7) | 9 (6.4) |
| Infections with hospitalisation | 21 (14.3) | 32 (21.6) | 28 (19.9) |
| CMV | 4 (2.7) | 4 (2.7) | 2 (1.4) |
| BKV | 1 (0.7) | 2 (1.4) | 2 (1.4) |
| Hospitalisation due to: | | | |
| Acute rejection | 9 (6.1) | 10 (6.8) | 8 (5.7) |
| Cardiovascular event | 4 (2.7) | 2 (1.4) | 1 (0.7) |
| GI event | 5 (3.4) | 4 (2.7) | 3 (2.1) |
| Malignancy | 2 (1.4) | 6 (4.1) | 1 (0.7) |
| Metabolic disorder | 2 (1.4) | 0 | 0 |
| *during FU (Mo12-24) | | | |

**Conclusions:** After 24Mo IS regimen using EVR with low-dose or without CNI-exposure is an efficacious and safe therapeutic approach offering the opportunity for an individualized IS.

*Funding:* Pharmaceutical Company Support - Novartis Pharma GmbH, Germany

## SA-PO1003

**APOLLO after 4 Years: Outcome on Renal Function of an Everolimus Based Therapy after Calcineurin Inhibitor Withdrawal in Maintenance Renal Transplant Recipients**

Wolfgang Arns,[1] Petra Reinke,[1] Claudia Sommerer,[1] Hermann G. Haller,[1] Oliver Witzke,[1] Barbara M. Suwelack,[1] Thomas Rath,[1] Daniel Baeumer,[2] Christoph May,[2] Eva-Maria Paulus,[2] Klemens Budde.[1]
*[1]APOLLO Study Group, Germany; [2]Novartis Pharma Nuremberg, Germany.*

**Background:** To assess renal function(RF), safety and efficacy of an Everolimus(EVR) regimen after Calcineurin-Inhibitor(CNI) withdrawal in maintenance kidney allograft recipients.

**Methods:** In this open-label, randomized(RDZ), controlled, multi-center trial 93 patients(pts) on stable immunosuppression consisting of CNI, Enteric-Coated Mycophenolate Sodium(EC-MPS) with or without steroids were RDZ to either continue CNI treatment + EC-MPS or convert to EVR + EC-MPS based regimen. After 12Mo core study pts were included in an observational follow-up(FU).

**Results:** 93 pts (mean time since last transplantation(Tx): 6.4years) were RDZ to either EVR(n=46) or CNI(n=47) regimen. 72(77%)pts completed Mo48visit. At Mo48 mean adj. GFR(Nankivell) in EVR pts was higher by +5.6 mL/min/1.73m2(95%CI:[-0.6;+11.8];p=0.08) vs CNI pts (ITT); mean GFR change from RDZ to Mo48: +5.7(95%CI:[-0.1;+11.5]) in EVR vs +0.1(95%CI:[-5.1;+5.3])mL/min/1.73m2 in CNI group (p=0.08). Mean eGFR of pts who remained to Mo48 on the assigned EVR regimen: +8.7ml/min(95%CI:[+16.0;+1.4]n=20;p=0.02) over CNI pts (n=31).

| eGFR Nankivell (ml/min/1.73m²) | EVR | CsA | Difference EVR/CsA | P-value |
|---|---|---|---|---|
| ITT pop. | n=32 | n=33 | Δ | |
| eGFR mean ± SD | 66.3 ± 17.6 | 60.6 ± 15.5 | 5.7 | 0.1307[b] |
| eGFR change[a], LS-mean[c] | +5.7 [-0.1;11.5] | +0.1 [-5.1;5.3] | 5.6 [-0.6;11.8] | 0.0762[c] |
| eGFR LS-mean[c] | 67.1 [61.3;72.9] | 61.5 [56.3;66.7] | 5.6 [-0.6;11.8] | 0.0762[c] |
| On-therapy pop. | n=20 | n=31 | Δ | |
| eGFR mean ± SD | 69.2 ± 14.9 | 60.8 ± 15.9 | 8.4 | 0.0704[b] |
| eGFR change[a], LS-mean[c] | +9.9 [2.5;17.2] | +1.2 [-4.1;6.4] | 8.7 [1.4;16.0] | 0.0216[c] |
| eGFR LS-mean[c] | 71.1 [63.8;78.4] | 62.4 [57.2;67.7] | 8.7 [1.4;16.0] | 0.0216[c] |
| [a]RandomizedTreatmentPeriod; [b]Wilcoxon Rank-Sum test; [c]ANCOVA LS-mean [95%CI] | | | | |

Mean trough levels: 84±44ng/ml CsA, 5.9±2.4ng/ml Tacrolimus, 6.7±2.0ng/ml EVR. 2deaths and 1graft loss occurred in CNI, 1death and 3graft losses in EVR, no BPAR in either group. Infections after Mo12: 23(50%) pts in EVR vs 20(43%) in CsA group. Of these were 3(7%) severe in EVR group vs 1(2%) in CNI group; none lead to hospitalization. No malignancy occurred in EVR group, 1(2%) in CNI group.

**Conclusions:** Late conversion to an EVR/EC-MPS regimen in maintenance KTx patients after CNI withdrawal is safe and associated with tendency towards better RF maintained for 4 years.

*Funding:* Pharmaceutical Company Support - Novartis Pharma GmbH Germany

## SA-PO1004

**Outcome on Renal Function, Efficacy and Safety in Living-Donor Kidney Transplant Recipients after Conversion from a Calcineurin Inhibitor to an Everolimus Based Regimen: Post Hoc Subgroup Analysis from the ZEUS Study**

Claudia Sommerer,[1] F. Lehner,[1] Wolfgang Arns,[1] Petra Reinke,[1] Ute Eisenberger,[1] Rudolf P. Wuthrich,[1] Martina Porstner,[2] Christoph May,[2] Eva-Maria Paulus,[2] Anja Susanne Muhlfeld,[1] Heiner H. Wolters,[1] Katharina M. Pressmar,[1] Rolf A. Stahl,[1] Oliver Witzke,[1] Klemens Budde.[1] *[1]ZEUS Study Group, Germany; [2]Novartis Pharma Nuremberg, Germany.*

**Background:** To study renal function and patient outcome in subgroup of living-donation (LD) kidney *de novo* transplant recipients after conversion from calcineurin inhibitor (CNI) therapy to an everolimus (EVR) based regimen.

**Methods:** *Post hoc* subgroup analysis on LD recipients from a prospective, open-label, controlled, multi-center study on renal transplant (Tx) patients that were randomized (RDZ) at month (Mo) 4.5 post Tx to an immunosuppressive regimen consisting of either EVR + enteric coated-mycophenolate sodium (EC-MPS ) or cyclosporine (CsA) plus EC-MPS.

**Results:** 300 kidney transplant recipients were RDZ to continue CsA or convert to EVR at 4.5Mo post Tx. Results from 80 LD recipients (EVR: N=42; CsA: N=38) were analyzed *post hoc*. In this subpopulation, adjusted estimated GFR at Mo12 (Nankivell formula) was 74.2 (95%CI [70.5;78.0]) mL/min/1.73m² for EVR pts vs 63.6 (95%CI [59.5;67.8])mL/min/1.73m²) for CsA pts, resulting in a difference of 10.6mL/min/1.73m² in favor of EVR group (p<0.001). From RDZ to Mo12, adjusted estimated GFR increased by a mean of 9.9 (95%CI [6.2, 13.7]) mL/min/1.73m² with EVR vs -0.6 (95%CI [-4.8, 3.5])mL/min/1.73m²) (p<0.001) in CsA subgroup. Mean trough levels at Mo12: 7.1±2.1ng/ml for EVR, 117±32ng/ml for CsA pts. In EVR group 6 BPAR episodes (5 of them Banff I), occurred vs 1 (Banff I) in the CsA group (p=0.109). One graft loss occurred in the CsA group, none with EVR. Discontinuation due to AEs occurred in 6 EVR pts (14.3%) and 5 CsA pts (13.2%) between RDZ and Mo12. Overall safety profile was similar between treatment groups.

**Conclusions:** These findings suggest that initiation of EVR with early elimination of CNI therapy in LD kidney transplant recipients is associated with a significant renal benefit at 12Mo post Tx without compromising safety and efficacy.

*Funding:* Pharmaceutical Company Support - Novartis Pharma Germany

## SA-PO1005

**Living Renal Donation: Emotional Status of the Donor** Claudia Sommerer,[1] Ralf A. Dikow,[2] Matthias Schaier,[1] Christian Morath,[1] Vedat Schwenger,[1] Martin G. Zeier.[1] *[1]Nephrology, Univ Hospital, Heidelberg, Germany; [2]Dialysis, Renal Center, Bruchsal, Germany.*

**Background:** The benefits of living kidney donation for recipients are well-known. Nevertheless, donor safety is of importance at all time. Data on the impact of living donation on the emotional status including depression and anxiety are rare.

**Methods:** In an open, prospective observational study, renal allograft donors were evaluated with respect to psychosocial and emotional outcome after living donation. Standardized questionnaires (HADS, SF-12) as well as additional questions related to living donation were used.

**Results:** Altogether, 128 renal allograft donors were evaluated (86 male, median age 49.1 ± 11.4 years, mean time after transplantation 3.7±3.8). None of the donors had any serious post-transplant complications and the renal function was stable. 13 donors (13%) assessed general health worse compared to prior to transplantation. Emotional role function was decreased in 8% of the donors. Concerning the emotional status, most of the patients demonstrated normal HADS anxiety scores, with pathological results in 6 patients (5%). Depression scale showed pathological results in 15 donors (13%). Patients with pathological anxiety scores were more likely to be parents, foreign origin and living alone. There was no difference concerning gender, age or renal function. Concerning the HADS depression scale no difference in the patient cohort with and without depression symptoms could be noticed. Health status was more often judged as worse in patients with pathological results in one of the sub-scales. Renal allograft function or recipient health had no relevant impact on the emotional status of the donor.

**Conclusions:** A minority of renal donors demonstrated and impact of renal donation on the emotional status or anxiety and depression symptoms. Health status was impacted by pathological results. Parenthood, foreign origin and living alone were identified as risk factors for anxiety symptoms.

## SA-PO1006

**Evolution of Allograft Fibrosis and Related Markers in Kidney Transplant Patients under Treatment with Cyclosporine and Everolimus** Luis Eduardo Becker,[1] Ruediger Waldherr,[2] Martin G. Zeier,[1] Claudia Sommerer.[1] *[1]Nephrology, Univ of Heidelberg, Heidelberg, Germany; [2]Pathology, Univ of Heidelberg.*

**Background:** In kidney transplant, protocols to avoid calcineurin inhibitor chronic toxicity are continuously developed in order to prevent allograft fibrosis and function loss. We analyzed the histological evolution of fibrosis and the expression of related markers after conversion from cyclosporine (CsA) to everolimus performed in stable kidney transplant patients.

**Methods:** Forty patients maintained on prednisone, sodium mycophenolate and CsA were included in our analysis. After 4.5 months of transplantation, patients were randomised to either continue the current CsA regimen (n = 21) or gradually convert from CsA to everolimus (n = 19). Histological sections of kidney biopsies obtained prior (baseline) and at least 6 months after randomization were digitally analyzed for kidney fibrosis (Masson's trichrome, Sirius Red) and for the immunohistochemical expression of related markers (transforming growth factor ß (TGFß), hypoxia inducible factor 1alpha (HIF1A) and platelet derived growth factor (PDGF)). Results are given as percent (%) of variation/year and presented as mean±SEM. Analysis was performed using the Student's t-test, $p<0.05$ was considered significant.

**Results:** Baseline characteristics were similar between groups. Time on target immunosuppression was 22±5 and 25±4 months in the CsA and in the everolimus groups, respectively. A tendency for a higher increase in the fibrotic area was seen in the CsA (8.5±5.5 %variation/year) compared to everolimus (0.4±2.5 %variation/year, p=0.2), which was confirmed by Sirius red. TGFß tubular expression reduced significantly in the everolimus group compared to baseline (-2.2±0.9% variation/year, p=0.03); the variation was also significantly different compared to the CsA group (+0.5±0.9% variation/year, p=0.04). Expression of both PDGF and HIF1A remained stable and was similar in both groups.

**Conclusions:** Conversion from CsA to everolimus in kidney transplant patients was associated with a tendency for a lower development of allograft fibrosis and a significant reduction of tubular TGFß expression on patients with stable allograft function.

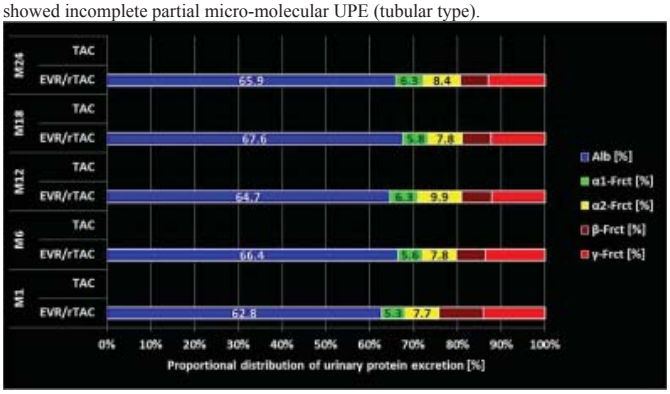
## SA-PO1007

**mTOR Inhibition and Evolution of Urinary Protein Excretion in Non-Renal Tranplant Patients – 24 Month Results from 719 De Novo Liver Transplant Patients** G. Junge, Sven Kohler, Ute Eisenberger, Heike Schwende, Wolfgang Arns. *H2304 Study Group.*

**Background:** The interplay of glomerular filtration and tubular absorption of proteins with various molecular weights defines pattern/magnitude of urinary protein excretion(UPE). Increased UPE(proteinuria) serves as clinical surrogate for renal injury and progressive damage affecting different parts of the nephron. mTOR-inhibitor(i) treatment has also been associated with increased UPE not only in KTx but also non-renal Tx recipients(R).

**Methods:** Data from study H2304(NCT00622869), a 24-month(M), randomized, multicenter study in 719 de novo LTxR comparing everolimus(EVR 3-8ng/mL) plus reduced tacrolimus(rTAC 3-5ng/mL) to standard TAC(sTAC-C 6-10ng/mL). Total daily UPE, measured as UP/UC ratio, as well as a set of different urinary proteins is described to allow a more detailed investigation of the origin/course of UPE in de novo LTxR receiving EVR.

**Results:** UPE was higher with EVR+rTAC compared to sTAC with highest values at M6 (290mg/d) followed by decrease to M12 and M24 (194mg/d) compared to 158mg/day in TAC-C at M24. UPE >500mg/d occurred in 18.1% of TAC-C vs 23.6% in EVR+rTAC (18.9% when EVR C0 was 3-8ng/mL). Analysis of urinary protein electrophoresis and levels of b2MG(12kDa), a1MG(26 kDa), Alb(70 kDa), TF(80 kDa), and IgG(150 kDa) suggest mixed glomerular/tubular UPE for TAC while patients on EVR with new proteinuria showed incomplete partial micro-molecular UPE (tubular type).




**Conclusions:** Clinical observations suggest that mTOR inhibition might be associated with increased UPE, potentially due to enhanced cell wall permeability and podocyte dysregulation. However, in case of mTORi facilitated CNI reduction the improvement in glomerular blood flow and consequently higher overall protein filtration in combination with mTOR-dependent reduction in tubular protein reabsorption may also contribute to increased UPE.

## SA-PO1008

**Surrogate Renal Function Endpoints for Predicting Long-Term Allograft Survival in Kidney Transplantation** G. Junge, Peter Bernhardt, Cesar Escrig, Helio Tedesco Silva. *For the A2309 Study Group, Novartis Pharma AG, Basel, Switzerland.*

**Background:** Despite the improvement of short-term kidney transplant (KTx) survival over the last decades, the main concern remains about the long-term outcome. However, classical primary endpoints in KTx clinical trials are still highly focused on short-term efficacy. A new approach to define study endpoints with clinical relevance in the current KTx setting is needed. We retrospectively examined different endpoints in the study A2309 to gain information on potential parameters that may predict long-term allograft survival for future verification.

**Methods:** This 24-month, multicenter, A2309 study randomized 833 de novo KTx patients to compare two regimens of everolimus (EVR; 1.5mg/day or 3.0mg/day) with reduced dose cyclosporine A (CsA) *vs.* enteric-coated mycophenolate sodium (EC-MPS; 1.44g/day) with standard dose CsA. Renal function was measured by estimated glomerular filtration rate (eGFR) using MDRD-4. The retrospective analyses used different cut-offs for eGFR to define renal function impairment. Efficacy was assessed as either treated biopsy-proven acute rejection (tBPAR), or the occurrence of any event of tBPAR, graft loss, or death. Different combinations of renal function and efficacy endpoints were analyzed.

**Results:** Fewer patients in the EVR group experienced the composite endpoint of impaired renal function or tBPAR and composite endpoint of eGFR cut-off or tBPAR, graft loss, or death (Table).

Table: Exploratory endpoints at mo 12 post-KTx

| Event at mo 12 | EVR 1.5 mg N=277 n/M (%) | EC-MPS N=277 n/M (%) | Risk Difference EVR 1.5 mg vs. EC-MPS (%) (95%CI) |
|---|---|---|---|
| eGFR ≤ 40 or tBPAR | 84/277 (30.3) | 96/277 (34.7) | -4.3 (-12.1, 3.5) |
| eGFR ≤ 50 or tBPAR | 117/277 (42.2) | 156/277 (56.3) | -14.1 (-22.3, -5.8) |
| eGFR ≤ 60 or tBPAR | 178/277 (64.3) | 205/277 (74.0) | -9.7 (-17.4, -2.1) |
| eGFR ≤ 40 or tBPAR/graft loss/death | 91/277 (32.9) | 99/277 (35.7) | -2.9 (-10.8, 5.0) |
| eGFR ≤ 50 or tBPAR/graft loss/death | 122/277 (44.0) | 157/277 (56.7) | -12.6 (-20.9, -4.4) |
| eGFR ≤ 60 or tBPAR/graft loss/death | 182/277 (65.7) | 206/277 (74.4) | -8.7 (-16.3, -1.1) |

CI, confidence interval; EC-MPS, enteric-coated mycophenolate sodium; eGFR, estimated glomerular filtration rate (mL/min/1.73m²); EVR, everolimus; KTx, kidney transplantation; mo, month; tBPAR, treated biopsy-proven acute rejection.

**Conclusions:** Endpoints to assess renal function impairment events in composite with efficacy failure allowed for the differentiation of the EVR 1.5 mg group *vs.* the EC-MPS group in the retrospective analysis of the A2309 study. These composites may provide a clinically meaningful approach to discriminate between immunosuppressive regimens in KTx. These endpoints need to be further explored in prospective trials.

**SA-PO1009**

**The Risk Factors for mTOR Inhibitors Associated Proteinuria in Kidney Transplant Recipients** Hung-Tien Kuo, Hung-Chun Chen. *Nephrology, Kaohsiung Medical Univ Hospital, Kaohsiung Medical Univ, Kaohsiung, Taiwan.*

**Background:** The use of mammalian target of rapamycin inhibitor (mTORI) after kidney transplantation has been associated with an increased risk of proteinuria. The purpose of this study is to investigate the risk factors for proteinuria after mTORI treatment in kidney transplant recipients in a Taiwan medical center.

**Methods:** A total of 55 adult (46.7±10.6 years old) deceased donor kidney transplant recipients who received mTORI treatment (sirolimus/everolimus 47/8, started at 351 days post-transplant in median) were enrolled. They had no overt-proteinuria before the initiation of mTORI treatment. The risk factors for mTORI-associated proteinuria were investigated using multivariate logistic regression analysis. The impact of mTORI-associated proteinuria on overall graft survival was investigated using multivariate Cox-regression analysis.

**Results:** Median follow-up after the initiation of mTORI treatment was 1603 days. During this time, there were 15 outcomes of new-onset proteinuria (27.3%) and 2 outcomes of overall graft failure (3.6%). After adjusting for major confounding factors in the logistic regression model, the independent risk factors for mTORI-associated proteinuria included renal function impairment (initial eGFR<30 vs. ≥60ml/min, odds ratio=91.7, $P<0.01$; 30-60 vs. ≥60ml/min, 3.1,$P=0.20$) and high body mass index (BMI≥25 vs. <25 Kg/m$^2$, 7.84, $P<0.05$). In the multivariate Cox regression model, mTORI-associated proteinuria was not significantly associated with the risk of overall graft failure.

**Conclusions:** In our study cohort, renal function impairment and obesity at the initiation of mTORI treatment were strong predictors for proteinuria. However, the impact of mTORI-related proteinuria on overall graft survival in kidney transplantation cannot be supported in the current analysis.

**SA-PO1010**

**Medication Safety: The Next Big Challenge to Improving Long-Term Outcomes in Kidney Transplantation?** David J. Taber, Justin Spivey, Nicole A. Pilch, John McGillicuddy, Kenneth Chavin, Frank Treiber, Prabhakar Baliga, Titte Srinivas. *MUSC, Charleston, SC.*

**Background:** Immunosuppressant regimens have significantly decreased acute rejection, but have potentially increased the risk of graft loss driven by infections, over-immunosuppression, adverse drug reactions, and medication errors.

**Methods:** This was a post-hoc analysis of a prospective randomized controlled trial that included adults that had a solitary renal transplant between 3/09-7/11. Data collection and analysis included sociodemographics, medications, adverse drug reactions, and medication errors. Patients were divided into groups based on the development of a clinical significant medication error, defined as a significant or severe medication error contributing to hospitalization, and analyzed for post-transplant clinical outcomes.

**Results:** 200 patients were included in this analysis; 233 medication errors were identified, with 64% of the cohort having at least one error. Immunosuppressant medications were associated with 48% of these errors. There was a total of 327 significant adverse drug events. Patients that experienced at least one med error had 1.8 times the risk of developing an adverse drug event (90% vs. 82%, p=0.128). 28 patients (12%) experienced a clinically significant medication error (CSME). Patients that experienced CSMEs had a statistically higher number of readmissions, cost of readmissions, and overall length of stay for these readmissions. These patients were more likely to experience graft failure (see Figure 1, CSME 22% vs. 10%, p =0.05); CSME was more strongly associated with graft loss as compared to acute rejections.

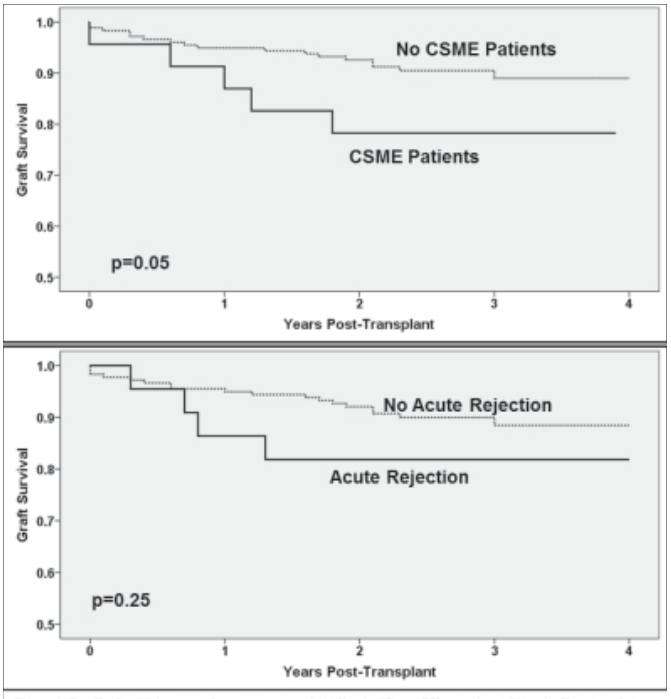



**Figure 1.** Top Kaplan-Meier curve demonstrates a statistically significant difference in graft survival between the CSME group and Non-CSME group. The bottom graph shows the lack of a strong association between acute rejection and graft survival suggesting a stronger association with medication errors and graft loss.

**Conclusions:** Clinically significant medication errors occur in one out of every eight kidney transplant recipients and lead to an increased risk hospital readmissions, cost of care and graft loss.

**SA-PO1011**

**Are Thiazide Diuretics Safe and Effective Antihypertensives in Kidney Transplantation?** David J. Taber, Titte Srinivas, Nicole A. Pilch, Beje S. Thomas, Maria N. Salazar, Kenneth Chavin, Prabhakar Baliga, Leonard Egede. *MUSC, Charleston, SC.*

**Background:** There is limited data assessing the safety and efficacy of thiazides as antihypertensives in kidney transplant (KTX) recipients.

**Methods:** This was a longitudinal retrospective cohort study of all KTX recipients transplanted between 2000-08 with follow-up through 2012. Patients were grouped based on receiving thiazide therapy. Safety and efficacy comparisons were made within thiazide patients and between groups.

**Results:** 1,093 KTX were included in this study (thiazide group: 108, control group: 985). KTX that received thiazides had a more aggressive HTN phenotype, demonstrated by being older (53±11 vs 48±13 yrs, p<0.001), more pre-KTX hypertension (97% vs 88%, p=0.004), diabetes (36% vs 27%, p=0.035), more post-KTX HTN medications (2.1±0.9 vs 1.4±1.0, p<0.001) and more HTN medication changes (10.9±4.4 vs 5.7±4.9, p<0.001, see Table 1). Despite this, means BPs were similar between thiazide and controls (139/77 vs 136/77) while receiving thiazide therapy. Safety analysis revealed thiazide patients were more likely to be readmitted to the hospital (70% vs. 53%, p<0.001), develop hyperkalemia (56% vs. 38%, p<0.001) or hypokalemia (28% vs. 18%, p=0.010), but had similar rates of hypotension, eGFRs, graft loss and death. After controlling for age and comorbidity differences, hospital readmissions were fairly similar between groups (OR 1.49, 95% CI 0.92–2.40). Within the thiazide cohort, paired repeated measures analysis demonstrated SBP (147±17 to 139±18, p<0.001) and DBP (79±9 to 77±11, p<0.001) were reduced after thiazide initiation.

| Outcome | Thiazide Patients n=108 | Control Patients n=985 | p-Value |
|---|---|---|---|
| Patient Survival | | | |
| One Year | 100% | 100% | |
| Three Year | 100% | 95% | 0.346 |
| Five Year | 95% | 91% | |
| Ten Year | 77% | 77% | |
| Graft Survival | | | |
| One Year | 100% | 100% | |
| Three Year | 97% | 90% | 0.160 |
| Five Year | 90% | 83% | |
| Ten Year | 67% | 65% | |
| Delayed Graft Function | 10% | 14% | 0.249 |
| Acute Rejection | 22% | 22% | 0.837 |
| Readmitted to Hospital Post-Transplant | 70% | 53% | **<0.001** |
| Reasons for Hospital Readmissions | | | |
| Infectious Issue | 26% | 18% | **0.030** |
| GI Issue | 11% | 9% | 0.535 |
| Rejection | 20% | 16% | 0.303 |
| Surgical Issue | 9% | 8% | 0.609 |
| Cardiovascular Event | 13% | 11% | 0.581 |
| Other | 50% | 35% | **0.003** |
| Developed at least one Episode of Hypotension (<90/60) | 13% | 10% | 0.384 |
| Hyperkalemia (>5 mEq/L) | 56% | 38% | **<0.001** |
| Severe Hyperkalemia (>6 mEq/L) | 5% | 5% | 0.985 |
| Hypokalemia (<3.5 mEq/L) | 28% | 18% | **0.010** |
| Severe Hypokalemia (3 mEq/L) | 6% | 4% | 0.299 |
| Mean Estimated GFR (mL/min/1.73 m$^2$) | 48±15 | 50±19 | 0.124 |
| Mean Serum Creatinine (mg/dL) | 1.7±0.5 | 1.8±0.7 | 0.177 |
| Mean Number of Anti-HTN Medications Used | 2.1±0.9 | 1.4±1.0 | **<0.001** |
| Total Number of Anti-HTN Medication Changes | 10.9±4.4 | 5.7±4.9 | **<0.001** |
| Number of Anti-HTN Medication Changes Per Year | 1.6±1.1 | 1.2±1.5 | **0.007** |
| Systolic BP at Transplant (mmHg) | 144±27 | 143±25 | 0.556 |
| Diastolic BP at Transplant (mmHg) | 77±15 | 78±15 | 0.577 |
| Mean Follow-Up Systolic BP (mmHg) | 142±15 | 136±16 | **<0.001** |
| Mean Follow-Up Diastolic BP (mmHg) | 78±8 | 77±9 | 0.861 |
| Follow-Up BP <130/80 mmHg | 32% | 36% | 0.219 |

**Conclusions:** Based on long-term outcomes, thiazides appear to be safe and effective antihypertensives in kidney transplantation; in the short-term, thiazides may increase the risk of hospitalization and potassium disturbances. Prospective RCT are warranted to confirm results.

## SA-PO1012

**Improving or Maintaining Renal Function over 5 Years with Belatacept or Cyclosporine (CsA): Insights from the BENEFIT and BENEFIT-EXT Long-Term Extension (LTE) Studies** F. Vincenti,[1] L. Rostaing,[2] A. Durrbach,[3] K. Rice,[4] L. Pupim,[5] J. Grinyo.[6] *[1]UCSF, USA; [2]Univ Hosp Toulouse, France; [3]Bicêtre Hosp, France; [4]Baylor Univ Med Ctr, USA; [5]Bristol-Myers Squibb, USA; [6]Univ Hosp Bellvitge, Spain.*

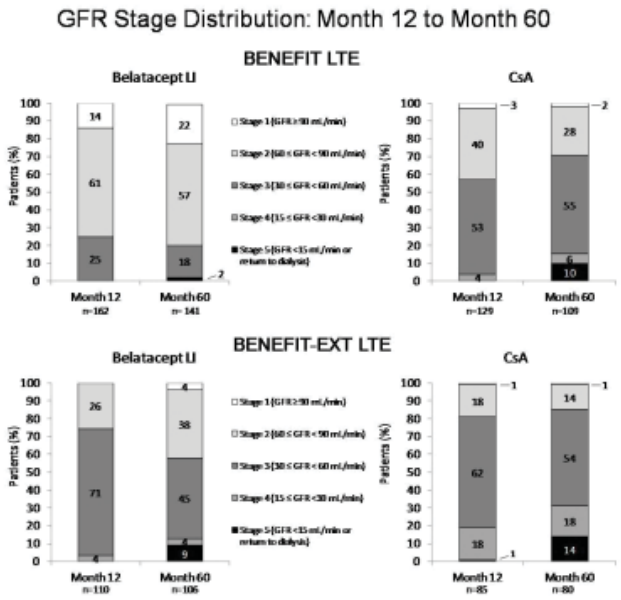
**Background:** In BENEFIT and BENEFIT-EXT, the early renal function benefit observed in belatacept(bela)-treated patients (pts) was maintained over 3 years with a consistent safety profile. Here we report renal function outcomes over 5 years in the LTE of both studies.

**Methods:** In both Phase III studies, adult kidney transplant recipients (living or SCD in BENEFIT; ECD in BENEFIT-EXT) were randomized to a more or less intensive (LI) regimen of bela or CsA. Pts entered the LTE after 3 years. GFR stages at Months (Mos) 12 and 60 are shown in the Figure. Post hoc analyses reported here assessed shifts in cGFR stage between Mos 12 and 60 in the LTE cohort, with the approved LI regimen vs. CsA. To be analysis eligible, patients in the LTE had to have a GFR value at Mo 12 and Mo 60. In BENEFIT, 139 of 165 pts in bela LI and 102 of 136 in CSA were eligible; in BENEFIT-EXT, 103 of 113 LI and 79 of 87 CSA were eligible.

**Results:** In BENEFIT, from Mo 12–60, 77 of 85 LI pts and 21 of 43 CsA pts in Stage 2 maintained or improved their GFR stage. Among Stage 3 pts, 33 of 35 LI and 40 of 52 CsA maintained or improved their GFR stage.

In BENEFIT-EXT, from Mo 12–60, 20 of 25 LI pts and 7 of 15 CsA pts in Stage 2 maintained or improved their GFR stage. Among Stage 3 pts, 63 of 74 LI and 38 of 50 CsA maintained or improved their GFR stage. Among Stage 4 pts, 3 of 4 LI and 7 of 12 CsA maintained or improved their GFR stage.

**Conclusions:** Despite modest sample sizes, these results suggest that patients treated with belatacept were more likely than CsA-treated patients to maintain or improve renal function over 60 Mos in both studies.




## SA-PO1013

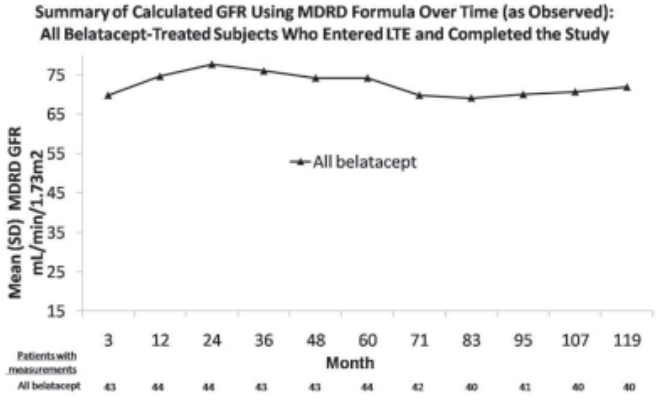
**A Decade of Treatment with Belatacept in Renal Transplantation: Final Results from the Long-Term Extension (LTE) of the Phase 2 Study** F. Vincenti,[1] C. Larsen,[2] J. Grinyo,[3] F. Mühlbacher,[4] G. Blancho,[5] Y. Vanrenterghem,[6] F. Lehner,[7] C. Jones-burton,[8] B. Charpentier.[9] *[1]UCSF, US; [2]Emory Univ; [3]Univ Hospital of Bellvitge, Spain; [4]Medical Univ of Vienna, Austria; [5]Univ Hospital, Nantes, France; [6]Univ Hospital Gasthuisberg, Belgium; [7]Medizinische Hochschule Hannover, Germany; [8]Bristol-Myers Squibb, US; [9]Univ Hospital of Bicêtre, France.*

**Background:** Results of the Phase 2 LTE of belatacept in kidney transplantation demonstrated a favorable safety profile & improved renal function vs cyclosporine (CsA) at 5 years (Vincenti F et al, *JASN* 2010;21(9):1587–96). Here we report the safety & efficacy of belatacept in the LTE up to study closure (9–13 years).

**Methods:** 218 patients were randomized to receive a more or less intensive regimen of belatacept (n=145) or CsA (n=73), with belatacept patients receiving treatment at 4- or 8-week intervals (5 mg/kg after 6 months). Only 9 CsA patients remained at the end of the study. 44 belatacept patients completed the study, & their results are presented here.

**Results:** Mean belatacept treatment duration for these 44 patients was 9.7± 0.4 years. Mean(SD) MDRD cGFR was 70(21) mL/min/1.73m$^2$ at Month 3 & 72(17) mL/min/1.73m$^2$ at the end of the study (Figure). There was 1 acute rejection episode in Year 9 (8-week dosing interval), & there were no deaths or graft losses. From randomization to study end, 82% of belatacept patients had serious AEs, 36% had serious infections, & 18% had malignancies (with no cases of PTLD). From randomization to end of study, 30% of patients missed no infusions, & 16% missed only 1 infusion.

**Conclusions:** While the sample sizes are limited in this self-selecting cohort, data suggest that the profile of belatacept is consistent over nearly a decade. Patients maintained renal function over ~10 years with no new safety findings & high treatment compliance.




GFR=glomerular filtration rate, MDRD=modification of diet in renal disease

## SA-PO1014

**Accuracy of Kidney Failure Risk Equations among Kidney Transplant Recipients** Brad C. Astor,[1,2] Brenda L. Muth,[1] Arjang Djamali.[1,3] *[1]Dept of Medicine, Univ of Wisconsin School of Medicine and Public Health, Madison, WI; [2]Dept of Population Health Sciences, Univ of Wisconsin School of Medicine and Public Health, Madison, WI; [3]Dept of Surgery, Univ of Wisconsin School of Medicine and Public Health, Madison, WI.*

**Background:** Accurate prediction of graft failure among kidney transplant recipients may identify patients at greatest risk and allow improved management and preparation for return to dialysis. Kidney failure risk equations have recently been developed and validated in native chronic kidney disease (CKD), but their utility in transplant recipients is unknown.

**Methods:** We tested the accuracy of the 3-variable (age, sex, estimated glomerular filtration rate) and 4-variable (urinary protein:creatinine ratio) to predict 3-year and 5-year graft survival in a cohort of 1,673 patients who received a kidney transplant at the University of Wisconsin from 1990-2009 and had survived one year post-transplant. C-statistic, net reclassification index (NRI) and integrated discrimination improvement (IDI) are reported. The NRI was calculated for risk groups of <5%, 5-10% and >10% for 3-year survival and <10%, 10-20%, and >20% for 5-year survival.

**Results:** A total of 91 grafts failed within 3 years and 134 failed within 5 years. The 4-variable model resulted in a significantly positive NRI for 3-year survival, but the C-statistics did not differ significantly. The 4-variable model resulted in a higher C-statistic and significantly positive NRI and IDI for 5-year survival.

| | C-statistic (3-variables) | C-statistic (4-variables) | p | NRI | IDI |
|---|---|---|---|---|---|
| 3-year graft survival | 0.7827 | 0.8052 | 0.14 | 7.7% (-2.7, 18.1) | 8.3% (5.2, 11.4) |
| 5-year graft survival | 0.7426 | 0.7746 | 0.01 | 18.4% (7.6, 29.3) | 10.1% (7.4, 12.9) |

**Conclusions:** The accuracy of both kidney failure risk equations was substantially lower in this population of kidney transplant recipients (C-statistics ≤ 0.80) than reported for native CKD populations (C-statistics ≥ 0.90). While the 4-variable equation, with the addition of urinary protein:creatinine ratio, significantly improved risk prediction, additional factors must be considered to provide accurate risk prediction in kidney transplant recipients.

## SA-PO1015

**Effect of Mycophenolate Mofetil Dose on Long-Term Kidney Allograft Function** Karlo K. Mihovilovic, Matija Pavlovic, Bojana Maksimovic, Mladen Knotek. *Clinical Hospital Merkur, Zagreb, HR, Croatia.*

**Background:** Studies have reported significant improvement in kidney function in patients on MMF with lower exposition to calcineurin inhibitors.The optimal long-term MMF dosing in patients maintained on contemporary low-dose calcineurin inhibitors is still undetermined. The aim of our study was to determine effect of MMF dose on kidney function over five years post-transplant.

**Methods:** A cohort of 171 transplant patients with kidney, kidney-pancreas and kidney-liver transplantation was included. Immunosuppression consisted of anti-IL2 or antithymocyte immunoglobulin induction with MMF, calcineurin inhibitors ± steroids. Estimated creatinine clearence (eCrcl) was calculated using Cockroft Gault formula. MMF dose at 1,2,3,4 and 5 years was correlated with eCrcl at respective time point. Univariate and multiple regression analyses were done to test relationship between independent variables and eCrcl. Data are shown as mean±SD, or median with range.

**Results:** 105 patients had kidney transplantation and 58 kidney-pancreas transplantation. Recipients were a mean of 42.3 ± 12.03 years old at the time of transplantation, 63 percent of them were male and all were Caucasians. Donors were a mean of 42.8 ± 16.2 years old. There were 46 living donor transplantations.

| | 1 yr | 2 yrs | 3 yrs | 4 yrs | 5 yrs |
|---|---|---|---|---|---|
| eCrcl (ml/min) | 61.5 (49-74.4) 171 | 68 (53.7-80.5) 169 | 67.8 (53.9-81) 130 | 67 (52.7-79.7) 92 | 72.2 (58-82.1) 61 |
| MMF dose (mg) | 2000 (500-4000) 171 | 2000 (500-4000) 164 | 2000 (500-3000) 123 | 2000 (720-3500) 87 | 2000 (500-3000) 59 |
| Tac. conc. (µg/L) | 7.7 ± 2.9 125 | 7 ± 7.5 123 | 6.1 ± 2.1 87 | 5.8 ± 2.3 53 | 5.1 ± 1.6 28 |
| Cyclo. conc. (µg/L) | 173 ± 54 43 | 127 ± 53 23 | 101 ± 43 41 | 80 ± 30 38 | 77 ± 25 33 |

eCrcl at 1,2,3,4 and 5 years post-transplant positively correlated with average MMF dose ($p<0.01$ for each time point). There was no correlation of eCrcl with tacrolimus or cyclosporin concentration at any time point. In multivariate analysis MMF dose remained strongly positively correlated with kidney function at all time points.

**Conclusions:** Higher MMF dose with lower calcineurin concentration is associated with better long-term renal function and may increase long-term survival of transplanted kidney.

## SA-PO1016

**Are We Reducing Tacrolimus Exposure Adequately after Kidney Transplantation? A 3-Year Analysis of the Mycophenolic Acid Observational REnal Transplant (MORE) Study** Fuad S. Shihab,[1] Ali Olyaei,[2] Anne Wiland,[3] Kevin M. McCague,[3] Larry Chan.[4] *[1]Univ of Utah School of Medicine, Salt Lake City, UT; [2]Oregon State Univ and Oregon Health & Science Univ, Portland, OR; [3]Novartis Pharmaceuticals Corporation, East Hanover, NJ; [4]Univ of Colorado School of Medicine, Aurora, CO.*

**Background:** It is unclear if tapering of tacrolimus (Tac) exposure in routine practice after kidney transplantation is adequate to minimize calcineurin inhibitor-related nephrotoxicity.

**Methods:** MORE was a prospective, observational study of *de novo* kidney transplant recipients receiving mycophenolic acid (MPA) at 40 US centers, and managed according to local practice. Tac $C_0$ levels and estimated GFR (eGFR, CKD-EPI) were analyzed up to 3 years post-transplant. Tac $C_0$ was graded as low, moderate or high (<6, 6–8 or >8ng/mL) and eGFR analyzed as <60 or ≥60mL/min/1.73m$^2$. Risk of eGFR <60 at 1, 2 or 3 years was analyzed based on Tac $C_0$ at 1 year and was adjusted for living donor, ethnicity, gender, delayed graft function, recipient age, PRA, HLA mismatches, expanded criteria donor, MPA dose at month 1 and CMV serology.

**Results:** 904 patients were analyzed. The % of patients with low/moderate/high Tac $C_0$ was 29%/36%/34% at year 1, 37%/36%/27% at year 2 and 39%/35%/27% at year 3. Incidence of biopsy-proven acute rejection at month 12 was 8.5%, 8.3% and 6.5% (n.s.) with low, moderate and high tac $C_0$. eGFR<60 was present in 55%, 51% and 54% at 1, 2 and 3 years. The adjusted odds ratio (AOR) for eGFR <60 at years 1, 2 and 3 in patients with high vs low Tac $C_0$ was 1.28 (95%CI 0.83-1.96, p=0.263), 1.75 (1.05-2.92; p=0.033) and 1.69 (0.88-3.25, p=0.115), respectively. AORs for eGFR<60 at years 1, 2 and 3 in the high vs moderate Tac $C_0$ groups were 1.24 (0.84-1.84; p=0.282), 1.13 (0.71-1.81; p=0.605) and 1.14 (0.62-2.08; p=0.680).

**Conclusions:** Over a quarter of patients were exposed to high Tac $C_0$ (>8ng/mL) up to 3 years after kidney transplantation, resulting in a lower eGFR at 2 years. Risk of low eGFR may be adversely affected by maintenance of unnecessarily high Tac exposure.

*Funding:* Pharmaceutical Company Support - Novartis

## SA-PO1017

**Pregnancy Outcomes in Kidney Recipients Who Discontinued Mycophenolic Acid Products prior to Conception** Serban Constantinescu,[1] Peter Axelrod,[1] Lisa Coscia,[2] Michael J. Moritz,[3] Vincent T. Armenti.[2] *[1]Temple Univ School of Medicine, Philadelphia, PA; [2]Gift of Life Institute, Philadelphia, PA; [3]Lehigh Valley Health Network, Allentown, PA.*

**Background:** The FDA changed the pregnancy category for mycophenolic acid products (MPA) from C to D in 2007. Transplant centers report discontinuing MPA in female kidney recipients anticipating pregnancy.

**Methods:** Data were collected by the National Transplantation Pregnancy Registry (NTPR) via questionnaires, phone interviews and medical records.

**Results:** 114 pregnancies were conceived on MPA, and in 163 pregnancies MPA was discontinued preconception (69% switched to another agent).

| | MPA exposure during early pregnancy | MPA discontinued preconception | p value |
|---|---|---|---|
| **Maternal Factors** | **n=114** | **n=163** | |
| Unplanned pregnancies | 62% | 19% | <0.001 |
| Transplant-to-conception (yrs) | 4.2±3.6 | 5.4±3.5 | 0.004 |
| Creatinine before pregnancy | 1.3±0.5 | 1.1±0.3 | <0.001 |
| Creatinine during pregnancy | 1.4±0.6 | 1.2±0.4 | 0.003 |
| Creatinine after pregnancy | 1.5±0.9 | 1.2±0.5 | 0.001 |
| Biopsy-proven rejection during pregnancy | 3% | 1% | NS |
| Graft loss within 2 yrs of delivery | 5% | 3% | NS |
| **Outcomes** | | | |
| Livebirth | 43% | 79% | <0.001 |
| Miscarriage | 52% | 19% | <0.001 |
| Stillbirth | 2% | 1% | NS |
| **Livebirths** | **n=50** | **n=133** | |
| Gestational age (wks) | 35.3±3.3 | 35.7±3.5 | NS |
| Birth weight (g) | 2344±753 | 2590±716 | 0.02 |
| **Birth defects** | 14%* | 6% | NS |

* 1 stillbirth with birth defects, not included

Recipients who discontinued MPA preconception had significantly longer transplant to conception intervals and lower creatinine levels before, during and after pregnancy. Pregnancy resulted in significantly higher rate of livebirths and lower incidence of birth defects. Rejection rate during pregnancy and graft loss within 2 yrs of delivery were similar between groups.

**Conclusions:** Pregnancy in kidney recipients who discontinued MPA preconception resulted in a significantly higher rate of livebirths, without an increase in rejection, graft loss or birth defects.

*Funding:* Pharmaceutical Company Support - Novartis Pharmaceuticals Corp., Astellas Pharma US, Inc., Pfizer Inc., Bristol-Myers Squibb Co.

## SA-PO1018

**Differences in Long-Term Kidney Transplantation Outcomes between Hispanic and Non-Hispanic Whites in the United States** Maria Cristina Arce,[1] Colin R. Lenihan,[1] Maria E. Montez-Rath,[2] Wolfgang C. Winkelmayer.[1] *[1]Div of Nephrology, Stanford Univ, Palo Alto, CA; [2]Div of General Medical Disciplines, Stanford Univ, Palo Alto, CA.*

**Background:** For most patients who develop kidney failure, kidney transplantation offers the greatest promise for restoring a healthy, productive life. It is widely known that disparities in long-term outcomes persist among racial and ethnic minorities in the United States, but little is known about the Hispanic minority population.

**Methods:** Using the U.S. Renal Data System, we identified 105,250 Caucasian patients who received their first kidney transplant between 1/1/1996-12/31/2010 and investigated long-term kidney graft survival by Hispanic ethnicity. Death and death-censored graft failure were analyzed using Cox proportional hazards model, stratified by year of transplant, to estimate unadjusted and adjusted cause-specific hazard ratios ($HR_{CS}$) and subdistribution (competing risk analysis) hazard ratios ($HR_{SD}$) with corresponding 95% confidence intervals (CI).

**Results:** A total of 11,003 (10.5%) patients experienced kidney graft failure during 544,257 person-years of follow-up. In the unadjusted analysis, the incidence rate of death-censored graft failure was 2.25/100 person-years in Hispanics versus 1.97/100 person-years in non-Hispanics. The incidence rate of death occurred in 3.04/100 person-years in Hispanics versus 4.13/100 person-years in non-Hispanic whites. In the adjusted analysis, we found that Hispanics undergoing kidney transplantation experienced improved survival (adjusted $HR_{CS}$=0.69; 95%CI, 0.66-0.72) but similar death-censored graft survival ($HR_{CS}$=0.96; 95%CI, 0.91-1.01) compared to non-Hispanic whites. When accounting for competing risks, the graft survival by ethnicity remained similar ($HR_{SD}$=0.99; 95%CI 0.94-1.05). The presence of diabetes and level of education did not modify this association.

**Conclusions:** Few studies have described long-term transplantation outcomes in this rapidly growing minority population burdened with not only socioeconomic challenges but cultural and language barriers as well. Overall, Hispanics were found to have similar kidney graft survival compared to non-Hispanic whites.

*Funding:* Other NIH Support - T32 DK007357-26

## SA-PO1019

**Changes in Graft Survival among Pediatric and Young Adult Recipients, 1992-2010** Bethany J. Foster,[1] Mourad Dahhou,[1] Xun Zhang,[1] Susan M. Samuel.[2] *[1]McGill Univ Health Centre, Montreal, Canada; [2]Alberta Children's Hospital, Calgary, Canada.*

**Background:** Adult kidney transplant recipients had significant improvements in graft survival from the late 1980s to mid-1990s, but only very modest changes thereafter. We aimed to determine if graft failure rates among pediatric recipients changed between 1992 and 2010.

**Methods:** Using United States Renal Data System (USRDS) data, we studied individuals who received a 1st kidney transplant at ≤21 years old between 1992 and 2010. We used Cox models to compare graft failure (death or graft loss) rates in individuals transplanted in 1992-1997 with those transplanted in 1998-2005, and 2006-2010. Models were stratified by age at transplant, and adjusted for sex, race, donor source, age, primary disease, socioeconomic status, and duration of dialysis before transplant.

**Results:** Graft failure rates were significantly lower in more recent time periods compared with 1992-1997. Improvements over time were significantly larger for those <5 years at transplant (interaction p=0.001).

| | 1992-1997 | 1998-2005 | 2006-2010 |
|---|---|---|---|
| **Age <5 years at transplant** | | | |
| **All patients** (n) | 601 | 926 | 630 |
| Graft failures per 100 person-years | 4.7 | 2.8 | 3.2 |
| Adjusted HR [95% CI] | 1.00 | 0.64 [0.52, 0.78] | 0.38 [0.27, 0.56] |
| **Patients with ≥1 year of graft function (n)** | 522 | 860 | 511 |
| Graft failures per 100 person-years | 3.8 | 2.1 | 1.7 |
| Adjusted HR [95% CI] | 1.00 | 0.68 [0.52, 0.87] | 0.52 [0.29, 0.91] |
| **Age ≥5 years at transplant** | | | |
| **All patients** (n) | 4011 | 5960 | 3736 |
| Graft failures per 100 person-years | 7.2 | 5.9 | 4.3 |
| Adjusted HR [95% CI] | 1.00 | 0.78 [0.74, 0.84] | 0.51 [0.46, 0.58] |
| **Patients with ≥1 year of graft function (n)** | 3599 | 5581 | 3033 |
| Graft failures per 100 person-years | 6.8 | 5.7 | 3.9 |
| Adjusted HR [95% CI] | 1.00 | 0.82 [0.76, 0.87] | 0.52 [0.45, 0.61] |

**Conclusions:** Graft failure rates have decreased substantially over calendar time among young recipients.

*Funding:* Clinical Revenue Support

## SA-PO1020

**Renal Transplantation in the Older Populations: 5 Years Single Centre Experience** Asmaa Y.M. Al-Chidadi, Mark Harber. *Centre for Nephrology, Royal Free Hospital Univ College London, Hampstead, London, United Kingdom.*

**Background:** Elderly patients are increasingly being considered for renal transplantation due to the ageing population. However there may be associated short term morbidity and mortality that is more apparent in the elderly.

**Methods:** We reviewed the course of 47 recipients, 65 years or older transplanted between the 1st of January 2008 and the 31st of December 2010. One year patients and graft survival were compared with 367 patients aged 64 years or younger transplanted during the same time period.

**Results:** Survival was significantly higher in the younger group at 97.55% vs. 91.67%, p=0.028. Graft loss was not significantly different between the two cohorts (4.92% vs. 4.17% in the older group, p=0.819). Initial hospitalization was not significantly different between the two groups as well as hospital stay in the first 3 months and the number of admissions in the first year. E-GFR on discharge was significantly higher among the younger cohort 43.26±24.1 vs. 32.22±20.3, p=0.03. E-GFR at one year however did not achieve significance; 52.35±22.25 vs. 46.06 ±21.28 in the older cohort, p=0.077. Delayed graft function was indicated in 60% in the older versus just over 30% in the younger group, p<0.001. On bivariate analysis the mean number of rejection episodes was nearly identical in the 2 cohorts (0.26±0.80 Vs. 0.25±0.48).

**Conclusions:** Perhaps surprisingly the initial length of hospitalization in the first year among the older cohort was only a little different from younger recipients, however there was a significant higher rate of mortality and delayed graft function in the older cohort. The slightly poorer eGFR among the older cohort although it wasn't statistically significant at one year might have been a reflection of allocating more marginal and significantly older donors compared to younger cohort. The significant increase in mortality is an important consideration when listing older patients for transplantation.

*Funding:* Government Support - Non-U.S.

## SA-PO1021

**Long Term (Visit to Visit) Instability Is an Independent Predictor of Progressive Renal Function Loss in Transplant Patients** Francesca Mallamaci, Pasquale Fabio Provenzano, Graziella D'arrigo, Rocco Tripepi, Giovanni Tripepi, Carmine Zoccali. *Clin. Epid. and Physiopath. of Renal Dis. and Hypert., CNR-IBIM, Reggio Calabria, Italy.*

**Background:** Hypertension is an established risk factor for renal function loss in transplant pts but it is unknown whether BP instability is an independent risk factor for the same outcome.

**Methods:** We tested the relationship between long term, visit-to-visit BP variability [expressed in terms of standard deviation (SD)] and the evolution of the GFR over-time (eGFR slope) in an unselected series of 154 clinically stable renal transplant patients followed up for a median time of 3.2 years (IQ range: 2.2-4.9 years). In a sub-sample of 86 pts, we also investigated the association between short term (24h) BP variability (assessed by ambulatory BP monitoring, ABPM) and the same renal outcome.

**Results:** Office BP was on average 131±10 /78±7 mmHg and the visit to visit variability (SD) was 7.1±5.1/5.2±3.2 mmHg. Visit to visit systolic BP (SBP) variability was inversely related to baseline eGFR (rho=-0.21, P=0.007) but was independent of average BP and other classical risk factors. During the follow-up, the eGFR decreased in 64 pts (-2.8±3.4 ml/min/1.73m2/year, range:-20.6 to -0.01) and increased in the remaining 90 pts (3.7±3.2, ml/min/1.73m2/year, 0.01 to 15.6). Overall, there was an inverse relationship between visit to visit SBP and DBP variabilities and renal function deterioration over time (rho=-0.17, P=0.035 and rho= -0.19, P=0.02) and these associations held true also after data adjustment for the average BP and baseline eGFR (partial rho=-0.19, P=0.02 and partial rho=-0.19, P=0.02). Short term systolic and diastolic BP variability was largely unrelated to eGFR slope.

**Conclusions:** Visit to visit BP systolic variability predicts GFR loss over-time in transplant pts. Such an effect is independent of underlying average BP and other risk factors. In contrast short term (24h) variability does not predict renal outcomes. These observations have prognostic and therapeutic implications. Trials aimed at minimizing visit to visit BP variability are needed to establish whether attenuation of visit to visit variability may improve renal outcomes in transplant pts.

*Funding:* Government Support - Non-U.S.

## SA-PO1022

**Communication between Community Nephrologists and the Transplant Centers: A Survey of Nephrologists** Sreedhar Devathi, Ahmad Aswad, Shahed Abbasi, Nasrollah Ghahramani. *Dept of Medicine, Div of Nephrology, PennState Hershey Medical Center, Hershey, PA.*

**Background:** Interactive communication between the primary nephrologist and the transplant center (TC) is crucial to the optimal care of the patient pre- and post- kidney transplant (KT). Ideally, the TC and the nephrologist should discuss the plans for transition of care prior to KT. We examined nephrologists' current practice and experience regarding patient-related communication with the TC.

**Methods:** Invitations were sent to 3180 nephrologists in the eastern US. 822 expressed interest, and 250 were randomly invited to complete a questionnaire about demographics, practice characteristics, and their communication with TC. A total of 216 surveys with complete responses were analyzed. Univariate analysis and stepwise logistic regression were performed.

**Results:** 90% indicated access to an attending physician in the TC to discuss patient-related issues. 50% call the TC about patients they are referring, 57% call to provide updates on new events about listed patients, and 81% to discuss the care of their transplanted patients. 87% are informed in a timely manner when their patient undergoes KT. 79% consider the information they receive from TC about their transplanted patients as adequate. In multivariate analysis, attending at least one yearly transplant-related CME was associated with higher likelihood to call the TC to refer patients (OR:3.77; p<0.0001) and to discuss the care of patients post-KT (OR:3.35; p=0.002). Nephrologists who attended more than 2 national nephrology meetings in a 5-year period were more likely to call to provide updates on listed patients (OR:3.25; p=0.0005). Nephrologists are less likely to call with updates if more than half of their patients are not employed (OR:0.43; p=0.02). Practice in groups of more than 10 increased the likelihood that a nephrologist will call the TC to refer patients (OR:2.30; p=0.03).

**Conclusions:** The majority of nephrologists are satisfied with the communication with the TC. The likelihood of communication is related to participation in continuing educational meetings, the overall composition of the patients and the number of nephrologists in the practice.

*Funding:* NIDDK Support

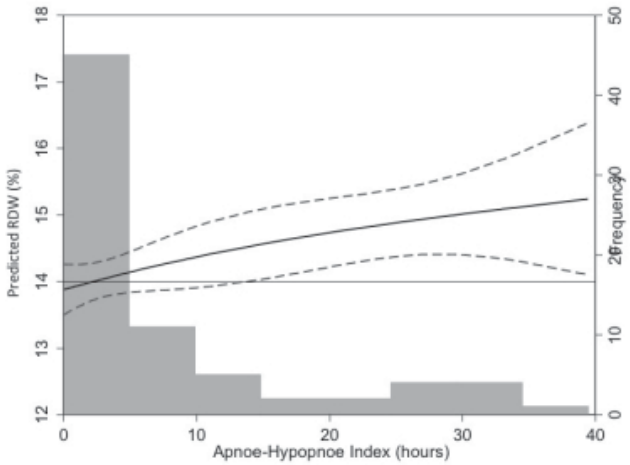
## SA-PO1023

**Red Cell Distribution Width Is Associated with Obstructive Sleep Apnea in Kidney Transplant Recipients** Miklos Zsolt Molnar,[1,2] Akos Ujszaszi,[3] Katalin Fornadi,[3] Marta Novak,[1,3] Istvan Mucsi.[4] *[1]Univ of Toronto, Toronto, Canada; [2]Nephrology and Hypertension, Univ of California, Irvine, Irvine, CA; [3]Semmelweis Univ, Budapest, Hungary; [4]McGill Univ Health Centre, Montreal, Canada.*

**Background:** Red cell distribution width (RDW), a marker of heterogeneity in the size of circulating erythrocytes. It is associated with mortality in various patient populations. We assess the association between Obstructive Sleep Apnea (OSA) and RDW in stable kidney transplant recipients to demonstrate if RDW is associated with intermittent hypoxemia generated by OSA.

**Methods:** Cross-sectional study of 100 kidney transplant patients who underwent polysomnography. Socio-demographic information and data about medication, comorbidity and laboratory parameters were collected.

**Results:** The mean age was 51±13 years, 43% were women, and the prevalence of diabetes was 19%. The mean RDW was 14±1%, and the median (interquartile range) Apnoe-Hypopnoe Index (AHI) was 3 (13). We found an incremental linear association between the AHI and RDW in unadjusted (B=0.027, 95%CI:0.012-0.04; p<0.001) linear regression models.




After adjustment for relevant factors, such as age, gender, eGFR, comorbidity, abdominal circumference, serum albumin, serum CRP, blood hemoglobin and soluble transferrin receptor levels, AHI was still associated with RDW (B=0.022, 95%CI:0.007-0.037; p=0.005).

**Conclusions:** RDW is associated with the apnoe-hypopnoe index, the objectively assessed measure of the severity of OSA. It is tempting to speculate RDW is increased in response to intermittent hypoxemia (a hallmark of OSA). Further studies will need to unravel if the link between RDW and mortality is a reflection of underlying OSA.

## SA-PO1024

**Prevalence of Primary Hyperaldosteronism in Renal Transplantation Is More Common Than Reported** Mahendra V. Govani, Cara Crone, Russell A. White, Alvin Wee, Islam A. Ghoneim, Joseph Whelan, Jay H. Weiss. *Kidney and Pancreas Transplantation, St. Vincent Hospital, Indianapolis, IN.*

**Background:** Despite several case reports of primary Hyperaldosteronism (PHA) diagnosed after renal transplantation, prevalence of this condition has not been studied in detail.

**Methods:** Between 1/1/2009 and 4/31/2013, 186 kidney alone transplants (146DD and 40LD) were performed at our center in 184 patients. On longitudinal follow-up, 7 patients were found to have refractory hypertension (≥3 antihypertensive drugs) and refractory hypokalemia (no potassium (K) restriction and supplemental K>40 mEq/day) without any obvious non-urinary K loss. Plasma aldosterone to renin ratio (PARR) was performed on these 7 patients; at the time all of them were on tacrolimus, and only one patient was on furosemide for CHF due to diastolic dysfunction. Demographics and outcomes were studied, and the patients were followed until 5/31/2013.

**Results:** PARR was >30 in 4 of 7(57.1%) patients (definite PHA), >20 in 2(28.6%) patients (probable PHA), and <5 in the remaining one.

| Variable | PHA Patients (N=6) |
|---|---|
| Age in years, median±SD(range) | 56.4±13.5(47.5-81.4) |
| African-American, n(%) | 3(50) |
| Male, n(%) | 4(67) |
| DM, n(%) | 6(100) |
| SCR, mg/dL, median±SD (range) | 1.5±0.3(1.4-2.2) |
| MDRD GFR, ml/min, median±SD (range) | 47.1±8.5(38.2-59.7) |
| PARR, median±SD (range) | 47±75(23-219) |

All 6 patients with PARR >20 were started on spironolactone; BP improved markedly(>30/15), and it was possible to stop or reduce other antihypertensive drugs in all of them. No patient had adrenal mass on CT scan of abdomen. Patient and graft survival were 100%; median serum creatinine (SCR) and GFR were 1.5 mg/dL and 47.1 ml/min respectively at median follow-up of 28.6±21.6 (range 1.7-53.5) months respectively. The patient with CHF improved (NYHA class III to class I), and furosemide was reduced from 80 to 20 mg/day.

**Conclusions:** Prevalence of PHA (definite and probable) is 3.3% in our cohort. All posttransplant patients with both refractory hypertension and hypokalemia (especially when patients are on tacrolimus) need to be tested for PAH.

*Funding:* Clinical Revenue Support

## SA-PO1025

**The Change of Circadian Rhythm of Blood Pressure after Successful Kidney Transplantation in End Stage Renal Disease** Myung Hyun Lee, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Republic of Korea.*

**Background:** The change of circadian rhythm after kidney transplantation (KT) is not fully studied. In this study, we prospectively investigated circadian rhythm before and after KT.

**Methods:** We performed 24 hour ambulatory blood pressure monitoring (ABPM) in 48 patients before KT and at 1 year after KT. Circadian rhythm was presented as dipper(ΔSBP ≥10%), non-dipper (0 < ΔSBP ≤10%), and reverse dipper (SBP nocturnal rise) according to the nocturnal reduction of systolic blood pressure (ΔSBP). We evaluated the pattern of circadian rhythm and mean blood pressure before and 1 year after KT.

**Results:** Mean blood pressure did not differ between before and after KT(124±19/81±13 vs.121±10/81±9 mmHg, P>0.05), but the number of anti-hypertensive medication was significantly decreased (1.7±1.2 vs 0.6 ±0.9, P <0.05). Before KT, 41 out of 48 patients (85%) showed abnormal circadian rhythm (non-dipper was 58% (28/48) and reverse dipper was 27% (13/48)) and dipper was only 13% (7/ 48). At 1 year from KT, the abnormal circadian rhythm did not improve in most patients. The proportion of non-dipper did not change compared to before KT and reverse dippers showed increasing pattern (27%(13/48) to 31%(15/48)). However, the proportion of dipper showeddecreasing pattern (15%(7/48) to 10%(5/48)). In individual analysis, 27 out of 28 non-dippers before KT still showed abnormal circadian rhythm (18 non-dippers and 9 reverse dippers at 1 year after KT) and only 1 changed to dipper. In 13 revere dippers before KT, only 2 patients converted to dipper and 11 patients still showed non-dipper(n=6) and reverse dipper(n=5). In 7 dippers before KT, only 2 patients remained in dipper, and another 5 showed abnormal circadian rhythm after KT (4 non-dippers and 1 reverse dipper).

**Conclusions:** In conclusion, overall status of blood pressure improved, but circadian rhythm showed deteriorating pattern at 1 year after transplantation.

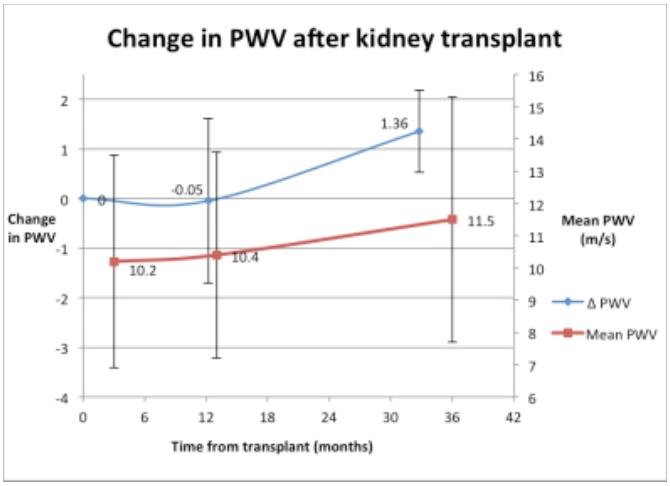
## SA-PO1026

**Initial Stabilization in Pulse Wave Velocity Is Followed by Rapid Worsening One Year after Kidney Transplantation** Fadi Tohme, Eduardo Cruz, Harald M. Stauss, Roberto S. Kalil. *Univ of Iowa Hospitals and Clinics, Iowa City, IA.*

**Background:** Cardiovascular (CV) disease is the leading cause of mortality in advanced CKD and after kidney transplantation (KT). Pulse wave velocity (PWV) is a highly sensitive and non-invasive predictor of CV risk. Our aim was to identify factors associated with a significant change in PWV after KT.

**Methods:** Kidney transplant recipients at an academic center were recruited between 2009 and 2012. Visits occurred at 3, 12, 24 or 36 months after transplant. Patients who did not have at least 2 sequential measurements of PWV were excluded. Data collected at each visit included age, gender, eGFR, blood pressure (BP), weight and carotid-femoral PWV, measured using the SphygmoCor system. Correlations were calculated using nonparametric Spearman's coefficient. Visits at 11-14 months and 21-37 months after transplant were compared using ANCOVA.

**Results:** 56 visits corresponding to 25 patients were included. Mean age was 50 [36-64] years and 72% were males. The absolute value of PWV had a positive correlation with age, SBP and weight (p<0.001) but not with gender (p=0.37), eGFR (p=0.7) or time elapsed from transplant (p=0.154). Sequential change of PWV (Δ-PWV) correlated with the time elapsed from transplant (p=0.01), but not with the change in SBP, eGFR or weight. ANCOVA analysis (using time between sequential measurements as a covariate) showed a significantly lower Δ-PWV in the 11-14 months group compared to the 21-37 months group (p=0.049.




**Conclusions:** PWV, a good marker of CV risk, initially stabilizes after KT but rapidly increase within a year of transplant. Those changes seen in PWV can not be explained by changes in SBP, GFR or weight after transplant. Persistent elevated PWV after 1 year from transplant may play a role in high rates of CV events post KT.

*Funding:* Other NIH Support - NHLBI

## SA-PO1027

**Arterial Stiffness and Changes in QTc Interval in Renal Transplantation Patients** Mehtap Erkmen Uyar,[1] Ugur Bal,[2] Zeynep Bal,[1] Emre Tutal,[1] Burak Say?n,[1] Orhan Guliyev,[1] Begum Erdem?r,[3] Siren Sezer.[1] *[1]Dept of Nephrology, Baskent Univ Faculty of Medicine, Ankara, Turkey; [2]Dept of Cardiology, Baskent Univ Faculty of Medicine, Ankara, Turkey; [3]Dept of Internal Medicine, Baskent Univ Faculty of Medicine, Ankara, Turkey.*

**Background:** Arterial stiffness plays an important role in cardiovascular diseases and is an independent predictor for cardiovascular mortality. The QTc interval has been reported to be increased and to be associated with high-risk ventricular arrhythmias and sudden death. Although renal transplantation improves survival, cardiovascular morbidity and mortality still remain as a significant problem compared with nonrenal populations. The aim of this study is to evaluate the association between the QTc interval changes and arterial stiffness in kidney transplant recipients.

**Methods:** 100 kidney transplant recipients from our renal transplant outpatient clinic were enrolled into the study. All patients were evaluated for clinical and biochemical parameters. Anthropometric and body composition analyses were performed for all patients. Body compositions were analyzed by using the Body Composition Analyzer (Tanita BC-420MA). PWv was determined from pressure tracing over carotid and femoral arteries using the SphygmoCor system. Pre- (retrospectively) and post-transplant electrocardiographic (ECG) evaluations were performed. Each QT interval was corrected for the patient's heart rate using Bazett's Formula.

**Results:** After renal transplantation maxQTc intervals (456.7 ms to 414 ms) and QTdc (54 ms to 34 ms) of all patients were significantly decreased. In post transplantation period, patients with high QTc intervals had significantly higher PWv (p:.009) and higher serum CRP levels (p:.001) than patients with QTc<440 ms. Patients with PWv≥7 m/s had significantly higher maxQTc interval decline than patients with PWv<7 m/s (p:.05, r:-.206).

**Conclusions:** High QTc interval after renal transplantation could be a predictor of arterial stiffness in renal transplant recipients. Electrocardiographic evaluation is seem to be a cheap and reliable way to detect arterial stiffness.

## SA-PO1028

**Anti-PLA2R Monitoring in Relapsing Membranous Nephropathy after Kidney Transplantation** Miguel Seras,[1] David San Segundo,[2] Marcos Lopez-Hoyos,[2] Emilio Rodrigo,[1] Juan Carlos Ruiz,[1] Gema Fernandez-Fresnedo,[1] Angel Luis M. De Francisco,[1] Manuel Arias-Rodriguez.[1] *[1]Nephrology, Univ Hospital Marques de Valdecilla, Santander, Spain; [2]Inmunology, Univ Hospital Marques de Valdecilla, Santander, Spain.*

**Background:** Idiopathic membranous nephropathy (MN) is an important cause of chronic kidney disease with relatively high incidence of relapse after transplantation. The antibodies against the phospholipase A2 receptor (anti-PLA2R) seem to play a key role in the pathogenesis and diagnosis of idiopathic MN. The aim of our study was to know the postransplant evolution of anti-PLA2R in patients with MN.

**Methods:** We selected 13 transplanted patients from our database with histological diagnosis of MN before transplantation and biopsies done under clinical suspicion of relapse or other pathologies after transplantation: 3 of them had histological confirmation of MN relapse, 2 in the first year and one after 5 years. We analyzed the sera of the 13 patients (from a prospectively updated sera bank) with indirect immunofluorescence for anti-PLA2R, on the day before transplantation, after 3 months and on the day of the confirmatory biopsy. We also analyzed the sera of 2 out of 3 MN relapsed cases after the administration of rituximab.

**Results:** 2 of the 3 MN relapses were positive before transplantation, maintaining high titles of anti-PLA2R in the 3rd month and when the biopsies confirming the relapses were done (11 and 13 months respectively). The test became negative after the administration of rituximab in both, with no correlation with proteinuria or clinical outcomes. The 3rd MN relapse was negative before and in the 3rd month after transplantation but became positive after five years, at the moment of histological diagnosis. Only 3 of the 10 patients who did not relapse had positive pretransplant anti-PLA2R, although at low titles, and they became negative in the first months after grafting.

**Conclusions:** Persistently high titles of anti-PLA2R after kidney transplantation are a good predictor of MN relapse. Monitoring after using rituximab is somewhat uncertain, because of no correlation with proteinuria or clinical outcomes.

## SA-PO1029

**The Natural History and Risk Factors Identified for Development of Skin Cancer in Renal Transplant Recipients** Rosa M. Montero,[1] Donald Choi,[2] Christopher Harland,[1] Mona Wahba.[1] *[1]Epsom & St. Helier Univ Hospitals NHS Trust, London, United Kingdom; [2]St. George's Univ of London, London, United Kingdom.*

**Background:** There is no consensus regarding the guidance and recommendations of skin cancer surveillance in Renal Transplant Recipients (RTRs). In this cross-section observational study all RTRs attending skin surveillance clinic were reviewed to determine the natural history of skin lesions occurring in RTRs and identify risk factors associated with development of these lesions.

**Methods:** A total of 125 RTRs were reviewed for diagnosis of de novo skin lesions in nurse led clinics between Sep 2010 and Nov 2011. Immunosuppressive regimen, Age, gender, and skin type were looked as potential risk factors. Spearman's correlation was used for data analysis.

**Results:** 13 Non Melanoma Skin Cancer (NMSC) and 17 Premalignant lesions (PMLs) were found in this cohort. Increasing age significantly correlated with the development of Bowens disease (BD) (p=0.03,r=0.20). Basal cell carcinoma (BCC) had a predilection for male RTRs (p=0.01). A bimodal distribution appeared in both NMSC and PML at <5yrs (mode for BCC: 3 yrs) and >10yrs post 1st renal transplant (RT). RTRs on Prednisolone (P)+ Cyclosporin correlated with development of PML; BD (p=0.01,r=0.246). The combination of P + Tacrolimus (FK) + Mycophenolate Mofetil correlated with the development of BCC (p=0.03,r=0.197). FK+ Azathioprine developed cases of BCC (p=0.03,r=0.194) and Actinic Keratosis (AK) (p=0.03,r=0.19). All of the skin lesions found occurred in those with skin types I-IV with BCC, AK and BD predominantly in the lower skin type (Spearman's correlation p<0.001,p=0.001,p=<0.01, respectively). NMSC and PML develop before 5 years and rise steadily with a similar rate of growth by 20 yrs post 1st RT.

**Conclusions:** Early screening is recommended in view of high detection rates <5yrs of NMSC and PML in RTRs post 1st RT. The high incidence of BCC in <5yrs post 1st RT suggests a role for rigorous pre-transplant screening as BCC are slow growing lesions. Risk factors for NMSC include skin types I-IV, length and combination of immunosuppression. We recommend skin surveillance from 3 years to 20 years should occur as a minimum in RTRs post 1st RT.

## SA-PO1030

**Clinical and Genetic Predictors of Cutaneous Squamous Cell Carcinoma in Kidney Transplant Recipients** M. Lee Sanders, Jason Karnes, Joshua C. Denny, Dan M. Roden, T. Alp Ikizler, Kelly A. Birdwell. *Vanderbilt Univ Medical Center.*

**Background:** Cutaneous squamous cell carcinoma (cSCC) occurs 100 times more frequently in kidney transplant recipients (KTRs) compared to the general population with more aggressive metastasis, a higher rate of recurrence, and an increased morbidity and mortality. We set out to identify clinical and genetic predictors which confer an increased risk for cSCC in this patient population.

**Methods:** We retrospectively identified 78 cSCC cases and 246 controls among Caucasian KTRs using a DNA biobank linked with de-identified electronic medical records (EMRs). We performed a genome wide association study (GWAS) for cSCC with 711,021 single nucleotide polymorphisms (SNPs) using the Illumina OMNI1 and OMNI5 platforms. Logistic regression was used to determine adjusted odds ratios for clinical predictors and SNP effects in a dominant model using SAS and PLINK.

**Results:** Age (OR 1.11 [1.08-1.15], p<0.0001), months of immunosuppression (OR 1.02 [1.01-1.024], p<0.0001) and inhaled tobacco use (OR 2.10 [1.07-4.09, p=0.030) were significant clinical predictors of cSCC development while gender and duration of dialysis were not. No SNPs reached nominal genome-wide significance ($p<10^{-8}$). The two top associations were rs2135976 in *TRPC3*, previously associated with glomerulonephritis and proteinuria (OR 9.13, $p=7.04x10^{-6}$) and rs373610, a nonsynonymous SNP in *OBSCN* previously associated with tobacco addiction (OR 5.90, $p=1.58x10^{-5}$).

**Conclusions:** Age, immunosuppression duration, and inhaled tobacco use were significant predictors for the development of cSCC in an EMR-based cohort of Caucasian KTRs. Genetic analysis suggests a role for the biologically plausible genes *TRPC3* and *OBSCN* in cSCC. Replication and better-powered studies are required to analyze these and other potential genetic predictors.

*Funding:* NIDDK Support, Other NIH Support - NIGMS, NCATS

**SA-PO1031**

**Six Years Posttransplant Follow-Up of the First Case Who Underwent Partial Bladder Transplantation with *En Bloc* Kidney Transplant in a Human** Noriko Sugawara,[1] Tatsuo Asano,[1] Kei Nishiyama,[1] Shoichiro Kanda,[1] Kiyonobu Ishizuka,[1] Masataka Hisano,[1] Hiroko Chikamoto,[1] Yuko Akioka,[1] Rie Yago,[2] Motoshi Hattori.[1] *[1]Dept of Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Dept of Urology, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** We have reported a 12-month-old girl who underwent partial bladder transplantation with *en bloc* kidney transplant (Kato et al. Am J Transplant 8: 1060, 2008). Because her native bladder was extremely small, bilateral kidneys were transplanted *en bloc* with their ureters connected to a patch of the donor bladder, which encompassed the bilateral ureterovesical junction (bladder patch technique). That was the first case report of transplanting a vascularized segment of the human urinary bladder.

Although this technique has several potential benefits including preserving the natural anti-reflux mechanism, there are some concerns. One potential concern is the inadequacy of blood supply to the donor bladder wall. Another concern is that the donor bladder wall is denervated and is not likely to contract. So, her bladder function was followed carefully.

**Methods:** She is routinely examined at our facility. At 6 years post transplant, a voiding cystourethrogram(VCUG), magnetic resonance urography, cystoscopy were performed. Here, we report a 6-years posttransplant follow-up of this case, especially focusing on her bladder function.

**Results:** Her native bladder capacity was 3 ml at the time of transplantation. At 6 years posttransplant, VCUG revealed that the bladder capacity increased to 140 ml, though the bladder deformed asymmetric shape. Both native and grafted bladders showed coordinate contractions, voiding pressure was 10 $cmH_2O$ and there were no residual urines. Reflux to the bilateral grafted kidneys was not observed. Cystoscopy revealed that the bladder had an adequate blood flow. She gained normal urinary continence and her serum creatinine was 0.4 mg/dl.

**Conclusions:** In conclusion, the bladder patch technique worked well in this child. Longer follow-up is needed to prove the efficacy of this technique. Also, additional cases with this technique are required to provide more accurate insight on its safety and efficacy.

**SA-PO1032**

**Do Outcomes of Living Donor Renal Allograft Recipients Differ in Patients on Peritoneal or Hemodialysis** Amit Gupta, Harsh Vardhan, Narayan Prasad, Anita Saxena, Raj K. Sharma. *Dept of Nephrology, SGPGIMS, Lucknow, Uttar Pradesh, India.*

**Background:** Studies in deceased donor renal transplantation show that patients on peritoneal dialysis (PD) have reduced incidence of post transplant delayed graft function (DGF) compared to patients on hemodialysis (HD). This study was undertaken to compare the outcomes of ***living donor renal transplantation*** in recipients who were on PD or HD in the pretransplant period.

**Methods:** This is a retrospective observational study.Inclusion criteria were end stage renal disease (ESRD) patients on PD who received living donor renal transplantation betweenJanuary 2002 to December 2011 at our institute and age & gender matched renal transplant patients who were on HD prior to transplantation.

**Results:** 45 patients on PD & HD were included in the study. Both groups were similar in terms of recipient's age & gender, donor's age & gender, diabetic & non diabetic status of the recipients & proportion of different blood groups. Immunosuppression protocol was similar in both groups. Duration of dialysis was significantly longer in PD patients. In the pretransplant period the infectious complications similar (15.4% in PD vs 17.8% in HD). Comorbidities was more in the PD patients but they had better residual renal function.Surgical complications (20% in PD vs 13.3% in HD), DGF (8.8% in PD vs 11% in HD), acute rejections [AR] (13.3% in PD vs 15% in HD) were similar. The mean eGFR values at 1 month (86.1±23.8 vs 77.9±15.3,p=0.22) and at last follow up (60.9±23.5 vs 60.3±18.8,p=0.94) was similar in both groups. Post transplant infections were similar. The mean cumulative patient survival was similar (PD : 84 mths vs HD : 85 mths). The 1, 2, 5 & 8 year patient survival in PD patients was 98%, 93%, 86% & 80% and in HD patients was 100%, 95%, 87% & 80% respectively. The overall mean cumulative death censored graft survival was similar (PD : 83 mths vs HD : 84 mths). The 1,2,5,& 8 years death censored graft survival of PD patients was 98%, 95%, 85% & 75% and in HD patients was 100%, 93%, 84% & 79% respectively.

**Conclusions:** The short & long term outcomes, AR & DGF of renal allograft recipients do not differ with modality of dialysis in living donor renal transplantation.

**SA-PO1033**

**Family Income and the Risk of Rejection, Graft Loss, and Death of Pediatric Renal Transplant Recipients** Gregory H. Gorman,[1] John C. McDonnell,[1] Brent L. Lechner,[1] Robert Nee,[2] Lawrence Agodoa,[3] Kevin C. Abbott.[2] *[1]Pediatrics, Walter Reed National Military Medical Center, Bethesda, MD; [2]Nephrology, Walter Reed National Military Medical Center, Bethesda, MD; [3]NIDDK, National Institutes of Health, Bethesda, MD.*

**Background:** Socioeconomic status influences health outcomes and access to care. Children with renal transplants have complex medical needs and may be particularly susceptible to income disparities.

**Methods:** Pediatric (<18 yrs old) renal transplant recipients from 1997-2006 were identified from United States Renal Data Service files and follow-up data through 2009 was obtained. Subjects were linked by zip code to median household income from the 2000 U.S. Census. Median family income was stratified by quintiles. Primary outcomes were death, graft loss due to rejection and all causes. Proportional hazards regression and Kaplan-Meier curves modeled risk based on income. Covariates considered included race, etiology of ESRD, age, race, gender, and donor type (deceased-donor vs. living-related).

**Results:** There were 7,781 pediatric renal transplants identified. Maximum follow-up was 11.7 years. 59% were male, 19% black, and congenital/urologic conditions accounted for 50% of ESRD etiologies. Mean age at transplant was 11.4 ± 5.1 yrs. 55% had living-related donors. Median annual household income was $39,359 (IQR $31,187-$51,101.) 1,951 (25%) lost their graft and 442 (5.6%) died. Of graft loss, (4.6%) were due primarily to rejection. In unadjusted regression with the highest income group as the reference, there was a 17% increased risk of mortality (HR 1.17 [95%CI 1.09-1.25; p<0.001]) and a 13% increase in risk of graft loss (HR 1.13 [95%CI 1.1-1.17; p<0.001]) for each successive decrease in income quintile. There was no relationship between graft loss due to rejection and median family income [HR 0.98 [95%CI 0.85-1.14; p=0.78]).

**Conclusions:** Those with the lowest income had increased risk of death and graft loss compared to those with the highest income. Differences in rates of graft rejection are not the primary mediators of this relationship. Policy makers should consider the effect of income when determining benefits for pediatric kidney transplant recipients.

**SA-PO1034**

**Mortality in Patients with a Failed Transplant after a Return to Dialysis** Zhyiar Abdulrahman, Nassim Kamar, Laure Esposito, F. Sallusto, I. Cardeau-desangles, Arnaud Dell Bello, Lionel Rostaing. *Dept of Nephrology, Dialysis, Transplantation, Toulouse Univ Hospital, Toulouse, France.*

**Background:** It is not clear whether mortality increases among those with kidney-allograft failure relative to those who start dialysis but have never received a transplant.

**Methods:** We compared mortality rates and its causes among 136 patients with a failed kidney allograft who returned to dialysis between January 2006 and December 2012 (at Toulouse University Hospital, France) with 532 *de novo* hemodialysis patients who had never received a transplant. Within failed-allograft patients, we also compared mortality rates of those who had lost their graft before or after the end of the first year post transplant, and of those who were or were not on a waiting list for a kidney transplant.

**Results:** Overall mortality was comparable between failed-allograft patients (22.8%) and *de novo* hemodialysis patients (24.1%; $p$=0.79). Mortality rates were also similar between patients who lost their allograft within 1 year post transplant (22.9%) and those who lost their allograft at >1 year (22.8%; $p$ =0.92). Failed-allograft patients who were not relisted for a kidney transplant had the highest mortality rate (40.8%) when compared to those patients who had been relisted (12.6%; $p$=0.0002) and hemodialysis transplant-naïve patients (24.1%, $p$=0.001). The lowest mortality rate (6%) was observed in failed-allograft patients who had a re-transplant when compared to those patients who were still on a waiting list (21.6%) and those who were not relisted (40.8%; $p$=0.0002). The major causes of mortality in the failed-allograft group ($n$=31) were cardiovascular (29%), others are cancer, gastrointestinal complications, and sepsis caused equal mortality rates (13% each). Causes of mortality in *de novo* hemodialysis patients ($n$=128) were similar.

**Conclusions:** Patients with a failed kidney allograft and transplant-naïve patients undergoing *de novo* hemodialysis had equivalent mortality risks and the causes of mortality were similar; the most frequent being cardiovascular. Failed-allograft patients who underwent re-transplantation had the lowest mortality rate regardless of their status vis-à-vis kidney transplantation.

**SA-PO1035**

**Elevated Fibroblast Growth Factor 23 Levels Are Associated with Chronic Rejection and Declining Kidney Transplant Function** Michael E. Seifert,[1,2] Aftab S. Chishti,[3] Myra L. Chiang,[4] David T. Selewski,[5] Debbie S. Gipson,[5] Keith A. Hruska.[2] *[1]Southern Illinois Univ; [2]Washington Univ; [3]Univ of Kentucky; [4]West Virginia Univ; [5]Univ of Michigan.*

**Background:** Chronic rejection (CR) is characterized by cumulative vascular injury that leads to kidney transplant failure. Chronic kidney disease-mineral bone disorder (CKD-MBD) contributes to vascular injury in native CKD, but its importance in CR is unknown. Circulating CKD-MBD factors such as fibroblast growth factor 23 (FGF23) have been associated with remote transplant loss and mortality but not directly with CR. We hypothesized that FGF23 and other biomarkers of CKD-MBD would serve as non-invasive biomarkers of CR.

**Methods:** Preliminary analysis of 26 pediatric kidney transplant recipients enrolled in an ongoing multicenter, case-control study of biomarkers of vascular injury in CR. Subjects with biopsy-proven chronic rejection were assigned to the CR group. Subjects were assigned to the No CR group if a recent biopsy (<6 months prior) revealed no abnormality or estimated glomerular filtration rate (eGFR) was > 90 mL/min/1.73$m^2$ (Schwartz calculation). We measured plasma FGF23, Dickkopf-related protein 1 (Dkk1), and sclerostin (SOST) levels at enrollment using ELISA and correlated levels with eGFR and CR.

**Results:** 10/26 (38%) of subjects had biopsy-proven CR. We detected variable FGF23 levels in the CR group but the mean plasma FGF23 was significantly higher vs. No CR (104 ± 93 pg/ml vs. 47 ± 19 pg/ml, p=0.03). FGF23 levels were inversely correlated with transplant function ($r^2$ -0.365, p=0.02). Using logistic regression we found that for every 5 pg/mL increase in FGF23 the risk for CR increased nearly two-fold, after adjusting for age and gender [OR 1.994 (95% CI 1.725, 5.175)]. FGF23 was a superior biomarker of CR when compared to eGFR [OR 0.737 (95% CI 0.516, 1.053)]. Dkk1 and SOST levels were similar between groups.

**Conclusions:** Plasma FGF23 is inversely correlated with eGFR and is a better non-invasive biomarker for CR. These findings will be further validated as we complete enrollment and analysis for this study. We speculate that FGF23 is a modifiable risk factor for CR that must be explored in a prospective study.

*Funding:* Other NIH Support - NIH UL1 TR000448; NIH KL2 TR000450

## SA-PO1036

**The Fall in FGF-23 Levels at One Year following Kidney Transplantation Ameliorates Acute Allograft Rejection Risk** Monique E. Cho,[1] William H. Chong,[2] Michael S. Ring,[1] Xiongce Zhao.[3] *[1]Kidney Disease Branch, NIDDK, NIH, Bethesda, MD; [2]Skeletal Clinical Studies Unit, NIDCR, Bethesda, MD; [3]Office of the Director, NIDDK, NIH, Bethesda, MD.*

**Background:** Although increased serum levels of fibroblast growth factor 23 (FGF23) predict progression of CKD and increased mortality risk, no published studies have addressed the role of FGF23 in incident renal transplant (tx) population.

**Methods:** In all 163 kidney and kidney/pancreas transplants performed in our center from 1999 -2007, 82 stored serum samples taken 0-3 months prior to tx (baseline) were available for analysis. Sixty of the 82 samples had repeat serum samples at one yr. We tested the hypothesis that the greater decline in FGF23 levels during the first yr is associated with decreased risk of allograft rejection and favorable allograft function. Because of non-parametric distribution of the data, Kruskal-Wallis test was used to investigate the relationship between FGF23 and renal allograft outcome.

**Results:** The median (IQR) baseline intact FGF23 was 4873 (589, 16041) pg/mL. At one-yr post tx, it decreased to 53 (36, 85) pg/mL, with a median reduction of 3305 (515, 15453) pg/mL. During the mean follow-up of 5.7±2 yrs, there were a total of 31 rejections (77% 1A/B, 23% 2A/B) in 24 patients (87% in the 1st yr). Of the 31 rejection episodes 18 were included in the analysis (6/24 patients did not have FGF23 decline data and those with multiple rejections were treated as one episode). The 5-yr median serum Cr was 1.5 (1.2, 1.8) mg/dL in 70 patients.Patients who experienced rejection had a median FGF23 decline of 1022 pg/mL while those without rejection had a median FGF23 decline of 4804 pg/mL, P=0.037. The baseline or 1-yr FGF23 level was not associated with the rejection risk. Those with greater reduction in FGF23 levels at 1 yr showed a trend for lower serum Cr at 5 yrs. There was no difference in the baseline serum PTH or phosphorus levels in the rejection vs. non-rejection group.

**Conclusions:** While greater FGF23 fall in the 1st yr may be associated with decreased allograft rejection risk, a larger study is needed to determine if FGF23 change independently predicts renal allograft outcome.

*Funding:* NIDDK Support

## SA-PO1037

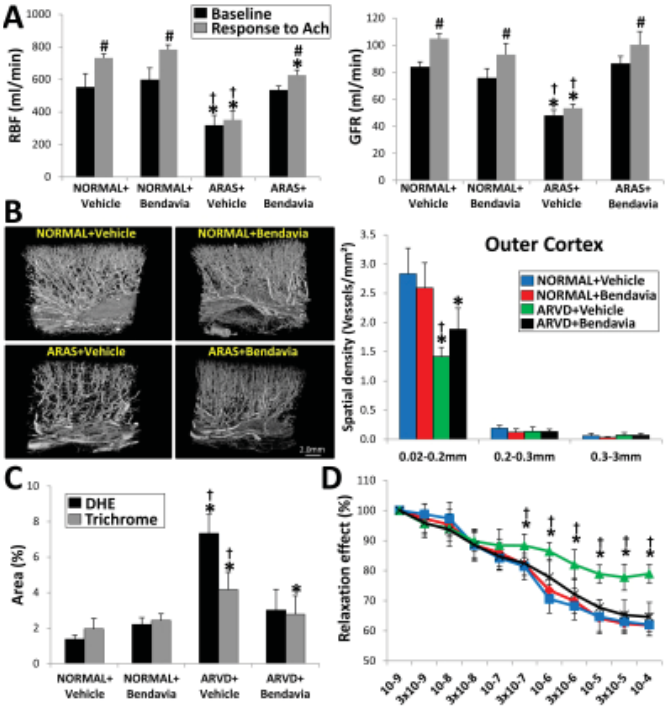
**Chronic Treatment with Bendavia Restores Renal Function in Swine Atherosclerotic Renal Artery Stenosis** Alfonso Eirin, Xin Zhang, James D. Krier, John A. Crane, John R. Woollard, Xiang-Yang Zhu, Stephen C. Textor, Amir Lerman, Lilach O. Lerman. *Mayo Clinic.*

**Background:** Few therapeutic options are available to blunt kidney injury in patients with Atherosclerotic Renal Artery Stenosis (ARAS). Bendavia, a novel tetrapeptide that prevents cardiolipin peroxidation in the inner mitochondrial membrane, improves renal outcomes in swine ARAS when infused adjunctively during revascularization. However, whether chronic treatment with Bendavia alone attenuates stenotic-kidney structural and functional deterioration remains unknown. We hypothesized that daily treatment with Bendavia would attenuate tissue injury and improve renal function in swine ARAS.

**Methods:** Four groups of pigs (n=7 each) after 6 weeks of ARAS or sham were treated for 4 additional weeks with SC injections of Bendavia (0.1mg/kg) or vehicle (5d/week). Single-kidney hemodynamics and function were studied using fast-CT, and microvascular architecture (micro-CT), oxidative stress (DHE), and fibrosis (trichrome) ex-vivo. Renal endothelial function (responses to acetylcholine [Ach]) was studied in vivo (CT) and ex vivo (renal arteries, organ bath).

**Results:** All ARAS pigs developed hypertension (mean arterial pressure=137.4±13.2mmHg). Renal blood flow (RBF) and glomerular filtration rate (GFR) were blunted in ARAS+Vehicle, but improved in ARAS+Bendavia. Loss of small outer cortical microvessels, oxidative stress, and fibrosis were ameliorated in Bendavia-treated pigs. Furthermore, Bendavia normalized renal endothelial function both in vivo and in vitro.

**Conclusions:** Chronic treatment with Bendavia in ARAS pigs improved stenotic kidney hemodynamic and function, and normalized vascular endothelial function in-vivo and ex-vivo. Microvascular loss, oxidative stress, and fibrosis were attenuated in ARAS+Bendavia, uncovering a unique therapeutic potential for Bendavia in restoring renal function in chronic experimental ARAS.




**A:** Single-kidney renal blood flow (RBF), glomerular filtration rate (GFR), and their responses to acetylcholine (Ach). **B:** Representative micro-CT images (left) and spatial density of outer cortical microvessels (right). **C:** Quantification of renal production of superoxide anion [dihydroethidium (DHE)] and tubulointerstitial fibrosis (trichrome). **D:** Endothelial-dependent relaxation of renal arterial segments from the study groups in response to increasing doses of Ach. *p<0.05 vs. NORMAL+Vehicle, †p<0.05 vs. ARAS+Bendavia. #p<0.05 vs. Baseline.

*Funding:* Pharmaceutical Company Support - Stealth Peptides Inc.

## SA-PO1038

**Mitochondrial Targeted Peptide Attenuate Myocardial Damage in Experimental Atherosclerotic Renovascular Disease** Alfonso Eirin, Xin Zhang, James D. Krier, Xiang-Yang Zhu, Stephen C. Textor, Amir Lerman, Lilach O. Lerman. *Mayo Clinic.*

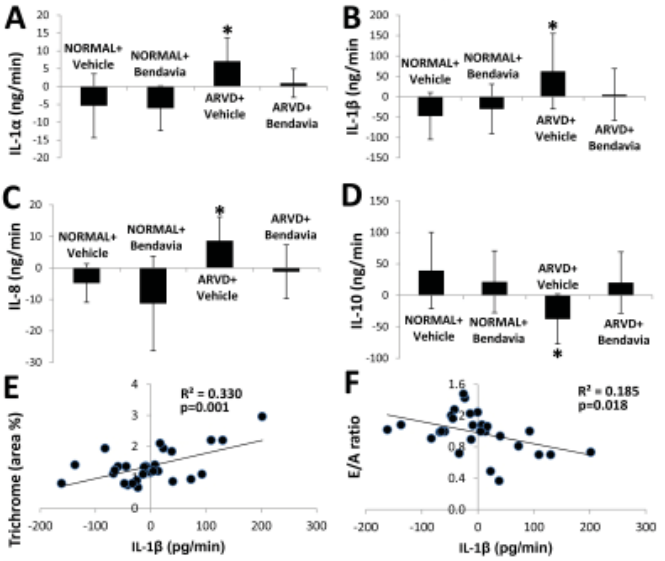
**Background:** Atherosclerotic renovascular disease (ARVD) leads to hypertension and abnormalities in cardiac structure and function, partly due to release of inflammatory cytokines from the post-stenotic kidney. Mitochondrial-targeted peptides (Bendavia) have shown novel reno- and cardio-protective properties in experimental ischemic disease. We hypothesized that chronic treatment with Bendavia would decrease cardiac remodeling and dysfunction in swine ARVD.

**Methods:** After 6 weeks of ARVD (unilateral renal artery stenosis and high-cholesterol diet) or control, pigs were treated for another 4 weeks with Bendavia (0.1mg/kg SC, 5d/week) or vehicle (n=7 each). Single-kidney and cardiac function were assessed by multidetector CT, and myocardial fibrosis by trichrome. Renal vein and inferior vena cava levels of interleukins (IL)1-α, 1β, 8, and 10 were measured by ELISA, and their net release calculated from their gradient.

**Results:** Blood pressure was similarly elevated in both ARVD groups, yet glomerular filtration rate (GFR), left ventricular muscle mass (LVMM), E/A ratio, and myocardial fibrosis normalized only in Bendavia-treated pigs (Table). Release of inflammatory IL was higher in the ARVD+Vehicle and correlated with myocardial fibrosis and diastolic dysfunction, but decreased in ARVD+Bendavia pigs (Figure).

**Conclusions:** Chronic treatment with Bendavia improved stenotic kidney function, and prevented myocardial hypertrophy, fibrosis, and diastolic dysfunction. These effects might be mediated partly by ameliorating renal dysfunction and inflammatory cytokines release, underscoring ubiquitous protective properties of Bendavia in chronic experimental ARVD.

| | NORMAL+ Vehicle | NORMAL+ Bendavia | ARVD+ Vehicle | ARVD+ Bendavia |
|---|---|---|---|---|
| Mean arterial pressure (mmHg) | 82.2±41.6 | 84.4±23.7 | 136.5±5.0* | 138.4±5.8* |
| GFR (ml/min) | 84.0±3.8 | 75.8±6.8 | 48.0±4.0*† | 86.6±11.2 |
| Ejection fraction (%) | 58.5±10.0 | 58.1±4.4 | 60.0±7.0 | 60.8±6.1 |
| LVMM (g/kg body weight) | 1.7±0.3 | 1.6±0.1 | 2.5±0.4*† | 1.8±0.1 |
| E/A ratio | 1.2±0.3 | 1.0±0.1 | 0.7±0.3*† | 1.0±0.2 |
| Trichrome (area %) | 0.9±0.2 | 1.2±0.2 | 2.2±0.5*† | 1.2±0.2 |




Net release of the inflammatory IL1-α, IL1-β, and IL-8 was higher in the ARVD kidney, and decreased in Bendavia-treated pigs (A-C). Anti-inflammatory IL-10 showed an opposite pattern. *p<0.05 vs. Normal; †p<0.05 vs. ARVD+Bendavia. Net release of IL1-β correlated directly with myocardial fibrosis (E), but inversely with diastolic dysfunction (F).

*Funding:* Pharmaceutical Company Support - Stealth Peptides Inc.

## SA-PO1039

**Renal and Vascular Safety after Renal Denervation Using the Symplicity Catheter in a Porcine Preclinical Model** Stefan Tuney, Robert J. Melder. *Cardiovascular Integrative Sciences, Medtronic Cardiovascular, Santa Rosa, CA.*

**Background:** Renal denervation (RDN) by radio frequency (RF) energy using the Symplicity Flex™ catheter significantly reduces blood pressure in patients with resistant hypertension. Preclinical studies to support the safety of RDN are necessary.

**Methods:** The renal and vascular safety following RDN by RF energy was evaluated quantitatively in normal swine at 7, 14, and 28 days by histological morphometric analysis and blood biochemistry. The success of RDN was confirmed by the significant drop in tissue norepinephrine values measured in the renal cortex. Naïve animals were used as controls for all time points.

**Results:** Histologically, the average circumferential extension of the RF lesion in the arterial media at 7 days was estimated at 28%. Quantitative morphometric analysis of the medial lesion extension confirmed this observation and yielded an average value of 26% (N=415 histological sections). Endothelialization was scored for each histological section semi-quantitatively and showed scores of 4 (>95% of the luminal surface covered by endothelium) for all sections at 7, 14 and 28 days. Quantitative morphometric analysis of endothelialization confined to the areas of RF lesions was performed using CD31 immunohistochemistry and demonstrated that the average percent endothelialization of the lesion area only at 7 days is >99%. Thin caged mural thrombi (completely endothelialized) were noted histologically in 5 out of 54 vessels at day 7 whereas none of the vessels at day 14 of 28 contained any thrombi. Histological assessment of serial kidney sections from all time points (N=1260 sections) failed to demonstrate presence of renal thrombo-embolization, infarction, foreign bodies, hemorrhage, microgranulomas and fibrosis. Furthermore, blood chemistry analysis showed that the treated animals did not develop significant deviations in renal physiological blood parameters (BUN, Creat, Ca, P, Na, K, Cl, BUN/Creat).

**Conclusions:** The data demonstrated that RDN via the Symplicity catheter results in consistent RF lesions with no compromise in vascular integrity, followed by a rapid vascular healing with no downstream anatomical or physiological consequences to the kidney.

*Funding:* Pharmaceutical Company Support - Medtronic, Inc

## SA-PO1040

**Angiotensin-(1-7) Modulates Renal Vascular Resistance through Inhibition of Mitogen-Activated Protein Kinase p38 in Apolipoprotein E Deficient Mice** Sebastian Alexander Potthoff, Lars C. Rump, Johannes Stegbauer. *Dept of Nephrology, Medical Faculty, Univ Düsseldorf, Düsseldorf, Germany.*

**Background:** ApoE KO mice are characterized by increased vasoconstrictor response. Previously, we have shown that chronic Ang-(1-7) treatment ameliorates endothelial dysfunction in apoE KO. However, the impact of Ang-(1-7) on vasoconstrictor response to Ang II is unknown.

**Methods:** To examine the role of Ang-(1-7) in vascular resistance in atherosclerosis, apoE KO and wild type (WT) mice fed on western diet were treated via osmotic minipumps either with Ang-(1-7) (82µg/kg/hr) or saline for 6 weeks. Mas/apoE double KO mice were used to investigate the role of the Ang-(1-7) recepter, Mas.

**Results:** Ang II induced renal pressor response was significantly increased in apoE KO compared to WT mice. Chronic Ang-(1-7) treatment attenuated AngII induced pressor response in apoE KO mice. Accordingly, phospho-MLC20 was increased in preglomerular vessels of apoE KO and reduced after Ang-(1-7) treatment. Ang-(1-7) treatment significantly decreases renal p47phox and urinary isoprostane-8 excretion. In addition, it reduced superoxide production in renal cortext tissue ex vivo. ROS is known to activate the MAPK p38. To test the role of p38-activation, we measured p38-activity and renal pressor response to AngII in the presence of SB203580, a p38-inhibitor. In preglomerular vessels, chronic Ang-(1-7) treatment restored the level of phospho-p38 almost to WT levels. Moreover, administration of SB203580 (5µmol/L) reduced renal pressor response to Ang II in apoE KO but not in Ang-(1-7) treated mice. Hence, chronic inhibition of p38 with BIRB796 (50mg/kg/day) attenuated the increased pressor response to Ang II in apoE KO mice. Chronic Ang-(1-7) treatment did not attenuated AngII induced pressor response nor did it decrease urinary isoprostane-8 excretion in in Mas / apoE double KO mice.

**Conclusions:** In summary, p38 plays a crucial role in regulating renal vascular resistance in apoE KO mice. Moreover, Ang-(1-7) attenuates pressor response to Ang II by reducing p38 activity mediated by reduction in ROS abundance, which is Mas-receptor dependent. Thus, this study reveals a new pathway how Ang-(1-7) modulates vascular function.

*Funding:* Government Support - Non-U.S.

## SA-PO1041

**The Macula Densa (Pro)renin Receptor Functions as an Amplifier of Renin Synthesis and Release** Anne Riquier-brison, Karie G. Villanueva, James L. Burford, Janos Peti-Peterdi. *Dept of Physiology and Biophysics, Univ of Southern California, Los Angeles, CA.*

**Background:** The (pro)renin receptor (P)RR was initially thought to be a new addition to the renin angiotensin system (RAS), but a recent paradigm shift questioned its association with the RAS and suggested new roles for (P)RR in organ development and cell differentiation. Recently, we found (P)RR expression in the basolateral cell membrane and signaling via MAP kinases ERK1/2 and p38 in macula densa (MD) cells which regulate renal hemodynamics and renin. Exogenous renin or prorenin added to the in vitro microperfused juxtaglomerular apparatus (JGA) preparation induced renin release which was inhibited by removing the MD or by the administration of the (P)RR blocker decoy peptide suggesting a role of MD (P)RR in renin control.

**Methods:** Here, we aimed to study the role of MD (P)RR in stimulating renin synthesis in vivo. We established a new mouse model with MD-specific conditional knockout (cKO) of the (P)RR by intercrossing nNOS-CreERT2 and floxed (P)RR mice.

**Results:** At baseline, blood pressure was not affected by removal of MD (P)RR (10 days following tamoxifen induction, SBP=112±9 mmHg in WT, 107±14 mmHg in (P)RR cKO). However, after 1 week on a combined low salt + captopril treatment (in order to challenge the RAS), normalized renin expression in whole kidney homogenates was significantly lower in (P)RR cKO animals (1.00±0.11 in wild type, 0.58±0.09 in (P)RR cKO, p=0.003). Similarly, microsomal prostaglandin E synthase (mPGES-1), a critical element of the MD renin release signaling cascade, was also lower in (P)RR cKO mice (1.00±0.10 in wild type, 0.58±0.08 in (P)RR cKO, p=0.005). A 20% decrease in COX2 expression in (P)RR cKO mice did not reach significance. Renin immunofluorescence confirmed reduced JGA renin expression in (P)RR cKO versus wild-type mice.

**Conclusions:** These results suggest the key role of the MD (P)RR in amplifying renin synthesis in vivo when the RAS is challenged. Also, this study confirmed the direct role and involvement of the (P)RR in the RAS consistent with the original paradigm.

*Funding:* NIDDK Support

## SA-PO1042

**Renal Distal Cortex Perfusion Reflects Cardiac Function in Chronic Kidney Disease** Arkadiusz Lubas, Robert Ryczek, Jerzy Smoszna, Stanislaw Niemczyk. *Military Institute of Medicine.*

**Background:** Disturbances of renal perfusion deregulate renal tissue oxygenation and nutrition causing the decrease of organ function. Properly preserved cardiac hemodynamic function is a key-factor for maintaining sufficient renal perfusion. The aim of the study was to evaluate a relationship between the renal cortex perfusion and cardiac functional and structural markers in patients with stable chronic kidney disease (CKD).

**Methods:** Twenty six patients (4 F; 22 M; age 50 ±17) with stable CKD (Cre-CKD-EPI 48.8 ±26.5 ml/min/1,73m$^2$) and a history of hypertension or heart failure were enrolled in the study. Serum Creatinine (Cre), Cystatin C (Cys), NT-pro brain natriuretc peptide (NTpBNP), Troponin I (TnI) were tested. Renal function was estimated according to Cre-

and Cys- based CKD-EPI formula. Echocardiographic examination, ABPM, estimation of Intima-Media Thickness (IMT), and mean arterial Perfusion intensity in Color Doppler (PixelFlux, Chameleon Software, Germany) of Total (TCP), Proximal (PCP) and Distal (DCP) renal Cortex were collected.

**Results:** DCP was significantly lower than PCP and TCP (0.071 ±0.66 cm/s vs 0.464 ±0.334 cm/s and 0.273 ±0.188 cm/s; p<0.001). TCP was significantly correlated with age (r=-0.47), Cys (r=-0.43), NTpBNP (r=-0.48), Cys-CKD-EPI (r=0.43), IMT (r=-0.43) and SBP (r=0.42). PCP was associated with age (r=-0.47), Cys (r=-0.50), Cre (r=-0.43), NTpBNP (r=-0.45), Cys-CKD-EPI (r=0.48), IMT (r=-0.42) and SBP (r=0.43). Whereas, only DCP was significantly correlated with left ventricle hemodynamic parameters: stroke volume (LVSV) (r=-0.59); cardiac output (LVCO) (r=-0.53), cardiac index (LVCI) (r=-0.56). In addition, DCP was associated with left ventricular mass index (LVMI) (r=-0.43) and NTpBNP (r=-0.45). In multiply stepwise regression analysis model (TnI, NTpBNP, Cre-CKD-EPI, Cys-CKD-EPI, LVEF, LVMI, LVSV, LVCO, LVCI, IMT and MAP) NTpBNP, LVCO and Cys-CKD-EPI independently influenced DCP ($R^2$=0.66, p=0.001).

**Conclusions:** Renal cortical perfusion is significantly associated with cardiac function in CKD patients. Ultrasound measurement of renal perfusion should be considered as a new tool in diagnosing cardio-renal interactions.

*Funding:* Clinical Revenue Support

## SA-PO1043

**Beraprost Sodium Suppresses Progression of Chronic Renal Failure by Protecting Renal Capillary Endothelial Cells and Improving Renal Hypoxia in Glomerulonephritis Rats** Shinichi Yamaguchi, Yasufumi Goto, Nahoko Sato, Hajimu Kurumatani, Mitsuko Miyamoto, Masateru Yamada. *Pharmaceutical Research Laboratories, Toray Industries Inc., Kanagawa, Japan.*

**Background:** Beraprost sodium (BPS), a chemically stable prostacyclin analog, has been reported to suppress the progression of chronic renal failure (CRF) in glomerulonephritis (GN) rats (Kidney Week 2012). Also, an international P-IIb/III study of BPS using a renal composite endpoint is on-going in Asia (China, Hong Kong, Japan, Malaysia, Republic of Korea, Taiwan and Thailand). In order to clarify the mechanism of the suppression of CRF progression, we examined whether BPS protects renal capillary endothelial cells and improves renal hypoxia in GN rats.

**Methods:** GN was induced to rats by injecting anti-glomerular basement membrane antibody. BPS administration (0.6mg/kg/day, p.o.) was started from day 15 after GN induction when serum creatinine levels were increased significantly. Glomerular flow rate (GFR) was measured by inulin clearance method. The number of renal capillary endothelial cells was quantitatively measured by immunohistochemistry with anti-aminopeptidase P antibody-positive area. Renal hypoxic region was evaluated by immunohistochemistry with anti-pimonidazole antibody-positive area.

**Results:** At day 15 after GN induction, GFR in GN rats was significantly decreased to 57% of normal rats, and further decreased to 11% at day 50. In BPS-treated GN rats, the decrease in GFR was completely inhibited until day 50. Anti-aminopeptidase P antibody-positive area in GN rats was decreased to 60% of normal rats at day 15, and further decreased to 44% at day 50. The decrease in the number of renal capillary endothelial cells was significantly prevented in BPS-treated GN rats. Furthermore, the anti-pimonidazole antibody-positive area was increased in GN rats at day 50. And, the increase in renal hypoxic region was also prevented by BPS administration.

**Conclusions:** In this study, BPS inhibited the decrease of GFR, protected renal capillary endothelial cells and improved renal hypoxia associated with CRF in GN rats. Therefore, it is suggested that the improving effect of BPS on renal hypoxia contributes to the suppression of CRF progression.

## SA-PO1044

**Renal Vein Inflammatory and Injury Biomarkers Are Associated with Net Renin Release and Persist Despite ACE/ARB Therapy in Human Atherosclerotic Renal Artery Stenosis (ARAS)** Sandra Herrmann, Ahmed Saad, Alfonso Eirin, Lilach O. Lerman, Stephen C. Textor. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** ARAS is known to reduce renal blood flow(RBF) and induce renin release, but the relationships between tissue inflammation and injury and renin release are poorly understood. The purpose of this study was to examine the renin release and renal injury as reflected by systemic and renal vein(RV) levels of the neutrophil gelatinase associated lipocalin(NGAL) and the inflammatory cytokines TNF-α and MCP-1 which are induced in inflammation and ischemia.

**Methods:** Inpatient studies were performed in ARAS(n=39), with more than 60% occlusion, or in essential hypertensives(EH)(n= 32), during fixed Na+ intake and ACE/ARBRx. We measured IVC and RV levels of NGAL,MCP-1 and TNF-α. Plasma renin activity(PRA) was measured and net contribution of renal vein renin(RVR) was determined as(Renal vein PRA-IVC PRA)/IVC PRA X 100%. RBF was measured with multidetector CT and GFR by iothalamate clearance.

**Results:** Renal blood flow, perfusion, and GFR were reduced in the STK. Renal vein levels of NGAL, TNF-α and MCP-1 were higher in ARAS than EH (Table). Net renin contribution was elevated in the STK and correlated with IVC NGAL (r=0.5, p=0.02) and MCP-1 (r=0.4, p=0.02) levels in the STK but not with TNF-α.

**Conclusions:** Occlusive renovascular disease triggers pressor mechanisms including renin release. These results demonstrate an association of net renin contribution of the STK and activation of inflamatory and injury pathways, despite therapeutic blockade of the renin-angiotensin system(RAAS). We interpret these data to indicate that RAAS blockade alone fails to inactivate inflammatory injury in this condition that likely requires additional anti-inflammatory measures to restore post-stenotic kidney function.

| | | ARAS | | |
|---|---|---|---|---|
| | EH | Stenotic Kidney | Contra-lateral Kidney | P value |
| Net Renin Contribution(%) | 23.1±36.2 | 86.42± 98.6* | 23.9±52.2* | 0.0005 |
| RV NGAL(ng/mL) | 66.9±37.5 | 134.1±52.3* | 129.7±48.3* | <0.0001 |
| RV MCP-1(pg/mL) | 152.8±72.1 | 188.4±67.6* | 171.7±68* | 0.05 |
| RV TNF-α (pg/mL) | 4.6±4.3 | 16.6±26.5* | 11.5±8.2* | <0.0001 |
| IVC NGAL(ng/mL) | 83.5±46.8 | 121.1±55.5* | | <0.027 |

P≤ 0.05 versus EH.

*Funding:* Other NIH Support - P01HL085307 from the National Heart, Lung, and Blood Institute and National Institutes of Health

## SA-PO1045

**Contrast-Enhanced Ultrasound Using Perflubutane Microbubble Agent Is Useful to Evaluate the Degree of Tubulointerstitial Injury** Kayori Tsuruoka, Takashi Yasuda, Tsutomu Sakurada, Sayuri Shirai, Yugo Shibagaki, Kenjiro Kimura. *Div of Nephrology and Hypertension, St. Marianna Univ School of Medicine, Kawasaki, Kanagawa, Japan.*

**Background:** Although we evaluate kidney function mostly by glomerular filtration rate, the degree of tubulointerstitial injury (TII) is more predictable of renal prognosis, but is harder to assess.We have reported that kidney contrast-enhanced ultrasound (CEUS) using Perflubutan is useful to evaluate renal microcirculation inpatients with chronic kidneydisease (CKD). To evaluate the possibility of assessing TII by CEUS, we reexamine the result of our previous study, by comparing CEUS parameters with kidney functional and histological findings.

**Methods:** We examined the parameters of CEUS in total of 23 CKD patients who underwent kidney biopsy. We first obtained the data of PSV (peak systolic velocity), EDV (end diastolic velocity), RI (resistive index) using color Doppler. For CEUS, we administered bolus i.v. (0.0075mg/kg) of perflubutane from median vein, then flushed with saline and recorded the data for ten minutes. We evaluated the contrast intensity using image analysis software, and drew a time intensity curve (TIC). Percutaneous kidney biopsy was obtained within one month after a CEUS and calculated the degree of tubuointerstitial injury.

**Results:** In patients with CKD, the time to reach the peak was delayed, peak intensity was low, intensity decreased earlier, and TIC was gentle, compared to those in patients with normal renal function.Because the change in the first 3 seconds from the start of cortical enhancement was remarkable, we assessed the slope of the TIC during this 3 seconds (CS-3 slope) that represents the intensity of the rising gradient. CS-3 slope had a strong correlation with the degree of tubulointerstitial injury (r=0.70, p<0.0001), and a mild correlation with eGFR (r=0.48, p=0.022). By multivariate analysis, CS-3 slope had significant correlations with RI, PSV, EDV.

**Conclusions:** Contrast-enhanced kidney ultrasound with perflubutane may be a useful tool to assess the degree of tubulointerstitial injury.

## SA-PO1046

**Involvement of ACE and ACE2 in Kidney and Cardiac Dysfunction in Rats with Heart Failure** Ravit Cohen,[1] Hoda Awad,[1] Niroz Abu-saleh,[1] Zaher Armaly,[2] Zaid Abassi.[1,3] *[1]Physiology, Faculty of Medicine, Technion, Haifa, Israel; [2]Nephrology, EMMS, Nazareth Hospital, Nazareth and Galilee Medical School-Bar Ilan Univ, Zafad, Israel; [3]Rambam Medical Center, Haifa, Israel.*

**Background:** The role of Angiotensin II (Ang II) in the pathogenesis of CHF has been well established. However, the involvement of Angiotensin 1-7 (Ang 1-7) and ACE2 in this phenomenon is largely unclear. This study examined (a) Expression of ACE and ACE2 in the renal and cardiac tissues of CHF rats, and (b) Acute and chronic renal and cardiac effects of Ang 1-7, AVE0991 (Ang 1-7 agonist), and A779 (Ang 1-7 antagonist) in these rats.

**Methods:** CHF was induced by surgical creation of aorto-caval fistula. ACE and ACE2 immunoreactivities were determined in the renal and cardiac tissues of CHF rats and their sham controls. In the acute protocol, the effects of incremental doses of Ang 1-7, AVE0991, or A779 on glomerular filtration (GFR), renal plasma flow (RPF), urinary flow (V), urinary sodium excretion (UNaV), and urinary excretion of cyclic GMP (UcGMP) were determined in these rats. In the chronic protocol, the effects of 4-week treatment with the above described compounds on kidney function and cardiac hypertrophy were evaluated in these animals.

**Results:** ACE and ACE2 immunoreactivities in the heart and kidney were ~2 fold higher in CHF rats. Acute infusion of either Ang 1-7 or AVE0991 at a high dose into CHF rats evoked significant increases in V, UNaV, GFR and RPF along enhanced UcGMP. In the chronic protocols, untreated CHF rats displayed lower cumulative V and UNaV than their controls. Chronic administration of Ang 1-7 and AVE0991 exerted significant diuretic and natriuretic effects in CHF rats, but not in sham. Serum Cr and Aldo levels were significantly higher in vehicle-treated CHF rats. Treatment with Ang 1-7 and AVE0991 reduced these parameters. Noteworthy, chronic administration of Ang 1-7 to CHF rats caused reduction of cardiac hypertrophy.

**Conclusions:** This study demonstrates that ACE and ACE2 are activated in CHF. Upregulation of ACE2 and Ang 1-7 with their beneficial actions may compose a compensatory response in face of the deleterious classic RAAS to prevent worsened renal and cardiac dysfunctions.

## SA-PO1047

**Long-Term Administration of Type-I Interferon Leads to Premature Atherosclerosis** Yanpeng Diao, Pui Lee, Rajesh Mohandas, Mark S. Segal. *Medicine, Univ of Florida, Gainesville, FL.*

**Background:** Disease states such as SLE, CMV, and HIV are associated with elevated type I interferon (IFN-I) and increased cardiovascular risk. We have demonstrated that IFN-I has detrimental effects on endothelial progenitor cells that could contribute to the atherosclerosis (AS). However, the relationship between IFN-I and smooth muscle progenitor cells (SMPC), cells derived from the bone marrow that can differentiate into mature SMC, has not been determined. We hypothesized that IFN-I could induce premature AS by increasing the number of SMPC in the bloodstream and increasing the number of immature SMC within the vasculature.

**Methods:** In vitro, SMPC isolated from wild-type (WT) and IFN receptor knock-out (IFNR-KO) animals, were cultured in medium plus or minus IFN-1. In vivo, two mice models of long-term IFN-I exposure (electroporation of an IFN-I plasmid, or Q 3 day IFN-I i.p. injection for 3 months) were studied and the number of SMPC and degree of AS were analyzed.

**Results:** The number of SMPC from WT mice was significantly increased when cultured in medium plus IFN-I. However, IFN-I had no effect in the SMPC from IFNR-KO mice. Increased SMPC numbers were also observed in mice treated with or that expressed IFN-I. In addition, atherosclerotic-like lesions were found in the arterial wall in WT mice expressing IFN-I. Within the lesions we observed CD34+/SM α-actin+ expressing cells.

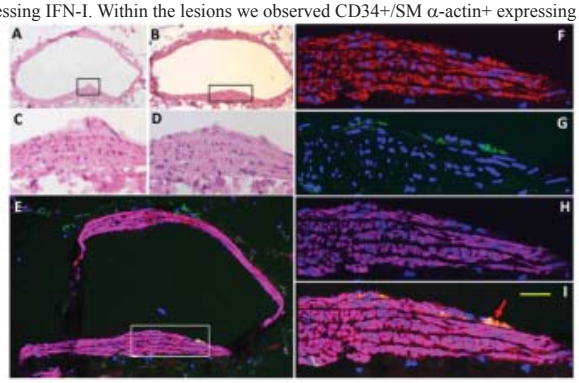



**Figure:** Atherosclerosis plaque-like lesion apparent in IFN-I treated mice. The aorta of mice whose gastrocnemius muscle were transduced with an IFN-I expressing plasmid were examined by H&E and confocal analysis, 3 months after transduction. Shown are low (20X, **A&B**) and high (40X, **C&D**) magnifications of the H&E stain from cross sections of the aortic bifurcation. Plaque-like lesions (**A&C**) and thickening of the vessel walls (**B&D**), indicated by the black box, were seen in IFN-I treated mice at much higher frequency (p=0.011) than in control mice. Immunohistochemical stain (with anti-smooth muscle(SM)-α-actin (red), anti-CD34 (green) and anti-SM myosin heavy chain (purple)) of an adjacent section to **B** was examined under low (20X, **E**) and high (63X, **F-I**) magnification by confocal analysis. A yellow, immature SMC, a cell that expresses SM-α-actin (red) and CD34 (green), but does express SM-MHC (purple), is indicated by the red arrow in I. Bar=25 μm.

These cells did not express SM myosin heavy chain, suggesting that they were immature SMC.

**Conclusions:** For the first time we demonstrated that elevated IFN-I levels could lead to an atherosclerosis-like lesion. In concert with our previous work, this work suggests a complex interplay between the bone marrow, circulating cells, and the vessel wall leading to IFN-I mediated atherosclerosis. This has profound implications for cardiovascular disease in a number of disease states.

## SA-PO1048

**Aryl Hydrocarbon Receptor Plays a Crucial Role for Uremic Toxin-Induced Vascular Inflammation** Shunsuke Ito,[1,2] Yoshiharu Itoh,[2] Masayuki Yoshida.[1] *[1]Tokyo Medical and Dental Univ; [2]Kureha Corporation.*

**Background:** Indoxyl sulfate has been know as one of the major components of uremic toxin in chronic kidney disease patients. The aryl hydrocarbon receptor (AhR) is a ligand-inducible transcription factor known to mediate the toxic effects of numerous environmental contaminants including indoxyl sulfate. In the present study, we investigated a potential role of AhR in indoxyl sulfate-induced leukocyte-endothelial interaction.

**Methods:** Human umbilical vein endothelial cells (HUVEC) were transfected with siRNA of AhR (siAhR) and then treated with indoxyl sulfate for 20 h, followed by stimulation with TNF-α for 4 h. Leukocyte (HL-60) adhesion assay was carried out under physiological flow condition. Protein expression and phosphorylation was evaluated by western blotting. Reactive oxygen species (ROS) production was investigated with CM-$H_2$DCFDA staining.

**Results:** siAhR significantly decreased indoxyl sulfate-enhanced leukocyte adhesion. Western blotting analysis revealed that siAhR abrogated indoxyl sulfate-enhanced expression of adhesion molecule such as E-selectin. To our surprise, though indoxyl sulfate enhanced activation of JNK and NF-κB, siAhR did not suppress their induction. siAhR did not affect indoxyl sulfate-induced ROS production. To assess the functional role of AhR in the indoxyl sulfate stimulated E-selectin gene induction, we conducted luciferase promoter assay. We found that the promoter region of E-selectin, corresponding to -153 to -146 bps, was essential for the effect of indoxyl sulfate mediated by AhR. Because the indoxyl sulfate response region is similar to AP-1 response element and cAMP response element (CRE), we investigated which transcription factors were activated by indoxyl sulfate. Mutation in the indoxyl sulfate response region to AP-1 response element did not affect indoxyl sulfate-induced E-selectin promoter activity, however, mutation to CRE decreased it. We also found that indoxyl sulfate induced AP-1 transcriptional activity but not that of CRE.

**Conclusions:** Our data suggest an important role for AhR in indoxyl sulfate-enhanced leukocyte-endothelial interaction and provides a novel mechanistic insight into the CKD-related vascular inflammation.

*Funding:* Government Support - Non-U.S.

## SA-PO1049

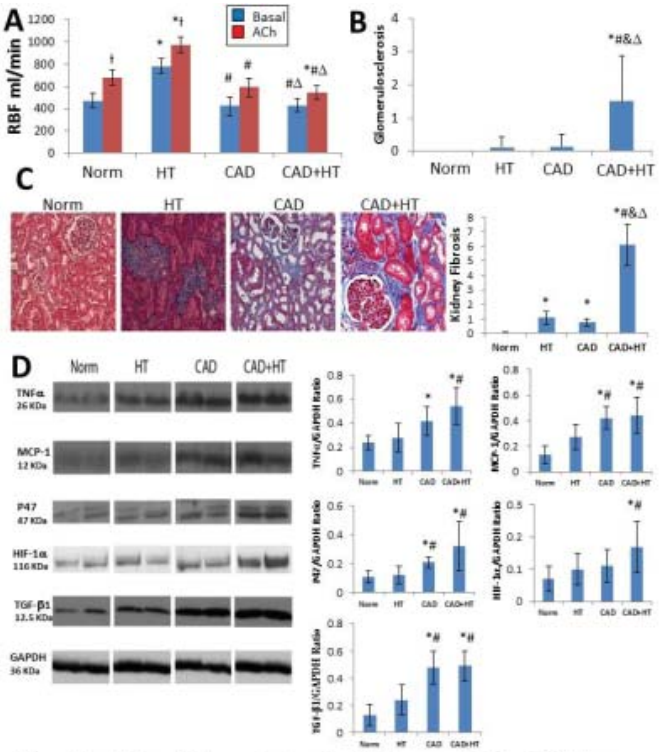
**Experimental Coronary Artery Disease Accelerates Kidney Fibrosis in Hypertensive Swine** Dong Sun, Alfonso Eirin, Xiang-Yang Zhu, Xin Zhang, John A. Crane, John R. Woollard, Amir Lerman, Lilach O. Lerman. *Mayo Clinic.*

**Background:** Patients with coronary artery disease (CAD) often have hypertension (HT). Cardiac dysfunction can impair kidney function, but if CAD aggravates renal injury is unknown. We hypothesized that CAD elicits kidney inflammation, which is exacerbated by HT.

**Methods:** Domestic pigs were randomized to normal, CAD, renovascular HT or CAD + HT (n=7, each). Cardiac and non-stenotic kidney function were measured by multidetector CT. Renal fibrosis and glomerular score were assessed by trichrome staining. Tumor necrosis factor (TNF)a, monocyte chemoattractant protein (MCP)-1, p47, hypoxia inducible factor (HIF)-1a and transforming growth factor (TGF)-b1 expressions were analyzed by western blotting.

**Results:** Mean arterial pressure increased in both HT and CAD+HT, and serum creatinine was elevated in CAD, HT and CAD+HT. Urine protein increased significantly in CAD+HT compared to normal and CAD. The levels of isoprostane were increased in CAD and CAD+HT compared to normal and HT. Compared with HT, basal renal blood flow (RBF) and cortical perfusion decreased in CAD and the non-stenotic kidney of CAD+HT, but their response to acetylcholine (ACh) was blunted only in CAD and CAD+HT. Basal medullary perfusion was similar among the four groups, but its response to Ach was blunted only in CAD+HT. MCP-1, p47 and TGF-b1 expressions were elevated in both CAD and CAD+HT compared with normal and HT, whereas TNFα and HIF-1a were increased only in CAD+HT than normal and HT. Furthermore, both glomerulosclerosis and fibrosis increased synergistically in CAD+HT compared to normal, CAD and HT (Figure).

**Conclusions:** CAD augments inflammation and increases systemic and renal oxidative stress and synergistically interacts with HT to impair RBF and increase kidney fibrosis. This interaction may contribution to the increased incidence of renal failure seen when CAD and HT coexist.




**A**-Renal Blood Flow; **B**-Glomerulosclerosis; **C**-Trichrome staining ; **D**-Western Blotting. $^{*}P<0.05$ compared with Norm; $^{\#}P<0.05$ compared with HT; $^{\&}P<0.05$ compared with CAD; $^{\dagger}P<0.05$ compared with baseline, $^{\Delta}P<0.05$ for synergistic interaction CAD*HT. Norm=Normal; HT=Hypertension; CAD=Coronary Artery Disease.

*Funding:* NIDDK Support

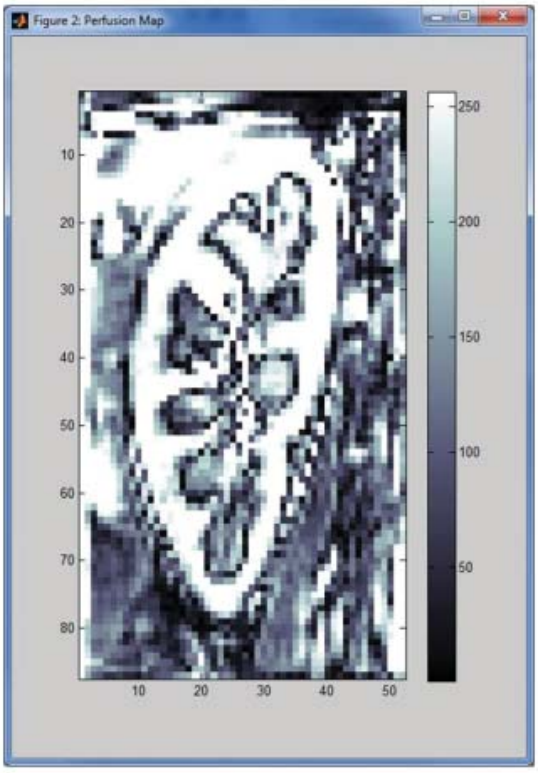
**SA-PO1050**

**Reproducibility of Renal Perfusion Measurements Using Arterial Spin Labelling MRI – A Healthy Volunteer Study** Keith Gillis,[2] Christie McComb,[2] Scott Morris,[2] Christian Delles,[2] Patrick B. Mark.[2] *[1]Glasgow Renal and Transplant Unit, Western Infirmary Glasgow, Glasgow, United Kingdom; [2]Univ of Glasgow, Glasgow, United Kingdom.*

**Background:** Accurate assessment of renal perfusion is a crucial part of measuring kidney function and allows assessment of renal hemodynamics in health and disease. Arterial spin labelling magnetic resonance imaging (ASL MRI) is a novel non invasive method of measuring renal function using paramagnetic properties of blood as endogenous contrast. We studied the reproducibility of ASL MRI in normal volunteers.

**Methods:** ASL MRI was performed in 12 healthy volunteers on 2 occasions 4-28 days apart on a 3 Tesla MRI scanner along with measurement of routine serum biochemistry. A flow sensitive alternating inversion recovery perfusion and true fast imaging with steady precession sequence (FAIR True-FISP) was utilised to obtain 5 kidney images on each occasion. Cortical and whole kidney perfusion was then calculated on 24 kidneys on 2 visits using automated analysis software.

Automated analysis of ASL MRI images




**Results:** All subjects (5 male, mean age 44 years) completed 2 ASL MRI studies. Mean estimated glomerular filtration rate was 98.3 ml/min by CKD-EPI formula. Mean cortical perfusion (CP) on visit 1 was 321 ml/min/100g whilst on visit 2 was 336ml/min/100g. Whole kidney perfusion (WKP) was 227ml/min/100g on visit 1 and 230ml/min/100g on visit 2. There was no significant difference between perfusion values measured in visit 1 and visit 2 (CP p=0.167; WKP p =0.659). Bland Altman plots showed no systematic bias when comparing perfusion measured at each visit, whilst the mean difference between visits was 13ml/min/100g for CP and 2.6ml/min/100g for WKP.

**Conclusions:** ASL MRI provides a repeatable method of measuring renal perfusion in healthy volunteers. Further research is required to determine if this is also the case in renal impairment, and if findings correlate with renal function.

*Funding:* Private Foundation Support

**SA-PO1051**

**Influence of Klotho Gene Polymorphisms on Vascular Klotho Expression and Cardiovascular Disease** Javier Donate,[1] Rafael Martínez,[2] Ernesto Martín,[1] Carmen Mora,[1] Mercedes Muros,[3] Violeta Cazaña,[1] Juan F. Navarro-Gonzalez.[1,4] *[1]Research Unit, Univ Hospital Ntra. Sra. de Candelaria, Santa Cruz de Tenerife, Spain; [2]Cardiac Surgery Service, Hospita Universitario de Canarias, La Laguna, Spain; [3]Clinical Analysis Service, Univ Hospital Ntra. Sra. de Candelaria, Santa Cruz de Tenerife, Spain; [4]Nephrology Service, Univ Hospital Ntra. Sra. de Candelaria, Santa Cruz de Tenerife, Spain.*

**Background:** Klotho protein has been associated with beneficial effects on cardiovascular (CV) health. The aim of our study was to analyze the relationship between Klotho gene polymorphisms, mRNA expression levels in the vascular wall and the presence of vascular disease.

**Methods:** We analyzed three single nucleotide polymorphisms (SNPs) of *Klotho* locus [F352V (rs9536314) (exon 2), G-395A (rs1207568) (promoter) and C1818T (rs564481) (exon 4)] in 111 patients undergoing coronary or valve replacement surgery. Gene expression studies were performed in fragments of thoracic aorta.

**Results:** G-395A polymorphism is related to Klotho levels in vascular tissue: Klotho mRNA levels were significantly lower in allele A carriers as compared with homozygous carriers of the G allele [0.19 ± 0.1 vs. 0.97 ± 0.17 (log relative units), P<0.0001]. These differences were not observed for the other two polymorphisms. When comparing the vascular klotho expression levels with clinical characteristics of patients, we observed a higher incidence of coronary artery disease (CAD) in subjects with lower mRNA Klotho expression (P=0.06). However, the G-395A polymorphism was not related with the incidence of CAD. By contrast, CAD and atherosclerotic disease were more frequent in patients carrying the minor allele of the F352 polymorphism (77.9%, P=0.08 and 89.8%, P=0.01, respectively). Finally, the incidence of valve calcification was higher in homozygous carriers of the C allele of C1818T polymorphism compared to carriers of the T allele (43% vs 19%, P=0.01).

**Conclusions:** Polymorphisms of the Klotho gene may influence tissue expression levels of this protein as well as may be related to the incidence of atherosclerotic vascular disease and valvular calcification.

**SA-PO1052**

**Protamine Sulfate and Hyaluronidase Reduce the Size-Selectivity, but Not the Charge-Selectivity, of the Rat Glomerular Filtration Barrier In Vivo** Kristinn Sverrisson, Josefin Axelsson, Anna Rippe, Bengt Rippe. *Dept of Nephrology, Clinical Sciences, Lund, Sweden.*

**Background:** The proteinuric actions of protamine sulfate (PS) have classically been, at least partly, attributed to alterations of the negatively charged glomerular endothelial glycocalyx (GC). To investigate whether the charge-selective properties of the glomerular filtration barrier (GFB) would be altered by PS we assessed the glomerular sieving of conventional, uncharged, polydispersed Ficoll (n-Ficoll) compared to charge modified, conformationally intact, anionic (carboxymethylated) Ficoll (a-Ficoll) before and after systemic infusions of PS in rats. For comparison we also investigated the impact of hyaluronidase (Hyase), postulated to partially degrade the glomerular GC, on GFB permeability.

**Methods:** In anaesthetized Wistar rats blood access was achieved and the left ureter was cannulated for urine collection. Rats were infused with either n-Ficoll or a-Ficoll before and during systemic infusions with either PS or Hyase in doses that minimally affected systemic blood pressure or the glomerular filtration rate. Plasma and urine samples were taken repeatedly and analyzed by high performance size exclusion chromatography (HPSEC) for assessing glomerular sieving coefficients (θ) for Ficoll (radius 13-80Å).

**Results:** As reported previously, we found a significant glomerular charge selectivity for Ficoll molecules of radius 20-45Å ($Ficoll_{20\text{-}45Å}$). PS infusions increased θ for $Ficoll_{50\text{-}80Å}$ markedly, and partly reversibly, for both n-Ficoll and a-Ficoll. For a-$Ficoll_{70Å}$ θ thus increased from $2.47 \times 10^{-5} \pm 1.1$ to $7.25 \times 10^{-5} \pm 1.1$ ($p<0.05$) at 15 min. However, neither PS nor Hyase had any effect on θ for n-$Ficoll_{20\text{-}45Å}$ or a-$Ficoll_{20\text{-}45Å}$, and thus had no effect on glomerular charge selectivity. Furthermore, contrary to PS, Hyase did not affect θ for a-$Ficoll_{50\text{-}80Å}$, whereas it caused a moderate increase in θ for large n-Ficoll molecules.

**Conclusions:** Systemically administered PS and Hyase decreased the size-selectivity of the rat GFB, but did not affect its charge selectivity, as indicated by the unaltered θ for a-$Ficoll_{20\text{-}45Å}$ after PS or Hyase infusions.

*Funding:* Government Support - Non-U.S.

**SA-PO1053**

**Continuous Erythropoietin Receptor Activator (C.E.R.A.) Improves Endothelial Function in Anemic Rats with Chronic Kidney Disease** Michinori Hirata, Ken-ichi Serizawa, Kenji Yogo, Yoshihito Tashiro, Koichi Endo. *Product Research Dept, Chugai Pharmaceutical Co., Ltd., Gotemba Shizuoka, Japan.*

**Background:** Endothelial dysfunction is closely associated with cardiovascular events in chronic kidney disease (CKD) patients. Continuous erythropoietin receptor activator (C.E.R.A.) is widely used for the treatment of anemia in CKD patients, but it is unclear whether C.E.R.A. has any endothelial protective effect. Therefore, in the present study, we assessed the influence of C.E.R.A. on endothelial function in 5/6 nephrectomized (Nx) rats.

**Methods:** C.E.R.A. (0.6 μg/kg, once every 2 weeks) was subcutaneously administered from 1 week after Nx. After 8 weeks of treatment, endothelial function was evaluated by

measurement of flow-mediated dilation (FMD) in the femoral artery of anesthetized rats by using a high-resolution ultrasound system. FMD was calculated from the change in diameter of the femoral artery (relative to baseline diameter) following reperfusion after 5 min ischemia of the iliac artery.

**Results:** Urinary protein (uTP) and blood pressure (BP) were increased in Nx rats. Femoral blood velocity instantaneously increased (i.e. reactive hyperemia) at reperfusion after 5 min of hind limb ischemia. Following reactive hyperemia, maximum arterial dilation (i.e. FMD) was observed at 0.5–1 min after reperfusion. FMD tended to negatively correlate with uTP and BP. In Nx rats treated with C.E.R.A., Hb increased to within the normal range, but uTP and BP did not show any significant change. C.E.R.A. significantly increased FMD in the femoral artery without changing reactive hyperemia. C.E.R.A. significantly decreased nitrotyrosine (a marker of peroxynitrite) in the femoral artery. In addition, C.E.R.A. enhanced phosphorylation of endothelial nitric oxide synthase (eNOS) in the femoral artery.

**Conclusions:** These results demonstrated that, in Nx rats, C.E.R.A. improved endothelial function with reduction of oxidative stress and improvement of eNOS activity.

## SA-PO1054

**Klotho Is Not Expressed Endogenously in Healthy and Uremic Vascular Tissue** Rik Mencke,[1] Joyce Struik,[2] Joris van Ark,[1] Melissa Verkaik,[3] Martin H. De Borst,[4] Marc G. Vervloet,[3] Jan-luuk Hillebrands.[1] *[1]Pathology, UMCG, Netherlands; [2]Physiology, VUmc, Netherlands; [3]Internal Medicine - Nephrology, VUmc, Netherlands; [4]Internal Medicine - Nephrology, UMCG, Netherlands.*

**Background:** Cardiovascular disease (CVD) is the leading cause of death in patients with chronic kidney disease (CKD), a disease state that is strongly associated with loss of renal klotho. Reversely, murine klotho deficiency also causes marked medial calcification. It is thought that klotho conveys a vasculoprotective effect, inhibiting calcification and the development of atherosclerosis. Klotho expression in the vessel wall itself, however, is still disputed.

**Methods:** We assessed klotho expression in healthy renal donor arteries (N=6), CKD (renal graft recipient) arteries (N=10), carotid endarterectomy specimens (N=4), healthy elastic arteries (N=9), and human aortic smooth muscle cells (HASMCs, N=3), using immunohistochemistry and immunofluorescence, Western blotting (WB), qRT-PCR, and mass spectrometry. We extensively validated antibody specificity, using multiple antibodies to compare staining patterns, and competition assays and WB with recombinant human klotho (RhKlotho).

**Results:** Using anti-klotho antibody KM2076, we detected strongly positive specific staining in healthy renal tissue, which was blocked by incubation with RhKlotho, and which co-localized partially with distal tubule marker Epithelial Membrane Antigen. In addition, renal klotho and RhKlotho could be detected with KM2076 on Western blot. However, we could not detect klotho with immunohistochemistry, immunofluorescence or Western blotting in any type of vascular tissue or in HASMCs. We could also not detect klotho by mass spectrometry in healthy donor and CKD arteries, nor find klotho mRNA with qRT-PCR.

**Conclusions:** In contrast to recently published data, using several independent and validated methods, we conclude that klotho is not expressed in healthy or atherosclerotic vascular tissue, nor in cultured HASMCs.The putative vasculoprotective effect of klotho most likely results from circulating klotho levels.

*Funding:* Private Foundation Support

## SA-PO1055

**Serum Cystatin C Is a Significant Diagnostic Marker of the Renal Resistive Index Detected on Doppler Ultrasound in Patients with Chronic Kidney Disease** Ayu Ogawa, Hitoshi Sugiyama, Masashi Kitagawa, Hiroshi Morinaga, Toshio Yamanari, Akifumi Onishi, Yoko Kikumoto, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Okayama Univ Graduate School of Medicine, Okayama, Japan.*

**Background:** Cystatin C (CysC) is a cysteine protease inhibitor produced by nearly all human cells whose serum level is a stronger predictor of the risk of cardiovascular events than that of creatinine. The resistive index (RI) on renal Doppler ultrasonography is a good indicator of renal vascular resistance as well as renal outcomes in patients with chronic kidney disease (CKD). However, it is unclear whether the serum CysC level is associated with signs of vascular dysfunction, such as renal RI in CKD patients.

**Methods:** We determined the levels of serum CysC in 73 CKD patients (median age: 57.0 years, male: 67.1%, diabetes: 9.6%) and investigated the relationship between the level of CysC and markers of vascular dysfunction, including the renal RI, ankle-brachial pulse wave velocity (baPWV), a marker of arterial stiffness, and intima-media thickness (IMT), a marker of atherosclerosis.

**Results:** The serum CysC level was significantly correlated with the renal RI ($P < 0.0001$), mean IMT ($P = 0.0499$) and baPWV ($P < 0.0001$). The serum CysC level was a significant determinant of the renal RI ($P = 0.0281$), but not the baPWV or mean IMT, in a multivariate regression analysis using a biomarker model. The multivariate odds ratio of the serum CysC level for a renal RI of 0.70, a level that predicts worse renal outcomes, was significant (4.04, $p = 0.0030$); however, the odds ratios for the baPWV and mean IMT were not significant. The area under the receiver-operating characteristic curve comparing the sensitivity and specificity of CysC for predicting the RI 0.70 was 0.926 ($P < 0.0001$) (cutoff value: 2.04 mg/L). The serum CysC level was significantly correlated with the level of albuminuria and inversely correlated with the eGFR, as previously reported.

**Conclusions:** The serum CysC level is independently associated with signs of vascular dysfunction, such as the renal RI, in patients with CKD. The study suggests that the serum CysC level serves as a significant marker of the renal RI in CKD patients.

## SA-PO1056

**Quantitative Measurement of Vascular Occlusion Is Associated with Tissue Hypoxia and Inflammatory Cytokines in Human Atherosclerotic Renal Artery Stenosis** Ahmed Saad, Sandra Herrmann, Alfonso Eirin, John A. Crane, Michael A. McKusick, James Glockner, Lilach O. Lerman, Stephen C. Textor. *Mayo Clinic.*

**Background:** Atherosclerotic renal artery stenosis (ARAS) reduces renal blood flow (RBF) and amplifies kidney hypoxia, but the relationship of degree of arterial stenosis and RBF reduction and renal injury remains poorly understood. We tested the hypothesis that RBF reduction and renal injury as reflected by renal vein levels of neutrophil gelatinase associated lipocalin (NGAL) would be magnified with more severe occlusive vascular disease in humans with ARAS.

**Methods:** Inpatient studies were performed in patients with ARAS (n=40, 71.3±11% (Range 48 , 89) occlusion by quantitative CT angiography) or essential hypertension (EH) (n=30), during fixed Na+ intake and ACE/ARB Rx. Single-kidney(SK) cortical/medullary perfusion, volume and RBF were measured using multidetector CT, and glomerular filtration rate (GFR) by iothalamate clearance. Tissue deoxyhemoglobin levels (R2*) and fractional kidney hypoxia (% of axial area with R2*>30/s) were measured by BOLD-MRI at 3T.

**Results:** SK-RBF, volume, cortical and medullary perfusion were reduced in the post-stenotic kidney, as was GFR. Renal vein NGAL and fractional hypoxia were higher in patients with ARAS than EH. The degree of arterial stenosis correlated directly with NGAL levels ($r = 0.49$, $P=0.0004$) and fractional hypoxia ($r= 0.33$, $P=0.01$) and inversely with the RBF, GFR, and kidney volume ($r=-0.59$, $P<0.0001$; $r =-0.66$, $P<0.0001$, and $r= -0.54$, $P<0.0001$).

| SK | ARAS N=40 | EH N=30 | *P-value* |
|---|---|---|---|
| NGAL (ng/ml) | 139±72 | 69.7±34 | <0.0001 |
| RBF(ml/min) | 215.9±135 | 399.2±174 | <0.0001 |
| Cortical perfusion (ml/min/mL tissue) | 2.4 ±0.8 | 3.4±1 | <0.0001 |
| Kidney volume (CT) mL | 104.6±44.3 | 143±32.8 | <0.0001 |
| Kidney hypoxia(R2*> 30/s) | 18.6±15 | 9.6±6.9 | 0.001 |
| GFR(ml/min/1.73m2) | 24.6±14 | 44.3±13.5 | <0.0001 |

**Conclusions:** Our results demonstrate that elevated renal venous markers of kidney injury (NGAL) and RBF in ARAS are correlated with the severity of arterial stenosis measured by CT. Therefore, although moderate blood flow reduction is often tolerated, severe stenosis is associated with active inflammatory injury in renovascular disease.

## SA-PO1057

**Early Detection of Renal Hemodynamic Dysfunction Using Sonographic Analysis in Drug-Induced Nephrotoxicity Rat Models** Tzongshi Lu,[1] Vivian Y. Chan,[2] Li-Li Hsiao.[1] *[1]Renal Div, Brigham and Women's Hospital, Boston, MA; [2]Harvard College, Cambridge, MA.*

**Background:** Drug-induced nephrotoxicity consists 19-25% of all cases of acute kidney injury (AKI). Conventional metrics such as serum creatinine (sCr) has been shown inadequate to detect nephrotoxicity before significant renal function loss and there is no robust method to accurately early detect hemodynamic in the course of drug-induced nephrotoxicity. To detect drug-induced kidney hemodynamic dysfunction in the early discovery phase would provide great benefit for better decision-making about candidate compounds and dose selection, as well as better design for clinical trials that will provide clear information about product benefit and safety. Real-time sonography combined with Color-Doppler imaging technique is the most advanced non-invasive tool to evaluate kidney status, and it is particularly useful for detecting early vascular occlusions and arterial stenosis in renal function analysis.

**Methods:** Rats were treated with Cisplatin (5 mg/kg b.w., single dose, i.p.) to induce AKI. Nephrotoxicity was assessed 6 days after Cisplatin treatment by measuring sCr and histopathology of kidney. Hemodynamic evaluation using Sonography combined with Color-Doppler imaging via Vevo2100 system (VisualSonic, Toronto, Canada).

**Results:** Our data showed successful drug-induced proximal tubular kidney injury by histological examination. Color-Doppler ultrasound study of AKI rats showed that resistive index (RI) and pulsatile index (PI) were increased; and peak-systolic velocity (mm/s), end-diastolic velocity (mm/s) and velocity-time integral (VTI, mm) were decreased at renal arteries. Importantly, these hemodynamic changes preceded the rise of sCr, the current gold-standard for renal function.

**Conclusions:** These observations suggest that RI, PI, VTI, peak-systolic velocity and end-diastolic velocity may serve as hemodynamic parameters to evaluate early phase of kidney injury. We suggest that this technical advance will provide an unprecedented opportunity for a non-invasive and sensitive method. Furthermore, the combination of these parameters (with histopathology and sCr) suggest their potential utility to study drug-induced dysfunction and nephrotoxicity.

*Funding:* Private Foundation Support

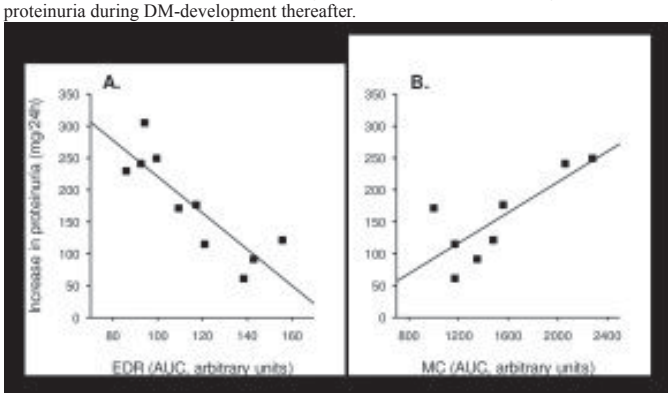
## SA-PO1058

**Proteinuria in Zucker Diabetic Fatty Rat Predicted by Small Renal Artery Function at Young Age** Mahdi Hamidi Shishavan, Leo E. Deelman, Sjoerd W. Landheer, Robert H. Henning, Hendrik Buikema. *Dept of Clinical Pharmacology, Univ Medical Center of Groningen, Groningen, Netherlands.*

**Background:** Severity of chronic kidney disease varies considerably among patients with similar risk profiles, such as obesity, diabetes (DM) and also be dependent on intrinsic factors.Early occurrence of vascular dysfunction in progressive renal disease has led us to question whether renal vascular function might condition the susceptibility of obese individuals to renal damage.

**Methods:** Obese Zucker diabetic fatty (ZDF) rats (n=10) at pre-DM state were subjected to unilateral nephrectomy (UNx) and followed for development of proteinuria until 10 weeks thereafter. At the moment of UNx, intrarenal arteries were isolated from extirpated kidneys and individually studied for endothelium-dependent relaxation (EDR) to acetylcholine and pressure-induced myogenic constriction (MC) responses. Linear regression analysis was performed of baseline EDR and MC (both expressed as AUC in arbitrary units) with increase in proteinuria (mg/24h).

**Results:** At the end, ZDF-UNx rats showed DM - i.e. increased blood glucose (18.5±3.2 mmol/L), $HbA_{1c}$ (5.9±0.6 %), cholesterol (5.4±0.6 mmol/L) and urinary glucose leakage (2.6±1.1 mmol/24h)- and associated renal injury including marked urinary protein excretion (190±25 mg/24h). Proteinuria was significantly predicted by renal artery function such that individuals with low EDR and/or high MC at baseline showed a higher increase in proteinuria during DM-development thereafter.




**Conclusions:** Proteinuria in ZDF rat is predicted by intrarenal artery function at young age. This seems of relevance as our society is becoming increasingly obese and developing associated health problems, such as diabetic nephropathy. Hence, tools that help to monitoring intrarenal vascular function could be useful to identify the individuals prone to the renal damage.

*Funding:* Government Support - Non-U.S.

## SA-PO1059

**Far-Infrared Induces Autophagy to Inhibit Advanced Glycation End Product-Induced Apoptosis in Vascular Endothelial Cells** Yung-Ho Hsu,[1] Tso Hsiao Chen,[2] Cheng-hsien Chen.[1,2] *[1]Div of Nephrology, Dept of Internal Medicine, Shuang Ho Hospital, Taipei Medical Univ, New Taipei, Taiwan; [2]Div of Nephrology, Dept of Internal Medicine, Wan Fang Hospital, Taipei Medical Univ, Taipei, Taiwan.*

**Background:** Diabetic vascular complication accounts for disabilities and high mortality rates in diabetic patients. The accumulation of advanced glycation end products (AGEs) in diabetic patients can trigger apoptotic changes in vascular endothelial cells and involves in the development and progression of micro- and macroangiopathy. Far-infrared radiation (FIR) therapy has been proved to be effective in increasing patency of dialysis vascular access.

**Methods:** Our study is to investigate the biological effects of FIR on AGEs-induced apoptosis in human umbilical vein endothelial cells (HUVECs).

**Results:** We found FIR exposure for 30 min significantly inhibited AGE-bovine serum albumin (BSA)-induced apoptosis in HUVECs. FIR exposure inhibited AGE-BAS-induced apoptotic signals, including cleaved caspase-3 increase and Bcl-xL decrease. We found that FIR exposure induced nuclear translocation of promyelocytic leukaemia zinc finger (PLZF) and phosphatidylinositol-3 kinase (PI3K) class III expression. PLZF siRNA transfection inhibited FIR-induced antiapoptotic effect and PI3K class III expression. Fluorescent staining showed that FIR exposure significantly increased autophagic activity in HUVECs. FIR exposure also induced the expression of autophagic markers, such as Beclin-1, Lamp1 and LC3-II. PLZF siRNA transfection reduced FIR-induced autophagic activity and the expression of Beclin-1, Lamp1 and LC3-II. Beclin-1 siRNA transfection and wortmannin, a PI3K inhibitor, blocked FIR-induced autophagic activity and the antiapoptotic effect of FIR in HUVECs. Additionally, we also found that autophagy promoted HUVECs to engulf AGE-BSA as revealed by immunofluorescence staining.

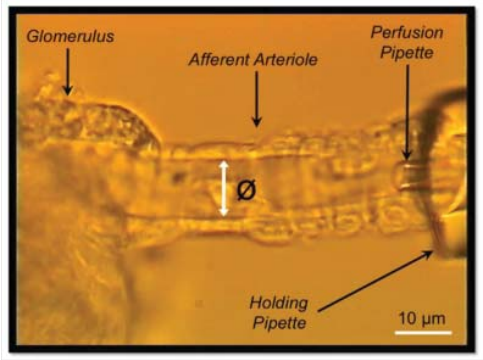
**Conclusions:** Our data suggest that FIR therapy induces autophagy to inhibit AGE-BSA-induced apoptosis in HUVECs.

## SA-PO1060

**Src Homology 2 Domain-Containing Protein B Deficient Mice Display an Increased Glomerular Filtration Rate and Augmented Renal Arteriolar Contractions to Both Adenosine and Ang II** Xiang Gao.[1] *[1]Medical Cell Biology, Uppsala Univ, Uppsala, Sweden; [2]Karolinska Institute, Stockholm, Sweden.*

**Background:** Src homology 2 domain-containing protein B (Shb) is an adapter protein whichregulates several signal transduction cascades and endothelial cell functions. The adenosine-angiotensin II (Ado-Ang II) interaction plays an important role in the regulation of glomerular filtration rate (GFR), vascular resistance and tubuloglomerular feedback. We used Shb-knockout ($Shb^{-/-}$) and wild-type ($Shb^{+/+}$) mice to investigate their GFR and effectiveness of Ado and Ang II to constrict renal resistance vessels.

**Methods:** GFR was measured in conscious $Shb^{-/-}$ and $Shb^{+/+}$ mice using FITC-inulin. Isotonic contractions were measured in isolated and perfused renal afferent arterioles from $Shb^{-/-}$ and $Shb^{+/+}$ mice.




Concentration responses to Ang II ($10^{-12}$ to $10^{-6}$M; 2 minutes each) doses or low-dose Ado ($10^{-8}$ mol/l; 15 min) alone, as well as Ado ($10^{-8}$ mol/l)in combination with cumulative application of Ang II ($10^{-12}$ to $10^{-6}$ mol/l; 2 minutes each) were studied in both genotypes.

**Results:** There was a significantly increased GFR (371 ± 12 µL/min, n=11) in $Shb^{-/-}$ comparing to $Shb^{+/+}$ (321 ± 11 µL/min, n=8) mice. The maximal arteriolar contraction to Ang II was significantly larger in $Shb^{-/-}$ (87 %; n=8) than in $Shb^{+/+}$ (54 %; n=8) mice. Low-dose Ado alone contracted afferent arterioles in both genotypes (6% in $Shb^{-/-}$ and 7% in $Shb^{+/+}$). Ado significantly enhanced Ang II constriction in afferent arterioles in both genotypes (to 93% in $Shb^{-/-}$ and to 72 % in $Shb^{+/+}$).

**Conclusions:** Low-dose adenosine augments Ang II arteriolar constriction effectiveness, which indicates Ado-Ang II interaction in both $Shb^{-/-}$ and $Shb^{+/+}$ mice. The absence of Shb increases GFR The underlying mechanisms remain to be resolved.

*Funding:* Government Support - Non-U.S.

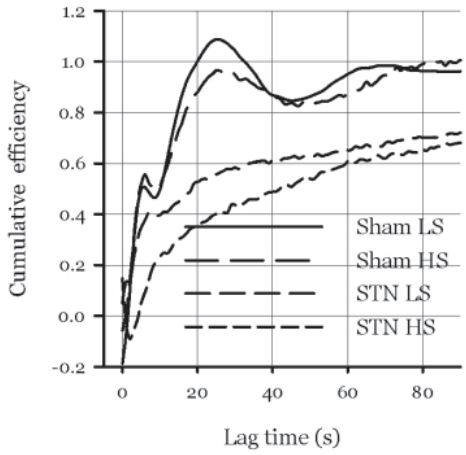
## SA-PO1061

**Dynamics of Renal Blood Flow Autoregulation in Early Remnant Kidney** Scott C. Thomson, Hai Pham, Prabhleen Singh. *Medicine, Univ of California and VASDHS, San Diego, CA.*

**Background:** Previous micropuncture data revealed that the early remnant kidney adapts to high NaCl diet by suppressing tubuloglomerular feedback (TGF), which permits distal delivery to increase. TGF normally accounts for half of RBF autoregulation.

**Methods:** Current studies tested for a signature of TGF suppression on dynamic RBF autoregulation at the whole kidney level in rats fed high (HS) or low (LS) NaCl diets for one week after 5/6 nephrectomy (STN) or sham surgery. (n=7-9 per group). RBF (Transonics transit time) and blood pressure (BP) were recorded at 1 Hz for 90-120 minutes. Impulse response functions were generated in MATLAB by best fit of current RBF to a linear combination of the last 240 values of BP. Autoregulatory step responses (same as accumulated fractional compensation for a unit step in BP) were obtained by integrating and normalizing to mean conductance. Values <0, 0, 1, >1 correspond to dilation, rigid conduit, perfect autoregulation, and super-autoregulation, respectively.

**Results:**




Several distinct features of the response varied among the groups as shown in the figure. Transient peaks and/or damped oscillations are consistent with simple negative feedback control with intrinsic delay and gain exceeding some threshold. Peaks at Lag<15s and >25s presumably owe to myogenic and TGF responses, respectively. Most of the difference among Sham LS, Sham HS, and STN LS can be explained by quantitative adjustment of the TGF gain. Unique to STN HS was a 3s vasodilatory phase followed by sluggish autoregulatory response.

**Conclusions:** Simple systems analysis revealed distinct components to the autoregulatory response that are individually affected by STN and dietary salt. Overall, the autoregulatory response is slower and salt-sensitive in STN.

*Funding:* Veterans Affairs Support

## SA-PO1062

**Diffusion Tensor Imaging: A Useful Tool in the Evaluation of Renal Artery Stenosis** Emiliana Ferramosca,[1] Laura Patregnani,[1] Caterina Gaudiano,[2] Fiorenza Busato,[2] Valeria Clementi,[3] Rita Golfieri,[2] Antonio Santoro.[1] *[1]Nephrology, Dialysis and Hypertension, S.Orsola-Malpighi Univ Hosp., Bologna, Italy; [2]Radiology Inst, S.Orsola-Malpighi Univ Hosp., Bologna, Italy; [3]GE Healthcare, Clin. Science Development Group, Bologna, Italy.*

**Background:** Diffusion Tensor Imaging (DTI) is an MR imaging technique used to show molecular diffusion.The apparent diffusion coefficient (ADC) combines the effects of capillary perfusion and water diffusion in the extracellular space. Fractional Anisotropy (FA) is a quantitative value that provides information on direction of water molecules flow within a tissue. DTI provides information on perfusion and diffusion simultaneously.

Aim of this study was to evaluate feasibility of DTI in detecting unilateral kidney alteration in pt with renal artery stenosis (RAS).

**Methods:** We studied 13 patients (pt) with RAS and 15 control subjects without any kidney disease. All subjects underwent morphological MRI followed by DTI sequence. Mean ADC and FA values of cortical and medullary Regions of Interest were calculated. The reference range for ADC and FA on cortex and medulla in the control group was defined as mean value±2SD. For each kidney in the pt with RAS the corresponding parameters were compared with the reference range.

**Results:** Reference ranges on controls were: 2.55±0.18 $\times 10^{-3}$ mm$^2$/s for cortical ADC, 2.25±0.24 $\times 10^{-3}$ mm$^2$/s for medullary ADC, 0.308±0.071 for cortical FA and 0.389±0.067 for medullary FA.

In 7 pt DTI parameters confirmed the unilateral alteration showed by the CE-MRI. In 1 pt DTI confirmed the bilateral alteration showed by the CE-MRI. In the other 5 pt DTI parameters suggested functional alterations but not corresponding to the side involvement showed by the CE-MRI.

**Conclusions:** DTI of the kidneys is a new application that allows us to study renal alterations. Previous studies demonstrated the feasibility of DTI in pt with CKD, showing lower ADC than in control subjects. This preliminary results suggest to increase the number of data to investigate the DTI ability to measure the functional alteration of each kidney, also by adding percentage of RAS and kidney volume data.Based on these data DTI could be useful on RAS study by evaluating functional alteration of each kidney.

## SA-PO1063

**Regulation of Mitochondrial Function and Dynamics in Early CKD** Prabhleen Singh, Hai Pham, Joanna Thomas, Scott C. Thomson. *UCSD&VASDHS.*

**Background:** Available therapies for CKD are inadequate in halting the progression of disease Intrarenal hypoxia has been identified as a major culprit in CKD progression. We have reported a high nephron oxygen consumption (QO2) leading to hypoxia in subtotal nephrectomy (STN) model of CKD.

**Methods:** In 1-week STN rats, mitochondrial QO2, ROS generation, ATP generation, mitochondrial dynamics (fission/fusion) and morphology were examined. Effects of HIF-1α activation by DMOG treatment were examined. Data presented as mean±sem.

**Results:** Cortical mitochondrial QO2 were not different between STN and controls, but mitochondria isolated from whole kidneys, showed elevated rates of both coupled and uncoupled respiration, $p<0.05$. ATP generation was not different despite elevated QO2, but increased superoxide production was seen in STN mitochondria, $p<0.05$. HIF-1α activation lowered both mitochondrial QO2 and ROS production. Imaging revealed longer lengths and elongated phenotype (fusion) in STN cortical (912±32 nm) vs. medullary (547±16 nm) mitochondria, which were fragmented (fission), $p<0.001$. STN cortical mitochondria had increased volume density (29±1%) compared to STN medullary (22±1%) or control cortical mitochondria (22±1%), $p<0.01$. Lastly, the cristae abundance, normalized to mitochondria outer membrane was increased in the STN cortical (2±0.1) compared to STN medullary (1.3±0.1) or control cortical mitochondria (1.3±0.1), $p<0.01$. Western blotting revealed increased mitofusin 2 (fusion protein) expression in STN cortex compared to control cortex and STN medulla ($p<0.05$) and decreased Fission 1 (fission protein) expression in STN vs. control cortex, but higher expression in STN vs. control medulla, $p<0.05$. Beclin-1 expression was significantly elevated in STN medulla vs. cortex and was lowered by DMOG treatment.

**Conclusions:** Elevated mitochondrial QO2 is observed in early STN medullary mitochondria, without expected increase in ATP generation, but associated with elevated ROS levels. Significant alterations in mitochondrial morphology and dynamics were also observed in STN medullary mitochondria, which could explain the mitochondrial dysfunction. HIF-1α activation ameliorated mitochondrial dysfunction, and its effects on mitochondrial dynamics are being examined.

*Funding:* NIDDK Support

## SA-PO1064

**Interferon-Gamma Increases Connexin-40 Expression and Affects Electrical Properties of Endothelial Cells** Branko Braam,[1,2] Wenqing Zhuang,[1] Steve Kulak,[1] R. Todd Alexander,[2,3] William A. Cupples.[4] *[1]Medicine/Nephrology, Univ of Alberta; [2]Physiology, Univ of Alberta; [3]Pediatrics, Univ of Alberta, Edmonton; [4]Physiology and Kinesiology, Simon Fraser Univ, Vancouver, Canada.*

**Background:** Connexin(CX)-37, -40 and -43 are part of the gap junctions between and participate in the electrochemical coupling of endothelial cells (EC). These properties have been implicated in vascular regulation, since the EC seem to form the 'electrical cable' for axial signal transmission in the microvasculature. We investigated whether CX expression responds to stimuli known to be involved in endothelial dysfunction.

**Methods:** Interferon-gamma (IFNγ) strongly induced CX-40 expression and subsequently was tested with respect to transcellular impedance. Human microvascular EC-1 (HMEC-1, CDC) in culture were subjected to partial vs. full confluence, normal vs. high glucose (5 vs. 25 mM), IL-6, IFNγ, TNFa, Ang II and DETA-NONOate. After 4h and 8h cells were harvested for RNA isolation, DNA degradation and RT-qPCR with specific primers.

**Results:** Of all those stimuli, IFNγ (1-30 ng/ml) consistently induced CX-40 expression at 4h and 8h dose dependently to 10-20 fold when corrected for the control (HPRT). TNFa and IL6 resulted in a small, significant depression of CX-37 expression at 4h. The induction of CX-40 by IFNγ was reproduced in HUVEC (less pronounced) and glomerular EC in culture. All other stimuli did not change expression of CX-37, CX-40 or CX-43. Two JAK inhibitors, EGCG (100-250 uM) and A-490 (100-250 uM) dose-dependently inhibited the induction of CX-40 expression by IFNγ. Subsequently, HMEC-1 were subjected to 10-30 ng/ml IFNγ for up to 48h and transcellular cell impedance monitored by cell-substrate impedance sensing (ECIS, Applied BioPhysics). IFNγ dose-dependently increased cell impedance significantly after 24h and this was blunted by A-490 (25 uM).

**Conclusions:** These experiments point to an important role of IFNγ to induce CX40 expression. IFNγ also seems to increase EC electrical impedance, which could 'insulate' the electrical cable. Experiments testing intercellular conductance are ongoing.

*Funding:* Government Support - Non-U.S.

## SA-PO1065

**Comparison of Combination Therapy with Irbesartan/Amlodipine and Irbesartan/Cilnidipine for Attenuation of Albuminuria in Rats with Streptozotocin-Induced Diabetic Nephropathy** Minoru Satoh, Yuko Nishi, Hiroyuki Kadoya, Tamaki Sasaki, Naoki Kashihara. *Dept of Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

**Background:** Managing hypertension is important for decreasing the risks of cardiovascular and renal diseases. Calcium channel blockers (CCBs) have varying suppressive effects on urinary albumin excretion. However, it is unknown whether this effect differs in combination therapy with angiotensin receptor blockers (ARBs). This study aimed to compare the efficacy of the combination therapy of irbesartan/amlodipine and irbesartan/cilnidipine on diabetic nephropathy.

**Methods:** Male Sprague-Dawley rats with streptozotocin–induced diabetes were treated with CCBs, amlodipine (2.0 mg/[kg·day]) or cilnidipine (2.0 mg/[kg·day]), with or without the ARB, irbesartan (20.0 mg/[kg·day]). In the acute protocol, changes in the diameters of glomerular afferent and efferent arterioles were examined by a charge-coupled device video microscope. In the chronic protocol, urinary albumin excretion, glomerular reactive oxygen species, and endothelial surface layer were evaluated.

**Results:** In the acute protocol, cilnidipine monotherapy caused a greater dilation in glomerular efferent arterioles than amlodipine monotherapy. In both groups, combination therapy with irbesartan induced comparable dilation in the glomerular efferent arterioles. In the chronic protocol, cilnidipine monotherapy suppressed albuminuria more than amlodipine monotherapy. However, the addition of irbesartan reduced the albumin excretion in both groups. In a group initiated on irbesartan monotherapy, addition of cilnidipine or amlodipine had no further effect on albuminuria reduction.

**Conclusions:** Cilnidipine monotherapy suppressed increased albuminuria more than amlodipine monotherapy. However, combination therapy with irbesartan led to a greater reduction in albuminuria in both treatment groups and with equal effectiveness. The combinations of irbesartan with cilnidipine or amlodipine are equally effective in reducing albuminuria in diabetic nephropathy.

*Funding:* Pharmaceutical Company Support - Dainippon Sumitomo Pharma, Private Foundation Support

## SA-PO1066

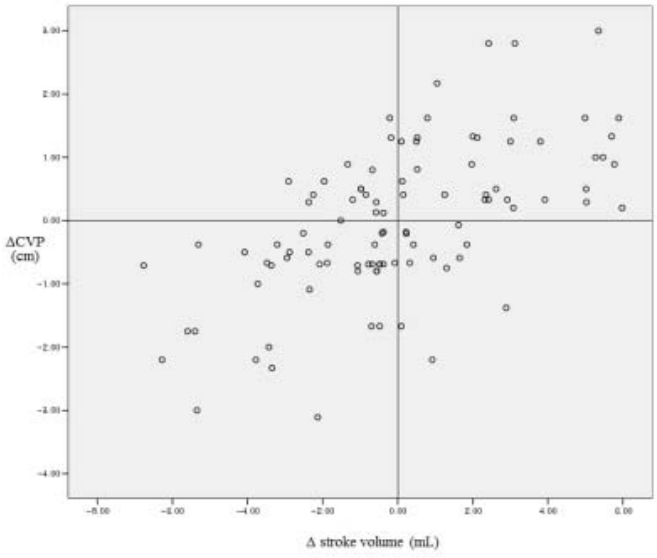
**Comparison of Central Venous Pressure with Cardiac Output Measured by Non-Invasive Cardiac Output Monitoring during Anesthesia for Kidney Transplant Surgery**

Joon Seok Oh,[1] Jin Ho Lee,[1] Seong Min Kim,[1] Yong Hun Sin,[1] Yong Ki Park,[2] Joong Kyung Kim.[1] *[1]Div of Nephrology, Internal Medicine, Bong Seng Memorial Hospital, Busan, Korea; [2]Div of Nephrology, Internal Medicine, Dongnae Bong Seng Hospital, Busan, Korea.*

**Background:** Patients with end-stage renal disease (ESRD) can be hemodynamically unstable because of frequent changes in peripheral resistance and blood volume during kidney transplant surgery. Cardiac output (CO) is a key variable when describing and treating the cardiovascular system. Thermodilution via a pulmonary artery catheter is the most frequently used method, but it lacks accuracy. Non-invasive cardiac output monitoring (NICOM) measures CO based on chest bioreactance. Validated data of NICOM in patients with kidney transplant surgery are lacking. So we compared central venous pressure (CVP) with cardiac output monitored via NICOM system during anesthesia for renal transplant surgery.

**Methods:** Stroke volume (SV) values using NICOM were recorded during anesthesia in patients with renal transplant surgery and CVP values were measured at the same time. The difference between measured and average values of CVP (ΔCVP) and SV (ΔSV) were calculated in each subject, because CVP values may be different depending on the person who measured. Correlation analysis was performed ΔSV with and ΔCVP.

**Results:** Twenty subjects were enrolled and the SV and CVP values were measured 100 times. There werepositive correlation with ΔSVand ΔCVP (R = 0.61, P = 0.00).




**Conclusions:** Stroke volume measured by NICOM and CVP showed a positive correlation. NICOM may be effective as non invasive method for circulating volume monitoring in patients with renal transplant surgery.

## SA-PO1067

**Angiotensin II Type 2 Receptor Activation Counteracts Angiotensin II-Induced Loss of Glomerular Permselectivity – A Multiphoton Microscopy Study**

Ina Maria Schiessl, Hayo Castrop. *Univ of Regensburg, Institute of Physiology, Regensburg, Germany.*

**Background:** In this study, we assess the acute effects of angiotensin II (AngII) on glomerular permselectivity for albumin.

**Methods:** Experiments were performed in Munich Wister Froemter rats using intravital microscopy. Alexa-Fluor-594 albumin was injected i.v. and fluorescence intensities were determined in glomerular capillaries and in Bowman's space to calculate the albumin glomerular sieving coefficient (GSC). GSC was measured before and during constant infusion of AngII (10 ng/min/kg BW).

**Results:** Baseline MAP averaged 99±5 mm Hg and stabilized at 137±5 mm Hg during AngII infusion. GSC averaged $0.00044\pm5*10^{-5}$ at baseline and increased by 386±44% after AngII infusion (p<.0001). During saline infusion, no changes in MAP or GSC were observed. Proximal tubular Alexa-Fluor-594 albumin uptake was markedly elevated during AngII infusion (518% of baseline vs. 218% in controls; p<.0001). Changes in GSC were abolished and reversed, when the AT1 antagonist losartan (9.9 µg/g BW) was injected before and after the start of the AngII infusion, respectively. Contrary to losartan, the AT2 antagonist PD123319 increased baseline GSC from $0.00052\pm4*10^{-5}$ to $0.00074\pm8*10^{-5}$ (p=.02), without altering MAP. During infusion of AngII in the presence of losartan, PD123319 increased albumin GSC from $0.00037\pm5.8*10^{-5}$ to 0.00115±.00015 (p=.001). There was a minor correlation between GSC and MAP, with the two variables increasing in parallel ($r^2$= .26). Pressure-independent effects of AngII on GSC were assessed after normalization of the renal perfusion pressure by pulling on a string loop, which was implanted around the aorta upstream of the branching of the renal arteries. With renal perfusion pressure kept constant (measurement in the femoral artery), GSC increased from 0.0007±.0002 to 0.0025±.0006 during AngII infusion, similar to what was seen without controlled pressure.

**Conclusions:** In summary, AngII increases the permeability of the glomerular filtration barrier for albumin. This effect is AT1 receptor-dependent and largely independent of changes in the renal perfusion pressure. AT2 receptor activation partially counteracts the proteinuric effects of the AT1 receptor.

*Funding:* Private Foundation Support

## SA-PO1068

**The Vascular Smooth Muscle Cell Calcium-Sensing Receptor Is Involved in Blood Pressure Regulation, Calcium-Homeostasis and Protection from Calcification**

Martin Schepelmann,[1] Polina L. Yarova,[1] Tom Davies,[1] Sarah C. Brennan,[1] Joao Z. Graca,[1,2] Wenhan Chang,[3] Daniel Bikle,[3] Donald T. Ward,[4] Ann E. Canfield,[4] William G. Richards,[5] David H. Edwards,[1] Sally A. Price,[2] Paul J. Kemp,[1] Daniela Riccardi.[1] *[1]Cardiff Univ, United Kingdom; [2]Astra Zeneca, Macclesfield, United Kingdom; [3]Dept of Medicine, Univ of California, San Francisco, CA; [4]Univ of Manchester, United Kingdom; [5]Amgen, Thousand Oaks, CA.*

**Background:** The extracellular Calcium-sensing Receptor (CaSR) is expressed in the vasculature, but the roles of the CaSR in vascular physiopathology still remain unclear.

**Methods:** We studied the phenotype of a mouse model of vascular smooth muscle cell (VSMC) targeted CaSR deletion by breeding exon 7 LoxP CaSR mice with SM22α-Cre mice.

**Results:** CaSR wild-type (WT) and knock-out (KO) mice showed no difference in weight, lifespan or reproductive capability. However, compared to WT control, 9 month old KO mice were hypotensive (90.3±2.8 vs. 80.5±2.8 mm Hg mean arterial pressure, p<0.05, N=6-11). Wire myography showed reduced phenylephrine-induced contraction and increased acetylcholine-induced relaxation of isolated KO aortae compared to WT control, suggesting a role for vascular CaSR in the control of arterial tone and hence blood pressure. $Ca^{2+}$ and FGF23 levels were significantly elevated in KO mice compared to WT ($Ca^{2+}$: 3.05±0.27 vs. 2.28±0.09 mM; FGF23: 384.4±83.5 vs. 145.0±11.0 pg/ml, p<0.01, N=6-11) indicating an influence of the vascular CaSR on $Ca^{2+}$/Pi homeostasis, a notion supported by the observation that KO mice showed significantly impaired bone mineral density. *In vitro*, VSMC cultured from KO mice – which show a ~60% reduction of CaSR staining intensity compared to WT (quantitative immunofluorescence) – exhibited a significantly greater predisposition to calcify in mineralising conditions (3 mM Pi and 1.8 mM $Ca^{2+}$) compared to WT control (24.60 vs. 0.99 AU, alizarin red densitometry, p<0.001, N=6-8).

**Conclusions:** These data point to an important physiological role of the vascular CaSR in blood pressure regulation and electrolyte homeostasis and to a possible protective role against vascular calcification in pathophysiology.

*Funding:* Government Support - Non-U.S.

## SA-PO1069

**Tubular Vegfa Is Required for Renal Microvasculature and Oxygen Sensing**

Henrik Dimke,[1] Matthew A. Sparks,[2] Sebastian Frische,[3] Thomas M. Coffman,[2] Susan E. Quaggin.[4] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; [2]Div of Nephrology and Dept of Medicine, Duke Univ, Durham, NC; [3]Dept of Biomedicine, Univ of Aarhus, Aarhus, Denmark; [4]Northwestern Univ, Feinberg School of Medicine, Chicago, IL.*

**Background:** Adequate renal oxygenation is pivotal for maintaining essential functions of the kidney. Vascular endothelial growth factor A (VEGFA) plays a key role in vascular formation and maintenance, processes that are critical for sufficient tissue perfusion and oxygenation.

**Methods:** Using a range of targeted transgenic mice models we determined the intrarenal localization of the Vegfa system and the functional consequences of *Vegfa* ablation in renal tubules.

**Results:** Here we show that *Vegfa* is expressed in select tubular epithelial cells, while expression of its receptor (*Kdr/Vegfr2*) is restricted largely to capillary beds. Early genetic ablation of tubular *Vegfa* allows the formation of a grossly normal kidney. However, microvascular density is markedly reduced, leading to increased renal hypoxia. As a consequence the mice develop pronounced polycythemia, elicited by an augmented renal production of erythropoietin. In kidney, expression of classical hypoxia-inducible factor-1 responsive transcripts remains unaltered; suggesting that erythropoiesis partly compensates the capillary rarefaction. An increase in diastolic blood pressure (112 ± 3.7 vs. 104 ± 1.6 mmHg) is present in mice lacking tubular Vegfa maintained on a normal diet, while systolic blood pressure (139 ± 2.8 vs. 135 ± 2.8 mmHg) does not change. The difference in diastolic pressure is ablated when dietary NaCl content changed or when angiotensin II is infused. Moreover, renal electrolyte excretion remains unperturbed by reductions in tubular Vegfa.

**Conclusions:** Tubular Vegfa is critical for adequate development of renal microvasuculature, a prerequisite for proper oxygenation of the kidney. Compensatory polycythemia likely prevents severe perturbations of renal function in this model. In addition, increased diastolic blood pressure may occur as a consequence of capillary rarefaction within the kidney.

*Funding:* Government Support - Non-U.S.

**SA-PO1070**

**Role of MicroRNA-126 in Asymmetric Dimethylarginine Induced Endothelial Dysfunction**

Filippo Martino,[1] Jan T. Kielstein,[2] Thomas Thum,[1] Johan M. Lorenzen.[2] *[1]Insitute of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Hannover, Germany; [2]Dept of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

**Background:** Asymmetric Dimethylarginine (ADMA), an endogenous inhibitor of NOS, is elevated in patients with chronic kidney disease and is known to contribute to endothelial dysfunction. It remains unclear if microRNAs have an impact on these detrimental effects. We investigated the role of microRNA-126, which is essential for endothelial biology in ADMA induced endothelial dysfunction.

**Methods:** We measured ADMA-Plasma levels in 39 patients with coronary artery disease (CAD) by ELISA assay and correlated them with circulating miR-126 levels assessed by qRT-PCR. We infused ADMA into healthy rats (250µMol ADMA/kg/day) and healthy human volunteers (0.1mg ADMA/kg/min, 40 minutes) and quantified plasma levels of different microRNAs. We investigated the underlying molecular mechanisms in vitro by using HUVECs. Stimulating them with ADMA, we quantified extra- and intracellular levels of miR-126. Transcriptional activation was assessed by electrophoretic mobility shift assay (EMSA).

**Results:** Plasma Levels of ADMA in CAD patients correlated inversely with levels of circulating miR-126 ($r=-0.52$; $p<0.001$). ADMA infusion reduced circulating levels of miR-126 in rats. Levels of circulating miR-126 in healthy human volunteers were significantly decreased after ADMA infusion ($p<0.05$), whereas other microRNAs were not significantly altered. We could detect a time dependent alteration of extra- and intracellular amounts of miR-126 (extra: decrease 24h $p<0.05$; intra: increase 2h $p<0.05$) in vitro. Changes of intracellular levels of miR-126 were not related to transcriptional activiation by ETS-1, which is known to regulate miR-126.

**Conclusions:** We were able to show that, miR-126, which is known to convey alarm signals to injured endothelium and thereby contribute to repair mechanisms and cellular survival, is regulated by ADMA. Therefore, detrimental effects of ADMA on endothelial function might be partly mediated by miR-126. These effects are rather a consequence of altered miR-126 trafficking (e.g. through exosomes/microvesicles) than transcriptional regulation.

**SA-PO1071**

**Angiopoietin-2 Induced Arterial Stiffness in Chronic Kidney Disease**

Fan-Chi Chang,[1,2,3] Yu-hsiang Chou,[1,4] Szu Yu Pan,[1] Yi-Ting Chen,[1,2,5] Wen-Chih Chiang,[1] Shuei-Liong Lin.[1,3] *[1]Renal Div, National Taiwan Univ Hospital, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ Hospital Chu-Tung Branch, Hsin-Chu, Taiwan; [3]Graduate Institute of Physiology, College of Medicine National Taiwan Univ, Taipei, Taiwan; [4]Dept of Internal Medicine, National Taiwan Univ Hospital Yun-Lin Branch, Yun-Lin, Taiwan; [5]Dept of Internal Medicine, E-DA Hospital, Kaohsiung, Taiwan.*

**Background:** Arterial stiffness is recognized detrimental to cardiovascular outcome. Given increased arterial stiffness in chronic kidney disease (CKD), we hypothesize that abnormal endothelial cell growth factor is implicated.

**Methods:** The cohort of CKD patients and animal models of CKD were studied.

**Results:** In the cohort of 416 CKD patients, plasma level of angiopoietin-2 was independently correlated with the severity of arterial stiffness assessed by pulse wave velocity. Using murine CKD models induced by 5/6 subtotal nephrectomy or unilateral ureteral obstruction, we demonstrated the increase of plasma angiopoietin-2. Angiopoietin-2 expression markedly increased in tubular epithelial cells of fibrotic kidney but decreased in other tissues including aorta and lung. Expression of collagen and pro-fibrotic genes in aortic vascular smooth muscle cells was increased in CKD mice or mice producing human angiopoietin-2. Angiopoietin-2 stimulated endothelial expression of monocyte chemokines and adhesion molecules, increased $Ly6C^{low}$ macrophage in aorta. Pro-fibrotic cytokine transforming growth factor b1 was increased in aortic endothelial cells and $Ly6C^{low}$ macrophages by angiopoietin-2. Angiopoietin-2 blockade not only attenuated expression of monocyte chemokines, pro-fibrotic cytokines, and collagen in aorta, but also decreased pulse pressure of mice after 5/6 subtotal nephrectomy.

**Conclusions:** This study identifies a link between fibrotic kidney and arterial stiffness through angiopoietin-2. Targeting angiopoietin-2 to attenuate inflammation and collagen expression may provide a novel therapy for cardiovascular disease in CKD.

*Funding:* Government Support - Non-U.S.

**SA-PO1072**

**The Identification of a Robust, High Capacity Albumin Transcytosis Pathway in Isolated Perfused Rabbit Tubules**

Yuehan Zhou,[1] Leileata M. Russo,[2] Lisa M. Satlin,[1] Wayne Comper.[2] *[1]Mount Sinai School of Medicine; [2]Exosome Diagnostics.*

**Background:** A high capacity tubular retrieval pathway for filtered albumin has long been proposed on the basis of the lack of charge selectivity of the glomerular filter, albumin excretion with the use of chemical tubular knock outs, chase studies of filtered albumin in vivo, and more recent studies of the high glomerular sieving coefficient measured for albumin by 2-photon microscopy. The aim of this study was to examine the ability of proximal tubules to efficiently take up and transcytose albumin at relatively high concentrations in the tubular lumen.

**Methods:** Following a 40min equilibration period, the isolated rabbit S1 proximal tubules, approximately 1mm in length, were perfused with Burg's solution containing Alexa 488 labelled albumin at a concentration of 3mg/ml and $^{14}$C-inulin at 10-15nl/min. The basolateral perfusion rate was 10ml/h and fractions were collected every 10min for 1h. The fractions were concentrated by a 30kD-cut-off membrane and both the retentate (intact albumin) and filtrate (degraded albumin) measured for fluorescence. Leakage from the basolateral bath to the lumen was estimated by volume flow Jv as measured by the difference in $^{14}$C-inulin concentration in the luminal collection fluid vs luminal perfusate and leakage from the lumen to the bath was estimated by radioactivity in the basolateral perfusate.

**Results:** In all studies we found significant amounts of intact albumin in the basolateral bath giving a mean±SD for steady-state transcytosis of 8.9±9.7ng/mmtubule.min (n=3). There was no detectable presence of $^{14}$C-inulin in the basolateral fractions nor was there negative Jv indicating no leakage. The presence of degraded albumin or free label in the basolateral perfusate was <5% of the intact albumin.

**Conclusions:** The proximal tubule has the capacity to efficiently retrieve relatively high concentrations of filtered albumin that are predicted to occur because of its high glomerular sieving coefficient. The mechanism of retrieval is not associated with fluid phase endocytosis.

*Funding:* Private Foundation Support

**SA-PO1073**

**Renin Vein Sampling in Patients with Refractory Hypertension and Non-Occlusive Renal Artery Stenosis**

Saravanan Balamuthusamy,[1,3] Bijin Thajudeen,[1] Raul Medina,[3] Evamaria Anvari,[1] Nishant B. Jalandhara.[2] *[1]Dept of Medicine, Univ of Arizona, Tucson; [2]Nephrology, Kansas Nephrology Physicians; [3]Nephrology and Hypertesnion, Angiocare, Tucson.*

**Background:** Less than 40% of patients with hypertension are being treated to target levels established by JNC-7. Refractory hypertension has been documented in atleast 10% of the patients with chronic hypertension. Elevated renin level under optimal conditions has been recorded in patients with refractory hypertension. The clinical significance of elevated renin in the absence of renin secreting tumors and occlusive RAS has not been analyzed in the past.

**Methods:** Three year retrospective comparative analysis of patients with refractory hypertension with elevated renin levels with non-occlusive RAS (NO RAS) and no RAS (no RAS) from two Nephrology and Hypertension practices was performed. Renal arteriogram was performed if there was evidence of renal artery stenosis on arterial Doppler based on flow or renal-aortic ratio criteria. Renin sample was obtained by selectively catheterizing the renal vein in an interventional lab. Renin angiotensin aldosterone blockers (RAAS) were discontinued 7 days before the procedure. Patients in the non-occlusive RAS group had <50% stenosis. Renal renin ratio (RRR) is calculated by dividing the renin level from the elevated to non-elevated side in patients with no RAS. The ratio in the NO RAS group was calculated by dividing the stenotic by non-stenotic side renin value.

**Results:** 22 patients met inclusion criteria for the analysis. The mean age was 61.7. There were more diabetics in the NO RAS group. The mean renin ratio in the NO RAS group was 3.1 as opposed to 0.8 in the non RAS group ($p<0.001$).

**Conclusions:** Renin ratio was significantly increased in refractory hypertension patients with non-occlusive renal artery stenosis when compared to patients who did not have any stenosis despite having elevated peripheral renin levels. The optimal treatment for NO RAS is unclear at this point with the exception of RAAS blockade and dihydropyridine calcium channel blockers.

**SA-PO1074**

**The Effect of Empagliflozin on Arterial Stiffness and Heart Rate Variability in Patients with Type 1 Diabetes**

David Cherney,[1] Bruce A. Perkins,[2] Nima Soleymanlou,[3] Ronnie Lok-Hang Har,[1] Nora M. Fagan,[4] Odd Erik Johansen,[5] Hans-Juergen Woerle,[5] Maximilian von Eynatten,[4] Uli Christian Broedl.[5] *[1]Univ Health Network, Univ of Toronto, Canada; [2]Mount Sinai Hospital, Univ of Toronto, Canada; [3]Boehringer Ingelheim Canada Ltd./Ltée, Canada; [4]Boehringer Ingelheim Pharmaceuticals, Inc.; [5]Boehringer Ingelheim Pharma GmbH & Co.KG, Germany.*

**Background:** Patients with type 1 diabetes (T1D) are at high risk for the development of hypertension. Hyperglycemia-mediated neurohormonal activation increases arterial stiffness, and is an important contributing factor for hypertension. Since the sodium glucose cotransport 2 (SGLT2) inhibitor empagliflozin lowers blood pressure and HbA1c, we hypothesized that this agent would reduce arterial stiffness and markers of sympathetic nervous system activity.

**Methods:** Blood pressure, arterial stiffness, heart rate variability (HRV) and circulating adrenergic mediators were measured during clamped euglycemia and hyperglycemia in 40 normotensive patients with T1D (NCT01392560). Studies were repeated after 8 weeks of empagliflozin (25 mg daily).

**Results:** During clamped euglycemic conditions, empagliflozin reduced systolic blood pressure (111±9 to 109±9 mmHg, p=0.0187), and augmentation indices at the radial (-52±16 to -57±17%, p<0.0001), carotid (+1.3±17.0 to -5.7±17.0%, p<0.0001) and aortic positions (+0.1±13.4 to -6.2±14.3%, p<0.0001) declined. Similar effects on arterial stiffness were observed during clamped hyperglycemia; however, blood pressure effects were not significant. Carotid-radial pulse wave velocity decreased significantly under both glycemic conditions (p≤0.0001), while declines in carotid-femoral pulse wave velocity were only significant during clamped hyperglycemia (5.7±1.1 to 5.2±0.9 m/s, p=0.0017). HRV, plasma noradrenaline and adrenaline remained unchanged under both glycemic conditions.

**Conclusions:** Empagliflozin reduces arterial stiffness in patients with uncomplicated T1D. The mechanisms responsible for this decline in arterial stiffness require further study and may relate to pleiotropic actions of SGLT2 inhibition, including glucose lowering, antihypertensive and weight reduction effects.

*Funding:* Pharmaceutical Company Support - Boehringer-Ingelheim

## SA-PO1075

### Differential Effects of Indoxyl Sulfate and Inorganic Phosphate in Murine Cerebral Endothelial Cells

Andréa Marques Stinghen,[1,2] Jean-marc Chillon,[1] Ziad Massy,[1,3] Agnes Boullier.[1] *[1]Inserm U1088, UPJV, Amiens, France; [2]UFPR, Curitiba, Brazil; [3]A.Paré Hospital, Paris, France.*

**Background:** Endothelial dysfunction, partly due to nitric oxide (NO) unavailability and increased oxidative stress, plays a key role in stroke in CKD patients. To explore the mechanisms, we evaluated the effects of two uremic toxins on cerebral endothelium function.

**Methods:** A murine cerebral endothelial cell line (bEnd.3) was treated with various concentrations of indoxyl sulfate (IS) and inorganic phosphate (Pi). Cell viability was assessed by MTT. NO, ROS and $O_2^{\circ-}$ productions were measured using DAF-FM (0.1µM), DCFH-DA (1µM) and DHE (10µM), respectively. N-acetyl-L-cysteine (NAC,10mM) and vitamin E (10µg/mL) were added prior to IS or Pi treatment. Peroxynitrite ($ONOO^-$) involvement was evaluated using ebselen, a peroxide scavenger. eNOS uncoupling was analyzed by adding 10µM tetrahydrobiopterin ($BH_4$).

**Results:** Cell viability decreases after 24h of IS or Pi treatment (P<0.01). Both toxins significantly reduce NO production (IS, P<0.05; Pi, P<0.001). IS dose-dependently induces ROS production in treated cells (P<0.001). Pi mediates a significant oxidative stress with an highest effect at 3mM (P<0.001). Antioxydants pretreatments significantly reduce ROS levels in cells treated with either IS or Pi (P<0.05 and P<0.001 respectively). IS reduces $O_2^{\circ-}$ production in a dose-dependent manner compared to control cells (P<0.001). This effect is also observed with 2mM Pi (P<0.001). Both antioxydants decrease $O_2^{\circ-}$ production compared to control cells with a greater effect in Pi-treated cells (P<0.001) than in IS-treated cells (P<0.05). Ebselen reduces ROS production induced by both toxins (P<0.001) suggesting a role of $ONOO^-$ in this process. Whereas BH4 addition has no effect on $O_2^{\circ-}$ and NO levels in IS-treated cells, it significantly reduces $O_2^{\circ-}$ and increases NO production in Pi-treated cells (P<0.001) suggesting an eNOS uncoupling induced by Pi.

**Conclusions:** This study shows for the first time that 2 uremic toxins induce cerebral endothelial dysfunction by two different mechanisms. They both decrease NO levels by inducing an oxidative stress. However, Pi seems to be more deleterious since it also induces eNOS uncoupling.

*Funding:* Government Support - Non-U.S.

**PUB001**

**Effect of Adipose-Derived Stem Cells Cultured with Astragaloside IV on the Cisplatin-Induced Renal Tubular Cells** Huiling Wang. *Div of Nephrology, Jimin Hospital, Shanghai, China.*

**Background:** To investigate the role and possible mechanisms of human adipose-derived mesenchymal stem cells (hADSCs) cultured with Astragaloside IV(Ast) to human renal tubular epithelial cells line (HKC) induced by cisplatin in vitro.

**Methods:** HKC cells were induced by different concentrations of cisplatinfor 24 hours. The proliferation activity and apoptosis of HKC were evaluated with CCK-8 and Flow Cytometer assay respectively. The influence of hADSCs on HKC was detected by transwell culture system, which was used to co-culture for 48 hours. After that,the apoptosis rate and proliferating of HKC were detected by TUNEL and PCNA assay respectively. Transwell culture system was used to test the migration effect of hADSC. The number of migrating cell was counted under a fluorescence microscope .

**Results:** As the increasing of concentrations of cisplatin, the number of HKC was decreasing, meanwhile the apoptosis and necrosis proportion of HKC was enlarging. ,When the density of cisplatin was smaller than 10umol/L,Compared to model group (no co-culture), the apoptotic rates of HKC co-cultured with hADSCs were decreased and the cell number increased obviously($P<0.05$). The effects on HKC were even more pronounced when hADSC cultured with 20mg/L Ast. When the density of cisplatin was bigger than 10umol/L, the proliferation activity and apoptotic rates of HKC were no significant difference between co-cultured group and model group ($P>0.05$). *f*Crystal violet staining show that hADSCs cultured with 20mg/L Ast can increase the number of cells crossing the membrane in the transwell culture system($P<0.05$) after co-cultured.

**Conclusions:** As the increasing of concentrations of cisplatin, the degree of HKC injury was increasing. Cisplatin-indcued HKC can facilitate the migration of hADSC in vitro and hADSCs cultured with 20mg/L Ast can increase the number of cells crossing the membrane. The hADSCs could lessen the apoptosis of HKC induced by cisplatin, especially cultured with 20mg/L Ast, when the density of cisplatin was smaller than 10umol/L. However,when the density was bigger than 10umol/L, it had no obvious effect on HKC.

*Funding:* Government Support - Non-U.S.

**PUB002**

**Urinary Neutrophil Gelatinase Associated Lipocalin (uNGAL) Is the Most Sensitive Biomarker of Acute Kidney Injury** Peter Hamar, Mária Godó Godó, Csaba Revesz, Tamás Kaucsár. *Institute of Pathophysiology, Semmelweis Univ, Budapest, Hungary.*

**Background:** Acute tubular necrosis (ATN) is the most common cause of ischemic acute kidney injury (AKI). Neutrophil gelatinase associated lipocalin (NGAL) is an accepted early biomarker of tubular epithelial cell injury and inflammation. We aimed to analyse the diagnostic role of NGAL in ischemia-reperfusion (IR) injury in detail.

**Methods:** We performed 10, 20 and 30 minutes of ischemia on the left kidneys with right nephrectomy in C57BL/6 mice. After 1, 3, 6, 15, 24, 48, 72 and 96 hours of reperfusion we examined NGAL renal expression (real-time PCR), localization (immunohisto on tissue microarray), plasma and urine protein levels (ELISA), tubular histology (PAS stain) and renal function by BUN. Inflammation was monitored by plasma IL-6 and p40 (IL-12, IL-23) (ELISA).

**Results:** After ischemia NGAL appeared in a punctuate form in juxtamedullary tubular cells (TEC) at the luminal pole and in tubular lumina. After 1 day reperfusion, NGAL mRNA and 24h urine protein level raised significantly (1.7 fold, $p<0.05$ and 6.3 fold, $p<0.01$, respectively) already after 10 minutes ischemia: not inducing any other renal damage marker. ATN ($p<0.01$), blood urea (BUN) (5.4 fold, $p<0.001$) and plasma NGAL (3.2 fold, $p<0.05$) was elevated only if the ischemia was minimum 20 min. Thirty min. ischemia lead to anuria and to severe ATN already after 1h ($p<0.01$), BUN at 3h (1.8 fold, $p<0.05$), renal NGAL mRNA at 6h (10 fold, $p<0.01$), plasma NGAL at 15 reperfusion (5.2 fold, $p<0.01$). In sham, NGAL renal mRNA and plasma level also increased (the most at 15h), but this elevation was minor vs the IR grop (50 and 5 times lower, respectively). Plasma inflammatory cytokines increased only temporarily. After 20 min ischemia, BUN and plasma NGAL returned to baseline, but renal NGAL mRNA remained elevated longer (13.9 fold, $p<0.05$).

**Conclusions:** NGAL was upregulated by IR and accumulated in apical vesicles of renal TEC. Thus, urine NGAL (if obtainable) is the most sensitive biomarker of tubular ischemic injury. As plasma NGAL could be influenced by other inflammatory processes, it should be used only in the absence of available urine sample.Funding:OTKA, K 81972.

*Funding:* Government Support - Non-U.S.

**PUB003**

**Activation of Proximal Tubule Sphingosine 1-Phosphate Receptor-1 Preserves Mitochondrial Function in Cisplatin-Induced Nephrotoxicity in Mice** Amandeep Bajwa,[1] Piotr Chroscicki,[1] Brandon M. Kenwood,[2] Hong Ye,[1] Diane L. Rosin,[2] Kyle L. Hoehn,[2] Mark D. Okusa.[1] *[1]Dept of Med./CIIR, Univ of Virginia, Charlottesville, VA; [2]Pharm., UVA.*

**Background:** S1P is a sphingolipid that is the natural ligand for a family of five G-protein coupled receptors (S1P1-5Rs), regulates cell survival and lymphocyte circulation. The pan S1PR agonist, FTY720 (fingolimod), attenuates ischemia-reperfusion injury by directly activating S1P1 on PT cells. In the current study we hypothesized that S1P1 activation protects kidneys from the nephrotoxic effects of C by preserving PT mitochondrial (Mt) function.

**Methods:** Renal proximal tubule (PT) S1P1KO (*PEPCK-CreS1pr1$^{fl/fl}$*) and control mice (*PEPCK-Cre)* received cisplatin (Cis-27 mg/kg; 20mcM) and renal injury was assessed on day 3 by monitoring plasma creatinine (Cr), inflammatory cell infiltration (by flow cytometry) and changes in pro-inflammatory cytokines (by real time RT-PCR). TKPTS cells were used for over-expression of S1P1.

**Results:** Compared to control mice (Cr: 0.79±0.02 mg/dl) C induced more kidney injury in PT S1P1 KO mice (1.22±0.13, $p<0.05$), and FTY720 reduced injury in control mice (0.47±0.06, $p<0.01$) but not in PT S1P1 KO mice (1.32 ±0.22, n.s.). Control or PT S1P1 KO mice treated with Cis had significantly higher levels of pro-inflammatory cytokines (CXCL1, MCP-1, TNF-$\alpha$ and IL-6) and increased neutrophil and macrophage infiltration in kidneys compared to vehicle-treated mice on day 3. FTY720 attenuated the increased immune cell infiltration and expression of cytokines in control but not PT S1P1 KO mice. Over-expression of S1P1 in TKPTS cells rendered cells resistant to Cis-induced cell death compared to empty vector controls. S1P1-overexpressing TKPTS cells had higher Mt respiratory capacity, Mt numbers (MitoSox/JC-1/Mitotracker) and more Mt fusion compared to control cells after Cis.

**Conclusions:** In summary, activation of S1P1 expressed in PT attenuates C-induced AKI by preserving Mt function. We conclude that FTY720 administration or increasing S1P1 expression might represent a novel strategy in the prevention of C-induced AKI. We are currently examining the mechanism of Mt control by S1P1 activation.

*Funding:* NIDDK Support

**PUB004**

**The Role of Autophagy in Radiocontrast Induced Nephropathy** Gang Jee Ko, So Yeon Bae, Mi-yeon Jung, Yu ah Hong, Heui-jung Pyo, Young-Joo Kwon. *Dept of Internal Medicine, Korea Univ College of Medicine, Seoul, Korea.*

**Background:** Radiocontrast induced nephropathy (CIN) is the third common cause of acute renal failure among inpatients. Although the number of patients taken the exams using radiocontrast is increasing, little has been progressed in the treatment for CIN. The pathophysiology of CIN was known as tubular injury with toxic effect by oxidative stress. Autophagy, which regulates cell death in physiological as well as in pathological conditions with various stress, was known to have a role in tubular injury of acute kidney injury related to cisplatin and ischemia. We investigated the role of autophagy in radiocontrast induced nephropathy.

**Methods:** Radiocontrast nephropathy was induced with male C57BL/6J mice by intraperitoneal injection of iohexol (Omnipaque). Prostanoids and nitric oxide (NO) synthesis was inhibited prior injection of iohexol with indomethacin and NO synthase inhibitor to accentuate tubular injury by radiocontrast. Mannitol was used for osmotic control of iohexol, and 3-methyladenine (MA) was used as an autophagy inhibitor. Tubular injury caused by iohexol was also examined in vitro model using rat tubular cells (NRK-52E).

**Results:** Increased autophagy after iohexol administration was demonstrated with increased LC3 II expression in damaged tubules of kidney. Serum creatinine and tubular injury measured in PAS staining were significantly increased at 24hrs after iohexol administration compared to controls, and they were worsen with autophagy inhibition by 3-MA (creatinine: saline *vs* manntol *vs* iohexol *vs* iohexol+3-MA, 0.26±0.01 *vs* 0.36±0.04 *vs* 0.91±0.19 *vs* 1.31±0.40 mg/dL, $p<0.05$). In vitro study also showed that decreased cell viability measured by MTT assay after iohexol treatment was accentuated with 3-MA pretreatment. Increased caspase 3 and 9 expression after iohexol administration was augmented by autophagy inhibition.

**Conclusions:** Autophagy was associated with radiocontrast induced nephropathy.

**PUB005**

**Evaluation of Ischemic Renal Injury after Nephron-Sparing Surgery Using Dynamic Renal Scintigraphy and L-Type Fatty Acid Binding** Tokunori Yamamoto, Hideki Mizuno, Norihisa Matsukawa, Momokazu Gotoh. *Urology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan.*

**Background:** Renal scintigraphy is an established method to quantitatively assess split renal function. L-type fatty acid binding protein (L-FABP) is a novel urinary biomarker to reflect acute kidney injury and may play a role in predicting the extent of ischemic renal injury following nephron-sparing surgery. We evaluated ischemic renal injury after nephron-sparing surgery using dynamic renal scintigraphy and urine level of L-FABP in treated kidney.

**Methods:** We performed open and laparoscopic nephron-sparing surgery for 7 patients with small renal mass and inserted ureteral catheter into the treated renal pelvis. Ischemic renal injury of the treated kidney was evaluated by comparing effective renal plasma flow (ERPF) estimated by radionuclide technetium Tc 99m-mercaptoacetyltriglycine (99mTc-MAG-3) renal scintigraphy before and 1 week after the operation. We calculated a previously described variable called baseline weighted differential (b-WD) of ERPF that represent the percentage of loss of kidney function, considering the baseline value with b-WD of ERPF= (1 week Postoperative ERPF- baseline ERPF)/ baseline ERPF. Urine was obtained from the ureteral catheter 4 hours and 24 hours after de-clamping of the renal artery. We defined induction rate of L-FABP by dividing the 24-hour level by the 4-hour level. The correlation between b-WD of ERPF and induction rate of L-FABP obtained from the ureteral catheter was assessed.

**Results:** There was a strong correlation($R2=0.633$) between b-WD of ERPF and the logarithm of induction rate of L-FABP. The proximal tubuli were stained by L-FABP.

**Conclusions:** The results of the present study show that L-FABP is an effective urinary biomarker to predict the extent of ischemic renal injury following nephron-sparing surgery.

**PUB006**

**The Expression of Canonical Transient Receptor Potential Channels 6 in Renal Cortex and Hippocampus of Rats after Intraperitoneal Injection of Silver Nanoparticles** Ye Liu, Zhuo Yang. *Medical College, Nankai Univ, Tianjin, China.*

**Background:** The canonical transient receptor potential channels (TRPC) are members of a large channel family known as TRP channels. TRPC6 serves a variety of functions in both brain and kidney. Recent years, the discovery that the TRPC6 mutation causes familial focal segmental glomerulose sclerosis (FSGS) has aroused great attention to the investigation of this ion channel. The aim of the present study was to observe the expression of TRPC6 in renal cortex and hippocampus during early postnatal development of normal rats, and after exposure to silver nanoparticles.

**Methods:** A dosage of 3mg/kg Ag-NPs was applied to 16 post weaning rats through intraperitoneal injection (Ag-NPs treated group). Other 16 rats of the same age were given the same volume of normal saline to treat as the control group. Eight rats from each group were randomly chosen and executed at the end of the first week, and then the kidneys and hippocampus were removed. Other rats kept on with the treatment of either Ag-NPs or normal saline until the end of the fourth week. Then the rats were executed and the samples were removed as before. Immunofluorescence and Western blotting methods were used to detect the expression alteration of TRPC6 in rats.

**Results:** Results showed that the expression of TRPC6 was detected in glomerulus and tubules of both control group and Ag-NPs treated group. A stable expression was detected in the rat renal cortex during postnatal maturation in control group , while in Ag-NPs treated group, the expression of TRPC6 was increased in rat kidney cortex, and this increased tendency was getting more obvious in rats, which exposed to Ag-NPs for 4 weeks. The expression of TRPC6 didn't show an obvious variation in hippocampus between control group and Ag-NPs treated group.

**Conclusions:** These in vivo experiments suggested that the expression of TRPC6 underwent very little change with the development of kidney and hippocampus in normal post weaning rats, while an increased expression of TRPC6 was found in Ag-NPs exposure rat group, which may be a potential threat for the renal function.

*Funding:* Government Support - Non-U.S.

**PUB007**

**In Vitro and In Vivo Study of Necropotosis in Renal Tubular Epithelial Cells** Xinling Liang, Jialun Luo, Yuan Han Chen, Fen Jiang. *Div of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

**Background:** Recently research show that there are 4 but not 3 major morphologies of cell death: apoptosis, autophagy, necrosis and a new way described recently: necroptosis which was our major concern that may lead us to a new target point of protecting tubular epithelial cells. Necroptosis death receptor signaling with receptor-interacting protein 1 or 3(RIP1 or RIP3) activation, with no caspase 8 activation and can be inhibited by necrostatins or genetic alteration of RIP1 and RIP3.

**Methods:** We have used a human HK-2 renal tubular epithelial cell line to investigate the necroptosis phenomenon in vitro. HK-2 cells were subjected to TNF-a followed by ATP depletion, and benzyloxycarbonyl-Val-Ala-Asp-fluoro-methylketone (zVAD–fmk) was added to block the activity of caspase-8. And tried to interfere the process. Based on the in vitro result, we made a further research with SD mouse. We used a unilateral nephrectomy+ischemia-reperfusion model (I/R model) and pretreated the mouse with nec-1, NSA and mdivi respectively and combined in different groups, along with parallel control groups were sited. Blood and tissue samples were drawn after the model conducted.

**Results:** Without interfere, the cells showing organelles inflation, fragmentation. However, if we pretreated the cells with a specific blocker nec-1, these phenomenon were inhibited obviously and cell viability were greatly improved ($P<0.05$) with a parallel lower expression in LC3-II. Electron microscopy conform that necroptosis exist and can be block.

We observed the result through pathology, western blot, laser scanning confocal microscope and found that the situation in vitro can be duplicated in vivo, the renal tubular epithelial cells did not commit autophagy, and will mainly behave a necroptosis like in the group which the I/R model animal were treated with mdivi.

**Conclusions:** By this research we establish a way to build a necroptosis cell line model and proved necroptosis blocking can be a protective factor to human HK-2 renal tubular epithelial cell line. Furthermore, nec-1 can also show its protective effects in vivo and play an import role in the AKI of SD mouse I/R model.

*Funding:* Government Support - Non-U.S.

**PUB008**

**Trps1 Promotes Kidney Repair following Ischemia-Reperfusion Injury by Regulating Proliferation and Re-Differentiation of Renal** Kehong Chen, Jurong Yang, Yani He. *Nephrology, Daping Hospital, Third Military Medical Univ, Chongqing, China.*

**Background:** Renal tubular epithelium cells (RTECs) are the main victims of acute kidney injury (AKI). Tubule repair following AKI involves epithelial de-differentiation followed by cell proliferation and re-differentiation. Trps1 is a transcription factor which plays a crucial role in the regulation of mesenchymal-epithelial transition in early stage of embryonic kidney development. The purpose of this study is to investigate the role of Trps1 in kidney repair following AKI.

**Methods:** Kidney IRI model was established by clipping bilateral renal pedicles for 45 min (moderate) or 60 min (severe). Ultrasound-microbubble-mediated adenovirus transfer technique was applied to contruct the Trps1 knockdown or over-expression rat model.

**Results:** Moderate and severe IRI rats showed similar renal dysfunction and the degree of renal tissue injury at 1d after injury. Renal tissue damage in severe IRI rats, not in moderate rats, continued to increased and reached the peak at 3d after ischemia. Trps1 expression and renal dysfunction and tubulointerstitial injury scores were negatively correlated. The number of $Trps1^{+}Idu^{+}$ cells and $Trps1^{+}Idu^{+}CldU^{+}$ cells was significantly higher than the predicted value. In severe IRI model, the percentage of PCNA-positive cells in Trps1 overexpression group was significantly higher than that of the control group at 5d after injury. Scr, BUN levels, renal injury scores and vimentin expression ascended significantly in Trps1 overexpression group while α-catenin expression descended at 3d and 7d after injury. In moderate IRI model, the percentage of PCNA-positive cells in Trps1 overexpression group was significantly lower than that of the control group at 3d after injury. Scr, BUN levels, renal injury scores and vimentin expression were attenuated significantly in Trps1 overexpression group while α-catenin expression were enhanced at 1d and 3d after injury.

**Conclusions:** Trps1 regulates proliferation and re-differentiation of renal tubular dedifferentiated cells and promotes kidney repair after AKI.

*Funding:* Government Support - Non-U.S.

**PUB009**

**Risk Prediction Models for Acute Kidney Injury Requiring Continuous Renal Replacement Therapy after Off-Pump Coronary Surgery** Hiromichi Suzuki,[1] Youhei Tsuchiya,[2] Isao Tsukamoto,[2] Hirokazu Okada,[1] Tsutomu Inoue,[1] Tsuneo Takenaka.[1] *[1]Nephrology, Saitama Medical Univ, Moroyama Machi Iruma Gun, Saitama, Japan; [2]Medical Engineering, Saitama Medical Univ, Moroyama Machi Iruma Gun, Saitama, Japan.*

**Background:** Patients who undergo coronary artery bypass grafting (CABG) are at increased risk for acute renal injury requiring continuous renal replacement therapy. Accurate models are needed to predict the individual risk. Recently, off-pump coronary artery bypass (OPCAB) grafting without cardiopulmonary bypass has become a less stressful surgical procedure for CABG. However, there are only a few papers on OPCAB. The purpose of this study was to assess the impact of OPCAB on the incidence of AKI requiring CRRT.

**Methods:** An observational study of 371 consecutive non dialysis patients who underwent isolated CABG using OPCAB was conducted. Among them, 42 patients needed CRRT due to AKI. Variables with $P<0.05$ in bivariate analysis collected from pre-operative data were entered in the multivariate and proportional hazards regression analysis for risk factors of independent AKI's requiring CRRT after OPCAB was carried out.

**Results:** The risk factors that were independently associated with AKI requiring CRRT were: presence of diabetes mellitus, estimated glomerular filtration rate (less than 45 ml/min/1.73 m2), serum albumin level (less than 3.6 g/dL), hemoglobin level (less than 11.5 g/dL) and use of intra aortic balloon pump.

**Conclusions:** In conclusion, our study will provide a decision aid for OPCAB and highlight the need for further validation and clinical testing of the risk prediction models for AKI requiring CRRT in patients undergoing OPCAB.

**PUB010**

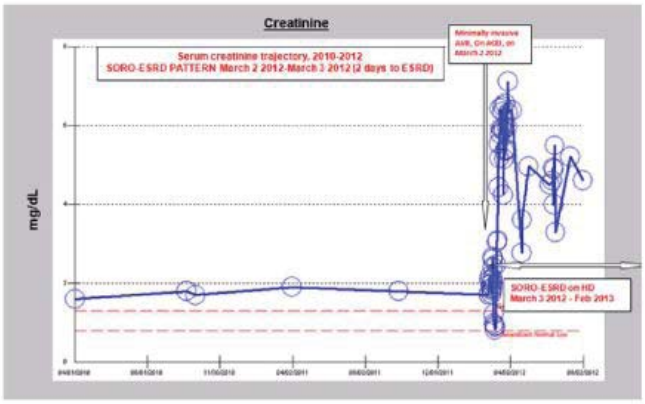
**The Rainbow Syndrome of AKI on CKD: From Full Recovery to Acute Irreversible Renal Failure or the Syndrome of Rapid Onset End-Stage Renal Disease – A Mayo Clinic Perspective** Macaulay A. Onuigbo,[1,2] Ngozi J. Achebe.[3] *[1]Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI; [3]Internal Medicine, Capital Medical Center, Olympia, WA.*

**Background:** Whereas nephrologists and physicians are aware of the phenomenon of acute kidney injury (AKI) on chronic kidney disease (CKD), the common consensus of usual full renal recovery is misguided. Anecdotal and objective evidence demonstrate that quite often, the reverse is the case, and renal recovery is either only partial, or worse still, never occurs, leading to rapid onset yet irreversible ESRD. The latter represents the new previously unrecognized syndrome of rapid onset end-stage renal disease (SORO-ESRD), that we first described in 2010, in the journal ***Renal Failure***. We describe four main patterns of renal outcomes of AKI on CKD as observed in a Mayo Clinic Health System Renal Unit.

**Methods:** Four case reports.

**Results:** Four patterns of renal outcomes following AKI on CKD:

- Full rapid recovery, in days to a few weeks, with terminal eGFR within ≤10% of baseline eGFR, or even higher.
- Partial recovery, in days to a few weeks, with terminal eGFR >10% below baseline eGFR.
- Rapid-onset yet irreversible ESRD, usually within 2-4 weeks, in native kidneys.
- Rapid-onset yet irreversible ESRD, usually withing 2-4 weeks, in renal allografts.




**Conclusions:** We have shown that renal outcomes following AKI on CKD is a spectrum of a continuum from quick complete recovery through to the rapid, unpredictable and precipitate progression to irreversible ESRD without any renal recovery ever. The latter pattern is the syndrome of rapid onset end-stage renal disease (SORO-ESRD), a previously unrecognized syndrome, that we first described in 2010. Undeniably, the variable renal outcomes following AKI on CKD is best described as the several colors of the rainbow.

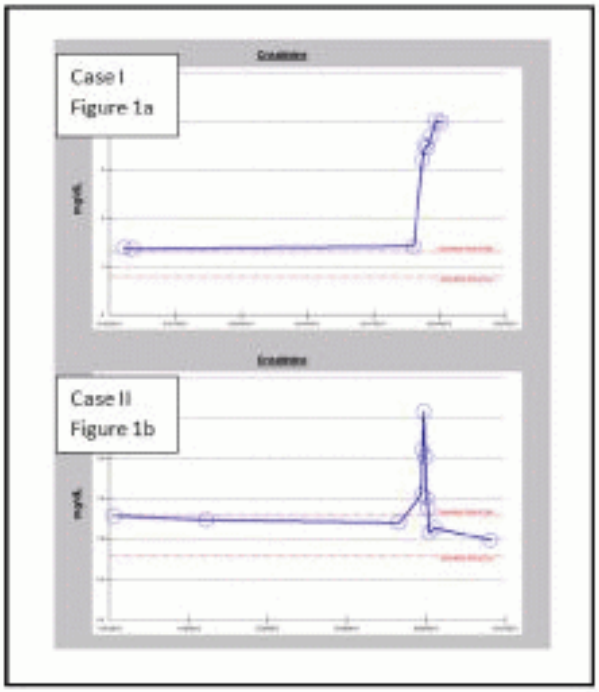
## PUB011

**Quadruple Whammy – A New Preventable Syndrome of Peri-Operative AKI on CKD in Patients on Concurrent Combination Triple Whammy Medications** Macaulay A. Onuigbo,[1,2] Belmarie P. Roman-maradiaga,[3] Ngozi J. Achebe.[4] *[1]Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, Eau Claire, WI; [3]Hospital Medicine, Mayo Clinic Health System, Eau Claire, Eau Claire, WI; [4]Internal Medicine, Capital Medical Center, Olympia, WA.*

**Background:** The potential nephrotoxicity from combination diuretics-ACEIs-NSAIDs, the 'triple whammy', is often unrecognized. In early 2013, we described accelerated post-operative AKI in patients on triple whammy, named 'quadruple whammy', in the ***British Medical Journal***.

**Methods:** Case reports.

**Results:** I. A 59-year old morbidly obese Caucasian male, hypertension, serum creatinine (SCr) 1.42 mg/dL (eGFR 54), developed accelerated oliguric AKI after elective right nephrectomy, peri-operative hypotension and anemia. He was on Lisinopril-Hydrochlorothiazide 20/25, Nabumetone (NSAID) 1000 mg daily. Within 24-hours, SCr more than doubled to 3.2 mg/dL (eGFR 22) with metabolic acidosis and hyperkalemia (Figure 1a). He was transfused; hypotension corrected. He was discharged on Metoprolol and Furosemide. SCr was 1.96 mg/dL (eGFR 37) three months later. II. A 46-year old morbidly obese Caucasian male, hypertension, SCr 1.21 mg/dL (eGFR 70), developed accelerated AKI after elective right hip arthroplasty, peri-operative hypotension and anemia. He was on Lisinopril 40 mg, Hydrochlorothiazide 25 mg daily, and had a preoperative dose of Celecoxib (cox II inh.). Within 36 hours, SCr more than doubled to 2.58 mg/dL (eGFR 28) with metabolic acidosis (Figure 1b). Hypotension was corrected. He was discharged on Amlodipine and Furosemide. SCr was 0.99 mg/dL (eGFR 85), one month later.




**Conclusions:** 'Quadruple Whammy', a new syndrome of preventable AKI with 'triple whammy', due to superimposition of peri-operative stressors especially hypotension is preventable, not uncommon, and calls for more pharmacovigilance.

## PUB012

**Contrast Induced Nephropathy after Transcatheter Arterial Chemoembolization in Hepatocellular Carcinoma** Kenichi Ishizawa,[1] Takamasa Ohki,[2] Takafumi Kanemitsu,[1] Tomoko Honda,[1] Masatomo Chikamori,[1] Ayako Tsuchiya,[1] Rika Miura,[1] Mai Sugahara,[1] Nobuo Toda,[2] Naobumi Mise.[1] *[1]Dept of Medicine, Div of Nephrology, Mitsui Memorial Hospital, Tokyo, Japan; [2]Dept of Medicine, Div of Gastroenterology, Mitsui Memorial Hospital, Tokyo, Japan.*

**Background:** The aim of this study was to determine the incidence and risk factors for contrast induced nephropathy (CIN) after transcatheter arterial chemoembolization (TACE) in patients with hepatocellular carcinoma (HCC) and coexisting chronic liver disease.

**Methods:** We retrospectively investigated 126 patients(97 male, age 70±9 years, estimated glomerular filtration rate (eGFR) 78±21 ml/min)with HCC who underwent TACE between January 2003 and January 2011. The first TACE treatment session of each patient was examined. CIN was defined as an increase in serum creatinine of ≥ 0.5mg/dl or ≥ 25% over baseline within 72 hours after TACE. Low-osmolality contrast medium, iopamidol, was administered in all patients.

**Results:** CIN developed in 4 patients (3.2%) and all recovered without renal replacement therapy. In a univariate analysis, age ($P<0.05$), HCC diameter more than 5-cm ($P<0.05$) and total bilirubin ($P<0.05$) influenced the development of CIN. Multivariate analysis including these 3 variables demonstrated that HCC diameter more than 5-cm ($P<0.05$) was a significant predictor of CIN. Between patients with and without CIN, the prevalence of chronic kidney disease (eGFR less than 60ml/min/1.73m$^2$, 50% vs. 18%, $P=0.11$) and diabetes (50% vs. 20%, $P=0.14$) was comparable. The mean dose of contrast medium was 100±45 ml.

**Conclusions:** CIN after TACE was associated with HCC size, but not with underlying kidney dysfunction. In the present study, the incident rate of CIN was lower than previously reported (9 ~ 24 %).

## PUB013

**M13-796 Study Design – A Phase 2b, Randomized, Double-Blind, Placebo-Controlled, Safety and Efficacy Trial of Multiple Dosing Regimens of ABT-719 for Prevention of Acute Kidney Injury in Patients Undergoing High Risk Cardiac Surgeries** Samina Khan,[1] Peter A. McCullough,[2] Lakhmir S. Chawla,[3] Thomas M. Beaver,[4] Elliott Bennett Guerrero,[5] Deli Wang,[1] Mark T. Houser.[1] *[1]AbbVie, IL; [2]Providence Park Hospital, MI; [3]George Washington Univ Medical Center, Washington, DC; [4]Univ of Florida, FL; [5]Duke Univ Medical Center, NC.*

**Background:** Prevention and treatment of acute kidney injury (AKI) is a significant unmet medical need. Patients undergoing high risk major surgeries are at increased risk for ischemia reperfusion (I/R)-induced AKI. ABT-719 is a novel melanocortin receptor agonist in development for prevention and treatment of AKI. In a Phase 2 study (CS007), 800 μg/kg of ABT-719 reduced the incidence of AKI defined by AKIN and RIFLE criteria, as well as

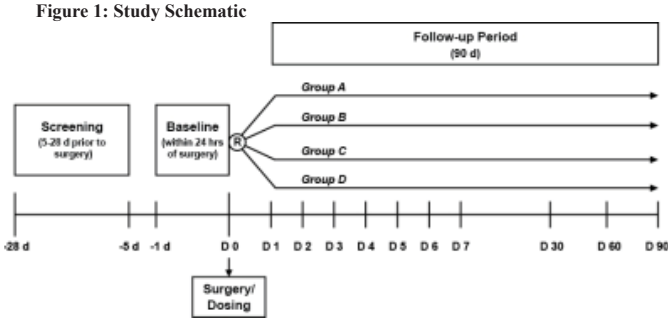
the 90 day composite outcomes. We describe the study design of M13-796 (ClinicalTrials.gov Identifier NCT01777165), a Phase 2b trial of ABT-719 in patients undergoing high risk on-pump cardiac surgeries.

**Methods:** This is a double-blind, placebo-controlled study. Subjects will be randomized at a 1:1:1:1 ratio into 4 treatment groups: placebo and ABT-719 at 800, 1600 or 2100 μg/kg (3, 5 or 6 bolus infusions, respectively). AKI will be evaluated according to the AKIN, RIFLE and KDIGO criteria with additional clinical outcomes of interest.

**Results:** The primary efficacy endpoint is the proportion of subjects developing AKI based on the AKIN criteria. The key secondary efficacy endpoint is the proportion of subjects developing at least one of the composite events: death, need for renal replacement therapy, or having a ≥ 25% reduction in eGFR at 90 days.

**Conclusions:** M13-796 is designed to further evaluate the efficacy of ABT-719 and optimal dosing strategy for prevention of AKI in patients undergoing high risk on-pump cardiac surgeries.

**Figure 1: Study Schematic**




*Funding:* Pharmaceutical Company Support - AbbVie

## PUB014

**Pseudo-Acute Renal Failure after Abdominal Hysterectomy** Muhammad K. Shakeel. *Nephrology and Internal Medicine of Anderson, Anderson, SC.*

**Background:** Intraperitoneal urinary leak from either urinary bladder or ureteral perforation should be considered in the diagnosis of acute kidney injury after gynaecological surgery.

**Methods:** I present a case of reversible acute renal failure after total abdominal hysterectomy. Patient developed intraperitoneal urinary bladder perforation. Subsequently she was noted to have elevated BUN and creatinine after 48 hours of surgery. Elevated creatinine occurred as a result of intraperitoneal urine leak and reabsorption through the peritoneum. Patient was taken to the OR and the leak was repaired.

**Results:** There was a prompt recovery of the serum biochemistry. Early recognition and surgical repair as opposed to dialysis therapy are warranted in such clinical setting. Nephrologist and gynaecologist should be aware of this diagnosis since pseudo-renal failure which may resolve without dialysis.

**Conclusions:** Early recognition and surgical repair as opposed to dialysis therapy are warranted in such clinical setting. Nephrologist and gynaecologist should be aware of this diagnosis as this condition may resolve without dialysis.

## PUB015

**ANA Negative Patient with Lupus Nephritis** Muhammad K. Shakeel. *Nephrology and Internal Medicine of Anderson, Anderson, SC.*

**Background:** I present a case of a 44-year-old male who presented with acute kidney injury and nephrotic syndrome.

**Methods:** Patient underwent a native kidney biopsy which showed immune complex mediated focal proliferative glomerulonephritis with 10% crescents consistent with lupus glomerulonephritis. Initial serology including ANA, complement levels and double stranded anti DNA antibodies were negative. Patient was treated with monthly pulse cyclophosamide and corticosteroids. Patient failed Cyclpphosamide and a repeat kidney biopsy was done which again showed cresentic glomerulonephritis consistent with lupus nephritis. Repeat serology including ANA complements were again negative. Patient was treated with rituximab times two doses. Patient remained nephrotic and there was no improvement in kidney function. Patient was started on haemodialysis and is doing well.

**Results:** The diagnosis of a SLE depends on patient's clinical and laboratory abnormalities. ANA is a diagnostic hallmark of SLE having a frequency of 95% in SLE patients. There are previous case reports of patients with clinical features of SLE having negative test for ANA. These patients appear to represent 1 to 5% of the SLE population. The age of onset and the female predominance are the same for ANA negative SLE as for ANA positive SLE. There are several possible explanations for negative ANA but there is no definitive explanation at this time.

**Conclusions:** My patient may be a rare case of ANA negative lupus nephritis and this suggests that ANA may not be required in the diagnosis of lupus nephritis.

## PUB016

**Acute Kidney Injury and Severe Hypokalemia in McKittrick-Wheelock Syndrome: Report of Two Cases** Claudio Angelini, Elisa Merizzoli, Paola Arosio, Manuel Alfredo Podesta', David Cucchiari, Salvatore Badalamenti. *Nephrology, Humanitas Clinical and Research Center, Rozzano, Milan, Italy.*

**Background:** McKittrick-Wheelock Syndrome (MWS) is a rare disorder characterized by the pentad: (1)secretory diarrhea induced by a colorectal villous adenoma, (2)pre-renal acute kidney injury (AKI), (3)metabolic acidosis, (4)hypokalemia and (5)hyponatremia.

**Methods:** Retrospective analysis of biochemical parameters from two patients admitted for AKI who were previously diagnosed with colorectal adenomas.

**Results:** Laboratory results were consistent with AKI, associated to dehydration, metabolic acidosis and hyponatremia in patients who had a previously normal renal function.

| | Case 1 | Case 2 |
|---|---|---|
| Age/Sex | 72/F | 83/M |
| Adenoma size | 15 cm | 15 cm |
| Creatinine | 11.7 | 4.8 |
| $Na^+$ | 122 | 124 |
| $K^+$ | 2.9 | 2.4 |
| $Cl^-$ | 96 | 94 |
| $HCO^{3-}$ | 14 | 13 |
| Anion Gap | +12 | +16 |

These parameters were rapidly corrected by intravenous infusion of saline. Both cases also showed severe hypokalemia, which was treated with intravenous KCl supplementation (80-90 mEq/day). All the attempts to wean the patients off this therapy resulted in hypokalemia relapse. Extra-renal potassium loss was confirmed by low urinary potassium levels: this finding, along with medical history and biochemical tests, led to MWS diagnosis.

**Conclusions:** MSW clinical picture ensues from the secretory diarrhea induced by colorectal villous adenomas. The adenomatous cells undergo a switch from an absorptive to a secretory phenotype, leading to ions and water loss. Non-anion gap metabolic acidosis results from the equal secretion of sodium, chloride and bicarbonate. When AKI develops, retention of organic acids can increase the anion gap. Patients can remain stable for years, until tumor secretion exceeds renal compensation, determining symptoms onset. MWS severity is proportional to the size and distal localization of adenomas, which prevents the reabsorption of secreted fluids. Although hypokalemia correction can only be achieved by surgical resection, prompt rehydration is usually enough to recover from AKI. Nevertheless, owing to MWS rarity, the diagnosis is often delayed, thus increasing the risk of complications.

## PUB017

**Effect OF Dialysate Fflow Direction on Solute Clearances during Continuous Rrenal Replacement Therapy** Jeong Chul Kim,[1,2] Flavio Basso,[1,2] Mauro Neri,[1] Alessandra Brendolan,[1] Massimo de Cal,[1,2] Monica Zanella,[1] Claudio Ronco.[1,2] *[1]Dept of Nephrology, St. Bortolo Hosp, Vicenza, Veneto, Italy; [2]IRRIV (International Renal Research Institute), Vicenza, Veneto, Italy.*

**Background:** Continuous renal replacement therapy (CRRT) is commonly used in critically ill patients with acute kidney injury (AKI). During CRRT, dialysate can be used to increase solute removal by diffusion. The connection of the dialysate fluid to the filter is usually applied such that dialysate fluid (Qd) flows in the opposite direction (counter-current) of blood to guarantee the maximum solute clearance by diffusion. However, there is no evidence about the superiority of counter-current on co-current configuration in CRRT. We performed a clinical study to investigate the effect of the direction of Qd on urea and creatinine removal.

**Methods:** We conducted a prospective study on adult patients admitted in ICU requiring CRRT. At this point ultrafiltration rate was set as zero and Qd was set as 2000 ml/h. Details of blood sampling procedure was represented in fig 1.


The CVVHDF were randomly initiated with co- or counter-current configuration for the first 50 minutes until it was switched to CVVHD. Urea (Ucl) and creatinine (CrCl) clearance were calculated to each flow configuration.

**Results:** Median value of UCl, CrCl and E/P ratio in co-current vs counter-current configuration were: 27.4 vs 33.3 ml/min, 26.7 vs 33.3 ml/min and 0.82 vs 1.0 respectively ($p< 0.001$).

**Conclusions:** Counter-current dialysate flow configuration during CRRT provides a higher solute clearance. However, advantages of each configuration should be balanced against the overall performance of the treatment and its simplicity in terms of treatment delivery and circuit handling procedures.

**PUB018**

**Modelling Acute Kidney Injury (AKI) Biomarkers** John W. Pickering,[1] Zoltan H. Endre.[1,2] *[1]Christchurch Kidney Research Group, Dept of Medicine, Univ of Otago, Christchurch, New Zealand; [2]Dept of Nephrology, Prince of Wales Clinical School, Univ of New South Wales, Sydney, Australia.*

**Background:** Many factors may modify the temporal profile of urinary AKI biomarkers, including type of biomarker and underlying CKD. We modelled these physiological differences to explore the effect of increased (hyperfiltration) or reduced GFR (CKD) on the biomarker time-course profiles.

**Methods:** Single nephron models of the time-courses of pre-formed (eg GGT) or, induced (eg NGAL) in tubular epithelial cells, or filtered and normally reabsorbed (eg Cystatin C) biomarkers were modelled based on known physiology. Excretion rates were combined using multiples of single nephron urine flow rates to compute urinary concentrations under scenarios of hyperfiltration, normofiltration, and reduced GFR. Whole kidney scenarios were then constructed for kidneys with normal or reduced nephron number.

**Results:** Preformed biomarker concentrations peaked earliest after insult with total excretion limited by pre-injury mass of biomarker, which in turn depended on the number of available nephrons and the rate of biomarker regeneration. Induced biomarkers peaked later and were influenced by nephron number but not limited by this. Filtered biomarker profiles varied with nephron number and GFR with extended time courses compared to other biomarkers.

**Conclusions:** Preformed biomarkers concentrations can directly quantify tubular injury, but must be measured very soon after renal insult. Induced biomarkers provide a longer window of assessment. Filtered biomarkers have the longest window of opportunity, and measure injury directly and indirectly measure reduced GFR.

*Funding:* Government Support - Non-U.S.

**PUB019**

**Epidemiology of Acute Kidney Injury in Indian Intensive Care Unit – Single Centre Study** Piyush Mathur,[1] Rajasekara Chakravarthi Madarasu,[1] Rusheendra Kambhampati,[1] Vikranth Reddy,[1] Santosh Hedau.[1] *[1]Nephrology, Care Hospital, Banjara Hills, Hyderabad, AP, India; [2]Nephrology, Care Hospital, Banjara Hills, Hyderabad, AP, India; [3]Nephrology, Care Hospital, Banjara Hills, Hyderabad, AP, India; [4]Nephrology, Care Hospital, Banjara Hills, Hyderabad, AP, India.*

**Background:** The incidence of acute kidney injury in intensive care units has increased in past few years but exact incidence is not known in because of many confounding factors.

**Methods:** Material: Single centre study conducted at tertiary care centre from March 2010 till May 2013. Method: Patients admitted in ICU, who developed AKI in 48 hours were included in study after taking consent. Demographic data was collected and patients younger than 18 years, patients with end stage kidney disease on maintenance hemodialysis, renal transplant recipients were excluded from study. Risk factors for AKI and APACHE score was calculated at enrollment.

**Results:** 350 patients were screened during study period for AKI in ICU's and 300 were included in study as they met AKIN criteria for AKI. 261 patients completed the follow up period of study. Out of these 261 patients who had AKI, 80 required renal replacement therapy. Of the 80 patients who required RRT, 40 were managed on hemodialysis, 37 on SLED and 3 were on CVVHDF. Major risk factors for AKI were old age and diabetes. APACHE 3 score was higher in those who had dialysis requiring AKI as compared to those who had AKI but not dialysis requiring. Multiple factors were responsible for AKI but most common etiology for AKI in Indian ICU setting was sepsis with septic shock. Others being drugs, cardiac surgery, contrast agents. The mortality of septic AKI and AKI due to other causes like cardiogenic, drug induced was significantly different.

**Conclusions:** Indian ICUs has high incidence of AKI which is mostly multifactorial. Sepsis is the most common etiology for AKI in a tertiary care ICU. Higher APACHE 3 score at admission to ICU is associated with higher incidence of dialysis requiring AKI.

**PUB020**

**Predictors of Contrast Induced Nephropathy after Coronary Angiogram in Nepalese Population** Sanjib Kumar Sharma. *Dept of Cardiology, College of Medical Sciences, Bharatpur.*

**Background:** The implications of radio-contrast induced nephropathy (CIN) are disastrous. In Nepal there is no published data on CIN**.** This study was undertaken to study the incidence of CIN and to identify risk factors (predictors) for the development of CIN in patients undergoing coronary angiography in tertiary care hospital.

**Methods:** The subject group consists of 540 patients undergoing coronary angiogram/ PCI in 2011 and 2012 enrolled by convenient sampling technique. Two hundreds ten patients were excluded from the study due either to incomplete data or death of the patients before completion of the study duration. Therefore, a total of 330 patients were enrolled in the study and analyzed. Serum creatinine values were measured before and at 24 hours, 48 hours and at 2 weeks of administration of contrast agents. Further measurements were performed in all CIN patients. Contrast-induced nephropathy was defined as an increase of >25% or >0.5 mg/dl in pre-PCI serum creatinine at or after 48 h after PCI. E-GFR was calculated by applying the 4 variables MDRD equation. Standard definitions were used to define the variables.

**Results:** Among the 330 patients studied, diabetes mellitus was present in 84 (28.81%), anemia in 120 (36.36%), e-GFR <60ml/min in 33 (10%), hypertension in 162 (49.09%), and heart failure in 48 (14.54%) patients at baseline. Twenty seven (8.18%) patients experienced CIN. The incidence of CIN in patients with baseline creatinine clearance < 60 ml/min was 45.45.9%. CIN developed in 10% of anemic and 12.5% diabetic patients. The amount of the contrast agent administered was similar for CIN and non-CIN patients (138.20±91.34ml vs. 175.56±118.86 ml; p =0.254). No correlation was found between the amount of contrast agent administered and the change of serum creatinine concentration. Multivariate logistic regression analysis found that baseline e-GFR and baseline hemoglobin were independent predictors for CIN.

**Conclusions:** In this first study of CIN in Nepal, the incident of CIN was 8.18%. Patients with preexisting kidney disease and anemia were at high risk of CIN.

**PUB021**

**A Case of Shunt Nephritis: Early Diagnosis and Management with a Complete Resolution of Acute Kidney Injury** Ibrahim Qaqish,[1] Pratik Shah.[2] *[1]St. Joseph's Hospital & Medical Center; [2]St. Joseph's Hospital & Medical Center.*

**Background:** Shunt nephritis is an immune-complex mediated glomerulonephritis associated with chronically infected ventriculoatrial shunts inserted for treatment of hydrocephalus. Renal outcome is good with early diagnosis and treatment. Delay in diagnosis can lead to irreversible ESRD.

**Methods:** 31-year-old male presented with two day history of worsening of his chronic headaches, blurry vision and fever.His past medical history was significant for ventriculoperitoneal shunt at age 16 weeks secondary to congenital hydrocephalus. His last revision was done 9 months ago when his ventriculoperitoneal shunt was changed to ventriculoatrial shunt. On physical examination only remarkable finding was temperature of 38.6 C. Labs showed hemoglobin of 10 gm/dl and serum creatinine of 2.2 mg/dl. Urine dipstick was positive for hematuria and proteinuria. Urine microscopy showed RBC casts. 24 hour urine protein was 590 mg/24hr. Cerebrospinal fluid (CSF) analysis revealed 172 nucleated cells with predominantly PMN. CSF culture and blood culture were positive for Propionibacterium acnes.Complement levels were low with C3 of 73 mg/dl and C4 of 12 mg/dl.Patient was treated with intravenous vancomycin and infected shunt was removed. Temporary external ventricular drain (EVD) was placed. Once infection cleared, a new shunt was placed and EVD was removed.At the time of discharge his serum creatinine was 1.4 mg/dl which normalized at 0.8 mg/dl two months as outpatient follow up.

**Conclusions:** Shunt nephritis was first described by Black et al in 1965. The overall incidence of ventricular shunt infection varies between 0.7 to 2.2 %. Patients with shunt infection typically present with fever, anemia, and cerebral manifestations i.e. headaches, blurry vision, and seizures. Renal manifestations include hematuria, proteinuria, renal insufficiency, and hypertension.Predominant finding on renal biopsy is membranoproliferative glomerulonephritis (MPGN). Decreased serum complement levels, presence of cryoglobulins or autoantibodies support the diagnosis of shunt nephritis. Treatment of shunt nephritis includes prompt removal of infected shunt and intravenous administration of antibiotics.

**PUB022**

**Protective Effect of N-Acetylcysteine on Contrast Induced Nephropathy in Patients with Stage 2-4 Chronic Kidney Disease** Irem Ari,[1] Mehmet T. Sezer,[2] Salih Inal,[2] Veysel Kidir,[2] Atila Altunta?.[2] *[1]Internal Medicine, Suleyman Demirel Univ School of Medicine, Isparta, Turkey; [2]Nephrology, Suleyman Demirel Univ School of Medicine, Isparta, Turkey.*

**Background:** There is a considerable interest in strategies to prevent contrast induced nephropathy (CIN) because of the increasing prevalence of renal insufficiency and the increasing use of imaging studies using contrast media (CM). The efficacy of N-acetylcysteine (NAC) for preventing CIN is uncertain. We aimed to investigate whether NAC might have a preventive role against the development of CIN in chronic kidney disease (CKD) patients.

**Methods:** The study included 60 stage 2-4 CKD patients undergoing investigations with CM. Patients were randomized into two groups. 2x1200 mg/day iv NAC and 1500 ml/day iv salin infusion were administered for three days starting one day before the procedure in the study group (n:30). Salin infusion was only given to the control group without altering other treatments of patients. Before the CM injection and two days after the procedure serum Cr, daily protein excretion, KIM-1, NGAL and MCP-1 levels were measured and Cr clearances were calculated.

**Results:** Baseline clinical and laboratory findings were similar in two groups.Although serum Cr levels were not significantly different between the two assessments in both groups; KIM-1 and MCP-1 levels increased significantly in the control group (253.03 ± 183.41 vs 388.57 ± 353.59 ; p:0.018 and 185.05 ± 114.66 vs 295.13 ± 274.49; p:0.026, respectively).In the study group, there was only a slight but not significant increase in MCP-1 and KIM-1 levels.

**Conclusions:** In conclusion, we assume that in CKD patients who are undergoing investigations with contrast medium administration, the use of NAC + iv salin infusion significantly reduces the risk of developing CIN. Secondly, KIM-1 and MCP-1 seem to be good surrogate markers for predicting renal tubulary injury.

**PUB023**

**A Strange Case of Hyperphosphatemia during Acute Kidney Injury** Davide Ricci, Raffaella Rizzo, Elena Mancini, Antonio Santoro. *Nephrology Dialysis Hypertension, Policlinico S.Orsola-Malpighi, Bologna, Italia, Italy.*

**Background:** Hyperphosphatemia (HPh) frequently accompanies acute kidney injury (AKI), due to the reduced urinary excretion. A severe HPh is rare, but may appear during cytolytic syndromes, often resulting in overt AKI. An inaccurate definition of the cause of HPh may lead to a wrong diagnosis of AKI.

**Methods:** A 77-y F patient was admitted in the Cardiology Unit because of NSTEMI. Renal function was normal (creatinine 0.85 mg/dl). The patient had a history of chronic obstructive pulmonary disease (COPD) and diverticulosis. After near 1 week, the patient needed enemas. After the administration, she developed copious diarrhoea, hypotension, bradycardia and syncope. In the following 24 hours urine output decreased and blood tests revealed stage 2 AKI (creatinine 1.97 mg/dl, urea 143 mg/dl, uric acid 9.9 mg/dl) with severe hyperphosphatemia (24.2 mg/dl). Inflammatory markers were elevated (leucocytes 23,950/mmc, N91%, PCT 121 ng/ml, PCR 12 mg/dl), as well as cytolysis parameters (CK 181 U/L, myoglobin 1384 ng/mL, LDH 318 U/L). An ultrasound scan showed a solid mass (8 cm) in the left kidney. A thorax scan excluded pulmonary infections.The patient appeared dehydrated.

**Results:** AKI secondary to a cytolytic syndrome and sepsis due to diverticulitis was supposed. In spite of the therapeutic measures, renal function further worsened. The patient was moved to our nephrological department where she was treated with hemodiafiltration. An accurate examination of the clinical history highlighted the administration of 2 enemas with high phosphate content (22 g/100 mL of sodium phosphate). Despite this therapeutic approach, the patient's general conditions worsened rapidly and she died 24 hours after, because of acute abdomen with septic shock (diverticulitis with intestinal perforation was the hypothesis).Autopsy was declined by the family.

**Conclusions:** Acute Phosphate Nephropathy (APN) was the final diagnosis. We can hypothesize a role of inflammation and altered intestinal wall permeability due to diverticulitis that could have favoured phosphate hyperadsorption. A major caution in the use of sodium containing enemas should be used, especially in patients with risk factors and several comorbidities.

**PUB024**

**Incidence, Risk Factors and Prevention of Contrast-Induced Nephropathy in a Medicine In-Patient Setting: Analysis of Current Practices at Veteran Administration Hospital** Aileen P. Tlamsa, Margaret Duffy, Shyam Patel, Osama El Shamy, Tekchand Ramchand, Jin K. Jung, Rakesh Malhotra. *UMDNJ-NJMS, Newark, NJ.*

**Background:** Contrast-induced nephropathy (CIN) is a form of acute kidney injury that occurs after administration of radio-contrast material. The aim of the study was to assess how well current CIN prevention guidelines are being adhered to in clinical practice.

**Methods:** We conducted a retrospective analysis of 245 patients admitted to the medicine floors at the Veteran Affairs (VA) hospital from Sept 2010 – Dec 2012 who received radio-contrast material during their stay. Serum creatinine (sCr) was measured at least once per 24 hour before and within 2 weeks after the radio contrast study. Only patients with minimum of 3 sCr readings were included in the analysis. CIN was defined as rise in serum creatinine ≥ 0.5 mg/dL or 25% relative increase from baseline sCR within 48 hours. The primary endpoint was to calculate the incidence of CIN in our study population. Secondary endpoint was to examine the adherence of CIN prevention guidelines which included use of IV Fluids and N-aceytylcysteine (NAC).

**Results:** The mean (SD) age of the study population was 66.4±12.1 years and 100% were male. The mean (SD) baseline sCr was 1.1±0.9 mg/dL and 14.7% had an eGFR <60 ml/min. Twenty four patients (24/245, 9.8%) developed CIN. The mean increase in sCr levels was 0.42±0.24 mg/dL. The patients who developed CIN had greater prevalence of chronic kidney disease, heart failure and liver disease (20.8% vs. 14.0%; 12.5% vs. 5.9% and 20.8% vs. 16.0%, respectively). 15/24 (62.5%) of the patients who developed CIN were documented to be on IV fluids. None of the 24 CIN patients was prescribed NAC prior to radiographic contrast procedure.

**Conclusions:** Based on our preliminary data, the incidence of CIN in hospitalized male VA medicine patients is high. Furthermore, the adherence to CIN prevention guidelines was suboptimal. Future studies would involve the implementation of a computerized reminder system in accordance with CIN prevention guidelines when ordering IV contrast studies in high risk patients.

**PUB025**

**Immunomodulation with a Selective Cytopheretic Device (SCD) Reduces Injury in a Pig Model of Intracranial Hemorrhage (ICH)** H.D. Humes,[1,2,3] D. Buffington,[1] A. Westover,[1] K. Johnston,[1] P. Smith.[1] *[1]Innovative BioTherapies; [2]Univ of Mich; [3]CytoPherx.*

**Background:** Inflammation has recently been recognized as a central contributor to the pathobiology of stroke and traumatic brain injury (TBI), indicating that therapies that target inflammation may provide a new approach to the treatment of the acute phase of this disease process, particularly for ICH as there are currently no interventive therapies for this subset of stroke patients. The SCD is a novel biomimetic device that has shown efficacy in preclinical models of acute and chronic diseases for which inflammation is an exacerbating factor. When placed in an extracorporeal blood circuit (EBC) with regional citrate anticoagulation (RCA), the low ionized calcium environment provides a simultaneous combination effect to sequester and alter the activity of the transiently bound leukocyte (LE). The safety/efficacy of this device has been demonstrated in clinical studies in ICU patients with ARF and MOF and is currently being evaluated in a US multicenter, randomized pivotal IDE trial. Pilot studies were initiated to determine the SCD effect on the acute inflammatory cascade in ICH.

**Methods:** The acute effects of SCD therapy were evaluated in a pig model of ICH for which thrombin was used as the inflammatory nidus. ICH was induced simultaneously to SCD therapy using an EBC with RCA. Therapy continued for 24 additional hours during which systemic inflammation was monitored by assay of systemic cytokines. Platelet activation and CD11b expression of LE populations were determined using flow cytometry. Most mortem coronal sections of frozen brain tissue were evaluated for LE infiltration, and neuronal injury was evaluated by histology. SCD effects on lesion size and brain edema were evaluated in treated animals (n=3) and compared to a contemporaneous control.

**Results:** SCD therapy resulted in a reduction in edema, reduced expression of neuronal injury markers and reduced LE infiltration as evaluated in animals sacrificed at 24h post ictus.

**Conclusions:** Pilot studies demonstrate that immunomodulation with the SCD represents a novel therapy that has the potential to improve outcomes associated with ICH.

*Funding:* Other U.S. Government Support

**PUB026**

**The Role of p66shc in Renal Toxicity of Oleic Acid** Istvan Arany,[1] Dustin Reed,[1] Luis A. Juncos,[2,3] Mehul P. Dixit.[1] *[1]Pediatrics, Div of Peditric Nephrology, Univ of Mississippi Medical Center, Jackson, MS; [2]Medicine, Univ of Mississippi Medical Center, Jackson, MS; [3]Physiology and Biophysics, Univ of Mississippi Medical Center, Jackson, MS.*

**Background:** Adult and childhood obesity is a growing problem: it is an independent risk factor in development of chronic kidney disease (CKD) and progression to end-stage kidney disease (ESRD). Pathologic consequences of obesity include non-esterified fatty acids (NEFAs)-induced oxidative stress and consequent injury of the kidney. Since the serine36 phosphorylated p66shc is a novel mediator of oxidative stress-dependent kidney injury we studied its role in oleic acid (OA)-induced oxidative stress and consequent injury in cultured renal proximal tubule cells (RPTCs).

**Methods:** Impact of OA or BSA-bound OA (OA-BSA) on production of reactive oxygen species (ROS), mitochondrial depolarization and cell injury (LDH release) was determined in RPTCS that harbor wild type (wt), knockdown (kd), Ser36-phosphorylation-(S36A) or cytochrome c binding-deficient (W134F) p66shc.

**Results:** OA increased ROS production via the mitochondria –and in less extent via NADPH oxidase- resulting in ROS-dependent mitochondrial depolarization. Further studies demonstrated that mitochondrial depolarization is responsible for injury of cultured cells. Knockdown of p66shc, mutation of its Ser36 or cytochrome c binding sites (S36A or W134F, respectively) attenuated adverse effects of OA. Interestingly, OA also stimulated the promoter of p66shc.

**Conclusions:** Our results revealed that OA increased expression, Ser36 phosphorylation and mitochondrial cytochrome *c* binding of p66shc that resulted in mitochondrial depolarization and consequent injury of RPTCs. Hence, these data may offer a novel mechanism by which obesity could lead to development of CKD and ultimately ESRD. Thus, manipulating this pathway may help to prevent obesity-associated renal lipotoxicity.

*Funding:* NIDDK Support

**PUB027**

**Hirsutella Sinensis Antagonize Aristolochic Acid-Induced Epithelial-Myofibroblast Transition through Inhibition of Snail** Xiao-yi Xu, Hong-liang Rui, Yan-yan Wang, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital, Capital Medical Univ, Beijing, China.*

**Background:** Cordyceps sinensis extracts were shown to inhibit aristolochic acid (AA)-induced renal fibrosis in rodent model. However, the molecular mechanism underlying this process has remained unclear. Hirsutella sinensis (HS) is the anamorphic mycelial form of cordyceps sinensis. This study is to investigate if HS could inhibit AA-induced renal tubular epithelial-myofibroblast transition (EMT) and the role of transcriptional factor, snail, in this process.

**Methods:** Cultured Human renal proximal tubular epithelial cells (HKC) were divided into the following four groups: control group, AA group, AA+ HS group and AA+HS+ SB415286 group. The effects of AA or/and HS on HKC proliferation and cytotoxicity were determined by MTT assay and LDH release assay, respectively. After treatment with AA or/and HS for 12h, the mRNA expression of α-smooth muscle actin, (α-SMA), cytokeratin-18 (CK-18), transforming growth factor-β1 (TGF-β1) and Snail was measured by real time RT-PCR. After treatment for 36h, the protein expression of α-SMA, CK-18, TGF-β1 and snail was measured by Western Blot.

**Results:** 1. 10umM AA or/and 10mg/L HS do not affect cell proliferation and have cytotoxicity on HKC. 2. Compared with the control group, AA stimulation up-regulates the expression of α-SMA and TGF-β1 and down-regulates the expression of CK-18. 3. AA stimulation also up-regulates the expression of snail, which is the key molecular involved in EMT. 4. HS could inhibit AA-induced overexpression of α-SMA, TGF-β1 and snail. HS also up-regulates expression of CK-18. 5. GSK-3β could mediate degradation of snail through phosphorylation of snail. SB415286 up-regulates of expression of snail through inhibiting GSK-3. Compared with AA+HS group, the protective effects of HS on AA stimulation were blocked in AA+HS+ SB415286 group.

**Conclusions:** HS could inhibit AA-induced renal tubular epithelial-myofibroblast transition and interstitial fibrosis. Such effect maybe exert through inhibition of Snail.

*Funding:* Government Support - Non-U.S.

**PUB028**

**Oxidized High-Density Lipoprotein Impairs Function of Human Proximal Tubular Cells via CD36** Changlin Mei, Xiang Gao. *Kidney Institute of PLA, Changzheng Hospital, Second Military Medical Univ, Shanghai, China.*

**Background:** The participation of tubular lesions in the progression of chronic kidney disease (CKD) is widely recognized, in which inflammation plays an important role. Previous studies reported the presence of oxidatively modified high-density lipoprotein (HDL) in the serum of CKD patients. Unlike native HDL, oxidized HDL is thought to be an adverse factor and may increase the production of inflammatory in chronic diseases, but the effect of oxidized HDL on renal tubular cell remains unclear.

**Methods:** Human proximal tubular cell line (HK-2) was cultured and stimulated with different concentrations of oxidized HDL (0-50 μg/mL) in the absence and presence of CD36 siRNA. Intracellular reactive oxygen species (ROS) levels, proinflammatory factors, apoptosis and migration in HK-2 cells were assayed. The expression of Src (tyr527 and tyr416), MAPK protein (p38, JNK and ERK) and NF-κB activity were also detected.

**Results:** Oxidized HDL enhanced ROS production and upregulated expression of proinflammatory factors including TNF-α, MCP-1 and RANTES in HK-2 cells dose-dependently. Incubation with oxidized HDL also increased apoptosis of HK-2 cells and reduced the ability of migration in a dose-dependent manner. Src-family kinase tyr527, MAPK and NF-κB were activated post oxidized HDL stimulation. All oxidized HDL-mediated effects on HK-2 cells could be significantly attenuated by pretreatment with CD36 siRNA transfection.

**Conclusions:** These findings suggest that oxidized HDL enhances proinflammatory properties and impairs HK-2 cells function largely via scavenger receptor CD36, which might be one of the major players responsible for the progression of chronic kidney disease.

*Funding:* Government Support - Non-U.S.

**PUB029**

**Inflammation Contributes to Podocyte Injuries and Exacerbates the Progression of Diabetic Nephropathy via HIPK2 Signal Pathway** Kun Ling Ma,[1] Yang Zhang,[1] Chang Xian Wang,[2] Jing Liu,[1] Wu Yu,[1] Bi-Cheng Liu.[1] *[1]Institute of Nephrology, Southeast Univ School of Medicine, Nanjing, China; [2]Infection Mamagement Dept, Southeast Univ School of Medicine, Nanjing, China.*

**Background:** Inflammation plays central roles in the progression of diabetic nephropathy. Homeo-domain interacting protein kinase 2 (HIPK2) is a conserved serine/threonine nuclear kinase. In response to infection, DNA damage, and oxidative stress, HIPK2 is activated and plays a crucial role in renal injuries. This study aimed to investigate whether inflammation exacerbates the progression of diabetic nephrology (DN) via HIPK2 signal pathway.

**Methods:** Twenty male db/db mice were randomly divided into two groups: Control (distilled water injection) or inflamed group (10% casein injection). Body weight and 24-hour urinary protein were measured every week. The plasma levels of serum amyloid A (SAA) and tumor necrotic factor-a (TNF-a) were checked by enzyme-linked immunosorbent assay. The morphological changes of renal pathology and ultra-microstructures were checked by pathological staining and electron microscope. Immunofluorescent staining and Western blotting were used to check the expression of podocyte specific proteins, inflammatory cytokines, and HIPK2 pathway related protein in kidneys.

**Results:** The 24-hour urinary protein, plasma levels of SAA and TNF-α, as well as the protein expression of inflammatory cytokines in kidneys were significantly increased in inflamed group compared to the control. Moreover, there were more significant mesangial expansion, collagen accumulation, and foot process effacement in inflamed group compared to the control. Further analysis showed that inflammation markedly decreased the expression of wilims tumor-1 and nephrin, which were specific biomarkers of podocyte, suggesting accelerating injuries of podocyte induced by inflammation. Interestingly, inflammation increased the expression of HIPK2 and decreased the expression of SIAH1, which was closely associated with accelerated renal injuries.

**Conclusions:** chronic inflammation contributes to podocyte injuries and exacerbates the progression of DN via the activation of HIPK2 signal pathway.

**PUB030**

**CCN3 Inhibits TGFβ1-Induced Non-Canonical Smad Signalling in Human Podocytes** Tarunkumar H. Madne,[1] Iain Macphee,[2] Mysore Keshavmurthy Phanish,[1] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]St. Georges, Univ of London, London, United Kingdom.*

**Background:** The pleiotropic growth factor TGFβ1 mediates some of its diverse effects by regulation of multiple signaling cascades as well as the recruitment of other growth factors such as CCN2/CTGF. Recently CCN3 has been identified as putative antagonist to CCN2/CTGF. ]In our work investigating the mechanism of TGFβ's effect on podocytes we have investigated novel signaling pathways activated by TGFβ1 in human podocytes in culture and their regulation by CCN3.

**Methods:** Experiments were conducted on conditionally immortalised human podocytes incubated with TGFβ1 (1.25 & 2.5 ng/mL). Western blotting analysis was performed to detect different levels of Smad and p38 expression and activation. Cells were co-incubated +/- CCN3 (360ng/mL).

**Results:** TGFβ1 induced canonical Smad signaling significantly phosphorylating Smad 2 & 3 at 1h ($p<0.05$). As we have previously reported p38 MAP kinase was also significantly activated by TGFβ1 [ASN 2012]. In addition significant phosphorylation of Smad1/5/8 was also observed ($p<0.05$). Following reports of a requirement for CCN2/CTGF for TGFβ1 activation of Smad1, we co-incubated the cells with the putative CCN2/CTGF antagonist CCN3. Incubation with CCN3 had no effect on activation of Smad2, 3 or p38 but significantly reduced Smad1/5/8 activation ($p<0.05$).

**Conclusions:** To our knowledge this is the first report of the activation of Smad1/5/8 in podocytes by TGFβ1. Our work with CCN3 suggests that endogenous CCN2/CTGF is expressed by podocytes and can mediate the activation of Smad1/5/8. Previous research demonstrated that Smad 1/5/8 activation can regulate collagen IV promoter activity. Hence, we are currently investigating the role of CCN2/CTGF mediated collagen IV expression in podocytes and propose this as a mechanism for glomerular basement membrane thickening observed in renal disease.

*Funding:* Government Support - Non-U.S.

**PUB031**

**Molecular Mechanisms of TNF-Receptor 1 Mediated Signaling Pathways in Human Peritoneal Mesothelial Cells** Julia Ranzinger,[1] Willie Lüdemann,[1] Danijela Heide,[1] Peter E. Scheurich,[2] Martin G. Zeier,[1] Vedat Schwenger.[1] *[1]Nephrology, Univ of Heidelberg, Heidelberg, Germany; [2]Institute of Cell Biology and Immunology, Univ of Stuttgart, Stuttgart, Germany.*

**Background:** Cellular apoptosis is involved in several physiological processes such as immunological responses and can be induced by the activation of death receptors including tumor necrosis factor receptor 1 (TNFR1). Human peritoneal mesothelial cells (HPMCs) play a central role in the context of inflammatory responses during peritoneal dialysis (PD) caused peritonitis. Although TNFR1 is expressed on HPMCs, TNF-induced apoptosis is inhibited in these cells. The aim of our present study is to investigate the molecular mechanisms of TNF-signaling in HPMCs.

**Methods:** HPMCs were isolated from omentum of healthy donors and dialysis fluid from patients undergoing PD. Flow cytometry was applied to determine the expression of TNFR1 on HPMCS from healthy donors in absence or presence of TNF and dialysis solution in comparison to the TNFR1 expression on cells from PD-patients. To investigate TNFR1-mediated signaling, HPMCs were either treated with dialysis solution or TNF and expression patterns of proteins involved in the TNFR1-signaling pathway (NFĸB, IĸBα, Caspase-3, $cFLIP_L$) were assessed by Western Blot and Immunocytochemistry.

**Results:** We show that incubation of HPMCs with dialysis solution leads to a significant up-regulation of TNFR1 on the cell surface. This correlates with elevated TNFR1-numbers on HPMCs from PD-patients. Concerning the molecular mechanisms of TNFR1-signaling, Western Blot experiments and Immunocytochemistry showed that incubation of HPMCs with dialysis solution results in increased levels of IĸBα protein. Furthermore, dialysis solution caused a reduction of $cFLIP_L$ protein amounts in the cells. In contrast, stimulation of the cells with TNF led to an increase in phosphorylated IĸBα and Procaspase-3.

**Conclusions:** This study shows that dialysis solution used in PD-treatment significantly up-regulates TNFR1 in HPMCs. Moreover, we demonstrate that the presence of dialysis solution affects TNF signaling at the proapoptotic signaling pathway by enhancement of IĸBα and reduction of $cFLIP_L$.

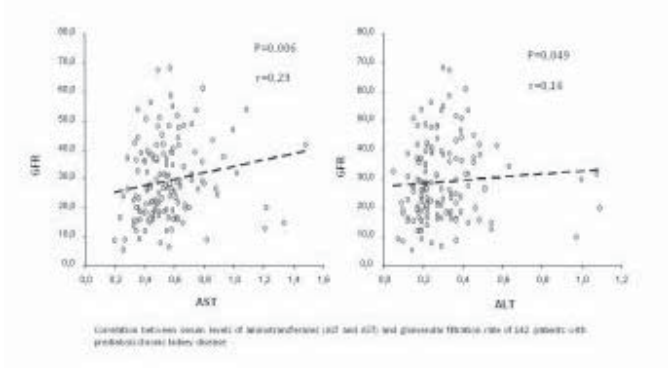
**PUB033**

**Evaluation of Serum Aminotransferases in Patients with Predialysis Chronic Kidney Disease** Luis H.B.C. Sette, Edmundo Pessoa Lopes. *Universidade Federal de Pernambuco.*

**Background:** Pacientes with chronic kidney disease (CKD) on hemodialysis have lower serum levels of aminotransferase than those with normal renal function and such a profile may compromise the diagnosis, clinical management and treatment of patients with CKD and liver disease.However, it is not known the behavior of these enzymes in patients with CKD and its relations with the GFR or the stages of CKD.

**Methods:** From September 2011 to December 2012, was held an observational and cross-sectional study in patients with chronic kidney disease (CKD) attended at the ambulatory of Nephrology at the Hospital of Clinics of the Federal University of Pernambuco (HC-UFPE).Renal function was assessed by estimated GFR using the formula proposed by Cockcroft-Gault. Patients were classified into stages of CKD, according to the definition of the Kidney Disease Outcomes Quality Initiative (K / DOQI).

**Results:** We evaluated 142 patients with CKD, 72 male (52.1%) with a mean age of 64 ± 16 years. The mean GFR was 29.1 ± 13 mL/min/1, 73m2. Patients showed the following distribution according to the stages of CKD: 15 (10.5%) patients stage 5; 70 (49.3%) stage 4, 54 stage 3 (38%) and 3 (2.1%) patients stage 2. The mean serum ALT and AST reduces in proportion to the decrease of GFR ($p = 0.006$ and $p = 0.049$, respectively).




Serum ALT levels were higher in the 57 patients with stages 2 and 3 compared to 85 patients at stages 4 and 5 ($p < 0.03$).

| | CHRONIC KIDNEY DISEASE | | |
|---|---|---|---|
| AMINOTRANSFERASES[a] | STAGES 2-3 | STAGES 4-5 | p-value |
| AST / ULN* | 0.546 (0.454; 0.632) | 0.494 (0.411; 0.603) | **0.066** |
| ALT / ULN* | 0.273 (0.212; 0.394) | 0.219(0.181; 0.341) | **0.030** |
| [a] Mann-Whitney test * Upper limite of normal | | | |

**Conclusions:** Serum ALT and AST correlated with GFR and the reduction of these enzime levels were in accordance with the progression of CKD.

## PUB034

**NGAL and NT-proBNP Levels in Type II Diabetic Patients with Macroproteinuria** Hulya Taskapan,[1] Mehmet Cagatay Taskapan,[2] Özkan Ulutas.[1] *[1]Nephrology, Inonu Univ Medical Faculty, Malatya, Turkey; [2]Biochemistry, Inonu Univ Medical Faculty, Malatya, Turkey.*

**Background:** In patients with heart failure plasma N terminal-pro brain natriuretic peptide (NT-proBNP) levels are correlated to urine Neutrophil gelatinase-associated lipocalin (NGAL) levels. We prospectively evaluated the relationship among glomerular filtration rate (eGFR), urine albumin to creatinine ratio, urine and serum NGAL levels and NT-proBNP in 20 tip 2 diabetic patients with macroalbuminuria at 4-month intervals.

**Methods:** In 20 diabetic patients with macroproteinuria, serum creatinine, albumine, N terminal-pro brain natriuretic peptide (NT-proBNP), serum and urine NGAL levels, urine microalbumin, urine creatinin were measured at the baseline, at the 4th month and at the 8th month. The baseline results of the patients were compared with those of 20 healthy, age and sex matched controls.

**Results:** Diabetic patients had higher urine and serum NGAL, NT-proBNP levels, and lower eGFR. The eGFRs at the baseline, the 4th and the 8th month were 29,6±12,0, 27,8±13,7 and 22,9±10,4mL/min/1.73 $m^2$, respectively. No significant change in urine NGAL levels was detected (p>0,05), wherease there were significantly increases in NTproBNP, serum NGAL levels, and urine albumin to creatinine ratio and significantly decrease in eGFR as the study progressed. (p<0,05). The baseline and the 4th month urine albumin to creatine ratio were positively correlated to NT-proBNP levels measured at the same periods (r:0,451; p:0,046; r:0,489; p:0,029 respectively). In all measurements urine albumin to creatine ratio were negatively correlated to serum albumin levels measured at the same periods (r:-0,792; p:0,000; r:-0,716; p:0,000; r:-0,531; p:0,016 respectively). The 4th month microalbumin to urine creatine ratio was positively correlated with serum NGAL (r:0,478; p:0,033). The 8th month eGFR was negatively associated with urine NGAL (r:-0,471; p:0,039). None of eGFR measurements was correlated with NT-proBNP (p>0,05). Neither serum NGAL nor urinary NGAL levels is associated with NT-proBNP levels(p>0,05).

**Conclusions:** Our findings showed an association between serum NT-proBNP levels and proteinuria in diabetic patients with macroalbuminuria but not with NGAL levels.

## PUB035

**Iron Management in Non Dialysis CKD Patients: The Italian Multicenter Prospective Study in Renal Clinics** Roberto Minutolo,[1] Francesco Locatelli,[2] Giuseppe Conte,[1] Luca De Nicola.[1] *[1]Nephrology, Second Univ of Naples, Naples; [2]A.Manzoni Hospital, Lecco, Italy; [3]For the RECORD-IT Study Group.*

**Background:** Knowledge on iron management in non-dialysis CKD (ND-CKD) patients regularly seen in renal clinics is scarce.

**Methods:** We prospectively evaluated iron management in two visits, performed 6 months apart, in 734 ND-CKD stage 3b-5 patients followed in 19 renal clinics from ≥6 months. Iron deficiency (ID) was defined as TSAT<20% and/or ferritin<100 ng/mL. Endpoints were the change of ID prevalence between basal and month-6 visit (primary) and the prevalence of clinical inertia to iron therapy, that is, the lack of iron prescription despite ID (secondary).

**Results:** Age was 69±13 yrs, GFR 28±10 mL/min/1.73$m^2$ and male gender, diabetes and prior CVD accounted for 57%, 30% and 30%, respectively. CRP [3.0 mg/L (0.5-3.8)], TSAT [24.3±9.5%] ferritin [102 ng/mL (54-193)] did not change at month 6. Table shows changes between the two visits by iron status at baseline (§P<0.05 vs ID, *P<0.05 vs basal).

| | No ID (N=292) | | ID (N=442) | |
|---|---|---|---|---|
| | Basal | Month 6 | Basal | Month 6 |
| Hb (g/dL) | 12.6±1.7§ | 12.5±1.7§ | 12.2±1.5 | 12.2±1.5 |
| Hb<11 g/dL (%) | 16.1 | 18.2 | 19.7 | 20.6 |
| TSAT<20% (%) | 0.0 | 23.3 | 55.9 | 48.0* |
| Ferritin<100 (%) | 0.0 | 12.7 | 81.1 | 69.3* |
| ESA use (%) | 29.1 | 33.2* | 26.9 | 30.1* |
| Iron use (%) | 14.0 | 16.8 | 26.2 | 29.4 |
| Clinical inertia to iron therapy (% of ID pts) | 0.0 | 80.0 | 75.7 | 89.6 |

ID was prevalent at baseline and month 6 (60% and 61%) and was associated to lower Hb (0.4 and 0.5 g/dL, respectively).While ESA use increased at month 6, iron supplementation (91±41 mg/day, oral in 97%) did not change in either group. Among ID patients only a minority received iron therapy at either visit despite low rates of intolerance (13%); consequently, 81% of patients had low TSAT and/or low ferritin at month 6. Clinical inertia to iron therapy was detected in 80% of patients developing ID and involved about 4 out of 5 ID patients at either visit.

**Conclusions:** Therefore, abating clinical inertia for iron therapy likely represents a main area of improvement in the nephrology management of renal anemia.

## PUB036

**25(OH)Vitamin D and Calcitriol: Dissimilar Actions on Left Ventricular Hypertrophy** Bruno C. Silva, Rosa M.A. Moyses, Manuel C. Castro, Rosilene M. Elias. *Nephrology Div, Univ of Sao Paulo, São Paulo, Brazil.*

**Background:** Hypovitaminosis D has been associated to cardiovascular (CV) morbidity and mortality both in general population and in patients with chronic kidney disease. Observational studies have shown that receiving any vitamin D (25(OH)vitamin D or calcitriol) reduced CV risk. Whether the effect of 25(OH)vitamin D (VitD) is independent of calcitriol is still controversial. The aim of this study was to evaluate the role of VitD levels on left ventricular hypertrophy (LVH) in patients on hemodialysis.

**Methods:** We performed a cross-sectional study, including 46 patients who underwent conventional chronic hemodialysis program at a single center facility (University of São Paulo). Clinical, laboratorial, demographic, and echocardiographic data were collected. LVH was categorized as LVH index (LVHI) > or < than 125g/$m^2$. Comparison between these 2 groups was performed using unpaired t tests. Multivariate analysis was also undertaken with LVHI as dependent variable.

**Results:** Of 46 patients, 26 were male, mean age of 47±18 years and dialysis vintage of 8.7 years (1 to 30 years). Mean hemoglobin levels were 11.3±1.5 g/dl and VitD levels of 26.1±12.4 ng/ml (82.6% had levels below 30ng/ml, and 41.6% below 15ng/ml). Nineteen patients (41.3%) were using calcitriol. In univariate analysis, VitD was the only significant variable associated to LVH, showing that levels were lower in patients with LVMI >125gm$^2$ (22.1±13.3 vs 29.1±10.8ng/ml, p=0.046). In backward stepwise logistic regression, VitD deficiency (<15ng/ml) was independently associated to LVH, when adjusting for calcitriol use, hemoglobin levels, and gender (HR 73.06, p=0.006).

| Variable | B | Sig. | Exp(B) |
|---|---|---|---|
| Gender | -0.319 | 0.678 | 0.727 |
| Hemoglobin | 0.485 | 0.086 | 1.62 |
| Calcitriol use | 1.934 | 0.114 | 6.92 |
| 25(OH) vitamin D deficiency | 4.291 | 0.006 | 73.06 |

Table 1. Logistic regression: dependent variable: LVHI

**Conclusions:** 25(OH)vitamin D deficiency is associated to reduced LVH, and its action seems to be independent of active vitamin D3 (calcitriol). Our results are supportive of prescribing VitD to patients on hemodialysis, even for those patients already taking calcitriol.

## PUB037

**The Efficacy and Usefulness of Darbepoetin Alpha in Patients with Chronic Kidney Disease: An Observational Clinical Trial** Noriaki Iino, Junichiro J. Kazama, Ichiei Narita. *Clinical Nephrology and Rheumatology, Niigata Graduate School of Medical and Dental Sciences, Niigata, Japan.*

**Background:** Conventional treatment with epoetin to manage anemia in chronic kidney disease, (CKD) is sometimes hard to reach target hemoglobin level defined by KDIGO guidelines. The aim of this study was to confirm the effectiveness of darbepoetin alpha (DA) on hemoglobin control in patients with CKD with preserved kidney function and to examine the relationship between anemia correction and occurrence of adverse events (i.e., cardiovascular disease, progressive decline in GFR).

**Methods:** An observational study was conducted in 202 patients with CKD who had anemia of hemoglobin levels below 11 g/dl. Patients with a malignancy, or an active gastrointestinal hemorrhage were excluded. Patients received darbepoetin alpha once every 4 weeks for 36 months intravenously or subcutaneously. Physicians adjusted a dose of DA based on manufacturer protocol for hemoglobin target (11-13 g/dl).

**Results:** At 3 months after starting darbepoetin alpha, the mean hemoglobin level increased from 9.6 g/dl to 10.4 g/dl. During follow up period, mean hemoglobin level maintained within target hemoglobin levels defined by KDIGO. To maintain target hemoglobin levels, physicians administered 80 to 100 μg of DA to the patients once every 4 weeks. During the study period, blood pressure remained relatively stable and good control. The mean change in hemoglobin from baseline at 3 months after initiating the study was significantly different between ESA-naive patients and those with previous epoetin-treatment. In the ESA-naive group, mean hemoglobin level rapidly raised to target levels and was maintained during the study period. On the other hand, mean hemoglobin levels slowly increased in previously epoetin-treated group. After 12 months of the investigation, differences of mean hemoglobin levels in the two groups finally diminished. However, to maintain same levels of hemoglobin, requirement dose of DA was significantly different between them.

**Conclusions:** Darbepoetin alpha once every 4 weeks successfully maintained stable hemoglobin levels in patients with CKD with preserved renal function,for prolong period.

*Funding:* Government Support - Non-U.S.

**PUB038**

**Prescribing in Renal Impairment within the Acute Hospital Setting – A Prospective, Observational Study** Michelle M. O'Shaughnessy,[1] Niamh Allen,[1] John O'Regan,[1] Edwina Louise Payne-danson,[2,3] Lise Mentré,[2,3] Dawn A. Davin,[2] Peter J. Lavin,[1] Tamasine C. Grimes.[2,3] *[1]Dept of Nephrology, Trinity Health Kidney Center, Tallaght Hospital, Dublin, Ireland; [2]Dept of Pharmacy, Tallaght Hospital, Dublin, Ireland; [3]Trinity College Univ, Dublin, Ireland.*

**Background:** Patients with renal impairment (RI) are susceptible to adverse drug reactions. We studied the nature and frequency of potentially inappropriate prescribing (PIP) amongst hospitalised patients with RI. We examined trends in, and risk factors for, PIP and measured agreement between renal prescribing guidelines.

**Methods:** This was a single-centre, prospective, observational study conducted between October 2012 and March 2013. Hospitalized patients with RI were stratified according to estimated GFR and randomly selected for study inclusion. Prescriptions were reviewed at three time-points (pre-admission, admission, discharge) and according to two renal prescribing guidelines, the British National Formulary (BNF) and the Renal Drug Handbook (RDH). Renal Risk Drugs (RRDs) and PIP were identified. Data were analysed using SPSS v20.

**Results:** 173 patients were studied, mean age 71, 52% male. During admission, 2,096 medications including 995 RRDs were prescribed. 93 cases of PIP by RDH (prevalence 39.8%) and 204 by BNF (prevalence 62.2%) were identified. Agreement between guidelines was fair, Cohen's k-coefficient 0.4, $p<0.05$. PIP prevalence increased as renal function deteriorated by BNF, but was similar across renal sub-groups by RDH. For patients with chronic kidney disease, PIP prevalence was lower prior to admission (26.7%) and at discharge (21.8%) than during admission (39.7%). Moderate RI was less commonly recognized and reported than severe RI. Pain, gout and diabetes were independent risk factors for PIP, whereas severity of RI, recognition of RI by treating team, and clinical pharmacy input were not.

**Conclusions:** PIP is common amongst hospitalized patients with RI, although prevalence rates vary depending upon severity of RI, referenced prescribing guideline, timing of assessment and patient co-morbidity. In particular, the impact of disagreement between renal prescribing guidelines upon clinical outcomes requires further study.

**PUB039**

**Anemia Management Practice in Chronic Kidney Disease in Eastern Europe** Andrzej Wiecek,[1] Lyudmila Biriukova,[2] Ivan Rychlik,[3] Gabriel Mircescu,[4] Jacek Lange,[5] Daniell Mitchell.[5] *[1]Medical Univ Silesia, Katowice, Poland; [2]RNRMU, Moscow, Russian Federation; [3]Fresenius Medical Care, Prague, Czech Republic; [4]Medical Univ, Bucharest, Romania; [5]Vifor Pharma, Glattbrugg, Switzerland.*

**Background:** Anemia and iron deficiency (ID) are frequent in chronic kidney disease (CKD) and should be managed with an erythropoiesis-stimulating agent (ESA) and iron. This study evaluated current practice in diagnosis and treatment of CKD-associated anemia in Eastern Europe.

**Methods:** Nephrologists from Bulgaria, Czech Republic, Poland, Romania, Russia, Slovakia, and Slovenia were surveyed (Jan-Feb 2012) for the use of diagnostic tests and therapies in their last 5 CKD patients (stage 3 or 4) that were treated for anemia in the prior 6 months. Data shown for all patients and range across countries.

**Results:** 163 nephrologists reported data of 855 anemic CKD patients (stage 3: 47% [26-59%], female: 52% [44-57%], mean age: 58y [52-65y]). At anemia diagnosis, 97% (94-100%) were tested for hemoglobin (Hb); 48% (23-71%) for serum ferritin and 46% (26-69%) for transferrin saturation (TSAT). Median Hb, ferritin and TSAT were 100g/L (88-110g/L), 84µg/L (12-102µg/L) and 19% (15-24%), respectively. Moderate-to-severe anemia (Hb ≤100g/L) was diagnosed in 67% (42-85%) of patients; 11% (1-36%) presented with Hb <80g/L. Insufficient iron for erythropoiesis (TSAT <20%) was seen in 65% (37-84%) and depleted iron stores (ferritin <100µg/L) in 56% (43-100%) of tested patients. Within 12 months prior the survey, 86% (80-94%) received iron treatment. Despite ESA use in 58% (37-87%), only 27% (9-53%) received intravenous iron. Notably, 17% of patients in the Czech Republic and 14% in Russia received intramuscular iron. Blood transfusions were given to 7% (1-11%) of patients.

**Conclusions:** Current anemia and iron status management in CKD patients varies substantially across Eastern Europe and is often not in line with recommendations. The high proportion of moderate-to-severe anemia and ID suggest insufficient iron status management. Similar findings in Western Europe suggest that improving awareness and implementation of guidelines for management of CKD-related anemia and ID is a pan-European if not a global need.

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**PUB041**

**Posterior Reversible Encephalopathy Syndrome in Patients with Chronic Kidney Disease** Marta Pereira, Maria Alice Gonçalves Fortes, Hugo Mário Silva, Natacha Rodrigues, Estela Nogueira, Antonio Gomes da Costa. *Nephrology and Kidney Transplantation, Hospital de Santa Maria, Lisbon, Portugal.*

**Background:** Posterior reversible encephalopathy syndrome (PRES) is a clinicoradiologic entity characterized by neurologic symptoms and typical neuroimaging transient changes. PRES is consequence of an endothelial dysfunction of the cerebrovascular barrier. It is associated with many conditions such as hypertension and uremia, and patients with chronic kidney disease (CKD) may have an increased risk for this syndrome.

**Methods:** We report three cases of PRES in patients with CKD on renal replacement therapy.

**Results:** The patients were three women, had a mean age of 20 years, two were in peritoneal dialysis and one in hemodialysis. The patient didn't have an autoimmune disease and weren't receiving immunosuppression. The presenting symptoms were bilateral blindness, loss of consciousness and seizures. All cases progressed to generalized tonic-clonic seizures and one patient was in status epilepticus at admission. All patients had abrupt increases in blood pressure. Hypertension was consequence of anti-hypertensive therapy withdraw in hemodialysis patient. In patients on peritoneal dialysis coexisted signs of failure of the technique with significant hypervolaemia. Brain magnetic resonance imaging (MRI) was performed in all patients and showed extensive bilateral white-matter abnormalities suggestive of edema, not only in the posterior regions of the cerebral hemispheres, but also in other cerebral areas, in the brain stem, and in the cerebellum. The patients were treated with hemodialysis, antihypertensive and antiepileptic drugs. Resolution of seizures occurred in three to four hours and complete neurologic symptoms were resolved in average after five days. In two patients the follow-up MRI revealed imagiologic significant improvement.

**Conclusions:** These cases emphasize the role of hypertension and hypervolaemia as inducing mechanisms of PRES and stress the importance of considering this syndrome in the differential diagnosis of seizures in CKD patients. This is essential since the early diagnosis and treatment may allow complete resolution of neurologic changes, without persistent cognitive sequelae.

**PUB042**

**Low Formic Acid Maybe Involved with Decreased ATP Production in ESRD Patients** Emiko Miyazawa,[1] Hiroyuki Terawaki,[2] Sadayoshi Ito,[1] Masaaki Nakayama.[2] *[1]Dept of Nephrology, Endocrinology, and Vascular Medicine, Tohoku Univ, Sendai, Japan; [2]Dept of Nephrology and Hypertension, Fukushima Medical Univ, Fukushima, Japan.*

**Background:** Formic acid (FA) is an essential substance for de novo synthesis of purine nucleotides. FA is combined with folic acid and incorporated into purine synthesis pathway as formyl-tetrahydrofolate. FA is used as a constituent of C2 and C8 of purine bodies. We previously reported that endogenous FA exists in human plasma. But only a limited number of reports examined about level of FA in ESRD and its physiological role.

**Methods:** Patients with ESRD (CKD 5 and CKD 5D; n=111) and predialysis (CKD 3a-4; n=51) were subjected for analysis of plasma FA and tryptophan (precursor of FA). FA and tryptophan were measured by high-performance liquid chromatography (HPLC). Adenosine triphosphate (ATP) levels were measured by luciferase luminescence method in (a) whole blood, (b) mononuclear cell, and (c) neutrophil after incubation for three hours with the addition of FA, and examined differential rates of increase.

**Results:** The levels of FA (mean ± SD) were 3.04 ± 1.09 mg/L in ESRD, 1.57 ± 1.10 mg/L in predialysis CKD, and 3.71 ± 1.16 mg/L in control ($P<0.05$). Rates of ATP change after incubation for 3 hours with the addition of FA were (a) 2.52 times, (b) 0.97 times, (c) 1.16 times the controls. Significant increases were seen in whole blood and neutrophil ($P=0.011$ and $P=0.0007$respectively), but ATP was not changed in mononuclear cell by t-test.

**Conclusions:** Taken together the result that plasma FA was lower in ESRD, and ATP level was significantly increased when cultured with the addition of FA to isolated neutrophil in healthy subject. It suggests that FA enhances not only purine metabolite but also ATP production. Lower FA maybe involved with the decreased ATP production in ESRD. The clinical relevance of this pathology needs to be addressed.

**PUB043**

**Clinical Utility of Reticulocyte Hemoglobin, a Parameter Obtained on High-End Hematology Analyzers, in the Diagnosis and Assessment of Functional Iron Deficiency in Predialysis Chronic Kidney Disease** Alan F. Almeida,[1] P. Gudsoorkar,[1] J. Kothari,[1] R. Sirsat,[1] S. Khodaiji,[2] K. Sehgal.[2] *[1]Nephrology, P.D.Hinduja Hospital, Mumbai, India; [2]Hematology, P.D.Hinduja Hospital, India.*

**Background:** Functional iron deficiency (FID) can complicate the management of the anemia of CKD. This study evaluates the utility of reticulocyte hemoglobin(Ret-He) as a marker for the assessment of FID.

**Methods:** CKD predialysis patients (Stage 3-5) with hemoglobin <11g%, transferrin saturation ≥20 % and Serum Ferritin >100ng/ml were included. They received intravenously 500 mg of ferric carboxymaltose. Soluble transferrin receptor assay (sTfr), Ret-He (Sysmex XE-2100), Ultrasensitive CRP, serum folate vitamin B12 measured before and Hb, Ret-He and sTfr was checked 1 month after IV iron therapy.

**Results:** 180 patients with CKD (stage 3-5) were screened. 47 enrolled in the study [19(40%) males, 28(60%) females] had a mean age of 46.6 years (S.D 13.7yrs).The most common renal diagnosis was CTID(36%) followed by CGN(32%). A majority were CKD stage 4(45%),followed by stage 5(38%). 4 weeks following iron infusion, a statistically significant increment in Hb ($p<0.001$), Ret-He increased from 23.58 (pre-infusion) to 24.55 (post-infusion)($p< 0.001$). sTfr assay showed a significant decrease with iron treatment ($p<0.001$). Further analysis of 7 patients with definite FID (confirmed by generating Thomas plot) showed a significant improvement in Hemoglobin and Ret-He.

**Conclusions:** Ret-He is a useful test to assess the status of hemoglobinization of RBC. A combination of Ret-He, Sr ferritin and sTfr assay along with the Thomas plot can help categorize the patients as FID. After categorization, the patients can be subjected to intervention in the form of oral iron / IV iron / Erythropoetin alone / Erythropoetin plus IV iron. The Thomas plot will help in the diagnosis and also the follow up of patients of anemia of renal disease. Whether Ret-He alone or in combination with other parameters, like sTfr assay and Sr ferritin, will require further studies with larger sample sizes.

*Funding:* Private Foundation Support

## PUB044

### The Activation of Mineralocorticoid Receptor in Adipose Tissues in CKD Rat Impaired Glucose Metabolism through Tissue ADMA Increase

Kozi Hosoya, Shu Wakino, Ayumi Yoshifuji, Hitoshi Minakuchi, Kazuhiro Hasegawa, Naoki Washida, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio Univ School of Medicine, Tokyo, Japan.*

**Background:** Recent studies revealed the influence of mineralocorticoid receptor (MR) activation by aldosterone (Aldo) and asymmetric dimethylarginine (ADMA) on the glucose metabolism.We examined whether the activation of MR and ADMA affected insulin resistance (IR) in chronic kidney disease (CKD).

**Methods:** We rendered SD rats renal insufficiency with 5/6th nephrectomy (Nx group) and were treated with MR blockade spironolactone (Spi group). The results of oral glucose tolerance test (OGTT) and intraperitoneal insulin tolerance test (IP-ITT) were compared among the groups. Tissue ADMA levels were measured by ELISA. In *in vitro* analysis, 3T3-L1 fibroblasts were differentiated with/without the addition of Spi.

**Results:** In OGTT, rats in Nx showed impaired glucose tolerance. In ITT, the decline in blood glucose was blunted. These changes were partially reversed in Spi. The phosphor-Akt level after insulin stimulation was attenuated in adipose tissue, which were restored in Spi. These changes were not observed in muscle and liver tissue. MR expression in the nuclear fraction was increased in the adipose tissues in Nx. The expression of SGK1 (Serum/glucocorticoid regulated kinase 1), MR target molecule was upregulated in Nx. These increase were blocked in Spi. The adipose tissue Aldo and ADMA levels were increased in Nx that were reduced in Spi. Both isoforms of DDAH1 and 2, ADMA-degrading enzymes, were downregulated in adipose tissues, which were reversed in Spi. In *in vitro* analysis, Aldo downregulated DDAH1 and DDAH2, which were reversed by the pretreatment with Spi. ADMA inhibited the increase in phospho-Akt levels after insulin stimulation in adipocytes.

**Conclusions:** In CKD, the activation of Aldo/MR pathway in the adipose tissue affected at least in part the insulin resistant state. The activation of adipose tissue MR downregulated DDAH and increased the levels of ADMA, which blocked the insulin signaling.

## PUB045

### Inflammatory Cytokine TNFalpha/IL-6 Related to Muscle Wasting and Up-Regulated Expression of Myostatin and Atrogin-1

Huiling Wang. *Nephrology Div, Jinmin Hospital, Shanghai, China.*

**Background:** Although plenty of research confirmed elevated systemic inflammation related to malnutrition in advanced chronic kidney disease (CKD), few clinic research revealed the mechanism of inflammatory cytokines impacted muscle wasting in patients with CKD. This study investigates the serum cytokines level and muscle wasting of patients with underlying maintenance hemodialysis (HD) and the expression of genes involved in the regulation of muscle mass and that for the inflammatory cytokine TNF-alpha/IL-6 in muscle biopsies.

**Methods:** HD patients were selected according to the enrolled standard as HD group, 30 healthy adults with gender and age matched as control(Ctl). We collected the biochemical and anthropometry data, measured inflammatory cytokine such as C-reactive protein( CRP), interleukin-6(IL-6) and tumor necrosis factor alpha(TNF alpha) by ELISA with their serum samples. Then we got muscle sample from 8 HD patients and 6non-HD patients, observed the muscle histology by light scope after HE staining, and analyzed the transcriptional levels of myostatin and Atrogin-1, TNFalpha; and IL-6 by real-time PCR.

**Results:** 42 patients enrolled in the study. The HD patients kept low level of body weight (68.8+-17.90Kg Ctl vs 58.16+-14.82Kg HD,P=0.003)and body mass index(BMI 23.56+-1.69Kg/m2 Ctl vs 21.52+-178Kg/m2 HD, P=0.026) ; and the plasma albumin showed lower in HD (45.47+-1.91g/L Ctl vs 42.35+-3.62g/L HD, P=0.006) though still in normal limits. The serum inflammation markers such as high sensitive C-protein, TNF alpha; and IL-6 showed elevated in HD compared with Ctl,which showed as hs-CRP1.32(0.11-10.21)mg/L Ctl vs 7.28(2.6-29.68)HD, P<0.001),TNFalpha; 154.8(74.8-298.1) pg/mL Ctl vs 92.6(220.1-1474.7)pg/mL HD, P<0.001); IL-6 1.37(0.03-6.31) Ctl pg/mL vs 2.55(0.13-28.37) pg/mL HD, P=0.004) The morphologic observation showed muscle fibers were tenuous and atrophy in an extent, the space between fibers was widen. The muscle fiber diameter was decreased compared with Ctl. We analyzed mRNA transcription of the muscle myostatin and Atrogin-1,the results showed myostatin mRNA levels was elevated 5.96 fold ; and the Atrogin-1 mRNA increased 2.43 fold respectively; and the TNFalpha;, IL-6 mRNA increased 8.37 fold and 3.36 fold respectively measured by RT-PCR.

**Conclusions:** CKD patients underlying hemodialysis present elevated inflammatory cytokines level and obviously muscle wasting. Up-regulation of TNFalpha and IL-6 expression, stimulating the transcription of myostatin and Atrogin-1, might be an important mechanism of muscle wasting in CKD.

*Funding:* Government Support - Non-U.S.

## PUB046

### Assessment of Coronary Artery Calcification in Patients of Coronary Artery Disease with and without Chronic Kidney Disease

Anil Kumar Yadav, Abhishek Kumar, Sunil Agarwal, Alpana Raizada, Shuchi Bhatt, Basu Dev Banerjee, Om Parkash Kalra. *Univ College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India.*

**Background:** Cardiovascular disease (CVD) is the main cause of morbidity and mortality in patients with chronic kidney disease (CKD). Vascular calcification, such as coronary artery calcification (CAC) is considered to be the causal link between them; however the role of Multidetector Computed Tomography (MDCT) for assessment of CAC is less well studied. In this study, we assessed CAC in patients of CAD with and without CKD using MDCT.

**Methods:** We conducted a cross sectional study, comprising of 75 patients, 25 in each of the 3 groups: Group I- CAD without CKD, Group II- CAD with CKD stage 3/4,and Group III- CAD with CKD stage 5 on maintenance hemodialysis (MHD) ≥3 months. Patients with diabetes mellitus were excluded. Assessment of CAC was done using MDCT by assigning a scoring system by Agatston as a CAC score. In addition, CAC score was correlated with calcium phosphorus product, intact parathyroid (iPTH) level, duration of CKD and duration of MHD.

**Results:** The mean CAC score was 75.05±193.35 (median 0), 163.62±399.17 (median 1) and 142.48±701.12 (median 0) in patients of Group I, II and III respectively. The CAC score was higher in patients with CKD; however, the difference was not significant ($p=0.792$). We found a positve correlation between CAC and duration of CKD in group II ($r=0.554$, $p=0.004$). Patients having CKD for ≤12 months duration had mean CAC score 24.56±65.17 and those having CKD for >12 months duration had mean CAC score 410.82±602.19. We also found a positive correlation of CAC with duration of dialysis ($r=0.605$, $p=0.001$). Patients who were on MHD for ≤12 months had mean CAC score 3.36±7.70 and those on MHD >12 months had mean CAC score 390.78±1168.99; however, in our study, there was no correlation between CAC and calcium phosphorus product and iPTH levels.

**Conclusions:** The coronary artery calcification was found to have a positive correlation with the duration of CKD and duration of maintenance hemodialysis. Further no correlation was found between CAC score and calcium phosphorus product and iPTH levels.

*Funding:* Government Support - Non-U.S.

## PUB047

### Does Impaired Renal Function Interfere with the Development of Coronary Collateral Circulation in Patients with Acute Myocardial Infarction?

Sang Heon Suh, Ha Yeon Kim, Yong Un Kang, Chang Seong Kim, Joon Seok Choi, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Depts of Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea.*

**Background:** It has been suggested that low estimated glomerular filtration rate (eGFR) was associated with poor collateral flows in patients with acute myocardial infarction (AMI). We investigated the association of impaired renal function with coronary collateral development. The association of coronary collateral circulation and impaired renal function with clinical outcomes of AMI was also examined.

**Methods:** We retrospectively analyzed 1,760 patients diagnosed with AMI between November, 2005 and March, 2009. eGFR < 60 mL/min/1.73m$^2$ was defined as impaired renal function. According to Rentrop classification, collaterals were considered present with scores ≥ 1 and were considered adequate with scores ≥ 2.

**Results:** Univariate analysis revealed eGFR was lower in patients with visible collateral circulation, while other high risk features of coronary artery disease (CAD), such as elevated hsCRP, N-terminal pro-brain natriuretic peptide, low thrombolysis in myocardial infarction (TIMI) grade before revascularization and low ejection fraction (EF) were also associated with the development of visible collateral flow. Multivariate logistic regression analysis, however, showed there is no significant association between impaired renal function and coronary collateral development, although other factors such as low TIMI grade before revascularization and low EF exerted significant impact on the development of coronary collateral vessels even after the adjustment of confounders. Cox proportional regression analysis for 24-month survival demonstrated impaired renal function, but not the development of any visible collateral, was one of the independent risk factors for all-cause mortality.

**Conclusions:** It is supposed the development of coronary collateral circulation is independent of renal function. Coronary collateral circulation might be mere a reflection of the severity of CAD rather than a guardian of myocardium against AMI.

**PUB048**

**Weekend Weight Gain in Late Stage Chronic Kidney Disease**
Steven Fishbane, Shailaja Chidella, Candice Halinski, Sofia Agoritsas. *Div of Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY.*

**Background:** Patients with late stage CKD (stages 4-5) are at high risk for volume related hospitalizations. Reduced salt and water excretion make control of oral intake important. We hypothesized that over weekends patients would have greater weight gain compared to weekdays because of a greater propensity to eat meals at restaurants, attend parties and other functions where dietary intake is difficult to manage.

**Methods:** This analysis is of patients participating in the Healthy Transitions in Late Stage Kidney Disease Program of the Janet and John Raggio Nephrology Institute of the North Shore-LIJ Heath System. Patients with CKD stages 4-5 are monitored, and one aspect is daily weights with computerized tracking of weight changes. Weight changes that reflect the weekend period (Saturday, Sunday and Monday weights) were compared to weight gain on weekdays. Patients were excluded if they did not weigh themselves more than 50% of eligible days.

**Results:** 96 patients with CKD stages 4-5 had 8,821 weights recorded during evaluation periods of 60-182 days. Most patients recorded weights on 70-90% of total possible days (44.4%), another 27.1% had weights on >90% of days and 28.5% between 50-70% of possible days. The mean weight change on weekend days was 0.14±0.16 lb/d compared to -0.10±0.11 lb/d during weekdays ($p<0.0001$). A weight gain greater than 2 pounds occurred on 10.6% of weekends (Sat-Mon) compared to 4.2% of weekdays (Tues-Fri) ($p<0.004$). A weight gain greater than 4 pounds occurred on 3.6% of weekends and 0.8% of weekdays ($P=0.004$).

**Conclusions:** We found that in late stage kidney disease (CKD stages 4-5) that weight gain on weekend days was greater than on weekdays. The risk for excessive weight gain (>2 pounds) was greater over weekends than during the week. Because fluid weight gain is poorly tolerated in late stage CKD, and may increase hospitalization risk, our findings suggest a need for further study of interventions for weekend fluid management.

*Funding:* Clinical Revenue Support

**PUB049**

**Test Battery to Assess Physical and Cognitive Function in Stage 3-4 Chronic Kidney Disease**
Kristen L. Jablonski,[1] Jamie Justice,[2] Douglas R. Seals,[2] Michel Chonchol.[1] *[1]1Div of Renal Diseases and Hypertension, Univ of Colorado Denver, Aurora, CO; [2]Dept of Integrative Physiology, Univ of Colorado Boulder, Boulder, CO.*

**Background:** Physical and cognitive function is impaired in non-dialysis dependent chronic kidney disease (CKD), but has not been well characterized using a battery of tests evaluating multiple domains in a given cohort. Furthermore, there is limited published data available regarding the utility of these tests in patients with CKD not requiring dialysis, despite wide validation in the general gerontology literature.

**Methods:** We compared 10 patients with stage 3-4 CKD (estimated glomerular filtration rate [eGFR] 29+4 mL/min/1.73 $m^2$) matched for age (59+3 years) and gender (8M/2F) to 10 healthy controls (eGFR 89+3 mL/min/1.73 $m^2$).

**Results:** Patients performed worse than controls on the 400 m walk (endurance; 196+5 vs. 308+25 seconds; $p<0.001$), timed up and go (TUG) (mobility; 5.6+0.3 vs. 9.3+0.6 seconds; $p<0.001$), rapid step test (balance; 41.2+1.3 vs. 70.1+6.2 seconds; $p<0.001$), hand-grip strength (strength; 43.6+2.2 vs. 30.5+3.2 kg; $p<0.005$), grooved pegboard (dexterity; 67.3+5.1 vs.125.9+24.3 seconds; $p<0.05$), and trailmaking test part A and B (cognitive function; A: 29.9+3.5 vs. 52.8+9.6 seconds; B: 64.4+8.2 vs. seconds 132.3+30.7; both $p<0.05$).

**Conclusions:** These results confirm physical and cognitive function impairments across multiple domains in patients with stage 3-4 CKD and establish the need for intervention strategies.

*Funding:* Other NIH Support - NIA R21 HL107105

**PUB050**

**Fibroblast Growth Factor 23 and Pulmonary Hypertension in Patients with Chronic Kidney Disease and Hemodialysis**
Farid M. Nakhoul,[1,3] Nadia Thawho, Ana Roth,[1] Farber Evgeny,[1] Aviva Peleg. *[1]Nephrology, Poriya Medical Center, Israel; [2]Diabetic Nephropathy Lab, Poriya Medical Center, Israel; [3]Faculty of Medicine, Bar Ilan Univ Galilee, Israel.*

**Background:** The pathogenesis of Pulmonary Hypertension in advanced chronic kidney disease and Hemodialysis is hypothesized to be explained by different mechanisms. A-V fistula, parathyroid hormone and hyperphosphatemia. A new player, the Fibroblast growth factor 23(FGF 23) regulates phosphorus metabolism and is a strong predictor of mortality in dialysis patients. FGF 23 is thought to be an early biomarker of disordered phosphorus metabolism in the early stages of chronic kidney disease (CKD), and can cause pulmonary hypertension propably via vascular calcification. our group had described high incidence pulmonary hypertension and increased mortality in HD patients.

**Methods:** Forty one CKD patients on hemodialysis treatment( CKD-D group), and 44 patients with CKD not on dialysis therapy and healthy group (21), were measured for total FGF-23, comparing to healthy control, by using ELISA ( Millipore, St. Charles, MI , USA ) , and arterial pulmonary pressure (PAP) by transthoracic echocardiography.

**Results:** FGF-23 was significantly higher in the CKD-D group compare to control (502.67 ± 575.93 pg/ml vs 8.58 ± 0.92 pg/ml, $p<0.0001$ (Mann-Whitney test). The CKD group had increased FGF-23, at 37.12 ± 90.633pg/ml, significantly higher than the control ($p<0.0001$). Between the CKD groups there was a significant difference ( $p<0.0001$). Both CKD groups, had significantly higher FGF23 level than the control ( $p<0.0001$).

CKD patients had a significantly lower PAP (33.281 ± 12.44 mmHg) than the CKD-D patients (39.23 ± 13.23 mmHg, $p=0.035$), creatinine (2.73 ±1.7 dl mg/dl, vs 6.8 mg/dL $p<0.0001$). Both with the same LVEDD and serum phosphate ($p=0.4$).

**Conclusions:** 1.Patients with CKD express increased levels of plasm FGF-23 compare to healthy control; 2. FGF-23 can be used as a biomarker for early CKD and high incidence of pulmonary hypertension and increased cardiovascular mortality; 3. CKD patients must be followed annualy for pulmonary hypertension by echocardiography.

*Funding:* Government Support - Non-U.S.

**PUB051**

**Barriers to Angiotensin Inhibitor Use in Advanced Chronic Kidney Disease**
Shayan Shirazian, Candace D. Grant, Joseph Mattana. *Medicine, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Angiotensin inhibition with an ACEi or ARB is recommended for the treatment of hypertension (HTN) in patients with chronic kidney disease (CKD) and proteinuria regardless of GFR or presence of diabetes (DM). In patients with advanced CKD, angiotensin inhibitors (Ais) may be more conservatively utilized due to known adverse effects. The objective of this study was to determine the frequency with which Ais are used in patients with HTN, proteinuria and stage 4 or 5 CKD and to identify reasons why they are not utilized.

**Methods:** In this cross-sectional study, electronic charts from patients with stage 4 or 5 CKD and HTN seen at an outpatient nephrology practice were reviewed. Eligible patients had moderately or severely increased albuminuria or urine protein to creatinine ratio greater than 150 mg/g at least once during the year prior to study. The frequency of AI use was determined and documented reasons for not using these medications were collected. Groups receiving and not receiving an AI were compared using chi-squared tests for categorical variables and unpaired t-tests for continuous variables.

**Results:** Two-hundred and four charts were reveiwed and 89 patients with HTN, stage 4 or 5 CKD and proteinuria were identified. Fifty-three percent had DM, 57% were male, 75.3% were white, the average age was 70 and the average estimated GFR was 20 mL/min/1.73$m^2$. Of these patients, 46 (52%) were not taking an AI. Documented reasons for not being on an AI included: hyperkalemia (15/46), AKI (9/46), cough or allergy/anaphylaxis (4/46) and no documented reason (18/46). There were no significant differences between the groups on and off an AI with regards to DM status, gender, ethnicity, age, or average estimated GFR.

**Conclusions:** In this study the frequency of AI use among patients with stage 4 or 5 CKD and proteinuria was low, with hyperkalemia and AKI being the most commonly documented reasons for non-use and for a substantial number no reason is given. These findings suggest that there may be substantial opportunities to increase AI use, and future efforts should focus on strategies for increasing tolerance to AI therapy in patients with advanced CKD rather than simply recommending their use.

**PUB052**

**Variables Associated with Increased Proteinuria in Patients with Advanced Chronic Kidney Disease**
Shayan Shirazian, Candace D. Grant, Joseph Mattana. *Medicine, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Reducing proteinuria is an important aim of therapies to slow the progression of chronic kidney disease (CKD) and typically entails aggressive use of ACEi/ARB therapy and blood pressure reduction. With advanced CKD however, markedly altered mineral, acid-base and hormone metabolism among other abnormalities might also exert adverse effects on proteinuria and contribute to CKD progression. Knowledge of such associations might help improve antiproteinuric strategies in this population.

**Methods:** We carried out a cross-sectional study of 187 adult patients with CKD-4 and 5 in an outpatient nephrology practice utilizing an electronic health record and examined the relationship between urine protein excretion and several laboratory variables which are commonly affected by advanced CKD.

**Results:** Urine protein-to-creatinine ratio (UPCR) was positively correlated with serum phosphorus ($r=0.218$, $p<0.01$) and serum intact parathyroid hormone levels ($r=0.181$, $p<0.01$). A negative correlation was found for UPCR and serum bicarbonate ($r=-0.242$, $p<0.01$) and serum 25-hydroxy vitamin D levels ($r=-0.283$, $p<0.01$). Serum albumin levels were negatively correlated with UPCR, both for the population as a whole ($r=-0.366$, $p<0.01$) as well as for patients with UPCR greater than or equal to 0.5 ($r=-0.389$, $p<0.01$), suggesting that the relationship with albumin is not restricted to only those patients with significant proteinuria.

**Conclusions:** These findings suggest that lower vitamin D levels, reduced serum bicarbonate, lower serum albumin levels, elevated phosphorus levels and elevated parathyroid hormone levels may be associated with higher urine protein excretion in patients with CKD-4 and 5. It is plausible that treating these abnormalities in the advanced CKD population might have additive favorable effects on urine protein excretion, though a cause and effect relationship is not clear. Future interventions, possibly through dedicated CKD clinics, should focus on intensive combined therapies to reduce proteinuria in patients with advanced CKD.

## PUB053

**Epoxyeicosatrienoic Acid Prevents Renal Fibrosis and Inflammation via Peroxisome Proliferators-Activated Receptor Activation in Obstructive Nephropathy** Jinu Kim,[1] Babu J. Padanilam.[1,2] *[1]Cellular and Integrative Physiology, Univ of Nebraska Medical Center, Omaha, NE; [2]Medicine, Div of Nephrology, Univ of Nebraska Medical Center, Omaha, NE.*

**Background:** Epoxyeicosatrienoic acids (EETs), lipid metabolites produced from arachidonic acid, have anti-inflammatory and profibrinolytic functions in the cardiovascular system. Soluble epoxide hydrolase (sEH) converts EETs to inactive dihydroxyeicosatrienoic acids. Based on previous studies, we hypothesized that genetic or pharmacological inhibition of sEH would attenuate tubulointerstitial fibrosis and inflammation induced by unilateral ureteral obstruction (UUO) in mouse kidneys.

**Methods:** UUO was performed by ligation of the left ureter near the kidney pelvis in sEH-knockout (*Ephx2*-KO) and wild-type (WT) male mice. In WT mice, sEH inhibitor *trans*-4-{4-[3-(4-trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid (*t*TUCB, 0.2 mg/d) or vehicle was administered by oral gavage.

**Results:** Genetic or pharmacological inhibition of sEH abolished tubulointerstitial fibrosis as demonstrated by attenuated profibrotic protein expression and myofibroblast formation. Inflammatory response was attenuated as demonstrated by decreased leukocyte influx and proinflammatory protein expression during UUO. Consistently, chronic administration of 11,12- or 14,15-EET regioisomers reduced tubulointerstitial fibrosis and inflammation in UUO-subjected WT mice kidneys. Activity in subtypes of peroxisome proliferators-activated receptor (PPAR), especially PPARγ, were decreased by UUO, but sEH inhibition or 11,12/14,15-EET treatment enhanced PPAR activity.

**Conclusions:** These data demonstrate that EETs anti-inflammatory and fibroprotective effects are mediated via PPAR activation. Our data suggest the potential use of sEH inhibitors or EETs in treating fibrotic diseases.

## PUB054

**Soap Bar Placebo for the Treatment of Nocturnal Leg Cramps in a Chronic Kidney Disease Clinic** Martin Sedlacek. *Dartmouth Hitchcock Medical Center, Lebanon, NH.*

**Background:** Nocturnal leg cramps are a frequent complaint in the chronic kidney disease (CKD) population that we screen for in our specialized CKD clinic. Putting a soap bar under the bed cover is a popular remedy which most likely acts as a placebo. In the absence of a specific treatment for leg cramps we suggest that our patients try the soap bar placebo. As there is no generally accepted objective assessment of leg cramps we record the assessment that patients make of their symptoms.

**Methods:** The electronic medical records of the most recent 100 consecutive patients with chronic kidney disease seen in our CKD clinic by one physician were analyzed.

**Results:** Of 100 patients there were 47 women and 53 men with a mean age of 71 years and an age range of 31 years to 93 years. 15% were in stage 3 CKD, 56% in stage 4 CKD and 29% in stage 5 CKD. 44% had diabetes mellitus. 58 patients had leg cramps (58%) and 30 of these tried out the soap bar and 7 were interested but had not tried yet. Of the remaining 21 patients some were content to use other remedies (tonic water, ice, magnesium, vitamin E, oxycodone, and saltine crackers) and some had only mild symptoms or had other reasons not to try the soap bar. There was a statistical tendency for muscle cramps to be more frequent in women and patients with diabetes mellitus and electrolyte abnormalities. Of the 30 patients who tried the soap bar 18 (60%) reported improvement of the leg cramps. The response was variable, ranging from mild to dramatic.

**Conclusions:** The pathogenesis of nocturnal leg cramps is unknown and likely involves muscle, peripheral and central nervous system. We found that besides correction of metabolic abnormalities and changing possible offending medications, soap bar placebo was an effective treatment for leg cramps in most patients who tried it. Interestingly, there was improvement of symptoms even though the placebo was suggested to patients openly as a placebo.

## PUB055

**Myoclonus in Renal Failure: Two Cases of Gabapentin Toxicity** Lili Chan,[1] Milisha Shah,[2] Mary M. Bridgeman,[2] Kenneth Kaufman,[1] Amay Parikh.[1] *[1]UMDNJ-Robert Wood Johnson Medical School; [2]Robert Wood Johnson Univ Hospital.*

**Background:** Antiepileptic drugs (AEDs) are used in the treatment of epilepsy, pain, and psychiatric disorders. Renal status may impact the efficacy and toxicity associated with AEDs. Gabapentin is an AED that is cleared solely by renal excretion and dosing requires consideration of the patient's renal function. We report 2 cases of myoclonic activity associated with gabapentin toxicity in the setting of renal disease and address treatment with dialysis.

**Methods:** Case 1: 78-year-old woman with congestive heart failure, history of thromboembolism, hypertension, diabetes mellitus, asthma and diabetic peripheral neuropathy presented with tremors involving her upper extremities for 3 days prior to admission. Evaluation revealed acute kidney injury (AKI) secondary to increased furosemide and lisinopril with hyperkalemia and azotemia. The patient was noted to have severe myoclonus. Prior to admission, the patient was being treated with gabapentin 900 mg total daily dose. The patient did not have any history of renal disease, but presented with an estimated glomerular filtration rate of 13 mL/min/1.73 m$^2$. With discontinuation of gabapentin and initiation of hemodialysis, marked improvement in her myoclonus occurred. Case 2: 55-year-old man with end-stage renal disease on peritoneal dialysis (PD), diabetes mellitus, hypertension, neuropathic pain, and peripheral vascular disease with toe gangrene on long term antibiotics presented for evaluation of diffuse body tremors, altered mental status and worsening leg infection. Gabapentin 600 mg total daily dose was initiated 3 days prior to presentation with myoclonus. The patient's PD treatment was increased from 4 to 6 exchanges daily. With increased dialysis and discontinuation of gabapentin, mental status and myoclonus improved.

**Conclusions:** Myoclonic spasms may occur as a complication of gabapentin toxicity, especially in the setting of renal dysfunction. In the cases reported, both hemodialysis and PD were effective in treating myoclonic activity in acute and chronic renal dysfunction. Gabapentin dosing needs to be adjusted in patients with chronic kidney disease and in patients at risk for developing AKI.

## PUB056

**Lithium Induced Chronic Tubulointerstitial Nephropathy; Rate of Progression and Prognostic Markers towards ESRD in the North-Eastern Part of the Netherlands: The Case for Early Referral** Annika M.A. Berends,[1] Frank G.H. van der Kleij,[1] Riko Nap,[1] Casper F.M. Franssen,[2] Judith J. Dasselaar.[1+2] *[1]Dept of Internal Medicine, Div of Nephrology, Scheper Hospital Emmen; [2]Univ Medical Center Groningen, Netherlands.*

**Background:** Lithium, the treatment of choice for bipolar disorder, is known for its renal toxicity. Current guidelines advise a nephrologic consult when kidney function becomes moderately impaired (CKD stage 3A, GFR 45–59 ml/min/1.73m$^2$). The purpose of this study was to examine the course of renal function, and the effects of nephrologic interventions on renal function of patients on lithium.

**Methods:** In this, partially, retrospective study, we included all patients using lithium that were referred to our hospital for renal function decline from 1995 up until now. Subjects are prospectively followed. CKD was defined as eGFR <60 mL/min/1.73m$^2$.

**Results:** Data on thirteen patients (30.8% male) was available up until now. At referral, mean age was 51 years (range 44-71), mean duration of lithium therapy 18±10 years, and mean follow up time 35 months (range 8-132). eGFR was inversely related to duration of lithium use at referral (r: -0.57 (95%CI [-0,85--0,025]), and to last follow-up (r: -0.73 (95%CI [-0,95--0,054]). CKD stage, after correction for age, gender and hypertension, was strongly associated with cumulative lithium exposure (p=0.0037). In 8 patients, lithium therapy was ceased (mean eGFR at cessation: 26±15); renal function improved in none, and four progressed to ESRD.

**Conclusions:** Lithium induced nephropathy proofs to be irreversible, despite cessation of lithium even at CKD stage 3A. Our data might entail an underestimation of known incidence rates of lithium induced chronic tubulointerstitial nephritis and its progression towards ESRD. Future studies are therefore indicated to evaluate the effects on renal function when the CKD stage threshold for nephrologic evaluation is lowered. And, because preserving mental wellbeing is a factor to keep in mind when caring for these patients, those studies should include continuation of lithium as well, since no acceptable alternative to lithium is available as yet.

*Funding:* Government Support - Non-U.S.

## PUB057

**Estimating Glomerular Filtration Rate in Chinese Patients with Type 2 Diabetes: A of Creatinine-Base Equations** Jin-xia Chen,[1] Chenggang Shi,[1] Jianhua Huang,[2] Xilian Qiu,[3] Cailian Cheng,[1] Xun Liu.[1] *[1]Div of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guanghzou, China; [2]Dept of Laboratory Medicine, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China; [3]Dept of Laboratory Medicine, The First Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China.*

**Background:** Diabetes has become a major cause of end-stage renal disease (ESRD). The aim of our study is to evaluate the equations based on serum creatininin type 2 diabetic patients in China.

**Methods:** A totle of 454 patients who were diagnosed with type 2 diabetes participated in the study. Comparisons of their efficiency to estimate GFR in the subjects were made between Cockcroft-Gault equation(CG), 6-variable Modification of Diet in Renal Disease equation(MDRD-1), 4-variable MDRD equation (MDRD-4), Reexpressed 6-variable MDRD equation (R-MDRD1), Reexpressed 6-variable MDRD equation (R-MDRD4), Mayo Clinic quadratic equation (MCQ), Chronic Kidney Disease Epidemiology Collaboration equation(CKD-EPI), Chinese equation, previously Japanes equation (Japan-1), new Japanese equation (Japan-2) by using the $^{99m}$Tc-DTPA –GFR to act as the standard GFR (sGFR).

**Results:** None of the equations had accuracy up to the 70% level with a deviation less than 30% from sGFR. The agreement limits of all the equations exceeded the prior acceptable tolerances defined as 60 ml/min per 1.73 m$^2$. When the overall performances were compared, GFR estimated by Japan-1 and Japan-2 demonstrated better results. Area under curve (AUC) of CKD-EPI is the largest. In the subgroup of sex, the accuracy of male is all higher than the female.When sGFR >60 mL/min per 1.73 m2, Japan-1 demonstrate higher accuracy. When sGFR <60 mL/min per 1.73 m2, the CG equation perform a better result.

**Conclusions:** This results displays that Japan-1 andJapan-2 is better than the other equations in overall performance. When sGFR is more than 60 mL/min per 1.73 m2 , the use of Japan-1 and Japan-2 may have better prediction of the GFR in diabetic patients, while if the patients sGFR is less than 60 mL/min per 1.73 m2 , the use of the CG equation is more accurate.

*Funding:* Government Support - Non-U.S.

**PUB058**

**The Renal Survival of Post-Nephrectomy in Kidney Cancer and Non-Kidney Cancer Patient – East Cost Malaysian Experiences**
Azreen Syazril Adnan, Azhar Amir Hamzah, Nurul Jannah Ambak, Nik Zamli Nik Jid. *Chronic Kidney Disease Resource Center, School of Medical Sciences, Kota Bharu, Kelantan, Malaysia.*

**Background:** Chronic Kidney Disease (CKD) is a recognized worldwide public health issue. It is associated with increased morbidity, mortality and poor quality of life. Nephrectomy remains the standard of care in treatment of renal masses, however its risks in developing CKD have not been widely studied in our population. This study id meant to examine postoperative CKD and acute kidney injury (AKI) in patients post nephrectomy.

**Methods:** 31 patients from Hospital Universiti Sains Malaysia, whom underwent nephrectomy from 1st January 1998 until 31st December 2011 were enrolled and followed by one year follow-up. Patients' medical records were retrieved and reviewed. Socio-demographic, clinical characteristics and onset of CKD (primary end point) and secondary end point was AKI status were recorded. The duration of freedom from onset of disease was measured from the date of surgery until the onset of event.Survival probabilities were analyzed using the Kaplan Meier Product-limit Estimates (percentages with 95% confidence intervals) and comparison of survival probability used log-rank test. Data entry and analyses were done using SPSS version 20 and Stata version 11 software.

**Results:** Patients age ranged from 2 to 72, with mean ± SD was 43.48 ±3.55 years old. Majority were Malays 26 (83.9%) and 29 (93.5%) underwent open surgery. Overall proportion of CKD was 3.2% and AKI 6.5%. The five-year overall, freedom from the CKD and AKI onset were 100% and 90.5% (95% CI: 67.0%, 97.6%) respectively. The median freedom of CKD and AKI onset cannot be determined because, the survival curve still higher than 50% cumulative survival probabilities. The freedom from CKD and AKI in non cancer nephrectomy group was significant compared to kidney cancer nephrectomy group with log-rank statistics: 4.00 (*p*-value: 0.046) and log rank statistics: 4.21 (*p*-value: 0.040) respectively. Patients underwent surgery because of non kidney cancer had better survival rates.

**Conclusions:** Non-kidney cancer nephrectomy patient shave better renal survival rates from CKD and AKI onset compared to kidney cancer nephrectomy.

*Funding:* Government Support - Non-U.S.

**PUB059**

**Abstract Withdrawn**

**PUB060**

**Correlation of 25(OH)D3 Levels with Diet, Serum Calcium, Phosphorus, and Parathyroid Hormone in Normals and in People with Chronic Kidney Disease Stages 1-4 from NHANES 2003-2006**
Linda W. Moore, A. Osama Gaber, Wadi N. Suki. *Surgery, The Methodist Hospital, Houston, TX.*

**Background:** Little is known about the prevalence of low vitamin D [25(OH)D3] levels in stages of chronic kidney disease (CKD) or the correlation of 25(OH)D3 with diet, serum calcium (Ca), phosphorus (P), or intact parathyroid hormone (iPTH) for level of kidney function. We examined 25(OH)D3 levels in a large sample of people with normal kidney function (NL) and CKD subjects, and explored its relationship to kidney function, diet, serum Ca, P, and PTH.

**Methods:** Subjects were from NHANES 2003-2006. Renal function was estimated using the CKD-Epi equation. Intake of dairy product servings, according to the Academy of Nutrition and Dietetics method, was estimated from a 24-hour dietary recall taken the morning of fasting blood sample collection for serum Ca, P, iPTH, and 25(OH)D3. Mean, SE, median, range, correlation (r), and p-value were calculated using complex survey statistics.

**Results:** Only 45% of NL (26.9% US adults) had a 25(OH)D3 level ≥30. 25(OH)D3 and iPTH levels in CKD were different from NL (Table). A positive correlation was present between the level of 25(OH)D3 and number of servings of dairy products (r=0.14), serum Ca (r=0.12) and P (r=0.03). Correlation with iPTH was negative (r=-0.25); all p<0.0001.

| Variable | NL | CKD1 | CKD2 | CKD3a | CKD3b | CKD4 | p-value |
|---|---|---|---|---|---|---|---|
| n | 5,644 | 219 | 252 | 1,116 | 452 | 123 | |
| 25(OH)D3, ng/mL, mean±SE | 23.5±0.4 | 21.3±0.9 | 20.2±0.5 | 25.1±0.4 | 24.2±0.6 | 20.7±1.0 | <.0001 |
| median, range | 23, 2-86 | 20, 4-68 | 19, 3-53 | 25, 3-72 | 24, 6-53 | 20, 3-51 | |
| iPTH, pg/mL, mean±SE | 41.3±0.4 | 44.5±1.9 | 47.4±1.6 | 48.7±0.9 | 60.4±2.1 | 110.9±10.7 | <.0001 |
| median, range | 38, 6-315 | 40, 12-156 | 42, 12-138 | 43, 6-306 | 52, 6-320 | 81, 10-491 | |

**Conclusions:** We conclude that 1) low 25(OH)D3 levels are prevalent in NL and CKD, 2) low levels are associated with elevated levels of iPTH and may explain the hypophosphatemia observed in NL, and 3) dietary intake of dairy products contributes significantly to serum 25(OH)D3.

**PUB061**

**Prevalence of Cardiovascular Risk Factors and Kidney Function Progression among a Developing World Chronic Kidney Disease Sample**
Jafar Al-Said, Teerath Kumar, Soni Murdeshwar. *Nephrology and Internal Medicine, Bahrain Specialist Hospital.*

**Background:** CKD patients had high cardiovascular (CV) mortality and morbidity. Identifying the specific population CV risk factors is detrimental in implementing a successful preventive medical plan. The eGFR drop 3-5 ml/min annually in CKD patients. Reduction in kidney function increases the CV burden. The aims of this study were to identify the CV risk factors profile among CKD outpatient population, and to determine the progression of kidney function and its related factors.

**Methods:** This is a retrospective analysis of adult CKD patient followed in our nephrology department, from Oct. 2003, for 8.5 years. Primary Glomerulonephritis, transplant recipients, and pregnant patients were excluded. Demographic variables and CV risk factors were collected from electronic charts. Calculated eGRF by CKD EPI was used as an estimate for kidney function on the first and last OPD visit. SPSS 18 was used for analysis.

**Results:** Total patients were 245. Mean follow up was 23.6 months (SE 1.6), mean age 58.7 years (SE 0.9), mean BMI 30.5 kg/m2 (SE 0.5). Male form 61% of the total population. Mean eGFR was 45.4 ml/min (SE1.4), and 51% of the populations were having CKD stage III. The average number of CV risk factors was 4 and 95% of the population was having two or more CV risk factors. Hypertension was the most common CV risk factor present among 91% of the population. DM was present among 60%. Hypertension was more prevalent with advanced CKD stage. Uric acid levels increases significantly with the stage of CKD. The eGFR over 2 years of management was stable for the total population. Improvement in eGFR was noticed of 2ml/min among CKD stage III and IV and eGFR increased 4ml/min in non-Hypertensive patients. Certain factors were related to final eGFR.

**Conclusions:** High Cardiovascular risk was found in CKD patients. HTN was the most common CV risk factors present in our CKD population. For the total population eGFR did not decline over two years of treatment; moreover it improved in non-HTN patients and patients with CKD stages III and IV.

**PUB062**

**Long-Term Mortality and Its Risk Factors in Patients with Lupus Nephritis: A Cohort of 158 Patients at a Single Center in Japan**
Ken Kayakabe, Keiju Hiromura, Hidekazu Ikeuchi, Noriyuki Sakurai, Toru Sakairi, Yoriaki Kaneko, Akito Maeshima, Yoshihisa Nojima. *Dept of Medicine and Clinical Science, Gunma Univ Graduate School of Medicine, Maebashi, Gunma, Japan.*

**Background:** Lupus nephritis (LN) is a major cause of morbidity and mortality in SLE patients. Although the mortality of LN patients has been reported from various countries, little is known about mortality and its risk factors in Japan.

**Methods:** One hundred fifty-eight LN patients who received renal biopsy at our hospital from 1975 to 2012 were retrospectively analyzed.

**Results:** The mean age was 36.2±13.4 years; 86.7% was female. The mean observation period was 9.8±7.6 years. Urinary protein was 4.0±4.1 g/gCr and eGFR was 79±33 ml/min/1.73m$^2$ at biopsy. ISN/RPS class was as follows; II 12.7%, III 14.6%, IV 41.8%, V 14.6%, III/IV+V 14.6%. During observation periods, 18 patients died; infection (n=5), uncontrolled lupus (n=3; CNS lupus, pulmonary hemorrhage and renal failure), malignancy (n=3), cardiovascular disease (n=2), gastrointestinal disease (n=2), and others (n=3; uncertain, suicide and traffic accident). Early death within 3 years after renal biopsy occurred in 4 patients; 2 in uncontrolled lupus (CNS lupus and pulmonary hemorrhage) and 2 in others (uncertain and suicide). The 5, 10 and 20-year survival rates were 95.5, 93.1 and 76.1%, respectively. The log-rank test showed that older age, complication with CNS lupus, diabetes mellitus and progression of kidney dysfunction (doubling serum creatinine or ESRD) were significantly associated with increased mortality. Neither eGFR at biopsy nor ISN/RPS class was associated with mortality. A multivariate stepwise Cox hazard model revealed independent risk factors for death as follows; older age [per 1-year increase; Hazard Ratio (HR) 1 .09], complication with CNS lupus [HR 14.1] and progression of kidney dysfunction [HR 5.49].

**Conclusions:** Long-term survival rate of our facility is comparable to those of previous reports. From our cohort, older age, complication with CNS lupus and progression of kidney dysfunction were found as independent risk factors for increased mortality of LN.

**PUB063**

**Epidemiology of Nephropathies in Irregular Immigrants in Northern Italy**
Francesco Quarello,[1] Cristiana Rollino,[1] Rosanna Coppo.[2] *[1]Nephrology, Osp. S. G. Bosco, Turin, Italy; [2]Nephrology, Osp. Inf. Regina Margherita, Turin, Italy.*

**Background:** There are about 544.000 irregular immigrants in Italy (out of 60 millions inhabitants). Nephropathies epidemiology in the birth areas of these people is unknown.

Aim of this study was to evaluate the prevalence of renal disease among irregular immigrants in Turin.

**Methods:** The survey was conducted in the health reception center for immigrants from January to December 2012. It was based on: 1) anamnestic evaluation through a questionnaire in 7 languages; 2) serum creatinine (SCr); 3) glomerular filtration rate (GFR-EPI); 4) urine examination; 5) blood pressure.

**Results:** Data of 494 people (287 women, 207 men), mean age 36.9±12 years, 167 black, 11 Asians, were examined. 50 subjects reported hematuria, 90 dysuria, 21 infectious diseases, 67 previous malaria. 23 patients were diabetic (4.6%); in 1 case diabetes was a new diagnosis. Familiarity for kidney disease was found in 4 patients (2 stones, 2 polycystic

nephropathy), hypertension in 29 (5.8%). 63 patients (12.7%) had microhematuria, 67 (13.5%) proteinuria, 15 (3%) both hematuria and proteinuria. Mean SCr was 0.79±0.25 mg/dl (GFR 109±23.7 ml/min/1.73mq); 13 patients had SCr >1.3 mg/dl (10of these also had urinary abnormalities and/or hypertension). We also found: 1 renal glycosuria, 1 schistosomiasis (Schistosoma mansoni), 4 renal stones. 99 patients (20%) were recalled: 69 came. Eventually, 10 patients (2%) were sent to the GP to monitor blood pressure or diabetes; 41 (8.2%) were integrated in a nephrologic follow-up: of these only 8 turned up to the second visit.

**Conclusions:** This survey on irregular immigrants in Northern Italy identified 10 patients (2%) to be monitored by the GP because of hypertension or diabetes and 41 (8.2%) to follow in a nephrologic context for possible ongoing nephropathies or for polycystic disease or stones. Moreover, 1 schistosomiasis, 1 new diabetes and 1 renal glycosuria were detected. The studied people are young: Italian peolple in the same age range have a lower frequency of nephropathy. Poor patients compliance prevented a correct survey and diagnosis. This represents a main issue for the outcome and for the consequent wasted resources.

## PUB064

**Chronic Kidney Disease and Risk of Cardiovascular Events and Death among Asian Adults** Cynthia Ciwei Lim,[1] Boon Wee Teo,[2] Wan Ting Tay,[3] Carol Y. Cheung,[3] Su-chi Lim,[4] Khuan Yew Chow,[5] Jeannette Lee,[6] E. Shyong Tai,[2] Tien Yin Wong,[7] Charumathi Sabanayagam.[3] *[1]Dept of Renal Medicine, Singapore General Hospital; [2]Dept of Medicine, National Univ of Singapore; [3]Singapore Eye Research Institute, Singapore; [4]Diabetes Center, Khoo Teck Puat Hospital; [5]National Registry of Diseases Office, Singapore; [6]School of Public Health, Yong Loo Lin School of Medicine, National Univ of Singapore; [7]Dept of Ophthalmology, National Univ of Singapore.*

**Background:** To examine the association between chronic kidney disease (CKD) and adverse outcomes in a multi-ethnic Asian population using data from two independent studies in Singapore.

**Methods:** A prospective cohort study of 7098 individuals who participated in two population-based studies conducted from 2004-2007: the Singapore Malay Eye Study (n=3148 Malay adults aged 40-80 years) and the Singapore Prospective Study Programme (n= 3950 Chinese, Malay and Indian adults aged ≥40 years) was conducted. CKD was assessed from estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (ACR). eGFR was categorized into ≥60, 45-59.9, <45 ml/min/1.73 $m^2$ and ACR into <30, 30-299, ≥300 mg/g. Outcomes were (1) combined incident cardiovascular disease (CVD) and CVD mortality, and (2) all-cause mortality identified by linkage with national disease/death registries.

**Results:** Over a median follow-up of 4.3 years, 4.6% developed CVD outcome and 6.1% died. Lower eGFR and higher ACR were associated with both outcomes in a dose-dependent manner. Compared to eGFR≥60, the adjusted HR (95% CI) of incident CVD/CVD mortality and all-cause mortality were 1.57 (1.08-2.77) and 1.99 (1.51-2.61) for eGFR<45; compared to ACR<30, the HR (95% CI) were 3.68 (2.10-6.47) and 2.01 (1.13-3.57) for ACR ≥300. In analysis stratified by age, the associations for both outcomes were stronger among those aged <65 years.

**Conclusions:** This study in a large multi-ethnic Asian cohort confirmed that eGFR and albuminuria were independent risk factors for CVD and mortality. Interventional studies are required to assess if modifying these factors will improve adverse outcomes.

*Funding:* Government Support - Non-U.S.

## PUB065

**Cardiovascular Risk Factor Profile, Hypertension Control, and Progression of Renal Function among Diabetic and Non-Diabetic in a Developing World Sample** Jafar Al-Said, Teerath Kumar, Soni Murdeshwar. *Nephrology and Internal Medicine, Bahrain Specialist Hospital, Bahrain.*

**Background:** CKD population has high CV risk factors. The major cause of death in CKD patients is Cardiovascular. Diabetes increases further the CV risk. In this study we wanted to:

1- Determine the CV risk factors among diabetic CKD patients and Compare them with non-diabetic patients.

2- Study the progression of kidney function among both subgroups and identify the factors correlated with the final kidney function.

**Methods:** All patients with CKD followed between Oct. 2003 and April 2012 were identified. Demographic and CV risk factors were collected from electronic files. Seventy seven matched diabetic and non-diabetic patients, for age, Gender and BMI, were selected.

| Variables | Diabetic CKD, Mean (SE) | Non Diabetic CKD, Mean (SE) | P |
|---|---|---|---|
| Age | 56.7(1.3) | 57.5(1.8) | NS |
| BMI | 29.2(0.8) | 28.3(0.6) | NS |
| Follow up months | 26.3(2.9) | 26.1(2.9) | NS |
| Urea mg/dl | 57.5(1.8) | 52.2(3.3) | NS |
| Creatinine mg/dl | 1.8(0.1) | 1.7(0.1) | NS |
| eGFR CKD EPI ml/min | 48.4(2.7) | 49.1(2.9) | NS |

The eGFR on the first and last clinic visit were estimated using CKD EPI equation. Progression of CKD over the follow up period was identified. The prevalence of CV risk factor and the renal function progression were tested using SPSS version 18.

**Results:** Total patients were 245. Mean follow up was 23.6 months (SE 1.6). In each subgroup 52 were males. Diabetic CKD subgroup has a mean of 5 CV risk factors as compared to 3 in non-DM. Hypertension was the most common risk factor. Hypertension and hyperlipidemia were more common in DM subgroup. Urea, Creatinine and eGFR in both subgroups were not significantly different on the first visit. There was no significant change in eGFR over the follow up. Certain factors were related with the last eGFR.

**Conclusions:** Diabetic CKD population has higher CV risk factors. Hypertension and Hyperlipidemia are the most common CV risk and more prevalent in diabetic CKD. The eGFR did not decrease over the follow up for both subgroups. We would like to share our population data with the global nephrology group.

## PUB066

**Ratings of Executive Functioning in Children and Young Adults with CKD** Stephen R. Hooper,[1] Jerilynn Radcliffe,[2] Divya Ganeshmurthy Moodalbail,[2] Nina Laney,[2] Abbas F. Jawad,[2] Susan L. Furth.[2] *[1]Univ of North Carolina School of Medicine, Chapel Hill, NC; [2]Children's Hospital of Philadelphia, Philadelphia, PA.*

**Background:** Cognitive deficits in individuals with mild to severe CKD are well documented; however, few studies have examined executive function in children and young adults with CKD. Such measurement approaches may provide a cost effective strategy for nephrologists working with this population and provide useful clinical information with respect to health care management for individuals with CKD.

**Methods:** Cross sectional observational study of 42 children and young adults with CKD (eGFR below 90 ml/min/1.73m2), ages 8 to 25 years. The primary instrument was the Behavior Rating Inventory for Executive Functioning (BRIEF) completed by either the parent, for subjects (ages 8 to 18), or the adolescent/young adult (ages 19 to 25). Scores across the two versions of the BRIEF were combined for comparison purposes. Initial data analyses included descriptive findings of the CKD sample, with elevated ratings of at least one standard deviation above the normative mean reflecting risk for impairment.

**Results:** Mean(SD) age and eGFR were 15(3) yrs and 42(23) ml/min/1.73m2. The sample was 64% male and 67% Caucasian. 29% had transplant and 5% were on dialysis after a failed transplant. Results showed that none of the Behavior Regulation scales were significantly elevated when compared to normative expectations, with the Behavioral Regulation Index (BRI) falling within the average range (BRI T-Score = 54.39). The Metacognitive Index (MI) and Global Executive Composite (GEC) were also within average range (MI T-Score = 59.64, GEC T-Score = 59.64), although weaknesses were noted for Working Memory, Initiate, and Monitoring scales with associated effect sizes greater than 0.5 SD.

**Conclusions:** These findings indicated the presence of mild executive dysfunction, particularly for behavioral initiation, self-monitoring, and working memory. It will be important for nephrologists to be aware of the executive capabilities of their patients, especially with respect to how these capabilities affect adherence to medical care and ongoing medical management.

*Funding:* Other U.S. Government Support

## PUB067

**Cross Sectional Findings from the German Chronic Kidney Disease Cohort** Stephanie Titze, Matthias Schmid, Anna Kottgen, Martin Busch, Katharina Paul, Claudia Sommerer, Georg Schlieper, Johan M. Lorenzen, Jan T. Kielstein, Thomas Sitter, Elke Schaeffner, Seema Baid-Agrawal, Karl F. Hilgers, Wolfram Gronwald, Jürgen Floege, Christoph Wanner, Florian Kronenberg, Kai-Uwe Eckardt. *On Behalf of the GCKD Investigators.*

**Background:** Chronic kidney disease (CKD) is increasingly recognized as a global health problem. Causes and prognosis differ markedly, but the reasons for the heterogeneity remain poorly understood. Throughout the recent years registry data have become available, but prospectively obtained data in patients with moderate CKD are still scarce.

**Methods:** The German Chronic Kidney Disease (GCKD) Study is a national cohort study with CKD of various etiologies; patients have been enrolled prospectively in cooperation with 169 nephrologists in Germany. A structured interview and clinical examination were performed and data from medical records obtained. Biosamples were acquired in standardized fashion. At the time of screening, male and female patients had to have an estimated GFR of 30-60 ml/min x 1.73 $m^2$ or overt proteinuria in the presence of an eGFR > 60 ml/min x 1.73 $m^2$.

**Results:** GCKD enrolled a total of 5217 CKD patients, 60% male and 40% female, with a mean age of 59 years and mean eGFR of 45 ml/min. Approx. 8% of the cohort were included due to overt proteinuria. Roughly 26% had undergone kidney biopsy. A significant proportion of patients had several underlying causes or unclear diagnosis. The most common causes were nephrosclerosis (38%) and diabetic nephropathy (27%). Immunological diseases were more present in younger patients. There was a high cardiovascular disease burden with approx. 20% history of CAD and approx. 10% of cerebrovascular or peripheral artery disease.

**Conclusions:** The GCKD Study has established one of the largest cohorts of non-dialysis dependent CKD patients worldwide. Baseline analysis confirms a high cardiovascular disease burden and shows uncertainties in identification of the underlying primary cause of kidney disease. The comprehensive data set of the GCKD-study will allow comparative and pooled analyses with other renal cohorts worldwide to validate observations and to identify important regional differences.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## PUB068

### Incidence of Proteinuria Is a Simple Sign of Poor Outcome in Cancer Patients Receiving Gemcitabine

Masaki Hara,[1] Minoru Ando,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Renal Div, Dept of Medicine, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan; [2]Dept IV of Internal Medicine, Tokyo Women's Medical Univ, Shinjuku-ku, Tokyo, Japan.*

**Background:** Gemcitabine (Gem) is a widely used as an anti-cancer drug. Gem administration induces glomerular thrombotic microangiopathy, resulting in the emergence of proteinuria and/or kidney dysfunction. This study attempted to ascertain both incidence of proteinuria and an association between incident proteinuria and mortality in Gem recipients.

**Methods:** A prospective cohort study was conducted in 60 non-proteinuric patients with pancreatic or biliary cancer (mean age, 67 years), who received the first single therapy of Gem and who lived over 6 months following the administration. Incident proteinuria was defined as dipstick test ≥ 1 +, persistent in at least two consecutive examinations within 6 months. Cumulative mortality was analyzed by the Kaplan-Meier method, stratified by presence and absence of incident proteinuria. Multivariable proportional hazards regression analysis was used to calculate hazard ratio (HR) with its 95% confidence interval (CI) for all-cause mortality, adjusted for age, gender, disease severity, and estimated glomerular filtration rate (eGFR).

**Results:** Incidence of proteinuria was 23.3% in the first 6 months, and mortality rate was 45.0% in the follow-up period (median, 251; range, 20 – 550 days). Cumulative mortality was significantly greater in patients who developed proteinuria (82.4%) than those who did not (38.2%) at the time of 450 days following the Gem administration. The HR (95% CI) of proteinuria incidence was 3.36 (1.44 – 7.49; P = 0.0060), as compared with the absence of incident proteinuria.

**Figure. Kaplan-Meier curves stratified by presence and absence of incident proteinuria**

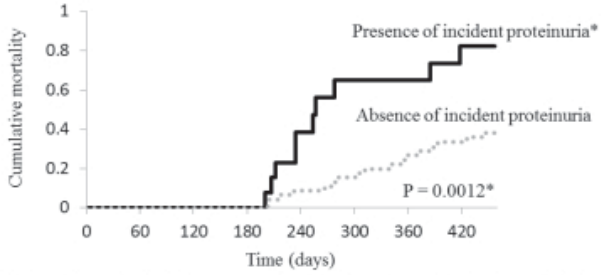



**Table. Adjusted risks of incidence of proteinuria for time to mortality in Gem recipients**

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| Variable | HR (95% CI) | P value | HR (95% CI) | P value |
| Age | 0.99 (0.94 - 1.04) | 0.7213 | 1.02 (0.97 - 1.09) | 0.3857 |
| Gender, men | 2.17 (0.99 – 5.07) | 0.0508 | 1.94 (0.86 – 4.69) | 0.1098 |
| Stage of the disease | 1.62 (1.03 - 2.91) | 0.0349 | 1.65 (1.02 – 3.01) | 0.0411 |
| eGFR | 1.01 (0.99 – 1.04) | 0.2184 | 1.01 (0.98 – 1.03) | 0.4533 |
| Incidence of proteinuria | 3.43 (1.49 – 7.45) | 0.0046 | 3.36 (1.44 – 7.49) | 0.0060 |

**Conclusions:** Incidence of proteinuria may be a harbinger of near-term death in Gem recipients.

## PUB069

### Sex and Racial Differences in End-Stage Renal Disease Risk in Diabetes

Margaret K. Yu,[1] Xiaobo Ding,[1] Bessie A. Young.[1,2] *[1]Univ of Washington; [2]VA Puget Sound Health Care System.*

**Background:** Chronic kidney disease (CKD) is more common in women than in men, however few studies have examined the relationship between sex and risk of end-stage renal disease (ESRD). Furthermore, although racial/ethnic minority women have a high burden of CKD risk factors, there is little information regarding ESRD risk by sex and race. The objective of this study is to assess sex and racial differences in 10-year incident ESRD risk in a primary care diabetic population.

**Methods:** The Pathways Study is a prospective, observational cohort of ambulatory, diabetic patients from a large managed care population in Seattle, WA. Self-reported race/ethnicity was used to categorize subjects as non-Hispanic white, non-Hispanic black, Asian, or other. Subjects were followed for 10 years for incident ESRD. Cox proportional hazards regression was used to estimate the risk of incident ESRD by sex and race/ethnicity, after adjustment for age, marital status, education, smoking, diabetes duration, hemoglobin A1c, baseline estimated glomerular filtration rate (eGFR), microalbuminuria, body mass index (BMI), and depression, taking into account mortality as a competing risk.

**Results:** Of the 3877 total subjects, 1864 (48.1%) were women. Racial composition did not vary by sex (79.4% non-Hispanic white, 8.4% non-Hispanic black, 8.0% Asian, and 4.2% other races). Women were less likely to be married or have a high school education and had higher BMI and baseline eGFR compared to men. 78 subjects developed ESRD over 10 years. Amongst women, Asians had the highest unadjusted incidence rates of ESRD (4.99 per 1000 person-years vs 3.51 in non-Hispanic blacks and 2.82 in non-Hispanic whites). Amongst men, non-Hispanic blacks had the highest incidence of ESRD (6.55 per 1000 person-years vs 3.45 in Asians, 3.37 in other races, and 2.62 in non-Hispanic whites). In the fully adjusted model, Asian women had an increased risk of incident ESRD (sub-hazard ratio 2.75, 95% CI 1.20-6.30) compared to non-Hispanic white men.

**Conclusions:** In this primary care diabetic population, Asian women had a greater risk of incident ESRD at 10 years compared to non-Hispanic white men. Asian women with diabetes may represent a high risk subgroup for ESRD.

*Funding:* NIDDK Support, Other NIH Support - NIMH, Veterans Affairs Support, Private Foundation Support

## PUB070

### Long-Term Outcome and Prognostic Factors of Idiopathic Membranous Nephropathy: Looking Back on the past Twenty Years

Rika Sakai,[1] Masao Kikuchi,[1] Yuji Sato,[1] Kazuo Kitamura,[1] Shouichi Fujimoto.[2] *[1]First Dept of Internal Medicine, Univ of Miyazaki, Miyazaki, Japan; [2]Dept of Hemovascular Medicine and Artificial Organs, Univ of Miyazaki, Miyazaki, Japan.*

**Background:** Idiopathic membranous nephropathy (IMN) is common cause of adult onset nephrotic syndrome. Although 30% patients showed spontaneous remission, 30-40% patients progress end-stage renal disease. This study addressed the long-term outcome and prognostic factors of IMN.

**Methods:** From 1992 to 2012, we diagnosed 100 patients with biopsy-proven IMN. 74 patients were followed up at least 1 year. We checked their drug history (prednisolone, cyclosporine, cyclophosphamide, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, statin, aspirin and warfarin) and complications (cerebrovascular accidents, cardiovascular accidents, malignancy). Present patient's status was divided into three groups by degrees of proteinuria: complete remission (CR), partial remission (PR) and nephrotic syndrome (NS).

**Results:** The mean age of studied patients was 59±13 years. The 5, 10 and 15 years of renal survival rate of IMN were 94%, 91% and 85%. During the follow up (average 107 months), 5(7%) patients reached end-stage renal disease. They significantly showed high levels of serum creatinine and total cholesterol at renal biopsy. In present, 39 patients (53%) are in CR, 19 patients (26%) in PR, 9 patients (12%) in NS. Patients in NS were significantly used more immunosuppressant. 5(7%) patients developed cardiovascular accidents and these patients had higher level of serum creatinine at renal biopsy, no drug history of cyclosporine, more drug history of aspirin or warfarin and more malignant disease. 7(9%) patients developed malignant disease and these patients had more cerebrovascular and cardiovascular accidents.

**Conclusions:** In our study, IMN patients with higher level of serum creatinine and total cholesterol at renal biopsy showed poor renal prognosis. Although there was no relationship between their drug histories with poor renal prognosis, non-use of cyclosporine was associated with cardiovascular accident. Further studies are necessary to clarify the effectiveness of treatment.

## PUB071

### Prevalence of Heart Failure in a Cohort of Patients with Chronic Kidney Disease: Results from the German Chronic Kidney Disease Study

Anna Kottgen,[1] Hanna Beck,[1] Stephanie Titze,[2] Silvia Huebner,[2] Martin Busch,[3] Florian Kronenberg,[4] Vera Krane,[5] Kai-Uwe Eckardt.[2] *[1]Univ of Freiburg, Germany; [2]Univ of Erlangen-Nürnberg, Germany; [3]Univ of Jena, Germany; [4]Univ of Innsbruck, Austria; [5]Univ of Würzburg, Germany.*

**Background:** Reduced kidney function is a risk factor for the development and progression of heart failure, but there is limited data on the prevalence of heart failure from large cohorts of patients with chronic kidney disease (CKD) of moderate severity. In this initial analysis, we examined the prevalence and correlates of self-reported heart failure in the German Chronic Kidney Disease (GCKD) Study.

**Methods:** The GCKD Study is a prospective observational cohort. Between 2009 and 2012, 5217 CKD patients aged 18 to 74 years with an eGFR of 30 to <60 ml/min/1.73m² or eGFR ≥60 and overt proteinuria during screening were enrolled. As part of a detailed standardized interview including cardiovascular disease history, patients were asked if they were suffering from heart failure. Patients not meeting inclusion criteria or with missing information were excluded from analysis. GFR was estimated from serum creatinine by the 4-variable MDRD Study equation. Factors associated with heart failure were identified using logistic regression.

**Results:** The overall prevalence of self-reported heart failure was 18% (18% among men and 17% among women). Heart failure prevalence was considerably higher in patients with coronary heart disease (43% vs. 11% without) and those with diabetes (26% vs. 13% without). Overall, heart failure prevalence increased with lower eGFR from 5% among patients with eGFR of ≥90 ml/min/1.73m², 13% for eGFR 60-89, 17% for eGFR 60-89, 20% for eGFR 30-44, to 21% for eGFR <30 ml/min/1.73m². The strongest associations of prevalent heart failure were observed with older age, presence of coronary heart disease, valvular heart disease, higher BMI, hypertension and diabetes.

**Conclusions:** Heart failure is common among patients with CKD, and constitutes a large burden of disease. Alternative definitions of heart failure using information from signs and symptoms of heart failure as well as from biomarkers will be evaluated.

*Funding:* Government Support - Non-U.S.

## PUB072

**Improved In-Hospital Morbidity and Mortality for Patients Treated by Hemodialysis Undergoing Orthopedic Procedures** David Bennett,[1] Ting-jung Pan,[3] Steven K. Magid,[3] Stephen Lyman,[3] Mayu Sasaki,[3] Jeffrey I. Silberzweig.[2] *[1]New York-Presbyterian Hospital-Weill Cornell Medical College; [2]The Rogosin Institute; [3]Hospital for Special Surgery.*

**Background:** The in-hospital post-operative medical course for patients with Chronic Kidney Disease (CKD) treated by hemodialysis (HD) undergoing orthopedic procedures has not been well reported. The few published reports document high rates of morbidity (up to 100%) and mortality (up to 28.5%), but have not clearly defined the patient population or focused on the in-hospital period.

**Methods:** We identified patients at a single institution between 2005-2008 and reviewed their charts for age, sex, comorbidities, complications, mortality, length of stay (LOS), cause of CKD, and discharge disposition. We compared our patients to the New York State (NYS) administrative database for mortality and LOS. Data was analyzed using two-sample t-tests for continuous variables and Fisher's exact tests for categorical data.

**Results:** 38 patients underwent 58 orthopedic procedures, most on the hip (46.6%) or knee (19.0%). Patients had been previously treated with HD for an average of 6.8 +/- 9.6 years. The most common causes of CKD were diabetic nephropathy (20.7%), hypertensive nephropathy (15.5%), and collagen vascular disease (13.8%). There were 17 post-operative complications in 14 patients (24.1%) and 1 in-hospital death occurred (1.7%). The average LOS was 9.1 +/- 5.0 days. Most patients were discharged to rehab (44.8%), but many went home (31.0%). The mean LOS was 2.2 days less than the NYS hospital average (95% CI: 0.8, 3.6, p-value=0.003) but the difference in mortality was not statistically significant (p-value >0.05).

**Conclusions:** The in-hospital post-operative medical course for patients with CKD treated by HD undergoing orthopedic procedures has not been well reported. By studying patients at a single institution, we have better defined the patient population and their post-operative course. Our results do not corroborate the high morbidity/mortality rate previously reported. Our mortality results do not significantly differ from other NYS hospitals.

## PUB073

**Albuminuria Is Associated with Diabetic Retinopathy in Ethnic Minorities** Amit J. Joshi,[1] Albert M. Osei,[1] Imran Tahir,[2] Bhavi Paresh Pandya,[3] Peter D. Hart.[1] *[1]Section of Nephrology, Stroger Hospital of Cook County, Chicago, IL; [2]Dept of Medicine, Stroger Hospital of Cook County, Chicago, IL; [3]Staten Island Univ Hospital, Staten Island, NY.*

**Background:** Diabetic nephropathy remains the most frequent cause of end-stage kidney disease (ESKD) in the United States. The association between the presence of diabetic retinopathy and diabetic nephropathy is well described; however, there are no data on whether presence of diabetic retinopathy is associated with progressive renal disease (defined as degree of albuminuria ± interval change in serum creatinine). We examined and herein describe the clinical characteristics of such patients at a large inner city hospital.

**Methods:** We retrospectively examined medical records of adult patients (≥18 years) with diabetes seen in the Ophthalmology clinic of our institution from Jan. and Feb. 2012. Data on baseline demographics, duration of diabetes, type of retinopathy, renal function, degree of albuminuria, use of renin-angiotensin-aldosterone system (RAAS) blockers, and other clinical parameters was collected.

**Results:** 245 patients had adequate data for clinical analysis. Mean age was 59.9 (±9.4) years and women accounted for 47% of the study cohort. Hispanics constituted 41%, African Americans 38%, Caucasians 9%, and other races 12%. 144(59%) patients had proliferative retinopathy, 73(30%) had nonproliferative retinopathy, 16(7%) patients had both proliferative and nonproliferative retinopathy; and 12 (4%) patients had no retinopathy. Longer duration of diabetes was associated with increased severity of retinopathy (P=0.0018). No difference was noted with respect to retinopathy severity and cerebrovascular disease, coronary artery disease or statin use. Degree of albuminuria influenced the severity of retinopathy (P 0.037). The presence of retinopathy had no impact on initial creatinine or interval change in creatinine (p value was insignificant) Also no ethnic difference was noted in terms of glycated hemoglobin level, age, initial creatinine, duration of diabetes, or use of RAAS blockers.

**Conclusions:** Albuminuria and longer duration of diabetes are associated with increased severity of retinopathy in ethnic minority patients.

## PUB074

**Risk Factors Associated with End-Stage Renal Disease and Fatality in Patients with Chronic Kidney Disease** Ya-Wen Chuang,[1,2] Cheng-Hsu Chen,[1] Fung-chang Sung.[2] *[1]Div of Nephrology, Dept of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; [2]Dept of Public Health, China Medical Univ, Taichung, Taiwan; [3]Taichung Tzu Chi General Hospital, Taichung.*

**Background:** With the highest prevalence of end-stage renal disease (ESRD) worldwide, there is no study for patients in Taiwan on the prognosis of chronic kidney disease (CKD) by stage. This study evaluated the risk of ESRD and fatality for patients with stage 3, 4 and 5 of CKD.

**Methods:** We identified 4702 patients with CKD of stages 3-5 from nephrology clinics at the Taichung Veteran General Hospital from December 2001 to December 2011. Patient prognosis data were extracted from the baseline to July 31, 2012, ESRD diagnosed, death or loss to follow-up. We used Cox models to identify risk factors associated with ESRD and deaths by CKD stage and age, and Kaplan-Meier method to measure the cumulative events of ESRD and death.

**Results:** Patients with CKD consisted of 64% of males, with 60% of patients aged >65 years and 39% having diabetes. The incidence of ESRD in CKD patients of stages 3, 4, 5 were 1.95%, 10.6%, 46.7%, with the fatalities of 4.56%, 6.23%, 4.69%, respectively. The elderly were at a lowered risk for ESRD, but at an elevated risk for death. Diabetic patients were at higher risk than non-diabetic patients for ESRD and death.

**Conclusions:** The risk of ESRD for patients with CKD increases rapidly with the advance of stage; but the differences in fatality are not so acute among the 3 stages of patients. The elderly CKD patients are less prone to ESRD, but at an elevated risk of death.

## PUB075

**The Asymptomatic Ankle-Brachial Index Abnormality Can Predict Poor Prognosis of Arteriosclerosis-Based Chronic Kidney Disease Patients** Yuji Kamijo, Koji Hashimoto, Makoto Harada, Taro Kanno, Makoto Higuchi. *Dept of Nephrology, Shinshu Univ School of Medicine, Matsumoto, Japan.*

**Background:** World Health Statistics 2013 indicated that average life of Japanese is the longest. Furthermore, Ministry of Health, Labour and Welfare reported that average life of Nagano prefecture's people is the longest in Japan; therefore characteristics of chronic kidney disease (CKD) in Nagano might differ, greatly be affected by arteriosclerotic lesions by ageing.

**Methods:** To reveal the arteriosclerotic effects, we investigated the ankle-brachial index (ABI) of CKD patients in Nagano (n=144, mean age 70) and followed up prospectively (2.3 years). We analyzed hazard ratio (HR) of ABI abnormality (ABI<1.0 or ABI≥1.3) influencing incidence of cardiovascular diseases (CVD) and worsening of kidney function, mortality, CVD-free survival, and all event-free survival, using multivariate Cox regression analyses. We analyzed also ABI-abnormality-related factors using multivariate logistic regression analyses.

**Results:** The mean eGFR and proteinuria were 41 ml/min/1.73m$^2$ and 0.5 g/gCr, respectively. Twenty six percent patients had well controlled diabetes mellitus (DM, mean HbA1c 6.1%), indicating a little contribution of DM nephropathy. We detected ABI abnormality in high rate (24%), and most patients were asymptomatic. Multivariate analyses demonstrated that ABI abnormality was the strongest risk factor of incidence of CVD (adjusted HR 5.3, 95%CI 1.8-16.2), CVD-free survival (adj-HR 4.4, 95%CI 1.7-11.5), and all event-free survival (adj-HR 3.1, 95%CI 1.3-7.0). The strongest risk factor of worsening of kidney function and that of mortality were GFR reduction and age, respectively. ABI abnormality was greatly related to the GFR reduction (Odds ratio 1.84, 95%CI 1.2-2.8), while it was not related to presence of DM.

**Conclusions:** This study suggests that asymptomatic ABI abnormality is highly detected in arteriosclerosis-based CKD patients, and strongly relates to lethal events and survival rate. GFR reduction is a very important independent risk factor of ABI abnormality; therefore we should conduct ABI measurement for all CKD patients regardless of presence of DM.

## PUB076

**Discontinuation of Angiotensin Converting Enzyme Inhibitors in Advanced Chronic Kidney Disease** Silvia Gonzalez S, Ines Castellano, Pedro J. Labrador, Jesús P. Marin, Clarencio Javier Cebrian andrada, Maria C. Jimenez, Vanesa Garcia-Bernalt, Sandra Gallego, Juan R. G?mez-Martino. *Nephrology, San Pedro de Alcántara Hospital, Cáceres, Spain.*

**Background:** Renin-angiotensin-aldosterone system (RAAS) inhibition has been used to slow chronic kidney disease (CKD) progression in diabetic (DN) and non-diabetic proteinuric nephropaties.

However, in advanced CKD, their withdrawal can delay the onset of renal replacement therapy (RRT).

The aim was to evaluate the effect of discontinuation of RAAS inhibitors in patients with CKD stage 4-5 and to assess K levels.

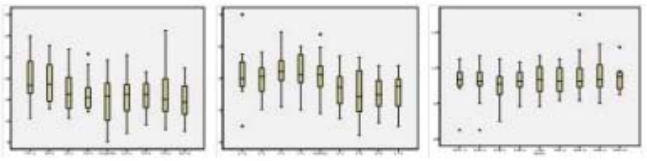
**Methods:** Observational prospective study made in patients who were being treated with angiotensin converting enzyme inhibitors (ACEi)/angiotensin receptors blockers (ARB) and whose estimated glomerular filtration rate (GFRe) was lower than 25 ml/min. We recorded creatinine and K in serum, GFRe (MDRD-4 IDMS) and mean arterial blood pressure (MAP) every 3 months for 1 year before and after stopping treatment. When it was necessary an alpha-adrenergic blocker or a calcium channel blocker was introduced.

**Results:** 27 patients were included (7 females, 20 males), mean age 73.7 ± 9.4 years. The aetiology of CKD was: vascular nephropathy (n = 10, 37%), DN (n = 9, 33.3%) chronic glomerulonephritis (n =2, 7.4%), chronic tubulointerstitial nephritis (n =2, 7.4%), autosomic policystic kidney disease (n =2, 7.4%), unknown aetiology (n =2, 7.4%).

Three patients started RRT 1, 2 and 11 months after stopping RAAS inhibitors, and one was lost to follow-up.

MAP, GFRe and K level when ACEi/ARB were stopped was 5.06 ± 0.59 mmol/l, 12.9 ± 4.8 ml/min and 92.3 ± 8.7 mmHg respectively.

MAP, GFRe and K levels 12 months before and after discontinuation of ACEi/ARB are shown in Figure 1.



[Figure 3]

**Conclusions:** Discontinuation of ACEi/ARB in patients with CKD stage 4-5 stabilized GFRe and improved K levels. There were no differences in MAP after stopping RAAS inhibitors.

## PUB077

**Renoprotective and Antiproteinuric Effect of Mineralocorticoid Receptor Blockers in Obese Patients with Proteinuric Nephropathy** Enrique Morales, Jorge Enrique Rojas-Rivera, Eduardo Gutierrez-martinez, Manuel Praga. *Nephrology, Hospital 12 de Octubre, Madrid, Spain.*

**Background:** Obesity is a risk factor for the development of renal failure (RF). Proteinuria (P) is an independent determinant of the progression of chronic kidney failure. Adipose tissue is a recognized source of components of the renin-angiotensin-aldosterone system (RAAS). The use of drugs which block the RAAS is unable to inhibit aldosterone long term. The aim of our study was to analyze the renoprotective effect of mineralocorticoid receptor blockers (MRB) in combination with other drugs that block the RAAS in obese patients with proteinuric nephropathy.

**Methods:** In January 2004, we began a prospective observational study in our hospital-based consultation. The management of spironolactone (S) patients with P> 1 g/24 hours being treated with other drugs blocking RAAS were divided according to body mass index (BMI): the group of obese (Ob) (BMI> 30 kg/m2) and a control group (C).

**Results:** Seventy-one patients were included. The mean age was 56.7 ± 15.1 years. The percentage of diabetics in both groups was greater than 50%. Thirty-two patients found within the Ob group, and 39 in group C. Initially there were no significant differences in renal function, P, blood pressure (BP), serum potassium levels and the percentage of drugs that block RAAS in both groups. After a follow-up of 28.9 (14-84) months, there was a reduction of 59.4% P in the Ob group (2.8 ± 2.1 to 1.3 ± 1.6 g/24hr, p <.05). The reduction of P was greater than 50% in 70 cases. The mean BP showed a significant decrease in their numbers (100.6 ± 9 to 92.1 ± 7.4 mmHg, p<.05). Renal function remained stable throughout the follow-up (56.8 ± 27 to 54.1 ± 30.9 ml/min). A slowing of renal function was observed since the introduction of E (3 to 1.4 ml/min/year, p 0.39). There gynecomastia in 9 cases (28.1%) in the Ob and only 2 cases (5.1%, p 0.008) in group C. The incidence of hyperkalemia was similar in both groups (6.3%).

**Conclusions:** MRB treatment in obese patients with different proteinuric kidney diseases induces a significant slowing in the progression of renal failure associated with a remarkable and sustained reduction in proteinuria with a few adverse events.

## PUB078

**Preliminary Examination of Chronic Kidney Disease Shared Medical Appointement Outcomes** Mohsen M. Elramah,[1] Henry N. Young,[2] Micah R. Chan.[1] *[1]UW Hospital and Clinics Dept of Nephrology; [2]Univ of Wisconsin School of Medicine and Public Health.*

**Background:** Chronic kidney disease(CKD) care can be challenging for multiple reasons . Our nephrologists implemented shared medical appointments (SMA) for CKD care in an effort to overcome such challenges. We examined if CKD SMAs resulted in similar outcomes in comparison to individual visits.

**Methods:** After IRB approval, we conducted a retrospective analysis of data from our database. Patients who received care in CKD shared medical appointments and individual visits from January 2011 to March 2013 were included. We compared the two groups on outcomes of,dialysis, mortality, transplantation, the change over time in CKD stage, glomerular filtration rate, intact parathyroid hormone , creatinine, phosphorus, proteinuria, and blood pressure.

**Results:** We analyzed 75 records; 23 SMA and 52 individual visits. SMA patients had a greater mean difference in PTH (M=40.1, SD=98.3) compared to individual visit patients (M=-4.9, SD=10.0) from 2011 to 2013 (t=-21, p=0.04).

| | **Group Visits (n=23)** n(%) or M(SD) | **Individual Visits (n=52)** n(%) or M(SD) |
|---|---|---|
| Dialysis in 2013 | 5 (22%) | 10 (19%) |
| Deceased in 2013 | 2 (9%) | 2 (4%) |
| Transplant | 1 (4%) | 1 (2%) |
| Change (2011 to 2013) | | |
| CKD stage (progression) | 5 (22%) | 13 (25%) |
| eGFR | -1.2 (10.2) | -2.5 (6.9) |
| iPTH | 40.1 (98.3) | -4.9 (61.7) |
| Creatinine (mg/dl) | 0.4 (1.0) | 0.3 (0.9) |
| Proteinuria (Ur P/C) | 0.2 (1.5) | 0.6 (3.0) |
| Phosphorus (mg/dl) | 0.3 (1.3) | 0.4 (1.1) |

Adjusting for potential confounders, this difference was no longer statistically significant. There were no differences in the proportions of patients who were on dialysis, deceased, or received a transplant between the two groups in 2013. Results also failed to indicate any differences in the changes across time for any other assessed outcome between the two groups.

**Conclusions:** This study demonstrates that CKD shared medical appointment clinical outcomes may be similar to individual visit outcomes. larger study designs are needed to confirm these initial findings.

## PUB079

**Chronic Kidney Disease (CKD) Is Distinct from End Stage Kidney Disease (ESKD)** Helen G. Healy,[1,2] Andrew John Mallett,[1,2,3] Zaimin Wang,[1,3] Anne Salisbury,[1,2,3] Robert G. Fassett,[1] Wendy E. Hoy.[1,3] *[1]CKD.QLD; [2]Dept of Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia; [3]Centre for Chronic Disease, School of Medicine, Univ of Queensland, Brisbane, Queensland, Australia.*

**Background:** CKD is the most common chronic disease in Australia. Inferences about its natural history have largely relied upon hypotheses from mortality and Renal Replacement Therapy (RRT) datasets.

**Methods:** Aim: To describe how characteristics of Australian CKD patients differ from those of Australian RRT populations. Primary diagnosis, age and sex of the first 2139 patients from 5 sites in the CKD.QLD Registry (CKD.QLD) were analysed and compared with the 2005-2010 (n=15,514) and 2011 (n=2,760) Australia and NewZealand Incident RRT cohorts. (ANZ RRT 2005-10, ANZ RRT 2011). Comparison was also made with the Australian dataset of people dying with ESKD not on RRT (n=10421, AIHW ESKD/nonRRT).

**Results:** The most common primary renal diagnoses in the CKD.QLD cohort were Miscellaneous (Misc, 26%), Diabetic Nephropathy (DN, 23%), Hypertension (HTN, 18%) and Uncertain (15%). In ANZ RRT 2005-10 and 2011 the most common primary renal diagnoses were DN (36%, 38%), Glomerulonephritis (GN, 23%, 22%), Misc (13%, 11%) and HTN (10%, 13%).

Mean age in CKD.QLD was 65.5 years and in ANZ RRT 2011 it was 60.3 years. The most common age group in both cohorts was 55-74 years (47%, 47%) however in AIHW ESKD/nonRRT cohort it was ≥75 years (87%).

45% of the CKD.QLD cohort, 39% of the ANZ RRT 2005-2010 and 53% of the AIHW/nonRRT ESKD cohorts were female.

**Conclusions:** There are differences in proportion and ranking of primary renal diagnoses between these CKD and RRT/ESKD cohorts. CKD patients are older and more frequently female then those undergoing RRT. Extrapolation from RRT cohorts for characterisation of CKD should be replaced with direct study of CKD cohorts. When paired with RRT and mortality cohort data this will provide information of greater accuracy and translational impact.

*Funding:* Government Support - Non-U.S.

## PUB080

**Left Atrial Volume Index as Useful Predictor for Decline of Kidney Function in CKD G3b and G4** Takeshi Yokoyama,[1] Mikako Hisamichi,[1] Masahiko Yazawa,[1] Nagayuki Kaneshiro,[1] Katsuomi Matsui,[2] Yusuke Konno,[1] Yugo Shibagaki,[2] Kenjiro Kimura.[2] *[1]Nephrology and Hypertension, Kawasaki Municipal Tama Hospital, Kawasaki, Kanagawa, Japan; [2]Nephrology and Hypertension, St. Marianna Univ School of Medicine, Kawasaki, Kanagawa, Japan.*

**Background:** Several studies have documented a strong relationship between CKD and accelerated cardiovascular disease. Although heart failure is known to associate with progression of CKD, little is known which parameters of ultrasound cardiography (UCG) are helpful to predict decline of kidney function (KF).

The aim of present study is to clarify the relationship between the rate of decline of KF and the parameters of UCG.

**Methods:** UCG was performed in 38 CKD G3b and G4 patients from April 2011 to December 2012, and the following parameters such as left atrial dimension (LAD), left atrial volume index (LAVi), end-diastolic wall thickness of the interventricular septum (IVSTd) and posterior wall (PWTd), and left ventricular mass index (LVMi) were measured. We calculated estimated GFR (eGFR) by the japanese GFR equation, and defined the rate of decline of KF as [(the latest eGFR – intial eGFR) / follow-up period]. We examined the correlations between the rate of decline of KF and the parameters of UCG.

**Results:** Average eGFR at UCG was 29.23 ± 8.47 ml/min/1.73m2 and average rate of decline of KF was -0.29± 0.74 ml/min/1.73m2/month. Rate of decline of KF was significantly correlated with LAD(r = -0.381), IVSTd + PWTd(r = -0.425), LVMi(r = -0.394) and LAVi(r = -0.455). Multiple regression analysis revealed that only LAVi was an independent predictor for decline of KF.

**Conclusions:** Among the parameters of UCG, LAVi was the most useful predictor for decline of KF in CKD G3b and G4.

**PUB081**

**The "Opt-Out" Recruitment Strategy: The SNORE Study**
Muna T. Canales,[1,2] Nicole Kay,[1] Areef Ishani,[3] I. David Weiner,[1,2] Richard Berry,[1,2] Rebecca Beyth.[1,2] *[1]Malcom-Randall VAMC, Gainesville, FL; [2]Univ of Florida, Gainesville, FL; [3]Minneapolis VAMC, Minneapolis, MN.*

**Background:** Recruitment in an unbiased fashion of adequate numbers of human subjects is key for scientifically sound and generalizable studies. We report initial results of an IRB-approved "opt-out" recruitment strategy that involves primary care provider (PCP) approval and easy "opt-out" options as part of the SNORE Study, an ongoing prospective study of sleep apnea and CKD progression.

**Methods:** With IRB approval, we identified veterans age 18-99 in the N. FL/S. GA Veterans Health System (NF/SGVHS) (2/12/2012-present) with at least 2 MDRD eGFRs between 15-44, at least 3 months apart; target enrollment = 250 over 2 years. We excluded those with dialysis, kidney transplant, PAP or $O_2$ therapy, active cancer, solid organ transplant or inability to give consent. Via e-mail, we requested permission from every PCP in the NF/SGVHS to contact their patient for enrollment; non-responders were contacted again by e-mail and then by phone. We invited eligible veterans in random order to participate via a letter with follow-up call within 1 week if no response. Letters stated that the PCP gave permission for us to contact them and provided a 1-800 number and an email to facilitate communication. Interested subjects presented for initial baseline visit during which they completed informed consent and baseline assessments.

**Results:** Of 141 PCPs across 13 clinical sites 103 gave approval, 6 refused, 11 wanted to be contacted for each patient, and 21 have not responded. To date 560 letters have been mailed. 130 veterans called in and, of these, 37 were interested in participation. We contacted the remaining 430 veterans and, of these, 222 were not interested, 105 were interested and 103 we could not reach. 75 study visits have been scheduled (13% of letters mailed out). Of those who scheduled a study visit, 86% completed enrollment as planned, 8% rescheduled and 6% cancelled before the visit. On average, 3-4 visits occur each week with anticipated completion of enrollment in < 2 years as planned.

**Conclusions:** An "opt-out" recruitment strategy with PCP approval and easy "opt-out" options met expectations of IRB and yielded adequate enrollment numbers for this study.

*Funding:* Veterans Affairs Support

**PUB082**

**Wine Intake Is Associated with Fewer Cardiovascular Events in the National Health and Nutrition Examination Survey (NHANES)**
Tapan Mehta,[1] Pamela Mettler,[2] Kim McFann,[2] Diana I. Jalal.[1] *[1]Div of Renal Diseases and Hypternsion, Univ of Colorado, Aurora, CO; [2]Colorado Biostatistics Consortium and Colorado School of Public Health, Univ of Colorado Denver, Aurora, CO.*

**Background:** Chronic Kidney disease (CKD) is associated with an increased risk of cardiovascular disease. Wine intake is associated with a lower risk of cardiovascular disease in non-CKD populations. Although kidney and cardiovascular disease share many risk factors, such as insulin resistance and inflammation, no study to this date has examined the potential relation between wine intake and CVD in individuals with CKD.

**Methods:** This was a cross sectional analysis of 5876 participants in NHANES 2003-2006. Wine intake was categorized as: none, <1 glass/day, ≥1 glass/day. Cardiovascular disease (CVD) was defined as history of: cardiovascular disease, angina, myocardial infarction, or stroke. The analysis was then stratified based on presence of CKD defined as estimated GFR < 60 mL/min/1.73m$^2$ or albumin/creatinine ratio ≥30 mg/g.

**Results:** Of the 5876 participants, 671 had a history of CVD, 187 ingested <1 wine glass/day, and 20 ingested ≥1 wine glass/day. The group with CVD was older, had lower HDL- cholesterol, greater waist circumference, and a greater proportion of males, in addition to a greater prevalence of diabetes, kidney disease, and hypertension. Those who drank less than <1 glass/day had 0.5 odds (95% CI 0.4, 0.62) of having CVD than those who drank no wine ($p < 0.0001$). This relation remained significant after adjusting for demographics and other CVD risk factors (OR 0.63; 95% C.I. 0.50, 0.79; $p<0.0001$). Among individuals with CKD (n=1263), the odds of CVD was 0.72 (95% C.I. 0.54, 0.96; $p=0.03$) for those drinking <1 wine glass/day compared to non- drinkers after adjusting for demographics and CVD risk factors. The odds of CVD were not significantly different for those who drank ≥ 1 wine glass/ day compared to non- wine drinkers.

**Conclusions:** These data suggest that wine intake is associated with reduced odds of CVD in the general population with and without CKD. Further studies are needed to understand the mechanisms underlying this association particularly in individuals with CKD.

*Funding:* NIDDK Support

**PUB083**

**Study of Apolipoprotein E Polymorphism across Different Stages of Chronic Kidney Disease**
Marcelo Costa Batista,[1,2,3] Andrei Alkmim Teixeira,[1] Mauro Sergio Martins Marrocos,[1] Maria Dalboni,[1] Beata Marie Redublo Quinto,[1] Maria Eugenia F. Canziani.[2] *[1]Nephrology Div, Universidade Federal de Sao Paulo, Sao Paulo, SP, Brazil; [2]Federal Univ of Sao Paulo, Sao Paulo, SP, Brazil.*

**Background:** The burden of chronic kidney disease (CKD) is epidemic. Apolipoprotein E (APOE) polymorphism has been associated with its progression although scarce data are available in South American ethnic groups. **Objective**: We aim to determine apolipoprotein E polymorphism (APOE) alleles and genotypes distribution across different stages of CKD patients.

**Methods:** In a single-center cross-sectional study, patients were divided into 3 groups. Group 1: estimated glomerular filtration rate (eGFR) higher than 60ml/min/1,73m$^2$ (n=189); Group 2: eGFR between 15 and 60ml/min/1,73m$^2$ (n=222); Group 3: patients on renal replacement therapy (RRT) (n=224). APOE alleles and genotypes distribution were analyzed in relation to the different stages of CKD. ApoE genotypes were determined by PCR-restriction fragment length polymorphism (RFLP) analysis.

**Results:** We studied 615 patients (317 men, 51.0%), 63.8% were white, 40.3% diabetic and 15.6% smokers. Mean age was 60 ± 14 years. The homozygous genotype e3/3 was the most frequent genotype in all three groups (Group 1: 121 (72.9%), Group 2: 161 (75.6%) and Group 3: 144 (71.6%), p: NS) and even after considering just CVD patients (Group 1: 31 (72.1%), Group 2: 62 (73.8%), Group 3: 58 (69%), p: NS). We did not observe difference on alleles or genotype distribution among the studied population along the different CKD stages. However, among diabetic patients, e2 allele was negatively associated with more advanced stages of CKD (Group 1: 7 (13.1%) OR: 3.36, 95%CI: 1.15,9.77; Group 2: 8 (7.1%) OR: 1.19, 95%CI:0.41,3.42; Group 3: 0 (0%) OR: 0.9, 95%CI:0.86,0.95. This progressive inclination was demonstrated across the 5 CKD stages.

**Conclusions:** In diabetic patients, the authors demonstrated a negative association between e2 allele prevalence and more advanced stages of CKD.

**PUB084**

**A Phase IV, Randomized, Blinded Single-Center Study of the Effects of Calcitriol and Paricalcitol on Vascular Calcification in Chronic Kidney Disease Stages 3 and 4-Vitamin D and Coronary Calcification Study (VCOR)**
Sylvia E. Rosas,[1] Wei Yang,[2] Harold Litt.[3] *[1]Medicine, Univ of Pennsylvania, Philadelphia, PA; [2]Biostatistics, Univ of Pennsylvania, Philadelphia; [3]Radiology, Univ of Pennsylvania, Philadelphia, PA.*

**Background:** In animal models, calcitriol significantly increased the serum calcium-phosphate product and aortic calcium content, while paricalcitol had no effect. The objective of this randomized clinical trial was to determine the differential effect of calcitriol and paracalcitol on vascular calcification in patients with chronic kidney disease (CKD).

**Methods:** Inclusion criteria included patients with CKD and secondary hyperparathyroidism (SHPT) with any CAC naive to activated vitamin D therapy. We used the Wilcoxon rank-sum test to compare CAC progression between groups using the absolute change in Agatston unit (AU)/time between scans. We also evaluated the square root difference in CAC volume [sqrt(post) – sqrt (pre)]. Progressors were defined as those with a difference in volume > 2.5.

**Results:** Forty-four participants were randomized. The mean age(standard deviation) was 65.6 (9.3). Fifty-nine were male and two-thirds were African American. Fifty-seven percent had a history of diabetes (DM). The mean and median CAC score were 318.2 (632.7) and 140.2 AU at baseline, respectively.

We did not find any difference in the median CAC progression between the calcitriol vs. paracalcitol arms (33.7 vs.64.1 31, p=0.75). The square root of the difference method yielded similar results (1.34 vs. 1.56, p=0.75). There were no statistical differences between progressors between groups [2(10.5%) vs. 7 (33.3%), p=0.09]. The median CAC progression in non-DM was similar between the two arms [57.3 vs. 38.3, p=1.0]. However, the median CAC progression in those with DM appeared higher in the paracalcitol group but did not achieve statistical significance. [31.6 vs. 111.1, p=0.2]. (p=0.04 for test of interaction).

**Conclusions:** In CKD patients with secondary hyperparathyroidism naïve to activated vitamin D therapy, we were unable to detect differences in CAC progression between the two therapies. The effect of diabetes on treatment group needs to be explored in future studies.

*Funding:* Pharmaceutical Company Support - Abbvie

**PUB085**

**Perceptions of Risks in Chronic Kidney Disease among Patients, Clinicians and Administrators**
Helen Chiu,[1] Navdeep Tangri,[2] Ognjenka Djurdjev,[3,4] Brendan J. Barrett,[5] Brenda Hemmelgarn,[6] Francois Madore,[7] Claudio Rigatto,[2] Norman Muirhead,[8] Manish M. Sood,[2] Catherine M. Clase,[9] Adeera Levin.[4,10] *[1]PHCRI; [2]U of M; [3]PHSA; [4]BCPRA; [5]MUN; [6]U of C; [7]U de M; [8]WU; [9]MU; [10]UBC, Canada.*

**Background:** Clinical trajectories of patients with chronic kidney disease (CKD) are highly variable. Understanding the appropriate risk thresholds and time horizons associated with predicting risks of key outcomes (renal failure, cardiovascular (CV) events & death) is important in facilitating decision-making and addressing the needs of the patients and care providers. We aimed to determine the importance of specific time frames for prediction of key outcomes from patients, nephrologists and renal administrators, and performed a needs assessment for risk prediction tools among these groups.

**Methods:** Online surveys were developed for each group of stakeholders and deployed nationally through the Kidney Foundation of Canada, provincial renal networks and the Canadian Kidney Knowledge Translation and Generation Network. Anonymous responses gathered over a 4-month period were descriptively analyzed.

**Results:** Over 270 respondents across Canada participated in the surveys. Patients deemed personal risks of needing renal replacement therapy and developing heart disease as similarly important over all time horizons (1-15 years). Both nephrologists and administrators felt that the time horizons of 1-5 years are the most relevant. The majority of nephrologists were not satisfied with their current ability to predict the progression to renal failure, CV events and death, with the ability to predict CV events and death being the most dissatisfying. All groups indicated willingness to use risk scores with improved accuracy, if available, to predict specific outcomes.

**Conclusions:** Patients, clinicians and administrators have slightly different time horizons that they deem important for predicting outcomes in CKD; however, all agree that there is a need to improve risk prediction. These perspectives from patients and other stakeholders provide valuable information for developing future research and tools for predicting patient-centered outcomes in the CKD population.

**PUB086**

**Patient Centered, Multidiciplinary Clinic for Pre-End Stage Renal Disease Care: A Process Improvement Project** Nicole Piero,[1] Julia Hennessy,[1] Loretta Simbartl,[1] Charuhas V. Thakar.[2] *[1]VA Medical Center; [2]Univ of Cincinnati.*

**Background:** Changes in U.S. healthcare system necessitates creative resource use while providing patient-centered care. A gap analysis within our hospital (2006-2010) revealed that 700/42,984 patients, who had ≥ 1 outpatient creatinine, had glomerular filtration rate (GFR) < 30 ml/min/1.73m2. 333/700 (48%) were evaluated in renal clinics, and 116/333 (35%) were seen by one additional clinic (nutrition, education, vascular access, social work) for ESRD preparation. We report a process improvement initiative by converting the need for multiple appointments to a multidisciplinary, pre-ESRD clinic.

**Methods:** Since Jan 2012, after a renal evaluation, patients with a GFR <30 are enrolled in this pre-ESRD clinic representing all disciplines, and led by a nurse practitioner. Patients learn about kidney disease, dialysis choices, transplant, nutrition, payment, advance directives, and conservative therapy. Since Oct 2012 patients also complete a quality of life (KDQOL) and nutrition evaluation.

**Results:** In a sample of 56 males mean age was 69 years, mean GFR of 20.5 ml/min/1.73m2. After pre-ESRD education, of the 56 patients 64% favored hemodialysis, 5% peritoneal dialysis, 7% no dialysis or hospice, and 24% were undecided. Outcomes for all 56 were: 25% have started hemodialysis (incident fistula rate = 36%), 7% died before dialysis, and remaining 68% are under follow-up care. 8/56 are being evaluated for kidney transplant. 19/56 completed the KDQOL of which 39% and 58% reported severe activity limitation or limitation to climb stairs respectively. 15/56 completed nutritional assessment: 67% ate out <2 times/week whereas 33% ate out 3 – 10 times per week; 34% ate > 4 oz meat portions/meal; and 60% ate frozen or canned food > 3 times/week. 15/56 patients completed 24-hour urine collection: Urea ranged 4.7-14.3 g/24h, sodium ranged 67-294 mmol/24h, and protein ranged 0-9.3 g/24h.

**Conclusions:** Over 1-year, such an initiative could result in an efficient use of resources by reducing the number of required appointments by over 50%. Moreover, a patient-centered program provides individualized care, and an avenue for nutritional intervention in patients with advanced CKD.

*Funding:* Veterans Affairs Support

**PUB087**

**Primary Care Physicians' Own Exercise Habits Influence Exercise Counseling for Patients with Chronic Kidney Disease** Yoshiyuki Morishita, Yasuhiro Ando, Shigeaki Muto, Eiji Kusano. *Nephrology, Internal Medicine, Jichi Medical Univ, Shimotsuke, Tochigi, Japan.*

**Background:** We investigated primary care physicians' exercise counseling practices for chronic kidney disease (CKD), and the association of these physicians' own exercise habits with exercise counseling.

**Methods:** The population of this cross-sectional study was 3310 medical doctors who graduated from Jichi Medical University from 1978 to 2012. The study instrument was a self-administered questionnaire to investigate their age class, specialty, workplace, exercise habits, and practices of exercise counseling for CKD.

**Results:** 581 (64.8%) medical doctors practiced the management of CKD among a total of 933 responses. These 581 medical doctors were defined as CKD primary care physicians and Their answers were analyzed. CKD primary care physicians' own exercise habits (frequencies and intensities) were as follows: frequencies: daily, 71 (12.1%); ≥2–3 times/week, 154 (26.5%); ≥1 time/week, 146 (25.1%); and ≤1 time/month, 176 (30.2%); intensities: high (≥6 Mets), 175 (30.1%); moderate (4–6 Mets), 132 (22.7%); mild (3–4 Mets), 188 (32.3%); very mild (<3 Mets), 47 (8.1%); and none, 37 (6.4%). CKD primary care physicians' exercise recommendation (general) levels for CKD patients were as follows: high, 31 (5.3%); moderate, 176 (29.7%); low, 256 (44.0%); and none, 92 (15.8%). The details of exercise recommendations (frequency and intensity) were as follows: frequency: daily, 23 (10.8%); ≥2–3 times/week, 144 (70.4%); ≥1 time/week, 25 (12.3%); and ≤1 time/month, 0 (0%); intensity: high (≥6 Mets), 1 (0.5%); moderate (4–6 Mets), 62 (30.5%); mild (3–4 Mets), 132 (66.0%); very mild (<3 Mets), 3 (1.5%); and none, 3 (1.5%). The CKD primary care physicians' exercise recommendations (general) for CKD patients were significantly related to their own exercise frequency ($p<0.01$), but they were not related to their age, specialty, workplace, or exercise intensity. Furthermore, the CKD primary care physicians' exercise recommendations (frequency ($p<0.01$) and intensity ($p=0.01$)) were related to their own exercise frequency.

**Conclusions:** CKD primary care physicians' own exercise habits influence their exercise counseling for CKD patients.

**PUB088**

**Kidney Health in Volunteers Recruited as Study Controls** Samer Rateb Abbas,[1,2] Cassandra Cartagena,[1] Fansan Zhu,[1] Peter Kotanko,[1] Nathan W. Levin,[1] Mary Carter,[1] Stephan Thijssen,[1] Caroline M. Williams,[1] Cesar Flores-Gama.[1] *[1]Nephrology, Renal Research Institute, New York, NY; [2]Nephrology, Beth Israel Medical Center, New York, NY.*

**Background:** Little is known about kidney function in volunteers recruited as healthy controls. Here we report kidney function in such individuals.

**Methods:** We solicited the participation of healthy volunteers using posters and newspapers. Telephone callers underwent a structured 22 questions to explore their eligibility. Volunteers who passed the telephone interview underwent clinical assessment (, height, weight, blood pressure) and measurement of serum creatinine, BUN, urinalysis) 1-4 weeks later. Estimated glomerular filtration rate (eGFR) was computed with the CKD-EPI equation. Albumin-creatinine ratio (ACR) was calculated as urine albumin/urine creatinine (mg/g). Systolic blood pressure SBP was recorded three consecutive times in a sitting position; subjects with average SBP > 140 mmHg were considered hypertensive.

**Results:** Based on the phone interview, almost 50% of 180 volunteers were excluded because of kidney, cardiac, or malignancy-related conditions. Ninety-one participants (age 57±10 years; 49 female) were eligible. Of these 18 participants were excluded, mostly because of absent blood and urine samples. Results obtained in the remaining 73 participants are shown in Table 1.

| | eGFR>90 N=20 | eGFR 60-89 N=43 | eGFR<60 N=10 | ACR>30 N=5 | Hypertensive N=9 |
|---|---|---|---|---|---|
| Black [N]<br>White [N] | 10<br>10 | 17<br>26 | 2<br>8 | 1<br>4 | 6<br>3 |
| Male [N]<br>Female [N] | 10<br>10 | 21<br>22 | 4<br>6 | 1<br>4 | 5<br>4 |
| Age [year] | 50±7 | 60±9 | 69±9 | 62±9 | 63±12 |

eGFR in mL/min/1.73 m²

Age was significantly different between eGFR groups ($p = 0.001$, 1-way ANOVA).

**Conclusions:** Our results indicate the presence of unrecognized kidney impairment in a notable fraction of normal healthy volunteers who identify themselves as healthy and who pass a telephone interview screening aimed at identifying and excluding non-healthy volunteers. Since decreased function of unknown cause is frequent in older people this should be considered in recruiting apparently healthy volunteers. Laboratory tests are indispensable to define healthy controls.

**PUB089**

**Prevalence of Chronic Kidney Disease in an Adult Population from Southern Brazil: Results of Campo Largo Study** Marcelo M. Nascimento,[1,3] Ana Paula Piccoli,[3] Miguel C. Riella.[2,3] *[1]Dept de Clinica Médica, Universidade Federal do Parana, Curitiba, Brazil; [2]Post Graduation Program, Pontifícia Universidade Católica do Parana, Curitiba, Brazil; [3]Pro-Renal Foundation, Scribner Institution, Curitiba, Brazil.*

**Background:** There is a rising incidence of chronic kidney disease (CKD) worldwide. However, in Latin America there are few studies which could estimate the real prevalence of CKD in the general population.

**Methods:** From April 2009 to December 2011 the Pro-Renal Foundation screened 5216 individuals for the presence of CKD in the city of Campo Largo (a city situated in the southern Brazil, with an estimated population of two hundred thousand people). Individuals younger than 18 years were excluded from the study. Personal and medical history data through a specifically designed questionnaire was obtained as well as blood and urine samples were collected to estimate eGFR with microalbuminuria (MA) status. All participants were weighed and had their height measured. The eGFR was estimated by Modification of Diet in Renal Disease MDRD equation formula.

**Results:** The total cohort included in this analysis was 5216 subjects. The mean +/- SD age of all participants was 46+/- 15 years (range 18-87 yrs) and 64 % of them were females. The mean eGFR of whole cohort was 89 +/- 22 mL/min/1.73 m² and the prevalence of MA and reduced eGFR (stage 3-5) was 4 % and 9%, respectively. The prevalence of MA and CKD were all significantly higher among adults with diabetes and hypertension. Finally, in multivariate analysis only age (HR=5.25 (95% CI; 4.18-7.60) ($p<0.001$), DM (HR= 1.78 (95% CI (1.35-3.34) ($p<0.001$), hypertension (HR= 1.78 (95% CI;1.35-3.34) ($p<0.001$), BMI (>30kg/m2) (HR= 1.81(95% CI ;1.34-3.45) ($p<0.01$) were independently predictors for the presence of CKD.

**Conclusions:** CKD risk factors must be addressed to all primary care physicians who take care of hypertensive and diabetic patients. Moreover, in Brazil, it is necessary to establish a health national strategy for the treatment and prevention of CKD.

**PUB090**

**Improving Chronic Kidney Disease Detection and Care in a High Risk Underserved Population** Miguel A. Vazquez,[1] Ruben Amarasingham,[2] Adriana Valencia,[1] Beverley Adams-huet,[1] Adeola O. Jaiyeola,[2] Blake R. Barker,[1] Noel O. Santini,[2] Ying Ma,[2] Javier A. Velazquez,[2] Vibin Roy,[1] Zhiyi Zhou,[2] George Oliver,[2] Brett Moran,[2] Christopher Y. Lu,[1] Robert D. Toto.[1] *[1]Internal Medicine, UT Southwestern Medical Center, Dallas, TX; [2]Parkland Hospital and Health Systems, Dallas, TX.*

**Background:** Many patients with chronic kidney disease (CKD) are not receiving treatments shown to be effective for improving outcomes. The main purpose of this study is to facilitate detection of CKD and implement established treatments for slowing the progression of CKD and its associated complications.

**Methods:** We developed a collaborative primary care and nephrology model for CKD care enhanced by a novel technology information platform, PIECES (Parkland Intelligent e-Coordination Evaluation System), that makes it possible to use information in the electronic health record and electronic databases to identify patients with CKD, implement evidence-based therapies and evaluate responses to interventions. CKD was defined by eGFR <60 ml/min and/or albuminuria or proteinuria. The expected number of study participants is 500, and 72 patients have been enrolled so far and provided baseline data.

**Results:** Initial review reveals 62% of patients in clinic with established CKD had not been previously diagnosed with CKD. Among the 72 patients enrolled so far, at initiation of the study less than 40% with CKD have blood pressure control within established guidelines. More than 75% of patients are receiving ACEIs and statins. The table below summarizes achievement of goals on recommended clinical measures at baseline.

| Clinical Measures | Percent at Goal (n=72) |
|---|---|
| Systolic BP <130 mmHg | 37% (n=26) |
| Diastolic BP <80 mmHg | 62% (n=44) |
| ACEI/ARB | 86% (n=62) |
| Statin | 75% (n=54) |

**Conclusions:** Using a primary care and nephrology collaborative model enhanced by novel information technology, we detected previously undiagnosed CKD. We also found a large proportion of patients are not getting the potential benefit of BP control and ACEi/ARB known to slow progression of CKD and prevent cardiovascular complications. We are now implementing accepted practices for CKD in our patients and monitoring prospectively compliance with clinical management.

*Funding:* NIDDK Support

## PUB091

**Serving the Underserved: A Case Study of Community Screening by the Kidney Disease Screening and Awareness Program (KDSAP)**

Michael Ho-Young Ahn,[1] Manjinder Singh Kandola,[1] Laura C. Polding,[1] Andrew J. Pan,[1] Ryan Lindeborg,[1] Jennie Kuo,[2] Kenneth Lim,[2] Tzongshi Lu,[2] Li-Li Hsiao.[2] *[1]Harvard College, Cambridge, MA; [2]Brigham and Women's Hospital, Boston, MA.*

**Background:** Chronic Kidney Disease (CKD) is a public health problem affecting 27 million Americans. Early detection of CKD can prevent premature kidney failure. The prevalence of CKD and its risk factors, including diabetes, hypertension, and obesity, have increased within minorities, in part due to less access to care and no insurance. The Kidney Disease Screening and Awareness Program (KDSAP) strives to reduce the incidence of CKD by reaching out to underserved communities and providing kidney health screenings free of charge.

**Methods:** The screening was held in New Brunswick, NJ, and was modeled after the National Kidney Foundation's Kidney Early Evaluation Program. Diagnostic evaluations included body mass index, blood pressure, blood glucose, and urinalysis. Participants were also asked to report their health insurance status. Student T-test and Fisher's exact test were used for data analysis.

**Results:** A total of 70 community members participated in the screening (66% Chinese, 21% Hispanic, 9% African American, and 4% White). Among the participants, there was a high prevalence of CKD risk factors: overweight or obese 61% (19 and 24, respectively); hypertension 61% (42), hyperglycemia 64% (45), hematuria 46% (32), proteinuria 9% (6) and both hematuria and proteinuria 6% (4). 57% of the participants lacked health insurance (40). Furthermore, 56% (22) of uninsured individuals presented with hematuria versus 32% (10) of those insured. Lack of insurance coverage was associated with 2.6-fold greater odds of developing hematuria ($p<0.05$).

**Conclusions:** We detected a high prevalence of obesity, hypertension, hyperglycemia and hematuria at a single kidney health screening event in New Brunswick, NJ. There is a significant correlation between the lack of health insurance and hematuria in this unique population. Ultimately, the data support that KDSAP's free community kidney health screening efforts can serve as an effective tool to detect CKD risk factors early, especially among underserved communities.

*Funding:* Private Foundation Support

## PUB092

**CKD Prevention: It Is Possible**

Maria Eva Baro-Salvador,[1] Carlos Sillero,[2] Sonia Baldoví.[2] *[1]Nephrology, Hospital de Torrevieja y Hospital de Vinalopo, Torrevieja/Elche, Alicante, Spain; [2]Nephrology, Hospital de Torrevieja.*

**Background:** Chronic Kidney Disease is an emerging problem that affect, by EPIRCE study , at the 10% of the adult spanish population. In our population, it represents more than 40,000 affected people and is for this reason that we needs to adapt to us at the new healthcare model scenario . Although various chronic diseases have developed chronic plans has not been so in the case of CKD as accurate a number of prerequisites such as continuity of care between primary and specialty, which is rarely given. We have developed a chronic patient care as the Pyramid of Kaiser, which stratifies the population by levels of chronicity. The correlation between these levels of chronicity and stages of CKD has allowed the development of a Plan for the ERC approach that collects from disease prevention to case management more complex levels.

Objective: Create a comprehensive plan for diagnosis, treatment and monitoring renal disease at its different stages from prevention to End-Stage Renal Disease (ESRD) homogeneously and to implement it in two departments of health (16 GP´s centers and two general hospitals).

**Methods:** We made a multidisciplinary working group for this purpose by defining a CKD Clinic Guide unified criteria and concise definition, treatment and referral of CKD easily accepted by all health personnel. Stratification of renal disease in different stages and their correlation with chronic patient stratification according to the Kaiser pyramid, has helped define and develop goals, interventions, algorithms and indicators.

**Results:** In February of 2013 took place the presentation to all health professionals in both departments (management staff, physicians and nurses). In March the program officially started and we hope to analyze initial results in August but in this moment , after 3 months, we have detected more than 700 news patients only in the Third Level .

**Conclusions:** Developing a comprehensive approach to Plan CKD which perform an adaptation of the diagnosis, treatment and monitoring early disease at all levels gets evenly reduce CKD progression from one stage to another with the resulting gain for the patient and the healthcare system.

*Funding:* Private Foundation Support

## PUB093

**A Screening Model of Correlation Factors for Type 2 Diabetes Mellitus with Massive Proteinuria**

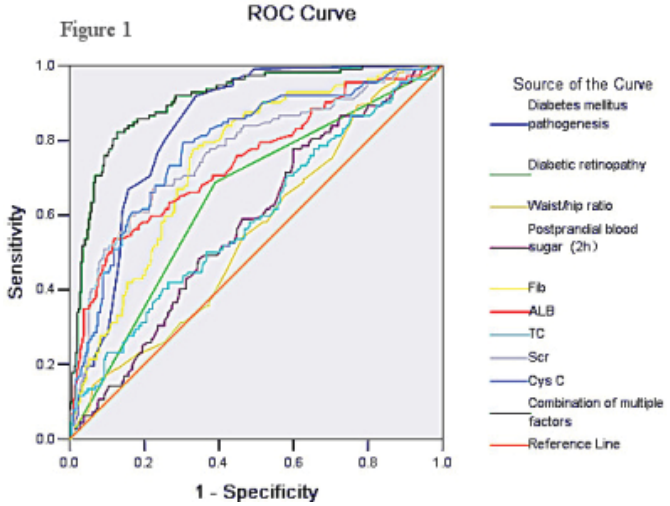
Wenbo Zhao, Yan-ru Chen, Jia-ling Rao, Hui Peng, Jun Zhang, Tan-qi Lou. *Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

**Background:** To establish the logistic regression screening model of the DKD with massive proteinuria crowd, and validate the effectiveness of the model.

**Methods:** Collected 605 type 2 diabete patients with microalbuminuria and massive proteinuria were treated during January 2008 to November 2012. 605 cases were randomized to the first group(404 cases) for modeling group, and the second(201 cases) to validation. The modeling group was including 292 cases with microalbuminuria and 122 with massive proteinuria. The eGFR was Calculated (MDRD Formula, all more than30 ml/min·1.73m$^2$). Statistical analysis used the SPSS15.0 software. Univariate and multivariate Logistic regression analysis were used to estabilsh regression equation.sensitivity and specificity of the model was analysised by ROC curves, diagnosis cut-off point was set on the maximum of youden index. Put the validation group into the model, to compare the difference of areas of ROC curve between model group and validation group with Z test.

**Results:** After multi-factor analysis, the duration of diabetes, DR, waist-to-hip ratio, postprandial blood glucose 2 h, Fib, ALB, TC, Scr, CysC were the 9 main relative factors for massive proteinuria. AUC of the combination of multiple main relative factors was largest (0.907), and the cut-off value was 0.3501 (Sensitivity 82.1%, Specificity 87.3%). The data of validation group applied to the model, the AUC was 0.850. To Compare the areas of ROC curves, that were no statistical difference (***P***>0.05).

Figure 1




**Conclusions:** The combination of multiple factors screening model of DKD with massive proteinuria was effective. The performance of the screening was stable. It can be used in clinical according to validation. Early prevention could be implement in the patients who are close to the diagnosis cut-off point.

*Funding:* Government Support - Non-U.S.

## PUB094

**Healthy Behavior Is Low in Patients with Pre-Dialysis Chronic Kidney Disease**

Julie A. Wright Nunes,[1] T. Alp Ikizler,[2] Kerri L. Cavanaugh.[2] *[1]Nephrology, Univ of Michigan, Ann Arbor, MI; [2]Nephrology, Vanderbilt, Nashville, TN.*

**Background:** Preventing chronic kidney disease (CKD) progression and complications could be enhanced by patient engagement in healthy behaviors.

**Methods:** Adult patients with CKD Stages 1-5 not on maintenance dialysis were enrolled in a cross-sectional study from April 2009 to October 2010. Patients were asked how many days over the past week they performed health behaviors on topics including healthy eating, medication adherence, smoking, and physical activity.

**Results:** 556 patients were enrolled. The mean (SD) age was 57 (16) years. 53% were male, 81% were Caucasian, 19% had limited health literacy, and 78% had CKD Stage 3-5. Twenty-two percent reported eating healthy, 16% ate at least 5 servings of vegetables/fruits, 10% avoided high fat foods, 14% had at least 30 minutes of daily physical activity, 80% took their medications exactly as prescribed, and 91% did not smoke. In univariate analysis older patients had a higher odds of reporting eating healthy every day (OR 1.03[CI 1.01,1.04]; $p<0.01$), including eating at least 5 servings of fruits/vegetables (1.02[1.01,1.04]; $p<0.01$). Non-white patients were less likely to report eating healthy (0.50[0.30,0.90];$p=0.03$) and to take medications as prescribed (0.50[0.30,0.80];$p<0.01$) versus white patients. In analyses adjusted for age, sex, race, kidney function, income, and health literacy, older patients continued to show higher odds (1.03[1.01,1.04]; $p<0.01$), and non-white race lower odds (0.50[0.30,1.004];$p=0.05$) of healthy eating. Older patients were less likely to report daily physical activity (0.98[0.96,0.99];$p=0.02$). Non-white patients had 0.50[0.30, 0.80];$p=0.01$) lower odds of taking medications as prescribed. Patients with an eGFR>60 had higher odds (2.80[1.09,7.20];$p=0.03$) of not smoking vs. patients with a lower eGFR.

**Conclusions:** Adherence to many health behaviors were low for our cohort of Stage 1-5 CKD patients not on dialysis. Older patients were more likely and non-white patients less likely, to report healthy eating behaviors. More work is needed to determine reasons for the racial differences in health behaviors and potential strategies to improve healthy behavior patterns.

*Funding:* NIDDK Support, Other NIH Support - T32, Private Foundation Support

## PUB095

**Improving Care of Patients with Kidney Disease: Use of Mobile Telephony to Improve Lifestyle and Adherence** Alfonso M. Cueto-Manzano,[1] Hector Gallardo-rincon,[2] Héctor R. Martínez Ramírez,[1] Laura Cortes-sanabria,[1] Enrique Rojas-Campos,[1] Petra Martínez,[3] Jose I. Cerrillos,[3] Jorge Andrade-Sierra,[3] Miguel Medina Perez.[3] *[1]UIMER, Hospital Especialidades, CMNO, IMSS, Guadalajara, Jalisco, Mexico; [2]Dirección de Soluciones Operativas, Instituto Carlos Slim de la Salud, Mexico, DF, Mexico; [3]Dept Nephrology, Hospital Especialidades, CMNO, IMSS, Guadalajara, Jalisco, Mexico.*

**Background:** Opportunities for new technologies (as mobile phones) to play formal role in health services are increasing. However, no study has been performed in kidney disease (KD). Aim: To develop and test a mobile phone program to improve lifestyle, adherence and clinical outcome of KD patients.

**Methods:** In collaboration, and with technology developed by the Carlos Slim Health Institute, a program based on mobile phone text messages and reminders was developed, and tested in a pilot study (Jun-Oct 2012). Text messages about KD risk factors (50), healthy lifestyle (40), and to improve adherence and follow-up appointments (35) were generated and sent to patients agreed to participate (recipients of a functioning kidney transplant performed at our Hospital, included when they were discharged). Patients were treated by their nephrologists according to clinical practice standards. A satisfaction questionnaire was applied at end of follow-up.

**Results:** Twenty-three patients (age 33±13 yrs, 43% women) had a follow-up of 58 (41-76) days. Patients received 0.8 (0.3-1) text messages/day, 3 (2-3) medication reminders/day, and 1.1 (0.3-2) appointment reminders/month. Clinical and biochemical data are shown in the table.

| Variable | Baseline | Final |
|---|---|---|
| Hemoglobin (g/dL) | 11 (10-13) | 13 (12-14)* |
| Creatinine (mg/dL) | 0.9 (0.8-1.1) | 1.0 (0.9-1.2) |
| Systolic Blood Pressure (mmHg) | 130 (118-137) | 120 (115-125)* |
| Diastolic Blood Pressure (mmHg) | 80 (72-82) | 78 (70-80) |

*$p<0.05$ vs baseline

In a 0-10 scale, patients considered utility of messages as 9.6±0.7, medication reminders 9.8±0.5, and appointment reminders 9.8±0.6.

**Conclusions:** More than 100 mobile phone messages were successfully developed and tested to improve lifestyle, adherence and clinical outcome of KD patients. Future research should address scalability of this intervention and explore association with changes in attitudes and behaviors.

## PUB096

**Chronic Kidney Disease Education and Impact on Dialysis Modality Choice and Dialysis Access** Doris Sofia Galina Quintero, Clare Nichols Lyas, Russell Griffin, Jane S. Davis, Dana Rizk, Eric L. Wallace. *Univ of Alabama at Birmingham.*

**Background:** Despite the morbidity and mortality associated with the initiation of dialysis with intravascular catheters (IVC), IVC rates of incident patients remains over 60%. Home modalities utilization remains low despite the associated benefits. Perhaps a contributing factor for these data is patient education. There is limited information about how Chronic Kidney Disease Education (CKDE) affects modality selection and placement of permanent access. Although education may be documented, its effectiveness is unclear. We analyze the effects of the UAB CKDE class on modality selection and permanent access placement.

**Methods:** Clinical and demographic data on all patients who attended CKDE class from 2010 through 2012 were collected through chart review. Data on all dialysis accesses placed up until June 2013 was collected on these patients utilizing a preexisting dialysis access database. Chi-square test and ANOVA statistical analysis were used for categorical and continuous variables respectively.

**Results:** 174 patients were analyzed. At the time of class attendance, patients had a mean glomerular filtration rate (GFR) of 19 ml/min/1.73 $m^2$ (Standard Deviation (SD) of 6). 50.3% were diabetic. 70/174 (40.2%) had no access placed at the time of analysis.This group had higher GFR compared to all other access types ($p<0.05$). Length of time from class to first access was 174 days (SD 192). Of patients with access, 11.5% had an IVC,12.5% an AV graft; 51% an AV fistula; and 25% a peritoneal dialysis (PD) catheter as first access. There was no difference in age, gender, or race with regards to type of access placed.

**Conclusions:** When compared to USRDS data, patients who attended CKDE class were significantly more likely to choose PD as their modality of choice and much less likely than national average to have an IVC as their initial dialysis access.

*Funding:* Other NIH Support - 5UL1R025777-Center for Clinical and Translational Sciences

## PUB097

**Cost Analysis of Participatory Educational Intervention for Prevention, Diagnosis and Treatment of Early Chronic Kidney Disease** Laura Cortes-sanabria, Victor Omar Frías-navarro, Héctor R. Martínez Ramírez, Alfonso M. Cueto-Manzano. *Unidad de Investigación Medica en Enfermedades Renales, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico.*

**Background:** Our group previously demonstrated that adequate training to family physicians (FP) successfully preserved renal function of patients with DM2. However, no previous study has evaluated the economic impact of such an educative intervention.

AIMS: To determine and evaluate the direct costs (DC) of an educative-participative intervention (EPI) to FP and a multidisciplinary team (MDT) addressed to increase their clinical competence (CC) in the management of patients with DM2 and early nephropathy (EN).

**Methods:** Economic evaluation study. EPI included review of Clinical Practice Guidelines of early CKD and discussion of real clinical cases, 5 h/wk during 3 months. CC was evaluated with a previously validated instrument, applied at the beginning and the end of the EPI (maximum value of CC 150 points). The pattern of service utilization was identified; costs were calculated for each activity, according to the Institutional Unit Cost System (IMSS). Only direct medical costs (DMC, all monetary costs incurred during the EPI) were considered, and they are shown in US dollar. *Statistical Analysis:* Mac-Nemar $Chi^2$, and Wilcoxon test. Bootstrap analysis to assess uncertainty in the estimates of CD. The cost-effectiveness ratio was estimated.

**Results:** The CC of the FP and MDT was significantly increased at the end of EPI (Basal 61 [41-71] vs final 89 [76-101], $P<0.0001$). The total CD distribution is shown in the table.

| Resource | Costs US Dollar | % |
|---|---|---|
| Time of doctor attendance visit | 32,714 | 95 |
| Printing of Clinical Practice Guidelines of early CKD | 1,279 | 2 |
| Office supplies | 378 | 0.6 |
| Printed materials | 257 | 0.4 |
| Teacher salaries | 403 | 1.0 |
| Audiovisual equipment | 721 | 1.0 |
| Total Cost | 35,746 | 100 |
| Average training cost per person | 760 | |

The cost-effectiveness ratio of the educational intervention was 0.89 (better result = 1.0).

**Conclusions:** Implementing an EPI in the primary health-care is a cost-effective measure to reduce the burden of kidney disease.

## PUB098

**National Kidney Centre Report on Kidney Screening Programm Risk Factor for Chronic Kidney Disease – A Survey from Ranibas, Gulmi Nepal** Rishi Kumar Kafle. *National Kidney Center, Kathmandu, Nepal.*

**Background:** National kidney center is providing dialysis to increasing numbers of patients every year. kidney disease screening programm along with awareness campaign is carried out from time to time.

**Methods:** Team of experts from National Kidney centre visited village Ranibas of Gulmi district to conduct kidney disease screening and awareness camp. Infrmation about camp was publishized through media before the team arrived there. On three consecutive days all who came for screening were evaluated. Every individual registered,his /her BMI, BP, was measured. Urine dipstick test for protein and sugar was done there only. survey form filled with the hep of volunteers to know medical history, personal habits especially water intake; smoking; tobacco chewing; alcohol consumption and exercise done by that individual. all data were entered into register for evaluation.

**Results:** More than 33 % of 731 persons examined were overweight
17 % of examined had raised blood pressure
around 5 % examined were suspected to have Diabetes
around 6% examined had proteinuria
majority had healthy kidneys
98% had access to aerated drinks and use it
94 % do not exercise except their regular work.

**Conclusions:** Even in a remote village of Nepal there are enough risk factors for development of kidney disease. Programm to educate not only about kidney diseases or others education to remain healthy is needed. Need to make people aware about their health and ways how they can prevent serious disease by simple screening method which is not costly.

*Funding:* Clinical Revenue Support

## PUB099

**High Self-Efficacy Is Associated with More Self-Care in Patients with Chronic Kidney Disease** H. Omer Ikizler, Jillian Molli Berkman, Kenneth A. Wallston, T. Alp Ikizler, Kerri L. Cavanaugh. *Vanderbilt Univ.*

**Background:** Self-care behaviors, dietary and medication adherence, physical activity, and health care engagement, are important to optimize chronic kidney disease (CKD) treatment. To identify potentially modifiable factors to promote effective self-care, we investigated the relationship between patients' self-perceived efficacy and frequency of CKD self-management behaviors.

**Methods:** Adult patients with CKD (eGFR<60) from one academic nephrology clinic were enrolled in a cross-sectional study from May 2012 to July 2013. Patient surveys assessed kidney knowledge, health literacy, frequency of CKD self-care, and their self-perceived efficacy in managing their kidney disease using the Perceived Kidney Disease Self-Management Scale (PKDSMS).

**Results:** Among 252 patients enrolled, mean (SD) age was 58 (12) years. 42% were male, and 83% were Caucasian. Mean (SD) self-efficacy score was 2.58 (0.73) out of 5; mean self-care score was 26.7 (6.4) out of 39. In univariate analysis, higher subjective numeracy (rho(r)=0.15, p<.05), and greater perceived (r=0.19, p<0.01) and objective kidney knowledge (r=0.23, p<0.01) were correlated with higher self-efficacy. Self-care behaviors were also correlated with higher self-efficacy: healthier food (r=0.22, p<0.01), physical activity (r=0.20, p<0.01), adhering to a kidney diet (r=0.14, p<0.05), not missing doctor visits (r=0.13, p<0.05), and taking medications as prescribed (r=0.22, p<0.01). Total overall self-care score was also positively correlated (r=0.33, p<0.01). In an analysis adjusted for age, gender, race, education, perceived and objective kidney knowledge, the association between self-efficacy and overall self-care behaviors persisted [β (95%CI): 0.21 (0.10 – 0.31); p<0.001]. Furthermore, higher self-efficacy was also associated with greater satisfaction with physician communication (r=0.30; p<0.01).

**Conclusions:** Self-efficacy is easily assessed and is moderately to strongly associated with a variety of self-care behaviors in chronic kidney disease. Interventions to improve self-efficacy specific to CKD may increase participation and effectiveness of self-care, enhance patient-centered communication, and ultimately improve clinical outcomes.

*Funding:* NIDDK Support, Private Foundation Support

## PUB100

**A Bioreactor as a Long Term Perfusion Model** Vera Jankowski,[1] Horst-Dieter Lemke,[2] Joachim Jankowski.[1] *[1]Charite, Berlin, Germany; [2]Excorlab, Obernburg, Germany.*

**Background:** Metabolic stimuli, pressures and hemodynamic flows are major mediators of vascular plasticity. In this study, we have developed an *in vitro* bioreactor to investigate cell-specific interactions, molecular mechanisms as well as time-dependent effects under physiological hemodynamic flow conditions. This bioreactor termed "*artificial artery*" can be used for screening of potential arterio-protective substances, pro-arteriogenic factors, and for investigating biomarkers of vascular diseases in CKD.

**Methods:** The bioreactor is built up out of 4 hollow fibre membranes colonised with primary human umbilical vein endothelial cells (HUVECs) on the inside and primary human arterial smooth muscle cells (HUASMCs) on the outside. By means of fluorescent staining as well as immunocytochemistry of reference proteins for endothelial cells and smooth muscle cells, a hydrophobic polypropylene membrane was identified as being the optimal polymer for co-colonisation.

**Results:** Applying defined arterial flow conditions, metabolic exchange, and cross-talk of endothelial and smooth muscle cells through the microporous structure of the hollow fibre, capillaries mimic physiological *in vivo* conditions of the vasculature and is therefore of high relevance for further studies. Chromatographic methods and subsequently mass spectrometry analyses as well as expression analyses of shear stress regulated genes proved our system to be viable and functional up to five days.

**Conclusions:** The "*artificial artery*" provides a solid *in vitro* model to test pharmacological active compounds for their impact on arterio-damaging or arterio-protective properties on vascular response in CKD.

## PUB101

**Renal-Coloboma Syndrome Associated with Arnold-Chiari Malformation Type 2 and Proximal Renal Tubular Acidosis** Heino R. Anto, Tatyana Tolchinsky, Fnu Jaipal. *Dept of Nephrology, St. John's Episcopal Hospital, Far Rockaway, NY.*

**Background:** A 28 y/o patient was admitted for corrective surgery involving Arnold-Chiari malformation type 2. Besides this malformation the patient also had optic disc dysplasia and syringomyelia and was treated with topiramate for seizures. On this admission laboratory serum values were as follows : sodium 140 mEq/L, potassium 3.9 mEq/L, chloride 116 mEq/L, co2 17 mEq/L, BUN 35 mg/dL, creatinine 2-3 mg/dL, and anion gap of 7. The patient was also found to have 3 + proteinuria, microhematuria, and hypoplasia of the left kidney on sonography.The patient ultimetly developed end-stage renal failure and was being evaluated for renal transplant.

This case exemplifies the characteristics of renal-coloboma syndrome. While this syndrome has been reported with Arnold-Chiari malformation type 1, we believe this is the first case reported with type 2. The progressive renal failure seen in this case was likely due to the development of focal segmental glomerulosclerosis, frequently seen in renal-coloboma syndrome and resulted from increased intra-glomerular pressure associated with renal hypoplasia, leading to ESRD and renal transplantation.

Renal coloboma syndrome is an autosomal dominant disorder due to a mutation of the PAX 2 gene located on chromosome 10 q 24.3- q 25.1.The PAX gene is vital for the proper development of both eyes and kidneys; and when mutated leads to improper development of these organs. The diagnosis is made by renal ultrasound, kidney biopsy, family history, opthalmologic exam, and genetic testing.

This patient also presented with renal tubular acidosis, a finding not associated with renal-coloboma syndrome, but likely due to topiramate; which inhibits carbonic anhydrase leading to reduced bicarbonate reabsorption by the proximal tubule.

## PUB102

**Evidence from Cytogenomic Aberrations Suggests Complicated Genetic Mechanisms in the Development of Multicystic Dysplastic Kidney** Tianjian Chen, Arivudainambi Ramalingam, Renfang Song, Adam T. Janssen, Graeme James Preston, Dominique Monlezun, Ihor V. Yosypiv. *Pediatrics, Tulane Univ, New Orleans, LA.*

**Background:** Multicystic dysplastic kidney (MCDK) is considered to be a nonheritable form of congenital anomaly of the kidney and urinary tract (CAKUT). However, several lines of evidence strongly suggested that genetic factors play a pathogenic contribution to the pathogenesis of MCDK. In our previous report (ASN 2012), three cytogenomic aberrations have been detected by arrayCGH in a pilot study from ten pediatric patients with isolated unilateral MCDK. In this work, we characterized the derivation of the aberrations.

**Methods:** DNA was isolated from patients and their biological parents. Primers and probes for quantitative PCR (qPCR) were designed for detection of the relative copy number on targeted regions within the aberrations. Two quantitative technologies, TaqMan assay and SYBR green assay, were performed on more than two targeted regions for each aberration with reference control. The relative copy number was calculated by ΔΔCt method.

**Results:** Deletion at 7p14.3 with size of 2.07 Mb was revealed to be inherited from patient's father. Duplication at 16p13.11-p12.3 with size of 3.28 Mb. This duplication was determined to derive from patient's mother. Parental origin of monosomy X was not studied.

**Conclusions:** The two aberrations were determined to be inherited from parents, indicating that much complicated molecular mechanisms play the role in kidney morphogenesis. The molecular products encoded in the two altered regions should be involved in the reciprocal interaction between the ureteric bud and the metanephrogenic mesenchyme, and be tightly regulated during kidney development. Thus, interpretation of cytogenomic alternations to be recessive or dominant is not enough. In addition to halploinsufficiency and dosage effects, incomplete penetrance, variable tissue expression, imprinting, and unmarked mutations in the deletion regions have to be explored. Detailed analysis of the embryonic transcription and interactions related to the genes located within these aberrations will provide a better understanding for the genetic mechanisms.

*Funding:* NIDDK Support

## PUB103

**Chronic Low-Level Lead Exposure in Young Mice Results in Abnormal Glomerular Development** John M. Basgen,[1] Christina Sobin.[2] *[1]Charles R. Drew Univ; [2]Univ of Texas, El Paso.*

**Background:** Long-term exposure to high levels of lead is a risk factor for chronic kidney disease. Few studies have examined glomeruli in children or in young mice exposed to low levels of lead. This study examines the effects of early chronic low-level exposure of lead on glomerular development in young mice.

**Methods:** Pups were exposed to 30 ppm (99.4% lead acetate) via dams' drinking water. Controls received water with sodium acetate. Pups were sacrificed at post-natal day 28 and organs fixed with 4% paraformaldehyde via transcardial perfusion. Kidneys were harvested, weighed, and processed for light and electron microscopy. Glomerular volumes were measured on light microscopy images using the Cavalieri priniciple. The volumes of the glomerular components podocyte, mesangium, and capillary lumen were calculated by first measuring the volume fraction of each glomerular component on low magnification electron microscopy images using manual point counting. Finally the respective volume fractions were multiplied by glomerular volume to obtain podocyte, mesangial, and glomerular capillary volumes. Twelve mice per group were studied with ten glomeruli per mouse measured.

**Results:** Mean lead blood levels in the control and 30 ppm groups were 0.03 and 3.42 μg/dl respectively. The mean kidney weight of the 30 ppm exposed mice (91±7 μg) was significantly less than that of the controls (105±16 μg), p<0.02. The mean glomerular volume of the 30 ppm exposed mice was significantly greater (86±8 $x10^3$ $\mu m^3$) than that of the control group (72±6 x $10^3$ $\mu m^3$), p<0.001. The mean podocyte volume of the lead exposed mice was significantly greater (26±4 $x10^3$ $\mu m^3$) than that of the control group (23±3 x $10^3$ $\mu m^3$), p<0.02. The mean mesangial volume of the lead exposed mice was significantly greater (17±3 $x10^3$ $\mu m^3$) than that of the control group (13±3 x $10^3$ $\mu m^3$), p<0.01. The mean glomerular capillary lumenal volume of the lead exposed mice was significantly greater (34±3 $x10^3$ $\mu m^3$) than that of the control group (27±3 x $10^3$ $\mu m^3$), p<0.001.

**Conclusions:** Early chronic exposure to low-level lead in mother's milk is associated with decreased kidney weight and abnormal glomerular development in young mice.

*Funding:* Other NIH Support - NICHID/NIH R21HD060120, NCRR/NIH 5G12RR008124, NCRR/NIH 1U54RR026138, Private Foundation Support

## PUB104

**Glomerular Development in the Chinese Experimental Miniature Pig** Xiang-Mei Chen, Yuansheng Xie, Qing-gang Li, Xueyuan Bai. *Dept of Nephrology, State Key Laboratory of Kidney Disease, General Hospital of Chinese People's Liberation Army, Beijing, China.*

**Background:** The pig, which shares more similarities in renal structure and function with humans than with other species, is an excellent model for the study of renal development. However, few studies have focused on glomerular development in pigs.

**Methods:** The morphological changes of glomerular development, the development of podocytes, endothelial and mesangial cells, and the interrelationship between these cells were systematically observed in Chinese experimental miniature pigs at 17 time points from embryonic day 28 (E28d) to postnatal day 21 (P21d).

**Results:** Immunofluorescence staining showed that diffuse WT1 expression was observed in the metanephric mesenchyme and then, in succession, in the renal vesicle, the comma-shaped body, the tail of the comma-shaped body, the lower aspect of the S-shaped body and the glomerular podocytes. CD31 was scattered throughout the fetal kidneys and then surrounded the developing renal vesicle and comma-shaped body. At the S-shaped body stage, CD31-positive cells migrated into the vascular cleft to form precapillary cords in the immature glomeruli and finally localized in the endothelium of mature glomeruli. α-SMA expression was not present in the early fetal kidney, renal vesicle or comma-shaped body, but appeared near the periphery of the S-shaped body at the early stage of the S-shaped body, streamed into the vascular cleft by the late S-shaped body stage, aggregated at the root of the developing glomerulus, migrated to the periphery of the glomerular tuft at the capillary loop stage, and finally localized to the mesenterium in the mature glomerulus.

**Conclusions:** Porcine podocytes arise from the metanephric mesenchyme and the development of podocytes and endothelial cells precedes the development of the mesangial cells. Cross-talk between cells in the glomerulus may be essential during the development of the porcine glomerular tuft.

*Funding:* Government Support - Non-U.S.

## PUB105

### Atorvastatin Improve Effects of Mesenchymal Stem Cells Treatment for Kidney Ischemia/Reperfusion Injury through Down-Regulation of TLR4 Expression

Jieru Cai, Xiaofang Yu, Bingyng Zhang, Yi Fang, Xiaoqiang Ding. *Nephrology of Dept, Zhongshan Hospital, Fudan Univ, Shanghai, China.*

**Background:** Statin has been shown to attenuate ischemia/reperfusion (I/R) injury and enhance efficacy of mesenchymal stem cells (MSCs) treatment. However, the components of the mechanism remain largely unknown. We aim to investigate whether atorvastatin regulates the expression of Toll-like receptor 4 (TLR4), a potential mediator of innate activation and inflammation, and facilitates therapeutic efficacy of MSCs in renal I/R injury.

**Methods:** The model of I/R injury was induced by bilateral renal pedicles clamp for 45 min. Immediately after reperfusion, CM-Dil labeled MSCs ($1\times10^6$ cells) or vehicle were administered into the animals, pretreated with or without Ator for 3 days, through the carotid artery.

**Results:** After 24h systemically administration following I/R, the combined treatment with Ator and MSCs (Ator+MSCs) markedly decreased the elevated level of serum creatinine and blood urea nitrogen, reduced the severity of renal damage. In addition, inhibited renal tubular cell apoptosis, and reduced oxidative stress were observed in the Ator+MSCs group compared with control or mono-therapy group. Furthermore, TLR4 expression and high-mobility group box 1 (HMGB1) were decreased and survivals of implanted MSCs were increased within the kidney of Ator+MSCs animals.

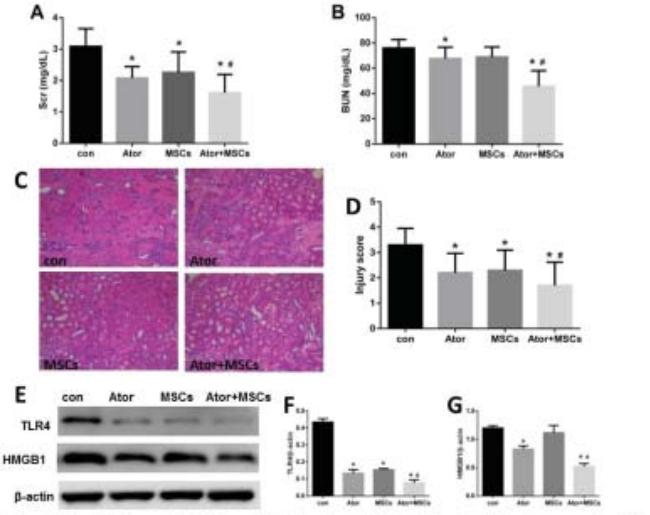



**Figure 1 Effect of Ator and/or MSCs on I/R-induced renal dysfunction.** Renal function was evaluated by determing (A) serum creatinine (Scr) and (B) blood urea nitrogen (BUN) levels post-I/R 24h. All the animals treated with Ator and/or MSCs showed less increasing in Scr and BUN levels than control group, especially in the combination of Ator and MSCs group. (C) representative photomicrographs of kidney sections showed that there were severe tubular injury occurred in the cortical and corticomedullary junction areas when the rat subjected to 45 min renal ischemia and 24 hours of reperfusion (HE staining, magnification×200). (D) The degree of renal injury was assessed via a semiquantitative method in the sections. Compared with control or MSCs alone, there were less injured score in group Ator+MSCs. (E-G) The inflammatory-regulating protein levels of TLR4 and HMGB1 were analyzed by Western blotting and representative scan of three independent experiments was shown. The expressions of HMGB1 and TLR4 were down-regulated in the treated group compared to the control group. The presence of the Ator further decreased the expression of the HMGB1 compared with the MSCs group ($P<0.05$) but not affected the TLR4 ($P=0.321$). *$P<0.05$, vs control group; #$P<0.05$, vs MSCs group.

**Conclusions:** Ator inhibit the apoptosis, oxidative stress, and inflammation in the kidney undergoing I/R injury, through down-regulate the expression of TLR4 and endogenous ligand HMGB1. Therefore, Ator pretreatment create a better environment for the survival of implanted MSCs and enhance their therapeutic potential.

## PUB106

### Secreted Factors from Adult Kidney Stem Cells Protect against Renal Ischemia-Reperfusion Injury

Kenji Tsuji, Shinji Kitamura, Hirofumi Makino. *Medecine and Clinical Science, Okayama Univ Graduate School of Medicine, Okayama, Japan.*

**Background:** Recently, stem cell therapies have been proposed as a novel therapy against acute kidney injury (AKI) on basic research. We have established adult stem / progenitor-like cells (KS cells) from S3 segment of renal proximal tubules (Kitamura S et al., FASEB J, 2005). We have previously reported that KS cells replaced in injured tubules directly and improved renal function after implantation in rat AKI models (Kinomura M et al., Cell transplantation, 2008). It is unclear whether the secreted factors from adult kidney stem / progenitor cells are effective or not. Here we examined whether the KS cell therapy system is not only the direct cell differentiation at injury place but also the secreted factors from KS cells.

**Methods:** SD rats were subjected to kidney ischemia/ reperfusion (I/R) injury. We separated the rats in three groups, KS cell supernatant (CS-KS) administrated group (Intraperitoneally administrated 3 hours after I/R; I/R-KS, n=3), vehicle group (I/R; n=3) and sham operated group (sham: n=3). They were sacrificed after 2 or 4 days, were evaluated renal function, tubulointerstitial injury, apoptosis, cell proliferation and inflammation.

**Results:** In I/R-KS group, we could observe significant suppression on urinary N-acetyl-b-D-glucosaminidase (NAG) level in 4 days compared to I/R group (I/R group v.s. I/R-KS group; 4.43±1.76 v.s.1.36±0.99 U/l, $p<0.01$). The cell proliferation increased significantly in I/R-KS group (I/R group v.s. I/R-KS group; Histone H3(+) cells: 24.3±7.9 v.s. 43.9±9.5/×40field).The apoptotic cells decreased in I/R-KS group (I/R group v.s. I/R-KS group; TUNEL(+) cells: 46.4±14.5 v.s. 25.3±13.0 / ×200field, $p<0.01$). Moreover, we could observe significant inflammation suppression in I/R-KS group compared to I/R group (F4/80(+) area: 4476±2433 v.s. 1577±1042 pixel/×40field, $p<0.01$). In addition, CS-KS significantly suppressed cisplatin-induced cell apoptosis in vitro. Immunoblot analysis revealed that CS-KS contained many factors, such as HGF.

**Conclusions:** Not only the direct cell differentiation but also secreted factors from adult kidney stem cells have the potential to protect against AKI and activate renal regeneration.

## PUB107

### Acute Hemodialysis Therapy in Neonates with Inborn Errors of Metabolism

Israel Eisenstein,[1,4] Mahdi Tarabeih,[1] Daniella Magen,[1,4] Shirley Pollack,[1,4] Hanna Mandel,[2,4] Gad Bar Joseph,[3,4] Israel Zelikovic.[1,4] *[1]Pediatric Nephrology, Rambam Medical Center; [2]Pediatric Metabolic Diseases, Rambam Medical Center; [3]Pediatric Intensive Care, Rambam Medical Center; [4]Faculty of Medicine, Technion, Haifa, Israel.*

**Background:** Inborn errors of metabolism (IEOM) can cause devastating damage to the neonatal brain if not promptly treated. The most efficient therapy for IEOM-induced metabolic crisis is hemodialysis (HD). Data on the use of HD in IEOM in neonates is scarce.

**Methods:** We analyzed the demographic, clinical, and biochemical data of all neonates with IEOM who were admitted to our Pediatric Intensive Care Unit between 2004-2013 with a metabolic crisis necessitating HD.

**Results:** Fourteen neonates (M:F 7/7; Arabs/Jews 13/1) with IEOM [6- Urea cycle defects (UCD), 4- maple syrup urine disease (MSUD), 2-mitochondrial cytopathies (MC), 2 - other] had uneventful delivery, and were admitted because of metabolic/neurological deterioration starting at 48h -14 days of age. Median age and weight of the infants at the initiation of HD was 6.3 days and 2999 g, respectively. HD was performed through double-lumen, 6.5- French, Gamcath® acute catheter inserted into the internal jugular vein; Gambro AK200 dialysis machine and tubing and Fresenius FXpaed® dialyzers were used. A total of 30 dialysis treatments (at least 2 per patient) were performed. The first (2 hrs) HD tretment markedly decreased (91.9%) mean plasma ammonia level in UCD and MC patients from 1071 to 86 μmol/L (nl: 53-90), and markedly decreased (90%) mean plasma leucine level in MSUD patients from 2408 to 230 μmol/L (nl: 70-240). The second (3-4 hrs) HD treatment decreased the mean rebounded ammonia and leucine levels from 433 μmol/L and 780 μmol/L to 69 μmol/L and 88 μmol/L, respectively. The HD procedure was free of complications, resulted in marked clinical improvement in 11 patients, and enabled the initiation of appropriate dietary/pharmacological therapy in all patients. Eleven (78.6%) of the infants survived, and 3 (21.4%) succumbed to their metabolic disease in the neonatal period.

**Conclusions:** HD in neonates with IEOM is safe, very efficient, and life-saving when performed by skilled personnel and in the appropriate setting.

*Funding:* Clinical Revenue Support, Government Support - Non-U.S.

## PUB108

### Predictive Factors of Graft Censored Failure in Pediatric Kidney Transplants

Ana Rocha, Liliana Rocha, Maria Faria, Conceição Mota. *Centro Hospitalar do Porto.*

**Background:** Kidney transplant in children's has shown steady improvement in graft survival outcome over the last decades. Using data obtained from the transplant registry of our Centre between 1984 and 2012, we assessed the independent determinants of graft failure using the Cox proportional hazards regression.

**Methods:** Altogether, 128 recipients aged <18 years at the time of kidney transplant and who had > 3 months graft survival were studied. Over 9.95 years of medium follow-up, 27 censored graft failures occurred. Censored graft survival at five, ten, fifteen and twenty years post-transplant was 93%, 82%, 70% and 63%, respectively. Studied factors included receptor and donor age, receptor gender, dialysis vintage, donor/recipient CMV

serology, panel reactive antibody percentage, human leucocyte antigen mismatching, previous transplant number, donor type [deceased vs. living donation], cold ischemia time, induction therapy with antithymocyte globulin, occurrence of acute tubular necrosis and development of acute rejection.

**Results:** Using univariate analysis, the significant predictors for graft censored failure were adult donor (P<0.001), higher receptor age (P=0.035), human leucocyte antigen mismatching (P=0.025), antithymocyte globulin induction (P=0.03) and development of acute rejection (P<0.001).Two factors independently predicted graft censored failure in multivariate analysis. The odds ratios for graft failure in patients with acute rejection and in children who received an organ of an adult were 3.744 and 4.962, respectively.

**Conclusions:** Pediatric recipients should receive the first priority for allografts from pediatric donors and acute rejection should be meticulously prevented.

## PUB109

### Efficacy and Safety of Eculizumab in Children with Dense Deposit Disease

Kiran K. Upadhyay, Richard N. Fine. *State Univ of New York at Stony Brook, NY.*

**Background:** Dense Deposit disease (DDD) is a rare cause of glomerulonephritis and is believed to result from dysregulated alternative complement pathway. Treatment options are limited and there is a high incidence of end stage renal disease if untreated. Eculizumab, a humanized monoclonal antibody, binds to the terminal complement, C5, and prevents formation of membrane attack complex (MAC) - C5b9, a potent proinflammatory agent.

**Methods:** We describe a 6 yo male with biopsy-proven DDD who was treated off-label with eculizumab soon after the diagnosis. The patient presented with nephrotic range proteinuria, microhematuria, C3 hypocomplementemia, with normal renal function and mild hypoalbuminemia without edema. Baseline serum soluble MAC (SC5b9) was elevated. Other tests included negative C3 nephritic factor, normal factor H & I levels, no autoantibodies to factor H & I, and no CD46, C3 or factor H mutation. After initial Rx with biweekly intravenous eculizumab, the proteinuria decreased from baseline urine protein to creatinine ratio (U P/C) of 5.6 to 0.7. SC5b9 normalized when checked after 3rd treatment. Subsequently, when the treatment frequency was changed to monthly after 5$^{th}$ treatment, the proteinuria started rising to U P/C of 3-4, necessitating initiation of ACE inhibitor therapy. SC5b9 level increased to pretreatment value when checked again during 10$^{th}$ infusion. The frequency was then changed back to biweekly and the treatment is ongoing. Renal function remains normal but the C3 hypocomplementemia persists. Repeat renal biopsy after 7$^{th}$ treatment showed no interval histopathological changes but with de novo staining for IgG in the same distribution as C3 and C5b9. Patient was fully immunized including meningococcal vaccine prior to the treatment. No serious adverse effects have been observed so far. To the best of our knowledge, this is the first youngest pediatric patient to receive eculizumab for DDD. It is yet to be seen whether eculizumab continues to be effective in this patient.

**Conclusions:** We conclude that biweekly eculizumab effectively inhibits the terminal complement cascade and may be useful in preventing progression of renal disease in DDD, especially in those who have elevated SC5b9 level.

## PUB110

### Typical or Atypical Hemolytic Uremic Syndrome? This Is the Question

Gianluigi Ardissino, Francesca Tel, Stefania Salardi, Sara Testa, Fabio Paglialonga, Nicolò Borsa, Ilaria Possenti, Silvana Tedeschi. *Center for HUS Prevention, Control and Management, Fondaz. IRCCS Ca' Granda Osp Maggiore Policlinico, Milan, Italy.*

**Background:** Hemolytic Uremic Syndrome (HUS) is commonly classified into 2 forms: typical HUS (tHUS) due to an infection by Shiga-toxin (Stx)-producing *E. coli* (STEC) and atypical HUS (aHUS), mainly caused by complement alternative pathway dysregulation. The classification relies upon the evidence or not of STEC infection: isolation of STEC, detection of Stx in stools and/or in blood and serotype specific LPS antibody. However, diarrhea can also represent a trigger for aHUS, leading to difficulties in identifying the most appropriate treatment. Herein, we report a case of HUS associated with bloody diarrhea, in which prompt lab investigations revealed a complement related protein gene mutation which guided the clinical choice of treating the patient with Eculizumab.

**Methods:** A 5-yrs-old girl, while on vacation on the Red Sea, was admitted to the local hospital with macrohematuria, severe bloody diarrhea and fever. Lab showed mild anemia, low PLT count and AKI. An antibiotic treatment was started. Two days later, for worsened clinical conditions, the child was referred to our Center. Bloody stools were negative for Stx *1 and 2*. Due to low C3 level (40 mg/dl) molecular biology for complement related genes was started and on day 6, a novel variant on MCP gene (p.Phe175Val) mutation was identified. The same day Eculizumab (600 mg) was administered with a rapid remission of the thrombotic microangiopathy and recovery of renal function. During the following 8 mos, no relapse occurred, despite the single dose of Eculizumab. The child is well with normal renal function.

**Conclusions:** This case suggests that proofs of STEC infection should be always actively seeked and documented. C3 level should also be determined in any patient with HUS. In diarrhea-associated HUS, if no clear evidence of STEC infection and/or if low C3 are found, aHUS triggered by diarrhea should be suspected and ruled in or out.

*Funding:* Private Foundation Support

## PUB111

### Epidemiology of Hemolytic Uremic Syndrome in Children in Northern Italy

Gianluigi Ardissino, Stefania Salardi, Sara Testa, Silvana Tedeschi, Valentina Paracchini, Francesca Tel, Ilaria Possenti. *Center for HUS Prevention, Control and Management, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico, Milan, Italy.*

**Background:** HUS is a rare microangiopathy characterized by platelet (PTL) consumption, hemolysis and renal damage. The most frequent type of HUS in children is associated with shigatoxin (Stx)-producing E. coli infection (STEC-HUS), but other atypical forms (aHUS) are occasionally seen, including those related to complement dysregulation. Despite the severity of the disease and the fact that it represents a leading cause of AKI in children, the general epidemiology of HUS is all but well documented.The aim of the study is providing reliable, population-based epidemiological.

**Methods:** Between 2001 and 2011, all incident cases with a first episode of HUS <18 yrs who present these conditions, were included: 1.PLT <150,000/mm$^3$ or evidence of PLT consumption; 2.Evidence of hemolysis; 3.Signs of renal damage. Overall and age-related crude incidence rates per 100,000 people were calculated from 2001 to 2011 using the population estimates issued by the Italian Institute of Statistics (ISTAT). The population at risk was defined as Lombardy residents aged ≤18 years.

**Results:** Ninety-eight HUS cases were identified during the 10 yrs; the overall annual incidence was 0.55/100,000 children ≤18 years of age (range 0.44-0.66). The incidence in patients aged <5 yrs was 1.2 cases per 100,000. aHUS accounted for 11.2% of the cases (incidence:0.069/100.000); among the 11 aHUS patients, 4 (36.4%) were caused by complement gene dysregulation (2 CFH, 1 MCP, 1 CFI), 3 (27.2%) were associated with methyl-malonic acidemia, 2 (18.2%) were associated with pneumonia, and 2 (18.2%) were of unknown origin. The overall case fatality rate was 4.1% (3.4% in STEC-HUS).

**Conclusions:** Our findings provide epidemiological data useful to healthcare planning in the area of HUS, particularly in relation to estimating the financial burden that health care providers might have to face for treating atypical HUS.

*Funding:* Private Foundation Support

## PUB112

### Pediatric Nephrotic Syndrome Practice Guidelines: Adoption and Outcomes

Debbie S. Gipson,[1] J. Troost,[2] Emily G. Herreshoff,[2] Courtney L. Harkness,[2] Susan F. Massengill,[2] Rasheed A. Gbadegesin,[2] Larry A. Greenbaum,[2] Ibrahim Shatat,[2] Yi Cai,[2] Gaurav Kapur,[2] Diane Hebert,[2] Michael J. Somers,[2] Howard Trachtman,[2] Priya J. Pais,[2] Michael E. Seifert,[2] David T. Selewski,[2] Jens W. Goebel,[2] John D. Mahan,[2] Heather E. Gross,[2] Darren Dewalt.[2] *[1]Univ of Michigan; [2]PROMIS Pediatric Study Network.*

**Background:** North American Clinical Practice Guidelines were published (2009) for childhood onset nephrotic syndrome (CONS). Based on systematic review, we expect 40% of incident patients to develop frequently relapsing/steroid dependent (FRNS/SDNS) and 25% steroid resistant (SRNS) disease. The objective of this study was to evaluate guideline adoption and outcomes.

**Methods:** 126 children with CONS, age 8 to 17 years, enrolled in the PROMIS II prospective longitudinal cohort from 14 institutions between 9/2010 - 3/2013, were included. Nephrologists were asked if they intended to follow the published guidelines and when the answer was 'no', the reason for alternate therapies was captured.

**Results:** In 103(82%) cases physicians reported the intent to follow guidelines, increasing from 73% in the initial 6 months to 100% at months 24-30(p=0.02). 23(18%) declined guideline recommendations secondary to provider preference (N=16), patient contraindications (N=3), secondary NS (N=2) and established resistance to guideline regimen (N=2). Outcomes are shown in Table 1.

**Conclusions:** Adoption of Clinical Practice Guidelines was high (82%) within 3 years from publication. The observed 69% combined FRNS/SDNS and SRNS outcomes in the setting of high guideline adoption rates, suggests focus areas for novel therapy development and generates estimates for standard of care outcomes for future trials.

| Patient Characteristics | Incident N=58 | Prevalent N=68 |
|---|---|---|
| **Baseline:** | | |
| Onset Age (Yr) | 13.6(2.6) | 6.5(4.6) |
| Black Race | 16(28%) | 19(28%) |
| Second Line Immunosuppression | 0(0%) | 24(35%) |
| **Follow Up (Mo)** | 9.0(6.3) | 7.3(6.1) |
| **Status at Follow Up:** | | |
| Never Relapsed | 8(19%) | 0(0%) |
| Infrequent Relapse | 5(12%) | 6(21%) |
| FRNS | 5(12%) | 1(4%) |
| SDNS | 10(24%) | 12(43%) |
| SRNS | 14(33%) | 9(32%) |
| Unknown (FU<12mo) | 16 | 40 |

*Funding:* Other NIH Support - 5-U01-AR052181-09

## PUB113

**The U.S. Pediatric Nephrology (PN) Workforce 2013** William A. Primack,[1] Kevin E.C. Meyers,[2] Suzanne Kirkwood,[3] Holly S. Ruch-Ross,[4] Carrie Radabaugh,[3] Larry A. Greenbaum.[5] *[1]UNC Kidney Center, Chapel Hill, NC; [2]CHOP, Philadelphia, PA; [3]AAP, Evanston, IL; [4], Chicago, IL; [5]Emory Children's Center, Atlanta, GA.*

**Background:** A shortage of pediatric nephrologists exists, yet the PN workforce is poorly characterized.

**Methods:** The AAP is conducting workforce surveys of all pediatric subspecialists (PSS). The first 44 questions are asked of all PSS to allow comparisons. Then 44 PN specific questions were created with help of the ASPN. The survey was sent electronically to all physicians who are either working as pediatric nephrologists or were ever US board eligible in PN.

**Results:** 500 responses (response rate 65.8%): 51% male. 68% US graduates. 396 are ABP Certified in PN. 409 practice PN full or part time. They spend 57% of their time in direct patient care, 14% administration, 12% clinical research, 11% teaching, 9% basic research. Mean work week is 54.5 ± 15.7 hrs (n=470). Respondents (n=405) spend 84% of their time in all aspects of PN with the rest of their time spent in non-nephrology administration, general peds, and in adult neph. 131 plan to decrease clinical work in the next 5 yrs due to 1 or more of the following: 59 are dissatisfied with work-life balance; 70 plan to retire; 13 for family reasons; 49 to increase nonclinical activities. 65% would probably or definitely choose PN as a career if they could do it over again, but 22% would not and 14% were unsure (n=455). 49% think that there should be a 2 year clinical as well as a 3 year academic track for fellowship, 34% did not and 17% were unsure (n=452). PN Division chiefs (n=110) and solo PN's (n=28) report that 52% have adequate PN staff, 47% inadequate. In the next 3 years, 92% anticipate recruiting for newly created PN positions, as well as 77% and 71% to replace retirees and departing PN's respectively.

**Conclusions:** 409 PN's spend 57% of their time in patient care, but 32% plan to decrease clinical work in next 5 yrs. Also 20% of trained PN's do not practice PN. An ongoing shortage of pediatric nephrologists is likely given the high numbers who will need to be replaced over the next 5 years. The PN workforce requires continuing needs assessment monitoring.

## PUB114

**Fighting Bloody Diarrhea for Shigatoxin-Associated Hemolytic Uremic Syndrome. Prevention and Mitigation: North Italian HUS Network** Gianluigi Ardissino, Francesca Tel, Sara Testa, Stefania Salardi, Rosaria Colombo, Ilaria Possenti. *Center for HUS Prevention, Control and Management, Fondazione IRCCS Ca' Granda Osp. Maggiore Policlinico, Milan, Italy.*

**Background:** Shigatoxin-associated Hemolytic Uremic Sindrome (STEC-HUS) still represents a major public health problem caused by an infection often presenting with bloody diarrhea.

**Methods:** Since May 2010 a Network connecting pediatric hospitals in Northern Italy (10 millions gp) was developed aimed at screening bloody diarrhea in children for Shigatoxin. Among the objectives of the Network is the early diagnosis of STEC-HUS thus reducing the time of referral to our Center and allowing the implementation of interventions measures to prevent or mitigate the renal complication. The present study compares the clinical characteristics at diagnosis of an equal number of STEC-HUS cases referred to our Center in the past (2007-2009) and during the last 2 years (2012-2013) in order to analyze the efficacy of the Network.

**Results:** Since Jan. 2012, 22 cases of STEC-HUS have been referred to our Center and these were compared to 22 historical cases (sequentially referred during 2007-2009). No significant difference was observed in gender and age distribution as well as in mean haemoglobin, LDH and platelet levels at onset while mean serum creatinine was significantly higher in the historical group (3.6 mg/dL with CI 1.8-5.4 vs 1.7 mg/dL with CI 1.1-2.4; $p < 0.05$).

**Conclusions:** The screening of bloody diarrhea, together with the increased awareness among pediatricians towards STEC-HUS, led to a remarkable advance in the diagnosis of the renal complication which can be estimated, based on creatinine, in as much as 24-48h. The anticipation in the diagnosis is the prerequisite for testing early intervention measures for mitigating the severity of STEC-HUS.

*Funding:* Private Foundation Support

## PUB115

**Coinfection in Children with Bloody Diarrhea Caused by Shigatoxin Producing *Escherichia coli*** Gianluigi Ardissino, Ilaria Possenti, Stefania Salardi, Rosaria Colombo, Francesca Tel, Sara Testa, Erminio Torresani. *Center for HUS Prevention, Control and Management, Fondazione IRCCS Ca' Granda Osp. Maggiore Policlinico, Milan, Italy.*

**Background:** Hemolytic Uremic Syndrome (HUS) is characterized by the triad of microangiopathic hemolytic anemia, platelet consumption and renal damage. Bloody diarrhoea, caused by Shiga-toxin producing *Escherichia coli* (STEC), is the most frequent prodromic sign of HUS (at least 57% of cases). Although rarely, other microorganisms have been reported as cause of HUS in children but their responsibility in the thrombotic microangiopathy is not clearly defined.

**Methods:** Since May 2010 a Network connecting pediatric hospitals in Northern Italy (10 millions gp) was developed aimed at early diagnosis of STEC-HUS. 930 children with bloody diarrhoea were screened and the rate of bacterial coinfection was analyzed.

**Results:** In Shigatoxin positive patients (n:52) the rate of coinfection (with either Salmonella or Campylobacter) was as high as 34.6% while coinfection between Salmonella and Campylobacter was as low as 0.5%.

**Conclusions:** It is speculated that infection by Salmonella or Campylobacter increase the risk of STEC infectionor viceversa. Based on our finding, the isolation of microrganisms other than STEC in HUS cases is expected to be unrare and it should not be necessarily regarded to as the etiological agent. In conclusion, in all HUS case it is of paramount importance to perform the full panel of available diagnostic tools for STEC (shigatoxin in stools and blood and serum antiLPS) to clearly define the etiological agent.

*Funding:* Private Foundation Support

## PUB116

**The Effects of Hydroxyurea (HU) or Chronic Blood Transfusion (CTx) on Sickle Cell Nephropathy (SCN) in Children** Valeriya M. Feygina,[1] Kathy Rey,[2] Miho Hatano,[2] Bandana Paudyal,[1] Anil K. Mongia,[1] Morris J. Schoeneman,[1] Vaishali Bansilal,[1] Scott Miller,[2] Hanan K. Tawadrous.[1] *[1]Pediatric Nephrology, SUNY Downstate Medical Center, Brooklyn, NY; [2]Pediatric Hematology/ Oncology, SUNY Downstate Medical Center, Brooklyn, NY.*

**Background:** Renal complications of sickle cell anemia (HbSS) include: a concentrating and acidification defect, hyperfiltration, proteinuria, renal scarring. No treatment is proven to slow progression of SCN. **Hypothesis:** HU or/and CTx may slow SCN progression in children with HbSS.

**Methods:** Data collected: age, sex, Hb, HbF, MCV, eGFR, fractional excretion of sodium (FeNa), transtubular potassium gradient (TTKG), fractional reabsorbtion of phosphorus. Osmolality and microalbuminuria/proteinuria were measured in a first morning urine after at least 6 hours of fasting. Student's t-test was used to compare parameters.

**Results:** 26 patients (50 % female) with HbSS were enrolled. 9 patients on CTx for primary and secondary stroke prevention and 17 on HU for acute recurrent vaso-occlusive complications. Treatment duration 56.1±45.9 and 26.7±10.7 months, patients age 13.5±9.1 and 10.8±3.8 yrs in CTx and HU group, respectively. HU dose: 24.7±1.9 mg/kg/24hr. Comparison of HU and CTx groups.

| | HU | CTx | P value |
|---|---|---|---|
| HbF (g/dL) | 13.5 +5.8 | 4.5+3.9 | 0.0005 |
| Retic (%) | 9.6+4.0 | 8.4+0.02 | 0.02 |
| MCV (fL) | 103.5+14.5 | 91.8+7.2 | 0.02 |
| Urine osmolality (mOsm/kg) | 433.9+110.7 | 479.6+92.3 | 0.15 |
| FeNa (%) | 0.32+0.2 | 0.38+0.3 | 0.25 |
| Reabsorb of phosphorus (%) | 95.3+2.9 | 95.7+1.5 | 0.34 |
| TTKG (%) | 4.05+2.9 | 3.10+1.6 | 0.27 |
| Microalb /cr (mg/mg) | 70.3 +125.1 | 83.3+101.5 | 0.43 |
| Protein/cr (mg/mg) | 0.2+0.4 | 0.19+0.1 | 0.47 |
| eGFR (ml/min/1.73m2) | 150.2+31.7 | 172.0+37.8 | 0.06 |

There was no significant difference in renal function between the groups but a trend toward less hyperfiltration in the HU group. 18.75 % and 20.8 % of patients have microalbuminuria and proteinuria, respectively, prevalences similar to that reported in untreated children.

**Conclusions:** Renal function is similar in children with HbSS receiving either HU or CTx, and both groups have a high prevalence of SCN.

## PUB117

**Functional Magnetic Resonance Imaging on Pediatric Renal Disease Patients** Nobuaki Takagi, Takayuki Shibano, Kohei Maekawa, Hiromu Mae, Masuji Hattori, Takakuni Tanizawa. *Dept of Pediatrics, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

**Background:** We have been evaluated renal disease children by estimated glomerular filtration rate (eGFR) or by invasive renal biopsy, which sometimes require general anesthesia. But in adult patients, Inoue et al. reported (JASN, 2011) that they evaluated renal hypoxia and fibrosis using functional Magnetic Resonance Imaging (MRI). It is known that apparent diffusion coefficient (ADC) by Diffusion-weighted (DW)-MRI reflects fibrillation and T2* by blood oxygen level-dependent (BOLD)-MRI reflects renal tissue hypoxia. It is reported that chronic kidney disease (CKD) stage5 patients' renal MRI showed low ADC and T2* compared to normal kidneys. Therefore, we now test the possibility of functional MRI on pediatric renal disease patients. For this purpose, we compared the ADC and T2* value of normal and renal disease children.

**Methods:** We tested DW- and BOLD-MRI to 10 normal children, whose urinary test was normal and had no history of renal disease (median age 12.5 y.o.), and 18 children who underwent renal biopsy 2days before MRI because of abnormal urinary test (median age 13.0 y.o., 8 IgAN, 5 TBMD, 2 nephrotic syndrome, 3 others). All patients were not CKD.

**Results:** ADC value of renal cortex was 1729.9±114.8m2/sec (normal control) vs. 1765.8±96.4m2/sec (patients, $p>0.05$), and T2* value of renal cortex was 175.9±19.7msec (normal control) vs. 190.1±24.3msec (patients, $p>0.05$). ADC value of renal medulla was 1800.3±121.5m2/sec (normal control) vs. 1750.1±105.0m2/sec (patients, $p>0.05$), and T2* value of renal medulla was 165.3±20.1msec (normal control) vs. 176.5±20.4msec (patients, $p>0.05$).

**Conclusions:** These results reveal that children with normal renal function and abnormal urinary test (proteinuria and/or hematuria) show normal functional MRI. That may because the patients had no or little renal fibrosis or tissue hypoxia. We may distinguish CKD and non-CKD patients by functional MRI in children as well as in adults. Also, we may expect renal fibrosis and ischemic change by these non-invasive functional MRI, if we could show the usefulness in children.

## PUB118

### Development of Oxabact® for the Treatment of Primary Hyperoxaluria

Elisabeth Lindner. *OxThera AB, Stockholm, Sweden.*

**Background:** Primary Hyperoxaluria (PH) is a rare inborn error of the glyoxylate metabolism characterised by severe hyperoxaluria (Uox >0.8 mmol/day/1.73m$^2$) with clinical presentation of nephrocalcinosis with or without recurrent calcium-oxalate urolithiasis and deteriorating renal function leading to ESRD.

**Methods:** Oxabact® therapy consists of lyophilised *Oxalobacter formigenes* in an enteric-coated capsule for oral administration. *O. formigenes* is an anaerobic bacterium which only utilizes oxalate as its substrate and is naturally suited to degrade oxalate in the human GI tract. Based on extensive animal and clinical data it is hypothesised that Oxabact® treatment can remove endogenously produced oxalate in patients with PH by enteric elimination, thereby lowering the disease´s detrimental effect on kidneys.

**Results:** The Oxabact® treatment has been evaluated in a series of clinical studies in PH patients. Although earlier Phase I/II studies showed a significant reduction of urinary and plasma oxalate these results were not reproduced in larger multi-centric double blind placebo controlled trials. The outcome of the these studies was found to be related to significantly reduced biological potency of freeze dried bacteria used in said studies. Extensive research and improvement in the manufacturing process for Oxabact® was done in the last two years to produce the final formulation (OC5) which has a hundred fold improvement in its potency (increased concentration of live cells, faster regeneration from freeze dried state and higher oxalate degradation activity) as compared to the product used in unsuccessful studies.

**Conclusions:** OC5 has been tested for safety and efficacy in animal models and is currently being tested in a pivotal randomised, placebo-controlled, double-blind, multi-centre study in patients with PH type 1. Following screening and baseline evaluations eligible subjects have been randomised 1:1 to receive OC5 or placebo for a period of eight weeks. The study end points will evaluate reduction of oxalate in 24h urine and plasma; quantification of *O.formigenes* in the feces and the safety and tolerability of treatment. Successful outcome of this trial is expected to provide a significant unmet medical intervention for PH patients.

*Funding:* Pharmaceutical Company Support - OxThera AB, Government Support - Non-U.S.

## PUB119

### Possible Pathogenic Roles of Granzyme B in Peripheral Blood Mononuclear Cells in Pediatric Patients with Idiopathic Nephrotic Syndrome

Yuko Akioka, Tatsuo Asano, Kei Nishiyama, Noriko Sugawara, Kiyonobu Ishizuka, Masataka Hisano, Hiroko Chikamoto, Motoshi Hattori. *Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan.*

**Background:** Granzyme B (GrB) is a serine protease released from cytotoxic lymphocytes. Recently, increased research has focused on extracellular GrB activity which contributes to be a loss of structural integrity through the cleavage of extracellular matrix in a number of chronic inflammatory conditions. Several clinical studies showed a correlation between GrB expression and disease severity. The purpose of this study was to examine the possible pathogenic roles of GrB in idiopathic nephrotic syndrome (NS).

**Methods:** We examined 41 patients with steroid sensitive nephrotic syndrome (SSNS) and 10 patients with steroid resistant NS (SRNS). Sex- and age-matched 11 healthy subjects were also included. Peripheral blood mononuclear cells were isolated and polychromic flow cytometric analysis was performed to evaluate the expression of GrB in CD3, CD4, CD8, CD19, CD56 cells. Plasma GrB level was determined with ELISA.

**Results:** In both SRNS and SSNS patients in relapse, the percentages of GrB-positive CD3 cells were significantly higher than those in healthy controls (11.6±9.0%, 15.6±10.2% vs. 5.1±3.1%, P<0.01, P<0.05, respectively). There were no differences in the percentages of GrB-positive cells in CD19 and CD56 cells between NS patients and controls. GrB expression in CD3 cells decreased accompanied with the remission in SSNS patients. The plasma levels of GrB in both SRNS and SSNS patients in relapse were not different from those in controls. The positive correlation between GrB-positive CD3 cells and selectivity index of urine was observed in NS patients (r=0.556, p=0.041).

**Conclusions:** Although further studies including measurement of urinary levels of GrB are clearly needed, our preliminary results suggest possible pathogenic roles of granzyme B in CD3 cells in pediatric patients with idiopathic NS.

## PUB120

### Do Immunosuppressed Renal Patients with Fever Need Routine Hospitalization?

Mauricio Romero Olvera, Rossana Baracco, Amrish Jain, Gaurav Kapur, Tej K. Mattoo. *Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI.*

**Background:** Immunosuppressed patients with high fever are often hospitalized until cultures are negative and temperature settles down. The objective of this study was to assess the need for such practice in our renal patients on immunosuppression.

**Methods:** This retrospective study included children 2 to 18 years old who were on immunosuppressive medications for nephrotic syndrome (NS), systemic lupus erythematosus (SLE) and renal transplantation (RT), admitted to Children's Hospital of Michigan from 2009 – 2012 for fever >38.3ºC. Patients with diabetes, cardio-respiratory disease or lupus flares were excluded. Patients were considered as having a significant clinical presentation (SCP) if emergency department evaluation revealed low blood pressure, tachycardia, abnormal cardiac or chest auscultation, abdominal tenderness, and abnormal test results. Those with normal examination except for high fever were considered as having insignificant clinical presentation (ICP).

**Results:** The study included 60 patients with 103 admissions. Mean age was 9.28 (SD+/- 4.66) years; 59.2% were males. Of the103 admissions, 52 were in patients with NS, 32 had RT, and 19 had SLE. Twenty four admissions had SCP(23.3%), which included 4 to PICU; 79 (76.6%) admissions had ICP.

| Table1 | SCP N = 24 | ICP N = 79 | Total |
|---|---|---|---|
| Bacterial infections N (%) | 20 (83.3) | 9 (11.4) | 29 (28.15) |
| Viral infections N (%) | 4(16.7) | 70 (88.6) | 74 (71.84) |
| Tmax mean (SD) | 102.1 (0.9) | 102.0 (1.1) | 102.04(1.02) |
| White blood cell count mean (SD) | 12.4 (6.9) | 11.3 (5.7) | 12.23(6.11) |

Of the 20 patients with bacterial infections/SCP, 8 had pneumonia, 5 had abscesses/cellulitis, 4 had UTIs(1 had E.coli bacteremia), and 1 had bacterial peritonitis, 1 C. difficile infection, and 1 sepsis episode due to S. pneumoniae. Of the 9 patients with bacterial infections/ICP, 5 had strep pharyngitis, and 2 had otitis media and 2 UTI.

**Conclusions:** Immunosuppressed renal patients with high fever and insignificant clinical findings do not need routine hospitalization; they could be sent home with appropriate counseling/follow-up after culture specimens are collected.

## PUB121

### Renal Involvement in Children with Sickle Cell Disease, Sickle Cell Trait, and Sickle Beta Thalassemia in Southern Iran

Mohammad Hossein Fallahzadeh,[1] Yusof Mojtahedi,[1] Mohammad Kazem Fallahzadeh,[1,2] Mohammad Amin Fallahzadeh,[1] Mitra Basiratnia,[1] Ghamar Hosseini Al-hashemi.[1] *[1]Dept of Pediatric Nephrology, Shiraz Univ of Medical Sciences, Shiraz, Islamic Republic of Iran; [2]Dept of Nephrology, LSUHSC, Shreveport, LA.*

**Background:** Different types of renal involvement have been reported in children with sickle disease, sickle cell trait and sickle beta thalassemia. There is not enough information about the renal manifestations of these diseases in Iranian children. The aim of our study was to evaluate the manifestations of renal involvement in children with sickle disease, sickle cell trait and sickle beta thalassemia.

**Methods:** Over a period of 1 year (2011-2012), 97 consecutive children (30 with sickle cell disease, 31 with sickle trait and 36 with sickle beta thalassemia) who were referred to us by pediatric hematologists were enrolled in this cross-sectional study. These children were evaluated by history, physical examination and laboratory workups including renal function panel, urinalysis and albumin/creatinine measurement in spot urine.

**Results:** Mean age of the enrolled patients was 8.8 ± 3.4 years (range: 3-18 years). The prevalence of their renal manifestations is listed in the following table:

| Clinical findings | Sickle cell disease (n=30) | Sickle cell trait (n=31) | Sickle beta thalassemia (n=36) |
|---|---|---|---|
| Enuresis | 8 (27%) | 7 (23%) | 5 (14%) |
| Microalbuminuria (albumin/creatinine ratio: 30-300 mg/g) | 4 (13%) | 5 (16%) | 5 (14%) |
| Macroalbuminuria (albumin/creatinine ratio: >300 mg/g) | 0 | 0 | 0 |
| Hypertension | 2 (7%) | 4 (13%) | 1 (3%) |
| Hematuria | 1 (3%) | 0 | 0 |
| Chronic kidney disease (defined as GFR<60 ml/min/1.73m$^2$) | 1 (3%) | 0 | 2 (6%) |

**Conclusions:** In contrast to previous reports, hematuria and macroalbuminuria were rare in our patients with sickle cell disease and its variants while enuresis, microalbuminuria and hypertension were more common.

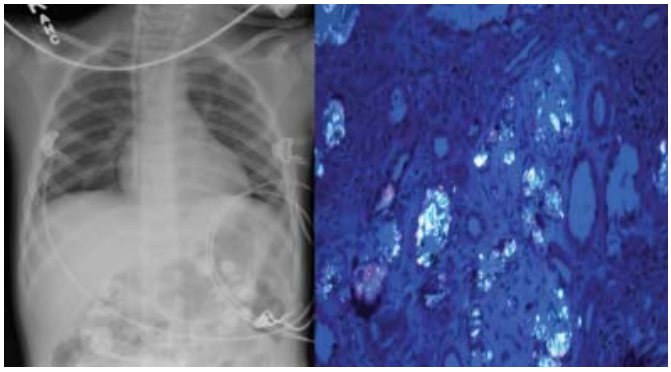
## PUB122

### Primary Hyperoxalosis Presenting with Anemia, Thrombocytopenia and Acute Renal Failure

Luis A. Ortiz. *Pediatric Nephrology - Pediatrics, Children's Hospital of Georgia, Augusta, GA.*

**Background:** Primary Hyperoxaluria (PH) is an AR inborn error of metabolism resulting in high oxalate production and excessive urine oxalate excretion, it is a deficiency in the liver peroxisomal enzyme alanine-glyoxylate aminotransferase that catalyzes the transamination of glyoxylate to glycine. Failure to detoxify glyoxylate results in its conversion into oxalate then the endogenous production and urinary oxalate and glycolate are elevated. It is 1% of pediatric ESRD.

**Methods:** Evaluation of a 5 y/o healthy w/g, she presented fatigue, abdominal pain and vomiting. Labs work-up showed Hgb 6.7, platelets 133; K 4.2, HCO3 14, BUN: 210mg, Cr 11.67mg**.** She was drinking one gallon of milk/day for the last 3 years. Her physical Exam was unremarkable with BP: 93/55 and UOP 2.3ml/kg/hr. CxR: bilateral nephrocalcinosis, DsDNAab, pANCA, cANCA were negative; Stool for E. coli 0157 H7 were negative; urine oxalate 335 [<129], glycoxylate (10.2 [<4]), Urine oxalate-creatinine ratio of 25.7 and serum oxalate level was 123.1 (<1.8). Renal biopsy on day # 3: abundant deposit of oxalate crystals compatible with Primary hyperoxaluria.



Serum Alanine: Glyoxylate Aminotransferase (AGXT) mutation full gene analysis was (+). Bone marrow biopsy, bone serial x-rays, eye exam were negative. Treatment with Low oxalate diet, PO fluid intake of 2 L/m2 day, pyrodixine (B6) 5mg/kg and daily hemodialysis (3-4 hours/day) avoided extra renal damage, but renal function was not recovered.

**Conclusions:** Clinical presentation in PH was very atypical. Nephrocalcinosis was a significant finding for the diagnosis. Early kidney Bx was the best procedure for final diagnosis (<36 hours). There was a delayed in the crystal precipitation in other tissues/organs because of the high amount of Calcium she was drinking with the milk. Aggressive dialysis with low oxalate diet was the best procedure to avoid oxalate crystal precipitation.

## PUB123

**Why Pediatricians Fail to Diagnose Hypertension: A Multicenter Survey**
Arend Bokenkamp,[1] Merijn Bijlsma,[2] Hester Blufpand,[2] Gert-jan Kaspers.[3] *[1]Pediatric Nephrology, VU Univ Medical Center, Amsterdam, Netherlands; [2]Pediatrics, VU Univ Medical Center, Amsterdam, Netherlands; [3]Pediatric Oncology, VU Univ Medical Center, Amsterdam, Netherlands.*

**Background:** It has become clear that adult hypertension begins in childhood and contributes to the development of cardiovascular disease. Early recognition and intervention may slow down the progression of elevated blood pressure (BP) to cardiovascular disease. Recent studies indicate that the majority of hypertensive cases are unrecognized. The purpose of this study was to elucidate why pediatricians fail to diagnose childhood hypertension, with special emphasis to the use of reference data. We hypothesized that pediatricians frequently omit to measure BP and do not routinely relate BP measurements to reference data.

**Methods:** Multicenter questionnaire survey among 197 pediatricians, pediatric residents and medical students in the Netherlands about their daily practice of BP measurement and the use of pediatric BP reference tables. Furthermore, respondents were asked to estimate BP percentiles and classify BP readings in 12 short clinical cases (6 normal BP, 2 pre-hypertension, 4 hypertension).

**Results:** 71% of physicians only measure BP during ambulatory visits if a child has risk factors for hypertension. After measuring BP, 65% compares the reading to reference data only if they suspect it to be elevated. However, their ability to rate a reading at its true value was limited. Forty-seven percent of the physicians categorized one or more of the prehypertensive or hypertensive cases as normal.

**Conclusions:** Most pediatricians only measure BP in certain circumstances, contrary to recommendations. After obtaining a BP measurement, the majority does not compare the reading to reference standards. Without the use of reference data, however, they commonly underestimate the BP percentile and potentially miss childhood hypertension. The introduction of automated blood pressure monitors that directly report blood pressure percentiles might improve the recognition of childhood hypertension.

## PUB124

**Genetic Control of Renin Angiotensin System and Extracellular Matrix-Related Protein Polymorphismsin Obese Preschool Children**
Kee Hwan Yoo, Hyung Eun Yim, In Sun Bae, Young Sook Hong, Joo Won Lee. *Pediatrics, Korea Univ Medical Center, Seoul, Korea.*

**Background:** We have recently shown that postnatal overnutrition in male rats leads to obesity and the acquired reset of the intra-renal renin angiotensin system (RAS) and of extracellular matrix (ECM)-linked moleculesduring renal maturation. Long-standing obesity, renal functional and structural impairment, and hypertension were programmed later in adulthood. This study was designed to investigate whether genetic polymorphisms of RAS and ECM-linked proteins were associated with the susceptibility to obesity in childhood.

**Methods:** We performed the genotyping of angiotensin converting enzyme (ACE), angiotensinogen (AGT), angiotensin type 2 receptor (AT2), matrix metalloproteinase (MMP)-9, and plasminogen activator inhibitor (PAI)-1 proteins in 46 obese, 48 overweight, and 96 control children using PCR-restriction fragment length polymorphism analysis.

**Results:** The mean age was 22.3 ± 1.5 months in controls, 17.3 ± 2.19 months in overweight children, and 24.2 ± 2.96 months in obese children. No differences among the groups were found with respect to age, sex, gestational age, birth weight, blood pressure levels, and serum levels of glucose and cholesterol. Obese children showed a lower incidence of ACE II genotype, compared to the controls (Control 46.8% Vs. Obese 15.6%, $P < 0.05$). Overweight children had a higher incidence of MM genotype in the AGT M235T polymorphism (Control 2.1% Vs. Overweight 16.7%, $P < 0.05$). In overweight children, the MMP-9 C1562T polymorphism revealed an increase in CC genotype (Control 66.3% Vs. Overweight 90.7%, $P < 0.05$) and a decrease in CT genotype (Control, 30.5% Vs. Overweight 9.3%, $P < 0.05$). In the case of combination of overweight and obese children, a higher incidence of MM genotype in the AGT M235T polymorphism was found compared to the controls (Control 2.1% Vs. Overweight + Obese 10.6%, $P < 0.05$). There were no genotype differences of the AT2 C3123A and the PAI-1 4G/5G polymorphism among the groups.

**Conclusions:** The ACE I/D, AGT M235T, and MMP-9 C1562Tpolymorphisms could be suitable genetic markers for development of overweight or obesity of childhood.

*Funding:* Government Support - Non-U.S.

## PUB125

**Eculizumab (Anti C5 Monoclonal Antibody) in a Child with Therapy Resistant Systemic Lupus Erythematodes**
Rosanna Coppo,[1] Alessandro Amore,[1] Licia Peruzzi,[1] Roberta Camilla,[1] Maria Elena Donadio,[1] Giuliana Guido,[1] Luca Vergano,[1] Silvana Martino,[2] Pier angelo Tovo.[2] *[1]Nephrology Dialysis Transplanation, Regina Margherita Hospital, Turin, Italy; [2]Pediatrics, Univ of Turin.*

**Methods:** A 4 y.o girl presented with extremely severe systemic lupus erythematodes (SLE) (SLEDAI score: 42; C4<4 mg/dl, C3 <20 mg/dl, ANA>1/640; anti DNA>400 UI/ml),lupus nephritis Class IV-G, e-GFR120ml/min/1.73m2, UP/UCr5mg/mg). The symptoms slightly improved after 3 MP pulses, prednisone, 9 plasmapheresis, cyclophosphamyde (withdrawn due to leukopenia) followed by cyclosporine. Three months later she worsened: e-GFR 60ml/min/1.73m2, UP/UCr 6 mg/mg. In spite of Rituximbab 1g/1.73m2 and MP pulse, SLEDAI increased to 63 (hypertension, seizures, numbness, O2 desaturation, pleural effusion). Platelets (Ptls) 55,000/mm3, aptoglobin <10 mg/dl, neg LAC, e-GFR 60ml/min/1.73 m2, UP/UCr 7mg/mg. After eculizumab 20 mg/Kg, she had impressive improvement in general conditions with normalization of Ptls, aptoglobin and e-GFR increase to 140 ml/min/1.73m2. Eculizumab was repeated over 2 months, then stopped. Rituximab was infused but immediately stopped for severe adverse reaction. A new attempt to use cyclophosphamyde was interrupted because of leukocytopenia. 9 plasmaphereses were performed, but renal conditions worsened (e-GFR 50 ml/min/1.73m2, UP/UCr 8) with a new drop in Ptls 70,000/mm3, aptoglobin <10 mg/dl (LAC neg). A repeated renal biopsy confirmed Class IV nephritis without any sign of thrombotic microangiopaty. However, when eculizumab was re-introduced (20 mg/Kg) a prompt improvement was observed with normalization of aptoglobin level, Ptls count and e-GFR. The child was followed for 4 months on maintenance treatment with cyclosporin, prednisone and eculizumab every other week. SLE data remained unchanged over the follow-up (C4 < 4 mg/dl, C3 30 mg/dl , ANA 1/640, Anti DNA >400 UI/ml), however e-GFR is 138 ml/min/1.73 m2, UP/Ucr 1 and SLEDAI score is reduced to 14 with good general conditions. Complement genetics and ADAMST are negative.

**Conclusions:** This is the first case reporting a beneficial effect of C5 inhibition as rescue therapy in a SLE refractory to the standard therapies.

## PUB126

**Hydrothorax, a Serious Complication of Infants on Peritoneal Dialysis – Can We Identify Risk Factors**
Michaela Gessner, Christina Taylan, Gesa Schalk, Rasmus J.C. Ehren, Lutz Thorsten Weber. *Dept of Child and Adolescent Medicine, Univ Hospital, Cologne, Germany.*

**Background:** Approximately 150 children suffer from end-stage renal disease requiring renal replacement therapy in Germany every year. Due to congenital kidney disease 2/3 of them are still in infancy at initiation of therapy, which is most commonly peritoneal dialysis (PD) in this age group. Besides infections another serious complication of this procedure is the development of a hydrothorax, possibly due to an embryonic remnant of a persisting pneumatoenteric recess or an infracardiac bursa, a passage connecting the peritoneal cavity to the right pleural space.

**Methods:** In this single center analysis all infants that had started PD in their first year of life between 2002 and 2012 were included (n=19). Infants with short-term PD treatment due to acute renal failure were excluded. All infants had a minimal duration of PD of four months. PD catheters were inserted by laparotomy. Median initiation of PD was at the ninth day of life (range: second day to ninth month).

**Results:** 3 of 19 infants were symptomatic with a hydrothorax at a median of 49.5 weeks (range: 33.6-72.1) after start of PD treatment, finally leading to long-term change of treatment modality in 2/3. All affected infants had been born prematurely. However, another 11 premature infants in this group did not show this complication. There was no hydrothorax in 5 term deliveries. Hydrothorax occurred independently from previous abdominal surgeries, the occurrence of catheter obstruction, birth weight, mode of delivery, APGAR score, age at initiation or duration of PD. However, this complication appeared in 3/6 preterm infants of Turkish origin.

**Conclusions:** This analysis failed to determine risk factors for the incident of hydrothorax after initiation of PD treatment in infants. The overall risk in our cohort was 15.9% and 21.4% in preterm infants, respectively, that is higher than reported in literature (3%), which reviews other cohort compositions regarding age of children. Since preventive actions do not exist, physicians must be aware of this serious complication for the purpose of early detection and treatment, especially in preterm infants.

## PUB127

**Reducing Central Venous Catheters in Children on Chronic Hemodialysis**
Rossana Baracco, Tej K. Mattoo, Amrish Jain, Gaurav Kapur, Rudolph P. Valentini. *Pediatrics, Children's Hospital of Michigan/Wayne State Univ, Detroit, MI.*

**Background:** Permanent vascular access is preferred over central venous catheters (CVC) for chronic hemodialysis (HD). However, CVCs remain the most commonly used access in children. The objective of this study was to assess the impact of increased arteriovenous fistula (AVF) use in our HD patients.

**Methods:** Retrospective chart review of children ages 1 to 18 on chronic HD from January 2001 to June 2012 at Children's Hospital of Michigan. Patients were divided into 3 time periods; 2001 to 2005; 2006 to 2009; and 2010 to 1012. A systematic approach to AVF placement was introduced in 2006 and a new, more experienced, vascular surgeon became available in 2010.

**Results:** There were 55 patients (67.3% male) that had an AVF placed. Of 65 AVF created, 41 (63.1%) were successfully used. AVF use rates increased and CVC use rates decreased with time; by the end of the third time period only 7.7% of HD patients were using a CVC. The number of successful AVF were significantly higher in the third time period. Primary patency rate was 42.9% at one year. Secondary patency rates were 96.8% at one year, 88.7% at 2 years and 76% at 5 years. Infection and hospitalization rates were higher for CVC compared to AVF (0.8 vs 0.1 infections per access-year and 1.0 versus 0.3 hospitalizations per access-year, respectively).

| | 2001 - 2005 | 2006 - 2009 | 2010 - 2012 |
|---|---|---|---|
| AVF use rates (%) | 33.3 | 42.9 | 76.9 |
| AVG use rates (%) | 16.7 | 35.7 | 15.4 |
| CVC use rates (%) | 50 | 21.4 | 7.7 |
| AVF N (%) | | | |
| Successful | 10 (52.6)* | 19 (57.6)* | 12 (92.3)* |
| Unsuccessful | 9 (47.4) | 14 (42.4) | 1 (7.7) |
| Demographics of successful AVF | | | |
| Age median in years (range) | 14 (10 - 17) | 15 (12 - 17) | 13.5 (3 - 18) |
| Weight median in kilograms (range) | 42.1 (27 - 82) | 61 (28 - 113.4) | 60.5 (14.7 - 110.7) |
| AVF=arteriovenous fistula, AVG=arteriovenous graft. *p≤0.05 | | | |

**Conclusions:** With a dedicated team committed to permanent vascular access placement, it is possible to decrease the use of CVCs and increase AVF use in children. Increased use of AVF decreases access related infection and hospitalization rates.

## PUB128

**Design of IM103-144: A Pediatric PK/Conversion Study with Belatacept to Assess PK, Safety, Tolerability, and Preliminary Efficacy in EBV-Seropositive Pediatric Renal Transplant Recipients**
Robert B. Ettenger,[1] Allan D. Kirk,[2] Sandra Amaral,[3] Vikas R. Dharnidharka,[4] Daniel Feig,[5] Bishu Ganguly,[6] Charlotte Jones-burton.[6] *[1]UCLA, US; [2]Emory Univ; [3]Children's Hosp Philadelphia; [4]Washington Univ, US; [5]Univ of Alabama, US; [6]BMS, US.*

**Background:** Belatacept(bela) is approved for use in prevention of acute rejection(AR) in adult EBV-seropositive(+) kidney transplant(TX) recipients. In Ph 3 trials, bela was associated with comparable pt & graft survival & superior renal function despite higher rates & grades of early AR vs cyclosporine. The current study, the first study of bela in children, will determine if similar results occur in pediatric kidney TX recipients. Study objectives are to establish dose & evaluate PK, efficacy, & safety in pediatric conversion.

**Methods:** In this global multicenter study, 54 kidney TX recipients, currently on CNI-based immunosuppression (IS), will be studied in 2 groups: adolescents(age 12-17y) & children(6-11y). Pts will be ≥1y post-TX, will have stable eGFR 45–75 ml/min/1.73m$^2$, & be EBV+ both at TX & start of study. Study will be conducted in 3 phases: 1) uncontrolled single dose phase(SD) to determine PK characteristics & appropriate dosing for bela in each age group; 2) multiple dose phase(MD): pts randomized 2:1 to convert from CNI to bela or remain on CNI for 12 months; 3) long term extension phase (LTE). Enrollment will be staggered, with adolescents (N=9) starting SD first; adolescent PK results will inform dosing in this group for MD & also in the younger group for SD (N=6). All pts in SD will continue to receive all maintenance IS (steroids optional). In MD, CNI will be discontinued for pts in the bela arm.

**Results:** PK & safety determinations will be assessed in all pts in SD & MD. Outcomes to be examined during MD & LTE include the evolution of the eGFR by the updated Schwartz formula, AR, graft loss, & safety (including PTLD) in bela & CNI arms. Medication adherence & quality of life will also be explored.

**Conclusions:** This controlled multicenter trial will provide dosing & PK information, as well as initial efficacy & safety information about bela use in stable EBV+ children & adolescents following kidney TX.

## PUB129

**Relationship between Birth Weight and Renal Volume in Children with and without Overweight**
Maria Eugenia V. Bianchi,[1] Gustavo A. Velasco,[1] Daniel Forlino.[2] *[1]Argentinian Northeast Kidney Foundation, Resistencia, Chaco, Argentina; [2]Radiology, Facultad de Medicina Unne, Corrientes, Argentina.*

**Background:** Determination of renal volume by ultrasound (ERV) is considered as an indirect indicator of the number of nephrons. As it know the low number of nephrons can be considered an independent risk factor for the development of hypertension, and taking into account that low birthweight has been associated with a low kidney volumen, we evaluate differences in renal volume in scholar children with and without overweight and its relationship to birthweight.

**Methods:** We performed a cross-sectional observational study with non-probability sampling, in children of school of the Province of Chaco, by his guardian consent. The variables considered were: Nutritional Status by BMI according to WHO tables (overweight and normal-weight), referred Birthweight, VRE and VRE corrected for body surface area (SC), used for them a portable ultrasound. Maximum diameters were recorded Right and Left Kidney in the longitudinal, transverse and anteroposterior axis. The VRE was calculated using the ellipsoid formula and SC was estimated by Du Bois formulation. Diameters and VRE, were associated with birthweight and actual nutritional status.

**Results:** We studied 84 children, after excluding those with morphologically abnormal kidneys and low weight. The mean age was 9.14 ± 2.36 (range 3-13 years), 36 were male (42.8 %), and the average SC was 1.20 ± 0.27 m$^2$, 46 were overweight (54,8%). The combined volume of both kidneys for children with overweight was 174.4 ± 40.9 ml and for those with normal weight was 134.4 ± 39.2 ml (p <0,05). Classified by birthweight (<3300 and ≥ 3300 g) in the normal weight group combined VRE were 148.6 ± 43.6 ml and 120.2 ± 29.0 ml (p <0.05) while in the overweigth group cambined VRE were 165.3 ± 29.6 ml and 180.8 ± 46.8 ml (p= NS).

**Conclusions:** .VRE differences found in the groups according to referred birthweight disappear in actually overweight children. We conclude, that overweigth enhanced the combined VRE masking the difference found in the low birthweigth.

*Funding:* Private Foundation Support

## PUB130

**Disease Burden of Cystinosis**
Larry A. Greenbaum,[1] Ben Cadieux.[2] *[1]Pediatric Nephrology, Emory Univ, Atlanta, GA; [2]Raptor Pharmaceuticals, Novato, CA.*

**Background:** Cystinosis is a rare autosomal recessive disorder with a variety of renal (Fanconi syndrome and CKD) and extrarenal complications. Cysteamine, which reduces lysosomal levels of cystine, is the only specific therapy, and prevents, attenuates or delays disease complications. However, Q6H dosing may limit adherence. The disease burden of cystinosis has been assessed via single center studies and among patients who travel to the NIH. However, these approaches may lead to significant selection bias. We report the long-term trajectory of cystinosis-related and comorbid disease among 9 pediatric Medicaid enrollees.

**Methods:** We examined 12 years (7/1/97 to 6/30/09) of Florida Medicaid claims data and identified 9 newly-diagnosed pts (6 male) with cystinosis (based on ICD-9 270 and initiation of cysteamine at ages 12-43 mos).

**Results:** Duration of claims data was 3.5-12 years. There were 231 diagnostic, procedural or claims-related complications possibly or probably related to cystinosis (22, 9.5%) involved an ED visit). There was an average of 3.4 claims per year (range 1.7 to 5.7/yr). 29 claims were related to CKD (including CKD IV for 2 pts at 13 yr; ESRD in 2 pts at 9 and 11 yrs; hyperparathyroidism in 3 pts). There were 2 pts with obstructive hydrocephalus; 1 with heart failure at 11 yrs. The majority (6/9 pts) had feeding problems; 4 had complications from g-tubes. There were 72 claims related to infections (UTIs x 28 in 7 pts; otitis media x 24 in 6 pts). Developmental issues were common: speech/language difficulties in 2 pts (1 pt with additional conduct disorder); ADHD in 1 pt; mental retardation and epilepsy in 1 pt.

**Conclusions:** Claims data analysis provides a complementary approach for assessing disease burden and long-term complications of cystinosis. We observed substantial cystinosis-related disease burden and unexpected comorbidities, including obstructive hydrocephalus, heart failure, and frequent UTIs. Future analysis will assess disease burden following the recent introduction of q12h dosing cysteamine, which has been found to improve QOL and is expected to improve adherence.

*Funding:* Pharmaceutical Company Support - Raptor Pharmaceuticals

## PUB131

**Adherence to Cysteamine Therapy in Cystinosis**
Larry A. Greenbaum,[1] Ben Cadieux,[2] David Lapidus.[3] *[1]Pediatric Nephrology, Emory Univ, Atlanta, GA; [2]Raptor Pharmaceuticals, Novato, CA; [3]LapidusData LLC, Brookline, MA.*

**Background:** Early initiation and adherence to cysteamine therapy are essential in delaying progression to ESRD in cystinosis. Immediate-release cysteamine (IR-C) requires chronic, lifelong Q6H dosing. Objective measures of long-term adherence are challenging to quantify.

**Methods:** We examined IMS data for adherence to IR-C in cystinosis patients. From 2002 to 2012, we identified 221 unique pts (104 male) ages 0 to 51 years with cystinosis (based on ICD-9 270.0 and claims for IR-C). Adherence was assessed by refill data and used to calculate a medication possession ratio (MPR).

**Results:** Twelve, 13, 11 and 9 new pts were diagnosed per year from 2008 to 2011. In a representative year (2011), 207 pts were distributed equally among younger age groups (born post IR-C): 0-10 (N = 75), 11-20 (N = 74); whereas it progressively declined for those born before IR-C approval: 21-30 (N = 52), 31-40 (N = 16), 41-50 (N = 3), 1 pt age 51.

The average duration of reliable IR-cysteamine claims data among all pts (N = 221) was 4.5 years, with an overall MPR of 79%. The MPR declined by age group: 88% (0-5 yrs), 83% (6-11 yrs), 79% (12-17 yrs), and 64% (>17 yrs). The percentage of pts who had a least 1 gap >180 days between IR-C fills increased by age group: 27%, 33%, 35% and 40%, respectively. In pts 0-5 yrs, a diagnosis of failure to thrive (22/34 pts) was associated with a higher MPR (95%) vs. those w/out this diagnosis (83%).

69 of 97 pts (71%) born before the approval of IR-cysteamine reached ESRD; 8 of 93 (9%) and 29 of 93 (31%) pts born from 1994 to 2006 achieved CKD stage IV and ESRD, respectively. The median age for ESRD post approval of IC-cysteamine was 12 yrs (range 1 – 16).

**Conclusions:** Claims data is a novel approach for assessing long-term adherence to cysteamine in patients with cystinosis. Adherence with IR-C according to IMS claims with cystinosis is low and declines with age. Future analysis will correlate adherence with health outcomes, and analyze changes in adherence following the recent introduction of delayed release cysteamine (q12h dosing formulation). Efforts to improve adherence in this population are needed.

*Funding:* Pharmaceutical Company Support - Raptor Pharmaceuticals

## PUB132

**Anhydramnios in Fetal Nephropathy: A Non Conventional Indication to Amnioinfusion. Second Work of Mercy: To Give Drink to the Thirsty**

Gianluigi Ardissino,[1] Roberto Fogliani,[2] Sara Testa,[1] Nicola Persico,[2] Francesca Tel,[1] Simona Boito,[2] Sarah Salmona,[2] Fabietti Isabella,[2] Maria albina Galli.[3] *[1]Pediatric Nephrology, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico; [2]Obtetrics and Ginecology, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico; [3]Pediatric Cardiology, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico.*

**Background:** Oligohydramnios, complicating 4-5.5% of all pregnancies, may occur in some fetal nephropaties. When present in association with fetal kidney diseases, it predicts a more severe postnatal prognosis for it anticipates congenital reduction of renal function. Most of congenital nephropathies are characterized, soon after birth, by significant poliuria and, consequently, by increased needs of fluid which, if unmet, are responsible for worsening of renal function. In case of oligohydramnios the fetus might not count on an adequate amount of drinking fluid possibly leading to fetal dehydration and consequent further reduced renal function.

**Methods:** A pregnant woman who presented with anhydramnios from the 32nd wk of gestation, with severe fetal bilateral hypodisplastic kidney and repeatedly documented empty bladder, was addressed to 2 amnioinfusions (saline total 750cc) for providing the fetus with the fluid normally swallowed during late pregnancy (around 100 cc/kg/day) with the working hypothesis of rehydrating the fetus, improving renal perfusion, preventing further deterioration of renal function and eventually for reversing fetal anuria.

**Results:** Soon after the amnioinfusions a significant increase in the bladder volume (from 18 to 54 mm3) was observed indicating that fetal diuresis had restarted; this was associated with a doubling of both right (from 0.6 to 1.4 mL) and left (from 0.4 to 1.1 mL) cardiac ouput.

**Conclusions:** It is speculated that anhydramnios in fetal nephropathies may be responsible for further reduction of renal function secondary to fetal dehydration and that amnioinfusion may revert this functional worsening.

## PUB133

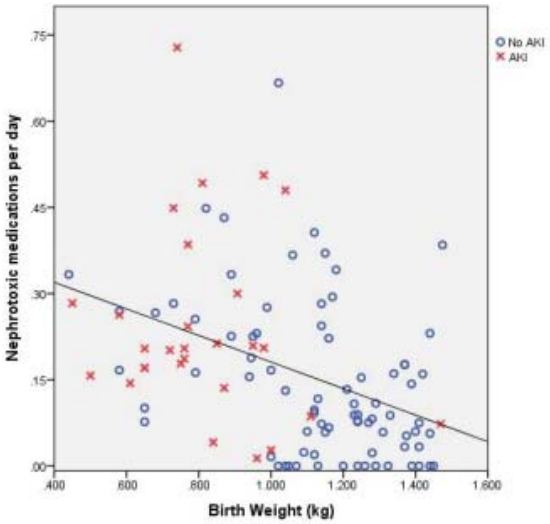
**Nephrotoxic Medication Exposure in Very Low Birth Weight Infants**

Erika Rhone,[1] J. Bryan Carmody,[1] Jonathan R. Swanson,[2] Jennifer Richardson Charlton.[1] *[1]Pediatrics, Div of Pediatric Nephrology, Univ of Virginia, Charlottesville, VA; [2]Pediatrics, Div of Neonatology, Univ of Virginia, Charlottesville, VA.*

**Background:** Nephrogenesis continues until 36 weeks gestation, meaning that many very low birth (VLBW) infants must complete development *ex utero*. While potentially nephrotoxic medications (NM) are commonly used in the neonatal intensive care unit, the extent of exposure among VLBW infants has not been established. We sought to quantify NM exposure among VLBW infants in a Level IIIc NICU.

**Methods:** We retrospectively reviewed the electronic medical record of VLBW infants (n=107) admitted from April 2011 to March 2012, excluding those admitted at >2 d of age or who died during hospitalization. Exposure to the following was calculated in person days: acyclovir, amikacin, amphotericin B, gentamicin, ibuprofen, indomethacin, iohexol, tobramycin, and vancomycin. AKI was assessed using the Kidney Disease: Improving Global Outcomes system, modified to include serum creatinine alone.

**Results:** Exposure to 1 or more NM occurred in 86.9%. The median number of NM was 2 (range 0-6). On average, each infant received 0.17 NM per day when accounting for length of stay. The most common exposures were gentamicin (86%), indomethacin (43%), and vancomycin (25%). Exposure to other NM occurred in <10%. There was an inverse linear relationship between birth weight and mean NM received per day ($\beta$=-0.231, $R^2$=0.169, p<0.001).




AKI occurred in 26.2%. Infants who experienced AKI received higher mean NM per day than those who did not (0.24 versus 0.15;p=0.003).

**Conclusions:** Exposure to NM in VLBW infants was common and occurred in nearly 90% of all NICU survivors. Infants received on average 1 NM per every 6 days spent in the NICU. The greatest nephrotoxic medication exposure occurred in the smallest, most immature infants and in those who experienced AKI.

## PUB134

**A Systematic Approach for the Management of Antenatal Hydronephrosis in Children**

Joana R. Dos Santos,[1] Tino D. Piscione,[1] Rulan S. Parekh,[1] Norman D. Rosenblum.[1] *[1]Nephrology, The Hospital for Sick Children, Toronto, Canada; [2]Urology, The Hospital for Sick Children, Toronto, Canada.*

**Background:** Hydronephrosis in children may require surgery to prevent deterioration in renal function; however, it is unclear which grading system will best predict outcomes. Our goal was to evaluate the accuracy of the Society for Fetal Urology (SFU) grading system compared to measurement of anteroposterior diameter of renal pelvis (APPD) at predicting need for surgery.

**Methods:** The study was a retrospective chart review of patients referred for antenatal hydronephrosis to Nephrology and/or Urology clinics between 2003-10 who had a first postnatal ultrasound < 3 months and at least minimum 1 year of follow-up.

**Results:** 1191 patients were screened; 526 met the inclusion criteria. In a subset of patients analyzed to date (N=416), 79.8% were male, 249 patients had unilateral, and 167 bilateral hydronephrosis. 115 patients underwent surgery (27.6%; mean age 454 ± 643 days; median 244; range 1-3764). APPD measurement at baseline demonstrated positive predictive value (PPV) for surgery of 50.8% (sensitivity 55.2%; specificity 79.7%) and SFU grading demonstrated a PPV for surgery of 54% (sensitivity 81.5% and specificity 73.7%). Logistic regression showed no statistical significance between APPD and SFU grading for predicting surgery at baseline (APPD: OR 2.72, CI 95% 2.06-3.06, p value 3; SFU: OR 3.1,CI 95% 2.5-3.8, p value 1.3). Combined APPD and SFU grading (Group 1: APPD <12 mm + SFU 0-2; Group 2: APPD< 12 mm + SFU 3-4; Group 3: APPD ≥ 12mm + SFU 0-2; and Group 4: APPD≥12mm+ SFU 3-4) showed higher PPV of 60% (sensitivity 34.2% and specificity 91.3%) but did not improve accuracy for predicting surgery when compared to SFU and APPD alone (OR 3.9, CI 95% 2.5-6.1, p value 1.9).

**Conclusions:** These data suggest that neither APPD or SFU grading alone, nor a grading system which combines both methods are good predictors for surgery at baseline. Our future analyses are aimed at determining whether accuracy for predicting surgery is improved by evaluating interval changes in APPD and/or SFU over time.

## PUB135

**Successful Remission of Treatment-Resistant Minimal Change Disease with Adrenocorticotropic Hormone Gel**

M. Khurram Faizan,[1] Ruchir S. Patel.[2] *[1]Dept of Pediatrics, Hasbro Children's Hospital; [2]Dept of Medicine, Rhode Island Hospital, Providence, RI.*

**Background:** Adrenocorticotropic hormone (ACTH) has historically been used to treat pediatric nephrotic syndrome (NS) and also received FDA approval for the treatment of NS in the 1950's. It fell out of favor with the advent of oral steroids. Recent case reports and small case series reporting the successful use of ACTH has rekindled interest in its use for treatment of NS resistant to conventional immunosuppression.

**Methods:** We report a 19 yr old Caucasian female with frequently relapsing, treatment resistant NS who achieved remission with purified porcine ACTH (ACTHAR Gel®) treatment. Patient was diagnosed with NS at age 10. Two renal biopsies 5 yrs apart showed minimal change disease. Clinical course was complicated by numerous relapses despite chronic steroid and CNI therapy, severe dyslipidemia, and episodes of anasarca requiring IV albumin/furosemide. PMH: Significant for splenic infarct at age 14 and restrictive eating disorder. ROS: Positive for intermittent swelling, fatigue, and anorexia. Medications: Prednisone 10 mg, Tacrolimus 4 mg BID, Losartan 25 mg, Lisinorpil 10 mg, Simvastatin

40 mg, Penicillin V 250 mg BID. Vital signs: BP 122/81, Pulse 104, afebrile. Wt 51 kg, BMI 20.64. Physical exam: No lower extremity edema. Table 1 shows labs at baseline and post-treatment. ACTH was started at 40 units SQ twice a week for 6 mths. Tacrolimus and prednisone were tapered.

**Results:** Our patient underwent prompt remission of proteinuria and marked improvement in lipid levels, did not experience any side-effects, and remains on ACTH (Table 1). Subjectively, she reported improvement in her mood, energy and self-esteem.

**Table 1:**

| | Before ACTH | 2 Wks | 4 Wks | 6 Wks | 10 Wks |
|---|---|---|---|---|---|
| Urine Protein/Creatinine Ratio | 5.35 | 0.66 | 0.68 | 0.24 | 0.12 |
| Albumin (g/dl) | 1.7 | | | 2.9 | 3.2 |
| Creatinine (mg/dl) | 0.82 | | | 1.01 | 0.69 |
| Cholesterol (mg/dl) | 506 | | | 403 | 240 |
| Triglyceride (mg/dl) | 717 | | | 324 | 172 |

**Conclusions:** Our report suggests that ACTH may be an important alternative in patients with challenging and difficult to treat NS due to minimal change disease. However, long term efficacy and side-effects remain unknown. Large scale prospective studies are needed to further validate our observation.

## PUB136

**Effect of AngiotensinII Type 1 Receptor Blocker on the 12-Lipoxygenase Activation and Slit Diaphragm Protein Expression in Type 2 Diabetic Rat Glomeruli** Zhong-gao Xu, Wan-ning Wang, Fu-zhe Ma, Tao Sun. *Dept of Nephrology, The First Hospital of Jilin Univ, Changchun, Jilin, China.*

**Background:** 12-lipoxygenase (12-LO) is implicated in the development of diabetic nephropathy, in which the proteinuria is thought to be associated with a decreased expression of glomerular slit diaphragm protein. Recently, it was demonstrated that nephrin expression, but not p-cadherin expression, varies among the different sizes of glomeruli under type 2 diabetic conditions. Furthermore, the decreased expression of nephrin in the hypertrophied glomeruli may be responsible for the albuminuria at the early stage of diabetes. In this study, we investigated the effect of angiotensinII(AngII) type 1 receptor blocker(ARB) on 12-LO activation and slit diaphragm protein expression in type 2 diabetic rat glomeruli.

**Methods:** Podocytes were stimulated by AngII for 24 hours. 12-LO product 12(S)-HETE and AngII were infused to rats by osmotic mini-pump for 1 week and 2 weeks respectively. Rats fed high fat diet were received low dose streptozotocin (STZ) to make type 2 diabetes and divided into 2 groups:low dose STZ(DN), low dose STZ+ARB treatment(Losartan). Rats fed regular chow were as control group (Ctrl). All rats were sacrificed after 6 weeks. Glomeruli were isolated with a sieving method, we used relative large glomeruli (on the 125μm sieve, not on the 75μm sieve). ELISA, RT-PCR and Western blot for related target were performed respectively.

**Results:** AngII increased 12(S)-HETE levels in podocytes and glomeruli. AngII levels in the glomeruli were significantly increased by 12(S)-HETE stimulation. Blood glucose, kidney/body weight and albuminuria were increased in DN compared with Ctrl. However, albuminuria, Kidney/body weight were decreased after Losartan treatment compared with DN. Increment of 12(S)-HETE content and decrement of nephrin and p-cadherin expression were observed in DN glomeruli compared with Ctrl.These abnormalities were prevented by administration of the Losartan.

**Conclusions:** These results indicate that ARB can ameliorate the progression of DN via upregulation of glomeruli nephrin and p-cadherin through inhibition of 12-LO activation in type 2 DN rat.

*Funding:* Other NIH Support - NSFC Support

## PUB137

**The Effect of High Glucose Exposure on Iron Induced Cytotoxicity and the Protective Role of Carnosine** Sibylle Jenny Hauske, Emmanouil Ntasis, Eleni Stamellou, Shiqi Zhang, Bernhard K. Krämer, Benito Yard. *Vth Medical Dept, Medical Faculty Mannheim, Univ of Heidelberg, Germany.*

**Background:** It is generally believed that diabetic complications are oxidative stress mediated. The production of mitochondrial derived reactive oxygen species (ROS) seems to play a pivotal role herein. Recently it has also been discussed that increased intracellular iron accumulation, occurring under high glucose conditions (HGC) may lead to additional oxidative damage.

**Methods:** In keeping with the postulated protective role of L-carnosine (CAR) in diabetic complications we sought to asses in this study: 1) if HG exposure makes cultured human umbilical vein endothelial cells (HUVEC) more susceptible to metal induced toxicity, 2) if this correlates with increased expression of known iron transporters (DMT-1, IREG, TFR), 3) If CAR is able to protect HUVEC in this respect and if so, do HUVEC express CAR transporters (PEPT1, PEPT2).

**Results:** HUVEC were cultured for 2 days under normal glucose conditions (NGC) or HGC (30mM) in the presence or absence of 20mM CAR. Hereafter the cells were challenged for 24h with different concentrations of FeCl3 without changing the culture conditions. Cell viability was not impaired under HGC nor associated with increased susceptibility to FeCl3. HGC down regulated the expression of IREG1 but did not change DMT1 and TFR expression. CAR showed a strong protective effect under NGC or HGC. The protective effect only occurred if CAR was present during FeCl3 challenge but not if CAR was only applied as pre-treatment. CAR did not quench ROS. Both PEPT1 and PEPT2 were poorly expressed on HUVEC. The expression was not changed under HGC.

**Conclusions:** Our data indicate that metal induced cytotoxicity is not enhanced under HGC. This is compatible with an unaltered expression of the iron transporters DMT-1 and TFR and with down regulation of IREG1. CAR displayed a strong protective effect on iron mediated toxicity. The protective effect was not related to quenching of ROS. In keeping with the poor expression of PEPT1 and PEPT2 on HUVEC our data indicate that the protective effect of CAR most likely resides outside the cells, possibly via a mechanism that involves iron chelation.

*Funding:* Government Support - Non-U.S.

## PUB138

**Aggravation of Diabetic Nephropathy in OLETF Rats with thy-1 Nephritis** Maho Watanabe, Hitoshi Nakashima, Kenji Ito, Yasuhiro Abe, Satoru Ogahara, Takao Saito. *Div of Nephrology and Rheumatology, Dept of Internal Medicine, Faculty of Medicine, Fukuoka Univ, Fukuoka, Japan.*

**Background:** OLETF rat was established as an animal model of human type 2 diabetes. We previously reported increase of urinary protein and aggravation of diabetic nephropathy when thy-1 nephritis was introduced into OLETF rats (Clin Exp Nephrol 15:22-29, 2011). In this study, we clarifiy the mechanisms for advance of diabetic nephropathy in OLETF rats by thy-1.

**Methods:** Forty week-old OLETF rats were divided into 2 groups. One mg/kg body weight of OX7, an anti-thy1.1 antibody, was administered in one group intravenously (Group T, n=14); the same volume of 0.9% saline in the other group as control (Group C, n=14). The rats were sacrificed after 25 weeks of the injection. Using the quantitative real-time polymerase chain reaction, we assayed the transcription levels of the Smad1, NF-κB,CREB(cAMP response element binding protein), GLUT1,RAGE as diabetic nephropathy related transcription factor , ICAM-1,MCP-1,Collagen type III, IV, VI, IL-6, IL-10, TGF-β, Angiotensin II,VEGF, collagens type IV and type VI, CD68 and TGF-beta in the kidney with immunohistochemical technique. The same procedures were peformed in 2 groups of LETO rats, a non-diabetic strain of OLETF rat.

**Results:** Relative abundance of ICAM-1 mRNA normalized by beta-actin were 0.52±0.06 and0.26±0.02 in Groups T and C, respectively ($p<0.05$). The abundances of Collagen type III expression were 0.57±0.11 and 0.35±0.05; Collagen typeVI were 0.18±0.03 and 0.12±0.01 , IL-6 were 2.2E-4±1.19E-4 and 5.19E-5±2.00E-5 in Groups T and C, respectively ($p<0.05$ in all assays). In Group T , Smad1, NF-κB, GLUT1, RAGE were higher than that in Group C, but they were not significant between groups T and C. Histologically, collagen type VI ollagen positive area were increased by OX7 administration.

**Conclusions:** The inflammatory changes induced by anti thy1.1 antibody may be irreversible in the diabetic conditions of OLETF rats and accerelate the production of collagens III and VI for aggravating diabetic nephropathy. These mechanisms may be applied to the advance of diabetic nephropathy by glomerulonephritis in human, eg., IgA nephropathy.

## PUB139

**Activation of Renin-Angiotensin System Is Involved in Fatty Acid Induced ER Stress in the Kidney** Chunling Li,[1] Yu Lin,[1] Renfei Luo,[1] Moshe Levi,[2] Tianxin Yang,[1,3] Weidong Wang.[1] *[1]Institute of Hypertension and Kidney Research, Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Renal Div, Univ of Colorado Denver, Aurora, CO; [3]Dept of Internal Medicine, Univ of Utah and Veteran Affairs Medical Center, Salt Lake City, UT.*

**Background:** Free fatty acids (FA) are critically involved in the pathogenesis of diabetic nephropathy (DN), and renal proximal tubule cells are an important site for the onset of DN. The objectives of this study were to elucidate the role of renin-angiotensin system (RAS) activation in FA-induced endoplasmic reticulum (ER) stress in cultured human proximal tubule epithelial cells (HK2) and in mice fed with high-fat diet.

**Results:** Treatment with saturated FA palmitic acid (PA, 1mM) for 24h induced HK2 ER stress, leading to an unfolded protein response as reflected by increased expressions of the ER chaperone BiP (4.23±0.54 in PA vs. 1.00±0.20 in controls, $p<0.05$) and proapoptotic transcription factor CHOP (9.06±0.36 in PA vs. 1.00±0.26 in controls, $p<0.05$) as evaluated by immunoblotting. PA treatment induced increased protein expression of IRE1alpha (3.44±0.46 in PA vs. 1.00±0.16 in controls, $p<0.05$) and phosphorylation of eIF2alpha. The mRNA levels of angiotensin type I receptor (AT1R), but not AT2R, was significantly increased in response to PA treatment. In contrast, unsaturated FA linoleic acid didn't induce ER stress in HK2 cells. The AT1R blocker valsartan or renin inhibitor aliskiren protected against PA-induced upregulation of BiP (1.70±0.36 in valsartan, 1.16±0.14 in aliskiren group), CHOP (2.27±0.14 in valsartan, 2.29±0.10 in aliskiren group) , IRE1alpha (1.18±0.18 in valsartan, 1.24±0.26 in aliskiren group), and phosphorylation of eIF2alpha in HK2 cells. The renal cortex of mice (C57BL/6) fed with high-fat diet for ten weeks showed increased protein expressions of BiP (4.73±0.27 in HF vs. 1.00±0.28 in control) and CHOP (1.43±0.15 in HF vs. 1.00±0.10 in control) in comparison to control mice, which were significantly blunted by the valsartan treatment.

**Conclusions:** It is suggested that the intrarenal RAS activation may play an important role in renal injury via mediating ER stress induced by fatty acid.

*Funding:* Government Support - Non-U.S.

## PUB140

**mPGES-2 Deletion Improves Glucose Metabolism but Enhances Streptozotocin-Induced Liver Toxicity** Ying Sun,[1,2] Zhanjun Jia,[1,2] Kevin Yang,[1] Mi Liu,[1] Tianxin Yang.[1,2] *[1]Internal Medicine, Univ of Utah, Salt Lake City, UT; [2]VA Medical Center, Salt Lake City, UT.*

**Background:** Microsomal prostaglandin E synthase-2 (mPGES-2) deletion doesn't influence PGE2 production in vivo and the function of this enzyme remains elusive.

**Methods:** We examined a possible role of mPGES-2 in glucose metabolism using mPGES-2 KO mice.

**Results:** mPGES-2 KO mice had elevated blood insulin (WT: 1.34±0.18 vs KO: 2.37±0.38ng/dl, p<0.05), moderately reduced random blood glucose(WT: 119.0±5.4 vs KO: 106.1±2.84mg/dl, p<0.05) and significantly improved glucose tolerance(GTT). Interestingly, at day 4 of streptozotocin (STZ) (120 mg/kg, i.p.) administration, mPGES-2 KO mice exhibited severe lethality (6 of 9 died and 3 of 9 were near-death),, but WT controls had no noticeable abnormality. The near-death KO mice consistently presented pale liver and extremely enlarged stomach. To avoid lethality, another experiment was done at day 3 of STZ. The blood glucose levels were similar between STZ treated KO and WT mice (WT: 291.8±49.5 vs KO: 278.0±40.8 mg/dl, p>0.05). However, these KO mice showed pale liver with severe global ballooning degeneration (PAS staining), in parallel with markedly elevated liver enzymes (ALT: WTSTZ 72.7±18.1 vs KOSTZ 2910±862.1u/L, p<0.05; AST: WTSTZ 250±54.5 vs KOSTZ 5430±1539.5u/l, p<0.05), and remarkable stomach expansion with extremely thin stomach wall (PAS). However, the morphology of other organs including kidney, pancreas and heart are largely normal. By qRT-PCR and western blotting, inductions of caspase-3, Bak and Bax were much greater in KO indicating a severe liver apoptosis. Meanwhile, the inflammatory markers of IL-1β, TNF-α, MCP-1 were much higher in KO. More interestingly, a liver-specific 50% increase of Glut2, a key transporter of STZ uptake, was found in KO mice. Finally, liver PGE2 content in KO mice had high baseline (KO:747.6.23±107.2 vs WT470.2±89.2pg/mg protein: p<0.05) and greater induction after STZ (KOSTZ: 4247.6±1124.5 vs WTSTZ: 1751.9±388.8 pg/mg protein, p<0.01).

**Conclusions:** mPGES-2 deletion significantly enhanced the blood insulin and improved glucose metabolism, but enhanced STZ-induced liver toxicity possibly via Glut2-mediated STZ uptake, independently of diabetes mellitus.

*Funding:* NIDDK Support, Veterans Affairs Support

## PUB141

**The Histopathology of Diabetic Nephropathy (DN) Emerges from Two Different Pathomechanisms: A Structure-Based Analysis** Wilhelm Kriz,[1] Hermann-Josef Groene.[2] *[1]Anatomy and Embryology, Medical Faculty Mannheim, Univ of Heidelberg, Mannheim, Germany; [2]Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany.*

**Background:** The glomerular pathology of DN comprises a mesangio-capillary scenario presenting as diffuse or nodular mesangial sclerosis and a podocyte-parietal cell scenario consisting of the usual changes following podocyte loss. How these two manifestations are related to each other is poorly understood. We postulate that they emerge from different mechanisms.

**Methods:** Biopsies from patients with DN were compared with the histopathology of experimental glomerular diseases.

**Results:** Hyperfiltration, glomerular hypertension and growth are the challenges to which podocytes are exposed in DN. Podocytes have been considered as pericytes counteracting transmural pressure gradients. Based on the insight that podocytes are lost as viable cells by detachment we conclude that the podocyte cytoskeleton mainly serves to fix podocytes to the GBM; a pressure counteracting role is unlikely. The major part of hydraulic resistance must be located upstream in the GBM correlating with the synthesis of a thickened GBM in DN as a protective response to increased pressure and flow.

Inside the GBM the changes are highly specific for DN; experimentally, identical changes were found in response to high serum levels of VEGF (Hakroush et al 2009, Am J Pathol 175:1877). Normally, glomerular capillaries are located on the surface of a lobule facing Bowman's space. This placing of capillaries beneath podocytes is likely a consequence that growth stimulation by VEGF exclusively comes from podocytes. In patients with DN serum levels of VEGF are elevated likely alternating with episodes of low VEGF. We hypothesize that "diffuse mesangial sclerosis" is due to random stimulation of capillary growth by high serum VEGF associated with repression of podocyte VEGF. "Nodular sclerosis" develops during episodes of low serum VEGF. VEGF from podocytes cannot reach remote capillaries in the mesangium followed by capillary collapse and sclerosis.

**Conclusions:** Podocyte loss results from increased mechanical stress, diffuse and nodular sclerosis develop from disturbances in VEGF availability.

*Funding:* Private Foundation Support

## PUB142

**The Researches on the Protection Mechanism of DanHong-Injection to Diabetic Rats** Li Wang. *Renal Dept, Sichuan Provincial People's Hospital.*

**Background:** To investigate the renoprotective effect of Dan Hong-injection on streptozocin (STZ)-induced diabetic rats, and explore the renoprotective mechanism by comparing the expression of vascular cell adhesion molecule-1(VCAM-1) and endothelial cell adhesion molecule (PECAM-1) in kidney before and after administration.

**Methods:** The unilateral nephrectomy model of SD rats were feed with high-fat diet for one month. 12 rats were used as the control group. The other rats were given a single dose of STZ. Forty rats with diabetic kidney disease model were randomly divided into two groups:Dan Hong-injection group (DH group, n=20), saline injection group (NS group, n=20). DH group were given Dan Hong-injection intravenously (2ml/kg) daily for two weeks; NS group, NR group were given saline intravenously (2ml/kg) daily for two weeks. After treatment, urine, blood and kidney were collected.

**Results:** At 2, 6, and 10 weeks after treatment, the blood glucose in both NS and DH groups were significantly increased. BUN, SCr , and Cys-C in DH group was lower than NS group at 2 weeks after treatment. At 2, 6, and 10 weeks after treatment, Cys-C in DH group was lower than NS group. 24h-TP in DH group was lower compared with that in NS group at 6,10 weeks after administration.The glomerular's volume of DH group significantly decreased compared with NS group at 6 (P=0.023),10(P=0.049)weeks after treatment. At 2, 6, and 10 weeks after administered, the IOD of VCAM-1 and PECAM-1 in DH group were significantly weaker than that in NS group(P5.The IOD of VCAM-1 was significantly positively correlated with both proteinuria (γ=0.59, P=0.001) and glomerular's volume. The IOD of PECAM-1 was also significantly positive correlated with both proteinuriaand glomerular's volume.

**Conclusions:** Danhong-injection alleviates the early renal injury, protects renal function and reduces urine protein, and has the potential to decrease the progress of kidney damage in DKD rats. Danhong-injection decrease the expression of PECAM-1 and VCAM-1 in kidney. It reveals that the renoprotective effect of Danhong-injection may be attributed to its anticoagulant effect, thus improving the hyper-filtration state in renal and the endothelial function of kidney.

## PUB143

**Down-Regulation of TRPM6 and NCC in Diabetic Nephropathy as a Principal Cause of Hypermagnesuric Hypomagnesemia in Diabetes** Kaori Takayanagi,[1] Hajime Hasegawa,[2] Takatsugu Iwashita,[2] Yosuke Tayama,[2] Juko Asakura,[2] Naohiko Anzai,[3] Yuya Shiota,[2] Saeko Seki,[2] Nobuyuki Onizawa,[2] Tetsuya Mitarai.[2] *[1]Kawagoe Ekimae Clinic, Kawagoe, Saitama, Japan; [2]Nephrol & Hypertens, Saitama Med Center, Saitama Med Univ, Kawagoe, Saitama, Japan; [3]Pharmacol & Toxicol, Dokkyo Med Univ School of Medicine, Mibu, Tochigi, Japan.*

**Background:** Hypermagnesuric hypomagnesemia is frequently appeared in diabetic patients, and is known to be highly related to the development of insulin resistance. The present study aimed to investigate the molecular mechanism of the hypermagnesuria in diabetic nephropathy, and the involvement of interstitial damage because renal interstitial damage is not rarely associated with hypermagnesuria.

**Methods:** Kidneys were obtained from male OLETF (F) and LETO (L) rats of 16, 24, and 34 weeks-old. Altered expression of Mg transporting molecules was assessed by immunohistochemistry and RT-PCR. Time-differential development of interstitial damage was assessed by histological and molecular biological analysis.

**Results:** Absolute Mg excretion in F were significantly high in 24 and 34 weeks (0.16±0.01 in 24L, 0.28±0.01 in 24F, 0.18±0.02 in 34L, 0.29±0.04 μg/min/100g BW in 34F), showing significant hypermagnesuria from the early stage of diabetic nephropathy. Gene expressions of TRPM6 and NCC were significantly reduced in F (reduction rate of TRPM6: 73.8% in 24 week, 78.3% in 34 week, NCC; 87.9% in 24 week, 75.3% in 34 week), whereas the expressions of claudin-16 in TAL were not significantly changed. Semi-quantitative analysis of immunohistochemical expression of both molecules were agreed with the reduced gene expression (reduction rate in TRPM6; 52.7% in 34 week, NCC; 40.8% in 34 week). Expression of molecular markers for interstitial damage, such as MCP-1, α-SMA were not different between F and L in all experimental periods in compatible with the histological assessment of interstitial damage.

**Conclusions:** Present study might show that down-regulated TRPM6 in DCT would be a principal cause of hypermagnesuria in diabetic nephropathy. Suppression of NCC might be involved in the TRPM6 down regulation. The elevation Mg excretion in diabetes might be independent of renal interstitial damage.

## PUB144

**Study of TGFB1 -509C>T Polymorphism Association with Diabetic Nephropathy Development in T1D Patients** Thamara R. de Melo,[1] Karla Souza,[1] Marcela Ururahy,[1] Yonara Monique Oliveira,[1] Melina Bezerra Loureiro,[1] Heglayne P. Silva,[1] João F. Bezerra,[1] Gustavo M. Oliveira,[1] Rosario D.C. Hirata,[2] Maria Das Graças Almeida,[2] Ricardo Fernando Arrais,[3] Sonia Q. Doi,[4] Mario Hiroyuki Hirata,[2] Adriana Augusto de Rezende.[1] *[1]Clinical and Toxicological Analysis, UFRN, Natal, RN, Brazil; [2]Clinical and Toxicological Analysis, USP, São Paulo, SP, Brazil; [3]Pediatrics, UFRN, Natal, RN, Brazil; [4]Medicine, USUHS, Bethesda, MD.*

**Background:** Diabetic nephropathy (DN) is the most commom cause of end-stage renal disease. DN manifestations may be a consequence of transforming growth factor beta 1 (TGF-β1) actions, since it promotes renal cell hypertrophy and stimulates extracellular matrix accumulation. In this way, we aimed to investigate *TGFB1* -509C>T polymorphism association with DN development patients with type 1 diabetes (T1D) from Natal-RN/Brazil.

**Methods:** *TGFB1* -509C>T polymorphism was analyzed in 156 T1D patients and 188 normoglycemic (NG) subjects, aged between 6 to 20 years. Glucose, glycated hemoglobin, creatinine, urea, albumin-to-creatinine ratio (ACR), serum lipids and *TGFB1* mRNA expression were evaluated.

**Results:** T1D patients showed increased levels of urea (mg/dL) [NG:22 (19-27) and T1D: 29 (22-33), p<0.001] and ACR (mg/g of creatinine) [NG:6 (4.7-7.9) and T1D: 7.7 (5.2-16.1), p<0.001], compared to NG subjetcs. No association was found for *TGFB1* -509C>T with markers of kidney damage development in T1D patients (Table 1).

Table 1 - Relationship between TGFB1 -509C>T genotypes and biochemical parameters of T1D patients

| Genotype | Glucose *mg/dL* | Glycated Hemoglobin *%* | Creatinine *mg/dL* | Urea *mg/dL* | RAC *mg/g of creatinine* | Total cholesterol *mg/dL* | *TGB1/Bactin* mRNA *fold-change* |
|---|---|---|---|---|---|---|---|
| GG+GA | 218.2±10.4 | 10.3±0.3 | 0.75±0.02 | 29.4±1.0 | 17.5±4.4 | 182±6.9 | 3.0±0.2 |
| AA | 260.6±30.6 | 11.6±0.8 | 0.79±0.04 | 29.8±1.8 | 30.5±10.8 | 169±17.0 | 3.6±0.5 |
| p-value | 0.135 | 0.136 | 0.137 | 0.861 | 0.272 | 0.499 | 0.356 |

Results are mean±SEM.

**Conclusions:** Our results show no evidence of association between *TGFB1* -509C>T polymorphism and markers of diabetic nephropathy development.

*Funding:* Government Support - Non-U.S.

## PUB145

**Anti-Apoptotic Effects of Green Tea Extracts on Streptozotocin-Induced Diabetic Nephropathy in Mice** Byung Chul Shin, Jong Hun Back. *Dept of Internal Medicine, Chosun Univ Hospital, Gwangju.*

**Background:** Diabetic nephropathy is the most common cause of end-stage renal disease and is characterized by glomerulosclerosis and tubulointerstitial fibrosis, associated with apoptosis. Green tea extracts (GTE) have antioxidant properties and is responsible for the renoprotection. We examined whether GTE could ameliorate the development of diabetic nephropathy and its role of antiapoptotoic effects.

**Methods:** The mice (n=40) were divided into 4 groups. Control group (n=10) was intraperitoneal (IP) injected 0.9% saline, Streptozotocin (STZ) group (n=10) was IP injected STZ 200mg/Kg and induced diabetic nephropathy. GTE group (n=10) was received 0.1% GTE by oral rout from 4 weeks to 16 weeks. STZ plus GTE group (n=10) was received with same dose. Serum glucose, blood urea nitrogen, serum creatinine, urine volume and urine protein amounts were measured at 16 weeks. Western blot assay of p53, caspase-3, cleaved caspase-3, caspase-7 were compared for the different groups. Histopathologic examination and immunohistochemical staining of mice kidney were performed.

**Results:** Compared with control group, STZ-group showed an increase in blood glucose, blood urea nitrogen, creatinine levels and urine protein amounts, and a decrease in body weight. All the above parameters were significantly reversed with GTE treatment. After STZ injection, there were a diabetic glomerulosclerosis with increased caspase-3 and caspase-7 activities. GTE-treated mice kidney showed a reduced expression of above parameters and the reserved pathologic findings.

**Conclusions:** GTE can inhibit progression of STZ-induced diabetic nephropathy by reductions in caspase-3 and caspase-7 activities. These results suggest that GTE ameliorates STZ-induced diabetic nephropathy by apoptosis suppression.

## PUB146

**Remission of Diabetes and Hypertension Accompanying Rapid Weight Reduction following Bariatric Surgery** Anil K. Mandal,[1] Linda M. Hiebert.[2] *[1]Univ of Florida, Gainesville, FL; [2]Univ of Saskatchewan, Saskatoon, Canada.*

**Background:** Obesity and diabetes go hand in hand. Diabetes control is difficult to accomplish because of insulin resistance. Hypertension control also requires many and high doses of antihypertensive drugs. Remission of diabetes following gastric bypass surgery is reported (Arch Surg 2011; 146: 744-750).

**Methods:** This study reports a 52y obese male with progressive weight increase and uncontrolled diabetes of 10-12 years, despite intensive insulin therapy and variable hypertension control despite 4 line therapy. Gastric bypass surgery was recommended. Baseline weight of 207 lbs.; increased to 356 lbs., fasting blood glucose (FBG) and 2h postprandial blood glucose (2hPPG) increased from 131 and 181 mg/dL to 147 and 263 mg/dL respectively, despite 60 units of glargine insulin twice daily and regular insulin 5 units with each meal. Blood pressures (BP) ranged from low 130/80 to high 180/100 mmHg. Roux-en-y-Gastric bypass surgery performed in November 2011.

**Results:** At last visit, 18 months later, weight was 230 lbs. with a total weight loss of 126 lbs. Sitting BP was 120/60 with reduced BP medication. FBG and 2hPPG were reduced to 102 and 85 mg/dL respectively without insulin therapy. $HbA_1c$ varied from 7.8% to 9.3% with a mean 8.3% before surgery to mean 5.5% 18 months after surgery. Renal function is maintained normal.

**Conclusions:** Massive weight reduction following gastric bypass surgery restores normoglycemia even in insulin treated, long-term diabetes and simplifies BP control. Gastric bypass surgery is not a conventional therapy but should be considered when weight reduction, diabetes and BP control are at a crossroad situation.

## PUB147

**Circulating Irisin Level in Human Diabetic Cohorts; 11 Year Retrospective Cohort Study** Jin Joo Cha,[1] Mi Jin Lee,[1] Young Youl Hyun,[2] Dae R. Cha,[1] Jung Eun Kim,[1] Mihwa Lee,[1] Hye Kyoung Song,[1] Young Sun Kang.[1] *[1]Nephrology, Korea Univ Ansan Hospital, Ansan, Kyunggido, Republic of Korea; [2]Nephrology, Sungkyunkwan Univ Kangbuk Samsung Hospital, Seoul, Republic of Korea.*

**Background:** Irisin, a novel myokine, has been proposed to induce browning of subcutaneous adipocytes. In humans, circulating irisin level was significantly decreased in obese and diabetic patients. We therefore, examined circulating irisin level and its associations with various complications of diabetes.

**Methods:** We performed a retrospective cohort analysis of 161 diabetic individuals followed up for approximately 11 years. Baseline circulating plasma level of irisin was measured using commercially available EIA kit. The associations between the circulating irisin with variable metabolic parameters, microvascular and macrovascular complications were evaluated.

**Results:** Total 161 patients (n=14, type 1 diabetes, n=147 type 2 diabetes, mean age 43,6±4.6, 66.8±10.4 respectively) were analyzed. There were no significant associations with circulating irisin levels with gender difference, the type of diabetes, body mass index, age, Patients with hypertension (n= 66) showed significantly decreased level of circulating irisin (649.09 ±661.9 vs 884.01± 792.27, p=0.04). There was no significant difference in irisin level among the CKD stages (using CKD-EPI equation), however there was a trend toward decrement as the stage advanced. (stage 1(n=62) 806.97±793.70, stage 2(n=58) 925.31±849.83, stage 3a(n=20) 690.75±522.96, stage 3b(n=12) 547.5±460.7, stage 4(n=5) 627.30±464.73, stage 5(n=4) 207.48±75.89). Binary logistic regression was performed to evaluate the relative risks of irisin level with the development of micro- and macrovascular complications. The irisin level did not affect the risks of macrovascular complications. However, incre! Ased level of irisin (log irisin) significantly decreased the risk of retinopathy (Unadjusted RR 0.688, 95% CI 0.516-0.918, p=0.01, Adjusted RR 0.677, 95% CI 0.487-0.942, p=0.02).

**Conclusions:** Circulating irisin level of diabetic patients was not affected by renal insufficiency nor did not affect diabetic nephropathy. It might have a predictive role in diabetic retinopathy.

## PUB148

**The Association of Glypican-4 with Diabetic Complications in Korean Population** Mi Jin Lee,[1] Jin Joo Cha,[1] Young Youl Hyun,[2] Dae R. Cha,[1] Jung Eun Kim,[1] Mihwa Lee,[1] Hye Kyoung Song,[1] Young Sun Kang.[1] *[1]Nephrology, Korea Univ Ansan Hospital, Ansan, Republic of Korea; [2]Nephrology, Sungkyunkwan Univ Kangbuk Samsung Hospital, Seoul, Republic of Korea.*

**Background:** Glypican-4(Gpc4) is an adipokine, which is known to be important for adipocyte differentiation by interacting with and regulating insulin receptor activation and its downstream signaling. Serum Gpc4 is known as a marker for BMI and insulin sensitivity in mice and human. But it is uncertain that the association of Gpc4 with diabetic complications.

**Methods:** We investigated the association of serum Gpc4 level with diabetic complications in a prospective cohort of 161 Korean diabetic patients from 2002 to 2013.

**Results:** In Univariate regression analysis, glycemic variability, estimated GFR was negatively correlated and urine albumin excretion and use of ACE inhibitor was positively correlated with serum Gpc4 level (all $p<0.05$). In multivariate regression analysis, the increase of serum Gpc4 level was associated with the decrease of glycemic variability independently ($p =0.05$). And the increase of serum Gpc4 level was associated with the increase of urine albumin excretion ($p =0.02$). The serum Gpc4 level was not associated with retinopathy, neuropathy, nephropathy, cardiovascular disease, cerebrovascular events. Also serum Gpc4 level did not increase depending on the CKD-epi stage.

**Conclusions:** Taken together, there is no relationship between diabetic complications and serum Gpc4 level. But more research is needed on the relationship between Gpc4 and diabetic complications.

## PUB149

**Effect of Ultrafiltration on Plasma Hemoglobin Concentration in Patients with End-Stage Renal Disease on Chronic Hemodialysis** Subir K. Paul, Shejuti Paul, Rajesh Boorgu, Narasimha Rao Boorgu, Jamie N. Cockrell. *Shoals Kidney and Hypertension Center, Florence, AL.*

**Background:** Anemia is highly prevalent in patients with end stage renal disease. It is common practice to measure plasma hemoglobin concentration in patients on hemodialysis before HD treatment begins. Therapeutic interventions including adjustment of Epogen dose and intravenous iron administration are undertaken based on these meaurements. We have found considerable variation in plasma hemoglobin level in these patients without any apparent clinical change, except for interdialytic weight gain. We hypothesize that the pre-ultrafiltration hemoglobin concentration in this patient population is artificially low because of plasma volume expansion. We propose that plasma hemoglobin level should be measured after ultrafiltration is completed. This study compares the plasma hemoglobin level in patients on chronic hemodialysis before and following ultrafiltration.

**Methods:** Our study included twenty five patients with end stage renal disease on chronic hemodialysis for at least 6 months duration with minimum interdialytic weight gain of three kilograms. Mean age was 59.08 years. Twelve were male and thirteen were female. Ten were white and fifteen were African American. Pre and post hemodialysis plasma hemoglobin concentration was determined in a standard laboratory. Statistical analysis was done with student's t-test.

**Results:** The mean interdialytic weight gain was 4.6 kilograms. Pre-ultrafiltration hemoglobin concentration was 11.32 +/- 0.27 g/dL. Post-ultrafiltration hemoglobin increased to 12.14 +/- 0.28 g/dL (P=0.04).

**Conclusions:** This preliminary data suggests that pre-ultrafiltration hemoglobin concentration in chronic hemodialysis patients may be artificially low because of plasma volume expansion associated with interdialytic fluid gain. Measurement of hemoglobin in this patient population should be done after hemodialysis when ultrafiltration is completed and euvolemia is achieved. This strategy may reduce pharmacological interventions for anemia management in these patients.

## PUB150

**Effect of Ultrafiltration on Serum Albumin in Patients with End Stage Renal Disease on Chronic Hemodialysis** Subir K. Paul, Shejuti Paul, Rajesh Boorgu, Narasimha Rao Boorgu, Jamie N. Cockrell. *Shoals Kidney and Hypertension Center, Florence, AL.*

**Background:** Malnutrition is prevalent in many hemodialysis patients with end stage renal disease on chronic hemodialysis. It is common practice to measure serum Albumin before the initiation of ultrafiltration and hemodialysis. Serum albumin has been found to vary considerably without any apparent clinical change except for interdialytic weight gain. We hypothesize that serum albumin in these patients is artificially low because of plasma volume expansion and should be measured following ultrafiltration.

**Methods:** Our study included 65 patients with end-stage renal disease on chronic hemodialysis for at least 6 months with minimum weight gain of 1 kg. Mean age was 61.17years. Thirty two patients were male. Thirty three were female. Thirty three were White. Thirty two were black. Ultrafiltration was done to reach pre dialysis dry weight for each patient. Serum albumin was measured pre and post ultrafiltration. Statistical analysis was done with student t-test.

**Results:** The mean weight gain was 3.66 kg. Pre-ultrafiltration albumin was 3.77 +/- 0.04 g/dL. Post-ultrafiltration albumin increased to 4.145 +/- 0.05 g/dl (P=0.0001).

**Conclusions:** This preliminary data suggests that pre-ultrafiltration albumin measurements in hemodialysis patients may be artificially low in patients with plasma volume expansion associated with interdialytic fluid gain. We propose that serum albumin should be measured after ultrafiltration when patients are relatively euvolemic. This strategy may be valuable for assessment of malnutrition in patients of end stage renal disease on chronic hemodialysis.

## PUB151

**Clinical Factors and Renal Function at the Start of Dialysis; Impact on Mortality in Incident Hemodialysis Patients** Elvira Bosch,[1] Eduardo Baamonde,[1] German Perez Suarez,[1] Cesar Garcia-canton,[2] Rita Guerra,[2] Dolores Checa.[2] *[1]Centro de Hemodialisis Avericum, Las Palmas de Gran Canaria, Spain; [2]Servicio de Nefrologia, Hospital Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain.*

**Background:** The goal of this study was to analyze whether clinical conditions and renal function at the start of renal replacement therapy (RRT) are related with mortality.

**Methods:** Retrospective study with 220 incident hemodialysis patients between 2007 and 2010 in Southern Gran Canaria. Patients' demographic, clinical and laboratory data were analyzed at the start of dialysis treatment. Glomerular filtration rate (GFR) was calculated by using the MDRD4 and Cockroft-Gault (CG) formula. Patients were classified into 3 different groups according to their GFR (MDRD4 > 15, 8-15 and < 8 mL/min). Survival rates were compared between groups.

**Results:** 65.5% male, 61.13 ± 13 years old, 58.6% diabetic and 17.7% with cardiovascular disease; 65.5% started with catheter, the mean age-adjusted Charlson comorbidity index (ACCI) was 6.2 ± 2.4 and the average MDRD4 at the start of hemodialyis was 10.67 ± 3.25 mL/min. Patients who started with MDRD4 < 8 mL/min were ($p< 0.05$) younger, with lower ACCI and higher incidence of catheter; they showed lower albumin and hemoglobin levels and higher urea and phosphorus levels. The median follow-up time was 30.9 months. Eighty patients died (17.7 % of them with early start and 3.8% with late start). The risk of death was significantly higher for patients with hypoalbuminemia, age > 65 years, higher ACCI, MDRD4 > 15 mL/min at the start, heart failure or history of ACV. After adjusting the model (Cox regression) the global risk of death was higher ($p< 0.05$) for patients who started with catheter (HR: 2.4 CI: 1.3- 4.4), higher ACCI (HR: 1.16 CI: 1.03-1.3), > 65 years (HR: 1.7 CI: 1.01-3.08) and for those who started with GF-MDRD4 > 15 mL/min (HR: 3.6 CI: 1.03-13).

**Conclusions:** Patients with a late RRT start were younger, with lower ACCI and with lower albumin and hemoglobin levels. Factors affecting the survival of incident hemodialysis patients are: early start, start with catheter, older age, the GF-MDRD4 and high ACCI.

## PUB152

**The Use of Continuous Measurement of Glucose Concentration in Interstitial Fluid in Hemodialysed Patients** Stanislaw Niemczyk,[1] Wojciech Klimm,[1] Katarzyna Pleskacz,[2] Bozena Pietrzak.[1] *[1]Internal Diseases, Nephrology and Dialysis, Military Institute of Medicine, Warsaw, Poland; [2]Information and Communication Technologies, Military Institute of Medicine, Warsaw, Poland.*

**Background:** Fluctuations of glucose concentration in serum are an important problem in hemodialysed (HD) patients with and without diabetes. The aim of this study is to compare the fluctuations of the glucose concentrations in interstitial fluid in hemodialysed patients with and without diabetes assessed by the method of continuous measurement.

**Methods:** The study involved 22 HD patients (9 women and 13 men) in two groups: with diabetes (GDM): 4 women and 8 men; and without diabetes (GN): 5 women and 5 men. The concentration of glucose was measured using a Real Time Guardian (Medtronic) monitoring system every 5 minutes. The study assumed 4-day monitoring.

**Results:** In the GDM 9319 measurements were performed (average 776.58/person). Total observation time was 752 hrs 35 min(average 62 hrs 42 min). In the GN 7079 measurements were performed (average 707.9).Total observation time was 589 hrs 55 min(average 58 hrs 59 min). In the GDM the average value of glucose concentration was 137.58 mg/dl and was higher than the GN by 30.28 ($p = 0.004$). The maximum values of glucose concentration were higher in the GDM. The duration of hyperglycemia in the GDM was by 61390 s significantly longer ($p = 0.0008$) compared with the GN. The groups of patients were analyzed for the frequency and the length of glycemia fluctuations. No differences in the number of hyperglycemia were observed.

**Conclusions:** Continuous measurement of interstitial glycemia seems to be a useful, accurate and safe method of the assessment of carbohydrate metabolism in HD patients. In patients with diabetes asymptomatic hyperglycemia are more frequent and longer. Episodes of hypoglycemia also occur in patients without diabetes.

## PUB153

**Lack of Effect of Standard or Adjusted Dialysate Potassium Concentration on Postdialysis Potassium Levels** Xenia P. Sumin, Michael F. Michelis, Maria V. DeVita. *Nephrology, Lenox Hill Hospital, New York, NY.*

**Background:** Controlling serum potassium concentration (K) is an important goal in maintenance hemodialysis (MHD) patients. Intermittent hemodialysis results in rapid fluctuations of serum K that can extend from pathologically high to low concentrations. Predialysis hyper- and hypokalemia have been implicated in increased death risk in MHD patients. Complications of dialysis in regard to serum K is largely a function of both K flux and the development of hypokalemia. The goal of this study was to examine the postdialysis serum K in patients with normal predialysis serum K when dialyzed against standard and adjusted K baths to assess the frequency and extent of postdialysis hypokalemia.

**Methods:** Monthly labs at a single outpatient dialysis center were screened to identify MHD patients with predialysis serum K in normal range (K≥3.7 and K<4.5mEq/L). Of 72 such patients identified, 11 patients were on 3K bath. Twenty-two of the remaining 61 patients, all on 2K bath, were chosen at random to compare to the 3K group. Postdialysis serum K levels were collected on all patients. Patient data on treatment time (min), blood flow (mL/min), and URR was also collected.

**Results:** Mean pretreatment K (4.05±0.22mEq/L) in 2K dialysate group was not different from the mean pretreatment K (4.00mEq/L±0.35mEq/L) in 3K dialysate group ($p=0.59$). Mean change between pre- and posttreatment serum K was -0.12 ±0.58 mEq/L for 2K dialysate group and -0.14±0.31mEq/L for 3K dialysate group ($p=0.94$). Mean posttreatment K (3.93±0.60mEq/L) in 2K dialysate group was not different from the mean postreatment K (3.86mEq/L±0.33mEq/L) in 3K dialysate group ($p=0.73$). Treatment time was longer in the 2K group, 214 versus 195 minutes ($p=0.04$). There was no difference in blood flow or URR between the groups. Though the 2K group had significantly longer treatment time, there was no correlation between change in K and either treatment time or blood flow.

**Conclusions:** There was no significant difference in postdialysis serum potassium levels among the 2K and 3K dialysate groups. It appears patients with normal predialysis K can be dialyzed against 2K bath with minimal risk for the development of severe hypokalemia.

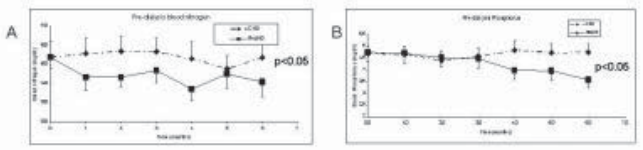
## PUB154

**Performances of the New Revaclear High Flux Dialyzers for Conventional HD Treatments** Fulvia Caligaris,[1] Maria Roseo,[1] Adina Misca,[1] Francesco Guarnieri,[2] Denis Steckiph.[2] *[1]ASL AT Ospedale, Asti, Italy; [2]Gambro, Italy.*

**Background:** High-flux dialyzers may delay long term clinical complications of dialysis. Aim of this study: to evaluate if standard HD with high-flux dialyzers Revaclear (Gambro) improves the removal of uremic toxin in stable HD patients respect to other low and high flux dialyzers.

**Methods:** ESRD patients on dialysis (Qb>250 ml/min) were retrospectively enrolled in a single center, controlled and observational study. The patients were treated on conventional HD (CHD: 19 low-flux, 5 high-flux; n=17 1,7m2, n=7 2,1m2) for 6 months and on high-flux HD with Revaclear dialyzers (RevHD: n=5 1,4m2, n=19 1,8m2) for further 6 months. Revaclear is equipped with a high water permeability PAES/PVP membrane, efficient and compact in size thanks to fiber microondulation and bundle density. Primary end-point: to evaluate the monthly pre-dialysis level of blood urea (Ur) and phosphorus (P). Secondary end-points: to evaluate the monthly pre-dialysis value of hemoglobin, hematocrit, Epo dose and 2-month eKt/V (Daugirdas).

**Results:** We analyzed 24 patients(10 F, age 68.4±12.0 years, HD vintage 6.8±6.1years; vascular access:11 FAV, 13 Tesio CVC). Mean Ur among the whole evaluation period was lower during RevHD(156±37 vs. 142±35mg/dl, $p<0.05$) and a different trend was found between the two periods since by the first month of observation($p<0,05$, Fig.1A). P was significantly lower during the RevHD period, both as mean value(4.65±1.81 vs. 4.19±1.88mg/dl, $p<0.05$) and as time-related trend($p<0.05$, Fig.1B). Other parameters did not show any significant difference (Fig.1C).

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**Conclusions:** Our findings in RevHD period were obtained with smaller surface filters on about 2/3 of the treatments respect to CHD, showing the high efficiency of Revaclear high-flux dialyzers on HD. Concerning the effect of Revaclear on hemoglobin levels and Epo dose, a further study on a larger population is needed.

## PUB155

**Is Acidosis Associated with a Better Survival in Hemodialysis Patients?**
Asmaa Y.M. Al-Chidadi, Abdelgalil Abdelrahman Ali. *Broomfield Hospital, Chelmsford, United Kingdom.*

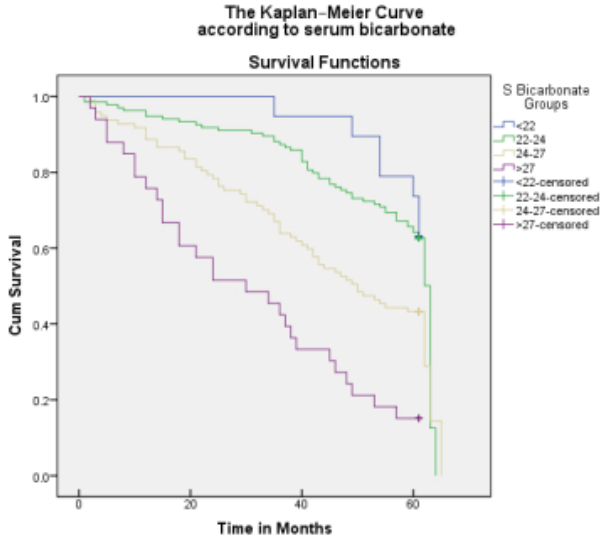
**Background:** Association between acidosis and survival in hemodialysis patients remains controversial.

**Methods:** Correlation between 5-years survival and time-averaged S. Bicarbonate (HCO3) among 293 adults on maintenance hemodialysis was studied. Patients were categorized into four groups according to S. HCO3 levels.

**Results:** CRP correlated positively while S. Phosphate correlated inversely with S.HCO3. Normalized protein nitrogen appearance (nPNA) showed an inverted U-shape correlation with S.HCO3. The mildly acidotic group were significantly younger and had a significantly higher BMI.

| S. HCO3 mmol/l | <22 | 22-24 | 25-27 | >27 | P-value |
|---|---|---|---|---|---|
| (n) | 19(6.5%) | 135(46.2%) | 97(33.2%) | 34(11.6%) | |
| S. HCO3 | 21.4±0.9 | 23.7 ±0.95 | 25.9±0.7 | 27.9±1.0 | **<0.005** |
| Age | 67.7 ± 10.8 | 64.9 ±15.8 | 69.6±15.3 | 73.1 ±12.1 | **0.01** |
| M(F) % | 4.6(2.1) | 32.6(14.7) | 24.2(9.8) | 6.3(5.6) | 0.26 |
| Mortality% | 2.5 | 19.6 | 20.4 | 10.2 | **<0.0001** |
| Kt/V | 0.95±0.2 | 1.1±0.4 | 1.2±0.4 | 1.0±0.2 | **0.009** |
| nPNA | 0.94±0.2 | 0.93 ±0.3 | 0.96±0.2 | 0.90±0.3 | 0.8 |
| BMI | 34.4±26.3 | 27.8±7.9 | 28.8±24.2 | 24.4±4.3 | **0.006** |
| CRP | 41.2±49.7 | 36.3±38.1 | 50.1±41.5 | 53.6±9.2 | **0.007** |
| S. Pi | 1.6±0.5 | 1.50±0.5 | 1.4±0.5 | 1.18±0.4 | **<0.005** |

**Survival was significantly higher** among the acidotic group, p<0.0001, despite a significantly lower Kt/V.




**Conclusions:** The higher survival among the acidotic group could be influenced by reverse epidemiology engendered by malnutrition and inflammation.

## PUB156

**Low Molecular Weight Heparin (LMWH) "Enoxaparin" as a Standard Anticoagulant in Hemodialysis Patients: A 15 Year Experience**
Bassam O. Bernieh,[1] Yousef Boobes,[1] Mohamed Raafat Al Hakim,[1] Qutaiba Hussain Daoud,[1] Hanan Eljack,[1] Nicholas T. Richards.[2] *[1]Nephrology, Tawam Hospital, Al Ain, United Arab Emirates; [2]SEHA Dialysis Services, Abu Dhabi, United Arab Emirates.*

**Background:** LMWHs are available since 25 years with wide use in the treatment and prevention of medical and surgical thromboembolic events. The use of LMWHs as anticoagulant in hemodialysis (HD) patients, is still not a routine or standard practice. We are using LMWH 'Enoxaparin' since 1998 as a standard anticoagulation in our HD unit, and in a previous publication (1), we defined the dose which would be used in HD (0.36 mg/kg), and the advantages of LWMH "Enoxaparin" over unfractionated heparin. **Aim of the study:** In the current study, we are reporting our 15 years experience of using "Enoxaparin" , evaluating the current used dose, the dose according to the type of vascular access, etiology of kidney disease (diabetic, none diabetic), and dialytic mode (HD, HDF).

**Methods:** This study includes 252 HD patients, mean age 56.6 (17) year, Male 145 (57.5 %). Mean duration on HD 50 (44.5) months. 139 (55%) were diabetics, 138 (55%) had AVF, 28 (11%) had AVG, and 86 (34%) had tunneled catheter (TC). 187 (74%) patients were on HD, and 65 (26%) were on line HDF.

**Results:** Total number of HD sessions during the 15 years use of "Enoxaparin" was 350,000. The average dose of "Enoxaparin" was 0.31 (0.14) mg/kg/session. 0.29 (0.13), 0.34 (0.17), and 0.34 (0.14) mg/kg/ session, were the average doses used in AVF, AVG, and TC respectively with ***p*=0.03**. The dose used in DM patients was 0.30 (0.13), and 0.34 (0.13) mg/kg in non DM, with ***p*=0.15**. Average dose in HD was o.31 (0.13), and in HDF 0.32 (0.15) mg/kg, ***p*=0.69**.

**Conclusions:** To the best of our knowledge this study is the longest, and the largest experience ever reported with the use of LMWH "Enoxaparin" as a standard anticoagulant in HD. The average dose used continued to be low and stable with time. Only the type of vascular access impact significantly the required dose.

(1) Bernieh B, et al. Blood Purif 2009;27:242–245.

## PUB157

**Successful Treatment of Metabolic Encephalopathy by Switching Peritoneal Dialysis to Hemodialysis**
Ayesha Waqar, Monalisa Joseph, Muhammad R. Syed, Azka Arif, Talal A. Khan, Muhammad W. Khattak, Sandeep Aggarwal, Nauman Shahid. *Drexel Univ College of Medicine, Philadelphia, PA.*

**Background:** End stage renal disease patients on dialysis can suffer from metabolic encephalopathy. More often than not the reason remains uncertain. We report a case of a patient in which the encephalopathy completely resolved by switching from peritoneal dialysis to hemodialysis.

**Methods:** Our patient was a 65-year-old Hispanic male, with history of end stage renal disease, diabetes mellitus type 2, hypertension, coronary artery disease, gout and peripheral vascular disease. He was on peritoneal dialysis for last one year and had been compliant with his treatment with adequate dialysis. He was admitted to the hospital with change in mental status. Prior to this admission he was completely independent and worked as mathematics teacher. As part of his work up, CT head did not reveal any new changes and lumbar puncture results were unremarkable. His EEG showed slowing of back ground activity suggestive of mild diffuse encephalopathy likely due to metabolic, toxic or infectious etiology. His TSH was elevated so he got IV synthroid . He had culture negative peritonitis which was treated with IV antibiotics to resolution. Despite treatment of the metabolic and infectious abnormalities and providing adequate dialysis (Kt/V 1.7), his mental status did not improve. He remained hospitalized for two months, without any improvement in mental status until his renal replacement therapy was switched to hemodialysis. We used F 180 dialyzer with Blood flow rate of 350 and Dialysate flow rate of 700 for his first session. Immediately after his first hemodialysis, a dramatic improvement of his mental status was noted. We continued hemodialysis and after his second session, he returned to his baseline, nine weeks after his initial presentation.

**Conclusions:** Although we do not have clear explanation for this but there is a possibility that hemodialysis may have some benefit in clearing middle molecules and some unknown substances in blood which may contribute to encephalopathy.

## PUB158

**Continuous Veno-Venous Hemodialysis (CVVHD) with Regional Citrate Anticoagulation (RCA) Is a Suitable and Safe Modality for Severe Acute Kidney Injury (AKI) Induced by Tumor Lysis Syndrome (TLS)**
Veronica T. Costa e Silva,[1] Elerson Costalonga,[1] Cilene Muniz de Alencar Soares,[1] James Hung,[1] Juliana Silva Bezerra,[1] Ana Paula Leandro Oliveira,[1] Luciane Oikawa,[1] Ludhmila Abrahão Hajjar,[2] Luis Yu,[1] Emmanuel A. Burdmann.[1] *[1]Nephrology Div, Sao Paulo State Cancer Institute - Univ of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Intensive Care Dept, Sao Paulo State Cancer Institute - Univ of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

**Background:** It has not been established which is the most suitable dialysis modality for TLS-induced AKI and there is no data on RCA protocol safety in these patients.

**Methods:** We prospectively analyzed 8 consecutive adult cancer TLS-induced AKI patients (Cairo-Bishop criteria) submitted to CVVHD for a minimum of 3 days in the Intensive Care Unit of a University tertiary hospital (Cancer Institute) from January 2010 to December 2011. RCA was performed according to an adaptation of Mehtas' protocol.

**Results:** Patients were 61±20 y, 55.6% male, 67% on vasopressors and none on mechanical ventilation. Six had hematologic diseases (leukemia or lymphoma) and 2 solid tumors (kidney cancer and hepatocellular carcinoma). Venous access was temporary triple lumen catheter (11 Fr). Blood flow was 180 (150 -180) ml/min, calcium cloride flow was 25ml/h (500mg/h), dialysis dose was 2000 ml/h and citrate dose was 21.5 (14.6 – 24.4) mmol/h. Laboratory tests at dialysis initiation were: uric acid (UA) 14.7 (10.5 – 20.6) mg/dL; phosphate (P) 7.25 (4.0 – 8.8) mg/dL; creatinine 5.3 (3.5 – 6.9) mg/dL; urea 166 (140 – 216 mg/dL); K 3.9 (3.6 – 4.7) mEq/L; bicarbonate (Bic) 19.4 (18.5 - 23) mEq/L; Cai 4.4 (4.0 – 4.7) mg/dL; lactic dehydrogenase 1107 (1012 – 1876) U/L. Laboratory tests after 24h of CVVHD were P 4.3 (3.7 – 5.8) mg/dL; Bic 23 (21.9 - 29.3) mEq/L. UA after 48 h of CVVHD was 3.7 (2.4 - 4.5) mg/dL. The Cai during CVVHD was maintained at 4.40 (4.1 – 4.9/minimum of 3.7 mg/dL). Low Ca (< 4.0 mg/dL) was observed in 66% of all measured Cai, but no patient presented related complications such as seizure or cardiac arrhythmias.

**Conclusions:** CVVHD with RCA was an efficient and safe dialysis modality for TLS-induced AKI.

## PUB159

**Adjusting Effective Replacement Fluid Sodium Level in Continuous Hemofiltration**
Carl P. Walther, Amber S. Podoll, Kevin W. Finkel. *Div of Renal Diseases & Hypertension, UTHealth Science Center at Houston, Houston, TX.*

**Background:** Management of dysnatremic patients with renal replacement therapy can be perilous because of rapid sodium correction if standard therapy fluids are used. We describe a means whereby patients receive CVVH with standard replacement fluid, and standard hypo- or hyperosmolar fluids are infused into the patient, either post-filter or at a separate venous access site, to adjust the effective replacement fluid sodium concentration.

**Methods:** A model was developed for calculating the "effective" sodium concentration of the replacement fluid in CVVH as the mean of the sodium concentrations of the input fluids, weighted for fluid rate. [$Na_{eff}$] represents desired effective replacement fluid sodium concentration, [$Na_R$] is the sodium concentration in the standard replacement fluid (usually 140 mEq/L), [$Na_A$] is the sodium concentration in the "adjustment" fluid, $Q_R$ is the standard replacement fluid rate, and $Q_A$ is the rate of "adjustment" fluid. [$Na_{eff}$] = ( [$Na_R$] x [$Q_R$] + [$Na_A$] x [$Q_A$] ) / $Q_R$ + $Q_A$. The equation can then be solved for the "adjustment" fluid rate: $Q_A = Q_R \times \{([Na_R] - [Na_{eff}]) / ([Na_{eff}] - [Na_A])\}$.

**Results:** By inputting values for the desired "effective" replacement sodium concentration, the replacement fluid flow rate, and the sodium concentration of the adjustment fluid used, the flow rate of the adjustment fluid is determined. As an example, the table below shows the calculated adjustment fluid rates for various desired effective sodium concentrations and CVVH replacement fluid rates where the adjustment fluid is 5% dextrose in water.

| Replacement fluid rate (ml/hr) | | 500 | 1000 | 2000 | 3000 | 5000 |
|---|---|---|---|---|---|---|
| | Effective Na concentration (mEq/L) | | | | | |
| 5% Dextrose infusion rate (ml/hr) | 100 | 200 | 400 | 800 | 1200 | 2000 |
| | 110 | 136 | 273 | 545 | 818 | 1364 |
| | 120 | 83 | 167 | 333 | 500 | 833 |
| | 130 | 38 | 77 | 154 | 231 | 385 |

**Conclusions:** We propose that using standard CVVH replacement fluid, and standard additional "adjustment" intravenous fluids (e.g., 5% Dextrose in water, 3% saline), the effective sodium concentration for CVVH can be easily adjusted to avoid excessive correction in dysnatremic patients.

## PUB160

### Metformin Associated Lactic Acidosis (MALA): A Long Term Single Center Experience

Andrea Angioi, Antonello Pani. *AOB Cagliari.*

**Background:** Metformin associated lactic acidosis (MALA) is a well-known side effect of Biguanides and in 13 years we observed an exponential increase in hospitalizations. Aim: investigate about the incidence in our area, the underlying causes, the survival in the short and long term.

**Methods:** We conducted a retrospective analysis retrieving 22 patients (pts) with MALA and acute renal failure admitted from Jan 2000 to Dec 2012. Inclusion criteria: type II diabetes, metformin intake, lactic acidosis (lactate≥5 mmol/L). Treatment: SLED hemodialysis(HD) with HCO3- buffer, based on lactate and electrolytes. In order to weigh the role of comorbid factors, we calculated a MALA risk score (MRS) to find relationships with mortality and survival curve dividing a low (MRS≤2-Gr.A) from a high risk group (MRS≥3-Gr.B). We followed them for a mean of 20.7 months; all discontinued the metformin. Then we obtained the MALA incidence in our area.

**Results:** Selected pts (M:F 1.4:1; mean age 67±9 yrs) showed: acute on chronic renal failure(59.1%); dehydration(86.4%); SIRS(50%); sepsis(27%); renal stones(13.6%); infusion of iodinated contrast(4.5%). Elevation of sCr(mean: 8.4±2.7mg/dL), BUN(mean: 104 ± 49.1 mg/dL), K+(mean: 6.0 ± 1.5 mEq/L), low pH(median: 7.06 ± 0.19) and serum lactate(median: 13.4 ± 6.2 mEq/L) were demonstrated at admission. Mortality was 22.7% after the 1st HD(100% of deaths), similar in both risk groups (2/5 in group B).

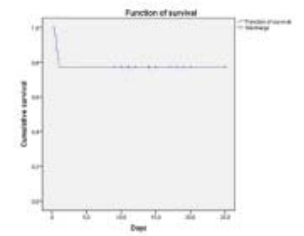



Figure 1A: Kaplan-Meier survival curve (admission – 25 days) of MALA patients. During the follow-up (mean 20.7 months), none of the patients who survived have experienced new episodes of lactic acidosis after discontinuing the drug.

Figure 1B: The MALA risk score: every shade of blue corresponds to a score, from +1 (light) to +8 (dark). Sepsis was considered as a SIRS (+1) plus an additional +1 score point. Red indicate deceased patients. CKD: Chronic Kidney Disease; SIRS: Systemic Inflammatory Response Syndrome

We found no absolute contraindications on drug use but none of pts was informed about the side effects. At follow-up, no episodes of lactic acidosis were observed. Incidence: lower=1.1/10.000(2000); higher=8.1/10.000 users(2010), especially in cold months (Jan-Mar).

**Conclusions:** Mortality(22.7%) was not related with the severity of the score. The lack in information of the patient facilitated the most of cases. The current spreading of MALA could be explained by renewed confidence in the drug, that is safe when properly prescribed.

## PUB161

### Multipass Hemodialysis (MPHD) – A Flexible Alternative in the ICU

James G. Heaf,[1] Mette K.M. Axelsen,[2] Robert Smith Pedersen.[3] *[1]Dept of Nephrology B, Copenhagen Univ Hospital at Herlev, Herlev, Denmark; [2]Institute of Public Health, Aarhus Univ Hospital, Aarhus, Denmark; [3]Flexdialysis A/S, Rungsted, Denmark.*

**Background:** Dialysis treatment of acute renal injury (AKI) varies between conventional HD for 4 hours to more gentle continuous renal replacement therapy (CRRT). We assessed available data to calculate whether MPHD, a batch system where the dialysate is recirculated, is a valid alternative.

**Methods:** Ten stable HD patients were dialyzed for 8 hours. The dialysate volume was 50% of the calculated water volume and the water flow 500 ml/min. Dialysate tests and blood tests before and after the filter, were taken at start and every hour thereafter. Dialysate volume and ultrafiltration was measured continuously.

**Results:** The initial S-Urea was 20.5 ± 7.6 mmol/l. Minimal S-Urea levels of 13.0 ± 4.7 mmol/l occurred after 5 hours, and thereafter rose to 14.0 ± 4.8 mmol/l. A total of 316 ± 131 mmol urea was eliminated. The dialysate volume was 22.9 ± 4.8 liter. The rise in S-Urea from 5 to 8 hours was 1 mmol/l corresponding to 0.33 mmol/hour. If MPHD had been used for 24 timer (CRRT), assuming constant urea generation, s-urea would have risen to 14 + (16 x 0.33) = 19.3 mmol/l. Assuming a terminal dialysate urea concentration of 19 mmol/l, a total of 435 mmol/ day can be removed. In cases of extreme hyperkalemia, e.g. rhabdomyolysis, a water flow of 500-800 ml/min and blood flow of 300-350 ml/min, with a dialysate potassium concentration of zero, is recommended. This would rapidly permit the removal of approximately 100 mmol of potassium. Continuing potassium problems would require new dialysate.

**Conclusions:** MPHD can be used both as CRRT and for intensive toxin elimination, with a very low water consumption. The principle is also applicable to areas where sustained low-efficiency daily dialysis (SLEDD) is used. Thus MPHD is not a low-efficiency system. This has logistical advantages.

*Funding:* Pharmaceutical Company Support - Flexdialysis A/S

## PUB162

### Comparison of 15% and 4% Citrate Anticoagulation in Plasma Exchange

Manja Antonic, Jadranka Buturovic-Ponikvar, Jakob Gubensek, Rafael Ponikvar. *Dept of Nephrology, Univ Medical Centre, Ljubljana, Slovenia.*

**Background:** To reduce infusion volume we introduced 15% citrate solution and compared its (micro)anticoagulant effect with 4% citrate solution in plasma exchange (PE).

**Methods:** In 18 patients, PE was performed using 4% or 15% trisodium citrate. Anticoagulant effect was evaluated by postfilter ionized calcium (iCa) (target range 0.2-0.4 mmol/l), postfilter hourglass clotting time and visually by assessing circuit after the procedure (on a 5-excellent anticoagulation to 1-total clotting scale). Sieving coefficient (SC) for immunoglobulin G was measured at the start and at the end of all procedures; the reduction of SC at the end was indicative of microanticoagulation failure at plasmafilter.

**Results:** 26 PEs were performed. Postfilter iCa was in a target range in both groups and was significantly lower in the 15% citrate group (0.37±0.02 vs. 0.4±0.02 mmol/l, p<0.05). The mean hourglass clotting time was similar (19±5 min in 15% citrate group vs. 16±2.3 min, p=0.22). Visual assessment showed excellent anticoagulation at plasmafilter and arterial bubble trap (score 4.9±0.3 in both groups) and almost excellent at venous bubble trap (4.6±0.8 and 4.7±0.6). On average, SC did not change significantly during the procedure in 15% citrate group (0.93±0.31 vs. 0.99±0.21, p=0.52) or in 4% citrate group (0.86±0.26 vs. 1.00±0.2, p=0.19). We observed a decrease in SC during 36% of procedures in 15% citrate group and during 50% of procedures in 4% citrate group (p=0.46). The infused volume of citrate per procedure was significantly lower in 15% citrate group (170±26 ml vs. 530±71 ml, p<0.001). No side effects were detected in either group.

**Conclusions:** 15% citrate showed similarly good anticoagulation as 4% citrate and allowed reduction of infused volume, which can be important in volume overloaded patients needing PE.

## PUB163

### Early Dialysis Initiation Based on Acute Kidney Injury Network Criteria in Critically Ill Asian Patients: A Prospective Cohort

Cynthia Ciwei Lim, Chieh-suai Tan, Manish Kaushik, Han Khim Tan. *Dept of Renal Medicine, Singapore General Hospital.*

**Background:** To compare patient mortality in intensive care unit (ICU) patients with acute kidney injury (AKI) and renal replacement therapy (RRT) initiated based on modified Acute Kidney Network (AKIN) creatinine and urine output criteria.

**Methods:** This was a single-center, prospective cohort study of medical and surgical ICU patients referred for AKI over 22 months. Indications for RRT were classified as Group A: "absolute" indications including serum potassium ≥6.0 mmol/L, serum urea ≥30 mmol/L, arterial pH <7.2, serum bicarbonate <10 mmol/L, acute pulmonary edema, acute uremic encephalopathy or pericarditis; Group B: modified AKIN stage 3 without "absolute" indications; Group C: AKIN Stages 1 or 2 without "absolute" indications. Demographic, clinical and biochemical data were collected prospectively from patient medical records. Patients with incomplete baseline data were excluded.

**Results:** There were 169 patients (mean age 59.2 ± 17.1 years; male 63.9%; diabetes mellitus 33.7%; hypertension 53.8%; ischemic heart disease 21.3%; medical ICU 72.2%; mean APACHE score 23.8 ± 6.0). Main AKI causes were sepsis (63.9%) and ischemia (34.9%). RRT was initiated in 121 patients. Complete data on RRT indications were available in 83 patients: Group A (n=26), B (n=26) and C (n=31). Those with RRT initiated in absence

of "absolute" indications were examined. Comparing Group B vs. C: baseline demographics and comorbidities were similar. There were no significant differences in premorbid MDRD eGFR (p=0.36); time from ICU admission to RRT initiation (p=0.51); CRRT modality (p=0.08); average CRRT effluent flow rate (p=0.23). There were no differences in ICU mortality (33.3% vs. 33.3%, p=0.85) or in-hospital mortality (35.7% vs. 28.6%, p=0.25).

**Conclusions:** In the absence of "absolute" indications, earlier RRT initiation based on AKIN criteria did not improve survival in critically ill patients. Controlled trials with well-defined dialysis criteria, including novel biomarkers, are required for further evaluation of optimal RRT timing.

*Funding:* Government Support - Non-U.S.

## PUB164

**Predictors of Mortality in Patients Treated with Continuous Veno-Venous Hemodialysis** Tanush Gupta,[1] Sahil Agrawal,[1] Jalaj Garg,[1] Nikhil Agrawal,[1] Savneek S. Chugh,[1] Dipak Chandy.[2] *[1]Internal Medicine, New York Medical College; [2]Pulmonary and Critical Care Medicine, New York Medical College.*

**Background:** Continuous Veno-Venous Hemodialysis (CVVHD) is a mode of renal replacement therapy utilized in critically ill patients when intermittent hemodialysis is contraindicated due to hemodynamic instability. The purpose of our study was to determine predictors of 7-day and in-hospital mortality in patients treated with CVVHD.

**Methods:** Study cohort included 60 consecutive adult patients (age≥18 years) requiring CVVHD in our Medical Intensive Care Unit (MICU). Acute Physiology and Chronic Health Evaluation II (APACHE II) score was calculated at the time of MICU admission. Primary outcome of our study was in-hospital mortality.

**Results:** The mean age of our study population (65% males, 35% females) was 60.7±13.7 years. The mean APACHE II score was 23.6±6.6 with mean time to initiate CVVHD being 9.2±6.6 hours. Mean duration of CVVHD in our study cohort was 7.2±5.7 days. 77% patients were started on CVVHD due to Acute Kidney Injury while remaining 23% had pre-existing end stage renal disease. Only 17.39% patients eventually recovered their renal function. The 7-day mortality in our analysis was 28.3% while in-hospital mortality was 71.7%. In unadjusted analysis, duration of CVVHD (OR 0.70, p = 0.004) and vasopressor support (OR 2.18, p 0.01) were found to be predictors of 7-day mortality. However, only duration of CVVHD (OR 0.63, p 0.007) predicted mortality in multimodel regression analysis. Similarly, recovery of renal function (OR 0.03, p 0.003) and APACHE II score (OR 1.12, p 0.03) were found to be predictors of inpatient mortality in unadjusted analysis. But, only recovery of renal function (OR 0.01, p 0.001) was associated with decreased inpatient mortality in multimodel regression analysis. Interestingly, time to initiate CVVHD did not predict both 7-day (OR 1.04, p=0.32) and in-hospital mortality (OR 0.99, p=0.77).

**Conclusions:** Duration of CVVHD and recovery of renal function were found to be predictors of 7-day and in-hospital mortality in critically ill patients respectively. Time to initiation of CVVHD had no association with mortality.

## PUB165

**AKI Requiring Dialysis: Shift CVVHD Technique Dose Results** Luis A. Concepcion. *Medicine, Texas A&M Healthscience Center Scott&White Hospital, Temple, TX.*

**Background:** The treatment of AKI requiring dialysis is controversial regarding the modality and dose.

**Methods:** Analysis of 18 month cohort of AKI patients treated with shift CVVHD(Nx Stage 8 hours 40L bicarbonate based dialysate per session)and conventional HD(4-6h Fresenius 4008Bicarbonate) demographics, laboratory data and survival obtained from the EMR, technical and monitoring details from the dialysis run sheets, pre/post BUN,KT/V,Urea reduction (URR), CVVHD dose (ml/kg/hr),standardized KT/V and EKR(equivalent renal clearance)per standard methods. Data as mean and standard deviation.

**Results:** 166 patients with AKI requiring dialysis (37.3% sepsis, 24% CV surgery, 38.7% other) Mortality 43.9%(48.3%sepsis 57.5% CV surgery 31.25% other) 8.31 (9.1) days in dialysis received 6.06(6.05)dialysis, median of 23 days in the hospital. 800 treatments analyzed.91.9%of treatments done in the ICU,78.4% were CVVHD 21.6%conventional dialysis. Age 56.1(17.4) years, weight 95.2(39.4)kg, dialyzed for 6.9(1.6) hours QB300 (40) ml/min ,dialysate K 2.83(0.5) mEq/L,Venous pressure 193(46.9)mmHg,Heparin 596.2 (1267) units,hypotension/hour 0.18(0.33)MAP predialysis 83.2(16.4) mmHg MAP post 83.5(15.7) mmHg,UF 2.6(1.7)L,UF/hour 394.6(274)ml/h ,per sesionURR 47.6 (13.2) % ,Kt/V 0.88(0.36) ,CVVHD dose 59.4(20.9)ml/kg/h , standarized KT/V 3.5(0.8), EKR 9.8(3.6)ml/min. Non survivors (p<0.05) : Older (59 vs 54.1y)longer dialysis times(7.2vs6.7h)lower MAP pre dialysis (77.9vs86.6mmHg) less UF per treatment (3.1vs3.4L) lower pre dialysis BUN(64vs 70 mg/dl)lower creatinine(3.4 vs 4.6mg/dl) higher CVVHD dose (63 vs 56 ml/kg/h) lower EKR (9.2vs10.1ml/min)Good correlation between kt/v,dose CVVHD,standardized KT/V and EKR .Inverse correlation between weight and kt/v ,dose of CVVHD standardized KT/V and EKR.Heavier patients(>95kg) have a better survival despite receive a lower dose of of CVVHD.

**Conclusions:** Shift CVVHD is a method of RRT that can be used for AKI requiring dialysis,the survival is similar to other methods. The dose of dialysis as measured (URR kt/v EKR) was lower in non survivors,dose of CVVHD(ml/kg/min)was higher on nonsurvivors suggesting that other factors affect the survival outcome (weight).

*Funding:* Clinical Revenue Support

## PUB166

**Renal Replacement Therapy in the Setting of Orthotopic Liver Transplant – A Retrospective Descriptive Study** Adetokunbo A. Taiwo, Amishi S. Desai, Jay L. Koyner. *Section of Nephrology, Univ of Chicago, Chicago, IL.*

**Background:** Electrolyte and acid-base disturbances are common during the intraoperative course of orthotopic liver transplantation (OLT) and are magnified in those with chronic kidney disease (CKD) or acute kidney injury (AKI). Some have argued that utilizing intraoperative continuous renal replacement therapy (CRRT) attenuates these fluctuations and improves patient outcomes.

**Methods:** We performed a retrospective, chart review on all OLT recipients receiving intraoperative CRRT between Sept. 1, 2010 and May 15, 2012 at the University of Chicago. Electrolyte and pH data were collected from different time points during OLT [the start of surgery (time 0), 25%, 50%, and 75% of the time into the surgery (time 25,50,75)], pre-op and post-op. Data was analyzed using paired t-test.

**Results:** Data from 11 OLT recipients was available for analysis). Patients were predominantly male (9, 82%), mean age was 51.5 years (range 38-70). Indication for CRRT was as follows: 5 AKI (45.4%), 2 AKI on CKD (18.2%), 2 hyperkalemia (18.2%), 1 volume overload (9.1%) and 1 ESRD (9.1%). Type 1 hepatorenal syndrome was diagnosed in 3 (27.3%) patients prior to OLT. Nine of eleven (81.8%) patients died prior to hospital discharge. With the exception of comparisons between sodium at time 0 and time 75 (P= 0.019), sodium at time 0 and post-op (P= 0.001) and ionized calcium at time 0 and time 25 (P=0.016), there were no statistically significant difference between the other electrolyte and pH values measured at the different time points. Table 1 – Mean solute and pH values (+/- standard deviation).

| | T0 | T25 | T50 | T75 | Pre-Op | Post-OP |
|---|---|---|---|---|---|---|
| Sodium | 139.27 (3.66) | 140.0 (4.49) | 140.64 (3.04) | 142.09 (4.25) | 138.82 (6.37) | 145.18 (4.26) |
| Potassium | 4.55 (0.83) | 4.80 (0.89) | 4.32 (0.57) | 3.98 (0.96) | 4.28 (0.51) | 4.07 (0.80) |
| Bicarb | 24.21 (2.94) | 22.65 (3.55) | 24.34 (5.01) | 26.0 (5.07) | 24.73 (4.29) | 25.82 (5.86) |
| Ionized Ca | 1.08 (0.09) | 0.89 (0.23) | 1.03 (0.18) | 1.10 (0.13) | | |
| pH | 7.36 (0.07) | 7.34 (0.86) | 7.32 (0.091) | 7.391 (0.06) | | 7.47 (0.07) |

**Conclusions:** Randomized control trials will be necessary to elucidate whether CRRT has a defined role intra-operatively in the outcomes of OLT.

## PUB167

**Improving AVF Rate: Effect on Hemodialysis Quality** Ayman Karkar,[1] Ahmed Chaballout,[2] Mohammed Abdelrahman,[1] Maher Haj ibrahim,[1] Mona Al Shubaili.[1] *[1]Kanoo Kidney Center, Dammam Medical Complex, 1, Dammam, Saudi Arabia; [2]Kidney Transplantation Dept, Riyadh, Saudi Arabia.*

**Background:** Vascular access (VA) is the life-line for patients on regular hemodialysis (HD), and the type of VA is associated with patient outcome. Tunneled catheters are associated with increased risk of luminal thrombosis, infection, hospitalization, and high cost. The native arterio-venous fistula (AVF) remains the best available VA option. The high catheter usage rate, increased incidence and prevalence of catheter-associated complications and the progressive increase in number of our ESRD patients in need for HD prompted us to follow the USA "Fistula First Initiative". Our aim was to avoid or reduce the rate of catheter insertion and improve the rate of AVF use, and study that effect on quality of dialysis and patient's outcome.

**Methods:** A vascular access program has been established in collaboration with an enthusiastic and professional vascular surgery team to manage 358 patients who have been on regular HD treatment for a period ranging from 1 to 252 months. The mean ± SD age of patients was 52±15 years with 62% male patients.

**Results:** Over a period of 2 years, 403 procedures were performed. These include 293 AVF and 56 AVG. Other procedures include 35 permanent catheter insertion, 8 AVF aneurysmectomy, removal of 6 AVG, embolectomy of 3 AVG, excision of 1 AVG lymphocele and ligation of 1 AVF. This program resulted in an increase in AVF rate from 35% to 82%, reduction in catheter rate from 62% down to 10%, reduction in infection rate from 6.6% to 0.6%, reduction in VA clotting from 5.1% to 1% and an increase in average blood flow rate from 214±32 to 298±37 ml/min (p<0.01). These results have been associated with improved average single pool Kt/V from 0.88±0.19 up to 1.28±0.2 (p<0.01), increased hemoglobin from 9.2±1.2 to 10.9±0.9 g/dl (p<0.01), improved serum albumin from 3.2±0.5 to 3.7±0.4 g/dl (p<0.05), reduction in administered erythropoietin dose by 16% and a significant drop in hospitalization rate from 6.1% down to 3.8%.

**Conclusions:** In conclusion, these results confirm the great benefit of AVF on quality of HD and patient outcome, and clearly affirm that AVF should always come first.

*Funding:* Clinical Revenue Support

## PUB168

**Rates of Vessel Mapping prior to Arteriovenous Fistula Placement Vary across the Mid-Atlantic United States** Janet R. Lynch,[1] W.G. Schenk,[2] Kim Deaver,[2] Ryan D. Evans.[3] *[1]Mid-Atlantic Renal Coalition, Richmond, VA; [2]Univ of Virginia; [3]Valley Nephrology Associates.*

**Background:** The Fistula First Breakthrough Initiative (FFBI) is a population-based intervention by the Centers for Medicare & Medicaid Services to increase appropriate arteriovenous fistula (AVF) use. Early referral for surgical evaluation and timely placement of a fistula, including prior vessel mapping, is encouraged. This study examines geographic variation in vessel mapping rates among Medicare beneficiaries. The relationship of vessel mapping to subsequent prevalent AVF use is examined in a sub-group of hemodialysis patients >=66 years treated by large dialysis organizations.

**Methods:** Using Medicare Part B claims for Maryland, Virginia, West Virginia and Washington, D.C., we identified AVF placements in 2011, matched these with prior vessel mapping claims in 2010 and 2011, and calculated rates of vessel mapping in hospital referral regions (HRRs) defined by the Dartmouth Atlas of Health Care. To examine the relationship between vessel mapping and prevalent AVF use, we matched claims with April 2012 FFBI prevalent patient-level vascular access data from large dialysis organizations. These linked data met the following criteria: (1) 2011 claim for AVF placement, (2) matched with FFBI prevalent access record, (3) patient >= 66 years of age at access claim, and (4) patient started dialysis in 2011. Chi-square was used to examine association.

**Results:** 50.4% (1,990/3,948) of AVF claims were preceded by vessel mapping claims. Across 17 HRRs within our geographic boundaries, the range was 23.3% to 85.8% for all patients (n=3,948) and 27.0% to 84.1% for patients >= 66 years (n=2,206). Four hundred eighty six patients met all criteria for examination of association between prior vessel mapping and AVF use. Of these, 71.4% were using an AVF in April 2012. Those with prior vessel mapping (n=260) had 74.6% AVF use and those with no prior vessel mapping (n=226) had 67.7% AVF use (p<0.1).

**Conclusions:** There may be an opportunity to improve fistula prevalence both by increasing rates of pre-operative vessel mapping as well as by reducing geographic variation in mapping.

*Funding:* Other U.S. Government Support

## PUB169

**Use of Gentamycin/Citrate Lock Solution Decreases Catheter Related Bacteremia** Grant Springman, John Hergenrother. *The Christ Hospital, Cincinnati, OH.*

**Background:** Infection remains a leading cause of morbidity and mortality in hemodialysis patients. Those particularly susceptible are accessed by tunneled hemodialysis catheters, which comprise a majority of the dialysis population. Antimicrobial lock solutions have previously demonstrated decreases in catheter related bacteremia (CRB) rates. An investigation of catheter related bacteremia rates with use of a gentamicin/citrate catheter locking solution (GCLS) was compared to the standard heparin catheter locking solution.

**Methods:** A prospective review evaluating all prevalent patients using a tunneled catheter for hemodialysis access was performed in an urban, tertiary care, hospital-based, outpatient hemodialysis unit from July 1 2008 to December 31 2008. Patients were assigned to the control group of the standard 2000 units/ml of heparin or the study group of a 5mg/dl gentamicin/4% trisodium citrate based on their dialysis schedule. Overall 53 patients were evaluated, 34 in the control group and 19 in the GCLS group. CRB was determined by positive blood cultures drawn on patients based on clinical suspicion for infection.

**Results:** Results demonstrated a CRB rate of 5.3% (1/19) in the experimental group compared to 26% (9/34) in the standard group, an absolute risk reduction of 21% ($p < 0.05$). Therefore, the GCLS was implemented for all patients treated in the hemodialysis unit. Data collection continued from 2009-2011, and CRB rates continued at low rates significantly less than historical controls. Rates decreased from 2.1 to 0.9 to 0.7 infections per 100 patient months of treatment, from 2009-2011 respectively.

**Conclusions:** Gentamicin/Citrate is an effective antimicrobial lock solution in tunneled dialysis catheters, significantly decreasing CRB rates.

*Funding:* Clinical Revenue Support

## PUB170

**Tunneled Dialysis Catheter Infection and Troponin-I Levels in End-Stage Renal Disease Patients** Kamel A. Gharaibeh,[1] Mihaly B. Tapolyai,[2] Eva Csongradi,[1] Tibor Fulop.[1] *[1]Dept of Medicine, Univ of Mississippi, Jackson, MS; [2]Dept of Medicine, Semmelweis Univ.*

**Background:** Elevated troponin-I levels in end-stage renal disease (ESRD) patients on dialysis are commonly attributed to ESRD itself. Our hypothesis was that infection of tunneled hemodialysis catheters (TDC) may be associated with subtle troponin-I elevations.

**Methods:** We performed additional data analysis on our consecutive 3-year experience (01/2007 - 12/2009) with bedside TDC removal at the University of Mississippi Renal Fellowship Program. Data was already collected for multiple patients and procedure-related variables. Data was analyzed with SPSS 19. and reported with means ±SD or medians 25-75% IQR for descriptive data; Pearson's correlation and independent-samples T test was utilized for statistical comparisons. The study was reviewed and approved by the University of Mississippi Human Research Office.

**Results:** Indication at the time of removal included bacteremia, fever or clinical sepsis with hemodynamic instability or respiratory failure. Of the 55 TDC removed, 63.6% took place on general floor, 12.7% in Emergency Department and 16.3% in Intensive Care Units, with a median time of 3 days [IQR 1-13] elapsed since admission or TDC placement. Four (7.2%) patients were hypothermic, 33 (60%) febrile or subfebrile (T>37 C°) at time of removal; 7 (12.7%) on vasoactive pressors. Peak C-reactive protein (available in 63.6% of cohort) measured 12.9±8.4 mg/dl (nl<0.49); mean troponin-I (34% available) was 0.534 ng/ml [IQR 0.03-0.9] (nl<0.034) and they did not correlate with each other. Troponin-I had no association with temperature, systolic and diastolic BP or clinical sepsis. However, troponin-I, as a continuous variable had a strong trend of association with proven bacteremia (p=0.075); furthermore, the association of troponin-I as a bivariate variable (normal/abnormal) with bacteremia has reach significance (Pearson's chi-square 0.049).

**Conclusions:** Our results strongly suggest that some of the elevated troponin-I levels in ESRD patients may be attributed to TDC-induced bacteremia. Additional investigations are warranted on this subject for definitive conclusion.

## PUB171

**Comparison of Survival and Hospitalization of Hemodialysis Patients Who Started Hemodialysis via Tunnelled or Non-Tunnelled Central Venous Catheter** Ji In Park,[1] Jung Pyo Lee,[2] Ji-Young Choi,[3] Yong-Lim Kim,[3] Yun Kyu Oh,[2] Dong Ki Kim,[1] Yon Su Kim,[1] Chun Soo Lim.[2] *[1]Seoul National Univ College of Medicine; [2]Seoul National Univ Boramae Medical Center; [3]Kyungpook National Univ Hospital.*

**Background:** Some of end-stage renal disease (ESRD) patients inevitably start dialysis via central venous catheter (CVC). Tunnelled central venous catheter (TCVC) is well known to be superior to non-tunnelled central venous catheter (NTCVC) in terms of lower risk of infection and catheter failure. In this study, we investigated whether the difference in the use of these two different types of catheter shows difference in the survival and vascular access related hospitalization or not.

**Methods:** We enrolled newly diagnosed ESRD patients and collected the data prospectively from August 2008 to March 2013. Laboratory and clinical variables with the type of catheter were documented, and hosppitalization and mortality were recorded as the outcome events.

**Results:** Among 1770 patients, 810 patients started hemodialysis via CVC, 629 patients with TCVC and 181 with NTCVC. Hospitalization due to catheter-related infection were higher in NTCVC group (2.21%) than in TCVC group (0.47%) (p=0.048). TCVC group also showed significantly better survival rate than NTCVC. In the multivariate analysis adjusting for age, liver disease and congestive heart failure, the type of catheter was still significantly associated with survival (p=0.021).

**Conclusions:** The data demonstrate the TCVC has an association with the lower hospitalization rate due to catheter-related infection and has the higher survival rate than the NTCVC. TCVC should be firstly considered in the patients who start hemodialysis without functioning vascular access.

## PUB172

**Renal Outcomes in PICC Patients** Rita L. McGill, Tarik Noureldeen, Sushil Ratnaparkhe. *Div of Nephrology, Allegheny General Hospital, Pittsburgh, PA.*

**Background:** A 2012 quality-improvement study identified peripherally-inserted central catheters (PICC) in 85/375 (22.6%) of our inpatients.

**Methods:** The hospital data system was examined to identify renal outcomes in these patients over the next 365 days; external data was not sought. AKI events were defined by KDIGO criteria, with elevated serum creatinine for 3 days required. Outcomes were classified as: non-recovery with dialysis, recovery after dialysis, recovery/stabilization without dialysis, or indeterminate.

**Results:** At baseline, 7/85 PICC patients were in ESRD and 18/85 had acute kidney injury (AKI); 60/85 patients were at their CKD baseline or had estimated GFR>60 mL/min/1.73m2. At least one additional AKI was seen in 11/18 patients. 6/18 required dialysis for initial AKI and 1 for subsequent AKI; 3 of these recovered. 21/60 patients without AKI at baseline developed episodes of AKI over 12 months, 3 of which required permanent dialysis. Overall 39/78 (50%) non-ESRD patients had initial and/or subsequent AKI, and 10/78 (12.8%) required dialysis.

| | AKI at baseline | No AKI at baseline |
|---|---|---|
| N | 18 | 60 |
| Non-recovery with dialysis | 4 | 3 |
| Recovery after dialysis | 3 | 0 |
| Indeterminate; no dialysis* | 0 | 3 |
| Recovery/stabilization without dialysis | 11 | 15 |
| Never AKI | --- | 39 |

*patient lost to follow-up or early death. Among 39 patients with initial or subsequent AKI, recovery or stabilization with or without dialysis could be documented in 29 cases. Final serum creatinine was significantly higher than baseline for these patients, (0.95+/-0.30 vs. 1.73+/-0.84, P-value<0.0001 for paired t-test, 95% CI for difference: 0.48-1.09).

**Conclusions:** Inpatients requiring PICC have a 50% risk for AKI and a 12.8% rate of requiring dialysis within a 12-month time-frame, despite methodological potential for low ascertainment. PICC patients who sustain AKI are at risk for accelerated deterioration of renal function, even when dialysis does not occur. Given the negative impact of PICC placement upon future hemodialysis vascular access, it may be reasonable to place all PICC lines in dominant arms, even when advanced CKD is not present, especially in patients with AKI.

## PUB173

**Prospective Observation of Effect of Vascular Access Types on C Reactive Protein, ESR and Fibrinogen in 203 Hemodialysis Patients** Ebadur Rahman, Nader Mohamed Omran, Naveed Aslam, Raees Farhan Mushtaq, Ahmed Soliman, Seddeg Younis, Dujanah Hassan Mousa. *Nephrology, PSMMC, Riyadh, Saudi Arabia.*

**Background:** In this study effect of arteriovenous fistula (AVF) and Permanent hemodialysis catheters (PC) on common inflammatory markers were observed in patients without any local or systemic infection.

**Methods:** Out of 350 patients dialyzing in Prince Sultan military medical city (PSMMC) 200 patients were included in this study. Patients with arterivenous graft and who had documented local or systemic infection by repeated cultures were excluded from this study. The patients included in this study had monthly blood cultures. Total observation period was 180 days. The average of monthly C reactive protein( CRP), ESR and fibrinogen was taken for analysis. All patients were dialyzed with same dialyzer (Hemoflow FX8O)

and heparin was used as anticoagulant. Patients with Hemoglobin above 12 or bellow 10 gm/dl were also excluded. Hemodialysis mode was used for all patients. SAS version 9.3 software was used for the data analysis.

**Results:** Mean age of observed group was 59.49 years. 55% were dialyzing with fistula and the rest with PC. Mean Kt/v in these two groups were statistically insignificant. There was no statistical significance between the PC and AVF regarding ESR and CRP protein level (P 0.696). However fibrinogen level was significantly lower in PC group (p 0.0311 *).

**Conclusions:** In non infected patients, CRP and ESR are not affected by access types, if the patients are dialyzed by same dialyzer using same anticoagulant. Fibrinogen level is significantly lower in PC group.

*Funding:* Government Support - Non-U.S.

## PUB174

### Presentation and Complications of Non-Thrombus-Related Fibrin Sheath Pulmonary Embolus following Tunnelled Dialysis Catheter Removal

Iain Moore, Mark Brady, Saeed Ahmed. *Renal Unit, City Hospitals Sunderland, Sunderland, United Kingdom.*

**Background:** We report an unusual complication of tunnelled dialysis catheter removal. Fibrin sheaths have a reported prevalence of 60-100% in association with tunnelled dialysis catheters. Fibrin sheath emboli have previously only been reported following line stripping, or in association with catheter related thrombus, whilst a prospective study has suggested that fibrin sheaths alone do not directly embolise following line removal.

**Methods:** A 25 year old male presented with advanced renal impairment of unknown cause. Dialysis was commenced via uncomplicated insertion of a right internal jugular tunnelled dialysis catheter. This remained his preferred choice of access whilst transplantation was pursued. After 9 months hemodialysis he presented with dialysis catheter sepsis. His line was removed without event. Temporary access was attempted via right internal jugular vein 24 hours later but failed; a temporary femoral dialysis catheter was inserted. Two days later he described left upper quadrant pain out of keeping with physical examination and observations. After several reviews, a computed tomography pulmonary angiogram (CTPA) was performed.

**Results:** CTPA demonstrated left sided pulmonary artery fibrin sheath embolus and no thrombus. Following involvement from the cardio-thoracic team he was managed conservatively and warfarinised. Within a week he reported new breathlessness. CTPA demonstrated unchanged sheath emboli, extended infarction and an expanded exudative pleural effusion. After a prolonged admission he recovered and was established on peritoneal dialysis.

**Conclusions:** Symptomatic non-thrombus-related fibrin sheath embolisation has not previously been reported. It is thought to be a rare complication; perhaps rarely diagnosed or clinically apparent. This episode may have been precipitated by access attempts at the site of recent removal. We recommend consideration be given to fibrin sheath embolisation in patients presenting with pleuritic chest pain, upper abdominal pain or new pleural effusions, after recent tunnelled dialysis catheter removal. A high index of suspicion should prevail if access attempts have been made at the recent removal site.

## PUB175

### Importance of Monitoring and Immediate Intervention for Vascular Access Optimization

Marco Mendes, Patricia Veigas, Fernanda Gomes, Ines Aires, Célia Gil, Patrícia Matias, Cristina Jorge, Manuel A. Ferreira. *Nephrology, Nephrocare Vila Franca de Xira, Lisboa, Portugal.*

**Background:** Programs of monitoring vascular access (VA), including angiographic and/or surgical intervention, have been performed in order to improve VA outcome. Our goal is to assess the impact of an immediate response from a vascular access center (VAC) on the VA of patients (pts) from a large hemodialysis center.

**Methods:** A retrospective analysis of the VA and their interventions in 2 periods (20 months each), before and after the implementation of an immediate response from VAC, was performed. Both populations were equivalent. 218 pts were included, with mean age (± SD) 67,2 ± 15,4 years, 50% female, 37,2% diabetics, with mean HD time of 61,5±58,4 months. Monthly determinations of VA flow (Qa) using the Blood Temperature Monitor-Fresenius Medical Care were performed. Indications for angiography were: A-V fistula (AVF)Qa < 200 ml/min; PTFE graft < 600 ml/min or a Qa drop > 50% in 2 consecutive months; significant high venous pressure (VP) and/or edema of VA limb and thrombosis of the VA.

**Results:** The results comparing Pre VAC with Post VAC are presented at Table 1.

| | Pre CAV | POS CAV | |
|---|---|---|---|
| Total average lengh of use of VA/AVF (years) | 2.76 (±3.85)/4.4 | 2.91 ( ±3.86)/4.1 | p>0.05 |
| VA in use | | | |
| Central venous catheter (CVC) (%) | 28.58 | 25.7 | |
| AVF (%) | 42.86 | 48.2 | |
| AV flow (Qa) (ml/min) | 1489.3 ( ±632.5) | 1164.7 ( ±448.4) | p<0.05 |
| Kt/v | 1.57 ( ±0.27) | 1.94 ( ± 0.35) | p<0.05 |
| Diagnostic angiography (%) | 0.82 | 2.22 | p>0.05 |
| Angiography without stent (%) | 13.47 | 24.76 | p>0.05 |
| Surgical review of the VA (%) | 18.37 | 26.98 | p>0.05 |
| Failure due to thrombosis (%) | 19.42 | 8.97 | p>0.05 |
| Hospitalization related to the VA (%) | 11.02 | 3.17 | p<0.05 |

**Conclusions:** Introduction of VAC was associated with decrease thrombosis rate (50%) and hospitalizations related to the AV (71%) at expenses of increased number of interventions. In the authors opinion, continuous monitoring of vascular access and a timely reference to the VAC is crucial for increasing AV longevity and reducing morbidity.

## PUB176

### Percutaneous Transluminal Angioplasty (PTA) for Dysfunctional, Non Maturing or Thrombosed AVF: A Local Experience

Silvia Mattei,[1] Gianluca Pignatelli,[2] Mattia Corradini,[1] Alfredo Stefani,[1] Achiropita Bovino,[1] Augusto Vaglio,[3] Francesco Iannuzzella,[1] Sonia Pasquali.[1] *[1]Nephrology and Dialysis Unit, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; [2]Interventional Cardiology, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; [3]Nephrology and Dialysis Unit, Univeristà Degli Studi, Parma, Italy.*

**Background:** The arteriovenous fistula (AVF) is considered the vascular access of choice and stenoses are the major cause of its dysfunction. Our study retrospectively evaluates the clinical course and treatment outcome of performing PTA for dysfunctional, non maturing or thrombosed AVF in our experience.

**Methods:** Between Jan. 2008 and Dec. 2012, 101 PTA procedures were performed on 73 patients (49 men; median age 67 years, range: 27-89) with dysfunctional (63%), non maturing (7%) or thrombosed (30%) AVF. 63 wrist radiocefalic [25 with latero-terminal (L-T), 38 with termino-terminal (T-T) anastomosis] and 10 elbow fistulae with median age of 12 months (range 1 month-13 years) were treated.

**Results:** In radiocefalic L-T AVF, stenoses were located in the iuxta-anastomotic segment in 11 patients, in anastomotic area in 5, in the venous outflow in 5, in central vein in 1, in multiple areas in 3; in radiocefalic T-T AVF the distribution was similar (iuxta-anastomotic 47%, anastomotic 21%, outflow vein 16%, central vein 5%, multiple areas (11%). In elbow fistulae the stenoses occurred in the outflow vein in 7 patients (70%) and around the anastomotic region in 3 patients (30%). Angiografic and clinical success was 88%; 17 patients required 28 repeat PTA for recurrent stenosis/thrombosis. 8 patients had small extravasation that required no further treatment, 1 patient had microembolism in second interdigital artery. Excluding initial failure, mean primary and secondary patency for AVF were 34.3 months (95% CI 26.4-42.1) and 41.1 months (95% CI 37.9-52.4); the primary and secondary cumulative patency rates at 12 months were 59% and 84% respectively.

**Conclusions:** PTA can effectively salvage dysfunctional, non maturing or thrombosed AVF. Since repeat angioplasty is often necessary to maintain function, careful surveillance is necessary.

## PUB177

### Doppler Window; Vascular Access Ultrasound Development Platform

William Weitzel,[1,2] Leo Koziol,[1] Joseph L. Bull,[1] John J. Pitre,[1] Alan Vollmer,[1] Grant H. Kruger.[1] *[1]Univ of Michigan, Ann Arbor, MI; [2]VA Medical Center, Ann Arbor, MI.*

**Background:** Extensive research using ultrasound has sought to improve vascular access care through pre-operative evaluation, post-operative monitoring, and dialysis access surveillance. With the vision of low-cost high-performance ultrasound becoming ubiquitous, we designed, fabricated, and tested a flexibly reconfigurable vascular access ultrasound development platform to further develop conventional Doppler and B-mode as well as advanced capabilities such as vascular elasticity imaging.

**Methods: Figure 1a** shows the transducer with magnetic drive sub-assemblies. The system includes transmit and receive circuits for signal capture, and wireless and USB communications hardware. A MIPS microcontroller (MCU) controls the high-level functions of the device, while complex programmable logic device (CPLDs) handle low-level sequencing of the ultrasound sub-systems. The MCU also controls the magnetic drive board, providing the sensor and feedback control for 2D scanning.

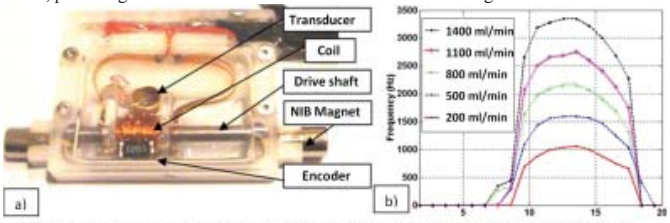



**Figure 1:** a) Vascular access ultrasound probe prototype, and b) gated-pulse wave Doppler flow measurements across flow phantom vessel using blood mimicking fluid.

**Results:** Velocity based measurements using the flow profile were obtained from our device in gated PW Doppler data acquisition mode; and PW flow estimation agreed with phantom flow for 4mm, 6mm, 8mm vessels from 0 to 1500 ml/min with an $R^2$ of 0.93, 0.93, and 0.96 respectively. **Figure 1b** illustrates the measured Doppler frequencies as the sample gate is transitioned across the vessel diameter at flow rates of 200, 500, 800, 1100 and 1400 ml/min. The expected parabolic profile can clearly be seen as well as a monotonic increase in frequency as volume flow is increased. The impact of pulsatility on flow measurement and edge detection algorithms were also tested and validated.

**Conclusions:** After accomplishing current engineering objectives, we are preparing to perform a pilot clinical study to validate our prototype for vascular applications for hemodialysis patients.

*Funding:* Other NIH Support - NIH grants 1R41HL110430 and 1R41HL112517, Private Foundation Support

**PUB178**

**Use of Cefuroxime in Treatment of Methicillin Sensitive *Staphylococcus aureus* in Haemodialysis Patient – A Single Centre Experience** Vijay Sundaram Thanaraj, Ajay Prabhakar Dhaygude. *Dept of Renal Medicine, Lancashire Teaching Hospital, Preston, Lancashire, United Kingdom.*

**Background:** Infection is one of the most common causes of mortality in haemodialysis patient especially in patients who dialyse via catheter. It is common practice to use Vancomycin in management of Methicillin sensitive *Staphylococcus aureus* (MSSA) infection because of the broad-spectrum cover and convenience of administration in haemodialysis patients. Flucloxacillin is an effective alternative but q.i.d. dosing poses compliance problem. Use of Vancomycin however is associated with number of limitations. The incidence of Vancomycin resistant Enterococcus is on the rise.It is less bactericidal compared with the cephalosporins and penicillins.Cefuroxime, a second generation Cephalosporin is effective against MSSA and offers advantage over Vancomycin as above.

**Methods:** We used intravenous Cefuroxime; 1.5 grams given at the end of each dialysis session for a period of four weeks in patients with MSSA infection in haemodialysis patients. Root cause analysis and appropriate investigations were done for all the patients with MSSA infection to exclude other potential sources of infection other than dialysis catheter. Repeat blood cultures and exit site swab were taken following treatment to exclude ongoing bacteraemia. Ten patients were diagnosed with MSSA infection between December 2009 to March 2013.

**Results:** None of the 10 patients, who were treated with Cefuroxime, relapsed following completion of the course of treatment. The mean follow up period was 398 days.

**Conclusions:** In this study Cefuroxime appears to be safe and effective alternative in management of MSSA infection in haemodialysis patients. First generation cephalosporin (cefazolin) has been used successfully, use of second generation cefuroxime has not been reported before. Although small number of patients were treated with cefuroxime, unlike cefazolin, we did not find any treatment failure and it may be superior to 1st generation cephalosporins. **Limitation:** Our study has small number of patients however we are proactive with 'fistula first' policy and incidence of catheter associated infections are very low in our centre. Further large studies are recommended.

**PUB179**

**The Research of Anatomic Relationships between the Internal Jugular Vein and the Common Carotid Artery under CT Scan** Yimin Zhang.[1] *[1]Div of Nephrology, The Sixth Hospital of Sun Yatsen Univ, Guangzhou, Guangdong, China; [2]Div of Nephrology, The Sixth Hospital of Sun Yatsen Univ, Guangzhou, Guangdong, China; [3]Div of Nephrology, The Sun Yatsen Memorial Hospital of Sun Yatsen Univ, Guangzhou, Guangdong, China.*

**Background:** Central venous cannulation through the internal jugular vein is necessary for the management of a variety of clinical conditions. The aim of this study was to investigate anatomic relationships between the internal jugular vein and the common carotid artery under CT enhancement scan at a different plane.

**Methods:** A total of 226 patients who were checked the neck by CT enhancement scan since January 2010 to July 2012.All patients were supine position and the heads were normotopia.To collect the anatomic relationships between the internal jugular vein and the common carotid artery at the high plane(the upper edge of the thyroid cartilage) and the median plane(the level of the cricoids cartilage)of all patients.The relationships were classified as anterior,anterolateral,anteromedial,posterior,posterolateral,posteromedial,medial,lateral or partially overlap,complete overlap,parallel.Categorical variables were presented as counts and were compared by the chi-squared test.

**Results:** There were 116 male patients(mean age was 52.7±13.9) and 110 female patients(mean age was 49.5±14.5). No patients showd the image with the internal jugular vein and the common carotid artery placed conversely.Two cases were anterior complete overlap on the lefe side(0.9%).At the high plane,the internal jugular veins were mainly posterior and posterolateral position(78.6%-83.9%),the relationships with the common carotid artery were mainly partially overlap(70.5%-71.4%). While at the median plane ,the internal jugular veins were mainly anterior and anterolateral position(72.3%,P=0.00 for both),the relationships with the common carotid artery were mainly parallel(56.2%-63.4%,P=0.00 for both).

**Conclusions:** The relationships between the internal jugular vein and the common carotid artery at the median plane had more proportion of parallel than at the high plane.

**PUB180**

**Turolidine Catheter Locking to Prevent Catheter Related Bloodstream Infections (CRBSI) in New Patients Undergoing Hemodialysis through Temporary Hemodialysis Catheter** Omer Sabir,[1] Nauman Tarif,[1] Rizwan Muhammad Sheikh,[2] Kashif Rafique,[1] Nabiha Rizvi.[1] *[1]Dept of Nephrology, Fatima Memorial Hospital, Lahore, Pakistan; [2]Dept of Nephrology, Hashmat Medical and Dental College, Gujrat, Pakistan.*

**Background:** Temporary dialysis catheter for as long as 4 weeks, is unfortunately the first hemodialysis access in majority of patients initiating HD. Many patients will continue HD with same catheter beyond even 4 weeks due to cost and lack of permanent access. Several Interdialytic catheter locking strategies have been devised to prevent catheter related blood stream infection (CRBSI). Taurolidine is a nontoxic broad-spectrum antimicrobial agent that can potentially eradicate CRBSI and has generted recent interest to prevent CRBSI.

**Methods:** Open labelled, randomized controlled study in incident 38 patients initiating HD with non cuffed temporary HD catheter.

**Results:** Final analysis of 36 patients revealed one patient in heparin group had symptoms strongly suggestive of CRBSI, however refused further work up. The point incidence rates of CRBSI was 2.7% or 1.1 per 1000 catheter days. 5 patients in taurolidne group and 4 in heparin group continued HD beyond 30 days till they were shifted to AVF, except one in taurolidne group who continued on the same catheter and died of CRBSI on 100th day.

**Conclusions:** Taurolidine 2% and heparin compared to heparin alone as catheter lock solution are similar in terms of incidence of CRBSI over 4 weeks. Catheter survival may be prolonged with taurolidine, due to possible effect on biofilm and antibacterial activity however this needs further study in temporary vascular access.

**PUB181**

**Vascular Access Types at the Initiation of Hemodialysis** Kashif Rafique, Omer Sabir, Nabiha Rizvi, Nauman Tarif. *Nephrology, Fatima Memorial Hospital, Pakistan.*

**Background:** Initial vascular access dictates the short and long term survival patterns of hemodialysis patterns. Permanent vascular access arteriovenous fistula (AVF) is not the first access in majority of our patients. To stress the need to follow the AVF first slogan among our physicians and patients we conducted this study.

**Methods:** Retrospective review of hemodialysis patients by chart review and patient interview was conducted over a period of 6 months at four different hemodialysis (HD) centers.

**Results:** Total 80 patients took part in the study. Males were 51(63%), mean age was 53.9±15.34. Temporary hemodialysis catheter was the most common vascular access (86.3%) in majority of patients, whereas AVF in 13.7 % of patients. most of the patients were diabetics and or hypertensive (81.3%). The time from the diagnosis of Chronic kidney disease to initiation of HD was 24.73±25.9 months. At the time of interview 77.5% had AVF, and primary failure was only in two patients (2.5%), secondary failure in 17 (21.3%). 5(6.3%) patients had arteriovenous graft, and permcath in 4(5%) as current vascular access . Temporary vascular access was still used for HD in 9(11.3 %). Mean duration of functioning AVF was 24.25±23.8 months.

**Conclusions:** Temporary vascular access is still the primary vascular access in majority of our patients and once AVF is created continues to have a successful outcome. There is a need for patient education and more stress among the nephrology community in our setup to highlight the Fistula first initiative.

**PUB182**

**Do Taurolidine-Heparin-Citrate Line Locks Offer Improved Outcomes to Heparin? – Interim Analysis following a Cohort Switch** Mark E. McClure, Richard W. Corbett, Rachna Bedi, Bhavini Patel, Natasha Gunawardana, Damien Ashby, Neill D. Duncan. *Dialysis Research Group, West London Renal and Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

**Background:** Catheter dysfunction and catheter-related infections remain a significant cause of morbidity in patients maintained on tunneled central venous catheters. Taurolidine-heparin-citrate line locks containing an antimicrobial (taurolidine) could potentially reduce the risk of catheter-related bacteraemia, although there are concerns that the reduced concentration of heparin (500 units/ml vs 5000 units/ml) may result in unacceptably high rates of catheter dysfunction. A small randomized control trial demonstrated increased catheter survival in the setting of catheter salvage, however, there is no published data relating to switching line locks in a prevalent haemodialysis population.

**Methods:** A retrospective comparison was performed of all patients in three dialysis units, who underwent a switch in their standard line lock from heparin to taurolidine-heparin-citrate. Patients who died or changed modality of renal replacement therapy were excluded. Comparison of a number of parameters including thrombolytic usage, line changes, catheter associated bacteraemia, Kt/V, blood flow rate and erythropoietin resistance was undertaken for three months prior to and following the switch.

**Results:** Data from 225 patients were included in the analysis over six months. There was a trend to a non-statistically significant reduction in catheter-related bacteraemia (8 vs 3 [pre- vs post]; p=0.12) and reduction in individuals requiring thrombolytic line locks (25 vs 18; p=0.26). There was also a low rate of catheter dysfunction requiring line change following the switch (2 vs 1). No difference could be demonstrated between any of the anaemia or dialytic parameters.

**Conclusions:** This retrospective study has not demonstrated any significant difference in a range of clinically relevant parameters following a switch from heparin to taurolidine-heparin-citrate line locks in a prevalent dialysis population. This study has not demonstrated increased levels of catheter dysfunction but requires further analysis at twelve months following the switch.

**PUB183**

**An Unusual Turn of the Wire** Roxana Neyra. *Ambulatory Surgery Center, Arizona Kidney Disease and Hypertension Center, Phoenix, AZ.*

**Background:** Every day, hundreds of hemodialysis (HD) catheters are inserted either electively or as an emergency to initiate HD and save patients from life threatening conditions. In the process, there are some immediate and long-term complications, such

as hematoma, bleeding, pneumothorax, and central venous stenosis respectively. A case of subcapsular hepatic hematoma post HD catheter insertion is presented.

**Methods:** 88-year-old female with past medical history significant for end stage renal disease on HD 3 times per week. She had refused arterio-venous fistula creation. Therefore, her vascular access was a right internal jugular hemodialysis catheter for about 4 years. Her catheter was exchanged about once a year. She presented to the outpatient surgery center once more for catheter exchange due to poor flows. The catheter was replaced successfully using two wires advanced to the Inferior Vena Cava (IVC). Shortly after the procedure she complained of abdominal pain, nausea and vomiting. At the same time hypotension and bradycardia was noted. Patient was immediately transferred to the hospital. She underwent emergent venogram of the IVC, and arteriogram of the hepatic artery that showed no bleeding or extravasation. A computerized tomography of the abdomen was performed and demonstrated a heterogeneous fluid collection in the right lobe of the liver suggestive of subcapsular hematoma. Patient was treated with supportive measures and was stabilized. Over the following days, she developed a myocardial infarction, sepsis and was transferred to hospice.

**Results:** An autopsy was performed, and showed no liver laceration, hepatic artery or IVC laceration, but confirmed the subcapsular hepatic hematoma.

**Conclusions:** a) Nontraumatic subcapsular hepatic hematoma is a rare complication of insertion of dialysis catheters due to the wire advancing through the hepatic veins. It can be managed by observation and supportive measures. It can be life threatening. b) There is a relative safety in advancing the wire to the (IVC) when inserting a catheter. The position of the tip of the wire needs to be monitored at all times during the procedure to prevent complications, like the one described here.

## PUB184

### Use of Rivaroxaban to Rescue Vascular Access in Hemodialysis Patients

Fabian A. Ortiz-Herbener. *Dept of Nephrology, Instituto Ecuatoriano de Dialisis y Trasplantes, Guayaquil, Guayas, Ecuador.*

**Background:** Rivaroxaban is a direct inhibitor of factor Xa. This novel oral anticoagulant has substantial renal clearance, with half-life prolonged in patients with chronic kidney disease. There is a lack of significant clinical experience of rivaroxaban use in hemodialysis patients.

**Methods:** This study aimed to investigate the effect of rivaroxaban as a compassionate treatment for vascular access dysfunction. A cohort of 5 patients with a history of at least four previous vascular access thrombosis was selected. Two patients received 10 mg QD and three patients received 15 mg QD with a follow up of four months. The INR was measured in a monthly basis, and the results were categorized as: “Good” (improvement in vascular access permeability), and “No Response” (when there is no improvement at all, and the patient required a new access). In addition, major side effects (bleeding from any cause) were stated as “Present” or “Not present”.

**Results:**

| | Dose (mg) | Baseline INR | Month 1 | Month 3 | Month 4 | Response | Side Effects |
|---|---|---|---|---|---|---|---|
| male (71) | 10 | 1.15 | 1.25 | 1.33 | 1.21 | No Response | Not Present |
| female (64) | 10 | 1.13 | 1.03 | 0.9 | 0.96 | No Response | Not Present |
| female (58) | 15 | 1.05 | 1.03 | 1.13 | 1.08 | Good | Not Present |
| male (53) | 15 | 0.98 | 1.13 | 1.12 | 0.97 | Good | Not Present |
| male (50) | 15 | 1.13 | 1.11 | 1.23 | 1.21 | Good | Not Present |

**Conclusions:** To our knowledge, this is the first clinical experience of Rivaroxaban in hemodialysis patients. Although it is a small number of cases, we found three patients with good response and no patients with major side effects. Larger studies are required to establish the safety profile of the drug in hemodialysis scenario.

## PUB185

### Persistent Variation in the Frequency of Payment for More Than Thrice Weekly Home Hemodialysis (HHD)

Richard Hirth, Adam S. Wilk, Wei Zhang, Tammie A. Nahra, John R.C. Wheeler, Kathryn Sleeman, Joseph M. Messana. *Univ of Michigan, Ann Arbor.*

**Background:** Regional fiscal intermediaries (FIs) and Medicare Administrative Contractors (MACs) that pay Medicare claims can authorize payment for >3 weekly treatments. Previous work showed FIs/MACs may contribute significantly to variation in HHD practice (Hirth et al. 2011). Due to bundling of drugs and lab tests on a flat, / treatment basis, each additional paid treatment results in greater incremental revenues under the expanded bundle dialysis prospective payment system (PPS) than under the prior system.

**Methods:** We examined trends in HHD payments and evidence that dialysis facilities may select FIs/MACs with favorable payment policies.We identified all Medicare HHD patients (pts) in 2009-2012 by FI/MAC. We used Ordinary Least Squares regression to relate FI/MAC with HHD pt treatments per month by year, adjusting for pt age, race, BMI, and 23 Medicare claims-derived comorbidities. We examined plots of this relationship and found heterogeneous trends, though most FIs/MACs authorized the same or fewer payments over time. We predicted modality choice in a linear probability model to test whether FI/MAC payment policy is associated with the likelihood of choosing HHD therapy.

**Results:** Despite significant variation across FIs/MACs in payments authorized for additional HHD treatments, persistence in these practices for some FIs/MACs, and stronger financial incentives for dialysis facilities, we find no evidence facilities with more favorable FIs/MACs provided more HHD. This suggests a HHD patient’s expected treatment frequency, conditional on clinical factors, may be a function of the FI/MAC processing his/her facility’s claims. This creates a natural experiment for identifying the effects of more frequent HHD treatment.

**Conclusions:** Average monthly HHD patient paid treatments peaked at 18.5 (12.1 for In-center HD pts) in 2010—ranging from 13.1 to 20.6 across FIs/MACs—and fell to 17.7 (12.3 IHD) in 2012—ranging from 12.8 to 21.2—perhaps due to increased scrutiny by FIs/MACs in light of the expanded PPS. The fraction of pts treated by HHD grew from 1.1% (0.3%-3.2%) in 2009 to 1.5% (0.7%-10.3%) in 2012.

*Funding:* Other U.S. Government Support

## PUB186

### A New System for the Short-Daily Hemodialysis Using Slow Sterile Dialysate Flow Rate

Walid Arkouche,[1] Jacky Potier,[2] Maurice Laville.[3] *[1]AURAL, Lyon, France; [2]Centre Hospitalier, Cherbourg, France; [3]Univ de Lyon, Pierre Benite, France.*

**Background:** PHYSIDIA S[3] is a new system for hemodialysis using slow sterile dialysate flow rate, without needing of a processing of water treatment. The mechanical characteristics of this machine are based on a new concept for the control of a precise dialysate flow. This system is especially intended for the short-daily home hemodialysis and obtained the marking of the European Community.

**Methods:** A clinical pilot multicentric trial is led in France before the marketing. The object of the study is to control the clinical tolerance of the patients and to analyze the impact of low dialysate volumes on the removal of solutes of different molecules. The ethic committee approved the protocol.

**Results:** Four patients dialyzed in two different centers ended the study : mean age 53.3 ± 20.7 years (mean ± SD); dry weight 69.9 ± 11.9 kg; BMI 26.3 ± 4.7; dialysis protocol 6 times per week x 2 hours by session. Polyethersulfone membrane is used (surface 1.9m²; KUF 75 ml/h/mmHg). The results of 12 sessions by patient (total 48 sessions) are summarized below (mean ± SD):

Dialysis session duration 116 ± 5 minutes; the total of volume of spent dialysate and of ultrafiltration volume by session 24.8 ± 1.1 L; flow rate of dialysate 195 ± 5 ml/min; total UF by session 1554 ± 437 ml; total of urea recovered in the dialysate 317 ± 62 mmol by session; spKt/V by session 0.71 ± 0.15; eKt/V by session 0.53 ± 0.11; Std Kt/V by week 2.66 ±0.45; β2microglobulin (β2m) Reduction Rate (RR) by session 49.3 ± 6.4%; total of β2m recovered in the dialysate 110 ± 32 mg by session; Phosphorus RR by session 50.0 ± 8.2%; total of phosphorus recovered in the dialysate 17.6 ± 5.2 mmol by session.

**Conclusions:** The patient tolerance is very satisfactory without particular event, with a clinical evolution as the well-known evolution of a short-daily hemodialysis. Results are satisfactory and correspond to the results described for the short-daily hemodialysis with low flow dialysate rate. The machine is easy to use, and was reliable for all the periods of the essays.

## PUB187

### Adequacy of Glycemic Control in Diabetic Dialysis Patients: A Single Center Prospective Observational Study Using Continuous Glucose Monitoring

Siddharth Sharma, Thomas T. Titus, Talib Kasim Aljumaily. *Nephrology, Gold Coast Hospital, Gold Coast, Queensland, Australia.*

**Background:** Most international guidelines recommend targeting HbA1c of <7% irrespective of the presence of chronic kidney disease. However, the correlation of HbA1c with glycaemic control in dialysis patients is contentious due to shortened red cell survival, frequent blood transfusions, iron deficiency and use of erythropoietins. Paradoxically, a large retrospective analysis of diabetic dialysis patients has shown worse outcomes in dialysis patients with HbA1c <5%. Interstitial glucose measurements using Continuous Glucose Monitors(CGM) correlates well with blood glucose using a glucometer even in dialysis patients. Hence, Continuous Glucose Monitoring could identify periods of significant hypoglycaemia in dialysis patients.

**Methods:** This is a single center observational study, conducted on all in-center diabetic dialysis patients. CGM was applied to each patient for a total of 6 days continuously. Patients underwent routine dialysis and kept a diary to record their food intake and episodes of hypoglycaemia. HbA1c was measured along with routine monthly dialysis bloods on each patient.

**Results:** There were no episodes of asymptomatic hypoglycemia in the study patients. There appeared to be better correlation between regular venous blood sugar reading than with HbA1C.

**Conclusions:** Asymptomatic hypoglycemia does not appear to be a factor in this small study. Regular blood sugar monitoring may be better marker for adequacy of glycemic control than HbA1C, in diabetic haemodialysis patients.

*Funding:* Pharmaceutical Company Support - Medtronic- supplied Continuous Glucose monitors for duration of study only

## PUB188

### Increased Levels of Tissue Factor, Adhesion Molecules, Nitric Oxide and Adiponectin in End Stage Renal Disease

Vinod K. Bansal,[1] Jawed Fareed,[2] Kristiyana Kaneva.[2] *[1]Nephrology, Loyola Univ Medical Center, Maywood, IL; [2]Pathology, Loyola Univ Medical Center, Maywood, IL.*

**Background:** End stage renal disease (ESRD) represents the fifth (final) stage of chronic kidney disease characterized by an established kidney failure (GFR <15 mL/min/1.73 m²). To further understand the pathophysiology of ESRD, this study was designed to measure the circulating levels of tissue factor (TF), adhesion molecules, such as p-selectin (P-Sel), soluble ICAM (s-ICAM), nitric oxide and adiponectin (AD).

**Methods:** This study included 119 ESRD patients on maintenance hemodialysis in conjunction with an ongoing IRB approved protocol on the profiling of inflammatory markers in this syndrome. Citrated blood plasma samples were collected from these patients prior to the routine dialysis session. Nitric oxide levels (NO) were measured using a commercial kit from R&D systems (Minneapolis, Minnesota) and ELISA based methods for TF, P-Sel, s-ICAM and adiponectin were also purchased from R&D systems. Chromogenic and thrombin substrate method were used to measure the anti-Xa activity thrombin generation.

**Results:** Tissue factor levels were found to be increased in the ESRD group (20.4±6.1pg/ml) vs the control (11.9±2.8pg/ml). The nitric oxide level was markedly higher in the ESRD group (32±17uM) vs the controls (7±3uM). The p-selectin levels were also elevated in the ESRD group (46±20ng/ml) vs the control (31±3ng/ml). The soluble ICAM levels were higher in the ESRD group (250±112ng/ml) vs the control (180±19ng/ml). Interestingly, the adiponectin levels were also increased in the ESRD group (19.2±9.3ug/ml) vs the control (11.2±4.1ug/ml). The pre-dialysis samples of the ESRD patients exhibited detectable levels of heparin.

**Conclusions:** These studies suggest that TF, NO, p-selectin and s-ICAM levels are increased in the ESRD patient. Despite the fact that a significant number of ESRD patients being diabetic; the AD levels were increased in this group. These results suggest that while ESRD represents a pro-inflammatory/hypercoagulable state, the repeated administration of heparin and other drugs may contribute to the regulation of the hemostatic process and inflammatory balance.

## PUB189

**Optimal Serum Phosphate and Calcium for Hemodialysis Patients Judging from Fibroblast Growth Factor-23 and Parathyroid Hormone**

Hiroshi Tanaka,[1] Tomoyuki Kita,[2] Kumi Okamoto,[2] Maki Mikami,[2] Rumi Sakai.[1] *[1]Ashiya Sakairumi Clinic, Ashiya, Hyogo, Japan; [2]Sakairumi Clinic, Kobe, Hyogo, Japan.*

**Background:** High dose hemodialysis, HDD improves mortality, blood pressure control, anemia, quality of life of hemodialysis patients. We have reported that HHD significantly decreased serum fibroblast growth factor-23, FGF-23, interleukin-6, IL-6, $\beta_2$-microglobulin, $\beta_2$-MG than standard dose hemodialysis, SDD. In this study we analyzed correlations between the above serum parameters including routine laboratory data of the patients with different dialysis doses.

**Methods:** We selected 206 hemodialysis patients, whose treatment modes were not changed at least 3 months. They were divided in two groups, whose hemodialysis product, HDP was higher or lower than 54 (ex. 6 hours/session and 3 times weekly). SDD patients consisted of 129 patients (53 women and 76 men, 66.8 ± 12.8 years old, mean ± s.d., HDP 40.9 ± 4.1) and HDD patients did of 77 patients (27 women and 50 men, 61.1 ± 11.4 years old, HDP 68.4 ± 14.5). Serum FGF-23, IL-6 and routine laboratory tests were measured before hemodialysis on the day of the longest interval between consecutive dialysis sessions. Correlation between variables was analyzed using Pearson product moment correlation coefficient and Student's t-distribution.

**Results:** Serum phosphates of SDD and HDD patients were 4.9 ± 1.3 and 4.4 ± 1.0 mg/dl, respectively with a significant difference. Their serum calciums were 8.9 ± 0.6 and 8.9 ± 0.6 mg/dl, respectively. Table shows correlation coefficients and p values of each laboratory data of all, SDD and HDD patients.

| | All patients | SDD patients | HDD patients |
|---|---|---|---|
| FGF-23 : P | r = 0.553, P<0.001 | r = 0.564, P<0.001 | r = 0.529, P<0.001 |
| FGF-23 : Ca | r = 0.269, P<0.005 | r = 0.244, P<0.001 | r = 0.225, P<0.05 |
| PTH : Ca | r =−0.270, P<0.005 | r =−0.278, P<0.001 | r =−0.290, P<0.05 |
| $\beta_2$-MG : IL-6 | r = 0.200, P<0.05 | r = 0.209, P<0.005 | n.s. |

**Conclusions:** In order to decrease serum FGF-23, serum phosphate should be as lower as possible avoiding morbid hypophosphatemia. Serum Calcium should be kept higher within the normal range regarding FGF-23 and parathyroid hormone, PTH.

## PUB190

**Kinetics of Cystatin C during Single Hemodialysis Treatments: Determining Its Diffusional and Convectional Clearance, Equilibration Ratio, and Volume of Distribution**

Shih-Han S. Huang, Benjamin Ka Thomson, Robert M. Lindsay. *Medicine, Nephrology, Western Univ, London Health Sciences Centre, London, Canada.*

**Background:** Recent studies have demonstrated that cystatin C is cleared by hemodialysis. In this study, we assessed cystatin C kinetics during single hemodialysis treatments and estimated its volume of distribution.

**Methods:** Nine patients undergoing 10 hemodialysis sessions were studied. Each patient received a 3-4 hours hemodialysis session, which included a 1-hour dialysis only treatment, a 1-hour ultrafiltration only treatment plus 1-2 hours of combined dialysis and ultrafiltration. Arterial and venous blood sampling to measure cystatin C, creatinine, and urea levels at each treatment interval was done to calculate the diffusional, convectional and combined clearances of each solute. The clearance values of the three markers were compared with each other. The cystatin C clearance as calculated by solute clearance equations was compared with that obtained from a one-compartmental mass balance model. The cystatin C volume of distribution, its 30-minutes rebound ratio and its equilibration ratio were also calculated.

**Results:** The mean age ± standard deviation (SD) of the patients was 57 ± 9.3 years. The mean ultrafiltration volume (L) was 1.9 ± 1.05 L. There was a statistically significant difference between the cystatin C clearance values as estimated by the equations and by the model (p<0.014). There was also statistically significant differences between cystatin C, urea and creatinine clearances by all the treatment modes (p<0.05). The estimated cystatin C volume of distribution ratio was 0.33 ± 0.12 of total-body water. The rebound ratio at 30 minutes post-hemodialysis session was 0.085 ± 0.0059, and the equilibration ratio was 1.17 ± 0.227.

**Conclusions:** This study confirmed our previous hypothesis that cystatin C is cleared by both diffusion and convection during hemodialysis. It appears to be distributed mainly in the extracellular space. Larger studies to assess its clinical value as a marker of middle molecular weight toxin clearance in dialysis patients are needed.

## PUB192

**Continuous Erythropoietin Receptor Activator (C.E.R.A.) Improve Iron Dysregulation and Hypercytokinemia in Patients on Maintenance Hemodialysis (MHD)**

Yukiko Hasuike,[1] Naoto Kakita,[2] Kiyoko Yamamoto,[1] Aritoshi Kida,[1] Mana Yahiro,[1] Takeshi Nakanishi.[1] *[1]Div Kidney and Dialysis, Dep. Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]Tanaka-Kitanoda Hospital, Sakai, Japan.*

**Background:** In MHD, the disturbance of iron regulation for erythropoiesis could cause hyporesponsiveness to erythropoiesis-stimulating agents (ESA). We previously reported the decrease in frataxin, an iron regulator, and an association between frataxin and inflammatory cytokines in MHD [Clin Exp Nephrol 2012]. In the present study, we evaluated whether the switching of ESA to C.E.R.A. affect frataxin expression as well as cytokines in MHD patients with ESA-hyporesponsive anemia.

**Methods:** In 92 MHD with low Hb (<10 g/dl), 50 patients who were treated with conventional ESA and 42 patients with darbepoetin alpha, ESA was switched to C.E.R.A. (Hb target level 10-11 g/dl). Hemoglobin, serum ferritin, hepcidin (HPLC-MS/MS), and interleukin-6 (IL-6, ELISA) and tumor necrosis factor-alpha (TNF-α, ELISA) as inflammatory makers were measured. Intravenous iron (iron sucrose 40 mg once) was administered when serum ferritin level was less than 75 ng/ml. In 34 patients, polymorphonuclear leukocytes were separated to measure the mRNA of ferroportin-1/GAPDH ratio and frataxin/GAPDH ratio (real-time PCR), and protein levels of frataxin (Dipstick assay).

**Results:** During 3 months, both mRNA and protein levels of frataxin were significantly increased (1.56: 1, p=0.0033; 1.18:1, p=0.0324; respectively), and ferroportin-1 mRNA was significantly elevated (2.91:1, p<0.0001) likewise. Serum hepcidin levels were significantly decreased compared with that at start (12.2±2.5 versus 45.3±5.9 ng/ml, p<0.0001), and serum levels of both IL-6 (11.0±2.2 versus 31.3±11.0 ng/l, p=0.0007) and TNF-α (6.29±0.70 versus 8.06±1.73 pg/ml, p=0.0148) were significantly reduced compared with the levels at start.

**Conclusions:** The intervention of switching to C.E.R.A. and iron might improve abnormality of frataxin expression, which could subsequently reduce inflammatory cytokines.

## PUB193

**Tryptophan Catabolism and Its Association with Sleep, Fatigue and Depressive Symptoms in Hemodialysis Patients**

Vanja Persic,[1] Rakesh Malhotra,[2] Laura Rosales,[2] Weifang Zhang,[3] Garry J. Handelman,[3] Caroline M. Williams,[2] Stephan Thijssen,[2] Fredric O. Finkelstein,[4] Mark L. Unruh,[5] Nathan W. Levin,[2] Peter Kotanko.[2] *[1]Univ Medical Center Ljubljana, Slovenia; [2]Renal Research Institute, New York; [3]Univ of Massachusetts, Lowell; [4]Yale Univ, New Haven; [5]Univ of New Mexico School of Medicine, Albuquerque.*

**Background:** Sleep and mood disorders are common in hemodialysis (HD) patients. Tryptophan (TRP) and its metabolites play prominent roles in neural pathways related to sleep, fatigue, and depressive symptoms. Here we report levels of TRP and its metabolite kynurenine (KYN) and their association with sleep health, non-physical fatigue and depressive symptoms in HD patients.

**Methods:** In this prospective pilot study we measured pre-dialysis levels of TRP and its metabolite kynurenine (KYN) by high-performance liquid chromatography with fluorometric detection. We performed a concurrent survey which included 3 self-reported questionnaires: the Medical Outcomes Study Sleep Scale (MOS-Sleep); the PROMIS Short form Fatigue 8a questionnaire (PROMIS-F); and the Patient Health Questionnaire (PHQ-9). Spearman rank correlation coefficient ($r_s$) was used to assess correlations.

**Results:** We enrolled 30 HD patients (age 58.8±13.3 yrs, 70% male, 63% African-American, 50% diabetics). Compared to levels reported in healthy controls, in our cohort TRP levels were decreased (35.3±8.1 μmol/L) and KYN levels were elevated (6.0±2.5 μmol/L). MOS-sleep scores confirmed poor sleep quality. PROMIS-F T score was 54.1±9.4, indicating a trend towards moderate fatigue. PHQ-9 score was 7.4±5.2, revealing the presence of mild depressive symptoms. TRP and KYN levels were not correlated with indicators of sleep health ($r_s$=0.045 and -0.027, respectively), fatigue ($r_s$=0.211 and 0.186, respectively), and depressive symptoms ($r_s$=0.181 and 0.046, respectively).

**Conclusions:** Our relatively small study indicates disturbed metabolism of the essential amino acid tryptophan, poor sleep health, fatigue, and depressive symptoms. A larger sample size is necessary to more comprehensively explore the potential spectrum of interactions between TRP status and patient reported outcomes.

**PUB194**

**Levocarnitine Administration Can Improve Left Ventricular Systolic Function in Hemodialysis Patients Diagnosed with Carnitine Deficiency** Masaki Aono, Yuzuru Sato. *Sato Junkankikanaika, Matsuyama, Ehime, Japan.*

**Background:** Since cardiac systolic dysfunction has been shown to be a risk factor for both mortality and the development of de novo CHF in patients on HD, a number of studies were performed to measure the effect of levocarnitine ( LC ) administration on systolic function in such patients. Cardiac dysfunction is also a contributing factor to the occurrence of dialysis-associated hypotension.

**Methods:** Over a three-month period, a daily dose of levocarnitine chloride (1800mg/day) was administered orally to 134 patients on HD who were diagnosed with carnitine deficiency. At the end of the three months, the patients were divided into 3 groups according to the concentration of plasma free carnitine (FC) as follows: group1: FC<30umol/L, N=22, group2:30<FC<72, N=13, group 3: FC>72, N=99. LVD and EF were measured by echocardiogram and PEP/ET was examined by systolic time interval using Vasera system in all patients before and after treatment. Blood pressure (BP) was measured at the start and the end of each HD session and averaged for a week before and after treatment. The lowest BP and maximum decrease of BP during HD were also measured. The frequency of use of vasopressors during the time of dialysis was compared among the patients in the groups.

**Results:** After administration of LC, the plasma concentration of FC increased in groups 2 and 3 significantly (from a mean ± SD of 22.5±4.7μmol/L to 45.8±11.3μmol/L in group 2, from 20.4±4.6μmol/L to 275.5±136.6μmol/L in group 3 ; paired t-test P<0.01). However, it did not increase significantly in group 1 (from 23.4±3.1 to 25.0 ± 4.6 μmol / L, n.s). PEP/ET improved significantly in group 3 (from 0.331 to 0.27, P<0.01), but remained unchanged in the other groups. LVD and LVEF did not change in any group. BP during dialysis did not show a significant change. The dosage of an occasional boosting agent to be used for dialysis hypotension significantly decreased in group 3, but remained unchanged in the other two groups.

**Conclusions:** This study suggested that levocarnitine administration can improve the left ventricular systolic function of patients on HD and reduce the frequency of HD-related hypotension.

**PUB195**

**Oral Iron Administration Corrects Renal Anemia Effectively without the Development of Adverse Reactions in Hemodialysis Patients Received Erythropoiesis-Stimulating Agents (ESA) at a Large Dose** Shigeru Miyazaki,[1] Noriko Saito,[1] Hiroki Takimoto,[1] Masaaki Shimotori,[1] Yutaka Tsubata,[1] Kozo Ikarashi,[1] Tetsuo Morioka,[1] Hisaki Shimada,[1] Tadashi Yamamoto.[2] *[1]Shinraku-en Hospital, Japan; [2]Niigata Univ, Japan.*

**Background:** ESA have an ability not only to stimulate erythropoiesis, but also decrease serum hepcidin, which disturbs iron utilization for erythropoiesis, resulting in anemia improvement and increase iron absorption. The additional intravenous iron, in association with an increase in ESA dose, can be effective in increasing hemoglobin levels over the short times. But efficacy of oral iron therapy is not well studied. We examined the effect of oral iron therapy on renal anemia and iron metabolism markers in hemodialysis(HD) patients receiving larger dose of ESA.

**Methods:** When ESA were increased in 18 HD patients from 8500±5200 to 22200±12500 U/week (Epoetin β equivalent) without iron supplementation, they developed microcytic anemia. Then they were treated with oral iron administration (Fe 100mg/day) for 12 weeks.We evaluated the following markers: hepcidin-25(HPC), growth differentiation factor 15(GDF15), soluble transfferin receptor(sTfR), 8-oxo-2'-deoxyguanosine (8-OHdG) and standard hematological parameters including high sensitive CRP (hs-CRP). Serum HPC level was determined by LS-MS/MS. GDF15, sTfR and 8-OHdG were measured by ELISA. Serum samples of 20 normal volunteers (N) were served as controls.

**Results:** Oral iron administration increased serum ferritin(ng/ml), Hb (g/dl) and MCV(fl) from 0W to 12W (14.6±9.2 to 39.1±14.9*, 8.5±1.3 to 12.4±2.1* and 79.6±5.7 to 90.7±6.5* respectively).HPC (ng/ml) was 0.6±0.8 at 0W in the HD, which was lower than N ( 11.1±11.9*). Then HPC was gradually increased to 17.5±30.5** at 12W. However, the level of HPC did not exceed that of N (17.5±30.5 vs 11.1±11.9, ns).Serum GDF15 (ng/ml) and sTfR (nmol/ml) levels were gradually decreased from 7.7±3.3 to 6.0±2.5* and from 48.0±13.4 to 39.5±8.8*, respectively.Hs-CRP (ng/ml) and 8-OHdG (ng/ml) levels were unchanged. *:p<0.01 **:p<0.05.

**Conclusions:** Oral iron therapy was very effective to improve renal anemia, and did not induce hyperferritinemia, hyperhepcidinemia nor oxidative stress state.

**PUB196**

**Do Patients on Chronic Hemodialysis Have a Suppressed Fever Response to S. aureus Bacteremia?** Eman Mohammad Shaban, George P. Bayliss, Leonard Mermel. *Dept of Medicine, Alpert Medical School, Brown Univ, Providence, RI.*

**Background:** We sought to determine whether hemodialysis patients with Staphylococcus aureus bacteremia have a suppressed febrile response compared to age matched, non-dialysis patients. We hypothesized that dialysis patients are more likely to have an afebrile bacteremia (<100.3°F) and lower maximal temperature over the first 48 hours than non-dialysis patients, potentially delaying treatment.

**Methods:** We identified patients on hemodialysis from the electronic medical record with methicillin-sensitive and methicillin-resistant S. aureus (MSSA and MRSA) bacteremia over a 5-five year period. We defined MSSA/MRSA bacteremia as a positive blood culture drawn either peripherally or from a dialysis catheter. Temperatures were recorded on presentation and over 48 hours. Cases included people age 18 or older on hemodialysis more than six weeks. We excluded as cases or controls patients on immunosuppressive drugs, with MSSA/MRSA infection other than in the bloodstream, or anyone who received antipyretic therapy within 6 hours before or 48 hours after the first positive blood culture.

**Results:** We identified 12 cases and 12 age- and gender-matched controls. Cases were more likely to suffer MRSA bacteremia than controls (7 cases versus 2 controls, p=0.04), but only 3 of 4 cases who screened positive for MRSA colonization developed MRSA bacteremia. Although mean temperatures were lower for dialysis patients with S. aureus bacteremia, the differences were not significant (temperature at initial blood draw = 99.8°F for cases versus 100.9°F for controls, p=0.2; Tmax over the next 48 hours = 99.4°F for cases versus 100.6°F for controls, p=0.13).

**Conclusions:** We found no significant difference in temperature at the time of presentation with S. aureus bacteremia or over the next 48 hours. But our study was underpowered for this outcome measurement. We found that chronic dialysis patients were more likely to present with MRSA bacteremia than non-dialysis patients, and this may have therapeutic implications for chronic dialysis patients in terms of initial selection of antimicrobial therapy.

*Funding:* Clinical Revenue Support

**PUB197**

**Relationships between Hemoglobin Stability and Inflammation in the Treatment of Renal Anemia with Epoetin β Given during the Initiation Period of Hemodialysis Treatment** Yumi Furuno, Masahito Tamura, Tetsu Miyamoto, Akihiro Kuma, Kenichiro Bando, Yoko Fujimoto, Emi Hasegawa, Ryota Serino, Yutaka Otsuji. *Dept of Nephrology, Univ of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

**Background:** In patients undergoing maintenance hemodialysis (HD), inflammation may cause resistance to erythropoiesis stimulating agents and is a high risk factor of cardiovascular diseases. Despite this relevance, relationships between inflammation and hemoglobin (Hb) stability during the initiation period of HD treatment have not been explored yet. We investigated correlations of Hb stability with inflammation in patients who newly initiated HD.

**Methods:** Epoetin β was administered to patients who initiated HD treatment in our university hospital, and the 1-year success rate of achieving the target Hb level and factors affecting the rate were prospectively examined.

**Results:** Among 139 patients initiating HD in our hospital from February 2008 to November 2010, 25 patients who provided written informed consent were involved in the study. The patients were consisted of 64% male, the average age was 61.4 years, and 60% had diabetic nephropathy. The mean values of laboratory tests at the initiation of HD treatment were as follows: Hb, 8.62 ± 1.47 g/dL; creatinine, 8.54 ± 1.41 mg/dL; albumin (Alb), 3.06 ± 0.71 g/dL; CRP, 0.78 ± 1.64 mg/dL; dose of epoetin β, 8460 ± 1492 IU/week. During the follow-up period, the mean Hb level was 10.70 ± 0.56 g/dL and the mean success rate of achieving the target Hb level (10-12 g/dL) was 70%. The high target Hb maintenance rate group had significantly higher mean Hb levels and significantly lower cardiothoracic ratio (CTR) during the period. Negative correlation was observed between the target Hb maintenance rate and CRP (r = -0.4445, p = 0.0296). Although the mean Hb change tended to exhibit an inverse relationship with Alb, the difference was not statistically significant.

**Conclusions:** Significant correlation of the target Hb maintenance rate with CRP and CTR suggested that inflammation may have effects on the management of renal anemia and prognosis even during the initiation period of HD treatment.

**PUB198**

**On-Line Hemodiafiltration and Control of Uremic Anemia** Ignace Mpio, Jean-christophe Szelag, Walid Arkouche. *AURAL, Lyon, France.*

**Background:** Control of anemia in chronic dialysis is a major regarding clinical outcomes. Nine months prospective cross-over study to determine if the advantages of the convective transfers of on line hemodiafiltration (ol-HDF) are associated with a better control of anemia than in conventional hemodialysis (CHD).

**Methods:** We studied 33 stable, dialyzed-patients transffered from CHD to ol-HDF (16 women (48%), mean age 73 ± 11 years, length of dialysis 7 ± 6 years). 24 (73%) were treated with erythropoiesis stimulating agents (ESA). Technical characteristics of CHD and ol-HDF: ultrapure water, blood flow ≥ 300 ml / min and high membrane permeability (area ≥ 1.9 m² and ultrafiltration coefficient ≥ 40 ml / h / mmHg). ol-HDF was mainly used in the post-dilution mode (volumes of substitution of 18 to 24 l.) Two phases were defined: phase A (CHD) and phase B (ol-HDF). Clinical and biological parameters of anemia were measured at Day 0 in phase A and after 9 months in phase B: ESA doses converted in doses of darbepoetin alpha (DA) per week, hemoglobin (Hb), erythropoiesis resistance index (ERI) (ERI = weekly dose of DA in μg / Hb in g/dl), CRP, albumin, ferritin, and delivered dialysis dose (KT).

**Results:** After 9 months in ol-HDF, the comparative analysis of the results phase A versus phase B reported: Hb (11.8 vs 11.7 g / dL); ERI (4.5 vs 4.1); doses of DA (53 vs 43 μg / wk, p <0.05); CRP (12 vs 9 mg / L, p <0.05); KT (47 vs 52 L); ferritin (353 vs. 453 μg / L); albumin (39 vs 38 g / L). In Phase B the ESA dose was decreased by 10 μg (20%) associated with a reduction of CRP, maintaining Hb and ERI levels equivalent to phase A without any difference in the martial and nutritional assessment. We found a significant correlation between delta DA and delta CRP but no correlation between KT and dose of DA.

**Conclusions:** ol-HDF is a non pro-inflammatory dialysis mode as reported by other authors and facilitates the response to ESA. The properties of the convective purification have implications in reducing other causes of erythropoietin resistance.

**PUB199**

**Impact of Angioaccess Type in Hemoglobin Levels, Iron Kinetics and Erythropoietin Dose in Two Hemodialysis Units of Fresenius Medical Care in Puebla City, Mexico** José Guillermo Pacheco Paredes, Carlos Colchero, Julio de León Ramirez Reyes, Karina Delfina Perez ayala. *Fresenius Medical Care, Puebla, Mexico.*

**Background:** An important issue in correcting anemia in hemodialysis patients is improving dialysis quality. It is demonstrated that in patients with chronic access has better outcome than patients with temporal access. The present study tries to demonstrate the relationship between hemoglobin levels, Epo alpha dose and iron kinetics with the type of angioaccess chronic or temporal in two separated units of hemodialysis in Puebla City, Mexico.

**Methods:** Data from medical files were analyzed that included hemoglobin levels, iron kinetics and recombinant erythropoietin (Epo) dose, by access type. STUDY DESIGN: Cohort longitudinal prospective study.

**Results:** 249 patients were studied in two-separated hemodialysis units with 6 months follow up from July 2012 to January 2013. The access type distribution was 47% with temporal angioaccess and 53% with chronic angioaccess. In patients with temporal access the hemoglobin levels were 10.6, g/dl serum ferritin were 434 and% transferrin saturation was 32%. For patients with chronic angioaccess the hemoglobin levels were 11g/dl, serum ferritin 396 and % ST 39. The distribution of Epo dose was not significantly different. 50 patients were lost for different causes. 209 patients with complete data were included in the study. The access type distribution was 75% with chronic angioaccess and 25% of the patients with temporal access. Hemoglobin levels for patients with temporal access were 10.9 ferritin levels 409 and %ST was 32%. For chronic access hemoglobin levels were 11.6 ferritin levels 361 and %ST 31%. 33 patients changed from temporal vascular access to chronic vascular access. These patients had an improvement in the hemoglobin levels and ferritin levels with no significantly change in Epo dose.

**Conclusions:** In our population the improvement of hemoglobin levels and the decrease in the ferritin levels were the most relevant factors associated to chronic angioaccess. Epo dose was not significantly changed during the 6-month follow up; nevertheless no patient with chronic angioaccess required an increase in Epo dose.

*Funding:* Private Foundation Support

**PUB200**

**Can the Use of a Novel Dialysis Bloodline Increase Haemoglobin and Reduce Erythropoetin Doses? Interim Results of an Ongoing Clinical Audit** Iain C. Macdougall,[1] Adam Rumjon,[1] Emmanuel Mangahis,[1] Thomas Ryzlewicz,[2] Franz-ferdinand Becker,[2] William Kilgallon.[2] *[1]Renal Unit, King's College Hospital, London, United Kingdom; [2]Oxyless Ltd, Reading, United Kingdom.*

**Background:** The Oxyless bloodline (patented in USA and Europe) has been designed to reduce the contact between blood and air during the haemodialysis (HD) process whilst still being able to remove additional air introduced during the set up and operation phases of dialysis, prior to entry to the dialyser. The hypothesis is that this novel design would reduce shear stresses on red cells and prolong their lifespan, potentially reducing dose requirements of ESAs as well as potentially other drugs such as iron and heparin.

**Methods:** A pilot audit was set up to test the hypothesis. Fifteen patients from the chronic haemodialysis unit were entered into a 12-month open-label, single cross-over, cohort prospective audit. The patients were all aged over 18 years, had been on HD for at least 3 months and all had A-V fistulae. Haemoglobin (Hb) was measured on a monthly basis and EPO doses were recorded. Clinical decisions regarding EPO doses were made independently in line with an established anaemia management protocol.

**Results:** Eight of the patients experienced intercurrent problems which let to them dropping out of the assessment. Drop out events included renal transplantation, gastrointestinal bleeding, episodes of acute inflammation, hospitalisation, and non-compliance. The remaining 7 patients demonstrated increases in haemoglobin (Hb) over a six month period of 1 g/dL. In 4 of the patients EPO requirements were reduced by 33% representing a total reduction of 9,000 IU/week, and a mean of 2,250 IU/week. Two patients have had no reduction to-date while one had a small increase at the start of the audit and has maintained that dose to-date. The patients have now entered into the cross-over phase.

**Conclusions:** As a result of these encouraging preliminary results, a Prospective European Multicentre Audit (PEMA) has been initiated to further evaluate the hypothesis that the use of the Oxyless bloodlines can improve the half-life of erythrocytes in dialysis patients and thereby reduce the requirements for ESAs, iron and heparin.

*Funding:* Pharmaceutical Company Support - Oxyless Ltd

**PUB201**

**Hepatitis C Cirrhosis and Iron Load in End Stage Renal Disease** Nancy M. Tran,[2] Devasmita Choudhury,[1,2] Geri Brown,[1,2] Terri W. Crook.[3] *[1]Internal Medicine, Dallas Veterans Affairs Medical Center, Dallas, TX; [2]Internal Medicine, Univ of Texas Southwestern Medical Center, Dallas, TX; [3]Pathology, Dallas Veterans Affairs Medical Center, Dallas, TX.*

**Background:** Hepatitis C (HCV) in patients with end stage renal disease(ESRD) is associated with increased cirrhosis, carcinoma and all-cause mortality. Whether or not an iron load given as part of dialysis anemia management can exacerbate fibrosis is uncertain.

**Methods:** To evaluate liver disease progression, we retrospectively compared levels of aspartate aminotransferase(AST), platelet count(plt), AST/plt ratio, albumin, and iron load over a period of 20 years, from January 1992 through July 2012, in patients with ESRD alone(group 1,n= 18), ESRD and HCV(group 2,n=24), and HCV alone(group 3,n=20). Using SPSS 11.0, continuous variables were analyzed with t-test and ANOVA; non-parametric variables were analyzed with Mann-Whitney test. The 3 groups were matched in terms of age, race, and gender.

**Results:** No difference was found in AST/plt ratio within or between groups(p=0.06) at start time (dialysis initiation in groups 1 and 2; liver biopsy in group 3). Over study duration, the plt count was lower(p =0.04) but other biomarkers remained stable within group 2. Over time, there was no difference in biochemical markers within group 1, and none except for albumin within group 3. At completion of study, the AST/plt ratio in group 2 was not statistically different than values in group 1(p=0.86) or group 3(p=0.13). The total amount of iron received (intravenously or via packed red blood cell transfusions) was similar between groups 1(mean 8.0g) and 2(mean 10.6g) (U=171, p=0.72).

**Conclusions:** Preliminary findings show that while plt count was decreased within group 2, the remainder of the biomarkers was not significantly different between groups 1 and 2 even in the setting of similar iron administration, which suggests a stable level of fibrosis. This finding could indicate that, despite their pre-existing liver disease, patients with ESRD/HCV may be able to tolerate the iron load. However, a larger cohort and review of other parameters (ie.years of HCV exposure, HCV viral load, stage/grade of cirrhosis) will need to be further investigated.

**PUB202**

**Erythrocyte Indices Changes during Erythropoiesis Stimulating Agent Therapy in Hemodialysis Patients** Kyungyoon Chang, Hyung Wook Kim, Dong Chan Jin, Hoon Suk Park. *Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, St.Vincent's Hospital, Republic of Korea.*

**Background:** Macrocytosis occurs in chronic hemodialysis patients. It may be a manifestation of abnormal rapid cellular maturation in states of massive erythropoietic stimulation due to high doses of erythropoiesis stimulating agent (ESA). The aim of this study is to access the prevalence of macrocytosis and to identify its clinical associations.

**Methods:** We conducted a single-center, retrospective study of 104 chronic stable hemodialysis patients during ESA. Blood samples collected monthly from January through December 2012. Macrocytosis was defined as a mean corpuscular volume (MCV) > 98 fl.

**Results:** The mean MCV was 94.0 ± 4.6 fl (range 79-112 fl). We identified high prevalence of macrocytosis (16%).The ΔHb positively correlated with the ESA/dry weight (Pearson correlation coefficient= 0.477, P<0.001, $r^2$=0.2188), albumin (Pearson correlation coefficient= 0.143, P<0.001, $r^2$=0.0204 ) and negatively with iron (Pearson correlation coefficient= -0.178, P<0.001, $r^2$=0.0315 ). The ΔMCV positively correlated with ΔHb (Pearson correlation coefficient= 0.378, P<0.001, $r^2$=0.1473 ) and ESA/dry weight (Pearson correlation coefficient= 0.212, P<0.001, $r^2$=0.0619). The ΔMCHC positively correlated with iron (Pearson correlation coefficient= 0.119, P=0.017, $r^2$=0.0137 ). Macrocytosis is related to the increased Hb and the dose of ESAs requirement in hemodialysis patients. There were no significant correlation between serum iron level and macrocytosis.

**Conclusions:** Macrocytosis may be associated with the increased Hb in hemodialysis patients during ESAs. The measuring of MCV will help access the response of ESAs. Future studies will need the relationship between erythropoietin resistance.

**PUB203**

**The Association between Hemoglobin Variability and Morbidity/Mortality of Cardiovascular (CV) Events in Maintenance Hemodialysis (MHD) Patients: A Prospective Observation** Yong Gu,[1,2] Jianying Niu,[1] Xiaojing Zhong.[1] *[1]Div of Nephrology, The Fifth People's Hospital of Shanghai, Fudan Univ, Shanghai, China; [2]Div of Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China.*

**Background:** The study aims to find the relationship between morbidity/mortality of CV events with different hemoglobin level and variability in MHD patients.

**Methods:** 80 MHD patients were recruited into this 9-month period prospective study, from June 2012 to February 2013. Hb lecel was measured monthly, while the rest serum biochemical tests were preformed every 3 months. The observational end point was death, CV events.

**Results:** The mean age is 60.8±12.79 years, ratio of male to female is 1.2:1, dialysis age is 4.27±3.09 years, 74 cases had hypertension while 24 had diabetes.

During the period , 6 cases developed cardiovascular events, 4 cases developed cerebrovascular events and 10 cases died.

The mean Hb level was 101.62±15.24g/l, mean standard deviation was 8.91±4.4g/l, mean coefficient variation was 0.09±0.05, mean differences among 9 times results was 7.83±4.1g/l, residual standard deviation was 7.58±3.97g/l, and the standard deviation across thresholds (110-120g/l) was 19.97±14.67g/l.

Of six patterns of Hb level fluctuation, consistently low (<110g/l) is the most common pattern (43%). The proportion of Hb variability varies from 53.8% to 87% depending on the definition of Hb variability used.

Analysis of multiple stepwise regressions showed the independent risk factors were the Hb standard deviation (OR=1.162, P=0.030), coefficient variation (%) (OR=1.17, P=0.009), mean differences between 9 times results (OR=1.292, P=0.006), while the Hb level was protective factor (OR=0.95, P=0.02) .

The COX regression analysis showed that the mean differences between 9 times results was an independent risk factor (HR=3.13, P=0.006) of death, while the Hb level was protective factor (HR=0.849, P=0.012).

**Conclusions:** Hb variability is very common in MHD patients, and the consistently low (<110g/l) is the most common Hb level fluctuation pattern in our center. Hb level is a protective factor for total clinical adverse events and death, Hb standard deviation and coefficient variation are risk factors for CV events.

*Funding:* Government Support - Non-U.S.

## PUB204

**Inflamation and CRP Chronic Haemodyalisis Patients** Cristobal Santacruz. *Nefrologia, Clinica de los Rinones Menydial, Quito, Pichincha, Ecuador.*

**Background:** We know that patients with CKD 5D have some conditions that predispose to be chronically inflamed and by this way their morbimortality is increased. Several markers of inflammation have been used to measure, but has not been able to determine which one of them are the best and which could be useful in our daily clinical practice to get decrease morbimortality. The aim of our study is to contribute in the interpretation of PCR and to determine patients in high risk.

**Methods:** 144 non selected patients 82 men (60%) and 62 women (40%), with a mean age of 53 years (range 16 to 83 years) treated with HD 44 months on average (range 3 to 161 months) were included in a base data when they began their treatments. All the personal data was fill in. They did treatments with standard bicarbonate, biocompatibility dialyzers, minimal heparinization. None reused dialyzer. We made some labs exams as ferritin, transferrin, albumin and quantitative CRP,EPO was used to maintain predialysis Hb level 11-12gr% . For this observational study we divided the patients into two groups:1. Not inflamed CRP <5 mg / L. 2.Inflamed CRP> 5 mg / L.

| VARIABLES | GROUP 1 CRP < 5 mg/dl | GROUP 2 CRP > 5 mg/dl | P |
|---|---|---|---|
| TOTAL | 74 (51%) | 70(49%) | |
| SEX DISTRIBUTION | 38 M (51%) 36 F (49%) | 44M (63%) 26 F (37%) | NS |
| AGES | 49 YEARS | 57 YEARS | 0,0086 |
| HAEMODIALYSIS TREATMENT TIME | 29 MONTHS | 58 MONTHS | NS |
| BMI | 23.7 | 23.8 | NS |
| FERRITINE | 1102 MG. % | 969 MG.% | NS |
| TRANSFERRIN | 369 MG.% | 361 MG.% | NS |
| ALBUMIN | 4.4 MG. % | 4.2 MG. % | NS |
| EPO UNITS MONTH | 16000 | 18000 | NS |

**Results:** 133 patients (94%) when assessed clinically were in good conditions regardless of the level of CRP, while 8 pctes (6%) considered clinically bad had an CRP average of 23. Associated comorbidities were: active lupus infection, severe heart disease and ischemic stroke.

**Conclusions:** 1. - 49% of HD patients specially men over 50 years old had high CRP level P=0,0086; 2. - The time spent in HD treatment, BMI, albumin, transferrin, ferritin and EPO doses doesn't have statistical significance to rise the CRP level; 3. - The clinical course of patients and complications are not related with CRP levels but help us to identify patients at risk.

## PUB205

**Anemia in Hemodialysis Patients: An Audit of Hemoglobin Targets and Erythropoiesis-Stimulating Agents Use** Samantha Jayne Owen, Adil Hazara, Sunil Bhandari. *Hull and East Yorkshire Hospitals NHS Trust, United Kingdom.*

**Background:** The objectives of anemia management in hemodialysis dependent (HD) patients have changed recently. We have reviewed the impact of Kidney Disease: Improving Global Outcomes (KDIGO) 2012 guidelines on anemia management in our HD population.

**Methods:** Hemoglobin concentration (Hb), Serum Ferritin (Fer), Platelet counts (PLT), ESA doses and blood pressures (BP) were reviewed retrospectively in all HD patients with reference to two separate time intervals: the last quarter of 2011 ('Oct-Dec 2011') and the same period in 2012 ('Oct-Dec 2012'). For Hb, BP and ESA doses, average values from multiple observations have been used whenever possible.

**Results:** 230 HD patients were studied. Mean age: 64 (range 19 – 91) years; Male 147 (64%); mean duration on hemodialysis: 7 (range 2 – 34) years.

Averages of Hb, Fer, PLT, ESA doses, BP and Hb/ESA are presented in table 1. There were no statistically significant differences in average Hb, Fer, systolic and diastolic BPs over the two time periods. ESA doses were lower by 0.5 micrograms(mcg)/patient/week. Reduction in PLT was statistically significant.

| | Oct-Dec 2012 | Oct-Dec 2011 | P value |
|---|---|---|---|
| Hemoglobin (grams/Litre) | 116 | 115 | 0.20 |
| Serum Ferritin (nanogram/millilitre) | 435.4 | 434.7 | 0.61 |
| Platelet count (x $10^6$ / Litre) | 221.6 | 233.1 | 0.02 |
| ESA dose/patient/week of Darbepoetin alfa (mcg) | 15.9 | 16.4 | 0.70 |
| Systolic BP (mmHg) | 132 | 129 | 0.11 |
| Diastolic BP (mmHg) | 73 | 71 | 0.09 |
| Haemoglobin/ESA [Hb/ESA] (g/L/mcg) | 7.32 | 7.04 | n/a |

**Table 1: Comparison of selected laboratory and clinical data in 230 haemodialysis patients over two time intervals (Oct-Dec 2012 versus Oct-Dec 2011).** The estimated annual cost saving on the account of lower ESA use was £36.40 (USD $56.94) per patient. Average Hb within the KDIGO recommended range of 90 to 115 grams/litre (g/L) was achieved in 127 (55%) patients during the most recent (Oct-Dec 2012) period.

**Conclusions:** KDIGO recommendations are gradually being implemented. Use of ESAs have declined slightly resulting in modest cost savings and possibly reduced PLT. Although average Hbs have not changed, guidelines may have helped focus attention towards reduced reliance on ESAs as demonstrated in our patients by increase in Hb/ESA ratio.

## PUB206

**Determining the Hemoglobin Target in Order to Avoid Transfusion** Romilkumar Patel, Adam E. Gaweda, Michael E. Brier. *Univ of Louisville.*

**Background:** The package insert for erythropoietin and recent comments from the FDA state "The goal is to individualize therapy and use the lowest ESA dose possible to reduce the need for red blood cell transfusion. We evaluate this goal through computer simulation.

**Methods:** We performed a simulation of hemoglobin (Hb) concentrations through time using the computer program Matlab. The following assumptions were made: 1) Hb level is chosen to buffer against routine blood loss 2) Routine blood loss is in the range of 1-3 grams Hb 3) Patients Hb cycle over a period of 6 months 4) Patients Hb cycle with an amplitude of 05. to 1.5 g/dL and 5) Transfusion threshold is 7 g/dL. Hb concentration was simulated usinga sin function with period 6 months and amplitudes of 0.5, 1.0 and 1.5 g/dL. Each week we applied a Hb drop of 1-3 g/dL and assessed the need for transfusion based on the Hb falling to the transfusion threshold. The probability of transfusion was then summed over 26 weeks.

**Results:** The results are shown in Table 1 as the Hb target needed to avoid any transfusion for routine blood losses.

| | | | Hb Buffer (g/dL) | | |
|---|---|---|---|---|---|
| Amplitude (g/dL) | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| 0.5 | 8.6 | 9.0 | 9.6 | 10.0 | 10.6 |
| 1.0 | 9.0 | 9.6 | 10.0 | 10.6 | 11.0 |
| 1.5 | 9.6 | 10.0 | 10.6 | 11.0 | 11.6 |

**Conclusions:** Defining the target Hb concentration for an individual patient requires the assessment of the patients variability in Hb concentration over time, likely blood loss one wishes to protect against, the threshold for transfusion for that patient, and for those patients with the highest variability, one must balance the risk of transfusion vs. high mean Hb concentrations. The results of this simulation can provide the necessary information to set individual targets.

*Funding:* NIDDK Support

## PUB207

**Playful Intervention Improves Nutritional Knowledge of Hyperphosphatemia in Hemodialysis Patients** Camila Machado de Barros,[1] Marina Pioltine,[2] Tânia Maria Marsulo Franciozi,[2] Thaís Barca Moraes,[2] Edeli Simioni Abreu,[2] Rosana Farah,[2] Bárbara Margareth Menardi Biavo,[1] Jacqueline Santos,[1] Elzo Ribeiro Júnior,[1] Carmen B. Tzanno-martins.[1] *[1]Nutrition, Grupo CHR, Brazil; [2]Nutrition, Universidade Presbiteriana Mackenzie, Brazil.*

**Background:** Chronic Kidney Disease (CKD) is a major public health problem and the control of serum phosphorus in these patients is recognized as an essential goal to be achieved in medical and nutritional treatment. This is due to the participation of this nutrient in the pathogenesis of secondary hyperparathyroidism, as well as in bone metabolism and bone mineralization. Objective: To evaluate the effects of a playful nutritional intervention on the knowledge regarding phosphorus of hyperphosphatemics patients undergoing dialysis.

**Methods:** A longitudinal study of 187 hemodialysis patients with hyperphosphatemia. In three nephrology clinics, located in São Paulo, Brazil. A nutrition dynamic was applied in the form of musical parody, composed by the researchers. All patients received, at the beginning of a given dialysis session, a pamphlet with the lyrics. The song featured guidelines on consumption of phosphorus in patients with CKD. The music was played and sung by patients and multidisciplinary team during the dialysis session. The impact of playful nutritional intervention was assessed by the questionnaire prepared by Nerbass et al. (2010). The questionnaire contains five questions of multiple alternatives on the knowledge of dietary phosphorus and it was applied before and after the dynamic.

**Results:** Knowledge regarding hyperphosphatemia increased in all three units when compared before and after nutritional intervention. There was an increase of 71.4% (± 16) to 80% (± 13), so that, in all questions, there was an increase in percentage of correct answers.

**Conclusions:** The nutritional intervention showed positive results on the knowledge of phosphorus in the diet, as well as the use of chelating agents to decrease the serum phosphorus level in hyperphosphatmics patients. In order to contribute to quality of life and life expectancy of these patients, we believe that further studies of nutritional intervention in dialysis patients are needed.

*Funding:* Private Foundation Support

## PUB209

**Quantifying and Predicting the Effect of Infection and Erythropoietin Usage on Hemoglobin Level in Dialysis Patients** Jenny Feng, Adam E. Gaweda, Michael E. Brier. *Univ of Louisville.*

**Background:** Infection contributes to anemia in dialysis patients with End Stage Renal Disease (ESRD). Erythropoietin(EPO) is recommend for improving hemoglobin(Hb) levels in such cases. However, hypo-responsiveness to EPO is evident during infection and there is a lack of evaluation of appropriate EPO dosages during infection.

**Methods:** The Hb levels of 46 dialysis patients with ESRD at the University of Louisville Kidney Disease Program Dialysis Unit were studied. They had stable Hb levels pre-infection, had at least 1 infectious event, and received increased dosages of EPO following infection. Hb levels from pre-infection, infection, and post-infection were averaged for each time period. EPO resistance was calculated. Patients who were hospitalized for their infections were compared to non-hospitalized patients.

**Results:** There were 27 infectious events that resulted in hospitalization and 15 infectious events that were treated at the dialysis unit. Hb levels decreased in both groups

with a greater degree of decrease in hospitalized, -2.01±0.92 g/dL, versus 1.67±1.32 g/dL in non-hospitalized. Duration of decreased Hb level was longer for hospitalized, 66.7±27.07 days, when compared to 59.53±33.66 days in non-hospitalized. The amount of time it took for Hb concentrations to recover and reach peak Hb concentrations was also longer for hospitalized, 77.40±45.41 days, when compared to 69.23±20.93 days non-hospitalized.

**Conclusions:** Infection contributes to hypo-responsivity to EPO in both hospitalized and non-hospitalized patients. Compared to non-hospitalized patients, hospitalized patients suffered from greater Hb decline and longer duration of EPO hypo-responsitivity. Aggressive EPO dose adjustment during and immediately following an infectious event may lead to overcompensation post-infection and Hb overshooting the target range. EPO dosages may need to be held constant or at least limit the dose excalation that occurs due to an anemia management protocol. Ongoing work involves identifying factors associated with poorer EPO response and clinical outcomes, and simulating patterns of hgb response to EPO in infection.

*Funding:* NIDDK Support

## PUB210

**Dysregulation of Thrombotic and Hemostatic Factors in End Stage Renal Disease** Vinod K. Bansal,[1] Debra Hoppensteadt,[2] Syed Mustafa Ahmed,[3] Jawed Fareed.[2] *[1]Nephrology, Loyola Univ Medical Center, Maywood, IL; [2]Pathology, Loyola Univ Medical Center, Maywood, IL; [3]Medicine, Loyola Univ Medical Center, Maywood, IL.*

**Background:** Increased cardiovascular events occur in ESRD patients on maintenance hemodialysis. The objective of this study was to utilize newly introduced cardiac biomarker chips and immunoenzymatic methods, profiling various biomarkers of inflammation and thrombogenesis in a defined ESRD patient population.

**Methods:** Blood samples from seventy two ESRD patients were drawn prior to maintenance hemodialysis. Commercially available ELISA kits for Tissue Plasminogen Activator – Plasminogen Activator Inhibitor Type-1 complex (tPA-PAI-1 complex), Plasminogen Activator Inhibitor Type-1 (PAI-1), Myeloperoxidase, (MPO), Thrombomodulin, (TM), Interleukin-1 beta (IL-1β), Anti-Annexin V, human sL-selectin, and Inter-Cellular Adhesion Molecule 1 (ICAM-1). Functional methods were used for measuring Antithrombin and Von Willebrand factor (vWF) activity were used. The biochip arrays (Randox, Evidence System, United Kingdom)for cardiac markers, included creatine kinase-MB (CK-MB), Myoglobin (Myo), Heart-type fatty acid binding protein (hFABP) and cardiac troponin I (cTn1). The cerebral array included C-reactive protein (CRP), D-Dimer (DDMER), Neuron Specific Enolase (NSE), Neutrophil Gelatinase-Associated Lipocalin (NGAL), soluble Tumor Necrosis Factor Receptor 1 (TNFRI) and Thrombodulin (TM).

**Results:** ESRD patients showed assay dependent decrease in markers such as tPA-PAI-1, Anti-Annexin V, L-selectin and Antithrombin activity (ranging from 18 to 46%). The levels for MPO, TM, CK-MB, MYO, FABP, CRP, DDMER, NSE, NGAL, TNFR1, and vWF activity all showed a significant increase in the ESRD patients (ranging from 25%-5587%). No changes were observed in the IL-1B, ICAM1, or cTn1 in ESRD patients compared to normal groups.

**Conclusions:** These studies underscore the role of inflammatory and thrombotic mediators in ESRD. The cardiac biochip array revealed a remarkable elevation of the FABP and as well as a notable increase in MYO. The cerebral array revealed a remarkable elevation of the TNFR1.

## PUB211

**Stable Serum Magnesium Levels in Chronic Hemodialysis Patients** Eduardo K. Lacson,[1] Weiling Wang,[1] Chinu M. Jani,[2] Franklin W. Maddux.[1] *[1]Fresenius Medical Care, North America, Waltham, MA; [2]Spectra Laboratories, Rockleigh, NJ.*

**Background:** Serum magnesium (sMg) has been reported to be stable within one year in Japanese patients. We investigated the stability of sMg and prescribed dialysate magnesium (dMg) in chronic hemodialysis (HD) patients.

**Methods:** All in-center HD patients treated as of 1/1/08 at Fresenius Medical Care North America facilities with Mg measured for period 1 (4/1 - 6/30, 2007) and period 2 (10/1 – 12/31, 2007) were included. The 3-month mean sMg and weighted mean dMg for both periods were calculated and age, gender, race, diabetes mellitus, vintage, vascular access and hospitalization from the prior 30 days for each period. Spectra assay variability (represented by twice the SD) indicates that ~95% of re-test samples will fall between ±0.2 mEq/L.

**Results:** 16,768 patients had mean age of 61.9 ±14.6 years, 53.7% males, 46.2% whites, 46.6% blacks, 7.2% other races, and 52.8% with diabetes, and dialysis vintage of 4.1 ± 3.7 years. The interquartile range (IQR) of delta sMg was -0.1 – 0.2 (Table). Subgroup analyses for age (<45, 45-54, 55-64, 65-75, and > 75 years), gender, race, diabetes mellitus, vintage (<=120, 121-1095, and >1095 days) and prior 30 days hospitalization did not reveal effect modification.

Distribution of sMg, dMg, and the difference between periods (delta).

| Units: mEq/L | Mean (SD) | Median (IQR) |
|---|---|---|
| sMg of Period 1 | 1.86 (0.31) | 1.8 (1.7 – 2.0) |
| sMg of Period2 | 1.89 (0.31) | 1.9 (1.7 – 2.1) |
| sMg Delta (P2-P1) | 0.03 (0.25) | 0 (-0.1 – 0.2) |
| (prescribed) dMg of Period 1 | 0.95 (0.19) | 1 (0.9 – 1.0) |
| (prescribed) dMg of Period2 | 0.97 (0.15) | 1 (1.0 – 1.0) |
| (prescribed) dMg Delta (P2-P1) | 0.03 (0.16) | 0 (0.0 – 0.01) |

**Conclusions:** Serum and prescribed dialysate magnesium levels were stable over a 6-9 month period in a national sample of American HD patients. The observed differences in sMg were no greater than the expected assay variability of this test.

## PUB212

**Are There Differences in Treatment for Acute Myocardial Infarctions among Patients with Chronic Kidney Disease Stage 3-5, End Stage Renal Disease, and Patients with Normal Kidney Function?** Ninad D. Parekh, Chadi Saifan, Elie El-Charabaty, Suzanne E. El Sayegh. *Dept of Medicine, Div of Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

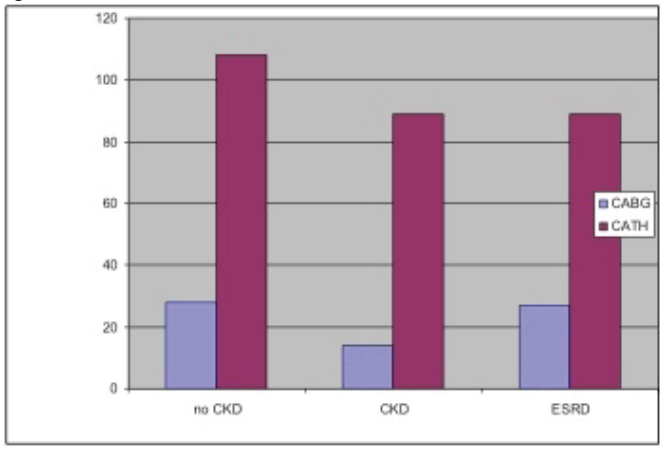
**Background:** Patients with CKD and/or ESRD have a higher risk of CAD and consequently higher morbidity and mortality from CAD. In this study, we aim to evaluate the differences in inpatient care of acute MI among patients with no CKD, patients with CKD and patients with ESRD.

**Methods:** All patient with ESRD on dialysis admitted with MI from 2007 to 2012 were matched with patients with CKD and patients with no CKD. The patients' characteristics including age, sex, risk factors, medications, length of stay, and need for a cardiac catheterization or CABG were collected.

**Results:** Mean ages were 68.2, 66.1 and 68.9 years, for the no CKD, CKD, and ESRD groups respectively. There were no statistically significant differences in medications, length of stay, and LVEF among all three groups. The admission diagnosis, the need for catheterization or CABG, and different risk factors are shown in table1.

| Category | N | NSTEMI | STEMI | Cath | CABG | DM | HTN |
|---|---|---|---|---|---|---|---|
| No CKD | 132 | 58% | 48% | 83% | 21.4% | 44.3% | 74% |
| CKD | 122 | 44.6% | 55.4% | 74.8% | 11.6% | 49.6% | 73% |
| ESRD | 122 | 62% | 38% | 73.6% | 22.% | 45.8% | 73.8% |

A t-test of two-sample assuming equal variances were done for the all three groups including the need for CABG or cardiac catheterization, and showed no statistically significant differences.




**Conclusions:** Contrary to other studies, our study did not show any statistical difference in treatment of MI among all three groups: patients with normal kidney function, with CKD or ESRD. We recommend that our finding should be verified in large multicenter study.

## PUB213

**Pulmonary Hypertension and Right Ventricular Dysfunction in Patients under Maintenance Hemodialysis** Lijun Zhao, Songmin Huang, Hong Tang, Ting Liang. *West China Hospital, Sichuan Univ, Chengdu, China.*

**Background:** When chronic hemodialysis treatment has been revealed to increased the systolic pulmonary artery pressure (sPAP),right ventricular dysfunction (RVD) is an important predictor of prognosis in patients with end-stage renal disease (ESRD).This study was to investigate the prevalence of pulmonary hypertension (PHT) and RVD among patients and the possible contributing factors for these clinical features.

**Methods:** Study population consisted of 67 patients under long-term hemodialysis (HD) therapy via arteriovenous (A-V) access in our HD center.Doppler echocardiography including myocardial performance index (MPI) of right ventricle and RV wall thickness were performed in all patients.The relationship of PHT and RV hypertrophy were analyzed by Pearson correltion analysis.The risk factors of PHT were analyzed by Logistic regression analysis.

**Results:** SPAP>35 mmHg which indicate PHT was found in 37.31% of patients,while 44.78% of patients had RVD.Patients were divided into non-PHT subgroup (n=42) and PHT group (n=25).Compared with the non-PHT subgroup, patients with PHT had a higher systolic blood pressure,while had lower levels of hemoglobin and Kt/V value.Patients in PHT subgroup demonstrated lower left ventricular ejection fraction (LVEF) than patients in non-PHT subgroup (65.5% *vs.* 60.1%,*p=0.04*). RVD,assessed by MPI,was higher in patients with PHT than those without PHT. Additionally,The former group showed a higher incidence of RV hypertrophy than those in non-PHT subgroup (9.5% *vs.* 32%,*p*=0.041). Pearson correlation analysis showed that sPAP was correlated with RV wall thickness positively (*r=0.394, p=0.01*).Logistic regression analysis revealed that LVEF contributed to the predictability of incidence of PHT ( regression coefficient *b*=-0. 096, *OR*=1.100, *p*=0. 01) as well as systolic blood pressure ( regression coefficient *b*= 0. 063,*OR*=0.940, *p*=0. 002).

**Conclusions:** The prospectively study demonstrated high incidences of PHT and RV dysfunction among ESRD patients under maintenance HD via surgically created native A-V access.The status of LV function,poor control of systolic blood pressure have played an important role in the mechanism of PHT,RVD in chronic uremia patients.

## PUB214

**The Association of Dental Health and All Cause and Cardiovascular Mortality in Hemodialysis Patients: Oral-D Study** Giovanni F.M. Strippoli,[1,2,3,4] Suetonia Palmer,[5] Marinella Ruospo,[1,2] Patrizia Natale,[1] Valeria Maria Saglimbene,[1,2] Michela Sciancalepore,[1] Letizia Gargano,[1] Fabio Pellegrini,[2] David W. Johnson,[6] Pauline J. Ford,[6] Jonathan C. Craig,[4] Paul Stroumza,[1] Luc Frantzen,[1] Miguel Rodrigues Leal,[1] Marietta Torok,[1] Anna Bednarek,[1] Jan Dulawa,[1] Eduardo Jorge Celia,[1] Ruben Gelfman,[1] Jorgen B.A. Hegbrant,[1] Charlotta Wollheim,[1] Staffan Schön,[1] Michele De Benedittis,[3] Massimo Petruzzi.[3] *[1]Diaverum; [2]Mario Negri Sud Consortium; [3]Univ of Bari; [4]Univ of Sydney; [5]Univ of Otago; [6]Univ of Queensland.*

**Background:** It has been hypothesized that oral disease may be associated with increased risks of death due to inflammation or as a general indicator of healthcare practices. We evaluated the association between dental status and the risk of all-cause and cardiovascular mortality in adults on hemodialysis.

**Methods: ORAL-D** is a multinational prospective cohort study of consecutive adults on hemodialysis in 75 outpatient clinics selected randomly from a dialysis network in Europe and South America. A dental surgeon evaluated dental status by using the DMFT (decayed, missing, filled, permanent teeth) score. Quality of dental health was defined based upon WHO criteria as low, moderate and high DMFT scores of <2.6, 2.7-4.4 and >4.4. We assessed survival at 8 months using centralized mortality data. We conducted analyses using Cox regression controlling for age, gender, previous cardiovascular events, income status, clinical performance measures, dialysis prescription and performance indicators, and depressive symptoms. Complete follow-up data at 12 months will be available by September 2013.

**Results:** 4720 hemodialysis patients in the participating clinics received a complete evaluation of their dental status and completed follow up. Median follow up was 8.0 (6.5 to 8.7) months and 344 (10%) died during follow up. Dental health (DMFT scores) had uncertain associations with all-cause or cardiovascular mortality.

**Conclusions:** Dental health has uncertain associations with all-cause and cardiovascular mortality in patients on hemodialysis. ORALD will be completed at the end of 1 year follow up by September 2013.

## PUB215

**Investigating the Relationship of Intradialytic BP Changed and ADMA** Aiqun Chen,[1] Hua Wu,[1] Ying Sun,[1] Deping Liu.[2] *[1]Nephrology, Beijing Hospital, Beijing, China; [2]Cardiology, Beijing Hospital, Beijing, China.*

**Background:** To observe the level of plasma asymmetric dimethylarginine (ADMA) and analyze the relationship between ADMA and the different intradialytic blood pressure change in MHD patients.

**Methods:** The blood pressure changed intra-dialysis was measured through ambulatory BP monitor. The 31 MHD patients with proper dry body weight were divided into intradialytic hypertention(BP increased >15mmHg, n=11), hypotention(BP decrease >20mmHg, n=12)and stable BP(n=8)groups. The plasma ADMA wsa detected by ELISA (Enzyme linked immunosorbent assay) method in pre- and post- HD. The possible effect factors were analysed and compared between the 3 groups.

**Results:** There were 17 male and 14 female in 31 MHD patients. The average age was 66.97±9.24 years. The average hemodialysis duration was 75.71±50.65 monthes. The plasma ADMA were markedly elevated in the beginning of HD and fell in the end of HD sessions (3.37±1.48umol/L vs 1.71±0.80 umol/L). Both before and after HD, the plasma ADMA levels were highest in intradialytic hypotensive patients (4.38±1.56 umol/L, 2.25±0.83 umol/L) than in stable BP and hypertensive patients(2.70±1.18umol/L, 1.32±0.60umol/L; 2.78±0.88umol/L and, 1.43±0.56umol/L; P=0.004 and 0.011). The pulse pressure was significantly higher in intradialytic hypertensive patients than that in stable BP and hypotensive patients (62.41±11.57mmHg, 47.98±13.30 mmHg and 44.56±8.30 mmHg, P=0.008 and 0.003). ALP, TC, LDL-C were higher in hypertensive and hypotensive patients than in stable BP patients (all P<0.05), but there was no significant difference between this two groups. Hs-CRP in hypotensive group was highest in 3 groups(8.43 ± 6.00mg/L, 4.85 ± 3.39 mg/L, 2.00±1.81mg/L, P= 0.092, 0.004).

**Conclusions:** ADMA are markedly elevated in MHD patients and with a significant fall in the end of the HD sessions. The plasma ADMA lever and hs-CRP in intradialytic hypotensive patients is the highest.

## PUB216

**Usefulness of NT-pro-BNP as a Marker of Overhydration and Alteration on Body Composition in Dialysis Patients. Effect of a Nutritional Intervention on NT-pro-BNP Levels** Maria astrid Rodriguez Gomez, María José Fernández-reyes, Manuel M. Heras, Alvaro Molina Ordas. *Nephrology, Hospital General de Segovia, Segovia, Spain.*

**Background:** NT-proBNP is a powerful predictor of risk of heart failure (HF) and mortality in patients with chronic kidney disease. Elevated levels of NT-proBNP in dialysis patients have been associated with heart disease and with extracellular water excess.

Analyze NT-proBNP to detect extracellular volume overload dependent on alterations in body composition and independent of cardiac history. Check if a nutritional intervention in malnourished patients would modify the body composition and NT-proBNP.

**Methods:** We evaluated the nutritional status in 40 stable dialysis patients, mean age 71 ± 10 years, time on dialysis of 49 ± 47 months, 40% with history of HF. Nutritional analysis included: biochemical parameters related to nutrition and inflammation; anthropometric parameters; body composition by electrical bioimpedance; and nutrient uptake. In 16 patients with nutritional deficiencies we started nutritional supplements. Two months later, the initial evaluation was repeated (including NT-proBNP).

**Results:** NT-proBNP correlates with history of HF (r = 0.30, p = 0.05) and with some body composition parameters related to extracellular water such as phase angle (r = -0.39 , p = 0.014), Na K exchange (r = 0.49, p = 0.001) and extracellular water percentage (r = 0.40, p = .01). In multiple regression analysis the model that better explains the levels of NT-pro-BNP is the one that includes the Na K exchange and the history of HF. There is a strong correlation between the NT-proBNP levels at baseline and after 2 months (r = 0.9, p <0.0001). Overall NT-proBNP did not change after the redetermination two months later. Excluding the 16 patients with HF history, we observed that proBNP levels in 10 patients with nutritional intervention significantly decreased, whereas in 14 patients without nutritional intervention remained stable.

**Conclusions:** NT-proBNP in dialysis patients is independently correlated with parameters of extracellular water overload. Nutritional intervention in malnourished patients without HF history can decrease the NT-pro-BNP levels.

## PUB217

**Preliminary Study on the Intradialytic Blood Pressure Variability and Influencing Factors in Maintenance Hemodialysis Patients** Aiqun Chen,[1] Ying Sun,[1] Hua Wu,[1] Deping Liu.[2] *[1]Nephrology, Beijing Hospital, Beijing, China; [2]Cardiology, Beijing Hospital, Beijing, China.*

**Background:** Objective To understand the blood pressure variability intra-dialysis and the influencing factors in MHD patients.

**Methods:** We enrolled 81 MHD patients into the study. Using dynamic BP monitor BP was measured intra-dialysis. BPV was estimated with the variation coefficient(CV) and standard deviation(SD). Patients are divided into two groups according to SBP-CV: high and low SBPV groups. The possible effect factors were analyzed and compared. And multivariate stepwise regression analysis was made between the SBPV and the influencing factor.

**Results:** There were 47 male and 34 female in 81 MHD patients. The average age was 62.8±13.1 years. The average dialysis duration was 62.00±47.50 monthes. The average SBP-CV in intradialysis was 8.12±3.16%. The patients were divided into CV>8.12% group(35 patients) and <8.12% group(46 patients). There were more female,longer dialysis duration,lower dry body weight in high SBPV group, but not significantly differences(P=0.13,0.11±0.08 respectively). Complicating of coronary artery disease(CAD) and T2 DM in low SBPV group(43.5%, 23.9%)was higher than in high SBPV group(25.7%, 20.0%)(P 0.099, 0.660). The incidence of intrahypertension and hypotension (20.0%, 25.7%) was higher in high SBPV group than in low SBPV group(8.7%, 6.5%)(P <0.009).The patients taking CCB were more (80.4%,65.7%)and lower dose of EPO(5358.7±4415.5U/ week, 6600.0±5270.0U/week)in low SBPV group than in high SBPV,but not statistical different(P 0.13±0.20). Serum creatinine, potassium, hs-CRP and ALP were higher in high SBPV group than that in low SBPV group (P 0.034, 0.005, 0.001 and 0.049 respectively). SBP-SD,CV and hsCRP were positively related (r = 0.756,0.464 ), SBP-CV and dialysis vintage were positively related (r = 0.211) by the multivariate stepwise regression analysis. There was no correlation between SD, CV and age, dry body weight, UF volume, dehydrate/ weight ratio, serume calcium, potassium, sodium, lipoprotein.

**Conclusions:** Intradialytic SBPV is positive associated with the inflammatory state and dialysis vintage in the MHD patients. There is no correlation between SBPV and UFV, dry weight.

## PUB218

**Association between Sleep-Disordered Breathing (SDB) and Brain Natriuretic Peptide (BNP) in Hemodialysis Patients** Fumiko Fukuuchi,[1] Takashi Kabaya.[2] *[1]Nephrology, Kumanomae Jin Clinic, Arakawa-ku, Tokyo, Japan; [2]Minmisenjyu Hospital, Japan.*

**Background:** A high prevalence of SDB has been reported in patients on hemodialysis. SDB has been associated with cardiovascular risk. SDB has important clinical implications in patients with congestive heart failure. Plasma BNP level is the independent prognostic factor in dialysis patients.

**Methods:** The aim of this study is to reveal the relationship between BNP and SDB.15 dialysis outpatients are included in this study and received sleep study with portable sleep apnea test equipment (LS-300, Fukuda Denshi Co., Ltd. Japan) for one night after dialysis. 4 dialysis outpatients are hospitalized and performed a sleep study with conventional Polysomunography (PSG) for two consecutive nights, before HD day and HD day. BNP, total protein, UN, creatinine, hematocrit were measured at the before and after dialysis. The echocardiography was also performed.

**Results:** Of 15 patients with LS-300, 13 patients were diagnosed as SDB. The average AHI was 26.7 ± 10.3 (mean ± SD)/ hour. Obstructive apnea was main. The BNP level was 418.2 ± 434.2 (mean ± SD) pg / ml, 278.8 ± 280.1 pg/ml, before and after dialysis, respectively. Correlation coefficient of BNP and AHI was 0.38, 0.37, before and after dialysis, respectively. In the case of whose pre-dialysis BNP level was more than 400pg/ml, the correlation coefficient of BNP and AHI was 0.72 ($p = 0.04$). Three of these patients were received sleep test again after Dry Weight reduction. AHI and BNP of them were improved. Of 4 patients with conventional PSG, all patients were diagnosed as SDB. 3 cases were severe, 1 case was mild grade of SDB. Obstructive apnea is main in both the day before dialysis and dialysis day. The AHI was improved after dialysis in three case of four. The BNP levels of 2 cases were more than 300pg/ml even after dialysis. In these 2 cases, AHI, maximum apnea time, minimum arterial oxygen saturation was improved in dialysis day.

**Conclusions:** Elevated BNP level is related to SDB in dialysis patients. Extracellular fluid volume overload is associated with SDB and the BNP level. SDB in dialysis patients can be expected to improve after dialysis if heart failure is suspected in the high level BNP.

## PUB219

**Diagnosis of Autonomic Dysfunction in Hemodialysis Patients Using Heart Rate Related Parameters in the Exercise Test** Jocemir R. Lugon, Maria A. Carreira, Marcio Galindo Kiuchi, Felipe M. Pena, Andre B. Nogueira, Ronaldo C. Rodrigues, Jorge P. Strogoff-de-Matos. *Medicine, Universidade Federal Fluminense, Niteroi, Rio de Janeiro, Brazil.*

**Background:** Hemodialysis (HD) patients have increased risk of sudden death problably due to impaired autonomic function. We aimed to evaluate the frequency of autonomic dysfunction in a cohort of HD patients in comparison to controls (C) employing the exercise test (ET).

**Methods:** Cross sectional study with HD patients from a single center. Sessions were performed thrice a week for 4 hours. ET was accomplished in a non-dialysis day using the treadmill ramp protocol. The test was symptom-limited with an active recovery period of 2 min. A set of heart rate (HR) related parameters were assessed at baseline, exercise and recovery.

**Results:** From 125 HD patients, 41 were selected for the study. They were 50±14 years old, 51% male, 66% non-white, and 7% diabetics. Forty-one non-dialysis C, age and gender matched were enrolled. At baseline, HR, systolic blood pressure (BP) and diastolic BP of HD and C patients were not different. At ET values were statistically different between HD and C groups for the following variables: chronotropic deficit (23.5±12.1 vs. 6.5±6.4%, P<0.001); chronotropic index (57.5±19.1 vs. 88.9±12.0, P<0.001); HR recovery (HRR) at the 1st min (11.9±9.1 vs. 19.6±8.6 bpm, P<0.001), 2nd min (21.3±12.3 vs. 34.1±10.5 bpm, P<0.001), 3rd min (33.8±21.3 vs. 53.2±18.0, bpm, P<0.001), and 5th min (40.8±21.3 vs.60.2±14.4 bpm, P<0.001); and R-R variability (SDNN) either during exercise (33.7±14.2 vs. 50.4±21.3 msec, P<0.001) or recovery (20.1±9.8 vs. 26.5±16.9, P=0.041). When patients under betablockers (HD 34% and C 17%) were analysed separately , chronotropic deficit (35.0±10.0 vs. 10.6±5.9bpm, P<0.001), chronotropic index (41.2±15.7 vs. 80.9±9.5, P<0.001) and HRR at the 3rd min (27.8±16.1 vs. 42.6±11.2 bpm, P=0.043) and 5th min (33.2±14.1 vs. 53.4±14.4, P=0,006) still discriminate between HD an C.

**Conclusions:** The ET was found useful for diagnosis of autonomic dysfunction in HD patients. The most discrimant parameters between HD and C groups were the chronotropic deficit, the chronotropic index and the HRR at the 3rd and 5th min, irrespective of the use of betablockers.

## PUB220

**Cinacalcet May Improve Oxidative DNA Damage in Maintenance Hemodialysis Patients – An Observational Study** Elif Ari Bakir. *Nephrology, Kartal Research and Training Hospital, Istanbul, Turkey.*

**Background:** The aim of the study was to evaluate the impact of cinacalcet on oxidative stress biomarkers, oxidative DNA damage (8-hydroxy-2'-deoxyguanosine/deoxyguanosine) (*8-OHdG/dG*) and endothelial function in HD patients.

**Methods:** Thirty chronic HD patients undergoing 60 mg/day cinacalcet treatment with a follow-up of 6 months and 30 healthy individuals were included in this prospective study. Plasma malondialdehyde (MDA) levels and *8-OHdG/dG* were determined as oxidative stress markers. Superoxide dismutase (SOD), paraoxanase (PON), catalase (CAT), carbonic anhydrase (CAN) and glutathione peroxidase (GPx) activites were measured as antioxidants.

**Results:** MDA levels were decreased; SOD, PON, CAT, CAN and GPx activites were increased after 6 months of cinacalcet treatment. Although CIMT remained stabile, there was a significant improvement in FMD% as well as a notable reduction trend in 8-OHdG/dG ratio after 6 months of treatment.

| | Hemodialysis Group-Baseline (n=30) | Hemodialysis Group-after 6 months (n=30) |
|---|---|---|
| Malondialdehyde (µmol/L) | 10,05 ± 5,74 | 5,71 ± 2,05¶ |
| 8-hydroxy-2´-deoxyguanosine/ deoxyguanosine | 2,47 ± 1,26 | 1,83 ± 0,83¶ |
| Superoxide dismutase (EU/mL) | 5,17 ± 1,82 | 7,29 ± 1,35¶ |
| Glutathione Peroxidase (EU/mL) | 7,62 ± 4,25 | 16,79 ± 7,63¶ |
| Paraoxanase (EU/mL) | 140,12 ± 18,54 | 183,95 ± 28,89¶ |
| Catalase (EU/gHb)–¹ | 24,60 ± 4,75 | 35,68 ± 4,48¶ |
| Carbonic anhydrase (EU/gHb)–¹ | 0,43 ± 0,22 | 1,45 ± 0,53¶ |
| Flow mediated dilatation (%) | 7,42 ± 2,08 | 9,64 ± 2,26¶ |
| Carotid artery intima-media thickness (mm) | 0,70 ± 0,11 | 0,69 ± 0,16 |

*¶: p<0.05 vs Hemodialysis group (Baseline).*

**Conclusions:** Cinacalcet improves oxidative stress, genomic damage, endothelial function and increases antioxidant protection in HD patients after 6 months of treatment.

## PUB221

**Left Ventricular Hypertrophy in End-Stage Renal Disease: Determinants and Impact on Myocardial Mechanics** Attila Kovács,[1] Mihaly B. Tapolyai,[2] Maria Faludi,[2] Klara Berta,[2] Bela Merkely.[1] *[1]Heart and Vascular Center; [2]Fresenius Medical Care.*

**Background:** Left ventricular (LV) hypertrophy is a major predictor of mortality in end-stage renal disease (ESRD). We sought to reveal the causes of increased LV mass in ESRD patients and to assess its influence on LV deformation using 3D echocardiography.

**Methods:** Forty-four patients (mean age 48±13 years, 54% men) with ESRD on maintenance hemodialysis (HD), without diabetes and any significant cardiac disease were investigated just before and immediately after HD, and compared to 46 controls (NC; 48±12 years, 48% men). Beyond conventional echo, 3D recordings were obtained using multi-beat reconstruction from 6 cardiac cycles (GE Vivid E9). LV mass indexed to body surface area (LVMi) were measured, and 3D speckle tracking analysis was performed to assess global longitudinal (LS), circumferential (CS), area (AS) and radial (RS) peak systolic strain (4D Auto LVQ). We also measured serum FGF-23 levels (Merck Millipore) and evaluated overhydration status by bioimpedance (Fresenius BCM). Data are presented as median(interquartile range).

**Results:** LVMi was remarkably increased in ESRD patients [136(46) vs. 71(8) g/m², p<0.001]. By multivariate linear regression ($R^2$=0.91, p<0.001), FGF-23 (β=0.41), total iron binding capacity (β=0.49), overhydration (β=0.29), systolic blood pressure (β=0.43) and bodyweight (β=0.39) were found to be independent predictors of LV mass. Strain values in all directions improved after HD [pre- vs. post-HD; LS: -20(3) vs. -21(6), CS: -20(4) vs. -22(7), AS: -33(5) vs. -35(10), RS: 50(12) vs. 53.5(20) %, all p<0.01]. While there was no difference in LS, the patients' pre-HD CS, AS and RS values were reduced, and after HD only CS increased enough to be similar compared to NC [NC LS: -20.5(3), CS: -21(3), AS -36(3), RS 61(8) %, all p<0.05]. LVMi inversely correlated with pre-HD CS (Spearman's ρ=0.63) and post-HD LS (ρ=0.64), CS (ρ=0.74) and AS (ρ=0.53, all p<0.05).

**Conclusions:** Beyond the chronic volume- and pressure overload, LV hypertrophy is determined by the endocrine effect of FGF-23. HD results in immediate improvement of LV function. The increase of LVMi correlates strongly with the reduction of 3D deformation parameters.

## PUB222

**Does This Adult Hemodialysis Patient without SIRS Require Blood Culture?** Daisuke Uchida,[1] Sho Sasaki,[1] Masahito Miyamoto,[2] Kenichiro Koitabashi,[4] Tomo Suzuki,[1] Hiroo Kawarazaki,[1] Yugo Shibagaki,[1] Kenjiro Kimura.[1] *[1]Internal / Div of Nephrology and Hypertension, St. Marianna Univ School of Medicine, Kawasaki, Kanagawa, Japan; [2]Nephrology, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan; [3]Medicine / Div of Nephrology, St. Luke's International Hospital, Tokyo, Japan.*

**Background:** Bacteremia is one of the most common causes of death in hemodialysis patients in Japan, but it is often difficult to diagnose it at the first hospital visit. The systemic inflammatory response syndrome (SIRS) is a powerful predictor of bacteremia in general population. However, it is not known if SIRS is also predictive of bacteremia in patients on hemodialysis.

**Methods:** We designed a multi-center retrospective observational study of adult hemodialysis outpatients who had 2 sets of blood cultures drawn for suspected infection at their first hospital visit from August 2011 to July 2012. Antibiotics administered prior to first visit and access other than arterio-venous fistula (AVF), which is the main vascular access in Japan, was excluded. Bacteremia was diagnosed by positive blood culture after the exclusion of contamination by a pre-set criteria. Clinical, biological and microbial data were obtained to evaluate the usefulness of SIRS as a predictor of bacteremia at first visit to the outpatient setting.

**Results:** Data was obtained from 279 participants. Vascular access other than AVF (N=51) and antibiotics administered prior to first visit (N=36) were excluded. Mean age of participants was 70 years, 68.2% were male, 49.5% were diabetic, and 28.1% had indwelling artificial objects. Among the 192 participants, 17.7% (N=34) had bacteremia and the most common infectious sites were endocarditis, osteomyelitis and AVF infection (N=4). The most common microbe was Staphylococcus aureus (N=11). 22.9% of those who met the

criteria for SIRS, and 12.5% of those who did not meet the criteria for SIRS had bacteremia (p=0.09). SIRS had a low sensitivity for bacteremia in hemodialysis patients (sensitivity 64.7%, specificity 53.2%, positive LR 1.38, negative LR 0.66).

**Conclusions:** SIRS may not be helpful in distinguishing adult AVF hemodialysis patients to exclude bacteremia.

## PUB223

**Annual Update on Elimination of Hemodialysis Related Bacteremia and Vascular Access Infection** Jafar Al-Said, Aimee Pagaduan, Soni Murdeshwar. *Nephrology and Internal Medicine, Bahrain Specialist Hospital, Bahrain.*

**Background:** Hemodialysis related bacteremia and dialysis blood stream infection are the major causes of morbidity and the second cause of mortality in ESRD population. Great efforts are spent to control and reduce hemodialysis related bacteremia and access infection. We have been following strict infection control protocol since the beginning of our Hemodialysis service. The aims of this study were to update the prevalence of Hemodialysis related Bacteremia and vascular access infection and to identify the outcome of the tight infection control protocol we have been following.

**Methods:** This study is a retrospective analysis for the outcome of hemodialysis infection protocol over 112 months from January 2004 till May 2013. All Hemodialysis sessions performed were included during that period. Patients' demographic and vascular access types were collected from the electronic data. Hemodialysis induced bacteremic infection per 100 patient month, admission rates for Hemodialysis related bacteremia per 1000 patient year and admission for vascular access infection were estimated.

**Results:** Total Patients were 147. Total Hemodialysis sessions were 8641. Mean age was 57.6 years (SE 1.3). Male gender formed 55%. Types of vascular access were; 56.3% cuffed tunneled catheters, 19.5% AV fistula, 18.2 AV grafts and 5.8% vascath. Estimated infection prevalence is shown below.

| Calculated Variables | Ratio |
|---|---|
| Hemodialysis bacteremic infection per month | 0.001 |
| Hemodialysis related blood stream infection per 100 patient month | 0.016 |
| Admission for hemodialysis related bacteremia per 1000 patient year | 0.4 |
| Admission for vascualr access infection over 112 months | Zero |

**Conclusions:** The tight infection control protocol followed in our unit had persistently eliminate HD related infection and vascular access infection. We are reporting significant lower infection rate as compared to all published international data. We would like to present and share this infection control protocol with other HD centers.

## PUB224

**The Role of Pre-Dialysis N-Terminal Probrain-Type Natriuretic Peptide for Volume Estimation in Haemodialysis Patients** Arkom Nongnuch,[1,2] Andrew Davenport.[2] *[1]Renal Unit, Dept of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol Univ, Bangkok, Thailand; [2]Centre for Nephrology, Royal Free Hospital, Univ College London, London, United Kingdom.*

**Background:** N Terminal Probrain Type Natriuretic Peptide (NTproBNP) is used to aid the diagnosis of heart failure. In haemodialysis patients, NTproBNP could be increased due to pre-existent cardiac disease, volume overload, and reduced by residual renal function. We examined the effects of volume status on predialysis NT-proBNP, by measuring body water with bioimpedance and estimating over hydration using the Fresenius BCM In Body manufacturer's equations.

**Methods:** We prospectively studied 158 stable chronic HD patients, attending their midweek dialysis session who had predialysis bioimpedance measured (In Body). Serum biochemistry, relevant demographic, biochemical and bioimpedance data was collected prior to the session.

**Results:** Predialysis log NT-proBNP was correlation with age, sex, race, dialysis vintage, history of valvular heart disease, myocardial infarction, coronary stenting, peripheral vascular disease, number of anti-hypertensive medications, predialysis systolic blood pressure, mean arterial blood pressure, haematocrit, albumin, CRP, B2 microglobulin and volume overload estimated by the In Body equation, but not diabetes, stroke, hypertension, ratio of predialysis ECW/TBW, and volume overload estimated by the Fresenius BCM equation. On multiple linear regression analysis, NTproBNP was significantly associated with history of cardiac valve disease, myocardial infarction, coronary stenting, number of anti-hypertensive medications, predialysis systolic blood pressure and log B2 microglobulin, and negatively with serum albumin and haematocrit.

| Parameters | B coefficient | SD | t | P value |
|---|---|---|---|---|
| History of valve disease | 0.558 | 0.19 | 2.8 | 0.006 |
| Previous myocardial infarction | 0.25 | 0.12 | 1.9 | 0.049 |
| Previous coronary artery stenting | 0.43 | 0.14 | 3.1 | 0.002 |
| Total No hypertensive medications | 0.1 | 0.04 | 2.27 | 0.024 |
| Predialysis systolic blood pressure | 0.007 | 0.002 | 4.1 | 0.000 |
| Predialysis haematocrit | -2.47 | 1.07 | -2.3 | 0.022 |
| Predialysis serum Albumin | -0.032 | 0.01 | -3.1 | 0.002 |
| Log B2 microglobulin | 0.996 | 0.31 | 3.13 | 0.002 |

**Conclusions:** Predialysis NT-proBNP is associated with pre-existent cardiovascular morbidity, anaemia and hypoalbuminaemia rather than estimates of overhydration.

*Funding:* Private Foundation Support

## PUB225

**Birth Weight and End-Stage Diabetic Nephropathy in Later Life: A Japanese Multicenter Study** Kei Nagai,[1] Chie Saito,[1] Soh Suzuki,[1] Masahiro Hagiwara,[1] Hirayasu Kai,[1] Joichi Usui,[1] Keigyou Yoh,[1] Shuichi Tsuruoka,[2] Kunihiro Yamagata.[1] *[1]Dept of Nephrology, Faculty of Medicine, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Dept of Nephrology, Faculty of Medicine, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

**Background:** Low birth weight is a surrogate marker for low nephron number, and an important risk factor for both the progression of end-stage renal disease (ESRD). However, most participants of previous study have been younger than 40, because little well-organized information on birth weight is available for the old general population as comparison subjects.

**Methods:** In January 2010 in 9 centers in Japan, 1130 Japanese patients undergoing maintenance hemodialysis were identified. Data was obtained from 230 patients (20.3%) by using a self-completed questionnaire containing questions about birth weight. The race and age-matched mean birth weight was calculated by using well-organized data from nationwide surveys. We divided our subjects into those with and without DM and used the median ages in each group (53.0 y for DM and 43.5 y for non-DM) and birth weight of each group was compared with age-matched mean birth weight.

**Results:** Older patients with DM had significantly lower birth weight than age-matched mean birth weight ( 2963±674* vs 3129±28, mean±SD, respectively, *P<0.05), whereas no significant differences between them existed in younger patients in the DM group ( 3191±697 vs 3145±54 ) or in any patients in the non-DM (younger; 3107±401 vs 3140±54, older; 3048±517 vs 3114±40 ) group which consists of 101 patients with chronic glomerulonephritis and 45 with miscellaneous diseases.

**Conclusions:** We considered older diabetic patients with a small number of glomeruli due to significantly lower birth weight may have more severe long-term nephron damage than older non-diabetic patients. It was also suggested that the patients with young-onset diabetic nephropathy may develop ESRD with birth weight-independent manner. Therefore, we propose to notice that lower-than-mean birth weight would be a risk factor for ESRD in patients with DM in later life.

## PUB226

**Morbimortality in Incident Hemodialysis Diabetic Patients in Southern Gran Canaria** Elvira Bosch,[1] Eduardo Baamonde,[1] German Perez Suarez,[2] Cesar Garcia-canton,[2] Marta Riaño-ruiz,[3] Ana Ramirez Puga,[2] Batista Fatima Garcia,[2] Agustin Toledo Gonzalez,[2] Mar Lago alonso,[2] Dolores Checa.[2] *[1]Centro de Hemodialisis Avericum, Las Palmas de Gran Canaria, Spain; [2]Servicio de Nefrologia, Hospital Insular de Gran Canaria, Las Palmas, Las Palmas de Gran Canaria, Spain; [3]Servicio de Bioquimica Clinica, Hospital Insular de Gran Canaria, Las Palmas, Las Palmas de Gran Canaria, Spain.*

**Background:** To describe the characteristics and prognosis of patients with diabetes mellitus (DM) who are incident in hemodialysis and to compare them with those of non diabetic (NoDM).

**Methods:** Retrospective study of incident hemodialysis (2007-2010). Subjects' baseline clinical and laboratory data, as well as data on preexisting comorbidity were collected. Hospitalizations, causes of admission and causes of death were compared between DM and NoDM patients.

**Results:** 220 patients (58.6% DM – 41.4% NoDM) were included. Diabetic patients had higher average age, BMI and age-adjusted Charlson's index (ACCI); they started HD with higher GFR and incidence of catheter and with lower levels of albumin, proteins and creatinine; they showed higher prevalence of ischemic cardiopathy, cerebrovascular accident (CVA), peripheral artery disease (PAD) and heart failure. The median follow up was 31 months (DM: 30.1 vs NoDM: 32.7 months p: 0.064). No differences were found in hospitalization rate (DM: 2.23 vs NoDM: 1.84; p:ns) or in the number of days in hospital (DM: 17.4 vs NoDM: 24.7 days; p:ns). Admissions due to PAD were more frequent among DM patients (p:0.000). DM patients had shorter survival time (49.1 vs 57.2 months; p: 0.045) and higher rates of global mortality (45.2% vs 24.2%; p:0.002) and mortality of cardiovascular cause (47.3% vs 24%; p: 0.046). Preexisting cardiovascular disease (HR: 3.26 CI: 1.58-6.71), age > 65 years (HR: 2.53 CI: 1.15-5.56) and starting with catheter (HR: 2.6 CI: 1.42-4.91) were the main risk factors (p< 0.05).

**Conclusions:** Diabetic patients start hemodialysis with higher GFR, ACCI and percentage of catheter and with poorer nutritional control. They have poorer prognosis, mainly determined by preexisting cardiovascular disease, age and starting with catheter.

## PUB227

**Survival Analysis in Korean Elderly Patients Initiating Dialysis: A National Population-Based Study** Jung-hwa Ryu,[1] Shina Lee,[1] Hyunwook Kim,[2] Seung-Jung Kim,[1] Duk-Hee Kang,[1] Kyu Bok Choi,[1] Dong-Ryeol Ryu.[1] *[1]Internal Medicine, School of Medicine, Ewha Womans Univ, Seoul, Republic of Korea; [2]Internal Medicine, Wonkwang Univ College of Medicine Sanbon Hospital, Gunpo, Republic of Korea.*

**Background:** Although the proportion of the elderly among incident end-stage renal disease (ESRD) patients has been increasing in Korea, there has been a lack of information on their outcome. Furthermore, there are some controversies about the appropriateness of dialysis initiation in elderly patients due to the dismal survival. This study aimed to assess the survival and to delineate predictors influencing on the all-cause mortality among elderly Korean patients initiating dialysis.

**Methods:** We included 11,301patients(6,138 men) aged 65years or older who initiated dialysis from January 1,2005 to December 31,2008 and followed up(median 37.8months:3.0-84.0months). Baseline demographic data, comorbidities and the information on mortality were obtained using the database of the Health Insurance Review & Assessment Service. The survival rate and the factors associated with mortality were analyzed.

**Results:** Unadjusted 24-month survival rate was 59.3% in all subjects, and it was decreased with the increment of age;66.5% among patients aged 65-69years, 59.0% among patients of 70-74years, 52.5% among patients of 75-79years, 48.5% among patients ofd 80-85years, and 30.2% among patients of 85years or older. In addition, the survival rate was significantly higher in female and in patients on hemodialysis than patients on peritoneal dialysis in intention-to-treat analysis. Multivariate Cox proportional hazard model revealed that age,sex,type of healthcare security system, and comorbidities:diabetes mellitus, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease,hemiparesis, liver disease, and any malignancy were independent predictors for mortality.

**Conclusions:** The survival rate in Korean elderly patients initiating dialysis was significantly associated with age, sex, and various comorbidities.With these results, individual counseling to dialysis initiation could be possible in elderly patients with chronic kidney disease.

## PUB228

**Risk Factors and Outcomes of Unplanned Hospitalization in Haemodialysis Patients – A Prospective Study from Greater Manchester East Sector Renal Network, UK** Shiv Bhutani,[1] Leonard Ebah,[1] Emily R. Keating,[1] Sheetal Asrani,[2] Marie McNulty,[1] Andrew Stevens,[1] Arvind Ponnusamy,[1] Sandip Mitra.[1] *[1]Renal Medicine, Manchester Royal Infirmary, Manchester, United Kingdom; [2]Stockbridge Village Health Centre, United Kingdom.*

**Background:** Hospitalization is a key prognostic indicator in Hemodialysis (HD) patients and has significant financial burden on renal networks. Registry data has shown links of hospitalisation in HD to various modifiable & unmodifiable risk factors, however prospective studies are distinctly lacking. This study investigated risk factors,outcomes & financial impact of Hospitalization (HZ) & rehospitalization (rHZ) for HD patients.

**Methods:** HZ data were collected prospectively from 4 satellite & central hub HD unit (April-September 2012). Electronic notes were used to collect data on demographics, comorbidity, causes & outcomes for HZ episodes.

**Results:** Among 413 HD patients, 148 episodes of HZ were recorded in 92 patients. 56 rehospitalisation (rHS) episodes observed in 36 patients. The annual HZ rate was 0.7 episodes per patient-year (ppy) & 0.27 ppy for rHS. Cardiovascular(25.5%), Infections(20%) and access related (13.5%) were the leading causes of HZ. 12.3% admissions (n=18) were potentially avoidable: fluid overload (6.8%), blood pressure crisis (2.7%), Catheter related infection (2.7%). Diabetes as comorbidity was the strongest independent predictor of HZ;OR 3.3 [1.6-6.8, p=0.001]. The mean age of patients was 60.7±15 & 57.7±15 yrs with similar gender ratios (m:f,1.2) for HZ & rHS respectively. 21% died within a year, a higher than expected mortality quoted for HD patients. Patients who were functionally less dependent more likely to be hospitalized (p=0.002) but longer length of stay was associated with worse functional status (r=0.31, p=0.0001).During the study period, 2031 inpatient days were utilised with an estimated annual equivalent cost of $2 million.

**Conclusions:** This prospective study demonstrates a high incidence of HZ amongst HD patients, with younger & diabetic patients most at risk. The Total HZ was associated with an increase in mortality and However, a proportion were due to potentially preventable causes, with significant outcome & cost implications.

## PUB229

**Cancer Treatment for Patients under Dialysis Therapy; Single Center Surveillance** Tatsuo Tsukamoto, Makoto Araki, Takeshi Matsubara, Motoko Yanagita. *Nephrology, Graduate School of Medicine, Kyoto Univ, Kyoto, Japan.*

**Background:** Cancer is the growing cause of death (approximately 10% of all mortality) in Japanese dialysis patients, whose population becomes older along the better survival rate.

**Methods:** In order to clarify the therapeutic choice of the cancer treatment and the outcome, we examined actual conditions of the therapy by retrospective chart review in Kyoto University Hospital, which possess both cancer center and dialysis unit. From 2007 to 2012, fifty-seven dialysis patients, who were 63±13 y.o. and 63.2% was male, were admitted to our hospital to receive a surgical operation, chemotherapy and/or radiation therapy.

**Results:** 40% of cancers were found within 5 years after the initiation of dialysis therapy. The most frequent cancer was derived from atrophic kidney and urinary tract (19.4% of male and 19.0% of female). Fifty of 57 patients were undergone radical operations, whose performance statuses were mostly 1. 13 patients (26%) had been treated with percutaneous cardiac intervention therapy prior to the operation. All patients were dialyzed the next day with intermittent or continuous blood purification method without serious adverse effects, such as severe hypotension, thrombosis and bleeding. The dry weight were reduced approximately 3% before the discharge. The average hospitalization was 29.9 days. Post-surgical infection was found in 9 patients, which were not larger than those of non-dialytic population. Half of the advanced cases were also treated with chemotherapy after the operation, whereas others were avoided to prescribe the chemotherapy because of their dialysis dependency. Thus, most dialysis patients had been successfully treated with single or combination therapy with careful administration of blood pressure, fluid volume, and cardiac function under the co-operation of oncologist and nephrologist, instead of fewer clinical information of cancer treatment in dialysis patients.

**Conclusions:** Further study, such as an intensive registry, would be required to establish the effective as well as safety therapeutic protocol for cancer patients under dialysis therapy.

*Funding:* Government Support - Non-U.S.

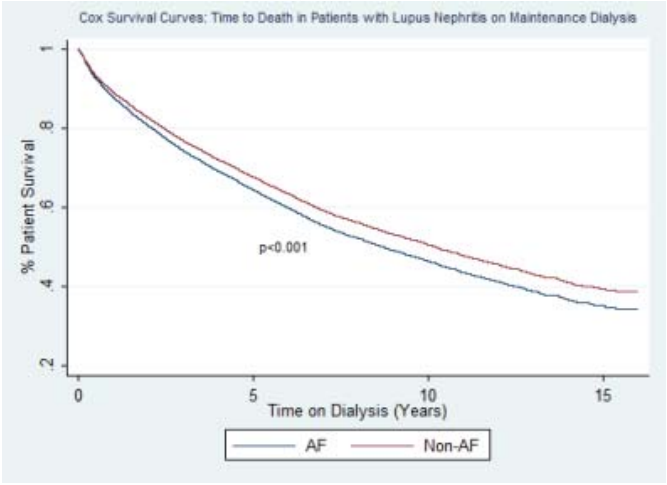
## PUB230

**The Impact of Race and Income on Mortality in Patients with Lupus Nephritis on Maintenance Dialysis** Robert Nee,[1] Jorge I. Martinez Osorio,[1] Lawrence Agodoa,[2] Christina M. Yuan,[1] Kevin C. Abbott.[1] *[1]Nephrology, Walter Reed National Military Medical Center, Bethesda, MD; [2]NIDDK, National Institutes of Health, Bethesda, MD.*

**Background:** An analysis of racial and income disparities on mortality in end-stage renal disease (ESRD) patients with lupus nephritis (LN) on maintenance dialysis has not been reported. We analyzed the United States Renal Data System database to assess the impact of race and income on mortality in the LN and non-LN cohorts.

**Methods:** We identified 12,352 patients with LN as the cause of ESRD in a retrospective cohort of 1,132,217 patients, initiated on chronic dialysis between January 1,1995 to December 31, 2006, followed until December 2, 2010. We merged data on median household income from the United States Census based on the ZIP code.

**Results:** In multivariate Cox regression analyses, African-American (AF) patients with LN (vs. non-AF) had an increased risk of death (adjusted hazard ratio [AHR], 1.10; 95% confidence interval [CI], 1.04-1.17).




Without adjusting for income, this racial disparity in the LN cohort was greater with an AHR 1.19 (95% CI, 1.12-1.25). Furthermore, analysis of interactions demonstrated that higher income quintiles were significantly associated with lower mortality rate in both LN and non-LN populations, regardless of race.

**Conclusions:** Contrary to the lower mortality rate among AF patients in the general ESRD population, AF with LN were at increased risk of death. This risk was attenuated but remained significant after adjusting for income. Income as an effect modifier for mortality risk, however, was not unique to the AF cohort with LN.

*The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy of the Department of the Army, the Department of the Navy, the Department of Defense, or the US government.*

## PUB231

**Effect of Bacteremia and Associated Risk Factors on Survival in Hemodialysis Patients** Puja Chebrolu,[1] Rhonda E. Colombo,[1] Stephanie L. Baer,[1,2] Mufaddal F. Kheda,[1] N. Stanley Nahman,[1,2] Kristina W. Kintziger.[1,2] *[1]Georgia Regents Univ, Augusta, GA; [2]Charlie Norwood Vet., Augusta, GA.*

**Background:** Bacteremia (BAC) is a major cause of morbidity and mortality in hemodialysis (HD) patients. We have previously shown that BAC in HD patients is significantly associated with many access-independent risk factors. However, the effect of BAC and/or associated risk factors on survival remains to be defined.

**Methods:** Incident HD cases from the United States Renal Data System for 2005-2008 were queried for a diagnosis of BAC and several potential clinical covariates using ICD-9 codes billed to Medicare. Cox proportional hazards regression was used to determine hazard of death in bivariable models. Covariates were classified by time of occurrence in relationship to a BAC diagnosis and HD initiation, and grouped according to infectious events, immunosuppressed conditions, demographic factors, and other comorbidities.

**Results:** In the 4-year period, 355,084 patients were available for analysis, and BAC was identified in 77,288 (21.8%). 208,397 patients (58.7%) were alive at the end of the period. Median time to death for patients with BAC was 455.7 days. Hazard ratio (HR) for death for BAC was 2.00 (95% CI 1.98, 2.02). Comorbidities which carried a higher HR than BAC were: age >65 years (HR 2.54 95% CI 2.51, 2.57), TPN (HR 2.29 95% CI 2.19, 2.39), pancytopenia (HR 2.11 95% CI 1.98, 2.25), and cirrhosis (HR 2.07 95% CI 2.01, 2.13), as well as infectious comorbidities such as candidemia and decubitus ulcer. These infectious conditions had the highest relative risks of bacteremia in our previous analyses as well. Among immunosuppressed comorbidities, diabetes was associated with a slight increase in hazard of death compared to non-diabetic patients (HR 1.22 95% CI 1.21, 1.23). In addition, SLE was negatively associated with hazard of death (HR 0.753 95% CI 0.71, 0.80) but carried a high relative risk of bacteremia (RR 1.77) in previous analyses.

**Conclusions:** Bacteremia is associated with a high risk of death in HD patients. Older age, TPN, pancytopenia, cirrhosis, and candidemia may carry a higher risk for mortality than bacteremia. Risk of bacteremia did not correlate with risk of death in patients with SLE.

## PUB232

**Can a Newly Started Dialysis Center Improve Patient's Quality of Life; Single Center Experience** Ihab El Madhoun, Rony Jose Pulikkan, Khalid Burghal, Omar Ahmad Alhasanat. *Renal Unit, Al Wakra Hospital, Al Wakra, Qatar.*

**Background:** Chronic diseases like chronic kidney disease can have a significant impact on patients quality of life (QoL)particularly when dialysis is indicated.

In our study, we assessed the impact of multidisciplinary approach on the QoL of haemodialysis patients in a new dialysis unit.

**Methods:** A multidisciplinary renal team of doctors, nurses, social worker and dietation managing patients in the newly started hospital. 13 patients were enrolled in the survey in a newly started dialysis center in Al Wakra Hospital, Qatar. All patients were assessed at the beginning of dialysis in the new unit using the WHOQOL-BREF questionnaire consisted of 26 questions representing 4 domains of physical health, psychological health, social relationship and environment. 7 patients reassessed after 3 months again.

| Domain | Facts Incorporated within domains |
|---|---|
| 1-Physical Health | Activities of daily living<br>Dependehnce on medicalinal substances and medical aids<br>Energy and fatigue<br>Mobility<br>Pain and discomfort<br>Sleep and rest<br>Work capacity |
| 2-Psychological | Bodily image and appearance<br>Negative feelings<br>Positive feelings<br>Self esteem<br>Spirituality/Religion/PersonalBeliefs<br>Thinking, Learning, memory and concentration |
| 3- Social relationships | Personal relationships<br>Social support<br>Sexual activity |
| 4- Environment | Financial resources<br>Freedom, physical safety and security<br>Health and social care; accessibilty and quality<br>Home environment<br>Opportunities for acquiring new informationa nd skills<br>Participation in and opportunities for recreation / leasure activities<br>Physical environment (pollution/ noise/ traffic/ climate)<br>Transport |

**Results:** 7 patients who had the chance to be assessed twice, have shown a significant improvement in their QoL after three months of treatment.

The remaining patients have shown a satisfactory level of QoL with space for some further improvement.

**Conclusions:** QoL is an important health care issue for dialysis patients. Newly established renal unit in a new hospital can still play a big role in improving patient's QoL. Multidisciplinary team approach is an important and strong tool in improving patients QoL.

## PUB233

**Impact of Changes in Dialysis Unit Structure and Procedures on Catheter Related Infections** Joseph Rossi Berger, Henry Quinones. *Nephrology, UT Southwestern Medical Center, Dallas, TX.*

**Background:** The Acute Dialysis Unit (ADU) at Parkland Health and Hospital System provides hemodialysis (HD) emergently to patients unfunded for outpatient dialysis. Catheter related bloodstream infections (CRBSI) are a significant source of morbidity in this group. We conducted this study to determine the impact of changes in ADU operational structure and procedures that were created to improve care and to decrease rates of CRBSI and infectious complications in our patients unfunded for outpatient dialysis.

**Methods:** We retrospectively analyzed all unfunded patients undergoing HD via a cuffed tunneled catheter during the 6 months before (period 1) and after (period 2) changes in the operational structure of the ADU. Changes included extended hours of operation, creation of an extra shift, increased levels of nursing staff, and hiring of 2 faculty nephrologists primarily responsible for care of unfunded patients. There was also a policy change that all CRBSIs requiring intravenous antibiotics be completed while inpatient rather than providing antibiotics when returning for urgent HD. We assessed the rates of overall CRBSI, Gram negative and polymicrobial infections, and complications (death, endocarditis, osteomyelitis) per 100 patient weeks of HD.

**Results:** There were 2392 patient weeks in perod 1 and 2578 patient weeks in period 2. There was a trend of reduction in overall CRBSI (3.22 v 2.33) and a significant reduction in Gram negative (2.30 v 0.85) and polymicrobial infections (1.30 v 0.27). There was not a significant difference in rate of complications (0.38 v 0.35).

| | Period 1 | Period 2 | P |
|---|---|---|---|
| Patient Weeks | 2392 | 2578 | |
| Overall CRBSI/100 pt weeks | 3.22 | 2.33 | 0.058 |
| Gram neagtive/100 pt weeks | 2.30 | 0.85 | <0.001 |
| Polymicrobial/100 pt weeks | 1.30 | 0.27 | <0.001 |
| Complications/100 pt weeks | 0.38 | 0.35 | 0.87 |

**Conclusions:** Changes in ADU structure and procedures were associated with a trend of reduced CRBSI rates and significant decrease in frequency of Gram negative and polymicrobial infections. These results support continued implementation of these practices and future study of other targeted interventions to further lower CRBSI rates.

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## PUB234

**Symptom Cluster, but Not Single Symptoms, Predicts Mortality in Dialysis Patients** Bård Waldum,[1,2] Amin Amro,[1] Fredrik Barth Brekke,[2] Nanna von der Lippe,[2] Ingrid Os.[1,2] *[1]Dept of Nephrology, Oslo Univ Hospital, Ullevål, Oslo, Norway; [2]Faculty of Medicine, Univ of Oslo, Oslo, Norway.*

**Background:** Bothersome symptoms are frequent and often co-occur in dialysis patients. Symptom burden is related to both health related quality of life (HRQOL) and depression. As HRQOL and depression are associated with mortality in dialysis patients, our aim was to investigate if symptom clusters predicted mortality in prevalent dialysis patients in Norway.

**Methods:** In a prospective observational cohort study including 301 prevalent dialysis patients, Kidney Disease and Quality of Life Short Form version 1.3 (KDQOL), SF-36, and Beck Depression Inventory (BDI) questionnaires were used. Based on KDQOL, 11 kidney-specific self-reported physical symptoms were entered into principal component analysis with varimax rotation to generate symptom clusters. Patients were categorized using quartile limits of symptom scores. Kaplan-Meier and multivariate Cox regression analyses were used for the survival analysis.

**Results:** Three symptom clusters were identified. The "uremic" cluster (nausea, lack of appetite, dizziness, feeling squeezed out, shortness of breath and chest pain) and "skin" cluster (itching and dry skin) were associated with all-cause mortality (log-rank p=.0001 and p=0.043). In multivariate analyses the quartile with most "uremic" symptoms had higher mortality rates compared to the three quartiles with less perceived symptoms (HR 2.47, 95% CI 1.44-4.22, P=0.001). No single symptoms were independently associated with mortality, neither were the other two symptom clusters.

**Conclusions:** "Uremic" symptoms independently predicted mortality in prevalent dialysis patients. Assessing co-occuring symptoms rather than single symptoms might help to identify dialysis patients at high risk for all-cause mortality. Additional research is warranted to determine whether symptom treatment may affect mortality rate.

*Funding:* Government Support - Non-U.S.

## PUB235

**The Prevalence and Risk Factors of Gallbladder Stone in Dialysis Patients** Xiaohong Tang, Ling Ji, Zi Li, Fei Liu, Wei Qin. *Div of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

**Background:** To investigate the prevalence of gallbladder stone in dialysis patients, clarify the prevalence difference between hemodialysis and peritoneal dialysis patients and analyzes the risk factors of it in dialysis patients.

**Methods:** 358 dialysis patients (126 hemodialysis patients and 232 peritoneal dialysis patients) and 376 pre-dialysis CKD stage 5 patients hospitalized in West China Hospital from January 2009 to October 2012 were included in this study. Abdominal ultrasound examination was performed to confirm the diagnosis of gallbladder stone. Data of hemoglobin (HGB), platelet (PLT), white blood cell (WBC), albumin (Alb), globulin (GLB), glucose (GLU), urea nitrogen (BUN), Creatinine (Cr), serum total cholesterol (CHOL), triglyceride (TG), low density lipoprotein (LDL), high-density lipoprotein (HDL), calcium (Ca), phosphorus (P) and parathyroid hormone (PTH) were collected. Prevalence of gallbladder stone in each team, then analysis risk factors of gallbladder stone in dialysis patients.

**Results:** The prevalence of gallbladder stone in dialysis patients was 23.5%, which is higher than the pre-dialysis CKD5 patients (14.4%, p=0.002) and the general population of China (10%, p<0.0005). The prevalence of gallbladder stone was higher in diabetic patients than non-diabetic patients, but with no statistical significance (p=0.076). Age, dialysis period, serum globulin and creatinine were significantly higher in dialysis patients with gallbladder stone (p<0.05). The prevalence of gallbladder stone in peritoneal dialysis patients was obviously higher than that of the hemodialysis patients (P < 0.05). The albumin level was significantly lower in peritoneal dialysis patients than the hemodialysis patients, while serum total cholesterol, low density lipoprotein cholesterol and low density lipoprotein cholesterol/high density lipoprotein were much higher. Logistic regression analysis showed that increasing age, women, diabetes mellitus and dialysis period were risk factors for gallbladder stone in dialysis patients

**Conclusions:** The prevalence of gallbladder stone in dialysis patients, especially the peritoneal dialysis patients, is higher than the general population. Risk factors for gallbladder stone in dialysis patients are: increasing age, women, diabetes mellitus, dyslipidemia, hypoalbuminemia and long dialysis period.

## PUB236

**Efficacy and Safety of Calcium Carbonate in Patients on Hemodialysis – A Pilot Study** Hui-juan Ma,[1] Yitong Yao,[2] Jianxiong Shi,[2] Ronglang Zhou,[2] Huanling Guo,[2] Xun Liu.[1] *[1]Div of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China; [2]Zhongshan School of Medicine, Sun Yat-sen Univ, Guangzhou, China.*

**Background:** Hyperphosphatemia in hemodialysis patients causes secondary hyperparathyroidism, and is associated with vascular calcification. Calcium carbonate ($CaCO_3$) is used broadly in China as phosphate binder to control the level of serum phosphorus.

**Methods:** A total of 19 hemodialysis patients with hyperphosphatemia (serum phosphorus > 5.5 mg/dL) were enrolled. All patients received a fixed dosage of $CaCO_3$ (3.0g/day, equivalent to 1.2g/day of elemental calcium). The effect and safety of $CaCO_3$ on serum phosphorus level were recorded at baseline and Weeks 4, 8 and 12. Statistical results are based on t-test (for one sample and two-group comparisons) and Fisher's Exact Test.

**Results:** Five patients were excluded including four of poor compliance and one receiving surgery. 14 patients completed the analysis. 10 were men and 4 were women, with a mean age of 48.7 ± 13.0 years who were on dialysis since 40 ± 26 months before the study. After 12 weeks of treatment, the mean level of serum phosphorus decreased significantly from 6.8 to 5.6 mg/dL (P <0.05). Eight patients (57.1%) achieved the NKF Kidney Disease Outcomes Quality Initiative target (serum phosphorus ≤ 5.5 mg/dL) and 4 (28.6%) achieved the Kidney Disease: Improving Global Outcomes target (serum phosphorus ≤ 4.5 mg/dL). In the subgroup with moderate hyperphosphatemia, the decreases of serum phosphorus in weeks 4, 8 and 12 were significantly relative to that in week 0, whereas in the subgroup with severe hyperphosphatemia, the respective changes revealed no significant differences. No serious adverse events related to treatment were reported.

**Conclusions:** $CaCO_3$ controlled serum phosphorus effectively in chronic hemodialysis patients with hyperphosphatemia. It may require larger dose of CaCO3 for the decrease of serum phosperus in the subgroup with severe hyperphosphatemia. The adverse events were mild in short term. Baseline serum phosphorus level affected the efficacy of $CaCO_3$.

HJM, YTY, JXS, RLZ, HLG contributed equally to the paper.

*Funding:* Government Support - Non-U.S.

## PUB237

### Albumin and Age Are Predictors of Early Mortality in Incident Hemodialysis Patients

André Fragoso,[1] Ana Pinho,[1] Jose António Lopes,[2] Antonio Gomes da Costa.[2] *[1]Nephrology Dept, Hospital de Faro, Faro, Portugal; [2]Nephrology Dept, Hospital Santa Maria, Lisboa, Portugal.*

**Background:** Despite improvements in dialysis care, incident hemodialysis (HD) patients have the highest mortality in the first months, particularly, elderly patients with multiple comorbidities. Recent prognostic tools to estimate 6-month survival in elderly incident dialysis patients have been validated and showed that decreased albumin levels and older age are important independently factors. The purpose of this study was to evaluate predictors of early mortality in incident HD patients.

**Methods:** In this prospective cohort study we included 188 chronic kidney disease patients beginning, permanently or temporarily, unplanned HD after hospital admission in 2012. Clinical and several laboratory parameters were determined for each patient at admission and the follow-up period was timed for 6-months from the first HD session. Our population was divided in two groups accordingly to the mortality 6-months after beginning HD: G-I (n=55) non-survivors and G-II (n=136) survivors.

**Results:** We found that G-I was older than 80 years old (p=0.001), showed more than six comorbidities (p= 0.0001), higher occurrence of cardiovascular disease (p=0.001) and recent hospital admissions (0.002). G-I also showed albumin level below than 2.5g/dl (p= 0.010). No differences were found between the two groups regarding history of diabetes and analytic levels of Hemoglobin or C Reactive Protein. In a cox multivariate analysis, two variables were independently associated with early mortality: age more than 80 years (hazard ratio [HR] 2.375; p= 0.002; 95% CI 1.36 to 4.14) and albumin levels below 2.5g/dl (HR 2.045; p= 0.013; 95% CI 1.16 to 3.60). Area under the curve for the resulting prognostic analysis of 6-months mortality was 0.68 (p= 0.0001; 95% CI 0.59 to 0.78).

**Conclusions:** In our study, an integrated 6-month prognostic tool based on decreased levels of albumin (under 2.5g/dl) and older patients (above 80 years old) accurate estimate early mortality. The instrument may be of value for researchers and clinicians to identify patients who have poor prognosis and consequently provide better care.

## PUB238

### Mortality Risk by Early versus Late Start of Hemodialysis

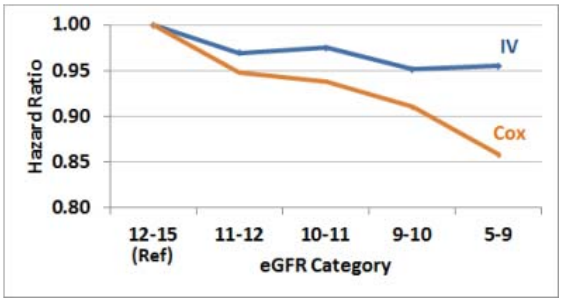
Bruce M. Robinson,[1] Yun Li,[1,2] Yan Jin,[1] Alissa Kapke,[1] Jeffrey Pearson,[1] Friedrich K. Port.[1] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]Univ of Michigan, Ann Arbor, MI.*

**Background:** In the US there has been a trend to starting dialysis at a higher eGFR over the past decade and before. Observational studies have found a positive association between eGFR at dialysis start and mortality, perhaps due to confounding by indication (CBI). The IDEAL randomized trial showed no association of eGFR at start with mortality, but had limited separation between early vs. late start. We aimed to reduce CBI by studying the practice of early versus late start.

**Methods:** The US Renal Data System ESRD database was used to identify 163,424 patients who initiated hemodialysis at dialysis facilities with at least 10 new patients in each year between 2006 and 2009. Mortality risk by eGFR (adjusted for demographics and comorbidity) was assessed using standard Cox regression models and an instrumental variable (IV) approach using physician as the instrument. The IV first stage used a linear model to explain continuous eGFR as a function of physician dummy variables; the second stage used a Cox model to explain mortality as a function of categorized predicted eGFR (from stage 1).

**Results:** The Cox models confirmed prior findings of a strong positive association of eGFR at start with mortality risk (higher mortality at higher eGFR); the IV approach showed an attenuated association, presumably by reducing CBI (Figure 1). Sensitivity analyses limited to patients with nephrology care for six months prior to starting dialysis and excluding patients who died in the first two months of dialysis were corroborative.

**Conclusions:** Contrary to US trends in increasing eGFR at dialysis start over time, no survival benefit could be detected to support a practice of generally starting dialysis earlier.




*Funding:* Private Foundation Support

## PUB239

### Peritoneal Dialysis in Nursing Home. Over 10 Years Experience

Valerio Vizzardi,[1] Massimo Sandrini,[1] Luigi Manili,[1] Laura Econimo,[1] Grazia Ventisette,[2] Giovanni Cancarini.[1] *[1]UO Nefrologia, Spedali Civili and Univ of Brescia, Brescia, Italy; [2]Fondazione di Cura, "Città di Gardone Val Trompia" ONLUS, Gardone Val Trompia, Italy.*

**Background:** Guests of nursing home (NH) often are extremely frail, poor self-sufficiency and high hospitalization rate. Hemodialysis (HD) in these patients faces several issues, e.g.: very old age, comorbidity, vascular access problems and logistic difficulties (mainly transportation). Peritoneal Dialysis (PD) is a possible treatment in not self-sufficient ESRD patients living in NH; in fact, trained health professionals are able to assist frail patients in these structures. In Italy there is a prevalence of about 40 patients (0,9%) doing PD in NH.

**Methods:** Retrospective analysis of clinical course and outcome in patients on PD treated in 2001 to 2013 in NH and followed-up by our Center.

**Results:** 24 pz (M/F=8/16) underwent PD in 8 NHs; mean age was 77±9 years (range: 56-94), median time on PD in NH was 5,5 months (I and III quartile: 1.9; 16.8; range: 1-81) with a total follow-up of 237 patient-months. Peritonitis occurred in 9 cases with a peritonitis rate of 1/26 patient-months; the causative microorganism were Gram negative in 67%, Gram positive in 11%, fungi in 11%, negative culture in 11%). The mean hospitalization was 10.8 days/patient year, but fourteen patients had no hospitalization during PD; the remaining 10 had a median of 14 days (I and III quartile: 14 and 37). Outcome: 21 deaths (age: 78±9 years; range: 56-95), 1 shift to HD (ultrafiltration failure). Today, two patients are still on PD.

| | |
|---|---|
| Albumin (g/dl) | 3.0±0.5 (range 2.2-3.7) |
| Hb (g/dl) | 10.8±1.6 (range 8.9-14) |
| Kt/V | 2.0±0.5 (range 1.5-3.4) |
| wCrCl (L/w/1.73 $m^2$) | 63±19 (range 34-121) |
| RRF (ml/min) | 2.1±2.1 (range 0-6.5) |

**Conclusions:** In patients on PD in nursing home, dialysis adequacy, nutrition and hemoglobin met targets acceptable for that kind of patients. Peritonitis rate was in the range suggested by ISPD. According to our experience, PD in NH is effective and safe. Moreover, PD allows the "home-management" of poli-comorbid and not self-sufficient ESRD patients.

## PUB240

### Intravenous Epogen in Peritoneal Dialysis: A Single Center Experience

Abdullah Hamad,[1] Kelly S. Jarvis,[2] Michelle G. Brickle,[2] Tarek M. Sobeih,[1] John Durham.[1] *[1]Palmetto Nephrology, Orangeburg, SC; [2]Davita Dialysis, Orangeburg, SC.*

**Background:** Epogen is commonly given subcutaneously (SQ) in Peritoneal Dialysis (PD) patients. The reason is being more potent and longer acting. We are reporting a retrospective evaluation of a single center experience with the use of Intravenous (IV) Epogen in anemia management in PD patients. The use of IV Epogen in anemia management has been the local practise at this center as a convenient way to deliver Epogen while having laboratory tests done with venous stick without having to do SQ stick. That practive provided an opportunity to evaluate its efficacy.

**Methods:** The records for all PD patients (total 34) were reviewed. 14 patients excluded (6 were not on any kind of erythropoietin stimulating agents and 3 had their Epogen on hold for over 1 month, 2 patients on PD less than one month, 2 patients on SQ Epogen). 20 PD patients were on PD more than a month and receiving IV Epogen weekly or every other week and included in the analysis. They were evaluated for Epogen dosing, Hemoglobin, Ferritin, Iron saturation, Albumin, Age and Sex.

**Results:** Their mean age was 48.3 years and were 9 males. Mean Ferritin was 683.6 mg/dL, iron sat was 41.5 % and albumin 3.52 mg/dL. Mean hemoglobin value for 1 month of follow up was 9.99 mg/dL, mean Epogen IV dose was 35400 units for PD patients on weekly dosing (7 patients) and 27600 units for the every other week dosing (13 patients).

**Conclusions:** It appears that IV Epogen provided for stable management of anemia and convenient dosing in PD patients. Most patients were maintained on an every other week schadule. Only one third of patients required more frequent and higher doses of Epogen. Despite what might be predicted, most patients can be successfully managed on every other week dosing regimine.

**PUB241**

**Kinetics of α-Amylase Activity in Mixture of Glucose Polymers: A Model for Degradation of Osmotic Agent** Jan T. Poleszczuk,[1] Elvia Garcia-lopez,[2] Bengt Lindholm,[2] Jacek Waniewski.[1] *[1]Nalecz Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland; [2]Depts of Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

**Background:** Glucose polymers (GP, as Icodextrin) are used as osmotic agents in peritoneal dialysis, and therefore the activity of α-amylase, the starch degrading enzyme, may have a substantial impact on the efficacy of the treatment. The kinetics of α-amylase activity is not easily predictable and the development of an experimental and mathematical framework for assessing α-amylase characteristics was necessary.

**Methods:** The reaction of porcine pancreatic α-amylase (PPA of 0, 25, 50, 100 U/L) and maltoheptaose (G7 of 2.5 and 5 mg/mL) in PBS was carried out *in vitro*. G7 and all its possible decay products were frequently measured by HPLC. Different hypothetical pathways of G7 decay were analyzed together with mathematical models for the second order reactions of PPA with GP and the derived generalized Michaelis-Menten equations. The model parameters were estimated.

**Results:** For all experimental conditions no trace of maltohexaose (G6) and only small quantities of glucose (G1) were found, what allowed us to eliminate a few possible G7 decay pathways. Further comparison of the experimental data with fitted model curves yielded a single decay pathway with maltotriose (G3), maltose (G2) and G1 as the final products and maltopentaose (G5), maltotetraose (G4) as both products and substrates for PPA. The corresponding mathematical model reproduced the experimental data with high accuracy. The cleavage of G4 was much slower than the digestion of higher oligosaccharides, G7 and G5, which had similar kinetic parameters. G4 was digested mostly to two G2 molecules, but also, with low rate, to G3 and G1. The intermediate products of the G7 decay (G5, G4), but not the final products, had substantial inhibition effect on PPA catalytic efficiency.

**Conclusions:** The proposed method with the application of mathematical modeling yielded important details about PPA activity without sophisticated experiments on oligosaccharides with labeled bonds. The obtained mathematical model may be generalized for describing the digestion of GP of arbitrary length.

*Funding:* Pharmaceutical Company Support - Kidney Foundation of Canada

**PUB242**

**Make Peritoneal Dialysis Patient Driving Their Therapy: Cornerstone of Our Program** Piyatida Chuengsaman. *CAPD Service and Training Center, Banphaeo Hospital (Public Organization), Prommitr Branch, Bangkok, Thailand.*

**Background:** Our center was established since 2008, the same year as implementing Thai PD First Policy, which make dialysis be fully subsided thus more accessible in Thailand. We are the main referral PD center in Bangkok. Now we have provided PD therapy for about 1,500 ESRD patients. We recognized the cornerstone of our program: "making our patients driving their therapy". All the activities provided here, put this concept at the core. Under the limited healthcare professional resources and budget constraint, we can provide dialysis to save lives with good quality and affordable.

**Methods:** We retrospectively reviewed number of patients, survival analysis, infection-related peritoneal dialysis complication, and number of healthcare professional in each time period.

**Results:** As shown all the detail in Table 1, despite the rising number of our patients, compare with the number of staff, we can provide acceptable outcome. 1 and 2 year patient survival is 79 and 68 percent. 1 and 2 year technique survival is 77 and 64 percent. Our peritonitis and exit site infection rate is 1 episode per 27 patient-month and 1 episode per 24 patient month, respectively. We need to make our patients driving the therapy, this make the patient acting as part of the provider. Implementing concept of self-management (assessment, monitoring, reinforcement), ensure adequate and tailoring program of training & re-training, good data management which guiding to real time action plan, are the three main strategies for our program.

| Year | 2008 | 2009 | 2010 | 2011 | 2012 | 2013[March] |
|---|---|---|---|---|---|---|
| Patients | 113 | 373 | 673 | 1062 | 1426 | 1463 |
| Nephrologists[Full-, Part-time] | [1, 0] | [2, 1] | [2, 1] | [1, 6] | [2, 6] | [2, 6] |
| PD Nurses | 1 | 4 | 7 | 7 | 9 | 9 |
| Healthcare Workers | 2 | 2 | 2 | 4 | 3 | 6 |
| Volunteer Patients | 3 | 5 | 6 | 6 | 5 | 5 |

**Conclusions:** Driving their own therapy under good center management gives peritoneal dialysis a form of renal replacement therapy which can be implemented despite the limited healthcare professional resources.

*Funding:* Government Support - Non-U.S.

**PUB243**

**The Clinical Investigation on Inflammatory Cytokine Profiles during Peritoneal Dialysis-Related Peritonitis** Guochun Chen, Jing Liu, Hong Liu, Feng Wen, Xiang Zhou, Fu-You Liu. *Dept of Nephrology, The Second Xiangya Hospital of Central South Univ, Changsha, Hunan, China.*

**Background:** Peritonitis is one of the key complications during peritoneal dialysis,this study was planned to detect new and sensitive inflammatory cytokines in order to improve its diagnosis.

**Methods:** 36 PD in and outpatient are enrolled in the study ,16 are with PD related peritonitis, others are in control.The overnight dialysate and other related clinical data are collected.Expression of IL-6,IL-17A, MCP-1,TNF-α,serum and dialysate hs-CRP were detected. pHPMC are cultured in vitro, then exposed respectively to different concentration(0, 2, 10, 20, 100ug/ml) of LPS and different time(10ug/ml 0, 3, 6, 12, 24h). The expression of CRP,IL-6,IL-8,IL-1β, TNF-α,LDH, ICAM-1 and TGF-β were examined.

**Results:** We found that, Compared with control, the dialysate concentration of IL-6, IL-17A, MCP-1, TNF-α and hs-CRP were significantly elevated in de novo PD patients with peritonitis. During recovery of peritonitis, expression of each above markers gradually decreased. The age, sex, BMI, primary disease, and dialysis duration showed no difference between peritonitis and control.The serum hs-CRP, white blood cell count, neutrophil ratio, dialyslate hs-CRP, LDH were significantly elevated in peritonitis PD patients compared with control (P< 0.05). The dialysate hs-CRP was significantly higher in the effluent of PD patients with peritonitis,which decreased significantly and rapidly after anti-infection therapy. And those patient withdrawed PD after peritonitis had relatively high hs-CRP level compared with normal.And dialysate hs-CRP was negatively correlated with serum albumin(>P=0.026), and serum hs-CRP was netively associated with BMI(P<0.05), serum albumin (r=0.665, P< 0.05) and total plasma protein(P<0.05).After HPMC was treated by series concentration of LPS, the mRNA expression of CRP,IL-6,IL-8,IL-1 β,TNF-α,LDH,ICAM-1and TGF- β were increased.

**Conclusions:** Our study indicated that dialysate hs-CRP,IL-6,IL-17A,MCP-1 and TNF-α may be early diagnosis markers of PD-related peritonitis,and dialysate hs-CRP could be a prognosis indicator.

**PUB244**

**Oral Ciprofloxacin and Intraperitoneal Cefazolin as Empirical Treatment for Peritoneal Dialysis (PD) Related Peritonitis: A 18 Years Experience** Tatiana Tanasiychuk, Daniel Kushnir, Alon Antebi, Jerome Marcusohn, Victor Frajewicki. *Dept of Nephrology and Hypertension, Carmel Medical Center, Haifa, Israel.*

**Background:** Peritonitis still remains a common and serious complication of PD. It is the primary cause of technique failure and it is associated with increased risk of death. According to the ISPD 2010 recommendations the most accepted empiric protocol includes a combination of Vancomycin/Cephalosporin for Gram-positive organisms and third-generation Cephalosporin or Aminoglycoside for Gram-negative bacteria. Cure rate widely varies between 44% to 85% but the initial empirical treatment of PD-related peritonitis is still under investigation.

**Methods:** We retrospectively evaluated all records of patients on PD with peritonitis between 1995 and 2012 treated in our Department. The protocol used in most of patients included an initial intraperitoneal instillation of Cefazoline (500 mg) and Ciprofloxacin (200 mg) or Ofloxacin (200 mg), followed by intraperitoneal Cefazoline (250 mg/bag) and oral Ciprofloxacin (500 mg) or Ofloxacin (200 mg) once daily.

**Results:** Records of 323 cases of peritonitis episodes in 143 patients on either Automated PD or Continuous Ambulatory PD were reviewed. More than one event was reported in 71 patients (250 cases). A single peritoneal infection was recorded in 72 (50%). Bacteriological data of 93 cases are missing. In 222 cases (97%) a single organism was isolated: 127 of Gram positive (57%) and 57 of Gram negative (26%) organisms. Methicillin resistant germs were detected in 69% of Gram positive and Pseudomonas in 33% of Gram negative infections. Fungal peritonitis was recorded in 9 cases. Thirty five cases were culture- negative. Overall treatment success rate was 73%. Total mortality was 4%, mainly (57%) related to Gram negative infection. Resolution rate was not significantly different in 98 cases with the use of Aminoglycosides (76%) than in the 211 cases where Quinolones were used(75.8%).

**Conclusions:** Empirical treatment with oral Quinolones and intraperitoneal Cefazolin is at least as good as the widely accepted combination of Vancomycin/Cephalosporin and third-generation Cephalosporin or Aminoglycoside in peritonitis catheter related.

**PUB245**

**Peritoneal Dialysis in HIV Seropositive End Stage Renal Disease Patients: Single Center Experiences** Piyatida Chuengsaman. *CAPD Service and Training Center, Banphaeo Hospital (Public Organization), Prommitr Branch, Bangkok, Thailand.*

**Background:** Our peritoneal dialysis program was established since 2008, now we have provided this therapy to about 1,500 end stage renal disease patients. In Thailand, HIV-seropositive end stage renal disease patients get limitation to access to hemodialysis, due to healthcare professional's concern about chance of viral transmission. Because peritoneal dialysis is a home-based therapy, this make the provider of this type of therapy feel more comfortable, thus make this therapy be more accesible for this group of patients.

**Methods:** We retrospectively reviewed our database. We compared the survival data, PD-related infection data, and other clinical parameter, between HIV -seropositive PD patients and overall center data.

**Results:** From February 2008 till September 2012, there were 1,366 patients which we have provided peritoneal dialysis. In this group of patients, there were 38 HIV-seropositive patients. At the end of September 2012, there were 13 active HIV-seropositive patients. Data about HIV status was available only for the ones who alive. According to HIV viral load, 11 out of 13 patients got very low copies of virus (less than 40 copies/ml). The patient's data is shown in Figure1.

| Patient number | Hb (g/dl) | HIV Duration (year) | ARV Duration (year) | BMI | Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | Albumin(g/dl) | Potassium (mmol/dl) | Lymphocyte (CD4+) Abs. Count | ViralLoad |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9.9 | 17 | 15 | 0 | 261 | 116 | 69 | 3.2 | 3.19 | 701 | <40 |
| 2 | 9.6 | 15 | 15 | 1 | 137 | 173 | 26 | 3.7 | 3.29 | 1365 | <40 |
| 3 | 11.1 | 1 | 1 | 0 | 215 | 206 | 61 | 4 | 4.36 | 1071 | <40 |
| 4 | 10.7 | 25 | 15 | 0 | 183 | 117 | 44 | 2.8 | 3.16 | 924 | <40 |
| 5 | 8.5 | 9 | 9 | 0 | 268 | 438 | 36 | 2.6 | 2.8 | 1080 | <40 |
| 6 | 7.6 | 9 | 0 | 1 | 121 | 99 | 38 | 3 | 3.43 | 527 | <40 |
| 7 | 8.4 | 1 | 1 | 0 | 222 | 238 | 23 | 3.1 | 2.46 | 1798 | 487 |
| 8 | 8.9 | 1 | 1 | 1 | 183 | 90 | 41 | 3.4 | 3.77 | 1239 | <40 |
| 9 | 14.9 | 1 | 1 | 0 | 320 | 71 | 131 | 4 | 4.21 | 1836 | <40 |
| 10 | 8.3 | 0 | 4 | 1 | 112 | 160 | 38 | 3.3 | 3.46 | 1150 | <40 |
| 11 | 9.8 | 18 | 17 | 0 | 242 | 411 | 42 | 3.8 | 3.79 | 1840 | <40 |
| 12 | 10.1 | 11 | 6 | 0 | 336 | 810 | 38 | 3.9 | 3.28 | 1096 | 186,339 |
| 13 | 9.6 | 2 | 2 | 1 | 181 | 128 | 28 | 3.1 | 2.8 | 2010 | <40 |
| 14 | 7.9 | 3 | 3 | 0 | 191 | 62 | 60 | 2.4 | 4.47 | 1333 | <40 |
| 15 | 9.6 | 10 | 10 | 1 | 283 | 268 | 32 | 3.7 | 2.56 | 2170 | <40 |

We also compare our overall center data with HIV-seropositive patients' data as shown in (Table 1).

| | Overal patients | HIV-seropositive patients |
|---|---|---|
| Number of Patients | 1,366 | 38 |
| 1,2 year patient survival | 81%, 69% | 68%, 60% |
| Peritonitis rate[1 episode per patient-month] | 28 | 56 |

**Conclusions:** HIV-seroposive ESRD patients, is an emerging problem in Thailand. Despite the concern about PD-related infectious complication. our data did not support this. Due to survival data is not as good as in HIV-seronegative ESRD patients, our program need to focus more to improve the outcome.

*Funding:* Government Support - Non-U.S.

## PUB246

**Combination Therapy with Peritoneal and Hemodialysis from the Initiation of Renal Replacement Therapy Preserve the Residual Renal Function and Peritoneal Function; Case Report** Atsushi Ueda,[1] Aki Hirayama,[2] Shigeru Owada,[3] Kei Nagai,[4] Kunihiro Yamagata.[4] *[1]Tsukuba Univ Hospital Hitachi Medical Education and Research Center, Japan; [2]Tsukuba Univ of Technology, Japan; [3]Asao Clinic, Japan; [4]Univ of Tsukuba, Japan.*

**Background:** In ASN 2012, we reported in a retrospective cohort study an advantage of the peritoneal and hemodialysis (PD+HD) combination therapy from the initiation of renal replacement therapy (RRT) on the patient's survival compared to PD or HD groups. We concluded this beneficial result was caused from the preservation of residual renal function (RRF) and serum albumin level.

**Methods:** This was the typical valuable case, a 51-year-old woman with diabetic nephropathy selected PD+HD therapy as the first dialysis method 10 years ago. The dialysis schedule consisted of five PD days and one HD without fluid removal a week. The Kt/V of RRF, PD and HD calculated by equivalent renal urea clearance were measured, and peritoneal equilibration test was performed every six months.

**Results:** At the beginning of this therapy, Kt/V of HD, PD and RRF was 0.81, 1.29, and 0.52, respectively. Interestingly, Kt/V of RRF increased to 1.29 at six months, it kept absolutely stable within 96 months and 0.44 at 120 months. The mean Kt/V of RRF, PD and HD was 0.85, 1.32 and 0.83, respectively. The mean for 10 years urine volume was 943 mL/day and the peritoneal fluid removal volume was 733 mL/day. The mean D/P Cr ratio was 0.52, peritoneal permeability did not increase for 10 years. Albumin level maintained stably, in addition anemia, inflammation and nutrition status kept as good control.

**Conclusions:** Since this combination therapy started at the initiation of RRT, fluid control could be kept well by urine and ultrafiltration of PD. A stable PET data indicated peritoneal resting might be expected to be beneficial on the preservation of peritoneal function. Finally, these effects could bring not only the long-term continuation of dialysis but also the improvement in anemia, inflammation and nutrition status. This case demonstrated that this new combination therapy was extremely useful for preserving both RRF and peritoneal function, indicating a good prognosis.

## PUB247

**Natural History of Cuff Externalization of Peritoneal Dialysis Catheter with Tunnel Infection** Ebadur Rahman, Nader Mohamed Omran, Naveed Aslam, Dujanah Hassan Mousa. *Nephrology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia.*

**Background:** Patients with multiple access failure on peritoneal dialysis (PD),surgical externalization of catheter cuff was done and outcomes were observed.

**Methods:** Study included four cases two of which were unfit for reinsertion of (PD) catheter or fistula creation.Two patients refused for removal of catheter. All patients had positive cultures from exit site. Exit sites of two patients were positive for Methicillin sensitive staphylococcus aureus (MSSA). The exit sites of other two were positive for methicillin-resistant staphylococcus aureus (MRSA) and Pseudomonas Aeruginosa respectively. External Cuff was removed surgically followed by oral fluclaxacillin for MSSA positive Patients. Intra peritoneal vancomycin was prescribed for the MRSA positive patient whereas ciprofloxacillin was prescribed for pseudomonas positive patient. Along with continuation of PD, twice weekly dressing with 3% saline was done for all patients. Three months prospective observation was made regarding wound healing, persistence of exit site infection and any episode of peritonitis. PD fluid cell count, culture and exit site swab culture was done weekly to diagnose any infectious episodes during this period. Additional cultures were taken in case of any suspicion of peritonitis or tunnel or exit infection. Adequacy of dialysis was assesed monthly.

**Results:** Mean age of this group is 68 years and two of them were females. None of the 4 patients had wound healing within this observation period. Exit site cultures were persistanly positive for two cases who had MSSA in the exit site and of them one developed peritonitis. Eventually catheters were removed for this two cases. For the remaining two cases the repeated post procedure cultures were negative and they had no episode of peritonitis and remained on PD. Catheters were eventually removed because of poor wound healing. Mean Weekly Kt/V of these patients before and after the procedure was 1.87 and 1.8 respectively.

**Conclusions:** Catheter loss of this procedure is 100% .50% patients with this procedure can remain in PD. Procedure can be used for patients with high co morbidity and with very poor life expectancy.

*Funding:* Government Support - Non-U.S.

## PUB248

**Urgent Start PD: Establishing a Center of Excellence as a Model of Delivery** Heather L. Henderson, Quresh T. Khairullah, Jukaku S. Tayeb, Robert Provenzano. *Nephrology, St. John Hospital & Medical Center, Detroit, MI.*

**Background:** PD is underutilized as a dialysis modality in the US. Currently ~7% of US dialysis patients use PD. First reported by Ghaffari and replicated by others, urgent start PD programs (catheter use ≤ 2 weeks post placement) have been shown to increase PD % with acceptable complication rates. Urgent start PD is difficult to deliver to all patients due to physician inexperience & commitment, adequate staffing (in hospital & PD units), and initial increased operational cost. Additionally, many argue that ESRD care in the US is structured around in-center HD and this places an undo burden on the majority of PD programs as to "ease of entry". To address this deficit, we developed a center of excellence (COE) to deliver urgent start PD and increase PD percentages in our region.

**Methods:** Successful PD outcomes are largely related to the experience of the nephrologists and PD nurses, which are typically higher in PD centers with larger patient populations. Unfortunately small PD centers far outnumber large centers making urgent start PD a nonviable option in many regions due to cost and staffing constraints. Our community based PD unit provides intermittent peritoneal dialysis (IPD) to urgent start patients referred by community based hospitals and nephrologists. Once tolerating full fill volumes, patients may be transferred back to their primary nephrologist at smaller PD units for continued care and management. Our intention is to increase patients into our urgent start program with plan for transfer back to their primary nephrologist once they no longer require IPD.

**Conclusions:** As integrated care penetrates the ESRD market, growing and maintaining PD populations will be critical. We have shown that instituting a centrally located PD COE gives more patients the opportunity to initiate PD that may otherwise not be possible and grow smaller PD centers nationwide. Transferring patients back to their primary nephrologist allows physician ownership and continuity of care, critical in an era of patient centered medical homes. Also by networking larger PD centers with smaller centers this may improve patient survival, outcomes, and drop-out rates.

*Funding:* Private Foundation Support

## PUB249

**Status of 835 CAPD Patients in 6 Peritoneal Dialysis Center in Hunan Province in China** Liu Yan, Hao Zhang, Ke Zhang. *Nephrology, The Third Xiangya Hospital in Central South Univ, Changsha, Hunan, China.*

**Background:** Hunan is in the middle of China, the number of Continuous Ambulatory Peritoneal Dialysis(CAPD) patients here counted for more than 19% in China. This study is to investigate the status of CAPD patients in 6 peritoneal dialysis centers in Hunan Province.

**Methods:** Cross-sectional study of general data, nutriture, inflammatory state and dialysis adequacy of 835 CAPD patients in 6 peritoneal dialysis centers in Hunan Province during July, 2012 to December 2012, and analysis the datas.

**Results:** 1. General data:sex ratio is 1.2:1,age is 51.91±14.27, dialysis age is 23.64±19.71 months, and the primary disease before three were chronic glomerulonephritis (59.2%), diabetic nephropathy (15.6%), benign arteriolar nephrosclerosis (12.7%). 35.2% patients controlled their blood pressure under 140/90mmHg, and blood pressure of patients with dialysis age more than 36 months significantly higher than less than 36 months. 2. Nutiture: the rate of anemia is 83.3%, hypoproyeinemia is 55.7%, serum phosphorus beyond control is 47% and hyperlipemia is 54.7%. The BMI of the patients is 22.56±3.02kg/$m^2$, 90% patients with mild to moderate malnutrition, calcium-phosphorus product is 41.24± 10.16$mg^2/dl^2$, and PTH of 45.8% patients is higher than 300pg/ml. Patients with dialysis age more than 36 months compared with less than 36 months, Hb is significantly lower(P<0.05), TG and LDL is significantly higher(P<0.05), SGA score is significantly higher, PTH is significantly higher(P<0.05).3. Dialysis adequacy: BUN is 21.25±9.25 mmol/L, CR 953.72±369.32 μmol/L, Kt/V为1.53±0.44, and BUN and CRE of patients with dialysis age more than 36 months significantly higher than that of less than 36 months. 4. Inflammatory state: CRP is 3.39±3.37mg/L, patients with dialysis age more than 36months higher than less than that less than 36 months(P<0.05).

**Conclusions:** As the dialysis age increases, anemia, malnutrition and inflammation become more serious in CAPD patients in Hunan. 2. The analysis of CAPD datas is important and practical guidance for clinnical work. Key Words: CAPD, general data, nutriture, inflammatory state, dialysis adequacy.

*Funding:* Clinical Revenue Support

## PUB250

**Simplifying the Identification of Changes in Residual Renal Function in Patients Treated with Peritoneal Dialysis** Matthew Miller,[1] K. Scott Brimble,[1] Chenglin Ye,[2] Brenda Hemmelgarn,[3] Arsh Jain,[5] Marcello Tonelli,[4] Michael Walsh.[1] *[1]Div of Nephrology, McMaster Univ, Hamilton, Canada; [2]Dept of Clinical Epidemiology and Biostatistics, McMaster Univ, Hamilton, Canada; [3]Div of Nephrology, Univ of Calgary, Calgary, Canada; [4]Div of Nephrology and Immunology, Univ of Alberta, Edmonton, Canada; [5]Div of Nephrology, Univ of Western Ontario, London, Canada.*

**Background:** Changes in residual renal function (RRF) may require changes in the peritoneal dialysis (PD) prescription. However, measuring RRF with a 24-hour urine collection is laborious for patients. In a retrospective multicentre cohort, we studied whether simplified measures of RRF adequately predict renal Kt/V (rKt/V) or significant changes in rKt/V.

**Methods:** We studied prevalent PD patients who had rKt/V >0.3 and at least 2 peritoneal adequacy tests done between 3 and 15 months apart without a change in modality of fill volume. We attempted to predict the follow-up rKt/V using multiple linear regression in which patient demographics, serum creatinine, serum urea and baseline rKt/V were potential predictors. We assessed candidate models by calculating mean bias of predictions and accepted the simplest model with a mean bias ≤0.2 as optimal. We also attempted to predict a clinically important change in rKt/V of 0.2 using only a change in serum creatinine using logistic regression.

**Results:** 388 patients were eligible for study. When predicting rKt/V, no model resulted in a mean bias of ≤0.2.

| Model Characteristics | Mean Aboslute Bias |
|---|---|
| Creatinine | 0.433 |
| Creatinine and BUN | 0.431 |
| Creatinine, BUN, age, and gender | 0.414 |
| Creatinine, BUN, age, gender, and initial rKt/V | 0.332 |

Change in serum creatinine was associated with a significant change in rKt/V (p<0.001) but had poor discrimination (area under the receiver operator curve 0.63).

**Conclusions:** We were unable to identify a simple method for accurately predicting Kt/V or significant changes in Kt/V. Further research is required to simplify the identification of patients with changes in RRF.

## PUB251

**Clincal Characteristics of Long-Term Survivors and Short-Term Survivors on Peritoneal Dialysis** Yiwen Li. *Kidney Disease Dept, ZheJiang Province Hospital.*

**Background:** To evaluate the characteristics of patients on long-term and short-term peritoneal dialysis (PD).

**Methods:** Patients who be treated with PD since 2006 to 2011 and received PD for at least one year in our Department of kidney disease were included in this study.According to dialysis duration, patients were divided into tree groups.Group A(long-term)was defined as patients survived on PD for more than 4 years.Group B(short-term)was defined as patients who died and Group C(short-term)was defined as switched to hemodialysis within less than 4 years.Demography, biochemical indexes, dialysis adequacy, residual renal function were compared between those groups.

**Results:** There were 50 patients in group A, 55 patients in group B and 90 patients in group C. Mean followed-up period of group A , group B and group C Was (58.33±10.03) months,(27.15±9.35) months and (41.65±12.80) months , respectively.Younger, fewer episodes of diabetic comorbidity(group A 4/50 vs group B 13/55, P<0.05) were found in group A.Compared to group B and gruopC, the level of semm albumin at the beginning of PD was much higher in group A [(38.74±4.65)g/L vs (36.15±4.85)g/L and vs (35.23±5.27) g/L, P<0.01)].Compared to group A and group B, the levels of blood sugar in group C is lower. The levels of CRP in group B is higher than in group A and group C(P<0.01 and P< 0.05). The levels of nPNA in group A is higher than in group B(P<0.05). The levels of TC, TG, hemoglobin, calcium, phosphate and iPTH were not significantly different between three groups. Renal Kt/V,renal Ccr and urinary volume at the beginning of dialysis were much higher in group A(P<0.05).Ultrafiltration volume and pulse pressure difference was less in group A(P<0.05). Peritonitis morbidity,peritoneal membrane transport,increased blood sugar morbidity were not significantly different between three groups.

**Conclusions:** In comparison to short-term survivors, long-term PD patients are characterized by being younger, less diabetic disease, higher level of serum albumin and Hemoglobin, higher volume of urine , lower level of CRP, better residual renal function , less ultrafiltraion volume and lower pulse pressure difference.

## PUB252

**Planned Dialysis, Not Early Referral, May Improve Quality of Life and Depression in Newly Diagnosed End Stage Renal Disease Patients: Comprehensive Prospective Study of the Clinical Research Center for End Stage Renal Disease (CRC ESRD) in Korea** Ji In Park,[1] Jin Ho Hwang,[1] Yong-Lim Kim,[3] Chun Soo Lim,[4] Yon Su Kim,[1] Jung Pyo Lee.[4] *[1]Internal Medicine, Seoul National Univ College of Medicine; [2]Internal Medicine, Hangang Sacred Heart Hospital; [3]Internal Medicine, Kyungpook National Univ Hospital; [4]Internal Medicine, Seoul Boramae Medical Center, Republic of Korea.*

**Background:** Health-related quality of life (HRQOL) has recently become an important issue, which was revealed to affect survival of end-stage renal disease (ESRD) patients. Few studies reported that early referral and planned dialysis improves HRQOL. Here, we investigated whether referral time and planned dialysis affects HRQOL by nationwide prospective cohort study.

**Methods:** We enrolled newly diagnosed 670 ESRD patients who responded to the survey on the HRQOL and depression at post-dialysis 3 month. We got follow-up data of 298 patients at post-dialysis 1 year. Early referral (ER) was defined as meeting with a nephrologist more than a year before dialysis, and planned dialysis as starting dialysis with functioning arteriovenous fistula or graft. Kidney Disease Quality of Life Short Form 36 (KDQOL-36) and Beck's Depression Inventory (BDI) were utilized to measure HRQOL and depression.

**Results:** Higher KDQOL-36 and lower BDI score correlate with significantly higher survival. Type of referral did not affect KDQOL-36 and BDI score at post 3 months and at post 1 year, neither. However, planned dialysis showed significant improvement in mental component score and symptom/problem list at 3 months (P<0.05). Furthermore, planned dialysis showed better quality of life at post dialysis 1 year in physical component score, symptom/problem list, and BDI (P<0.05). These associations were significant after adjusting for age, sex, laboratory results and social factors.

**Conclusions:** Planned dialysis significantly improved HRQOL and depression of ESRD patients in short and long period of time. However, timing of referral didn't affect them. This suggests that nephrologists should prepare dialysis at proper timing and make an effort to improve HRQOL of early referred patients.

## PUB253

**Value of Anion Gap, Serum Bicarbonate, Strong Ion Difference and Strong Ion Gap in the Diagnosis of Lactic Acidosis** Seyed-ali Sadjadi, Navin Jaipaul, James I. McMillan, Mohammad Sharif. *Nephrology, Jerry L Pettis VA Medical Center, Loma Linda, CA.*

**Background:** Timely detection of presence of lactic acidosis (LA) is of great importance in emergency rooms, operating rooms and intensive care units. Various methods have been proposed to look for LA, but none of them has been found to be reliable. Due to this, we decided to compare the utility of albumin corrected AG, serum bicarbonate, strong ion difference apparent(SIDa) and strong ion difference effective (SIDe) and strong ion gap (SIG) for the diagnosis of LA.

**Methods:** Our patient cohort included 299 patients, with LA who had a serum lactate ≥4 meq/L who were either seen in the emergency room or intensive care units. To simulate the acute care setting, we calculated SIDa as the sum of Na + K + Ca - Cl in meq/L and SIDe as, CO2 + albumin gram per liter * 0.3252, ignoring serum Mg, PO4 and arterial blood gases. Correction factor of AG for albumin was 2.5 meq/g/dL. SIG was the difference between SIDa and SIDe.

**Results:** AG, uncorrected for serum albumin was 13 ± 5.4(normal range 3 to 11), corrected for albumin 16.8 ± 5.6 meq/L, SIDa 41.5 ± 8.4 meq/L, SIG 13.5 ± 2 meq/L. Serum CO2 < 24 was present in 228/299, AG > 11 meq/L in 174/229, AG corrected for albumin (AGc) was >11 in 297/299, SIDa <40 meq/L in 122/279, SIG >5, in 281/299. Sensitivity of bicaronate <24, AG>11, AGc >11, SID <40, SIG >5 meq/L was 75.2, 58, 99.3, 40.8, and 93.9% respectively. The correlation between serum lactate and AGc was 0.521, lactate vs SIG 0.534, and SIG vs AGc was 86.35%. Table 1 shows patient characteristics.

| Parameter | Value |
|---|---|
| mean age, years | 67 |
| Na meq/L | 137.9 ±5.8 |
| K , meq/L | 4.5 ± 1 |
| Cl, meq/L | 105 ± 7.3 |
| CO2, meq/L | 19.9 ± 6 |
| Ca mg/dL | 8.3 ± 1.2 |
| Albumin g/dL | 2.5 ± 0.9 |

**Conclusions:** Although uncorrected anion gap has a low sensitivity for detecting lactic acidosis, AG corrected albumin and SIG have a high sensitivity for detecting LA. However the correlation between currently avaliable parameters with lactic acid level is poor. This emphasizes need to include several parameters rather than a single one.

*Funding:* Veterans Affairs Support

## PUB254

**Hyponatremia in Hospitalized Patients: Management Discordance** Chelsea Estrada, Michael Petry, Adebayo Shakir Adewale, David L. Luftman, Nand K. Wadhwa. *Nephrology/Medicine, Stony Brook Medicine, Stony Brook, NY.*

**Background:** Hyponatremia is the most common electrolyte abnormality in hospitalized patients and carries significant morbidity and mortality.

**Methods:** We investigated if hyponatremia is managed appropriately in our academic tertiary care center. We reviewed 1344 serial electronic medical records retrospectively on 3 different medical services, over a six month period. Hyponatremia was defined as 2 or more serum sodium (Na) <135 mEq/L.

**Results:** 141 (10.5%) patients (85 M and 56 F, mean age 65.9 yrs) were found to be hyponatremic during their hospitalization. 84 (59.5%) had mild hyponatremia (Serum Na 131-134), 46 (32.6%) had moderate (Na 126-130) and 11 (7.8%) had severe (<126). In the hyponatremic group, mean serum sodium on discharge (133.8) was unchanged to admission (133.7, p=.56). Standard diagnostic criteria, serum osmolarity, urine osmolarity and urine electrolytes were not checked in 74/84 (88.1%), 34/46 (73.9%) and 6/11 (54.5%) of the hyponatremic patients in the mild, moderate and severe groups respectively. The mean lowest sodium was 128.6 in those that had any diagnostic work up done, compared to 130.9 in the group that had none (p<.001). At least one management discordance (either giving IV NaCl 0.9 % solution with diuretics or with a salt/fluid restricted diet) was seen in 52 (37%) of the hyponatremic patients. No significant difference in the management discordance was seen in the mild versus the severe groups. Length of stay was longer in the hyponatremic patients with management discordance than the patients without. Importantly, mean length of stay was 7.7 days in the hyponatremic groups compared to 4.3 days in the normonatremic group (P<.001).

**Conclusions:** Diagnostic workup was significantly less comprehensive for patients with mild hyponatremia compared to those with moderate or severe hyponatremia. Longer length of stay had both a positive correlation with lower serum sodium levels and with greater Δ Na levels, indicating that larger fluctuations in sodium levels may be an indicator of disease severity. Hyponatremia management remains suboptimal and further studies are needed to understand it and possible educational avenues to improve it.

## PUB255

**Interim Analysis of a Multi-National, Observational Safety Study of Tolvaptan in the Treatment of SIADH** Christian Stefan Haas,[1] Volker Rolf Burst,[2] Isabelle A. Runkle de la Vega,[3] Edward Jude,[4] Jayadave Shakher,[5] Duncan Gould,[6] Robert Goedde.[7] *[1]Univ of Luebeck, Luebeck, Germany; [2]Univ of Cologne, Cologne, Germany; [3]Hospital Clínico San Carlos, Madrid, Spain; [4]Tameside General Hospital, Ashton-under-Lyne, United Kingdom; [5]Birmingham Heartlands Hospital, Birmingham, United Kingdom; [6]Otsuka Pharmaceutical Europe Ltd., Uxbridge, United Kingdom; [7]Otsuka Frankfurt Research Institute GmbH, Frankfurt, Germany.*

**Background:** Hyponatremia (HN) is the commonest electrolyte disorder in in-patients with SIADH. Tolvaptan, a selective vasopressin-2 receptor antagonist, is the only vaptan approved in Europe for SIADH. Despite its good safety profile in prospective studies there is concern of possible rapid sodium correction in a real-life setting and increased risk of osmotic demyelination syndrome (ODS). Study objective was to assess tolvaptan drug utilization patterns and safety in clinical practice.

**Methods:** Prospective observational study in 7 European countries (65 sites). In-hospital prescription data and tolvaptan usage were studied. HN-associated symptoms, co-morbidities, co-medication and course of serum sodium were studied.

**Results:** Data from 664 prescriptions were available; 86% SIADH. The course of 252 patients was analyzed; 96% had a diagnosis of SIADH. Co-morbidities included neoplasms (47%), psychiatric disorders (21%), disorders of nervous (26%) or respiratory system (22%). Median age was 72 years (range 26-96). At baseline, 49% of patients had HN symptoms. Serum sodium at baseline, day 4 and week 4 was 123.2±6.5 (mean±SD), 131.8±5.6 and 134.0±6.5 mmol/L, respectively. Maximum daily dose ranged from 3.75 to 60mg (median 15mg). Most patients received tolvaptan daily, some received it less frequently. Treatment length varied from 1 to 857 days (median 17 days). Rapid correction of HN ($\Delta S_{Na} \geq 12$ mmol/L/24h) occurred in 15% of patients, no cases of ODS occurred.

**Conclusions:** In routine medical practice tolvaptan: is used for the indication of SIADH in the majority of cases; is an effective treatment for HN; may result in overly rapid correction of HN. Despite occurrence of rapid correction ODS has not been observed in this study to date.

*Funding:* Pharmaceutical Company Support - Otsuka Pharma Europe Ltd.

## PUB256

**Aquaporin-10 May Not Be Physiologically Important in Mammals as Some Are Pseudogenes** Kenichi Ishibashi,[1] Ryouya Koma,[1] Kenma Nozaki,[1] Yasuko Tanaka,[1] Yoshiyuki Morishita.[3] *[1]Medical Physiology, Meiji Pharmaceutical Univ, Kiyose, Tokyo, Japan; [2]Nephrology, Jichi Medical School, Shimotuke, Tochigi, Japan.*

**Background:** The role of AQP10 is unclear as we reported previously that mouse AQP10 is a pseudogene (BBRC 294: 630, 2002). Human authentic AQP10 is located intracellularly at enterochromaffin cells of the small intestine, while its non-functioning splice-variant at the brush border membranes. We speculated that AQP10 will be a pseudogene in some other animals.

**Methods:** We searched genome databases for AQP10 orthologues and sequenced them with the analyses of their mRNA expressions by RT-PCR. We also analyzed the AQP10 gene of 100 people by direct exon sequencing from the genomic DNA.

**Results:** We found that the AQP10 of some mammals has insertions and deletions in the exons, including cattle (Bos taurus), sheep (Ovis aries) and goat (Capra hircus). In bovine AQP10, exon1 and 5 lack 'C' and 'CTT''CA' respectively to be frame-shifted and prematurely terminated, which was confirmed by the direct all exon sequencing of the genomic DNA. The PCR primer sets for exon1/2 and exon4/5 revealed only genomic bands without bands for mRNA in the duodenum and jejunum. Similar results were obtained by the analyses of the sheep and the goat AQP10s. Since above animals are derived from pig ancestors, swine (Sus scrofa) AQP10 was also analyzed to find that the exons were complete and expressed in the jejunum. Therefore, AQP10 might turn to a pseudogene around 65 million years ago when the cattle evolved. Although the rat (rattus norvegicus) AQP10 mRNA is complete, it was not detected in all the GI tracts, kidney, testis, uterus, eye, and skin, suggesting that rat AQP10 is also a pseudogene as mouse AQP10 is. The dog (Canis familiaris) AQP10 mRNA was detected not only in the small intestine and stomach but also in the cecum and colon where enterochromaffin cells are absent. No mutation was detected in the exons of AQP10 in hundred humans.

**Conclusions:** AQP10, selectively expressed in the GI tracts, may have lost its role or been replaced by other aquaglyceroporins, AQP3 and AQP7 in some branches of mammals.

*Funding:* Government Support - Non-U.S.

## PUB257

**Symptomatic Chronic Hyponatremia in a Young Female due to Nephrogenic Syndrome of Inappropriate Antidiuresis: Response to Tolvaptan** Vimal Chadha, Uri S. Alon. *Nephrology, Children's Mercy Hospitals and Clinics, Kansas City, MO.*

**Background:** First reported in 2005 in two unrelated male infants, nephrogenic syndrome of inappropriate antidiuresis (NSIAD) was found to be caused by gain-of-function mutations in the V2 vasopressin receptor (V2R). Since then a few other cases including two families with this syndrome have been reported. Transmitted in an X-linked mode, all reported cases so far have been of males, with abnormal water loading test in female relatives. All reported patients had missense mutations in the V2R gene and these patients did not respond to Tolvaptan (inhibitor of AVPR2) where it was tried.

**Methods:** Seventeen year old previously healthy girl presented with dizziness, and short-term memory loss, and was found to have serum sodium (S. $Na^+$) of 128 mEq/L. Her clinical picture was suggestive of SIADH (euvolemia, low serum osmolality, increased urinary osmolality and sodium level) but extensive investigations failed to delineate any underlying cause for SIADH. Kidney, thyroid and adrenal function tests were normal; urine drug screen was negative. Review of medical records revealed normal S. $Na^+$ of 142 mEq/L over a decade ago. She was managed as possible SIADH patient, with water restriction that resulted in modest improvement in S. $Na^+$ to 133 mEq/L. A month later her symptoms recurred with a drop in her S. $Na^+$ to 128 mEq/L. Serum ADH levels were undetectable on both occasions as confirmed in two different labs. Later, her S. $Na^+$ dropped to 119 mEq/L when she was given water prior to a renal ultrasound. She was suspected to have NSIAD and blood was sent for DNA sequencing of V2R gene, that did not show any mutation. She responded to Tolvaptan 7.5 mg daily and her current S. $Na^+$ is stable between 140 to 145 mEq/L with urine osmolality < 200 mOsm/kg.

**Conclusions:** All previously reported cases of NSIAD were males with activating mutations of V2R. Tolvaptan has not been effective when used. This is a first case report of NSIAD in a female with symptomatic hyponatremia that responded to Tolvaptan therapy. The case suggests (1) possible yet unknown mutations or mechanisms of water balance dysregulation resulting in NSIAD, (2) trial of Tolvaptan in cases of NSAID.

## PUB258

**Unexpectedly High Frequency of Reset Osmostat and Cerebral-Renal Salt Wasting in Nonedematous Hyponatremia: Value of Determining Total, Extracellular and Intracellular Water Volumes and Fractional Excretion of Urate** John K. Maesaka,[1] Louis J. Imbriano,[1] Joseph Mattana,[1] Dympna Gallagher.[2] *[1]Medicine, Winthrop-Univ Hospital, Mineola, NY; [2]Body Composition Unit, St. Lukes-Roosevelt Hospital, New York, NY.*

**Background:** The volume approach to the differential diagnosis of hyponatremia (HN) has hampered insights into its etiology.

**Methods:** We measured total body (TBW) and extracellular (ECW) water volumes by deuterium and sodium bromide, respectively, in 23 of 57 HN patients with a wide spectrum of comorbidities, fractional excretion (FE) of urate, normal 4-11%, and urine osmolality in random urine and during saline infusion. We used the following criteria: normal to high water volumes (WV) for reset osmostat (RO) and SIADH, low WV for renal salt wasting (RSW), normal FEurate and dilute random urine for RO, increased FEurate for SIADH and RSW, normalization of FEurate after correcting HN for SIADH, persistently increased FEurate after correcting HN for RSW and induction of dilute urine by saline for RSW because of removal of volume stimulus for ADH.

**Results:** The following data and conclusions with ample literature support were obtained: 1) 19 with normal FEurate had RO-8 with dilute random urine and 6 with increased WV. 2) 19 with increased FEurate had RSW-9 had decreased WV, 9 had dilute urines induced by saline (not seen in SIADH), 16 had no evidence of cerebral disease and 6 had persistently increased FEurate after correcting the HN. 3) 12 with increased FEurate had SIADH, 8 had increased WV, and 3 had normal FEurate after correction of HN. 4) 7 with high FEurate and HN did not have enough data to differentiate SIADH from RSW.

**Conclusions:** Determination of FEurate is superior to the volume approach in HN; RSW is known to be much more common than SIADH in neurosurgical patients; the unexpectedly high prevalence of RO and RSW in a non-neurosurgical population creates a need to change cerebral salt wasting to RSW because RSW would otherwise not be considered in the absence of cerebral disease; patients with RO and SIADH are hypervolemic, not euvolemic; RO is a separate clinical entity from SIADH because of a normal FEurate and predictable response to water-loading.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical-US

**PUB259**

**Ineffectiveness of Volume Approach to Hyponatremia: Proposal of New Approach** Louis J. Imbriano,[1] Dympna Gallagher,[2] Nobuyuki (Bill) Miyawaki,[1] Joseph Mattana,[1] John K. Maesaka.[1] *[1]Medicine, Winthrop-Univ Hospital, Mineola, NY; [2]Body Composition Unit, St. Lukes-Roosevelt Hospital, New York, NY.*

**Background:** There is awareness that the volume approach to evaluation of HN has been unsuccessful. We present 4 cases of hyponatremia (HN) that support this viewpoint.

**Methods:** Two females, ages 64 and 84 and 2 males, ages 71 and 73 yrs. met criteria for SIADH and renal salt wasting (RSW).

**Results:** Serum sodium (Na) ranged from 116-121 mmol/L, concentrated urine, urine Na 23-95 mmol/L, increased fractional excretion (FE) of urate, 16.8-22.7% (normal 4-11%) and normal renal, adrenal and thyroid function. Two non-edematous females, 1 had Lyme disease and 1 had hypertension; 1 male had 2+ edema of both legs due to inferior vena caval obstruction by stage IV B cell lymphoma. All 3 patients had postural hypotension and tachycardia. In 2 patients, saline infusion induced excretion of dilute urines, plasma ADH being appropriately undetectable in the edematous patient with lymphoma; FEurate remained increased after correction of HN in both females that was consistent with RSW as compared to normalization in SIADH. Total (TBW) and extracellular body water (ECW) as determined by deuterium and sodium bromide, respectively, were decreased in the 2 nonedematous females. The increased FEurate, which persisted after correction of HN, dilution of urine with saline infusion with appropriate inhibition of ADH in the edematous patient, postural hypotension with reflex tachycardia, and decreased TBW, ECW and intracellular water in the two nonedematous patients collectively support the diagnosis of RSW. The fourth patient had pleural effusion and edema after cardiac bypass surgery with increased FEurate of 19.3%, which normalized to 6% after correcting the HN as in SIADH and not RSW. A high FEurate is very unusual in edematous patients, usually <4%. None of the patients had cerebral disease.

**Conclusions:** RSW was noted in 3 patients-2 by critical volume studies and 1 had edema; SIADH can occur with edema and pleural effusion. None had clinical cerebral disease which justifies proposal to change cerebral to RSW. We demonstrate the superiority of determining FEurate to the volume approach in HN.

*Funding:* Pharmaceutical Company Support - Otsuka Pharmaceutical-US

**PUB260**

**Cost-Utility Analysis of Tolvaptan versus Water Restriction for Euvolemic or Hypervolemic Hyponatremia** David J. Yoo, Lisa K. Prince, Dustin J. Little, Robert Nee, Christina M. Yuan. *Walter Reed National Military Medical Center.*

**Background:** Hyponatremia is associated with increased morbidity and mortality. Tolvaptan, an oral vasopressin (V2) receptor antagonist, increases serum sodium concentration vs. placebo in subjects with mild to moderate hyponatremia (SALT-1/SALT-2 trials, NEJM 2006), with increased scores on the Mental Component Summary of the SF-12 General Health Survey. We performed a cost-utility analysis of tolvaptan vs. water restriction based on data from the SALT Trials.

**Methods:** A computer-based decision model was developed using TreeAge Pro software (Williamstown, MA) to represent the costs and effectiveness of tolvaptan 30 mg daily vs. water restriction alone for outpatient treatment of euvolemic/hypervolemic hyponatremia. Costs, utility indices, and probabilities were obtained from literature review. Model time horizon was 30 days. A one-day hospital admission was required for tolvaptan initiation. Probabilities of resolving hyponatremia, and utilities for resolved (0.68) and unresolved (0.55) hyponatremia were obtained from the SALT trials (Cyr, et al. Abstract 25379. ISPOR. 2009). The primary endpoint was the incremental cost-effectiveness ratio (ICER) in dollars per quality-adjusted year of life (QALY) gained.

**Results:** The ICER was $2,800,000 per QALY gained, using a cost of $250 per 30 mg tolvaptan. One-way sensitivity analysis, using an arbitrary willingness to pay (WTP) threshold of $1,000,000, demonstrated water restriction to be the cost-effective option unless the utility of resolved hyponatremia was > 0.902, or the cost of tolvaptan was < $71.22/day. Water restriction was more cost-effective than tolvaptan in 100% of model iterations using Monte-Carlo probabilistic sensitivity analysis. The expected value of perfect information was $0.00, at a WTP $1,000,000.

**Conclusions:** Tolvaptan is not cost-effective as compared to water restriction alone for the treatment of outpatient euvolemic/hypervolemic hyponatremia, because of the requirement for hospital admission and the daily cost of the drug. This analysis may help to guide decisions regarding adoption of tolvaptan in this setting.

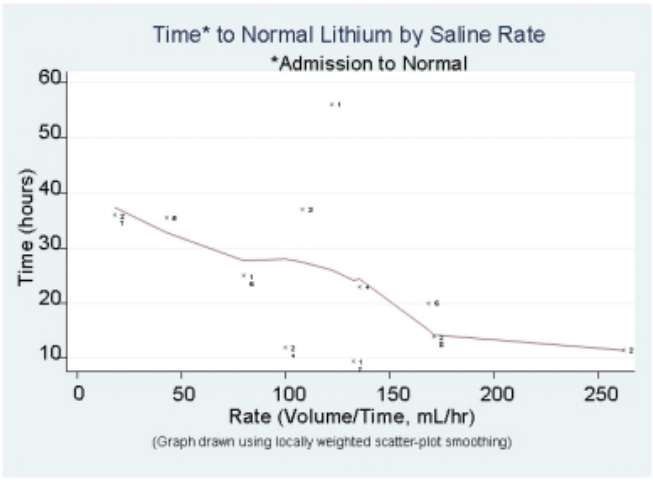
**PUB261**

**Saline Diuresis for Lithium Intoxication Revisited 30 Years Later** Wei Boon Ooi, Francisco Hernandez Munoz, Alina Livshits, Monique Carreno, Alexander B. Knee, Anthony E. Poindexter, Gregory Lee Braden. *Dept of Medicine, Baystate Medical Center, Springfield, MA.*

**Background:** Reviews and textbooks do not recommend saline diuresis as therapy for lithium (Li) intoxication. Past case reports ('70s) showed forced saline diuresis (>250ml/hour 0.9% NaCl) may be helpful. We now describe patients with Li intoxication & correlate saline administration with clinical outcomes.

**Methods:** Patients were identified by ICD-9 codes from 2007-2012. Exclusion criteria included incomplete medical records & patients who were dialysed. Time from admission to normal serum Li level (≤1.0mmol/L), Tn, was used as surrogate marker for Li clearance. Main outcomes were rate of Li clearance due to saline diuresis & potential complications of saline diuresis.

**Results:** From 19 patients, 8 were excluded (4 dialysed, 4 incomplete records). Median age was 49 years. The majority (82%) were female & white. Bipolar disorder was the most common psychiatric diagnosis. Median baseline renal function was 132 ml/min (Cockroft-Gault). Five out of eleven patients had chronic intoxication. Symptoms at presentation included decreased level of consciousness, coarse tremors & tachycardia. Median peak Li level was 2.4 mmol/L. Median total saline administration from admission to Tn was 2400mL. Median saline rate from admission to Tn was 122.3ml/hour. Spearman correlation between saline rate & time to normal serum Li level was -0.546. Tn was less than 24 hours in 3 patients receiving >150ml/hr of saline. No patient had adverse complications from saline administration.




**Conclusions:** There was a trend for more rapid improvement of serum Li levels in lithium intoxicated patients with greater rates of saline administration although it was not statistically significant given our small sample size. Prospective studies are needed to re-examine the efficacy of forced saline diuresis in Li intoxication.

**PUB262**

**Statistical Model for Evaluating Efficacy of Oral Zirconium Silicate in Treatment of Hyperkalemia** Philip T. Lavin,[2] Stephen R. Ash,[1] Henrik S. Rasmussen,[1] Alvaro F. Guillem,[1] Fiona Stavros,[1] Donald Jeffrey Keyser,[1] Robert Alexander,[1] Roland Winger.[1] *[1]ZS Pharma Inc., Coppell, TX; [2]Boston Biostatistics Research Fdn, Boston, MA.*

**Background:** A Phase 2 proof of principle RCT (ZS-002) was conducted by ZS Pharma to evaluate three doses (0.3g tid, 3g tid, 10g tid) of active therapy and placebo control in 90 patients with renal impairment to evaluate the reduction in serum potassium (s-K) during the first 48 hours following therapy initiation. A total of 12 post-baseline assessments were available for evaluation and modeling including an averaged baseline s-K over the prior 24 hours.

**Methods:** A longitudinal model (SAS PROC MIXED) was pre-defined in accordance with regulatory requirements. The model evaluated the log s-K as a means to stabilize variance and to represent the anticipated drug effect during the 48 hour assessment. The model included all post-baseline observations including the actual hour when the data were collected. Time and time-treatment interactions were included in the model with subject as a random effect. The model accommodated all available intent-to-treat data.

**Results:** The overall goodness of fit of the model was excellent with p<0.0001. The model-derived standard deviation (SD) estimate was 0.102, which was within 10% of the assumed SD. Statistical significance was established for the two highest doses relative to placebo control. The study power exceeded 80% to detect the preplanned 10% relative difference between active dose versus control in a pre-defined hierarchy from highest to lowest dose.Alternative post hoc models were investigated, however all other models were unable to match the goodness of fit of the following pre-defined model: */** Model for 48 hour endpoint **/ ods output Solutionf=mod_48; **procmixed**data=labs; class trtgrp subject; model val=timpnt trtgrp*timpnt/solution; random intercept timpnt/subject=subject*trtgrp type=un;**run**;* Data will be presented on the ability of the model to predict the patient-specific 48-hour outcome based on the pre-treatment baseline and the randomized dose group as a means to avoid hypokalemia.

**Conclusions:** A highly efficient model for the analysis of 48-hour s-K data has been identified.

*Funding:* Pharmaceutical Company Support - ZS Pharma, Inc.

**PUB263**

**Fractional Excretion of Urate Provides Insights into Pathophysiology of Hyponatremia in Addison's Disease and Myxedema** James Drakakis,[1] Louis J. Imbriano,[1] Naheed Ansari,[2] Joseph Mattana,[1] John K. Maesaka.[1] *[1]Dept of Medicine, Winthrop Univ Hospital, Mineola, NY; [2]Dept of Medicine, Jacobi Medical Center, Bronx, NY.*

**Background:** Fractional excretion (FE) of urate provides insights into the etiology of hyponatremia (HN). Low FEurate of <5% is most common in edematous states such as heart failure and in volume depleted patients with normal renal function. Normal FEurate

is most common in reset osmostat, while FEurate is increased in cerebral-renal salt wasting (RSW) and SIADH.

**Methods:** We present a patient with Addison's disease (AD) and another with myxedema (MX), where FEurate provided insight into the pathophysiology of HN. A 51 year old female with AD was admitted with low blood pressure, serum sodium (SNa) 119, potassium 5.4 mmol/L, low AM cortisol 3.1ug/dl, high ACTH 865.1 pg/ml, urine (U) Na 116 mmol/L, urine osmolality (Uosm) 537 mosm/kg and low FEurate of 2 and 3% (normal 4-11%). A 74 year old male with MX was admitted with SNa 116, UNa 44 mmol/L, Uosm 300 mosm/kg, high FEurate of 26.6%, TSH 39.7 mIU/L and free T4 0.53 ng/dL. Both had normal renal function.

**Results:** HN in AD is associated with an appropriate increase in plasma ADH secretion due to extracellular volume (ECV) depletion resulting from defective distal tubule (DT) Na transport with intact function of the proximal tubule (PT) where urate is exclusively transported. This induces a prerenal state with low FEurate. In MX, high FEurate is consistent with RSW and SIADH. Studies in MX rats demonstrate a 17.1% reduction in ECV as determined by inulin space and a 28% reduction in PT Na reabsorption by micropuncture methodology. These data are consistent with the increase in FElithium from 29 to 42% when plasma from patients with RSW was infused into rats. This suggests a major defect in PT Na transport in RSW since lithium is transported 1:1 with Na in the PT and no transport in the DT.

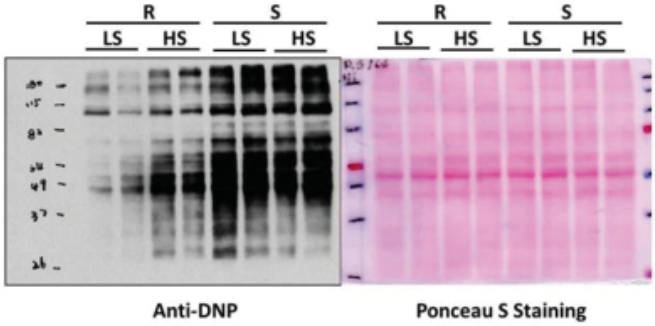
**Conclusions:** The HN in AD and MX is due to an appropriate increase in ADH induced by ECF volume depletion, defective Na transport in the DT in AD and PT in MX. The low FEurate in AD is consistent with intact PT function whereas the high FEurate in MX is consistent with a major defect in PT solute transport.

## PUB264

**The Implication of Overstimulation of Redox-Sensitive Na/K-ATPase Signaling in Dahl Salt Sensitivity** Yanling Yan,[1,3] Anna P. Shapiro,[2] Steven T. Haller,[2] Muhammad A. Chaudhry,[1] Jiang Tian,[2] Zi-jian Xie,[2] Deepak K. Malhotra,[2] Nader G. Abraham,[1] Joseph I. Shapiro,[1,2] Jiang Liu.[1] *[1]Marshall Univ JCE School of Medicine, Huntington, WV; [2]Univ of Toledo College of Medicine, Toledo, OH; [3]Yanshan Univ, Qinhuangdao, China.*

**Background:** Impairment of renal proximal tubule (RPT) Na/K-ATPase signaling contributes to Dahl salt-sensitive hypertension, but there is no difference in α1 gene coding, ouabain-sensitivity, and α1 expression between Dahl salt-resistant (R) and salt-sensitive (S) rats.

**Results:** A high salt diet (2% NaCl for 7 days) significantly stimulated RPT Na/K-ATPase signaling, protein carbonylation (whole cell lysate and Na/K-ATPase α1 subunit) and heme-oxygenase-1 (HO-1) expression in isolated RPTs in Dahl R but not S rats (12-14 week old). Compared to age- and gender-matched R rats fed with a low salt diet (0.3% NaCl), isolated RPTs from the S rats have a significantly higher basal level of protein carbonylation and HO-1 that do not respond to a high salt stimulation.




In isolated R rat PRTs, ouabain (10 μM)-stimulated α1 carbonylation, HO-1 expression and Na/K-ATPase signaling were significantly attenuated by pre-treatment with the antioxidant *N*-acetyl-L-cysteine (NAC, 10mM, 30min). However, the effect of ouabain on carbonylation was not observed in overstimulated S rats.

In porcine RPT LLC-PK1 cells, ouabain-stimulated protein carbonylation of the a1 subunit and HO-1 expression depend on Na/K-ATPase signaling, suggesting a redox-sensitive Na/K-ATPase signaling as well as a dynamic cellular adaptation (HO-1 and alike) to counterbalance ouabain-induced oxidative stress.

**Conclusions:** Oxidative stress affects RPT Na/K-ATPase signaling and sodium handling. Overstimulation of ROS/carbonylation in the S rats at baseline might desensitize the Na/K-ATPase signaling that is no longer responding to a high salt diet or ouabain.

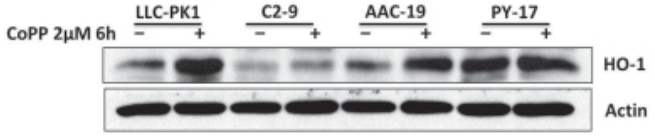
*Funding:* Other NIH Support - NIH RO1 HL-109015 to Z.X. and J.I.S. and HL105649 to J.T.

## PUB265

**The Involvement of the Na/K-ATPase in HO-1 Induction by Ouabain and CoPP** Yanling Yan,[1,3] Anna P. Shapiro,[2] Muhammad A. Chaudhry,[1] Zi-jian Xie,[2] Deepak K. Malhotra,[2] Nader G. Abraham,[1] Joseph I. Shapiro,[1,2] Jiang Liu.[1] *[1]Marshall Univ JCE School of Medicine, Huntington, WV; [2]Univ of Toledo College of Medicine, Toledo, OH; [3]Yanshan Univ, Qinhuangdao, China.*

**Background:** Both CoPP (Cobalt protoporphyrin, an inducer of heme-oxygenase-1, HO-1) and ouabain induce HO-1 expression in porcine renal proximal tubule LLC-PK1 cells.

**Results:** In LLC-PK1 cells, ouabain-stimulated c-Src activation, ROS generation and protein carbonylation of the Na/K-ATPase α1 subunit were significantly attenuated by the antioxidant *N*-acetyl-L-cysteine (NAC) and disruption of the Na/K-ATPase signaling. Interestingly, both ouabain and CoPP also significantly induced HO-1 expression in LLC-PK1 cells. To determine the role of the Na/K-ATPase and its signaling, we used three stable cell lines generated from LLC-PK1 cells, PY-17 (the Na/K-ATPase α1 subunit knock-down cells), C2-9 (caveolin-1 knockout cells), and AAC-19 (PY-17 cells only expressing rat α1) cells. Both PY-17 and C2-9 show disrupted Na/K-ATPase signaling. Both ouabain and CoPP significantly induced HO-1 expression in AAC-19 cell as seen in LLC-PK1 cells. However, depression of Na/K-ATPase expression and disruption of Na/K-ATPase signaling not only prevented ouabain-induced HO-1 expression, but also prevented CoPP-induced HO-1 expression.




Induction of HO-1 by CoPP partially but significantly attenuated ouabain-induced c-Src activation.

**Conclusions:** In LLC-PK1 cells, ouabain stimulated a dynamic antioxidant adaptation (HO-1 and alike) to counterbalance ouabain-induced oxidative stress. Furthermore, the Na/K-ATPase and its signaling function as a functional receptor of CoPP-induced HO-1 expression.

*Funding:* Other NIH Support - NIH RO1 HL-109015 to Z.X. and J.I.S.

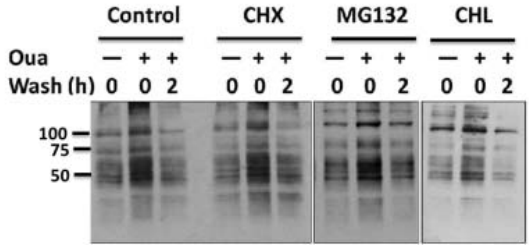
## PUB266

**Ouabain-Induced Protein Carbonylation of the Na/K-ATPase α1 Subunit Is Reversible in LLC-PK1 Cells** Yanling Yan,[1,3] Anna P. Shapiro,[2] Muhammad A. Chaudhry,[1] Zi-jian Xie,[2] Deepak K. Malhotra,[2] Nader G. Abraham,[1] Joseph I. Shapiro,[1,2] Jiang Liu.[1] *[1]Marshall Univ JCE School of Medicine, Huntington, WV; [2]Univ of Toledo College of Medicine, Toledo, OH; [3]Yanshan Univ, Qinhuangdao, China.*

**Background:** In LLC-PK1 cells, ouabain-induced protein carbonylation of the Na/K-ATPase α1 subunit is involved in the Na/K-ATPase signaling and sodium reabsorption.

**Results:** To assess whether ouabain-induced protein carbonylation is reversible, LLC-PK1 cells were treated with ouabain (100nM, 1h) to induce protein carbonylation of the α1 subunit, and then either collected as ouabain-treated (Oua) or washed to remove un-bound ouabain (by extensive washing with pre-warmed culture medium) and further cultured in ouabain-free medium for 2h (Oua+wash). Comparing to control and ouabain-treated (Oua) cells, ouabain-induced protein carbonylation of the α1 subunit was reversed by removal of un-bound ouabain.

To test if this carbonylation/decarbonylation process is due to *de novo* protein synthesis or degradation, LLC-PK1 cells were pre-treated for 2h with different inhibitors and aforementioned experiment was repeated in the presence of the inhibitors. The inhibitors used included protein biosynthesis inhibitor cycloheximide (20μg/ml), proteasome inhibitor MG132 (10μM), and lysosomotropic weak base agent chloroquine (100μM). In comparison with experiments without inhibitors, the decreased carbonylation of the α1 subunit was largely independent of *de novo* protein synthesis and degradation through lysosome and proteasome pathways, leading us to speculate a likely enzyme-driven mechanism of the removal of the carbonyl group, by either a known enzyme system or an unidentified enzyme-like protein.




**Conclusions:** Ouabain-induced protein carbonylation of the α1 subunit was reversible that is largely independent of *de novo* protein synthesis and degradation.

*Funding:* Other NIH Support - NIH RO1 HL-109015 to Z.X. and J.I.S.

## PUB267

**Abnormal Urinary Excretion of ENaCγ after Hypertonic Saline Infusion in Chronic Kidney Disease** Janni Majgaard Jensen,[1,2] Frank H. Mose,[1] Jesper N. Bech,[1] Erling B. Pedersen.[1] *[1]Depts of Medical Research and Medicine, Holstebro Regional Hospital, Holstebro, Denmark; [2]Aarhus Univ, Denmark.*

**Background:** Patients with chronic kidney disease (CKD) have a diminished capacity to concentrate urine. We wanted to test the hypothesis that this phenomenon is caused by abnormal activity in the aquaporin2 water channels and/or the epithelial sodium channels in the distal nephron.

**Methods:** The study comprised of 23 patients with CKD and 24 healthy controls, who consumed a 4- day standardized diet (120 mmol sodium/day) prior to the study day. At baseline conditions and after 3 % hypertonic saline infusion, we measured urinary

concentrations of AQP2 (u-AQP2) and gamma subunit of ENaC (u-ENaCγ,) free water clearance ($C_{H2O}$), urinary osmolality (u-Osm) and fractional excretion of sodium ($FE_{Na}$). GFR was measured by constant infusion clearance technique using $^{51}$Cr-EDTA as reference substance. Plasma concentrations of vasopressin (AVP), renin (PRC), Angiotensin II (AngII) and Aldosterone (Aldo) were determined by radioimmunoassay (RIA). Extracellular body fluid volume (ECV) was calculated by Body Composition Monitor.

**Results:** At baseline, GFR was 34 ml/min in patients and 89 ml/min in controls. CKD patients had a high u-ENaCγ, $FE_{Na,}$ PRC, p-Aldo and p-AVP and a low $C_{H2O}$compared to controls. No differences in u-AQP2 or ECV were measured between patients and controls. Hypertonic saline infusion caused an increase in u-ENaCγ in controls ($p<0.05$), but there was no change in u-ENaCγ in patients. Controls had a higher increase in u-osm and a larger decrease in $C_{H2O}$ compared to patients, but the increase in u-AQP2, $FE_{Na}$ and p-AVP were similar. PRC, AngII and Aldo decreased in both groups all though levels were higher in patients. ECV increased to the same extent in both groups.

**Conclusions:** In response to hypertonic saline, u-ENaCγ increased in controls and did not change in CKD patients. This abnormal response in u-ENaCγ in CKD patients might be caused by a less efficient decrease in reabsorption of sodium in the proximal tubules and subsequently no need for adjustment in the distal nephron via ENaC.

*Funding:* Private Foundation Support, Government Support - Non-U.S.

## PUB268

**Hypomagnesemia in Critically Ill Patients with Sepsis** Ashish Kataria,[1] Daniel W. Ross,[1] Nirav Mehta,[1] Myriam Kline,[2] Kenar D. Jhaveri,[1] Steven Fishbane.[1] *[1]Kidney Diseases and Hypertension, Hofstra NSLIJ School of Medicine, NY; [2]Biostatistics, Feinstein Institute of Medical Research.*

**Background:** Hypomagnesemia has been reported in 20% to 65% of patients in an ICU setting. Our study aimed to evaluate the frequency of hypomagnesemia in critically ill patients with sepsis, determine clinical predictors for its development, and to ascertain how hypomagnesemia affects patient outcomes in the ICU setting.

**Methods:** A single center, retrospective cohort study was conducted. All ICU patients with sepsis within the last year were evaluated. A group with normal Mg levels was compared to low Mg group. Baseline demographics, medications use, and lab data were collected. Primary outcome was the frequency of patients that developed hypomagnesemia. The adverse outcomes studied from were: ventilator requirement , days on ventilator, ICU length of stay and mortality during the hospital stay. Mg level of <1.6mg/dl was used as the cutoff level. Logistic regression was used to model hypomagnesemia as a function of key demographic clinical variables.

**Results:** Hypomagnesemia was observed in 23% of (n=99) studied patients. There were no significant differences between the groups at baseline on any demographic or laboratory variables. Risk factors in developing hypomagnesemia such as diarrhea, chemotherapy use, use of PPI's and diuretic were not significantly associated with the development of hypomagnesemia. Nevertheless, 77% patients who had low Mg levels were administered PPI's as compared to 53% in the normal Mg group, (p value=0.76). The development of hypomagnesemia was not associated with ventilator use, length of ventilator dependence, or length of stay in ICU and mortality.

**Conclusions:** While low Mg was extremely common in the ICU pateints with sepsis, it did not affect their overall ICU stay and mortality. Despite the absence of significant associations of low Mg levels with the studied risk factors and clinical outcomes, the development of hypomagnesemia in 23% of ICU patients having sepsis during their ICU stay warrants a prospective study with a larger sample size to ascertain its relationship to clinical outcomes in this specific patient population.

## PUB269

**The Effects of Tolvapton on Severe Chronic Kidney Disease Patients with Congestive Heart Failure** Kiyoto Koibuchi,[1] Kentaro Tanaka,[3] Toshiyuki Aoki,[1] Moriatsu Miyagi,[1] Ken Sakai,[2] Sonoo Mizuiri.[2] *[1]Dept of Nephrology, Saiseikai Yokohama-City Eastern Hospital, Yokohama, Kanagawa, Japan; [2]Dept of Nephrology, Toho Univ School of Medicine, Ota-ku, Tokyo, Japan; [3]Div of Diabetes and Metabolism, The Institute for Adult Diseases, Asahi Life Foundation, Chiyoda-ku, Tokyo, Japan.*

**Background:** Tolvaptan is a selective vasopressin receptor 2 antagonist and dose-dependent drug used to treat congestive heart failure (CHF) as diuretic. It is known that tolvaptan increases excretion of excess fluids and improves blood sodium levels in patients with heart failure without affecting renal function compared to conventional diuretics. However, few studies examined the effects of tolvaptan for patients with severe chronic kidney disease (CKD). The aim of the study is to examine the effect of tolvaptan on patients with severe CKD.

**Methods:** We analyzed CKD patients with less than 30 of estimated glomerular filtration rate (eGFR) who had CHF. Those of all patients have already treated conventional diuretics, but CHF worsened. We administrated tolvaptan for the patients with CHF in admission. In order to evaluate the effect of tolvapton, we examined urinary volume, body weight, serum sodium and eGFR for three days after administration. The patients who changed the dose of conventional diuretics in observation period were excluded.

**Results:** We evaluated 52 patients consisted of 32 men and 20 women with a mean age of 77.5 ± 10.9 years and a mean eGFR of 17.8 ± 7.4 ml/min/1.73m2 at admission. The mean dose of tolvaptan was 12.0±4.0mg. The urinary volume increased from 1141.1±539.5 ml/day to 1864.5±1008.2 ml/day ($p<0.001$, Wilcoxon signed-rank test). Body weight improved from 59.7 ± 17.6 kg to 57.8 ± 17.0 kg ($p<0.01$, Wilcoxon signed-rank test). Serum sodium elevated from 132 ± 6.3 mEq/l to 139.4 ± 6.4 mEq/l ($p=0.03$, ANOVA and Dunnett's testing). eGFR (17.8±7.4 at baseline) remained 17.1±8.7 after administration ($p=0.34$, Wilcoxon signed-rank test).

**Conclusions:** Tolvaptan is effective diuretic even in sever CKD patient , resistance to conventional diuretic, without worsening renal function.

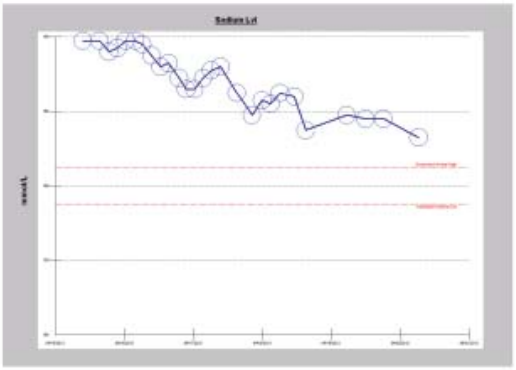
## PUB270

**A New Step-Ladder Approach to Treating Severe Symptomatic Chronic Hypernatremia to Mitigate Catastrophic Cerebral Events** Macaulay A. Onuigbo,[1,2] Ngozi J. Achebe.[3] *[1]Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI; [3]Internal Medicine, Capital Medical Center, Olympia, WA.*

**Background:** Hypernatremia is associated with hyperosmolality and hypertonicity of plasma and intracellular space. The brain and other tissues therefore adapt to avoid pathologic cell shrinkage/dehydration by synthesizing idiogenic osmoles. Severe hypernatremia is much less common than hyponatremia so physicians are less aware of the implications of over-rapid correction of chronic hypernatremia. We describe a new step-ladder approach to managing severe hypernatremia.

**Methods:** Case report.

**Results:** An unresponsive 80 year old Caucasian male patient, a Group Home resident, with cognitive developmental delay from birth, central diabetes insipidus with chronic hypernatremia was admitted with fever, pneumonia, volume depletion and severe hypernatremia (179 mEq/L). In addition to intravenous antibiotics, he required daily intermittent guided free water repletion therapy with intravenous 5% dextrose (100-250 cc/hr) to achieve a step-ladder correction of Na over 72-96 hours. Inhaled DDAVP was added about hospital day 4. This way, the over-rapid correction of chronic hypernatremia by >10-12 mEq/L/day was avoided, thus mitigating the potential but often unrecognized risk of cerebral edema. He was later discharged at baseline mental status, with a Na of 146 mEq/L.



**Conclusions:** With adaptive processes in severe hypernatremia, over-rapid correction produces rapid fluid movement into the brain cells and the development of cerebral edema. Most experts recommend 10-12 mEq/L/day correction. We have described a step-ladder approach of safely managing severe hypernatremia using guided intermittent intravenous 5% dextrose in an unconscious patient. Acute hypernatremia (<12 hours) should be corrected quickly; however, when in doubt, go slow.

## PUB271

**Aggressive Therapy Fails to Influence Outcomes in Hospitalized Hypernatremic Geriatric Patients** Muhammad R. Toor, Anjali Singla, Xenia P. Sumin, Maria V. DeVita, Michael F. Michelis. *Nephrology, NSLIJ-Lenox Hill Hospital, New York, NY.*

**Background:** Hypernatremia is a common electrolyte disorder and is associated with adverse outcomes such as increased length of stay and increased mortality. Prior studies have suggested a variety of factors that may result in undesirable outcomes.

**Methods:** A 3 month retrospective chart review was performed on hospitalized patients over the age of 18 with a serum sodium level >150 mmol/L. This study was designed to characterize the rate of sodium correction, the level of monitoring as indicated by ward setting, whether or not a nephrologist was involved in the case, the type of fluids used, and other relevant data and whether any of these variables influenced patient outcomes.

**Results:** Of 28 patients with hypernatremia, 5 were excluded because of data deficiencies. Of the remaining 23 patients studied, the mean age was 82.6 years old and the mean initial sodium level was 152.3 mmol/L (range: 151–156 mmol/L). Ten (43%) patients were demented and twelve (52%) were on tube feeding. The overall mortality rate was 65%. Sodium level was corrected (≤145 mmol/L) in 15 (65%) patients. Correction did not influence rates of survival (33% corrected vs. 38% not corrected, p=0.99). Seven patients corrected in ≤2 days and 8 in ≥3 days but these differences did not affect survival. Thirteen of 14 patients admitted to or transferred to the ICU expired whereas 2 of 9 patients not in the ICU expired (p=0.001, RR 6.93). Obtaining a nephrology consult did not affect patient mortality (p=0.69). The patients received various IVF and/or oral fluids, including NS, ½ NS, D5W, and oral water, which appeared appropriate to their clinical status. The mean length of stay for all patients was 20.4 days and longer for those who remained alive, 23.6 days.

**Conclusions:** Hypernatremia is associated with a poor prognosis and outcomes are still disappointing despite appropriate rates of correction, intensive monitoring, and the involvement of a medical specialist. This indicates that the disease state associated with hypernatremia appears to be of paramount importance in regard to patient outcomes and early attention to the primary process is essential.

## PUB272

### An Unusual Case of Hyperkalemia Refractory to Hemodialysis

Sindhu Mallik Arjun, Gary Noroian, Robert Mark Black. *Div of Nephrology, Saint Vincent Hospital, Worcester, MA.*

**Background:** Pseudohyperkalemia is typically caused by hemolysis, thrombocytosis or traumatic blood drawing. We report a case of a patient with severe hyperammonemia-associated hyperkalemia.

**Methods:** An 80 year old man presented to our institution with critical aortic stenosis. Before his aortic valve replacement, his serum creatinine was 1.02 mg/dL and his serum potassium concentration was 3.0 meq/L. His post-op course required pressor support and mechanical ventilation. He developed anuric acute kidney injury with a creatinine rise to 4.63 mg/dL. His urine sediment was consistent with acute tubular necrosis. Intermittent hemodialysis was initiated. His serum potassium level remained normal for the first two weeks of dialysis. It then rose to 6.1meq/L. He was dialyzed, but his potassium level two hours after hemodialysis was 6.7meq/L. A repeat potassium level was 7.3meq/L. The plasma level was also high. He was dialyzed again that evening. One hour into the dialysis treatment, the K fell to 5.7meq/L and it was 3.8meq/L at the end of the treatment. Blood pressure was lower and he had a very mild rise in the hepatic transaminases. An ammonia level was 572 umol/L (normal = 19–87). His ammonia level fell during the dialysis treatment, correlating with the decrease in the serum potassium level. While hepatic transaminases rose progressively over the next 24 hours, the acute hyperkalemia and hyperammonemia preceded these changes.

**Conclusions:** We hypothesize that the elevated ammonia (ammonium) level interfered with the serum potassium measurement in our patient. Extremely high serum ammonia levels (>400umol/L), were found to interfere with serum potassium measurements obtained using the OsmetechOPTI critical care analyzer[1]. The proposed mechanism is the cross reactivity of the $NH4^+$ ions with the $K^+$ selective ionophores. Pseudohyperkalemia should be considered in patients with refractory hyperkalemia and very high blood ammonia levels.

1. Carayannopoulos MO, Whilhite TR, Reddy L, Landt M, Smith CH, Dietzen DJ.Equimolar ammonia interference in potassium measurement on the Osmetech OPTI Critical Care Analyzer. ClinChem 2006;52:1603-1604.

## PUB273

### Factors Associated with Calf 100 kHz Impedance in Healthy Subjects

Samer Rateb Abbas,[1,2] Fansan Zhu,[1] Cesar Flores-Gama,[1] Cassandra Cartagena,[1] Caroline M. Williams,[1] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Nephrology, Renal Research Institute, New York, NY; [2]Nephrology, Beth Israel Medical Center, New York, NY.*

**Background:** Calf bioimpedance is used in hemodialysis patients to assess fluid status. Advantages of calf over whole body bioimpedance are ease of use and reliable evaluation of fluid status [Zhu, Physiol. Meas. 2008]. The aim of this study was to investigate associations between body weight, body mass index (BMI), age, gender and race and calf impedance (Zo) at 100 kHz in healthy subjects.

**Methods:** We measured Zo at 100 kHz (ZOE device; Noninvasive Medical Technologies; Las Vegas, NV) in adult subjects 40 to 80 years of age and eGFR > 60 mL/min/1.73 $m^2$ to create reference values. Zo was measured with leg horizontal in sitting subjects 5 minutes after placement of 4 electrodes; measurement took 30 seconds. Simple linear regression analysis was used to quantitate the associations between Zo and body weight, BMI, and age. Groups (genders; black vs. non-black) were compared by unpaired t-test.

**Results:** We studied 62 subjects (age 56±9 years; 31 males; 26 Blacks). Zo was not associated with body weight, BMI, age, gender and race figure A and B.

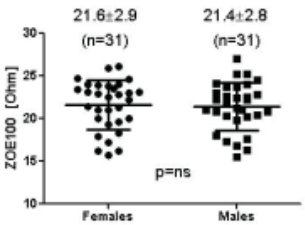



Figure A

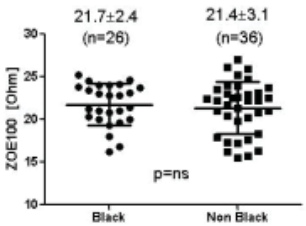



Figure B

**Conclusions:** In healthy subjects calf Zo measurement at 100 kHz is unrelated to key anthropometric and demographic characteristics, thus allowing the construction of Zo reference values irrespective of age, race, gender, body weight, and BMI.

## PUB274

### Dialysis Improved the Survival Rate of Patients with Severe Hyperkalemia

Do Hyoung Kim,[1] Jung Pyo Lee,[2] Jung Nam An,[3] Jin Ho Hwang,[3] Yon Su Kim,[3] Yun Kyu Oh,[2,3] Chun Soo Lim.[2,3] *[1]Dept of Internal Medicine, Chung-Ang Univ College of Medicine, Republic of Korea; [2]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Republic of Korea; [3]Dept of Internal Medicine, Seoul National Univ College of Medicine, Republic of Korea.*

**Background:** Severe hyperkalemia, with potassium levels ≥ 6.5 mEq/L, is a serious complication with high mortality in the hospitalized population. The purpose of this study is to evaluate the efficacy of renal replacement therapy (RRT) and the impact of the timing of RRT in patients with severe hyperkalemia.

**Methods:** We collected clinical data from 909 patients diagnosed with severe hyperkalemia. All were hospitalized between August 2007 and July 2010. The diagnosis of severe hyperkalemia was made either at the time of admission or during the period of hospitalization. Early dialysis (ED) was defined if RRT was started within 12 hours from the diagnosis of severe hyperkalemia. 135 patients (14.9%) who received RRT were compared with 774 patients who received only medical treatment.

**Results:** Of the 909 patients, 156 were end-stage renal disease (ESRD) patients with RRT and 753 were non-ESRD patients. The survival rate of RRT group was better than that of medical treatment group (Log Rank test, P=0.010). This association was also significant after adjusting for age, gender, disease severity, kidney function, and potassium level (HR 0.628, 95% CI 0.419-0.939, P=0.024). In subgroup analysis, RRT increased patients' survival rate in ESRD patients, however, it could not improve survival rate in non-ESRD patients. In non-ESRD patients, RRT group had significantly higher potassium levels and more comobidities than medical treatment group. The average time from diagnosis to dialysis was 9.1 ± 9.6 hours. The mortality rate of ED group was significantly higher than that of late dialysis (LD) group (P=0.007). Potassium level of ED group was higher that of LD group (ED vs. LD, 7.15 ± 0.74 vs 6.86 ± 0.53 mEq/L, P = 0.037).

**Conclusions:** Renal replacement therapy with medical treatment improved clinical outcomes in patients with severe hyperkalemia. But, timing of dialysis treatment could not affect the patients' survival.

## PUB275

### AVP-Induced Increase in AQP2 and p-AQP2 Is Blunted in Heart Failure during Cardiac Remodelling and Is Associated with Decreased AT1R Abundance in Rat Kidney

Sophie Constantin Lütken,[1] Jorgen Frokiaer,[2] Soren Nielsen.[1] *[1]The Water and Salt Research Center, Dept of Biomedicine -Anatomy, Aarhus Univ, Aarhus, Denmark; [2]The Water and Salt Research Center, Institute of Clinical Medicine, Aarhus Univ Hospital, Aarhus, Denmark.*

**Background:** The effects of early HF on a standard sodium diet, sodium restricted diet, and sodium restricted diet in combination with long-term 1-desamino-8-D-arginine vasopressin (DDAVP) treatment. Focus were the AVP-angiotensin II receptor (AT1R interactions with the (pro)renin receptor in regulation of the water channels AQP2, AQP1, AQP3 and AQP4 in the inner medullary (IM) collecting duct of rat kidney.

**Methods:** LAD-ligation was performed on male Wistar rats. 10 days after operation the rats were allocated to 6 groups for the last 7 days: Sham (N = 11), HF (N = 11), sodium restricted sham (LS-sham, N = 11), sodium restricted HF (LS-HF, N = 11), sodium restricted sham + DDAVP (LS sham+D, N = 12), and sodium restricted HF + DDAVP (LS-HF+D, N = 12). EF < 50% was the inclusion criteria for LAD ligated rats. DDAVP dose was 0.5 ng/h/day for 7 days. The rats were sacrificed after 17 days, before cardiac remodeling was completed.

**Results:** Early standard sodium HF and LS-HF increased AQP4 but blunted increase in AQP2 and p(Ser256)-AQP2 in the presence of hyponatremia, hypoosmolality and decreased fractional excretion of sodium in spite of increased V2R abundance. LS-HF+D exhibited decreased creatinine clearance, severe HF (EF = 41 ± 2), severe hyponatremia, and hypoosmolality. IM AQP2, p(Ser256)-AQP2, AQP3, and AQP4 decreased vs. LS sham and LS sham+D. LS-sham and LS-HF+D decreased (P)RR. (P)RR was only increased in LS-HF. All HF groups exhibited decreased AT1R levels compared with sham operated rats.

**Conclusions:** Early HF 17 days after LAD-ligation is associated with blunted increase in AQP2 and p(Ser256)-AQP2 in spite of hyponatremia, hypoosmolality, and increased inner medullary V2R expression. Decreased AT1R levels likely plays a role in the transduction of these effects. The present study also suggest an additional crosstalk between the renal collecting duct (P)RR and plasma AVP which might be of importance in development of chronic HF.

*Funding:* Other NIH Support - the intramural budget of the National Heart, Lung, and Blood Institute, National Institutes of Health, Private Foundation Support

## PUB276

### Young Soul with Old Bones

Payaswini Vasanth, Amsalu Erko. *UT Southwestern Residency Program at Seton Family of Hospitals, Austin, TX.*

**Background:** Acid–base balance plays an important role in calcium homeostasis and bone metabolism. We present a case of osteoporosis with an objective to discuss partial defect in renal acidification as a preventable cause of osteoporosis.

**Methods:** A 37 year-old male was evaluated for fatigue for 2 years, was diagnosed for depression and was treated with fluoxetine. While on this medication, he developed insomnia which led to sleep deprivation seizure, and he experienced acute back pain after the seizures. The physical exam was unremarkable except for tenderness in lumbar spine. A lumbar x-ray revealed a T5-T6 compression fracture with diffuse osteopenia. A subsequent DEXA scan showed a T-score of -2.4 of lumbar spine. Urinary studies showed a pH of

7, calcium of 637 mg/24hr (normal range:100-300 mg/24hr), citric acid of 149 mg/24hr (normal range: 320-1240 mg/24hr), presence of amorphous and calcium phosphate crystals, and a positive urinary anion/gap. Serum chemistries were normal, except for a potassium level of 3.3 mmol/L and a bicarbonate of 22 mmol/L. A diagnosis of incomplete distal tubular acidosis was made based on the persistently high urine pH even after a trial of diuretics, and the presence of hypercalciuria and hypocituruia in the setting of mild non-gap acidosis. A workup for major causes of RTA and osteoporosis were negative. The patient received potassium citrate, calcium and vitamin D supplements. A DEXA scan after a year showed a increase of 3.8% in bone density of the lumbar spine.

**Conclusions:** Adults with incomplete distal RTA (iDRTA) have a partial defect in renal acidification. The relationship between iDRTA and low bone mineral density has been proposed but not proven based on current data. Although iDRTA patients do not have persistent systemic acidosis, they may develop mild to moderate acidosis episodically due to high acid load (as in high protein diet), and their limited ability to excrete it. Such excessive acid is buffered by the bone causing its resorption and demineralization. Treating these patients with alkaline therapy, like potassium citrate, has shown to prevent, delay or reverse metabolic bone disease. Incomplete iDRTA should be suspected in patients with unexplained osteopenia or osteoporosis.

## PUB277

**Traumatic Encephalopathy Leading to Cerebral Salt Wasting and Hyponatremia with Permanent Brain Damage** Allen I. Arieff. *Medicine, Univ of California, Sausalito, CA.*

**Background:** 1.5 to 2 million USA civilians per year suffer traumatic brain injury. 4.5% of these sustain a chronic and severe disabling condition. It has not been previously noted that cerebral salt wasting, which often complicates head trauma. could lead to hyponatremia, and severe brain damage.

**Methods:** 14 individuals who suffered acute traumatic encephalopathy {(motor vehicle accident (10), falls (7)} were admitted and had neuroimaging showing closed head injury.

**Results:** Brain lesions were subdural hematoma (6), diffuse cerebral edema (5), cerebral infarct (3). All received isotonic fluids and all developed hyponatremia after 57±32 hours. Plasma Na fell from 138±4 to 119±7 mmol/L. All were initially diagnosed as having SIADH (urine osmolality > 40400 mOsm/kg). All were initially treated with fluid restriction. Over 24 Hr the mean BP declined from 135/63 mm Hg to (92/48 mm Hg). Urine Na (184±66 mmol/day) was significantly above control (113±65 mmol/day, $p < 0.01$). The systolic BP was restored to above 100 mm Hg by IV infusion of 0.9% NaCl. Two patients suffered hypoxic brain damage and died while the other 12 recovered. The 12 surviving patients were eventually maintained on oral NaCl tablets (mean 2.5 gm/day).

**Conclusions:** Closed head trauma can lead to hyponatremia secondary to a Na wasting syndrome; b) fluid restriction can lead to shock, worsening of hyponatremia and hypoxic brain damage; c) the Na wasting can eventually be controlled with oral NaCl tablets.

## PUB278

**Downregulation of RAGE Suppresses Cyst Growth in an ADPKD Mouse Model** Jong Hoon Park, Eun Young Park, Bo Hye Kim, Eun Sun Chang. *Dept of Biological Science, Sookmyung Women's Univ, Seoul, Korea.*

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder. Although a myriad of research groups have attempted to identify a new therapeutic target for ADPKD, no drug has worked well in clinical trials.

**Methods:** Our research group has focused on the receptor for advanced glycation end products (RAGE) gene as a novel target for ADPKD. This gene is involved in inflammation and cell proliferation. We have already confirmed that blocking RAGE function attenuates cyst growth *in vitro*. Based on this previous investigation, our group examined the effect of RAGE on cyst enlargement *in vivo*. PC2R mice, a severe ADPKD mouse model that we generated, were utilized. An adenovirus containing anti-RAGE shRNA was injected intravenously into this model.

**Results:** We observed that RAGE gene knockdown resulted in a loss of kidney weight and volume. Additionally, the cystic area that originated from different nephron segments decreased in size from downregulation of the RAGE gene. Blood urea nitrogen values tended to be lower after inhibiting RAGE, but the decrease was not significant.

**Conclusions:** We suggest that RAGE-related signaling may be closely associated with PKD and that RAGE could be a new potential therapeutic target for PKD.

## PUB279

**The Relationship between Liver Cyst Volume and QOL in ADPKD** Satoru Muto,[1] Masahiko Ando,[2] Saori Nishio,[3] Ichiei Narita,[4] Kouichi Kamura,[5] Toshio Mochizuki,[6] Ken Tsuchiya,[6] Kazuhiko Tsuruya,[7] Yoshifumi Ubara,[8] Kikuo Nutahara,[9] Shigeo Horie.[10] *[1]Urology, Teikyo Univ, Tokyo, Japan; [2]Center for Advanced Medicine and Clinical Research, Nagoya Univ, Nagoya, Japan; [3]The 2nd Dept of Internal Medicine, Hokkaido Univ, Sapporo, Japan; [4]The 2nd Dept of Internal Medicine, Niigata Univ, Niigata, Japan; [5]Urology, Chiba East Hospital, Chiba, Japan; [6]Nephrology, Tokyo Woman's Medical Univ, Tokyo, Japan; [7]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [8]Nephrology, Toranomon Hospital, Tokyo, Japan; [9]Urology, Kyorin Univ, Mitaka, Japan; [10]Urology, Juntendo Univ, Tokyo, Japan.*

**Background:** Although it is well known that ADPKD patients with large liver cysts have a significant decrement in QOL, there are few reports that clearly demonstrate the relationship between the size of liver cysts and QOL. Therefore, we started the prospective longitudinal study to clear the impact of liver cysts on QOL. We will report the compiling data at the time of enrollment in this study.

**Methods:** We divided the included ADPKD patients into 4 groups (group A;<25%, group B; 25-49%, group C; 50-75%, group D; >75%) according to liver cysts-parenchyma ratio. QOL was measured by FANLTC + FACT-Hep additional concerns. We compared QOL scores and several clinical parameters between groups during 3 years. We reported the compiling data at the time of enrollment in this study.

**Results:** We included 82 patients in this study. Number of patients in group A, B, C, and D was 31, 14, 14, and 23, respectively. Although there was no significant differences in AST (p=0.107), ALT (p=0.925), serum albumin (p=0.212) between 4 groups, platelet count was significantly decreased along with the extension of cysts volume (p=0.030). Overall, mean FANLTC score and FACT-Hep were 71.8 ± 12.5, and 32.4 ± 5.8, respectively. FANLTC (p=0.017) and FACT-Hep (p=0.003) were significantly decreased with the increasing cyst volume.

**Conclusions:** In this cross-sectional report, we could clear the relationship between liver cyst volume and QOL in ADPKD patients. We will show the long-term influence on QOL in this ongoing prospective longitudinal study.

## PUB280

**The Effect of Native Nephrectomy on Blood Pressure and Graft Outcome after Kidney Transplantation in ADPKD** Jo Hyung Ah,[1] Hayne C. Park,[1,2] Hyun Suk Kim,[1] Miyeun Han,[1] Hyuk Huh,[1] Jong Cheol Jeong,[1,2] Kook-Hwan Oh,[1] Jaeseok Yang,[2] Tai Yeon Koo,[2] Young-Hwan Hwang,[3] Curie Ahn.[1,2] *[1]Dept of Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [2]Transplantation Center, Seoul National Univ Hospital, Seoul, Korea; [3]Dept of Internal Medicine, Eulji General Hospital.*

**Background:** We often experience blood pressure decrement after nephrectomy in autosomal dominant polycystic kidney disease (ADPKD) patients. However, few studies have addressed the effect of native nephrectomy on blood pressure and graft outcome after kidney transplantation in ADPKD patients.

Purpose of this study was to determine the effect of simultaneous native nephrectomy on blood pressure and graft outcome after kidney transplantation in ADPKD patients.

**Methods:** We conducted retrospective study in ADPKD patients who underwent kidney transplantation from 1999 to 2012. Cases with simultaneous native nephrectomy (n=26) were compared with non-nephrectomy (n=16). Primary outcome was graft function 1 year after kidney transplantation. The secondary outcomes were patient and graft survival, post-operative hypotensive events and changes in blood pressure.

**Results:** Simultaneous native nephrectomy had no association with graft function at 3, 6, 12 months after kidney transplantation.

There were more frequent hypotensive events in the simultaneous nephrectomy group than controls (64.7% in unilateral, 77.7% in bilateral nephrectomy group vs. 37.5 % in controls; p=0.045). Graft function at post-transplant 1 year was higher in patients with hypotensive events than those without events (67.2 vs. 57.3 mL/min/1.73 $m^2$; p=0.02). The proportion of patients who had more than 3 anti-hypertensives was lower in patients with hypotensive events than those without events (5.5% vs.12.5% at 6 months; p=0.599).

**Conclusions:** Simultaneous native nephrectomy had no influence on graft outcome. Although hypotensive events occurred more frequently in the simultaneous nephrectomy group, patients with hypotensive events had better graft function than those without events. Better blood pressure control with fewer medication may explain the better graft function in patients with hypotensive events group.

## PUB281

**The Prevalence of Simple Renal Cyst in China and Its Correlative Factors** Jian Hui Yang. *Renal Div, Zhejiang Provincial People's Hospital.*

**Background:** Present study was to understand the prevalence of simple renal cysts in Jinan and to find the possible risk factors of them.

**Methods:** Abdominal sonography was performed in 10016 adults who received regular health check-up from February 2007 to May 2011. Their age, gender, blood pressure, BMI, values of serum cholesterol, glucose and creatinine, urine analysis, smoking habit and sonographic features were analyzed.

**Results:** The overall prevalence of simple renal cysts was 10.7%, ranging from 2.38% in the 2nd to 35.29% in the 7th or later decade of life. The incidence of simple renal cysts were associated with male gender, higher systolic blood pressure, older ages ($p < 0.001$). The age of individuals with cysts was significantly older than those without cysts (57.65 ± 13.35 vs. 47.78 ± 12.40 years; $p < 0.001$). Male-to-female ratio was 2.81 (15.14% vs. 5.38%; $p < 0.001$). The majority of cysts were solitary (82.3%). The diameter of cysts ranged from 40-8000 mm ( 20.89 ± 12.62 mm). The mean size of cysts in every age group was not statistically different. Factors significantly associated with simple renal cysts were age (odds ratio [OR], 4.37; $p < 0.001$), gender (OR, 0.32; $p < 0.001$), serum creatinine (OR, 11.77; $p = 0.001$), proteinuria (OR, 3.11; $p = 0.004$), renal stone (OR, 2.47; $p = 0.006$), and smoking (OR, 2.80; $p < 0.001$). However, in multivariate analysis, except proteinuria, all of the above factors were significantly related to the occurrence of simple renal cysts.

**Conclusions:** The overall prevalence of simple renal cysts in China was 10.7%. Age, sex, renal stone, serum creatinine, and smoking were found to be risk factors for the presence of simple renal cysts.

**PUB282**

**UMOD Polymorphism rs12917707 Is Not Associated with Severe or Stable IgA Nephropathy in a Large Caucasian Cohort** Miriana Dinic, Ingrid Masson, Nicolas Maillard. *Nephrology-Renal Transplantation Dept, CHU St. Etienne, St. Etienne, France.*

**Background:** The progression of IgA nephropathy (IgAN) varies among individuals and leads to a 20% global risk of End Stage Renal Failure. Genetic factors are involved in the physiopathology of IgAN. Genome-wide studies have recently addressed the genetic variants underlying impaired renal function and Chronic Kidney Disease (CKD) in the general population. Single Nucleotide Polymorphisms (SNPs) in UMOD gene were significantly associated with CKD and increased serum creatinine. The minor T allele of rs12917707 was linked with a 20% reduced risk of CKD. In this study,we tested the hypothesis that UMOD polymorphism rs12917707 is associated with severe outcome in IgAN.

**Methods:** We constituted two groups of Caucasian patients based on the disease phenotype. The first group was composed of IgAN cases with severe outcome as indicated by terminal renal failure requiring kidney transplantation. The second group was composed of stable cases of IgAN defined by a diagnostic made since at least 10 years and eGFR>60ml/min/1.73m². A third, control group, consisted of caucasian healthy volunteers.We performed TaqMan SNP genotyping assays and compared allele frequencies and genotype distributions.

**Results:**

| genotypes and allele of rs12917707 | severe cases (n,%)N=263 | stable cases (n,%) N=188 | controls (n,%) N=345 | p* | p** | p*** | p**** |
|---|---|---|---|---|---|---|---|
| GG | 180(68.4) | 133 (70.7) | 245 (69.9) | 0.91 | 0.69 | 0.88 | 0.85 |
| GT | 73 (27.8) | 49 (26.1) | 95 (27.5) | | | | |
| TT | 10 (3.8) | 6 (3.2) | 9 (2.6) | | | | |
| G | 80 | 84 | 84 | 0.69 | 0.58 | 0.84 | 0.58 |
| T | 20 | 16 | 16 | | | | |

Allele frequencies and genotype distribution among severe cases of IgAN,stable cases of IgAN and healthy volunteers.P*: comparison between the 3 cohorts; p**: comparison between severe cases and controls; p***: comparison between stable cases and controls; p****: comparison between severe and stable cases.

**Conclusions:** No difference in allele frequences of UMOD polymorphism rs12917707 was found between 2 groups of IgAN cases as to disease phenotype.This result tends to suggest that UMOD gene is not involved in IgAN progression.

*Funding:* Clinical Revenue Support

**PUB283**

**Enoxaparin-Induced Fatal Spontaneous Hemorrhage: The Case for Revising Dosing Guidelines for the Elderly** Tarek H. Naguib. *Internal Medicine, Texas Tech Univ Health Sciences Center, Amarillo, TX.*

**Background:** Enoxaparin-induced hemorrhage may prove fatal. The literature shows that elderly persons with kidney disease are especially at risk for this complication. They were previously recommended to receive individualized enoxaparin dosing. However, the dosing guidelines still recommend double dose for patients with eGFR of 30 compared to 29 mL/min, despite the lack of significant difference between the 2 values and the risk of bleeding from this abritrary dosing.

This case report reflects the complex factors that build the case for specific safety recommendations in the geriatric population with kidney disease.

**Methods:** A 78-year-old woman, who was admitted with heart failure due to aortic valve incompetence, was placed on enoxaparin due to atrial fibrillation. While on spironolactone and olmesartan, serum creatinine increased from 1.1 to 1.77 mg/dL and eGFR declined from 46 to 29 mL/min (Cockroft-Gault formula, weight is 69 Kg). Olmesartan was discontinued and the patient was discharged, on the same enoxaparin dose, to return in 1 week for valve replacement surgery. After 3 days, she was readmitted with severe retroperitoneal hemorrhage, confirmed by ultrasound. Despite fresh frozen plasma, 7 units of blood transfusion, intravenous fluids, and pressors, she expired within 24 hours.

**Conclusions:** This case highlights lack of safety of the current dosing guidelines for enoxaparin in elderly persons with chronic kidney disease. The following is recommended to decrease the risk of death in this population.

1. All persons aged 75 years or older shall be monitored with measuring anti-factor Xa levels. The same should be done for those aged 60 to 74, whenever the eGFR is at 35 mL/min (rather than below 30 mL/min, allowing for eGFR variability among different formulas). Clinicians are encouraged, but not required, to use anti factor Xa monitoring, whenever eGFR declines near 35 mL/min, regardless of age.

2. Clinicians shall avoid the use of intravenous contrast media and renin-aldosterone system antagonists, as possible, whenever enoxaparin is prescribed.

3. Pharmacy services shall flag medical records to ensure appropriate dosing that is consistent with the above recommendations.

**PUB284**

**Hyponatremia: When the Kidneys Flunk the Polypharmacy Tolerance Test** Jack Rubin, Heena M. Contractor. *Medicine, Los Alamitos Hospital, Los Alamitos, CA.*

**Background:** Depression in the elderly is common ranging in incidence up to 10%. Many new medications are available to treat the depressed patient and they are often used in combination. The elderly are often on medications for hypertension, diabetes, arthritis and congestive heart failure. We describe a patient who illustrates how rapidly and with the best of intentions one can move from routine care into a serious medical mishap.

**Methods:** Ms XX, age 74, was admitted from the emergency room with a diagnosis of depression. Her hospital medications and pertinent studies are listed by days from admission. Medications given between day 1 and 7 are listed below: Lexapro day 1 and 2; Zoloft day 1 to 4; Buproprion day 1 to 4; Buspar day 1 to 4; Protonix day 1 to 4; losartan day 1 to 4; Hydrochlorothiazide day 2 to 4; Amlodipine day 2 to 4 dicyclomine day 1 to 4; Lasix and 3% saline were given day 6. Lab studies: Na mmol/L: day 2: 144, day 4 : 127, day 5 : 109 , day 6 :129, day 7 : 137; Hg g/100 ml: day: 1: 12.5, day 4: 11.3, day 5:11.0, day 6: 11.5.

**Results:** Treatment was initiated by withdrawing the offending medications, restricting fluids and initiating Lasix and hypertonic saline. After return of her serum Na to the normal range Effexor (Venlafaxine) was introduced day 9 and and gabapentin day 20. The sodium remained stable throughout the remaining treatment. Although Lexapro, Zoloft, Protonix and hydrochlorothiazide are known to associate with hyponatremia and are all potentially implicated drugs, the rapidity of change in the serum sodium was eye-opening. The mechanism of hyponatremia remains speculative but the hemoglobin dropped approximately 10 % suggesting at least some element of hemodilution from acute fluid retention.

**Conclusions:** In summary we illustrate a case of a 74 year old female with psychiatric and medical co-morbidities whose treatment rapidly induced hyponatremia. Occasionally the kidneys fail the multiple medication test.

*Funding:* Clinical Revenue Support

**PUB285**

**Frail and Elderly Patient Outcomes on Dialysis (FEPOD): A 2-Part Study** Edwina A. Brown, Lina Johansson. *Imperial College Renal and Transplant Centre, London, United Kingdom.*

**Background:** The majority of frail older patients with ESRD are on haemodialysis (HD). In Europe, assisted peritoneal dialysis (aPD), with paid assistance or carers, is increasingly available for frail patients in their home. Little is known, though, about outcomes (mortality, morbidity and quality of life) of older people on aPD compared to HD.

**Methods:** Eligible patients on aPD (require assistance for PD and ≥60 years) are matched to HD patients by age + 2 years, sex, diabetes status, time on dialysis, ethnicity and Index of Deprivation. Study design: 2 Part study: Part 1 cross-sectional (11 UK centres, 50 aPD, completion August 2013) and Part 2 longitudinal (18 UK centres, 100 aPD). Both assess quality of life through questionnaires (including Hospital Anxiety and Depression Scale, SF-12, Palliative Outcome Symptom Scale), falls, social support, frailty and activities of daily living. Part 2 assesses healthcare use and survival over 2 years. Complete Part 1 data with multiple regression will be presented at meeting.

**Results:** Preliminary results for some measures from Part 1:

| | FEPOD 1 -aPD | FEPOD 1 - HD |
|---|---|---|
| Number | 25 | 14 |
| Age in years, mean (SD) | 71.7 (7.3) | 70.9 (7.2) |
| Diabetes, n (%) | 16 (64) | 9 (64) |
| Lives alone, n(%) | 6 (24%) | 2 (14%) |
| Frailty score, median (IQR); severely frail = 7 | 4.9 (1.0) | 4.4 (1.3) |
| Barthel Index (activities of daily living), mean (SD); normal = 100 | 83.0 (17.1) | 87.5 (11.1) |
| Timed up and go, mean (SD) (normal <10 sec) | 24.1 (13.6) | 23.4 (11.6) |
| Illness intrusion rating score, mean (SD); lower = less intrusion | 38.0 (17.2) | 52.4 (24.7) |
| HADS score >8 (probable depression) n, (%) | 11 (44%) | 4 (29%) |
| Renal Treatment Satisfaction, mean (SD); higher=greater satisfaction | 50.0 (9.5) | 53.1 (7.6) |

**Conclusions:** This is the first study to compare outcomes on aPD and HD. Results will provide valuable data to the understanding of how older frail patients manage on different dialysis options. Comparison of healthcare use and survival will be determined over the next 2 years in Part 2.

*Funding:* Private Foundation Support

**PUB286**

**Clinical Features of Elderly Patients with Diabetic Nephropathy Diagnosed by Renal Biopsy** Manuel M. Heras,[1] Ana Saiz,[2] Pedro Iglesias,[3] María José Fernández-reyes,[1] Maria astrid Rodriguez Gomez,[1] Alvaro Molina Ordas.[1] *[1]Nephrology, Hospital General, Segovia, Spain; [2]Pathology, Ramón y Cajal Hospital, Madrid, Spain; [3]Endocrinology, Hospital Ramón y Cajal, Madrid, Spain.*

**Background:** Diabetic nephropathy (DN) is the primary global cause of terminal nephropathy. Little is known about clinical features of elderly patients with DN diagnosed by renal biopsy (DNB).

**Methods:** A retrospective observational study at the General Hospital Segovia, between 2002-2012 was performed. 218 native renal biopsy (RB) patients were evaluated: 25 had DNB.

**Results:** Mean age of the entire NDB group was 66.0±11.0 years (range 39-83; 60% males). Hypertension 95.2%.The main indication for RB was nephrotic syndrome (NS) (68%) followed by acute kidney injury (AKI) (20%). Mean HbA1c was 7.1±1.0%, duration of the diabetes mellitus (DM) was 8.9±5.0 years. In 2 patients DNB was confirmed in dialysis. Study according to age in Table 1.

| Years | <65 (n=13) | ≥65 (n=12) | *p* |
|---|---|---|---|
| Age (years) | 57.0±7.0 | 75.8±4.0 | 0.000 |
| Gender (male/female) (%) | 77/23 | 42/58 | NS |
| Years evolution DM | 9.2±6.2 | 8.6±5.8 | NS |
| ACE inhibitors / ARB(%) | 77/54 | 58/33 | NS |
| ACE inhibitors+ARB (%) | 38 | 25 | NS |
| Oral antidiabetics (%) | 61 | 33 | NS |
| Insulin (%) | 46 | 58 | NS |
| Oral antidiabetics+insulin (%) | 23 | 8 | NS |
| Creatinine at diagnosis (mg/dl) | 2.1±1.3 | 2.2±1.2 | NS |
| Albumin (g/dL) | 3.3±0.7 | 3.0±0.8 | NS |
| Hemoglobin A1c (%) | 7.1±1.0 | 7.2±1.0 | NS |
| Proteinuria (g/24hours) | 5.7±3.0 | 7.7±6.0 | NS |
| Creatinine clearance (ml/min) | 53.4±41.0 | 35.2±12.0 | NS |

ARB: angiotensin receptor blocker; ACE: angiotensin-converting enzyme; NS: Not significant.

**Conclusions:** In our population, elderly NDB patients show the same clinical features than adult NDB patients. Both groups are patients with long-standing DM with nephrotic proteinuria, NS and/or AKI.

## PUB287

**Hematologic Parameters in Hemodialysis Patients Provide Insight on Anemia of Aging and Could Potentially Impact Anemia Treatment in End Stage Renal Disease** Namrata Baxi, Krystal Hunter, Amanda Logan, William D. Sirover. *Cooper Medical School of Rowan Univ.*

**Background:** Elderly patients comprise the most rapidly growing group of end stage renal disease (ESRD) patients. Unexplained anemia of the elderly (UAE) is also now recognized as a distinct clinical entity in this patient population. Decreased erythropoietin levels, impaired bone marrow activity, and increased inflammation have been implicated as possible causes.

Delineating the cause(s) may lead to a change in treatment practices. Increased erythropoietin (EPO) dose requirement in older ESRD patients could occur if bone marrow erythropoietic activity decreases and/or inflammation increases with age. In light of EPO-associated toxicity, if these two pathophysiologic changes occur, consideration of lowering hemoglobin (Hb) target may be warranted. We studied the effect of age on EPO dose requirement in a prevalent hemodialysis population.

**Methods:** Hematologic data on 179 hemodialysis (HD) patients was analyzed. Patients were 18 years or older and on HD for at least 3 months. Individual EPO dose was calculated as mean weekly dose over a 3-month period.Mann-Whitney was used to assess the relation between age (< and > 65 years old) and median EPO dose expressed as units/week (wk), units/wk/kg, or units/wk/Hb.

**Results:** No correlation existed between age and EPO dose expressed as units/wk (9,441 vs 10,560, p value = 0.48), units/wk/kg (107.83 vs 137.98, p= 0.22), or units/wk/Hb (827.66 vs 930.45, p= 0.57).

**Conclusions:** Building on previous literature, age does not appear to affect EPO dose or EPO resistance in HD patients. If impaired bone marrow activity or inflammation were causative factors in UAE, then age would have been expected to be associated with these EPO parameters. Therefore, by exclusion, these results indicate that UAE may be due to decreased EPO levels. Hypoxia inducible factor levels have been shown to decrease with age and may account for the decrease in EPO levels and subsequent development of UAE in general. In HD patients, exogenous EPO may effectively treat UAE.

*Funding:* Private Foundation Support

## PUB288

**Masked Hypertension and White Coat Hypertension Are Common and Chronic Conditions in CKD Patients Receiving Antihypertensive Therapy: Implications for Hypertension Management if Hypertension Control Is Based Only on Clinic Blood Pressure** Christopher Valentine, Ravish Shah, Daniel J. Birmingham, Lee A. Hebert. *Medicine, Ohio State Univ Wexner Med Ctr, Columbus, OH.*

**Background:** Masked hypertension (MH) is defined as normal blood pressure (BP) in the clinic but hypertension at home (e.g., assessed by home BP monitoring (HBPM). White coat hypertension (WCH) is the opposite of MH. MH increases cardiovascular (CV) risk. The risk of WCH is less clear. However, there is recent evidence that treated WCH can increase risk because BP is overcontrolled.

**Methods:** We assessed MH and WCH in 33 pts followed in our Nephrology Clinical Trials Unit (NCTU) with a diagnosis of hypertension, receiving anti-hypertension therapy, and performing HBPM morning and evening at least 10 times per mo, using calibrated equipment and proper technique. NCTU BP was measured each 2-3 mo by Omron. There were 103 to 767 home BP measurements per pt, median 301. Follow-up was 4 to 15 mo, median 10 mo.

**Results:**

| | | Blood pressure, mm Hg | | | BP Difference | |
|---|---|---|---|---|---|---|
| H type | CKD | Clinic | Home AM | Home PM | Clinic-Home | |
| MH | | 128.3 | 136.7 | 143.1 | 11.6 | p = 0.002 |
| N=8 | N=3 | ±7.6 | ±10.0 | ±4.2 | ±6.5 | |
| WCH | | 139.0 | 124.2 | 122.0 | -15.9 | p = 0.040 |
| N=4 | N=3 | ±4.3 | ±8.7 | ±5.8 | ±9.1 | |
| Neither | | 124.7 | 125.2 | 123.9 | -0.2 | p = 0.89 |
| N=21 | N=13 | ±4.6 | ±8.4 | ±6.4 | ±5.3 | |

The table shows that MH and WCH are chronic and common in CKD and non-CKD pts receiving BP therapy. If hypertension control is based only on clinic BP, MH will go untreated and WCH may be over treated. The prevalence of MH and WCH and their association with CKD shown here is comparable to that of previous reports. In MH the difference between clinic and home BP is large and associated with increased 10-yr CV risk. In WCH the difference is also large. It could increase CV risk by BP overcontrol.

**Conclusions:** A BP control strategy that uses only clinic BP will undercontrol BP in MH and overcontrol BP in WCH, creating risk. HBPM should be used to direct BP therapy in both CKD and non-CKD patients.

*Funding:* NIDDK Support

## PUB289

**Metabolic Safety Profile of Thiazides in Patients with Diabetes Mellitus Type 2** R.P. Niraula,[1] A. Jittirat,[1] C.S. Huang,[1] S. Sarani,[1] D. Vo,[1] P.T.T. Pham,[2] P.C. Pham.[1] *[1]Nephrology and Hypertension, UCLA-Olive View Medical Center, Sylmar, CA; [2]Kidney and Pancreas Transplant Program, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

**Background:** Thiazides remain the mainstay of antihypertensive therapy in the general population. Traditional concerns for thiazide induced glucose intolerance and dyslipidemia may deter its routine use in patients with concurrent hypertension and diabetes mellitus type 2 (DM2). We analyze various metabolic indices among DM2 patients with and without chronic thiazide use.

**Methods:** In this retrospective study, electronic records from patients with both DM2 and hypertension who were evaluated for any reason at UCLA-Olive View Medical Center during the months of January 2011 – January 2012 were collected. Inclusion criteria: all patients with ICD-9 codes 250.00 to 250.93 for diabetes and 401.0 to 404.93 for hypertension, who had at least 3 documented blood pressure measurements, estimated glomerular filtration rates (eGFR) by the 4 variabled-MDRD formula, lipid profiles, hemoglobin, and A1C over the span of the previous 6 months and as available up to 24 months.

**Results:** 769 patients were included: 389 received thiazide diuretics; Thiazide group had significantly higher systolic blood pressure: 137+14 vs 131+12 mm Hg, p<0.0001, lower initial estimated glomerular filtration rate (eGFR): 93+27 vs. 100+26 mL/min/1.73m$^2$, and heavier weight: 87.4+21.2 vs 83.3+18.3 Kg, p=0.004. Differences in other biochemical values are as shown.

| | Follow-up duration (days) | Hb (gm/dL) | Initial A1C (%) | Final A1C (%) | HDL (mg/dL) | LDL (mg/dL) | eGFR change (mL/min/1.73m$^2$) over duration of follow-up |
|---|---|---|---|---|---|---|---|
| No thiazides (n=380; 65% female; Age=56±10 years) | 635±151 | 13.2±1.4 | 8.3±1.7 | 8.4±1.8 | 42±12 | 96±29 | -3.0±19 |
| Thiazides (n=389; 73% female; Age=58±8 years) | 621±151 | 13.2±1.3 | 8.3±1.8 | 8.3±1.8 | 43±12 | 97±26 | -3.8±18 |
| p-value | 0.2 | 0.7 | 0.9 | 0.3 | 0.3 | 0.6 | 0.6 |

**Conclusions:** There were no detectable adverse effects in terms of A1C change, lipid profile difference, hemoglobin, or decline in eGFR in diabetic patients treated with thiazides compared to those who did not receive the medication.

## PUB290

**Arterial Stiffness and Central Aortic Blood Pressure in Indian Chronic Kidney Disease Population** Ashok Kirpalani, Hardik Shah, Dilip Kirpalani, Ranveer Choudhary, Jay Patel. *Nephrology, Bombay Hospital, Mumbai, Maharashtra, India.*

**Background:** To estimate noninvasively arterial stiffness and central hemodynamics in CKD patients and analyse the epidemiological data in various subgroups.

**Methods:** 50 CKD patients included .Basic epidemiological and related information obtained. Brachial BP measured by sphygmomanometer. Applanation tonometer (Sphygmocor) device applied on the radial, carotid and femoral artery and measurements obtained.

**Results:** 48 % patients < 50 years (Group A), 52 % > 50 years (Group B) .13 patients had DM, 12 IHD ,12 had CVA.19 in CKD stages 1-4 ,31 on hemodialysis. Mean brachial Systolic BP (BSBP) 138.4 + 13.5 and Diastolic BP (BDBP) 86.1 + 6.2 mmHg. Mean Central Aortic Systolic BP (CASBP) 133.7 + 10.1 and diastolic BP (CADBP) 88.5 + 4.4 mmHg. Mean Central aortic Pulse Pressure(CAPP) - 45.4 + 5.1 mmHg. There was no significant difference between the mean brachial and central aortic pressures (p= 0.9). In 78% subjects, the CASBP was higher than the normal for age values. However, the CADBP was higher than normal for age values in only 56 %. Augmentation Index (AIx) was 24.7 + 3.4 while Mean Carotid-femoral pulse wave velocity (cf-PWV) was 11.1 + 3.2 m/s. In diabetics, AIx (33.5 + 3.1) and cfPWV (12.5 + 3.3 m/s) were significantly higher (p< 0.05) than the nondiabetic patients (AIx : 21.6 + 2.4 and cfPWV :10.6 + 2.9 m/s) although brachial blood pressures in both groups were similar. Group A had significantly higher (p < 0.05) CASBP(135.8 + 8.1 mmHg) as well as CADBP (91.6 + 5.8 mmHg) readings as compared to Group B (CASBP 131.8 +7.4 ; CADBP 85.7 + 5.1 mmHg).

**Conclusions:** 1. Patients with CKD have higher central aortic pressure, AIx well as higher cfPWV than the normal values in the general population. This first study done in Indian CKD patients confirms that CKD is associated with stiff arteries.

2.Diabetic CKD patients as well as the younger CKD population have accelerated vascular ageing. This predisposes them to greater risk of CVD inspite of similar brachial BP. Preventive CVD strategies can thus be aggressively targeted at this subgroup.

## PUB291

**Association between Renal Function (MDRD-4 versus CKD-EPI) and Cardiovascular Risk. Prospective Observational Study**

Francisco Javier Lavilla, Carmen Calderon Gonzalez, Nuria Garcia-Fernandez, Paloma L. Martin Moreno, Jose Maria Mora Gutierrez, Diana Lopez Espinosa, Pedro Errasti. *Nephrology, Univ Clinic of Navarra, Pamplona, Navarra, Spain.*

**Background:** To study the relationship between cardiovascular risk (CVR) and renal function in healthy people .

**Methods:** 235 patients were studied in prospective cardiovascular risk study (5 to 10 years). Mean age 48.39 years (SD 0.69). CVR (PROCAM-PR-, FRAMINGHAM-FR, SCORE score-SC-and cardiovascular-event probability-SCE),renal function (CKD-EPI and MDRD-4),metabolism parameters (cholesterol, triglycerides, glucose, uric acid), cardiovascular risk markers (von Willebrand factor-antigen-VWF, fibrinogen -F- and C- reactive protein -CRP-), albuminuria (albumin/creatinine urinary-IALBCR-), carotid intima-thickness(IM) and E/A echocardiography index were evaluated. Statistical analysis with SPSS 15.0.

**Results:** Observed greater association between CKD-EPI and CVR scores (PR r = -0.281, FR r = -0.432, SC r = -0.551, SCE r = -0.468) than with MDRD-4 ( PR r = -0.223, FR r = -0.235, SC r = -0.322, SCE r = -0.261) (p<0.005). CKD-EPI was better associated with IM thickness (r = -0.359), E /A ratio (r = 0.401) and uric acid (r = -0.357), than MDRD-4 (r = -0.170), (r = 0.308) and ( r = -0.249). The association of both formulas is similar with IALBCR. The best FG stratified according CKD-EPI stratified better GFR with CVR, showing significant increase below 90 ml / min (p <0.001). This effect depends on the age, associated with GFR (p <0.001), showing significant drop in people older than 50 years.

| CKD-EPI | >100 | 100-90 | 89-80 | 79-70 | <69 |
|---|---|---|---|---|---|
| PR | 0.64 | 1.7 | 5.23 | 6.61 | 8.12 |
| FR | 5.27 | 5.66 | 14.3 | 16.39 | 21.72 |
| SC | 19.3 | 23.7 | 31.2 | 32.6 | 39.1 |
| SCE | 0.46 | 0.59 | 1.41 | 1.76 | 3.10 |
| Age (years) | 41 | 40 | 50 | 52 | 67 |

PR: PROCAM. FR: FRAMINGHAN; SC: SCORE, CE: SCORE PROBABILITY CARDIOVASCULAR EVENT.

**Conclusions:** In healthy population there are association between CVR and renal function. GFR below 90 ml/min in aged over 50 years is associated with significant increase CVR. CKD-EPI allows better assessing the relationship between renal function and cardiovascular risk.

## PUB292

**The Decreased Expression of Nephrin and CD2-Associated Protein in Patients with Hypertensive Kidney Injury**

Zilong Li,[1,2] Wei Wang,[1] Juan Wang,[1] Hua Zhou,[3] Lining Wang.[1,2] *[1]Dept of Nephrology, First Affiliated Hospital of China Medical Univ, Shenyang, China; [2]Institute of Nephropathology, China Medical Univ, Shenyang, China; [3]NIDDK/NIH.*

**Background:** Given that nephrin and CD2-associated protein (CD2AP), podocyte associated proteins, play important roles in maintenance of glomerular structual integrity, the study focused on the expression changes of nephrin and CD2AP induced by hypertensive kidney injury in patients with proteinuria.

**Methods:** The involved patients were divided into two groups as following. Hypertensive group: patients with hypertension and proteinuria who were diagnosed as hypertensive kidney injury via kidney biopsy. Control group: kidney trauma patients without hypertension and proteinuria. Kidney biopsy section taken from hypertensive group and normal kidney tissues taken from control group via urologic surgical procedures were fixed with immersion-fixation method. Then immunohistochemistry stainings were performed with HE, DAB, immunofluorescence, uranyl acetate, and observed by light microscopy,confocal laser scanning microscopy and immunoelectron microscopy.

**Results:** In control group, the capillary loops were smooth and plump. Nephrin and CD2AP were observed staining along the glomerular capillary loops continuously and evenly. However, in hypertensive group the capillary loops became wizened, and the Bowman´s space and luminal spaces of the proximal or distal tubules became more widely opened in the renal cortices. Additionally, nephrin and CD2AP decreased and stained intermittently. Through the immunoelectron microscopy, different degrees of foot processes effacement were observed in hypertensive group, nephrin and CD2AP decreased and stained weakly along the podocyte basal membrane, while in control group, they distributed evenly in podocytes.

**Conclusions:** Desregulation of podocyte associated proteins induced by hypertension may be an important cause leading to development of proteinuria and decline of renal function in hypertensive kidney injury patients.

## PUB293

**Blood Pressure Control among Diabetic CKD Patient in a Developing World Sample**

Jafar Al-Said, Teerath Kumar, Soni Murdeshwar. *Bahrain Specialist Hospital.*

**Background:** Hypertension is the most common CV risk factor. The presence of Diabetes with Hypertension increases the risk further. Well control BP reduces the CV risk significantly. The aims from this study were to:

1. Identify the prevalence of Hypertension among CKD population.
2. Determine the percentage of successful BP control among diabetic and non-diabetic CKD patients and the factors correlated with uncontrolled BP.

**Methods:** In this retrospective study all CKD patient followed for 9.5 years in our Nephrology department were identified. Demographic factors were collected from electronic files. Matched diabetic and non-diabetic CKD samples for age, gender and BMI were selected. BP measurement on the last visit was recorded. Patients with BP more than 140/90 mmHg were considered uncontrolled. Independent variables correlated with uncontrolled BP were tested using SPSS 18. Multivariate regression equation was generated for these variables.

**Results:** Total CKD population was 245 patients. Mean follow up was 23.6 months (SE 1.6), mean age was 58.7 years (SE 0.9), mean BMI was 30.5kg/m2 (SE 0.5). Males constitute 61%. Mean eGFR was 45.4ml/min (SE 1.4) and 51% of the populations were CKD stage III. We matched 77 diabetic with non-diabetic patients. HTN was present in 88% of which 59% had controlled BP on the last visit. Uncontrolled BP was present in 47% of the diabetic as compared to 28% in non-diabetic (p 0.019). Factors related with uncontrolled BP are:

| Variables | Correlation Coefficient | P |
|---|---|---|
| Age | 0.188 | 0.024 |
| DM | 0.19 | 0.019 |
| Stroke | 0.19 | 0.017 |
| Systolic BP on first visit | 0.36 | 0.0001 |
| B-Blocker | 0.2 | 0.014 |
| Alpha / B-blocker | 0.2 | 0.013 |
| Total CV risk factors | 0.23 | 0.005 |
| Number of Anti HTN medications | 0.3 | 0.0001 |
| Vasodilators | 0.2 | 0.009 |
| Nitroglycerin | 0.3 | 0.0001 |

Systolic BP on presentation and Nitroglycerin, in multivariate regression model with RR 0.27, were the only significant factors correlated with uncontrolled BP.

**Conclusions:** Hypertension was highly prevalent among diabetic CKD patients. It was less controlled among this subgroup as compared to non-diabetic. Several factors were identified to correlate with uncontrolled BP.

## PUB294

**Usefulness of 24h-Ambulatory Blood Pressure Monitoring in Discharged Patients with Chronic Kidney Disease**

Patrick Saudan, Belen Ponte, Sophie M. De Seigneux, Pierre-Yves F. Martin. *Nephrology Unit, Dept of Medical Specialties, Geneva Univ Hospitals, Geneva City, Geneva, Switzerland.*

**Background:** Optimal blood pressure (BP) control is mandatory in patients with chronic kidney disease (CKD). These patients are often discharged from hospital with controlled BP based on ward measurements. Whether this control is satisfactory within the outpatient setting remains unknown.

**Methods:** 82 patients enrolled in the Implicate Study (ClinicalTrials.gov: NCT00929760) and with ward SBP < 140 mm Hg at discharge had an 24h-ambulatory blood pressure monitoring measurement (ABPM) within the following 3 months. Target BP values at daytime and nighttime were < 135/85 and 120/70 mm Hg respectively. Dipping was a night to day ratio < 0.90.

**Results:** There were 69% males and 27% diabetics**.** Mean age was 69 (9) years, mean eGFR was 33 (9) ml/mn. Seventy-one % of the patients were treated with ACEIs/ARBs and 62% with diuretics. Mean daytime ABP were 135/81(18/12) mm Hg and mean nighttime ABP were 124/72(18/13) mm Hg. Dipping was present in 45% of the patients. Target daytime and nighttime BP were met in 54% and 38% of the patients respectively. Only 28% could achieve both daytime and nightime BP targets.

**Conclusions:** In stage IIIB-IV CKD patients, ward good BP control did not predict the achievement of BP targets measured by ABPM within the outpatient setting. 24h-ABPM should be routinely used in CKD patients recently discharged from hospital in order to achieve BP targets, especially during nighttime.

## PUB295

**Influence of Central Blood Pressure on Significant Proteinuria in Chronic Kidney Disease**

Vinaya R. Soundararajan, Ramesh Soundararajan. *Nephrology, Associates in Nephrology, Chicago, IL.*

**Background:** Central aortic blood pressures are thought to be more reflective of the actual pressures the vital organs including the kidney and heart are subjected to rather than the brachial blood pressures. There is limited data regarding the effect of central aortic blood pressure on proteinuria in patients with chronic kidney disease (CKD).

**Methods:** We decided to study a selected population of our CKD patients in an office setting by evaluating the central aortic pressures, including systolic, diastolic, pulse pressure, and augmentation index, and their correlation to significant proteinuria. For this study, we defined significant proteinuria as having greater than 1 Gm of protein per Gm of creatinine. Central aortic blood pressure was measured using SphygmoCor XCEL PWA from ATCor Medical (Itasca, IL). The data set included 37 patients with CKD between the stages of II and IV, both diabetics and non-diabetics.

**Results:** The central diastolic pressure for patients without significant proteinuria was **68.9 mmHg**, which was found to be significantly lower than that for patients with proteinuria > 1gm/gm of creatinine **77.3 mmHg** (p=.039). However, the other central BP parameters did not display a significant correlation with significant proteinuria. We also studied the influence of antihypertensive medications (ace inhibitors, angiotensin receptor blockers, dihydropyridine calcium channel blockers, and non-dihydropyridine calcium channel blockers) on central systolic pressures and surprisingly found no significance between the type of medication and the resulting central pressure.

**Conclusions:** Unlike brachial pressures, where the control of systolic BP seems to correlate with proteinuria, this study determined that among the different central pressure parameters the central diastolic pressure is the best predictor of degree of significant

proteinuria in CKD patients. Further studies can be conducted with a greater sample size and for varying degrees of proteinuria to determine whether or not controlling central blood pressure can decrease the degree of proteinuria.

## PUB296

**Add-On Administration of HCTZ to the Preceding ARB Treatment Can Attain the Lower Sodium Balance Leading to the Successful Antihypertensive Effects for Daytime and Night-Time**

Daisuke Fuwa,[1] Michio Fukuda,[1] Toshiyuki Miura,[1] Shuichi Watanabe,[1] Hiroyuki Togawa,[1] Yoshiaki Ogiyama,[1] Yukako Isobe,[1] Hiroyuki Kobori.[2,3] *[1]Cardio-Renal Medicine and Hypertension, Nagoya City Univ, Japan; [2]Tulane Univ Hypertension and Renal COE; [3]Dept of Pharmacology, Kagawa Univ, Japan.*

**Background:** Previously, we have reported that the angiotensin receptor blockers (ARBs) or thiazide diuretics (HCTZ) can restore the circadian blood pressure (BP) rhythm. On this study, we investigated to demonstrate that an add-on administration of HCTZ to the preceding ARB treatment can further attain the lower sodium balance leading to the successful antihypertensive effects for daytime and night-time.

**Methods:** Major inclusion criteria were as follows: 1) chronic kidney disease (CKD, K/DOQI), 2) previous treatment with ARB (valsartan, 80 mg/day) for ≥ 2 months, and 3) office BP > 130/80 mmHg.

**Results:** At the interim analysis, two men and three women (69 ± 11 years old, GFR 65 ± 16 ml/min/1.73m$^2$), were studied before and 8-wk add-on treatment of HCTZ (12.5 mg/day) to the ARB. Filtered sodium load ($S_{Na}$ x GFR, 16,590 ± 6,310 → 10,510 ± 3,905 mmol/day, p = 0.01), tubular sodium reabsorption ($t_{Na}$, 16,430 ± 6,330 → 10,370 ± 3,890 mmol/day, p = 0.01), and fractional sodium reabsorption [$t_{Na}$/($S_{Na}$ x GFR), 98.9 ± 1.0 → 98.5 ± 1.1%, p = 0.009] were all decreased. Urinary excretions of protein (2.1 → 1.1 g/day, p = 0.03) was attenuated, but urinary excretion of angiotensinogen ($U_{AGT}V$, 610 ± 940 → 840 ± 1,138 mg/g Cr, p = 0.2) was increased. BP were lowered for both daytime [131/78 → 121/77 (SBP, p = 0.02; DBP, p = 0.01), and night-time [126/72 → 118/67 (SBP, p = 0.01; DBP, p = 0.01)]. Nocturnal BP dip was determined by the increase in daytime natriuresis.

**Conclusions:** Add-on administration of HCTZ to the preceding ARB treatment can attain the lower sodium balance leading to the successful antihypertensive effects for daytime and night-time, accompanied by the increase in $U_{AGT}V$, which was the indicator of the intrarenal renin-angiotensin system status. Careful studies are needed to investigate whether the increase in $U_{AGT}V$ is merely the result from lowering the sodium balance, or it is the risk for CKD progression.

## PUB297

**Increase in Daytime Natriuresis during the ARB Treatment Is Not Attributed to the BP Reduction of the Night before**

Toshiyuki Miura,[1] Daisuke Fuwa,[1] Shuichi Watanabe,[1] Hiroyuki Togawa,[1] Yoshiaki Ogiyama,[1] Yukako Isobe,[1] Tadashi Ichikawa,[1] Shirasawa Yuichi,[1] Yoshida Atsuhiro,[1] Michio Fukuda,[1] Genjiro Kimura.[2] *[1]Cardio-Renal Medicine and Hypertension, Nagoya City Univ, Japan; [2]Asahi Rosai Hospital, Japan Labour Health and Welfare Organization.*

**Background:** Previously, we have reported that the angiotensin receptor blocker (ARB), olmesartan can increase the natriuresis during the daytime to attain a lower sodium balance, and can restore the circadian blood pressure (BP) rhythm. However, the details should be pursued. The increase in daytime natriuresis can be explained by the two mechanisms as follows: 1) ARB can inhibit the tubular sodium reabsorption; and 2) ARB can lower the night-time BP resulting in the attenuation of the night-time natriuresis, and thus the daytime natriuresis compensatorily increase. If the latter holds, the decrease in the night-time BP can precede the increase in daytime natriuresis during the acute phase of the treatment (not steady state).

**Methods:** We studied which comes first the increase in daytime natriuresis, or the decrease in night-time BP, in seven patients with CKD (five men, two women, 52±19year-old, GFR 68±64 ml/min$^2$) before and within 2 days after commencing the ARB.

**Results:** At baseline, two out of the seven patients exhibited dipper circadian BP rhythm, whereas five had non-dipper BP rhythm. Although their BP rhythm had been dipper type before the treatment, the two patients exhibited the increase in daytime natriuresis on the first day after starting ARB. One of the five non-dippers demonstrated the increase in natriuresis first day of the treatment and on that day, the rhythm was restored into dipper pattern. Other four patients, who were non-dippers at baseline, remained as the non-dippers 2 days after the institution of treatment. However, all of these four patients demonstrated the increase in daytime natriuresis within 2 days after starting treatment (1 day for three patients; 2 days for one patient) even though circadian BP rhythm was not restored.

**Conclusions:** These findings suggest that the increase in daytime natriuresis during the ARB treatment is not attributed to the BP reduction of the night before.

## PUB298

**L-arginine Improves Endothelial Function, Independent of Arginine Transport System, in Chronic Renal Failure Female Rats**

Idit F. Schwartz, Doron Schwartz. *Nephrology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.*

**Background:** Endothelial cell dysfunction (ECD) is a common feature of chronic renal failure (CRF). Defective nitric oxide (NO) generation due to decreased endothelial nitric oxide synthase (eNOS) activity is a crucial parameter characterizing ECD. Decreased activity of cationic amino acid transporter-1 (CAT-1), the selective arginine transporter of eNOS, has been shown to inhibit eNOS in uremia. Recently, we failed to demonstrate a decrease in glomerular arginine transport in uremic female rats. The current experiments were designed to determine whether the sexual dimorphism which characterizes the glomerular arginine transport system in uremia is a generalized phenomenon.

**Methods:** Contractile and vasodilatory responses, ultrastructural changes, and measures of the L-arginine NO system were performed in thoracic aortas of female rats subjected to 5/6 nephrectomy.

**Results:** Contractile response to KCl was significantly reduced in CRF dames when compared to healthy rats while arginine administration abolished this decline. Aortic rings contraction by Phenylephrine was unchanged in all experimental groups. Acetyl Choline induced vasodilation was significantly impaired in the aortas from CRF rats compared with controls. L-Arginine normalized the capacity of aortic vasodilation in CRF rats. The decrease in both cGMP generation, a measure of eNOS activity and abundance of aortic eNOS and phosphorylated eNOS observed in CRF rats were completely abolished by L-arginine while both arginine transport and CAT-1 protein abundance did not differ in all experimental groups.

**Conclusions:** Arginine administration has a profound beneficial effect on ECD independent of cellular arginine uptake, in CRF female rats.

*Funding:* Government Support - Non-U.S.

## PUB299

**Relationship of Asymmetric Dimethylarginine and Whole Blood Viscosity in Normal Subjects and Patients with Chronic Renal Failure**

Mary S. Hammes,[1] Sydeaka Watson,[2] Fredric L. Coe,[1] Faris A. Ahmed,[1] Promila Dhar.[3] *[1]Nephrology, Univ of Chicago, Chicago, IL; [2]Health Studies, Univ of Chicago, Chicago, IL; [3]Biomedical Engineering, Univ of Chicago, Chicago, IL.*

**Background:** In chronic renal disease (CKD), vascular disease involves the microcirculation: Endothelial dysfunction as manifested by high vascular resistance and resultant hypertension. Key regulators of endothelial function include nitric oxide (NO), its antagonist, asymmetric dimethylarginine (ADMA), and whole blood viscosity (WBV). Since ADMA is a counter regulator of NO, and NO rises with increased blood viscosity, we reasoned that ADMA rises with WBV. The aim was to determine the relationship of ADMA and WBV in normal subjects and patients with CKD.

**Methods:** 51 subjects, 20 controls, 11 renal transplant recipients, 10 CKD and 10 end-stage renal disease (ESRD) patients had blood samples drawn for WBV, Hematocrit, and ADMA. WBV was measured using a Brookfield DV-II+ cone-plate viscometer. Hematocrit was measured using CritSpin. ADMA was assayed by ELISA. The significance between groups was compared by boxplots and analysis of variance. Tukey's test was used to examine pair wise comparisons. The linear relationship was shown by regression lines and correlation coefficients.

**Results:** ADMA was elevated in all groups with renal failure when compared to controls ($p < 0.05$). Control subjects showed a positive correlation ADMA and WBV($r = 0.56$, $p = 0.011$). Subjects who received a renal transplant had a negative correlation between ADMA and WBV ($r = -0.96$, $p < 0.01$). Extremely high ADMA values in subjects with ESRD and CKD were observed. The difference in ADMA comparing pre-dialysis to post-dialysis conditions was positive ($p < 0.05$.

**Conclusions:** There is a positive correlation of ADMA with WBV in normal subjects and conversely, an inverse relationship between ADMA and WBV in tansplant recipients with well-functioning grafts. There was a disruption of the ADMA–WBV relationship with high fixed values of ADMA in patients with CKD and ESRD. Dialysis uniformly increases ADMA. This investigation provides an understanding of factors that influence endothelial cell function.

*Funding:* NIDDK Support, Other NIH Support - NIDDK and NIH award RO1DK090769

## PUB300

**Parathyroid Hypertensive Factor Binds to Endothelial Type 1a Receptors In Vitro**

Larry A. Slomowitz,[1] Brian E. Peerce.[2] *[1]DuoPhos LP, Friendswood, TX; [2]Neuroscience and Cell Biology, UTMB, Galveston, TX.*

**Background:** Dahl salt sensitive rats fed a high salt diet (8% $Na^+$) did not develop hypertension when phosphorus (Pi) was restricted either by reduction in dietary phosphorus (from 0.67% to 0.2%) or when intestinal absorption was reduced with a NaPi 2b inhibitor (2'-fluorophosphophloretin). On a restricted Pi diet, the major hormonal effect of reduced dietary Pi was a 50% reduction in PHF (parathyroid hypertensive factor). Previous studies suggested that PHF affects $K^+$ channel activity and $Ca^{2+}$ channel activity therefore we examined the effect of PHF on SK channels (small conductance $Ca^{2+}$-activated $K^+$ channels), voltage gated $K^+$ channels, tetrodotoxin-sensitive $Na^+$ channels, and L-type $Ca^{2+}$ channels activities and hormone receptors involved in the regulation of blood pressure.

**Methods:** Hormone receptor or channel ligand binding (agonist or antagonist) was determined using a high-throughput system. Binding of radioactive ligand in the presence or absence of PHF was determined by liquid scintillation counting. Specific versus non-specific ligand binding was determined in the presence of a 100-fold excess of cold ligand. A single concentration of PHF (1 μM) was used in all of the experiments. The results are means + S.E. of triplicate determinations.

**Results:** Neither form of PHF ($PHF_S$ or $PHF_L$) affected agonist or antagonist binding to the channels examined. Neither form of PHF ($PHF_S$ or $PHF_L$) affected agonist or antagonist equilibrium binding (4 hours) to adrenergic receptors (α and β) to angiotensin receptors ($AT_1$ and $AT_2$), or to norepinephrine transporter. $PHF_S$ increased equilibrium [$^{125}$I] endothelin-1 binding to ET-A receptors (31% + 5%) but not to ET-B receptors. $PHF_L$ increased binding to ET-A receptors 22% + 3%.

**Conclusions:** $PHF_S$ increased endothelin-1 binding to ET-A receptors in equilibrium binding assays. ANG II and $PHF_S$ share greater than 80% amino acid homology. ANG-II has been reported to enhance ET-1 binding to ET-A receptors and it appears that $PHF_S$ may mimic this effect.

*Funding:* NIDDK Support, Other U.S. Government Support

## PUB301

### Serum Asymmetric Dimethylarginine and Nitric Oxide Levels and Their Correlation with Kidney Functions in Turkish Patients with Acute Ischemic Stroke

Hakki Yilmaz, Huseyin tugrul Celik, Ali Akcay, Nuket Bavbek, Ayse Mukadder Bilgic. *Turgut Ozal Univ, School of Medicine, Ankara, Turkey.*

**Background:** Nitric oxide synthase (NOS) is present in the brain and cerebral arteries and enables the synthesis of nitric oxide (NO), which plays a critical role in brain perfusion. Asymmetrical dimethyl arginine (ADMA) is an endogenous NOS inhibitor. The goal of this study was to examine ADMA as an indicator of endothelial dysfunction of renal functions in patients with acute ischemic stroke and determine if a correlation between ADMA and NO levels or L-arginine/ADMA ratios exists.

**Methods:** Fifty-two patients and 48 healthy controls were included in this study. ADMA and Arginine levels were measured with an Applied Biosystems MDS SCIEX API 3200 LC-MS/MS system device in the ESI mode and an Aglient Eclipse XDB-C18 column. NO was measured with a Nitrate/Nitrite colorimetric assay kit.

**Results:** The mean serum ADMA level was 0.46 ± 0.13μM for the patients and 0.40 ± 0.11μM for the controls.The mean NO level was 2.78 ± 1.59 μM for the patients group and 4.34 ± 2.70 μM for the controls. The ADMA levels for the patient group were significantly higher than for the control group (p=0.011); the NO levels for the patients were significantly lower than for the controls (p<0.001).There was a negative correlation between the NO levels and age (r = -0.251), and a positive correlation between NO levels eGFR (r = 0.223). In addition, there was a positive correlation between ADMA and creatinine (r=0.224) and a negative correlation between ADMA and eGFR (r= -0.216).

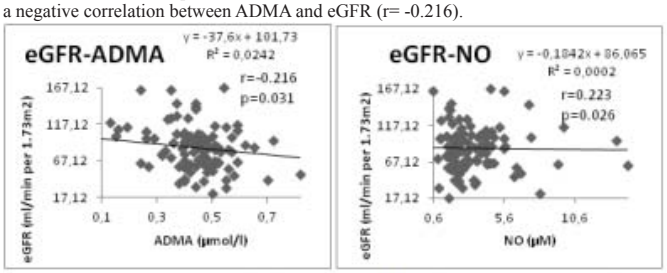



Figure 1. eGFR-ADMA correlation (left), eGFR- NO correlation (right).

**Conclusions:** Increased plasma levels of the NOS inhibitor ADMA and decreased levels of NO were found to be independent risk factors for ischemic stroke. Decreased NO levels cause vasoconstriction and may be important in the pathogenesis of ischemic stroke.

## PUB302

### Regulation of Human Endothelial Cells by Vitamin D Metabolites

Jessica L. Sea,[1] Tae Wuk Ko,[1] Eileen W. Tsai,[2] Isidro B. Salusky,[2] Martin Hewison.[1] *[1]Dept of Orthopaedic Surgery, Univ of California Los Angeles, Los Angeles, CA; [2]Dept of Pediatrics, Univ of California Los Angeles, Los Angeles, CA.*

**Background:** Vitamin D-deficiency in humans has been linked to aberrant vascular function but the possible mechanisms for this remain unclear. In human umbilical vein endothelial cells (HUVEC) effects of inactive 25-hydroxyvitamin D (25D) and active 1,25-dihydroxyvitamin D (1,25D) have been reported, but only following immune/inflammatory challenge. In current studies we have assessed 25D and 1,25D responses in HUVEC and human aortic endothelial cells (HAEC) and compared this to an established 25D/1,25D target cell, human monocytes.

**Methods:** Primary cultures of HUVEC, HAEC and human monocytes were cultured in the presence of 25D, 1,25, as well as native vitamin D (VitD) and 24,25-dihydroxyvitamin D for 6, 12 or 24 hrs. Cells were then lysed to extract RNA and expression of various target genes assessed by qRT-PCR. Western blot analysis were carried out to assess changes in protein expression.

**Results:** HAEC showed similar patterns of mRNA expression for the vitamin D-activation enzyme CYP27B1 and vitamin D receptor (VDR) relative to monocytes, whereas HUVEC exhibit lower levels of CYP27B1 and VDR. At 6 hours of culture, HAEC treated with LPS showed induction of CYP27B1 expression, whereas HUVEC treated with LPS led to a decrease in CYP27B1. Both HAEC and HUVEC treated with TNFα showed induction of VEGF with the effect taking place at 6 hours in HAEC but not until after 12 hours in HUVEC. At 6 hours HAEC showed little response to vitamin D metabolites but after 12 hours of culture HAEC treated with 25D, 1,25D and 24,25D showed decreased expression of VEGF. This was also seen in HUVEC with the effect being most pronounced after 24 hours.

**Conclusions:** These in vitro data suggest key differences between HAEC and HUVEC responses to LPS and suggest HAEC are more sensitive to vitamin D metabolites relative to HUVEC which exhibit more delayed responses to treatments of 25D, 1,25D and 24,25D. Direct effects of vitamin D metabolites on VEGF in both HAEC and HUVEC suggest that vascular endothelial cells may be highly sensitive to changes in patient vitamin D status.

*Funding:* NIDDK Support, Other NIH Support - Ruth L. Kirschstein National Research Service Award T32HL69766

## PUB303

### Association between Renal Microangiopathy and Brachial Artery Flow-Mediated Vasodilation in Patients with Chronic Kidney Disease

Tsuyoshi Miyagi,[1] Kentaro Kohagura,[1] Yusuke Ohya,[1] Kunitoshi Iseki.[2] *[1]Dept of Cardiovascular Medicine, Nephrology and Neurology, Univ of the Ryukyus, Nishihara, Okinawa, Japan; [2]Dialysis Unit, Univ Hospital of the Ryukyus, Nishihara, Okinawa, Japan.*

**Background:** Endothelial dysfunction is associated with progression of chronic kidney disease (CKD) and cardiovascular disease (CVD). However, underlying mechanism of this association is unknown. Pre-glomerular microvessel damage could be responsible for albuminuria, and hypertension, both of which are risk factor for progression of CKD and CVD. We examined the cross-sectional association between renal microvessel damage and brachial artery endothelial function using renal biopsy specimen.

**Methods:** Small arterial intimal thickening and arteriolar hyalinosis were assessed by semi quantitative grading for small artery among 172 patients with CKD (95 men and 77 women), who underwent renal biopsy. Vascular endothelial cell function was assessed by flow mediated dilatation (FMD) of forearm.

**Results:** The mean of age, blood pressure (BP), estimated glomerular filtration rate (eGFR), urinary protein, %FMD, and mean grade of arterial intimal thickening were 45y.o, 120/74 mmHg, 71.4 ml/min/1.73m$^2$, 1.75 g/gCr, 5.9%, and 2.3, respectively. %FMD was positively correlated with eGFR and negatively correlated with age, uric acid, HbA1c, and index of arterial intimal thickening. Multivariate regression analysis showed that %FMD was negatively correlated with max grade of arterial intimal thickening or arteriolar hyalinosis, respectively even after adjustment for age, sex, mean arterial BP, eGFR and HbA1c.

**Conclusions:** The results of present study suggested that renal microangiopathy might be associated with systemic endothelial dysfunction of macro-vessel, independent of classical CVD risk factors. This association might be involved in the link between endothelial dysfunction and progression of CKD and CVD.

## PUB304

### Dysfunctional Contractility and Endothelium Dependent Relaxation Mediated by Reactive Oxygen Species and Nitric Oxide Insufficiency in Microvessels from Renal Failure Mice

Dan Wang,[1] Cheng Wang,[1,2] Christopher S. Wilcox.[1] *[1]Hypertension, Kidney and Vascular Research Center, Georgetown Univ, Washington, DC; [2]Div of Nephrology, The Third Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

**Background:** Cardiovascular disease (CVD) is major complication in chronic renal failure (CRF). Endothelial dysfunction is a starting point for vascular change. We hypothesized that reactive oxygen species (ROS) and insufficiency of nitric oxide (NO) mediated abnormal microvascular contraction and endothelial function in renal reduced mass (RRM) mice.

**Methods:** Mesenteric arterioles (MAs) were isolated from C57BL/6 mice after sham-operation (Sham) or RRM (5/6 nephrectomy) for 3 months (n=6/group), mounted on a wire myograph. Relaxations were tested to sodium nitroprusside (endothelium-independent relaxation, EIR) and acetylcholine (ACh) in preconstricted MAs for: endothelium-dependent relaxation (EDR); endothelium-derived relaxation factor (EDRF; NOS-dependent relaxation); endothelium-derived hyperpolarizing factor (EDHF; $K^+$ channel dependent relaxation). Contractions were tested to endothelium-derived contracting factor (EDCF; ACh induced contraction with blocked endothelial relaxation pathways); phenylephrine (PE); U-46,619 and endothelin-1 (ET-1). NO activity (DAF-FM fluorescence) and ROS generation (tempo-9-AC fluorescence) were measured by fluorescence microscope equipped with RatioMaster system. Data present as mean ±SEM.

**Results:** The MAs from RRM mice had diminished EDR (54 ±5 *vs.* 77±3%; P<0.01), EDRF (13±5 *vs.* 27±4%; P<0.01) and NO activity (0.18 ± 0.05 *vs.* 0.36± 0.04 units; P<0.05), but not in EDHF and EIR , and developed an EDCF (14±1 vs. 8±1%; P<0.05) and enhanced ACh-induced ROS (0.17±0.03 *vs.* 0.06±0.02 units; P<0.05). Contractions were enhanced to U-46, 619 (107±4 *vs.*87±6%, P<0.05) and ET-1 (108±7 *vs.* 89±4%, P<0.05), but not to PE.

**Conclusions:** Mice with RRM model developed defective microvascular EDR and EDRF, a new ACh-induced EDCF and enhanced contraction responses. These profound microvascular disturbances mediated by excessed ROS generation and diminished NO may contribute to the development of premature CVD in CRF.

*Equal contribution of work from DW and CW.

*Funding:* NIDDK Support

## PUB305

### Arteriovenous Fistula Leads to Elevated Pulmonary Vascular Resistance in Hemodialysis Patients with Pulmonary Hypertension

Saranya Buppajarntham,[1] Rapeepat Lekkham.[2] *[1]Medicine, Albert Einstein Medical Center, Philadelphia, PA; [2]Nephrology, Albert Einstein Medical Center, Philadelphia, PA.*

**Background:** An incidence of pulmonary hypertension in ESRD is rising along with the increment of mortality rate. The mechanism of pulmonary hypertension in hemodialysis patients has been proposed as the effects of high cardiac output, which cause by arterio-

venous fistula (AVF) that functions as an artificial shunt. We hypothesized that after AVF created, changing of systemic hemodynamics and circulating vasoactive substances leads to increase pulmonary vascular resistance and pulmonary hypertension subsequently.

**Methods:** We conducted a retrospective study in hemodialysis patients, who have had two right heart catheterization (RHC) from January 2002 through March 2013. After 71 charts reviewed, we recruited 19 patients with AVF and 13 patients with non-AVF as a dialysis access. The progression of hemodynamic parameters between two catheterizations was examined. Statistical analysis was done by paired two way t-test.

**Results:** In AVF group with mean pulmonary arterial pressure of second RHC more than 25 mmHg, pulmonary vascular resistance was increased (p=0.07), while mean arterial blood pressure and systemic vascular resistance were decreased (p=0.04 and 0.08 respectively). In both AVF and non-AVF groups, there was no difference in cardiac output or pulmonary capillary wedge pressure from two RHC. In non-AVF group, no difference between pulmonary vascular resistance from two RHC was observed.

**Conclusions:** Although, cardiac output was unchanged, pulmonary vascular resistance was elevated after AVF created. We proposed elevated pulmonary vascular resistance as a major mechanism of PHT in hemodialysis patients with AVF and the increase of pulmonary vascular resistance is not affected by increased cardiac output.

## PUB306

**Gone but Not Forgotten** Hyma V. Polimera,[1] Sachinkumar B. Kanagali,[2] Robert Pursell,[3] David Leh.[4] *[1]Internal Medicine, St. Luke's Univ Health Network, Bethlehem, PA; [2]Internal Medicine, St. Luke's Univ Health Network, Bethlehem, PA; [3]Nephrology, St. Luke's Univ Health Network, Bethlehem, PA; [4]Internal Medicine, St. Luke's Univ Health Network, Bethlehem, PA.*

**Background:** Fibromuscular dysplasia (FMD) is a non-inflammatory, non-atherosclerotic disorder of medium size arteries of unknown etiology that can lead to arterial stenosis, aneurysm, and dissection.

**Methods:** A healthy 40 year old man was admitted with acute onset of severe, sharp, left flank pain. CT scan of the abdomen, with contrast, revealed acute, left renal infarcts and subacute/chronic right sided infarcts. A digital subtraction angiogram showed duplication of the right renal artery and abnormal, beaded left renal arterial vasculature and multiple thrombi; findings consistent with FMD. Follow up abdominal aortogram showed an aneurysm and spontaneous dissection of the left renal artery. The aneurysm was successfully embolized using detachable bioactive coils. The patient was started on IV heparin and Coumadin, which was to be continued for 6 months, with close follow up of his kidney function. After an uneventful six year hiatus, the patient presented with severe, right flank pain. CT, with angiogram, of the abdomen revealed right, main renal artery dissection with infarction and abnormalities at the origin of the accessory right renal artery. A diffuse abnormality of the celiac artery, previously normal, was described. The patient was anticoagulated with IV heparin and Coumadin and conservatively managed for the arterial dissection. Follow up includes quarterly serum creatinine levels and annual renal duplex ultrasound.

**Conclusions:** Since first described in 1938, there has been little progress in understanding the pathogenesis and outcomes of FMD. The clinical course is highly variable, ranging from the asymptomatic to the symptomatic with hypertension or with grave complications like dissections, aneurysmal ruptures with organ infractions or stroke depending on the severity and arterial segment involved. Though more common in young women, FMD should be considered in male patients presenting with flank pain and hypertension.

## PUB307

**Oral Administration of Calcium Carbonate Is a Risk for Coronary Artery Disease in Japanese Hemodialysis Patients in a Dose-Dependent Manner** Chisako Nakano, Yoko Shima, Naoko Morikage, Masafumi Yamato, Akira Wada, Takahito Ito. *Div of Nephrology, Osaka National Hospital, Osaka, Japan.*

**Background:** Cardiovascular disease is the leading cause of hospital admission and death among patients with end-stage kidney disease. Several mechanisms are thought to contribute to vascular calcification and subsequent cardiovascular disease. It is known that calcium administration accerelates artery calcification and that cardiovascular events. In Japan, however, calcium carbonate is often prescribed as a phosphate binder to treat hyperphosphatemia.

We studied the relationship between cardiovascular disease (CVD) and oral calcium supplementation in Japanese patients under maintenance hemodialysis.

**Methods:** We performed a single-center case-control study using 90 individuals (68.6 ± 10.5 y, 67 males) who underwent maintenance hemodialysis while hospitalized in our hospital from December 1, 2011 to April 30, 2013. Blood analysis was done at the time of hospitalization or within one month prior to hospitalization. The case and control groups comprised 34 patients hospitalized for examination and/or treatment of coronary artery disease and 56 patients hospitalized for other medical issues, respectively. One with any previous history of CVD was excluded from the control group.

**Results:** More amount of calcium carbonate was administered in the case group than in the control at the time of hospitalization (2.25 ± 1.50 vs. 1.31 ± 1.19 g/day, P=0.0034), whereas there was no statistical difference regarding serum albumin, inorganic phosphate, albumin-corrected calcium, calcium-phosphate product, intact PTH or C-reactive protein. Dialysis vintage was significantly longer in the case than in the control (64.2 [29.0 – 132.7] vs. 35.7 [15.8 – 69.7], P=0.0458).</a> Male sex was a significant risk (P=0.0046) but diabetes was marginal (P=0.0612). Age was not significant. Odds ratio of calcium carbonate administration (per 1 gram increment) for CAD was 1.70 [95%CI: 1.22 – 2.45] (non-adjusted), and 1.65 [1.18 – 2.40] (adjusted by dialysis vintage and sex).

**Conclusions:** Calcium carbonate may be a risk factor of CVD-related hospitalization in a dose-dependent manner.

## PUB308

**Characteristics of Phosphate Binders Dissolution** Heather Busby,[2] John Durham,[1] Tarek M. Sobeih,[1] Abdullah Hamad.[1] *[1]Palmetto Nephrology, Orangeburg, SC; [2]Davita Dialysis, Orangeburg, SC.*

**Background:** Controlling hyperphosphatemia continues to be a challenge in dialysis patients. We evaluated for qualitative differences in the in-vitro dissolution times and characteristics of several calcium and non-calcium based phosphate binders in the acidic medium of vinegar to determine if these changes might correlate with the clinical responses to these binders.

**Methods:** Commercial phosphate binders were placed in 3 ounces of distilled white vinegar and observed for 20 minutes in transparent containers. Time to dissolution and its qualitative characteristics were visually monitored by two observers.

**Results:** Results are summarized in the attached table.

| | |
|---|---|
| Tums 1000 mg crushed | effervescent, very fast, mild increase in total volume, dissolved in 4 minutes |
| Calcium Acetate 667 mg powder (1) | slow and mild reaction, 8 minutes to dissolve |
| Renvela 2400 mg powder | effervescent, large volume expansion and dissolved in 1 minute |
| Fosrenol 1000 mg crushed | solution slowly became cloudy over 20 minutes |
| Phoslyra 667 mg (liquid) | immediatly dissolved woth no reaction |

1- calcium acetate was first removed the gel cap and crushed.

**Conclusions:** Different binders have different >in-vitro dissolution times and characteristics. That can affect the speed of binding to phosphorus and consequentially the importance of timing of taking medicine in relationship to food. In this regard the large and rapid expansion of volume with Renagel powder may explain some of its gastric and intestinal side effects.

## PUB309

**Hypomagnesemia Associated with Malignancy Induced Hypercalcemia** Wasay Humayun, Aparna Rao. *Vanguard Internal Medicine, Macneal Hospital, Berwyn, IL.*

**Background:** We report a case of a 81 y/o female patient who was admitted after a fall at home. Pt was found to have severe persistent hypomagnesemia despite attempts for repletion and hypercalcemia on labs.

**Methods:** Patient's detailed history was obtained a comprehensive physical exam was done. Patient's chart and records reviewed from an OSH as well. Daily labs and imaging were reviewed along with speaking to the Endocrinologist and Nephrologist on the case.

**Results:** Significant labs show her calcium was elevated at 11.1 and her magnesium level was 1.2. Her intact PTH was less than 7 and a Vitamin 1,25 OH level was 114. Patient also had a CT abdomen which showed a 8 cm mass near the pancreas which was biopsied to show a diffuse large B-cell lymphoma.

**Conclusions:** Patient's persistent hypomagnesemia was likely renal in etiology etiology. The utility of Fractional excretion of magnesium, which was calculated to be 3.7, helped differentiate renal from GI cause. The possible renal etiologies included: Loop diuretics, Barters syndrome, Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), Autosomal Dominant hypocalcemia. . Patient's hypercalcemia likely secondary to the newly diagnosed lymphoma an interesting contributing factor. Hypercalcemia may result in hypomagnesemia at least in part from the fact that calcium and magnesium functionally compete for transport in the thick ascending limb of the loop of Henle. A variety of mechanisms may contribute to the reduction in magnesium reabsorption resulting from hypercalcemia. One well known mechanism is facilitated by the basolateral calcium-sensing receptor (CaSR) in the thick ascending limb: This leads to an inhibition of the apical ROMK channels. Also hypercalcemia can decrease in serum magnesium by decreased paracellular transport of calcium.

## PUB310

**Amphotercin Induced Pseudeohyperphosphatemia** Snezana H. Mijovic-Das,[1] Darius Mason,[2] Muhammad A. Ashraf,[1] Wadad S. Mneimneh.[1] *[1]Nephrology, Albany Medical College, Albany, NY; [2]Pharmacy, College of Pharmacy, Albany, NY.*

**Background:** Previous studies suggest that liposomal Amphoteracin-B (LAMB) may interfere with inorganic phosphate (Pi) lab measurements, causing pseudohyperphosphatemia (PHP). LAMB-associated PHP is not well recognized and may lead to unwarranted workup and therapy. We present a case of a previously healthy 47-year-old male patient with a baseline Scr of 0.9 mg/dl, who was diagnosed with cryptococcal meningitis. He was treated with LAMB and subsequently developed PHP.

**Methods:** Pi measurements were obtained by endpoint assay (EPA) using two different instruments (Beckman Coulter Unicel DXC and Dimension Vista 500). Lipid ultracentrifugation (LUC) was applied on one of the samples. For further quality control we serially measured Pi levels with LAMB-spiked calibration solutions, using both EPA and kinetic assay (KA), reproducing the variation pattern of Pi levels described in the literature.

**Results:** Table1 shows values of serum Pi, Scr and LAMB doses, throughout hospitalization.

| Day Post-admission | 2-7 | 8-9 | 10-11 | 12-15 | 16-17 | 18-24 |
|---|---|---|---|---|---|---|
| LAMB (mg/day) | 440 | 220 | 350 | 440 | 220 | 350 |
| Scr (mg/dl) | 1.5-2.9 | 2.9-2.3-2.0 | 2.0-1.7 | 1.7-2.0 | 2.0-2.2-2.0 | 2.0-1.8-1.5 |
| Pi (mg/dL) | 5.3-6.6 | 6.6-6.4-5.2 | 5.2-4.6 | 4.6-5.6 | 5.6-5.5-5.8 | 5.8-6.0-4.7 |

The patient's elevated Pi levels could not be explained by the degree of renal dysfunction reflected by Scr increase, whereas they directly correlated with LAMB doses, suggesting an interference effect. Previous studies had similarly shown erroneous linear elevation of Pi levels with increasing LAMB concentrations. The interference effect was supported by: Correction of PHP by either LUC or lipemic clearing agents (Microcon®, LipoCLear®); Concurrent Pi measurement using EPA and KA; Ineffectiveness of PO4 binders to resolve PHP. Of note, LUC failed to correct Pi level in our case, but this single-sample result is inconclusive.

**Conclusions:** LAMB-associated PHP can lead to extensive and unwarranted workup. Awareness of this drug-assay interaction can prevent inappropriate therapy such as dietary restriction, phosphate binders, hemodialysis, or altering the choice of ideal antifungal regimen.

## PUB311

**Ethanol Injections for Management of Severe Hyperparathyroidism: Case Reports** Ivana Lazich, Marc J. Alonzo, Carolyn Kirchgessner Donaldson, Stuart M. Sprague. *NorthShore Univ Health System, Univ of Chicago, Evanston, IL.*

**Background:** Uncontrolled hyperparathyroidism in the setting of CKD or primary hyperparathyroid adenoma remains a therapeutic challenge despite the developing pharmacotherapy. We evaluated the utility of percutaneous ethanol injection therapy (PEIT) for the management of severe hyperparathyroidism with hypercalcemia in patients with nodular parathyroid hyperplasia.

**Methods:** A total of nine patients with elevated PTH and calcium concentrations and discrete parathyroid nodules were evaluated. Parathyroid nodules were identified and localized via Doppler ultrasound (US) and 98% ethanol (<1 ml) was injected via US guidance. A successful procedure was defined as decrease in PTH > 50% over a minimum of 6 months. To determine if response was a function of nodular size, mean nodule size was compared between responders and nonresponders (T-test).

**Results:** Five patients had uncontrolled tertiary hyperparathyroidism (either ESRD on HD or post renal transplant) and four had primary hyperparathyroidism. This population included seven Caucasians and seven females with mean age of 56 ± 4.4 years. PTH concentrations, prior the procedure, ranged between 88 - 2922 pg/ml and serum calcium ranged 10 - 12.7 mg/dl. Five of the nine patients were considered responders. Mean nodule size in responder group was 1.28 mm and in nonresponder group was 1.86 mm (NS). Of the patients who responded to treatment only two had normalization of PTH, whereas all of them had normalization of serum calcium concentrations.

**Conclusions:** This preliminary experience demonstrates that some patients with severe hyperparathyroidism with hypercalcemia could be controlled with PEIT. The procedure, however, is limited to selective group of patients who have readily identified US evidence of parathyroid adenomas. Our evaluation did not show impact of nodular size on outcome as reported previously, however, our sample was small. The risk is minimal but the potential downside is the need for repetitive interventions until desired results have been achieved. Further studies should be performed to evaluate this as a viable option to surgery or in those who are not a surgical candidate.

*Funding:* NIDDK Support

## PUB312

**25-Hydroxy-Vitamin D3 Concentration Detection for Screening of Early Renal Damage in Diabetic Kidney Disease** Wenbo Zhao,[1] Bo Huang,[2] Cailian Cheng,[1] Xun Liu,[1] Zengchun Ye,[1] Tan-qi Lou.[1] *[1]Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Nephrology, The Sichuan Academy of Medical Sciences &Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

**Background:** To detect of the type 2 diabetic nephropathy patients' 25-hydroxy-vitamin D3 concentration, and analyze whether the concentration applies to screening of diabetic nephropathy early renal damage.

**Methods:** We collected the clinical data and blood samples of hospitalized patients with type 2 diabetes. The material is assessed according to eGFR (﹥30 ml/min·1.73m$^2$, MDRD Formula) and proteinuria combined diagnostic criteria of DKD. 52 cases were acquired, consisting of 23 males and 29 females, divided into normal proteinuria group (31 cases) with average (56.29±13.84) years old, and microalbuminuria group (21 cases) with average (60.29±13.24) years old. The quantitative determination of serum 25(OH) VitD3 level (IDS Corp., England) is further performed adopting ELISA. The 25(OH) VitD3 level of normal proteinuria group is (44.51±19.87)ng/ml, while that of (31.7±10.03) ng/ml for microalbuminuria group (P<0.05). SPSS 15.0 is used for statistical analysis. Multi-factors are brought into the variables, then applying the ROC curve method.

**Results:** Multi-factor analysis demonstrates that ALB, UA and 25 (OH) VitD3 are the three main relevant factors. The areas under the curves of these three main relevant factors are 0.698, 0.770 and 0.707 respectively. The underlying area of ALB+UA+ 25(OH) VitD3 combined curve (ALB+UA+ 25 (OH) VitD3) is 0.909, which is the maximum, and also greater than the area of 0.814 combining (ALB+UA).

figure1

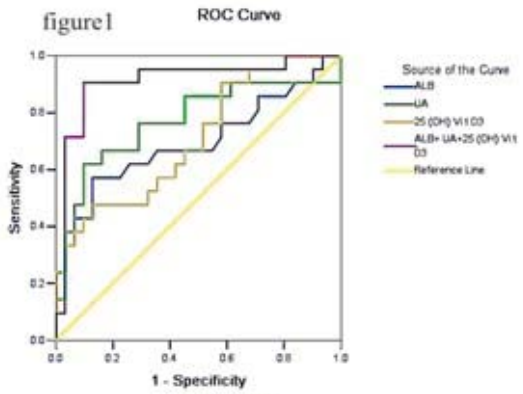
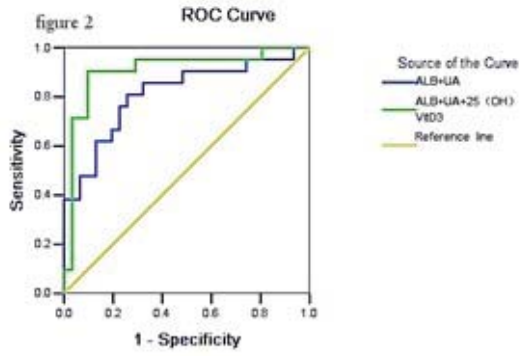



figure 2




**Conclusions:** The concentration of 25(OH) VitD3 in microalbuminuria group is lower. Multi-factorial ROC curve has a larger area. 25(OH) VitD3 can be considered as a cofactor for early screening in the period of microalbuminuria.

*Funding:* Government Support - Non-U.S.

## PUB313

**Dialysate-Magnesium Regulates Serum-Magnesium Regulates PTH** Bernd A. Winterberg. *Dept of Nephrology, Marienhospital, Emsdetten, Germany.*

**Background:** The magnesium balance is a function of absorption and excretion.

The excretion of Mg is almost exclusively renal, is determined by the glomerular filtration rate and decreases with increasing renal impairment. In dialysis patients, magnesium is eliminated via the dialysate.

Hemodialysis patients typically have a mild hypermagnesemia (1.03 to 1.5 mmol / l, normal range 0.62 to 1.03 mmol / l).

The serum magnesium in hemodialysis patients depends on the magnesium concentration in the dialysate. If you increase it, increases the serum magnesium. Hypermagnesemia below 1.5 mmol / l is usually asymptomatic, from 2-3 mmol / l connect symptoms such as lethargy and dizziness, at even higher values, loss of tendon reflexes, bradycardia, paralysis, apnea and cardiac arrest .

Positive effects of hypermagnesemia in dialysis patients: an increase in myocardial contractility, prevention of intradialytic hypotension, lowering elevated PTH levels, inhibition of vascular calcification.

Hypermagnesemia has a suppressive effect on PTH secretion. There is an inverse correlation between intact PTH and serum magnesium regardless of serum calcium and serum phosphate.

The PTH levels in dialysis patients can be regulated by modification of dialysate magnesium. If the PTH level is below the recommended lower limit of 150 micrograms / ml, it will increase by the use of low dialysate magnesium. If it is above the recommended upper limit of 300 micrograms / ml, it will decrease by increasing the dialysate magnesium.

**Methods:** In a separate investigation dialysis patients(n=24) were treated with a dialysate magnesium of 0.5 mmol / l ( or 1.0 mmol / l).

**Results:** In these patients before HD the mean serum magnesium was 0.81 + / - 0.22 mmol / l, n = 17 (or 1.3 + / - 0.19 mmol / l, n = 7) and after HD the mean serum magnesium was 0.77 + / - 0.14 mmol / l (or 1 , 37 + / - 0.05 mmol / l) .

**Conclusions:** Regulation of serum magnesium by modification of dialysate magnesium lead to serum magnesium concentrations (low or high) in the safe range, offering the chance to regulate PTH levels, to reduce vascular calcification, left ventricular hypertrophy and cardiovascular mortality.

**PUB314**

**A Rare Cause of Tumor-Induced Osteomalacia: Metastatic Prostate Cancer**
Abdul Mubeen Mohammed,[1] Faheemuddin A. Ahmed.[2] *[1]Swedish Covenant Hospital, Chicago, IL; [2]Medical College of Wisconsin, Milwaukee, WI.*

**Background:** We present a rare case of severe asymptomatic hypophosphatemia in a patient with metastatic prostate cancer. It was associated with significant renal phosphate wasting as a consequence of high levels of fibroblast growth factor (FGF-23). We hypothesize that the metastatic prostate cancer caused high levels of FGF-23, resulting in tumor-induced osteomalacia.

**Methods:** A 63-year-old man with history of metastatic prostate cancer was evaluated for phosphorus level of less than 1 mg/dL for four months. He was asymptomatic. His past oncologic history was significant for Gleason 6 score prostate cancer diagnosed seven years ago. Patient subsequently underwent radical prostatectomy, adjuvant hormonal therapy and eventually radiation. PSA declined from a peak of 60 ng/mL to 0.08, but started increasing again. Adjuvant hormonal therapy was restarted with LHRH and bicalutamide. Follow-up staging scans revealed progressive metastatic disease and he was started on Docetaxel, three months prior to admission. Physical examination was unremarkable. Laboratory evaluation showed a calcium level of 8.4 mg/dL; 1,25 Vitamin D level 55 pg/mL and intact PTH level 182 pg/mL. Urine studies revealed renal phosphate wasting with fractional excretion of phosphorus of 60%. Serum FGF-23 levels were significantly elevated at 505 RU/ml (normal range of less than 180 RU/ml). Bone survey showed abnormal diffuse axial and proximal appendicular skeletal metastatic disease. Further work-up with Octreotide scintigraphy scan to locate for neuroendocrine tumor activity was negative. Patient was continued on potassium phosphorus supplements (10 tabs/day) with calcitriol. Subsequent phosphorus levels were at lower end of normal range.

**Conclusions:** Tumor induced osteomalacia due to metastatic prostate cancer is rarely described in literature. FGF-23 released from the tumor cells impairs phosphate reabsorption and 1α-hydroxylation of 25-hydroxyvitamin D. This leads to hypophosphatemia and usually low levels of 1,25-dihydroxy vitamin D. As the particular lesion could not be located with the octreotide scan, medical management was offered which included phosphate supplements and active vitamin D.

**PUB315**

**Phosphate Binder Powder Formulations: Perspectives from the Renal Care Community**
J. Brian Copley,[1] Elizabeth J. Lindley,[2] Maria Cruz Casal,[3] Susan Rogers,[4] Jitka Pancirova,[5] Jennifer Kernc,[1] Denis Fouque.[6] *[1]Shire Pharmaceuticals, Wayne, PA; [2]Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom; [3]Hospital 12 de Octubre, Madrid, Spain; [4]Codia Waterland, Pumerend, Netherlands; [5]EDTNA/ERCA Secretariat, Prague, Czech Republic; [6]Centre Hospitalier Lyon-Sud and CENS, Lyon, France.*

**Background:** Phosphate binders are commonly used in tablet form to help patients with chronic kidney disease limit their absorption of dietary phosphorus. Alternative formulations, such as powders, provide choice and may facilitate medication adherence. A survey of renal nurses and dietitians was conducted in order to examine their perceptions of the relative benefits and drawbacks of a powder formulation.

**Methods:** Renal nurses and dietitians (n = 83) in the Netherlands, Spain, Sweden and the UK were asked to complete a survey in September and October 2012.

**Results:** The most frequently reported (38/83 responders, 46%) perception of powder formulations was that they are likely to be beneficial because they are easier for the patient to take, particularly if the patient experiences difficulties chewing or swallowing tablets. This perception was reported by a similar proportion of nurses (14/35, 40%) and dietitians (22/48, 46%). When a powder formulation is administered with food, the associated reduced need for additional fluid intake was perceived as another benefit (7/83, 8%). The most frequently reported perceived drawbacks of powder formulations were poor palatability (16/84, 19%) and administration of inaccurate doses (15/83, 18%). A greater proportion of dietitians than nurses perceived that the drawbacks of the powder were poor palatability (11/48, 23%, and 5/35, 14%, respectively) and administration of inaccurate doses (12/48, 25%, and 3/35, 9%, respectively). Another perceived drawback of powder was increased fluid intake required when a formulation is administered as an oral suspension (11/83, 13%).

**Conclusions:** Phosphate binder powder formulations were generally perceived favorably in the renal community surveyed, mainly because they were considered to be easier to take. The perceptions evaluated here may assist in the development of future phosphate binder formulations.

*Funding:* Pharmaceutical Company Support - Shire Pharmaceuticals

**PUB316**

**Assessment of Serum Calcium (Ca), Phosphorous (P) and Parathyroid (PTH) Hormone Levels According to K/DOQI Guidelines in Hemodialysis Patients at a Tertiary Care Hospital of a Developing Country**
Ayesha Mahmood Malik,[1] Hafiz I. Ahmad,[1] Syed Rizwan Bokhari,[1] Muhammad Zaman Khan Assir,[1] Arif Asif.[2] *[1]Dept of Nephrology, Allama Iqbal Medical College/ Jinnah Hospital, Lahore, Punjab, Pakistan; [2]Div of Nephrology and Hypertension, Albany Medical College, Albany, NY.*

**Background:** Studies have shown that achievement of bone mineral metabolism targets as recommended by K/DOQI guidelines is universally poor. Patients in developing countries receive less than optimal dialysis treatment. A cross sectional study was performed to assess these markers in our patient population receiving dialysis at a tertiary care hospital.

**Methods:** Demographic data of patients were collected and pre dialysis blood samples were drawn for serum calcium, phosphorous, alkaline phosphatase, intact PTH and albumin. Ca x P product was also calculated. The results were analyzed and compared with K/DOQI guidelines targets.

**Results:** Of the 66 ESRD patients, 41(62%) were males with mean age 42.3±5 years. The percentage of patients who achieved K/DOQI target ranges of calcium, phosphorus, PTH and CaxP product were 40.9%, 43.9%, 19.7% and 77.3% respectively. Values above the target ranges of calcium, phosphorus, PTH were seen in 12.1%, 36.4% and 59.1% respectively, while values below target ranges of calcium, phosphorus, PTH were found in 47%, 19.7% and 21.2% patients respectively. Abnormal Ca x P was seen in 23% patients.

**Conclusions:** Most of the patients receiving dialysis at our center have their values of calcium, phosphorus, alkaline phosphatase, PTH and Ca x P product out of the range recommended by K/DOQI guidelines. These results are similar to those achieved by the patients in developed countries.

**PUB317**

**The Efficacy and Safety of Lanthanum Carbonate on Chronic Kidney Disease Mineral and Bone Disorder in Patients with Dialysis: A Systematic Review**
Ling Ji, Zi Li, Fei Liu, Xiaohong Tang, Junming Fan. *Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan, China.*

**Background:** Hyperphosphatemia is a common complication in patients with end-stage renal disease (ESRD) requiring dialysis. Elevated serum phosphorus concentration is an established independent risk factor for increased mortality. Lanthanum carbonate (LC) is a potent new, non-aluminum, non-calcium phosphate binder. This systematic review evaluated the efficacy and safety of LC in dialysis patients.

**Methods:** Search methods: MEDLINE, EMBASE, the Cochrane Renal Group's Specialized Register and CENTRAL were searched from . 2000 to Dec. 2012 for relevant studies. Selection criteria: all randomized controlled trials (RCTs) and quasi-RCTs that assessed the efficacy and safety of LC in hemodialysis or peritoneal dialysis patients. Data collection and analysis: two authors independently reviewed search results and extracted data. Analysis was performed using the statistical software Review Manager 5.1.

**Results:** Twelve RCTs were identified and retained for this review, with 3029 patients included. There was no statistic difference between two groups in all-cause mortality. Due to the limited trials, there was not enough evidence to show the priority of LC in lowering vascular calcification or cardiovascular events, improving bone morphology or metabolism or improving bone turn over parameters. LC can significantly decrease serum phosphorus level and Ca×P product, increase the control rate of serum phosphorus compared with placebo. Nevertheless, there was no difference in phosphorus level, the control rate of serum phosphorus, iPTH and Ca×P product compared with calcium carbonate, but sith a significant lower level serum calcium. People treated with LC appeared a higher rate of vomiting and lower risk of hypercalcemia, diarrhea, intra-dialytic hypotension. For the incidence of other side-effects, meat-analysis showed no significant difference.

**Conclusions:** LC has a well efficacy in lowering serum phosphorus level and iPTH without increasing the serum calcium level. LC was safe for dialysis patients especially in patients with high risk of hypercalcemia except a higher incidence of vomiting.

**PUB318**

**Body Mass Index and Survival in Patients with Incident Kidney Stone Disease**
Jie Tang,[1] John R. Holmen,[2] Kim McFann,[1] Michel Chonchol.[1] *[1]Medicine, Univ of Colorado, Denver, CO; [2]Intermountain Health Care, Murray, UT.*

**Background:** Kidney stone disease is associated with obesity and metabolic syndrome. However, the impact of obesity on survival among kidney stone formers is not clear.

**Methods:** Incident kidney stone formers were identified from Intermountain Healthcare Data Warehouse and were defined as patients having first ever diagnosis of kidney stone disease using ICD9 codes 592.0, 592.1, 592.9 & 594.0 assigned from 1994 to 2013. Mortalities were determined using state and national death registries. Logistic regression models were used to test the associations of body mass index (BMI) with cardiovascular mortality (CVM) and all-cause mortality (ACM).

**Results:** Among 21,752 incident stone formers, 959 (5%) had BMI <20, 4,767 (22%) had BMI 20-24, 7,011 (32%) had BMI 25-29, and 9,015 (41%) had BMI ≥30. There were a total of 1,835 all-cause and 874 cardiovascular deaths during a median follow-up of 7.0 years. Higher BMI was associated with lower tobacco and alcohol use, lower glomerular filtration rate (GFR), and was associated with higher risk of prevalent hypertension, diabetes, and dyslipidemia. After adjusting for age, sex, race, tobacco history, alcohol history, diabetes, hypertension, cardiovascular disease history, dyslipidemia, and GFR, BMI <20 (vs. 20-24) was not associated with a significantly increased odds of CVM, odds ratio (OR) 1.5 (95% CI 0.9-2.5), p=0.11, but was associated with a significantly increased odds of ACM, OR 1.9 (95% CI 1.3-2.7), p=0.0005. BMI 25-29 (vs. 20-24) was associated with a reduced trend of CVM, OR 0.8 (95% CI 0.6-1.0), p=0.08, and was associated with a significantly reduced odds of ACM, OR 0.7 (95% CI 0.5-0.8), p=0.0002. BMI >30 (vs. 20-24) was associated with a significantly reduced odds of CVM, OR 0.6 (95% CI 0.4-0.8), p=0.0005, as well as ACM, OR 0.5 (95% CI 0.4-0.6), p<0.0001.

**Conclusions:** Higher BMI appears to be associated with better survival in patients with incident kidney stone disease. The cause of this finding remains to be determined.

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**PUB319**

**Lithogenic Risks in Calcium Oxalate Nephrolithiasis Familial Members** Thasinas Dissayabutra,[1] Jakkaphan Rattanaphan,[1] Thanida Chiramongkolsiri,[1] Talerngsak Kanjanabuch,[2] Piyaratana Tosukhowong.[1] *[1]Biochemistry, Chulalongkorn Univ, Pathumwan, Bangkok, Thailand; [2]Medicine, Chulalongkorn Univ, Pathumwan, Bangkok, Thailand.*

**Background:** Many evidences suggested that nephrolithiasis familial members are at risk, although no pathologic gene has yet defined. This study aims to discover the epigenetic risk factors in calcium oxalate nephrolithiasis family.

**Methods:** 146 participants, including 28 stone-removed nephrolithiasis patients and 45 of their first-degree descendants, as well as 73 healthy volunteers were enrolled. Medical history about urologic disease, blood and 24-hour urine were collected. Urinary calcium, magnesium, potassium, and sodium were measured by inductively coupled plasma mass spectrometry (ICP), oxalate and citrate by capillary electrophoresis, and sulfated glycosaminoglycans (sGAGs) by dye binding assay. Statistical analyses were testing by SPSS (IBM, USA) version 16.0.

**Results:** Compared to healthy population, nephrolithiasis patients had elevated urinary protein (336.6 *vs.* 30.4 mg/day, $p$=0.002), calcium (5.8 *vs*. 3.1 mEq/day, $p$=0.005), oxalate (43.7 *vs*. 12.7 mg/day, $p$<0.001) together with decreased urinary citrate (66.2 *vs.* 185.9 mg/day, $p$<0.001) and sGAGs (5.3 *vs*. 48.5 µg/ml, $p$<0.001). While the nephrolithiasis descendants had the following parameters being in-between of 2 groups: proteinuria (67.0+7.0 mg/day, $p$<0.001), diminished urinary citrate (113.6 mg/day, $p$=0.001) and sGAGs (32.29 µg/ml, $p$<0.001), all of which were more obviously changed by age. The descendant also had lower kaliuria level (19.89 *vs*. 26.49, $p$=0.001) compared to the controls. No other urinary indices or clinical characteristics showed any significant differences. Adjusted odd ratios of the nephrolithiasis for urinary protein, citrate, and sGAGs were 1.024 (1.012-1.036), 0.993 (0.989-0.997) and 0.936 (0.914-0.958), respectively.

**Conclusions:** Nephrolithiasis patients and their first-degree descendants, although no stone-forming, have elevated proteinuria as well as depleted urinary citrate and sGAGs compared to healthy controls. Stone imaging and primary prophylaxis should be warranted in the relatives with abnormalities of urinary protein, citrate, or sGAGs.

*Funding:* Government Support - Non-U.S.

**PUB320**

**Serum 25-Hydroxyvitamin D Concentration and Survival among Incident Kidney Stone Formers** Jie Tang,[1] Pamela Mettler,[1] John R. Holmen,[2] Michel Chonchol.[1] *[1]Univ of Colorado, Denver, CO; [2]Intermountain Healthcare, Murray, UT.*

**Background:** Nutritional vitamin D deficiency is highly prevalent in kidney stone formers. However, its impact on survivial among incident kidney stone (IKS) formers is unknown.

**Methods:** IKS formers were identified from Intermountain Healthcare Data Warehouse and were defined as patients having first ever diagnosis of kidney stone disease using ICD9 codes 592 & 594.0 assigned from 1994 to 2013. All-cause mortalities were determined using state and national death registries. Logistic regression models were used to test the association of serum 25-hydroxyvitamin D (25D) concentration with all-cause mortality.

**Results:** Among 1,045 IKS formers, 569 (54%) had serum 25D concentrations ≤30 ng/ml (mean=21 ng/ml), 476 (46%) had serum 25D concentrations >30 ng/ml (mean=43 ng/ml). Lower serum 25D concentration was associated with lower body mass index and higher likelihood of tobacco & alcohol exposures, but was not associated with increased risks of prevalent hypertension, diabetes, dyslipidemia and cardiovascular disease. After adjusting for age, gender, tobacco & alcohol use, diabetes, hypertension, dyslipidemia, cardiovascular disease, and GFR, serum 25D concentration >30 ng/ml was not associated with a significantly reduced odds of all-cause mortality when compared to serum 25D concentration ≤30 ng/ml, odds ratio (OR) 1.74 (95% CI 0.88 - 3.45), p=0.11. Higher serum 25D concentration (per 1 ng/ml increase) was also not associated with a reduced odds of all-cause mortality, OR 1.02 (95% CI 1.00-1.04), p=0.06.

**Conclusions:** Higher serum 25D concentration does not appear to modify survival in patients with IKS disease. This is likely due to the fact that kidney stone formers often have normal or above-normal active vitamin D levels.

*Funding:* NIDDK Support

**PUB321**

**Taking into Consideration Migrant Patients' Cross-Cultural Features during Patient Educational Therapy for Patients** Abdallah Guerraoui,[1] Guillaume Jean,[2] Agnes Caillette-beaudoin,[1] Corinne Isnard-Bagnis.[3] *[1]Nephrologie Dialyse, Calydial, Vienne, France; [2]Nephrologie Dialyse, Nephrocare, Lyon, France; [3]Patient Education, Univ Pierre et Marie Curie, Paris, France.*

**Background:** This work is based on the results of a qualitative research dealing with the educational nurse's (EN) activity during a patient education session with migrant patients. More precisely, in this article we intend to report on the way the educational nurse takes into consideration all the cross-cultural factors and their repercussion/negative impact on the EN practice.

**Methods:** This research is uses a method which stems from social sciences called "the simple auto confrontation. The process consists in filming the interaction between the EN and the patients during the educational diagnosis. This video-taped interaction is recorded and coded according to L Bardin's method of content analysis. An auto confrontation between the EN and the researcher is carried out. This second interaction is also filmed, recorded and coded according to the same method.

**Results:** We demonstrate how difficulties stemming from differences in presentations, beliefs and cultures between the EN and the migrant patient have a significant impact on the consultation. We will highlight the ability of both the EN and the migrant patient to adapt their interactions to new cross cultural realities. The EN adaptation is closely linked to the individual's career path and their formation. Therefore, this work includes operationalizing the analysis in order to make the best use of educational therapy for patient (ETP) interviews.

**Conclusions:** When EN take into consideration each patient's culture, their life path and their individual itinerary, this enhances the success of an ETP programme. Hence, this qualitative research on the cross cultural aspects of the ETP falls within a more global dimension which enables us to better analyze how the medical culture influences relationships with both migrant and non-migrant patients. The differences between these two cultures (the doctor or nurse and the patient's cultures) lead to misunderstandings and divergences which complicate the health care provider the doctor-patient relationship.

**PUB322**

**Three Children Developing Enlarged and, Echogenic Kidneys while Receiving Circulatory Support from Pediatric Ventricular Assist Device** Atul Poudel,[1] Richard E. Neiberger.[2] *[1]Dept of Pediatric Nephrology, Univ of Florida, Gainesville, FL; [2]Dept of Pediatric Nephrology, Univ of Florida, Gainesville, FL.*

**Background:** In last decade pediatric ventricular assist device (VAD) specially designed for small children have been increasing implanted in North America. Prospective trials have shown pediatric VAD decreases the mortality of patient on heart transplant list and superior to extracorporeal membrane oxygenation (ECMO). After Food and Drug Adminstration approval of pediatric VAD, Berlin Heart EXCOR in 2011, more use of this device is expected in future. We are presenting unexpected renal findings developing in three children receiving circulatory support using Pediatric VAD ( Berlin Heart Excor).

**Methods:** The mechanisms of heart failure in these children included: 1) Hypoplastic ventricle with aortic stenosis; 2) Anomalous coronary artery arising from the pulmonary artery and 3) Idiopathic progressive heart failure. During circulatory support with VAD each child had coagulation times maintained at 2-3 times normal. Each child had normal or mildly reduced renal function.Each of these children had imaging studies (Ultrasound or Computed tomography) while receiving VAD support which demonstrated enlarged echogenic kidneys.These children subsequently died and autopsy studies of two children showed multiple bilateral kidney infracts, parenchymal congestion and arterial thrombosis.

**Conclusions:** Our case series shows that thromboembolism of renal artery may be a cause of enlarged echogenic kidneys in patients on pediatric ventricular assist device.

**PUB323**

**A Case of Warfarin Related Nephropathy** Nicole Bautista, William Whittier. *Rush Univ Medical Center, Chicago, IL.*

**Background:** Warfarin was approved in 1954 and currently is the most prescribed anticoagulant medication. Warfarin related nephropathy (WRN) is a recently described condition that involves acute kidney injury, supratherapeutic international normalized ratio (INR), and tubular obstruction with red blood cells (RBCs) and RBC casts. WRN is associated with significant morbidity as many patients progress to end stage renal disease (ESRD) despite normalization of the INR. We report a case of WRN where the acute kidney injury improves after cessation of warfarin.

**Methods:** A 64 year-old African American man presented with a two week history of gross hematuria. He had a history of chronic hepatitis C, CHF, well-controlled HIV, and an aortic arch atheroma and was initiated on warfarin 8 months prior to presentation. He had a baseline creatinine of 0.9 mg/dl and a urinalysis without blood or protein. Serial INR levels were in the therapeutic range. On presentation, he was found to have an INR of 7.3, acute kidney injury with a creatinine of 4.2 mg/dl, and his urinalysis showed large blood, >200 RBCs, and no protein. A renal biopsy revealed acute tubular necrosis, widespread tubular RBC casts, and no tubulointerstitial nephritis. There was mild mesangial hypercellularity and the IF had trace deposits of C3 and IgG. The EM showed normal glomerular basement membrane thickness (470 nm) without electron dense deposits, and normal endothelial and podocyte structures. There were no electron dense deposits in the mesangium. There were no clinical or pathologic etiologies that could explain the presence of tubular RBC casts and he was diagnosed with WRN. Warfarin therapy was discontinued, aspirin started, and at one year follow-up his creatinine had improved to 2.3 mg/dl. His urinalysis revealed no protein, trace blood, and 4-10 RBCs.

**Conclusions:** The diagnostic criteria for WRN includes acute kidney injury, supratherapeutic INR, and the presence of tubular RBCs and RBC casts on kidney biopsy. Often renal function does not recover and progression to ESRD is common, even after cessation of warfarin. This report highlights that cessation of warfarin therapy can be associated with improvement or stabilization in renal function.

## PUB324

**Heavy Chain Deposition Disease** Sarah Margaret Moran,[1] Maeve P. Crowley,[2] Marek J. Mazur.[1] *[1]Nephrology, Cork Univ Hospital, Ireland; [2]Haematology, Cork Univ Hospital, Ireland.*

**Background:** Heavy chain deposition disease (HCDD) is the least common of the plasma cell dyscrasias. 24 have been described in the literature to date. We report the presentation, diagnosis, treatment and complications of the 25th report patient. HCDD is an important but rare cause of renal impairment in association with plasma cell dyscrasia.

**Methods:** A 56 year old male was referred to Nephrology OPD for evaluation of hypertension and elevated creatinine of 2.9mg/dL (eGFR 26mls/min/1.73m2). History revealed a nine month history of hypertension and dyslipidemia treated with rosuvastatin and perindopril. Examination revealed BP 177/105mmHg, nil else of note. Urinary protein excretion was 6.4 g/24 hrs, serum total protein x g/L, albumin 31g/L. Thin-layer agarose electrophoresis revealed a monoclonal IgG kappa band, creatinine concentration 2.6mg/dL. Bone marrow aspiration showed 12% plasma cells, bone marrow biopsy 10-15% atypical plasma cell infiltrate. Renal biopsy revealed nodular glomerulosclerosis, PAS positive, Congo Red negative, electron microscopy reveals amorphous granular electron dense deposits. Treatment was initiated with bortezomib, cyclophosphamide and dexamethasone and subsequent stem cell harvest and transplantation. Stem cell harvest was complicated by AKI and urosepsis requiring renal replacement therapy. Repeat biopsy was morphologically similar to the initial biopsy, unfortunately baseline creatinine increased to 5.8mg/dL.

**Conclusions:** HCDD is rare cause of renal dysfunction in plasma cell dyscrasia, which is caused by a deletion of the CH1 domain. Series to date have been limited by small numbers. Reported treatments include bortezomib, cyclophosphamide, dexamethasone, chlorambucil, melphalan and stem cell transplantation.

## PUB325

**A Case of Minimal Change Disease in a Patient with Churg-Strauss Syndrome** Philippe Lachance, David Philibert. *Nephrology, CHUQ, Quebec, Canada.*

**Background:** Renal involvement in Churg-Strauss syndrome (CSS) ranges from 22 to 88%. Nephrotic syndrome is however rarely described and no case of minimal change disease (MCD) has ever been reported. We herein present the case of a young man who presented with this entity.

**Methods:** A 38 year-old male with a past medical history of CSS based on corticosteroid-dependant asthma, migrating pulmonary infiltrates, colitis, rash and peripheral eosinophilia presented to hospital on January 2012 with a two weeks history of leg edema. Laboratory studies revealed slight anemia, marked hypoalbuminemia, normal creatinine and a significant anti-PR3 level of 13.1 . The 24-hr urinary protein excretion was 13.4 g. The sediment revealed red cell casts. Renal biopsy was performed and showed 9 glomeruli with diffuse effacement of foot processes and no other anomaly, which was compatible with MCD. Prednisone 1mg/kg was instituted with improvement of proteinuria and edema but 2 relapses occurred within 6 months as corticosteroids were decreased to 40 mg/d. Cyclophosphamide was given for 3 months and then switched to azathioprine. Remission occurred while on Azathioprine.

**Conclusions:** The most common renal histological finding in CSS is necrotizing crescentic glomerulonephritis. Other forms of renal disease have also been reported including tubulointerstitial nephritis and IgA disease. Nephrotic range proteinuria in the context of CSS is extremely rare and the histopathologic lesion associated with this anomaly is focal and segmental glomerulosclerosis. To our knowledge it is the first case of MCD in the context of CSS reported in the literature. However, we cannot definitively exclude an unsampled FSGS. Regarding treatment, corticosteroids alone were first used as they are the mainstay of treatment in MCD. Because of established steroid dependance, cyclophosphamide and azathioprine were given as is usually done in ANCA associated vasculitis. Steroids were decreased to 10mg/d but to date the patient has not been weaned off. In conclusion, we herein reported the first case of MCD associated with CSS. Dual therapy with prednisone and cyclophosphamide and then Azathioprine seems more effective than prednisone alone at achieving remission in this scenario.

*Funding:* Clinical Revenue Support

## PUB326

**Invasive Pulmonary Aspergillosis in a Patient with Kidney Transplant** Chadi Saifan, Elie El-Charabaty, Rabih Nasr, Chetana Rondla, Ninad D. Parekh, Suzanne E. El Sayegh. *Medicine, Staten Island Univ Hospital, Staten Island, NY.*

**Background:** Invasive pulmonary aspergillosis is a serious medical condition with a high morbidity and mortality (40%). Risk factors include immunosupprive therapy. While patients with solid organ transplants are at increased risk for such complication, it is thought to be lower in kidney transplant recipients.

**Methods:** A 56 year old female with history of deceased donor kidney transplant 3 months before presented complaining of low GI bleed. Patient's hospital course after her kidney transplant was complicated by an IVC tear and AAA aneurysm s/p repair that led to her graft loss and she was back on hemodialysis. By the time she presented to the hospital, she was still on tacrolimus, mycophenolate mofetil, prednisone, bactrim, and valgancilovir. Her physical exam revealed a BP of 85/50, temp101, with decreased breath sounds. Labs showed a BUN 74, Cr 1.92, K 2.8, WBC 0.96, Hb 10.7, and a platelet count 57,000. She was admitted to the ICU, was given blood transfusion, neupogen and was started on IV pressors. CT chest showed a bilateral basilar consolidation and a right pneumothorax. bronchoalveolar lavage grew fungal elements suggestive of aspergillus spp., and pseudomonas aeruginosa carbapenem sensitive. She was transferred to outside hospital and was treated with IV voriconazole, liposomal amphotericin B and wide spectrum antibiotics, but the patient subsequently expired after two weeks because of septicemia and respiratory failure.

**Conclusions:** Invasive aspergillosis appear to manifest in kidney transplant patients with pulmonary or CNS infection. A retrospective study by Abbott et Al in 2001 found that aspergillus pneumonia is the second cause of fungal infections in 33,479 of kidney transplant patients after candida esophagitis. No randomized clinical trials have been done regarding the treatment of invasive aspergillosis, but voriconazole and amphotericin B have been used in previous cases with relative success. Physicians should be aware of the interactions of the antifungal therapies with calcineurin inhibitors and should adjust the dose based on the creatinine clearance.

## PUB327

**Ifosfamide-Induced Nephrotoxicity: A Reversible Complication in Cancer Patients** Chadi Saifan, Rabih Nasr, Elie El-Charabaty, Ninad D. Parekh, Chetana Rondla, Suzanne E. El Sayegh. *Medicine, Staten Island Univ Hospital.*

**Background:** Ifosfamide is a synthetic analog of cyclophosphamide that is used in combination with other anti-neoplastic agents in the treatment of metastatic germ-cell testicular cancer and some sarcomas. Ifosfamide causes significant nephrotoxicity mainly due to direct tubular injury.

**Methods:** A 65 year old female with previous history of metastatic sarcomatous uterine cancer who was admitted to the hospital. She received ifosfamide chemotherapy 2 days prior. Her physical examination was benign. During the hospital stay she developed electrolyte abnormalities including hypokalemia, hypomagnesemia, and hypophosphatemia (see table) that required repletion. Urine analysis demonstrated increased 24 hours protein (1575mg/24hr) and a magnesium of (164.25 mg/24h) excretion. Phosphorus had normalized by the time of outpatient follow-up (4.1 mg/dl), as well as the serum creatinine. Patient's electrolyte abnormalities were thought to be due to proximal tubular dysfunction secondary to ifosfamide chemotherapy with associated acute kidney injury.

| | BUN | Creatinine | K | Mg | P |
|---|---|---|---|---|---|
| Day1 | 19 | 1.08 | 2.5 | 1.7 | 2.9 |
| Day2 | 20 | 1.16 | 3.4 | 1.9 | 1.8 |
| Day3 | 18 | 1.2 | 3 | 1.9 | N/A |
| Day4 | 14 | 0.96 | 2.8 | 2.2 | 1.9 |
| Day5 | 12 | 0.92 | 4.7 | 1.9 | 1.6 |

**Conclusions:** Nephrotoxicity caused by ifosfamide manifests by onset by one or more of the following tubular dysfunction sign including hypophosphatemia, glucosuria, aminoaciduria, hypokalemia and hypomagnesemia. Ifosfamide therapy can also cause a reduction in GFR which is usually mild unless given in conjunction with another nephrotoxin such as cisplatin. The risk factors for ifosfamide nephrotoxicity are cumulative dose, age under four to five years, and cisplatin therapy. Mesna and N-acetylcysteine, have been evaluated but their efficacy is unproven. For patients receiveing ifosfamide, we recommend telemetry monitoring and continuous intravenous hydration with electrolytes repletion to minimize the side effects. Physicians should be aware of the possible complications due to ifosfamide.

## PUB328

**Thrombotic Microangiopathy (TMA) and Lupus Nephritis: Two Hits, One Organ** Sumaira Talib Shaikh,[1] Ruthanna S. Seidel,[2] Micah R. Chan,[1] Laura J. Maursetter.[1] *[1]Div of Nephrology, Univ of Wisconsin School of Medicine and Public Health, Madison, WI; [2]Div of Internal Medicine, Univ of Wisconsin School of Medicine and Public Health, Madison, Wi.*

**Background:** The prevalence of TMA in patients with systemic lupus erythematosus (SLE) is rare (1%). Recognizing TMA in patients with SLE is difficult because these 2 diseases have many overlapping features.

**Methods:** Our 28 year old patient with SLE, who was previously maintained on hydroxychloroquine, presented with confusion, platelet count of 5 K/uL and creatinine of 1.2 mg/dL. Her urinalysis revealed 21-50 RBCs, RBC casts and 3+ proteinuria(UPC 2.88). Antiphospholid antibody syndrome (APS) was ruled out based on negative antiphospholipid antibodies and later negative anticardiolipin antibodies. She was started on antivirals for possible encephalitis as lumbar puncture could not be performed. A lupus flare and TTP remained high on the differential. MRI findings were not consistent with lupus cerebritis and given there were schistocytes on peripheral smear, plasmapheresis was initiated. ADAMTS13 activity results, later confirmed the diagnosis of TTP (less than 5%). Her creatinine continued to rise and peaked at 2 mg/dl. A renal biopsy was performed on day 5 when platelet count improved to 100 K/uL. The biopsy revealed TMA and class III lupus glomerulonephritis. She was started on mycophenolate in addition to her prednisone. Her mental status improved. She was discharged on hospital day 9 with a creatinine of 1.1mg/dL and a platelet count of 182K/uL. At her follow up in the kidney clinic 1 month later, her Cr improved to 0.97, UPC decreased to 0.43, platelet count was 282 K/uL and ADAMTS 13 actvity improved to 96%. She continues to be treated with mycophenolate and hydroxychloroquine for her Lupus nephritis.

**Conclusions:** The incidence of TTP is increased in patients with SLE, although the mechanism of the association is not known. TTP can be difficult to differentiate from APS or a lupus flare. Early diagnosis is vital, as early treatment can be life saving.

**PUB329**

**Culture-Negative Subacute Endocarditis Presenting with Immune Complex Glomerulonephritis and Stroke in the Young** Ekamol Tantisattamo,[1] Royden S. Young,[2] Chuong Dinh.[2] *[1]Renal Div, Emory Univ School of Medicine, Atlanta, GA; [2]Medicine, Univ of Hawaii John A. Burns School of Medicine, Honolulu, HI.*

**Background:** Endocarditis is not only difficult to diagnose, but is responsible for considerable morbidity and mortality if not treated in a timely fashion. Negative blood cultures are not uncommon, and diagnosis is sometimes heavily reliant on a good history and physical examination. Although immunologic and embolic phenomena are rare, these classic clinical findings remain important clues to the diagnosis. We report a case of a man presenting with glomerulonephritis and embolic stroke. Echocardiography and positive blood cultures later confirmed the diagnosis of endocarditis.

**Methods: Case Description:** A 32-year-old man with recent stroke presented with seizures and right arm numbness 3 months ago. He had acute kidney injury (AKI), hematuria, and highly positive serum streptozyme. He had no residual weakness and was discharged with phenytoin. Renal function spontaneously returned to normal. Two months later, hemoglobin dropped to 5.3 g/dl. He had fevers and was empiricallytreated with piperacillin/tazobactam. Blood cultures later grew *Leuconostoc spp.* On the day of admission, he developed seizures, right hemiparesis, and expressive aphasia. Head CT scan revealed a large left MCA stroke. He again had a profound anemia consistent with anemia of chronic disease. He had AKI, positive streptozyme, and hypocomplementemia. Urine exam showed RBC cast and dysmorphic RBC. Therefore, transesophageal echocardiogram was performed and revealed mitral valve vegetations with perivalvular abscesses. He underwent mitral valve replacement. His renal function remained stable but did not return to baseline, and right-sided weakness persisted.

**Conclusions:** Our patient likely had endocarditis since 3 months ago when he developed AKI and positive streptozyme. In addition to embolic stroke, intermittent fevers and glomerulonephritis with elevated streptozyme raise a suspicion for endocarditis. Clinical presentations of endocarditis are varied. Stroke in the young with glomerulonephritis from immunologic phenomenon and unexplained positive streptozyme should prompt a search for endocarditis.

**PUB330**

**Idiopatic Retroperitoneal Fibrosis Successfully Treated with Rituximab** Carmela Pino, Andrea Guarnieri, Serena Bainotti, Elisabetta Moggia, Paola Inguaggiato, Alfonso Pacitti. *Nephrology and Dialysis Unit, S. Croce - Carle Hospital, Cuneo, Italy.*

**Background:** Retroperitoneal fibrosis (RF) is a rare condition characterized by the presence of inflammatory and fibrous retroperitoneal tissue that encases the ureters or abdominal organs. The goals of therapy are to relieve the obstruction caused by fibrosis, stop the progression of the fibrotic process, and to prevent recurrence.

A variety of medical therapies, including glucocorticoids, immunosuppressants, and tamoxifene, used alone or in combination, are available for treatment. The optimal strategy for disease recurrence is unclear. We describe a case of recurrent idiopathic RF successfully treated with anti-CD20 monoclonal antibody Rituximab.

**Methods:** A 44 year old man was admitted to our unit in 01/2008 for obstructive acute renal failure. Abdominal CT showed a RF encasing left kidney (unfunctiong at nuclear imaging), aorta and right ureter. A ureteral stent was positioned in right ureter resolving hydronephrosis with normalization of renal function. Diagnosis of RF was made by laparoscopic biopsy. Since 06/2008 to 06/2009 he was treated with prednisone alone (initial dose 1 mg/Kg/day reducing to 5 mg/day) with partial response (less but still present fibrosis around right ureter) at abdominal CT and positron emission tomography (PET/CT). Since 06/2010 to 10/2011 we added full dose of methotexate (15 mg/week) without response. Since 10/2011 to 01/2013 treatment was shifted to tamoxifen 10 mg bid plus prednisone 5 mg/day without response again. During this period creatinine was in normal range and ureteral stent was changed every six months. In 01/2013 (with PET/CT positive) we treated with a course of Rituximab (375 mg/mq every week for four doses). In 05/2013 PET/CT was negative and ureteral stent was removed.

**Conclusions:** RF is a rare condition usually responsive to steroid treatment but some patient may be steroid-refractory; other immunosuppressants are not of proven superiority and their effectiveness in unknown. Because B cells infiltrate lesions anti-CD20 antibody may be a therapeutical option: our patient affected by RF non responsive to steroids and second and third line therapy showed a complete response with rituximab.

**PUB331**

**Effect or Side Effect** Maria Saleem Khan, Rakesh Kilari. *Medicine, Loyola Univ Medical Center, Chicago, IL.*

**Background:** Atypical hemolytic-uremic syndrome(aHUS) is a rare and life threatening complement mediated disorder characterized by nonimmune hemolytic anemia, thrombocytopenia and renal impairment. In late 2011, eculizumab, a monoclonal anti-C5 antibody was approved by the FDA for aHUS. We describe a case of aHUS treated with eculizumab, but this could not be continued due to side effects.

**Methods:** A 51year old female with history of HTN presented with minimal urine output for 2 days. She also had several episodes of vomiting and diarrhea which had resolved few days prior to presentation. Her physical exam was unremarkable. Serum creatinine was 8.6 mg/dl. FeNa 3.9%, FeUrea 36.8%. Random prot/Cr ratio was 1.1 g/g. LDH was 2140 and haptoglobin <15. Her initial hemoglobin was 11.8g/dl, but was down trending, and was 7.3g/dl on hospital day 3. DAT-Coombs test was negative. Initial Platelet count was 60,000/UL. She was noted to have significant number of schistocytes on peripheral smear and concern was raised for hemolytic uremic syndrome (HUS). There was no improvement in renal function or Urine output with IV fluids. Renal biopsy confirmed thrombotic microangiopathic changes, with some features of acute tubular necrosis as well. Stool was negative for shigatoxin/O157:H7 genotype. ADAMTS13 activity was within normal limits. Pt was initiated on dialysis and plasmapheresis, with some improvement in urine output, stabilization of hemoglobin and resolution of thrombocytopenia. Several weeks after discharge, renal function improved, but she continued to have modest evidence of hemolysis, and was started on eculizumab, with positive response, but had to be stopped after few months, due to liver enzyme abnormalities, weight gain and fatigue.

**Conclusions:** The high morbidity and mortality of aHUS indicates a severe and unmet medical need. Eculizumab which is the only treatment to offer a highly specific complement-targeted therapeutic option for patients with aHUS has revolutionized the treatment approach. However, treatment had to be stopped in our patient due to intolerance. Although this novel drug has shown early promising results, not every patient may be able to be tolerate it. More research is needed to help develop additional treatment options for aHUS.

**PUB332**

**Tenofovir Induced Acute Kidney Injury in a Patient with Nephrotic-Range Proteinuria** Omid Bakhtar, Nduka-obi Ossai, Machaiah M. Madhrira. *Dept of Nephrology, Univ of Arizona, Tucson, AZ.*

**Background:** Tenofovir induced acute kidney injury has been reported, in association with proximal tubular dysfunction, osteomalacia, and non-nephrotic proteinuria. Commonly seen risk factors include preexisting renal insufficiency, older age, and concomitant use of protease inhibitors. However, we report a case of tenofovir induced nephrotoxicity presenting with nephrotic-range proteinuria that successfully resolved after discontinuation of the offending drug.

**Methods:** A 46-year-old white male with HIV diagnosed in 2002 (CD4 1297 and undetectable viral load) currently on Atripla with Stage 2/3 CKD due to chronic NSAIDS presents for evaluation of an increase in creatinine from 1.2mg/dL to 1.6mg/dL in a 3 month period. On physical examination, BP is 111/77, mucous membranes moist and no peripheral edema. Laboratory results were significant for creatinine of 1.6mg/dL, eGFR 57, urine sodium < 20, albumin/creatinine 841mg/g, and protein to creatinine ratio of 3.09 g/g. Urinalysis showed glucosuria with normal serum glucose of 102 and urine sediment demonstrated granular casts 4/lpf, tubular epithelial cells, oval fat bodies, and no dysmorphic RBCs. SPEP, UPEP, C3/4, ANCA, RF, antiGBMAb, ANA, antidsDNAAb, RPR, and hepatitis profile werenegative. A high fractional excretion of phosphate and uric acid, hypophosphatemia, hypouricosemia, and aminoacidouria were also demonstrated. He was subsequently scheduled for a renal biopsy, which demonstrated ATN possibly secondary to tenofovir exposure. After withdrawal of tenofovir, albumin/creatinine decreased to 126 mg/g and protein to creatinine ratio decreased to 0.5 g/g, serum creatinine was 0.8mg/dL and eGFR >60.

**Conclusions:** This case provides organization of evidence of a rare case of tenofovir induced nephrotoxicity presenting with nephrotic-range proteinuria. In the light of this case, it is important to consider this unique presentation.

**PUB333**

**Warfarin-Induced Calciphylaxis** Chadi Saifan, Elie El-Charabaty, Chetana Rondla, Ninad D. Parekh, Suzanne E. El Sayegh. *Staten Island Univ Hospital.*

**Background:** Calciphylaxis has been a challenging complication of ESRD with unknown underlying mechanism. Several risk factors were identified such as hyperphosphatemia, hypercalemia, hyperparthyoidism, low serum albumin levels, history of warfarin therapy.

**Methods:** A 56 y/o female with DMII, morbid obesity (BMI =48kg/m$^2$), DVT and PE on warfarin for 3 years, chronic kidney disease stage IV and peripheral vascular disease presented with s/p fall and worsening AKI (baseline creatinine 2.42). The patient had drop in hemoglobin, and warfarin was held for 4 days for suspicion of blood loss, and then restarted on day 6 (INR=1.6) after placement of Ash Catheter and institution of dialysis. She developed a right foot blister on day 7 and bilateral thighs blisters on day 10.

Labs were Ca 7.4 mg/dL-Albumin 2.9 g/dL, IP 10.3 mg/dL, creatinine 7.68 mg/dL BUN 98 mg/dL. The skin became mottled and the lesions had worsened progressively over 3 days to necrotic eschar. Extensive workup for vasculitis was negative. Patient had a right lower extremity eschar lesion of 75 x 20 cm, and a left lower extremity 75x20 cm and abdominal lesion of 10x5x5. Patient was taken in charge by the burn specialist, underwent skin debridement and wound care but failed all resuscitation efforts on d 42. Skin biopsy specimen showed a portion of skin and subcutaenous tissue with diffuse necrosis. Multiple small arteries showing calcification of the tunica media. This pattern is strongly suggestive of calciphylaxis.

**Conclusions:** We hypothesise that the reintroduction of warfarin in patients with ESRD induces an imbalance favoring calcification in the vessels in a previously sensitized conditions, which set our patient up for a catastrophic acceleration of vascular calcification. Understanding the pathophysiology of this process will certainly enhance our treatment approach and reduce the morbidity and mortality of this devastating condition.

## PUB334

**Acute Kidney Injury with the Histologic Diagnosis of Osmotic Nephrosis without an Identifiable Cause** Jun Sohn,[1] Ashok P. Chaudhari,[1] Jinil Yoo.[2] *[1]Nephrology, Metropolitan Hospital, New York, NY; [2]Nephrology, Montefiore Medical Center - Wakefield Campus, Bronx, NY.*

**Background:** Osmotic nephrosis is a histologic pattern of acute tubular injury, characterized by marked vacuolization and swelling of renal tubular cells, mostly related to IV infusion of non-metabolizable macromolecules such as hydroxyethyl starch or IV immunoglobulin. We present a case of acute kidney injury (AKI) with the histologic diagnosis of osmotic nephrosis without an identifiable cause.

**Methods:** A 74 year old man with type 2 DM and hypertension was admitted with pneumonia, and treated initially with IV ceftriaxone and oral doxycycline, then IV ampicillin/sulbactam. During the 10-day's hospitalization, he did not receive other IV medications, IV colloids, or IV contrast, and was discharged with serum creatinine (Cr) 1.2 mg/dL. 4 days later, he was readmitted because of oliguric AKI (Cr 9 mg/dL). He denied taking any unusual foods, solutions, or medicines except his regular glipizide, metformin, and lisinopril. Physical examinations on admission revealed BP 125/65 mmHg, no edema. UA was positive for trace protein, RBC 2~4/HPF, and coarse granular casts. Serologic studies and kidney sonogram were not remarkable. The kidney biopsy revealed prominent swelling and vacuolization of proximal tubular cells without any remarkable glomerular changes, imparting "osmotic nephrosis". Cr plateaued to 11.2mg/dL, then started to improve only with gentle IV hydration to 1.7mg/dL on discharge.

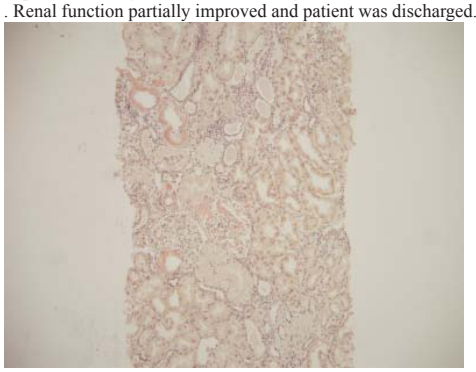
**Conclusions:** Our patient developed oliguric AKI with histologic features of osmotic nephrosis just after being discharged from the hospital, without receiving any known non-metabolizable macromolecules or substances. Histologic "look alikes" to osmotic nephrosis have been described in calcineurin-inhibitor toxicity, foam cells in lipid storage, hypokalemia, ischemic tubular injury, or ethylene glycol intoxication. However the histologic findings of our patient conformed to the description of a classical osmotic nephrosis, but without an identifiable case.

## PUB335

**Muckle-Wells Syndrome and Secondary Amyloidosis (AA) with Nephropathy** Tariq Javed, Shirisha Bodana, Catherine G. Staffeld-Coit, Abdul Moiz. *Nephrology, Ochsner Clinic Foundation, New Orleans, LA.*

**Background:** Incidence of AA amyloidosis in Muckle-Wells syndrome is 25 percent. Muckle-Wells syndrome consists of periodic fever, urticaria, arthralgia and deafness. Muckle-Wells syndrome is transmitted by an autosomal dominant trait. In clinical practice, renal failure due to amyloidosis and Muckle-Wells syndrome is rare and should be considered as differential diagnosis in difficult cases of renal failure.

**Methods:** A 75 years old white male with PMH of Arthritis, Skin rash presented to ER with nausea, vomiting and abdominal pain for 3 weeks. Review of system is positive for joint pain, skin rash from childhood, and hearing loss for couple of years. Family history is positive for same symptoms of arthritis and skin rash in a daughter and a son. Physical exam shows normal vital signs, short stature, hearing loss, left wrist swelling and tenderness and diffuse skin rash. Initial labaroratory work up shows CBC with white blood cells 10.18, hemoglobin 10.6, platelets 298, chemistry shows sodium 142, k 4.8, Cl 111, bicarb 20, BUN 44, creatinine 2.8, calcium 8.8, albumin 1.7. Creatinine on 2/26/2012 was 0.9 and on 7/26/12 was 2.9. Urine collection for 24 hours showed proteinuria with 6.3 gm per day. Renal ultrasound was unremarkable. Renal biopsy showed findings AA amyloidosis with mild chronicity. Rheumatological work up was negative. He was clinically diagnosed with Muckle-Wells syndrome because of episodic skin rash, arthralgias and generalized pain, fatigue, intermittent fever, late-onset sensorineural hearing loss. Genetics Consult was requested, they agreed with clinical diagnosis and they recommended NLRP3 gene testing . Renal function partially improved and patient was discharged.



**Conclusions:** Renal failure due to amyloidosis secondary to Muckle-Wells syndrome is rare and should be considered as differential diagnosis in difficult cases of renal failure.

## PUB336

**Transient False Positive Hepatitis B Surface Antigenemia following Hepatitis B Vaccine (Recombinant) Administration** Timothy A. Williams,[1] Cynthia F. Frazier,[2] Daniel F. Balkovetz.[1,2,3] *[1]Medicine and Nephrology, Univ of Alabama at Birmingham, Birmingham, AL; [2]Dialysis, Birmingham VA Medical Center, Birmingham, AL; [3]Medicine and Nephrology, Birmingham VA Medical Center and Univ of Alabama at Birmingham, Birmingham, AL.*

**Background:** Hepatitis B vaccination is mandatory for all hepatitis B surface antigen (HBsAg)-negative patients on hemodialysis.

**Methods:** A case of transient HBsAg positivity was detected in a previously HBsAg negative hemodialysis patient 2 days after the administration of recombinant hepatitis B vaccine (Engerix-Bä). The patient was placed on hemodialysis isolation while further confirmatory testing was conducted. The hemodialysis unit's infection control practices were reviewed and the patient and family members were interviewed for possible exposure and risk factors. The initial positive HBsAg test result caused concern and anxiety for the patient, family, and staff. Confirmatory testing including a repeat HBsAg and hepatitis viral DNA was negative and indicated the earlier HBsAg result was false positive. A review of the literature found case reports in 1996 indicating transient HBsAg positivity (lasting less than 21 days) following administration of the hepatitis B vaccine. The University of Iowa also reported three cases of false positive HBsAg tests due to recent hepatitis B vaccine administration. The possibility of false positive HBsAg testing following administration of the hepatitis B vaccine is not mentioned in the vaccine package insert. Further review found evidence to support the practice that HBsAg should not be tested within ~30 days of administration of the hepatitis B vaccine.

**Conclusions:** This case is presented to increase awareness of this phenomenon to health care providers. To prevent this phenomenon, HBsAg testing should be deferred until approximately 30 days after administration of the recombinant hepatitis B vaccine.

*Funding:* Veterans Affairs Support

## PUB337

**A Grave Case of Acute Weakness: Thyrotoxic Hypokalemic Periodic Paralysis** Mamta Shah, Rahul Mutneja. *Internal Medicine, Univ of Connecticut, Farmington, CT.*

**Background:** Thyrotoxic hypokalemic periodic paralysis (THPP) is a rare complication of thyrotoxicosis. Usually associated with Grave's disease, it can also be seen in other forms of thyrotoxicosis like toxic nodular goiter, drug induced, use of thyroxine containing weight loss supplements and thyroiditis. Common in males (male to female ratio of 20:1), the typical presentation includes recurrent episodes of paresis, in the presence of low potassium levels usually precipitated by heavy meals, stress or strenuous exercise. Patients may also present with life threatening arrthymias secondary to hypokalemia.

**Methods:** A 31 year old Hispanic male with no significant past medical history presented with acute onset bilateral lower extremity weakness. He was unable to get out of bed and had no sensation in his legs. Patient gave history of strenuous exercise and lower extremity cramps the day prior. In the emergency room, patient was anxious, tachycardic and exam was significant for 2/5 strength in both lower extremities proximally and 4/5 distally, 1+ reflexes and inability to walk. He was found to have potassium of 1.7mEq/L. EKG showed sinus tachycardia. He was given 80 mEq's of potassium with subsequent improvement of symptoms. Further work up showed TSH of 0.04Uu/ml, free T4 of 4.54pg/ml, free T3 of 10.2pg/ml. Thyroglobulin antibody was negative. A thyroid uptake scan showed increased uptake of 54% and findings consistent with Grave's disease. Patient was managed with propranolol and propylthiouracil and he returned to full strength in 48 hours. He subsequently underwent radioactive iodine ablation, following which he was discharged to home with follow up.

**Conclusions:** THPP is an important differential of acute weakness and should be kept in mind for all patients presenting with a similar picture. Recognition of this is important since treatment is easy and provides quick and early relief of symptoms. Almost all patients show response to potassium replacement and management of thyrotoxicosis with non selective beta blockers like propranolol and anti thyroid agents. Definitive treatment in the form of radioactive iodine or thyroidectomy should be considered especially in cases refractory to medical management.

## PUB338

**Successful Treatment of Telaprevir in Saudi Patient with Hepatitis C on Hemodialysis** Mamdouh Abdulghafour Nada. *Nephrology-Hepatology, Prince Sultan Military Medical City, Riydh, Saudi Arabia.*

**Background:** Telaprevir is a hepatitis C vInterferon remains contraindicated post-renal transplant; therefore, it is recommended to treat HCV infection in patients with concomitant kidney disease prior to kidney transplantationirus protease inhibitor that is both a substrate and an inhibitor of CYP3A.

**Methods:** Our patient is a 44 year old male Saudi with ESRD Secondary to Chronic Pyelonephritis on Hemodialysis since 1990. Status post living non-related kidney transplant in India which survived for 4 years. Another attempt for a transplant was a cadaveric renal transplant which was removed 2 weeks after transplantation due to perinephric hematoma and renal vein thrombosis. The patient had previously received treatment for the hepatitis by taking PEG-IFN as a single treatment, but failed to respond. Another attempt to cure him was done by administering a dual medication of PEG-IF with Ribavirin. According to the kidney biopsy, the patient was considered to have grade 2 chronic hepatitis stage 2 associated with HCV. Abdominal ultrasound was unremarkable for the liver. Hepatitis C virus PCR (HCV viral load) was 197,731 IU/ml, genotype 1A. The patient had a

compensated liver function with normal liver enzymes. So the patient was considered to be a non - responder with a non - cirrhotic liver genotype 1. Therefore the team decided that this patient fulfilled the criteria to start the triple medication for 12 weeks as Telaprevir 750 mg every 8 hrs in combination with PEG-IFN alpha 130 mcg once weekly plus Ribavirin 200 mg three times per week. Then we administered dual medication for 36 weeks as PEG-IFN alpha plus Ribavirin.

**Results:** After 12 weeks of triple combination therapy, the patient was considered a responder (he achieved undetectable HCV RNA at weeks 4 and 12) and the treatment still ongoing.

**Conclusions:** The team concluded that triple therapy with a first generation protease inhibitor could be the new standard for the treatment of HCV patients with ESRD on hemodialysis. To confirm our findings, this study must be performed on a larger scale to clarify the efficacy of this therapy.

*Funding:* Other NIH Support - prince sultan military medical city, Government Support - Non-U.S.

## PUB339

**Tendon Rupture and Calcification: Consequence of Tertiary Hyperparathyroidism in CKD 5** Ankur Sharma, Andrew I. Chin, Shubha Ananthakrishnan. *Dept of Internal Medicine, Nephrology Div, Univ of California, Davis, Sacramento, CA.*

**Background:** Tendon ruptures usually occur in athletic patients aged below 40 years, much more following sport injuries. Several systemic diseases predispose patients to spontaneous tendon rupture and calcification such as CKD5d, hyperparathyroidism, diabetes, rheumatoid arthritis, and SLE. Spontaneous rupture of quadriceps or patellar tendons represents a rare entity.

**Methods:** We present 2 cases of CKD5 patients with complications from renal osteodystrophy. Case 1, is a 26 year old man with CKD5resumed to be secondary to neonatal injury who started Peritoneal dialysis at age 8, eventually transplanted from a living donor. He lost the allograft due to medication noncompliance and started hemodialysis at age 21. He developed progressive pain in his ankles and difficulty in ambulation. A CT scan revealed heavily calcified achilles tendon bilaterally. He had high PTH (peak 1593pg/ml) for 5 years. The patient underwent an elective tendon transfer procedure bilaterally with successful outcome. Case2 is a 27 year old man with CKD5 presumed due to congenital renal malformation who received a pre-emptive transplant from a living donor at 6 years. He lost the allograft due to medication noncompliance at age 23 and was started on PD. He has high PTH for 6 years (peaked at 1688pg/ml). He presented to the hospital with bilateral knee pain which began suddenly after he tried to sit up and heard a loud "pop" and was unable to bear weight. MRI showed near complete tear of the quadriceps tendons bilaterally which was repaired and he subsequently underwent elective parathyroidectomy 4 weeks later.

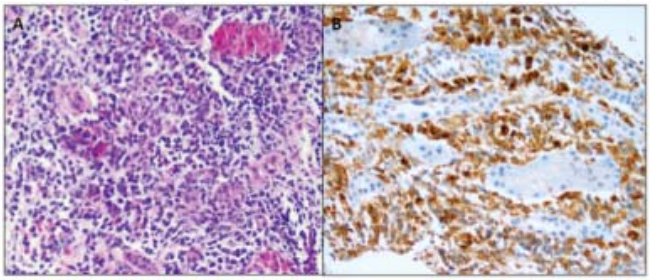
**Conclusions:** In absence of trauma, generally an underlying condition leads to abnormal tendons that rupture under normal stress. Risk factors include Diabetes, hyperparathyroidism, chronic renal failure, obesity, SLE, steroid use and gout. Medline review by Mrugeshlumar of 66 patients showed that younger patients were most likely to have CKD5 (43%) and hyperparathyroidism. In the review, only the duration CKD and duration of dialysis was related with spontaneous rupture. Both of our patients had CKD for over 15 years with dialysis for more than 4 years.

## PUB340

**Primary Renal Manifestation of Acute Myeloid Leukemia: A Case Report** Sana Waheed,[1] Duvuru Geetha,[1] Naima Carter-Monroe,[2] Lois J. Arend,[2] Satish Shanbhag.[1] *[1]Dept of Medicine, Johns Hopkins Univ, Baltimore, MD; [2]Dept of Pathology, Johns Hopkins Univ, Baltimore, MD.*

**Background:** Kidneys can be a site of extra medullary involvement in leukemias, typically occurring late in the disease course. We describe a case of acute myeloid leukemia (AML) with renal involvement as a presenting feature.

**Methods:** 62-year-old female with history of Diabetes Mellitus, hypertension and breast cancer s/p mastectomy had new onset anemia, thrombocytopenia and acute renal failure. She had recently taken ibuprofen and doubled her dose of losartan. Her physical exam was notable for a fine maculopapular rash over bilateral knees. Labs were significant for sodium 134 mEq/L, potassium 4.8 mEq/L, BUN 46mg/dL, serum creatinine 3.6mg/dL (baseline 0.9mg/dl),hemoglobin 7.7g/dL,platelets 81000/mm$^3$ and LDH 1234 IU/L. Proteinuria (2.53 g/g of creatinine) was present on urinalysis without hematuria or pyuria.Renal ultrasound showed echogenic 13 cm kidneys bilaterally. Renal biopsy revealed extensive interstitial infiltration by atypical myelocytic cells in a background of severe acute tubular injury.




After a dry tap on a bone marrow biopsy, a core specimen showed focal involvement by similar atypical myeloid cells and patient was started on chemotherapy for AML. Her kidney function improved to baseline without needing renal replacement therapy.

**Conclusions:** Renal involvement including dense infiltration of kidneys (often bilateral involving both cortex and medulla) is seen in up to 34% of patients diagnosed with AML, acute lymphocytic leukemia and non-Hodgkin's lymphoma. Most cases are seen in patients after a diagnosis of AML has been confirmed in contrast to our case where dense infiltration of the kidneys with myeloid blasts was noted first leading to bone marrow biopsy revealing focal bone marrow involvement.

## PUB341

**A Case of IgA1-κ Monoclonal Immunoglobulin Deposition Disease in Alcoholic Liver Cirrhosis** Naoko Nakaosa, Akira Fukui, Nobuo Tsuboi, Kentaro Koike, Yoichi Miyazaki, Makoto Ogura, Tetsuya Kawamura, Takashi Yokoo. *Div of Kidney and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Hepatic glomerulosclerosis, also termed hepatic IgA nephropathy, typically presents with chronic mild alterations in glomeruli. A novel form of proliferative glomerulonephritis related to glomerular deposition of monoclonal IgG mimicking immune-complex glomerulonephritis, termed proliferative glomerulonephritis with monoclonal IgG deposits (PGNMID), is recently described.

**Methods:** The patient was a 60-year-old male with a medical history of alcoholic liver cirrhosis since he was 50 years old. He had an episode of hepatic encephalopathy 4 years before admission, when his serum creatinine (Cr) level was within normal range. On admission, the patient showed leg edema and his serum Cr level was 1.69mg/dl. He presented nephrotic syndrome with urinary protein excretion of 4.7g/day and serum albumin level of 2.3mg/dl, and the urinalysis showed microscopic hematuria. Serum IgA level was 507mg/dl, and serum complement levels were normal. Serum anti-neutrophil cytoplasmic antibodies, anti-nuclear antibodies, cryoglobulins, and hepatitis virus B and C were all negative. Serum immunoelectrophoresis showed no evident monoclonal protein. Renal biopsy showed diffuse mesangial proliferation together with doubling of the glomerular basement membrane. Cellular crescents were also observed in about 20% of the glomeruli. Immunofluorescence (IF) showed granular deposition of IgA and C3 along the glomerular capillary walls. IF findings of IgA subtypes and light chains showed only positive for IgA1 and κ. Granular electron dense deposits were observed in the subendothelial areas. These findings led us to the diagnosis of monoclonal IgA1-κ deposition disease.

**Conclusions:** The clinicopathological features of our case are compatible with those of PGNMID. In this case, monoclonal IgA1-κ deposits may be involved in the formation of acute glomerular lesions which is atypical in hepatic glomerulosclerosis. Lastly, our case suggests an importance of IF assays for immunoglobulin subtypes and light chains in the differential diagnosis of glomerular immunoglobulin deposition diseases.

## PUB342

**Nontuberculous Mycobacterial Exit-Site Infection in Patients on Peritoneal Dialysis: Report of Three Cases** Kana Asari, Nanae Matsuo, Masatsugu Nakao, Yudo Tanno, Izumi Yamamoto, Ichiro Ohkido, Keitaro Yokoyama, Tatsuo Hosoya, Takashi Yokoo. *Div of Nephrology and Hypertension, Dept of Internal Medicine, Jikei Univ School of Medicine, Japan.*

**Background:** Nontuberculous mycobacteria (NTM) are an uncommon cause of peritoneal dialysis(PD)-associated infections. A standard diagnostic procedure and treatment regimen for NTM infection have not yet been established. We report here three different cases of NTM exit-site infections (ESI) in PD patients.

**Methods:** [Case 1] A 42-year-old female on PD due to IgA nephropathy, presented with refractory ESI extend beyond the external cuff. The catheter was removed and the new one was placed at the opposite site simultaneously. Nine days after the operation, she presented peritonitis. The catheter was removed immediately. From the catheter culture at the time of operation, the acid-fast bacillus smear was positive, and *M. abscessus* was identified. She was treated with Imipenem/Cilastatin(IPM/CS), Amikacin(AMK) and Clarithromycin(CAM), and her condition impoved rapidly. [Case 2] A 75-year-old male on PD due to nephrosclerosis, presented with refractory tunnel infection beyond the external cuff. The exit-site culture grew a NTM, identified as *M.abscessus*. The catheter was removed, and he temporarily swiched to hemodialysis. He continued undergoing IPM/CS, AMK, CAM therapy and cleansing open wound therapy. After we confirmed that the wound culture was negative for 4 weeks after the PD catheter removal, the catheter was replaced again. He remains on PD with no evidence of tunnel infection or peritonitis. [Case 3] A 70-year-old male on PD due to nephrosclerosis, developed tunnel infection due to NTM, which did not extend beyond the external cuff. Partial catheter reimplantation was perfomed and no relapse have been observed.

**Conclusions:** Each of the 3 cases that we present here had different process of treatment. These cases are highly suggestive to consider timing of surgical treatment and what surgery is appropriate for each patient. Based on our experience and other cases reported previously, we discuss appropriate diagnosis and treatment strategies for the management of PD-associated NTM infections.

## PUB343

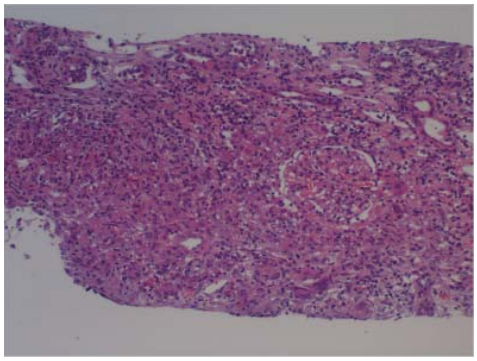
**Sarcoidosis Manifesting as Acute Kidney Injury** Mandeep Singh, Roberto L. Collazo, Sreevalli Pariti. *Methodist Dallas Medical Center.*

**Background:** Granulomatous interstitial nephritis is a rare manifestation of Sarcoidosis. We report a case of Sarcoidsis presenting as acute kidney injury.

**Methods:** A 26 y/o male with past medical history of pancytopenia and hepatosplenomegaly was admitted for evaluation of acute kidney injury.On initial

evaluation he had sub-nephrotic range proteinuria.Worsening of his renal function prompted kidney biopsy which showed granulomatous interstitial nephritis with focal acute tubular injury,classic noncaseating granulomas.Patient was started on pulse dose steroids with dramatic improvement in his renal function.ACE levels were normal,CT scan of chest did not reveal any hilar adenopathy. This case was a rare presentation of Sarcoidosis manifestion as acute kidney injury in the absence of overt systemic sarcoidosis,normal levels of ACE and dramatic response to steroid therapy.

**Conclusions:**



## PUB344

**Emphysematous Pyelonephritis after Plasmapheresis in a Renal Transplant Patient** Rapeepat Lekkham, Rasib Raja. *Medicine, Albert Einstein Medical Center, Philadelphia, PA.*

**Background:** Emphysematous pyelonephritis(EPN) is a rare but devastating complication after renal transplantation. It carries the risk of graft loss and is associated with a high mortality. The majority of cases reported thus far have undergone graft nephrectomy. Here we present a case of EPN in a renal allograft after plasmapheresis for recurrent FSGS that was successfully treated conservatively.

**Methods:** A 49-year-old women received a second living-related renal allograft from her son in April 2009. Her first transplanted kidney from deceased donor in 2002 failed from acute rejection and required allograft nephrectomy. Her native kidney disease was FSGS. 3 weeks prior to the admission, she developed acute generalized edema with nephrotic range proteinuria with allograft dysfunction. Plasmapheresis was initiated after her allograft biopsy confirmed diagnosis of recurrent FSGS. After the 9th sessions, she developed fever, tachycardia, leukocytosis and thrombocytopenia. CT scan of the abdomen revealed focal medial enlargement of the transplant kidney, with a moderate sized area of gas-filled collection in the parenchymal with small subcapsular gas. The possible emergent need for transplant nephrectomy was discussed, but she decided to preserve the graft with medical management. Urgent intervention with percutaneous abscess drainage and empiric broad-spectrum antibiotic coverage with IV aztreonam and tobramycin (changed to ciprofloxacin later on) helped stabilize the patient's condition. Mycophenolate mofetil and tacrolimus were held except for low dose prednisone. The urine culture, fluid from the drain and blood culture all grew Klebsiella pneumoniae. The drain was removed on day 23rd, the antibiotic was continued for total of 14days. Repeat U/S 1 month later showed normal renal allograft parenchyma and collecting system, her allograft function was back to her baseline as well.

**Conclusions:** Plasmapheresis in renal transplant patient may increase risk of EPN. However, timely diagnosis and early conservative intervention with aggressive intravenous antibiotics and percutaneous drainage will help in preservation of renal allograft and may be able to avoid graft nephrectomy like in our patient.

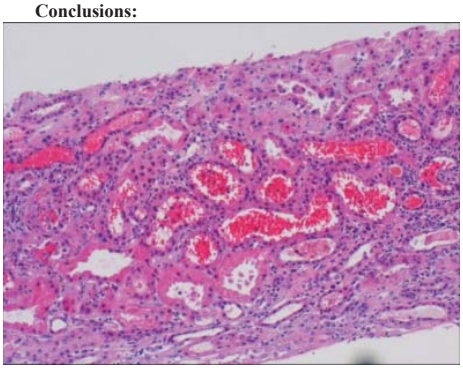
## PUB345

**A Rare Case of Anti GBM Disease in Alpha 1 Antitrypsin Deficiency** Mandeep Singh,[1] Roberto L. Collazo,[2] Sreevalli Pariti.[3] *[1]Nephrology, Methodist Dallas Medical Center, Dallas, TX; [2]Nephrology, Methodist Dallas Medical Center, Dallas, TX; [3]Nephrology, Methodist Dallas Medical Center, Dallas, TX.*

**Background:** We report a rare case of Anti GBM disease manifesting as acute kidney injury in a patient with history of liver cirrhosis secondary to alpha 1 antitrypsin deficiency.

**Methods:** A 61 y/o female with past medical history of liver cirrhosis due to alpha 1 antitrypsin deficiency admitted to the hospital for acute kidney injury with elevated BUN and ceatinine found on outpatient labs.Patient had significant eosinophiluria and peripheral eosinophilia on admission.Her worsening renal function prompted kidney biopsy which showed acute interstitisl nephritis,tubular luminal hemorrhage with glomerular capillary linear IgG deposition.Patient started on i.v steroids for acute interstistisl nephritis but her renal function kept deteriorating and further investigations showed extremely high anti GBM antibodies levels.This was a rare case of non crescentic anti GBM disease in a patient with alpha 1 antitrypsin deficiency.

**Conclusions:**



## PUB346

**Nephritic Syndrome due to Mixed Cryoglobulinemia Non Hepatitis C-Related: Report of 3 Cases** Carla Queiroz Neves, Alline S. A. Oliveira, Camila Barbosa L. Oliveira, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Serviço de Nefrologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

**Background:** Mixed Cryoglobulinemia is a rare cause of glomerular disease and Membranoproliferative Glomerulonephritis (MPGN) is the most frequent histological finding. The association with hepatitis C (HCV) infection is approximately 90-100% of the patients.

**Methods:** We retrospectively analyzed 3 patients with Nephritic Syndrome (NS), positive serum cryoglobulins, negative serology and viral load by polymerase chain reaction (PCR) for HCV, between 2011 and 2013. Epidemiology, clinical manifestations and outcomes are described in table 1.

| | Patient 1 | Patient 2 | Patient 3 |
|---|---|---|---|
| Age | 36 | 64 | 47 |
| Sex | female | female | male |
| Nephrotic syndrome | yes | no | yes |
| Hematuria | yes | yes | yes |
| Proteinuria (g/day) | 4.0 | 0.8 | 9.8 |
| Serum Creatinine (mg/dL) | 0.6 | 1.1 | 0.8 |
| C3 (90-180) (mg/dL) | 81 | 66 | 108 |
| C4 (10-40) (mg/dL) | 0 | 3 | 1 |
| FAN | neg | 1:320 | neg |
| ANTI-HCV (ELISA) | neg | neg | neg |
| PCR HCV-RNA | neg | neg | neg |
| HBV (ELISA) | neg | neg | neg |
| HIV (ELISA) | neg | neg | neg |
| Serum cryoglobulins | pos | pos | pos |
| Malignancy | mantle cell lymphoma | no | no |
| Renal Biopsy | MPGN | MPGN | no |
| Steroids | yes | yes | yes |
| Cyclophosphamide | yes | yes | yes |
| Riruximab | yes | yes | no |
| Plasmapheresis | yes | yes | yes |
| Follow-up time (months) | 20 | 17 | 10 |
| Dialysis | yes | yes | yes |
| Time to start dialysis (months) | 5 | 11 | 2 |
| Outcome | death | death | ESRD |

All patients underwent metilprednisolone pulse, followed by prednisone 1mg/kg and cyclophosphamide (CYC) pulse and plasmapheresis. Two patients underwent rituximab therapy, one due to lymphoma treatment and the other to CYC toxicity.

**Conclusions:** We described 3 cases of mixed cryoglobulinemia non-HCV related associated with NS. Prognosis was unfavorable and all patients required dialysis. According to literature, non-infectious cryoglobulinemia with renal involvement carries a poor prognosis despite treatment instituted as shown in this series of cases.

## PUB347

**Atypical Presentation and Evolution of a Single Case of Goodpasture Syndrome** Tiago Assis Pereira, Jorge Dickson, Jose Diogo Barata. *Nephrology Dept, Hospital Santa Cruz, Lisbon, Portugal.*

**Background:** Goodpasture Syndrome (GPS) is a clinical syndrome characterized by rapidly progressive glomerulonephritis and hemorrhagic pulmonary involvement. There is clinical consensus for aggressive treatment with cyclophosphamide (CF), high dose corticosteroids and coadjuvant plasmapheresis in most cases. Other immunosuppressive agents have been used occasionally, in specific or refractory cases.

**Methods:** We report a 25-year-old white man, smoker, who presented with macroscopic haematuria and haemoptysis. On admission, nephrotic syndrome was diagnosed and further investigation was initiated. The investigation came up positive only for the presence of anti-glomerular basement membrane (GBM) antibodies and renal biopsy was compatible with anti-GBM disease. Accordingly, CF, corticotherapy and plasmapheresis was initiated, with partial recovery and negativation of anti-GBM antibodies. Patient was discharged on oral prednisone and CF therapy. One week later, a relapse of macroscopic haematuria, nephrotic range proteinuria and pulmonary radiologic findings; the laboratory workout unveiled for the first time disturbed renal function. Facing a very early clinical relapse under aggressive

imunosupression, the initial diagnosis was challenged and laboratorial and histological study was repeated: there was a re-positivation of anti-GBM antibodies and crescentic glomerular findings on biopsy. The initial diagnosis was reaffirmed, plasmapheresis was resumed and Mofetil Mycophenolate (MMF) was replaced for CF, with good clinical and laboratorial response. MMF was maintained for 2 years and then weaned. With 8 years of follow-up, the patient has remained asymptomatic, renal function and nephrotic syndrome fully recovered.

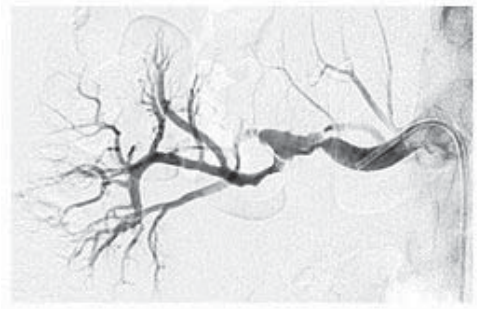
**Conclusions:** This case of GPS is atypical both for its nephrotic presentation and successful response to MMF, after relapse under CF. To the best of our knowledge, only two cases of successful therapy with MMF after failure of CF were reported. Based on our findings, GPS should be considered in the differential diagnosis of nephrotic syndrome with pulmonary involvement and additional study is needed to validate MMF as a therapeutic option.

## PUB348

**Delayed Intervention in Acute Renovascular Thromboembolic Disease Could Salvage Renal Function** Suyash Sharma, Hemant J. Mehta, Anup Chaudhari. *Nephrology, Lilavati Hospital, Mumbai, Maharashtra, India.*

**Background:** An acute renal arterial occlusion can happen due to in-situ thrombus or embolism. Unless immediately treated, it can lead to renal infarction. We are presenting a case where there was a delay in diagnosis, still renal function could be salvaged partially with prompt intervention.

**Methods:** 40/M, Non-smoker on routine health check up 7 years ago has BP of 130/94 mm Hg and S. Creatinine 1.3mg/dl. Presented to a gastroenterologist after 15 days with pain in right upper abdomen. S. Bilirubin: 1.8, direct 1.0mg/dl; all other laboratory investigations and USG abdomen were normal. Since the severe pain persisted after 1 week, CT abdomen with CT abdominal angiography was done, which showed right renal infarction and raised possibility of right renovascular cause. The patient was referred to our hospital. BP on admission 170/130 mm/Hg and except for mild tenderness over right lumbar area, no other clinical signs present. DSA showed right renal artery dissection, thrombus and right renal lower pole infarction.



The question was: Will an intervention be able to salvage the right renal function after 1 week? Right renal angioplasty with stenting was performed, the BP stabilized. One week later (and until now), right kidney GFR remains at 26ml/min on DTPA renogram and S. Creatinine value normal. BP is well controlled.

**Conclusions:** There is a dilemma about the outcome of interventions when the acute thromboembolic events of renal artery are diagnosed late. We have presented a case where, in spite of a delay of 1 week from the onset of symptoms to diagnosis and endovascular intervention, right kidney function could be salvaged partially. This case report should encourage clinicians to enforce immediate radiological intervention, especially in the patient at risk. Even if diagnostic procedures are delayed, radiological intervention should be taken into account.

## PUB349

**New Genetic Variant of ADPKD-2** Tetiana Litvinchuk, Tetyana L. Vasylyeva. *Pediatrics, TTUHSC, Amarillo, TX.*

**Background:** ADPKD is an autosomal dominant renal cyst disorder due to mutations in genes coding for polycystin1(PKD1, on ch 16p13.3) and polycystin 2 (PKD2, on ch 4q13-23), and PKD3 gene (gene unmapped).It is also associated with TSC2/PKD1 contiguous gene syndrome. ADPKD is usually inherited but new mutations without a family history occur in 10% of the cases.

**Methods:** A 17 y.o. boy was followed since he was a 13 y.o. for bilateral cystic kidney disease, hypertension and obesity. The diagnosis was an accidental finding during abdominal CT at age 13 to rule out appendicitis Labs: BUN-14, Cr-0.77, electrolytes-within normal limits. Ultrasonogram: The right kidney measures 11.3x5.2cm. The left kidney 11.2x5.2cm. There is a cluster of cysts about the upper pole of the right kidney measuring 2.4x1.9x3.4cm and identified cysts of the right kidney measuring 1.9x1.6x1.7cm and 1.7x1.2x2.3cm ,and of the left kidney measuring 1.7x1.4x2.0cm. No hydronephrosis and a good corticomedullary differentiation. Impression: Bilateral renal cysts. Because of parental history of bilateral renal cysts, PKD1 and PKD2, genetic testing was ordered.

**Results:** PKD2 variant 1:3 bp deletion of TGT was found; nucleotide position: 1602_1604; codon position: 512-513; mRNA reading frame maintained. ADPKD2 disease is often present later in life. A variety of genetic defects have been described including frameshift, deletion, and missense mutations in patients with ADPKD. Most families have an abnormality on chromosome 16 in the PKD1 locus that is tightly linked to the alpha-globin gene locus (85-90% of cases).Most of the remaining patients have a defect in the PKD2 locus on chromosome 4. (10% of cases). PKD1 and PKD2 encode proteins called polycystin-1 and polycystin-2. Mutated proteins are involved in cell differentiation, polarization, proliferation and membrane transport.This is a case of new genetic mutation, which probably causes a development of ADPKD2 in this patient during a childhood.

**Conclusions:** A smaller number of patients have a defect in the PKD2 locus on chromosome 4 (resulting in PKD2 disease) and a few families have an undefined defect. There are not known published cases on this genetic variant of ADPKD2 cystic kidney disease. In this case the disease is present unusually early in life.

## PUB350

**A Case of Cardiac Tamponade and Proteinuria: Is There an Etiological Relationship?** Vasanthi Balaraman, Shyam Ravisankar, Robert D. Zenenberg. *Dept of Medicine, Saint Barnabas Medical Center, Livingston, NJ.*

**Background:** Cardiac tamponade, as a cause of proteinuria has not been described in the literature. We demonstrate a temporal relationship between the onset of proteinuria with cardiac tamponade and its resolution subsequent to pericardial window on two occasions in the same patient.

**Methods:** A 75 year old lady with pancreatic adenocarcinoma was admitted for acute kidney injury and proteinuria. Physical examination was unremarkable.Laboratory data showed serum creatinine of 2.33 mg/dl (baseline creatinine 0.75-1.20 mg/dl) and proteinuria of 300 mg/dl on urinalysis. The spot urine protein to creatinine ratio was 3 gram/g creatinine. On day 3, she developed shortness of breath and palpitations. Echocardiogram showed a large pericardial effusion with early cardiac tamponade. Pericardial window was performed and 500cc of clear fluid was drained. Cytology revealed adenocarcinoma. Serial measurements of her spot urine protein to creatinine ratio decreased from 4.39 to 3.6 to 2.3 to 1.66 g/g creatinine subsequently. Four months after discharge, she was admitted with shortness of breath and was diagnosed with cardiac tamponade. She had proteinuria of 100 mg/dl on dipstick. Her proteinuria completely resolved after the pericardial window.

**Conclusions:** In acute febrile illnesses and subsequent to vigorous exercise, one can see proteinuria related to hemodynamic stress. In Orthostatic proteinuria, Cardiorenal syndrome and Nutcracker phenomenon (left renal vein traverses under the superior mesenteric artery and becomes trapped between aorta and superior mesenteric artery), there is increased efferent glomerular vasoconstriction, increased glomerular back pressure with increased angiotensin II release and resultant proteinuria. Our case of cardiac tamponade can be categorized within a subset of "Type I" cardio-renal syndrome. Glomerular capillary hemodynamic changes due to renal venous back-pressure and elevated AT-II levels may influence glomerular permselectivity to macromolecules and can evoke proteinuria. The relationship between proteinuria and cardiac tamponade has not been specifically studied to our knowledge and warrants further research.

## PUB351

**Type B Lactic Acidosis in Multiple Myeloma** Luis Daniel Torres, Sreevalli Pariti. *Nephrology Dept, Methodist Dallas Medical Center, Dallas, TX.*

**Background:** Type B lactic acidosis is a rare manifestation related to underlying diseases, errors in metabolism, medications, intoxication and rarely in some hematologic malignancies and solid tumors. Only 3 cases of Type B lactic acidosis in multiple myeloma have been reported worldwide, and of those 3, only 2 reported in the U.S.

**Methods:** 62 year old female who was admitted due to back pain, weakness and difficulty breathing. Upon admission she was found to be volume depleted, with acute kidney injury, severe hypercalcemia and high anion gap metabolic acidosis and pancytopenia. Treatment was initiated with aggressive volume expansion and broad spectrum antibiotic. Once her volume status improved and no evidence of infection, lactic acid levels remained high. Bone marrow biopsy showed atypical plasmacytosis consistent with multiple myeloma. During her stay,her blood pressure remained normal and responded well to volume expansion with excellent urinary output. No evidence of tissue hypoperfusion was ever showed.She was treated aggressively for her hypercalcemia as well. Days later, once her serum creatinine improved to baseline, chemotherapy was started with bortezomib,dexamethasone and cyclophosphamide. On top of that, zoledronic acid and thiamine was added. It wasn't until chemotherapy started that her lactic acid levels decreased slowly in the next 3 days from 12.4mmol/L to 4.2mmol/L.

**Conclusions:** This case illustrates how the high lactic acid levels were not associated with any episode of tissue hypoperfusion or hypotension, and it was more related to high cell turnover seen in severe hematologic malignancies such as leukemias,high grade lymphomas and rarely in multiple myeloma. Our case was unique because our patient followed all her laboratory findings typical of multiple myeloma except the high lactic acid resistant to volume expansion, antibiotics and good renal perfusion.it wasn't until chemotherapy was started when levels dropped progressively, making this, type B lactic acidosis related to multiple myeloma.

## PUB352

**Markedly Nephrotic Rapidly Progressive Glomerulonephritis** Lisa Aimee Hechanova. *Nephrology, Loma Linda Univ Medical Center, Loma Linda, CA.*

**Background:** Seronegative pauci-immune glomerulonephritis is much more common than traditionally thought, with an incidence of about 30%, instead of the previously thought 10-15%. Seronegative pauci-immune glomerulonephritis has a much more aggressive presentation and worse prognosis than seropositive pauci-immune glomerulonephritis, and thus is essential to diagnose and treat early. Herein is presented a severe case of seronegative pauci-immune glomerulonephritis.

**Methods:** A 79-year-old black male with longstanding HTN, DM2, CKD stage 3 presented with several weeks fatigue, malaise, decreased appetite, unintentional 10lbs weight loss, worsened SOB. On review of his prior labs, his creatinine worsened from his baseline of 2.0mg/dL to 4.2mg/dL over a ten month span, then rapidly progressed from 4.2mg/dL to 14.3mg/dL over a one month span. He was found to have iron deficiency anemia with hemoglobin 7.1. Spot urine protein/creatinine ratio 35.5g/g, SPEP negative, UPEP unable to be obtained due to progression to anuria, immunofixation electrophoresis was negative, skeletal survey with no focal lytic or blastic bony lesions. Anti-GBM negative, cANCA/pANCA negative, hepatitis panel negative, compliment normal. Renal ultrasound showed 11cm kidneys. Renal biopsy showed acute crescentic GN, moderate active and chronic tubulointerstitial nephritis, mild to moderate interstitial fibrosis and tubular atrophy. Immunoflorescence was negative, and electron microscopy did not show any electron dense deposits.

**Conclusions:** Seronegative pauci-immune GN is much more common than previously thought, with an incidence of about 30%. Age of onset is generally younger: 39.7 +/- 17yrs vs 57.6 +/- 14yrs with seropositive pauci-immune GN. Only 20% will have constitutional symptoms vs 67% with seropositive pauci-immune GN. Renal failure is more dramatic, and patients generally have a worse renal survival rate. Proteinuria is also more severe with average urinary protein excretion is 5.5g/24hrs in seronegative pauci-immune GN vs 2.2g/24hrs in seropositive pauci-immune GN. Thus it is imperative that one be aware of the high proportion of pauci-immune glomerulonephritis which can be seronegative, and be very aggressive about diagnosis and treatment.

## PUB353

**Hyponatremia Associated with Unilateral Hand Weakness and Numbness** Fazel Dinary, Buthayna Dinary, Merugu Srinivas, Keyvan Ravakhah. *Internal Medicine/Nephrology, St. Vincent Medical Center, Cleveland, OH.*

**Background:** The key clinical feature in this case is making the diagnosis of apical lung cancer (Pancoast tumor) in a patient with brachial plexopathy and to recognize the association between SIADH as a paraneoplastic syndrome and non-small cell lung cancer (NSCLC).

**Methods:** A 62 year old mechanist male presented with 3 months h/o worsening right hand numbness and weakness with difficulty operating machinery at work. He reported anorexia, unintentional weight loss of about 20 lbs over 3 months and mild chronic cough with no fever, night sweats, hemoptysis or dyspnea. He denied neck pain, headaches or dizziness. His vitals were normal with BMI of 17. His grip strength in right hand was 4/5 with atrophy of the interossei muscles). Sensation to pinprick was reduced along the right C7, C8 and T1 dermatomes. Initial LABS was normal except serum sodium of 121mmol/L. R. shoulder and chest x-ray showed R. apical pleural based density suggestive of a soft tissue mass lesion. CT scan and MRI of the chest showed large R. apical superior sulcus pulmonary mass (Pancoast tumor) with extensive spread to proximal R. first and second ribs, R. brachial plexus nerve roots. Histopathology showed a poorly differentiated NSCLC . Tumor was staged IV (T3N2M1a). Workup for hyponatremia showed serum osmolality of 254 mOsmol/kg, urine osmolality of 451 mOmol/kg, and urinary sodium of 69mmol/L. TSH, LFT and cosyntropin stimulation test were all normal. Results were consistent with SIADH. His initial treatment consisted of pain control and fluid restriction. At 4 months follow up, the hyponatremia resolved and reported partial improvement in his right hand weakness.

**Conclusions:** Tumor-associated SIADH in majority of the cases is caused by small cell lung cancer (SCLC). NSCLC is shown to be responsible for a small proportion with few cases reported in the literature. The optimal therapy for SIADH is to treat the underlying malignant disease which may improve this paraneoplastic condition. Although SIADH is rare in NSCLC, careful history and early workup for hyponatremia in patient with weight loss and brachial plexopathy helps early detection and prevent progression of cancer.

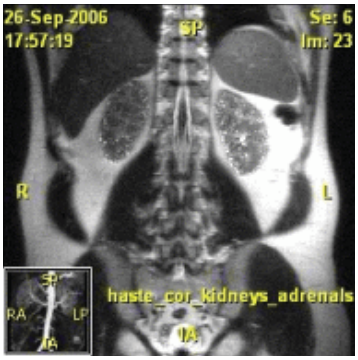
## PUB354

**Lithium Nephropathy Presenting in CKD, DI, and Bilateral Cystic Kidney Disease** Sami Safadi, Vicente E. Torres. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** Lithium is used for the treatment of bipolar disorder. A frequent side effect is renal toxicity. The major biochemical action of lithium is inhibition of glycogen synthase kinase-3, an enzyme that has an important role in the regulation of vasopressin action in the renal collecting ducts.

Lithium nephrotoxicity can be divided into three main categories: nephrogenic diabetes insipidus, acute intoxication, and CKD.

**Methods:** A 56 y/o male with a past medical history significant for bipolar disorder, hypertension, and obstructive sleep apnea presented to our clinic for a second opinion regarding chronic kidney disease, and bilateral cysts on his kidneys. He was diagnosed with CKD several years ago when a routine laboratory workup revealed a serum creatinine of 1.7 mg/dl. He also had a sub-nephrotic range proteinuria around 1.5 gm/day. The patient had bipolar disorder and received lithium for 20 years. The dose was adjusted based on his blood levels. His lithium was stopped 16 ago. Exam: The patient was afebrile; his blood pressure was 133/85. He looked comfortable, and his examination was unremarkable. Laboratories: He had an elevated serum creatinine of 2.6 mg/dL with an estimated GFR of 26 ml/min. His serum sodium was 146 mmol/L. His urine osmolarity was low indicating the presence of diabetes insipidus. He has a nephrotic range proteinuria. Imaging: MRI done several years ago showed a diffusely abnormal appearance of the kidneys with numerous tiny cysts throughout both kidneys. The cysts were less than 5 mm in size and more pronounced in the periphery of the kidneys near the corticomedullary junctions.




**Conclusions:** Nephrogenic DI is the most common adverse effect of lithium. The concentrating defect and natriuretic effect develop within weeks of lithium initiation. After years of lithium exposure, full-blown nephropathy can develop, characterized by decreased GFR and CKD.

## PUB355

**Familial Hyperkalemia** R.R. Malchira,[1] A.C. Ortiz-Guerrero,[1] S. Sarani,[1] P.T.T. Pham,[2] P.C. Pham.[1] *[1]Nephrology, Olive View MC; [2]Kidney & Pancreas Transplant, UCLA, LA, CA.*

**Background:** We present a case of hyperkalemia of undiagnosed etiology requiring multiple unwarranted admissions prior to his presentation to our institution.

**Methods:** A 22 year old man was referred for an incidental serum potassium (K) of 7.7 mmol/L. He denied musculoskeletal symptoms, palpitations, high K intake, medication use, strenuous activity, or constipation. Family history: hyperkalemia in maternal grandmother, great-aunt, and uncle. Evaluation: Serum Na 137 mmol/L, K 6.8 mmol/L (non-hemolyzed), Cl 110 mmol/L, bicarbonate 23 mg/dL, creatinine 0.93 mg/dL, glucose 108 mg/dL. EKG: no peaked T waves. Physical exam: afebrile, blood pressure 124/66 mmHg, heart rate 80 bpm. Well-nourished and developed, comfortable, moist oral mucosa. Heart, lungs, abdomen, extremities were unremarkable. Familial hyperkalemia due to K leakage from red blood cells with cooling to or below room temperature was ruled out (serial K measurements with blood cooling). Subsequent evaluation: urine transtubular potassium gradient: 3.3, random urine K concentrations of 17.8 mmol/L and 5.5 mmol/L with corresponding serum K of 5.7 and 5.6 mmol/L respectively on 2 different days. Blood gas consistent with non-gap metabolic acidosis. Hyperkalemia was suspected to be due to reduced renal secretion. Possible causes: inhibition or reduced function/expression of the Na epithelial channel (ENaC), hypoaldosteronism or pseudohypoaldosteronism, reduced ROMK activity and/or expression, or urinary stasis. Investigation for medication induced inhibition of ENaC, aldosterone, and ROMK was negative. Kidney, bladder ultrasound: no obstruction or retention. Plasma renin activity: 0.35 ng/mL/h (range 0.25-5.82), aldosterone: 7 ng/dL. Both were considered low in the presence of hyperkalemia. Relative hyporenin hypoaldosteronism was entertained. Patient responded minimally to a trial of fludrocortisone and high salt intake (K 5.7-5.8 mmol/L), but markedly to hydrochlorothiazide (K 4.7 mmol/L).

**Conclusions:** Our evaluation suggested Gordon's syndrome, likely due to WNK1 or WNK4 mutations. A systematic approach to the evaluation of hyperkalemia can lead to the correct diagnosis and treatment, hence avoidance of unnecessary hospitalizations.

## PUB356

**Spontaneous Clearance of Hepatitis B Surface Antigenemia in a Chronic Hemodialysis Patient** Chinmay P. Patel,[1] Divya Monga,[1] Mohini Alexander,[1] John D. Wagner,[1] Joseph Mattana.[2] *[1]Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY; [2]Nephrology, Winthrop Univ Hospital, Mineola, NY.*

**Background:** Hepatitis B virus (HBV) infection is a substantial global health problem. For a variety of reasons, patients with end stage renal disease (ESRD) on hemodialysis (HD) are at increased risk of acquiring HBV. Because of cellular immunity disturbances, clearance of HBsAg is an unusual event in a HD patient with chronic HBV infection. We report a patient who was HBsAg positive for 9 years and then spontaneously seroconverted and offer a hypothesis for its occurrence.

**Methods:** A 62-year-old woman with ESRD from diabetic nephropathy was started on HD and found to be HBsAg positive. Her anti-HBs Ab was negative and liver enzymes were in the normal range. She underwent arterio-venous graft (AVG) placement and developed multiple episodes of thrombosis requiring revisions. Two years after starting dialysis she developed staphylococcal bacteremia due to infection of the AVG site. She received antibiotic therapy and underwent partial excision of the AVG. Cultures from the excised AVG revealed Staphylococcus aureus and Pseudomonas aeruginosa. Patient subsequently developed multiple episodes of AVG infection requiring aggressive antibiotic therapy. After approximately 10 years of starting HD, her HBsAg testing came back negative for the first time. Her anti-HBc was positive, anti-HBs was negative, HBeAg was negative, anti-HBe was positive, HBV DNA PCR was <60 IU/mL and anti-HCV was negative. Two years later her anti-HBs titer became positive at >100 and after 4 years her HBsAg has continued to remain negative.

**Conclusions:** In our patient we hypothesize that the AV graft infection may have provided a strong stimulus to her immune system. This may have resulted in substantial amounts of TNF-alpha secretion which reduced Treg-mediated suppression of the anti-HBV response, thus helping her clear the chronic HBV infection. A better understanding of the mechanism by which this occurs might help develop therapeutic approaches to help HBsAg positive HD patients clear this infection.

## PUB357

### Transplant Renal Artery Stenosis Causing Hypertension and Acute Renal Failure Without Biopsy Evidence of Acute Tubular Necrosis

Talal K. Mahmood, Iasmina Craici, Kenneth E. Kokko, Steven Wagner. *Internal Medicine Nephrology, Univ of Mississippi Medical Center, Jackson, MS.*

**Background:** Transplant renal artery stenosis (TRAS) is a potentially treatable and reversible cause of post-transplant hypertension and allograft dysfunction. It generally occurs 3 months to 2 years post-transplant, and its prevalence varies from 1-23% depending on the diagnostic criteria and technique used. Renal hypoperfusion & renin-angiotensin system activation lead to ischemia & fluid retention.

**Methods:** A 50-year-old African American male with ESRD secondary to hypertension and type II diabetes underwent deceased donor ECD kidney transplant. Post-operative course was complicated by delayed graft function before achieving a nadir creatinine of 2.3. He was admitted 4 months post-transplant with hypertensive emergency, volume overload & acute kidney injury (BUN 55, Creatinine 3.7). He was also noted to have pancytopenia (WBC 3.8, Hb 8.4, PLT 75). Transplant renal ultrasound showed turbulent flow at the anastomosis. Allograft biopsy showed evidence of atypical thrombotic microangiopathy, including amorphous material in the glomerular capillaries, without significant evidence of acute tubular necrosis. He failed therapy with plasma exchange and was noted to have continued worsening of renal function with progressive oliguria. Dialysis was initiated secondary to hyperkalemia & volume overload. Repeat allograft biopsy showed no significant abnormality, and previously noted amorphous material in the glomerular capillaries was no longer present. Repeat ultrasound again showed turbulent flow in the region of anastomosis concerning for kinking. He underwent renal artery angiogram, angioplasty, and stent placement. Urine output increased immediately after the procedure and renal function started to improve without the need for further dialysis. Nine months after transplant, his creatinine is 1.85 and hypertension is well controlled with clonidine, metoprolol, and nifedipine.

**Conclusions:** This case illustrates that TRAS can cause severe allograft dysfunction requiring dialysis without biopsy evidence of acute tubular necrosis, and that timely intervention can lead to complete recovery of renal function.

## PUB358

### Renal Failure in an Adult Niemann-Pick Disease Type E

Cailian Cheng, Zhenda Zheng, Chenggang Shi, Xun Liu, Wenbo Zhao, Tan-qi Lou. *Dept of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, China.*

**Background:** Niemann-Pick (NP) disease is an autosomal recessive lipid-storage disorder. At least five forms (types A to E) of NP disease have been identified. However, NP disease type E is uncommon and seldom reported.

**Methods:** A 43-year-old woman was admitted to our hospital complaining of abdominal distension, weakness, and anorexiawith a weight loss of 5 kg in a month. Laboratory findings were: hemoglobin level was 9.5 g/dl. All autoimmunity markers were negative. HIV and hepatitis B and C serologies were negative. Alanine transaminase and aspartate aminotransferase were normal.Laboratory tests confirmed glomerulopathy with renal failure, with serum creatinin 3.7 mg/dl, serum albumin 31 g/L, proteinuria 2.5 g/24 h, and hematuria 20000 RBC/min.Abdominal ultrasonography showed parenchyma of the liver and renal cortex showed diffuse increased echogenicity with the paraumbilical vein open and large amounts of ascites. Microscopic study of a kidney biopsy identified widespread cytoplasmic vacuolation of endothelial and visceral epithelial cells in the glomerulus. Widespread vacuolar degeneration, focal dissolve and desquamation in the proximal convoluted tubule, protein casts, and interstitial diffused foam cells were observed, but chronic changes such as interstitial fibrosis or tubular atrophy were absent. Inflammatory cell infiltration was not seen. Electron microscopy showed massive lipidic deposits in endothelial cells and podocytes. Liver biopsy showed widespread foam cells.Bone marrow examination identified the presence of NP cells. Acid sphingomyelinase activity was normal in leukocytes and chitotriosidase activity was moderately elevated at 768.7 µmol/L/h. Albumin, erythropoietin,and vitamins were administered. Abdominocentesis and aspirated ascites were performed to alleviate abdominal distention. With these treatments, the patient achieved clinical improvement of symptoms. In follow-up visits, symptoms did not rapidly progress.

*Funding:* Government Support - Non-U.S.

## PUB359

### Mesangial IgA Deposition in Tip Lesion Focal Segmental Glomerulosclerosis

Ingrid Calliste, Liliana E. Rios Rojas, Shayan Shirazian, Joseph Mattana, James Drakakis. *Dept of Medicine, Winthrop Univ Hospital, Mineola, NY.*

**Background:** IgA Nephropathy is the most common cause of glomerulonephritis worldwide, with hypertension, hematuria, minimal proteinuria, and elevated serum creatinine as some of the presenting features. More rarely, mesangial deposition of IgA has been described as being concurrent with the glomerular lesion of minimal change disease (MCD). We describe a case of nephrotic syndrome, where the incidental dual glomerulopathy consisted of mild mesangial IgA nephropathy and the tip lesion variant of focal segmental glomerulosclerosis (FSGS), an entity which closely approximates MCD.

**Methods:** A 53 year old male presented to the emergency department with the complaint of abrupt onset lower extremity swelling. In addition to elevated blood pressure, he was found to have a serum albumin of 2.7g/dL and a normal serum creatinine. Urinalysis showed 4+ protein, 2+ blood and 4 red blood cells oer high powered field. He admitted to occasional nonsteroidal anti-inflammatory drug use as a fairly long-standing practice. Loop diuretic and angiotensin converting enyzme inhibitor therapy were initiated. Given the clinical and laboratory findings, percutaneous renal biopsy was undertaken. Histological analysis showed the glomerular tip lesion variant of FSGS and mesangial hypercellualrity with dominant staining for IgA.

**Conclusions:** Prior literature has described the concurrence of MCD and mild mesangial IgA nephropathy. This case illustrates a more overlap, in which the nephrotic entity is not MCD, but rather the closely related tip lesion variant of FSGS. The course of these nephrotic lesions is typically favorable, being exquisitely steroid responsive with low risk of progressive renal failure. Whether the concurrent expression of mesangial IgA deposition is purely coincidental, or instead is a manifestation of a mechanistic relationship between these disorders, remains to be determined.

## PUB360

### Hyponatremia due to the Syndrome of Inappropriate Antidiuretic Hormone (SIADH) following Administration of Intrathecal Methotrexate (IT MTX)

Fernanda Payan Schober, Gerald A. Hladik. *Nephrology, Univ of North Carolina, Chapel Hill, NC.*

**Background:** IT MTX has been routinely used in treatment of primary central nervous system (CNS) lymphoma, leptomeningeal metastatic disease, and as CNS prophylaxis in patients with non-CNS lymphoma. This case illustrates a possible link between IT MTX and SIADH.

**Methods:** A 50 year old man with diffuse large B-Cell lymphoma with CNS involvement was treated with IT MTX and Rituximab. He completed 2 of 6 cycles without complications and MRI of the brain showed response to treatment with decreased size and near resolution of CNS lesions. During the third cycle of therapy he was noted to develop acute hyponatremia. Serum sodium was 135mEq/L on admission. IT MTX was administered on day 4, which was followed by a fall in serum sodium from 133 mEq/L on day 5 to 120mEq/L on day 6. Notably, he had been receiving intravenous sodium bicarbonate to decrease the risk of MTX nephrotoxicity. He endorsed nausea, but had no other complaints. On physical examination he was alert and oriented. Blood pressure was 129/87 without postural changes. There was no edema and remainder of the examination was normal. Additional laboratory studies revealed serum osmolality 262mOsm/kg, serum creatinine 0.63mg/dL, urine sodium 127mEq/L and urine osmolality 459mOsm/kg. Serum thyroid-stimulating hormone and cortisol levels were normal. The findings were felt to be most consistent with SIADH. Intravenous sodium bicarbonate was discontinued. He was placed on a 1L fluid restriction and furosemide 20mg daily was started. Over the next 2 days the serum sodium improved to 124 mEq/L and nausea resolved. Two days later, serum sodium was 129mEq/L.

**Conclusions:** The time-related onset of SIADH following administration of IT MTX in this patient supports IT MTX as a possible cause of this syndrome. The diagnosis of lymphoma-related SIADH is less likely given the near resolution of CNS lesions on MRI, absence of hyponatremia on admission, and onset of hyponatremia following chemotherapy administration. Close monitoring of the serum sodium level in patients receiving IT MTX is therefore warranted, particularly when administered in conjunction with intravenous hydration.

## PUB361

### Coexisitng Acute Interstitial Nephritis and Atheroembolic Renal Disease

Nima Rabbani, Omid Bakhtar, Machaiah M. Madhrira. *Dept of Nephrology, Univ of Arizona, Tucson, AZ.*

**Background:** Acute interstitial nephritis is characterized by an inflammatory process induced by drugs, infections, or a systemic disease. The syndrome is important, in part, because it can cause a decline in creatinine clearance. However, we report a unique case of concomitant acute interstitial nephritis and atheroembolic renal disease.

**Methods:** A 63 year old male with a past medical history of ckd 3 with a baseline creatinine of 1.1-1.2mg/dl, hypertension, COPD, CAD with CABG was admitted with shortness of breath. He was diagnosed with CHF exacerbation, pneumonia and a left foot ulcer. He was prescribed antibiotics during his hospital course and discharged with a creatinine of 1.2 mg/dl. He was then readmitted at an outside facility with chest pain and had a cardiac catheterization with an unknown creatinine at discharge. He was subsequently readmitted for a worsening left ankle ulcer and was diagnosed with osteomyelitis. His creatinine was 3.5 mg/dl during that hospital stay and his AKI was determined to be due to nsaid use and was recommended supportive care. The patient was then started on iv antibiotics for 8 weeks (cefuroxime) and discharged home.

**Results:** On a subsequent follow up in the clinic, his BP was 118/64, mucous membranes moist and no peripheral edema was present. The rest of his physical exam was unremarkable. Laboratory results were significant for creatinine of 4.8mg/dL and a protein to creatinine ratio of 1.2 g/g. ANA, spep/upep, hepatitis panel, C3, C4, and ANCA levels were negative. An increased absolute eosinophilic count of 1.75% was demonstrated. Renal US showed no hydronephrosis with right kidney measured 10.4 cm and left kidney 12.1 cm. Renal biopsy was pursued which subsequently demonstrated changes consistent with acute interstitial

nephritis and atheroembolic renal disease. The patient's renal function progressed to a creatinine of 5.3 mg/dl in which renal function stabilized to 2.0-2.7 mg/dl with egfr ~35 ml/min after a two year therapy of steroids.

**Conclusions:** This case provides corroboration of evidence of a presentation of acute interstitial nephritis and atheroembolic renal disease.

## PUB362

**Long Term Follow Up of Proteinuria Associated with Vascular Endothelial Growth Factor Receptor (VEGF-R) Inhibition** Advayanand Shirsalkar, Anuradha Wadhwa. *Loyola Univ Medical Center.*

**Background:** High incidence of proteinuria and hypertension with VEGF-R inhibitors is well known but data regarding long-term follow up and optimal management of these side effects is lacking. Development of proteinuria can result in discontinuation or alteration of therapy, limiting treatment options for patients with malignancy. We describe long term follow up of a challenging case treated with VEGF-R inhibitors presenting with proteinuria and difficult to control hypertension.

**Methods:** A 48 year-old man with right nephrectomy for renal cell carcinoma was found to have metastatic disease to brain and lungs during routine follow up. Over a course of 4 years, patient was treated with multiple chemotherapeutic agents (including VEGF-R inhibitors- sunitinib, sorafenib, pazopanib) due to disease progression and/or limiting side effects. Prior to this therapy, the patient had no protein on urine dipstick. This increased to 1+ to 2+ protein soon after treatment with sunitinib was initiated and persisted when therapy was changed to sorafenib. Patient was then switched to pazopanib and proteinuria increased to 3+ on dipstick and 9 g on 24 hour urine collection. Given disease progression while on pazopanib and development of nephrotic syndrome, decision was made to stop the agent. Proteinuria decreased to 2 g/g within 4 weeks of discontinuing pazopanib. Patient was subsequently treated with sunitinib followed by axitinib. After complete cessation of VEGF-R inhibitor therapy in January 2013, proteinuria persisted but remained stable. Patient required close renal follow up for management of difficult to control blood pressure (2 additional antihypertensive agents required).

**Conclusions:** This challenging case with persistent proteinuria and difficult to control hypertension was successfully managed with a multidisciplinary team approach. Data regarding long term follow up and management of proteinuria and hypertension in patients treated with VEGF-R inhibitors is lacking. Reporting of this data with a larger series of patients ought to be made so that nephrologists may make evidence-based recommendations on managing these patients.

## PUB363

**Nodular Glomerulosclerosis of Diabetic Nephropathy Superimposed with Crescentic Glomerulonephritis** Kathleen Joy Paredes Garcia, Kristin Mercado Luzentales, Beatrice P. Concepcion. *Section of Nephrology, Philippine General Hospital, Manila, Philippines.*

**Background:** Diabetic nephropathy is a clinical diagnosis in a patient with long standing history of diabetes presenting with proteinuria, ultrasound findings ruling out other cause of renal insufficiency, signs of other end organ damage, and progressive decline in GFR at an average rate of 4ml/min annually. Deviation from the usual clinical picture should prompt a search for alternative diagnoses.

**Methods:** 34 year old male, diabetic for 5 years, poorly controlled with, nephropathy and retinopathy, presented with sudden onset of anuria, rapid decline in estimated GFR, and normal systemic findings apart from hypertensive urgency. Urinalysis showed active urinary sediments (pyuria, hematuria, WBC, coarse granular and waxy casts) and nephrotic range proteinuria. Impression was ARF from RPGN, Chronic Kidney disease secondary to diabetic kidney disease with chronic glomerulonephritis. HBsAg, anti-HCV, pANCA, cANCA and anti-nuclear antibody test were negative. Complement factor C3 was low. He underwent hemodialysis, methylprednisolone pulse therapy and kidney biopsy. Renal function failed to improve after treatment. Renal biopsy showed crescentic glomerulonephritis with 12% cellular crescents; diabetic nephropathy, nodular glomerulosclerosis; moderate interstitial fibrosis and tubular atrophy with 88% global sclerosis and 12% segmental sclerosis; arteriosclerosis and hyaline arteriolosclerosis. Immunofluorescence showed negative immunoglobulin staining, consistent with pauci-immune GN.

**Conclusions:** Studies show that among biopsied diabetic patients, the most common glomerular diseases superimposed with diabetic glomerulosclerosis (DGS) are membranous GN, IgA nephropathy, and post-infectious GN. Acute renal failure (ARF) from rapidly progressive glomerulonephritis (RPGN) on top of DGS is rare and occurs among older patients. Progressive decline in kidney function is almost inevitable in DM nephropathy. Physicians should have a high index of suspicion in looking for alternative causes of renal deterioration in this population.

## PUB364

**A Case Report of Aplastic Anemia Developed in a Peritoneal Dialysis Patient due to Lupus Nephritis** Hae Min Lee, Yong Kyun Kim, Euy Jin Choi, Ho Cheol Song. *Div of Nephrology, Dept of Internal Medicine, Bucheon St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Republic of Korea.*

**Background:** Aplastic anemia (AA) is often accompanied with systemic lupus erythematosus (SLE). Extrarenal manifestations of lupus nephritis became gradual complete or partial resolution after development of end-stage renal disease. We describe a rare case report of severe AA developed in a peritoneal dialysis patient due to lupus nephritis who was treated successfully with cyclophosphamide and steroid.

**Methods:** A 26-year-old woman who has maintained continuous ambulatory peritoneal dialysis since 2 years ago visited our outpatient clinic because of general weakness. She was diagnosed with SLE and lupus nephritis before 5 years then got treatment with cyclophosphamide and rituximab. Her vital sign was stable at the time of visiting. Her hemoglobin level and platelet count declined from 11.6 to 7.5 g/dL and from 1.3 x $10^{11}$ to 1.6 x $10^{10}$ /L within 5 weeks. Her absolute neutrophil count decreased from 3.87 x $10^{9}$ to 1.89 x $10^{9}$ /L 2 days after hospitalization. Bone marrow revealed less than 5% of cellularity and all the erythroid and granulocytic precursors and megakaryocytes are markedly decreased in number. She was diagnosed with severe AA. Her serum anti-DNA antibody level fell from > 50 at the diagnosis of SLE to 23.27 IU/mL. After diagnosis with AA, she had gotten treatment with 5 times of cyclophosphamide and 1 time of steroid pulse. During the course of treatment with maintenance low dose prednisolone, her white blood cell and platelet counts have been normal and hemoglobin level was maintained between 9.5 and 11.5 g/dL with erythropoiesis stimulating agent (ESA).

**Conclusions:** Although severe AA is rarely developed in patient with ESRD due to lupus nephritis whose lupus activity fell over 50 %, the patient successfully treated with formal immunosuppressive therapy of AA with cyclophosphamide. We do not hesitate to evaluate bone marrow study if anemia is acutely aggravated or poorly responded to ESA with or without decrement of other cellular linage in ESRD patients due to lupus nephritis even though their lupus activity is low.

## PUB365

**Treatment of Calcific Uremic Arteriolopathy in Peritoneal Dialysis Patient** Neelima Chilukuri.[1,2] *[1]Nephrology, Metropolitan Hospital Center, New York, NY; [2]Nephrology, SUNY Downstate Medical Center, New York, NY.*

**Background:** Calcific Uremic Arteriolopathy ( Calciphylaxis) is medial calcification of arterioles leading to ischemia and subcutaneous necrosis commonly seen in ESRD patients on chronic dialysis, and there are no controlled studies that compare its treatment modalities.

**Methods:** We are presenting a rare case of calciphylaxis in a caucasian female who is 50 years old and was started on peritoneal dialysis recently, she has past medical history of Hypertension, ESRD secondary to Post streptococcal Glomerulonephritis. She presented with painful nodules on her lower extremities, even before she was started on peritoneal dialysis, one of the lesions which ulcerated was biopsied and was confirmed to be calciphylaxis, her labs around the time of diagnosis calcium was 10.5, phosphorus was above 9.0, PTH was above 3000, BUN 92, Cr 6.08. She was dialysed adequately by PD, she was given sevelamer as phosphate binder, was treated with cinacalcet for secondary hyperparathyroidism, she was told to avoid all calcium supplements, her calcium levels came down to 9.5, phosphorus to 5.5 and PTH to 1318, her ulcer on her lower extremity was aggressively treated with local wound care to avoid superinfection, her lesions gradually started healing , we did not give sodium thiosulphate since patient was on peritoneal dialysis.

**Conclusions:** There are no studies that would compare different treatment strategies for calciphylaxis, except for few case reports, where sodium thiosulphate was used successfully in hemodialysis patients after each dialysis, which acts as chelator and antioxidant along with decreasing calcium phosphate product less than 55 and treating secondary hyperparathyroidism, in our patient we could not use sodium thiosulphate , if given oral its not very effective, if given intraperitoneal has risk of chemical peritonitis as reported in one case report, and if given Intravenous will be dialysed by PD and would not be effective. Its still a question whether and how to use sodium thiosulphate in PD patients.

## PUB366

**Successful Treatment with Tocilizumab in a Case of Lupus Nephritis Complicated with Nuropsychiatric Complications** Oonishi Takahiro. *Nephrology, Ise Red Cross Hospital.*

**Background:** Neuropsychiatric complications of SLE (NPSLE) have been reported to occur in 14-75% of SLE patients at any time during in disease course. The features of this condition may include seizures, stroke, depression, psychosis and cognitive disorders. Neuropsychiatric complications are associated with increased morbidity and mortality of SLE patients. In NPSLE immunoregulatory cytokines ,such IL-6, IL-10,

IFN,IL-1 and TNF-α were extensively examined. On the other hand TCZ was reported could effectively block IL-6 in patients with SLE.(Gabor G. Illei1, Arthritis Rheumatism 2010) To assess that TCZ might be a therapeutic option for SLE, especially in case complicated with NPSLE.

**Methods:** Case report 45-year-old woman with nephrotic syndrome was diagnosed lupus nephritis in January 2012. Pathological examination of a renal biopsy specimen revealed membranous lupus nephritis (Class V). After renal biopsy she had seizure with consciousness loss. Her cerebrospinal fluid (CSF) displayed a leukocyte count of 9/μl ,a sugar level 40 mg/dl, a protein level of 60mg/dl, serum anti Sm antibody 83.8 positive anti-ribosomal P was positive. MRI detected abnormal signal in ponse and basal ganglia. She was diagnosed LN with NPSLE. Remission induced by treatment with high dose of prednisolone and cyclophosphamide was followed by relapse within 1 year. Although her condition was resistant to immunosuppressive drugs, her symptoms inflammatory response and quality of life measures were successfully improved by tocilizumab, a humanized anti-interleukin-6 receptor antibody. IL-6 is expressed in a wide variety of cell lineages, including monocyte/macrophages, T cell, fibroblasts, and endothelial cells, in response to diverse stimuli. Although a significant role for IL-6 in CNS during NPSLE is not clearly elucidated, elevated IL-6 in the CNS could be relevant in active NPSLE. TCZ may be a therapeutic option for SLE, especially in case complicated with NPSLE.

## PUB367

**Long-Term Intravenous Vancomycin Hydrochloride Induced Erythema** Eleni Chelioti,[1] Maria Sotiraki,[1] Alexia Papalexandrou,[1] Evangelia Gkalitsiou,[1] Evdokia Efthimiou,[1] Kassiani Manoloudaki,[2] Maria Tsilivigou.[1] *[1]Dept of Nephrologr, General Hospital of Piraeus, Athens, Greece; [2]Dept of Pathology, General Hospital of Piraeous, Athens, Greece.*

**Background:** Erythema on face and on upper body (red neck or red man syndrome), with hypotension and flushing is reported as an adverse reaction on intravenous (iv) Vancomycin Hydrocloride(VAN-HCL) in >10% of cases. Erythema is a rare hypersensitivity infusion-related reaction to VAN-HCL. It is often associated with rapid infusion of the first dose and 94% of patients are undergoing treatment less than a month. We report a case of generalized erythema due to iv VAN-HCL.

**Methods:** A 72 years-old man with a history of chronic kidney disease and long-term indwelling urinary catheter due to prostatic hypertrophy presented in a uremic state that necessitated urgent initiation of dialysis treatment. After few hemodialysis his renal function was improved. The patient experienced fever and presented recurrent urinary infections due to Enterococcus falcium, Acinetobacter baumani and Enterobacter aerogenes. Also,the patient presented bloodstream infections by methicillin-resistant Staphylococcus epidermidis, haemolyticus and capitis and treated by iv VAN-HCL. His clinical condition remained critical and in more than a month of continuous iv VAN-HCL, a symmetric generalized, total body, erythematous rash appeared with prominent scaling and desquamation. Skin swabs were taken of the erosions and a skin biopsy was performed. Skin biopsy was consistent with a diagnosis of drug-vancomycin-reaction dermatitis. Stopped using VAN-HCL and started corticosteroids, resulted to amelioration of his clinical status. Unfortunately, our patient became septicemic and died on month after onset.

**Conclusions:** Long term iv use of VAN-HCL is associated with erythema in only 5% of cases. Erythema can be generalized with scaling, affecting greater than 90% of the total body surface area. It appears in less than a month treatment on i.v. VAN-HCL, most commonly in male patients, of a mean age of 60+. Our patients had many predisposing factors for the development of erythema. Since today 114 people have erythema when taking i.v. VAN-HCL and only 4 cases have reported on 2012.

## PUB368

**Belatacept for Kidney Transplant Recipients with Baseline Low Blood Pressure: A New Indication?** Swati Mehta, John R. Lee, Thangamani Muthukumar, Manikkam Suthanthiran. *Nephrology and Hypertension, New York Presbyterian Hospital- Weill Cornell.*

**Background:** Calcineurin inhibitors(CNI) are the mainstay of immunosuppressive medications in transplantation. Unfortunately, acute tubular injury secondary to their vasoconstrictive properties is a common phenomenon.This may be particularly severe in kidney transplant recipients with low blood pressures, an identified risk factor for primary non-function (Webber et al.Transplantation 2005;93:54-60). Belatacept (CTLA-4-Ig fusion protein) is being evaluated as a CNI sparing agent in kidney transplant recipients. We report a case of a kidney transplant recipient with acute tubular injury attributed to tacrolimus that improved remarkably after replacing tacrolimus with belatacept.

**Methods:** A 64yr old male with ESRD secondary to presumed iatrogenic phosphate nephropathy received a living unrelated renal transplantation. He had been on home hemodialysis for two years with systolic blood pressures (SBP) in the 90-mmHg range. A pretransplant donor CDC crossmatch and DSAscreen with HLA single antigen beads were negative. Initial immunosuppression consisted of thymoglobulin induction and maintenance therapy with tacrolimus and mycophenolate mofetil. Following uneventful kidney transplantation, creatinine (Cr) decreased from 5.7 to 1.7 mg/dl on post op day (POD)#2. His course was complicated by hypotension, not responding to IV saline. Tacrolimus was initiated on POD#2 and reached a level of 14 ng/mL on POD#4. His Cr rose from 1.7 to 3.2 mg/dl on POD#6. Renal ultrasound was negative and biopsy revealed widespread tubular vacuolization consistent with CNI toxicity. Tacrolimus was discontinued and he was switched to belatacept. Patient continued on mycophenolic acid and started on tapering prednisone. Patient's Cr peaked at 4.2mg/dl on POD#8 but decreased to 1.7mg/dl by POD#14. His most recent Cr at 5-month follow up was 1.6mg/dl.

**Conclusions:** Belatacept was utilized as a salvage therapy to prevent further CNI-associated acute tubular injury. Belatacept conversion may be a novel approach for kidney transplant recipients with low mean arterial blood pressures and particularly sensitive to CNI-associated vasoconstriction and consequent renal injury.

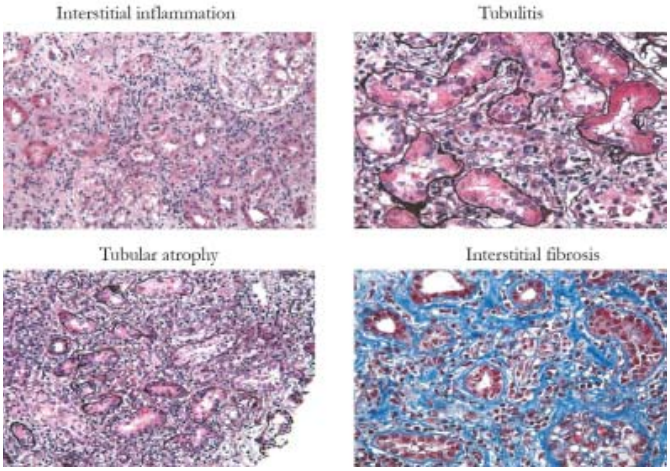
## PUB369

**Omeprazole-Induced Acute Tubulo-Interstitial Nephritis** Rakesh Malhotra,[1] Srujana Polsani,[2] Anjali Acharya,[2] Zaher Hamadeh.[2] *[1]UMDNJ; [2]Jacobi Medical Center.*

**Background:** Proton pump inhibitors (PPIs) are widely prescribed to treat acid-related gastrointestinal disorders. We report a case of reversible acute renal failure due to acute tubulointerstitial nephritis (AIN), confirmed by histology of a renal biopsy, associated with taking omeprazole.

**Methods:** A 70-year-old female presented with complain of generalized weakness and abdominal pain for last 2 months. She had a past medical history for HTN, diabetes, and CKD (baseline sCr 1.6 mg/dL). Physical examination revealed pale conjunctivae. There was no pedal edema, skin rash, petechiae or purpura. Lab results showed elevated BUN of 53 mg/dL and sCR of 2.4 mg/dl. Her Hb was 11g/dL. Urinalysis revealed proteinuria and eosinophiluria. Complements were normal. Serological markers for Hep B, Hep C, and HIV were negative, and no cryoglobulins were detected. Renal ultrasonography was normal. Patient was started on IV fluids however her sCr kept worsening with peak sCr of 4.1 mg/dL. Nephrology consult was obtained and kidney biopsy performed. Biopsy results showed interstitial inflammation with active tubulitis and acute tubal injury. Biopsy also showed chronic features including interstitial fibrosis and tubular atrophy. Overall, findings were consistent with severe interstitial nephritis. The patient had initiated treatment with omeprazole 3 months prior to admission. Omeprazole was discontinued in view of the diagnosis of AIN. Patient was started on oral prednisone for 6-8 weeks with further tapering. The patient sCr gradually improved with subsequently serum creatinine concentration leveled off at 1.2 mg/dl.

**Conclusions:** The pathogenesis of omeprazole-associated AIN is unclear and may be related to the involvement of both humoral and cellular immune mechanisms. Physicians should be aware of this disorder. Accurate and timely diagnosis and withdrawal of the offending drug can prevent potentially life-threatening renal failure.

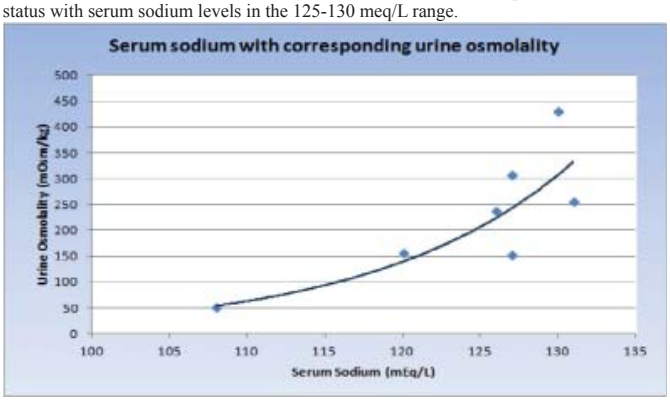



## PUB370

**A Case of Hyponatremia Secondary to Reset Osmostat** Rushi K. Nayak. *Dept of Nephrology, St. Luke's-Roosevelt Hospital Center, New York, NY.*

**Background:** The syndrome of inappropriate anti-diuretic hormone (SIADH), and its reset osmostat variant, result in impaired water metabolism and hyponatremia. Alternatively, the ADH response is maintained in psychogenic polydipsia, in which a massive water ingestion overwhelms the kidney's diluting abilities, resulting in water retention. Here we present a case of hyponatremia related to reset osmostat, unmasked by psychogenic polydipsia.

**Methods:** A 62 y/o male with history of schizophrenia, non-compliant with antipsychotics, presented with altered mental status for 3 days. In addition to poor memory and inability to care for himself, he was also noted to be drinking large quantities of water. He was admitted 2 months prior for acute confusion with severe hyponatremia secondary to massive water ingestion. On initial evaluation, patient was awake but disoriented, afebrile, and hemodynamically stable. Laboratory evaluation was significant for serum Na 120 meq/L, serum osmolarity 250 mOsm/kg, BUN 11, and Cr 1.2. Thyroid and cortisol studies were unremarkable. Urine osm was found to be 157, with UNa of 18. Review of labs from prior admission revealed serum Na of 107meq/L with a corresponding Uosm of 54 mosm/kg. During current admission, patient was treated with water restriction; serial measurements of serum Na and urine osm are shown. Patient had improvement in mental status with serum sodium levels in the 125-130 meq/L range.




**Conclusions:** Reset osmostat is a variant of the SIADH marked by disordered ADH regulation. ADH levels can be maximally suppressed at a basal hyponatremic state, but rise quickly with serum sodium long before the hyponatremia is corrected. As shown in Figure 1, this patient demonstrated an ability to maximally dilute the urine when his serum sodium reaches a value of 107 meq/L, but ADH levels quickly rise as serum sodium levels approach 120 meq/L. The end result is a persistent hyponatremic state.

**PUB371**

**An Overlooked Entity: Warfarin Related Nephropathy – A Case Report** Manjunath Kottalgi, Pragnesh J. Patel, Amber S. Podoll, Kevin W. Finkel. *Internal Medicine, Renal Disease and Hypertension, Univ of Texas Medical School, Houston, TX.*

**Background:** Warfarin has long been used as the oral anticoagulant of choice for treatment, primary and secondary prophylaxis in patients with various pro-thrombotic states that can lead to thromboembolic events. Several adverse effects of warfarin overdose on renal function have been described in the recent literature including glomerular hemorrhage, acute tubular injury with occlusive red blood cell casts[3] and interstitial nephritis[2,8]. Hematuria in patients on warfarin therapy has been described as early as in 1964 by Reilly et al. The incidence, population at risk and outcomes has been described recently by Brodsky et al in a prospective study[3]. Warfarin-related nephropathy has been an overlooked entity; but it can have a significant impact on morbidity and mortality, more so in patients with chronic kidney disease[3].

**Methods:** A 68 year old female was recently started on warfarin anticoagulation for an acute pulmonary embolism one week prior to presentation. On readmission, she complained of fatigue, tremors of hands, decreasing urine output and dysuria. She denied any fever, chills, nausea, vomiting, abdominal pain, hematuria, prior history of renal disease or use of NSAIDS. She was initially hypoglycemic. She had an acute rise in her serum creatinine from 0.8 mg/dl to 8.0 mg/dl along with a correlated rise in INR from 1.11 to 5.67 over 9 days. She was oliguric at presentation with urine output of 20ml/hr. Urine analysis showed 6 rbcs/hpf, 42 wbcs/hpf, moderate blood, mild proteinuria. Urine culture was negative. Biopsy was deferred due to coagulopathy. Subsequently along with correction of INR with vitamin K, her renal functions improved over the course of 7 days to a creatinine of 1.7mg/dl and eGFR of 31ml/min/1.73m2 at discharge. She never required renal replacement therapy.

**Conclusions:** Considering the large population on warfarin therapy clinicians should have a heightened awareness of this entity. This calls for a larger prospective study to identify at risk populations, guidelines to monitor renal functions while being treated with warfarin, precautions and therapies to minimize the risk of warfarin- related nephropathy.

**PUB372**

**Crescentic IgA Nephropathy (IgAN) in the Renal Allograft: A Case Report** Shashikant Patel, Samir Parikh, Jon R. Von Visger. *Dept of Nephrology, The Ohio State Univ Medical Center, Columbus, OH.*

**Background:** Aggressive IgAN is rare in the renal allograft. We report a case of recurrent IgAN that manifested as a necrotizing and crescentic glomerulonephritis (GN).

**Methods:** 31 year old male received deceased donor renal transplant (Tx) after developing ESRD due to IgAN. Induction therapy included ATG, steroids (5 days) and sirolimus. Nine months after Tx he developed acute cellular and antibody mediated rejection with mild IgA staining which improved with steroids. Graft function again worsened and repeat biopsy showed crescentic GN but no IgA. Therapy was started but due to worsening disease a 3rd biopsy was done and showed crescentic IgAN. Graft function returned to baseline after treatment with oral cyclophosphamide and steroids.


**Conclusions:** We report a case of crescentic IgAN in a renal allograft successfully treated with oral cyclophosphamide. Crescentic IgAN in the renal allograft is rare. Reports from our institution suggest steroid free regimen and sirolimus may increase risk. Treatment with cyclophosphamide appears to be effective with response similar to native IgAN. Crescentic IgAN is a devastating manifestation that can cause graft failure. Early recognition and aggressive treatment provides the best chance for graft survival.

**PUB373**

**BK Nephropathy or Rejection** Sarah Khan, Bhupinder Sangha, Scott McRight, Neeraj Singh, Adrian P. Sequeira. *LSU Health Sciences Centre, Shreveport, LA.*

**Background:** BK virus nephropathy may mimic acute cellular rejection histologically. We present a kidney transplant recipient with worsening renal function 2 years post-transplant. Her kidney biopsy showed lymphocytic cellular infiltrate, with weakly positive BK stain and a negative plasma BK virus PCR, presenting a diagnostic dilemma.

**Methods:** A 53 year old female with a deceased donor kidney transplant presented with slowly rising serum creatinine. She was on tacrolimus 2mg twice daily, mycophenolate mofetil 360 mg twice daily and prednisone 2.5 mg daily for maintenance. Her baseline serum creatinine was 1.5 mg/dl, had slowly risen to 2.3 mg/dl over 6 months. She was treated for biopsy proven BK nephropathy 15 months earlier, with plasma BK virus at 135,000 copies/ml. Her immunosuppression was reduced and serum creatinine returned to baseline with resolved viremia. On current admission, plasma BK virus PCR was undetectable (<5000 copies/ml, Mayo Clinic Laboratories). Kidney biopsy showed intense lymphocytic cellular infiltrate, no viral cytopathic changes and weakly positive BK stain. Donor specific antibodies was negative. Banff 1B acute cellular rejection was diagnosed; managed with IV steroids. Serum creatinine improved to 1.7 mg/dl at 2 weeks, but again worsened to 3.0 mg/dl a month later. Repeat biopsy showed persistent cellular infiltrate, and a stronger BK virus stain than previous. Plasma BK virus PCR was positive at 18,200 copies/ml. Diagnosis of BK nephropathy was made and immunosuppression was reduced with improvement in both serum creatinine and BK viremia.

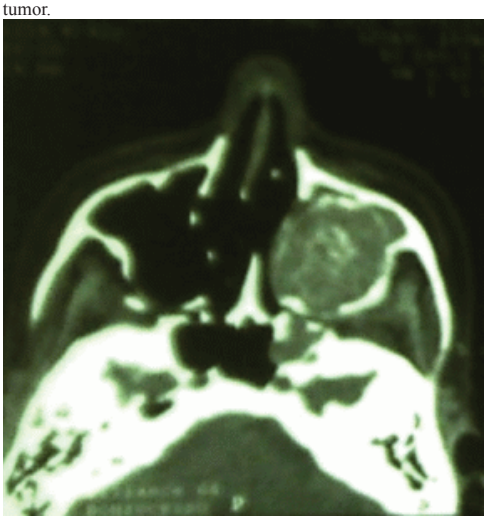
**Conclusions:** This case underlines the difficulty differentiating BK nephropathy from acute rejection, specially in absence of BK viremia, cytopathic changes; and weakly positive stain. Negative plasma BK virus PCR is reported to have a 100% negative predictive value for ruling out BK nephropathy. Our case highlights that BK nephropathy may occur at serum BK virus levels below the threshold levels reported by some laboratories. It is unclear whether our patient had a genotype variant causing falsely low BK virus PCR. Finally, if weak staining for BK virus has the same significance as intense staining, and whether some genotype variants may cause a weak BK virus staining is unclear.

**PUB374**

**Multiple Brown Tumors as a Complication of Hyperparathyroidism in a Patient with Chronic Kidney Disease** Guilherme Fonseca Mendes, Talita Mourao Chaves Corrica, Talita Cardoso Proenca, Tatiana Santos, Maria izabel Neves de Holanda Barbosa, Luiz Fernando Christiani. *Nephrology, Hospital Federal de Bonsucesso, Rio de Janeiro, Brazil.*

**Background:** Brown tumors are benign lesions with mass effect and intense osteoclastic activity caused by primary or secondary hyperparathyroidism. It is observed in 1.5-13% of secondary hyperparathyroidism.This should be managed with active Vitamin D and calcimimetics to avoid serious complications.

**Methods:** We report a case of a 42yo african-american female patient referred to our department for evaluation of hyperparathyroidism and chronic kidney disease managed by hemodialysis for 12 years. She was using calcitriol 3mcg 3x/week and Calcium 2g/day. Due to history of multiple kidney stones leading to left side nephrectomy, we considered the diagnosis of primary hyperparathyroidism. The physical exam was relevant for several deformities, especially left eye proptosis, chest enlargement and phalanx resorption. On chest x-ray was seen multiple masses close to chest wall; the skull, chest and spine CT performed showed multiple inflating lesions in ribs, severe spinal compression and orbital tumor.



The 99TC parathyroid scintigraphy evidentiate two hypercaptating areas, suggesting hyperplasia. Laboratory workup was relevant for: PTHi: 1771 pg/mL; ALP: 1318 U/L; P: 4,6 mg/dL; Ca: 8,8 mg/dL. She underwent total parathyroidectomy with subcutaneous implant, improving significantly her quality of life and laboratory findings. The brown tumors still, but are being followed with CT and had no reduction in 4 months.

**Conclusions:** Several complications may be associated with hyperparathyroidism. In this patient, the lack of an early diagnosis and the development of chronic kidney disease contributed to this level of deformity. Thus, hyperparathyroidism, either primary or secondary, must be controlled aggressively to avoid this level of complication.

**PUB375**

**Posterior Reversible Encephalopathy Syndrome in an Adult Patient with Thrombotic Microangiopathy and Diffuse Alveolar Hemorrhage** Zhongguang Yang, John Doran, Titilayo O. Ilori. *Nephrology, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** Posterior reversible encephalopathy syndrome (PRES) is a neurologic condition characterized by headache, altered consciousness, seizures, and cortical visual loss with typical MRI features.Its pathogenesis is unclear. Renal failure and hypertension

are known risk factors. Limited PRES cases associated with immunosuppressive drugs including cyclophosphamide (CYC) and steroids were reported. We present an interesting case of biopsy-proven thrombotic microangiopathy (TMA) who developed PRES.

**Methods:** A 29 year old male with ESRD and biopsy proven TMA presented developed diffuse alveolar hemorrhage. The etiology of alveolar hemorrhage was unclear. He was stared on plasmapharesis followed by high dose prednisone and IV CYC monthly. Patient was admitted with hypertensive emergency with pulmonary edema. He received 2nd dose of CYC 20 days before admission. Before hemodialysis started, he suddenly developed altered consciousness, headache, and seizure lasting 2 minutes together with visual loss. Head CT demonstrated hypodense foci in bilateral posterior occipital lobes. CT angiogram of head and neck failed show blood vessel abnormalities. Brain MRI revealed T2- and fluid-attenuated inversion recovery (FLAIR)-weighted hyperdense lesions in bilateral parieto-occipital lobes. EEG showed mild generalized slowing of the background without focal features. PRES was diagnosed and treated with hemodialysis and aggressive antihypertensive therapy as well as keppra.

**Conclusions:** PRES is a rare finding in patients with glomerulonephritis and more commonly seen in lupus. It has also been reported in few cases of atypical HUS and thrombotic thrombocytopenic purpura. We believe our patient may have atypical HUS secondary to mutations in the complement pathway, genetic testing was not done. CYC, steroids and uncontrolled hypertension might also be causative factors for PRES. CYC may cause water toxicity; corticosteroids may lead to hypertension and fluid overload. Early recognition and intervention with aggressive antihypertensive therapy and hemodialysis will improve outcome and avoid permanent brain injury and sequela.

## PUB376

**Disseminated Primary Varicella Infection in Adult Renal Transplant Patient** Ramandeep S. Banga, Maria Aurora C. Posadas, Dannah Wray, Titte Srinivas. *MUSC.*

**Background:** Immunosuppressed patients are susceptible to disseminated varicella (VZV) due to impaired cell-mediated immunity. We present a case of primary varicella in an adult patient with kidney transplant.

**Methods:** A 37 year old African-American male who received a cadaveric renal transplant 3 years ago presented with rash. His maintenance immunosuppression included tacrolimus, mycophenolate mofetil (MMF), and prednisone. He denied any history of recent travel or VZV in the past. His nephew was recently diagnosed with VZV. The patient presented with diffuse vesiculo-papular rash spreading from his scalp, face, arms, trunk, and groin over a period of three days. He had no rashes on his hands and feet. Direct fluorescence antibody testing performed on the serous fluid from his vesicle was positive for VZV. His VZV IgM was high (5.47) while IgG was negative. His VZV PCR was 173,000 copies/ml. HSV, HIV, hepatitis B, and hepatitis C serologies were all negative. The patient's MMF was held. He was treated with Acyclovir 10mg/kg IV q8 hours for 7 days. Soon after starting treatment, the patients vesiculo-papular rashes evolved into crusting lesions.

**Conclusions:** Since the introduction of Varicella vaccine in 1995, only approximately 2-4% of adult solid organ transplant (SOT) recipients are seronegative for VZV and therefore susceptible to primary varicella infection. Currently, there are no guidelines for using varicella vaccine in patients with end-stage renal disease. Early diagnosis and aggressive treatment with acyclovir effectively alters the clinical course and could be life saving. Varicella vaccination has been shown to reduce morbidity in VZV seronegative recipients and should be considered in ESRD patients anticipating kidney transplant. VZV seronegative transplant patients should also be counseled on precautions to take to reduce the risk of disease transmission from recently vaccinated or infected contacts.

## PUB377

**Subclinical Transient Immune-Complex Mediated Glomerulonephritis in Living Donor Kidney** Manpreet Singh, Jean M. Francis, Sandeep Ghai. *Renal Section, Boston Univ School of Medicine, Boston, MA.*

**Background:** Kidney transplantation is the best available therapy for patients with end stage renal disease (ESRD) with live kidney donation having better long-term outcomes. Selection criteria for live donors should be very strict to prevent any long-term complications after kidney donation. We report a case of young living donor with transient immune complex (IC) mediated glomerulonephritis(GN) noted as an incidental finding on zero time allograft biopsy. We emphasize the importance of a systematic approach to donor evaluation.

**Methods:** A 31 year old female donated a kidney to her father who has ERSD from diabetic nephropathy. She has a history of well controlled skin psoriasis . She was deemed a good candidate byrheumatology with a negative workup. Her workup was completely normal, importantly she had two normal UA without proteinuria and hematuria and two 24-urine collections with normal creatinine clearance and normal proteinuria. Her physical Exam was unremarkable. A zero time biopsy was performed per our protocol which showed diffuse mild glomerular hypoperfusion without an active inflammatory process, There was mild chronic changes and a focal finding of IC deposits predominantly distributed in the mesangium with immune-fluorescence positive for C3, IgM, trace IgA and IgG. A repeat biopsy of the graft was done 3 months after transplant, which showed persistence of the immune deposits. Both donor and recipient remain totally asymptomatic.

**Conclusions:** Despite the extensive workup performed in our patient to rule out the possibility of any potential kidney disease, her glomerular lesion remained silent and subclinical. Our patient likely had a transient IC mediated (GN) in the setting of a skin infection prior to kidney donation. Her skin infection was successfully treated, and she continues to do well without any serologic evidence of any disease process, and her kidney function remains normal without proteinuria and hematuria. This case underlies that a complete and thorough evaluation remains a difficult process. A systematic approach should be used on a case by case basis and follow up of all donors should be regimented.

## PUB378

**Overlapping Syndrome of ANCA Vasculitis and Lupus Nephritis** Noemie C. Juaire, Paramita Mukherjee. *Div of Nephrology, Univ of Washington, Seattle, WA.*

**Background:** ANCA associated vasculitis is rarely seen to occur simultaneously with lupus nephritis (LN). We present a case of an elderly man with no prior history of kidney disease, who presented with general malaise and acute kidney injury and was found to have lupus nephritis and ANCA related vasculitis concurrently.

**Methods:** 73 year old Caucasian man with known hypertension presented to his primary care doctor with anorexia, nausea and vomiting over 2 weeks and 30 pound weight loss over last 1 year. He had no prior history of kidney disease. Physical exam was remarkable only for a blood pressure of 143/83. Initial workup showed blood urea nitrogen of 118mg/dL and creatinine of 10.9mg/dL. His creatinine 1 year back was normal. Urine analysis was notable for 3+ blood and 3+ protein. Numerous monomorphic red blood cells (RBC) and few dysmorphic RBC were seen on urine sediment. Urine protein to creatinine ratio was 1.5 grams/gram, C3 was low at 75mg/dL, C4 was normal, cryoglobulins, hepatitis and human immunodeficiency virus serologies were negative. Autoimmune workup revealed p-ANCA positive at 1:320 and myeloperoxidase autoantibodies at >100. Interestingly, anti-dsDNA , anti-cardiolipin IgM and IgG were significantly elevated at 45 IU/mL, 87.3 U/mL and 101.7 U/mL respectively. On hospital day 3, he was started on dialysis. Renal biopsy done on hospital day 6 showed findings consistent with concurrent LN and ANCA vasculitis. Subsequently, he was started on plasmapheresis, cyclophosphamide and pulse dose steroids. He had partial renal recovery and both plasmapheresis and dialysis were stopped after 2 weeks.

**Conclusions:** Although, cases of concurrent LN and ANCA related vasculitis reported in the literature is very low, their association occurs more frequently than just the coincidental occurrence of unrelated diseases. Both these diseases are mediated by a T helper1 immune mechanism suggesting a possible pathogenic link. Therefore, it is important to consider both ANCA vasculitis and LN in patients with high suspicion of glomerulonephritis associated with renal failure.

## PUB379

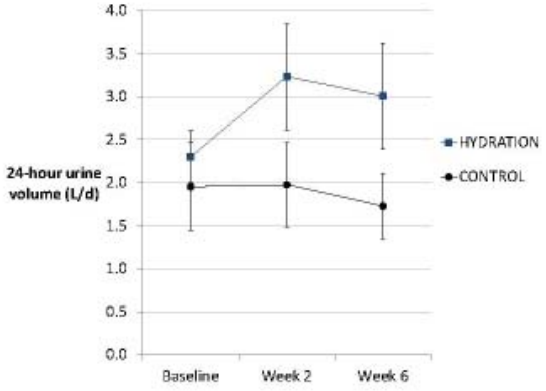
**The Chronic Kidney Disease Water Intake Trial: Results from a Pilot Study** William F. Clark, Jessica M. Sontrop, Shih-Han S. Huang, Louise M. Moist, Andrew A. House, Matthew A. Weir, Amit X. Garg. *Nephrology, London Health Sciences Centre, Canada.*

**Background:** Evidence from animal and human studies suggests a beneficial effect of increased water intake on kidney function. Prior to conducting a large trial on the effect of increased water intake on kidney function in chronic kidney disease (CKD) patients, we conducted a pilot trial to examine the safety and feasibility of increasing water intake in CKD patients.

**Methods:** In this randomized pilot trial, 29 adults with stage 3 CKD were randomly assigned, in a 2:1 ratio, to a hydration intervention (n=18) or control group (n=11) (Canada, 2012-2013). Participants in the hydration group were instructed to drink 1.0 to 1.5 L water per day for 6 weeks (in addition to usual consumed beverages), depending on sex and weight. Patients in the control group were instructed to maintain their current fluid intake. Participants collected 24-hr urine samples at baseline, 2- and 6-weeks after randomization.

**Results:** Participants were an average of 61 years (SD 14), 63% male, and 81% Caucasian; 54% had diabetes and 86% had hypertension. Average eGFR at baseline was 40 ml/min/1.73m$^2$(SD 11 ml/min/1.73m$^2$); the median albumin to creatinine ratio was 19 mg/mmol (IQR: 6-74 mg/mmol). Between baseline and 6-weeks, 24-hour urine volume increased by 0.7 L/d in the hydration group and decreased by 0.3 L/d in the control group (between-group difference in change 0.9 L/d; p=0.002) (**Figure 1**). Average plasma sodium was 138 mmol/L in both groups at 6 weeks and remained above 130 mmol/L for all patients at all time points. No changes in electrolytes, osmolality, or eGFR were observed. Health-related quality of life remained similar between groups. No serious adverse events were reported nor observed.

**Conclusions:** Patients with stage 3 CKD can safely and successfully increase daily water intake up to 1.5 L/day in addition to usual daily fluid intake.




*Funding:* Pharmaceutical Company Support - Danone Research

**PUB380**

**Uric Acid Lowering in Metabolic Syndrome Patients Utilizing a Phytochemical Approach** Günter Siegel,[1,2] Janine Berkholz,[1] Karl Winkler,[3] Eugeny Ermilov.[1] *[1]CharitéCrossOver, Charité - Univ Clinic Berlin, Berlin, Germany; [2]Biomedical Center, Univ of Uppsala, Uppsala, Sweden; [3]Institute of Clinical Chemistry, Univ Clinic Freiburg, Freiburg, Germany.*

**Background:** As a rule, hyperuricaemia leaves to think of gout, renal insufficiency and/or especially metabolic syndrome (MetS). We sought to determine whether uric acid (URAC) could be lowered in MetS patients by a phytochemical approach. In a 2-month clinical pilot study with Ginkgo biloba (EGb761, 2×120 mg/d) on 11 MetS patients, a novel biomarker spectrum made point-of-care theranostics feasible.

**Methods:** Photometric methods, ELISAs, EIAs, ellipsometry.

**Results:** The ratio oxLDL/LDL was reduced by 21.0% (p<0.002), 8-iso-PGF$_{2\alpha}$ 39.8% (p<0.002), MPO 29.6% (p<0.0137), IL-6 12.9% (p<0.0407), hs-CRP 39.3% (p<0.0049), Lp(a) 26.3% (p<0.001), MMP-9 32.9% (p<0.042), insulin 9.4% (p<0.042), HOMA-IR 14.0% (p<0.0244), nanoplaque formation 14.3% (p<0.0077), nanoplaque size 23.4% (p<0.0004), whereas SOD was augmented 17.7% (p<0.0095), GPx 11.6% (p<0.001), cAMP 43.5% (p<0.001), and cGMP 32.9% (p<0.001). Since none of the patients had a manifest kidney disease, we evaluated URAC. Fasting morning URAC was reduced by 10.6% (p<0.0033) from 315.7 to 285.2 µmol/L (p<0.0046). Because ginkgo is not an ASS- and allopurinol-like agent, we looked out for a mechanistic explanation. URAC was directly correlated to SOD,GPx,MPO,Lp(a),IL-6,hs-CRP,TGFβ$_1$,insulin,HOMA-IR,VLDL,AST,ALP, and inversely to nanoplaque formation k$_{Ca2-3/Lp}$,ALT,GGT,CREA. Thus, changes in cytokine pattern and beneficial effects on oxidative stress parameters could unravel diminution of URAC.

**Conclusions:** Ginkgo had beneficial effects on a multitude of inflammatory, oxidative stress, diabetic and arteriosclerotic biomarkers. URAC reached reference values in all patients. Thus, ginkgo may be used as complementary drug in the treatment of MetS and gout patients.

| Biomarker | ΔURAC [µmol/L] | |
|---|---|---|
| | r | p< |
| ΔSOD [U/mL] | 0.660 | 0.037 |
| ΔGPx [U/mL] | 0.708 | 0.049 |
| ΔMPO [ng/mL] | 0.718 | 0.044 |
| ΔLp(a) [mg/dL] | 0.733 | 0.024 |
| ΔIL-6 [pg/mL] | 0.658 | 0.053 |
| Δhs-CRP [mg/L] | 0.844 | 0.008 |
| ΔTGFβ$_1$ [ng/mL] | 0.630 | 0.068 |
| ΔInsulin [mU/L] | 0.746 | 0.013 |
| ΔHOMA-IR [mU/L×mg/dL] | 0.728 | 0.026 |
| ΔVLDL [mg/dL] | 0.720 | 0.028 |
| ΔNanoplaque formation [1] | -0.711 | 0.014 |
| ΔAST [U/L] | 0.743 | 0.034 |
| ΔALT [U/L] | -0.739 | 0.036 |
| ΔALP [U/L] | 0.786 | 0.020 |
| ΔGGT [U/L] | -0.700 | 0.035 |
| ΔCREA [µmol/L] | -0.884 | 0.008 |

*Funding:* Pharmaceutical Company Support - Research Grant by Schwabe Pharmaceuticals, Modest

**PUB381**

**Supplemental Knowledge of an Elevated Creatinine** James Francis Dylewski,[1] Christopher Neil Marshall,[1] Robert Pursell.[2] *[1]Internal Medicine, St. Luke's Univ Hospital and Health Network, Bethlehem, PA; [2]Nephrology, St. Luke's Univ Hospital and Health Network, Bethlehem, PA.*

**Background:** Serum creatinine (Cr) is conventionally used as a surrogate marker to estimate glomerular filtration rate and therefore kidney function. There are many causes for an elevated Cr, but the majority are due to decreased creatinine elimination resulting from kidney injury. However, this is not the only cause.

**Methods:** A 27-year-old Caucasian male presented for evaluation of flank pain of several days duration. It was found that his Cr was 5.4 mg/dl and BUN 34. The patient denied any urinary symptoms & denied taking any medications including NSAIDs or anabolic steroids. He did, however, relate he was taking arginine & creatine supplements for athletic enhancement. His urinalysis was bland in dip & microscopic examination. His CPK was 184 U/L & tests including CBC, ASO titer, C3, C4, CH50, ANA, HIV antibody, & hepatitis panel were all normal. Additionally, urine drug screen, eosinophils, & myoglobin were also normal. A retroperitoneal ultrasound showed normal sized kidneys without hydronephrosis, calculi, or masses. CT of the abdomen did not demonstrate any abnormalities. The patient was given intravenous fluids & monitored closely. With discontinuation of the arginine & creatine supplements, his Cr fell significantly to 1.3 mg/dL, which was felt to be his baseline.

**Conclusions:** Creatine is an endogenous nitrogen based organic acid that is synthesized from arginine & serves as an energy source for skeletal muscles, particularly when phosphorylated. In muscle cells, creatine & creatine phosphate are converted to creatinine as part of degradation. Creatine monohydrate is a common supplement used by athletes and is readily oxidized to creatine in the body. Consequently, with large exogenous intake of creatine & arginine, Cr could be elevated. This is a case of a patient who chronically ingested arginine & creatine that developed what appeared to be acute kidney injury from these substances. The supplements were stopped & his Cr improved accordingly. Review of the literature yielded a few cases of similar occurrences with the use of creatine supplements.

**PUB382**

**L-Arginine or L-Citrulline Supplementation Does Not Protect Diabetic Renal Injury** Alaa S. Awad,[1] Hanning You,[1] Ting Gao,[1] Timothy K. Cooper,[2] Sidney M. Morris.[3] *[1]Medicine, Penn State Univ College of Medicine, Hershey, PA; [2]Comparative Medicine, Penn State Univ College of Medicine, Hershey, PA; [3]Microbiology and Molecular Genetics, Univ of Pittsburgh, Pittsburgh, PA.*

**Background:** Our recent publication showed that pharmacological blockade of arginases confers kidney protection in diabetic nephropathy via a nitric oxide synthase 3 (NOS3)-dependent mechanism. Arginases compete with NOS for the common substrate L-arginine. L-arginine or L-citrulline supplementation showed beneficial effect in patients with hypertension, angina and erectile dysfunction by improving endothelial function with enhanced NO synthesis. Therefore, we hypothesized that L-arginine or L-citrulline supplementation would prevent or reduce diabetic renal complication.

**Methods:** DBA mice injected with multiple low doses of vehicle or streptozotocin (STZ; 50 mg/kg ip for 5 days) were provided drinking water (DW) supplemented with L-arginine, L-citrulline, isonitrogenous nonessential amino acid control or no amino acids (DW control) for 9 weeks.

**Results:** Diabetic mice with DW control showed significant increases in urine albumin excretion ($p<0.05$), albumin/creatinine ratio ($p<0.05$), kidney weight/body weight ratio ($p<0.05$), plasma creatinine ($p<0.05$), blood urea nitrogen ($p<0.05$), glomerular histopathological changes ($p<0.001$), and kidney macrophage recruitment ($p<0.001$) compared to normal mice after 9 weeks of diabetes. Isonitrogenous amino acid supplementation in diabetic mice was similar to DW control diabetic mice. Interestingly, neither L-arginine nor L-citrulline supplementation in diabetic mice affected any of these renal parameters despite increasing kidney arginine levels (6- and 8-fold; respectively).

**Conclusions:** These findings indicate that chronic L-arginase or L-citrulline supplementation does not prevent or reduce diabetic renal injury.

*Funding:* NIDDK Support

## PUB383

**The Influence of Age on the Changes in Reduced Glutathione (GSH) Levels in Mitochondria from Rat Kidney Cortex and Medulla following Dietary Supplementation with N-Acetylcysteine (NAC)** Marianna J. Zamlauski-Tucker, Bingwei Ye. *Physiology & Health Science, Ball State Univ, Muncie, IN.*

**Background:** Dietary supplementation is beneficial since it increases the level of GSH, the principal antioxidant inside cells, and protects cells against oxidative injury. A previous study reported that mitochondrial GSH levels in kidneys from young rats did not change with dietary supplementation. The present study investigated whether rat age influenced the effect of supplementation with NAC on mitochondrial and cytosolic GSH levels in the kidney.

**Methods:** Young Experimental (i.e., 3 months of age) and Old Experimental (i.e., 22 months of age) female Lewis rats were given NAC (200 mg/Kg body wt) via intraperitoneal injection for one week. Age-matched Control rats were not given any supplementation. At the end of the treatment period, the kidneys were harvested and separated into cortical and medullary sections. The sections were further separated into cytosolic and mitochondrial fractions by differential centrifugation. GSH levels in the fractions were measured using a spectrophotometric assay. There were 6 to 8 rats in each group and statistical comparisions between similar aged rats were done using a Student's t test.

**Results:** There were significant increases in mitochondrial and cytosolic GSH levels in kidney cortex and medulla from both Young and Old rats.

| | | Young | | Old | |
|---|---|---|---|---|---|
| | | Control | Experimental | Control | Experimental |
| Cytosol | | | | | |
| umol/g kid wet wt | Cortex | 5.5 ± 0.4 | 7.7 ± 0.6* | 5.6 ± 0.6 | 11.4 ± 0.4* |
| | Medulla | 3.1 ± 0.2 | 9.2 ± 0.2* | 3.2 ± 0.6 | 7.9 ± 0.4* |
| | | | | | |
| Mitochondria | | | | | |
| nmol/ g kid wet wt | Cortex | 100 ± 6 | 133 ± 6* | 128 ± 23 | 198 ± 13* |
| | Medulla | 94 ± 7 | 130 ± 8* | 124 ± 23 | 240 ± 12* |

All date expressed as X + SEM. * significantly different from age-matched Contol.

**Conclusions:** Rat age does not influence the increase in either mitochondrial or cytosolic GSH levels observed in rat kidneys following exogenous dietary supplementation with NAC.

## PUB384

**Health Literacy and Serum Albumin in Veterans on Chronic Hemodialysis** Anil Ramesh, Michael J. Fischer, Anuradha Wadhwa, Elisa J. Gordon. *Nephrology, Hines VA Medical Center, Hines, IL.*

**Background:** Adults with end-stage kidney disease (ESKD) suffer many metabolic complications. Controlling albumin and serum potassium requires dietary control and understanding of dietary counseling. Inadequate health literacy (HL) could impair the ability of patients to understand and follow simple instructions related to dietary health. However, little is known about whether health literacy contributes to patients' ability to maintain nutrition/electrolyte balance.

**Methods:** We conducted a cross-sectional study involving semi-structured interviews with Veterans with ESKD requiring chronic hemodialysis at one VA outpatient dialysis unit between 2011-2013. HL was assessed by the Short Form Test of Functional Health Literacy Assessment (S-TOFHLA), and inadequate/marginal HL was defined by a score of < 67. Adequate HL was assessed by a score of > 68. Serum laboratory values including albumin, potassium and sodium were obtained from the electronic Health Record (EHR) at time of interview. T- Test was used to assess association between laboratory values and HL.

**Results:** Among 43 patients (88% participation rate), 19 (44%) had inadequate/marginal HL. Serum albumin mean concentration was 3.70 (standard deviation 0.41). Patients with adequate HL had serum albumin concentrations of 3.81 (standard deviation 0.43) compared with 3.56 (standard deviation 0.33) in patients with marginal/low HL ($p=0.044$). Potassium and sodium were not significantly related to HL ($p>0.05$).

**Conclusions:** Low health literacy is associated with lower serum albumin levels, suggesting that health literacy may impact nutritional status in patients with ESKD. Nutrition counseling should be provided to accommodate ESKD patients of all literacy levels.

*Funding:* Veterans Affairs Support

## PUB385

**Effect of Intradialytic Parenteral Nutrition (IDPN) on Nutrition Status in Hemodialysis Patients** Zvi Barnea, Sara Blumberg Benyamini, Relu Cernes, Biro Alexander, Ze'ev Katzir. *Nephrology, Wolfson Medical Center, Holon, Israel.*

**Background:** Malnutrition is common in hemodialysis patients and is associated with an increase in morbidity and mortality. Intradialytic parenteral nutrition (IDPN) has been proposed to improve nutritional status and outcome in malnourished patients. The aim of this study was to evaluate the effect of IDPN in patients suffering from nutrition deterioration.

**Methods:** We have evaluated nutrition monthly score, based on six biochemical parameters (albumin, creatinine, urea, cholesterol, Kt/V, C-Reactive Protein) and weight change (paper in preparation) in 28 malnourished hemodialysis patients. We calculated the slope of three subsequent monthly scores prior to beginning of IDPN treatment and three months following beginning of treatment. We used paired t test to compare score, slope, and each of the score components before and after three months of IDPN treatment.

**Results:** In 28 HD patients, three months following beginning of treatment, IDPN treatment has stopped nutritional deterioration: Average nutrition score was significantly improved. Slope values of three subsequent monthly scores turned positive compared to a negative slope before treatment. IDPN treatment induced a significant increase in albumin, urea and post dialytic weight. Creatinine, cholesterol, CRP, Kt/V were not significantly changed.

| Nutrition parameters | Average value three months before IDPN | Average value three months after IDPN | P value |
|---|---|---|---|
| Nutrition score | 67 (7.77) | 70 (8.37) | 0.0426 |
| Slope | -5.14 (1.03) | 1.73 (0.83) | <0.0001 |
| Albumin (mg/dL) | 3.13 (0.49) | 3.25 (0.4) | 0.0454 |
| Creatinine (mg/dL) | 5.71 (1.29) | 5.94 (1.24) | 0.156 |
| Urea (mg/dL) | 91.24 (28.4) | 106.0 (26.88) | 0.0023 |
| Cholesterol (mg/dL) | 135.9 (34.17) | 138.2 (5.85) | 0.247 |
| Percent post dialysis weight change | -0.3 (0.1) | 0.4 (0.02) | 0.038 |
| Kt/V | 1.32 (0.3) | 1.29 (0.6) | 0.15 |

**Conclusions:** IDPN treatment improved nutrition parameters, score and slope of nutrition status, and stopped nutritional deterioration in HD patients.

## PUB386

**Effect of an Increased Water Intake in DNA Adducts Formation and Urinary Mutagenicity in Smokers: A Randomised Controlled Trial** Inmaculada Buendia Jimenez,[1] Pascaline Richardot,[1] Pascaline P.P. Picard,[1] Glenn Talaska,[2] Michel De Meo,[3] Gerard Friedlander.[4] *[1]Danone Research, Palaiseau, France; [2]Dept of Environmental Health, Univ of Cincinnati, Cincinnati, OH; [3]Laboratoire Mutagenèse Environnementale, Univ de la Méditerranée and IMBE, Marseille, France; [4]Institute for European Expertise in Physiology, Paris, France.*

**Background:** The association between fluid intake and bladder cancer risk remains controversial since epidemiological studies has produced mixed results. To date, very little is known about to which extent the amount of water intake influences the action of excreted toxics upon the urinary system. We performed a proof of concept trial to investigate the effect of water intake on mutagenesis in smokers, a high risk population for bladder cancer.

**Methods:** Monocentric randomized controlled trial. Inclusion criteria: Male subjects aged 20-45 y/o, smokers (at least 15 cigarettes per day) and small drinkers (≤1 L of fluid/day; ≤500 mL of water/day; urinary 24-hour urinary volume 700 mOsmol/kg). Exclusion criteria: History of diseases which could affect the results of the study or under treatment which could modify measurements of the study. Primary outcome: 4-ABP DNA adducts formation in exfoliated bladder cells in 24-hour urine collection. Secondary outcome: urinary mutagenicity in 24-hour urine. Study design: Subjects were allocated in two groups: Test group: Subjects had to consume 1.5 L daily of the study product (EVIAN®) on top of their usual water intake for 50 days. Control group: Subjects had to continue their usual lifestyle habits.

**Results:** 65 subjects were randomized. Mean age was 30 y/o and mean cigarettes per day was 20. A slight decrease in adducts formation was observed between baseline and last visit but no statistically significant difference was demonstrated between the groups.

Conversely, urinary mutagenicity significantly decreased.

**Conclusions:** Our study shows that increasing water intake decreases urinary mutagenicity. However, it is not confirmed by urinary adducts formation. Further research with a larger sample size or longer intervention period would be necessary to address the impact of water intake on this parameter.

*Funding:* Pharmaceutical Company Support - Danone Research

## PUB387

**Providing Snacks during Dialysis Treatment Contributes to an Adequate Protein-Energy Intake in Hemodialysis Patients** Geertrude I. Struijk-wielinga,[1] Floor Neelemaat,[1] Peter Jm Weijs,[1] Pieter M. Ter Wee.[2] *[1]Nutrition & Dietetics, Internal Medicine, VU Univ Medical Centre, Amsterdam, Netherlands; [2]Dept of Nephrology, VU Univ Medical Centre, Amsterdam, Netherlands.*

**Background:** Protein-energy wasting (PEW) is a strong predictor of mortality in hemodialysis patients. Although PEW is also caused by non nutritional conditions, nutritional support targeting adequate protein intake has been shown to improve PEW and

patient outcome. Previously we found that our patients, consuming an in-center meal and snack during dialysis treatment were still short of 15 grams of protein and 240 kcal per day. Aim: to investigate a 6 week intervention of a snack with education focused on adequate protein-energy intake particularly on dialysis days.

**Methods:** Hemodialysis patients in a university hospital, dialysed three times per week, were offered a choice out of 7 different snacks (±15 g protein, 240 kcal). Indirect calorimetry and physical activity level were measured to provide daily energy needs. 24 hour dietary recall was completed for 2 dialysis days both at baseline (t0) and after 6 weeks (t6).

**Results:** 28 patients (15 men), age 57±12 year (mean ± SD), BMI 24±4 kg/m$^2$were enrolled. At t0 protein intake was 78% of estimated requirements (65±25 g/d, p<0.01), and increased to 100% (85±29, p<0.002) at t6. At t0 energy intake was 84% of energy requirements (1693±535 kcal/d, p<0.007) and increased to 99% (1989±606, p<0.033) at t6. At t0 8 patients out of 25 (32%) achieved their protein goal, at t6 13 patients (57%) (p<0.003).

**Conclusions:** Protein and energy intake of hemodialysis patients on dialysis days is insufficient and can be improved by providing a snack (±15 g protein, 240 kcal) and education during dialysis treatment.

## PUB388

### Validation of Objective Malnutrition Inflammation Scores in Pediatric and Adolescent Cohort on Chronic Maintenance Dialysis

Franca M. Iorember, Oluwatoyin F. Bamgbola. *Pediatrics, LSU Health Science Center, New Orleans, LA.*

**Background:** Altered body composition and uremic milieu often confound accuracy of single-item diagnostic tools [including anthropometry]. In addition, shared pathophysiologic process makes it impractical to dissociate burden of nutritional deficiency and pro-oxidative inflammation in dialysis patients. Hence, composite score of malnutrition-inflammation morbidity [MIM] has been validated in adult dialysis patients. However, despite greater burden of chronic uremia, similar tools are not widely available for pediatric use. Objective: To assess the validity of composite scoring of clinical indices of malnutrition-inflammation morbidity in a pediatric dialysis cohort.

**Methods:** We derived composite scores [OMI] from quantitative indices of renal pathology, nutrition, dialysis adequacy, protein catabolism, and dialysis modality. We avoided items that required [cognitive] subjective responses in the questionnaire. We assessed reliability by a test-retest method and measured validity by examining predictive relationship of the indices with serum c-reactive protein [CRP] in a multiple regression analysis.

**Results:** Twenty patients on chronic dialysis with a mean age of 12.8 years were enrolled. Eight subjects with elevated CRP [> 0.3 mg/dl] had higher scores for MIM. Patients on hemodialysis [HD] had greater OMI score than those on peritoneal dialysis [PD]. Test-retest score showed high degree of reliability [typical error 0.07; r = 0.95]. Diagnostic indices of MIM were predictive of elevated serum CRP in a regression analysis [$R^2$ = 0.9, F = 4.3, p = 0.03]. Sensitivity of OMI score was 62.5%, specificity 83%, accuracy 75% and precision was 71%.

**Conclusions:** Anthropometric and laboratory indices of nutritional status are often confounded by proximate events [e.g. inflammation, fluid retention] in dialysis patients. Composite scoring system may be more accurate and cost effective. Unlike Subjective Global Assessment instrument in adults, we attempted to improve accuracy and enhance universal applicability by avoiding cognitive input [subjective responses] from patients. Large scale population studies are needed to optimize clinical effectiveness and refinement of the diagnostic tools.

## PUB389

### Effect of Appetite and Body Mass Index on Energy Intake in Chronic Kidney Disease Stage 3 and 4 Patients

Anita Saxena,[1] Amit Gupta,[1] Raj K. Sharma,[1] Reena Choudhary.[1] *[1]Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, India; [2]Nephrology, Madras Medical Mission, Chennai, TN, India; [3]Inst#1; [4]Inst#1.*

**Background:** Assessment of nutritional status in chronic kidney disease requires multiple markers.

Objective: To assess effect of appetite on nutritional status of predialysis patients on first visit to nephrology center.

**Methods:** Three day dietary intake of 141 stage 3 and 4 CKD patients was recorded. Appetite was classified as normal, average, poor and anorexic. Body mass index was classified as normal, underweight, severely, underweight and overweight.

**Results:** Serum albumin was 2.9± 0.9 g/dL; protein 5.7 ±1.40; and proteinuria 3.5 ±1.4g. Of 140 patients, 52.8% had normal BMI, 15% were underweight, 5.71% were severely underweight and 27.1% overweight. BMI correlated (p .000) with energy, protein, carbohydrate, creatinine, serum protein, proteinuria and SGA scores. There was difference in dietary intake in BMI groups in weight (p .000), appetite (.004), energy (.004) and carbohydrate (.003). Dietary intake was: energy 18.8±8.9 kcal/kg; protein 0.7±.33 g/kg , fat 0.34±.4g/kg and carbohydrate 3.0±1.4/kg. Based on BMI classification, energy intake was: normal 18.8 ±9.0, underweight 19.3±10.0, severely underweight 16.3±11.1, overweight 19.0±7.8kcal/kg. Protein intake was: normal 0.73 ±.34; underweight 0.81±0.4, severely underweight 0.67±.44, overweight 0.63±.20 g/kg.

Appetite correlated (p .000) with weight, BMI, dietary energy, protein, fat , serum albumin and creatinine. Based on appetite groups energy intake was: normal 29.9±4.6; average 20.4±.4, poor 18.2±5.8 and anorexic 10.1±5.5 kcal/kg and protein intake was: normal 1.0±.3; average 0.75±.2; poor 0.68±.2 and anorexic 0.43±.2.

**Conclusions:** 47.2% patients were underweight. Energy intake was low. Protein intake was as required for prediialysis patients. Serum albumin and serum protein were low. Loss of appetite, low energy intake and presence of proteinuria may have a synergistic effect and cause malnutrition in CKD stage 3 and 4 patients. Inadequate energy intake, poor appetite, body mass index and low serum albumin and protein are markers of malnutrition in predialysis population.

## PUB390

### Is Free Testosterone Concentration a Prognostic Factor of Survival in Chronic Renal Failure?

Stanislaw Niemczyk,[1] Katarzyna Szamotulska,[2] Longin Niemczyk,[3] Malgorzata Gomolka.[1] *[1]Military Institute of Medicine; [2]National Research Institute of Mother and Child; [3]Medical Univ of Warsaw.*

**Background:** Persons with chronic kidney disease (CKD) experience low quality of life and increased cardiovascular mortality. Understanding the reasons of poor outcomes in renal failure seems to be an important challenge.

Aims. 1) evaluation of total (T) and free testosterone (fT) concentrations in pre-dialysis patients and patients treated with different methods of dialysis 2) assessment of relationship of T and fT concentration with biochemical parameters, which are sensitive to renal failure 3) evaluation of prognostic importance of T and fT levels in predicting long-term survival of CKD patients.

**Methods:** Four groups of men were examined: 14 hemodialysed (HD), 13 on peritoneal dialysis (PD), 9 pre-dialysis and 8 healthy aged 56±17, 53±15, 68±12, 43±10 years respectively. The concentrations of T, SHBG, TBG, prealbumin, albumin, CRP, Hb and gasometry were measured. FT was estimated from formula.

**Results:** T concentration [ng/ml] was 3.98±1.76 in HD, 5.46±1.95 in PD, 4.02±1.26 in pre-dialysis and 6.40±2.43 in healthy subjects (p=0.035) , fT [ng/ml] 0.065±0.025, 0.091±0.041, 0.060±0.014, 0.104±0.043, respectively (p=0.004). T and fT were negatively correlated with age in all studied groups. There was also observed a negative significant association between T concentration and body mass index (p=0.013) , a negative marginally significant association between T concentration and HCO3 concentration (p=0.085), and a negative significant association between fT concentration and HCO3 concentration (p=0.006). Within six years from the baseline, there were 5 deaths in HD, 1 in PD and 3 in pre-dialysis. In univariate analysis, both age (p=0.002) and fT (p=0.001) were related to survival probability. However, when both variables were entered into the model only fT was marginally significant (p=0.094).

**Conclusions:** The concentration of free testosterone was lower in hemodialysis and pre-dialysis patients than in healthy subjects. Free testosterone is related to metabolic acidosis and in chronic renal failure may prognose long-term survival better than age.

## PUB391

### Olive Oil in the Treatment of Constipation of Patients on Hemodialysis

Christiane Ishikawa Ramos, Aline Fátima Andrade De Lima, Daniela Gimenes Grilli, Giovana Sertori, Maria A. Kamimura, Lilian Cuppari. *Nutrition Program, Div of Nephrology, Federal Univ of São Paulo, São Paulo, Brazil.*

**Background:** Constipation is a common gastrointestinal symptom in patients with chronic kidney disease (CKD), especially those on hemodialysis (HD). Although effective and safe treatment for constipation has not been established, mineral oil is commonly used due to its laxative effect. Edible oils, such as olive oil, could also be an alternative for the management of constipation of these patients. Therefore we aimed to investigate the effect of olive oil in comparison to mineral oil on constipation of patients with CKD on HD.

**Methods:** This was a double-blind randomized controlled trial conducted for 4 weeks (wk4). Constipation was diagnosed by the Roma III criterion.Thirty-one patients (16M, 52.1±11.0 years) diagnosed as constipated were randomized to mineral oil (MG; n=16) or olive oil (OG; n=15) groups. The initial dose of each oil was 4ml/day and was adjusted as needed.

**Results:** There was no difference in total oil used between the groups during the follow-up (MG=143.5 ± 51.5 ml, OG=148.5 ± 42.7 m, p=0.67). As shown in the table, the decrease in the frequency of the symptoms of constipation, evaluated by Roma III criterion, was similar between mineral oil and olive oil groups.

| | Mineral% | | Olive% | |
|---|---|---|---|---|
| | baseline | wk4 | baseline | wk4 |
| < 3 evacuations/week | 50.0 | 6.3* | 40.0 | 13.3* |
| Straining on defecation | 68.8 | 18.8* | 60.0 | 20.0* |
| Lumpy or hard stools | 75.0 | 18.8* | 86.7 | 26.7 |
| Incomplete evacuation | 87.5 | 18.8 | 100.0 | 26.7 |
| Anorectal obstruction | 68.8 | 25.0 | 73.3 | 6.7* |
| Manual maneuvers | 12.5 | 0.0* | 13.3 | 0.0* |
| Abdominal pain | 56.3 | 18.8* | 26.7 | 6.7* |
| *p<0.05; baseline vs wk4. | | | | |

At the end of follow-up in 57% and 60% of the patients of mineral oil and olive oil groups, respectively, constipation was resolved (p=0.83).

**Conclusions:** Olive oil seems to be an effective and health alternative in the treatment of the constipation of HD patients.

**PUB392**

**Investigation of ANCA Induced Activation of Human Neutrophils Using High Content Analysis** Alice M. Coughlan,[1] Vincent P. O'Reilly,[1] Anthony Mitchell Davies,[2] Julie M. Williams,[3] Mark Alan Little.[1] *[1]Trinity Health Kidney Centre, Trinity College Dublin, Ireland; [2]The Irish National Center for High Content Screening and Analysis, Trinity College Dublin, Ireland; [3]School of Immunity and Infection, Univ of Birmingham, United Kingdom.*

**Background:** ANCA vasculitis is associated with the presence of autoantibodies that are capable of stimulating neutrophils in vivo. There is marked variability in stimulatory capacity both between donors and IgG preparations. However, this phenomenon is difficult to study due to inter-assay variability.

**Methods:** To overcome this variability in ANCA induced neutrophil activation we used high content analysis (HCA) to screen IgG from vasculitis patients and controls for their ability to activate healthy donor neutrophils. Neutrophils from up to 8 donors were stimulated with a panel of protein-G purified IgG in 384 well plates. Activation was accessed by measuring actin rearrangement (phalloidin stain) and superoxide production (Dihydrorhodamine 123) by automated fluorescence microscopy using an InCell Analyser 1000™.

**Results:** We were able to simultaneously assess the stimulatory potential of IgG from 22 patients or controls against neutrophils from 8 healthy donors. Neutrophil responses to ANCA varied greatly. Some donor neutrophils were poor responders to all ANCA preparations. Furthermore, ANCA preparations that did activate neutrophils, did so in a donor dependent manner. The DHR assay produced a more robust and reproducible signal than the phalloidin stain.

**Conclusions:** Using robotic fluid handlers and the InCell Analyser™ to miniaturise and automate ANCA neutrophil assays allowed us to overcome the inter-assay variability observed when neutrophil donors are assessed on different days and facilitated testing of a large number of IgG preparations simultaneously.

*Funding:* Government Support - Non-U.S.

**PUB393**

**Effection of IL-17 on the Proliferation and IgA1 Underglycosylation of B Cells** Junming Fan,[1,2] Jiaru Lin,[2] Fugang Li,[2] Li Liu.[2] *[1]Dept of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China; [2]Div of Nephrology, Dept of Internal Chinese Medicine, Luzhou Medical College, Luzhou, Sichuan, China.*

**Background:** IL-17, a Th17 cell-derived proinflammatory molecule, has been found to play an important role in the pathogenesis of autoimmune diseases. IL-17 may be associate with IgA nephropathy (IgAN). Wherefor it has been not known whether IL-17 exerts a direct effect on B Lymphocytes in IgA nephropathy. This study was to investigate the effection ofIL-17 on the proliferation and underglycosylation of IgA1 in a B Lymphocytes line.

**Methods:** DAKIKI, was cultured and stimulated by IL-17. For dose-dependent test, cell was cultured for 48h with different doses (5ng/ml, 10ng/ml, 20ng/ml, 40ng/ml, 80ng/ml, 160ng/ml, 320ng/ml) of IL-17. For time-dependent test, cell was cultured for 24, 48 and 72h. The cell proliferation was examined by CCK-8 assay. The IgA1 concentration and galactosylation in supernatant tested by ELISA and HAA lectin *binding assay*.

**Results:** 1.The result of CCK-8 showed that the proliferation of DAKIKI cells in group with 10ng/ml,20ng/ml,40ng/ml concentration were statistical significance obviously increased ($P<0.05$) compared with medium group. 2. The ELISA showed that the DAKIKI cells could secreted more IgA1 than control after induceded by IL-17. The levels of IgA1 secreted by DAKIKI cells in IL-17 incubation were gradually increased with the dose- and time-dependent. 3. The HAA lectin showed that, the glycosylation of IgA1 were decreased with dose- and time-dependent manner after 48 hours incubation with IL-17.

**Conclusions:** IL-17 could induced the B cell proliferation, IgA1 secretion and IgA1 underglycosylationin in DAKIKI cells with a dose-dependent and time-dependent manner. The results suggested that the possible mechanism IL-17 participation the pathogenesis of IgA nephropathy was associated with B lymphocyte secreting much IgA1 and underglycosylation of IgA1.

*Funding:* Government Support - Non-U.S.

**PUB394**

**CD19_CD5_ B Cells in Primary Nephropathy** Hilmi Umut Unal,[1] Ali Kilinc,[2] Mahmut Ilker Yilmaz.[3] *[1]Nephrology, Gulhane Military Medical School, Ankara, Etlik, Turkey; [2]Internal Medicine, Gulhane Military Medical School, Ankara, Etlik, Turkey; [3]Nephrology, Gulhane Military Medical School, Ankara, Etlik, Turkey.*

**Background:** In developing countries, IgA nephropathy is the most common cause of the primary glomerulonephritis. As can be seen at any age, it most frequently occurs between the ages of 20 and 30. The etiology of this disease is unknown. Focal segmental glomerulosclerosis (FSGS) has become an important lesion found to underlie the nephrotic syndrome in adults . Primary FSGS is one of the causes of idiopathic nephrotic syndrome that may be etiologically related to but more severe than minimal change disease.CD19CD5 B cells are important producers of IgA and contribute to several autoimmune diseases, they may play an important role in IgA nephropathy.In this study, peripheral Lymphocyte subgroups CD-5-19 with primary glomerulonephritis (IgA ve primary FSGS) evaluated the relationship between response to treatment and don't response to treatment.

**Methods:** In the 6-month follow-up responses to the treatment of patients which as a result of renal bx FSGS (primary) or IgA reported that CD5- CD19 values evaluated retrospectively. Lymphocyte subgroups were measured by flow cytometry in 31 patients. The results of the patients between two groups who do not respond and respond to treatment were compared.A complete response is a reduction in proteinuria to <200 to 300 mg/day. A partial response in patients presenting with nephrotic range proteinuria is a reduction of ≥50 percent, and to less than 3.5 g/day.

**Results:** There was no significant difference between the lymphocyte sub-groups in two groups.

**Conclusions:** In humans, elevated numbers of CD19CD5 B cells have been reported in patients with Sjogren syndrome and rheumatoid arthritis. CD19CD5 B cells are the main IgA producers in mucosal tissues. As few as 5% of the circulating B cells are CD5 B cells in normal adults. Further researches need doing on use of flow cytometry results for the activity of the disease and disease in response to treatment.

**PUB395**

**The Involvement of Histone Acetylation in Kidney Injury of Dahl Salt-Sensitive Rats** Kumiko Io,[1] Tomoya Nishino,[1] Shinichi Abe,[1] Yoko Obata,[1] Takehiko Koji,[2] Shigeru Kohno.[1] *[1]Second Dept of Internal Medicine, Nagasaki Univ School of Medicine, Nagasaki City, Nagasaki Prefecture, Japan; [2]Dept of Histology and Cell Biology, Nagasaki Univ School of Medicine, Nagasaki City, Nagasaki Prefecture, Japan.*

**Background:** Renal sclerosis is caused by arteriosclerosis associated with long-term hypertension. Although epigenetics, which is an acquired regulation of gene expression, has recently been reported to be related to the progression of hypertension, it is unclear whether epigenetics is associated with the progression of renal sclerosis. Therefore, we investigated the relationship between epignetics, especially histone acetylation, and renal sclerosis.

**Methods:** 6 week-old male Dahl salt-sensitive (DS) rats were used as models of renal sclerosis. Rats were divided into 3 groups: (i) rats fed normal salt diet defined as NS group, (ii) rats fed high salt diet defined as HS group, (iii) HS group administered curcumin, a histone acetyltransferase inhibitor, daily defined as HS+C group. After 6 week from the start of salt load, the kidneys of these rats were dissected out. Morphologic changes were assessed by Masson's Trichrome staining. ED-1 which is a marker of macrophage, Monocyte Chemotactic Protein-1 (MCP-1) and histone acetylation were assessed by immunohistochemistry or by Western blotting.

**Results:** Compared with NS group, HS and HS+C groups showed a significant increase of systolic blood pressure from the 2 weeks of salt load. Serum creatinine increased markedly in HS group at the 6 week, while the increase of serum creatinine was suppressed in HS+C group. In HS group, interstitial fibrosis and glomerular sclerosis were observed and the numbers of ED-1 or MCP-1 positive cells were increased significantly more than those in NS group at the 6 week. On the other hand, HS+C group showed the inhibition of these inflammation and fibrosis. Histone acetylation was more increased in HS group than that in NS group, whereas the administration of curcumin decreased histone acetylation in the kidney.

**Conclusions:** Our results suggested that histone acetylation was involved in progression of hypertensive kidney injury in DS rats.

**PUB396**

**The Effects of AST-120 Treatment on Uremia-Induced Disruption of Colonic Epithelial Tight Junctions and the Associated Systemic Inflammation** Hoang Anh Nguyen,[1] Jun Yuan,[2] Nosratola D. Vaziri.[2] *[1]UCI Dept of Internal Medicine; [2]UCI Div of Nephrology & Hypertension.*

**Background:** The primary functions of the intestinal mucosa are absorption of nutrients, secretion of waste products, and serving as a barrier to prevent absorption of waste products and entry of luminal microorganisms and their harmful by-products in the host's internal milieu. This study was designed to test the hypothesis that oral administration of the activated charcoal (AST-120) may attenuate uremia-induced depletion of the intestinal tight junction key protein constituents ZO1, occludin, and claudin-1 via absorption of ammonia and its subsequent conversion to the highly caustic ammonium hydroxide.

**Methods:** Male Sprague-Dawley rats were randomized into control (CTL), experimental (CRF), and AST-120 treatment groups for two weeks. The CTL group consumed regular diet. Two CRF groups were rendered uremic by addition of 0.7% adenine to their food, and one of these CRF groups was exposed to AST-120 for two weeks. Then, they were euthanized and the colons were harvested and processed for the expression of the key constituents of the tight junctional proteins using Western blot analysis and immunohistological staining.

**Results:** The Western blot revealed that the CRF group showed a significantly marked reduction in protein expressions of ZO1 ($p < 0.001$), occludin ($p < 0.01$), and claudin-1 ($p < 0.01$) compared to the CTL group. However, activated charcoal (CRF + AST) treatment group showed significant increase in protein expression only for ZO1 ($p < 0.05$). Immunohistological staining indicated that the colonic tissues have increased wall thickness and accumulation of mononuclear leukocytes in the lamina propria and microvilli in the CRF animals. With AST-120 treatment, the inflammatory markers in the colonic tissues decreased compared to the CRF group.

**Conclusions:** Uremia results in depletion of the key protein constituents of the colonic tight junction leading to impairing intestinal barrier function and contributing to the systemic inflammation. Activated charcoal was effective in attenuating uremia-induced depletion of colonic epithelial tight junction and the associated systemic oxidative stress and inflammation.

## PUB397

**Isolation of MicroRNA from the Proximal Tubules of Archival Renal Biopsies Utilising Laser Capture Microdissection**

Geraint James Rees Dingley,[1,2] Juan Mason,[1] Sara Kathryn Campbell,[1,2] Paul Steven Bass,[3] Jane Elizabeth Collins.[2] *[1]Wessex Renal and Transplant Service, Portsmouth Hospitals NHS Trust, United Kingdom; [2]Clinical Experimental Sciences, Univ of Southampton, United Kingdom; [3]Dept of Cellular Pathology, Royal Free Hospital, London, United Kingdom.*

**Background:** MicroRNAs are short noncoding RNAs that play important roles in regulating mammalian gene expression by inducing posttranscriptional gene repression by blocking protein translation or mRNA degradation. Altered regulation of miRNAs are implicated in mechanisms of tubulointerstitial fibrosis in renal disease and are differentially expressed in different cell populations within the kidney. To understand the role of microRNAs in the kidney it is desirable to quantify microRNA expression from individual cell types.

Due to the serious risk of bleeding associated with renal biopsy, it is not ethically advisable to take additional biopsies for research. The archive of paraffin-embedded biopsy specimens therefore offers an invaluable source of diseased human tissue for molecular interrogation. Laser Capture Micro dissection (LCM) provides a method to isolate miRNAs from specific cell types within biopsies.

**Methods:** Serial sections were cut through renal biopsy and normal kidney blocks which had been fixed in formalin and embedded in paraffin-wax for a similar period of time. Sections were stained with lectins to accurately identify the proximal tubules prior to LCM. RNA was extracted from the samples using a commercially available kit which was modified to optimise RNA yields. MicroRNA expression was quantified with RT-PCR.

**Results:** In proximal tubules of patients with moderate to severe diabetic nephropathy, miRNAs 192 and 194 were consistently downregulated at a statistically significant level compared with morphologically normal proximal tubules from healthy tissue. Three other microRNAs were also differentially expressed at statistically significant levels.

**Conclusions:** This result is consistent with published data using in situ hybridisation and demonstrates that LCM of archival biopsy samples is a reliable and powerful quantitative tool in the study of cell specific microRNAs in healthy and diseased tissues.

## PUB398

**Bone-Derived Mesenchymal Stromal Cells from HIV Transgenic Mice Exhibit Altered Proliferation, Differentiation Capacity and Paracrine Functions along with Impaired Therapeutic Potential in Kidney Injury**

Kang Cheng, Xiqian Lan, Partab Rai, Andrei Plagov, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical Center, Great Neck, NY.*

**Background:** Mesenchymal stem cells (MSCs) secrete paracrine factors that could be cytoprotective and serve roles in immunoregulation during tissue injury. Although MSCs express HIV receptors, and co-receptors, and are susceptible to HIV infection, whether HIV-1 may affect biological properties of MSCs is not clear. We hypothesize that MSCs derived from Tg26 mice will have compromised paracrine function. To test our hypotheis, we evaluated cellular proliferation, differentiation and paracrine functions of MSCs isolated from compact bones of healthy control mice and HIV-1 transgenic (Tg26) mice.

**Methods:** MSCs were isolated from compact bones of control and HIV transgenic (Tg26) mice and their properties were characterized (Exp Mole Pathol, 2013). Control or HIV-MSCs were administered 24 hours prior to administration of cisplatin (12 mg/Kg, intraperitoneal) in mice (6 mice in each group). Total RNA was extracted from HIV-MScs and probed for HIV genes. The ability of control and HIV- MSCs to protect against cisplatin toxicity was also studied in cultured renal tubular cells.

**Results:** We successfully isolated MSCs from healthy mice and Tg26 HIV-1 transgenic mice and found the latter expressed viral Nef, Vpu, NL4-3 and Vif genes. The proliferation and differentiation of Tg26 HIV-1 MSCs was inferior to MSCs from healthy mice. Moreover, transplantation of Tg26 HIV-1 MSCs less effectively improved outcomes compared with healthy MSCs in mice with acute kidney injury. Also, Tg26 HIV-1 MSCs secreted multiple cytokines, but at significantly lower levels than healthy MSCs, which resulted in failure of conditioned medium from these MSCs to protect cultured renal tubular cells from cisplatin toxicity.

**Conclusions:** HIV-1 had adverse biological effects on MSCs extending to their proliferation, differentiation, function, and therapeutic potential. These findings will help in advancing mechanistical insight in renal injury and repair in the setting of HIV-1 infection.

*Funding:* NIDDK Support

## PUB399

**Milk Fat Globule-Epidermal Growth Factor-8 Modulates Tissue Fibrosis and Renal Damage in Ureteral Obstruction**

Jean-Francois Cailhier, Marie-joelle Brissette. *Medicine, Institut du Cancer de Montreal, CRCHUM, Montreal, Canada.*

**Background:** Renal diseases are characterized by tubulointerstitial fibrosis due to the loss of renal parenchymal cells. In the early phases of inflammation, macrophages kill renal parenchymal cells leading to renal fibrosis. Mediators released by the apoptotic renal resident cells play a crucial role in the modification of the renal microenvironment. We recently demonstrated that milk fat globule-epidermal growth factor E8 (MFG-E8) was released by apoptotic epithelial and endothelial cells, resulting in a reduced pro-inflammatory cytokine production by macrophages. Therefore, we propose to study the role of MFG-E8 on the modulation tissue damage and the inflammatory response in unilateral ureteral obstruction (UUO).

**Methods:** C57BL/6 WT or MFG-E8 KO mice underwent unilateral ureteral ligation for 3, 7 and 14 days to evaluate tissue damage and macrophage phenotype. MFG-E8 (30μg/kg) was administered intra-peritoneally 1 day before and every 3 days subsequently. Kidneys were harvested and stained with Sirius Red (collagen fibers) and with aSMA (myofibroblasts) to evaluate fibrosis. We also used PAS to evaluate tissue damage. F4/80 and iNOS staining were used to assess macrophage phenotype.

**Results:** MFG-E8 administration reduced kidney damage and fibrosis compared to control, moreover, its absence in MFG-E8 KO mice was associated with a worse outcome. Furthermore, the presence of MFG-E8 was associated a decreased number of pro-inflammatory M1 macrophages.

**Conclusions:** This suggests that MFG-E8 (prior to any MFG-E8 release by apoptotic cells) can attenuate tissue damage induced by UUO through modulation of the inflammatory response.

*Funding:* Private Foundation Support

## PUB400

**Hydrochlorothiazide Is Superior to Furosemide as a Renoprotective and Antiproliferative Add-On to Losartan in the Remnant Kidney Model**

Simone C.A. Arias, Renata A. Souza, Claudia R. Sena, Camilla Fanelli, Denise M.A.C. Malheiros, Clarice K. Fujihara, Roberto Zatz. *Univ of Sao Paulo, Brazil.*

**Background:** We showed previously that a losartan+hydrochlorothiazide (L+H) association arrests renal injury in the remnant kidney model (Nx) even when started after renal injury is already established. Here we investigated whether this is due solely to a diuretic effect.

**Methods:** Munich-Wistar rats (n=61) underwent Nx. One month later, tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/d), glomerulosclerosis index (GSI), % glomerular collagen-1 (%COLL), %interstitial (INT) α-smooth muscle actin (%SMA), INT PCNA (PCNA, cells/mm$^2$), and glomerular volume ($V_G$, x $10^6$ μm$^3$) were evaluated in 17 rats ($\mathbf{Nx_{pre}}$). The remaining rats were divided in: **Nx** (untreated); $\mathbf{Nx_{LH}}$ (L, 50 mg/Kg/d + H, 6 mg/Kg/d); and $\mathbf{Nx_{LF}}$ (L + Furosemide (F), 20 mg/Kg/d, the highest nontoxic F dose), and followed for 7 months, when all measurements were repeated.

**Results:**

| | TCP | ALB | GSI | %COLL | %SMA | PCNA | $V_G$ |
|---|---|---|---|---|---|---|---|
| $Nx_{pre}$ | 198±5 | 91±7 | 16±3 | 12±2 | 4±1 | 145±17 | 0.7±0.1 |
| Nx | 200±14 | 203±26[a] | 327±38[a] | 24±3[a] | 9±1[a] | 163±21 | 1.4±0.1[a] |
| $Nx_{LH}$ | 145±6[ab] | 26±4[ab] | 15±5[b] | 10±2[b] | 3±1[b] | 34±3[ab] | 1.3±0.5[a] |
| $Nx_{LF}$ | 167±6[abc] | 90±18[bc] | 40±13[abc] | 19±3[c] | 5±1[bc] | 86±10[abc] | 1.6±0.1[ac] |

Mean ± SE, [a, b, c] $p<0.05$ vs. $Nx_{Pre}$, Nx, and $Nx_{LH}$, respectively

Survival was 35% in Nx, 100% in $Nx_{LH}$ and 82% in $Nx_{LF}$. L+H arrested GS, SMA and COLL, and reversed TCP, ALB, and interstitial PCNA. Likewise, L+F partially reversed hypertension and exerted a renoprotective effect, which nevertheless was always less pronounced than with L+H. In addition, glomerular volume was increased in L+F compared with L+H.

**Conclusions:** The striking renoprotection afforded by L+H treatment in the Nx model is at least in part associated with effects unrelated to its diuretic action, such as limited glomerular hypertrophy, regression of interstitial proliferation and arrested inflammation. Thiazide add-on can exert superior renoprotection even in advanced chronic kidney disease.

FAPESP/CNPq.

*Funding:* Government Support - Non-U.S.

## PUB401

**Early Glomerular Crescents Consist of the Accumulation of Podocytes That Undergo Detachment from the GBM**

Wilhelm Kriz,[1] Isao Shirato.[2] *[1]Anatomy and Embryology, Medical Faculty Mannheim, Univ of Heidelberg, Mannheim, Germany; [2]Internal Medicine, Nephrology, Juntendo Univ, School of Medicine, Tokyo, Japan.*

**Background:** Cellular crescents are a prominent feature encountered in many glomerular diseases. The pathogenesis is poorly understood. Frequently, the hypothesis is favored that breaks in the GBM allow the leakage of plasma and cells triggering cell proliferation in Bowman's space (Ryu et al 2012, J Pathol 228: 482 i.a.). Focal ruptures of the GBM in crescents have been shown but evidence that such gaps precede and represent the cause of crescent formation is lacking. Since many years we have known that podocytes are shedded from the GBM and excreted as viable cells with the urine in patients with kidney diseases (Hara et al. 1998, Am J Nephrol 18: 35; Vogelmann et al. 2003, Am J Physiol Renal Physiol 285: F40 i.a.)Meanwhile, the detachment of podocytes from the GBM has been documented by TEM as a gradual process of viable cells (Kriz et al. 2013, Am J Physiol Renal Physiol 304: F333). We pursued the idea that detaching podocytes accumulate in Bowman's space forming clusters corresponding to a cellular crescent.

**Methods:** We studied the process of podocyte detachment in several models of glomerular diseases (including growth stimulation by FGF2, Masugi GN, PAN nephrosis) by LM in 1mm serial sections and TEM.

**Results:** We found that the process of podocyte disconnection from the GBM is extremely gradual comprising hundreds of individual steps. Portions of a certain podocyte may be found to be fully disconnected, others were seen in intermediate stages, still others had close contact to the GBM. Prolongation of this process was achieved by podocytes that established new contacts to adjacent podocytes, which were still connected to the GBM.

In addition, contacts to parietal cells were regularly seen. These manoeuvres prevented detached podocytes from being spoiled into the urine and led to their accumulation in Bowman's space.

**Conclusions:** Cellular crescents consist of detaching and detached podocytes linked to each other as well as to the tuft and Bowman's capsule.

*Funding:* Private Foundation Support

**PUB402**

**Influence of Klotho in Cognitive Deficit Linked to Chronic Kidney Disease (CKD) on Animal Model of CKD (5/6 Nephrectomy)** Sabrina Degaspari,[1] Carmen B. Tzanno-martins,[2] Clarice K. Fujihara,[3] Roberto Zatz,[3] Tania Araujo Viel,[4] Ana Elisa Bohmer,[1] Cristoforo Scavone,[1] Elisa M. Kawamoto.[1,5] *[1]Dept of Pharmacology, Univ of São Paulo - USP, Brazil; [2]CINE, São Paulo, Brazil; [3]Faculdade de Medicina, USP, Brazil; [4]School of Arts, Sciences and Humanities, USP, Brazil; [5]Laboratory of Neurosciences, NIH.*

**Background:** CKD is believed to have a negative impact on cognitive function. Recent studies have reported changes in serum klotho with aging, and levels of this protein are strongly influenced by renal function. Also, it may be involved in the cognitive impairment (CI) found in CKD. The aim of the present study is to evaluate the correlation between CI and levels of Klotho in serum and hippocampus and frontal cortex in 5/6 nephrectomized rats (Nx).

**Methods:** Forty Munich-Wistar rats underwent 5/6 nephrectomy (Sham rats were used as control groups). After 30 days of surgery, all animals were evaluated using different behavior tests (Fear Conditional Test and New Object Recognition Test) during five months. Nx animals which had only 25% or less of the markers of Sham group in the behavior tests were included in the CI group (Nx-CI). At the end of this period, blood and brain samples were collected. All samples were used to analyze Klotho levels by ELISA test.

**Results:** After Nx surgery, rats showed higher levels of creatinine (1.13±0.05) compared to Sham group (0.65±0.05), we found an increase of glomerulosclerosis in Nx group (9.96±1.9) than in Sham rats (0.92±0.9), and an important interstitial expansion in Nx group (6.64±0.8) comparing with Sham rats (0.04±0.01). Forty six percent of Nx rats (Nx-CI) showed impaired memory retention (novel object recognition test and contextual fear conditioning). After 4 months from Nx, we observed that serum Klotho was lower in Nx-CI than in Sham and Nx rats. Our data also show a decreased in frontal cortex Klotho in Nx-CI group comparing with Sham and Nx rats, however, hippocampus Klotho levels showed no difference between groups.

**Conclusions:** Together, the present data suggest that changes in Klotho signaling plays a key role in the development of cognitive changes associated with chronic renal failure.

*Funding:* Government Support - Non-U.S.

**PUB404**

**Iron Reduction Suppresses Renal Tubulointerstitial Fibrosis in Mice with Unilateral Ureteral Obstruction** Iori Ozono,[2] Yasumasa Ikeda,[1] Shoji Kagami,[3] Toshiaki Tamaki.[1] *[1]Pharmacology, Institute of Health Biosciences, The Univ of Tokushima Graduate School, Tokushima, Japan; [2]Student Laboratory, Tokushima Univ, Tokushima; [3]Pediatrics, Institute of Health Biosciences, The Univ of Tokushima Graduate School, Tokushima.*

**Background:** Renal fibrosis plays an important role in onset and progression of chronic renal diseases. We have clarified that iron is involved in the pathological conditions on obesity, atherosclerosis and diabetic nephropathy. Although several underlying mechanisms of renal fibrosis have been shown, the role of iron in the process of renal fibrosis remains unclear.

**Methods:** o investigate iron role in renal fibrosis, we employed mice with unilateral ureter obstruction (UUO) and mice with sham operation as control. Mice with UUO were divided into 2 groups, treatment with deferoxamine (DFO), an iron chelator, or vehicle.

**Results:** At 1 week after surgery, renal interstitial fibrosis, as same as increased mRNA expression of collagens, were increased in mice with UUO and these changes were suppressed by DFO treatment. UUO-induced macrophage infiltration was reduced in UUO mice with DFO. UUO-induced expression of inflammatory cytokines and extracellular matrixes were ameliorated with iron reduction. Iron chelation inhibited the activated signaling of transforming growth factor-ß1-Smad3 pathway in mice with UUO operation. UUO-induced renal superoxide production and $p22^{phox}$ expression was attenuated by DFO treatment. Additionally, renal expressions of interleukin-1ß and NOD-like receptor, pyrin domain containing 3 were induced in mice with UUO, these changes were ameliorated by DFO treatment. In renal iron transporters, protein expressions of ferroportin and ferritins were upregulated, and transferrin receptor-1 and divalent metal transporter-1 were down-regulated in mice with UUO compared to sham operated mice although no difference of renal iron content was seen between mice with sham and mice with UUO.

**Conclusions:** These results have suggested iron is involved in the progression of renal tubulointerstitial fibrosis through oxidative stress and inflammatory changes, therefore the iron reduction potentiates to be a new therapeutic approach for renal fibrosis.

*Funding:* Government Support - Non-U.S.

**PUB405**

**Sphingosine-1-Phosphate Receptor 1 and 3 Are Pivotal Mediators of Renal Fibrosis** Shunji Shiohira, Takumi Yoshida, Hidekazu Sugiura, Miki Nishida, Kosaku Nitta, Ken Tsuchiya. *Dept of Medicine Four, Tokyo Women's Medical Univ, Shinjuku, Tokyo, Japan.*

**Background:** Sphingosine-1-phosphate (S1P) has been suggested to be involved in the mechanism of renal fibrosis that functions through Sphingosine-1-phosphate receptor (S1PR) signaling pathways. Five subtypes of S1PRs have been identified. There have been reports of fibrosis and S1P in each cell in each organ, and differences in the effects of S1PRs have been reported in each organ. To get more insight into roles for S1P and receptor subtype effects *in vitro*, we performed siRNAs knockdown of receptor subtypes (1 and 3) and S1PR1 agonist and S1PR3 antagonist.

**Methods:** Normal rat kidney interstitial fibroblast (NRK-49F) cells were stimulated with exogenous S1P. Then the morphological changes of the NRK-49F cells after stimulation by S1P were examined. The growth and migration of cultured cells was quantified by using CL-Quant software to analyze time-lapse images in a Nikon BioStation CT. The real-time images of cell migration were monitored for 2 days. And the expressions (mRNA/Western blotting) of a-SMA, E-cadherin, collagen type 1 (COL1), collagen type 4 (COL4), TIMP1 and PAI1 were examined. To specify the kidney specific signal pathway, siRNAs targeted to S1P receptor subtypes (1 and 3) were generated. And also NRK-49F cells were stimulated with S1P after the addition of S1PR1 agonist and S1PR3 antagonist were evaluated.

**Results:** S1P stimulated fibrosis of NRK-49F cells in a dose- and time-dependent manner as previously observed, and induced morphological changes of NRK-49F cells. Migration of NRK49F cells was accelerated and increased a-SMA, COL1, COL4, TIMP1, and PAI1 and decreased E-cadherin expression levels were observed in the S1P-stimulated cells. S1PR3 siRNA transfection to NRK-49F cells attenuated S1P-induced cell growth, cell migration, and the expression of fibrotic markers. And also in the presence of S1PR1 agonist and S1PR3 antagonist, fibrosis and migration induced by S1P were suppressed.

**Conclusions:** These results suggest that S1P signaling mediated by S1PR1 and S1PR3 results in renal fibrosis.

**PUB406**

**Medullary Fibrosis Assessment in Human Renal Biopsies: Correlation with Cortical Fibrosis** Alton Brad Farris,[1] Diane H. Lawson,[1] Cynthia Na Cohen,[1] Seymour Rosen.[2] *[1]Emory Univ; [2]Harvard Univ/Beth Israel Deaconess Medical Center.*

**Background:** Renal tubulointerstitial injury evaluation is important in both native and transplant kidneys; however, past studies have primarily focused on the renal cortex. To study medullary fibrotic injury, interstitial fibrosis (IF) was quantitated in both the renal cortex.

**Methods:** Native renal biopsies (n=37), included in the study if they contained medullary tissue, were scanned into digital whole slide images (WSIs); and IF was quantitated on trichrome stains and collagen III (CIII) immunohistochemistry using a positive pixel count algorithm. The algorithm was also used to quantitate the % composed of BM material on a PAS stain. IF was also measured by subtracting the PAS BM% from the trichrome (Trichrome%–PAS%, referred to as T–P) in order to account for BM material also detected on trichrome staining. Pathologist visual assessment of %IF was also obtained.

**Results:** IF showed a wide range by the different measurement modalities (e.g., 20-61% for all tissue by trichrome IF%). Image analysis of trichrome and T-P WSIs correlated for all anatomic compartments (r=0.66–0.98, p all <0.0001). Visual measures also showed a wide range, from 15-90% for all tissue and a significant correlation with all of the trichrome and T-P measures. CIII showed a range of 9.5-47% for all tissue and correlated between compartments (r=0.74, p=0.0001 for all tissue vs. cortex, r=0.89, P<0.0001 for all tissue vs. medulla, and r=0.71, P=0.0003 for cortex vs. medulla); however, CIII did not show a significant relationship with any of the other IF measures.

**Conclusions:** Trichrome and T-P WSI analysis correlated between the cortex, medulla, and entire tissue, and with visual assessment. In this study, CIII deposition correlated between compartments but not with other IF measures, suggesting the importance of additional components in extracellular matrix composition. Overall, medullary measurements may show utility in larger studies prospectively correlating medullary IF with renal function in both human and animal models; and medullary measurements could prove useful in drug studies, clinical trials, and evaluation of biopsies containing only medulla.

**PUB407**

**Chronic Nicotine Attenuates Pro-Fibrotic Signal-Dependent Induction of HO-1: Its Role in Exacerbation of Renal Fibrosis** Istvan Arany, Dustin Reed, Robert Kampen, Luis A. Juncos. *Univ of Mississippi Medical Center.*

**Background:** There is a strong epidemiological association between smoking and risk of development and accelerated progression of chronic kidney diseases (CKD). Since plasma levels of TGFβ1 –a known mediator of renal fibrosis- are elevated in smokers, smoking may contribute to progression of CKD by augmenting tubulointerstitial fibrosis. On the other hand, HO-1 is an important factor that controls pro-fibrotic signaling. Here, we tested the hypothesis whether chronic nicotine (Ch-NIC) exposure exacerbates renal fibrosis by mitigating anti-fibrotic effects of HO-1.

**Methods:** A mouse model of unilateral ureteral obstruction (UUO) was employed to assess effect of chronic NIC exposure on interstitial fibrosis as well as expression of HO-1 and mediators of fibrosis (TGFβ1, IL-6) in the obstructed kidneys of mice exposed or not

to Ch-NIC. Contralateral kidneys served as controls. In vitro, the impact of chronic NIC on TGFβ1-mediated activation of HO-1 and alpha-smooth muscle actin (αSMA: a marker of fibroblast activation) promoters were studied in renal interstitial fibroblasts (NRK49F).

**Results:** UUO significantly increased tubulointerstitial fibrosis (αSMA expression and Masson trichrome staining), expression of TGFβ1, IL-6 and HO-1 in the obstructed kidneys. Ch-NIC exposure augmented fibrosis and expression of IL-6 but not TGFβ1 while decreased HO-1. In vitro, chronic NIC or IL-6 augmented TGFβ1-mediated activation of the αSMA but attenuated the HO-1 promoter. Activation of endogenous HO-1 by cobalt protoporphyrin (CoPP) or exogenous overexpression of HO-1 greatly diminished promoter activity of αSMA.

**Conclusions:** Our results suggest that chronic NIC augments promoter activity of the αSMA gene through IL-6-dependent suppression of HO-1 induction. Further studies are on the way to determine whether this effect is associated with suppression of the antioxidant response element or other cis-acting element of the HO-1 promoter. Manipulation of those pathways may present therapeutic means to ameliorate adverse effects of chronic NIC/smoking on renal interstitial fibrosis.

*Funding:* NIDDK Support

## PUB408

**Synergistic Interplay of the Canonical Wnt and TGFβ Pathways Induces Fibrosis** Omar H. Maarouf,[1] Derek Paul DiRocco,[1] Deepika Rangarajan,[2] Benjamin D. Humphreys.[1] *[1]Medicine/Renal, Brigham and Women's Hospital, Boston, MA; [2]Biotechnology, SRM Univ, Chennai, India.*

**Background:** Fibrosis is the morphologic hallmark of CKD and is characterized by exaggerated accumulation of collagen and emergence of myofibroblasts. Both the TGFβ and canonical Wnt signaling pathways are activated during kidney fibrosis, but whether these pathways interact during myofibroblast activation is unknown. We hypothesize that these pathways synergize, through β-catenin activation, to drive fibrotic gene expression in myofibroblasts.

**Methods:** We used two cell culture myofibroblast models: immortalized NRK49F cells and primary human dermal fibroblasts. The Wnt pathway was modulated pharmacologically, and the ability of TGFβ alone or with β-catenin stabilization to drive a luciferase-based β-catenin activity reporter (BAR) was assessed. Levels of activated myofibroblast transcripts, like fibronectin and αSMA, were also measured.

**Results:** TGFβ independently induced β-catenin activation in both NRK-49F cells and primary human dermal fibroblasts. Blocking β-catenin activation with XAV939, a tankyrase inhibitor which induces β-catenin degradation, repressed TGFβ-induced myofibroblast gene expression (fibronectin and αSMA). Stimulation of the Wnt pathway with the GSK3β inhibitor CHIR99021 induced fibronectin production. Combining TGFβ and CHIR99021 at sub-threshold doses led to an increase in the fibrotic phenotype by inducing fibronectin production and αSMA expression. Using the BAR system, sub-threshold doses of TGFβ and CHIR99021 were synergistic in inducing β-catenin pathway activation in a dose- and time-dependent fashion.

**Conclusions:** In fibroblast cell lines, the TGFβ and canonical Wnt pathways synergize to induce β-catenin activity, fibronectin expression and αSMA expression. Future efforts will focus on understanding whether β-catenin is required for myofibroblast gene expression induced by TGFβ, either alone or in combination with Wnt ligands.

*Funding:* Other NIH Support - T32

## PUB409

**Is Galectin-3 Responsible for Microvascular Dysfunction in Uremia?** Andrew Duncan Stewart Findlay, Steven Michael Harwood, Julius Edward Kieswich, Magdi Yaqoob. *Translational Medicine and Theraputics, William Harvey Research, London, United Kingdom.*

**Background:** Microvascular dysfunction is universal in uraemia and contributes to cardiovascular mortality. The aetiology of uremic microvascular dysfunction remains elusive. We investigated microvascular dysfunction in experimental chronic uraemia by intravital microscopy (IVM) of leucocyte endothelial interactions in cremasteric postcapillary venules. We aimed to establish if the pro-adherent leucocyte cell surface protein Galectin-3 – (which is elevated in uremic leucocytes) would augment the interaction.

**Methods:** Wild Type (WT) C57Bl mice subject to 4 weeks 0.25%Adenine diet (AD n=8) or Sham Diet (SD n=6) underwent cremasteric IVM. Leucocyte rolling velocity, adhesion and transmigration were assessed. Galectin-3 Knockout (G3KO) mice were then subjected to 4 weeks AD (n=5) vs 4 weeks SD (n=3) and had Cremasteric IVM performed.

**Results:** WT AD fed mice had significantly more cell adhesion vs WT SD (p=0.008), Cell rolling velocity and emigration were not significantly altered. In Galectin-3 KO on AD the cell adhesion was reduced to equivalent WT and G3KO SD levels. Interestingly the G3KO on AD had a milder uraemic phenotype than WT on AD with significantly higher body weights and lower plasma creatinine.

**Conclusions:** Leucocyte adhesion on post capillary venules is increased in experimental uremia which is either directly or indirectly regulated by Galectin-3 interactions. The aetiology of AD induced chronic kidney disease is initially by Adenine deposition in renal tubules but also of secondary macrophage infiltration and tubulointerstitial fibrosis. A reduction in the uremic phenotype is likely to be due to a reduction in macrophage infiltration as Galectin-3 is a chemoattractant for monocytes and activates macrophages towards a pro-fibrogenic M2 phenotype.

*Funding:* Clinical Revenue Support

## PUB410

**Pressure Promotes Fibrotic Responses in Renal Tubular Cells through miR-328-Mediated CD44 Up-Regulation** Tso Hsiao Chen, Cheng-hsien Chen. *Internal Medicine, Taipei Medical Univ-Wan Fang Medical Center, Taipei, Taiwan.*

**Background:** Pressure force is an important mechanism contributing to the induction and progression of tubulointerstitial fibrogenesis in ureteric obstruction.

**Methods:** In this study, we set up an in vitro pressure culture system, and investigated microRNA expression alterations correlate with pressure treatment in rat renal tubular cells (NRK-52E). When NRK-52E cells were cultured in the pressure culture system, 60 mmHg of pressure induced the expression of connective tissue growth factor (CTGF), transforming growth factor (TGF)-β, and fibronectin.

**Results:** MicroRNA array assays showed that pressure reduced miR-328 at the initial stage of pressurization. The expression level of miR-328 in pressurized cells significantly decreased within 2 h, recovered at 4 h, and increased at 8 h. Potential target of miR-328 has been reported to be human CD44. We identified a potential target sequence of miR-328 in rat CD44 3'-UTR as proven by 3'-UTR analysis. Protein analysis demonstrated that expression of CD44 was up-regulated at the initial stage of pressurization. CD44 siRNA transfection significantly reduced pressure-induced fibronectin in NRK-52E cells. However, CTGF expression was not influenced by CD44 siRNA transfection. In normal cells, pressure reduced E-cadherin, an important EMT marker, at 4 and 8 h. CD44 siRNA transfection induced E-cadherin in pressurized cells at 4 and 8 h. In UUO animal model, we also found CD44 increased in renal tubular cells in slight dilated tubules.

**Conclusions:** Our results suggest that miR-328-mediated CD44 up-regulation play an important role in pressure-induced fibrotic responses in renal tubular cells.

*Funding:* Government Support - Non-U.S.

## PUB411

**Responses of Human Podocytec Grown on Different Cellular Matrices** Tarunkumar H. Madne, Mysore Keshavmurthy Phanish, Mark Edward Dockrell. *SWT Institute for Renal Research, London, United Kingdom.*

**Background:** Cell phenotype and survival is strongly regulated by interaction with the extracellular matrix proteins, particularly those of a basement membrane. The glomerular basement membrane (GBM) is dynamic structure made up of protein secreted by endothelial cells and podocytes. In renal disease there are changes to the quantity and quality of GBM proteins contributing to the progression of disease such as fibrosis. We previously described TGFβ1-induced EDA+Fn expression in human podocytes. In this study we investigate response of podocyte culture grown on GBM proteins such as collagen IV and cellular and plasma fibronectin.

We aim to investigate the role of different matrices on podocyte phenotype and responses to TGFβ1.

**Methods:** Human podocyte were grown dishes coated with different matrices; collagen IV (Col IV), cellular fibronectin containing the EDA Exon (EDA+ Fn), plasma fibronectin, which lacks the EDA Exon, (EDA –Fn). Expression of EDA+Fn, synaptopodin and alpha-SMA was assessed by RT-PCR and Q-PCR in the presence and absence of TGFβ1 (2.5ng/ml).

**Results:** Marked changes in podocyte morphology were observed by light microscopy in cells grown on the different matrices. A decrease of ~40% in synaptopodin expression was observed in cells grown on cellular Fn. The expression alpha-SMA mRNA did not significantly change at this time point. Investigating the effects of different cellular matrix protein on TGFβ-mediated responses demonstrated that the TGFβ1-mediated increase in expression of EDA+Fn was completely abolished in cells grown on collagen IV. The effect of TGFβ on synaptopodin was also greatly affected by the growth surface, with opposite effects seen on collagen as compared to fibronectin. Activation of receptor Smads is unaffected by different matrices.

**Conclusions:** Alteration of the constituents of the GBM is likely to significantly alter podocyte phenotype and cellular responses to growth factors involved in podocytopathies, such as TGFβ.

*Funding:* Government Support - Non-U.S.

## PUB412

**Upregulation of Protein Phosphatase 2A Activity by Nitration in Endothelial Cells Is Associated with Renal Fibrosis** Min Han, Yuanjun Deng, Ping Liu, Gang Xu. *Tongji Hospital, Tongji Medical College, Huazhong Univ of Science and Technology.*

**Background:** Previous studies show that endothelial cells may acquire smooth muscle–like phenotype and contribute to extracellular matrix deposition in renal fibrosis. The underlying mechanism, however, remains elusive.Here,we hypothesize that a protein post-transcriptional modification of PP2A may have profound effects on the process of extracellular matrix deposition resulted from endothelial cells.

**Methods:** The expression of VE-cadherin and α-SMA were detected by using western blot, immunofluorescence and flow cytometry 72h after human umbilical vein endothelial cell (HUVEC) were treated with TGF-β1. PP2Ac (PP2A catalytic subunit) nitration was determined by Immunoprecipitation (IP) after TGF-β1 stimulation. Further, we used tandem mass spectrometry to map the nitration sites of PP2Ac treated with Peroxinitrite (a nitric oxide with superoxide- product) in vitro. Site-specific liquid chromatography-mass spectrometry quantitative analyses were designed to specifically determine the order of the nitration sites.

**Results:** After the stimulation of TGF-β1, certain subsets of endothelial cells lose endothelial characteristics and transformed into smooth muscle-like cells characterized by loss of endothelial marker VE-cadherin, and gain mesenchymal markerα-SMA. TGF-β1 stimulation upregulated PP2A activity companied with increased PP2Ac nitration. Tandem mass spectrometry identified that of the fifteen tyrosine residues in PP2Ac, Tyr-284, Tyr-218, Tyr-265, and Tyr-267, Tyr-127 and Tyr-130 were nitrated. Site-specific liquid chromatography-mass spectrometry quantitative analyses demonstrated that the favored modification site following exposure to peroxynitrite is Tyr-284. And these profibrotic effects were significantly attenuated by adding okadaic acid and NADPH oxidase inhibitor apocynin.

**Conclusions:** Nitric oxide with superoxide pathway can regulate PP2A modification under oxidative stress conditions, which modulate TGF-β1 induced phenotype change mechanisms involved in profibrotic effects. This can augment extracellular matrix deposition and fibrosis linked to renal fibrosis.

## PUB413

**Effects of Membrane Cholesterol Content and the Anionic Amphipath Trinitrophenol on Mechanical Activation of TRPC6 Channels in Podocytes**

Stuart E. Dryer,[1,2] Cory Wilson,[1] Marc Thomas Anderson.[1] *[1]Biology and Biochemistry, Univ of Houston, Houston, TX; [2]Nephrology, Baylor College of Medicine, Houston, TX.*

**Background:** We have previously shown that TRPC6 channels can be activated by mechanical stimuli in cultured podocytes and in podocytes in isolated glomerulus preparations. Mechanical activation of TRPC6 in podocytes persists after inhibition of phospholipases or G proteins. Knockdown of the podocin increased TRPC6 activation by mechanical stimuli, but suppressed activation by diacylglycerol analogs[1].

**Methods:** Whole-cell recordings from mouse podocyte cell lines and filipin staining of membrane cholesterol.

**Results:** Filipin staining showed that cholesterol occurred in distinct patches, often arranged in an elliptical pattern on the surface of immortalized mouse podocytes. This same spatial pattern of filipin staining persisted in podocin knockdown cells[1], but filipin staining was eliminated after 18-24 hr treatment with 10 mM methyl-β-cyclodextrin (MBCD). MBCD treatment caused a marked increase in hypoosomotic stretch-activation of TRPC6 in podocytes. Conversely, it is possible to increase membrane cholesterol content by pre-equilibrating MBCD with cholesterol in a 20:1 molar ratio. A 24-hr exposure to this reagent caused nearly complete inhibition of hypoosmotic stretch-activation of TRPC6. Exposure to 10 mM MBCD for just 20 min caused a marked increase in stretch-activation of TRPC6. It is possible that this brief treatment with MBCD selectively depletes cholesterol from the outer leaflet resulting in microfolding of the membrane. A similar effect was evoked by treating cells for 20 min with the anionic amphipath trinitrophenol (200 μM), which is known to cause crenelation of the plasma membrane[2].

**Conclusions:** These data suggest that cholesterol content regulates membrane mechanical properties in podocytes and the transmission of force to TRPC6 channel complexes. This is consistent with our observation that podocin interactions suppress mechanical activation of TRPC6 channels in podocytes[1].

1. Anderson et al. (2013) Am J Physiol-Cell Physiol in press.
2. Sheetz and Singer (1974) Proc Natl Acad Sci USA 71: 4457-61.

*Funding:* Pharmaceutical Company Support - Pfizer Inc.

## PUB414

**Mechanistic Studies of a Chinese Herbal Medicine Prescription for Treatment of IgA Nephropathy**

Zhi Qiang Huang,[1] Yiping Chen,[2] Nicolas Maillard,[1] Stacy D. Hall,[1] Bruce A. Julian,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Medicine, Longhua Hospital Affliated to Shanghai Univ of Traditional Chinese Medicine, Shanghai, China.*

**Background:** Immune complexes (IC) consisting of galactose-deficient IgA1 (Gd-IgA1) and anti-Gd-IgA1 IgG induce protein-kinase signaling, including PDGF pathway, and proliferation in human mesangial cells (MC). Thus, blocking IC-mediated activation of MC is of therapeutic significance. Chinese herbal medicine prescription LH-1 improves hematuria and proteinuria in IgA nephropathy (IgAN) patients, but the mechanism is unknown. In this study, we assessed the effects of LH-1 on proliferation and signaling in MC induced by IC or PDGF.

**Methods:** Powder of LH-1 water extract was reconstituted in water before use. Cultured, quiescent MC were incubated with *in vitro*-formed IC or with PDGF (10 ng/ml) for 24 hr in the presence or absence of LH-1. Proliferation of MC was assessed by $^{3}$H-thymidine uptake. Cells were also stimulated with IC or PDGF for 15 min with or without LH-1 and cell signaling was analyzed using SDS-PAGE/Western blotting with phospho-Tyr-antibody or phospho-ERK1/2 and total ERK1/2 antibodies. Quiescent MC, without treatment or treated with only LH-1, were used as control.

**Results:** IC induced MC proliferation and LH-1 blocked this stimulatory effect in a dose-dependent manner. Phosphorylation of multiple Tyr-containing proteins, including ERK1/2, was enhanced by IC. LH-1 inhibited activation of Tyr-phosphorylation in a dose-dependent manner. PDGF increased MC proliferation and enhanced Tyr-phosphorylation, including ERK1/2. LH-1 blocked these stimulatory effects of PDGF as well. LH-1 treatment did not affect quiescent MC at the concentrations ≤400 μg/ml.

**Conclusions:** LH-1 inhibited MC proliferation and the enhanced Tyr-phosphorylation induced by IC or PDGF. Thus, LH-1 may contain potent Tyr-kinase inhibitor(s) that may provide compounds for development of disease-specific treatment of IgAN.

*Funding:* NIDDK Support

## PUB415

**Transcriptome Based Analysis of Nephrotic Syndrome for Therapeutic Target Identification**

Sebastian Martini,[1] J. B. Hodgin,[2] Viji Nair,[1] Wenjun Ju,[1] Celine C. Berthier,[1] Ann Randolph,[1] Clemens D. Cohen,[3] Matthias Kretzler.[1] *[1]Nephrology, Univ of Michigan; [2]Pathology, Univ of Michigan; [3]Nephrology, Univ of Zurich.*

**Background:** Focal Segmental Glomerulosclerosis (FSGS), Membranous Nephropathy (MN), or Minimal-change Disease (MCD) share ultra-structural and functional glomerular alterations. We have generated comprehensive glomerular gene expression profiles to define regulatory networks of the glomerular filter. These signatures can be mined for patterns of known drug targets.

We hypothesized that:

1.Gene expression signatures in FSGS, MN, and MCD reflect specific disease-relevant mechanisms.

2.Transcripts that are differentially expressed in all three diseases allow for detailed description of common molecular mechanisms of filtration barrier dysfunction.

3.A Connectivity Map (CMaP) analysis of the shared transcriptome can identify transcriptional networks to be targeted by established therapeutic compounds.

**Methods:** Fifty nine glomerular gene expression profiles of NS patients from the ERCB were compared to 31 controls. Enriched pathways were defined among differentially expressed genes individually for each disease and for the shared transcriptome. Potential drug candidates capable of either enhancing or reversing the transcriptional changes seen among genes of the shared transcriptome were identified using Connectivity Map.

**Results:** Pathway analysis highlighted novel and known pathways for each disease, i.e.: Lymphocyte/interferon-related pathways for FSGS; NFkappaB-signaling pathway for MCD as well as antigen presentation pathways/PLA2R1 for MN. CMaP identified drugs with nephrotoxic properties that recapitulated the transcriptional changes seen in NS disease in in vitro systems, while compounds with known beneficial effects on renal function (i.e. PI3K-inhibitor LY294002 in model systems) reversed these transcriptional changes *in vitro*.

**Conclusions:** Our studies have catalogued transcriptional changes and identified known and novel pathways that may play a role in the pathogenesis of nephrotic syndrome diseases. The cross-sectional analysis of these diseases also allowed for screening of therapeutic and toxicological profiles of candidate drugs in NS.

*Funding:* NIDDK Support, Other NIH Support - NEPTUNE

## PUB416

**IgA and Immune Complex Profile of an IgA-Dominant Post-Infectious Secondary Glomerulonephritis**

Eric L. Wallace,[1] Nicolas Maillard,[3] Hiroyuki Ueda,[3] Stacy D. Hall,[3] Huma Fatima,[2] Jan Novak,[3] Bruce A. Julian.[1] *[1]Div of Nephrology, UAB, Birmingham, AL; [2]Dept of Pathology, UAB, Birmingham, AL; [3]Dept of Microbiology, UAB, Birmingham, AL.*

**Background:** A 67-year old woman, baseline creatinine 1.0 mg/dL, developed a gallbladder-fossa infection after gallbladder resection for adenocarcinoma. Admission creatinine was 1.9 mg/dL with a bland urinary sediment. The infection cleared with antibiotics, and creatinine improved to 1.3 mg/dL at discharge. She was readmitted with acute renal failure, nephritic urinary sediment, 3.3 g/g proteinuria, leg edema, and hypertension. She had diarrhea later attributed to *Clostridium difficile*-induced colitis. Due to a rapidly rising creatinine, renal biopsy was performed. Light microscopy showed marked mesangial and endocapillary hypercellularity; immunofluorescence microscopy showed dominant IgA staining with less staining for IgG in the mesangium and capillary loops; electron microscopy showed large subendothelial and mesangial deposits consistent with immune deposits of a post-infectious glomerulonephritis (PIGN). She became dialysis dependent. Because the pathogenesis of IgA-dominant PIGN is unknown, we undertook additional laboratory studies.

**Methods:** Serum levels of IgA and galactose-deficient IgA1 were measured by ELISA. Western blot after SDS-PAGE separation of serum IgA under non-reducing conditions determined the ratio of monomeric and polymeric IgA. Serum (native or IgA- or IgG-depleted) was fractionated by size-exclusion chromatography, and fractions were added to cultures of human mesangial cells and proliferation assessed by thymidine incorporation. Fractions were assessed by ELISA for IgA and IgG profiles.

**Results:** Serum level of total IgA was markedly elevated, with normal polymeric-to-monomeric ratio of IgA. Total serum level of galactose-deficient IgA1 was elevated. Large-molecular-mass circulating immune complexes containing IgA and IgG stimulated proliferation of mesangial cells.

**Conclusions:** For this patient with IgA nephropathy associated with colitis due to *Clostridium difficile*, an increased serum level of galactose-deficient IgA1 likely led to formation of circulating nephritogenic IgA-IgG immune complexes.

*Funding:* NIDDK Support

## PUB417

**Evaluation of Disease State of 200 Patients with Various Glomerulonephritis by Claudin1, CD68 Positive Cells and the mRNA Levels of M1 and M2 Macrophages in Urine** Kojiro Yamamoto,[1] Takashi Oda,[1] Takahiro Uchida,[1] Atsushi Watanabe,[1] Hanako Takechi,[1] Naoki Oshima,[1] Yutaka Sakurai,[2] Hiroo Kumagai.[1] *[1]Nephrology, National Defense Medical Collage, Tokorozawa, Saitama, Japan; [2]Preventive Medicine and Public Health, National Defense Medical Collage, Tokorozawa, Saitama, Japan.*

**Background:** Urine test is non-invasive and can reflect more general condition of the whole kidney than findings of renal biopsy.

**Methods:** We collected morning urine samples from 200 patients for kidney biopsy from 2008 to 2011. Logistic regression analysis was performed with the finding of renal biopsy as dependent variable (0 = normal; 1 = crescent existed) and with claudin1and CD68 positive cell count as predictor variables. And the relations of claudin1 and CD68 positive cell count in urinary sediment with urinary protein or red blood cell were examined. Secondly, we evaluated phenotypes of the macrophage in urinary sediment. The relationship between relative levels of target mRNAs and the histological changes such as crescent formation (CF) (exist or none) or global sclerosis rate was evaluated.

**Results:** Logistic analysis of claudin1 and CD68 positive cell count with the presence of CF revealed ROC curve with the area under the curve (AUC) of 0.60 and 0.70, respectively, both of which were statistically significant. There was significant positive trend between the claudin1 positive cell count and the urinary protein, but no trend was found between the CD68 positive cell count and the urinary protein. Both claudin1 and CD68 positive cell count showed significant positive correlation with the urinary red blood cell. The relative mRNA levels of the M2 makers (CD163, CD204, CD206, IL-10) were significantly higher in patients with than without CF. On the other hand, the relative mRNA levels of M1 markers (iNOS, IL-6) were similar in both patients with and without CF.

**Conclusions:** These data suggest that the claudin1, CD68 positive cell count and quantification of the mRNA of the M2 markers of urinary sediment are useful for the evaluation of the state of the glomerular disease and that claudin1 reflects parietal epithelial damage not only by glomerular inflammation but also by urinary protein.

## PUB418

**Pentraxin 3 Expression in Renal Tissue of HIV Positive and HIV Negative Patients with Glomerular Diseases** Manuela Nebuloni,[1] Angelita Ferri,[2] Antonella Tosoni,[2] Pietro Zerbi,[1] Irina Edu,[2] Giovanni Belgiojoso.[2] *[1]Pathology Unit, DIBIC, Univ Milan, Milan, Italy; [2]Path. Unit, L.Sacco Hospital.*

**Background:** Pentraxin 3 (PTX3) is involved in inflammation and regulation of innate immunity. It has been detected in renal tissue of patients with glomerulonephritis (GNs), suggesting a possible role in glomerular and tubular injury. On the other side, HIV infection *per se* is a causative agent/eventual cofactor for GNs.

Aim of the study is to evaluate the additional role of HIV infection in PTX3 expression as a modulatory factor in tissue injury in different GNs.

**Methods:** Renal biopsies from 35 HIV + and 46 HIV - patients (pts) with various types of GNs were tested for PTX3 by immunohistochemistry. Immunostaining distribution and intensity were considered. Immune complex related GNs (IC) and non immune complex GNs (NIC), in HIV+ and HIV- groups were compared. The Mann Whitney U test was employed for statistical analysis of results.

| Diagnosis | HIV+ | HIV- |
|---|---|---|
| IgA Nephropathy* | 4 | 8 |
| Lupus-like GN* | 4 | - |
| Lupus GN* | - | 6 |
| Membranoproliferative GN* | 4 | 7 |
| Membranous GN* | 4 | 9 |
| Diabetic nephropathy | 4 | 4 |
| Minimal changes | 2 | 2 |
| Focal Segmental Glomerulosclerosis | 12 | 11 |

distribution of cases in single Gns. *: IC group.

**Results:** PTX3 immunostaining was localized in the interstitium and in the cytoplasm of some endothelial cells and macrophages. IC vs NIC GNs, irrespective of HIV infection: no statistical difference. IC GNs and NIC GNs, HIV- pts: no statistical difference. IC vs NIC GNs, HIV+ pts: p=0.02. PTX3 expression in IC GNs higher than in NIC GNS. IC GNS, HIV+ pts, vs IC GNs, HIV- pts: p=0.04. PTX3 expression in HIV+ pts higher than in HIV- pts.

**Conclusions:** HIV infection within IC GNs group enhances PTX3 expression in glomerular diseases. Moreover, the presence of IC *per se* is an increasing factor for PTX3 expression among HIV+ pts. Our results suggest that PTX3 can modulate tissue injury in glomerular diseases but its exact role is unclear.

## PUB419

**Clinicopathological Spectrum of Kidney Diseases in Patients Treated with Vascular Endothelial Growth Factor Inhibitor for Cancer Therapy** Joichi Usui,[1,2] Ilya Glezerman,[3] Chandra B. Chandran,[4] Steven Salvatore,[1] Carlos D. Flombaum,[3] Surya V. Seshan.[1] *[1]Dept of Pathology, Weill Cornell Medical College, Cornell Univ, New York, NY; [2]Univ of Tsukuba, Japan; [3]Renal Service, Dept of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; [4]Nephrology Div, Dept of Medicine, St. Joseph's Regional Medical Center, Paterson, NJ.*

**Background:** Recently, cancer therapies have been supplemented by vascular endothelial growth factor(VEGF) inhibitors as anti-angiogenic agents. The present work discloses the spectrum of pathological features in VEGF inhibitor-associated kidney disease.

**Methods:** Clinicopathological findings of kidney disease were retrospectively studied in 5 cancer patients treated with VEGF inhibitors.

**Results:** Four cases received bevacizumab(anti-VEGF-A) and one was given sorafenib(tyrosine kinase inhibitor affecting VEGFR2), with chemotherapeutic agents. All patients presented with acute kidney injury(serum Cr 1.6-6mg/dl), hypertension and/or proteinuria(none to 2.6g/day). All kidney biopsies showed endothelial injury of varying severity, including 2 with typical active features of thrombotic microangiopathy(TMA). Evidence of chronic endothelial injury and vascular sclerosis were also observed. Furthermore, acute tubular injury with focal necrosis was seen in all cases. While administration of VEGF inhibitor was discontinued in 4 cases, it was resumed for 5 more doses, following steroid therapy in 1 case. Cessation of VEGF inhibitor was successful in reversing anemia and led to improvement of hypertension and proteinuria in 4 of the 5 cases. One case with active and severe TMA progressed to ESRD, but renal function of other 4 cases improved(final serum Cr 0.5-1.6mg/dl).

**Conclusions:** A range of renal pathologic lesions secondary to endothelial injury are noted often accompanied by acute tubular damage following anti-VEGF therapy, the most severe being TMA. While most of the clinical manifestations are reversible with discontinuation of therapy, the role of other nephrotoxic chemotherapeutic agents in enhancing renal injury and other host factors with possible poor outcome should be considered.

## PUB420

**Analysis of AKT/mTOR Signaling Pathway in the Renal Biopsy Tissue of Patients with Glomerulonephropathy by Luminex xMAP Technology** Hong Liu, Lujun Xu, Xiang Zhou, Fu-You Liu. *Dept of Nephrology, The Second Xiangya Hospital of Central South Univ, Changsha, Hunan, China.*

**Background:** To investigate role of AKT-mTOR signaling in the pathogenesis of glomerulonephropathy by the xMAP technology.

**Methods:** Kidney tissues were obtained from 25 patients who were diagnosed as IgA nephropathy, idiopathic membranous nephropathy , idiopathic FSGS or minor lesions nephropathy. The activation of AKT-mTOR signaling was determinied by the xMAP technology. The correlation between AKT-mTOR and the clinical indicators was analyzed.

**Results:** 1. Compared with minor lesions nephropathy group, the level of hematuria of the IgAN group was higher(P=0.03). The level of pAKT, pGSK3?,pmTOR of the kidney tissue of the IgAN group was increased , and the level of pPTEN of the kidney tissue of was decreased than the minor lesions nephropathy group( P=0.003). Hematuria was positively correlated to pAKT, pGSK3?,pmTOR , and negatively correlated to pPTEN. 2. Compared with minor lesions nephropathy group, the level of cholestasis, triglycerides,proteinuria and hematuria of the idiopathic membranous nephropathy group was higher(P=0.04). The level of pAKT, pmTOR,pp70s6K of the kidney tissue of the idiopathic membranous nephropathy group was increased than the minor lesions nephropathy group( P=0.04). Cholestasis, triglycerides, proteinuria and hematuria were positively correlated to pAKT, pmTOR and pp70s6K. 3. Compared with minor lesions nephropathy group, the level of systolic pressure,mean arterial pressure ,proteinuria and hematuria of the of idiopathic FSGS group was higher(P=0.004,0.036,0.004, 0.006). The level of pAKT of the kidney tissue of the idiopathic FSGS group was increased and pPTEN was decreased than the minor lesions nephropathy group( P=0.04). Systolic pressure,mean arterial pressure, proteinuria and hematuria were positively correlated to pAKT ,and systolic pressure ,mean arterial pressure and proteinuria were negatively correlated to pPTEN.

**Conclusions:** We find that Akt-mTOR pathway might play a role in the pathogenesis of IgAN,IMN and iFSGS. Our research indicated the potential for the application of luminex xMAP technology to explore more signaling pathways related to chronic kidney disease.

## PUB421

**Henoch-SchÖlein Purpura Nephritis and IgA Nephropathy in Children: A Comparison Pathological Features Using "Oxford Classification of IgA Nephropathy" and Clinical Correlation** Xu Zeng,[1] Murty Adabala,[2] David G. Bostwick,[1] Deloar Hossain,[1] Tej K. Mattoo.[2] *[1]Nephrocor, Bostwick Laboratories, Orlando, FL; [2]Pediatric Nephrology, Children Hospital of Michigan, Detroit, MI.*

**Background:** Henoch-SchÖlein purpura nephritis (HSPN) and IgA nephropathy (IgAN) are common in children with overlapping clinical, genetic and immunological features. Both entities are characterized by IgA deposits in glomeruli. However, a systemic approach to compare both diseases is lacking. The objective of this study was to assess the benefit of applying the newly described Oxford classification of IgA nephropathy", to distinguishing these two entities.

**Methods: R**enal biopsies in Children's Hospital of Michigan during 2004-2010 with HSPN and IgAN were reviewed. The MEST scheme of "Oxford classification of IgA nephropathy" was applied to measure mesangial cellularity, endocapillary proliferation, segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis. Active glomerular lesions, i.e. the highest number of mesangial cells, percentage of glomeruli with endocapillary proliferation, cellular crescent and segmental necrosis were measured. Hypertension, protein/creatinine ratio, hematuria and estimate glomerular filtration rate were compared. Student t-test (P<0.05 is considered to be statistical significance).

**Results:** A total of 23 HSPN (F:M=12:11) and 26 IgAN (F:M=11:15) were included. The HSPN patients were younger than those with IgAN (7.8 3.3 vs 12.4 3.2, year-old, p<0.05). Patients with HSPN had more mesangial cells (11 3.2 vs 8.3 2.4), higher percentages of glomeruli with endocapillary proliferation (14.9 2.2 vs 4.9 8.2), cellular crescents (10.4 1.2 vs 4.3 2.8) and segmental necroses (6.6 1.3 vs 0.7 1.1, all P<0.05). There was no significant difference between HSPN and IgAN in MEST scheme (P>0.05). There is higher, but not significant difference in protein/creatinine ratio between HSPN and IgAN.

**Conclusions:** HSPN has more extensive mesangial/endocapillary proliferation and higher percentage of glomeruli with cellular crescents and segmental necrosis, thus more severe glomerular damage. These findings further characterize pediatric HSPN and will benefit to the clinical management.

## PUB422

### Histologic and Clinical Progression of ANCA Associated Glomerulonephritis

Naima Carter-Monroe, Lois J. Arend, S. Bagnasco, Lorraine C. Racusen, Duvuru Geetha. *Johns Hopkins School of Medicine.*

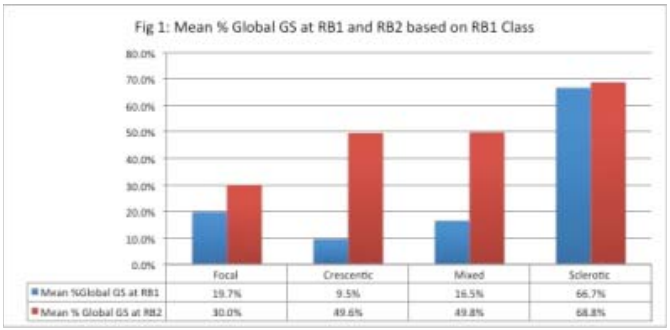
**Background:** Histologic correlates with outcomes in ANCA associated glomerulonephritis (ANCA-GN) based on IWGRP class are limited to findings on initial biopsy. Analysis of evolution of these changes over time would lead to better understanding of salvageable lesions.

**Methods:** Cases of ANCA-GN diagnosed from 1996 to 2009 with at least one follow up biopsy were identified. Clinical data at the time of initial (RB1) and follow up (RB2) biopsy were collected. Biopsies were categorized as Focal, Crescentic, Mixed, or Sclerotic (IWGRP classification for AAV-GN) based on original pathology reports.

**Results:** Eighteen patients (mean age: 57 years, 50% males, 83% Caucasian, 50%p-ANCA, 33% c-ANCA) had RB2 at a mean of 47 months after RB1. RB2 was performed for suspected relapse (n=13), persistent hematuria (n=3) and CKD (n=2). Most cases were classified on RB1 as Focal (n=7) or Mixed (n=7), and showed mixed progression, with 3 Focal cases unchanged and 2 becoming Sclerotic; equal Mixed cases were classified as Focal or Sclerotic on RB2 (n=3 each). Among 7 patients who reached ESRD at a mean of 24 months post RB2, 5 patients progressed to Sclerotic and 2 were classified as Focal. (Table 1). Although Crescentic cases demonstrated the lowest mean % global glomerulosclerosis (GS) at RB1, they showed greater progression to GS at RB2 when compared to Focal cases (Fig. 1).

Table 1:RB1 and RB2 Class with Progression to ESRD

| RB1 Class (n) | RB2 Class | ESRD (n) |
|---|---|---|
| Focal (7) | Absent | 0 |
| | Focal | 2 |
| | Mixed | 0 |
| | Sclerotic | 1 |
| Crescentic (3) | Focal | 0 |
| | Sclerotic | 2 |
| Mixed (7) | Focal | 1 |
| | Crescentic | 0 |
| | Sclerotic | 1 |
| Sclerotic (1) | Sclerotic | 1 |




**Conclusions:** All classes at initial biopsy showed progression to ESRD. The IWGRP class changes seen in follow up biopsy suggests that some active lesions revert to normal phenotype with therapy while others progress to a chronic pattern.

## PUB423

### The Anti-Aging Gene Klotho Represents a Potential Predictive Marker of Metastasis in Renal Cell Carcinoma (RCC)

Margherita Gigante,[1] Paola Pontrelli,[1] Giuseppe Stefano Netti,[2] C. Divella,[1] Cesira Cafiero,[1] Matteo Accetturo,[1] Simona Simone,[1] G. Grandaliano,[2] Loreto Gesualdo,[1] E. Ranieri,[2] G. Stallone.[2] *[1]DETO, Univ of Bari, Italy; [2]Med and Surg Sciences Dept, Univ of Foggia, Italy.*

**Background:** The identification of new therapeutic and prognostic biomarkers for clear cell renal cell carcinoma (ccRCC) is needed since most patients appear in advanced stages of disease at diagnosis. The renoprotective antiaging gene Klotho works as a tumor suppressor in different human cancers. Aim of our study was to evaluate Klotho expression in tissue and serum of RCC pts and correlate it with disease progression.

**Methods:** We used a genome wide screening of the human exons expressed in 11 ccRCC vs paired adjacent non-tumoral tissue by microarray. Klotho expression in tumor tissue of primitive and metastatic RCC pts, was studied using quantitative RT-PCR and immunohistochemistry and the soluble serum levels were titrated in 35 primary RCC, 25 metatatic RCC and 15 healthy donors, by sandwich ELISA. Comparisons of variables among different groups were performed by Student's t-test and Mann-Whitney U-test. Frequencies were compared by chi-squared test. Kaplan–Meier estimates were used to generate overall patient survival curves and differences were assessed by log-rank test.

**Results:** Ingenuity pathway analysis revealed a set of genes modulated in ccRCC (FDR<5% and f.c.≥2), associated with inflammatory response, cancer and renal disorders. Among them, Klotho was strongly down-regulated in all ccRCC (f.c.:-41,9, p=0.0007) respect to paired normal renal tissue (NT). Both at gene and protein level, Klotho significantly decreased in RCC vs NT and the down-regulation was more evident in metastatic RCC than primitive RCC. This trend was also observed in serum samples, where circulating Klotho significantly decreased in RCC when compared with healthy donors and it was dramatically decreased in metastatic RCC than primitive RCC sera (p<0.001). Lower serum levels of KLOTHO protein at RCC onset are related to worse clinical presentation and outcome.

**Conclusions:** The decrease of Klotho correlates with the progression of RCC and suggest a key role of Klotho in the onset of cancer metastasis.

## PUB424

### Proteomic Profile of Retained Proteins from Hemodiafiltration with On-Line Endogenous Reinfusion (HFR) Cartridge

Mauro Atti,[1] Marialuisa Caiazzo,[1] Giuseppe Palladino,[1] Aurora Cuoghi,[3] Elisa Bellei,[3] Emanuela Monari,[3] Stefania Bergamini,[3] Aldo Tomasi,[3] Francesco Bruni.[2] *[1]Scientific Affairs, Bellco S.r.l., Mirandola, Modena, Italy; [2]Nephrology and Dialysis, Civil Hospital Madonna del Soccorso, San Benedetto del Tronto, Ascoli Piceno, Italy; [3]Dept of Diagnostic, Clinical and Public Health Medicine, Univ of Modena and Reggio Emilia, Modena, Italy.*

**Background:** Hemodiafiltration with on-line endogenous reinfusion (HFR) is a dialytic method, which combines the processes of diffusion, convection and adsorption. The performance of this system is linked to the optimal combination of the membrane permeability and cartridge resin bed.

Lupus nephritis (LN) remains one of the most severe manifestation of systemic lupus erythematous (LES), associated with considerable morbidity and mortality.

In this preliminary study, ESI-QTOF Mass Spectrometer was used for protein identification of ultrafiltrate (UF) and for the protein captured by resin bed, obtained from one dialysedpatient with LN.

**Methods:** Plasma, UF (pre and post cartdrige) of one patient with LN treated with HFR, were collected at the 15 min and at 235 min of the dyalitic session. The cartridge utilized during treatment, containing styrenic resin, was opened and the proteins kept by the resin were eluted by incubation O/N with 60% ACN and 1%TFA. Samples was desalted and separated by SDS-page, interesting band was picked and "in-gel" triptic digested before ESI-QTOF analysis.

**Results:** ESI-QTOF analysis of eluted protein resulted in the identification of several biomarker of kidney injury in LN, such as Retinol binding protein 4, Neutrophil gelatinase-associated lipocain, and Cystatin-C (and TRFE, A1AG1, PTGDS, TTHY). Moreover we identify several fragment of Immunoglobulin, that are implicated in the etiopathogenesis of LES.

**Conclusions:** The results of this study demonstrate that, styrenic resin retain several proteins implicated in the Lupus nephritis pathogenesis the corresponding bands in the UF disappear confirming the remotion of this proteins from the cartridge.

## PUB425

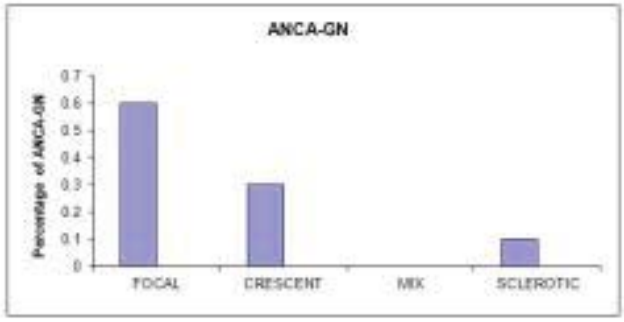
### Pediatric Anti-Neutrophil Cytoplasmic Antibody (ANCA)-Associated Glomerulonephritis (ANCA-GN) Is Predominately Focal

Xu Zeng, Guillermo A. Herrera, Deloar Hossain, David G. Bostwick. *Nephrocor, Bostwick Laboratories, Orlando, FL.*

**Background:** ANCA-GN is the most common cause of rapidly progressive GN and diagnosis is made mainly by renal biopsy. The majority of cases of ANCA-GN occur in adult patients. The recent published "histopathologic classification of ANCA-GN" divides ANCA-GN into four categories, including focal, crescentic, mixed, and sclerotic, corresponding to the order of severity of renal impairment. Most of adult patients belong to the crescentic group, which has highly active renal disease and severely reduced renal function. Less is known about pediatric ANCA-GN.

**Methods:** Renal biopsies with the diagnosis of ANCA-GN in children were reviewed. Based on the predominance of normal glomeruli, cellular crescents, and globally sclerotic glomeruli, each ANCA-GN case was categorized as mentioned above. The percentage of each group was recorded. In addition, the activity index, the percent of cellular crescent and segmental necrosis, as well as background renal function, global sclerotic glomeruli and tubular atrophy/interstitial fibrosis, were also recorded.

**Results:** A total of 10 ANCA-GNs (F:M=1:1) were diagnosed in children adolescents (age 12.8 ±3.0). The majority were focal (6/10, 60%); four (30%) were crescentic, 1 was sclerotic (1/10, 10%) and none was mixed. The average percentage of crescents, necrosis, global sclerotic and tubular atrophy/interstitial fibrosis was 29.3%, 17.3%, 11.9% and 15, respectively.




**Conclusions:** Our findings indicate that pediatric ANCA-GN is predominately focal and has a relatively favorable outcome with mild activity including a small percentage of cellular crescents and segmental necrosis. Renal function at the time of biopsy was relatively good with a low number of globally sclerotic glomeruli and low tubular atrophy and interstitial fibrosis.

## PUB426

**Result of the Online Registry for Early Detection and Care of Lupus Nephritis and Other Glomerular Diseases in the Colombian Caribbean Region** Gustavo Jose Aroca Martinez,[1,2,3,4] Andres A. Cadena,[1,2,4] Raul Garcia,[1,3] Antonio Iglesias,[4,5] Eder Augusto Hernandez Ruiz,[3] Erick Estrada.[2] *[1]Nefrologia, Clinica de la Costa, Barranquilla, Atlantico, Colombia; [2]Medicina, Universidad Simon Bolivar, Barranquilla, Atlantico, Colombia; [3]Medicina Interna, Universidad del Norte, Barranquilla, Atlantico, Colombia; [4]Nefrologia, Nefrologos del Caribe, Barranquilla, Atlantico, Colombia; [5]Reumatologia, Universidad Nacional, Bogota, Cundinamarca, Colombia.*

**Background:** The registry of the Latin American Society of Nephrology reports that 19,3% of cases of chronic kidney disease(CKD) in the region are due to Lupus Nephritis(LN) and Primary Glomerulonephritis(PGN). In the Colombian Caribbean region, there is a paucity of epidemiological data and many patients develop CKD secondary to delays in diagnosis and treatments. The purpose of this project is to create an online database that allows physicians to access in a opportune manner all the information of patients who are being actively worked up for LN and other PGN.

**Methods:** The NEFRORED database (href="http://www.nefrored.org/) was created to access the information of patients being worked up for PGN and LN. The information include demographics, clinical, laboratory findings and renal pathology results.

**Results:** Currently the network covers 7 states in the northern side of Colombia. Collection of data started on January 2008 and continues to May 2013, 616 biopsies were included; 40.7 % of the biopsies were reported as LN and the 59.2% to PGN. 83% of the patients are mestizo and 81% are female. 65% of the biopsies with LN had an activity index between 1 and 8 and 33% between 9 and 17. In PGN the most common diagnosis were IgA GN(24.2%), Membranous GN(21.6%), Mesangial Proliferative GN(14.7%), Membranoproliferative GN(11.5%), Focal Segmental GN(9%), Minimal Change GN(4.3%), Rapidly Progressive GN(4.3%) and others(11.7%).

**Conclusions:** The online Registry for Early detection and care of LN and other GN diseases in the Colombian Caribbean Region improves communication between all the physicians treating patients with complex kidney diseases, the information stored in the database is an important tool to establish the prevalence, geographical distribution and to facilitate the management of patients with glomerular diseases.

*Funding:* Private Foundation Support

## PUB427

**Clinico-Pathological Features and Outcomes of Not Otherwise Specified (NOS) Variant in Focal Segmental Glomerulosclerosis (FSGS) Compared with NOS Variant in IgA Nephropathy (IgaN)** Lectícia Jorge, Lilian P.F. Carmo, Aline Lázara Resende, Elerson Costalonga, Leonardo Abreu Testagrossa, Denise M.A.C. Malheiros, Cristiane Bitencourt Dias, Rui Toledo Barros, Viktoria Woronik. *Nephrology, Univ of São Paulo, Brazil.*

**Background:** Columbia Classification(CC) of FSGS discloses five variants with different progression rate to CKD and NOS variant is the most common form in primary FSGS. Columbia criteria can be applied to IgAN although not usual. It is largely unknown if one histological phenotype (NOS)expressed in two different glomerular diseases(FSGS and IgAN) affects clinical disease expression and outcomes.The aim of our study was to compare clinical profile and outcomes of ours NOS FSGS patients with NOS IgAN.

**Methods:** A retrospective analysis was carried out on all patients with biopsy-proven IgAN(n=165) or primary FSGS(n=80) between 1999-09. Biopsies were reviewed and classified according to CC, NOS subtypes was selected for analysis. Twenty-one patients with NOS FSGS and 25 patients with NOS IgA met inclusion criteria of age>18 years, biopsies containing at least 8 glomeruli and follow up longer than 1 year. The endpoint was defined as a variation of glomerular filtration rate(GFR) per year(ΔGFR/y), calculated as the difference between final and initial eGFR adjusted by follow-up time for each patient.

**Results:** The data are shown in Table 1.

| N | NOS FSGS(21) | NOS IgAN(23) |
|---|---|---|
| AGE(y) | 33±18 | 38±15 |
| Female | 13(62%) | 15(60%) |
| eGFRi(ml/min) | 58±29 | 43±27 |
| Proteinuriai(g/day)* | 11±5.1 | 2.5±1.5 |
| Hematuria* | 6(28%) | 21(84%) |
| Vascular Lesions* | 4(21%) | 17(65%) |
| Follow up*(m) | 53±41 | 87±34 |
| eGFRend(ml/min)* | 55±35 | 34±29 |
| ΔeGFR/year(ml/min) | -4.2±22 | -3.6±10 |
| ESRD | 3(14%) | 9(36%) |

Results are shown as mean±SD or n(%) *p<0.05

**Conclusions:** This study showed that NOS FSGS and NOS IgAN were different regarding clinical presentation. The NOS FSGS group are more nephrotic and less hematuria and vascular lesions. However the rate of progression to chronic kidney disease did not differ between the two groups, as shown in the data of ΔeGFR/y.

*Funding:* Government Support - Non-U.S.

## PUB428

**Clinical Course and Long-Term Follow-Up of 50 Patients with Idiopathic Membranous Nephropathy: Retrospective Cohort** Camila Barbosa L. Oliveira, Alline S. A. Oliveira, Luis H.B.C. Sette, Carla Queiroz Neves, Clarissa Jacob Barros Carvalho, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

**Background:** Natural course of idiopathic membranous nephropathy (IMN) is variable, and data related to prognosis were performed 2 or 3 decades ago, when antiproteinuric and immunosuppressive therapies were less well established and efficient than in present times. The aim of this study is to evaluate the clinical course and long-term prognosis of patients with IMN.

**Methods:** We conducted a retrospective cohort of adult patients with IMN and nephrotic syndrome between 1997 and 2012. Complete remission (CR) and partial remission (PR) were evaluated. Time to doubling creatinine (Cr), Cr clearance (Cl) < 30 ml/min/1.73m$^2$ and renal replacement therapy (RRT) were also analyzed. Patients were evaluated until RRT, last clinical visit or lost of follow up.

**Results:** Demographic data, clinical and prognostic characteristics are shown in Table 1.

| **Baseline characteristics** | **N = 50** |
|---|---|
| Mean age, years | 42.4 ± 16.6 |
| Male gender, n (%) | 31 (62) |
| SCr, mg/dL | 1.1 ± 0.5 |
| CrCl, ml/min/1.73m$^2$ | 72.2 ± 20.7 |
| Serum albumine, mg/dL | 2.0 ± 0.81 |
| Proteinuria, g/day | 7.6 ± 4.6 |
| Hypertension, n (%) | 33 (66) |
| ACEI/ARB, n (%) | 46 (92) |
| Cyclosporine, n (%) | 33 (66) |
| Follow-up, months | 55.1 ± 41.9 |
| **Outcomes** | |
| Any PR, n (%) | 21 (42) |
| Any CR, n (%) | 15 (30) |
| Relapse, n (%) | 14 (28) |
| Doubling creatinne, n (%) | 14 (28) |
| CrCl < 30 ml/min/1,73m$^2$ | 17 (34) |
| RRT, n (%) | 5 (10) |

Developing any remission was associated with a reduced risk of doubling Cr (OR 0.24, p=0.041), CrCl < 30ml/min/1,73m$^2$ (OR 0.05, p<0.001) and requirement for RRT (OR 0.07, p=0.018). Suffering a relapse after remission was not significantly associated with an increased risk of doubling Cr (p=0.495), CrCl < 30 ml/min/1,73m$^2$ (p=0.327) or RRT (p=0.523).

**Conclusions:** We found that most of the patients developed PR or CR during follow-up and achieving remission was the most significant factor associated with a reduced risk of RRT.

## PUB429

**Tacrolimus/Rituximab: A Promising Combination for Corticoresistant Idiopathic Nephrotic Syndrome in Adults** Michelle Elias, Charlene Levi, Sophie Chauvet, Elsa Guiard, Claire Trivin, Christian Jacquot, Eric Thervet, Alexandre Karras. *Nephrology, Hopital Europeen Georges Pompidou, Paris.*

**Background:** Resistance to corticosteroids is difficult to overcome when treating idiopathic nephrotic syndrome (INS), namely minimal change disease (MCD) and focal and segmental glomerulosclerosis (FSGS). Rituximab and calcineurin-inhibitors are efficient drugs for the treatment of steroid-dependent or frequently relapsing INS. However, there

is a lack of data on the effect of these treatments on corticoresistant (CR)-INS. This pilot study was conducted in order to test the efficacy of the tacrolimus/rituximab association in adults with CR-INS.

**Methods:** A total of 7 patients with CR INS (3 MCD, 4 FSGS) were enrolled in this study: 4 were corticoresistant from the beginning, 3 had developed resistance after a INS relapse. All patients had been unsuccessfully treated with 1mg/Kg/day of corticoids for an average of 16 weeks prior to the introduction of the immunosuppressive regimen. Our protocol treatment combined tacrolimus (target levels 4 to 8 ng/ml) and 4 weekly injections of rituximab (375mg/m2/wk). Complete remission (CR) was defined by an albuminemia above 30g/L and proteinuria below 0,05g/mmol of creatininuria. Patients were considered in partial remission (PR) if albuminemia was above 25g/L and proteinuria below 0,25g/mmol of creatininuria.

**Results:** After a mean follow up of 22 weeks, 4 patients showed CR and 3 were in PR, allowing a rapid tapering of corticosteroids. No significant side effects were observed, such as serious infectious disease or acute deterioration of renal function. Only 1 relapse was noted and was concomitant with a rise in the CD19+ B-cell levels.

| | Before RTX | At 6 months |
|---|---|---|
| Serum creatinin (µmol/l) | 104 (34-260) | 89.3 (76-126) |
| Serum Albumin (g/l) | 18.6 (4-32) | 36.6 (28-47) |
| Proteinuria (g/mmolCreat) | 0.858 (0.535-1.200) | 0.08 (0-0.200) |
| Steroids (mg/day) | 65.7 (20-80) | 8.9 (7.5-10) |

**Conclusions:** The Tacrolimus/Rituximab combination seems to be an efficient strategy for the treatment of patients with corticoresitant INS. Nevertheless, further controlled studies are necessary to confirm our preliminary data.

## PUB430

**Assessment of Predictive Factors for Resistance to Tonsillectomy and Steroid Pulse Therapy in Patients with IgA Nephropathy** Hiroaki Kikuchi, Hiroyuki Tanaka, Teiichi Tamura. *Dept of Nephrology, Yokosuka Kyosai Hospital, Kanagwa, Japan.*

**Background:** Tonsillectomy plus steroid pulse (TSP) therapy has been proposed as an effective method for obtaining clinical remission (CR), defined as negative proteinuria, in IgA nephropathy (IgAN) patients. However, it remains a challenge to predict outcomes for IgAN patients receiving TSP therapy. The purpose of this study was to identify the clinical and histological factors which affect obtaining of CR in IgAN patients treated by TSP therapy.

**Methods:** We retrospectively investigated 48 IgAN patients who were followed up for more than 18 months after TSP therapy between 2005 and 2012. Patients were divided into two groups according to whether CR was obtained (CR, n=28) or not (non-CR, n=20) 12 months after TSP therapy. The base line clinical characteristics and histological findings were compared between two groups.

**Results:** Univariate analysis revealed that there was a significant difference between non-CR and CR subgroups in amount of proteinuria (1.54±1.19 versus 0.94±0.89 g/day, p=0.04), male to female ratio (13/7 versus 8/20, p=0.01) and presence of chronic lesions including global sclerosis, segmental sclerosis and fibrous crescent (p=0.01). A multivariable logistic analysis demonstrated that resistance to TSP therapy depends on age at onset (p=0.03), presence of severe proteinuria (>0.5g/day) (p=0.01) and sex (p=0.01). Presence of chronic lesions was not significant in a multivariable analysis.

**Conclusions:** Older age, male and severe proteinuria were identified as predictive factors for resistance to TSP therapy in patient with IgAN. IgAN patients with these factors may require adjunctive therapy otherwise obtaining CR 12 months after TSP therapy.

## PUB431

**Successful Use of ACTHAR in Patient with Nephrotic Syndrome and Oral Steroid Intolerance** E.J. Barney,[1] E. McCann,[1] S. Sarani,[1] C.S. Huang,[1] P.C. Pham.[1] *[1]Nephrology and Hypertension, Olive View MC; [2]Kidney and Pancreas Transplant, UCLA, Los Angeles, CA.*

**Background:** ACTHAR gel (repository corticotropin injection) has been reported to successfully treat various resistant nephrotic syndromes including idiopathic membranous glomerulonephropathy (MGN), focal segmental glomerulosclerosis (FSGS), and IgA nephropathy (IgAN).

**Methods:** We report a case of nephrotic syndrome where patient successfully responded to ACTHAR gel after developing severe depression with oral prednisone. A 66 year old Filipino lady with a solitary left kidney due to donor right nephrectomy over 20 years prior, hypertension, dyslipidemia, and fully treated papillary thyroid carcinoma (currently cancer free), who presents with increasing urine protein to creatinine ratio (UPCR) of 4.9 g/g creatinine. HIV, RPR, hepatitis B and C screen, C3, C4, CH50, ANA, ANCA, and serum and urine protein electrophoresis and immunofixation were negative. Routine malignancy screen appropriate for age and LDH were negative. As patient only has a single kidney, no biopsy was performed. She was treated empirically with oral prednisone at 60 mg daily for the presumed top three differential diagnoses of FSGS, IgAN, or MGN (based on clinical history) after risks and benefits were fully explained. At 1- and 2- month-follow-up, her proteinuria decreased to 1.8 g/g Cr. Unfortunately, patient reported intolerable severe depression including crying spells, feeling of doom alternating with anxiety, and easy bruising, and self-discontinued the prednisone. Her proteinuria increased back up to 5.2 g/g Cr in the subsequent 5 months. After a full discussion with patient, she agreed to undergo a trial therapy with ACTHAR gel at 80 units/mL subcutaneously twice weekly in addition to losartan and simvastatin. Follow-up at 2- and 4 months revealed a gradual fall in UPCR to 1.5 and 0.95 g/g Cr, respectively. Patient reports feeling well without any mood altering or easy bruising effects.

**Conclusions:** ACTHAR gel may be considered in patients with nephrotic syndromes who cannot tolerate severe mood altering and easy bruising side effects of oral prednisone, but whose kidney disease is steroid-responsive.

## PUB432

**Clinicopathological Characteristics of Japanese Patients with ANCA-Positive Microscopic Polyangiitis with Relapse and Infection** Kiyoki Kitagawa,[1] Shinji Kitajima,[2] Tadashi Toyama,[2] Yasunori Iwata,[2] Norihiko Sakai,[2] Kengo Furuichi,[2] Takashi Wada.[2] *[1]Div of Internal Medicine, Kanazawa Medical Center, Kanazawa, Ishikawa, Japan; [2]Div of Nephrology, Kanazawa Univ Hospital, Kanazawa, Ishikawa, Japan.*

**Background:** Relapse and infection are key prognostic factor for ANCA-positive microscopic polyangiitis (mPA). We examined clinicopathological characteristics in mPA patients with relapse and infection.

**Methods:** A total of 46 patients who were diagnosed as mPA with crescentic glomerulonephritis and achieved remission from 2002 to 2012 was examined in this study. Follow-up period was from initial treatment to relapse / infection or March 31, 2013 (mean period 1152 days).

**Results:** Ten patients (22%) were relapsed and 16 patients (35%) developed infection during the follow-up period (Relapse group: mean age 63.6±10.6, female 8, male 2. Non-relapse group: mean age 67.0±9.3, female 16, male 20. Infectious group: mean age 69.1±7.4, female 10, male 6. Non-infectious group: mean age 65.6±10.6, female 14, male 16). The levels of vasculitis damage index (VDI) at remission were higher in relapse group than non-relapse group (Relapse group 6 (5-6) (median and IQ ranges (25th and 75th percentiles)), non-relapse group 2 (2-4), p<0.01). Cyclophosphamide (CY) therapy for remission induction was frequently used in infectious group (Infection group 43%, non-infection group 17%, p<0.05). In the analysis using the Cox regression model, the levels of VDI were related to relapse (HR 3.32, 95% CI 1.23-8.93, p<0.05), and the use of CY was related to infection (HR 4.07, 95% CI 1.32-12.5, p<0.05). Neither clinical findings (urinalysis, serum creatinine, CRP, ANCA level, and dose of prednisolone) nor pathological findings (% of crescentic formation and the intensity of interstitial fibrosis) at initial treatment had statistical difference between each group.

**Conclusions:** In conclusion, the levels of VDI at remission were related to relapse, and the use of CY therapy was related to infection in patients with mPA.

*Funding:* Government Support - Non-U.S.

## PUB433

**Renal Survival in Anti-Glomerular Basement Membrane Antibody Disease** Jason M. Kidd, Patrick H. Nachman. *UNC Kidney Center, UNC Hospitals, Chapel Hill, NC.*

**Background:** Anti-Glomerular basement membrane antibody (anti-GBM) disease presents with renal failure and is often accompanied by pulmonary hemorrhage. We studied our inception cohort to compare our patient population and outcomes to those that have been previously reported as a method of quality assurance.

**Methods:** Inception cohort study of 25 patients with diagnosis of anti-GBM disease based on renal biopsy. Age, sex, race, smoking history, serum creatinine on admission, need for dialysis during first admission, presence of pulmonary hemorrhage, ANCA positivity, renal biopsy results and renal survival were recorded.

**Results:** 25 patients were studied. Median age was 47 years (interquartile range (IQR): 24.5, 62) 44% were female, 90% Caucasian (based on available data), 48% had a history of tobacco use. Median serum creatinine on admission was 6.5 mg/dl, median estimated GFR by MDRD equation was 8 ml/min/1.73m^2 (IQR: 5,16) and 36.8% were ANCA positive. 44% of patients had pulmonary hemorrhage. 76% needed hemodialysis during their initial presentation. 96% of patients received glucocorticoids, 88% received plasmapheresis with a median of 6 treatments and 80% received cyclophosphamide. All patients who had 100% crescents on renal biopsy became dialysis dependent. All patients survived to hospital discharge. 87% (20/23) had reached end stage kidney disease (on dialysis or had received transplant) at last follow up.

**Conclusions:** Patients with anti-GBM disease frequently present with very severe renal failure, either due to the fulminant nature of the disease, or to delay in diagnosis. Our retrospective study confirms the previously reported poor renal outcomes of patients with anti-GBM and severe renal failure (Cr > 5.7mg/dL )(1). Our current therapy appears effective at preventing death from pulmonary hemorrhage, but is insufficient in reversing renal failure. Early diagnosis and perhaps more intensive therapy are needed to improve renal recovery.

[1] Levy JB et al. Long Term Outcome of Anti-Glomerular Basement Antibody Disease Treated with Plasma Exchange and Immunosuppression. Ann Intern Med. 2001; 134: 1033-42.

## PUB434

**Clinical Outcome of ANCA-Related Nephritis: Single Center Experience** Arzu Velioglu,[1] Serdar Osman Nalcaci,[1] Gurdal Birdal,[1] Derya Oztas,[1] Izzet Hakki Arikan,[1] Mehmet Koc,[1] Haner Direskeneli,[2] Serhan Tuglular,[1] Cetin Ozener.[1] *[1]Nephrology, Marmara Univ School of Medicine, Istanbul, Turkey; [2]Rheumatology, Marmara Univ School of Medicine, Istanbul, Turkey.*

**Background:** Standard therapeutic regimens for ANCA-related glomerulonephritis have not been established. Treatment usually planned individually depending on disease status. In patients required hemodialysis at the beginning, response to treatment may vary

according to the intensity of the treatment. In this retrospective study, we reviewed the clinical course, treatment and outcome of patients with ANCA-related glomerulonephritis.

**Methods:** We evaluated 28 patients with ANCA-related glomerulonephritis (mean age: 54.8±13.8 years, F/M: 9/19). Clinical characteristics and outcomes of patients were investigated.

**Results:** Wegener's Granulomatosis in 14 patients, microscopic polyangiitis in 14 patients had been diagnosed. Baseline creatinine level was 3.8±3.5 mg/dl. 21 patients (75%) had active urinary sediment. 15 patients (53%) were needed hemodialysis at the beginning. Crescentic glomerulonephritis was found in 21 patients. All patients received steroid treatment. 23 patients were received intravenous cyclophosphamide. 13 patients also underwent plasmapheresis. The mean follow-up time was 40.7±44.8 months. End-stage renal disease (ESRD) developed in 6 (21%) patients. 5 patients (17%) died due to infectious complications (pneumonia in 4, cytomegalovirus disease in 1). ESRD and death were significantly higher in patient required hemodialysis compared to the patients did not required hemodialysis (6/15 vs. 0/13, p=0.013; 5/15 vs. 0/13, p=0.031). There was no need for hemodialysis after plasmapheresis in 9 of 15 patients required hemodialysis. After the treatment, mean creatinine level was stabilized at 2.4±2.2 mg/dl.

**Conclusions:** Initial hemodialysis requirement is the most important factor for ESRD development and death in ANCA-related glomerulonephritis. Plasmapheresis in addition to steroid and cyclophosphamide improves the renal outcome in these patients. However, infectious complications, especially in terms of pneumonia, should be closely followed.

## PUB435

**Clinical Factors Affecting Cardio-Ankle Vascular Index (CAVI) in Patients with IgA Nephropathy of Low CAVI Values** Hideo Okonogi, Keita Hirano, Akihiro Shimizu, Nobuo Tsuboi, Yoichi Miyazaki, Makoto Ogura, Tetsuya Kawamura, Takashi Yokoo. *Div of Kidney and Hypertension, Dept of Internal Medicine, The Jikei Univ School of Medicine, Tokyo, Japan.*

**Background:** Cardio-ankle vascular index (CAVI) is commonly used as a non-invasive indicator of arterial stiffness. In general, CAVI of 9 or more is considered significant for assessment of atherosclerosis. In our previous study concerning IgA nephropathy (IgAN), hyaline change of afferent arterioles (HAA), which is one of the factors predicting adverse outcome, significantly correlated with CAVI, and the threshold of CAVI for diagnosis of the presence of HAA was 7.55 by receiver-operating characteristic analysis. Therefore, we examined the clinical parameters affecting CAVI in patients with IgAN of low CAVI values, i.e. those without marked atherosclerosis.

**Methods:** Thirty-three IgAN patients with CAVI of 8.5 or less at the time of renal biopsy were included. The relationship between CAVI and clinical findings including serum levels of inflammatory parameters were analyzed.

**Results:** CAVI significantly correlated with systolic blood pressure (BP) ($r=0.407$, $p<0.05$). Moreover, when patients were categorized into two groups by the CAVI=7.55, CAVI significantly correlated with lower estimated glomerular filtration rate (eGFR) (44 vs 67 ml/min/1.73m$^2$, $p<0.05$), higher uric acid (UA) (7.5 vs 6.2 mg/dl, $p<0.05$), and higher lipoprotein(a) (LP(a)) (39 vs 14 mg/dl, $p<0.05$). However, CAVI did not correlate with proteinuria, HbA1c, LDL-C, non HDL-C, remnant-like particle cholesterol, high-sensitivity C-reactive protein, D-dimer, and thrombomodulin, while these parameters correlated with CAVI in previous studies including patients with higher CAVI values.

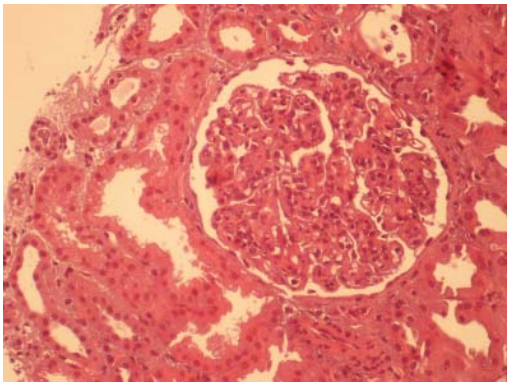
**Conclusions:** These results indicate that systolic BP, UA and Lp(a) correlate with CAVI in patients with IgAN of low CAVI values and, therefore, these clinical factors may be involved in formation of HAA in IgAN.

## PUB436

**Acute Kidney Injury and Diuretic-Resistant Anasarca in a Case of Idiopathic Membranous Glomerulonephritis** Claudio Angelini, Albania Calvetta, David Cucchiari, Manuel Alfredo Podesta', Elisa Merizzoli, Salvatore Badalamenti. *Humanitas Clinical and Research Center, Italy.*

**Background:** Membranous glomerulonephritis (MGN) is the most common cause of nephrotic syndrome in adult patients. Acute kidney injury (AKI) in this setting is a rare occurrence, usually related to complications of nephrotic syndrome (renal veins thrombosis), drugs toxicity (NSAIDs), or ensues on the basis of a superimposed immunologic process (anti-GBM, crescentic GN).

**Methods:** A 51 years-old woman presented to our unit with anasarca, developed in the previous two weeks. Laboratory tests revealed normal renal function (creatinine 0.7 mg/dL), nephrotic-range proteinuria (44 g/day) and macrohematuria. A renal biopsy was performed, showing diffuse thickening of capillary loops with focal endocapillary proliferation, focal tubular atrophy and interstitial fibrosis, but no signs of extracapillary proliferation.



Immunofluorescence showed parietal deposits of IgG and C3, confirming MGN diagnosis. An autoimmune panel and HBV/HCV screening tested negative. These findings and anti-PLA2R negativity raised the likelihood of paraneoplastic MGN: an accurate screening for malignancies was carried out, but resulted negative. Intravenous methylprednisolone (IVM) boluses were administered. During hospitalization, the patient developed AKI (creatinine up to 4.4 mg/dL), requiring hemodialysis.

**Conclusions:** AKI may have been caused by interstitial edema and tubular cells swelling, which have been described in some pediatric cases of AKI in minimal change disease. This hypothesis could not be confirmed by histology because these changes most likely occurred only after the biopsy was performed. Owing to the low gastrointestinal absorption rate, due to mucosal edema, IVM administration was maintained for the first month of therapy. Complete recovery from AKI was obtained, and proteinuria dropped to 22 g/day.

## PUB437

**Comparative Study between Primary and Secondary Focal Segmental Glomerulosclerosis** Vijay Sundaram Thanaraj, Muhammad Nauman Hashmi, Arvind Ponnusamy, Ajay Prabhakar Dhaygude. *Deaprtment of Renal Medicine, Lancashire Teaching Hospital, Preston, Lancashire, United Kingdom.*

**Background:** Focal Segmental Glomerulosclerosis (FSGS) is a common histological diagnosis encompassing variety of clinical presentations. Main types include primary and secondary. Immunohistochemistry shows IgM and C3 deposition in primary FSGS which are typically absent in secondary FSGS. Full-blown nephrotic presentation (proteinuria, oedema, hyperlipidaemia, and hypoalbuminaemia) is rare in secondary FSGS and main stay of treatment is rennin angiotensin aldosterone system (RAAS) blockade. While Nephrotic syndrome is common in primary FSGS and relentless progression to end stage renal disease (ESRD) is common though immunosuppression has been used with success for treatment. Although both primary and secondary FSGS are common, published data lacks comparative studies. In this study we compared degree of proteinuria, serum albumin, response to RAAS blockade, incidence of thrombo-embolism and ESRD.

**Methods:** This is a retrospective review of 51 patients with biopsy proven FSGS diagnosed between 1997 – 2008. Data regarding demographics, laboratory parameters, treatment details, incidence of thrombo-embolism and patient outcomes were collected.

**Results:** Average age at presentation was 50.33 ± 10.26 years and 54% were male. The follow up period was 2678.30 ±1627.6 days.

RAAS blockade therapy was initiated in 90% of secondary FSGS and in 76.19% of primary FSGS. Differences in the clinical features, laboratory parameters and outcomes are presented in Table 1.

| Parametres | Primary FSGS | Secondary FSGS | P value (paired t test) |
|---|---|---|---|
| Baseline Creatinine (umol/L) | 203.9±198.3 | 165.4 ±83.42 | P < 0.0001 |
| Baseline Proteinuria (grams/24hrs) | 7.07±5.5 | 2.5±1.5 | P < 0.0001 |
| Baseline Albumin (g/L) | 31.87±8.91 | 41±2.8 | P < 0.0001 |
| Pedal Oedema | 64% | 40% | |
| Incidence of VTE | 7.14% | 0% | |
| Progression to ESRD | 40.5% | 20% | |

**Conclusions:** 1] Classical nephrotic presentation was more common in Primary FSGS;
2] Response to RAAS blockade was superior in Secondary FSGS
3] Thrombo-embolism was absent in Secondary FSGS.

**Limitations:**
1] Relatively small sample size;
2] Retrospective study.

## PUB438

**Proteinuria in Severe Lupus Nephritis Correlates with Diffuse Foot Process Effacement** Preeti Gurnani,[1] David J. Cimbaluk,[2] Edmund J. Lewis,[1] William Whittier.[1] [1]*Nephrology, Rush Univ Medical Center, Chicago, IL;* [2]*Pathology, Rush Univ Medical Center, Chicago, IL.*

**Background:** Among the lesions of lupus nephritis, membranous glomerulonephritis (GN), mesangial GN, and minimal change glomerulopathy have all been reported to occur in patients with nephrotic range proteinuria associated with diffuse foot process effacement (FPE). This podocytopathy has not been described in other forms of lupus nephritis. The purpose of this study was to determine if any histopathologic characteristics correlate with nephrotic range proteinuria in severe lupus nephritis.

**Methods:** We conducted a retrospective clinicopathologic study of patients with lupus nephritis (N=265) at Rush University Medical Center. Mesangial and Membranous lupus GN were excluded. In patients with severe lupus nephritis (defined as ≥ 50% active segmental lesions, ISN/RPS Class III, IVs; and diffuse global lesions, ISN/RPS IVg) (N=63), demographic and clinical data were recorded and analyzed. Light, fluorescence (IF), and electron microscopy (EM) was used to detail the histologic characteristics and degree of FPE.

**Results:** Twenty-six patients (41%) had nephrotic range proteinuria (>3 grams proteinuria/gram creatinine [g/g]) and thirty-seven (59%) demonstrated subnephrotic range proteinuria (≤3g/g). Proteinuria was greater in the nephrotic patients (6.3±4.4 g/g compared to 1.3±0.9 g/g, p<0.001). There was a greater degree of FPE seen in the nephrotic compared to subnephrotic patients (foot process width [FPW], 1991±1655 nanometers (nm) compared to 939±467 nm, p<0.001). There was a positive correlation between FPE and proteinuria, r=0.694, p<0.0001. In ISN/RPS Lupus Class III and IVs, the proteinuria was less compared to Class IVg (2.5±3.0 g/g compared to 5.5±5.0 g/g, p=0.004). FPW in biopsies with Class III and IVs was also less (Class III, 1105± 712 nm compared to Class IV, 1991±1840 nm, p=0.008).

**Conclusions:** In severe lupus nephritis, a distinguishing morphologic feature in patients with nephrotic range proteinuria is diffuse foot process effacement. Given a lack of difference in other histologic characteristics, nephrotic range proteinuria in severe lupus nephritis may be a manifestation of concomitant podocytopathy.

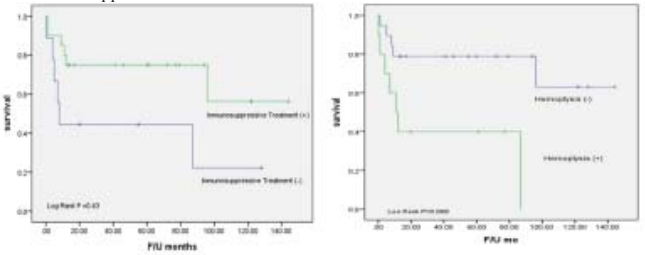
## PUB439

**Clinical Features and Outcome in Patients with ANCA-Associated Vasculitis (AAV) with Renal Involvement in Korea: An 12 Year Retrospective Single Center Study** Hyun Chul Chung, Jong Soo Lee, Jongha Park. *Univ of Ulsan College of Medicine, Ulsan Univ Hospital, Ulsan, Korea.*

**Background:** Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are major causes of rapid progressive glomerulonephritis. But they are not common in Korea, therefore little is known about their clinical features and outcome.

**Methods:** We respectively investigated 29 Korean patients with renal involvement in relation to AAV during 2001-2012.

**Results:** This study comprises 2 GPA, 22 MPA or 5 Renal limited vasculitis(RLV) patiensts (mean age 61.22±13.67 years), follow up for a median of 41 mo(0.1 – 144). ANCA was detected by ELISA in 28 (96.6%) of patient, of whom 23 had MPO-ANCA, 3 had PR3-ANCA, 2 had both, and one patient had negative. BVAS and lung involvement at diagnosis were 17.1±6.4 and 55.2%. Four patients(13.8%) were diagnosed after maintenance dialysis more than one month. Other twelve patients (41.4%) required dialysis at initial admission period, and 7 patients among them treated with immunosuppressive drug. 4 patients were able to be taken off dialysis during treatment. Twenty patients treated with prednisolone, cyclophosphamide and/or plasmapheresis, and nine patients received supportive care and/or hemodialysis. During follow up period, 17 relapses in 11 patients were recorded. Eleven patients (37.9%) resulted in death and other ten patients (34.5%) developed end-stage renal disease during their disease course. Mortality was associated with presence of hemoptysis, and immunosuppressive treatment.




**Conclusions:** Immunosuppressive treatment decreased the risk of mortality (RR, 0.38; CI, 0.11 to 1.33) and hemoptysis increased the risk of mortality (RR, 6.05; CI, 1.06 to 34.42) in Korean AAV patients.

## PUB440

**High Prevalence of Thyroid Diseases in Patients with Glomerulonephritis** Mohammad Kazem Fallahzadeh,[1,2] Sedighe Jafarian,[1] Ali Zamani,[1] Joan Blondin,[2] Mohammad Mahdi Sagheb.[1] *[1]Nephrology Research Center, Shiraz Univ of Medical Sciences, Shiraz, Islamic Republic of Iran; [2]Dept of Medicine, LSUHSC, Shreveport, LA.*

**Background:** There are few reports on the association of thyroid diseases with different types of glomerulonephritis (GN). However, this association has not been evaluated in a systematic study so far. The aim of this study was to evaluate the prevalence of thyroid diseases in patients with different types of GN.

**Methods:** All patients who had renal biopsy at our center in 2012 and were diagnosed with primary GN were invited to participate in this case-control study. All cases of secondary GN were excluded. A similar number of age and sex matched subjects from general population were enrolled as controls. Thyroid function test was done for all consenting cases and controls.

**Results:** Of all invited patients with primary GN, 98 (male/female: 46/52; mean age: 38.4±11.9 years) were enrolled as cases; 98 subjects (male/female: 46/52; mean age: 38.7±11.7 years) from general population were enrolled as controls. Cases and controls were similar in terms of age and sex (p=0.83 and p=1.0, respectively). Of 98 controls, 86 were euthyroid, 8 were hypothyroid and 4 had subclinical hypothyroidism. The prevalence of different types of GN and different types of thyroid diseases in cases are listed in the following table:

| | Euthyroidism | Hypo-thyroidism | Subclinical Hypothyroidism | Subclinical Hyperthyroidism | Hyper-thyroidism |
|---|---|---|---|---|---|
| Membranous GN (n=39) | 21 | 8 | 10 | 0 | 0 |
| Mesangioproliferative GN (n=22) | 17 | 3 | 1 | 1 | 0 |
| Focal Segmental Glomerulosclerosis (n=18) | 13 | 3 | 1 | 1 | 0 |
| Membranoproliferative GN (n=13) | 7 | 2 | 3 | 0 | 1 |
| Minimal Change Disease (n=5) | 2 | 1 | 1 | 0 | 1 |
| IgA Nephropathy (n=1) | 0 | 0 | 1 | 0 | 0 |
| Total | 60 | 17 | 17 | 2 | 2 |

The total prevalence of thyroid diseases was significantly higher in patients with GN compared with controls (39% vs 12%, p<0.001). Moreover, thyroid diseases were more common in some types of GN like minimal change disease (60%), membranous GN (46%) and membranoproliferative GN (46%).

**Conclusions:** Compared with general population, thyroid diseases are more common in patients with GN, particularly in some types like minimal change disease, membranous GN and membranoproliferative GN.

## PUB441

**Patient Satisfaction after Electronic Health Record Implementation in a Nephrology Outpatient Setting** Tarik Noureldeen, Neeharika Muddana, Annie Culver, Swati Arora, Kalathil K. Sureshkumar, Richard J. Marcus, Barbara A. Clark. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

**Background:** Electronic health records (EHR) are currently being implemented throughout the nation. The goal is to improve the quality of health care by providing efficient care, reduce medical errors and increase overall patient's satisfaction.

**Methods:** We conducted anonymous cross-sectional patient surveys from Nov 2011 to Dec 2012 during 3 phases of EHR implementation (pre, start and post-EHR). Surveys were done in the domains of satisfaction, care, distraction, waiting time and visit length. The primary objective was to assess patient's satisfaction with physician's visit with respect to EHR implementation.

**Results:** A total of 187 surveys were obtained. Visit satisfaction decreased (P=0.02), exam room wait time increased (P=0.009), and visit length increased (P=0.03) after EHR implementation along with a trend towards perception of improved care as shown in the table.

| | Pre-EHR (n=52) | Start-EHR (n=41) | Post-EHR (n=94) | p value |
|---|---|---|---|---|
| | | | | |
| Visit Satisfaction (-2 to 2) | 1.9±0.6 | 1.7±0.4 | 1.6±0.7 | 0.02 |
| Improve Care (-2 to 2) | 0.4±1.2 | 0.8±1.1 | 0.8±1.1 | 0.12 |
| MD Distraction (-2 to 2) | –1.6±0.9 | –1.4±1.0 | –1.5±0.9 | 0.39 |
| Waiting Room Wait (min) | 8±7 | 11±12 | 9±10 | 0.40 |
| Exam Room Wait (min) | 9±6 | 12±9 | 15±15 | 0.009 |
| Visit Length (min) | 33±19 | 37±17 | 42±19 | 0.03 |
| MD Charting (min) | 10±10 | 13±13 | 14±12 | 0.21 |

**Conclusions:** After implementation of EHR in our nephrology outpatient clinic, patient's wait time in the exam room and length of visit increased significantly. We believe this could be related to the learning curve phenomenon regarding EHR use among staff. Overall patient satisfaction with the visit decreased as result. We plan to repeat the survey after EHR has been implemented for a year to get past the learning curve. Despite these observations, it is encouraging to note a trend towards improved patient perception regarding overall care.

## PUB442

**Utility of CD4+ T Lymphocyte Counts to Determine Need for PJP Prophylaxis in ANCA Vasculitis** William Franklin Pendergraft,[1,2,3] Andrew P. Murphy,[3] Karen A. Laliberte,[3] John Niles.[2,3] *[1]Joint Nephrology Fellowship Program, Massachusetts General Hospital (MGH) and Brigham and Women's Hospital, Boston, MA; [2]Div of Nephrology, MGH, Boston, MA; [3]Vasculitis and Glomerulonephritis Clinic, MGH, Boston, MA.*

**Background:** Our group currently manages over 400 patients with anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis, the majority of which were exposed previously to numerous immunosuppressants and currently receive rituximab every four to six months to maintain durable remission. Prophylactic antibiosis is employed universally in these patients to prevent *Pneumocystis jirovecii* pneumonia (PJP). It is unclear if PJP prophylaxis is needed once patients are transitioned to rituximab alone, and also if CD4+ T cell counts can be used to guide the use of prophylaxis. We sought to investigate the levels of CD4+ T cells in our cohort.

**Methods:** We reviewed absolute CD3+CD4+ T cell counts in patients with ANCA vasculitis in our cohort who were undergoing rituximab-induced continuous B cell depletion. Additional standard clinical and serologic parameters were measured and compared.

**Results:** 215 patients treated by our group from April 2006 through June 2013 underwent continuous CD20-positive B cell depletion with rituximab. None of these patients developed PJP. Furthermore, there has not been a case of PJP in an ANCA vasculitis patient in the past fifteen years in our center. CD3+CD4+ T cell counts were measured in 59 ANCA vasculitis patients (64% (n=38) MPO-ANCA, 56% (n=33) women). Nine of these patients

were not receiving rituximab. B cells were undetectable in the rituximab group. Median absolute CD3+CD4+ count was 461 cells/mm$^3$ (S.D. 284, normal range 348-1456 cells/mm$^3$). Four patients had CD3+CD4+ T cell counts < 200 cells/mm$^3$.

**Conclusions:** CD3+CD4+ T cells were within normal limits in the majority of patients tested despite a history of prior cytotoxic therapy. These preliminary results suggest that cessation of PJP prophylaxis in patients receiving RTX monotherapy could be considered.

*Funding:* Clinical Revenue Support

## PUB443

**Pneumococcal Sepsis in Patients with Systemic Lupus Erythematosus**

Tingting Li, Anitha Vijayan. *Renal Div, Washington Univ, St. Louis, MO.*

**Background:** Severe infections caused by *Streptococcus pneumoniae* are an important cause of morbidity and mortality in pts with systemic lupus erythematosus (SLE). Immunosuppressive therapy (IS), functional asplenia, hypocomplementemia, defective opsonization and phagocytosis, impaired humoral and cellular immunity, and nephritis may contribute to increased susceptibility and severity of pneumococcal infections in this pt population. We aim to describe pt characteristics, clinical presentation and outcome of SLE pts with pneumococcal sepsis (PS).

**Methods:** After retrospectively identifying all cases of PS in SLE pts over a 13-year period at a single center using ICD-9 codes, we conducted a thorough chart review.

**Results:** Thirteen cases were identified in 11 pts between 2000 and 2013. One was male, 7 AA, 4 white, aged 19-60. Ten cases had active SLE, of which 7 had nephritis. Complement levels were low in 8 cases and not available in 3. IgG level was measured in 6 pts and was low in 1. Six pts were on steroids (10-60 mg/d) on presentation with 2 taking concurrent mycophenolate. One pt was on leflunomide. The rest were not on IS. Three cases had prior splenectomy. The rest had normal-sized spleen on imaging. Blood smear examination in 2 pts did not suggest functional asplenia. Three of 4 cases who had prior Pneumovax® were vaccinated >5 years prior to presentation. All cases of PS were confirmed with positive cultures (12/13 in blood and 1/13 in pericardial fluid). Twelve required ICU stay and 9 had septic shock. Sources of PS included tubo-ovarian abscess (1), pneumonia/empyema (3), pericarditis (1), meningitis (2), endocarditis (2), cellulitis (2), septic arthritis (2). Infection source was not identified in 2 cases. Two pts had recurrent PS, 5 and 12 years after the initial episode. 10/11 pts survived.

**Conclusions:** Pts with SLE are susceptible to PS, even during periods of lupus inactivity or in the absence of IS therapy, implicating the role of immune dysfunction in SLE. Physicians should have heightened awareness for PS in this pt population. Pneumovax® should be recommended for all SLE pts and repeat vaccination should be given after 5 years. Per recent CDC recommendation, vaccination with Prevnar 13® should also be a consideration.

## PUB444

**Analyses of Renal Dysfunction and Hyperuricemia in Patients with Chronic Hepatitis C Receiving Telaprevir-Based Triple Therapy**

Satoru Ogahara,[1] Kenji Ito,[1] Yasuhiro Abe,[1] Maho Watanabe,[1] Takao Saito,[2] Hitoshi Nakashima.[1] *[1]Div of Nephrology and Rheumatology, Internal Medicine, Fukuoka Univ School of Medicine, Fukuoka, Japan; [2]General Medical Research Center, Fukuoka Univ School of Medicine, Fukuoka, Japan.*

**Background:** Telaprevir (TVR) are available in combination with peginterferon and ribavirin for the treatment of chronic hepatitis C. It was reported that TVR caused a limited case of transient and reversible increase in serum uric acid (UA) and decrease in eGFR in patients who were enrolled in phase 3 trial. Recently, reports of severe renal dysfunction and hyperuricemia were increasing in post-marketing surveillance. We evaluated the association between the background and adverse effects especially, renal dysfunction and hyperuricemia in patients treated by telaprevir-based triple therapy (TT).

**Methods:** Fifty seven patients were administrated TVR. We measured the change of eGFR and serum UA, the percent of fractional excretion of UA (FEua), %recovery of eGFR and other laboratory findings.

**Results:** All patients decreased eGFR after TT including subclinical states. The decrease change rate of eGFR was divided into quartiles (Q1: 70-48%, Q2: 47-39, Q3: 38-32, Q4: 31-12.There were no association between quartiles of the change of eGFR and background of patients on administration (age, sex, eGFR, hemoglobin, albumin, UA). Compared with Q1, which had a 77% recovery of eGFR, there were lower in Q3 (91%, $p<0.01$) and Q4 (99%, $p<0.01$). Compared with Q1, which had a 93% increased change of UA, there were higher in Q3 (60%, $p=0.024$) and Q4 (60%, $p=0.027$). A mean FEua had 3.95±1.55% after TT. There were no association between FEua and quartiles of change of eGFR. Yet one case of patients had a renal hypouricemia (UA:0.4mg/dl), eGFR was decreased as well as other patients.

**Conclusions:** Renal dysfunction and hyperuricemia frequently occur after TT. The stronger a patient occurs renal dysfunction, the longer a patient has a time of renal recovery. Hyperuricemia appears to be the cause of lower UA excretion. Renal dysfunction is independent of hyperuricemia. It should be noted that eGFR is frequently measured after TT. We need to try uricosuric drug such as benzbromarone.

## PUB445

**Use of Monitoring Equipment and Fluoroscopy in the Insertion of Haemodialysis Permanent Catheter Essential or Ancillary – A Four Year Prospective Comparative Study**

Ebadur Rahman, Naveed Aslam, Nader Mohamed Omran, Seddeg Younis, Ahmed Soliman, Raees Farhan Mushtaq, Dujanah Hassan Mousa. *Nephrology, PSMMC.*

**Background:** Monitoring equipments are used during the insertion of central lines and permanent catheters in hospital settings however, in proficient hands these equipments might not be essential. Hence a comparative study was done in Riyadh Military Hospital.

**Methods:** 388 consecutive patients were included in the study from June 2009 to June 2013. The Hemodialysis permanent catheter insertion was done by two groups: Nephrologists without monitoring equipment or fluoroscopy and vascular surgeons with monitoring equipment and fluoroscopy. Blood pressure, pulse and ECG were monitored by the monitoring equipment. The overall immediate complications and failure rates between the two groups were analyzed by SAS Software version 9.2. The unstable patients (with hypotension, with pacemaker or arrhythmia) were not included.

**Results:** The median age of the study group was 59 years and 52% patients were male. 51% of the cases were done by nephrologists. Overall complications and failure rates were less than 1% in both the groups. Minor complications like Hematoma exit site oozing were also less than 1% in both groups. There were no fatal complications in either of the groups. Each group had one major complication ( pneumothorax) which did not require any intervention. Statistical analysis was not significant regarding complications and failure rates between the two groups.

**Conclusions:** Monitoring equipments are used universally for catheter insertion but did not show to improve procedure related morbidity in stable patients.

*Funding:* Government Support - Non-U.S.

## PUB446

**Successful Reduction in Interdialytic Weight Gain Using Decreased Sodium Dialysate Concentration**

Madhavi Katikaneni, Carol J. Blanchard, Roger F. Carbajal Mendoza, Donald I. Baumstein, Ashok P. Chaudhari. *Nephrology, Metropolitan Hospital, New York, NY.*

**Background:** Excess Interdialytic weight gain (IDWG) is associated with increased morbidity and mortality in hemodialysis patients. As a quality improvement project in a outpatient hemodialysis unit we sought to reduce IDWG by lowering dialysate sodium after first trying patient counseling. Aim was to decrease the IDWG by 1lb in patients with IDWG>6lb in 3 months time.

**Methods:** 14 out of total 19 patients on the first 2 shifts of Monday Wednesday Friday group had greater than 6lb of IDWG on Monday after the 3 day interdialytic interval. Of those 14 patients 8 are male and 6 are female with age range 38 to 73 yrs and average dialysis vintage of 3.7yrs. 86% have diabetes, 100% have hypertension, and 78% have heart failure. 9 are Hispanic, 4 African American and 1 Korean. Average number of antihypertensive medications is 3. For 2 months patients were counseled by staff using various methods. 5 out of 14 (36%) patients achieved the goal of decreasing IDWG with counseling alone. Subsequently in 8 out of 14 patients dialysate sodium was reduced to 138 meq/L from 140meq/L. One patient was excluded due to hypotension at baseline.

**Results:** In 1st and 3rd week 5 out of 8 (62%) of them reached the target. In 2nd and 4th week 4 out of 8 (50%) of them reached the target. No hypotensive episodes were noted. Antihypertensives were unchanged. In 36% of patients goal of lower IDWG was achieved by counseling. In those who did not improve with counseling at least 50% of those patients achieved the goal by reducing dialysate sodium. Taken together in 9 out 14 (64%) achieved reduction in IDWG with our intervention.

| | Number of patients | Average IDWG(lbs) for 14pts |
|---|---|---|
| Initial | 14 | 7.9 |
| After counseling phase | 9 | 7.48 |
| After counseling plus sodium 138meq/L dialysate | 5 | 6.78 |

Table 1. Patients with excess IDWG

**Conclusions:** There is no established standard of acceptable IDWG but excess weight gain leads to more fluid removal increasing the complications. In our study we found that decreasing dialysate sodium increases goal of lesser IDWG beyond that obtained through patient education and staff support.

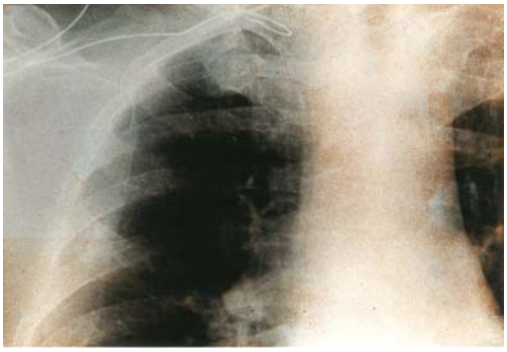
## PUB447

**Knot a Routine Central Line: Case Report of a Tangled Guidewire**

Sunny Kar, Pran M. Kar. *Pran M Kar MD PA, Orlando, FL.*

**Background:** Central venous catheter placement is a common procedure. Complications resulting from line placement are not uncommon. This is a case report of a knotted guidewire.

**Methods:** The patient is a 74 year old male with a history of end status renal disease (ESRD) who presented to the ER with a clotted graft in his left arm. His lab work revealed a potassium level of 6.1 mEq/L, last hemodialysis was 48 hours prior. A decision to insert a catheter for emergency dialysis was made. Due to failed attempts to cannulate his internal jugular, a subclavian catheter placement was planned. Complications including, but not limited to, bleeding, infection, pneumothorax, and death were explained to the patient and an informed consent was obtained. Using the Seldinger technique the subclavian vein was cannulated. The guidewire was inserted through the introducer and a slight resistance was felt after 13 cm of insertion. The introducer was then withdrawn and replaced with a dilator. Retraction of the guide was met with significant resistance. An X-ray revealed a tangled wire.



The guidewire was extracted in the operating room using a larger bore dilator.

**Conclusions:** Compilation rate for all lines is as high as 19%, and range from cardiac conduction abnormalities to embolization of a broken wire. Guidewire tangling is an unusual complication, marked by resistance during wire advancement. It is proposed that use of excess force can worsen the knots or kinks that may have formed. Caution should be extended to dilator insertion to avoid agitation of the guide. It is proposed that to reduce potential complications, guidewires should not be advanced beyond 18 cm. We caution that the guidewire be neither retracted nor advanced with force if any resistance is encountered during placement. Instead, use of a larger bore dilator should be encouraged to recover damaged guidewires. We propose that standard consents be modified to include guidewire related morbidity.

## PUB448

### Can Renal Artery Revascularisation Be Performed Safely as a Day-Case Procedure?

Richard W. Corbett, Alok Singh Chauhan, Peter Hill. *Imperial College Renal and Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

**Background:** Percutaneous renal artery revascularisation continues to have a role in the treatment of both native and transplant renal artery disease. Given the complications associated with this intervention, patients have traditionally been observed for 24 hours prior to discharge. This study was designed to assess whether an early discharge after 6 hours of observation could be performed without an impact on the incidence of significant complications.

**Methods:** All individuals undergoing out-patient percutaneous renal artery revascularisation within our centre were retrospectively identified over two periods of four months, the initial period with patients observed for at least 24 hours and the subsequent second period with these procedures performed as a day-case. Blood pressure, serum creatinine and haemoglobin results were obtained for all patients both prior to and at an interval of five to ten days following the procedure. Requirements for readmission or re-intervention were also examined.

**Results:** 32 interventions (12 transplant, 20 native) were performed in the initial period and 23 interventions (8 transplant and 15 native) during the day-case four months. In the latter group three individuals were admitted for overnight observation while one individual required admission for treatment of pulmonary oedema that developed directly following angiography. In neither group was there a requirement for re-admission, re-intervention nor was there identified pseudo-aneurysm formation or symptomatic hypotension. In the initial group Δ sCr (change in serum creatinine at time of clinic follow-up from pre-procedure) [mean ± sd] was 3.3 ± 16.7 μmol/l compared to 10.4 ± 23.1 μmol/l in the day-case group (p = 0.06). Equally, no difference in change of haemoglobin or systolic blood pressure was seen between the groups.

**Conclusions:** In this small study no differences in complications could be demonstrated between individuals discharged at six hours as compared to 24 hours following percutaneous intervention to either native or transplant renal arteries. Though limited by small numbers this study would support a move to performing these procedures as a day-case.

## PUB449

### Pharmacokinetic Study of Cefazolin in Short-Daily Hemodialysis

Katie Palmer,[1] Sarbjit Vanita Jassal,[1] Robert M. Richardson,[1] Marisa Battistella.[1,3] *[1]Univ Health Network, Toronto, Canada; [2]Univ of Toronto, Toronto, Canada.*

**Background:** A number of centres across Canada offer short-daily hemodialysis (SDHD) treatments. Dialysis sessions are on average two hours in duration performed six times per week. As with intermittent hemodialysis, infections requiring treatment with cefazolin are common. To date, cefazolin pharmacokinetics have not been described in patients undergoing SDHD. The primary objective of this study was to investigate the effect of SDHD on the pharmacokinetics of cefazolin.

**Methods:** Prospective, observational, single-centred study. Five non-infected adults undergoing SDHD were enrolled. Participants received a 1 g intravenous infusion of cefazolin after SDHD on study day one, and a second dose after SDHD on day two. To determine the concentration of cefazolin, six blood samples were drawn at 0, 1, 2, 2.3, 4, and 24 hours after initiation of dialysis on day two, and two dialysate samples were drawn at 1 and 2 hours after initiation of dialysis on day two. Samples were analyzed using high-performance liquid chromatography (HPLC) and pharmacokinetic parameters were determined.

**Results:** Median off-dialysis clearance was 3.6 mL/min (IQR: 2.6-4.8 mL/min) and median on-dialysis clearance was 41.9 mL/min (IQR: 37.7-87.3 mL/min). Median off-dialysis half-life was 25.8 hours (IQR: 22.7-27.3 hr) as compared to a median on-dialysis half-life of 1.6 hours (IQR: 1.6-2.2 hr). The percent removal of cefazolin during dialysis varied between patients, ranging from 34.2 to 93.3 % with a median of 56.2% (IQR: 34.2-56.2%).

**Conclusions:** Estimated cefazolin dialysis clearance is higher than previous estimates with thrice weekly regimens. Current dosing recommendations appear to achieve serum drug concentrations that meet existing standards for microbiological eradication. Further study is warranted in a larger cohort of patients.

## PUB450

### Biopsy-Derived Immortalized Human Proximal Tubule Cells: A New Model to Study the Role of Pharmacogenetics in CNI-Associated Nephrotoxicity

Noel Knops,[1,4] Dirk R. Kuypers,[2] Rosalinde Masereeuw,[3] Elena N. Levtchenko.[1,4] *[1]Pediatric Nephrology, UZ Leuven, Leuven, Belgium; [2]Nephrology, UZ Leuven, Belgium; [3]Pharmacology and Toxicology, Radboud Univ, Nijmegen, Netherlands; [4]Labarotory for Pediatrics, Dept of Development & Regeneration, KU Leuven, Leuven, Belgium.*

**Background:** Calcineurin inhibitors (CNI) constitute the basis of most immunosuppressive regimes in transplantation, but are associated with the development of histological lesions leading to kidney failure. CNI's are metabolized by CYP3A and excreted by Pgp (*MDR1*), primarily in the gut and liver but also in renal proximal tubule cells (PTC). Clinical studies demonstrated a relation between common variants of *CYP3A5*/*MDR1* genes and CNI-associated nephrotoxicity (CNIT). The mechanism is unknown. Here we established a model of human PTC that can be used to study the pathogenesis of CNIT.

**Methods:** A method was developed to culture cells from a protocol biopsy in renal allograft recipients. Primary cells were transfected with SV40T and hTERT virus for conditional immortalization and differentiation. Subclones were selected based upon specific PTC markers (AQP1 and CD13) using Western Blot (WB) and FACS. Light and scanning electron microscopy were performed to detect PTC morphology. PCR and sequencing was used to assess genotype, next to quantative RT-PCR and WB. CYP3A5 activity was assessed by midazolam(MDZ) hydroxylation using LC-MS and Pgp activity by calcein accumulation.

**Results:** From 27 out of 38 biopsies cell lines were generated. Based upon genotype, 10 subclones with PTC biomarkers, cobblestone appearance and brush border microvilli were selected. CYP3A5 and Pgp mRNA and protein expression was confirmed. *CYP3A5*1*carriers had increased 1OH/4OH MDZ formation v (0,83 vs 0,41; p<0,05). Pgp activity was confirmed by 138% (95% CI: 113-163) calcein accumulation, but not related to *MDR1 3435C>T* genotype.

**Conclusions:** PTC cell lines can be generated from a kidney biopsy and demonstrate functional expression of genes involved in CNI metabolism after immortalization. Differences in protein function were detected for *CYP3A5* but not *MDR1* genotypes. This in vitro model can be used to study the role of pharmacogenetic variation in CNIT.

*Funding:* Pharmaceutical Company Support - Astellas

## PUB451

### Significance of Serum Cholesterol Concentration for the Cyclosporine Treatment of Idiopathic Membranous Nephropathy with Steroid Resistant Neprhotic Syndrome

Takao Saito,[1] Yoshie Sasatomi,[2] Satoru Ogahara,[2] Maho Watanabe,[2] Hitoshi Nakashima.[2] *[1]General Medical Research Center, Fukuoka Univ School of Medicine, Fukuoka, Japan; [2]Div of Nephrology and Rheumatology, Fukuoka Univ School of Medicine, Fukuoka, Japan.*

**Background:** For the treatment of idiopathic membranous nephropathy (IMN) with steroid resistant nephrotic synodrome (SRNS), the combination of prednisolone (PSL) and cyclosporine (CYA) is one of the important tools, and therapeutic drug monitoring (TDM) of CYA is useful for predicting the effect. Based on data of receiver operating characteristic (ROC) curves, we proposed 600 ng/mL for the therapeutic target point of C2 (ASN 2009). However, this propose was criticized, because CYA may be influenced by various factors in the nephrotic condition. Now we compare C2 with several factors statistically and consider TDM of CYA again.

**Methods:** Thirty-seven IMN patients with SRNS were treated with PSL+CyA for 48 weeks. PSL was initially prescribed at 40mg/day and tapered. CyA was given at 3mg/kg BW/day. Biochemical data in sera and 24 hours urine protein (UP) were assayed repeatedly. The remission status of nephrotic syndrome were indicated by UP. C2 was monitored more than 4 times and the average was calculated. Significance of these factors for complete remission (CR) and influence of laboratory data on C2 were analyzed by logistic and multiple regression models, respectively. A ROC curve of each factor was drawn for CR.

**Results:** C2 was significantly related to CR (p=0.010) and was not influenced by any laboratory data. Although the cut-off point of C2 for CR (614.5 mg/dL) was determined from area under the curve of ROC (AUC/ROC) (0.731, 95%CI 0.557-0.905, p=0.022), but not so reliable for clinical use because of low likelihood ratio 3.2. When the subjects were limited to patients with TC<340mg/dL (N=25), however, AUC/ROC was increased into 0.868 (I 0.712-1.000, p=0.003) and likelihood ratio 7.3 was enough practically.

**Conclusions:** It is known that pharmacokinetics of CYA is affected by the plasma lipid concetration. Our study suggests that TDM of CYA is more available for the treatment when CYA is administered in the comparatively low level of serum cholesterol.

*Funding:* Clinical Revenue Support, Government Support - Non-U.S.

## PUB452

**Uremic Serum Inhibits the Function of Multiple Human Drug Transporters**

Catherine K. Yeung,[1,2] Danny D. Shen,[1,3] Kenneth E. Thummel,[3] Jane J. Huang,[4] Jonathan Himmelfarb.[2] *[1]Dept of Pharmacy, Univ of Washington, Seattle, WA; [2]Kidney Research Institute, Univ of Washington, Seattle, WA; [3]Dept of Pharmaceutics, Univ of Washington, Seattle, WA; [4]Optivia Biotechnology Inc., Menlo Park, CA.*

**Background:** In vivo pharmacokinetic studies suggest that uremia alters drug transport and metabolism; however the underlying molecular mechanisms remain poorly defined, especially in human subjects. The purpose of the study was to determine whether human uremic whole serum and serum ultrafiltrate inhibit the transport of substrate by P-gp, BCRP, OAT1, OAT3, OCT2, OATP1B1 or OATP1B3 transporter proteins.

**Methods:** For this study, MDCK-II cells expressing human solute carrier transporters OAT1, OAT3, OCT2, OATP1B1, OATP1B3, or ATP-binding cassette transporter MDR1 and Caco-2 cells expressing the ATP-binding cassette transporter BCRP were utilized. Probe substrates for each transporter were OCT2: [$^{14}$C]-metformin, OAT1: [$^{3}$H]-p-aminohippurate, OAT3: [$^{3}$H]-estrone-3-sulfate, OATP1B1: [$^{3}$H]-estradiol-17β-D-glucuronide, OATP1B3: [$^{3}$H]-CCK-8, BCRP: [$^{3}$H]-genistein, and P-gp: [$^{3}$H]-digoxin. Appropriate positive controls for inhibition were included with each set of incubations. Pooled uremic whole serum and serum ultrafiltrate were evaluated at a concentration of 10% in standard incubation medium.

**Results:** Changes in transport of probe substrates were observed between control serum and uremic serum for OATP1B1, OAT1, and P-glycoprotein. This difference was attenuated by removal of protein bound uremic constituents. No change was observed between control serum and uremic serum (whole/ultrafiltrate) for transporters OAT1B3, OAT3, OCT2, or BCRP.

**Conclusions:** We have shown that uremic toxins can alter the in vitro activity of multiple specific drug transporters and that the changes in activity are due mostly to protein bound constituents. These alterations in transporter activity may explain some of the pharmacokinetic variability observed in subjects with CKD and uremia.

*Funding:* Other NIH Support - KL2 TR000421 (CKY) and UH2 TR000504

## PUB453

**Dialysis Adequacy Measures and Dialytic Removal of Levofloxacin and Gentamicin**

Brian S. Decker,[1] Mary Chambers,[1] Kevin M. Sowinski.[2] *[1]Indiana Univ School of Medicine; [2]Purdue Univ School of Pharmacy.*

**Background:** Short-daily hemodialysis (SDHD) is emerging as an alternative to conventional, thrice-weekly hemodialysis due to its putative physiological benefits. However, there is a paucity of data regarding the appropriate dosing of medications in patients receiving SDHD and the ability of dialysis adequacy measures to predict drug removal.The purpose of this study was to investigate the ability of urea to predict levofloxacin and gentamicin removal in SDHD.

**Methods:** Six non-infected anuric adults treated with SDHD were studied. Following the completion of a subject's first SDHD, levofloxacin IV 250 mg and gentamicin 2 mg/Kg were administered. Blood samples for the determination of levofloxacin, gentamicin and urea were obtained before and during the next intradialytic period and for 4 hours after the dialysis session. Blood samples were processed and the serum stored at -80$^{0}$C until analysis. Levofloxacin concentrations were determined by microbiologic assay and urea and gentamicin concentrations by standard clinical methods. Regression analysis was utilized to investigate the relationship between urea removal and drug removal. The study was approved by the IUPUI IRB and all subjects gave written informed consent prior to study.

**Results:** Five men and one woman were studied. All subjects received SDHD with a new CT dialyzer (Exeltra 150, Baxter Healthcare, Inc.). SDHD dialysis operating characteristics were: dialysis time: 2.5 (2-2.5 hours) and Qb: 500 (450-500) mL/min. Mean urea and drug removal are shown in the table below (SD: standard deviation; RR: reduction ratio; e: equilibrated; sp: single pool). There was no statistically significant relationship between levofloxacin or gentamicin reduction ratios and measures of dialysis adequacy.

| | Urea RR | spKt/V | eKt/V | Levofloxacin RR | Levofloxacin eRR | Gentamicin RR | Gentamicin eRR |
|---|---|---|---|---|---|---|---|
| Mean | 0.412 | 0.611 | 0.518 | 0.605 | 0.460 | 0.656 | 0.503 |
| SD | 0.025 | 0.049 | 0.034 | 0.103 | 0.295 | 0.086 | 0.022 |

**Conclusions:** Dialysis adequacy measures do not predict dialytic removal of levofloxacin or gentamicin.

*Funding:* Private Foundation Support

## PUB454

**Effect of Hemodialysis on Phenytoin**

Robert Zoël Bell,[1] Sarah Bezzaoucha,[1] Jean-Philippe Lafrance,[1] Louis-Philippe Laurin,[2] Michel Vallee,[1] Vincent Pichette.[1] *[1]Nephrology, Hopital Maisonneuve-Rosemont, Montreal, Canada; [2]UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC.*

**Background:** Phenytoin is one of the main drugs used in patients undergoing dialysis as treatment of various types of epileptic seizures. Several studies have revealed that the binding of phenytoin to albumin is dramatically decreased in patients with chronic kidney disease. This phenomenon could lead to increased free plasma levels and toxicity. However, no study has yet evaluated the effects of dialysis on phenytoin-protein binding and plasma concentrations.

**Methods:** The plasma protein binding of phenytoin was evaluated in 10 hemodialysis-patients on phenytoin maintenance therapy. Total and free phenytoin serum concentrations were collected before and after hemodialysis sessions. All the patients were dialyzed 4 hours 3 times a week with high-flux polysulfon filters.

**Results:** In all the patients, the unbound phenytoin fractions, calculated from total and free phenytoin plasma concentrations, were significantly decreased in postdialysis serum compared to predialysis serum in all patients, with a mean value of 25 ± 0.04 % and 14 ± 0.03, respectively ( n=10, p <0.001). The mean free phenytoin predialysis concentration decreased from 6.86 ± 3.14 µmol/L to 3.90 ± 2.03 µmol/L post-dialysis (n=10, p <0.001), while the mean concentration of total phenytoin did not vary.

**Conclusions:** Our results clearly demonstrate that a dialysis session has a major impact on binding parameters that may decrease the anticonvulsant effect of phenytoin after dialysis sessions. Knowledge of these pharmacokinetics effects should be considered in the management of hemodialysis patients in order to achieve higher desired plasma free phenytoin concentrations and prevent seizures that may be associated with subtherapeutic levels.

*Funding:* Government Support - Non-U.S.

## PUB455

**Racial Differences in the Risk of Tenofovir Related Nephrotoxicity**

Srujana Polsani,[1] Sumit Mohan,[2] Jyotsana Thakkar,[1] Ali G. Gharavi,[2] Anjali Acharya.[1] *[1]Nephrology, Jacobi Medical Center, Bronx, NY; [2]Nephrology, Columbia Univ, New York, NY.*

**Background:** Tenofovir is an effective first line agent in the treatment of human immunodeficiency virus (HIV) infection. Although Tenofovir is thought to have a low overall toxicity profile, numerous case reports and studies in literature have shown that it can cause proximal tubular injury and Fanconi syndrome. There is limited information looking at its effect on phosphate wasting.

**Methods:** We reviewed charts for a 101 HIV patients (44% male, 45% Black, average age 46 with a deviation of 12 years) receiving care at the outpatient HIV clinic at Jacobi Medical Center. Our cohort includes 85 pts who received tenofovir for an average of 4.4±3 years.

**Results:** Exposure to tenofovir resulted in overt hypophosphatemia in only 4% of patients – all of them were females with somewhat longer exposure to tenofovir (5.8±3.5 vs 4.3±3.1 yrs, p=ns) who were also receiving other HAART agents. Patients exposed to tenofovir had a small but significant drop in the serum phosphate (3.5±0.6 vs 3.3±0.5, p=0.01) which on subgroup analysis appeared to remain significant only among non-Black patients (3.40.6 vs 3.2±0.5, p=0.03) but not among Black pts (3.5±0.6 vs 3.3±0.6, p=0.23).

**Conclusions:** Our results suggest possible racial pharmacokinetic differences, which may be protective. Blacks appear to be less prone to developing tenofovir related nephrotoxicity as evidenced by hypophosphatemia. Hypophosphatemia is likely related to increased urinary phosphate. This subtle change appears to be an early change that precedes the development of glycosuria. These findings are being confirmed in a larger cohort.

## PUB456

**Therapeutic Effect and Safety of Ginseng in Chronic Cyclosporine Treatment**

Kyoung Chan Doh,[1] Long Jin,[1] Shang Guo Piao,[1] Jian Jin,[1] Seong Beom Heo,[1] Sun Woo Lim,[1] Byung Ha Chung,[1,2] Chul Woo Yang.[1,2,3] *[1]CRCID & Transplant Research Center, The Catholic Univ of Korea, Republic of Korea; [2]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

**Background:** Ginseng is a popular traditional herbal medicine and has been used since ancient times. However, the study associated with ginseng effect on CsA treatment has not yet been performed. Therefore, we investigated the following issues;First,whether ginseng treatment influence immune response. Second,whether ginseng protect CsA-induced renal and pancreatic injury. Third,whether ginseng leads to unwanted drugs interactions.

**Methods:** We evaluated population of Th1,Th2,Th17and Treg in treatment ofCsA and ginseng in mouse splenocyte. Allogenic Tcell proliferation was also performed byMLR assay. Next, mice were treated with CsA(30 mg/kg) and ginseng(0.2,0.4g/kg) for4 weeks. Then, we measured renal function,histopathology,IPGTT,serum insulin,islet size, and oxidative stress marker(8-OHdG) in serum and urine. To confirm pharmacological interaction between CsA and ginseng, we measured CsA level usingLC-MS/MS in whole blood, and tissues.

**Results:** Cotreatment of ginseng with CsA decreased the population of Th17 and Th1. But, the population of IL-4 and Treg was increased by addition of ginseng. MLR results showed ginseng treatment decreased T cell proliferation in dose dependent manner. Four weeks of CsA treatment caused renal dysfunction,typical interstitial fibrosis,high level of blood glucose level,low level of insulin,and reduction of isle size. However, addition of ginseng on CsA recovered above parameters compared with CsA alone. These changes were accompanied by decreased the level of 8-OHdG. There was no difference in the CsA level between CsA alone and CsA+ginseng treated group in bloodand tissues.

**Conclusions:** Cotreatment of ginseng with CsA suppressed pathogenic Tcell proliferation and elicited immune tolerance. Chronically, addition of ginseng has protective effect in CsA-induced renal and pancreatic injury via decreasing oxidative stress without drug interactions. Our results provide good evidence that supplement of ginseng has significant effectiveness and safety in transplant patient receiving CsA.

*Funding:* Government Support - Non-U.S.

## PUB457

**Effects of Omega-3 Polyunsaturated Fatty Acid on Renal Function and Proteinuria in Kidney Transplant Recipients** Tariq Shah,[1,2,3,4] Don Vu,[1,2,3] Robert Naraghi,[1,2] Elizabeth Cadag,[2] Caron Hutchinson,[3] David I. Min.[2] *[1]Transplant Research Institute; [2]Saint Vincent Medical Center; [3]Western Univ of Health Sciences; [4]Univ of Southern California.*

**Background:** Omega-3 polyunsaturated fatty acid (O-3PUFA, fish oil) may slow the progression of kidney disease and reduce proteinuria in kidney transplant recipients (KTR). However, evidence from clinical trials is inconsistent. The aim of this study is to assess the effects of O-3PUFA therapy on renal function and proteinuria in KTR.

**Methods:** 137 allograft recipients who received O-3PUFA therapy between 2009 and 2012 at Saint Vincent Medical Center (SVMC) were studied in a retrospective study design. EGFR measured by the Cockcroft-Galt method and daily proteinuria calculated by the random urine protein/creatinine ratio were collected at various time intervals for each participant for 1 year. Statistical analysis was done using a paired t-test for changes in eGFR and proteinuria from baseline, and a p-value <0.05 was of statistical significance.

**Results:** O-3PUFA therapy started an average 6.8 months post- transplantation with a mean daily dose of 3.0±1.4 g/day. The mean eGFR ml/min (±SD)at baseline, 1, 3, 6, 9 and 12 months during O-3PUFA therapy were 51.8 ±22.6, 55.8±19.8, 57.9±19.7, 57.1±18.5, 55.8±19.5 and 56.4±20.1*, respectively (all p<0.05 except for *). Daily mean urine protein excretion g/day (±SD) were 2.60 ±4.28, 1.76 ±2.77, 1.68 ±2.92, 1.74 ±3.28, 1.11 ±2.13 and 1.07±2.02 at baseline, 1, 3, 6, 9 and 12 months, respectively (all p>0.05). Any significant side effects from O-3PUFA were not observed during study period.

**Conclusions:** In our study, O-3PUFA therapy significantly improves renal function measured by eGFR at one month, of which effects last up to 9 months. However, O-3PUFA therapy did not affect proteinuria in KTR at any time during therapy. Well-designed, prospective, randomized, controlled clinical trials are needed to further examine the potential benefits of O-3PUFA in kidney transplant recipients.

## PUB458

**Outcomes of Kidney Transplantation with the Use of Reduced-Dose Rabbit Antithymocyte Globulin Induction and Steroid Avoidance Maintenance Immunosuppression: A Single Center Experience** Sunn Sunn H. Thaw,[1] Nyan Pyae,[1] Susan Puumala,[2] Adit S. Mahale.[3] *[1]Dept of Internal Medicine, Univ of North Dakota, Fargo, ND; [2]Center for Health Outcomes and Prevention Research, Sanford Research, Sioux Falls, SD; [3]Dept of Nephrology, Sanford Health, Fargo, ND.*

**Background:** Induction and maintenance immunosuppression regimens in renal transplantation are largely variable. We analyzed the outcomes of kidney transplant recipients who had reduced-dose rabbit antithymocyte globulin (rATG) for induction and steroid-free maintenance immunosuppression in our center.

**Methods:** A group of 108 kidney transplant patients treated at our institution scheduled to receive reduced-dose rATG as induction therapy and treated with a steroid-free maintenance therapy were analyzed for graft and overall survival using the Kaplan-Meier method. We also explored prognostic indicators within this group to assess differences in graft and overall survival through a Cox regression model.

**Results:** Both graft and overall survival were favorable with one-year survival estimates of 100% and 98% respectively. No differences were found for either graft or overall survival for donor type, human leucocyte antigen (HLA) mismatch score, peak panel reactive antibody (PRA) > 50, or body mass index. A significant difference was found by racial groups, with Native Americans having lower graft survival (hazard ratio [HR], 27.61; p=0.001) compared to Caucasians, but no difference was found between these two groups for overall survival (HR, 1.05; p=0.98). Age at the time of transplant was associated with overall survival (HR, 1.14; p=0.05), but not with graft survival (HR, 0.99; p=0.66). Prevalence of diabetes and peak PRA levels >50 were significantly different between Native Americans and Caucasians (81.8% vs. 24.7%, p <0.001) and (45.5% vs. 11.8%, p=0.01) respectively.

**Conclusions:** Native American kidney transplant patients had lower graft survival compared to Caucasian patients with the use of reduced-dose rATG induction and steroid-free maintenance immunosuppression based on our observation. Higher prevalence of diabetes and higher peak PRA levels could contribute to this disparity in the Native American recipients.

## PUB459

**Acute Graft Pyelonephritis in Renal Transplant Recipients: Risk Factors, Graft Outcome and Association with Acute Rejection** Prem P. Varma,[1] Ashok Kumar Hooda,[1] Sonia Badwal.[2] *[1]Dept of Nephrology, Army Hospital (Research & Referral), New Delhi, Delhi, India; [2]Dept of Nephrology, Army Hospital (Research & Referral), New Delhi, Delhi, India; [3]Dept of Pathology, AFMC, Pune, Maharashtra, India.*

**Background:** Urinary tract infection is a common infection in the renal transplant recipients. However the risk factors and long term outcome of acute graft pyelonephritis (AGPN) remain unclear. Aim- To study the impact of graft pyelonephritis on graft outcome.

**Methods:** In this prospective study from 2002-2011, all transplant recipients were followed for development of UTI. Graft PN was diagnosed if patient had fever, graft tenderness, leucocytosis, pyuria, bacteruria, + Graft dysfunction. The risk factors for AGPN were analysed and impact of AGPN on graft outcome was studied.

**Results:** Of the 556 transplant recipients, 32 patients were diagnosed to have acute graft pyelonephritis. 30/32 patients had graft dysfunction and after 2 weeks of parenteral antibiotics, 26 of them with persisting graft dysfunction underwent biopsies. 7/26 patients revealed evidence of concomitant acute rejection. Anti-rejection therapy did not alter the course of these patients (p< 0.05). Both early as well as late AGPN had poor outcomes with late AGPN being worse off (p=0.02 , p< 0.01 respectively). Despite 4-6 weeks of antibiotics followed by prophylaxis, 75% of patients relapsed requiring repeat courses of antibiotics. The statistically significant risk factors identified in this study were HCV infection and pre-transplant history of UTI (p=0.01 & p=0.01 respectively). In a pilot study of controls, a 70% incidence of post-transplant vesicoureteric reflux (VUR) was demonstrable on MCU (18/26); however, VUR didn't predict AGPN in them (p=0.84). The effect of net immunosuppression in the causation of AGPN was highlighted by the lower incidence demonstrated in the latter half (10% vs 2%) by lowering the doses of steroids and calcineurin inhibitors (p<0.05) .

**Conclusions:** Both early and late graft pyelonephritis especially if associated with graft dysfunction has poor outcome.

## PUB460

**An Environmental Scan of Kidney Transplant Referral Practices in Georgia, North Carolina and South Carolina Dialysis Units** Teri Browne,[1] M. Ahinee Amamoo,[2] Rachel E. Patzer,[3] Leighann Sauls,[2] Jenna Krisher,[2] Stephen O. Pastan.[3] *[1]College of Social Work, Univ of South Carolina, Columbia, SC; [2]Southeastern Kidney Council, Raleigh, NC; [3]Emory Transplant Center, Emory Univ School of Medicine, Atlanta, GA.*

**Background:** The Southeastern United States has the lowest kidney transplant (KTx) rates in the country. To most effectively promote more kidney transplant referrals among dialysis units in the area, the Southeastern KTx Coalition conducted an environmental scan to determine existing dialysis provider beliefs, attitudes and practices related to KTx in their unit.

**Methods:** Every dialysis unit in GA, NC & SC (n=586) was invited to participate in a survey about facility practices in helping dialysis patients get a Ktx. 51% (n=297) of facilities completed the survey. Data were analyzed with descriptive statistics and bivariate and multivariate analyses.

**Results:** The majority of surveys were completed by nurse managers (48%) or social workers (25%). 90% of respondents reported that they were comfortable discussing KTx with their patients, and over 80% have a protocol in place for KTx education. Only 19% of respondents report that 50% or more of patients are interested in KTx as a treatment option, and 87% of respondents report that < 50% of their patients have success in navigating the pathway to KTx. Respondents identified the following barriers to patients receiving a KTx: Lack of patient education materials (34%), unclear KTx center requirements (22%), insufficient patient social support (61%), patient transportation (74%) and patient financial status (89%).

**Conclusions:** This is the first study in the Southeastern United States to survey dialysis units to determine the dialysis facility-level barriers and attitudes about KTx. Results suggest that dialysis professionals are equipped to explore KTx with patients (in that they feel comfortable doing so and have a process in place); however, patient and organizational- level barriers still exist that impede Ktx parity in this region. This study can help inform future research and program development to improve dialysis patient outcomes related to Ktx.

*Funding:* NIDDK Support, Other NIH Support - NIMHD

## PUB461

**Factors Associated with Preemptive Referral for Kidney Transplant Evaluation** Mohua Basu, Brendan P. Lovasik, Justin D. Schrager, Nancy G. Kutner, Rachel E. Patzer. *Emory Univ School of Medicine.*

**Background:** While kidney transplantation (KTx) prior to initiation of dialysis is the optimal treatment for end-stage renal disease (ESRD), racial and socioeconomic disparities exist in access to preemptive KTx. Little is known about factors associated with referral for KTx evaluation prior to initiation of dialysis, or preemptive referral.

**Methods:** We examined factors associated with preemptive referral in patients with no previous transplant referred to a Southeastern KTx center for KTx evaluation from 2005-2010. KTx center referral data were linked to United States Renal Data System baseline and follow-up data through September 2011. Multivariable logistic regression was used to examine the association between patient factors and preemptive referral.

**Results:** Of 4,914 referred patients, 934 (19%) were preemptively referred. In models adjusted for patient demographic, clinical, and socioeconomic factors, white (vs. Black) patients, patients who received pre-ESRD nephrology care, and patients with private (vs. public) insurance had a greater odds of preemptive referral (Table). Female sex, glomerulonephritis as cause of ESRD, erythropoietin use, and higher albumin and hemoglobin levels were also significantly associated with access to preemptive referral (Table).

**Table: Factors Significantly Associated with Preemptive Referral (Adjusted Model)**

| | OR (95% CI) | P-Value |
|---|---|---|
| Female (vs. Male) Sex | 1.20 (1.0-1.4) | 0.0333 |
| White (vs. Black) Race | 2.27 (1.6-2.5) | <.0001 |
| ESRD Etiology: Glomerulonephritis (vs. Diabetes) | 1.39 (1.1-1.8) | 0.0192 |
| Pre-ESRD Nephrology Care | 5.75 (4.6-7.1) | <.0001 |
| Erythropoietin Use | 1.25 (1.1-1.5) | 0.0135 |
| Low Albumin (<3.5 mg/dL) | 0.52 (0.4-0.6) | <.0001 |
| Low Hemoglobin (<10 mg/dL) | 0.83 (0.7-1.0) | .0370 |
| Private (vs. Public) Insurance | 2.68 (2.3-3.2) | <.0001 |

**Conclusions:** Minority race, pre-ESRD nephrology care, private insurance, and other patient factors play a role in preemptive referral for KTx. Interventions to increase preemptive referral may reduce racial and socioeconomic disparities in KTx.

## PUB462

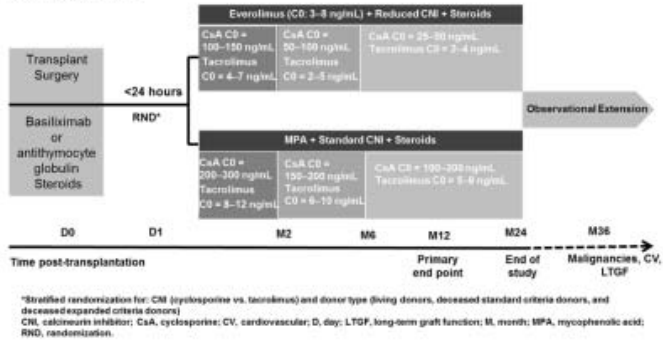
**The TRANSFORM Trial Design: A Large Randomized, Multicenter, Open-Label Study of Everolimus with Reduced Calcineurin Inhibitors in *De Novo* Renal Transplantation** Titte Srinivas, Julio Pascual, Steven J. Chadban, Franco Citterio, Mitchell L. Henry, Christophe M. Legendre, Federico Oppenheimer, Helio Tedesco Silva, F. Vincenti, Yoshihiko Watarai, J. M. Hexham, Peter Bernhardt, Martin G. Zeier. *For TRANSFORM Study.*

**Background:** Immunosuppressive regimens allowing both freedom from rejection and reduction calcineurin inhibitors (CNI) exposure and their associated nephrotoxicity remain an unmet need in kidney transplantation (KTx). Here, we present a phase IV study, TRANSFORM, designed to evaluate the efficacy and safety of everolimus (EVR) plus reduced CNI exposure in comparison to mycophenolic acid (MPA) plus standard CNI exposure in de novo KTx recipients (KTxR).

**Methods:** TRANSFORM, a 24-month (mo), multicenter, open-label study will be conducted across 200 centers worldwide. Approximately >2000 KTxR will be randomized (1:1) to receive either EVR (C0, 3–8 ng/mL) with reduced CNI exposure or MPA with standard CNI exposure, all with basiliximab or antithymocyte globulin induction and steroids (Figure 1).The primary endpoint will be the composite of (i) treated biopsy-proven acute rejection (tBPAR) rate or (ii) proportion of KTxR with estimated glomerular filtration rate <50 mL/min/1.73m$^2$ (eGFR;MDRD4) at mo 12 post-Tx. The key secondary endpoint is composite efficacy failure rate (tBPAR, graft loss, or death) at mo 12 post-Tx. These endpoints will be also analyzed at mo 24. Patients completing 24 mo of treatment will be eligible to participate in an observational extension study for a further 36 mo with outcomes analyzed up to 5 years including eGFR, patient and graft survival, and incident cardiovascular disease, malignancy, and infection.

**Results:**

Figure 1: Study Design




*Stratified randomization for: CNI (cyclosporine vs. tacrolimus) and donor type (living donors, deceased standard criteria donors, and deceased expanded criteria donors)
CNI, calcineurin inhibitor; CsA, cyclosporine; CV, cardiovascular; D, day; LTGF, long-term graft function; M, month; MPA, mycophenolic acid; RND, randomization.

**Conclusions:** TRANSFORM will be the largest clinical trial in KTx and is designed to demonstrate the short- and long-term benefits of de novo CNI-minimization with an EVR-based regimen.

*Funding:* Pharmaceutical Company Support - Novartis

## PUB463

**A Patient-Centered Approach to Exploring Kidney Transplant Promotion in the Southeastern United States** Teri Browne,[1] M. Ahinee Amamoo,[2] Rachel E. Patzer,[3] Jenna Krisher,[2] Henry Well,[4] Stephen O. Pastan.[3] *[1]College of Social Work, Univ of South Carolina, Columbia, SC; [2]Southeastern Kidney Council, Raleigh, NC; [3]Emory Transplant Center, Emory Univ School of Medicine, Atlanta, GA; [4]National Kidney Foundation of GA, NC & SC, Columbia, SC.*

**Background:** Disparities in access to kidney transplantation is a significant problem in the United States, and even worse in the Southeastern region. To determine perceived barriers and facilitators to kidney transplant in the Southeastern United States (and thereby respond to patient needs in program planning), we conducted three patient focus groups in Georgia, North Carolina and South Carolina.

**Methods:** We conducted three focus groups of kidney disease patients in GA, NC & SC. Forty participants were recruited from attendees at a kidney patient educational event hosted by the National Kidney Foundation. An interview guide was used by the group facilitators to explore patient interest and personal experience related to kidney transplantation. A constant comparative method was used to identify themes that emerged from a line-by-line review of the focus group transcripts.

**Results:** Of the 40 participants, 14 (48.3%) were male, 25 (87%) were African American, and the majority (46%) were on dialysis for more than two years. Participants described five main barriers to getting a kidney transplant: financial, medical, informational, attitudinal, and the composition and behaviors of the dialysis team, medical providers, and others in their social networks. They identified finances, younger age, information, attitudes and beliefs, and helpful medical professionals and others as facilitators for getting a kidney transplant.

**Conclusions:** This study is the first of its kind to explore the barriers and facilitators of getting a kidney transplant in the area of the United States with the greatest kidney transplant disparity. The study findings provide the basis for the development of targeted interventions that can improve kidney transplant parity in a way that is patient-centered.

*Funding:* NIDDK Support, Other NIH Support - NIMHD

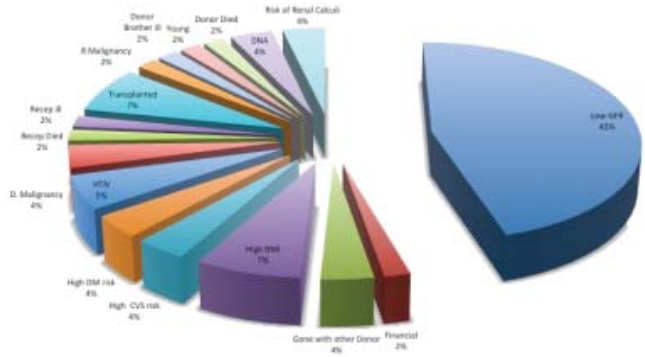
## PUB464

**Live Donor Evaluation, Why Do Potential Donors Not Proceed?** Maharajan Raman, Rachel Middleton, Grahame N. Wood. *Renal Medicine, Salford Royal Foundation NHS Trust, United Kingdom.*

**Background:** Live donation represents 38% of Kidney transplants in the United Kingdom. Live donation has become a treatment of choice. Donor welfare is paramount. Vigilant donor care and management is essential to inspire public confidence. We evaluated the reasons for unsuitability and outcomes following live donor assessment.

**Methods:** Retrospectively analysis of 109 donors who were referred to the consultant led live donor clinic following initial assessment in the Nurse lead clinic between the year 2009 and 2011. 49 of these donors (45%) were found to be unsuitable for donation. Evaluated donor demographics, relationship to recipient, past medical history, reasons for unsuitability, recipient eGFR and change in modality during this period.

**Results:** The average donor age was observed to be 47 years and majority of them were Caucasians (91%). Hypertension (19%) was the commonest past medical history followed by mental health issues (11%)and high cholesterol (10%). 31% of the recipients were in the pre-dialysis group and 69% were on RRT. The average eGFR among the pre-dialysis group was observed to be 12ml/min. 6% changed modality form pre-dialysis to requiring RRT during the assessment period. The chart below shows the various reasons for unsuitability.




Following the assessment of these unsuitable donors 8% were placed in the CKD register, 4% were referred to the Geneticist and 2% of the donors were either referred to Cardiologist, Gastroenterologist, Gynecologist or Psychologist due to abnormal findings discovered during the live donor assessment period.

**Conclusions:** In our cohort of donors we found low isotopic GFR to be the commonest reason for unsuitability followed by high BMI and hypertension. Hence isotopic GFR can be used as an effective screening and a cost saving tool. Incidental findings discovered among the unsuitable donors during this assessment period were appropriately managed.

## PUB465

**Patient Navigation to Increase Kidney Transplant Evaluation Completion in High-Risk Patients** Mohua Basu, Rachel Koval, Jennie P. Perryman, Kevin M. Clark, Dawn L. Fletcher, Perry Dykes, Lisa Petgrave-nelson, Rachel E. Patzer. *Emory Transplant Center.*

**Background:** Racial and socioeconomic disparities exist in the rate of kidney transplant (KTx) evaluation completion among referred patients. An ongoing pilot study will assess the effectiveness of a patient navigation (PN) intervention to increase the KTx evaluation completion rate and to decrease the time from KTx evaluation to candidacy decision in high-risk patients at a Southeastern KTx center.

**Methods:** We created a risk assessment tool that uses patient demographic and clinical factors to estimate probability of waitlisting for patients referred for KTx evaluation based on center-level and national surveillance follow-up data. Patients with <40% probability of waitlisting were classified as high-risk and randomly assigned to receive PN or standard of care during KTx evaluation. Kaplan-Meier methods and Cox models will be used to assess the KTx evaluation completion rate and time from evaluation to candidacy decision for patients receiving PN compared to standard of care.

**Results:** Since initiation of the study in January 2013, 213 (34%) patients referred for KTx evaluation were classified as high-risk. Currently 50 high-risk patients have been assigned to receive PN and 47 the standard of care. Characteristics of referred patients appear below (Table).

**Table: Patient Characteristics by Risk Group**

| | Total N=635 | High Risk N=213 (33.5%) | Low Risk N=422 (66.5%) |
|---|---|---|---|
| **African American** | 68.7% | 79.3% | 63.4% |
| **Less than High School Education** | 14.7% | 35.2% | 4.5% |
| **Private Insurance** | 47.7% | 9.4% | 67.1% |
| **Married** | 50.1% | 26.1% | 62.1% |
| **Non-English Speaker** | 2.5% | 7.6% | 0% |
| **Mean BMI** | 29.9± 7.3 | 31.9 ± 8.2 | 29.0 ± 6.6 |

**Conclusions:** Preliminary data support the feasibility of identifying high-risk patients and intervening with PN. The effectiveness of PN to increase the KTx evaluation completion rate and reduce disparities in KTx access will be evaluated upon study completion. Results will inform decisions about implementation of a long-term PN program in the KTx center.

*Funding:* Private Foundation Support

## PUB466

**Tacrolimus Dose and Trough Blood Levels in a U.S. Kidney Transplant Population** Brett Pinsky,[1] Mary Helen Tran,[2] Aylin A. Riedel.[1] *[1]OptumInsight, Eden Prairie, MN; [2]Novartis Pharmaceuticals Corp, East Hanover, NJ.*

**Background:** The goal of this study was to examine tacrolimus (TAC) dose over time and by TAC trough levels in kidney transplant (KTX) patients.

**Methods:** A retrospective analysis of transplanted kidney and a prescription claim for TAC (tacrolimus) between 01Jan2004 and 31Aug2011 was conducted using a large US healthcare claims database. The date of the first TAC claim was defined as the index date. All patients were required to have continuous enrollment for 1 year prior (pre-index) and a minimum of 6 months following (post-index). Evidence of KTX was defined in the pre-index period as at least one claim with a diagnosis or procedure code for KTX. Incident cases were defined as having evidence of transplant surgery during the pre-index period; all other cases were defined as prevalent. Patients had ≥1 lab result measuring TAC trough level in the post-index period. Average TAC trough levels were examined from index date in three month intervals through the first year. The average daily dose of TAC was examined during the same intervals and in relation to trough level.

**Results:** Study sample included 531 incident and 336 prevalent KTX patients with a TAC trough result. Depending on time frame, 96% to 73% of patients had a TAC fill in the same time period as an available TAC trough result. Over the first year the percentage of patients with TAC levels ≥7.5 ng/ml decreased from 46% to 41%. In the prevalent group, the percentage of patients with TAC levels ≥7.5 ng/ml is 40%. Table 1 examines the shift in the population's TAC trough levels over time and the relationship between dose and TAC trough level.

Table 1. Tacrolimus Trough Level and Dose over time

| | Tacrolimus Trough Level | | | | |
|---|---|---|---|---|---|
| | 0 – 4.799 ng/mL | 4.8 – 6.199 ng/mL | 6.2 – 7.499 ng/mL | 7.5 – 9.399 ng/mL | 9.4 – 20 ng/mL |
| *Incident Transplant* | | | | | |
| **3 Months (N)** | **92 (18%)** | **92 (18%)** | **94 (18%)** | **91 (18%)** | **145 (28%)** |
| TAC daily dose (mg) | 7.78 | 7.28 | 6.91 | 7.32 | 7.55 |
| **6 Months (N)** | **77 (17%)** | **108 (24%)** | **86 (19%)** | **96 (22%)** | **79 (18%)** |
| TAC daily dose (mg) | 5.92 | 7.45 | 6.52 | 7.27 | 6.25 |
| **9 Months (N)** | **88 (20%)** | **100 (23%)** | **88 (20%)** | **87 (20%)** | **72 (17%)** |
| TAC daily dose (mg) | 5.74 | 6.62 | 6.34 | 7.46 | 6.56 |
| **12 Months (N)** | **70 (18%)** | **90 (23%)** | **70 (18%)** | **95 (24%)** | **65 (17%)** |
| TAC daily dose (mg) | 5.86 | 5.99 | 6.34 | 6.95 | 6.27 |
| *Prevalent Transplants* | | | | | |
| **Over 12 Months* (N)** | **209 (18%)** | **260 (23%)** | **206 (18%)** | **248 (22%)** | **229 (20%)** |
| TAC daily dose (mg) | 5.06 | 5.50 | 6.12 | 5.94 | 5.78 |

*In the prevalent transplant cohort, TAC blood levels and TAC daily dose were all calculated at 3, 6, 9 and 12 months following the index date to identify a temporal relationship. The time periods were combined and classified as over 12 months since the timing in the prevalent cohort is happenstance.

**Conclusions:** TAC dose and trough level appear to change over time as evidenced by decreasing dosage and levels. A significant number of patients remain at a TAC lever >7.5 ng/ml or higher. This suggests that TAC management is not routinely occurring.

*Funding:* Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

## PUB467

**Epidemiology of Infections Requiring Hospitalisation during Long Term Follow-Up in Kidney-Transplant Patients** Laure Champion,[1] Christel Renoux,[2] Christine Randoux,[1] Caroline Du Halgouet,[1] Latifa Azeroual,[1] Denis Glotz,[3] Francois Vrtovsnik,[1] Eric Daugas.[1] *[1]Nephrology, Bichat Hospital, Paris, France; [2]McGill Univ, Montreal, Canada; [3]Kidney Transplantation, Saint Louis Hospital, Paris, France.*

**Background:** Are there more infections requiring hospitalisation (IRH) following renal transplantation nowadays than previously reported?

**Methods:** We performed a retrospective cohort study of 314 consecutive renal transplant recipients from 1999 to 2012. We stratified the cohort further by date of the renal transplantation (P1:1999-2003: n=61; P2:2004-2007: n=89; P3:2008-2012: n=164). Data regarding the IRH, including bacterial, viral, parasite and fungal infections, were collected. The main objectives of the study were to estimate the incidence rate of IRH (events/100 patients year), the median time to the first IRH in the 3 time periods (P1 being the reference period) and the risk factors of IRH using survival analysis.

**Results:** The patients who underwent a transplant during P3 were older, had more cardiovascular risks factors, higher title of pre-transplant antibodies, and received an RATG induction therapy more often. At 3 and 5 years, the graft and patient survival rates were 95%-89% and 95%-89% respectively, with a growing incidence of acute rejection from P1 to P3 (1.69; 5. 62; 6. 83). Overall, 172 (54. 8%) patients developed at least one IRH in a total of 381 during a median follow-up of 4.18 years. The median time for the occurrence of the first IRH was shorter for most recent transplant recipients: P1 : 9.53 years (IC-95% : 3.40 to non estimable) ; P2 : 2.83 years (1.30-4.55) ; P3 : 1.74 years (0.95-3.88) ($p<0.05$). Incidence rate of IRH adjusted for sex and age (3.38; 9.1; 14.03), opportunist infection (0.90; 5.04; 8.48), late infection (8.3; 22.16; 36.05) and death rate increased over time ($p<0.001$ for all). In a multivariate Cox regression analysis, the potential risk factors for IRH were age, time under dialysis before transplant, induction by RATG, corticoid use, and a transplant after 2004.

**Conclusions:** The incidence rate of IRH increases over time.This higher incidence might be related to different patient profiles and immunosuppressive protocols including RATG.

## PUB468

**Why Do Patients Forget to Take Their Immunosuppression Medications and Can a Smartphone App Help?** Ajay K. Israni,[1] Brian J. Kasel,[2] Winston Anderson Wildebush,[2] Bertram L. Kasiske,[1] Chihhung Jason Wang.[3] *[1]Medicine, Hennepin County Medical Center (HCMC), Univ of Minneosta (UMN), Minneapolis, MN; [2]Minneapolis Medical Research Foundation (MMRF), Minneapolis, MN; [3]Stanford Univ, Stanford, CA.*

**Background:** Kidney transplant recipients must adhere to their immunosuppressive medication regimen. However non-adherence remains a major problem post-transplantation.

**Methods:** We performed a qualitative study that included home or work place visits and interviews with kidney transplant recipients from a single urban, transplant center. The goal was to determine how patients remembered to take their medications, and to assess patients' perception and beliefs about adherence to immunosuppressive medications and barriers to medication adherence. Patients also reviewed screen shots of a prototype app to be used in smartphones. All transcripts were transcribed and coded for qualitative data analysis.

**Results:** We conducted home or work place visits with sixteen patients; an average of 3 visits were conducted per patient. The visits showed that most patients have incorporated medication use into their daily lives. However any minor deviation from daily routines results in non-adherence. The qualitative surveys revealed that transplant recipients understood the importance of taking their immunosuppressive medications and described various factors that motivated them to take their medications. However, patients reported barriers to adherence such as: forgetting to take or renew medications, procrastination, being short on money, having depression, distractions, or change in daily routine, forgetting medications while away from home, alcohol use and falling asleep before taking evening dose. All patients were interested in using the app. However, they reported potential barriers to using the app such as: cell phone being turned off or not with the patient, ignoring reminders from the app, app reminders becoming too annoying and app is inflexible about timing of reminders.

**Conclusions:** Although kidney transplant recipients understood the importance of medication adherence, there were significant barriers to maintaining adherence. Patients also reported interest in using a smartphone app.

## PUB469

**Clinical Epidemiology of Resistant Hypertension in Renal Transplant Patients** Francesca Mallamaci, Vincenzo Panuccio, Rocco Tripepi, Giovanna Parlongo, Maria Carmela Versace, Raffaele Politi, Carmine Zoccali. *CNR-IBIM, Clin Epid and Physiopath of Renal Diseases and Hypert., Reggio Calabria, Italy.*

**Background:** Only scattered information exists on treatment-resistant hypertension (RH) in transplant pts and the issue has never been assessed according to rigorous ABPM criteria.

**Methods:** We assessed the problem in unselected series of 219 renal transplant pts followed up according to recommendations by the Am Soc of Transplantation (AST, JASN 11: S1–S86, 2000) and in two well matched groups of stage 2-5 CKD patients. We applied the stringent diagnostic criterion for RH by NICE (Mean daytime BP>135/85 mmHg despite treatment with 3 drugs) and the standard JNC-VII criterion for RH.

**Results:** The prevalence of RH (NICE criteria) was substantially less in renal transplant pts (5 patient/219, i.e. 2.3%) than in the first CKD group (8.7%, $p=0.03$). Coherently with this finding, comparison of the transplant pts group with the second CKD group by the JNC VII criterion (Transplant Pts 1.1% vs CKD-B 11.9%, $p<0.001$) confimed a substantially lower prevalence of RH in transplant pts. Further analyses in a sub-group (n= 165) of CKD pts strictly matched to renal transplant pts for the GFR again showed a substantially lower prevalence of RH in renal transplant pts (1.1% vs 7.9% in CKD pts $p=0.002$). The prevalence of RH across these groups went along with the number of visits (12 visits/year in transplant pts vs 1- 2 visits/year in the two CKD pts groups).The low prevalence of RH in transplant patients went along with a lower frequency ($P<0.001$) of uncontrolled hypertension in this population.

**Conclusions:** Notwithstanding the use of pro-hypertensive drugs lik calcineurin inhibitors and the surge obesity after renal transplantation, the prevalence of RH is remarkably lower in renal transplant pts than in well matched CKD pts. Such a low prevalence goes along with the intense follow-up (number of visits) after renal transplantation. Effective hypertension control can be achieved in a substantial number of renal transplant pts underscoring the relevance of intensified follow up on BP control in this population.

**PUB470**

**Lower Incidence of Post-Transplant Diabetes Mellitus after Kidney Transplantation with Redused Tacrolimus Levels and Angiotensin Receptor Blockers** Mediha Boran,[1] Mertay Boran,[2] Ertay Boran,[3] Faruk Gonenc.[4] *[1]Dept of Nephrology, Hemodialysis and Transplantation, Turkiye Higher Education Hospital, Ankara, Turkey; [2]Clinic of Thoracic Surgery, Cankiri State Hospital, Cankiri, Turkey; [3]Dept of Anesthesiology and Reanimation, Izmir Ataturk Education and Research Hospital, Izmir, Turkey; [4]Dept of Urology, Turkiye Higher Education Hospital, Ankara, Turkey.*

**Background:** Post-transplant diabetes mellitus (PTDM) is an undesirable consequence of transplantation, affecting approximately 12-54% of renal transplant recipients. The objectives of the present study were to assess the incidence of PTDM and the factors that were associated with the development of this complication in kidney transplant recipients maintained on reduced tacrolimus (TAC) based immunosuppressive regimen and angiotensin receptor blockers (ARB) therapy.

**Methods:** The study was a retrospective analysis of a prospectively maintained database that compared clinical outcomes of 137 kidney allograft recipients transplanted in a single center from 2004 to 2011 receiving reduced TAC, mycophenolate mofetil (MMF) and minimal prednisone and ARB. In an attempt to detect risk factors predisposing to PTDM, data were compared between 12 recipients with PTDM and 125 recipients without PTDM.

**Results:** In this study, we selected consecutive 137 kidney transplant recipients receiving TAC, MMF and steroid. PTDM occurred in 12 of the recipients (9.6%) 30 days after transplantation. A multivariate analysis including variables which have significant effect on PTDM risk in univariate analysis revealed that recipients who were overweight (>65kg) (p=0.004) and have target TAC trough levels (p=0.005) have significant risk of PTDM.

**Conclusions:** In our study, kidney transplant recipients with reduced dose of TAC and steroid and adittional ARB therapy showed low incidence of PTDM. . The incidence of PTDM was associated with the doses of immunosuppression and overweight of the kidney transplant recipients. These findings demonstrated that PTDM is more consistent with an insulin resistance than with an insulin- deficient state. PTDM was not representing a risk for poor graft and recipient outcomes.

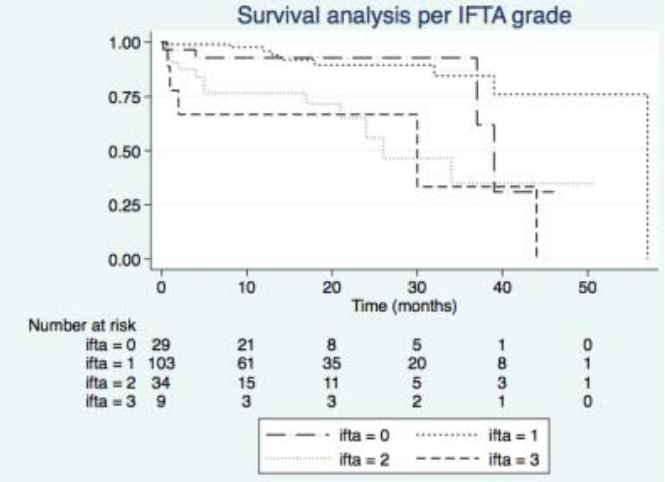
**PUB471**

**Interstitial Fibrosis and Tubular Atrophy as a Predictor of Median Renal Allograft Survival** Ana Paula Rossi,[1] Maxwell Yisheng Li,[2] Mahendra Mangray,[1] Douglas M. Dressel,[3] John P. Vella.[1] *[1]Div of Nephrology and Transplantation, Maine Medical Center, Portland; [2]Dept of Medicine, Medstar Georgetown Univ Hospital, Washington, DC; [3]Dept of Pathology, Spectrum Medical Group, ME.*

**Background:** Many patients with failing grafts start dialysis without adequate access and experience high morbidity and mortality. Better allograft prognostic tools may allow improved preparation for ESRD.

**Methods:** Retrospective review of 175 kidney transplant recipients undergoing for cause graft biopsies. IFTA was graded according to the Banff criteria. Main outcome was graft loss. Kaplan Meier curves and Cox proportional hazards model were used for analyzing graft survival.

**Results:** Mean follow up time was 16 ±13 months. Thirty patients (17%) lost their grafts. Mean graft age was 47 ±51 vs 25 ±48 months for lost vs functioning grafts (p0.02). Subjects with graft loss were younger (41 ±16 vs 48 ±14 years, p0.03), had a higher creatinine ([Cr] 3.3 ±2.1 vs. 2.3 ±1.3 mg/dL, p0.002) and IFTA grade (p<0.001), and were more likely to have antibody mediated rejection (34.8 vs 15.4%, p0.03) than subjects with no graft loss. There were no differences in immunosuppressives, antihypertensives or history of prior rejection. Median graft survival was 39, 57, 26 and 30 months for IFTA grade 0, I, II, and III respectively (p<0.0001). On univariate analysis, higher IFTA score (HR 0.52, p0.3; HR 2.66, p0.09; and HR 4.09, p0.04 for IFTA grade I, II and III respectively, IFTA=0 as reference), graft age (HR 1.01, p0.06) and higher Cr (HR 1.17, p0.01) were associated with a greater risk of graft loss. After adjusting for age, gender, donor type, graft age, Cr, concurrent rejection and IFTA grade, only Cr (p0.03) conferred greater risk of graft loss.




**Conclusions:** Median graft survival from biopsy is significantly shorter for grafts with IFTA grade II or higher compared to lower degrees of fibrosis.

**PUB472**

**What Factors Influence Renal Transplant Wait List Status** Mary McCarthy, Sarah Margaret Moran, William D. Plant. *Nephrology, Cork Univ Hospital, Ireland.*

**Background:** Renal transplantation (RTX) is the optimum form of renal replacement therapy (RRT) for End Stage Kidney Disease (ESKD) patients. Not all patients are suitable for listing; a variety of processes are necessary for patients to transition onto the wait-list; the reasons why a patient is not listed at a particular time point may vary; standardisation of processes may streamline future wait-listing strategies.

**Methods:** A cross-sectional single-centre study was performed. Departmental ESKD Registry identified all patients treated by any form of long-term dialysis on 31/12/12. Low clearance patients and patients with failing RTX were not included. Transplant wait-list status, transplant history, age, gender, ESKD vintage, modality of RRT, and reasons for current status were established by electronic and paper record review, supplemented by supervising Consultant interviews.

**Results:** 167 patients were identified. Mean age was 61 (range 26-90, median 62) years. Mean ESKD duration was 58 (range 0–386, median 36) months. 22 (13%) had had previous transplants. 134(80%) were undergoing centre haemodialysis, 6(4%) home haemodialysis, and 27(16%) home peritoneal dialysis.

| | N | % |
|---|---|---|
| On RTX List (Active/Suspended) | 50 (47/3) | 30% (28%/2%) |
| Working Up for RTX List (Ongoing / Ongoing – previous medical barrier improved/Suspended - medical barrier) | 43 (19/6/18) | 26% (11%/4%/11%) |
| Unwilling to consent to RTX despite offer to workup | 14 | 8% |
| Advised that permanent medical barrier to joining RTX List exists | 58 | 35% |
| Unclear as to RTX List status | 2 | 1% |

Table 1 indicates the point prevalence on the RTX wait-list on 31/12/12. For those working up for addition to the list, transient medical barriers were important factors leading to delays in completing wait-listing. Patients availing of Home Therapies (58%) were more likely to be RTX wait-listed than those on Centre-based Therapies (23%).

**Conclusions:** A definitive conclusion to RTX wait-listing processes had been attained for 73% of patients. A highly variable matrix of reasons for non-conclusion in the 26% still undergoing workup was identified on Consultant interview – these processes may benefit from structured and prioritised attention.

**PUB473**

**Clinical Usefulness of AKIN Criteria in Deceased Donor Kidney Transplantation** Myung Hyun Lee, Jong Hoon Lee, Keun Suk Yang, Yul Hee Cho, Ji Hyun Yu, Seun Deuk Hwang, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Republic of Korea.*

**Background:** In this study, we analyzed the renal graft function and graft survival after Kidney transplantation (KT) from deceased donors with acute kidney injury (AKI) defined using AKIN (Acute Kidney Injury Network) criteria.

**Methods:** We analyzed 157 deceased donor kidney grafts transplanted at our institution between September 1996 and December 2012. Donors were divided into non-AKI (n=112) and AKI groups(n=45) according to the AKIN classification. Out of 45 AKI donors, 35 donors(22%) were included in stage 1, 9(6%) in stage 2 and 1(0.6%) in stage 3 categories. We compared the incidence of delayed graft function(DGF), the change of allograft function assessed by MDRD-eGFR until 1 year from KT and graft failure between AKI and non-AKI groups.

**Results:** The proportion of deceased donors with diabetes, hypertension and old age (>50 years) did not differ between AKI and non-AKI groups. The development of DGF was significantly higher in the AKI group than in the non-AKI group recipients (12.3% vs 8.3%, p<0.05). Independent predictors of DGF were AKI defined by AKIN criteria(OR 4.94, p=0.001) and PRA(panel reactive antibody) percentage(OR 1.01, p=0.009) in multivariate

analysis. Allograft function until 6 months from KT showed significantly deteriorated pattern in AKI group compared to non-AKI group as well (9.12±5.69 vs 19.37±15.61, 43.74±24.83 vs 58.65±26.73, 53.29±32.3 vs 61.34±20.81, 54.97±18.72 vs 61.26±18.05, 56.39±19.09 vs 62.09±18.18, p<0.05; MDRD-eGFR on 3 day, 2weak, 1month, 3months and 6months in AKI and non-AKI group, respectively). However, allograft function at 12 months from KT did not differ between two groups(58.92±20.63 vs 63.07±23.57, p>0.05; MDRD-eGFR in AKI and non-AKI group, respectively). Graft failure also did not differ between two groups (2% vs 7.4%, p >0.05, AKI and non-AKI group, respectively).

**Conclusions:** In conclusion, definition of AKI according to AKIN criteria in deceased donor is useful to predict the development of DGF and the change of allograft function after KT.

## PUB474

### Immunosuppressive Regimen Adjustment Has No Effect on Serum Cystatin C Concentration in Patients with Severe Pulmonary Infection after Renal Transplantation

Fei Liu. *Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

**Background:** Assesment of renal function is of vital importance for patients with renal transplants. Cystatin C has the characteristics of an ideal marker to assess renal function.

**Methods:** A descriptive, analytical and prospective study was conducted between January 1st,2010 and January 31th, 2013 on 27 renal transplant recipients with severe pulmonary infection.

**Results:** Immunosuppressive drugs was abolished in 11cases of them and the methylprednisolone 40 mg only used by intravenous drips once a day. The forbidden course of immunosuppression was 7 to 20 days. The dose of immunosuppressive drugs was decreased in 13cases of them. No patients had rejection during the forbidden or reduced course of immunosuppression. The serum cystatin C concentration of 27 patients were 2.02±0.99 mg/L before adjustment and 2.21±1.12 mg/L after adjustment. There was no statistically significant difference between before and after adjustment of immunosuppressive regimen for serum cystatin C concentration (P=0.053).

**Conclusions:** Our results showed no significant difference for serum cystatin C was obtained between before and after adjustment of immunosuppressive regimen. Adjustment of immunosuppressive regimen was effective and safe for patients with severe infection after renal transplantation. Serum cystatin C may be considered as a sensitive predictive parameter of kidney function in renal transplant recipients with infection.

## PUB475

### Improvement in Renal Function in High Immunologic Risk Kidney Transplant Recipients Switched from Tacrolimus to Belatacept

Gaurav Gupta,[1] Stacey Posner,[2] Dhiren Kumar,[1] Qing Ren,[1] Marc P. Posner,[2] Amit Sharma,[2] Anne L. King.[1] *[1]Nephrology, Virginia Commonwealth Univ, Richmond, VA; [2]Transplant Surgery, Virginia Commonwealth Univ, Richmond, VA.*

**Background:** Belatacept might be an alternative to Calcineurin Inhibitors (CNI) to avoid short and long-term nephrotoxicity. Prior data on low immunologic risk de-novo kidney transplant recipients (KTx) switched from a CNI to belatacept demonstrated improved renal function. There is no literature on the use of belatacept for sensitized patients or regrafts.

**Methods:** All patients were confirmed to be EBV seropositive. Tacrolimus (tac) was tapered and belatacept initiated based upon prior published protocol (Grinyo et al., Transpl Int. 2012 Oct;25). Mycophenolate mofetil dose was increased to a dose of 2-2.5g/d to minimize risk of rejection.

**Results:** Five patients, of whom 60% (3/5) were African-American, were switched from tac to belatacept and followed for a median of 4.8 mths (range=3-7 mths). Clinical features are listed below:

| Pt N0. | cPRA (%) | Regraft (Y/N) | Index Biopsy | Time Post-KTx (mths) | Peak SCr(mg/dL) | Current SCr (mg/dL) |
|---|---|---|---|---|---|---|
| 1 | 23 | N | IFTA Gd II + isometric vacuolization | 2.2 | 3.3 | 1.5 |
| 2 | 0 | N | IFTA Gd III | 3.0 | 2.7 | 2.1 |
| 3 | 99 | Y | IFTA Gd II + isometric vacuolization | 8.7 | 1.9 | 1.6 |
| 4 | 97 | Y | IFTA Gd II | 25.7 | 3.0 | 1.5 |
| 5 | 0 | N | IFTA Gd III | 4.0 | 3.2 | 1.8 |

IFTA Gd: Interstitial fibrosis and tubular atrophy Grade (per Banff criteria)

Renal function improved significantly ($p$=0.02) from a mean serum creatinine (SCr)of 2.8±0.6mg/dL prior to the switch to a mean SCr of 1.7±0.2mg/dL at most recent follow-up. No rejection episodes were diagnosed. One patient had an infectious complication (CMV re-activation).

**Conclusions:** In this preliminary report we show a significant improvement in renal function in five patients, the majority of whom were high immunologic risk, when switched from tac to belatacept. Improved renal function was noted even in patients with chronic allograft fibrosis without evidence of acute CNI toxicity. Further follow-up and protocol biopsies are needed to ensure safety and wider applicability of this approach.

## PUB476

### Metformin Use in Kidney Transplant Recipients in the United States

Ajay K. Israni,[1,2] Sally K. Gustafson,[1] Jon J. Snyder,[1] Bertram L. Kasiske.[1,2] *[1]Scientific Registry of Transplant Recipients, Minneapolis, MN; [2]Medicine, Hennepin County Medical Center (HCMC), Univ of Minnesota (UMN).*

**Background:** Metformin is contraindicated in patients with "renal dysfunction (e.g., as suggested by serum creatinine levels ≥1.5 mg/dL [males], ≥1.4 mg/dL [females] or abnormal creatinine clearance)" (US Food and Drug Administration) due to the risk of lactic acidosis. The prevalence of metformin use in kidney transplant recipients (KTX) in the US is not known.

**Methods:** We linked Scientific Registry of Transplant Recipient data for all incident KTX occurring between 2001 and 2012 with pharmacy claims from a database constructed by IMS Health. We then excluded records that were inconsistent between the two databases based on gender, date of birth or state of residence; and required at least one pharmacy claim post-transplant. The final dataset included 123,533 KTX, which represented 82.1% of the initial match and 63.9% of all incident KTX recipients.

**Results:** There were 4,291 KTX (3.5% of final dataset) with at least one claim for metformin post-transplant and the number of claims for metformin ranged from 1 to 111 per KTX. Over 50% of these KTX had 5 or more claims. The first claim for Metformin was at a mean of 1116 ± 950 days post-transplant and the creatinine level prior to this first claim was 1.45 mg/dl ± 1.22 (Interquartile range, IQR 1-1.5). Characteristics of KTX on metformin versus the remaining 119,242 not on metformin are shown in Table.

**Baseline characteristics of kidney transplant recipients on metformin versus those not on metformin**

| Characteristics | On Metformin, n (%) | Not on Metformin, n (%) | p-value |
|---|---|---|---|
| Age in years | | | |
| 18 to 34 | 262 (6%) | 13,851 (12%) | <0.001 |
| 35 to 49 | 1225 (29%) | 36,763 (31%) | |
| 50 to 64 | 2142 (50%) | 49,993 (42%) | |
| 65 + | 662 (15%) | 18,635 (16%) | |
| | | | |
| Gender: Female | 1,990 (46%) | 46,620 (39%) | <0.001 |
| | | | |
| Mean Creatinine at discharge in mg/dl ± S.D. | 2.4 ± 2.12 | 2.8 ± 2.5 | <0.001 |

**Conclusions:** Metformin has been used in 3.5% of KTX. The KTX on metformin had a lower creatinine than those not on metformin. The KTX on metformin will be compared for morbidity and mortality with diabetic KTX not on metformin.

*Funding:* Other U.S. Government Support

## PUB477

### Kidney Transplant in Patients with History of Cardiac Surgery

Salvador Roberto Lopez, Pamela Vázquez, Francisco E. Rodríguez Castellanos. *Nephrology, Instituto Nacional de Cardiologia, Mexico DF, Mexico.*

**Background:** The survival of patients who undergo kidney transplant (KT) has improved considerably over the past three decades, death with a functioning kidney is a major reason for graft loss. Few published studies address renal graft and patient survival in patients with a history of cardiac surgery (HCS), which is important due to pretransplant cardiac disease (CD) is a major risk factor for developing post transplant CD.

**Methods:** Between 1995-2012, 600 KT were performed in our center including 7 cases with HCS We compared those patients with 21 patients without HCS We recorded renal function parameters at baseline (at discharge of KT), 6 and 12 months of follow up. In patients with HCS we analyzed echocardiogram parameters before and 1 year after KT.

**Results:** The age was significantly higher in HCS group. In HCS group the principal etiology of ESRD was diabetes (57.1%) Concerning cardiac surgery procedures, most patients (57.1%) underwent CABG and the rest of the group (42.9%) required valve replacement including one case with three cardiac valve replacement. All patients underwent first KT. The median time between cardiac surgery and KT was 8 months (25-75th IQR: 7-20). At 12 months of follow up serum creatinine were no significantly different between groups (1.4±0.12 vs.1.5±0.4 mg/dl in HCS and no-HCS group respectively). One-year patient and graft survival in both groups was 100%. In HCS group the ejection fraction increased after KT (45.3±13.5 vs. 51.3±18.5%, p=NS, before and one year after KT respectively).

**Conclusions:** In dialysis patients, the estimated survival after CRV is 40-54% and after CABG is 56% at 2-year. Debska reported a one year survival of 93% in 16 dialysis patients who had HCS and underwent KT. We found one-year graft and patient survival of 100% which is better compared with others reports. At one year of follow up we observed improvement of ejection fraction, no significant maybe due to sample size. Kidney allograft function was similar between groups at the end of follow up, thus it seems probable that patients with HCS have a favorable long term prognosis. We consider that KT is a safe procedure in patients with HCS, therefore such patients could be included in KT programs.

## PUB478

### Early Changes in Plasma Creatinine Predicts One Year Graft Function following Kidney Transplantation

Nicoline Valentina Krogstrup,[1] Bo M. Bibby,[2] Camilla Aulbjerg,[1] Bente Jespersen,[1] Henrik Birn.[1] *[1]Dept of Renal Medicine, Aarhus Univ Hospital, Denmark; [2]Dept of Biostatistics, Aarhus Univ, Denmark.*

**Background:** Much research aims to improve long term kidney graft function. Post transplant dialysis due to delayed graft function is associated with poorer long term graft survival. Little is known about the long term importance of initial graft recovery in patients

not requiring dialysis. This study evaluates the association between the velocity of initial kidney graft recovery estimated by the change in plasma creatinine (p-creatinine) and graft function after one year.

**Methods:** A single center, observational, cohorte study including 100 kidney transplants followed for one year at Aarhus University Hospital. P-creatinine was registered immediate before transplantation, daily the first seven days post transplant and finally after one year along with relevant patient characteristics. Patients with no improvement in graft function or requiring dialysis within the first week of transplantation were excluded. A total of 80 patients remained in the analysis. Time to a 50% drop in p-creatinine after transplantation was calculated based on either an exponential or logistic decrease of p-creatinine over time, depending on which model fitted the single patient data better. A multiple linear regression model was used to analyse the association between the time to a 50% drop in p-creatinine and p-creatinine after one year.

**Results:** The time to a 50% drop in p-creatinine correlated positively with p-creatinine at one year ($r$=0.31, beta=0.148, p=0.0052). The correlation persisted when corrected for donor type and recipient age, initial p-creatinine level, sex, number of rejections and use of calcineurin inhibitors (p=0.0468).

**Conclusions:** The initial change in p-creatinine is independently associated with one year kidney graft function indicating 1) that non dialysis dependent differences in early graft function may be important for long term outcome and 2) that this may be used as surrogate marker in studies aimed at enhancing long term outcome by improvement of early graft function.

## PUB479

### Basal Creatinine as Predictor of Graft Dysfunction a Postransplant Year

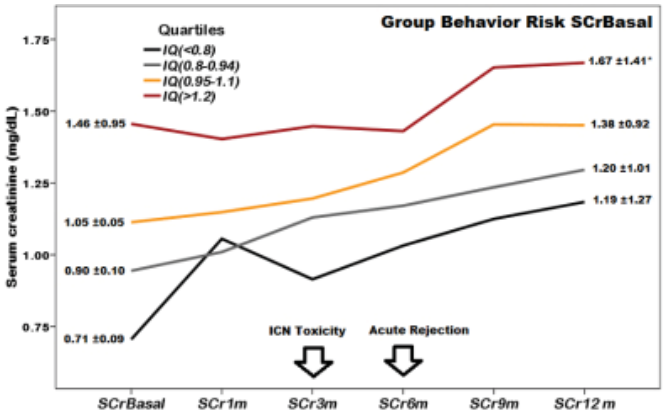
Gerardo Gilberto Azúa Díaz, Benjamin Gomez-Navarro, Enrique Rojas-Campos, Alfonso M. Cueto-Manzano. *Nefrologia, IMSS, Guadalajara, Jalisco, Mexico.*

**Background:** In renal transplant (RT) an increase of serum creatinine (SCr) ≥1.5mg/dl at 1yr of RT predict graft loss at 3yrs in almost 20%. The objective was to establish whether the creatinine at discharge postsurgical unit (SCrbasal) could predict the presence of graft dysfunction (GD) at 1yr post-RT in a Mexican population.

**Methods:** This is a retrospective cohort study performed between January 2009-June 2011 in 561 RT receptors; GD was defined as SCr ≥1.5mg/dl at 1yr.

**Results:** 71% were male receptors, living related donor in 92%, negative crossmath 16.34%. Patients were divided according to SCrbasal values in quartiles. Risk SCrbasal was defined as that with a value >1.2mg/dl because showed a value of SCr of 1.67mg/dl at 1yr post-RT. Changes in SCr and comparison between quartiles variables are show in table and figure (*p<0.005, **p<0.001).

| Variables:(n) | IQ(<0.8)(139) | IQ(0.8-0.94)(78) | IQ(0.95-1.1)(165) | IQ(>1.2)(179) |
|---|---|---|---|---|
| Receptor Age(yr) | 25.5±10.8 | 32.5±15.5 | 26.1±10.5 | 28±10.7 |
| GD Immediately after RT(%) | 1 | 8 | 10 | 14* |
| Time Dialysis(m) | 28.5±26.9 | 25.3±22.4 | 24.9±22.0 | 25±25.4 |
| HLA Antigens Matched(n) | 3.3±1.7 | 3.1±1.7 | 3.6±1.5 | 3.2±1.5 |
| MMF-TAC-PDN(%) | 83 | 87 | 87 | 87 |
| Basiliximab Induction(%) | 56 | 58 | 60 | 62 |
| Acute Rejection (AR)(%) | 25 | 22 | 34 | 42* |
| ICN Toxicity(%) | 48 | 35 | 54 | 51* |
| SCrBasal | 0.71±0.09 | 0.90±0.10 | 1.05±0.05 | 1.46±0.95** |
| SCr 6m | 1.03±0.83 | 1.07±0.29 | 1.26±0.52 | 1.42±0.47** |
| SCr 12m | 1.19±1.27 | 1.20±1.01 | 1.38±0.92 | 1.67±1.41* |




**Conclusions:** In receptors RT, SCrBasal concentration >1.2mg/dl, predicts GD at 1yr-RT ($X^2$62.4, p<0.0001) with a relative risk (RR) of 4.47 (IC95% 2.9-6.8). The factors involved were 1) GD immediately after RT (RR 28.27, IC95% 10.1-82.9) and 2) Gender male (RR 2.52, IC95% 1.6-4.0). This group had a higher number of events of ICN toxicity and AR in the first yr-RT.

## PUB480

### Late Onset De Novo Thrombotic Microangiopathy after Solid Organ Transplant

Mohamad Alhosaini, Krishna Pothugunta, Susan H. Hou, Kavitha Vellanki. *Div of Nephrology and Hypertension, Loyola Univ Medical Center, Maywood, IL.*

**Background:** De novo thrombotic micoangiopathy (TMA) is a well-described complication with calcineurin inhibitors (CNI) and mammalian target of rapamycin inhibitors (mTORI). The vast majority of the reported cases occurred within the first year post solid organ transplant. Here we report our center's experience with late onset TMA.

**Methods:** Since March 2010, we had 4 patients with solid organ transplant develop late onset de novo TMA. The patient characteristics are as shown in Table 1.

| patient | age/sex | type of transplant | etiology | transplant date | baseline immunosuppression | baseline cr (mg/dl) |
|---|---|---|---|---|---|---|
| 1 | 32 male | lung | Cystic fibrosis | 5/2009 | tacrolimus, sirolimus | 2.2 |
| 2 | 49 female | kidney | lupus | 7/2003 | tacrolimus, prednisone | 4.2 |
| 3 | 42 male | kdiney | Alport | 4/2001 | tacrolimus, mycophenolate mofetil, prednisone | 3.9 |
| 4 | 48 female | heart | dilated cardiomyopathy | 12/2010 | tacrolimus, sirolimus, mycophenolate sodium | 2.0 |

**Results:** The mean and the median times of developing TMA were: 82 and 80.5 months respectively post transplant. All patients had acute renal failure, hemolytic anemia, thrombocytopenia and schistocytes. Important characteristics and outcomes are summarized in Figure 1.

| Patient | Time to diagnosis (month) | Tacrolimus level (ng/ml) | Cr at presentation (mg/dl) | Plasmapheresis | Renal outcome | Alternative immunosuppression |
|---|---|---|---|---|---|---|
| 1 | 46 | 41.5 | 7 | Yes | HD for 3 months then recovery | Cyclosporine/mycophenolate mofetil |
| 2 | 115 | 2.4 | 9.7 | No | Permanent HD | Mycophenolate sodium/prednisone |
| 3 | 140 | 7 | 9.2 | No | Permanent HD | Cyclosporine/mycophenolate sodium/prednisone |
| 4 | 27 | 8.5 | 4.1 | No | Recovery | Sirolimus/mycophenolate sodium/ Prednisone |

**Conclusions:** Late onset TMA should be considered in the differential diagnosis of acute kidney injury in solid organ transplant patients on CNI or mTORI therapy for immunosuppression after solid organ transplant.

## PUB481

### Indian Transplantation of Human Organs Act (THOA) and Rules: Practical Problems

Bharat V. Shah. *Nephrology, Global Hospital, Mumbai, Maharashtra, India.*

**Background:** Transplantation of Human Organs Act (THOA) was passed to regularize organ transplant and prevent commercial dealing in transplant. While this has been achieved to a great extent, it has made living donor transplant very difficult and deceased donor transplant has not picked up. The aim of this study is to highlight with examples the problems faced in transplantation due to THOA and rules.

**Methods:** A detailed study of THOA and rules and their amendments has been made (http://www.orbo.org.in/Doc/Ann%204%20THOA%20ACT%202011.pdf). Further, check-list of documents to be submitted as per Directorate of Health Services, is studied. The problems are divided into those pertaining to regulation of professionals and institutions and those pertaining to patients to obtain approval of Authorization committee.

**Results:** Problems for professionals and institutions: 1. If a professional registered at 1 center wishes to perform transplants at another center, he/she again has to apply from that center – a process which takes months. 2. An institution is recognized for transplant for 5 years. For renewal, If the inspection is delayed by the committee, beyond expiry date, transplants cannot be performed at that center until inspection and renewal of recognition.

Problems for patients: 1. For LDT, a) Police verification report of recipient's and donor's address and criminal records report of unrelated and out of state transplants. This is necessary even if a passport is provided as proof of residence. b) If patient or donor or both are not domicile of the state, NOC of concerned state authorization committee to be obtained. c) The Authorization committee often questions medical evaluation of the transplant team.

2. For DDT the act requires removal of organ to be done in a recognized center. As per this act, if a family wishes to donate organs, if the deceased person is not in a center recognized for transplant, he/she has to be moved to a recognized center.

**Conclusions:** The Indian Transplant Act has resulted in severe hardships to many patients with end stage organ disease. In many cases it has lead to substantial delay and increase in avoidable expense, morbidity and mortality.

## PUB482

### Comparing 10 Year Renal Function Outcomes in Patients with Live Donor and Deceased Donor Liver Transplant: A Single Center Retrospective Study

Shaifali Sandal, Sandesh Parajuli, Anirban Bose. *Nephrology, Univ of Rochester, Rochester, NY.*

**Background:** Incidence of chronic kidney disease is approximately 25% by 10 years after an orthotopic liver transplantation (OLT). Long term survival of patients undergoing living donor liver transplant (LDLT) compared to those undergoing deceased donor liver transplant (DDLT) have yielded mixed results and no literature exists comparing the long

term renal outcomes in this patient population. LDLT have become rare at our institution. The purpose of this study is to compare long term renal outcomes in patients who underwent LDLT and DDLT.

**Methods:** A retrospective analysis of patient who received an OLT in years 2000 and 2001 was conducted. Data was obtained from databases UNOS, EPIC and OTTR.

**Results:** A total of 220 OLT were performed in the years 2000 and 2001, of which 82 were LDLT and 137 were DDLT. 1 combined liver and kidney transplant was excluded. Overall patient survival was: 87.7% at 6 months (87.8% for LDLT and 87.6% for DDLT), 84.5% at 1 year (84.1% for LDLT and 84.7% for DDLT), 69.9% at 5 years (74.4% for LDLT and 67.1% for DDLT) and 53% at 10 years (57.3% for LDLT and 50.3% for DDLT). Graft survival was: 80.8% at 6 months (80.5% for LDLT and 81% for DDLT), 76.2% at 1 year (75.6% for LDLT and 76.6% for DDLT), 61.6% at 5 years (63.4% for LDLT and 60.6% for DDLT) and 47.5% at 10 years (47.6% for LDLT and 47.4% for DDLT). 9.1% patients needed some form of RRT prior to the transplant and 25.1% in the immediate post transplant period. Our preliminary data suggest no significant difference in the average GFR of patients who received LDLT versus DDLT at 6 months, 1 year, 5 years and 10 years. We await more robust statistical analysis of this data.

**Conclusions:** Preliminary data shows that patients who receive LDLT and DDLT may have similar 10 year renal function outcomes.

## PUB483

### The Effect of Weight Loss Surgery before Kidney Transplant on Recipient Outcomes

Julie Ann T. Linatoc, Haidr Sabah Agha, Qing Ren. *Virginia Commonwealth Univ.*

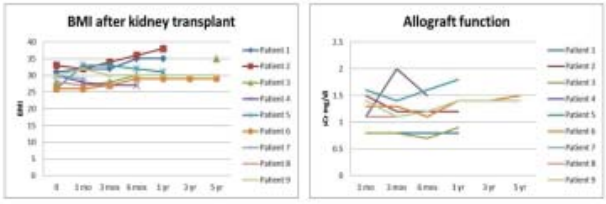
**Background:** Obese patients with a body mass index (BMI) greater than 35 are often denied for kidney transplantation. Obesity is associated with poor graft and patient survival and higher risk of wound infection. Weight loss surgery before kidney transplant may be a solution in this clinical scenario.

**Methods:** We retrospectively studied patients from January 2004 to June 2013 who underwent weight loss surgery, either laparoscopic gastric banding (LGB) or Roux-en-Y gastric bypass (GBP) prior to kidney transplant.

**Results:** Our series included 9 patients. Eight of 9 patients had a BMI below 30 at the time of kidney transplant and maintained BMI less than 35 post-transplant. Waiting time for 4 deceased donor (DD) kidney transplants was between 2 to 11 months. Only 1 of 5 recipients who were not diabetic prior to transplant developed new onset diabetes mellitus. All patients have excellent allograft kidney function with no wound infection or cardiovascular complications.

| Patient | Age (yrs)/ Sex | Weight loss surgery type | Donor Type | Waiting time (mos) | Wound infection | CV events | NODAT |
|---|---|---|---|---|---|---|---|
| 1 | 48/F | LGB | LD | 6 | no | no | N/A |
| 2 | 52/M | LGB | LD | 3 | no | no | N/A |
| 3 | 42/F | LGB | LD | 3 | no | no | no |
| 4 | 63/F | GBP | LD | 10 | no | no | no |
| 5 | 37/M | LGB | DD | 7 | no | no | no |
| 6 | 36/F | GBP | LD | 3 | no | no | N/A |
| 7 | 57/M | LGB | DD | 3 | no | no | N/A |
| 8 | 46/M | GBP | DD | 11 | no | no | yes |
| 9 | 57/F | GBP | DD | 2 | no | no | no |

LD, living donor; CV, cardiovascular; NODAT, new onset diabetes mellitus after transplant; N/A, not applicable, patient was diabetic prior to transplant




**Conclusions:** Weight loss surgery before kidney transplant could be used effectively to control obesity and allow these obese renal failure patients to have the opportunity of successful kidney transplantation with excellent outcomes.

## PUB484

### Perceived Bias in the Kidney Allocation System Predicts Mortality among Black Hemodialysis Patients

Avrum Gillespie,[1] Vladimir Ouzienko,[2] Jeanne Dreier,[1] Heather Hammer,[3] Teri Browne,[4] Zoran Obradovic.[2] *[1]Nephrology, Hypertension, and Kidney Transplantation, Temple Univ School of Medicine, Philadelphia, PA; [2]Center for Data Analytics and Biomedical Informatics, Temple Univ, Philadelphia, PA; [3]Abt SRBI, Silver Springs, MD; [4]College of Social Work, Univ of South Carolina, SC.*

**Background:** Perceived racism has been associated with disparities in renal transplantation and poorer health outcomes for African American hemodialysis patients. This study aimed to determine if perception of the kidney transplant allocation system as racially biased predicts mortality independent of biomarker data in a cohort of urban, African American hemodialysis patients.

**Methods:** In this study of the 101 end-stage renal disease (ESRD) patients who self-identified as black or African American in a hemodialysis clinic survey, perceived racism and other subjective variables were measured with the Dialysis Patient Transplant Questionnaire (DPTQ) and merged with prospective biomarker and mortality data. The predictive association with mortality was measured using chi-square, Fisher's exact test, and multivariate logistic regression.

**Results:** Twenty-two patients (21.8%) died during the study period. Fisher's exact tests showed that patients who thought the kidney transplant allocation system was definitely or mostly biased against blacks were 2.49 times more likely to die compared to those less certain (P=0.001). Serum albumin was also associated with mortality, however, in general, patients that percieved racism were more likely to die independent of their biomarkers.

**Conclusions:** This is the first study to validate the link between perceived racism in the kidney allocation system among African American hemodialysis patients.

## PUB485

### Effect of mTOR Inhibitors in Patients with Severe Posttransplantation Encapsulating Peritoneal Sclerosis

Edin Colic,[1] Claus Bistrup,[1] Helle C. Thiesson,[1] Niels Marcussen,[2] Martin Tepel.[1] *[1]Nephrology, Odense Univ Hospital, Odense, Denmark; [2]Pathology, Odense Univ Hospital, Odense, Denmark.*

**Background:** Posttransplantation encapsulating peritoneal sclerosis is a live threatening complication seen in patients after kidney transplantation who had previously been treated with peritoneal dialysis. In the present study we investigated the effects of mTOR inhibitors on outcome in patients with severe posttransplantation encapsulating peritoneal sclerosis.

**Methods:** Using the transplantation registry of the Odense University Hospital of Southern Denmark, we identified all patients with kidney transplantation between 2006 and 2012. The risks of encapsulating peritoneal sclerosis as diagnosed by radiological or histological signs and need for laparotomic or laparoscopic interventions were estimated. In addition, the effects of treatment with mTOR inhibitors during follow up were evaluated. Major outcome measures were patients' survival, graft survival, number of acute rejections and relapse of encapsulating peritoneal sclerosis.

**Results:** A total of 341 transplanted patients were evaluated. Five (4 female and 1 male) out of 94 patients with previous peritoneal dialysis (5.3 percent) developed posttransplantation encapsulating peritoneal sclerosis. Median age was 54 years (range, 46-59). Median duration of peritoneal dialysis was 63 months (range, 36-107). Median time for development of encapsulating peritoneal sclerosis after transplantation was 13 months (range, 1-38). 1 patient was shifted to rapamycine, whereas 4 patients were shifted to everolimus. During a median follow up period of 21 months, 2 patients developed a relapse of encapsulating peritoneal sclerosis necessitating additional surgical interventions. Graft loss was observed in 2 other patients. There were no acute rejections. All 5 patients are still alive.

**Conclusions:** This retrospective observational study in patients with severe posttransplantation encapsulating peritoneal sclerosis with need of surgical intervention shows that shifting from calcineurin inhibitors to mTOR inhibitors did not ameliorate ongoing fibrosis. However, altered speed of progression can not be excluded.

## PUB486

### Assessing the Quality of Deceased Donor Kidneys by Simple and More Accurate Prognostic Factors

Ana Pinho,[1] Inês Castro Ferreira,[2] Isabel Tavares,[2] Susana Moreira Norton.[2] *[1]Nephrology, Faro Hospital, Faro, Portugal; [2]Nephrology, São Jão Hospital, Oporto, Portugal.*

**Background:** Since the increasing use of lesser quality kidneys, both survival benefit and a quality of life advantage of kidney transplantation over dialysis is know a difficult question of patients to their transplant team. In order to clarify, several Donor Risk Indexes (KDRI)were derived from many potential donor factors that need to be validated. The aim of this study was to determine which donor factors predict poorer transplant outcome.

**Methods:** Data from transplant registry on 273 adult recipients of adult deceased donor kidney transplants, performed consecutively between 2009 and 2012 were analyzed. Donor factors potentially influencing transplant outcome were investigated using Cox regression, adjusting for significant recipient and transplant factors.

**Results:** Donor hypertension was the most significant factor predicting poor transplant outcome (hazard ratio [HR] 4.96; 95% confidence interval [CI] 1.81 to 13.55)*).* The cause of death by cerebrovascular accident (HR 3.85; 95% CI 1.49 to 9.92) and Donor age (HR for a 10-yr increase 1.44; 95% CI 1.1 to 1.78*)* were also associated with increased risk with poorer outcomes up to 4 years posttransplant. Other donor factors including diabetes history, longer hospital stay before death, use of adrenaline, previous infection and terminal creatinine were not significant.

**Conclusions:** This finding corroborates the relevance of donor risk factors, such as age, cause of death and hypertension; that may be of value for researchers and clinicians in provide a clinically useful tool that help with organ allocation and informed consent.

## PUB487

**Complications Associated with Failed Pediatric Renal Allograft**
Murty Adabala,[1] Tej K. Mattoo,[1] Gaurav Kapur,[1] Rossana Baracco,[1] Amrish Jain.[1] *[1]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [2]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [3]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [4]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI; [5]Pediatric Nephrology, Children's Hospital of Michigan, Detroit, MI.*

**Background:** NAPRTCS data reports 18-30% graft loss in the 1st 5 years post transplant. The failed graft in adult is associated with increased morbidity and mortality. However, there is paucity of pediatric reports on complications associated with failed renal allograft and their outcome.

**Methods:** Retrospectively reviewed medical records of patients with failed renal transplant over last 10 years (2001-2011). For study purpose, graft intolerance was defined by presence of chronic inflammatory state, pain at the graft site or hematuria.

**Results:** 11 grafts were lost in 10 patients( 50% male, 60% African American). Obstructive uropathy and FSGS accounted for 30% each for the primary renal disease. Mean age at transplant was 9.6y. Graft intolerance was noted in 9/11 (81%) grafts leading to nephrectomy in 8/11 (72%). The most common indication for the transplant nephrectomy was recurrent abdominal pain with hematuria.

| Variables Patients | Age at transplant (years) | Time to dialysis | Graft intolerance | Nephrectomy | Indication for nephrectomy |
|---|---|---|---|---|---|
| 1 | 10.1 | 5y | Y | Y | Persistent Systemic Inflammation |
| 2 | 10 | 5y | Y | Y | AP+H |
| 3 | 11.7 | 1 day | Y | Y | Surgical |
| 4 | 14.5 | 1.4y | Y | Y | AP+H |
| 5 | 11 | 2.3y | Y | Y | AP+H |
| 6 | 6.1 | 2.5y | Y | Y | AP+H |
| 7 | 9.3 | 2.6y | Y | N | |
| 8 | 8.8 | 1 day | Y | Y | Hyper acute Rejection |
| 9a | 6.7 | 1 day | Y | Y | Hyper acute Rejection |
| 9b | 11.11 | 5.7y | N | N | |
| 10 | 6.7 | 2.1y | N | N | |

Y = Yes, N = No

**Conclusions:** The complications associated with failed renal graft are common and a high percentage of our patients required allograft nephrectomy. More studies are needed to further define the complications of failed renal allograft and the indications for elective transplant nephrectomy in such patients.

## PUB488

**Effect of Valganciclovir Prophylaxis on Cytomegalovirus Disease in Kidney Transplant Recipients**
Gopal Basu, Golla Sudhakar, Riyaz A. Asad, Anna T. Valson, Anjali Mohapatra, Suceena Alexander, Shibu Jacob, Vinoi George David, Santosh Varughese, Chakko Korula Jacob, Veerasamy Tamilarasi. *Nephrology, Christian Medical College, Vellore, India.*

**Background:** Valganciclvoir (ValG) prophylaxis modifies the cytomegalovirus (CMV) disease pattern in kidney transplant recipients (KTRs). We aimed to study the efficacy of ValG prophylaxis in preventing CMV disease, the difference in CMV disease pattern and the effect of CMV disease and ValG on transplant survival.

**Methods:** All KTRs who underwent transplantation at Christian Medical College, Vellore between Jan 2006 to Dec 2011 were studied. KTRs from 2007 were offered ValG prophylaxis for first 3 months. The outcomes studied were CMV disease, transplant survival, pattern of CMV Disease and coincident Infections. Analysis was performed using standard statistical methods.

**Results:** Of the 470 KTRs studied (mean age =36.5±12.1 years; M:F = 3.3:1), 9.6% received deceased donor grafts, Basiliximab induction in 62.7% with prednisolone + Tacrolimus + Mycophenolate in 81.6% and ValG prophylaxis (3 months) in 62.4%. The overall incidence of CMV disease was 14.9 % . ValG prophylaxis effectively reduced CMV disease (10.9% vs. 21.5%; p<0.01; NNT=10), delaying its onset (median time of 11.9 vs 3.7 months) and reduced the degree of viremia. However,ValG prophylaxis was not associated with any difference in tissue invasive disease, recurrence or CMV associated mortality. There was however, a reduction in coincident infections such as Post transplant TB, varicella zoster virus and Hepatitis C virus infections as well as mortality. Graft survival was better in ValG group (p <0.01) despite no difference in rejection rates, probably associated with effective immunosuppression as well as control of the indirect effects of CMV. Within the ValG group also, CMV disease was significantly and independently associated with co-incident infection and mortality indicating a generalised immunosuppressed state.

**Conclusions:** ValG prophylaxis reduces incidence of CMV disease by 50%, delays its onset, decreases viremia and is associated with better graft and patient survival probably by controlling both the direct and indirect effects of CMV in KTRs.

## PUB489

**Combination Therapy of Leflunomide and Ciprofloxacin for Treatment of (Suspected) Polyomavirus BK-Associated Nephropathy in Kidney Transplant Recipient Lacking Graft Biopsy (Single Center Experience)**
Seddeg Younis, Dujanah Hassan Mousa, Ebadur Rahman, Naveed Aslam, Raees Farhan Mushtaq, Mamdouh Abdulghafour Nada, Nader Mohamed Omran, Ahmed Soliman. *Prince Sultan Military Medical City, Riyadh, Saudi Arabia.*

**Background:** Polyoma Virus type BK Nephropathy (BKVN) occurs in 1-10% of Renal Transplant Recipients. Progression to Graft Failure in the First year is up to 70%. Immunosuppression Reduction (ISR) is the Treatment of BKVN. Diagnosis of BKVN is Based on BKV Replication in Blood and Histological Evidence of BKV Involvement. In this Prospective Study, we Reported 7 Patients with Graft dysfunction and Significantly High BK Viral Load, declined to do Graft Biopsy, were treated Blindly as BKVN, with addition of Leflunomide & Ciprofloxacine to Improve Graft Survival.

**Methods:** Blood BKV Quantitative Polymerase Chain Reaction (PCR) and Graft Biopsy for all Patients with Unexplained Graft dysfunction. Between Jan 2011 to May 2012. Seven Patients Presented with Slowly Rising Creatinine and high BKV Viral Load, refused to do Graft Biopsy. The Median Ceartinine at Baseline was 121µmol/l (68-174), and the Median Peak Viral Load was $7X10^5$copies/ml ($1.2X10^4$-$3X10^6$). They were treated with ISR and Combination of Leflunomide and Ciprofloxacine. Leflunomide Loading dose was 40mg daily for One Week, Lowered to 20mg Until Complete Viral Clearance or Progressive Reduction in Viral Load, then Continued at 10mg. Ciprofloxacine, 500mg twice daily for 6 Week. The treatment Monitored by Serial Measurement of LFT, FBC, Serum Creatinine, and BKV PCR. Graft Function and Viral Clearance were Followed.

**Results:** After 22 months (19-22) follow up, Median creatinine was 152µml/l (84-273). Creatinine was Stable in 3/7 (42.9%) and deteriorated in 4/7(57.1%), but there was no Graft Loss on follow up. Complete viral clearance Occurred in 2/7, and Significant Reduction in 5/7(p<0.0001). There were no Serious Adverse effects.

**Conclusions:** Despite Good Response to Treatment of (Suspected) BKVN with ISR and Combination Therapy of Low Dose Leflunomide and Ciprofloxacine the Decline in Graft is not Determined, this Highlights the Importance of Graft Biopsy in the Diagnosis of BKVN and Alternative Etiology.

*Funding:* Government Support - Non-U.S.

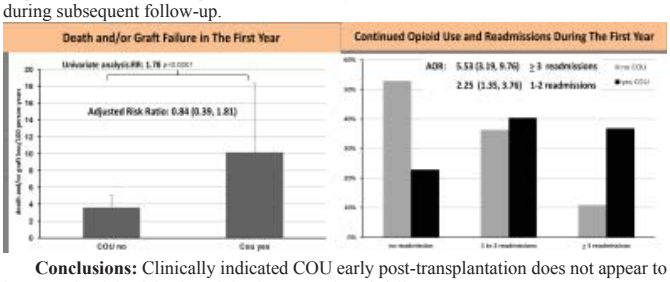
## PUB490

**Chronic Opioid Use, Pain and Outcomes of Kidney Transplantation**
Satyarth Kulshrestha,[1] Fidel Barrantes,[2] Milagros D. Samaniego-Picota,[1] Fu L. Luan.[1] *[1]Div of Nephrology, Univ of Michigan, Ann Arbor, MI; [2]Nephrology, Presbyterian Kidney Transplant Center, Albuquerque, NM.*

**Background:** Chronic opioid usage (COU) is relatively common among patients with end stage renal disease qualified for kidney transplantation. The prevalence of continued COU after kidney transplantation and its impact on transplant outcomes remains unknown.

**Methods:** We conducted a retrospective single center study to describe the prevalence of COU during the first year after transplantation, to identify the predictors of COU and to determine the effect of COU on patient and kidney transplant survival.

**Results:** Among 1045 kidney transplant patients, 114 (10.8%) had COU during the first year. The most common cause of pain was surgery-related (39.9%) and the most commonly prescribed opioid analgesic was hydrocodone (59.2%). A history of COU prior to transplantation was the strongest predictor of COU in the first year post-transplantation. Compared to patients without COU, COU patients had 2 to 5 folds higher risk of hospital readmission during the first year (AOR 2.25, 95% CI 1.35, 3.76, p=0.002 for 1 or 2 readmissions, and AOR 5.52, 95% CI 3.13, 9.76, p<0.0001 for >3 readmissions, respectively), but similar patient and kidney graft survival, both within the first year and during subsequent follow-up.




**Conclusions:** Clinically indicated COU early post-transplantation does not appear to increase the risk of death and death censored graft failure.

## PUB491

**Comparison of Cadaveric Kidney Transplantation from In-Center and External Hospital Donors**
Ajin Cho, Hye Ryoun Jang, Jung Eun Lee, Wooseong Huh, Yoon-Goo Kim, Ha Young Oh, Dae Joong Kim. *Internal Medicine, Samsung Medical Center, Sungkyunkwan Univ School of Medicine, Seoul, Korea.*

**Background:** Renal transplantation is the best treatment modality for end-stage renal disease. We investigated the effects of cadaver donor sources, inside vs. outside of transplantation center, on renal allograft and patient survival.

**Methods:** We retrospectively analyzed 190 cadaver kidney transplants carried out in our center from January 2000 to December 2009, 136 cadaver harvested in our transplant center and 54 harvested in external hospital. All patients received induction therapy and standard triple immunosuppressive protocols with cyclosporine or taclorimus (FK506), mycophenolate-mofetil, and prednisone. Variables on this study were donor and recipient age, sex, body mass index (BMI), presence of diabetes mellitus, HLA mismatching, dialysis duration, cold ischemic time, donor from external hospital, acute rejection episodes, delayed graft function (DGF) and panel reactive antibody (PRA) > 30%. Graft and patient survival were assessed with the Kaplan-Meier method and the significance of possible variables with the Cox proportional hazard model.

**Results:** Donor and recipient ages, recipient BMI, duration of dialysis, presence of DGF and PRA >30% were not significantly different. Cold ischemic time was shorter in recipients of cadaver from our center, 288 min, than external hospital, 388 min (P<0.001). Twenty recipients lost their grafts (14 cadaver from external hospital and 6 cadaver from our center). Graft survival at 1, 3, and 5 years were 99.2%, 97.3%, and 95.5% for our center donor while 98.1%, 88.9%, and 86.2% for external hospital donor transplants (P=0.01). Total of 8 patients died. (2 from external hospital and 6 from our center ). No difference in patient survival rates was found. Acute rejection episodes (hazard ratio [HR] =13.8; P<0.001) and location of harvest (HR =7.0; P=0.02) were independent associating factors for graft failure.

**Conclusions:** Graft survival rate of cadaveric transplants from external hospital donor was lower than that from donor harvested in our center. Acute rejection episodes and location of harvest were significant factors on graft survival.

## PUB492

### Adult Kidney Transplantation with Allografts from Pediatric Cardiac Death Donors in China – A Single-Center Experience

Ying Xu, Jianghua Chen. *The Kidney Disease Center, The Firtst Affiliated Hospital, College of Medcine, Zhejiang Univ, Hangzhou, Zhejiang, China.*

**Background:** Current transplant experience with cardiac death donors (DCD) organs has focused on the adult donor population, little is known about the outcome of pediatric donor used in adult recipients. The abstract is aimed to report clinical outcomes of kidney transplantation from pediatric DCD in China, and to investigate its feasibility to expand the organ donor pool.

**Methods:** We retrospectively reviewed the clinical data of 45 DCD kidneys from 27 donors from 1st January 2012 to 30th November 2012 in our center. Recipients were followed for patient and graft survival.

**Results:** There were seven pediatric DCD donors (age < or = 18) and twenty adult DCD donors (age > 18 years). All the eleven recipients who received the pediatric donors were adults. The age of the pediatric DCD donors ranged from 31 months to 17 years old (31 months, 50 months, 12 years, 13 years, 14 years, 15 years and 16 years respectively). The average weight of the donated kidneys was 126 grams (ranged from 46 to 160 grams). The incidence of delayed graft function was 18.2% (2/11); these two recipients received the kidneys from the youngest donor (31 months, 46 grams), their allograft function recovered to normal range within 3 months post-transplant. The function of the allograft from the 50 months-old donor recovered with a slow rate to the normal range. All other recipients survived with good allograft function.

**Conclusions:** Although kidney transplantations from younger pediatric DCD donors showed a higher rate of DGF with a longer duration of graft recovery, we achieved favorable short-term clinical outcome in terms of graft survival and function. Donation after pediatric cardiac death could expand the organ donor pool in China.

*Funding:* Government Support - Non-U.S.

## PUB493

### The Effect of Kidney Transplantation on Left Ventricular Function and Valvular Regurgitation in End Stage Renal Disease Patients

Prabhakar Doddi,[1] Harbir Singh Kohli,[1] Ajay Bahl.[2] *[1]Nephrology, PGIMER, Chandigarh, India; [2]Cardiology, PGIMER, Chandigarh, India.*

**Background:** Most patients with chronic kidney disease suffer frequently from cardiac abnormalities including valvular disorders and left ventricular(LV) systolic and diastolic dysfunction. At present, little information is available regarding the effect of kidney transplantation on LV function and valvular regurgitation in End Stage Renal Disease (ESRD) patients.

**Methods:** This is a prospective study in which 250 patients with ESRD on maintenance hemodialysis were enrolled between July 2010 to June 2011 and 30 patients were studied who subsequently underwent kidney transplantation. Base line LV function and valvular abnormalities were evaluated by using conventional echocardiography 4 weeks before kidney transplantation and same was repeated at 12 weeks after transplantation.

**Results:** In this study we found that 4 weeks prior to renal transplant, out of 30 patients, 15(50%) had low ejection fraction (EF), 13(43.3%) patients had moderate and 1(3.3%) patient had severe mitral regurgitation (MR). There were 13 (43.3%) patients with moderate tricuspid regurgitation (TR) and 2(6.7%) with severe TR. Twelve weeks post transplantation it was observed that there was significant improvement in EF(p<0.001) and valvular regurgitation(P<0.001 for MR and 0.001 for TR). Of 13 patients with moderate MR, 6 improved to mild MR and in rest 7 MR disappeared. One patient with severe MR had complete disappearance of MR after transplantation. Of 13 patients with moderate TR, 8 improved to mild, in 4 TR disappeared and one patient it persisted. Out of 2 patients with severe TR, one improved to mild and in other TR disappeared. Of 15 patients with low EF, 14 improved.

**Conclusions:** In this study we found that there was significant improvement in LVEF and valvular regurgitation after kidney transplantation and it implies that poor LV function and valvular regurgitation should not be contraindications for kidney transplantation.

## PUB494

### Impact of the Declaration of Istanbul on the Dynamics of Renal Transplantations in Oman

Nabil Mohsin, Ehab Mohamed Hassan, Emily Militsala, Issa A.L. Salmi. *Nephrology, Royal Hospital, Muscat, Oman.*

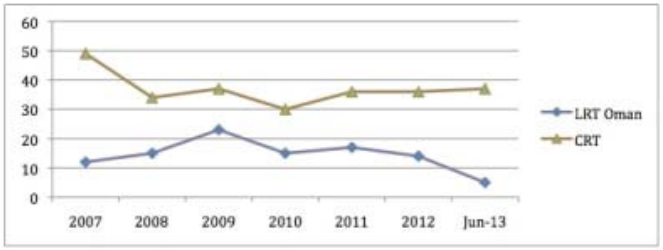
**Background:** Organ shortage is a universal thorny issue. Living related transplantations (LRT) are offered free of charge for all citizens. Law bans commercial transplants. Commercial renal transplants (CRT) are still practiced in some low-income countries. The declaration of Istanbul (DoI) formulated in April 2008 constitutes the global framework for transplantation practice rejects and combats this practice. We analyzed the impact of the DoI on LRT and CRT dynamics in Oman.

**Methods:** Registry data analysis for the period 2007 till June 2013 of local LRT and CRT. The chi-square proportion test was applied to look for significance before (year 2007) and after the DoI (2008-june 2013). We used Stata12.1,Tx, USA.

**Results:** Dynamics of Living related (LRT) and Commercial (CRT) transplants in Oman before and after DoI are shown on the table below:

| | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 06/2013 |
|---|---|---|---|---|---|---|---|
| LRT | 12 | 15 | 23 | 15 | 17 | 14 | 5 |
| CRT | 49 | 34 | 37 | 30 | 36 | 36 | 37 |
| LRT/CRT (p-value) | 0.24* | 0.24/0.44 (0.06) | 0.24/0.62 **(<0.001)** | 0.24/0.50 **(0.02)** | 0.24/0.47 **(0.03)** | 0.24/0.39 (0.15) | 0.24/0.14 (0.2) |

In 2007 the proportion of LRT to CRT was 24%. The proportion increased significantly to reach a peak of 62% in 2009. Also, CRT from Pakistan decreased from 84% in 2007 to 38% and 51% in 2008 and 2009 respectively. Painfully, Commercialism resurged, grows and affects negatively the local program.




**Conclusions:** Oman enjoyed a significant positive impact of the DoI till 2011. However, the effect seems to lessen due to the resurgence of commercial transplants. Mechanisms of resurgence should be analyzed meticulously. Concomitantly, an active deceased donor program and further development of the living donor program to ensure self-sufficiency would be effective means to deter this phenomenon and reinforce the Declaration.

*Funding:* Government Support - Non-U.S.

## PUB495

### Long-Term Effects of Renal Transplantation on Cardiac Autonomic Dysfunction

Eduardo Cruz,[1] Fadi Tohme,[1] Roberto S. Kalil,[1] Harald M. Stauss.[2] *[1]Medicine/Nephrology, Univ of Iowa; [2]Exercise Physiology, Univ of Iowa.*

**Background:** It has been suggested that uremic autonomic neuropathy in patients with end-stage renal disease (ESRD) contributes to cardiac autonomic dysfunction that has been associated with increased cardiovascular morbidity and mortality. With this regard, it has been demonstrated that reduced low frequency to high frequency (LF/HF) ratio of heart rate variability (HRV) is associated with a higher probability of cardiovascular disease events in patients with chronic kidney disease (Chandra P. et al., 2011). We hypothesized that renal transplantation (RTX) in patients with ESRD will improves autonomic dysfunction (indicated by an increase in the LF/HF ratio of HRV) and, thus, reduces the probability of cardiovascular disease events.

**Methods:** To test this hypothesis, we studied cardiac autonomic function by frequency domain HRV analysis (power spectral analysis) in patients with ESRD before and 3 months, 12 months, and 36 months following renal transplantation.

**Results:** 28 patients were studied. Mean age was 51.6 ±17.4 years with 20 males. Mean eGFR was 56.4 ±17.9. Low frequency to high frequency ratio of HRV increased from 1.1±0.6 before RTX to 1.9±0.5 at 3 months and 3.2±0.7 at 1 year after RTX (P=0.07 vs. before RTX). However, 3 years after renal transplant, the LF/HF ratio of HRV was no longer significantly elevated compared to before RTX (1.7±0.4).

**Conclusions:** Our results indicate that renal transplantation improves cardiac autonomic function only temporarily (for less than 3 years). Other factors than improved cardiac autonomic function appear to contribute to the beneficial long-term effects of RTX on the reduction in cardiovascular events. Further larger, prospective studies are needed to examine the impact of cardiac autonomic dysfunction on long-term outcomes of kidney transplantation.

*Funding:* Other NIH Support - NHLBI

**PUB496**

**10-Year Survival of Kidney Graft: Analysis of Predictive Factors** Francesca K. Martino,[1] Daniela Dissegna,[1] Tefanos Chiaramonte,[1] Claudio Ronco.[1,2] *[1]Nephrology, San Bortolo Hospital, Vicenza, Italy; [2]IRRIV, Vicenza, Italy.*

**Background:** The quality of graft plays an important role in the outcome of kidney transplant (KT). On the other hand, the possible complications following KT could impact on the kidney function in the long-term. This study investigates specific influence of pre-implantation and after-implantation features in the prediction of KT survival in the short and long period.

**Methods:** We performed a retrospective study on 72 patients, who received renal graft from January 2000 to December 2002. For all patients the following parameters were obtained: pre-implantation kidney biopsy with Karpinski score, duration of cold ischemia, donor's age, requirement of dialysis treatment after the KT, presence of early rejection in the first 6 months after KT, creatinine level after 1 week, 1 month, 1 year, and 10 years from KT and survival of KT. Kaplan-Meier methods was used for survival analysis. Logistic regression model was employed to evaluate the variable related with delayed allograft function. Cox regression models addressed the time to graft survival. All reported p-values were two sided, and statistical significance was set at $p < 0.05$. All statistical tests were performed with SPSS version 17.

**Results:** The median age of donor was 47 years (IQR 32-56). Delayed graft function and early reject occurred in 20.8% and 15.3% of case, respectively. The median biopsy score was 2 (IQR 1-3). Karpinski score and duration of cold ischemia were predictors of dialysis need (OR 2.5, p=0.014 and OR 0.035, p=0.03, respectively). The median follow-up duration was 120 month (IQR 38.75-120). Five and 10-year survival free from graft failure estimates were 83.7% and 78.6%, respectively. In the long-term, the survival of KT was predicted only by dialysis need after KT (HR 3.84 p=0.016), level of creatinine in the first month (HR 1.91 p<0.001) and presence of early rejection (HR 4.012 p 0.02). Conversely, Karpinski score was not predictive(HR 0.88, p=0.539).

**Conclusions:** The clinical features such as delayed graft function and early reject seem to have an important influence on long-term graft survival.

**PUB497**

**Mineral Bone Density and Cardiovascular Survival in Kidney Transplant Patients** Francesca K. Martino,[1] Daniela Dissegna,[1] Tefanos Chiaramonte,[1] Claudio Ronco.[1,2] *[1]San Bortolo Hospital, Vicenza, Italy; [2]IRRIV, Vicenza, Italy.*

**Background:** Previous studies demonstrated an association between low bone mineral density (BMD) and increased risk of death both in men and women. In kidney transplantation (KT) there is a different profile of risk to develop osteoporosis. Moreover, vascular calcification and arterial stiffness are inversely correlated with BMD and are independent predictors of cardiovascular mortality. The aim of the present study was to examine the pattern of cardiovascular mortality risk related with BMD in kidney recipients.

**Methods:** We studied the relationship between BMD and cardiovascular death among 123 patients undergoing KT from 1990 to 1999 who had DXA during the same period at all the following skeletal site: hip, spine and middle arm. Baseline data included general information about the patient and specifically about KT such as immunosuppressive therapy, level of creatinine, level of PTH life, age, presence of comorbidity, and BMD measured using DXA. Cox regression models addressed the time to patient survival. All reported p-values were two sided, and statistical significance was set at $p < 0.05$. All statistical tests were performed with SPSS version 17.

**Results:** Patients were followed for a median period of 15 years (IQR 9,5-15). During follow-up, 42 (34.1%) patients died, 17 (13.8%) due to cardiovascular disease. The median value of BMD at hip, spine, and middle arm were 0.74 (IQR 0.67-0.85), 0.89 (IQR 0.8-0.99), and 0.71 (IQR 0.65-0.8), respectively. BMD level at hip site was associated with increased mortality (OR 0.036 p=0.038), while BMD at spine and at middle arm were not predictors of cardiovascular mortality (OR 0.096 p=0.068 and OR 0.447 p=0.689, respectively).

**Conclusions:** Low BMD at hip is associated with a substantial excess risk of cardiovascular death compared to low BMD at spine and middle arm.

**PUB498**

**Patient Factors Contribute to Compliance for Renal Transplant Recipient Evaluation** Asha M. Alex, Mohini Alexander, Meredith Akerman, Eric Siskind, Poornima Ramadas, Prathik Krishnan, Prejith P. Rajendran, Qazi Nuaman Masood, Kellie R. Calderon, Ernesto P. Molmenti, Madhu C. Bhaskaran. *Kidney Transplant Center, North Shore LIJ Health System, Manhasset, NY.*

**Background:** This study sought to examine the different factors that may prevent patients from completing their workup for receiving a renal transplant.

**Methods:** We retrospectively reviewed 170 patients who did not complete their pretransplant evaluation. Records of patients, who were either on dialysis or had less than 20 ml/min creatinine clearance (pre-emptive transplantation), were reviewed to identify a variety of factors which may have influenced the likelihood of completing their workup.

**Results:** 56% of pre-emptive patients completed their work up, while 36% of dialysis patients completed their work up. The data revealed that the factors contributing towards patients completing their workup included intrinsic motivation (4 times more likely), lack of specific medical co morbidities ( 3 times more likely), and patients not on dialysis (2 times more likely). Among the patients on dialysis, the factors which contributed towards them completing their work up included intrinsic motivation (5 times more likely), absence of cardiovascular complications (4 times more likely). When comparing patients on dialysis to patients not on dialysis, there was a significant difference between the two groups' in terms of distance from their respective homes to the transplant center, their level of education, and presence of specific medical co-morbidities.

**Conclusions:** We believe that through targeted interventions such as timely referral, providing appropriate educational resources and development of adequate support systems, all patients with advanced CKD, including those on dialysis, can be motivated to complete their pre-transplant workup.

**PUB499**

**Early Belatacept Conversion for Slow Renal Allograft Function** David Wojciechowski, Sindhu Chandran, F. Vincenti. *Medicine-Nephrology, UCSF, San Francisco, CA.*

**Background:** Slow renal allograft function (SGF), which we define as the need for HD or a Cr >2 mg/dL >14 days post-transplant, has a negative impact on long-term graft outcome. Tacrolimus is postulated to prolong SGF. We investigated belatacept conversion from tacrolimus as a novel treatment strategy for SGF.

**Methods:** Retrospective single center analysis of the first 8 patients converted to belatacept for SGF (Group 1) compared to a historical control group of 16 SGF patients remaining on tacrolimus (Group 2) matched for the following variables: type of kidney transplant, cause of ESRD, and CIT. Primary outcome: eGFR at 6 months; secondary outcomes: rates of rejection and infection.

**Results:** Table: Baseline demographic and transplant characteristics.

| | Group 1 (8) | Group 2 (16) | P Value |
|---|---|---|---|
| **Race, n** | | | 0.19 |
| White | 1 | 7 | |
| African-American | 4 | 3 | |
| Asian | 3 | 6 | |
| **Male, n** | 5 | 14 | 0.29 |
| **Age, years (SD)** | 56.4 (14.8) | 57 (13.2) | 0.92 |
| **Donor type, n** | | | 0.24 |
| Standard | 5 | 13 | |
| ECD/DCD | 3 | 3 | |
| **ESRD cause, n** | | | 1.0 |
| DM | 1 | 2 | |
| HTN | 1 | 2 | |
| GN | 3 | 6 | |
| Other | 3 | 6 | |
| **cPRA, mean (SD)** | 26.8 (31.7) | 7.7 (22) | 0.1 |
| **CIT, hours (SD)** | 10.6 (4) | 10.3 (4) | 0.86 |
| **Induction agent, n** | | | 0.67 |
| rATG | 6 | 10 | |
| Basiliximab | 2 | 6 | |
| **Steroid maintenance, n** | 8 | 16 | |
| **Tacrolimus mean POD initiation (SD)** | 2.3 (1.4) | 2.8 (2) | 0.53 |
| **Tacrolimus mean trough (SD)** | | | |
| Week 2 | 9.9 (4.2) | 6.8 (2.2) | 0.03 |
| Week 4 | 7.7 (1.6) | 9.2 (3.3) | 0.24 |
| Month 3 | | 8.9 (2.4) | |
| Month 6 | | 7.2 (1.3) | |

Mean POD of conversion was 46.6 (range: 21-74). Mean number of HD treatments (SD) for Groups 1 and 2 was 8.4 (8.3) and 6.6 (6.5), respectively (P = 0.57). Mean eGFR mL/min/1.73m$^2$ (SD) for Groups 1 and 2 were 54.3 (35.4) and 45 (21.7; P = 0.43) at 3-months and 56.1 (28) and 46.8 (21.6; P = 0.38) at 6-months post-transplant, respectively. No clinical rejection occurred, however subclinical rejection occurred in 3 patients in Group 1 (all type 1a) and 1 patient in Group 2 (type 2a), all diagnosed on a 6-month post-transplant surveillance biopsy. Similar rates of CMV and BK viremia, and UTIs occurred between the groups.

**Conclusions:** Belatacept conversion for SGF is a novel strategy that resulted in numerically higher eGFR 6 months post-transplant compared to a historical control group. Expansion of this cohort with longer follow-up is necessary to further define efficacy and safety.

*Funding:* Clinical Revenue Support

**PUB500**

**Early Identification of Skin Cancer in Renal Transplant Recipients through a Nurse-Led Skin Surveillance Service** Donald Choi,[2] Rosa M. Montero,[1] Christopher Harland,[1] Mona Wahba.[1] *[1]Epsom & St. Helier Univ Hospitals NHS Trust, London, United Kingdom; [2]St. George's Univ of London, London, United Kingdom.*

**Background:** Non-melanoma skin cancer (NMSC), has become one of the major causes of morbidity and mortality in renal transplant recipients (RTRs), with a 20 fold increase of NMSC rates in RTRs. Previous studies predominantly address the incidence of these cancers in white populations while little is available to compare ethnically diverse populations such as that found in London. This study was to determine the incidence of skin lesions occurring in RTRs and the impacts of skin surveillance screening clinics.

**Methods:** 152 RTRs were seen in our renal and transplant unit nurse-led skin screening clinic between September 2010 and November 2011, 27 were excluded (16 did not attend, 5 declined, 3 reviewed elsewhere, 1 death, 1 notes were unavailable). A total of 125 RTRs were reviewed for diagnosis of de novo skin lesions in consultant/nurse led clinics. Diagnoses of de novo skin lesions, demographics and clinical data were collected retrospectively from patients' notes.

**Results:** Male to female ratio 2:1, mean age 53.4yrs. 65% White, 17.6% Asian, 15.2% Black and 1.6% not stated. NMSC were found in 13 patients (12 basal cell carcinoma, 1 squamous cell carcinoma), no melanoma was found. There were 17 pre-malignant lesions

(PMLs) in 15 RTRs (11 actinic keratosis, 5 Bowen's disease, 1 lentigo maligna). Total NMSC in this cohort was 10.4% with total PML of 12%. The incidence of NMSC in this cohort was 6.8%, and PMLs of 9%. Both NMSC and PML occurred in less than 5 years and more than 10 years post 1st renal transplant.

**Conclusions:** The incidence of NMSC and PMLs in this cohort supports the need for skin surveillance screening in RTRs. It also indicates both NMSC and PML are present in RTRs within 5 years of transplant and would support early screening post transplant. Long term follow up should also be considered in view of NMSC and PML detected at more than 10 year post transplantation.This nurse-ledservice with early detection and relatively simple and inexpensive treatments represents a cost effective way of post-operative skin management for RTRs and warrants further economic investigation.

**PUB501**

**New Onset Diabetes after Transplantation Has No Impact on Clinical Outcomes – A Retrospective Single Center Study** Raja Mohammed Kaja Kamal, Rosa M. Montero, Khandaker Jubair Islam, Simona Petkovic-miletic, Mona Wahba. *Epsom & St. Helier Univ Hospitals NHS Trust.*

**Background:** New Onset Diabetes After Transplantation(NODAT) is a complication following renal transplantation(RT) that has been reported to lead to reduced graft function and increased morbidity and mortality. Single centre study looking at the occurrence of NODAT and its effects on overall transplant function.

**Methods:** Retrospective study collecting data on 250 transplant patients from 2007-2012 at a single centre. PreRT oral glucose tolerance test (OGTT), were recorded. NODAT was diagnosed if random blood glucose was >11.1mmol/L.

**Results:** 19/250(7.6%) developed NODAT.15 White, 3 Asian, 1 Black. 14 male:5 female. The median age at time of NODAT diagnosis was 56.6 years with a median time of developing NODAT 3.7months post RT. 5/19(26.3%) had an impaired preRT oral glucose tolerance test(OGTT). The eGFR at 1, 2 and 3 years was not affected by NODAT and was comparable with those that did not develop NODAT. Median HbA1c at time of diagnosis was 52mmol/mol that increased to 58.5 by 1 year($p>0.05$). The median weight at time of diagnosis was 75.7kg. Weight did not correlate with OGTT or HbA1c in the follow up period. Median UPCR at the time of diagnosis of NODAT was 40.4mg/mmol and stabilised at 1 year post RT. 14/19(73.7%) patients were on immunosuppressive regimens with prednisolone(P) at time of diagnosis with NODAT.14/160(8.8%) RT on P based regimens developed NODAT whereas 5/90(5.6%) on P free regimen developed NODAT($p=0.08$). Treatment with Insulin or oral hypoglycaemics had no effect on eGFR as good HbA1C levels were achieved. A decrease in UPCR was seen in those with insulin treatment that was independent to eGFR. PreRT dialysis modality showed no predisposition to the development of NODAT. NODAT had no effect on renal or patient survival at 1, 2 and 3 years post RT.

**Conclusions:** Our data suggests that in our predominantly white population NODAT has no influence on clinical outcome. P based regimen does not appear to increase the risk of developing NODAT. Optimal control of diabetes and proteinuria following NODAT appears important for good clinical outcome, this needs to be confirmed in a study with a longer follow up period.

**PUB502**

**Hypercalcaemia Post Renal Transplantation Does Not Affect Renal Transplant Function: A Retrospective Single Centre Study** Khandaker Jubair Islam, Rosa M. Montero, Raja Mohammed Kaja Kamal, Simona Petkovic-miletic, Mona Wahba. *Epsom & St. Helier Univ Hospitals NHS Trust.*

**Background:** Hypercalcaemia post renal transplantation (RT) has been previously described as a consequence of hyperparathyroidism. Previous studies have reported a loss of eGFR with hypercalcaemia post RT.

**Aim:** This retrospective study determined the natural history of hypercalcaemia and the effect of cinacalcet post RT.

**Methods:** 210 patients' notes were reviewed between 2007-2012. Corrected calcium (Ca), parathyroid hormone (PTH), phosphate (P) levels were collected over time together with their eGFR. Hypercalcaemia was defined as corrected calcium >2.60mmol/L at 1 month post RT.

**Results:** 13/210 (6.2%) developed hypercalcaemia by 1 month post RT, 1/13 was hypercalcaemic pre-transplant. 10 White, 2 Asian and 1 not stated. 7 male; 6 female. No significant difference in eGFR between normocalcaemic and hypercalcaemic patients 1, 2 and 3 years post RT. A decrease in PTH was seen in the hypercalcaemic patients following RT though they continued to have raised PTH irrespective of their high Ca levels. 6/13 were started on Cinacalcet for treatment of hypercalcaemia, 4 of these had parathyroid adenomas. Cinacalcet improved eGFR and reduced PTH in these patients. There was a sharp decline in P at 3 months that was maintained over the 3 years. A decline in eGFR was seen in 1 cinacalcet treated patient whose hypercalcaemia continued. 6/210 (2.9%) were hypercalcaemic pre-RT with only 1 of these continuing to be hypercalcaemic 1 month post RT.

**Conclusions:** PreRT hypercalcaemia does not predict post RT Ca levels. High Ca levels at 1 month post RT are maintained at 1 year with no effect on eGFR. eGFR is unaffected by hypercalcaemia up to 3 years later. Treatment with Cinacalcet suggests a beneficial effect on eGFR whilst reducing PTH. This cohort had small numbers on this treatment and further studies are needed to confirm these initial findings. The effects of maintained hypercalcaemia on cardiac function, vascular calcification and bone status have not been addressed in this study and may warrant investigation to its possible implication.

**PUB503**

**A Comparison of the Effects of Hypercalcaemia Post Renal Transplantation in Live and Deceased Donor Transplants; a Retrospective Single Centre Study** Rosa M. Montero, Khandaker Jubair Islam, Raja Mohammed Kaja Kamal, Simona Petkovic-miletic, Mona Wahba. *Epsom & St. Helier Univ Hospitals NHS Trust.*

**Background:** Hypercalcaemia post renal transplantation(RT) has previously been described. It is unclear whether this differs in those with live donor(LD) or deceased donor(DCD) transplants.

**Aim:** A retrospective study to determine whether the natural history of hypercalcaemia differs in LD or DCD RT.

**Methods:** 210 patients' notes were reviewed between 2007-2012. Corrected calcium(Ca), parathyroid hormone(PTH), phosphate(P) levels were collected over time, with their eGFR. Immunosuppression regimen was recorded. Hypercalcaemia was defined as corrected calcium >2.60mmol/L at 1 month post RT.

**Results:** 13/210(6.2%) developed hypercalcaemia by 1 month post RT, 6 were LD and 7 DCD. Live: 3 male; 3 female, 4 white, 1 Asian 1 not stated. Median age 52 years. 3/6 adenomas, 2 haemodialysis(HD), 4 peritoneal dialysis(PD) preRT. 1/6 was hypercalcaemic preRT Median Ca 2.66(2.61-2.92). 3 had raised PTH preRT with 3 on cinacalcet and no vitamin D. 2 were given cinacalcet post RT. eGFR at 1 year was not significantly different from eGFR post 1 month RT. DCD: 4 male;3 female, 6 White, 1 Asian. Median 65 years old. 2/7 had adenomas, 1 a parathyroidectomy. 4 were on HD, 3 PD preRT. No DCD were hypercalcaemic preRT Median Ca 2.74(2.68-2.94). All had raised PTH preRT with 2 on cinacalcet and 3 on vitamin D. 2 were given cinacalcet post RT. Those whose eGFRs were seen to improve had lower Ca levels at 3 year follow up compared with post 1 month calcium. eGFR did not correlate with corrected calcium post RT. There was no significant difference in eGFR or Ca between groups. Both groups had a decrease in PTH and P levels by 3 months that was sustained to 3 years.

**Conclusions:** LD that were hypercalcaemic post RT had a higher number of parathyroid adenomas. DCD had a higher proportion of patients with hypercalcaemia preRT that continued compared with LD. Hypercalcaemia appeared to decrease over time. DCD had less adenomas with hypercalcaemia occurring post RT. We would recommend surveillance for parathyroid adenomas in those found to be hypercalcaemic post LD RT with close follow up to those hypercalcaemic preRT undergoing DCD.

**PUB504**

**Outcomes of Non Compliance with Immunosuppression after Renal Transplantation: Comparison between High and Low Immune Risk Patients** Ernesto P. Molmenti, Jasmeet Kaur, Eric Siskind, Antonette S. Flecha, Kellie R. Calderon, Ankita Sagar, Mohini Alexander, Asha M. Alex, Madhu C. Bhaskaran. *Kidney Transplant Center, North Shore LIJ Health System, Manhasset, NY.*

**Background:** Late acute rejection is a challenge faced by transplant nephrologists & non compliance is a leading factor for rejection. Ensuring compliance reduces the risk of non compliance related late acute rejection (NCR) in transplant recipients & improves allograft outcome.

**Methods:** Review of case records revealed first episode NCR in 19 recipients. High immunological risk factors (HIR) evaluated include 3 out of 6 HLA mismatches; prior failed transplants; antibody mediated rejection; HO desensitization, prior rejection , preexisting allograft dysfunction and appropriateness of therapy instituted. Outcome of treatment were - recovery of function or need for RRT (dialysis or transplant).

**Results:** 8 patients were female and 11 male, age 31 to 65, ABO compatible. Two had prior episodes of acute rejections. Eight recipients had no HIR factors prior to the episode of NCR. In HIR group, 81% NCR lead to renal replacement therapy (RRT). On the other hand only 12% required RRT in the low risk group (*$p<0.005$).

| | High Immune Risk=11 | Low Immune Risk=8 |
|---|---|---|
| Recovered | 2 | 7 |
| Not Recovered | 9 | 1 |

**Conclusions:** At least 1 HIR factor leads to high need for RRT after NCR in our limited data series. Low risk group did not need RRT despite NCR in 88% of cases. In our limited analysis HIR appeared to be a significant predictor of irreversible NCR. Noncompliance related late acute rejection may be a preventable cause of end stage renal disease in HIR recipients. Targeted intervention in this subgroup may offer significant benefit to allograft survival.

**PUB505**

**Duration and Magnitude of BK Viremia Post Renal Transplant and Its Impact on Allograft Function** Asha M. Alex, Mohini Alexander, Madhu C. Bhaskaran, Ernesto P. Molmenti, Eric Siskind. *Nephrology, North Shore Univ Hospital, Great Neck, NY.*

**Background:** With the use of induction therapy and potent immunosuppressive regimens, BK virus has become an important cause of allograft dysfunction in kidney transplant recipients. Prospective monitoring for BK viremia is now a routine practice in post transplant follow-up. Significance of duration and magnitude of BK viremia, and its influence on allograft function is unclear. The aim of the study was to evaluate the impact of duration and magnitude of BK viremia on allograft function in kidney transplant recipients.

**Methods:** We retrospectively reviewed blood BK PCR for recipients 48 months post-transplant. For the purpose of this study, we classified BK viremia according to viral copies as follows: Low grade transient (<5000 lasting less than 3 months); Low grade persistent (<5000 lasting more than 3 months); High grade transient (>5000 lasting less than 3 months); High grade persistent (>5000 viral copies lasting more than 3 months).

**Results:** Records of 78 transplant recipients were reviewed. 13% demonstrated BK viremia. Of these, 20% showed evidence of allograft dysfunction, while 80% did not. Of the total sample, 3% showed allograft dysfunction attributable to BK virus nephropathy (BKVN) on biopsy, and these patients belonged to the high-grade persistent group. Patients with BKVN are almost always found to have BK viremia. Our analysis of a limited sample revealed that a persistent high-grade viremia is probably more likely to be associated with BKVN, unlike transient BK viremia detected on prospective monitoring without allograft dysfunction. The impact of low-grade persistent viremia remains to be determined.

| | Patients with BK viremia (%) | Total Patients (%) |
|---|---|---|
| Low grade transient | 30 | 3.84 |
| Low grade persistent | 0 | 0 |
| High grade transient | 40 | 5.12 |
| High grade persistent | 20 | 2.56 |

**Conclusions:** High-grade persistent BK viremia post transplant with allograft dysfunction is most likely suggestive of BKVN. Transient BK viremia without allograft dysfunction may be less likely to be associated with BKVN.

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# KEYWORD INDEX

The number refers to the location of the body of the abstract in the publication section.

## HI-OR01

**Acute Kidney Injury from Off-Pump or On-Pump Coronary Bypass Grafting and Kidney Function One Year Later** Amit X. Garg,[1] Philip J. Devereaux,[2] Salim Yusuf,[2] Meaghan S. Cuerden,[1] Chirag R. Parikh,[3] Steven G. Coca,[3] Michael Walsh,[2] Richard J Cook,[3] Richard P. Whitlock,[2] Richard J Novick,[3] Yongning Ou,[2] Xiangbin Pan,[3] Sirish Parvathaneni,[3] Andre Lamy.[2] *[1]London Kidney Clinical Research Unit, London, ON, Canada; [2]Population Health Research Institute, Hamilton, ON, Canada; [3]On Behalf of the CORONARY Investigators.*

**Background:** Most acute kidney injury (AKI) observed in hospital is defined by sudden mild or moderate increases in serum creatinine which may persist for several days. It is unknown whether an intervention which reduces the risk of such AKI better preserves long term kidney function.

**Methods:** Within the CORONARY trial we assessed kidney function in 2932 patients (from 63 sites, 16 countries) who were randomly assigned to coronary-artery bypass grafting (CABG) either with a beating-heart technique (off-pump) or with cardiopulmonary bypass (on-pump) [CORONARY kidney protocol published BMJ Open 2012; 2:e001080]. The primary outcomes were AKI within 30 days of surgery (□ 50% increase in serum creatinine from pre-randomization value) and loss of kidney function at one year (□ 20% loss in estimated glomerular filtration rate from pre-randomization value).

**Results:** Off-pump (n=1472) vs. on-pump (n=1460) CABG reduced the risk of AKI (17.5% vs. 20.8%, relative risk 0.83 (95% confidence interval [CI] 0.72 to 0.95); p=0.01); however, there was no significant difference between the two groups in the loss of kidney function at one year (17.0% vs. 15.3%, relative risk 1.09 (95% CI 0.93 to 1.28); p=0.29). Results were consistent with multiple alternate continuous and categorical definitions of AKI or kidney function loss.

**Conclusions:** The use of off-pump vs. on-pump CABG reduced the risk of post-operative AKI with no significant difference in kidney function between the two groups at one year. The findings emphasize that an intervention which reduces the risk of mild AKI may not necessarily improve long term kidney function.

*Funding:* Government Support - Non-U.S.

## HI-OR02

**The Effect of Bardoxolone Methyl in Patients with Type 2 Diabetes Mellitus and Stage 4 Chronic Kidney Disease** Glenn M. Chertow,[1] Tadao Akizawa,[2] Paul Audhya,[3] George L. Bakris,[4] Melanie Chin,[3] Heidi Christ-schmidt,[5] Angie Goldsberry,[3] Hiddo Jan Lambers Heerspink,[6] Mark T. Houser,[7] Melissa Krauth,[3] John J. Mcmurray,[8] Colin John Meyer,[3] Hans-Henrik Parving,[9] Giuseppe Remuzzi,[10] Robert D. Toto,[11] Nosratola D. Vaziri,[12] Christoph Wanner,[13] Janet Wittes,[5] Danielle Wrolstad,[5] Dick de Zeeuw.[6] *[1]Stanford U.; [2]Showa U.; [3]Reata; [4]U. Chicago; [5]Statistics Collaborative; [6]U. Groningen; [7]AbbVie; [8]U. Glasgow; [9]U. Copenhagen; [10]IRCCS - Mario Negri; [11]U. TX SW Med Ctr; [12]U. CA; [13]U. Würzburg.*

**Background:** Although inhibitors of the renin-angiotensin-aldosterone system can slow the progression of diabetic kidney disease, residual risk is high. Whether antioxidant-inflammation modulators further reduce this risk is unknown.

**Methods:** We randomized 2185 patients with type 2 diabetes mellitus (T2DM) and CKD stage 4 (estimated glomerular filtration rate (eGFR) 15 to <30 mL/min/1.73m$^2$) to bardoxolone methyl 20 mg daily or placebo. The primary composite outcome was end-stage renal disease (ESRD) or cardiovascular death.

**Results:** The Sponsor and Steering Committee terminated the trial as recommended by the Independent Data Monitoring Committee; median follow-up was 9 months. Sixty-nine of 1088 (6.3%) patients randomized to bardoxolone methyl and 69 of 1097 (6.3%) patients randomized to placebo experienced a primary composite outcome (hazard ratio 0.98, 95% confidence interval (95% CI) 0.70 to 1.37, p=0.92). In the bardoxolone methyl group, 43 patients developed ESRD and 27 experienced cardiovascular death; in the placebo group, 51 patients developed ESRD and 19 experienced cardiovascular death. Ninety-six patients were hospitalized with or died from heart failure in the bardoxolone methyl group compared with 55 in the placebo group (hazard ratio 1.83, 95% CI 1.32 to 2.55, p<0.001). Estimated GFR (6.4 mL/min/1.73m$^2$, 95% CI 5.9 to 6.9 mL/min/1.73m$^2$), blood pressure and albuminuria increased significantly and body weight decreased significantly in the bardoxolone methyl group relative to placebo.

**Conclusions:** In patients with T2DM and stage 4 CKD, bardoxolone methyl did not reduce the risk of ESRD or cardiovascular death. More frequent cardiovascular events prompted termination of the trial.

*Funding:* Pharmaceutical Company Support - Reata Pharmaceuticals, AbbVie, Inc.

## HI-OR03

**Combined Angiotensin Inhibition for Treatment of Diabetic Nephropathy: VA Nephron D** Linda F. Fried,[1] Nicholas Emanuele,[2] Jane Hongyuan Zhang,[3] Mary Brophy,[4] Todd Conner,[5] William Duckworth,[6] David J. Leehey,[2] Peter A. McCullough,[7] Theresa Z. O'Connor,[3] Paul M. Palevsky,[1] Robert F. Reilly,[8] Stephen L. Seliger,[9] Stuart Warren,[5] Suzanne Watnick,[10] Peter Peduzzi,[3] Peter Guarino.[3] *[1]VA Pittsburgh HCS, Pittsburgh, PA; [2]Hines VA, Hines, IL; [3]Cooperative Studies Program Coordinating Center, VA Connecticut HCS, West Haven, CT; [4]VA Boston HCS, Boston, MA; [5]VA Cooperative Studies Program Research Pharmacy, Albuquerque, NM; [6]Carl T. Hayden VA Medical Center, Phoenix, AZ; [7]St John Providence Health System, Novi, MI; [8]VA North Texas HCS, Dallas, TX; [9]VA Maryland HCS, Baltimore, MD; [10]Portland VA Medical System, Portland, OR.*

**Background:** Combination therapy with angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) decreases proteinuria; however its safety and impact on progression of kidney disease is uncertain.

**Methods:** Patients with type 2 diabetes, urine albumin excretion □300 mg/g creatinine and estimated glomerular filtration rate (eGFR) 30-89.9 ml/min/1.73m$^2$ were treated with losartan 100 mg/day and then randomized to lisinopril (10 to 40 mg/day) or placebo. The primary endpoint was the composite of change in eGFR (decline of □30 ml/min/1.73m$^2$ if initial eGFR □60 or □50% decline if initial eGFR <60 ml/min/1.73m$^2$), end-stage renal disease (ESRD), or death. Change in eGFR or ESRD was a secondary renal endpoint. Safety outcomes included mortality, hyperkalemia, and acute kidney injury.

**Results:** The study was stopped early for safety concerns. Among 1448 randomized patients with a median follow-up of 2.2 years, there were 152 primary endpoints in the monotherapy arm and 132 in the combination arm [HR 0.88 (95% CI 0.70 to 1.12), p=0.30]. A trend towards benefit in renal endpoint [HR 0.78 (0.58 to 1.05), p=0.10] decreased with time (non-proportionality p-value = 0.02). There was no benefit on mortality [HR 1.04 (0.73 to 1.49), p= 0.75] nor cardiovascular events. Combination therapy increased the risk of hyperkalemia [6.3 vs. 2.6 per 100 person years, p< 0.001] and acute kidney injury [12.2 vs. 6.7 per 100 person years, p<0.001].

**Conclusions:** Combination ACEI and ARB therapy is associated with an increased risk of adverse events in patients with diabetic nephropathy.

*Funding:* Veterans Administration Support, Pharmaceutical Company Support - Merck (study drug donation)

## HI-OR04

**Hypertension in Hemodialysis Patients Treated with Atenolol or Lisinopril (HDPAL): A Randomized Controlled Trial** Rajiv Agarwal,[1,2] Arjun D. Sinha,[1,2] Maria K Pappas,[1,2] Terri N Abraham,[1,2] Getachew G. Tegegne.[1,2] *[1]Medicine, Indiana University, Indianapolis, IN; [2]Medicine, VAMC, Indianapolis, IN.*

**Background:** To evaluate the comparative safety and efficacy of an ACE inhibitor-based to a β-blocker-based antihypertensive treatment, we conducted a randomized, open-label, parallel-group, single-center trial.

**Methods:** Subjects on maintenance hemodialysis with echocardiographic left-ventricular hypertrophy and hypertension confirmed by interdialytic 44-hour ambulatory blood pressure (BP) monitoring were randomized to lisinopril (LIS) (n=100) or atenolol (ATL) (n=100) each administered 3 times weekly after dialysis. Home BP was controlled to <140/90 mmHg. The primary outcome was the change in left ventricular mass index (LVMI) from baseline to 12 months.

**Results:** Average age was 52.7 y, 33% were women, and 85% black. At baseline, 44h ambulatory BP was 151.5/87.1 mmHg in the ATL group. BP was similar in the LIS group, improved over time in both groups, and no statistical difference between drugs was noted. An independent data safety monitoring board recommended termination of the study when a clear signal for cardiovascular safety emerged. Serious cardiovascular events in the ATL group occurred in 14 subjects who had 18 events (22.2/100 patient-years (PY)) and in the LIS group in 26 subjects who had 40 events (54/100 PY; incidence rate ratio (IRR) 2.29 (95% CI 1.28-4.24, p=0.003). Combined serious adverse event of myocardial infarction, stroke, hospitalization for heart failure or cardiovascular death in the ATL group occurred in 10 subjects who had 11 events (13.5/100 PY) and in the LIS group in 17 subjects who had 23 events (31.0/100 PY; IRR 2.29 (p=0.021). Hospitalizations for heart failure were worse in LIS group (IRR 3.13, p=0.021). All-cause hospitalizations in the ATL group occurred in 37 subjects who had 73 hospitalizations (89.9/100 PY) and in the LIS group in 59 subjects who had 107 hospitalizations (144.3/100 PY; IRR 1.61 (95% CI 1.18 -2.19, p=0.002). LVMI improved with time; no difference between drugs was noted.

**Conclusions:** Atenolol-based antihypertensive therapy may be superior to lisinopril-based therapy in preventing cardiovascular morbidity and all-cause hospitalizations.

*Funding:* NIDDK Support

**Underline represents presenting author/disclosure.**

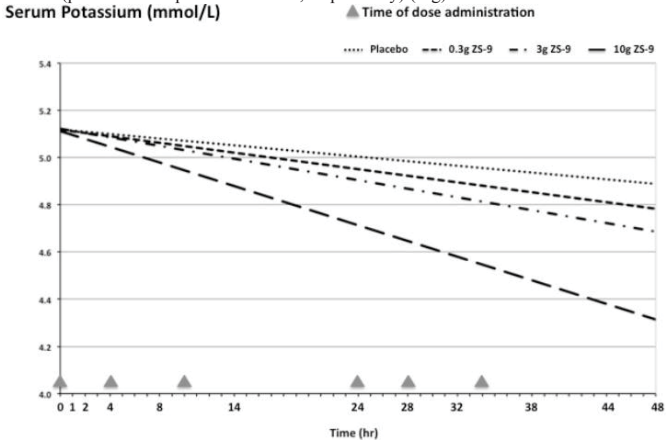
## HI-OR05

**Safety and Efficacy of ZS 9, a Novel Selective Cation Trap, for Treatment of Hyperkalemia in CKD Patients** Stephen R. Ash,[1] Bhupinder Singh,[2] Philip T. Lavin,[3] Fiona Stavros,[4] Henrik S. Rasmussen.[4] *[1]Indiana University, Lafayette, IN; [2]Southwest Clinical Research Institute, Tempe, AZ; [3]Boston Biostatistics Research Foundation, Framingham, MA; [4]ZS Pharma Inc., Fort Worth, TX.*

**Background:** Hyperkalemia is associated with significant mortality and limits use of cardio- and reno-protective RAASi, yet no reliably safe and effective treatment for this condition exits. ZS-9 is a selective cation exchanger designed to preferentially entrap excess potassium. A Phase 2 randomized, double-blind PBO-controlled trial (RCT) assessed ZS-9's safety and efficacy in CKD pts. with mild-to-moderate hyperkalemia.

**Methods:** Eligible pts. (N=90) with eGFR 30-60 mL/min/1.73 $m^2$ and serum potassium [s-$K^+$] 5-6 mmol/L on normal diets were randomized 2:1 to ZS-9 (0.3g [n=12], 3g [n=24], and 10g [n=24]) or PBO (n=30), given TID orally on an in-patient basis with regular meals. The primary efficacy endpoint was the rate of s-$K^+$ decrease over the first 48 hr, using longitudinal modeling to achieve a best fit to the data.

**Results:** ZS-9 was well tolerated and no serious AEs were reported; no patients withdrew. Only one ZS-9 AE (mild constipation, 3g) was considered at least possibly drug related. No significant hypokalemia, hypomagnesemia, or hypocalcemia was observed. At baseline, mean s-$K^+$ was 5.1 mmol/L across groups. ZS-9 demonstrated significant dose-dependent reductions in s-$K^+$; the primary efficacy endpoint was met in the 10g and 3g cohorts ($p<0.0001$ and $p=0.048$ vs PBO, respectively) (Fig).




Serum $K^+$ decreased 1 hr after the first dose of ZS-9 10g ($p=0.022$ vs PBO), and maximal mean reduction from baseline was 0.92 mmol/L after 38 hr ($p<0.001$ vs PBO), without affecting serum or urinary sodium.

**Conclusions:** In CKD patients with hyperkalemia, ZS-9 was safe and well tolerated and led to a rapid and sustained s-$K^+$ reduction. Consequently, ZS-9 is being evaluated in a large Phase 3 RCT.

## HI-OR06

**Treatment of Lupus Nephritis with Abatacept Plus Low-Dose Pulse Cyclophosphamide: The Results of the ACCESS Trial** Brad H. Rovin,[1] The Access Trial Investigators.[2] *[1]Internal Medicine, Ohio State University Wexner Medical Center, Columbus, OH; [2]The Immune Tolerance Network.*

**Background:** CTLA4Ig (abatacept, ABA) plus intravenous cyclophosphamide (IVC) act synergistically to treat murine lupus nephritis (LN). The ACCESS trial tested low-dose (i.e. Euro-lupus) IVC ± ABA in human LN to determine if complete renal response (CRR) rate could be improved with ABA, and if CRR in ABA-treated patients could be maintained without maintenance immunosuppression (IS).

**Methods:** ACCESS enrolled patients with Class III or IV (±V) LN and a urine protein to creatinine ratio (UPCR) >1. All patients were given a steroid taper and IVC (500 mg q 2 weeks X6) followed by azathioprine (AZA, 2mg/kg/d). Patients were randomized to placebo (n=68) or ABA (n=66) at weeks 0, 2, 4 and then monthly. The primary outcome was CRR at week 24, defined as UPCR <0.5, serum creatinine normal or within 25% of baseline, and prednisone □10mg/d. At week 24 AZA was stopped in ABA patients who had CRR, but continued in controls who had CRR. This group was followed to week 52.

**Results:** The ACCESS cohort was 39% African American (AA), 40% Hispanic/Mestizo, and 19% European American. Control and ABA patients were well-matched for baseline clinical characteristics. At week 24 CRR occurred in 33% ABA and 31% of control, and CRR+PRR was 59% in both arms. CRR occurred in 33% of AA ABA patients and 16% of AA control patients (p=NS). There were no statistically significant differences between study arms in anti-dsDNA or complement levels, frequency of serious or infectious adverse events, or withdrawals. Among complete responders at week 24, 50% (11/22) and 62% (13/21) of ABA and control patients respectively, still met CRR criteria at week 52 (p=NS).

**Conclusions:** ABA+IVC did not improve CRR over IVC alone. There was no difference in the number of ABA and control patients who maintained CRR to 1 year despite discontinuation of maintenance IS in the ABA group and continued AZA in the control group, suggesting ABA-treated patients may not need maintenance IS. ACCESS also showed, for the first time, that low-dose IVC may have applicability in racially and ethnically diverse LN patients. The CRR of >30% at 24 weeks is higher than any recent LN trial.

*Funding:* Other NIH Support - Immune Tolerance Network

## HI-OR07

***APOL1* Risk Variants, Race, and Progression of Chronic Kidney Disease** Afshin Parsa,[1] Wen Hong Linda Kao,[2] the AASK and CRIC Collaborative Research Groups.[1,2] *[1]Nephrology, University of Maryland School of Medicine; [2]Epidemiology, Johns Hopkins Bloomberg School of Public Health.*

**Background:** African Americans (AAs) are at substantially increased risk for end-stage renal disease (ESRD) compared to European Americans (EAs).

**Methods:** We examined the effects of *APOL1* variants on chronic kidney disease (CKD) progression separately in the African American Study of Kidney Disease and Hypertension (AASK) and the Chronic Renal Insufficiency Cohort Study (CRIC). AASK enrolled 693 AAs with CKD attributed to hypertension. CRIC enrolled EAs and AAs with CKD (n=2955, 46% with diabetes). *APOL1* risk group was defined by two copies of the high-risk *APOL1* variants. In AASK, primary outcomes were ESRD and a composite of ESRD or doubling of serum creatinine. In CRIC, primary outcomes were the slope in estimated glomerular filtration rate (eGFR) and the composite of ESRD or halving of eGFR from baseline.

**Results:** In AASK, the primary outcome occurred in 58% of participants in the *APOL1* risk group and 37% in the *APOL1* non-risk group [adjusted hazard ratio (HR)=1.88,P<0.001]. There was no interaction between *APOL1* status and trial interventions (3 drugs and 2 BP targets) or baseline proteinuria.

In CRIC, the adjusted rate of decline in eGFR was greater among AAs in the *APOL1* risk group than EAs in participants with diabetes (-1.32 ml/min/1.73$m^2$/year, P<0.001) and participants without diabetes (-1.11 ml/min/1.73$m^2$/year, P<0.001). The rate of decline in eGFR was similar among AAs in the *APOL1* non-risk group and EAs. Among participants with diabetes, the HR of a renal event was 1.95 (95% CI: 1.39-2.73) in AAs in the *APOL1* risk group and 1.40 (95% CI: 1.10-1.78) in AAs in the non-risk group, each compared to EAs. Corresponding HRs among participants without diabetes were 2.68 (95% CI: 1.78-4.05) and 1.57 (95% CI: 1.11-2.21).

**Conclusions:** *APOL1* renal risk variants increase CKD progression in AAs, even in the setting of well-controlled BP. These variants explain, in part, the markedly increased risk of ESRD in AAs compared to EAs, regardless of diabetes status. In the context of prior studies, our results suggest that *APOL1* risk variants increase CKD progression in AAs, irrespective of CKD etiology.

*Funding:* NIDDK Support

## SA-PO1076

**A Randomized Clinical Trial to Evaluate the Efficacy of Cinacalcet to Correct Hypercalcemia in Renal Transplant Recipients with Autonomous Hyperparathyroidism** Pieter Evenepoel,[1] Kerry Cooper,[2] Hallvard Holdaas,[3] Piergiorgio Messa,[4] Georges J. Mourad,[5] Klaus Olgaard,[6] Boleslaw Rutkowski,[7] Heidi M. Schaefer,[8] Hongjie Deng,[2] Jose-Vicente Torregrosa,[9] Rudolf P. Wuthrich,[10] Susan V. Yue.[2] *[1]U Ziekenhuizen, Belgium; [2]Amgen, USA; [3]Oslo U Hospital, Norway; [4]Fondazione Ca' Granda IRCCS Policlinico, Italy; [5]U of Montpellier Hopital Lapeyronie, France; [6]U of Copenhagen, Denmark; [7]Medical U of Gdansk, Poland; [8]Vanderbilt U Medical Center; [9]U of Barcelona, Spain; [10]U Hospital Zurich, Switzerland.*

**Background:** Autonomous hyperparathyroidism (HPT) after kidney transplantation (KTx) is associated with elevations of serum calcium (Ca) and reductions in serum phosphorus (P). Cinacalcet (Cin) has been shown to reduce Ca and increase P levels in several open-label, single arm studies.

**Methods:** 114 KTx recipients (□18 years) with autonomous HPT were randomized 1:1 to receive Cin (n=57):placebo (n=57) to evaluate the primary endpoint: correction of hypercalcemia (Ca < 10.2 mg/dL); secondary endpoints: to assess the percent change in bone mineral density (BMD) at the femoral neck and the change in serum P levels. Randomization was stratified by baseline corrected total serum Ca levels: □11.2 mg/dL (2.80 mmol/L; n=45) and >11.2 mg/dL (n=69). Efficacy assessment for Ca, parathyroid hormone and P occurred between wks 21 and 26 and wk 52 for BMD.

**Results:** Demographic distribution was similar across groups: 55% male, 82% white, with median (min, max) age of 53 (24, 76) years. The proportion of subjects achieving the primary endpoint was 78.9% and 3.5% for the Cin and placebo groups (p <0.001). BMD mean (SD) percent change from baseline to wk 52 was 2.16 (7.74)% and 0.73 (4.40)% for the Cin and placebo groups, respectively (n.s.). There was a nominally significant difference ($p<0.001$) for the observed increase in P from baseline to efficacy assessment in the Cin vs the placebo group. No new safety risks were identified.

**Conclusions:** This is the largest randomized trial with Cin in KTx subjects. Data support the efficacy of cinacalcet for the treatment of hypercalcemia in KTx subjects with autonomous HPT.

*Funding:* Pharmaceutical Company Support - Amgen Inc.

## SA-PO1077

**18 Month Results from a Randomised Trial of Mycophenolate Mofetil versus Cyclophosphamide for Remission Induction of Severe ANCA-Associated Vasculitis: MYCYC** Rachel B. Jones,[1] Lorraine Harper,[2] Michael Walsh,[3] David R.W. Jayne.[1] *[1]Addenbrooke's Hospital; [2]University of Birmingham; [3]McMaster University.*

**Background:** Mycophenolate mofetil (MMF) is a potential alternative to cyclophosphamide (CYC) for remission induction of ANCA-associated vasculitis (AAV). We present the 18 month data of an international randomised, controlled trial comparing MMF to CYC as initial therapy in severe AAV.

**Underline represents presenting author/disclosure.**

**Methods:** Patients with newly diagnosed severe AAV were randomised to receive 3 to 6 months of induction with MMF 2-3 g/day (n=70) or 6-10 pulses of IV CYC 15 mg/kg (n=70). Both groups received the same tapering prednisolone regimen and azathioprine therapy after remission induction. The primary outcome was remission at 6 months defined as no disease activity for □4 weeks without excess prednisolone. We considered MMF non-inferior to IV CYC if the lower bound of the 90% confidence interval (CI) for remission was no more the 12% lower for MMF.

**Results:** The groups were similar at baseline. The primary endpoint occurred in 51 (73%) MMF vs 52 (74%) CYC (risk difference -1%, 90% CI -14 to 11%; p=0.08 for non-inferiority). The secondary remission endpoint occurred in 63/70(90%) MMF vs 56/70 (80%) CYC (risk difference 10%, 90% CI 0 to 20%; p<0.001 for non-inferiority). Although, cumulative prednisolone dosing at 6 months did not differ (p>0.9), there was slightly more noncompliance at low doses in the MMF group. At 18 months, 21 patients (35%) in the MMF group and 12 (22%) in the CYC group relapsed (risk ratio 1.57, 95% CI 0.87 to 2.94; p=0.12). Other key secondary outcomes did not differ significantly between groups: estimated glomerular filtration rate (mean difference 2.6 ml/min, 95% CI -7.6 to 12.9; p=0.62), serious adverse events (risk ratio 1.25, 95% CI 0.86 to 1.81; p=0.23), serious infections (risk ratio 1.5, 95% CI 0.78 to 2.88; p=0.22) or deaths (risk ratio 1.25, 95% CI 0.35 to 4.46; p=0.73).

**Conclusions:** Although remission induction rates were similar, we did not demonstrate non-inferiority of MMF vs IV CYC and MMF was not substantially safer. MMF may offer an alternative to cyclophosphamide in select patients but requires particular attention to disease activity and glucocorticoid dosing.

*Funding:* Pharmaceutical Company Support - Roche

## SA-PO1078

**Continuous, Maintenance Iron Therapy Using Soluble Ferric Pyrophosphate Citrate Chelate (SFP, Triferic™) Infusion via Hemodialysate in CKD-HD: Phase III CRUISE Studies**

Steven Fishbane,[3] Ajay K. Singh,[2] Raymond D. Pratt,[1] Vivian H. Lin,[1] Carrie D. Guss,[1] Ajay Gupta.[1] *[1]R&D, Rockwell Medical, Wixom, MI; [2]Nephrology, Brigham and Women's Hospital, Boston, MA; [3]Nephrology, North Shore - Long Island Jewish Health System, Inc., Manhasset, NY.*

**Background:** SFP (Triferic™), a novel, unique, carbohydrate-free, complex iron salt delivered via hemodialysate donates iron rapidly and directly to apotransferrin, bypassing the RE system. The safety and efficacy of this novel dialysate delivery method for maintaining iron balance in adult, CKD-HD, was examined in two identical RCTs.

**Methods:** Iron replete HD patients were randomized to either placebo (standard iron-free dialysate) or dialysate containing SFP at 2 μM (110 μg iron/L), with every hemodialysis for up to 48 weeks while ESA dose changes and IV/oral iron were prohibited. Subjects meeting pre-specified criteria for a change in anemia management were advanced to SFP-only extension stage.

**Results:** The safety profile and tolerability of SFP were similar to placebo with no anaphylactoid reactions in over 20,000 administrations. AE and SAE were similar and there was no increase in intradialytic hypotension, CV events or infections. No deaths were attributed to SFP.

The primary endpoint was mean change in Hgb from baseline to the end of treatment (last 1/6th of randomized phase), analyzed using ANCOVA with baseline Hgb as covariate. Hgb was maintained with SFP, while placebo demonstrated a statistically significant decline.

| | CRUISE-1 | | CRUISE 2 | |
|---|---|---|---|---|
| | SFP N=148 | Placebo N=151 | SFP N=142 | SFP N=144 |
| Baseline Hgb g/dL | 10.96 | 10.90 | 10.96 | 10.93 |
| LS Mean Change from Baseline g/dL (SE) | 0.06 (0.115) | -0.30 (0.114) | -0.05 (0.108) | -0.40 (0.109) |
| Difference from Placebo LS Mean g/dL (SE) | 0.36 (0.14) | | 0.36 (0.14) | |
| 95% CI | 0.08, 0.63 | | 0.08, 0.63 | |
| P-value | **0.011** | | **0.011** | |

SFP reliably delivered iron which was rapidly cleared and maintained reticulocyte Hgb and ferritin while placebo experienced significant declines.

**Conclusions:** In HD patients, the novel iron salt-SFP infused by hemodialysis for up to 48 weeks is well tolerated with a safety profile similar to placebo. SFP is effective in maintaining hemoglobin by preventing iron deficiency without inducing iron overload.

## SA-PO1079

**FIND-CKD: A 56-Week Randomized Trial of Intravenous Ferric Carboxymaltose versus Oral Iron in Anemic Patients with Chronic Kidney Disease and Iron Deficiency**

Iain C. Macdougall,[1] Andreas H. Bock,[2] Fernando Carrera,[2] Kai-Uwe Eckardt,[2] Carlo A. Gaillard,[2] David B. Van Wyck,[2] Bernard Roubert,[3] Jacqueline G. Nolen,[3] Simon D. Roger.[2] *[1]King's College Hospital, London, United Kingdom; [2]On behalf of the FIND-CKD Study Investigators; [3]Vifor Pharma, Switzerland.*

**Background:** The optimal iron regimen in non-dialysis CKD (ND-CKD) is unknown.

**Methods:** FIND-CKD was a 56-week, open-label, multicenter, prospective, randomized study of 626 anemic patients with ND-CKD and iron deficiency not receiving an erythropoiesis-stimulating agent (ESA). Patients were randomized (1:1:2) to ferric carboxymaltose (FCM) targeting a higher (400–600μg/L) or lower (100–200μg/L) ferritin level, or oral iron. The primary endpoint was time to initiation of other anemia management (ESA, other iron therapy, or blood transfusion) or hemoglobin (Hb) trigger of 2 consecutive values <10g/dL during weeks 8–52.

**Results:** Time to initiation of other anemia management or Hb trigger was significantly longer with FCM targeting a higher ferritin level vs oral iron (mean [SE] 313 [8] days vs 287 [8] days [p=0.035]; hazard ratio 0.67; 95% CI 0.46, 0.98) but not vs FCM targeting a lower ferritin level. The increase in mean Hb from baseline to month 12 was greater with high ferritin FCM vs oral iron (p=0.014). Hb increased by □1g/dL in more patients with high ferritin FCM (56.9%) vs low ferritin FCM (34.2%, p<0.001) or oral iron (32.1%, p<0.001). High ferritin FCM was associated with a significantly greater increase in ferritin than oral iron, and a similar increase in transferrin saturation. Change in eGFR was minimal and similar in each group. No renal, cardiovascular, or infectious toxicity was observed. Rates of adverse events, serious adverse events, and deaths were comparable between groups. Intolerance led to study discontinuation in 0.7% of high ferritin FCM, 1.3% of low ferritin FCM and 7.5% oral iron patients (p=0.002 vs high ferritin FCM).

**Conclusions:** IV FCM targeting a ferritin range of 400–600μg/L significantly delayed the requirement for ESA therapy or other anemia therapy compared to oral iron in anemic patients with ND-CKD and iron deficiency. High target FCM led to a greater Hb increase than oral iron, maintained Hb, and was well tolerated.

*Funding:* Pharmaceutical Company Support - Vifor Pharma

## SA-PO1080

**Increased Duration and Dose of Prednisolone (PSL) Treatment Does Not Reduce Relapses in Childhood Nephrotic Syndrome**

Norishige Yoshikawa,[1] Koichi Nakanishi,[1] Mari Saito Oba,[2] Mayumi Sako,[3] Yasuo Ohashi,[4] Kazumoto Iijima.[5] *[1]Wakayama Medical Univ., Japan; [2]Yokohama City Univ.; [3]National Center for Child Health and Development; [4]Univ. of Tokyo; [5]Kobe Univ.*

**Background:** Although prolonged initial steroid treatment has been reported to decrease the risks of relapse in pediatric patients with steroid-sensitive nephrotic syndrome (SSNS), the initial treatment approach varies considerably. A Cochrane review concluded that although treating patients for up to 7 month(m)s appears more effective than 2 m-therapy in achieving sustained remissions, additional well designed and adequately powered randomized clinical trials are required.

**Methods:** We evaluated the hypothesis that prolonging a 2-m initial PSL therapy to 6 ms using an increasing cumulative dose would not reduce the incidence of frequently relapsing (FR) NS. We conducted a multicenter, randomized, open-label non-inferiority trial at 91 hospitals in Japan and compared a 2-m initial PSL treatment (cumulative dose 2240 mg/m$^2$) with a 6-m treatment (3885 mg/m$^2$). The primary endpoint was time to FRNS. A prespecified non-inferiority margin was hazard ratio (HR) of 1.3 and significant level was one-sided 5%. We randomly assigned 255 children with an initial episode of SSNS either 2-m treatment(n=128) or 6-m treatment (n=127).The trial medication consisted of initial PSL treatment and relapse PSL treatment as specified in the protocol. Enrolled patients received this for a total of 24 ms.

**Results:** The FRNS-free rates at 24 ms were 56.2% (95% confidence interval [CI], 47.0-64.4%) in the 2-m treatment group and 50.8% (95% CI, 41.4-59.4%) in the 6-m treatment group. The HR was 0.86 (90% CI, 0.64-1.16) and met the non-inferior margin (p = 0.011). There was no significant difference in the number of relapses during the 24-m intervention period (1.25 vs. 1.30/person-years; relapse rate ratio 0.94, 95% CI 0.71-1.22, p = 0.649). The incidence of central obesity in the 6-m treatment group was higher than in the 2-m treatment group (p = 0.03).

**Conclusions:** This trial shows that extending initial PSL treatment from 2 to 6 ms with an increasing dose does not reduce the incidence of FRNS.

*Funding:* Government Support - Non-U.S.

## SA-PO1081

**The Antialbuminuric Effects of an Aldosterone Blocker in Hypertensive Patients with Albuminuria: A Double-Blinded, Randomized, Placebo-Controlled (EVALUATE) Trial**

Toshiro Fujita,[1] Shunya Uchida,[2] George Seki,[3] Shinya Kaname.[4] *[1]Department of Epigenetics, RCAST, University of Tokyo, Tokyo, Japan; [2]Department of Nephrology, Teikyo University, Tokyo, Japan; [3]Department of Nephrology & Endocrinology, University of Tokyo, Tokyo, Japan; [4]Department of Nephrology, Kyorin University, Tokyo, Japan.*

**Background:** Results of many animal studies but a few human studies suggest that mineralocorticoid receptor (MR) blockades can inhibit albuminuria and renal damage in chronic kidney disease (CDK) patients. The aim of this trial was to evaluate the potential renoprotective capacity of the MR blockade in non-diabetic patients with hypertension and proteinuria who were already receiving the angiotensin receptor blockers (ARB) or angiotensin-converting enzyme inhibitors (ACE-I) and optimal treatment for hypertension.

**Methods:** We evaluated the antialbuminuric effect of the MR blockade by adding treatment of low dose (50 mg/day) of eplerenone to optimal antihypertensive therapy including in non-diabetic hypertensive CKD patients who had blood pressure [BP] of 130-180/80-100 mmHg and albuminuria (urinary albumin/creatinine [Cr] ratio: 30-600 mg/g). The patients had estimated glomerular filtration rate □50 mL/min/1.73m$^2$. We enrolled 314 patients in this multicenter, double-blinded, randomized, placebo-controlled trial (UMIN#000001803). The primary outcome was a percent reduction in urinary albumin/Cr, as measured in an early-morning sample, at 12 months.

**Results:** Treatment with 50 mg of eplerenone, as compared with placebo, reduced the mean urinary albumin/Cr (-17.8±99.5 [mean±SD] % vs. 8.7±109.4%, P<0.05). A small, but significant (both, p<0.05), difference in systolic and diastolic BP was seen between the treatment groups by the end of the study period: 138.7±11.16 to 126.8±11.52 mmHg in the eplerenone group and 138.7±12.58 to 129.4±12.87 mmHg in the placebo group. Serum potassium was similar between the two groups (4.32±0.40 vs. 4.17±0.50 mEq/L, NS), and total numbers of adverse events were also similar in the groups.

**Underline represents presenting author/disclosure.**

**Conclusions:** Low dose of eplerenone may have renoprotective effects in non-diabetic patients with hypertension and nephropathy who are receiving ARB or ACE-I.

*Funding:* Pharmaceutical Company Support - Pfizer

## SA-PO1082

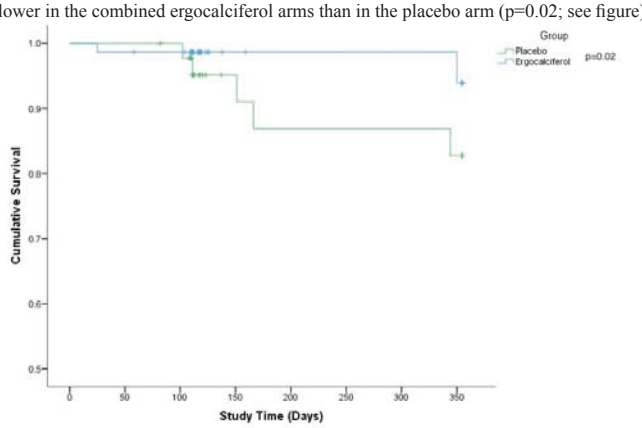
**The DIVINE Trial: Dialysis Infection and Vitamin D in New England**

Ishir Bhan, Dorothy A Dobens, Caitlin A. Trottier, Julia Beth Wenger, Hector Tamez, Joseph James Deferio, Kathryn J. Lucchesi, Ravi I. Thadhani. *Division of Nephrology, Massachusetts General Hospital, Boston, MA.*

**Background:** The role of nutritional vitamin D in ESRD remains uncertain. We performed a randomized, double-blind placebo-controlled trial to assess the safety and efficacy of ergocalciferol in chronic hemodialysis patients.

**Methods:** We randomized 105 incident dialysis patients with 25(OH)D levels □ 32 ng/mL from 32 centers in the northeast US to ergocalciferol 50,000 IU weekly (n=36), monthly (n=33), or placebo (n=36) for a 12 week treatment period. Follow-up was at 20 weeks and one year. The primary outcome was 25(OH)D levels at 12 weeks. Secondary outcomes included markers of mineral metabolism, hospitalizations, and one year mortality.

**Results:** Baseline features and active vitamin D use were similar across the groups. Most subjects were white (62.9%) and male (78.1%). The mean age was 57 ± 17 years. Mean 25(OH)D levels at 12 weeks were significantly higher than placebo (27.3 ng/mL ± 2.3) in the weekly (49.8 ng/mL ± 2.3; p<0.001) and monthly (38.3 ng/mL ± 2.4; p=0.001) treatment arms. 25(OH)D sufficiency (>32 ng/mL) was achieved in 90.9%, 64.5%, and 35.3% of patients receiving weekly ergocalciferol, monthly, and placebo, respectively. There were no differences between groups in serum calcium, phosphorus, or PTH. Overall hospitalizations (p=0.64), hospitalizations for infection (p=0.32) and cardiovascular hospitalizations (p=0.47) did not differ between groups. All-cause mortality at one year was significantly lower in the combined ergocalciferol arms than in the placebo arm (p=0.02; see figure).




**Conclusions:** Ergocalciferol can safely and effectively increase 25(OH)D levels in incident dialysis patients. Preliminary analysis suggests a lower one-year mortality rate among ergocalciferol-treated patients compared with placebo.

Clinicaltrials.gov NCT99782099, Funded by NIDDK R01DK09143.

*Funding:* NIDDK Support

## SA-PO1083

**Prospective Randomized Controlled Trial of Individualized Dialysate Cooling to Provide Brain and Cardiac Protection in Haemodialysis Patients**

Chris W. McIntyre, Mohamed Tarek Eldehni, Aghogho Odudu. *Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, University of Nottingham, Derby, United Kingdom.*

**Background:** Conventional haemodialysis (HD) results in significant circulatory stress producing recurrent and cumulative ischaemic injury to the heart and brain. Cool dialysate temperature (DT) is safe, well tolerated and acutely protects against HD induced cardiac stunning. This study aimed to evaluate the use of DT (with conventional thrice-weekly HD) to provide neuro and cardio-protection.

**Methods:** 49 incident HD patients were randomized (1:1) to receive either conventional HD or DT (-0.5°C below tympanic temperature). Study duration was 12 months. Patients underwent cardiac MR and Diffusion Tensor MR Imaging of the brain to assess white matter (WM) ultrastructural injury (with neurocognitive testing) at baseline and 12 months.

**Results:** At baseline, HD patients exhibited significant diffuse WM damage, well correlated with degree of neurocognitive impairment. DT resulted in significantly better haemodynamic response to HD, c.f. control treatment. Voxel based tract base spatial statistics (TBSS) analysis demonstrated progression of diffuse sub-acute changes in the brain WM (higher fractional anisotropy (FA) and lower diffusivity values p<0.05) -exclusively in the control group. DT provided protection against further brain injury. Axial diffusivity (AD) and radial diffusivity (RD) were computed separately from the eigenvalues of the diffusion tensor. The prospective changes were exclusively in radial diffusivity, a pattern pathognomonic of ischaemic injury. There was also evidence of cardio-protection, with DT being associated with a preserved pattern of segmental function and significant effects on changes in left ventricular mass (Control-LVMI increased by 3 ±14g/m$^2$, DT-reduced by 6 ±11 g/m$^2$, P=0.04).

**Conclusions:** HD results in functionally significant brain and cardiac injury. Dialysate cooling is well tolerated and effectively abrogates these effects. This intervention can be delivered without additional cost and is universally applicable. This study justifies further larger scale testing of dialysis based interventions (directed at iatrogenic HD induced organ injury).

*Funding:* Government Support - Non-U.S.

## SA-PO1084

**A Randomized Controlled Multicenter Trial of a Heparin-Grafted Polyacrylonitrile Membrane for No-Heparin Hemodialysis versus Standard-of-Care: Results of the HepZero Study**

Maurice Laville,[1] Marc Dorval,[2] Joan Fort,[3] Renaud Fay,[5] Frederique Moureau,[4] Nathalie Loughraieb,[4] Patrick Rossignol.[5] *[1]Nephrology Department, Lyon-Sud Hospital Centre, Lyon University, Lyon, France; [2]Dr-Georges-L.-Dumont University Hospital Centre, Moncton, Nouveau-Brunswick, Canada; [3]Nephrology Department, University Hospital Vall d'Hebron, Autonomous University of Barcelona, Spain; [4]Gambro-Hospal, Meyzieu, France; [5]Nancy-University Hospital, Lorraine University & ALTIR, Nancy, France.*

**Background:** Heparin is used in most patients on maintenance hemodialysis (MHD), but no-heparin HD (NH-HD) is occasionally needed to decrease bleeding risk. A new dialyzer, (Evodial®, Gambro-Hospal, Meyzieu, France) contains a heparin-grafted polyacrylonitrile membrane (HGPM) and may allow safe and efficient NH-HD.

**Methods:** The HepZero study NCT01318486 is the first large randomized controlled trial comparing no-heparin strategies. This multicenter international open-label trial was designed to demonstrate non inferiority (ultimately superiority if demonstrated) in two parallel groups comprising 251 MHD patients requiring NH-HD. Patients were randomly allocated for up to three NH-HD sessions, to be treated with either HGPM, or standard-of-care (Controls) including regular saline flushes or on-line predilution. The first NH-HD session was considered successful when there was neither complete occlusion of air traps or dialyzer; nor additional saline flushes, changes of dialyzer or bloodlines, or premature termination.

**Results:** It is found that current standard-of-care results in high failure rates (50%). Success rate in the HGPM arm was higher than in Controls (68.5% vs. 50.4 %, p = 0.003), consistent for all standard-of-care modalities. Absolute HGPM vs Controls arms difference was 18.2%, with a lower bound of the one-tailed 95% confidence interval (O-T, 95% CI) equal to + 10%. Hypothesis of the non-inferiority at 15%-level was accepted, although superiority at the 15%-level was not reached.

**Conclusions:** Hence, HGPM achieves 18% higher successful anticoagulation-free HD session compared to both saline flushes and on-line pre-dilution, and is shown to be firmly non-inferior to standard-of-care.

*Funding:* Pharmaceutical Company Support - Gambro

## SA-PO1085

**A Two-Part Trial of Patiromer for the Treatment of Hyperkalemia in Chronic Kidney Disease Subjects on Renin Angiotensin Aldosterone System Inhibition**

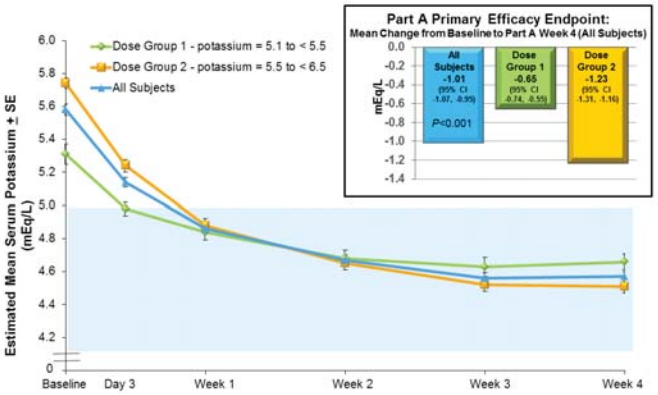
Matthew R. Weir,[1] George L. Bakris,[2] Martha Mayo,[3] Yuri Stasiv,[3] Heidi Christ-Schmidt,[4] Janet Wittes,[4] Lance Berman.[3] *[1]University of Maryland; [2]University of Chicago Medicine; [3]Relypsa; [4]Statistics Collaborative.*

**Background:** Hyperkalemia (HK) affects patients with chronic kidney disease (CKD), especially when treated with renin angiotensin aldosterone system inhibitors (RAASi). Patiromer (RLY5016), a novel ion exchange polymer with good gastrointestinal tolerability, lowered serum potassium ($K^+$) with daily administration in clinical trials. This multicenter international study in 243 hyperkalemic subjects with an eGFR 15 to < 60 mL/min/1.73m$^2$ with/without diabetes and on RAASi medication, was a two-part Phase 3 study: Part A, the 4-week single-blind treatment study, assessed patiromer for the treatment of HK; Part B, the 8-week placebo-controlled randomized withdrawal study, assessed whether chronic treatment with patiromer prevented recurrent HK.

**Methods:** Two subgroups were defined by screening serum $K^+$: 5.1 to < 5.5 (n=92) and 5.5 to < 6.5 mEq/L (n=151). The Part A primary outcome was change in serum $K^+$ from Part A Baseline to Part A Week 4 (all subjects). The Part B primary outcome was the between group difference in the change in serum $K^+$ from Part B Baseline to Part B Week 4. Secondary outcomes included the proportion of subjects with a serum $K^+$ in the target range at Part A Week 4 and the proportion of subjects with recurrent HK in Part B. An additional outcome was the proportion of subjects with modified RAASi treatment due to recurring HK.

**Results:**

**Underline represents presenting author/disclosure.**




*Final presentation will include population characteristics; Part B endpoints and safety data.

The proportion of subjects with a serum $K^+$ in the target range (3.8 to < 5.1 mEq/L) at Part A Week 4 was 76% (95% CI 70, 81).

**Conclusions:** Results from Part A demonstrate the efficacy of patiromer in treating HK.

*Funding:* Pharmaceutical Company Support - Relypsa, Inc.

## SA-O1086

**Effects of Atorvastatin on Renal Function in Patients with Dyslipidemia and Chronic Kidney Disease: ASsessment of Clinical Usefulness in CKD Patients with Atorvastatin (ASUCA) Trial** Genjiro Kimura,[1] Kenji Ueshima,[2] Masato Kasahara,[2] Sachiko Tanaka,[2] Shinji Yasuno,[2] Akira Fujimoto,[2] Tosiya Sato,[3] Shinji Kosugi,[4] Kazuwa Nakao.[5] *[1]Asahi Rosai Hospital, Owariasahi, Japan; [2]Department of EBM Research, Institute of Advancement of Clinical and Translational Science, Kyoto University Hospital, Kyoto, Japan; [3]Department of Biostatistics, Kyoto University School of Public Health, Kyoto, Japan; [4]Department of Medical Ethics/Medical Genetics, Kyoto University School of Public Health, Kyoto, Japan; [5]Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

**Background:** The effects of a strong statin, atorvastatin, on renal function was examined in patients with dyslipidemia and CKD.

**Methods:** The ASUCA trial was a prospective, multi-center, open-labeled, randomized study to compare the reno-protective effects between lipid-lowering agents other than statins (control group, Group C) and atorvastatin (atorvastatin group, Group A) under diet therapy in 349 patients with dyslipidemia (LDL-C □ 100 mg/dL in subjects taking lipid-lowering agents other than statins or □ 140 mg/dL) and CKD (eGFR < 60 mL/min/1.73m$^2$). Target of LDL-C was less than 100 mg/dL. The primary endpoint was the change in eGFR after 2 years of treatment.

**Results:** The patients were randomly assigned to either Group C or A, and the FAS population consisted of 166 patients in Group C and 168 patients in Group A (mean age: 63.1 vs 63.2 y.o.; mean LDL-C: 145.9 vs 142.2 mg/dL; median urinary albumin/creatinine ratio: 29.1 vs 24.9 mg/gCr, respectively). LDL-C was significantly reduced in Group A compared with Group C. Changes in mean eGFR were -2.6 mL/min/1.73m$^2$ (from 54.4 to 51.9) in Group C and -2.3 mL/min/1.73m$^2$ (from 56.2 to 53.9) in Group A, showing no difference between the two groups (P=0.85). Similarly, the changes in albuminuria were not different.

**Conclusions:** The ASUCA trial indicates no significant difference between atorvastatin and non-statin in the effect on renal function after 2 years of treatment in patients with dyslipidemia and CKD.

*Funding:* Pharmaceutical Company Support - The ASUCA trial was funded by EBM Research Center, Kyoto University Graduate School of Medicine with an unrestricted grant from Pfizer Co., LTD.

## SA-PO1087

**A Randomised, Placebo-Controlled Trial of Oxpentifylline on Erythropoiesis Stimulating Agent Resistance in Anemic Patients with Chronic Kidney Disease: The HERO Trial** Sunil V. Badve.[1,2] *[1]Nephrology, Princess Alexandra Hospital, Brisbane, QLD, Australia; [2]The Australasian Kidney Trials Network, Brisbane, QLD, Australia.*

**Background:** Erythropoiesis stimulating agent (ESA)-resistant anemia is common in chronic kidney disease (CKD) and associated with increased risks of hospitalization, cardiovascular events and mortality. Oxpentifylline shows promise as a treatment for ESA-resistant anemia but has not been rigorously evaluated.

**Methods:** In this multi-centre, prospective, double-blind, randomised, placebo-controlled trial, adult patients with stage 4 or 5 CKD, and ESA-resistant anemia (defined as hemoglobin [Hb] concentration □120 g/L and ESA resistance index [ERI] □1.0 IU/kg/week/g Hb for erythropoietin-treated patients and □0.005 mg/kg/week/g Hb for darbepoeitin-treated patients), were randomized to oxpentifylline (400 mg daily; N=26) or matching placebo (control N=27) for 4 months. The primary outcome was ERI. Secondary outcomes were Hb concentration, ESA dosage, iron stores, blood transfusion requirement, adverse events, costs and quality of life.

**Results:** ERI was not significantly different between the two groups, although the mean value was 15% lower in oxpentifylline-treated patients (adjusted mean difference -0.39 IU/kg/g, 95% CI -0.89 to 0.10, p=0.12). Hb concentrations were significantly increased in the oxpentifylline group (mean difference 7.6 g/L, 95% CI 1.7 to 13.5, p=0.01), despite no significant change in ESA dosage (-20.8 IU/kg/week, 95% CI -67.2 to 25.7, p=0.37). No interaction was observed for treatment group by C-reactive protein sub-group. No differences were observed between the two groups with respect to blood transfusion requirements or adverse events.

**Conclusions:** Oxpentifylline did not significantly modify ESA resistance, but did safely increase Hb concentration without a change in ESA dosage. Oxpentifylline offers a method by which Hb concentration can be increased in ESA-resistant anaemia without needing to increase ESA dose.

*Funding:* Pharmaceutical Company Support - Roche Foundation for Anaemia Research (RoFAR), Amgen, Janssen-Cilag, Government Support - Non-U.S.

## SA-PO1089

**Risk and Severity of Hyperkalemia with Combined Angiotensin Antagonism in Diabetic Nephropathy – The VA-NEPHROND Study** Stephen L. Seliger,[1] Jane Hongyuan Zhang,[4] Nicholas Emanuele,[3] Paul M. Palevsky,[2] Linda F. Fried.[2] *[1]Medicine, Baltimore VA, Baltimore, MD; [2]Medicine, Pittsburgh VA, Pittsburgh, MD; [3]Medicine, Hines VA, Hines, IL; [4]Coordinating Ctr, VA Co-Operative Studies, West Haven, CT.*

**Background:** Greater angiotensin antagonism may increase the risk of hyperkalemia in renal disease. Few studies have compared the risk and severity of hyperkalemia with dual-angiotensin antagonism in diabetic nephropathy patients.

**Methods:** We performed a secondary analysis of the VA-NEPHROND study, a randomized clinical trial comparing losartan monotherapy to losartan + lisinopril combination therapy in patients with type 2 diabetes, overt albuminuria, and stage 2-3 CKD. Hyperkalemia was a pre-specified safety outcome defined as a potassium>6 mEq/L or requiring emergency care or dialysis. Cox survival regression was used to identify factors predictive of hyperkalemia, and to test for interaction between treatment group and clinical factors. Severity and management of hyperkalemia was compared between the groups.

**Results:** Among 1448 participants, there were 104 with hyperkalemic events over a median 2.2 years. Those randomized to losartan+lisinopril had a 177% greater risk of hyperkalemia (HR=2.77, 95%CI=1.79, 4.28). This excess risk from combination therapy was not modified by race, baseline potassium, heart failure, use of diuretics, albuminuria, eGFR, use of insulin, or HbA1C (p>0.2 for all tests of interaction). Among all participants, other factors associated with hyperkalemia were initial eGFR (per mL/min/1.73m$^2$: HR=0.97, p<.001) and initial potassium concentration (per 1-mEq/L increment, HR=3.78, p<.001). Among hyperkalemic cases, the losartan + lisinopril group had higher peak potassium (6.60±0.50 vs. 6.33±0.56 mEq/L, p=0.017), were more likely to be referred for emergency care (69% vs. 50%, p=.057), to be treated with IV calcium (25% vs. 9.4%, p=.07) and insulin (34.7% vs. 15.6% p=.047), and to have study medications permanently discontinued (40.4% vs. 18.8%, p=.033).

**Conclusions:** Dual angiotensin antagonism in overt diabetic nephropathy markedly increases both the risk and severity of hyperkalemia, and this excess risk is similar regardless of renal function, co-morbdiity, initial potassium and concurrent diuretic use.

*Funding:* Veterans Administration Support

## SA-PO1090

**A Comparison of Short-Term Exposure of Once-Daily Extended Release Tacrolimus (Advagraf®) and Twice-Daily Tacrolimus (Prograf®) on 24 Hour Renal Hemodynamics and Function in Healthy Volunteers** David Cherney, Jeffrey S. Zaltzman. *University of Toronto.*

**Background:** Calcineurin inhibitor (CNI) nephrotoxicity remains an ongoing concern in organ transplant recipients, however the pathophysiology of this nephrotoxicity is unclear. Previous studies have demonstrated that after administration of Neoral® there is a strong correlation between $C_{max}$ and decreases in renal perfusion and GFR, possibly contributing to long-term nephrotoxicity. This response is attenuated with both tacrolimus formulations, the twice-daily Prograf®(P) and the once daily Advagraf®(A), the latter demonstrating similar $AUC_{24}$, but a different PK profile, with a lower $C_{max}$ and no second peak.

**Methods:** Healthy, normotensive, adult male volunteers (n=19) were randomized to receive once daily A and twice daily P in a prospective, randomized, open label, cross over design. Baseline and drug studies included 24 hr effective renal plasma flow (ERPF), and 24 hr GFR by PAH and inulin clearances. Renal blood flow (RBF), filtration fraction (FF) and renal vascular resistance (RVR) were then calculated and all measurements were repeated over a number of time points throughout the 24 hour period. Tacrolimus dosing was initiated at 0.15 mg/kg/d with TDMs titrated to [$C_0$] of 8-12 ng/ml. All studies were repeated on day 10 and 20 of A/P or P/A.

**Underline represents presenting author/disclosure.**

**Results:** Despite similar CNI exposure, RBF remained higher in the Advagraf versus the Prograf phase of the trial (p=0.037, Table 1).

| | Baseline | Prograf | Advagraf |
|---|---|---|---|
| [Co]ng/ml | - | 11.3±1.8 | 10.9±2.2 |
| AUC (24 hour*mg/ml/1.73m2) | - | 385±79 | 410±66 |
| ERPF (24 hour*ml/min/1.73m2) | 14468±1648 | 14544±2125 | 15761±2985* |
| RBF (24 hour*ml/min/1.73m2) | 24834±3037 | 24306±2987 | 26506±4703** |
| GFR (24 hour*ml/min/1.73m2) | 2625±202 | 2598±217 | 2751±327 |
| FF (24 hour) | 4.4±0.4 | 4.4±0.7 | 4.3±0.8 |
| RVR (24 hour*mmHg/L/min/1.73m2) | 2.03±0.33 | 2.11±0.41 | 1.96±0.46 |

* p=0.046, **p=0.037 for advagraf vs prograf

**Conclusions:** Renal hemodynamic effects characteristic of CNI are attenuated with Advagraf® compared to Prograf®, which may correlate with the differing PK profile of these CNIs. Possible explanations for apparent improvements over baseline are being investigated.

*Funding:* Pharmaceutical Company Support - Astellas

## SA-PO1091

**Changes in Cardiac Structure and Function after Revascularization versus Medical Therapy for Renal Artery Stenosis: The ASTRAL Heart Echocardiography Sub-Study** Darren Green,[1] Natalie Ives,[2] Kelly Handley,[2] Philip A. Kalra.[1] *[1]Salford Royal Hospital, United Kingdom; [2]University of Birmingham, United Kingdom.*

**Background:** The ASTRAL trial showed no difference in clinical outcomes between medical therapy and revascularization for atherosclerotic renal vascular disease (ARVD). Here we report a sub-study using echocardiography to assess differences in cardiac structure and function at 12 months.

**Methods:** ASTRAL patients from 7 participating centres underwent echocardiography at baseline and 12 months after randomization. Changes (mean ± standard deviation) in left ventricular ejection fraction (LVEF), left ventricular mass (LVM), left atrial diameter (LAD), aortic root diameter (AoRD), E:A and E deceleration time (EDT) were compared between study arms.

**Results:** 92 patients were included (50 medical vs 42 revascularization). There were no statistical differences in baseline co-morbidities and clinical characteristics between the groups (mean age 71 vs 70 years, eGFR 43 vs 45mL/min, systolic blood pressure 152 vs 146 mmHg, number of antihypertensives 3.0 vs 2.9) except that more medical patients were on a statin (93% versus 76%, p=0.03).

Echocardiography showed no statistical difference between arms in any echocardiographic parameter at baseline (LVEF medical 54±11% vs revasc 54±9%, LVM 203±37g vs 202±34g, LAD 3.8±0.5cm vs 3.9±0.5cm). Change in LVEF at 12 months was greater in medical patients: ΔLVEF medical 0.8±8.7% vs revasc -2.8±6.8% (p=0.049). In a multivariate model including age, blood pressure, renal function, degree of stenosis, beta blockade and ACE-inhibitor use this was no longer significant. There were no significant differences between arms for: ΔLVM -2.9±33.1 vs -1.7±38.9g, ΔLAD 0.1±0.4 vs 0.01±0.5cm, ΔAoRD 0.002±0.3 vs 0.1±0.3cm, ΔE:A 0.0±0.6 vs 0.03±0.7, ΔEDT -1.1±55.5 vs -9.0±70.2ms.

**Conclusions:** This sub-study did not show any significant differences in cardiac structure and function accompanying renal revascularization in ASTRAL. Limitations include sample size, relative insensitivity of echocardiography, as well as clinical heterogeneity of the patient population as described in the main study.

This abstract is presented by the authors on behalf of the ASTRAL Heart Collaborators.

## SA-PO1092

**Four-Week Safety, Efficacy and Pharmacodynamic Study of Hypoxia-Inducible Factor (HIF)-Prolyl Hydroxylase Inhibitor GSK1278863 in Anemic Hemodialysis Subjects Switching from Recombinant Human Erythropoietin** Amy M. Meadowcroft, Louis Holdstock, Rayma Maier, Delyth Jones, Brendan Johnson, Alexander Ralph Cobitz, John J. Lepore. *GlaxoSmithKline, Research Triangle Park, NC.*

**Background:** Hypoxia inducible factor (HIF)-prolyl hydroxylase inhibitors (PHIs), such as GSK1278863, are an emerging class of oral agents for treatment of chronic kidney disease (CKD) anemia. PHIs inhibit HIF-prolyl hydroxylases, resulting in accumulation of HIFα transcription factor, thus stimulating pathways activated during hypoxia, including erythropoiesis and iron mobilization.

**Methods:** This 4-week, randomized, blinded (with respect to GSK1278863 dose), active-controlled study examined the relationship between GSK1278863 dose and hemoglobin (Hgb) level in 83 hemodialysis-dependent (HDD) subjects with anemia of CKD previously receiving a stable dose of recombinant human erythropoietin (rhEPO). Effects of GSK1278863 on circulating levels of erythropoietin (EPO) and vascular endothelial growth factor (VEGF) were also explored. Subjects with a stable baseline Hgb of 9.5-12.0g/dL were randomized to stop rhEPO and receive once daily 0.5, 2 or 5mg GSK1278863 or to continue on rhEPO.

**Results:** Baseline Hgb (overall mean 10.8g/dL) was similar across treatment groups. Switching from rhEPO to 5 mg GSK1278863 had a similar effect on Hgb as continuing rhEPO, maintaining stable Hgb over 4 weeks (mean change from baseline at Week 4: 5mg: -0.08g/dL; rhEPO: -0.25g/dL), whereas switching to 0.5 or 2 mg GSK1278863 resulted in a decline in Hgb (0.5mg: -1.06g/dL; 2mg: -0.93g/dL). The maximum observed circulating EPO levels with GSK1278863 were markedly lower than those in the rhEPO group (0.5mg: 13.9U/L, 2mg: 12.7U/L; 5mg: 24.7U/L; rhEPO: 424.9U/L). No significant change in VEGF was observed in any group. GSK1278863 was safe and well tolerated.

**Conclusions:** These data inform the Hgb dose-response relationship of GSK1278863 in anemic HDD subjects after being switched from a stable dose of rhEPO and demonstrate that GSK1278863 can maintain Hgb with minimal effects on EPO levels and without significantly elevating VEGF.

*Funding:* Pharmaceutical Company Support - GlaxoSmithKline

## SA-PO1093

**Randomized Controlled Trial on the Effects of a Six-Month Intradialytic Physical ACTIvity Program and Adequate NUTritional Support on Protein-Energy Wasting, Physical Functioning and Quality of Life in Chronic Hemodialysis Patients ACTINUT** Dan Hristea,[1] Anne Paris,[1] Justine Magnard,[2] Thibault Deschamps.[2] *[1]Dialysis, ECHO, Nantes, France; [2]STAPS, Nantes, France.*

**Background:** The aim of this randomized controlled trial was to investigate the effects of a six-month intra-dialytic physical ACTIvity program and adequate NUTritional support on protein-energy wasting (PEW), physical functioning and quality of life in chronic hemodialysis patients.

**Methods:** 210 patients of 2 dialysis units in Nantes, France were screened for the presence of PEW according to criteria of the ISRNM. 20 Patients were randomized in an exercise and nutrition group (Ex +N) or nutrition only group (N). In both groups the prescription of nutritional supplements (oral or IV) were adapted according to the dietary record in order to attain goals set by the European guidelines for nutrition in terms of protein and energy intake. In addition in EX+N group patients a 6-months progressive submaximal intra-dialytic cycling exercise program was conducted. Primary outcome was to compare the number of patients having reached remission of PEW in N+EX vs. N group at 6 months.

**Results:** 20 patients (11 men) were randomized. Mean age was 70. 9 years (range 52 – 89) in Ex+ N and 63.6 (50-92) in N group.

Preliminary results at 2 months:

No serious adverse effects occurred. No significant effect on nutritional (albumin, prealbumin) or anthropometric parameters (lean body mass measured by bioimpedance spectroscopy, body mass index) could be observed in the two groups. Performance in the six-minute walk test increased in N+EX group from (217 ± 102.92 *vs* 257.75 ± 83.37 meters; p=0.04) and tended to decrease in N group. Moreover a significant increase of the Physical (PCS)(+26.27% ; p=0.005) and Mental (+ 22.64% ) Component Scale of the SF-36 was observed in N+EX patients while N patients had a significant decrease of PCS (-18.14% ; p=0.01).

**Conclusions:** Combining intradialytic exercise and nutritional support is safe and increases significantly physical performances and quality of life in malnourished frail dialysis patients. The successful completion of this current trial may give precious clues about the potential of exercise in the treatment of PEW.

## SA-PO1094

**The Pharmacokinetics of Laquinimod and Mycophenolate Mofetil during Treatment of Active Lupus Nephritis** David R.W. Jayne,[1] Gerald B. Appel,[2] Daniel Tak Mao Chan,[3] Dorit Mimrod,[4] Ofer Spiegelstein,[4] Hadas Barkay,[4] Roberta Weiss,[5] David Wofsy.[6] *[1]Addenbrooke's Hospital, London, United Kingdom; [2]Columbia University, New York, New York; [3]University of Hong Kong, Hong Kong; [4]Teva Pharmaceuticals, Netanya, Israel; [5]Teva Pharmaceuticals, Frazer, PA; [6]University of California at San Francisco, San Francisco, CA.*

**Background:** A recently completed phase 2a trial evaluated the safety, tolerability & clinical effect of two doses of laquinimod (LAQ, 0.5mg and 1.0mg) vs. placebo in patients with active Lupus Nephritis (LN) receiving mycophenolate mofetil (MMF) and corticosteroids. Although the trial was not powered to conclusively assess efficacy, percent change from baseline to week 24 suggest possible improvement in eGFR (placebo vs. 0.5mg vs. 1mg LAQ: 12.1±20.2 vs. 18.0±30.7 vs. 24.3±28.8), and 24-h UPCR (-23.1±74.7 vs. -49.0±62.1 vs. -46.7±37.0, respectively). There was no difference between treatment groups in frequency of AEs or SAEs. Here, we describe analyses between renal function and the PK of LAQ in patients with active LN and the influence of LAQ on the PK of MMF.

**Methods:** Patients with active LN were randomized to 24 wk treatment with LAQ 0.5mg/day (n=16), LAQ 1mg/day (n=15), or placebo (n=15). Plasma levels of LAQ and MPA (mycophenolic acid) were obtained at week 4.

**Results:** LAQ exposure in LN patients was similar to previously observed values in multiple sclerosis patients. No meaningful change of LAQ PK parameters (Cmax, Cmin, $AUC_{0-24}$) was seen across renal function categories (6 patients normal renal function; 9 mild renal insufficiency, 9 moderate, and 5 severe). Plasma MPA exposure (mean±SD of Cmax [μg/mL], Cmin [μg/mL], and $AUC_{0-12}$ [h*μg/mL]) was similar in placebo (12.5±5.4, 2.50±2.5, 38.6±19.3) and LAQ-treated patients at doses of 0.5mg (12.9±6.7, 3.24±5.2, 36.7±15.7) and 1.0mg (13.0±9.5, 2.67±2.0, 42.7±19.6), respectively.

**Conclusions:** Laquinimod pharmacokinetics was not affected by renal insufficiency nor altered by the co-administration of MMF. MMF pharmacokinetics was unchanged in the presence of LAQ, suggesting that LAQ can be safely given with MMF and corticosteroids for treating patients with active LN without requiring dose modification.

*Funding:* Pharmaceutical Company Support - Teva Pharmaceuticals

**Underline represents presenting author/disclosure.**

**SA-PO1095**

## Universal Prophylaxis for Cytomegalovirus in Pediatric Kidney Transplantation in Developing Country: Medellin, Colombia

Liliana Rubio, Jorge Henao, Joaquin Rodelo Ceballos. *Pediatric Nephrology, Hospital Universitario San Vicente Fundacion, Medellin, Antioquia, Colombia.*

**Background:** Cytomegalovirus affects more than 50% of transplant recipients, is one of most common complications, including infection with opportunistic pathogens and allograft rejection.

Objetive determine the effectiveness of prophylaxis with ganciclovir and valganciclovir for 3 months in pediatric kidney transplantation in one year of follow-up because data in pediatrics are limited.

**Methods:** This prospective cohort study included 34 kidney transplant, between December 2005 and April 2013. 82,4% and 100% respectively, received iv ganciclovir, 10mg/kg/day, dose 166 mg/day during 9,5 days followed by valganciclovir, using the following formula: (mg) = 7 x body surface area ($m^2$) x creatinine clearance (mL/min per 1, 73 $m^2$) the dose was 462mg day for 99 days, adjusted for renal function.

**Results:** (71%) were males, median age was 8 years and 97% were the cadaveric origin. Most kidney transplant recipients received induction therapy with thymoglobulin (n=27) other induction therapy included basiliximab (n=7). Maintenance immunosuppression consisted of tacrolimus, prednisone plus mycophenolate; Regarding CMV antibody status, 25 patients (73,5%) were D+/R+, 4(11,7%) were D-/R+, and 5 (14,8%) were D+/R-).5,8 % patients with serology D+/R- had CMV disease and 5,8% with serology D-/R+ had CMV disease for an incidence of 11,76 % and the late- onset CMV disease was 8%, The bivariate analysis showed that the highest risk group for CMV disease was D+ R-.(p=0.05).

| Variable | | Disease n (%) | No Disease n (%) | Chi squared | Significance |
|---|---|---|---|---|---|
| IgG Donante CMV | positive | 2 (50%) | 28 93,3 % | 6,38 | 0.05 |
| | negative | 2 (50%) | 2 6,7 % | | |
| IgG receptor CMV | positiva | 2 (50%) | 27 90 % | 4,5 | 0.09 |
| | negativa | 2 (50%) | 3 10 % | | |
| Dose timoglobulin | | 2,67 DS(0,57) | 3,40 DS(0,9) | 1,34 | 0.19 |

There was no mortality or graft loss secundary to infection, either resistance and difference in creatinine in the 1 year.

**Conclusions:** The main preventive strategy against CMV in pediatric renal transplantation is the universal prophylaxis and extension to 200 days is a benefit to prevent late onset CMV disease.

**SA-PO1096**

## Acute Kidney Injury (AKI) Associated with Combined Angiotensin Antagonism in Patients with Diabetic Nephropathy – A Secondary Analysis of the VA NEPHRON-D Study

Paul M. Palevsky,[1] Jane Hongyuan Zhang,[2] Stephen L. Seliger,[3] Nicholas Emanuele,[4] Linda F. Fried.[1] *[1]VA Pittsburgh HCS, Pittsburgh, PA; [2]CSP Coordinating Center, VA Connecticut HCS, West Haven, CT; [3]VA Maryland HCS, Baltimore, MD; [4]Hines VAMC, Hines, IL.*

**Background:** Dual blockade of the renin-angiotensin system (RAS) is associated with an increased risk of acute kidney injury (AKI); however the etiology, severity and outcome of AKI have not been carefully evaluated.

**Methods:** We performed a secondary analysis of the VA NEPHRON-D study, a RCT comparing losartan monotherapy (MT) to losartan/lisinopril combination therapy (CT) in patients with type 2 diabetes, overt albuminuria, and stage 2-3 CKD. Data were collected on etiology, severity and outcomes of episodes of AKI. Cox regression was used to identify predictive factors and test interactions with treatment group.

**Results:** 295 episodes of AKI occurred in 210 of 1448 (14.1%) study participants; 105 in 80 MT patients (6.7 events/100 person-years) and 190 in 130 CT patients (12.2 events/100 person-years; p<0.001). The maximal serum creatinine was <2x baseline in 54.4% of episodes in MT and 57.6% of episodes in CT (p=0.18); 12 episodes required dialysis in MT and 17 in CT. AKI was attributed to prerenal azotemia in 73.1% and 76.3%, respectively, and to ATN in 24.0% and 24.7% of episodes, respectively, in MT and CT. Predictors of AKI included albuminuria >1 g/g creatinine, lower baseline eGFR, and treatment group. Recovery of kidney function was similar in both arms, although mortality after AKI was higher in MT patients (15.0% vs. 4.7%; p=0.009). Patients who developed AKI were more likely to progress to the primary study endpoint of death, ESRD or CKD progression (HR: 1.78; 95% CI: 1.34-2.36; p<0.001). Among patients with AKI, the hazard for the endpoint was lower with CT than MT (HR: 0.60; 95% CI: 0.37-0.98; p=0.04).

**Conclusions:** Dual blockade of the RAS increases the risk but not the severity of AKI in patients with overt diabetic nephropathy as compared to monotherapy. Although the hazard for death, ESRD or CKD progression was higher in patients on monotherapy who developed AKI, the higher frequency of AKI in the combination therapy arm may have attenuated its potential benefit on progression of CKD.

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**Underline represents presenting author/disclosure.**